ENCYCLOPEDIA OF TOXICOLOGY, FOUR-VOLUME SET, 1-4

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Second Edition

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Description

The second edition of the **Encyclopedia of Toxicology** continues its comprehensive survey of toxicology. This new edition continues to present entries devoted to key concepts and specific chemicals. There has been an increase in entries devoted to international organizations and well-known toxic-related incidents such as Love Canal and Chernobyl. Along with the traditional

scientifically based entries, new articles focus on the societal implications of toxicological knowledge including environmental crimes, chemical and biological warfare in ancient times, and a history of the U.S. environmental movement. With more than 1150 entries, this second edition has been expanded in length, breadth and depth, and provides an extensive overview of the many facets of toxicology. Also available online via ScienceDirect – featuring extensive browsing, searching, and internal cross-referencing between articles in the work, plus dynamic linking to journal articles and abstract databases, making navigation flexible and easy. For more information, pricing options and availability visit www.info.sciencedirect.com.

Overview of all books

Audience
Toxicologists, pharmacologists, drug companies, toxicology testing labs, libraries, poison control centers, physicians, legal and regulatory professionals (EPA, government), and chemists.

Contents
Esterases Absorption Academy of Toxicological Sciences Acceptable Daily Intake (ADI) Accutane ACE Inhibitors Acenaphthene Acephate Acetaldehyde Acetamide Acetaminophen Acetamiprid Acetic Acid Acetone Acetonitrile Acetylaminofluorene Acetylcholine Acetylene Acetylsalicylic Acid Acids Aconitum Species Acrolein Acrylamide Acrylic Acid Acrylonitrile Adamsite Adiponitrile Aerosols Aflatoxin Agency for Toxic Substances and Disease Registry Agent 15 Agent Orange Aggregate Exposures Alachlor Alar Albuterol Alcoholic Beverages and Alcoholism Aldicarb Aldrin Algae Alkalies Alkyl Halides Allyl Alcohol Allyl Formate a-Methylfentanyl a-Naphthyl Thiourea Aluminum (Al) Aluminum Phosphide Amdro American Academy of

Clinical Toxicology American Association of Poison Control Centers American Board of Toxicology American College of Medical Toxicology American College of Toxicology American Conference of Governmental Industrial Hygienists American Industrial Hygiene Association Ames Test 4-Aminobiphenyl Aminoglycosides 4-Aminopyridine Amiodarone Amitraz Ammonia Ammonium Nitrate Ammonium Perchlorate Amphetamine Amphibians Amyl Nitrate Anabolic Steroids Analytical Toxicology Androgens Anesthetic Agents Aniline Animal Models "Animals, Poisonous and Venomous" Antagonism Anthracene Anthrax Anticholinergics Antimony (Sb) Antimony Trioxide Anxiolytics Apoptosis Aquatic Ecotoxicology Aramite Arsenic (As) Arsine Arum Asbestos Ascorbic Acid Aspartame Astemizole Atrazine Atropine Avermectin Avian Ecotoxicology Azamethiphos Azothioprine Azinphos-Methyl Bacillus cereus Bacillus thuringiensis BAL (British Antilewisite) Baneberry "Barbiturates, Long-Acting" "Barbiturates, Short-Acting" Barium (Ba) Baycol Baygon BCNU (Bischloroethyl Nitrosourea) Behavioral Toxicology Belladonna Alkaloids Benadryl Benchmark Dose Benomyl Benzene Benzene Hexachloride Benzidine Benzo(ghi)perylene Benzodiazepines Benzo[a]pyrene Benzyl Alcohol Benzyl Benzoate Benz[a]anthracene Beryllium (Be) Beta Blockers Biguanides Bioaccumulation Biocompatibility Bioconcentration Bioinformatics Biological Exposure Index Biological Warfare and Terrorism: Toxins and Other Agents Biomagnification "Biomarkers, Environmental" "Biomarkers, Human Health" Biomonitoring Biotransformation Bismuth (Bi) Bisphenol A Bleach Blood Boric Acid Boron (B) Botulinum Toxin Brodifacoum Bromethalin Bromine Bromobenzene Bromobenzylcyanide Bromadialone Bromoform Bromotrichloromethane

x

Phosphoric Acid Phosphorus Photoallergens Photochemical Oxidants Phthalate Ester Plasticizers Physical Hazards Picloram Picric Acid Piperazine Piperonyl Butoxide "Plants, Poisonous" Platinum (Pt) Plutonium (Pu) Poinsettia Poisoning Emergencies in Humans Pokeweed Pollutant Release and Transfer Registries (PRTRs) Pollution Prevention Act "Pollution, Air" "Pollution, Air Indoor" "Pollution, Soil" "Pollution, Water" Polybrominated Biphenyls (PBBs) Polybrominated Diphenyl Ethers (PBDEs) Polychlorinated Biphenyls (PCBs) Polycyclic Aromatic Amines Polycyclic Aromatic Hydrocarbons (PAHs) Polyethylene Glycol Polymers Potassium (K) Potassium Iodide Primidone Procainamide Prometryn Propachlor Propane Propanil Propargite Propazine Propene Propionic Acid Proposition 65 Propoxur Propoxyphene Propylene Glycol Propylene Oxide Prostaglandins Proteomics Prunus Species Pseudoephedrine Psychological Indices of Toxicity Public Health Service Puromycin PUVA Pyrene Pyrethrins/Pyrethroids Pyridine Pyridostigmine Pyridoxine Pyriminil Pyrrolizidine Alkaloids QT Interval Quinidine Quinine Quinoline Quinone "Radiation Toxicology, Ionizing and Non-Ionizing" Radium Radon Ranitidine Red Dye No. 2 Red Phosphorous Red Squill Red Tide Reference Concentration (RfC) Reference Dose (RfD) "Reproductive System, Female" "Reproductive System, Male" Research Institute for Fragrance Materials (RIFM) Reserpine Resistance to Toxicants Resource Conservation and Recovery Act Respiratory Tract Rhodium Rhododendron Genus Rhubarb Riboflavin Rifampin "Risk Assessment, Ecological" "Risk Assessment, Human Health" Risk Characterization Risk Communication Risk Management Risk Perception Rotenone Saccharin Safe Drinking Water Act Safety Pharmacology Saint John's Wort Salicylates Salmonella Sarin Saxitoxin Scombroid

"Toxicity Testing, Mutagenicity" "Toxicity Testing, Reproductive" "Toxicity Testing, Sensitization" "Toxicity, Acute" "Toxicity, Chronic" "Toxicity, Subchronic" Toxicology "Toxicology, Education and Careers" "Toxicology, History of" Trade Associations Transgenic Animals Triadimefon Trichlorfon Trichloroethane Trichloroethylene Tricyclic Antidepressants Trifluralin Trihalomethanes Trinitrotoluenes Tungsten Turpentine Uncertainty Analysis Uncertainty Factors UNEP Chemicals Uranium (U) Urea Urethane United States Pharmacopoeia (USP) V-Gas Valproic Acid Vanadium Vanillin VE Veterinary Toxicology VG Vinyl Acetate Vinyl Bromide Vinyl Chloride Vinylidene Chloride Virtually Safe Dose (VSD) Vitamin A Vitamin D Vitamin E VM Volatile Organic Compounds (VOC) VX Warfarin Wisteria Workplace Environmental Exposure Levels (WEELs) Xenobiotics Xylene Xyrem Yew Yohimbine Zinc (Zn) Zinc Oxide "Safety Testing, Clinical Studies" "Toxicity Testing, Validation" Genetically Engineered Products Global Environmental Change Pharmaceuticals in the Environment Aneuploidy Tacrine Selamectin Minamata Great Smog of London Itai-Itai N-methylpyrrolidone Peptide Coupling Agents DNA Phosphoramidites Occupational Exposure Limits Arts and Crafts Materials and Processes National Center for Environmental Health (NCEH) Curare Department of Defense Diazoaminobenzene Department of Energy (DOE) Drinking Water Criteria Environmental Crimes Grain Incidents Iatrogenic Disease Immediately Dangerous to Life and Health (IDLH) values "Hazardous Chemicals, Import/Export of" Islip Garbage Barge Mad Cow Disease Oxalates Perfluorooctanoic Acid (PFOA) Persisent Organic Pollutants (POPs) Risk Based Corrective Action (RBCA) Recommended Exposure Limits (REL) Sulfates Texas City Disaster United States Department of Agriculture (USDA) Silent

Spring Love Canal Exxon Valdez Donora Chernobyl Wildlife Toxicology Three Mile Island Cuyahoga River Material Safety Data Sheets and Chemical Hazard Communication Society for Chemical Hazard Communication Killer Lakes Times Beach Valley of the Drums Perfluoroisobutene Riot Control Agents Redbook European Centre for Ecotoxicology and Toxicology of Chemicals Toxicology Excellence for Risk Assessment (TERA) Estrogen Mimics "S-(1,2-dichlorovinyl)-L-cysteine" "Diabetes, Effect of Toxicity" Fetal Alcohol Syndrome Heat Shock Proteins Cell Cycle Trans Fatty Acids Biocides Alkanolamines Lanthanide Series of Metals International Organization of the Flavor Industry (IOFI) International Union of Pure and Applied Chemistry Cesium Nanotechnology Nails (of the Fingers and Toes) Famous Poisoners and Poisoning Cases "Regulation, Toxicology and" "Toxicology, Intuitive" Hair Methyl Isocyanate Bhopal Seveso Ancient Warfare and Toxicology Inert Ingriedients Bioremediation Bioremediation Cancer Chemotherapeutic Agents Homobatrachotoxin Chemical Warfare During WW1 Chemical Warfare Delivery Systems Toxicology Forum Nerve Agents Blister Agents/Vesicants G-Series Nerve Agents V-Series Nerve Agents: Other than VX

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Editor-in-Chief

Philip Wexler, *National Library of Medicine, Bethesda, MD, USA*

Associate Editors

Dedication

For my son Jake and my parents Yetty and Will, with love, appreciation, and respect.

FOREWORD

It gives me great pleasure to once again have the opportunity to introduce the *Encyclopedia of Toxicology* to its users. The second edition is a worthy successor to the first, expanded and refined, which will serve the toxicology community well. Particularly in these days when specialization tends to narrow the individual focus, it brings a real understanding of the entire scope and function of the science of toxicology.

The changes evident at the publication of the first edition have continued at an accelerated pace. At that time it was clear that toxicology, over a period of four or five decades, had changed from a largely descriptive science based on *in vivo* toxicity to one that included all aspects of modern biology and chemistry, from molecular biology to sophisticated instrumental analysis. The philosophical basis had shifted from routine risk analysis based primarily on pathological or *in vivo* toxicological endpoints to one that emphasized mechanisms of toxic action at the organ, cellular, and molecular levels. All of this brought about an explosion in the toxicological literature.

Since then, the techniques of molecular biology have played an increasing role in the elucidation of toxic mechanisms, in the study of xenobiotic metabolism, in the development of safer and more useful drugs and other chemicals, and in the development of biomarkers of exposure and effect, to mention only a few of the more important aspects impacted by these techniques. Analytical chemistry has continued to develop to the point that vanishing small quantities of xenobiotics can be detected, quantities so small that their toxicological impact is likely to remain unknown for the immediate future. While the application of all of this new science to risk assessment remains problematical, since the latter is still largely based on mathematical models rather than toxicological science, progress in both human health risk assessment and environmental risk assessment is also evident.

What has not changed, however, is the need for the toxicological literature to serve many masters. Given the eclectic nature both of the methodological roots and the practical needs served by toxicology, general works are needed more than ever. Works such as the *Encyclopedia of Toxicology* play a critical role at an important intermediate level, more detailed than dictionaries while remaining accessible to the generalist in risk assessment, regulation, teaching, and consultation as well as specialists seeking information beyond the narrow confines of their specialty. It will also serve as an important role for nontoxicologists who need to know more of the philosophy, methods, and uses of this science.

In summary, this is an important and outstanding contribution that no serious toxicologist or library serving toxicologists can afford to be without.

Ernest Hodgson
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PREFACE

Time passes, but the need for toxicological understanding persists. As much as we might wish for the end of poverty, ignorance, hunger, and exposure to hazardous chemicals, and as much as we work toward these goals, the challenges are formidable, and the end is not in sight. Chemicals and finished products made from chemicals continue to play an ever-present part in our lives. Although it is not evident that the benefits of chemicals always outweigh their risks, there is little doubt that a wide spectrum of chemicals and drugs has enhanced both the duration and quality of our lives. That said, certain of them, in certain situations, are clearly harmful to certain people. Among the fruits of toxicologists' labors is information on how best to eliminate, reduce, or prevent such harm.

The discipline of toxicology has made considerable strides in the 7 years since the first edition of this encyclopedia was published. The understanding of molecular toxicology continues to advance rapidly. Indeed, it is often much easier to generate the data than to find the time to adequately evaluate it. Genomic, proteomic, and other 'omic' technologies are helping us unravel the complex connection between exposure to environmental chemicals and susceptibility to disease. The US National Center for Toxicogenomics, dedicated to research on informatics and computational toxicology, was established in 2000. As a result of this and other research, much more sophisticated approaches are now available for ascertaining chemical safety, and investigating structure–activity relationships. In addition, analytical instrumentation has become more highly refined and sensitive, making it easier to detect and quantitate even smaller amounts of contaminants in biological systems and the environment.

With greater consumer (especially Western) acceptance of complementary and alternative medicine, more people than ever before are being exposed to a vast array of herbal and other plant-based medicinal products. Although toxicologists have always recognized that 'natural' does not necessarily equate with 'safe', not much has been done to assess the hazards of herbal supplements and their interactions with other chemicals. This is beginning to change.

Chemical, biological, and nuclear warfare have always been subjects of interest, sometimes as practical matters, and more often as academic ones. In the light of the events of September 11, 2001, there has been an increased urgency in learning more about nonconventional warfare and its agents, how they operate, and how to protect ourselves from their effects. Toxicology has found itself broadening its scope to deal with this resurgent type of weaponry.

The scope of what constitutes hazards waste, an ever-present downside of the benefits we derive from the manufacture, processing, and use of chemicals and their products, continues to expand as technology moves forward. In the US two million tons of electronic products, including 50 million computers and 130 million cellphones, are disposed of every year. According to the International Association of Electronic Recylers, this number will more than triple by 2010. With such quantities in landfills and rivers, there are bound to be consequences for our air and water. Potential toxicants include lead, cadmium, and beryllium.

Alternatives to animal studies no longer represent a toxicological sideline. While whole animal testing is unlikely to disappear soon, if ever, other methods of determining hazard and safety are increasingly being embraced by the toxicology community and becoming part of mainstream chemical evaluations. *In vitro* approaches (e.g., using cell culture or skin irritation potential) and *in silico* approaches (i.e., using computer programs to estimate toxic properties based on existing data for similar chemicals with or without supplemental chemical and physical property data) are both generating increasing amounts of toxicity information.

The marketplace is seeing an increase in products utilizing nanotechnologies, and nanotechnology research and development is on the upswing. The United States has had an official National Nanotechnology Initiative since 2001. A start has also been made by federal agencies and universities in assessing the environmental and health effects of nanomaterials.

Greater insight into chemical exposures, both actual and anticipated, is helping to develop a more focused picture of the risks these exposures present to humans and the environment. Growing cooperation between toxicologists and exposure assessors is proving vital to strengthening the scientific basis of risk assessment, thus giving risk assessors and managers more credible tools to address the control of chemical hazards.

At the global level, there have been important strides in the control and management of chemicals. The 10-year followup to the Rio Earth Summit, the World Summit on Sustainable Development, was held in 2002 in Johannesburg, South Africa. Among the targets it set was to use and produce chemicals by 2020 in ways that do not lead to significant adverse effects on human health and the environment.

The Stockholm Convention to protect human health and the environment from persistent organic pollutants (POPs) became binding on May 17, 2004. POPs tend to be toxic, persistent, accumulative, and capable of traveling long distances in the environment. This Convention seeks to eliminate or restrict the production and use of such chemicals. The Kyoto Protocol, designed to decrease greenhouse gas emissions, has now become an international law, despite the resistance of several countries.

The United States hosts a vibrant and growing community of toxicology professionals who perform innovative toxicological research, and scientists in other countries are making their presence felt equally. Global information sharing and collaborations among these investigators are growing, facilitated by the increased accessibility of the Internet and its enhanced technologies. Significant work is proceeding under the auspices of multinational bodies such as Organisation for Economic Co-operation and Development, the European Commission, and the International Program on Chemical Safety.

Efforts to harmonize and link data and information on toxic chemicals throughout the world have been multiplying. The Globally Harmonized System (GHS) of classification and labeling of chemicals has been adopted and is ready for implementation. This will provide a consistent and coherent approach to identifying hazardous chemicals, as well as provide information on such hazards and protective measures to exposed populations. Meanwhile in the European Union, a regulatory framework known as REACH (Registration, Evaluation and Authorization of Chemicals) has been proposed for the registration of chemical substances manufactured or imported in quantities greater than one ton per year.

Last, but not least, the role that poisons played in personal and political intrigues and vendettas, although it may have peaked with Borgias, by no means ended there. A case in point was the 2004 presidential elections in Ukraine. After a bitterly contested battle for the presidency of Ukraine, Viktor Yushchenko emerged victorious and was inaugurated in January 2005, a happy day for democracy, but with a toxic twist. Yushchenko, according to physicians, suffered severe facial disfigurement (chloracne) and other ailments by being poisoned with large dose of dioxins, allegedly mixed in some soup he consumed. Fortunately he is recovering gradually. Although the full story has not yet emerged, political motivations are suspected.

This second edition has grown from 749 entries submitted by 200 authors to 1057 entries contributed by 392 authors. Virtually all the entries from the first edition have been updated and in some cases entirely new versions of these entries have been written. Among the 308 topics appearing for the first time in this edition are avian ecotoxicology, benchmark dose, biocides, computational toxicology, cancer potency factors, metabonomics, chemical accidents, Monte Carlo analysis, nonlethal chemical weapons, invertebrate ecotoxicology, drugs of abuse, cancer chemotherapeutic agents, and consumer products. Many entries devoted to specific chemicals are also brand new to this edition and the international scope of organizations included has been broadened. Entries describing a number of well-known toxin-related incidents, e.g., Love Canal, Times Beach, Chernobyl, and Three-Mile Island, have been added. In addition to the scientific-based entries, others focus on the societal implications of toxicological knowledge. Among them are Toxicology in Culture, Environmental Crimes, Notorious Poisoners and Poisoning Cases Chemical and Biological Warfare in Ancient Times, and a History of the US Environmental Movement. Thus, this new edition has been expanded in length, breadth, and depth and provides an extensive overview of the many facets of toxicology.

Philip Wexler

PREFACE TO THE FIRST EDITION

There are many fine general and specialized monographs on toxicology, most of which are addressed to toxicologists and students in the field and a few to laypeople. This encyclopedia of toxicology does not presume to replace any of them but rather is intended to fulfill the toxicology information needs of new audiences by taking a different organizational approach and assuming a middle ground in the level of presentation by borrowing elements of both primer and treatise.

The encyclopedia is broad-ranging in scope, although it does not aspire to be exhaustive. The idea was to look at basic, critical, and controversial elements in toxicology, which are those elements that are essential to an understanding of the subject's scientific underpinnings and societal ramifications. As such, the encyclopedia had to cover not only key concepts, such as dose response, mechanism of action, testing procedures, endpoint responses, and target sites, but also individual chemicals and classes of chemicals. Despite the strong chemical emphasis of the book, we had to look at concepts such as radiation and noise, and beyond the emphasis on the science of toxicology, we had to look at history, laws, regulation, education, organizations, and databases. The encyclopedia also needed to consider environmental and ecological toxicology to somewhat counterbalance the acknowledged emphasis on laboratory animals and humans because, in the end, all our connections run deep.

In terms of the chemicals, we the editors of this book made a personal selection based on our own knowledge of those with relatively high toxicity, exposure, production, controversy, newsworthiness, or other interest. The chemicals do not represent a merger of regulatory lists or databases of chemicals; they are what we consider to be, for one reason or another, chemicals of concern to toxicology. The book was not intended as a large-scale compendium of toxic chemicals, several of which already exist.

In the tradition of many standard encyclopedias, scientific and otherwise, the encyclopedia is organized entirely alphabetically. Other than in a few useful but smaller scale dictionaries, this style of arrangement has not been done before for toxicology. This organization, along with a detailed index and extensive cross-references, should help the reader quickly arrive at the needed information.

Next, although this book should be of use to the practicing toxicologist, it is geared more to others who, in the course of their work, study, or for general interest, need to know about toxicology. This would include the scientific community in general, physicians, legal and regulatory professionals, and laypeople with some scientific background. Toxicologists needing to brush up on or get a quick review of a subject other than their own specialty would also benefit from it, but toxicologists seeking an in-depth treatment should instead consult a specialized monograph or journal literature.

The encyclopedia is meant to give relatively succinct overviews of sometimes very complex subjects. Formal references and footnotes were dispensed with because these seemed less relevant to the encyclopedia's goals than a simple list of recommended readings designed to lead the reader to more detailed information on a particular subject entry. The entry on Information Resources leads readers to print and electronic sources of information in toxicology.

First and foremost, thanks go to the Associate Editors and contributors, whose efforts are here in print. Yale Altman and Linda Marshall, earlier Acquisitions Editors for the books, were of great assistance in getting the project off the ground. Tari Paschall, the current Acquisitions Editor, and Monique Larson, Senior Production Editor, both of Academic Press, have with great expertise and efficiency brought it to fruition. Organization and formatting of the original entry manuscripts were handled with skill, patience, and poise by Mary Hall with the help of Christen Bosh and Jennifer Brewster.

My work on the *Encyclopedia of Toxicology* was undertaken as a private citizen, not as a government employee. The views expressed are strictly my own. No official support or endorsement by the US National Library of Medicine or any other agency of the US Federal Government was provided or should be inferred.

Philip Wexler

ACKNOWLEDGMENTS

This book, as is all too easy to discern, is not a one-man operation, and doubtlessly could not be one and still encompass the same breadth and depth. Above all, I bow, tip my hat, and throw roses in appreciation, to the nine associate editors Bruce D Anderson, Ann de Peyster, Shayne C Gad, Pertti J Hakkinen, Michael A Kamrin, Betty J Locey, Harihara M Mehendale, Carey N Pope, Lee R Shugart and the authors of this work. There is no exaggerating their importance in this collaboration. We were the prototypical occasionally disputative but affectionate family engaged in a common single-minded goal – self-preservation. Secondarily, we had an encyclopedia to produce cooperatively, and managed to engage in the process with good humor and without punching each other silly. Such are the advantages of online interaction. We survived, relatively intact, in good spirits, and on speaking terms, even after our few in-person meetings. And rest assured, no transfer of funds was involved in Dr Ernie Hodgson's flattering and much appreciated foreword.

On the publisher (Elsevier) end, Tari Paschall, experienced in the production of the first edition, ushered this second edition through its formative stages to the point where we had a stable process and a clear direction. She handed the baton to Judy Meyer, the new Publishing Editor for the encyclopedia, who deftly kept us on course, and hydrated, up to the finish line. Another baton pass shortly before the production process was from Nick Panissidi of Elsevier's San Diego Office to Michael Bevan in Oxford. Nick set up the Encyclopedia Website and initial editorial ground rules. Michael brought the editorial details to fruition and got us into and through production with hardly a scar. I would like to thank the many other unknown to me Elsevier staff who have worked diligently on other aspects of the book, including marketing. I have had great support from many colleagues. Dr Jack Snyder, Associate Director of the Division of Specialized Information Services at the National Library of Medicine, and Jeanne Goshorn, Chief of the Biomedical Information Services Branch of the same division, in particular, have been unflagging boosters of my efforts.

And finally, on the home front, I am certain that my dog, Chi-Chi, barked less than she would have, and my bird, Hercules, moderated his screeching, in consideration of my work on the encyclopedia. As for my teenage son, Jake, he probably bugged me more on account of it, but we are old hands at knowing how to annoy each other with relish.

Notes on the Glossary

Reprinted from the IUPAC 'Glossary for Chemists of Terms used in Toxicology' and the IUPAC 'Glossary of Terms used in Toxicokinetics', with permission from the International Union of Pure and Applied Chemistry.

In order that the *Encyclopedia of Toxicology* may be useful to as wide a readership as possible, a Glossary of key terms has been provided by the publisher. For the purpose of the article text itself, it is important to use the established technical vocabulary of the science of toxicology, in the interest of accuracy, brevity, and consistency.

However, it is possible that some of these technical terms will not be entirely familiar to the nonprofessional readers of this encyclopedia. Therefore, in the interest of greater understanding for those readers – and also for the possible benefit of professional readers consulting material outside their own area of expertise – the Glossary defines a selected group of several hundred terms. These terms occur frequently within a variety of articles in the encyclopedia and thus can be said to represent a core vocabulary of the field of toxicology. The definitions are presented in a concise, accessible format, based on the use of the term in the context of the encyclopedia.

Notes on the Subject Index

To save in the index, the following abbreviations have been used:

ADI	acceptable daily intake
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CSAF	chemical-specific adjustment factors
DDT	dichloro-diphenyl-trichloro-ethane
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
GCP	good clinical practice
GLP	good laboratory practice
ICH	International Conference on Harmonization
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
OPIDN	organophosphate-induced delayed neurotoxicity
QSARs	quantitative structure-activity relationships
SSRIs	selective serotonin reuptake inhibitors
WHO	World Health Organization

A

Aberrations of Chromosomes *See* Chromosome Aberrations.

Absorption

Jules Brodeur and Robert Tardif

Introduction

Absorption is the process by which a chemical crosses the various membrane barriers of the body before it enters the bloodstream. The main sites of entry are the gastrointestinal tract, the lungs, and the skin. In drug therapy, other convenient, but more rarely used, portals of entry are the intravenous, subcutaneous, and intramuscular routes.

The absorption of a chemical from the site of exposure is regulated by the biologic membrane surrounding the various cells that line the tissue compartments of the body. The membrane is composed principally of phospholipids forming an oriented bilayer, 7–9 nm thick. The more polar hydrophilic (attracted to water) ends of the phospholipids project into the aqueous media on each side of the membrane, and the hydrophobic (repelled by water) fatty acid tails form a barrier to water in the inner space of the membrane. Proteins are embedded throughout the lipid bilayer and have various functions. One of these is to act as active carriers for certain molecules across the membrane. Proteins can also form pathways or small pores through the membrane, serving as aqueous channels and allowing passage of water across them.

Before discussing absorption in more detail, it is important to consider mechanisms by which chemicals cross membranes. These mechanisms are of interest not only for absorption but also for all other processes (distribution, biotransformation, and excretion) involved in the disposition of chemicals because they also require passage through membranes.

Chemicals can cross membranes by one or more of the following mechanisms: passive diffusion, facilitated diffusion, active transport, filtration, and endocytosis.

Passive Diffusion

This is the mechanism by which lipophilic (hydrophobic) uncharged molecules find a passage across the membrane by solubilizing within the lipids of the membrane. The driving force for this process is the concentration gradient of the chemical between each side of the membrane, allowing molecules to be transported from the side with higher concentration to the side with lower concentration. Passive diffusion, therefore, requires no energy expenditure by the cell; it is not saturable or subject to competition between molecules.

Factors that govern passive diffusion are:

1. *The lipid solubility of a chemical*: This is a characteristic that is usually expressed in terms of the ability of the chemical to distribute between separate oil and water phases. The more a chemical dissolves in oil, or its substitute octanol, the more lipid-soluble it is and the more easily it will cross membranes.
2. *The electrical charge (degree of ionization) of a chemical*: As a rule, chemicals that are electrically neutral permeate more easily through the lipid phase of a membrane by virtue of their higher degree of lipid solubility. For several therapeutic agents that are weakly charged molecules, the pH of the aqueous environment will have considerable influence on the degree of ionization of the chemicals and hence on their lipid solubility and membrane permeation.
3. *The molecular size of a chemical*: Passive diffusion is normally limited to molecules whose molecular weight does not exceed 500 Da. However, a small molecule will cross membranes more rapidly than a larger one of equal lipophilicity.

Facilitated Diffusion

Facilitated diffusion is very similar to passive diffusion with the difference that transfer across membranes is assisted by the participation of carrier proteins embedded in the membrane bilayer. Again, the direction of passage will be from the side of the membrane with high concentration of a chemical to the side with low concentration; this also occurs without energy expenditure by the cell. Such a process is somewhat specific in the sense that it applies to molecules that are able to bind to a carrier protein. Absorption of nutrients such as glucose and amino acids across the epithelial membrane of the gastrointestinal tract occurs by facilitated diffusion. Since a finite number of carriers are available for transport, the process is saturable at high concentrations of the transported molecules and competition for transport may occur between molecules of similar structure.

Active Transport

Active transport requires a specialized carrier molecule, a protein, and the expenditure of cellular energy; transfer across membranes can therefore occur against a concentration gradient. The carrier system is selective for certain structural features of chemicals, namely their ionized state, whether anionic, cationic, or neutral. Recent advances in the understanding of active transport have led to the characterization of several families of carriers. Such carrier systems are saturable. In addition, molecules with similar structural features may compete for transport by a given carrier.

Active transport is of limited importance for absorption of chemicals; it plays an important role, however, in the elimination of chemicals by the liver and the kidneys.

Filtration

Small water-soluble and small charged molecules, such as methanol and salts, respectively, may cross the gastrointestinal epithelial membrane through minute pores or water channels (<4 nm) in the membrane. Filtration is also an important function for urinary excretion. Renal glomeruli possess rather large pores (~ 70 nm) that allow passage into the urine of various solutes contained in blood, including small proteins.

Endocytosis

Endocytosis is a specialized form of transport by which very large molecules and insoluble materials are engulfed by invagination of the absorptive cell membrane, forming intracellular vesicles. This process is responsible for the absorption of certain dyes by mucosal cells of the duodenum (pinocytosis). In the lung, alveolar macrophages scavenge insoluble particles, such as asbestos fibers, and may transport them into the lymphatic circulation (phagocytosis).

Absorption by the Gastrointestinal Tract

The major role of the gastrointestinal tract is to provide for efficient absorption of essential nutrients contained in ingested foods and liquids. It is also an important route for absorption of drugs and toxicants. The entire surface of the gastrointestinal tract is very large, being 200 times that of the body surface; the barrier between the contents of the tract and the blood vessels is easily crossed, consisting essentially of an epithelium only one cell thick. The anatomy of the gastrointestinal tract is illustrated in **Figure 1**. Absorption occurs mostly by passive diffusion of lipid-soluble, electrically neutral (nonionized) molecules.

The degree of ionization of many therapeutic drugs, which are usually weak electrolytes, is directly dependent upon the pH of the gastrointestinal content. The pH will therefore have considerable influence on the absorption of such chemicals; absorption will occur at sites where the drugs are present as neutral molecules. At the low acidic pH of the stomach (1–3), most weak organic acids such as

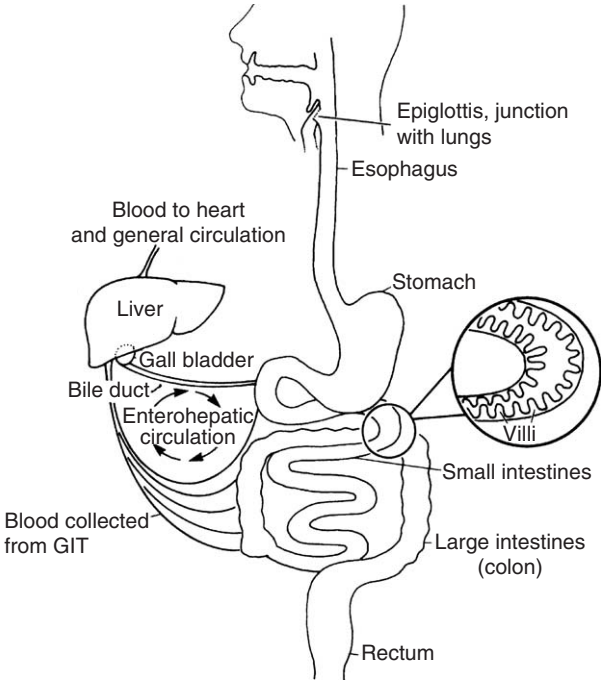

Figure 1 The anatomy of the gastrointestinal tract. (Reproduced from Smith RP (1992) The anatomy of the gastrointestinal tract. *A Primer of Environmental Toxicology*, p. 70. Philadelphia: Lea & Febiger, with permission from Lea & Febiger.)

acetylsalicylic acid will be nonionized and will diffuse passively across the gastric mucosa at a rate that will be proportional to the concentration gradient of the nonionized form. On the other hand, weak organic bases will diffuse more easily through the mucosa of the small intestine in which pH is higher (5–8). However, the bulk of absorption does not necessarily occur at the site where pH is optimal for electrical neutrality of the molecules. The very large surface area of the small intestine, due to the presence of finger-like projections, namely the villi and the microvilli, favors the diffusion of substances even at pH values for which the degree of ionization is not maximal; as a consequence, the small intestine is the region of the gastrointestinal tract that is most effective in the absorption of chemicals.

A small number of chemicals may be absorbed using facilitated diffusion (antimetabolic nucleotides), active transport (lead and 5-fluouracil), or pinocytosis (dyes and bacterial endotoxins).

Chemicals that reach the bloodstream by absorption through the gastrointestinal tract will move, via the portal circulation, directly to the liver, where they will normally undergo metabolic biotransformation to more or less active chemical forms, even before they gain access to the various tissues of the body; this phenomenon is known as the first-pass effect.

Among factors that may modify gastrointestinal absorption of ingested chemicals, the presence of food in the tract is one of the most important. The presence of food in the stomach will delay the absorption of weak organic acids at that site. The presence of lipid-rich food will delay the emptying of the gastric content into the intestine and thus also delay the absorption of chemicals. Conversely, an empty stomach facilitates absorption, a situation that is almost always beneficial in drug therapy.

Chemical interactions in the gastrointestinal tract between nutrients and drugs may considerably reduce the absorption of some drugs: calcium ions from dairy products form insoluble and therefore nonabsorbable complexes with the antibiotic tetracycline. On the other hand, certain drugs are irritants to the gastrointestinal tract (nonsteroidal anti-inflammatory drugs and potassium chloride tablets) and must be ingested with food.

Enterohepatic circulation provides an example of a special case of intestinal absorption. Certain chemicals, like methyl mercury, after undergoing biotransformation in the liver, are excreted into the intestine via the bile. They then can be reabsorbed in the intestine, sometimes after enzymatic modification by intestinal bacteria. This process can markedly prolong the stay of chemicals in the body. It can be interrupted by antibiotics that destroy the intestinal bacterial flora.

Absorption through the Skin

Normal skin represents an effective, but not perfect, barrier against the entry of chemicals present in the environment. There are two major structural components to the skin – the epidermis and the dermis (**Figure 2**).

The epidermis is formed of several layers of cells, with the outermost layers, $\sim 10\,\mu m$ thick, consisting of dried dead cells forming the stratum corneum. The latter, whose cells are rich in a filament-shaped protein called keratin, represents the major structural component of the barrier to passage of chemicals through the skin. Chemicals may move through the various cell layers of the epidermis by passive diffusion, more slowly through the stratum corneum, but more rapidly through the inner layers of live epidermal cells (stratum granulosum, stratum spinosum, and stratum germinativum).

The epidermis rests upon and is anchored onto a much thicker base of connective and fatty tissues, the dermis, whose major structural components are proteins called collagen and elastin; these proteins provide the skin with tensile strength and elasticity. The dermis also contains small blood vessels (capillaries), nerve endings, sebaceous glands, sweat glands, and hair follicles. Small pores in the epidermis that allow passage for sweat and sebum glands, as well as hair shafts, are not an important route of entry for chemicals. Once a chemical has crossed the epidermis by passive diffusion and gained access to the dermis, diffusion into the bloodstream occurs rapidly.

The stratum corneum is much thicker in areas where considerable pressure and repeated friction occur, like palms and soles; absorption is therefore much slower in these areas. Conversely, the stratum corneum is extremely thin on the skin of the scrotum. In general, skin surfaces of the ventral aspect of the body represent barriers that are easier to cross than those of the dorsal aspect.

Mechanical damage to the stratum corneum by cuts or abrasions of the skin or chemical injury by local irritation with acids or alkalis, for example, is likely to facilitate the entry of chemicals through the skin. This may also be the case in subjects suffering from certain skin diseases.

Lipid-soluble chemicals like organophosphate insecticides, tetraethyl lead, certain organic solvents, and certain dyes like aniline are relatively well absorbed through the skin. Percutaneous absorption is facilitated by increasing peripheral dermal blood

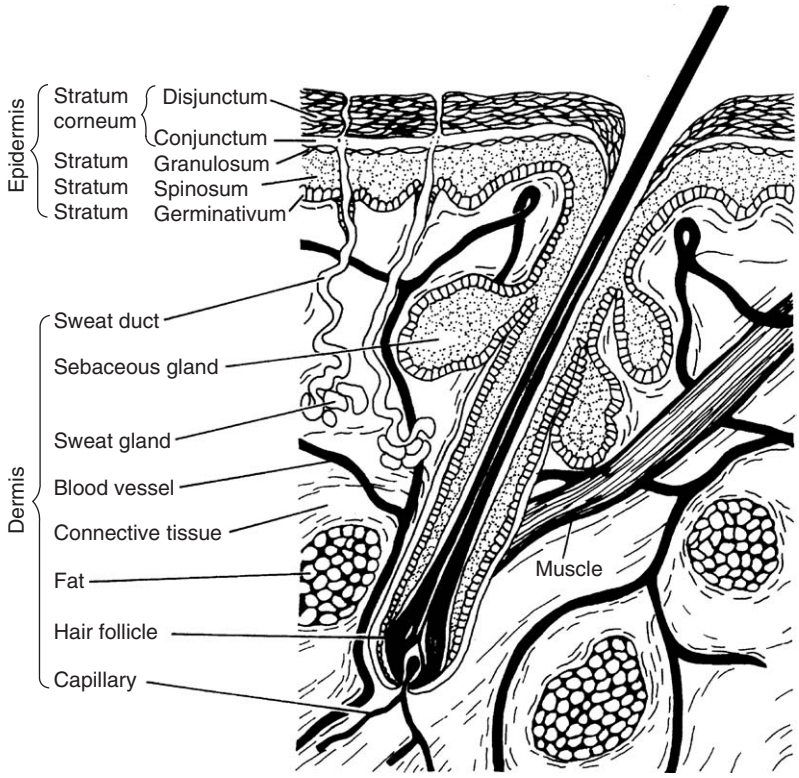

Figure 2 The organization of the skin as a biologic barrier. (Reproduced from Smith RP (1992) The organization of the skin as a biological barrier. *A Primer of Environmental Toxicology*, p. 73. Philadelphia: Lea & Febiger, with permission from Lea & Febiger.)

flow, as might occur when the ambient temperature is elevated. Under the same conditions, and in the presence of elevated sweating, the degree of hydration of the skin will increase considerably, enhancing the permeability of the stratum corneum to foreign chemicals; this observation is of special interest to workers in occupational settings.

Absorption by the Lung

The fundamental physiologic role of the lung is to allow gas exchange, extracting oxygen from the ambient air and eliminating carbon dioxide as a catabolic waste. When performing this function, the human adult lung is exposed each day to $\sim 10\,000$ l of more or less contaminated air. The lung can therefore become an important portal of entry for airborne chemicals present in the environment.

Extraneous substances are presented to the lung as gases or vapors or as liquid or solid particles; following inhalation, they may reach various regions of the respiratory tract, where some fraction of them will undergo absorption into the bloodstream; the remaining part will be either deposited locally or eliminated by exhalation even before being absorbed.

In terms of its anatomical and functional relationship with the contaminated atmospheric environment, the respiratory tract can be divided into three regions: the nasopharyngeal, the tracheobronchiolar, and the alveolar regions (**Figure 3**). The major part of the absorptive process takes place in the alveolar region, due principally to its large surface area ($80\,m^2$ in an adult human) and the extreme thinness of the cellular barrier ($<1\,\mu m$) between the air-side of the alveolar sac (lined with epithelial cells) and the lumen of the lung capillaries (lined with endothelial cells).

When discussing absorption of chemicals through the respiratory tract, it is practical to consider separately gases and vapors, on the one hand, and particles on the other hand.

Gases and Vapors

How much and at what location a contaminant gas or vapor will be absorbed in the respiratory tract is determined primarily by the solubility of the contaminant. The more water-soluble agents (sulfur dioxide and ketonic solvents) may dissolve in the aqueous fluid lining the cells of the more proximal region of the respiratory tree, even before they reach the alveolar region. They may then undergo absorption by passive diffusion or passage through membrane pores. When, in addition, water-soluble contaminants

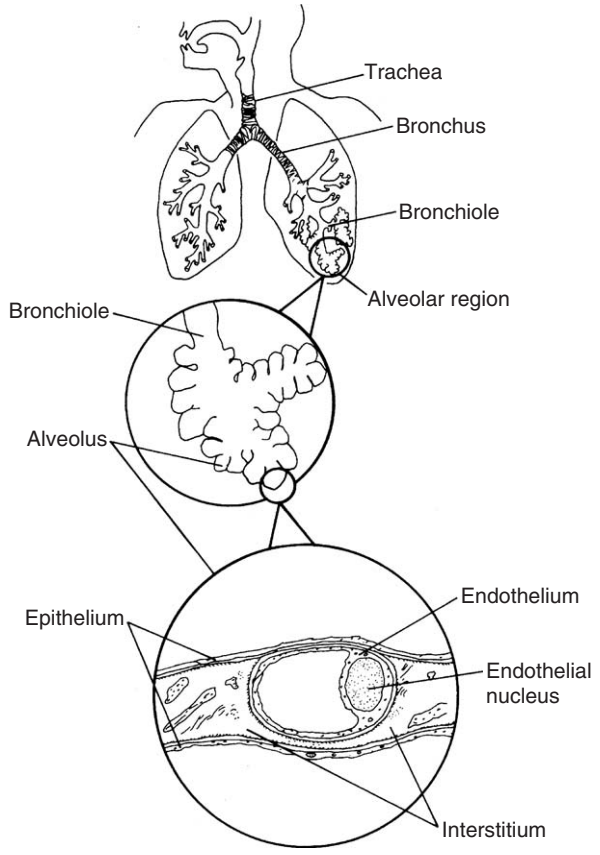

Figure 3 The anatomy of the respiratory tract from trachea to alveolus. (Reproduced from Smith RP (1992) The anatomy of the respiratory tract from trachea to alveolus. *A Primer of Environmental Toxicology*, p. 67. Philadelphia: Lea & Febiger, with permission from Lea & Febiger.)

are very reactive substances, like formaldehyde, they may form stable molecular complexes with cell components as proximally as the nasopharyngeal region. By virtue of these mechanisms, the alveolar region of the lung is partially protected against potential injury by certain gases and vapors.

Lipid-soluble contaminants diffuse passively through the thin alveolar–vascular cell barrier of the alveolar sac and then dissolve into the blood according to the ability of the contaminant to partition between alveolar air and circulating blood. Substances that are very soluble in blood are rapidly transported into the bloodstream. For these substances, like styrene and xylene, the amount absorbed will be greatly enhanced by increasing the rate and the depth of respiration, as is likely to happen when doing strenuous physical work. On the other hand, substances that are poorly soluble in blood have limited capacity for absorption due to rapid saturation of blood. For these substances, like the solvents cyclohexane and methyl chloroform, the amount absorbed may be increased only by increasing the blood perfusion rate in the lung; that is, by enhancing the replacement of saturated blood circulating in the lung capillaries. This can be achieved, for example, when doing work requiring heavy muscular activity.

Particles

Liquid (sulfuric acid and cutting fluids) and solid (silica dusts, asbestos fibers, and microorganisms) particles may become airborne and form respirable aerosols. According to their size and diameter, inhaled particles may be deposited in different anatomical regions of the respiratory system. Once deposited, particles may dissolve locally or may undergo removal to other regions of the respiratory tree.

The surface of the cells lining the tracheobronchial tree and the surface of most of the cells lining the nasopharyngeal region are covered with a layer of relatively thick mucous material; in the alveolar region, cells are lined with a thin film of fluid. The aqueous environment provided by these surface liquids favors at least partial dissolution and eventually absorption of water-soluble particles, especially those present as liquid droplets. Various defense mechanisms may help to remove less soluble particles from their site of deposition.

Particles larger than 5 μm in diameter are usually deposited by inertial impaction on the surface of the nasopharyngeal airways. They may be removed by coughing, sneezing, or nose wiping.

Particles with diameters between 1 and 5 μm are deposited in the tracheobronchial region as a result of either inertial impaction at airway bifurcations or gravitational sedimentation onto other airway surfaces. Undissolved particles may then be removed by the action of the mucociliary defense system working as an escalator; particles trapped in the mucus are propelled toward the pharynx by the action of thin cilia located on the surface membrane of specialized cells. Once in the pharynx, the particles may be swallowed. The efficiency of the escalator defense system may be greatly impaired by various environmental contaminants, like sulfur dioxide, ozone, and cigarette smoke that are known to paralyze the activity of the ciliated cells and consequently the upward movement of the mucus.

Particles ranging between 0.1 and 1.0 μm in diameter reach the alveolar region, where they finally hit cellular walls as a result of their random movement within minute air sacs. Removal of particles in this region of the lung is much less efficient. Some of the particles may eventually reach the tracheobronchiolar escalator system, either as engulfed

material within alveolar macrophages or as naked particles transported by the slow movement of the fluid lining the alveoli. Other possible mechanisms involve transport of the particles into the lymphatic system, either within macrophages or by direct diffusion through the intercellular space of the alveolar wall.

Particles smaller than 0.1 μm are not usually deposited in the lung, entering and exiting the airways together with inhaled and exhaled air.

Often, particulate matter acts as a carrier for gases, vapors, and fumes adsorbed onto their surface (solid particles) or dissolved within them (liquid particles); this increases the residence time of such pollutants in specific areas of the lung and imposes an additional task on the pulmonary defense mechanisms.

The most striking example of this synergistic effect is the one observed between sulfur dioxide, a respiratory tract irritant, and suspended particles, both being typical components of urban air pollution. This explains why current guideline values for exposure to sulfur dioxide in the presence of particulate matter are lower than those for exposure to sulfur dioxide alone. Similar concerns can be expressed for combinations comprising exhaust particles from diesel engines and certain carcinogens like polycyclic aromatic hydrocarbons, as well as cigarette smoke and certain other carcinogens like aromatic amines.

Chemicals absorbed by the lung reach the systemic circulation directly and are therefore immediately available for distribution to the various tissues of the body – brain, kidneys, liver, muscles, skin, bones, and others.

See also: Biotransformation; Distribution; Excretion; Exposure; Gastrointestinal System; Modifying Factors of Toxicity; Pharmacokinetics/Toxicokinetics; Respiratory Tract; Skin; Toxicity Testing, Dermal; Toxicity Testing, Inhalation.

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Acceptable Daily Intake (ADI)

Jaya Chilakapati and Harihara M Mehendale

The acceptable daily intake (ADI) is commonly defined as the amount of a chemical to which a person can be exposed, on a daily basis over an extended period of time, usually a lifetime without suffering a deleterious effect. It represents a daily intake level of a chemical in humans that is associated with minimal or no risk of adverse effects. It is a numerical estimate of daily oral exposure to the human population, including sensitive subgroups such as children, that is not likely to cause harmful effects during a lifetime. The ADI is expressed in milligrams of the chemical, as it appears in the food, per kilogram of body weight per day ($mg\,kg^{-1}\,day^{-1}$). The Environmental Protection Agency (EPA) refers to such an exposure level as the risk reference dose (RfD) in order to avoid any implication that any exposure to a toxic material is 'acceptable'. RfDs are generally used for health effects that are thought to have a threshold or low dose limit for producing effects. The ADI concept has often been used as a tool in reaching risk management decisions such as establishing allowable levels of contaminants in foodstuffs and water.

ADI is derived from an experimentally determined 'no-observed-adverse-effect level (NOAEL)'. An NOAEL is an experimentally determined dose at which there is no statistically or biologically significant indication of the toxic effect of concern. In an experiment with several NOAELs, the regulatory focus is normally on the highest one, leading to the common usage of the term NOAEL as the highest experimentally determined dose without a statistically or biologically significant adverse effect. In cases in which a NOAEL has not been demonstrated experimentally, the term 'lowest-observed-adverse-effect level (LOAEL)' is used.

ADI values are typically calculated from NOAEL values by dividing by uncertainty (UF) and/or modifying factors (MFs):

$$\text{ADI (human dose)} = \text{NOAEL (experimental dose)}/(\text{UF} \times \text{MF})$$

In principle, these safety factors (SFs) allow for intraspecies and interspecies (animal to human) variation with default values of 10. An additional uncertainty factor can be used to account for experimental inadequacies; for example, to extrapolate

from short-exposure-duration studies to a situation more relevant for chronic study or to account for inadequate numbers of animals or other experimental limitations. Traditionally, a safety factor of 100 would be used for RfD calculations to extrapolate from a well-conducted animal bioassay (10-fold factor for animal to human) and to account for human variability in response (10-fold factor human-to-human variability).

Modifying factors can be used to adjust the uncertainty factors if data on mechanisms, pharmacokinetics, and the relevance of the animal response to human risk justify such modifications. For example, if there is kinetic information suggesting that rat and human metabolisms are very similar for a particular compound, producing the same active target metabolite, then, rather than using a 10-fold uncertainty factor to divide the NOAEL from the animal toxicity study to obtain a human relevant RfD, a factor of 3 for that uncertainty factor might be used. Of particular interest is the new extra 10-fold Food Quality and Protection Act (FQPA) factor, added to ensure protection of infants and children.

For other chemicals, with databases that are less complete (for example, those for which only the results of subchronic studies are available), an additional factor of 10 might be judged to be more appropriate leading to an SF of 1000. For certain other chemicals, based on well-characterized responses in sensitive humans, an SF as small as 1 might be selected, as in the case of the effect of fluoride on human teeth.

Some scientists interpret the absence of widespread effects in the exposed human populations as evidence of the adequacy of the SFs traditionally employed.

The RfD approach represents a generally accepted (Food and Drug Administration, National Academy of Sciences (NAS), and EPA) method for setting lifetime exposure limits for humans, and the use of 10-fold uncertainty factors has some experimental support.

Limitations of RfD

However, there are several limitations in the RfD approach, the net result of which is that exposures resulting in the same RfD do not imply the same level of risk for all chemicals. In addition, the RfD approach does not make use of dose–response information. There are also difficulties in the implications of specific UFs. The default value of 10 for the interspecies UF is a reasonable assumption in some cases, but in other cases may not be appropriate. Too narrow a focus on the NOAEL means that information on the shape of the dose–response curve is ignored. Such data could be important in estimating levels of concern for public safety. Guidelines have not been developed to take into account the fact that some studies have used larger (smaller) numbers of animals and, hence, are generally more (less) reliable than other studies.

The ADI is generally viewed by risk assessors as a 'soft' estimate, whose bounds of uncertainty can span an order of magnitude. That is, within reasonable limits, while exposures somewhat higher than the ADI are associated with increased probability of adverse effects, that probability is not a certainty. Similarly, while the ADI is seen as a level at which the probability of adverse effects is low, the absence of all risk to all people cannot be assured at this level.

See also: Benchmark Dose.

Further Reading

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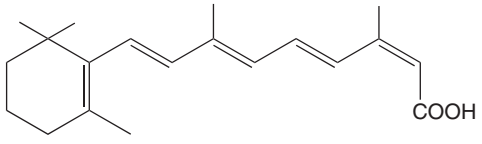
Accutane

Russell Barbare

- Chemical Abstracts Service Registry Number: CAS 4759-48-2
- Synonyms: Isotretinoin; 13-*cis*-Retinoic acid; 2-*cis*-Vitamin A acid; Ro-4-3780; Isotrex
- Chemical Formula: $C_{20}H_{28}O_2$
- Chemical Structure:



Uses

Isotretinoin is approved for use in the treatment of severe recalcitrant nodular acne and psoriasis, and is also used to treat keratinization disorders and some skin cancers.

Background Information

Isotretinoin is a retinoid, the class of natural and synthetic compounds that exhibit vitamin A activity. It is a naturally occurring metabolite of vitamin A that inhibits sebum production. The US Food and Drug Administration classifies it as Pregnancy Risk Category X.

Exposure Routes and Pathways

Ingestion is the most common route of exposure, and capsules are the only form currently produced.

Toxicokinetics

The apparent time lag between oral administration and appearance in systemic circulation is 30 min to 2 h. Absorption is approximately three times greater when taken with a high-fat meal as opposed to fasting, although the half-life is $\sim$21 h either way. Once in the body, isotretinoin binds to plasma proteins, especially albumin, at a rate greater than 99.9%. In humans, it readily undergoes reversible isomerization and irreversible oxidation; the exposure to these metabolites is more than three times greater than to the parent form. *In vitro* studies have indicated that the converted forms may have higher retinoid activity, but the clinical significance of this is unknown. ^{14}C studies have indicated that the half-life of the all drug activity in blood is $\sim$90 h. There was no statistically significant difference in exposure to any of the compounds between adults and patients 12–15 years of age. Excretion occurs in both feces and urine in approximately equal amounts, and overdosage in men can result in trace amounts in their semen. It is unknown whether it is excreted in human breast milk. It is metabolized by the liver, with the parent form having a terminal elimination half-life of 10–20 h.

Mechanism of Toxicity

Retinoids increase cellular mitotic activity, DNA and RNA synthesis, and protein synthesis. The primary toxicity of concern is female-mediated teratogenesis. Isotretinoin alters cell differentiation and placement in developing fetuses that are exposed to it in the first 3 weeks. Any exposed fetus has an increased change of spontaneously aborting or dying and may develop external or internal abnormalities. Cases of IQ less than 85 have been reported without other noted abnormalities. There is no accurate way to determine if a fetus has been exposed, so the safety recommendations are for potentially fertile females to not be pregnant or get pregnant within 30 days before or after exposure or at any time during exposure. External abnormalities have included skull, ear, and eye abnormalities such as cleft palate, absent external auditory canals, or microphthalmia. Noted internal changes have included abnormalities in the central nervous system such as hydrocephalus and microcephaly, abnormalities in the cardiovascular system or thymus gland, and parathyroid hormone deficiency. Even though it is unknown whether isotretinoin is excreted in human breast milk, breastfeeding should be avoided for the same period as pregnancy.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats and mice the oral LD_{50} of isotretinoin is $>4000\,mg\,kg^{-1}$; in rabbits it is $\sim1960\,mg\,kg^{-1}$.

Human

Overdosage can produce headache or abdominal pain, vomiting, dizziness, irregular muscular coordination, facial flushing, or drying and cracking of the lips, but all symptoms pass quickly and with no known long-term effects. An acute toxic dose has not been established – doses up to 1600 mg in an adult and 63 mg kg^{-1} in a child have resulted in only mild toxicity.

Chronic Toxicity (or Exposure)

Animal

Accutane is a potent rat and rabbit developmental toxin (teratogen). Testicular atrophy and evidence of lower spermatogenesis was noted in dogs given isotretinoin for 30 weeks at 20 or 60 $mg\,kg^{-1}\,day^{-1}$. Fischer 344 rats dosed at 8 or 32 $mg\,kg^{-1}\,day^{-1}$ for over 18 months had a dose-related raised incidence of pheochromocytoma, an adrenal gland tumor. The relevance in man is unknown since this animal develops spontaneous pheochromocytoma at a significant rate.

Human

Any level of exposure may be teratogenic, so potentially fertile females must not be pregnant or get

pregnant within 30 days before, during, and after exposure (see Mechanism of Toxicity). Other effects that often require monitoring are psychiatric disorders, including depression and suicidal thoughts, and benign intercranial hypotension, which can lead to headache, visual disturbances, or nausea and vomiting. These disorders may not stop upon discontinuation and should be evaluated by a professional. Dose-dependent adverse effects on the skin and mucous membranes may include inflammation or cracking of the lips, dry eyes, nosebleeding, irritation of the palpebral conjunctiva, and redness or dryness of the skin. Less common effects on the same organ systems include hair loss, photosensitivity, formation of granular tissue, or dark adaptation dysfunction. Colonization and, rarely, infection by *Staphylococcus aureus* can also occur. Hyperlipidemia is reported in 25% of treated patients during therapeutic courses of treatment on a systemic level, with the most common effect being increased triglyceride levels. There may also be increased cholesterol levels, raising of low-density lipoprotein levels, or lowering of high-density lipoprotein levels. Long-term treatments can generate several skeletal side effects including joint or lower back pain, bone hypertrophy, ossification at tendinous insertions, and lowered bone density. Children may experience premature closure of the epiphyseals. Tests of sperm count and motility in man have shown no significant changes.

Clinical Management

Roche Pharmaceuticals has produced the System to Manage Accutane Related Teratogenicity™ (S.M.A.R.T.™) and the Accutane Pregnancy Prevention Protocol (PPP) to be used in conjunction with the prescription of Accutane. Management of toxic effects involves monitoring by the appropriate specialist and discontinuation of the exposure where indicated. Isotretinoin-related depression may require long-term monitoring.

Exposure Standards and Guidelines

The recommended therapeutic dosage is 0.5–1.0 $mg\,kg^{-1}\,day^{-1}$ in two doses per day taken with food for 15–20 weeks.

See also: Developmental Toxicology; Photoallergens; Vitamin A.

Further Reading

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Relevant Website

http://www.rocheusa.com – Roche Pharmaceuticals, Accutane® Website for the United States.

ACE Inhibitors

Henry A Spiller

This article is a revision of the previous print edition article by Daniel J Cobaugh, volume 1, pp. 7–9, © 1998, Elsevier Inc.

- Representative Chemicals: Benazepril, Lotensin®; Capropril, Capoten®; Enalapril, Vasotec®; Enalaprilat, Vasotec IV®; Fosinopril, Monopril®; Lisinopril, Prinivil®; Zestril®; Quinapril, Accupril®; Ramipril, Altace®
- Chemical Abstracts Service Registry Numbers: CAS 86541-75-5; CAS 62571-86-2; CAS 75847-73-3; CAS 84680-54-6; CAS 888 89-14-9; CAS 76547-98-3; CAS 85441-61-8; CAS 87333-19-5
- Chemical/Pharmaceutical/Other Class: Angiotensin-converting enzyme (ACE) inhibitors
- Chemical Formulas: Benazepril, $C_{24}H_{28}N_2O_5$; Captopril, $C_9H_{15}NO_3S$; Enalapril, $C_{20}H_{28}N_20_5$; Enalaprilat, $C_{18}H_{24}N_2O_5 \cdot 2H_2O$; Fosinopril, $C_{30}H_{46}NO_7P$; Lisinopril, $C_{21}H_{31}N_3O_5 \cdot 2H_2O$; Quinapril, $C_{25}H_{30}N_2O_5$; Ramipril, $C_{23}H_{32}N_2O_5$

Uses

Angiotensin-converting enzyme (ACE) inhibitors are used in the management of hypertension and congestive heart failure.

Exposure Routes and Pathways

Ingestion is the most common route for both accidental and intentional exposures. Enalaprilat is

available for parenteral administration and toxicity could occur via this route.

Toxicokinetics

The extent of oral absorption varies from 25% (lisinopril) to 75% (captopril). The rate of absorption also varies from 0.5 h (captopril and enalopril) to 7 h (lisinopril). Reported volumes of distribution range from $0.7\,l\,kg^{-1}$ (captopril) to $1.8\,l\,kg^{-1}$ (lisinopril). All of the ACE inhibitors, except for captopril and lisinopril, are metabolized in the liver to active metabolites. Excretion is via both the urine and the feces. The half-life ranges from 1.3 h (enalapril) to 17 h (ramipril).

Mechanism of Toxicity

The ACE inhibitors affect the rennin–angiotensin system. This system has effects on blood pressure as well as fluids and electrolyte balance. Renin modulates the formation of angiotensin I from angiotensinogen. Angiotensin I is then converted via angiotensin-converting enzyme to angiotensin II. Angiotensin II is a potent vasoconstrictor that also causes increased aldosterone secretion. Aldosterone is responsible for sodium and water retention. The ACE inhibitors interfere with the conversion of angiotensin I to angiotensin II and, therefore, cause vasodilation as well as loss of sodium and water. Literature supporting a relationship between angiotensin and the beta endorphins exists. Angiotensin II is thought to be inhibited by endogenous beta endorphin. *In vitro* studies have demonstrated that captopril can inhibit encephalinase, the enzyme that degrades endorphins. Interference with endorphin metabolism may result in prolonged effects from these opiate-like neurotransmitters. Also, the opiate antagonist naloxone is thought to interfere with beta-endorphin inhibition of angiotensin II. An interaction between angiotensin and bradykinin may also exist. ACE is identical to kinase IT, which is responsible for inactivation of bradykinins. Accumulation of bradykinins may cause a decrease in blood pressure by a direct vasodilatory mechanism or through stimulation of prostaglandin release and/or synthesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

There are limited data, but accidental ingestion of small amount of ACE inhibitors by companion animals would not expected to be a problem.

Human

The clinical effects observed following ACE inhibitor poisoning or overdose are a direct extension of their therapeutic effects and would be expected to manifest in 1–2 h postingestion. Ingestions involving small amounts of ACE inhibitors may result in limited or no toxic effects. Clinical effects that may occur include hypotension with or without a reflex tachycardia and changes in level of consciousness that are directly related to vascular changes. Only a few cases of profound hypotension have been reported. In each of these cases, blood pressure returned to normal within 24 h of ingestion. One death has been attributed to an ACE inhibitor. This was in a 75-year-old male who ingested captopril and the calcium channel blocker diltiazem. Because this was a coingestion, it is not certain that captopril was the primary cause of death.

Chronic Toxicity (or Exposure)

Animal

Carcinogenicity studies carried out over years have not demonstrated any increased tumor incidence. No teratogenic effects have been documented in mice despite large chronic doses (e.g., 625 times the maximum daily dose of lisinopril on days 6–15 of gestation).

Human

Adverse effects observed at therapeutic doses include cough, dermal reactions, blood dyscrasias, bronchospasm, and hypogeusia. Angioedema has been reported, but does not appear to be an IgG related immune response. Reversible renal failure has been reported with chronic therapy. Clinical effects that may occur include hypotension with or without a reflex tachycardia, changes in level of consciousness that are directly related to vascular changes, and hyperkalemia. Hyperkalemia can occur as a response to sodium loss. Delayed hypotension, at 19 and 25 h, has been observed following ingestion of captopril.

In Vitro Toxicity Data

Lisinopril, captopril, quinapril, and benazepril have been studied for mutagenicity using a variety of methods and none have documented evidence of mutagenicity.

Clinical Management

Supportive care, including airway management as well as cardiac and blood pressure monitoring,

should be provided to unstable patients. Ingestion of small amounts of an ACE inhibitor in children can be managed with observation at home. Following ingestion of a toxic amount of these agents or recent ingestions involving toxic coingestants, activated charcoal can be utilized to decontaminate the stomach. Hypotension following ACE inhibitor ingestion has been managed with fluids alone or in combination with vasopressors such as dopamine. A limited number of case reports exist that describe a need for dopamine to treat hypotension. If profound hypotension resistant to dopamine were to occur, other vasopressors, such as epinephrine and norepinephrine, can be used. Laboratory analysis should be used to monitor electrolytes, especially sodium and potassium. ACE inhibitor serum concentrations are not readily available and have little if any clinical utility. Because ACE inhibitors may potentiate the effects of the opiate-like beta endorphins, some authors have suggested the use of naloxone to reverse their toxicities. Successes and failures with naloxone have been described in case reports. Because naloxone has limited adverse effects, its use could be considered in the management of serious ACE inhibitor toxicity. One case report describes the use of the experimental exogenous angiotensin II to counter severe ACE inhibitor toxicity. The pharmacokinetic characteristics of the ACE inhibitors, limited protein binding, and small volume of distribution make them amenable to hemodialysis. Because major morbidity is rare with these agents, the need for dialysis is questionable.

Angioedema with potential for airway obstruction may not respond to epinephrine and antihistamines. Rapid intubation to protect the airway may be necessary.

Environmental Fate

No information is currently available on breakdown in soil, groundwater, or surface water. ACE inhibitors are excreted into breast milk in trace amounts. Captopril is distributed into milk in concentrations of ~1% of those in maternal blood.

See also: Charcoal; Prostaglandins.

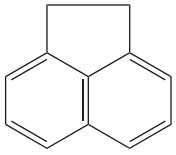
Further Reading

Augenstein WL, Kulig KW, and Rumack BH (1988) Captopril overdose resulting in hypotension. *Journal of the American Medical Association* 259: 3302–3305.

Acenaphthene

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 83-32-9
- Synonyms: 1,2-Dihydroacenaphthylene; 1,8-Dihydroacenaphthalene; 1,8-Ethylenenaphthalene; Acenaphthylene; Naphthyleneethylene; Periethylenenaphthalene; Ethylenenaphthylene
- Chemical/Pharmaceutical/Other Class: Arene belonging to the class of polycyclic aromatic hydrocarbons
- Chemical Structure:



Uses

Acenaphthene is a chemical intermediate used to produce naphthalimide dyes, which are used as fluorescent whitening agents, and used in manufacturing plastics, insecticides, and fungicides.

Background Information

Acenaphthene is a component of crude oil and a product of combustion, which may be produced and released to the environment during natural fires. Emissions from petroleum refining, coal tar distillation, coal combustion, and diesel fueled engines are the major contributors of acenaphthene to the environment. Acenaphthene is used as a chemical intermediate and may be released to the environment via manufacturing effluents and the disposal of manufacturing waste by-products. Because of the widespread use of acenaphthene in a variety of products, acenaphthene may also be released to the environment through landfills, municipal waste

water treatment facilities, and waste incinerators. Acenaphthene should biodegrade rapidly in the environment. The reported biodegradation half-lives for acenaphthene in aerobic soil and surface waters range from 10 to 60 and from 1 to 25 days, respectively. However, acenaphthene may persist under anaerobic conditions or at high concentration due to toxicity to microorganisms. Acenaphthene is not expected to hydrolyze or bioconcentrate in the environment; yet, it should undergo direct photolysis in sunlight environmental media. Acenaphthene is expected to exist entirely in the vapor phase in ambient air.

Exposure Routes and Pathways

Skin contact is the most common accidental exposure pathway. Acenaphthene may irritate or burn skin. Exposure can also be through ingestion or inhalation. Its vapor can be poisonous if inhaled.

Toxicokinetics

The half-life of acenaphthene in the bluegill fish is less than 1 day. A *Beijerinckia* species and a mutant strain, *Beijerinckia* species strain B8/36, were shown to oxidize acenaphthene. Both organisms oxidize acenaphthene to the same spectrum of metabolites, which included 1-acenaphthenol, 1-acenaphthenone, 1,2-acenaphthenediol, acenaphthenequinone, and a compound that was tentatively identified as 1,2-dihydroxyacenaphthylene.

Mechanism of Toxicity

5-Nitroacenaphthene causes toxicity by the reduction of the nitro function to the corresponding hydroxylamine. These arylhydroxylamines may be either direct-acting mutagens or may become so following nonenzymic conversion to aryl nitronium ions or they may be esterified to the corresponding electrophilic hydroxamic acid esters. Acenaphthene can bind to hemoglobin to cause methemoglobinemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acenaphthene can cause hepatotoxicity in rats and mice. Little information is available regarding acute exposure to acenaphthene. It is biotransformed in the liver. On the basis of a mouse oral subchronic study in which hepatotoxicity was seen as the major effect, the no-observed-adverse-effect level and the lowest-observed-adverse-effect level were 175 and $350\,mg\,kg^{-1}\,day^{-1}$, respectively.

Human

Acenaphthene can be irritating to eyes, skin, and mucous membrane. Acenaphthene may be poisonous if inhaled or absorbed through skin. The vapor may cause dizziness or suffocation. Acenaphthene may cause vomiting if swallowed in large quantity. It can cause methemoglobinemia.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to acenaphthene at a level of $12 \pm 1.5\,mg\,m^{-3}$ for 4 h a day, 6 days per week for 5 months showed toxic effects on the blood, lung, and glandular constituents. The bronchial epithelium showed hyperplasia and metaplasia, which may have been symptoms of the pneumonia that killed a large number of rats during the study. No signs of malignancy appeared during the 8 month postexposure observation period.

Human

Chronic human exposure data are not available. Currently, acenaphthene is under review by US Environmental Protection Agency for evidence of human carcinogenic potential. This does not imply that this agent is necessarily a carcinogen. The nitroderivative of acenaphthene (5-nitroacenaphthene) is a possible carcinogen to humans.

In Vitro Toxicity Data

Acenaphthene is devoid of any mutagenic activity in *Salmonella typhimurium* (TA 98) assay. The nitroderivatives of acenaphthene have tumorigenic potential.

Clinical Management

The victim should be moved to fresh air and emergency medical care should be provided. If the victim is not breathing, artificial respiration should be provided; if breathing is difficult, oxygen should be administered. In case of contact with the eyes, the eyes should be flushed immediately with running water for at least 15 min. Affected skin should be washed with soap and water. Contaminated clothing and shoes should be removed and isolated at the site. If methemoglobinemia occurs and is severe, treatment with methylene blue and oxygen is recommended.

Environmental Fate

The reported biodegradation half-lives for acenaphthene in aerobic soil and surface waters range from 10 to 60 and from 1 to 25 days, respectively. However, acenaphthene may persist under anaerobic conditions or at high concentrations due to toxicity to microorganisms. Acenaphthene is not expected to hydrolyze or bioconcentrate in the environment; yet, it should undergo direct photolysis in sunlit environmental media. A calculated K_{oc} range of 2065–3230 indicates acenaphthene will be slightly mobile in soil. In aquatic systems, acenaphthene can partition from the water column to organic matter contained in sediments and suspended solids. A Henry's law constant of 1.55×10^{-4} atm m^3 mol^{-1} at 25°C suggests volatilization of acenaphthene from environmental waters may be important. The volatilization half-lives from a model river and a model pond, the latter considers the effect of adsorption, have been estimated to be 11 h and 39 days, respectively. Acenaphthene is expected to exist entirely in the vapor phase in ambient air. In the atmosphere, the reaction with photochemically produced hydroxyl radicals (half-life of 7.2 h) is likely to be an important fate process. The most probable human exposure would be occupational exposure, which may occur through dermal contact or inhalation at places where acenaphthene is produced or used. Atmospheric workplace exposures have been documented. Nonoccupational exposures would most likely occur via urban atmospheres, contaminated drinking water supplies, and recreational contaminated waterways.

Ecotoxicology

Treatment of cherry-mazzard hybrid seeds with acenaphthene powder for 10 h inhibited the seed germination and seedling growth. Treatment of *Allium cepa* root meristem cells with acenaphthene vapor for 12–96 h caused anomalies leading to random development of cells. Acute toxicity value for bluegill fish was 1700 UG l^{-1} in freshwater and the toxicity to sheepshed minnow was 2230 UG l^{-1} in saltwater.

Exposure Standards and Guidelines

CERCLA reportable quantities: Persons in charge of vessels or facilities are required to notify the National Response Center (NRC) immediately, when there is a release of this designated hazardous substance, in an amount equal to or greater than its reportable quantity of 100 lb or 45.4 kg. State drinking water guidelines: Minnesota 400 µg l^{-1} and Florida 20 µg l^{-1}.

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

Byrne M *et al.* (1985) Government Reports, Announcements & Index, Issue 21. *Exposure and Risk Assessment for Benzo(a)pyrene and Other Polycyclic Aromatic Hydrocarbons*, vol. 3.

Harris J, Perwak J, and Coons S (1985) Government Reports, Announcements & Index Issue 21. *Exposure and Risk Assessment for Benzo(a)pyrene and Other Polycyclic Aromatic Hydrocarbons*, vol. 1.

Health & Welfare Canada (1979) *Polycyclic Aromatic Hydrocarbons*, Report No. 80-EHD-50.

Staples CA and Werner AF (1985) Priority pollutant assessment in the USA: Scientific and regulatory implications. *Toxic Substances* 6(4): 186–200.

US Department of Health & Human Services/Agency for Toxic Substances Disease Registry (1995) *Toxicological Profile for Polycyclic Aromatic Hydrocarbons* (Update), NTIS# PB/95/264370.

USEPA (1979) *Health Assessment Document: Polycyclic Organic Matter*, EPA-600/9-79-008.

USEPA (1980a) *Ambient Water Quality Criteria Doc: Acenaphthene* (Draft).

USEPA (1980b) *Ambient Water Quality Criteria Doc: Polynuclear Aromatic Hydrocarbons* (Draft).

Wilkins ES and Wilkins MG (1985.) Review of toxicity of gases emitted from combustion pyrolysis of municipal and industrial wastes. *Journal of Environmental Science and Health – Part A* 20(2).

Relevant Website

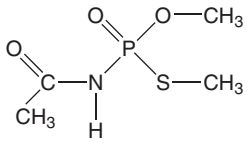
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Acenaphthene.

Acephate

Subramanya Karanth

- Chemical Abstracts Service Registry Number: CAS 30560-19-1
- Synonyms: Asataf; Aimthane; Chrevron RE 12420; Kitron; Orthene; Ortril; Pillarthene
- Chemical/Pharmaceutical/Other Class: Organophosphorus insecticide
- Chemical Formula: $C_4H_{10}NO_3PS$

- Chemical Structure:



Uses

Acephate is registered for use on a variety of field, fruit, and vegetable crops (e.g., cotton, tobacco, cranberries, mint). It is also used commonly in food handling establishments, on ornamental plants (cut flowers), and in and around residential and commercial buildings for the control of roaches and fire ants. It is effective against a wide range of biting and sucking insects, especially aphids.

Exposure Routes and Pathways

Common routes of acephate exposure include ingestion and inhalation.

Toxicokinetics

Acephate is converted to another organophosphorus compound, methamidophos, in the body. Studies with ^{14}C-acephate in mammals have shown 75% of the parent compound eliminated in the urine. Other major metabolites include *O*,*S*-dimethyl phosphorothioate (DMPT, 5%) and *S*-methyl acetyl phosphoramidothioate (5%).

Mechanism of Toxicity

Acephate exerts its toxicity by inhibiting the enzyme acetylcholinesterase in the synapse and neuromuscular junctions, which leads to accumulation of the neurotransmitter acetylcholine and overstimulation of postsynaptic receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acephate is moderately toxic to mammals with an acute oral LD_{50} of 850–950 mg kg^{-1} in rats, whereas its metabolite methamidophos is highly toxic to mammals. The common symptoms of acephate poisoning include salivation, nasal discharge, vomiting, diarrhea, nausea, blurred vision, difficulty in breathing, headache, and muscle weakness. Convulsions, coma, and death may occur in cases of severe acute poisoning.

Human

Acephate exposure can result in cholinesterase inhibition, which causes overstimulation of the nervous system. Acephate is nonirritating to skin and slightly irritating to the eyes but is not a skin sensitizer.

Chronic Toxicity (or Exposure)

Animal

Chronic studies in rats and dogs have shown cholinesterase inhibition. No pathological changes were observed in rats following 3 month exposure to 30 mg kg^{-1} dosage of acephate.

Human

Acephate is classified as a possible human carcinogen. Since acephate is used both on food crops and other common residential areas, risks of human exposures through multiple routes are high. Based on cholinesterase inhibition studies in rats, the no-observed-adverse-effect level for chronic dietary exposure is 0.12 mg kg^{-1} day^{-1}. Agricultural workers who are involved in mixing, formulation, and application may be at higher risk of exposure.

Clinical Management

In case of dermal exposure, the contaminated area should be washed with plenty of water or showered using soap and shampoo. Eyes should be flushed with water repeatedly for several minutes. Contaminated clothing should be removed and the airway cleared. In case of ingestion, vomiting should be induced. Atropine treatment should be initiated immediately to counteract muscarinic effects. Atropine (adults and children >12 years: 2–4 mg; children <12 years: 0.05–0.1 mg) treatment should be repeated every 15 min until oral and bronchial secretions are controlled and atropinization is achieved. The duration and dosage of atropine treatment should be slowly reduced as the condition of the patient improves. Pralidoxime should be administered slowly at the recommended dosage (adults and children >12 years: 1–2 g; children <12 years: 20–50 mg by IV infusion in 100 ml saline at $\sim$0.2 g min^{-1}). This dosage can be repeated at every 1–2 h intervals initially and at 10–12 h intervals later depending on the condition of the patient. Periodic medical examination and care is required depending on the degree of exposure.

Environmental Fate

Acephate is readily degraded in soil by microorganisms and in water it undergoes rapid hydrolysis.

Its half-life is less than 3 and 6 days under aerobic and anaerobic conditions, respectively. CO_2 is the major metabolite following microbial degradation in soil.

Ecotoxicology

Both acephate and its metabolite, methamidophos, pose a high acute and chronic risk to birds. Studies in insects have shown that acephate is highly toxic to honey bees and other beneficial insects. Methamidophos is also very highly toxic to freshwater invertebrates.

Exposure Standards and Guidelines

The chronic reference dose for acephate is $0.0012\,mg\,kg^{-1}\,day^{-1}$ while the accepted daily intake is $0.03\,mg\,kg^{-1}\,day^{-1}$.

See also: Acetylcholine; Methamidophos; Neurotoxicity; Organophosphates; Pesticides.

Relevant Websites

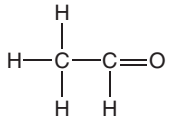
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Acetaldehyde

John Sanseverino

This article is a revision of the previous print edition article by Edward Kerfoot, volume 1, pp. 10–11, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 75-07-0
- Synonyms: Acetic aldehyde; Acetylaldehyde; Ethylaldehyde; Ethanal
- Chemical/Pharmaceutical/Other Class: Aldehydes
- Chemical Formula: C_2H_4O
- Chemical Structure:



Uses

Acetaldehyde is used in the manufacturing of various chemicals such as acetic acid, pyridine, peracetic acid, pentaerythritol, 1,3-butylene glycol, and chloral. It is also used in the silvering of mirrors, leather tanning, fuel compositions, preservatives, paper processing, glues, cosmetics, dyes, plastics, and rubber. Natural sources of acetaldehyde include metabolic intermediate in higher plants, alcohol fermentation, and sugar decomposition in the body. Anthropogenic sources include vehicle exhaust, fuel oil and coal, organic chemical manufacturing.

Exposure Routes and Pathways

Industrial exposures to acetaldehyde are most likely to occur by inhalation with potential for skin and eye contact. Accidental ingestion is also possible. Acetaldehyde is produced from the metabolism of ethanol in the body.

Toxicokinetics

Following inhalation exposure, acetaldehyde is deposited in the nasal cavity and upper respiratory tract, and eventually some traces can be absorbed into the blood and be distributed throughout the body. The uptake of acetaldehyde in the nasal cavity is influenced by its solubility and inspiratory flow rate. Perhaps acetaldehyde uptake in the nasal tissue is dependent on its reaction with tissue substrates that become depleted at high exposure concentrations. Acetaldehyde vapor can be metabolized in the nasal cavity by the mixed-function oxidase and carboxylesterase systems. The first metabolite of ethanol metabolism is acetaldehyde. Metabolism takes place in the liver to a number of metabolites and some unchanged acetaldehyde that can be excreted in the urine. Most of the free acetaldehyde is excreted in the exhaled breath.

Mechanism of Toxicity

Acetaldehyde is soluble in the mucous membranes of the upper respiratory tract causing irritation of the sensory nerve endings. There is also depression of the mucociliary defense system. The direct action of acetaldehyde in the skin and eyes is the result of irritation to these tissues.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for acetaldehyde in rats has been reported to be $1930\,mg\,kg^{-1}$ and the 4 h LC_{50} is

approximately 13 300 ppm. Acetaldehyde is a severe eye irritant to rabbits at 40 mg and mildly irritating to rabbit skin at 500 mg. Rats were exposed to acetaldehyde concentrations ranging from 400 to 5000 ppm in a 4 week subchronic inhalation study at 6 h day^{-1}, 5 day week^{-1}. At 1000 and 2200 ppm, the rats exhibited growth retardation, polyuria, and nasal epithelial degeneration. At 400 ppm, there was slight degeneration of the olfactory epithelium.

Human

Inhalation exposures to acetaldehyde can result in irritation of the upper respiratory tract. Inhalation at concentrations ranging from 100 to 200 ppm can cause irritation to the mucous membranes. Skin and eye contact with liquid acetaldehyde can produce a burning sensation, lacrimation, and blurred vision. Unacclimated subjects experienced eye irritation at 50 ppm after a 15 min exposure. Some more sensitive persons exhibited eye irritation at 25 ppm for a 15 min exposure.

Chronic Toxicity (or Exposure)

Animal

A 52 week chronic inhalation study in hamsters exposed to 1500 ppm acetaldehyde produced growth retardation, slight anemia, increased enzyme and protein content in the urine, and increased kidney weight. There were distinct histopathological changes in the nasal mucosa and trachea, including hyperplasia, squamous cell metaplasia, and inflammation.

Inhalation exposure to acetaldehyde has produced nasal tumors in rats and laryngeal tumors in hamsters. Male and female rats were exposed to acetaldehyde 6 h day^{-1}, 5 day week^{-1} for 28 months at concentrations of 0, 750, 1500, or 3000 ppm. A concentration-related incidence of squamous cell carcinomas of the respiratory epithelium was observed in both male and female rats. A statistically significant number of adenocarcinomas occurred in the olfactory epithelium of both sexes of rats exposed at all three acetaldehyde concentrations. Male and female hamsters were exposed to acetaldehyde 7 h day^{-1}, 5 day week^{-1} at concentrations gradually reduced from 2500 to 1650 ppm for 52 weeks. Both sexes of acetaldehyde-exposed hamsters developed laryngeal tumors consisting of squamous cell carcinomas and adenosquamous cell carcinomas.

Data from studies with rats suggest that acetaldehyde is teratogenic. Fetuses from dams injected intraperitoneally with acetaldehyde concentrations ranging from 50 to 100 mg kg^{-1} on day 10, 11, or 12 of gestation produced a significant increase in fetal resorptions, growth retardation, and an increase in malformations, including digital anomalies, cranial and facial malformations, and delayed skeletogenesis. It was concluded that acetaldehyde interfered with placental function via the maternal–placental nutrient exchange, resulting in retarded growth.

Data from some animal studies suggest that acetaldehyde is teratogenic. According to the American Conference of Governmental Industrial Hygienists (ACGIH), the recent identification of nasal and laryngeal carcinomas indicated that acetaldehyde should be considered an A3 animal carcinogen.

Human

Acetaldehyde, produced from the metabolism of ethanol, may also be responsible for localized cancers, brain damage in prenatal infants, and growth suppression (in chicken embryos). Acetaldehyde, as a direct result of ethanol metabolism in the body, has been implicated in alcoholic cardiomyopathy and cancer of the digestive tract. The levels of acetaldehyde in blood are directly correlated with ethanol consumption.

In Vitro Toxicity Data

Acetaldehyde has been shown to induce mutagenic changes in many assays. In mammalian *in vitro* assays, acetaldehyde produced sister chromatid exchanges and chromosomal breaks and aberrations in mammalian *in vitro* assays.

Clinical Management

Exposures by inhalation should be monitored for respiratory tract irritation, bronchitis, or pneumonitis. Humidified supplemental 100% oxygen should be administered. Gastric lavage may be indicated soon after ingestion of acetaldehyde followed by administration of activated charcoal slurry mixed with a saline cathartic or sorbitol. Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. If eye irritation, pain, swelling, lacrimation, or photophobia persists, the patient should be seen in a health care facility.

Exposure Standards and Guidelines

A short-term exposure limit ceiling of 25 ppm for acetaldehyde was recommended to prevent excessive eye irritation, lacrimation, and potential injury to the respiratory tract.

See also: Respiratory Tract.

Further Reading

Eriksson C and Peter J (2001) The role of acetaldehyde in the actions of alcohol (Update 2000). *Alcoholism: Clinical and Experimental Research* 25(5, Suppl.): 15S–32S.

Verschueren K (2000) *Handbook of Environmental Data on Organic Chemicals*, 3rd edn. New York: Wiley.

World Health Organization (1995) *Acetaldehyde*. Geneva: World Health Organization.

Acetamide

Gerald L Kennedy

- Chemical Abstracts Service Registry Number: CAS 60-35-5
- Synonyms: Acetic acid amide; Ethanamide; Methane-carboxamide
- Chemical/Pharmaceutical/Other Class: Amide, aliphatic; Organic solvent; Volatile organic compound
- Chemical Formula: CH_3CONH_2

Uses

As a dipolar solvent, acetamide finds many uses as a solvent for both inorganic and organic compounds. The solvency has led to widespread uses in industry including applications in cryoscopy, soldering, and the textile industry. The neutral and amphoteric characteristics allow its use as an antacid in the lacquer, explosives, and cosmetics industries. Its hygroscopic properties make it useful as a plasticizer in coatings, fixtures, cloth, and leather, and as a humectant for paper. It is also a raw material in organic synthesis of methylamine and thioacetamide and as an intermediate in preparation of medicines, insecticides, and plastics.

Exposure Routes and Pathways

Acetamide may be inhaled, swallowed, or absorbed through the skin. The chemical is considered to be mildly irritating to the skin and eyes. In its usual application, inhalation is the most common route of exposure, although dermal contact is always possible.

Toxicokinetics

Oral administration of acetamide in the rat is followed by absorption and 62% is excreted into the urine unchanged in 24 h. Likewise, a large proportion of an oral dose is excreted in the urine unchanged by the dog and cat. In sheep, absorption of an oral dose is followed by metabolism to CO_2 within 7–12 h. Sequential demethylation of methylacetamide results in acetamide production by rat liver but it is not clear whether this occurs in man. Acetamide is a metabolite of the antiprotozoal drugs metronidazole and ornidazole.

Mechanism of Toxicity

The mechanism of toxicity of acetamide is not known; the response profile is quite different from the better studied dimethyl derivative.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in rodents ranges from 1 to 7 g kg^{-1} and intravenous LD_{50} in mice and rats is 10 g kg^{-1}. No acute lethality information is available following either dermal or inhalation exposures. Acetamide is not a developmental toxicant and is generally inactive in genetic toxicity tests.

Human

No reports could be found in the literature concerning acute toxicity of acetamide in humans.

Chronic Toxicity (or Exposure)

Animal

Liver cancers were produced in rats following oral administration of relatively large amounts of acetamide. The liver appears to be the target of acetamide toxicity although the animal experiments have been limited in the range of endpoints studied.

Human

No reports could be found in the literature concerning the potential human health effects of chronic acetamide exposure.

Clinical Management

Exposed persons should be removed to fresh air and medical attention sought as needed for any breathing difficulty. If swallowed, several glasses of water should be given to dilute the chemical; medical attention is needed if large amounts are ingested. For skin contact, the exposed area should be washed with soap and water; medical attention should be sought if irritation develops. For eye contact, water should be used to flush for at least 15 min while lifting the lower and upper eyelids occasionally; immediate medical attention should be sought.

Environmental Fate

Acetamide will exist as a vapor in the ambient atmosphere. Atmospheric degradation occurs by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 7.6 days. If released to soil, acetamide is expected to have very high mobility and is not expected to adsorb to suspended solids and sediment. Experiments suggest that this chemical may break down in the environment through biodegradation and not through hydrolysis. Volatilization from water surfaces is not expected to be an important fate process based on this compound's estimated Henry's law constant. The potential for bioconcentration in aquatic organisms is low.

Exposure Standards and Guidelines

US Environmental Protection Agency: Listed as a hazardous air pollutant under the Clean Air Act of 1990.

Occupational Safety and Health Administration: No permissible exposure limit (as of October 2003).

International Agency for Research on Cancer: Classified as a 2B carcinogen (probable human carcinogen with sufficient evidence in laboratory animals).

See also: Clean Air Act (CAA), US; Methylamine; Metronidazole; Thioacetamide.

Further Reading

Kennedy GL Jr. (1986) Biological effects of acetamide, formamide, and their monomethyl and dimethyl derivatives. *Critical Reviews in Toxicology* 17: 129–182.

Kennedy GL Jr. (2001) Biological effects of acetamide, formamide, and their monomethyl and dimethyl derivatives: An update. *Critical Reviews in Toxicology* 31: 139–222.

Relevant Website

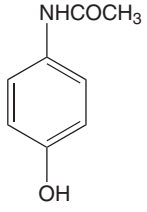
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Acetamide.

Acetaminophen

Kartik Shankar and Harihara M Mehendale

This article is a revision of the previous print edition article by Edward P Krenzelok, volume 1, pp. 11–13, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 103-90-2
- Synonyms: APAP; 4′-Hydroxyacetanilide; *p*-Hydroxyacetanilide; Acetamide *N*-(4-hydroxyphenyl); *N*-Acetyl-*p*-aminophenol; *N*-Acetyl-*p*-aminophenol; *p*-Acetamidophenol; 4-Acetamidophenol; 4-Acetaminophenol; Paracetamol; Tylenol
- Chemical/Pharmaceutical/Other Class: Acetaminophen is a synthetic nonopioid congener of acetanilide in the *p*-aminophenol class
- Chemical Formula: $C_8H_9NO_2$
- Chemical Structure:



Uses

Acetaminophen is a nonnarcotic analgesic and antipyretic drug. It is used to relieve pain of moderate intensity, such as usually occurs in headache and in many muscle, joint, and peripheral nerve disorders. Headaches are one of the most common indications for the use of acetaminophen. Acetaminophen is used to treat acute tension-headaches and mild to moderate

migraine, especially in combination with caffeine and aspirin. Acetaminophen is indicated in chronic pain associated with rheumatoid arthritis, back or hip pain, osteoarthritis, dental pain, or acute pain due to soft-tissue injury. Acetaminophen is a suitable substitute for aspirin for its analgesic or antipyretic uses in cases where aspirin is contraindicated (gastric bleeding) or when the prolongation of bleeding time caused by aspirin would be a disadvantage. Acetaminophen has been used in studies of pain relief following obstetric and gynecological procedures including Caesarean section, hysterectomy, tubal ligation, primary dysmenorrhoea, and termination of pregnancy. Acetaminophen is also used to manage chronic pain of cancer, postpartum and postoperative pain after minor surgery. In a double blind crossover study, the analgesic oral butorphanol and acetaminophen in combination, showed additive analgesic effects against moderate to severe pain due to metastatic carcinoma over that of individual drug. Acetaminophen is also widely used as an antipyretic drug to reduce fever.

Background Information

Acetaminophen can be found as the active ingredient in more than 100 over-the-counter products and a number of prescription drugs, alone or in combination with other drugs. The pharmacology and toxicology of this drug has been extensively studied and reviewed. Acetaminophen has been the subject of more than 30 000 articles in medical literature since 1966. The first clinical use of acetaminophen dates back to 1893 by von Mering (and subsequently by Hinsberg and Treupel, 1894) as an effective antipyretic with comparable pharmacological effects to antipyrine and phenacetin. However, after a hiatus of almost half a century, acetaminophen was rediscovered as the major metabolite of phenacetin and acetanilide in man and was marketed in the United States as a combination with aspirin and caffeine in 1950. In the 1960s and 1970s concerns about gastrointestinal adverse effects of aspirin and methemoglobinemia of acetanilide only led to increased popularity of acetaminophen as a generally safe antipyretic analgesic. Hepatotoxicity of acetaminophen began to be reported in the late 1960s and has been a topic of intense scientific evaluation to this day. The impact of acetaminophen-induced liver toxicity, accidental or otherwise, will be taken up in later sections.

Exposure Routes and Pathways

Acetaminophen is available in several dosage forms including tablets, capsules, syrups, elixirs, and suppositories. Oral ingestion is the most common route for both accidental and intentional exposure to acetaminophen.

Toxicokinetics

Absorption of acetaminophen occurs in the gastrointestinal tract primarily by passive nonionic diffusion and is highly dependent on the several factors including dose, presence of food and other chemicals, mucosal blood flow, age, body weight, time of day, and coexisting disease conditions. At pharmacological doses acetaminophen is absorbed rapidly with $\sim$75–95% of the therapeutic oral dose being recovered in the urine by 12–24 h as unchanged acetaminophen or metabolite. A large number of studies have evaluated the pharmacokinetic parameters of acetaminophen in man after oral or intravenous dosing. Most studies consistently report volume of distribution to be between 0.8 and 1 l kg^{-1}. Total clearance and plasma half-life with therapeutic doses in healthy subjects were usually 3–5 ml min kg^{-1} and 1–3 h, respectively. After suprapharmacological or toxic doses absorption may be delayed after producing peak blood concentrations at $\sim$4 h postingestion. In man, the majority of acetaminophen is metabolized in the liver to glucuronide and sulfate conjugates that are eliminated in the urine. Estimates in man from urinary metabolites report 50–60% as glucuronide conjugate, 25–35% as sulfate conjugate, and between 2% and 5% of cysteine and mercapturate conjugates each. In young children, the sulfate conjugate predominates. The water-soluble glucuronide and sulfate conjugates are eliminated via the kidneys. Approximately 2–5% is eliminated in the urine as unchanged acetaminophen. The half-life of therapeutic dose is 1–3 h. In overdose patients, this may be increased to more than 4 h and may even exceed 12 h in patients with severe acetaminophen-induced liver toxicity.

Mechanism of Toxicity

Although major part of the ingested dose of acetaminophen is detoxified, a very small proportion of acetaminophen is metabolized via the cytochrome P450-mixed function oxidase pathway to a highly reactive *N*-acetyl-*p*-benzoquinoneimine (NAPQI). The toxic intermediate NAPQI is normally detoxified by endogenous glutathione to cysteine and mercapturic acid conjugates and excreted in the urine. Recent studies have shown that hepatic P450 s, CYP2E1 and, to a lesser extent CYP1A2 are responsible for conversion of acetaminophen to NAPQI. In acetaminophen overdosage, the amount of NAPQI

increases and depletes endogenous glutathione stores. Time course studies have shown that covalent binding of reactive NAPQI and subsequent toxicity occurs only after cellular glutathione stores are reduced by 70% or more of normal. Mitochondrial dysfunction and damage can be seen as early as 15 min after a toxic dose in mice, suggesting that this may be a critical to cellular necrosis. The NAPQI is then thought to covalently bind to critical cellular macromolecules in hepatocytes and cause cell death. Recent proteomic studies have identified at least 20 known proteins that are covalently modified by the reactive acetaminophen metabolite. Hepatic necrosis as a consequence of hepatocellular death then results in development of clinical and laboratory findings consistent with liver failure. A similar mechanism is postulated for the renal damage that occurs in some patients who suffer from acetaminophen toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

A large body of evidence is available examining the acute toxicity of acetaminophen in animal models. Mice and rats have been widely used to study the toxic effects of acetaminophen. Since the rat is relatively resistant, the mouse has been the most widely used species to study both the mechanisms of acetaminophen toxicity and to examine chemicals that potentiate or protect from the toxicity. Hepatotoxicity and nephrotoxicity are the two main effects associated with acute overdose of acetaminophen. Of these, death in most species is due to acute hepatic failure. LD_{50} values range from 350 to 4500 mg kg^{-1} depending on the species and the route of acetaminophen administration, mice (LD_{50} 350–600 mg kg^{-1}) being more far more sensitive than rats, guinea pigs, and rabbits ($LD_{50} > 3$ g kg^{-1}). Death occurs within 12 h after acetaminophen exposure. In mice after a toxic dose, general findings in addition to the severe hepatic necrosis, include necrotic changes in the kidney, bronchiolar epithelium, testes, lymphoid follicles of the spleen, and small intestine. Cats are particularly susceptible to acetaminophen intoxication because of their impaired glucuronic acid conjugation mechanism and saturation of their sulfate conjugation pathway. The clinical signs associated with experimental acetaminophen administration to cats included cyanosis followed by anemic hemoglobinuria, icterus, and facial edema. Laboratory findings in acetaminophen poisoned cats include methemoglobinemia and an elevated serum alanine aminotransferase activity.

Human

Hepatotoxicity is the primary toxic insult from acute acetaminophen overdose. Acetaminophen overdose accounts for more than 56 000 emergency room visits and is implicated in ~50% of all acute liver failure in the United States (US Acute Liver Failure (ALF) Study Group). Exposure to toxic doses of acetaminophen may be intentional (suicidal) or unintentional (accidental). Recent data from Parkland Hospital suggests that greater percentages of unintentional overdose victims suffer from fatal consequences compared to persons attempting suicide (with acetaminophen) primarily due to their characteristic late presentation. Data from the US ALF Study Group shows that unintentional overdoses (which are more frequent in liver failure cases) were also larger (median dose of 34 g) compared to suicidal doses, being consumed over several preceding days. There is no clear agreement on a maximum tolerated dose of acetaminophen. Most people tolerate 4–8 g day^{-1} of acetaminophen without any hepatotoxic incidence. However, the risk of severe liver injury may be quite high above the 4 g day^{-1} dose, especially in a group of individuals due to indeterminate idiosyncratic reasons.

The typical clinical manifestations are secondary to hepatic damage. Plasma concentrations should be obtained to determine the probability of acetaminophen-induced hepatotoxicity. The Rumack–Matthews nomogram is used to assess the risk of hepatotoxicity. Levels in excess of 200 μg ml^{-1} of acetaminophen at 4 h postingestion are associated with high probability of development of hepatotoxicity. A second treatment line 25% lower than the original '200' line was added at the request of the Food and Drug Administration (FDA) in 1976. The clinical presentation follows four distinct phases. Gastrointestinal irritation, nausea and vomiting are present in the first 24 h postingestion. The second stage (24–48 h) postingestion is characterized by the resolution of the initial symptoms, accompanied by elevations of hepatic transaminases. Cases that progress to stage three develop hypoglycemia, coagulopathies, jaundice, and symptoms consistent with hepatic failure. Surviving patients go through a fourth stage of recovery. As toxicity develops half-life becomes prolonged and transaminases rise and fall. In instances where reliable history of time of ingestion is not available calculations of body burden may be useful in deciding treatment.

Chronic Toxicity (or Exposure)

Animal

In a 2 year feed study, there was no evidence of carcinogenic activity of acetaminophen in male F344/N

rats that received 600, 3000, or 6000 ppm acetaminophen for 104 weeks. There was equivocal evidence of carcinogenic activity in female F344/N rats based on increased incidences of mononuclear cell leukemia. Overall, there is inadequate evidence in experimental animals for the carcinogenicity of acetaminophen and is not classifiable as to its carcinogenicity. Acetaminophen was nonmutagenic in the *Salmonella*/mammalian microsome assay at concentrations ranging from 0.1 to 50 mg per plate. In a study to examine effect of acetaminophen on reproduction and fertility, no changes in the number of pups/litter, viability, or in adjusted pup weight were found. Acetaminophen in the diet of Swiss mice reduced weight gain during nursing. Fertility endpoints (ability to bear normal numbers of normal-weight young) were generally not affected.

Human

There is inadequate evidence in humans for the carcinogenicity of acetaminophen and is therefore not classifiable. The chronic ingestion of excessive amounts of acetaminophen may produce similar toxicity as a large acute dose but in a more insidious fashion. Age, chronic alcohol abuse, and preexisting disease may be contributing factors. The American Academy of Pediatrics considers use of acetaminophen safe during breast-feeding and is classified as a category B chemical by the FDA (studies in laboratory animals have not demonstrated a fetal risk, but there are no controlled studies in pregnant women). Acetaminophen should be given with care to patients with impaired kidney or liver function. Care should also be taken when giving acetaminophen to patients taking other drugs that affect the liver.

In Vitro Toxicity Data

Acetaminophen causes cytotoxicity in several cell types; however, the most widely studied cytotoxicity of acetaminophen is in primary hepatocytes or hepatocyte cell lines.

Cytotoxicity in Hepatic Cells

Primary hepatocytes from rats, mice, hamsters, rabbits, dogs, pigs, monkeys, and humans have been shown to be susceptible to acetaminophen *in vitro*. The cytotoxicity of acetaminophen varies considerably depending on species, presumably due to differences in bioactivation and glutathione status. The most obvious morphological effect of acetaminophen in isolated primary hepatocytes is blebbing of the cell membrane. However, electron microscopy has shown that toxicity is associated with progressive loss of microvilli, mitochondrial abnormalities and appearance of myeloid bodies. Exposure of primary mouse hepatocytes to concentrations of acetaminophen above 1 mmol l^{-1}, led to significant lactate dehydrogenase leakage as early as 3 h. Cytotoxicity of acetaminophen has also been examined using standard liver cell lines including, PC12 cells, HepG2 cells, H4IIEC3G$^-$ cells, among other cell lines. Immortalized hepatocyte cultures, in many cases, lose their ability to bioactivate acetaminophen and hence are resistant to toxicity. Transient or consistent overexpression of P450 enzymes (CYP2E1 and/or CYP1A2) leads to increased cytotoxicity of acetaminophen. Acetaminophen is also cytotoxic in cultures of rat liver sinusoidal endothelial cells, Kupffer cells and mouse fibroblasts.

Cytotoxicity in Other Cells

The cytotoxicity of acetaminophen has been demonstrated in cultures of HeLa cells, L929 and 3T3 murine fibroblasts, chick embryo neurons, rat embryonic and skeletal muscle, peripheral blood lymphocytes, and lung and dermal cells. In addition cytotoxicity of acetaminophen has been evaluated in BF-2 fish cell line (see section on 'Ecotoxicology').

Clinical Management

Activated charcoal or other gastrointestinal decontamination procedures can be utilized when deemed necessary. Induction of emesis is not recommended as prolonged emesis may interfere with *N*-acetyl cysteine (NAC) therapy. The Rumack–Matthews nomogram is utilized to identify proper course of treatment. Blood acetaminophen concentrations of 200 mg l^{-1} (or higher) at 4 h postingestion indicate severe risk of hepatic failure and are treated with standard NAC treatment regimen. NAC is a glutathione substitute and prevents hepatic damage by quenching the reactive NAPQI. An oral loading dose of 140 mg kg^{-1} (as a 5% solution in soft drink or juice) is followed by 70 mg kg^{-1} given orally as a (5% solution in soft drink or juice) every 4 h for an additional 17 doses. An alternative intravenous dosing protocol (20 h regimen) for NAC (Acetadote) can also be used in patients where oral NAC administration is not possible. A loading dose of 150 mg kg^{-1} NAC (in 200 ml of 5% dextrose in water) is administered over 15 min, followed by 50 mg kg^{-1} NAC (in 500 ml of 5% dextrose) over the next 4 h. A final dose of 100 mg kg^{-1} NAC is administered in 1000 ml of 5% dextrose over a 16 h period. A longer 72 h treatment regimen with intravenous NAC is recommended in the United States.

Basic and advanced life-support measures should be utilized as required by the condition of the patient.

Environmental Fate

Acetaminophen was found to be inherently biodegradable and has no bioaccumulation potential. No other information about the environmental fate of acetaminophen is currently available.

Ecotoxicology

The acute toxicity of acetaminophen has been examined in several aquatic species. LC_{50} value in brine shrimp (*Artemia salina*) examining mortality was reported to be $3820\,\mu mol\,l^{-1}$. EC_{50} for immobility over a 24 h experiment using water flea (*Daphnia magna*) was $367\,\mu mol\,l^{-1}$. Acetaminophen is classified as not toxic or only slightly to moderately toxic to all fish (fathead minnow, *Pimephales promelas*) and zooplankton species tested. The crustacean fairy shrimp (*Streptocephalus proboscideus*) appears to be highly sensitive to acetaminophen (average LC_{50} of $196\,\mu g\,l^{-1}$).

Other Hazards

Acetaminophen is stable under ordinary conditions of use and storage. In the presence of heat and water, acetaminophen will hydrolyze into acetic acid and *p*-aminophenol. Incineration may produce carbon monoxide, carbon dioxide, nitrogen oxides.

Flammability: As with most organic solids, fire is possible at elevated temperatures or by contact with an ignition source.

Explosivity: Fine dust dispersed in air in sufficient concentrations and in the presence of an ignition source, is a potential dust explosion hazard. Minimum concentration for explosion is $0.25\,oz.\,ft^{-3}$. The recommended fire extinguishing media is water spray, dry chemical, alcohol foam, or carbon dioxide. Acetaminophen is capable of generating a static electrical charge. Processes involving dumping of acetaminophen into flammable liquid, inert atmosphere in the vessels, or temperatures of flammable liquid should be maintained below its flashpoint.

Exposure Standards and Guidelines

Therapeutic exposure: Total daily dose of acetaminophen should not exceed 4 g. Dosages of acetaminophen over $4–8\,g\,day^{-1}$ over long periods of time may be associated with higher risk of liver toxicity. Acetaminophen should not be administered for more than 10 days or to young children except upon advice of physician.

Occupational exposure: Mallinckrodt recommends an airborne exposure limit of $5\,mg\,m^{-3}$.

Miscellaneous

A special mention of interaction of acetaminophen with alcohol consumption is warranted. Large numbers of reports in scientific literature and public media suggest that a potentially high risk of liver toxicity due to acetaminophen exists when consumed following alcohol intake. In a recent review, however, Dr. Barry Rumack suggests that only chronic heavy drinkers may be at greater risk following an overdose of acetaminophen and that no potentiation of toxicity occurs at therapeutic doses.

See also: Phenazopyridine.

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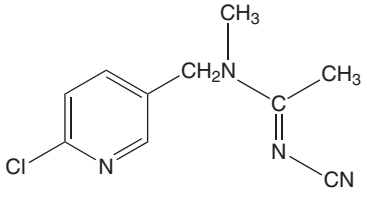
Relevant Websites

http://bulkpharm.mallinckrodt.com – Acetaminophen Material Safety Data Sheet, Mallinckrodt.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Acetaminophen.

Acetamiprid

David Wallace

- Chemical Abstracts Service Registry Number: CAS 135410-20-7
- Synonyms: Mospilan; Assail
- Chemical/Pharmaceutical/Other Class: Neonicotinoid (pyridylmethylamine) insecticide
- Chemical Formula: $C_{10}H_{11}ClN_4$
- Chemical Structure:



Uses

Acetamiprid is used as an insecticide to control sucking-type insects on leafy vegetables and fruits. In many instances, these insects may be resistant to the effects of organophosphorus and other conventional insecticides.

Exposure Routes and Pathways

The primary route of exposure is via diet (food and water). Occupational exposure for individuals who work with this insecticide can occur via dermal contact or inhalation.

Toxicokinetics

Acetamiprid is rapidly and extensively metabolized. Metabolites in urine account for 79–86% of the administered dose. Only 3–7% of acetamiprid is collected unchanged in the urine and feces. Demethylation by phase I biotransformation is the major pathway, with 6-chloronicotinic acid being the major metabolite. Compounds can then undergo phase II transformation with glycine conjugation representing the major pathway.

Mechanism of Toxicity

The primary mechanism of acetamiprid toxicity against insects is due to its action at nicotinic cholinergic receptors. The unique nature of the neonicotinoids as insecticides is that the negatively charged cyano (or nitro) group will specifically interact with a cationic binding region that is unique to insects. This action will convey selectivity of action against insects and leave mammalian nicotinic receptors relatively unaffected.

Acute and Short-Term Toxicity (or Exposure)

There is little evidence for acetamiprid toxicity in vertebrates. There is some evidence for contact exposure, dermal irritation, and stomach poisoning following oral ingestion.

Animal

Acute studies in laboratory animals, mainly rats, have demonstrated relatively low toxicity potential for acetamiprid. Oral ingestion appears to elicit the most severe toxicological responses. At dosages in excess of $140\,mg\,kg^{-1}$, acetamiprid elicited neurotoxic signs, with animals exhibiting disorders of movement and posture. Surviving animals were free of signs on the following day. Acetamiprid was only slightly toxic following inhalation (LC_{50} $>1.15\,mg\,l^{-1}$) and weakly toxic following dermal

administration (LD_{50} $>2000\,mg\,kg^{-1}$). There was minimal or no irritation of eyes or skin. Some metabolites of acetamiprid exhibited greater toxicity than the parent compound.

Human

No evidence is available for assessing human outcomes following acute exposure to acetamiprid. Signs associated with acute exposure and limits of exposure have been established using data from animal studies. Due to the selectivity of acetamiprid for insect nicotinic cholinergic receptors, little human toxicity is expected.

Chronic Toxicity (or Exposure)

Examination of chronic exposure to acetamiprid has utilized animals and little data on chronic human exposure is available. The selectivity of acetamiprid for insect nicotinic receptors would suggest minimal toxicity following chronic exposure.

Animal

Chronic dietary administration of acetamiprid resulted in reduced body and organ weight. Higher doses resulted in neurological dysfunction. There was evidence for teratogenic potential in animal studies.

Human

No evidence is available for assessing human outcomes following chronic exposure to acetamiprid. Symptoms associated with chronic exposure and limits of exposure have been established using data from animal studies. Due to the selectivity of acetamiprid for insect nicotinic cholinergic receptors, little human toxicity is expected however.

In Vitro Toxicity Data

There were no positive results in genotoxicity studies using bacterial or mammalian cell assays.

Clinical Management

There are no guidelines for acetamiprid toxicity outside of symptomatic control.

Environmental Fate

Acetamiprid exhibits a very short half-life in soil. It is rapidly degraded by aerobic metabolism. Acetamiprid is stable to hydrolysis at environmental temperatures and it photodegrades slowly in water. It is transformed moderately rapidly in aerobic aquatic environments, but only slowly in anaerobic aquatic systems. There appears to be minimal effects on drinking water and due to the rapid breakdown has not demonstrated the ability to bioaccumulate in wildlife. Due to the rapid breakdown of acetamiprid it is not expected to be persistent in the environment. Metabolites of acetamiprid will pose a greater risk to the environment, but additional work is needed to determine the fate and toxicity of acetamiprid metabolites.

Ecotoxicology

Due to the rapid breakdown of acetamiprid, there is minimal risk to fish or wildlife. Proper labeling could alleviate any additional risk. Specificity for insects significantly reduces any additional toxicity to nontarget organisms.

Exposure Standards and Guidelines

The Environmental Protection Agency has established guidelines for toxicological dose and endpoints for acetamiprid. Using the no-observed-adverse-effect level (NOAEL) and uncertainty factor (UF), the reference dose (RfD) can be calculated. For acute dietary ingestion for infants and children, $NOAEL = 10\,mg\,kg^{-1}$; $RfD = 0.10\,mg\,kg^{-1}\,day^{-1}$. Chronic dietary exposure for all populations, $NOAEL = 7.1\,mg\,kg^{-1}$; $RfD = 0.07\,mg\,kg^{-1}\,day^{-1}$. Short- and intermediate-term incidental exposure to infants and children, $NOAEL = 15\,mg\,kg^{-1}\,day^{-1}$ and for adults $NOAEL = 17.9\,mg\,kg^{-1}\,day^{-1}$. Long-term dermal exposure $NOAEL = 7.1\,mg\,kg^{-1}\,day^{-1}$ with dermal absorption of 30%. Short- and intermediate-term inhalation exposure $NOAEL = 17.9\,mg\,kg^{-1}\,day^{-1}$ and for long-term inhalation exposure, $NOAEL = 7.1\,mg\,kg^{-1}\,day^{-1}$.

See also: Imidacloprid; Neonicotinoids; Nithiazine; Pesticides.

Further Reading

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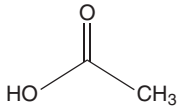
http://www.epa.gov – United States Environmental Protection Agency Federal Register of Environmental Documents; see also United States Environmental Protection Agency: Pesticide Fact Sheet.

Acetic Acid

Sanjay Chanda

- Chemical Abstracts Service Registry Number: CAS 64-19-7
- Synonyms: Glacial acetic acid; Acido acetico; Vinegar acid; Methanecarboxylic acid; Ethanoic acid; Pyroligneous acid
- Chemical/Pharmaceutical/Other Class: Pharmaceutical aid (acidifier)
- Chemical Structure:



Uses

Acetic acid is used in the manufacture of various acetates, acetyl compounds, cellulose acetate, acetate rayons, plastics, and rubber. It is also used in tanning, as laundry sour, in printing calico, and in dyeing silk. It is an acidulant and preservative in food. It is a solvent for gums, resins, volatile oils, and many other substances. Acetic acid is widely used in commercial organic synthesis.

Background Information

Acetic acid is present throughout nature as a normal metabolite of both plants and animals. Acetic acid may also be released to the environment in a variety of waste effluents, in emissions from combustion processes, and in exhaust from gasoline and diesel engines.

Exposure Routes and Pathways

Contact with skin and ingestion are the most common exposure pathways.

Toxicokinetics

Acetic acid is absorbed from the gastrointestinal (GI) tract and through the lung. Acetic acid is readily metabolized by most tissues and may give rise to the production of ketone bodies as intermediates. *In vitro* experiments have demonstrated that acetate is incorporated into phospholipids, neutral lipids, sterols, and saturated and unsaturated fatty acids in a variety of human and animal tissue preparations.

Mechanism of Toxicity

Acetic acid causes toxicity by coagulative necrosis; that is, the acid denatures all tissue protein to form an acid proteinate. As a result, both structural and enzymatic proteins are denatured and cell lysis is blocked. Therefore, cell morphology is not greatly interrupted. In addition, an ester is formed which delays further corrosive damage and helps reduce systemic absorption. Thus, damage, especially with small quantities of acid, is frequently limited to local sites of injury to the skin or the GI tract rather than the systemic response.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acetic acid is corrosive to skin and gastric mucosa. Repetitive exposure to acetic acid may cause erosion of dental enamel, bronchitis, and eye irritation. Bronchopneumonia and pulmonary edema may develop following acute overexposure. LC_{50} in guinea pig and mouse by inhalation is 5000 ppm h^{-1} and LD_{50} in rat by oral route is 3.53 g kg^{-1}.

Human

Acetic acid is corrosive to skin and gastric mucosa. Repetitive exposure to acetic acid may cause erosion of dental enamel, bronchitis, and eye irritation. Bronchopneumonia and pulmonary edema may develop following acute overexposure.

Chronic Toxicity (or Exposure)

Human

Chronic exposure may result in pharyngitis and catarrhal bronchitis. Ingestion, though not likely to

BLANK

Acetone

Lee R Shugart

- Chemical Abstracts Service Registry Number: CAS 67-64-1
- Synonyms: Dimethyl ketone; 2-Propanone; Dimethylketal
- Chemical/Pharmaceutical/Other Class: Ketone
- Chemical Formula: $(CH_3)_2CO$

Uses

Acetone is obtained by fermentation or chemical synthesis and is used to make plastic, fibers, drugs, and other chemicals. It is also used to dissolve fats, oils, waxes, resins, rubber, plastics, lacquers, varnishes, and rubber cements. In the laboratory, it is used to extract various substances from animal and plant tissues and as a dehydrating agent.

Background Information

Acetone occurs naturally in plants, trees, volcanic gases, forest fires, and as a product of the breakdown of body fat. It is present in vehicle exhaust, tobacco smoke, and landfill sites. Industrial processes contribute more acetone to the environment than natural processes.

Exposure Routes and Pathways

Exposure to acetone results mostly from breathing air, drinking water, or coming in contact with products or soil that contains acetone. Significant numbers of workers are potentially exposed to acetone. The general population may be exposed through the use of products such as paints, adhesives, cosmetics, and rubber cement.

Toxicokinetics

Acetone that enters the blood is carried to all organs in the body. Small amounts are enzymatically converted to nonharmful substances in the liver.

Mechanism of Toxicity

Acetone acts mainly as an irritant affecting the eyes, nose, throat, and respiratory tract.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral rat LD_{50}: 5800 mg kg^{-1}; inhalation rat LC_{50}: 50 100 mg m^{-3}; irritation eye rabbit, standard draize, 20 mg severe.

Human

Breathing moderate levels of acetone for short periods of time can cause headaches, light-headedness, and confusion with an increase in pulse rate. Vomiting, unconsciousness, and possibly coma can accompany high levels of exposure. Swallowing very high levels of acetone can result in unconsciousness and damage to the skin in the mouth.

Symptoms following acute acetone ingestion include nausea, vomiting, gastric hemorrhage, sedation, respiratory depression, ataxia, and paresthesia. Depression resembles alcoholic stupor, but its onset is quicker than that with ethanol. Coughing and bronchial irritation may be the only clues to ingestion of quantities that are too small to produce sedation. Hyperglycemia and ketonemia with acidosis that resembles acute diabetic coma may be present.

Chronic Toxicity (or Exposure)

Animal

Kidney, liver, and nerve damage with an increase in birth defects and lowered ability to reproduce (males only) were noted in animals exposed for long periods of time.

Human

It is not know if humans would experience the same effects observed in animals. The relevance to humans of the liver, reproductive, and developmental effects observed in animal studies is not known, and these endpoints have not been sufficiently examined in humans. Not considered genotoxic or mutagenic. Acetone has not been classified as a carcinogen and studies of workers exposed to acetone found no significant risk of death from cancer. Prolonged or repeated skin contact may produce severe dermatitis.

In Vitro Toxicity Data

Acetone (reagent grade) was evaluated by the standard plate incorporation method in the Ames *Salmonella* reverse mutation assay with strains TA98, TA100, TA1535, TA1537, and TA1538.

Experiments were done in triplicate with and without metabolic activation (S9 fractions from Aroclor-treated Sprague–Dawley rats). Results were negative in these strains.

Clinical Management

If inhaled and breathing is difficult, the person is moved to fresh air and administered oxygen. For skin contact, the area is washed with water. For eye contact, water is used for flushing.

Environmental Fate

Acetone is highly volatile and enters the environment mainly via the atmosphere where it may be moderately degraded by photolysis, react with photochemically produced hydroxyl radicals or be removed by wet deposition. Acetone may be biodegraded when released into the soil, but because it is miscible in water, it may leach into existing groundwater but is not expected to significantly bioaccumulate.

Ecotoxicology

Acetone is not expected to be toxic to aquatic life. The LC_{50}/96 h values for fish are over 100 mg l^{-1}.

Other Hazards

Acetone is a volatile and an extremely flammable liquid. Vapor may cause flash fire.

Exposure Standards and Guideline

Occupational Safety and Health Administration Permissible Exposure Limit: 1000 ppm (time-weighted average).

See also: Pollution, Air; Skin.

Further Reading

Arts JH (2002) An analysis of human response to the irritancy of acetone vapors. *Critical Reviews in Toxicology* 32(1): 43–66.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Acetone.
http://www.inchem.org – Acetone: Environmental Health Criteria (from the International Program on Chemical Safety).

Acetonitrile

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 75-05-8
- Synonyms: Cyanomethane; Ethane nitrile; Ethanenitrile; Ethyl nitrile; Methanecarbonitrile; Methyl cyanide
- Chemical/Pharmaceutical/Other Class: Organic solvent; Cyanogen; Nitrile
- Chemical Formula: C_2H_3N

Uses

Acetonitrile is used in the chemical industry as an intermediary in the synthesis of several chemicals and products such as acetophene, thiamine, acetamidine, and α-naphthaleneacetic acid, nitrogen containing compounds, acrylic fibers, nitrile rubber, pesticides, pharmaceuticals, perfumes, and lithium batteries. It is also used as a polar solvent for both organic and inorganic compounds and in non-aqueous titrations.

Exposure Routes and Pathways

Exposure to acetonitrile can occur through the oral, dermal, and inhalation routes. Symptoms of poisoning have been observed in persons exposed through these three routes.

Toxicokinetics

Acetonitrile can be acutely lethal when absorbed in high doses. Acetonitrile is metabolized to a hydroxyl metabolite by cytochrome P450 in the liver. Subsequent metabolism through catalase enzymes produces hydrogen cyanide. Once metabolized, the mechanism of action is the same as expected for

cyanide poisoning. Onset of cyanide poisoning may be delayed 8 or more hours as metabolism is required to produce the cyanide metabolite. Toxicity may be prolonged for up to 3 days in some cases.

Mechanism of Toxicity

Acetonitrile is slowly metabolized by cytochrome P450 in the liver to produce hydrogen cyanide. Toxicity is produced by the combined effect of circulating acetonitrile and cyanide. Cyanide exerts its toxicological effects by disrupting oxygen utilization at the cellular level. The disruption results in decreased oxygen utilization by body tissues and lactic acidosis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal susceptibility to acetonitrile varies by animal species and route of administration. Overall, animal susceptibility is mediated by the animal's ability to absorb and metabolize acetonitrile into its toxic metabolite, hydrogen cyanide.

Atmospheres containing up to 32 000 ppm acetonitrile are lethal to dogs. In rats, the oral LD_{50} has been measured to range from 200 mg kg^{-1} (in young rats) to 3800 mg kg^{-1} (age unspecified), whereas the inhalation LC_{50} has been determined to be 7500 ppm following an 8 h exposure. The acute dermal lethal dose has been investigated in rabbits. The LD_{50} through the dermal route has been determined to be 980 mg kg^{-1}. Subchronic exposures to low acetonitrile concentrations in the air (665 ppm or less) produced pulmonary inflammation and minor changes in body weights, hematocrit, hemoglobin, and liver and kidney functions.

Human

Toxicological effects of acetonitrile are usually delayed as the chemical has to be metabolized to cyanide. However, exposure to high doses may result in rapidly developing loss of consciousness and respiratory failure.

Signs and symptoms of exposure will be determined by the dose of acetonitrile. Onset of symptoms can be expected to be delayed from 2–12 h as acetonitrile is slowly metabolized to its toxic metabolite, cyanide. Exposure to low doses will produce nausea, salivation, vomiting, headache, and lethargy. Exposure to higher doses may produce cyanide intoxication characterized by extreme weakness, lethargy, respiratory depression, metabolic acidosis, tachycardia, shock, coma, seizures, and possibly death.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to acetonitrile in air at concentrations ranging from 166 to 665 ppm for 7 h per day for up to 90 days showed no-observed-effects at doses below 330 ppm. At the maximum dose tested (665 ppm), pulmonary inflammation as well as minor kidney and liver changes were noted in some animals.

Dogs and monkeys exposed to acetonitrile in air for 91 days showed minor variations in body weight, hematocrit, and hemoglobin. The animals were dosed acetonitrile at concentrations averaging 350 ppm for 7 h day^{-1}, 3 days week^{-1}. Autopsy of the animals revealed some cerebral hemorrhaging as well as pigment-bearing macrophages in some animals.

Male and female rats were exposed to acetonitrile by inhalation at doses ranging from 0 to 400 ppm for 6 h day^{-1}, 5 days week^{-1} for 2 years. Results of the study were inconclusive regarding the carcinogenic activity of acetonitrile as there was only a marginal increased incidence of hepatocellular adenomas and carcinomas in male rats. Furthermore, there was no evidence of carcinogenic activity in the female rats even at exposures as high as 400 ppm. In a similar study using male and female mice exposed to acetonitrile at doses ranging from 0 to 200 ppm by inhalation for 6 h day^{-1}, 5 days week^{-1} for 2 years, no carcinogenic activity was noted in the animals and doses tested.

The toxicological effects of acetonitrile have been attributed to the direct effects of the intact molecule combined with the effects of metabolically generated cyanide ions.

Human

No reports were found on the chronic toxicological effects of acetonitrile in humans.

In Vitro Toxicity Data

In vitro studies using rat liver microsomes have demonstrated that the conversion of acetonitrile to cyanide is mediated by cytochrome P450 (P-450IIE1).

Acetonitrile was tested for mutagenicity in the *Salmonella*/microsome preincubation assay. The tests were conducted using up to five *Salmonella* strains and in the presence and absence of rat or hamster liver S-9. All tests were negative for mutagenicity

including those run at the maximum dose tested (10 mg per plate).

Clinical Management

The major goal of treatment is to maintain respiration, blood circulation, and vital signs and to prevent further absorption of acetonitrile into the systemic circulation. If ingested, absorption can be prevented or minimized by instituting gastric lavage or by giving activated charcoal and a cathartic. Gastric lavage is effective only if performed soon after ingestion.

Treatment of acetonitrile poisoning is similar to that of cyanide poisoning. This includes immediate therapy with 100% oxygen and assisted ventilation, if necessary. Seizures can be controlled by giving diazepam, phenobarbital, or phenytoin intravenously at appropriate doses. Therapy should also include correction of the metabolic acidosis and to combat cyanide poisoning. Cyanide poisoning is treated by the intravenous administration of sodium nitrite and sodium thiosulfate. Care should be taken to maintain treatment for as long as acetonitrile is being metabolized to cyanide.

Environmental Fate

If released to ambient air, acetonitrile will remain in the vapor phase where it will be degraded through reaction with photochemically produced hydroxyl radicals. The half-life of acetonitrile in ambient air has been estimated to be $\sim$620 days. If released to soil, acetonitrile is expected to volatilize rapidly. Biodegradation in soil is not expected to be a major degradation pathway. If released to water, acetonitrile is not likely to adsorb to soil and sediment particles. Acetonitrile is expected to be removed from water bodies through volatilization as the chemical hydrolysis and bioaccumulation potential for this chemical are low.

Ecotoxicology

Toxicity thresholds for protozoa, bacteria, and green algae have been measured to range from 520 $mg\,l^{-1}$ for *Microcystis aeruginosa* (algae) to 7300 $mg\,l^{-1}$ for *Scenedesmus quadricauda* (green algae).

The LC_{50} for acetonitrile in fathead minnow (*Pimephales promelas*) has been measured to be $\sim$1640 $mg\,l^{-1}$ per 96 h in a flowthrough bioassay.

Other Hazards

Acetonitrile is highly flammable and will ignite in the presence of flames, sparks, or sufficient heat. Acetonitrile vapors may combine with air to form explosive mixtures.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration permissible exposure limit = 40 ppm (70 $mg\,m^{-3}$).
- American Conference of Governmental Industrial Hygienists (ACGIH) 8 h time-weighted average = 40 ppm.
- ACGIH short-term exposure limit = 60 ppm.
- National Institute for Occupational Safety and Health immediately dangerous to life or health value = 500 ppm.
- The US Environmental Protection Agency's Integrated Risk Information System has published a reference concentration for acetonitrile of 0.06 $mg\,m^{-3}$.
- Florida State Drinking Water Standard = 500 $\mu g\,l^{-1}$.

See also: American Conference of Governmental Industrial Hygienists; Cyanide; National Institute for Occupational Safety and Health; Occupational Safety and Health Act, US.

Further Reading

Ellenhorn MJ and Barceloux DG (eds.) (1988) *Medical Toxicology, Diagnosis and Treatment of Human Poisoning*. New York: Elsevier.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Acetonitrile.

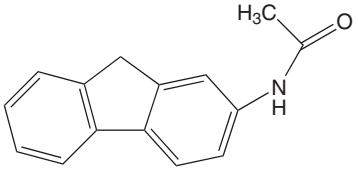
http://www.epa.gov – Chemical Summary for Acetonitrile (from the US Environmental Protection Agency).

http://www.osha-slc.gov – Safety and Health Topics: Acetonitrile (from the US Occupational Safety and Health Administration).

Acetylaminofluorene

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 28322-02-3
- Synonyms: *N*-Fluorene-4-yl-acetamide; *N*-4-Fluorenylacetamide; *N*-Fluoren-4-yl-acetamide; 4-Acetylaminofluorene; 2-Acetylaminofluorene
- Chemical/Pharmaceutical/Other Class: Aromatic amine
- Chemical Structure:



Uses

Acetylaminoflourene is found as a contaminant in coal gasification processes. It has no known use.

Background Information

2-Acetylaminofluorene is used frequently by biochemists and technicians. These persons may be exposed to acetylaminofluorene. The occupations at greatest risk to acetylaminofluorene exposure are organic chemists, chemical stockroom workers, and biomedical researchers. Although neither National Institute of Occupational Safety and Health (NIOSH) nor Occupational Safety and Health Administration (OSHA) has estimated the number of US workers exposed to acetylaminofluorene, perhaps fewer than 1000 workers in 200 laboratories may come in contact with this.

Exposure Routes and Pathways

Skin contact is the most common accidental exposure pathway. Acetylaminoflourene emits toxic fumes of nitrous oxides when heated to decompose and can be toxic when inhaled.

Toxicokinetics

Acetylaminofluorene is biotransformed in the liver. 2-Acetylaminofluorene can stimulate cytochrome P450 1A1 isozyme (CYP1A1) activity, inducing both CYP1A1 and CYP1A2 proteins, whereas 4-acetylaminofluorene modestly increases CYP1A2 but does not influence CYP1A1.

Mechanism of Toxicity

4-Acetylaminofluorene is not carcinogenic; 2-acetylaminofluorene is carcinogenic. 2-Acetylaminofluorene can be metabolized to form *N*-hydroxyacetylaminofluorene and 2-aminofluorene, which may covalently bind to the DNA and macromolecules. Ring hydroxylation, however, leads to the formation and excretion of water-soluble conjugates (e.g., glucuronides) of the respective hydroxylated metabolites and detoxification.

Acute and Short-Term Toxicity (or Exposure)

Animal

A single dose of acetylaminofluorene at 0.1 mg kg^{-1} when injected into mice (DD strain) on gestation days 8–15 produced mainly skeletal defects, cleft lips, cleft palates, and cerebral hernias. Fisher 344 rats were fed 0.06% acetylaminofluorene in diet for 4 weeks and then on control diet for 1 week. This schedule was carried out for three cycles (12 weeks). A smaller group was also treated for only one cycle. Rats treated for three cycles showed high incidence of liver, testis, and zymbal gland tumors. No tumors observed in rats treated for one cycle. The LD_{50} of 4-acetylaminofluorene in mice is 364 mg kg^{-1} by the intraperitoneal route. When fed to male rats (0.05% of diet) for 3 or 4 weeks, 4-acetylaminofluorene caused proliferation of agranular endoplasmic reticulum and glycogen depletion in hepatocytes. The same treatment when continued for 10 months produces conspicuous morphological alterations in pancreatic granular endoplasmic reticulum together with mitochondrial damage and focal cytoplasmic degradation.

Human

Human exposure data are not available. 2-Acetylaminofluorene is thought to be carcinogenic to humans.

Chronic Toxicity (or Exposure)

Animal

Different species respond differently to chronic administration of 2-fluoroacetylamine. Guinea pigs and

monkeys fail to develop tumors after treatment. Bladder and liver tumors have been induced in dogs. Liver tumors (but not bladder tumors) have been induced in chickens, fish, cats, and hamsters. Bladder tumors (not liver tumors) have been induced in rabbits. There were also variations in different colonies of inbred animals.

Human

Human exposure data are not available. 2-Acetylaminofluorene is thought to be carcinogenic to humans.

In Vitro Toxicity Data

The mutagenicity of *N*-2-fluorenylacetamide (NFA) was evaluated in *Salmonella* tester strains TA98, TA100, TA1535, TA1537, and TA1538 (Ames test), both in the presence and absence of added metabolic activation by aroclor-induced rat liver S9 fraction. Based on the results of preliminary bacterial toxicity determinations, NFA, diluted with dimethyl sulfoxide (DMSO), was tested for mutagenicity at concentrations up to 1 mg per plate using the plate incorporation assay. NFA caused a positive response in strains TA1535, TA100, and TA98 following metabolic activation.

Clinical Management

In case of contact with the eyes, the eyes should be immediately flushed with running water for at least 15 min. Affected skin should be washed with soap and water. If vapor is inhaled, the victim should be moved to fresh air and emergency medical care provided. If the victim is not breathing, artificial respiration should be provided; if breathing is difficult, oxygen should be administered. Contaminated clothing and shoes should be removed and isolated at the site.

Environmental Fate

Release of 2-acetylaminofluorene to the environment from artificial sources is probably not significant since less than 20 lb $year^{-1}$ of this compound are consumed in the United States. If released to soil, 2-acetylaminofluorene is expected to have low mobility. Chemical hydrolysis, oxidation, and volatilization are not expected to be significant. If released to water, 2-acetylaminofluorene may undergo direct photolysis and is expected to strongly adsorb to suspended solids and sediments. Chemical hydrolysis, oxidation, volatilization, and bioaccumulation are not expected to be significant. If released to the atmosphere, 2-acetylaminofluorene may undergo vapor phase adsorption to airborne particulate matter, it may react with photochemically generated hydroxyl radicals (estimated vapor phase half-life = 5.92 h) or it may undergo direct photolysis.

Ecotoxicology

No aquatic toxicology data available.

Other Hazards

Acetylaminofluorene can be easily ignited by heat, sparks, or flames. Vapors may form explosive mixtures with air. Vapors may travel to source of ignition and flash back. Most vapors are heavier than air. They can spread along ground and collect in low or confined areas (sewers, basements, tanks). Vapor explosion hazard exists indoors, outdoors, or in sewers.

Exposure Standards and Guidelines

As per OSHA, workers' exposure to 2-acetylaminofluorene is to be controlled through the required use of engineering controls, work practices, and personal protective equipment, including respirators. Identified as an occupational carcinogen without establishing a permissible exposure limit. NIOSH considers 2-acetylaminofluorene to be a potential occupational carcinogen. NIOSH usually recommends that occupational exposures to carcinogens be limited to the lowest feasible concentration.

See also: Carcinogenesis; Cytochrome P-450.

Further Reading

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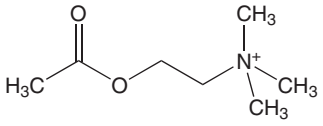
Shepard TH (1980) *Catalog of Teratogenic Agents*, 3rd edn., p. 148. Baltimore, MD: Johns Hopkins University Press.

Sigala F, Kostopanagiotou G, Andreadou I, *et al.* (2004) Histological and lipid peroxidation changes after administration of 2-acetylaminofluorene in a rat liver injury model following selective periportal and pericentral damage. *Toxicology* 196: 155–163.

Acetylcholine

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 51-84-3
- Synonyms: Acecoline; Choline acetate; Arterocholine; 2-(Acetoxy)-*N*,*N*,*N*-trimethylethanaminium; Ethanaminium; 2-(Acetyloxy)-*N*,*N*,*N*-trimethyl
- Chemical/Pharmaceutical/Other Class: Neurohumoral transmitter
- Chemical Structure:



Uses

Acetylcholine is present naturally in the body. Commercial drugs used as cholinergic agonists mimic the action of acetylcholine.

Background Information

Acetylcholine is the endogenous neurotransmitter at cholinergic synapses and neuroeffector junctions in the central and peripheral nervous systems. The actions of acetylcholine are mediated through nicotinic and muscarinic cholinergic receptors, which transduce signals via distinct mechanisms.

Exposure Routes and Pathways

Acetylcholine is present in the body as a neurohumoral transmitter. Acetylcholinesterase is the enzyme responsible for breakdown of acetylcholine in the synapse. Any drug classified as cholinergic agonist (which mimics the action of acetylcholine) or anticholinesterase agent (e.g., organophosphorus pesticides, which block the action of acetylcholinesterase and hence stop the breakdown of acetylcholine in the synapse) can increase the level of acetylcholine in the body. The most common exposure pathways for the cholinergic agonists are ingestion or contact to the eye. Acetylcholine chloride is available as an intraocular solution, methacholine chloride is available as a powder, bethanechol chloride is available as tablets, and carbachol is available as an ophthalmic solution. Common exposure pathways to anticholinesterase agents are ingestion, dermal or ocular contact, or inhalation.

Toxicokinetics

Acetylcholine is broken down by the acetylcholinesterase enzyme to choline and acetate. The time required for hydrolysis of acetylcholine is less than a millisecond. If the enzyme is depleted or inhibited, then excessive acetylcholine accumulation in the body can cause toxicity. Symptoms are salivation, lacrimation, urination, diarrhea, muscle tremor, and fasciculation.

Mechanism of Toxicity

Cholinergic agents can increase the acetylcholine level at the synaptic junction and cause rapid firing of the postsynaptic membrane. Anti-acetylcholinesterase agents block the acetylcholinesterase enzyme and thus increase the acetylcholine level in the synapse causing rapid firing of the postsynaptic membrane.

Acute and Short-Term Toxicity (or Exposure)

Animal

The clinical signs of excess acetylcholine at nerve endings mimic hyperactivity of the parasympathetic nervous system. Signs relative to the alimentary tract include excess salivation, lacrimation, abdominal pain, vomiting, intestinal hypermotility, and diarrhea. The muscarinic effects of acetylcholine cause bronchoconstriction and an increase in bronchial secretions. The nicotinic effects of acetylcholine consist of involuntary irregular, violent muscle contractions and weakness of voluntary muscles. Death occurs as a result of respiratory failure.

Human

Acetycholine agents are contraindicated in persons with asthma, hyperthyroidism, coronary insufficiency, and peptic ulcer. The bronchoconstrictor effect may precipitate asthma, hyperthyroid patients may develop atrial fibrillation, hypotension induced by these agents can reduce coronary blood flow, and gastric acid secretion caused by these agents can aggravate the symptoms of peptic ulcer. Excessive acetylcholine can also cause flushing, sweating, bradycardia, hypotension, abdominal cramps, belching, diarrhea, sensation of tightness in the urinary

bladder, involuntary defecation and urination, penile erection, difficulty in visual accommodation, headache, salivation, and lacrimation. It can also cause paralysis of the respiratory muscles. Central nervous system effects include ataxia, confusion, slurred speech, loss of reflexes, Cheyne–Stokes respiration, and finally coma. The time of death after a single acute exposure ranges from 5 min to 24 h depending on the route, dose, and agent of exposure (among other factors).

Chronic Toxicity (or Exposure)

Animal

Animals may lose weight due to the inability to feed and drink because of muscular weakness. Clinical signs in birds include goose stepping, ataxia, wing spasms, wing droop, dyspnea (difficulty in breathing), tenesmus (spasm of anal sphincter), diarrhea, salivation, lacrimation, ptosis (drooping) of the eyelids, and wing-beat convulsions. Susceptibility to organophosphate toxicity varies greatly among individuals of any species and can be increased by frequent repeated mild exposure, which results in greater susceptibility due to exhaustion of the body's store of cholinesterase. No definite postmortem changes are seen and when present are usually secondary to the symptoms and include pulmonary edema, asphyxia, gastroenteritis, and rarely kidney and liver degeneration.

Human

Chronic toxicity can cause polyneuritis, which starts with mild sensory disturbances, ataxia, weakness, and ready fatigability of legs, accompanied by fasciculation, muscle twitching, and tenderness to palpitation. In severe cases, the weakness may progress eventually to complete flaccid paralysis that, over the course of weeks or months, is often succeeded by a spastic paralysis with a concomitant exaggeration of reflexes. During these phases, muscles show marked wasting.

Clinical Management

Exposure should be terminated as soon as possible either by removal of the patient or by fitting the patient with a gas mask if the atmosphere remains contaminated. Contaminated clothing should be removed immediately; the skin and mouth should be washed with copious amounts of water. Gastric lavage should be conducted if necessary. Artificial respiration should be administered if required, and administration of oxygen may be necessary. If the convulsion persists, diazepam (5–10 mg intravenously) or sodium thiopental (2.5% intravenously) should be administered, and the patient should be treated for shock. Atropine should be administered in sufficiently large doses, but atropine is without any effect against peripheral neuromuscular activation and subsequent paralysis. Pralidoxime (1 or 2 g infused intravenously) should be administered for all the peripheral effects.

See also: A-Esterases; Anticholinergics; Cholinesterase Inhibition; Neurotoxicity; Organophosphates; Respiratory Tract.

Further Reading

Brown JH and Taylor P (1996) Muscarinic receptor agonists and antagonists. In: Hardman JG and Limbird LE (eds.) *Goodman and Gillman's Pharmacological Basis of Therapeutics*, 9th edn., pp. 141–160. New York: McGraw-Hill.

Foye WO, Lemke TL, and Williams DA (1995) *Principles of Medicinal Chemistry*, 4th edn. Baltimore and London: William & Wilkins.

Acetylcholinesterase *See* Cholinesterase Inhibition.

Acetylene

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 74-86-2
- Synonyms: Acetylene; Ethine; Ethyne; Narcylen; Welding gas
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon (C_nH_{2n-2})
- Chemical Formula: C_2H_2
- Chemical Structure: H–C≡C–H

Uses

Major producers manufacture acetylene by either the partial oxidation of natural gas or as a coproduct of the thermal cracking of ethylene; minor producers manufacture acetylene from calcium carbide. About 80% of production is used as a closed system intermediate in the manufacture of acetylene black as well as acetylenic and vinyl derivatives used in a variety of applications such as the manufacture of plastics. The remaining 20% is used primarily in oxyacetylene torches for welding and metal cutting. Although acetylene was used as an anesthetic in the early 1900s, this use has fallen into disfavor due to the explosive properties of acetylene.

Exposure Routes and Pathways

Due to its physical properties as a gas, its method of production and almost exclusive use as a closed system intermediate, potential industrial exposures are limited almost exclusively to inhalation. With the exception of torches, acetylene is not used in consumer products.

Toxicokinetics

Absorption of acetylene is driven by its partial pressure in respired air. Following inhalation, acetylene rapidly enters the blood by diffusion and is distributed to body organs in approximate proportion to their rate of perfusion. Acetylene crosses the blood–brain barrier, producing central nervous system effects characteristic of asphyxia. Diffusion is reversed upon the elimination of exposure and acetylene is excreted unchanged primarily via exhalation from the lungs, with some elimination via the urine.

Mechanism of Toxicity

Acetylene is a simple asphyxiant; a physiologically inert gas that can deplete the atmosphere of available oxygen when present in high concentrations and thereby deprive the tissues of necessary oxygen. Signs of asphyxia are noted when the atmospheric oxygen concentration is reduced to 16% or less. The tissues that are most sensitive to hypoxia are the brain and heart.

Acute and Short-Term Toxicity (or Exposure)

Animal

In dog studies, acetylene in oxygen rapidly produces anesthesia at 500 000 ppm, which becomes pronounced at 750 000–900 000 ppm. Recovery is rapid with no apparent effects. Administration of acetylene in oxygen to the cat reduces respiration and increases blood pressure at levels between 400 000 and 800 000 ppm. In the rabbit, these effects on respiration and blood pressure are noted between 600 000 and 800 000 ppm. Repeated exposures of multiple species to acetylene at concentrations $\leqslant$ 800 000 ppm acetylene did not result in cellular injury to the heart, lungs, liver, kidney, or spleen.

Human

Acetylene is not toxic below its lower explosive limit of 25 000 ppm. In the presence of adequate oxygen, acetylene causes a slight intoxication at 100 000 ppm, which leads to a staggering gait and general incoordination as the concentration increases to 300 000 ppm. Unconsciousness occurs in 5–7 min at $\sim$350 000 ppm. Full anesthesia occurs at 800 000 ppm. Anesthetic concentrations of acetylene do not affect heart, liver, or kidney function but do result in an increase in blood pressure.

Chronic Toxicity (or Exposure)

Human

Despite its long use as an industrial chemical and anesthetic, there are no epidemiological data linking acetylene exposure to deleterious health effects.

In Vitro Toxicity Data

Acetylene was not mutagenic in an Ames assay conducted both in the presence and absence of metabolic activation.

Clinical Management

The exposed individual should be removed from the toxic environment and given 100% humidified supplemental oxygen with assisted ventilation as required. If hypoxia has been prolonged, the patient should be evaluated for neurologic sequelae and supportive treatment provided. Dermal exposure to liquid acetylene should be treated as indicated for frostbite injury.

Environmental Fate

Acetylene is a gas that will partition almost exclusively to the air where it is degraded by reaction with hydroxyl radicals (13 day half-life). Although soluble in water (1230 mg l^{-1} at 25°C), acetylene is not

expected to accumulate in this medium due to its rapid volatilization to air.

Ecotoxicology

Due to its partitioning to air, exposure of aquatic receptors to acetylene does not pose a significant environmental risk and few studies have been conducted in this area. Limited experimental and modeling data indicate that the range of LC_{50} and EC_{50} values for acetylene in fish, aquatic invertebrates, and algae is 200–500 mg l^{-1}.

Other Hazards

Acetylene is a reactive material that poses a fire and explosion hazard. Its lower and upper explosive limits in air are 2.5% and 93%, respectively. Acetylene reacts with active metals (e.g., copper, silver, and mercury) to form explosive acetylide compounds. Acetylene manufactured from calcium carbide can contain impurities such as phosphine and arsine that are responsible for the ethereal to garlic-like odor of commercial acetylene and pose a greater human health risk than acetylene alone. Maintaining acetylene levels below 3000 ppm can minimize these secondary toxic effects.

Exposure Standards and Guidelines

Acetylene is recognized internationally as a simple asphyxiant. The American Conference of Governmental Industrial Hygienists recommends that atmospheres containing acetylene have a minimum oxygen concentration of 18%. The National Institute of Occupational Safety and Health has a recommended exposure limit for acetylene of 2500 ppm as a ceiling.

See also: Copper; Mercury; Silver; Toxicity Testing, Dermal; Toxicity Testing, Inhalation.

Further Reading

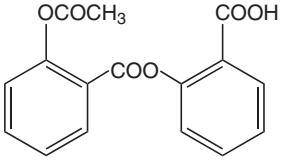
Budavari S (ed.) (1989) *The Merck Index – Encyclopedia of Chemicals, Drugs and Biologicals*, p. 14. Rahway, NJ: Merck and Co., Inc.

Acetylsalicylic Acid

Christopher P Holstege

This article is a revision of the previous print edition article by Sanjay Chanda, volume 1, pp. 21–22, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 50-78-2
- Synonyms: 2-(Acetyloxy)benzoic acid; 2-Carboxyphenyl ester; Aspirin
- Chemical/Pharmaceutical/Other Class: Salicylates
- Chemical Structure:



Uses

Aspirin is an analgesic, antipyretic, and anti-inflammatory agent.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures, but dermal and rectal exposures have also been reported.

Toxicokinetics

Absorption of aspirin occurs by passive diffusion across the gastrointestinal membrane and is influenced by gastric pH. The presence of food delays the absorption of aspirin. Aspirin-induced pylorospasm, gastric outlet obstruction, and concretions also delay the absorption. At therapeutic doses, aspirin is found within the plasma within 30 min, peak levels are obtained within 2 h, protein binding is 90%, and the volume of distribution is less than 0.2 l kg^{-1}. In overdosage, levels may not peak for over 12 h, the protein binding decreases to less than 75%, and the volume of distribution increases to over 0.3 l kg^{-1}. Aspirin exhibits Michaelis–Menton kinetics: the elimination half-life is ~15–20 min at therapeutic doses (first-order kinetics) and as long as 20 h in overdosage (zero-order kinetics). Aspirin is

metabolized primarily by the hepatic endoplasmic reticulum and mitochondria to salicyluric acid, ether glucuronide, and ester glucuronide. The metabolites are excreted in the urine. Approximately 10% of aspirin is excreted as free salicylic acid.

Mechanism of Toxicity

In salicylate toxicity, nausea, vomiting, and abdominal discomfort occur due to both local gastric irritation and stimulation of the medullary chemoreceptor trigger zone. Salicylates increase sensitivity to carbon dioxide in the medulla oblongata, thereby inducing hyperventilation, decreasing P_{CO_2}, and causing respiratory alkalosis. A compensatory increase in the renal excretion of bicarbonate leads to the loss of potassium and sodium in the urine. A metabolic acidosis may follow due to the accumulation of organic acids. As a result, aspirin may produce a mixed acid–base abnormality consisting of both respiratory alkalosis and metabolic acidosis. Salicylates uncouple oxidative phosphorylation, resulting in a failure to produce adenosine triphosphate while at the same time increasing oxygen utilization and carbon dioxide production. This results in an increase in heat production. Salicylates also interfere with glucose metabolism and gluconeogenesis. Salicylates may also profoundly decrease brain glucose concentrations despite normoglycemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may manifest toxicity to salicylates with signs and symptoms similar to those seen in humans. These may include fever, hyperpnea, seizures, respiratory alkalosis, metabolic acidosis, gastric hemorrhage, and kidney damage. Methemoglobinemia has also been seen in animals following salicylate toxicity. Activated charcoal has been used in animals. Methylene blue or ascorbic acid may be utilized for the treatment of methemoglobinemia.

Human

Nausea, vomiting, tinnitus, and hyperventilation are seen early in toxicity. As severity of toxicity increases, intractable vomiting, hyperthermia, hypotension, tachycardia, confusion, coma, seizures, pulmonary edema, acute renal failure, and death may occur. Hyperglycemia may be seen early, whereas hypoglycemia may occur later in toxicity. Acid–base disturbances such as respiratory alkalosis and/or metabolic acidosis may be noted. Signs and symptoms of salicylate toxicity may be noted as blood levels rise over $30\,mg\,dl^{-1}$.

Chronic Toxicity (or Exposure)

Animal

Daily doses of acetylsalicylic acid in cats produced toxic hepatitis, vomiting, weight loss, poor appetite in the low-dose group ($33–63\,mg\,kg^{-1}$) and anemia, gastric lesions, and death in the high-dose group ($81–130\,mg\,kg^{-1}$). High doses of aspirin given to mice on day 6 of gestation produced large incidence of lethal deformities.

Human

Chronic salicylism presents clinically in a similar fashion to the acute situation, although it is often associated with a delay in diagnosis, and a higher morbidity and mortality. Chronic salicylism is more often associated with pronounced hyperventilation, dehydration, pulmonary edema, renal failure, coma, seizures, and acidosis. Chronic salicylism can occur at serum salicylate levels as low as $15\,mg\,dl^{-1}$.

In Vitro Toxicity Data

Acetylsalicylic acid has been tested for mutagenicity using a variety of models and has not demonstrated mutagenic activity in concentrations of 0.01–50 mg per plate.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be considered in the patient with appropriate airway protection. In general, a single dose of activated charcoal should be considered in patients who have had substantial ingestions. Acetylsalicylic acid ingestions can result in substantial delays in absorption; therefore, charcoal may be given even up to 8 h postingestion and more than one dose of charcoal may be considered to prevent further drug absorption. Careful correction of fluid and electrolyte abnormalities is essential. The clinician should insure adequate urine output, but forced diuresis should be avoided. Administration of intravenous sodium bicarbonate should be considered in patients manifesting signs and symptoms of salicylate toxicity. Hemodialysis effectively increases clearance and improves fluid/electrolyte balance. This extracorporeal method of elimination should be considered in patients with acute mental status changes, renal failure, intractable acidosis, pulmonary edema, severe fluid imbalance, or acute serum salicylate levels over $100\,mg\,dl^{-1}$ or in patients with chronic salicylate overdose, who have symptoms and serum levels $>60\,mg\,dl^{-1}$.

Environmental Fate

As in humans, the environmental fate of acetylsalicylic acid is pH dependent. Above pH 5.5, acetylsalicylic acid will be the predominant form seen. Anions generally do not volatilize or undergo adsorption to the extent of their neutral counterparts. Although information is limited, it is expected that acetylsalicylic acid should biodegrade under anaerobic conditions and photodegrade in unlit soil surfaces.

See also: Ascorbic Acid; Charcoal; Salicylates.

Further Reading

Chapman BJ and Proudfoot AT (1989) Adult salicylate poisoning: Deaths and outcome in patients with high plasma salicylate concentrations. *Quarterly Journal of Medicine* 72: 699–707.

Done AK (1960) Salicylate intoxication: Significance of measurements of salicylates in blood in cases of acute ingestion. *Pediatrics* 26: 800–807.

Temple AR (1981) Acute and chronic effects of aspirin toxicity and their treatment. *Archives of Internal Medicine* 141: 364–369.

Acids

Sanjay Chanda

Uses

Acids have a wide range of uses. The specific use depends on the specific acid.

Exposure Routes and Pathways

Dermal contact, inhalation, and ingestion are the most common exposure pathways.

Toxicokinetics

The toxicokinetics depends on the specific type of acid.

Mechanism of Toxicity

Acids cause toxicity by coagulative necrosis; that is, the acid denatures all tissue protein to form an acid proteinate. As a result both structural and enzymatic proteins are denatured and cell lysis is blocked. Therefore, cell morphology is not greatly interrupted. In addition, an ester is formed which delays further corrosive damage and helps reduce systemic absorption. Thus, damage, especially with small quantities of acid, is frequently limited to local sites of injury to the skin or the gastrointestinal tract, rather than the systemic response.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acids are corrosive to skin and mucosal surfaces. Repetitive ingestion of acids may induce mucosal forestomach hyperplasia.

Human

Acids are corrosive to skin and mucosal surfaces. Repetitive ingestion of acids may induce mucosal forestomach hyperplasia.

Chronic Toxicity (or Exposure)

Animal

Acids are corrosive to skin and mucosal surfaces. Repetitive ingestion of acids may induce mucosal forestomach hyperplasia.

Human

Acids are corrosive to skin and mucosal surfaces. Repetitive ingestion of acids may induce mucosal forestomach hyperplasia.

Clinical Management

Exposure should be terminated as soon as possible by removal of the patient to fresh air. The skin, eyes, and mouth should be washed with copious amounts of water. A 15–20 min wash may be necessary to neutralize and remove all residual traces of the contaminant. Contaminated clothing and jewelry should be removed and isolated. Contact lenses should be removed from the eye to avoid prolonged contact of the acid with the area. A mild soap solution may be used for washing the skin and as an aid to neutralize the acid, but it should not be placed into the eye. No cream, ointment, or dressing should be applied to the affected area. Emesis should be avoided in case of ingestion. If a large quantity has been swallowed, then gastric lavage should be considered. Dilution with water may be effective for small quantities swallowed. Under no circumstances should carbonated beverages be used because of

large quantities of carbon dioxide gas released that distends the stomach.

Ecotoxicology

Varies depending on the type of acid.

Other Hazards

Varies depending on the type of acid.

Exposure Standards and Guidelines

Varies depending on the type of acid.

See also: Alkalies; Corrosives; Gastrointestinal System; Skin.

Further Reading

Timbrell J (2002) *Introduction to Toxicology*, 3rd edn. London: Taylor and Francis.

Aconitum Species

Christine Stork and Jeanna Marraffa

This article is a revision of the previous print edition article by Lanita B Myers, volume 1, pp. 23–24, © 1998, Elsevier Inc.

- SYNONYMS: *Aconitum napellus*; Monkshood; Wolfsbane; Helmet flower; Friar's cap; Soldier's cap; Aconite

Uses

Aconitum spp. are perennial herbs with a blackish tuberous rootstock that gives rise to several palmate or cleft leaves. Wild plants often have blue-mauve flowers. Cultivated flowers range in color from rich blue to dark purple, purple, white, or yellow. They are bilaterally symmetrical with five-membered flowers; the uppermost is shaped like a large, downward-opening hood. This feature gives the genus its name and distinguishes it from the larkspur. It grows from 1 to 6 ft high. The ripe follicles contain many seeds. *Aconitum* spp. occur naturally in the northern temperature zones of North America, Great Britain, Europe, and Asia. It usually prefers shady, moist places. Many cultivated forms and species are grown widely outdoors and in gardens.

The toxicity of any particular aconitine-containing plant varies depending on the amount of diterpenes versus the number of norditerpenes in relation to the amount of esterification of the norditerpenes. All parts of the plant contain toxic alkaloids, with the content and composition of these varying throughout the year. It is most toxic in its preflowering stage.

Exposure Routes and Pathways

The most common route of exposure is ingestion of any parts of the plant. The roots and seeds are the most toxic parts, but the whole plant is poisonous. Symptoms can occur after dermal exposure although this route is much less frequent. This is because *Aconitum* spp. can be rapidly absorbed through mucous membranes and even intact skin.

Toxicokinetics

Aconitine is rapidly absorbed after ingestion, usually within a few minutes. Absorption also occurs with dermal contact.

Mechanism of Toxicity

Aconitum spp. contain potent steroid alkaloids including aconitine, mesaconitine, and jesaconitine, the three major toxins. The main alkaloid of these plants is aconitine, a highly toxic alkaloid.

Aconitine binds to voltage-gated sodium channels, prolonging sodium current influx, slowing repolarization, and permanently activates cardiac muscle and voltage-dependent nervous tissue receptors, resulting in cardiac and neurologic toxicity. The powerful cardiac agent 'aconite' has vagal activity that causes slowing of the heart. The cardiac toxicity resembles that of cardiac glycosides with atrioventricular conduction blockade and increased ventricular automaticity inducing a variety of rapid ventricular rates, including premature ventricular contractions to ventricular fibrillation and torsades de pointes.

Acute and Short-Term Toxicity (or Exposure)

Animal

General symptoms in animals primarily include vomiting, colic, bloating, bradycardia, bradypnea, muscle weakness, paralysis, and dilated pupils. Death is usually due to cardiopulmonary failure. No specific antidote or treatment is available. The

estimated lethal dose of aconitine is 2 or 3 mg in a dog and 10 or 12 mg in a horse.

Human

Aconitine's minimal lethal dose is 3–6 mg.One gram of fresh *Aconitum napellus* may contain 2–20 mg of aconitine. Therefore, small amounts of this plant can be lethal. The effects produced by aconite poisoning are similar to that of veratrum alkaloids (veratrine) with the exception of the paresthesias being more prominent and persistent. A burning sensation and tingling of the mouth, lips, tongue, and throat occur almost instantly, within 10–20 min. This is usually followed within 2–6 h by nausea, salivation, violent emesis, generalized paresthesias, weakness, and extreme pain. Colicky diarrhea, skeletal muscle paralysis, cardiac rhythm disturbances, convulsions, and death may follow in up to 8 h. Cardiac toxicity often complicates serious aconitine poisoning with hypotension, conduction delays, and dysrythmias within 6 h. Respiratory paralysis is often the cause of death.

Chronic Toxicity (or Exposure)

Human

Chronic toxicity is not expected.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Decontamination with syrup of ipecac is essentially contraindicated due to extensive vomiting, rapid onset of symptoms, and possible respiratory paralysis. Gastric decontamination with activated charcoal may be considered for substantial recent ingestions. Fluid and electrolytes need to be frequently monitored and replaced as necessary secondary to vomiting and diarrhea.

Treatment is symptomatic and supportive after decontamination. Since toxicity is unpredictable due to alkaloid variability, observation for 2–4 h is recommended. Symptomatic patients should be hospitalized for 24 h with cardiac monitoring.

Bradycardia is usually responsive to atropine. For hypotension, intravenous fluids should be administered and if unsuccessful, vasopressor therapy should be initiated. Most arrhythmias are refractory to drug management; however, treatment should be guided by electrocardiographic changes. Sodium bicarbonate has theoretical disadvantages because of the sodium channel opening. There is no specific antidote. No specific laboratory tests are available.

See also: Atropine; Plants, Poisonous.

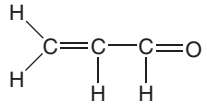
Further Reading

Agarwal B, Agarwal R, and Misra D (1977) Malignant arrhythmias induced by accidental aconite poisoning. *Indian Heart Journal* 29: 246–248.

Acrolein

James M Garrison

- Chemical Abstracts Service Registry Number: CAS 107-02-8
- Synonyms: Acrylaldehyde; Acrylic aldehyde; Allyl aldehyde; Aqualin; Ethylene aldehyde; 2-Propenal
- Chemical/Pharmaceutical/Other Class: Aldehydes
- Chemical Formula: C_3H_4O
- Chemical Structure:



Uses

Acrolein is used as an intermediate in the manufacture of glycerol, polyurethane, polyester resins, methionine, pharmaceuticals, and herbicides, and has been used in military poison gas mixtures.

Exposure Routes and Pathways

Acrolein is found in low levels in many foods. In addition, it is produced as a by-product of combustion of organic compounds, being present in smoke of all kinds, including cigarette smoke and combustion products from petrochemical fuels. Significant exposures to acrolein are most likely to occur by inhalation with potential for skin and eye contact. Ingestion is also possible.

Toxicokinetics

Following inhalation exposure, acrolein can be deposited in the nasal cavity and respiratory tract, where the majority is retained irreversibly due to its high tissue reactivity. Eventually, some traces can be absorbed into the blood and be distributed throughout the body. The uptake of acrolein in the nasal cavity is influenced by its solubility and inspiratory flow rate. Glutathione conjugation is the dominant detoxification pathway for acrolein. Further metabolism takes place in the liver resulting in glycidaldehyde and a number of metabolites that can be excreted in the urine as well as some unchanged acrolein. Most of the free acrolein is excreted in the exhaled breath.

Mechanism of Toxicity

Acrolein is soluble in the mucous membranes of the upper respiratory tract causing irritation of the sensory nerve endings. There is also depression of the mucociliary defense system. The direct action of acrolein on the skin and eyes is the result of irritation to these tissues.

Acute and Short-Term Toxicity (or Exposure)

The respiratory system is the primary target of acrolein toxicity, although dermal and ocular effects also occur. Acute exposure to acrolein results in irritation to the upper respiratory tract, skin, and eyes. Few other organ systems have been shown to exhibit significant effects from exposure.

Animal

In animal studies, the lowest oral LD_{50} for acrolein is $7\,mg\,kg^{-1}$ in rabbits. An animal inhalation exposure study at 10 ppm for 3.5 h resulted in respiratory irritation in cats. Another inhalation study on rats exposed to 8 ppm for 4 h resulted in the death of one animal while all the animals died at 16 ppm. A subchronic inhalation study with rats exposed to 4 ppm, $6\,h\,day^{-1}$, for 60 days resulted in 32/57 animal deaths due to bronchiolar necrosis and focal emphysema. The dermal LD_{50} has been reported to range from 160 to $1000\,mg\,kg^{-1}$ body weight in rabbits.

Human

Irritation to the mucous membranes occurs at as low as 0.25 ppm within 5 min and marked irritation of the eyes and nose at 1 ppm for 5 min. Fatalities have occurred at exposures to concentrations of 150 ppm for 10 min, resulting in pulmonary edema and tracheobronchitis. The lowest lethal concentration reported is 10 ppm, and the IDLH is 5 ppm. Chronic health effects, such as emphysema, can occur as a result of short-term acrolein exposure, and may last months or years.

Liquid splashes to the eye can cause corneal damage and exposures to concentrations of 0.25 ppm may cause eye irritation, lacrimation, conjunctivitis, lid edema, fibrinous or purulent discharge, and corneal injury. Splashes to the skin can result in dermal irritation, edema, and, in some cases, epidermal necrosis.

Chronic Toxicity (or Exposure)

Animal

A chronic inhalation study in rats indicates that exposure to 8 ppm acrolein for $1\,h\,day^{-1}$ for 18 months can result in emphysematous areas in the alveoli. Hamsters exposed to 4 ppm, $1\,h\,day^{-1}$, 5 days $week^{-1}$ for 12 months exhibited inflammation and epithelial metaplasia in the nasal cavity. Hamsters continued to show treatment-related effects in the nasal cavity 6 months after exposure was terminated.

There is limited evidence of acrolein carcinogenicity in animal studies, but glycidaldehyde, a potential metabolite of acrolein, is considered to be carcinogenic.

Human

Skin irritation with erythema, edema, and sensitization can occur from prolonged or repeated contact with acrolein. There is inadequate evidence in humans for chronic toxicity or carcinogenicity.

Acrolein is a weak sensitizing agent; however, the TLV – TWA of 0.1 ppm is sufficiently low to minimize irritation to most exposed individuals and a 15 min STEL of 0.3 ppm is also recommended.

In Vitro Toxicity Data

When tested in the *Salmonella* assay, acrolein was weakly positive. It was not mutagenic in the dominant lethal assay in the mouse and in the *Drosophila* sex-linked recessive lethal test, and negative for chromosome aberrations when tested in cultured Chinese hamster ovary cells; however, there was an increase in the frequency of sister-chromatid exchanges.

Clinical Management

Exposures by inhalation should be monitored for nasal and respiratory tract irritation, bronchitis, or pneumonitis. Humidified supplemental 100% oxygen should be administered. Because acrolein-induced tissue damage is slow to heal, follow-up monitoring of respiratory function may be warranted if damage to the respiratory tract is suspected.

Gastric lavage may be indicated soon after ingestion of acrolein followed by administration of activated charcoal slurry mixed with saline cathartic or sorbitol. Oxygen, in combination with intubation and mechanical ventilation, may be required in severe cases. Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. If eye irritation, pain, swelling, lacrimation, or photophobia persist, the patient should be seen in a health care facility.

Environmental Fate

Acrolein may be released to the environment from combustion processes or in effluents. Because it is a highly reactive compound, it is unstable in the environment and unlikely to persist. The half-life in air is predicted to be 15–20 h, and half-lives of dilute acrolein in water have been shown to be 1–3 days or less.

Other Hazards

Acrolein is highly reactive and is likely to polymerize violently/explosively into dimethylaniline in the presence of strong acids or bases. Care should be taken to prevent mixing with amines, sulfur dioxide, metal salts, and oxidants. In addition, acrolein is sensitive to heat, light, and air unless an inhibitor such as dimethyl sulfoxide (DMSO) is added; however, the stabilizing effects of inhibitors are usually short-lived.

Exposure Standards and Guidelines

Occupational exposure standards for acrolein include the following:

- US OSHA permissible exposure limit (PEL) time-weighted average (TWA) value of 0.1 ppm ($0.25\,mg\,m^{-3}$); short-term exposure limit (STEL) value of 0.3 ppm ($0.8\,mg\,m^{-3}$), not to exceed 15 min.
- US ACGIH threshold limit value (TLV) – TWA of 0.1 ppm; STEL of 0.3 ppm.

Miscellaneous

Acrolein is a colorless to yellowish liquid with a piercing, unpleasant odor.

See also: Combustion Toxicology; Respiratory Tract.

Further Reading

Beauchamp RO Jr., Andjelkovich DA, Kligerman AD, Morgan KT, and Heck HD (1985) A critical review of the literature on acrolein toxicity. *Critical Reviews in Toxicology* 14: 309–380.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Acrolein.
http://www.state.nj.us – New Jersey Hazardous Substances Fact Sheet.

Acrylamide

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 79-06-01
- Synonyms: Acrylic amide; Propenamide
- Chemical/Pharmaceutical/Other Class: Amide
- Chemical Formula: C_3H_5NO
- Chemical Structure: $H_2C=CH–CO–NH_2$

Uses

The primary use of acrylamide is in the production of polyacrylamide homopolymers and copolymers with nonionic, cationic, or anionic properties. Polyacrylamides are used as flocculants in wastewater treatment plants, as coagulants in the treatment of potable water, as fiber and pigment binders in the paper industry, as thickeners in soaps and personal grooming preparations, and as sizing agents in the permanent press fabric industry. Acrylamide monomer is used to

produce grouts and soil stabilizers for the construction of tunnels, dams, foundations, and roadways as well as acrylamide gels used in biotechnology laboratories.

Exposure Routes and Pathways

Exposures to acrylamide monomer are most likely to occur in the occupational setting via dermal contact with solutions of acrylamide monomer and via inhalation of the dry monomer or aerosols of acrylamide solutions. These exposures may occur during the manufacture of acrylamide and polyacrylamides, during grouting activities, and during laboratory preparation of polyacrylamide gels. The general public may be exposed to acrylamide monomer via drinking water if not removed by the water treatment process following use of polyacrylamide flocculants.

Toxicokinetics

Acrylamide is well absorbed via the gastrointestinal and respiratory tracts. It is also well absorbed through the skin but less rapidly than through the gastrointestinal tract; a significant portion of the dermally applied dose remains in the skin. Upon absorption into the blood, acrylamide is rapidly distributed throughout the body with an apparent volume of distribution equal to total body water. With the exception of plasma, erythrocytes, and testes, acrylamide and glycidamide do not exhibit preferential bioconcentration in any body tissue.

Acrylamide is rapidly metabolized to the epoxide, glycidamide, via cytochrome P450 oxidation. Both the parent and metabolite exhibit half-lives in the rat of $\sim$2 h. In rats, the conversion of acrylamide to glycidamide is saturable with 50% conversion at low doses and 13% conversion at 100 mg kg^{-1}. Both substances are detoxified by conjugation with glutathione; glycidamide is also detoxified by hydrolysis, presumably via epoxide hydrolase. While it appears that metabolism is qualitatively similar among species, quantitative differences exist depending on species and dose. Limited data indicate that the conversion of acrylamide to glycidamide is about twofold greater in the mouse than rat and that humans convert at about a twofold lower rate than the rat does.

Acrylamide is excreted primarily via the kidneys. About 60% of the administered dose appears in the urine within the first 24 h of exposure. Metabolites of acrylamide constitute the majority of the dose excreted in the urine; only $\sim$2% of the dose is excreted as the parent compound. Acrylamide and/or its metabolites are subject to enterohepatic circulation; $\sim$6% of the applied dose is eliminated in the feces. About 5% of the dose is expired as CO_2.

Mechanism of Toxicity

Although the mechanism of acrylamide toxicity is unknown, glycidamide may mediate the genotoxicity associated with acrylamide exposure. While both acrylamide and glycidamide bind to hemoglobin *in vivo*, only glycidamide forms adducts with DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Studies in several animal species indicate that acute exposures to acrylamide cause dose-related neurotoxic effects. Acrylamide has been observed to produce testicular lesions at high dose levels that also result in neurotoxicity. The oral LD_{50} for acrylamide in mice, rats, rabbits, and guinea pigs ranges between 107 and 203 mg kg^{-1}; the dermal LD_{50} in rabbits is $\sim$1150 mg kg^{-1}. Acrylamide produces equivocal responses in skin irritation tests but is irritating to the eye. Acrylamide is also a skin sensitizer. *In vivo* genotoxicity studies of somatic cells indicate that acrylamide produces clastogenic effects rather than gene mutations. In germ cells, acrylamide is both mutagenic and clastogenic and is capable of causing heritable translocations in mice.

Human

Upon chronic exposure, acrylamide produces a motor and sensory polyneuropathy in which the distal regions of the longest and largest axons appear to be preferentially affected. These effects may be manifested by weakness, paresthesias, fatigue, as well as decreased pinprick sensation and reflexes. Recovery generally occurs within a year following cessation of exposure although severe exposures may result in permanent peripheral nerve damage. Acrylamide can irritate the skin and may also cause allergic contact dermatitis.

Chronic Toxicity (or Exposure)

Animal

In a lifetime drinking water study, rats were exposed to acrylamide in drinking water at doses of 0, 0.01, 0.1, 0.5, or 2 mg kg^{-1} day^{-1}. At the two highest doses, acrylamide produced increased incidences of benign and malignant tumors in a variety of organs (e.g., thyroid and adrenal glands, testicular tunica, uterus, and mammary glands). The principal noncarcinogenic effect associated with these chronic exposures was degenerative changes in peripheral and optic nerves as well as the lateral geniculate

nucleus; peripheral nerve lesions were observed histopathologically at $2\,mg\,kg^{-1}$ but not at $0.5\,mg\,kg^{-1}$. Degenerative changes in the testes have been described at neurotoxic doses. No effects on fertility were observed in a two-generation rat reproduction study in which parental animals of each generation were exposed to $5\,mg\,kg^{-1}\,day^{-1}$ acrylamide for 10–11 weeks while in another study the fertility of male rats was impaired by exposure to $\geqslant 15\,mg\,kg^{-1}$ for 5 days. There is no evidence that acrylamide produces selective developmental toxicity in rodents as such effects were associated with maternal toxicity.

Human

Available epidemiological studies have not provided a significant link between acrylamide exposures and increases in the incidence of cancer.

In Vitro Toxicity Data

Acrylamide is not mutagenic in standard bacterial assays either in the presence or absence of metabolic activation; in contrast, glycidamide causes mutations in bacteria without addition of an exogenous metabolic system. Acrylamide is clastogenic in mammalian cells both with and without metabolic activation. These results suggest that acrylamide is a direct acting mutagen, probably causing clastogenic effects rather than gene mutations.

Clinical Management

Clinical management involves removal from exposure and treatment of symptoms.

Environmental Fate

At room temperature, acrylamide is a crystalline solid that slowly sublimes. When released into the environment, acrylamide is expected to partition almost exclusively to water (>99.9%) with only trace amounts to air (<0.1%), soil (<0.1%) and sediment (<0.05%). Acrylamide is very soluble in water ($2155\,g\,l^{-1}$ at 30 C) with little propensity to volatilize to air (Henry's law constant of $3 \times 10^{-5}\,Pa\,m^3\,mol^{-1}$). In water, acrylamide is removed primarily by biodegradation. At 15 days postapplication, 75% ($2\,mg\,l^{-1}$ acrylamide) to 100% ($0.5\,mg\,l^{-1}$ acrylamide) is degraded. In air, acrylamide will be removed by reaction with photochemically produced hydroxyl radicals (8.3 h half-life). In soil, acrylamide is biodegraded with an estimated half-life of 30 days. Based on its relatively low octanol–water partition coefficient ($\log K_{ow}$ of ~ -1.0) and measured bioconcentration factor in aquatic organisms (<1), acrylamide does not pose a significant bioaccumulation hazard.

Ecotoxicology

Acrylamide is moderately toxic to aquatic organisms. In a series of studies, acrylamide exhibited a 96 h LC_{50} value in four freshwater fish of 100–$180\,mg\,l^{-1}$, a 48 h LC_{50} (immobilization) value of $98\,mg\,l^{-1}$ in an aquatic invertebrate (*Daphnia magna*), and a 72 h EC_{50} (growth inhibition) value of $33.8\,mg\,l^{-1}$ in freshwater algae (*Selenastrum capricornutum*).

Other Hazards

Acrylamide is reactive but stable at room temperature. It can polymerize violently when heated to its melting point (84.5°C) or under ultraviolet light.

Exposure Standards and Guidelines

International occupational exposure limits (OEL) for acrylamide generally range from 0.03 to 0.3 ppm as an 8 h time-weighted average (TWA), with 0.03 ppm being the predominant value and the TWA OEL established by the American Conference of Governmental Industrial Hygienists (ACGIH). The US Occupational Safety and Health Administration lists a permissible exposure limit of 1 ppm for acrylamide. The National Institute of Occupational Safety and Health has a recommended exposure limit of 0.1 ppm as a 10 h TWA. Acrylamide is classified as *possibly carcinogenic to humans* (Group 2B) by the International Agency for Research on Cancer and as *reasonably anticipated to be a human carcinogen* by the US National Toxicology Program.

See also: Neurotoxicity; Pollution, Water; Polymers.

Further Reading

Ellenhorn MJ, Schonwald S, Ordog G, and Wasserberger J (1997) *Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning*, 2nd edn., pp. 1672–1673. Baltimore, MD: Williams and Wilkins.

LoPachin RM (2004) The changing view of acrylamide neurotoxicity. *Neurotoxicology* 25(4): 617–630.

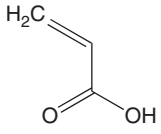
Ruden C (2004) Acrylamide and cancer risk: Expert risk assessments and the public debate. *Food and Chemical Toxicology* 42(3): 335–349.

Acrylates *See* Acrylic Acid; Ethyl Acrylate; Methyl Acrylate.

Acrylic Acid

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 79-10-7
- Synonyms: Acroleic acid; Ethylenecarboxylic acid; Propene acid; Propenoic acid; Vinylformic acid; 2-Propenoic acid; RCRA waste number U008; UN 2218 (DOT)
- Chemical/Pharmaceutical/Other Class: Copolymer
- Chemical Structure:



Uses

Acrylic acid derivatives treated with heparin are used to coat surfaces of clinical equipment. Acrylic acid is also used as a copolymer component in aerosol hair spray, in plastics, in molding powder for signs, in paint formulations, in leather finishing, in paper coatings, and in latex applications to prevent premature coagulation. It is also used in the production of hydrogels used for contact lenses.

Background Information

For more than decades, acrylic acid has served as an essential building block in the production of some of our most commonly used industrial and consumer products. Approximately two-thirds of the acrylic acid manufactured in the United States is used to produce acrylic esters – methyl acrylate, butyl acrylate, ethyl acrylate, and 2-ethylhexyl acrylate – which, when polymerized, are ingredients in paints, coatings, textiles, adhesives, plastics, and many other applications. The remaining one-third of the acrylic acid is used to produce polyacrylic acid, or cross-linked polyacrylic acid compounds, which have been successfully used in the manufacture of hygienic products, detergents, and waste water treatment chemicals.

Exposure Routes and Pathways

Inhalation, skin and eye contact, and ingestion are the most common exposure pathways. Acrylic acid is available as a colorless liquid.

Toxicokinetics

The excretion half-life of acrylic acid has been found to be 40 min. Both *in vivo* and *in vitro* studies of acrylic acid metabolism have produced strong evidence that the metabolism proceeds by a mitochondrial biochemical pathway for propionic acid metabolism that normally functions in the body at the final stages of breakdown of fatty acids and the production of intermediates for the tricarboxylic acid cycle. It is primarily excreted as carbon dioxide through the lungs. 3-Hydroxypropionate has been found to be a major metabolite. Part of acrylic acid also binds to glutathione and is excreted as the cysteine conjugate in the urine. Some part of acrylic acid can also be converted to acrylyl-CoA and reacts with glutathione to be excreted as cysteine conjugate.

Mechanism of Toxicity

Acrylic acid causes toxicity by rapid polymerization in the presence of light, heat, and oxygen and thereby interfering with the incorporation of thymidine into DNA and uracil into RNA and inhibits protein synthesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acrylic acid has been tested on mice, rats, and rabbits. The toxicity of acrylic acid in animals is similar to that found in humans.

Human

Acrylic acid is corrosive to skin. Acrylic acid vapor can cause moderate to severe skin and eye irritation.

It can also cause forestomach edema. Acute exposure can be corrosive to the skin, eyes, nose, and mucous membranes of the upper respiratory and gastrointestinal tracts. Inhalation of vapors may produce burning sensation, cough, nasal discharge, sore throat, labored breathing, headache, nausea, vomiting, confusion, dizziness, and unconsciousness.

Chronic Toxicity (or Exposure)

Animal

Animals exposed via chronic inhalation developed lethargy, weight loss, kidney abnormalities, embryotoxicity, and inflammation to the upper respiratory tract and gastric mucosa.

Human

Repetitive exposure to acrylic acid may induce mucosal forestomach hyperplasia.

Clinical Management

Exposure should be terminated as soon as possible by moving the victim to fresh air. The skin, eyes, and mouth should be washed with copious amounts of water. Contaminated clothing should be removed and isolated. The victim should be kept calm and normal body temperature should be maintained. Artificial respiration should be provided if the breathing has stopped. Treatment is usually symptomatic.

Environmental Fate

Acrylic acid's production and use in the manufacture of plastics, paint formulations, leather finishings, paper coatings, and in medicine and dentistry for dental plates, artificial teeth, and orthopedic cement may result in its release to the environment through various waste streams. Acrylic acid has also been identified in nine species of chlorophyceae algae, 10 species of rhodophyceae algae, and in the rumen fluid of sheep. If released to air, a vapor pressure of 3.97 mmHg at 25 °C indicates acrylic acid will exist solely as a vapor in the ambient atmosphere. Vapor-phase acrylic acid will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 2 days. If released to soil, acrylic acid is expected to have very high mobility. Volatilization from moist soil surfaces is expected to be slow. Acrylic acid may potentially volatilize from dry soil surfaces based upon its vapor pressure. If released into water, acrylic acid is not expected to adsorb to suspended solids and sediment in the water column. Biodegradation under both aerobic and anaerobic conditions is expected to occur.

Exposure Standards and Guidelines

Occupational Safety and Health Administration: 8 h time-weighted average (TWA) is 2 ppm. Worker exposure levels may exceed three times the threshold limit value (TLV) – TWA for no more than a total of 30 min during a work day, and under no circumstances should they exceed five times the TLV – TWA, provided that the TLV – TWA is not exceeded. National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit – 10 h TWA: 2 ppm ($6\,mg\,m^{-3}$).

See also: Polymers.

Relevant Websites

http://www.epa.gov – Acrylic Acid (from the US EPA's Technology Transfer Network Air Toxics Website).

http://www.inchem.org – Acrylic Acid (Environmental Health Criteria 191) from the International Programme on Chemical Safety, 1997.

Acrylonitrile

Raja S Mangipudy and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 107-13-1
- Synonyms: Acritet; Carbacryl; Propenenitrile; Ventox; Vinyl cyanide; TL 314
- Chemical/Pharmaceutical/Other Class: Industrial chemical; Solvent
- Chemical Formula: C_3H_3N

Uses

Acrylonitrile is used in the manufacture of acrylic fibers and in the plastic surface coatings and adhesive industries. It is also used as a pesticide/fumigant. It is a chemical intermediate in the synthesis of antioxidants, pharmaceutical dyes, surface-active agents, and in reactions requiring the cyanoethyl group.

Exposure Routes and Pathways

Accidental exposure can occur via dermal contact, ingestion, or inhalation. Acrylonitrile is found in cigarette smoke. It does not occur naturally.

Toxicokinetics

Acrylonitrile is absorbed by way of inhalation, ingestion, and percutaneously. Rats treated with [^{14}C]acrylonitrile via oral or intravenous route produced radioactivity in the blood, liver, kidneys, lungs, adrenal cortex, and stomach mucosa. Significant amounts are retained in the plasma. Acrylonitrile is metabolized to a lesser extent in humans than in rodents. Acrylonitrile metabolism in humans follows first-order kinetics and acrylonitrile has a half-life of $\sim$8 h. The elimination of acrylonitrile from the plasma of rats is biphasic, with a half-life of 3.5–5.8 and 50–77 h in the a and b phases, respectively.

There are four major pathways of metabolism for acrylonitrile: formation of glucuronides, direct reaction with glutathione to form cyanoethyl mercapturic acid, direct reaction with the thiol groups of proteins, and epoxidation to 2-cyanoethylene oxide. *N*-Acetyl-*S*-(2-cyanoethyl)-L-cysteine is a major urinary metabolite in human volunteers exposed to 5–10 mg.

Mechanism of Toxicity

Acrylonitrile owes some of its toxicity to cyanide generation, which inhibits cellular respiration. Preinduction of microsomal mixed function oxidase (MFO) with Arochlor 1254 greatly enhanced the toxicity of acrylonitrile and caused a threefold increase in cyanide levels in rats. Therefore, metabolic activation appears to be necessary in the toxicity of acrylonitrile. The direct reaction of acrylonitrile with the SH groups of proteins and its epoxide metabolite are also expected to be responsible for its effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute animal tests in rats, mice, rabbits, and guinea pigs have demonstrated acrylonitrile to have high acute toxicity from inhalation and high to extreme acute toxicity from oral or dermal exposure. No information is available on the reproductive or developmental effects of acrylonitrile in humans.

Fetal malformations (including short tail, missing vertebrae, short trunk, omphalocele, and hemivertebra) have been reported in rats exposed to acrylonitrile by inhalation. In mice orally exposed to acrylonitrile, degenerative changes in testicular tubules and decreased sperm count were observed.

Human

Workers exposed via inhalation to high levels of acrylonitrile for less than an hour experienced mucous membrane irritation, headaches, nausea, feelings of apprehension and nervous irritability. Low-grade anemia, leukocytosis, kidney irritation, and mild jaundice were also observed in the workers, with these effects subsiding with the ending of exposure. Symptoms associated with acrylonitrile poisoning include limb weakness, labored and irregular breathing, dizziness and impaired judgment, cyanosis, nausea, collapse, and convulsions. A child died after being exposed to acrylonitrile by inhalation, suffering from respiratory malfunction, lip cyanosis, and tachycardia before death. Several adults exposed to the same concentration of acrylonitrile exhibited eye irritation, but no toxic effects. Acute dermal exposure may cause severe burns to the skin in humans.

Chronic Toxicity (or Exposure)

Animal

In rats chronically exposed by inhalation, degenerative and inflammatory changes in the respiratory epithelium of the nasal turbinates and effects on brain cells have been observed. In several studies, an increased incidence of tumors has been observed in rats exposed by inhalation, drinking water, and gavage. Astrocytomas in the brain and spinal cord and tumors of the Zymbal gland (in the ear canal) have been most frequently reported, as well as tumors of the stomach, tongue, small intestine in males and females, and mammary gland in females. The reference concentration (RfC) for acrylonitrile is 0.002 mg m^{-3} based on degeneration and inflammation of nasal respiratory epithelium in rats. The Environmental Protection Agency (EPA) has calculated a provisional reference dose (RfD) of 0.001 milligrams per kilogram body weight per day (mg kg^{-1} day^{-1}) for acrylonitrile based on decreased sperm counts in mice.

Human

Headaches, fatigue, nausea, and weakness have been frequently reported in chronically (long-term) exposed workers. A statistically significant increase in the incidence of lung cancer has been reported in several studies of chronically exposed workers. However, some of these studies contain deficiencies such as lack of exposure information, short

follow-up, and confounding factors. EPA has classified acrylonitrile as a Group B1, probable human carcinogen (cancer-causing agent).

Clinical Management

In oral exposure, gastric lavage may be performed soon after ingestion or in patients who are comatose or at risk of convulsing. The volume of lavage return should approximate the volume given. Charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, may be administered. The usual charcoal dose is 30–100 g in adults and 15–30 g in children (1 or 2 g kg^{-1} in infants). In case of inhalation exposure, the patient must be moved to fresh air for respiratory distress. If cough or difficulty in breathing develops, evaluation for respiratory tract irritation, bronchitis, or pneumonitis must be performed. For eye exposure, eyes must be washed with copious amounts of tepid water for at least 15 min. If irritation, pain, lacrimation, or photophobia persists, the patient should be removed to a health care facility. For dermal exposure, the exposed area must be washed thoroughly with soap and water.

Environmental Fate

Acrylonitrile is both readily volatile in air and highly soluble in water. These characteristics determine the behavior of acrylonitrile in the environment. The principal pathway leading to the degradation of acrylonitrile in air is photooxidation, mainly by reaction with hydroxyl radicals (OH). Acrylonitrile may also be oxidized by other atmospheric components such as ozone and oxygen. Very little is known about the nonbiologically mediated transformation of acrylonitirle in water. It is oxidized by strong oxidants such as chlorine used to disinfect water. Acrylonitrile is readily degraded by aerobic microorganisms in water.

Other Hazards

Acrylonitrile is a reactive chemical that polymerizes spontaneously, when heated, or in the presence of a strong alkali unless it is inhibited, usually with ethylhydroquinone. It can explode when exposed to flame. It attacks copper. It is incompatible and reactive with strong oxidizers, acids and alkalis; bromine; and amines.

Exposure Standards and Guidelines

- Immediately dangerous to life or health (IDLH): Ca (85 ppm)
- Threshold limit value time-weighted average (TLV TWA): 2 ppm confirmed animal carcinogen (skin)
- Emergency Response Planning Guideline (ERPG)-1: 25 ppm
  - ERPG-2: 35 ppm
  - ERPG-3: 75 ppm
- National Institute for Occupational Safety and Health recommended exposure limit (NIOSH REL): Ca TWA 1 ppm C 10 ppm (15 min) (skin)

See also: Combustion Toxicology; Cyanide; Polymers; Respiratory Tract.

Further Reading

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Sakurai H (2000) Carcinogenicity and other health effects of acrylonitrile with reference to occupational exposure limit. *Industrial Health* 38(2): 165–180.

Starr TB, Gause C, Youk AO, Stone R, Marsh GM, and Collins JJ (2004) A risk assessment for occupational acrylonitrile exposure using epidemiology data. *Risk Analysis* 24(3): 587–601.

Swaen GM, Bloemen LJ, Twisk J, *et al.* (2004) Mortality update of workers exposed to acrylonitrile in The Netherlands. *Journal of Occupational and Environmental Medicine* 46(7): 691–698.

Thier R, Lewalter J, and Bolt HM (2000) Species differences in acrylonitrile metabolism and toxicity between experimental animals and humans based on observations in human accidental poisonings. *Archives of Toxicology* 74(4/5): 184–189.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Acrylonitrile.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Acrylonitrile.

Acute Toxicity *See* Toxicity, Acute.

Adiponitrile

Shashi Ramaiah and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 111-69-3
- Synonyms: Adipic acid dinitrile; Adipic acid nitrile; 1,4-Dicyanobutane; Hexanedinitrile; Tetramethylene cyanide
- Chemical Formula: $C_6H_8N_2$

Uses

Adiponitrile is a starting chemical intended for synthesis of hexamethylenediamine to make nylon, corrosion inhibitors, and rubber accelerators; it is also used for the synthesis of adipoguanamine, which is used as an extractant for aromatic hydrocarbons.

Background Information

Adiponitrile is an odorless, oily colorless liquid, which decomposes on heating and reacts violently with strong oxidants. Upon burning, adiponitrile produces highly toxic hydrogen cyanide.

Exposure Routes and Pathways

Adiponitrile may be inhaled, swallowed, or absorbed through skin.

Adiponitrile could potentially be released to the environment in the effluent or emissions from plants manufacturing adiponitrile, hexamethyelenediamine, or nylon-66. If released to soil, aerobic biodegradation may be an important removal mechanism. Although adiponitrile has the potential to undergo extensive leaching, biodegradation should limit movement through soil. Volatilization from soil surfaces is not expected to be significant. If released to water, aerobic biodegradation may again be an important removal mechanism.

Toxicokinetics

Seventy percent of the dose ($\sim 50\,mg\,kg^{-1}$) administered subcutaneously to guinea pigs was eliminated as thiocyanate in urine. After application of adiponitrile to depilated skin, skin penetration was suggested by increased thiocyanate in urine. Greater quantities were absorbed when skin was abraded. Based on the ratio between administered adiponitrile dose and quantity of cyanide detected, it was shown that a greater part of the dose was metabolized to cyanide. Cyanide thus released is the principle cause of toxicity.

Mechanism of Toxicity

Adiponitrile's mechanism of toxicity is similar to cyanide because it can potentially liberate cyanide in the body spontaneously. It forms a stable complex with ferric iron in the cytochrome oxidase enzymes, thereby inhibiting cellular respiration. Cyanide affects primarily the central nervous system (CNS), producing early stimulation followed by depression. It initially stimulates the peripheral chemoreceptors (causing increased respiration) and the carotid bodies (thereby slowing the heart). Early CNS, respiratory, and myocardial depression result in decreased oxygenation of the blood and decreased cardiac output. These effects produce both stagnation and hypoxemic hypoxia in addition to cytotoxic hypoxia from inhibition of mitochondrial cytochrome oxidase.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} is $155\,mg\,kg^{-1}$ in rats, $172\,mg\,kg^{-1}$ in mice, and $22\,mg\,kg^{-1}$ in rabbits. The subcutaneous LD_{50} in the guinea pig is $50\,mg\,kg^{-1}$.

Human

Vapors are irritating to the eyes and respiratory system at higher concentrations. Humans may experience tightness in the chest, headache, and weakness with difficulty in standing, and vertigo, cyanotic, rapid respirations, low blood pressures, and tachycardia. Mental confusion, tonic clonic contractions of limbs and facial muscles, irregular heartbeat, coma, and death may occur after exposure to higher

concentrations. Contact with skin and eyes may cause burns. Adiponitrile may be fatal if absorbed through skin, inhaled, or swallowed. The American Conference of Governmental Industrial Hygienists' threshold limit value is 2 ppm (with a skin designation indicating the potential significant contribution to the overall exposure by the cutaneous route). Short-term inhalation limits are not available.

Chronic Toxicity (or Exposure)

Animal

Adiponitrile was negative for mutagenicity in *Salmonella* with or without bioactivation. Adiponitrile has not been tested for its ability to cause cancer in animals.

Human

Upon repeated or chronic exposure, adiponitrile may have effects on the blood and adrenals, resulting in anemia and tissue lesions. Adiponitrile is not classifiable as a human carcinogen because no human data are available.

In Vitro Toxicity Data

There are no *in vitro* toxicity data available for adiponitrile.

Clinical Management

Emergency Treatment

The affected person should be removed from exposure to adiponitrile immediately. Contaminated clothes should be removed and the patient sponged to avoid any absorption through skin. Immediate cardiopulmonary resuscitation should be administered. If the victim breathes with difficulty, oxygen should be given. In case of ocular contact, the eyes should be flushed with copious amounts of water for at least 20 min. In cases of ingestion, vomiting should be induced. Mouth-to-mouth resuscitation should be avoided in order to prevent self-poisoning.

Medical Treatment

The goal of medical treatment is to eliminate the cyanide formed in the body. Sodium nitrate, amylnitrate, and thiosulfate should be administered. Sodium nitrite should be administered intravenously very slowly. Amylnitrite can also be inhaled from ampoules. Later, sodium thiosulfate should be administered.

Sodium nitrate reacts with hemoglobin in the red blood cells forming methemoglobin, which in turn can react with the free cyanide ion forming cyanmethemoglobin, thereby binding free cyanide and preventing its reaction with cytochrome oxidase enzymes in the cells. Cyanmethemoglobin dissociates slowly into free cyanide plus methemoglobin. The cyanide released by dissociation of cyanmethemoglobin then reacts with the thiosulfate ion forming thiocyanate, a relatively nontoxic compound that is excreted in the urine.

Ecotoxicology

There is no aquatic toxicity information available for adiponitrile.

Miscellaneous

Adiponitrile must be stored to avoid contact with oxidizing agents such as perchlorates, peroxides, permanganates, and fluorine, since violent reactions can occur. Adiponitrile is not compatible with strong acids and reducing agents. Adiponitrile should be stored in tightly closed containers in a cool, well-ventilated area.

Adiponitrile is produced as an intermediate or final product by a process covered under regulatory performance standards that have been promulgated to protect the atmosphere from equipment leaks of volatile organic compounds (VOCs) in the synthetic organic chemical manufacturing industry (SOCMI). The intended effect of these standards is to require all newly constructed, modified, and reconstructed SOCMI process units to use the best demonstrated system of continuous emission reduction for equipment leaks of VOCs, considering costs, non-air quality health, and environment impact and energy requirements.

See also: Cyanide; Volatile Organic Compounds (VOC).

Further Reading

Smith LW and Kennedy GL Jr. (1982) Inhalation toxicity of adiponitrile in rats. *Toxicology and Applied Pharmacology* 65: 257–263.

Relevant Website

http://www.state.nj.us – New Jersey Hazardous Substances Fact Sheet.

Aerosols

Raja S Mangipudy

- DESCRIPTION: Aerosols consist of very finely subdivided liquid or solid particles dispersed in and surrounded by a gas
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aerosols are systems ranging from those of colloidal nature to systems consisting of 'therapeutic packages'. Aerosols are classified as follows:

  (A) Liquified-gas systems
  Two-phase: space-spray; surface-coating; dispersion or suspension
  Three-phase: two-layer; foam; stabilized; quick-breaking
  (B) Compressed-gas systems
  Solid-stream dispensing
  Foam dispensing
  Spray dispensing
  (C) Separation of propellant from concentrate systems
  Piston type
  Flexible type
  Atomizer type
  Mechanical systems
  Latex diaphragm

Uses

Many therapeutically active ingredients are administered or applied to the body by means of the aerosol dosage form, including agents such as epinephrine, isoproterenol, antibiotics, antiseptics, steroids, and ergotamine. Oral aerosols have been used for the symptomatic treatment of asthma as well as for the treatment of migraine headaches, whereas topical aerosols find use in numerous dermatological manifestations.

Human Toxicity

The following pertains to the general evaluation and treatment of individuals exposed to potentially toxic chemicals via aerosols.

A. General evaluation

1. Exposed individuals should have a careful, thorough medical history and physical examination performed, looking for any abnormalities. Exposure to chemicals with a strong odor often results in such nonspecific symptoms as headache, dizziness, weakness, and nausea.

B. Irritation

1. Many chemicals cause irritation of the eyes, skin, and respiratory tract. In severe cases respiratory tract irritation can progress to acute respiratory distress syndrome (ARDS)/acute lung injury, which may be delayed in onset for up to 24–72 h in some cases.
2. Irritation or burns of the esophagus or gastrointestinal tract are also possible if caustic or irritant chemicals are ingested.

C. Hypersensitivity

1. A number of chemical agents produce an allergic hypersensitivity dermatitis or asthma with bronchospasm and wheezing with chronic exposure.

The inflammability and toxicity of the propellant needs to be considered. Additionally, the topical effects of the propellants must be determined.

Clinical Management

1. Supportive care must be instituted for patients accidentally exposed to aerosol contents via topical, inhalation, or oral routes. A number of chemicals produce abnormalities of the hematopoietic system, liver, and kidneys. Monitoring complete blood count, urinalysis, and liver and kidney function tests is suggested for patients with significant exposure.
2. If respiratory tract irritation or respiratory depression is evident, monitor arterial blood gases, chest X-ray, and pulmonary function tests.

See also: Corticosteroids.

A-Esterases

Lester Grant Sultatos

The phosphorothioate insecticides, such as chlorpyrifos and methyl parathion (**Figure 1**), are some of the most commonly used organophosphorus insecticides in the United States. Interestingly, these compounds have little capacity to inhibit the enzyme acetylcholinesterase and are, therefore, not highly toxic themselves. However, they are converted by the liver to potent acetylcholinesterase inhibitors termed oxons (such as chlorpyrifos oxon and methyl paraoxon; **Figure 1**), which are responsible for the toxicities observed following exposure to phosphorothioate insecticides. Once the oxons have been produced from the parent insecticides, one of the ways in which these highly toxic compounds can be metabolized by a variety of species is through their hydrolysis by an enzyme(s) termed A-esterase(s) (**Figure 2**). Since the products of these hydrolysis reactions are usually of low toxicity, A-esterase(s) catalyzes the detoxification of these oxons. Consequently, A-esterase(s) likely plays an important role in the protection of mammals against phosphorothioate insecticide toxicity. For example, although paraoxon and chlorpyrifos oxon have about the same capacity to inhibit acetylcholinesterase, the insecticide chlorpyrifos is about 10 times less toxic to laboratory mice and rats than is parathion, probably because chlorpyrifos oxon is detoxified much more avidly by A-esterase(s).

The term A-esterase originally referred to an enzyme(s) in the serum that metabolized carboxylic esters and was insensitive to inhibition by organophosphates (in contrast to the B-esterases, which are inhibited by organophosphates). Later this activity was shown to be associated with the detoxification of paraoxon, leading to the use of the term A-esterase to refer to enzymes that hydrolyze organophosphates. Since the original discovery of A-esterase, several enzymes have been identified, which can hydrolyze certain organophosphates, and hence have been referred to as A-esterases, even though it is currently not known if they also hydrolyze carboxylic esters.

Currently, there is much confusion regarding the nomenclature of A-esterase(s), and the term A-esterase is by no means universally endorsed. This enzyme(s) has been referred to by many different names, including paraoxonase, aryl-ester hydrolase, arylesterase, organophosphate hydrolase, organophosphorus compound hydrolase, and organophosphorus acid anhydrolase. Part of the confusion in the classification of this enzyme(s) appears to result from the presence of different forms (which may or may not be related) within an organism, as well as different forms within different species. For example, an enzyme that can detoxify paraoxon has been isolated from the bacteria *Pseudomonas diminuta*. This enzyme requires zinc for activity and can also detoxify the parent phosphorothioate insecticide parathion. In contrast, mammals seem to have at least two kinds of enzymes that could be called A-esterase, neither of which can detoxify parathion. The first detoxifies the compound diisopropylfluorophosphate and requires magnesium, manganese, or cobalt for activity. The second detoxifies oxons, such as chlorpyrifos oxon and paraoxon, and requires calcium for activity.

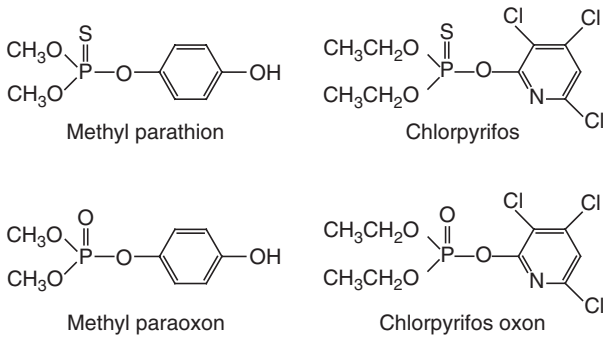

Figure 1 Structures of the phosphorothioate insecticides chlorpyrifos and methyl parathion and their corresponding oxygen analogs.

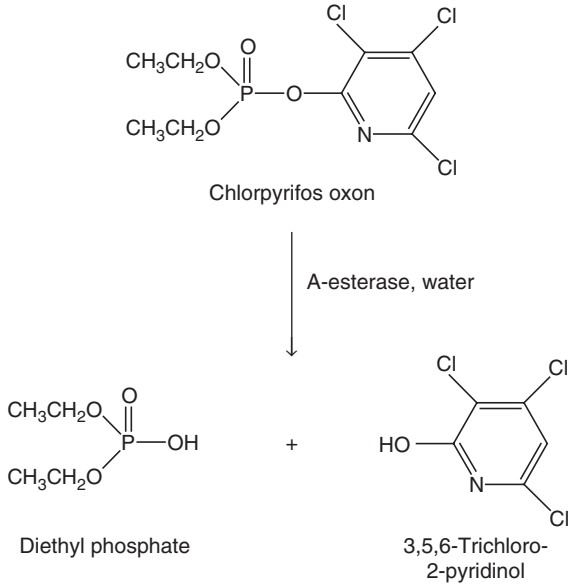

Figure 2 Hydrolysis of chlorpyrifos oxon by A-esterase. Although water is also involved in the reaction, it is usually ignored because it is present at an extremely high and constant concentration.

Although the term A-esterase has been sometimes applied to all these enzymes, it is more often used to refer to the enzyme(s) that requires calcium and detoxifies the oxygen analogs of phosphorothioate insecticides.

Considerable species differences in A-esterase activity exist, ranging from very low or nonexistent in certain birds and fish, to very high in rabbits. Species differences in A-esterase activity could account, at least in part, for species differences in the relative sensitivity to certain phosphorothioate insecticides. For example, birds are much more susceptible to the toxicity of pirimiphos methyl than are mammals.

In mammals, A-esterase(s) has been identified in several tissues, with the highest activity usually found in the blood and liver. It is now known that the liver synthesizes A-esterase(s) and secretes it into the blood. Within the past decade, the A-esterase that has been most characterized has been referred to as paraoxonase (named for its capacity to hydrolyze paraoxon). Interest in human paraoxonase has been fueled by the documentation of the existence of genetic polymorphisms that control its catalytic activity as well as its expression levels. While human paraoxonase has been shown to be encoded by at least three genes, designated *PON1*, *PON2*, and *PON3*; *PON1*, encoding for the protein PON1, has been the most studied. For example, the PON1 Q/R polymorphism of position 192 has been shown to affect catalytic activity in a substrate-dependent manner. The PON_{R192} isoform hydrolyzes paraoxon at a greater rate than does the PON_{Q192} isoform, while the opposite relationship has been observed for the subtrates diazonon, sarin, and soman. Other polymorphisms in the noncoding region of *PON1* have been shown to alter PON1 expression levels.

The identification of paraoxonase genetic polymorphisms has led to much discussion in the literature regarding the role that these polymorphisms might play in determination of individual susceptibility to organophosphorus insecticide toxicity. The issue of the toxicological significance of PON1 polymorphisms is confounded by evidence that suggests this enzyme might not play much of a role in the detoxification of oxons following exposure to the parent insecticide. Although administration of purified enzyme to animals can protect against insecticide toxicity, various lines of evidence suggest that endogenous enzyme plays a limited or negligible role following organophosphorus insecticide exposures and that other detoxification pathways are more significant. Moreover, the limited epidemiological studies that have examined possible relationships between PON1 activity and susceptibility to organophosphorus insecticide toxicity have reported mixed results and are, therefore, at present, inconclusive. Consequently, the toxicological significance of the human PON1 genetic polymorphisms is currently not known.

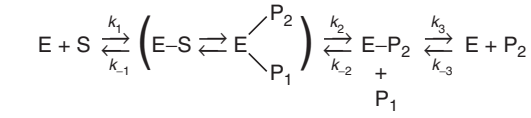

Figure 3 Kinetic mechanism for the interaction of paraoxon (S) with A-esterase (E) or acetylcholinesterase (E). *p*-Nitrophenol (P_1) is the first product released, whereas diethyl phosphate (P_2) is the second.

As outlined in **Figure 3**, the hydrolysis of paraoxon by human serum A-esterase(s) is very similar to the phosphorylation of B-esterases, such as acetylcholinesterase, by paraoxon. Both reactions involve an initial binding of paraoxon to the enzyme, followed by a rapid conformational change that produces diethyl phosphate and *p*-nitrophenol from paraoxon. *p*-Nitrophenol is quickly released from the enzyme, leaving diethyl phosphate covalently bound to enzyme. At this point, A-esterase quickly releases diethyl phosphate as a result of interacting with a water molecule. However, B-esterases, such as acetylcholinesterase, retain the diethyl phosphate for a much longer period of time, thereby resulting in inhibition of the enzyme.

While A-esterase(s) and B-esterases interact kinetically with paraoxon in a similar fashion (**Figure 3**), the molecular events occurring at their active sites during catalysis are probably very different. The active site of B-esterases such as acetylcholinesterase has been well characterized and contains a serine residue that is phosphorylated by paraoxon at the hydroxyl group. In contrast, the active site of A-esterase(s) has not been studied as extensively, but it likely does not contain a serine residue that participates in the hydrolysis of paraoxon. Additionally, A-esterase(s) requires a divalent cation like calcium for activity, whereas B-esterases do not.

For many decades, the function of mammalian A-esterase(s) was unknown. More recent studies, however, are perhaps uncovering a physiological role for this enzyme. Serum PON1 has been shown to be closely associated with high-density lipoproteins, and it might contribute to the antioxidant protection for low-density lipoprotein oxidation. Interestingly, when fed a high fat diet $PON1^{-/-}$ mice (knockout mice without PON1) exhibited larger atherosclerotic lesions compared to wild type mice. Furthermore, high-density lipoproteins from these knockout mice did not protect against low-density lipoprotein

oxidation *in vitro*. While some human studies have linked certain PON1 polymorphisms with an increased incidence of cardiovascular disease, others have reported the opposite. Further studies are required in order to better characterize possible roles for PON1 (and other isoforms) in cardiovascular disease.

See also: Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates.

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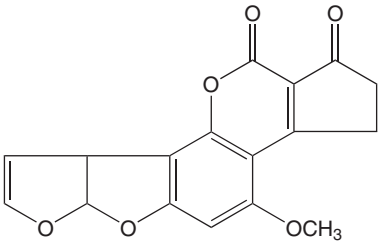
Reiner E, Aldridge WN, and Hoskin FCG (eds.) (1989) *Enzymes Hydrolyzing Organophosphorus Compounds*. West Sussex, UK: Ellis Horwood.

Aflatoxin

Raja S Mangipudy and Harihara M Mehendale

- Synonyms: Aflatoxins B1; B2; B3; B4; G1; G2; M1; M2
- Chemical Structure:



Background Information

Aflatoxins are naturally occurring bisfuranocoumarin compounds produced from the molds *Aspergillus flavus* and *Aspergillus parasiticus*. The aflatoxins are highly fluorescent. The 'B' refers to blue, the 'G' signifies green fluorescence. 'M' aflatoxins are fungal metabolites present in milk. Aflatoxin B1 is the most potent. Aflatoxins are contaminants in corn, peanuts, tree nuts, cotton seed, and certain meats. They have also been found in hypoallergenic milk.

Exposure Routes and Pathways

Ingestion and dermal contact are possible routes of exposure.

Toxicokinetics

Aflatoxins are well absorbed orally. Exposure to human skin results in slow absorption. Aflatoxins are rapidly cleared from blood. Sixty-five percent of an initial dose of aflatoxin B1 is removed from the blood within 90 min and excreted primarily in the bile. The plasma half-life of aflatoxin is short, and it is excreted slowly as multiple moieties as a result of extensive metabolism. When estimated in human liver homogenates, the parent compound had an estimated half-life of 13 min.

Aflatoxins are metabolized by the NADPH-dependent enzyme system using cytochrome P450. *In vitro* liver metabolism studies have shown five different types of metabolic pathways for aflatoxin B1: reduction, hydroxylation, hydration, *O*-demethylation, and epoxidation. All of these products contain hydroxide groups that allow them to be conjugated with glucuronic acid and sulfate, thus becoming detoxified.

Mechanism of Toxicity

Aflatoxins combine with DNA, suppressing DNA and RNA synthesis. This leads to structural changes in cell nucleoli and reduction of protein synthesis. Formation of reactive DNA adducts causes cancer.

Acute and Short-Term Toxicity (or Exposure)

Animal

Aflatoxins are carcinogenic in animals. The carcinogenic potential seems to be increased in malnutrition, especially pyridoxine deficiency. It has been proposed that aflatoxin B1-2,3-oxide (metabolite of aflatoxin B1) is the actual carcinogen.

The *in vitro* exposure of rat embryos to aflatoxin B1 induced neural tube defects. Aflatoxins have also

been shown to be teratogenic in hamsters and mice, causing neural tube closure defects, microcephaly, umbilical hernia, and cleft palate. Although negative teratogenicity studies exist for rats, mice, and commercial livestock, the negative studies involve long-term feeding exposures while the positive studies involve acute exposure, suggesting high doses and maternal toxicity may play a role in adverse effects on the offspring. Aflatoxin may be a transplacental carcinogen in the rat. Analysis of aflatoxins in maternal and cord blood samples has also demonstrated the transplacental transport of aflatoxins in humans. Although suspected of playing a role in the early onset of liver cancer in some populations, prenatal exposure has not been demonstrated to be a significant route of exposure to the aflatoxins. Aflatoxins and active carcinogenic metabolites are excreted in breast milk. An estimate of the percentage of the oral dose excreted in milk ranged from 0.09% to 0.43%.

A small number of studies have reported that male rats fed aflatoxins developed testicular degeneration and impaired spermatogenesis, although no clear association with aflatoxins and clinical infertility was uncovered in one of these studies.

Chronic Toxicity (or Exposure)

Human

Aflatoxin poisoning is difficult to diagnose early in humans. The first clinical symptoms are anorexia and weight loss. Aflatoxins are associated with hepatocellular damage and necrosis, cholestasis, hepatomas, acute hepatitis, periportal fibrosis, hemorrhage, jaundice, fatty liver changes, cirrhosis in malnourished children, and Kwashiorkor. There is evidence of transplacental transport of aflatoxin by the fetoplacental unit. Aflatoxins are proven human carcinogens.

In Vitro Toxicity Data

The *in vitro* exposure of rat embryos to aflatoxin B1 induced neural tube defects.

Clinical Management

Acute aflatoxin toxicity should be treated with decontamination procedures and good supportive care. With chronic ingestions, the primary treatment remains supportive in nature. Elevation of serum alkaline phosphatase is a good indicator of aflatoxin toxicity.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Immune System; Mycotoxins; Toxicity Testing, Mutagenicity; Veterinary Toxicology.

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Aflatoxin.

Aggregate Exposures

Jeffrey H Driver

Chemicals and other agents (biological, physical), both natural and man-made, may be released into the environment as a result of their production and use and, through dispersion and transport processes, may be present in food, water, soil, indoor and outdoor air, and other media (e.g., residential turf, indoor carpet, clothing). Thus, humans may be exposed to these agents by one or more routes (ingestion, inhalation, dermal absorption) and from one or more sources. Aggregate exposure assessments involve estimating the magnitude and frequency of exposure to a given agent by ingestion, inhalation, and dermal absorption for a defined population, taking into account reliable information on occurrence of the agent in all relevant media (**Figure 1**).

Exposure assessment methodologies in the past have often focused on a single source and/or route of exposure. However, it has become increasingly apparent in recent years that, for some chemicals, significant exposures may occur by more than one route and from more than one source. Therefore, integrated aggregate assessment methods have been

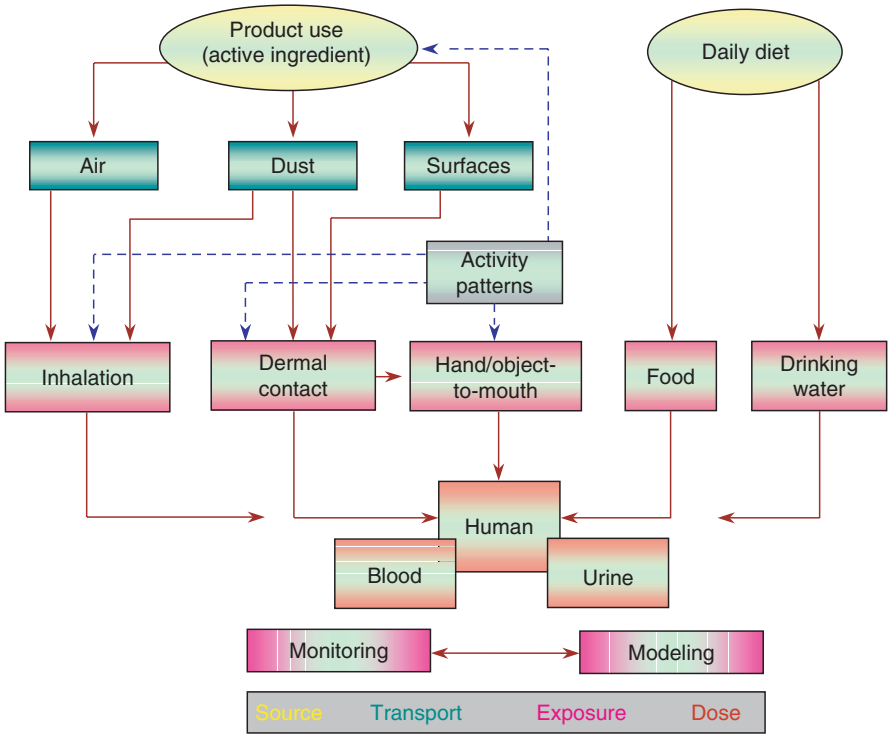

Figure 1 Components of multi-pathway, multi-route aggregate exposure assessment.

developed, and continue to be refined, to facilitate aggregate exposure assessments for these chemicals. Additional impetus has been provided by regulatory mandates, e.g., the US Food Quality Protection Act (FQPA) of 1996. FQPA requires a standard of safety for pesticide food tolerances, that is, reasonable certainty of no harm, resulting from aggregate exposure to the pesticide chemical residue, including anticipated dietary exposures and other relevant exposures for which there is reliable information (e.g., pesticide products applied in and around residences).

Aggregate exposure assessment has two complementary objectives. First, it seeks to estimate the dose that an individual will receive from exposures by all relevant routes and from all sources and pathways under given circumstances. Such an estimate of individual dose may vary as a function of space and time (e.g., at different locations, on different days). Second, it seeks to portray the range of individual doses that may be received in a well-defined population of such individuals as a distribution, reflecting the influence of varying individual characteristics (e.g., age, sex, ethnicity, place of residence, occupation). The process is based on an aggregate exposure model, which is generally a methodology incorporating mathematical algorithms for combining exposure input data from various sources to derive an exposure estimate. Exposure models often include procedures for estimating data that are not or cannot be measured directly. As with any model, exposure models are only as valid as the input parameters, data, and assumptions. Their accuracy and precision ultimately must be evaluated, at least, in part, by comparison with whatever data from measurements are available. In an aggregate exposure model, the exposure from each source is described by a set of equations. Many of the components of these equations have values that are variable (from individual to individual, from day to day or season to season, from sample to sample) and/or uncertain. These components of the exposure equations can be described by probability distributions that reflect the relative frequency of the different values for the variable components and the relative likelihood of the different possible values for the uncertain components. Exposure values from the various sources and/or routes need not be additive; whatever mathematical function is physiologically and toxicologically appropriate should be used to aggregate these values for an individual. Also, aggregate modeling should provide, where appropriate, for correlation among variables and ensuring that the component values (and the aggregate exposure estimate) for an individual are internally consistent. Input variable distributions for aggregate exposure assessments can be presented not only for a single day but also for

individuals or populations over time. In fact, the dimension of time may play a particularly important role in aggregate exposure assessments for many agents, such as chemical pesticides, where exposures may be seasonal based on pest pressures.

Aggregate assessments require consideration of indirect exposure measurements (e.g., dermal exposure by measurement of residues that are transferable from a surface such as residential turf to clothing, skin, or relevant surrogate media; inhalation exposure by measurement of air concentration in the breathing zones of individuals; ingestion exposure by measurement of concentrations in water, food, etc., ingested by an individual), and where available, direct exposure measurements (e.g., measurement of the concentration of the chemical or its biotransformation products in biological tissues or fluids).

Therefore, to estimate or measure aggregate exposures, relevant and reliable data are required. Further, aggregate exposure assessment methods and modeling tools are needed that more accurately reflect real-life situations (in contrast to methods and models that are based on very conservative assumptions and may lead to less realistic and sometimes gross overestimates of exposure).

Estimating aggregate exposure risk for a single agent, such as a chemical pesticide, brings to the forefront the need for both input data quality objectives and exposure estimate (model output) interpretation. Interpretation can be facilitated by comparing estimates of aggregate exposure to population-based or situation-specific biological monitoring data, which reflect aggregate measure of total absorbed dose. Aggregate exposure assessments typically require the use of data from indirect measurements and modeling at some level.

There is an emerging body of evidence that suggests person-to-person differences in exposure play an important role in the variability and uncertainty associated with risk assessments for chemicals (and other agents). The traditional or standard default approaches used in human health risk assessment often do not effectively evaluate interindividual variation and may underestimate the impact of chemical exposures on particular groups of individuals. Traditional approaches must be refined to adequately account for temporal variation in factors that contribute to complex aggregate exposure patterns (e.g., chemical-specific exposure media concentrations and time–activity interactions by humans) involving multiple, intermittent exposures.

Longitudinal exposure assessment methods and measurements have emerged to address temporal and spatial aspects of aggregate exposures. Longitudinal studies are being implemented to address the following:

- Temporal human time–activity patterns.
- Temporal dietary (food consumption) surveys.
- Temporal consumer and professional product use in and around the home (which addresses the potential co-occurrence of two or more exposures to a given chemical during the same toxicologically relevant time period).
- Population-based and situation-specific (e.g., reentry of residents onto pesticide-treated lawns) biological monitoring surveys.
- Integrated aggregate exposure monitoring programs (e.g., concurrent product use surveys, dietary surveys, and exposure media measurements).
- The development of assessments of exposure that include refined evaluations of the variability and uncertainty associated with aggregate exposure estimates.

See also: Exposure; Exposure Assessment; Exposure Criteria; Food Quality Protection Act, US; Mixtures, Toxicology and Risk Assessment.

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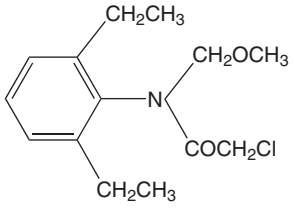
Air Pollution *See* Pollution, Air.

Alachlor

Raja S Mangipudy and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 15972-60-8
- Synonyms: Alachlore; Alanex; Alanox; Alatox 480; Lasso; Lasagrin
- Chemical/Pharmaceutical/Other Class: Herbicide
- Chemical Structure:



Uses

Alachlor is a pre-emergence herbicide registered by Monsanto in 1969. It is used as an herbicide for grasses, broadleaf seeds, corn, sorghum, soybeans, peanuts, cotton, vegetables, and forage crops.

Exposure Routes and Pathways

Dermal exposure is most common, although exposure via oral/parenteral route and ocular contact are also possible.

Toxicokinetics

Alachlor is absorbed orally. Dermal absorption may be linear over time for the duration of exposure. Excretion via kidneys is the major route of elimination.

Mechanism of Toxicity

This agent is a mucous membrane irritant. The exact mechanism of potential teratogenic changes is still being investigated. In mammals, alachlor appears to form conjugates with glucuronic acid, sulfate, and mercapturic acid. Sister chromatid exchanges have been demonstrated in human lymphocytes *in vivo* as well as dose-dependent chromosomal aberrations *in vitro* in human lymphocytes.

Acute and Short-Term Toxicity (or Exposure)

Animal

Alachlor is a slightly toxic herbicide. The LD_{50} of alachlor in rats is between 930 and 1350 mg kg^{-1}. In the mouse, the LD_{50} is between 1910 and 2310 mg kg^{-1}. The dermal LD_{50} in rabbits is 13 300 mg kg^{-1}, but some of the formulated materials can be more toxic, with dermal LD_{50} values ranging from 7800 to 16 000 mg kg^{-1}. Skin irritation is slight to moderate. The inhalation LC_{50} in rats is reportedly greater than 23.4 mg l^{-1} for 6 h of exposure. High oral doses (150 or 400 mg kg^{-1} day^{-1}) fed to rats during gestation resulted in maternal and fetal toxicity, but there was no indication that reproduction was affected. Alachlor does not appear to cause reproductive effects. Doses of up to 150 mg kg^{-1} day^{-1} fed to rabbits on days 7 through 19 of pregnancy did not result in any birth defects. Similar studies in rats at doses up to 400 mg kg^{-1} day^{-1} did not result in birth defects, but toxic effects in the mothers and offspring were seen at the highest dose. These data indicate that alachlor is not likely to cause birth defects.

Human

No specific information on the acute toxicity of alachlor in humans is available.

Chronic Toxicity (or Exposure)

Animal

A 90-day study on rats and dogs given diets containing low to moderate amounts of alachlor (1–100 $mg\,kg^{-1}\,day^{-1}$) showed no adverse effects. However, a 6-month dog study showed liver toxicity at all doses above 5 $mg\,kg^{-1}\,day^{-1}$, and a 1-year study established that above 1 $mg\,kg^{-1}\,day^{-1}$, alachlor causes effects in the liver, spleen, and kidney. In 2-year rat studies, doses above 2.5 $mg\,kg^{-1}\,day^{-1}$ caused irreversible degeneration of the iris and related eye structures.

Rats given high doses of alachlor developed stomach, thyroid, and nasal turbinate tumors. An 18-month mouse study with doses from 26 to 260 $mg\,kg^{-1}\,day^{-1}$ showed an increase of lung tumors at the highest dose for females but not males. Because of inconsistencies in these studies, the oncogenic potential of alachlor is uncertain.

Human

No specific information on the chronic toxicity of alachlor in humans is available.

In Vitro Toxicity Data

Alachlor does not appear to be mutagenic. Mutagenicity assays with a variety of microbial strains at numerous concentrations of alachlor were all negative.

Clinical Management

There are few acute symptoms. Treatment is symptomatic and supportive. There are no specific antidotes. In cases of oral exposure, measures to decrease absorption may be useful. Emesis may be induced after careful consideration. For dermal exposure, decontamination by washing the exposed area thoroughly with soap and water is recommended. In cases of inhalation exposure, the victim must be moved to fresh air and monitored for respiratory distress. In cases of eye exposure, the eyes should be irrigated with copious amounts of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the person should be seen in a health care facility.

Environmental Fate

Alachlor has a low persistence in soil, with a half-life of ~8 days. The main means of degradation is by soil microbes. It has moderate mobility in sandy and silty soils, and thus can migrate to groundwater. The largest groundwater testing program for a pesticide, the National Alachlor Well Water Survey, was conducted throughout the last half of the 1980s. Over 6 million private and domestic wells were tested for the presence of alachlor. Less than 1% of all of the wells had detectable levels of alachlor. In the wells where the compound was detected, concentrations ranged from 0.1 to 1.0 $\mu g\,l^{-1}$, with the majority having concentrations ~0.2 $\mu g\,l^{-1}$. Alachlor breaks down rapidly in natural water, primarily due to the action of microorganisms. The breakdown rate is much slower in water with no oxygen. Absorption is primarily by germinating shoots and it is readily translocated throughout the plant. Higher concentrations appear in the vegetative parts than in the reproductive parts of the plant. Alachlor is rapidly metabolized to water-soluble products in plants. It is almost completely metabolized within 10 days.

Other Hazards

Alachlor is moderately toxic to fish. The bioaccumulation factor in the channel catfish is 5.8 times the ambient water concentration, indicating that alachlor is not expected to accumulate appreciably in aquatic organisms. It is phytotoxic to sugar beet and cucurbits. The Material Safety Data Sheet should always be referred to for detailed information on handling and disposal.

Exposure Standards and Guidelines

- Acceptable daily intake is 0.0025 $mg\,kg^{-1}\,day^{-1}$.
- Maximum contaminant level is 0.002 $mg\,l^{-1}$.
- Reference dose is 0.01 $mg\,kg^{-1}\,day^{-1}$.

See also: Pesticides.

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Alar

Raja S Mangipudy

- SYNONYMS: Aminozide; Daminozide; DMSA; B-995; Kylar; Aminocide
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organic acid
- CHEMICAL STRUCTURE: $HOOCCH_2CH_2CONHN(CH_3)_2$

Uses

Alar is used as a translocated plant growth regulator. It reduces internode elongation; induces heat, drought, and frost resistance; and produces darker foliage and stronger stems. It also produces earlier and multiple flowers and fruits. A spray is often applied at the rate of 1500–10 000 ppm. It is systemic (i.e., it is taken up by the fruit). Its residues cannot be washed off or removed by peeling. Use of alar in apples caused environmental concern a few years ago; it has now been banned in the United States.

Exposure Routes and Pathways

Dermal contact and ingestion are routes of exposure.

Toxicokinetics

A breakdown product of alar is an asymmetrical 1,1-dimethylhydrazine and is excreted renally.

Mechanism of Toxicity

The growth retardant action has been attributed to formation of 1,1-dimethylhydrazine, which inhibits tryptamine oxidation by pea epicotyl homogenates.

Acute and Short-Term Toxicity (or Exposure)

Animal

The primary toxic effects seen in animals include ptosis, central nervous system (CNS) depression, gastrointestinal irritation, and possibly liver functional abnormalities.

Human

There are little data on mammalian toxicity. No human case reports are available. Based on animal data, alar should be low in toxicity. The US Environmental Protection Agency has determined that alar does not represent an imminent health hazard.

Clinical Management

No human cases have been reported so treatment recommendations are speculative. Dermal contamination probably requires no treatment other than decontamination. For gastric contamination caused by swallowing, treatment by emesis, gastric lavage, and/or activated charcoal may be indicated. Patients should be monitored for CNS depression, ptosis (drooping eyelid), and liver functional abnormalities if significant amounts (>8 g) have been ingested.

See also: Acids; Pesticides.

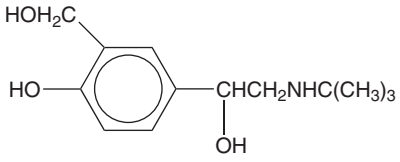
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Alar.

Albuterol

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 18559-94-9
- Synonyms: Salbutamol; Ventolin; Proventil; Apo-Salvent; Novo-Salmol; Albuterol sulfate; Volmax
- Chemical/Pharmaceutical/Other Class: Selective β_2-adrenergic agonist
- Chemical Formula: $C_{13}H_{21}O_3$
- Chemical Structure:



Uses

Albuterol is used as a bronchodilator in the treatment of asthma. Albuterol is also used in the prevention of premature labor. Off label uses include treatment of hyperkalemia.

Mechanism of Action

β-Adrenergic receptors mediate the effects of the sympathetic nervous system throughout the body. β_2-Receptors are found on vascular, bronchial, gastrointestinal, and uterine smooth muscle as well as skeletal muscle, hepatocytes, and also the myocardium. Albuteral stimulates adenyl cyclase which catalyzes cyclic adenosine monophosphate (AMP) from adenosine triphosphate (ATP). This mediates bronchdilation and smooth muscle relaxation through activation of protein kinases, leading to phosphorylastion of proteins, which in turn increases bound intracellular calcium. The reduced availability of intracellular ionized calcium inhibits actin-myosin linkage, leading to the relaxation of smooth muscle. β_2-Adrenergic receptors in the lung also inhibit secretions and decrease histamine release. Stimulation of β_2-adrenergic receptors found on the uterine smooth muscle inhibits the onset of labor.

Routes of Administration

Albuterol is available as tablets and as syrup for oral use, as solution and as sulfate for inhalation, as solution for injection, and as solution for intravenous infusion for parenteral use. Inhaled albuterol has been found to be more effective and less toxic than alternative forms.

Toxicokinetics

Nebulized albuterol has been found more effective than systemic administration. Oral albuterol is readily absorbed from the gut. There is significant first-pass conjugation with 50% bioavailability of an ingested (oral) dose. From 21% to 30% of an inhaled dose is available for absorption. Only $\sim$3% of an oral inhaled dose reaches the lungs. With a nebulizer, $\sim$10–20% is absorbed. Parenteral absorption is 100%. Sulfate conjugation is the primary metabolic pathway; it is transformed in the liver. There appears to be no direct biotransformation of albuterol in the lungs. Most of an inhaled dose is deposited on the pharynx after inhalation and then swallowed.

The volume of distribution is 156 ± 38 l and the plasma protein binding is 8%. Albuterol, as both the sulfate and sulfate conjugates (metabolite and unchanged drug), is eliminated via the kidneys. With oral dosing, 28% of albuterol is excreted unchanged in the urine and 64–80% unchanged with intravenous dosing. Albuterol follows first-order kinetics. Total plasma clearance is $0.41\,\mathrm{l\,h^{-1}\,kg^{-1}}$ and renal clearance is $0.28\,\mathrm{l\,h^{-1}\,kg^{-1}}$. The half-life is 3–5 h with oral dosing, 2–7 h with inhalation, and 5.5–6.9 h with intravenous dosing. Maximum brochodilation occurs within 15–30 min.

Side Effects

Tachycardia occurs as a reflex to the drop in mean arterial pressure (MAP) or as a result of β-1 stimulus. β-Adrenergic receptors in the locus ceruleus also regulate norepnephrine-induced inhibitory effects, resulting in agitation, restlessness, and hand tremor. Stimulation of nonpulmonary β_2 receptors may lead to an increase in heart rate, QT_c interval prolongation, nonspecific T-wave changes, skeletal muscle tremor, and slight increases in blood glucose and nonesterified fatty acids. Hypokalemia is more pronounced in patients receiving intravenous albuterol. Hypotension is also known to occur mostly in overdose. The buildup of cyclic AMP in the liver stimulates glycogenolysis and an increase in serum glucose. In skeletal muscle, this process results in increased lactate production. Direct stimulus of sodium/potassium ATPase in skeletal muscle produces a shift

of potassium from the extracellular space to the intracellular space. Relaxation of smooth muscle produces a dilation of the vasculature supplying skeletal muscle, which results in a drop in diastolic and MAP. Myocardial ischemia and infarction have been associated with excessive tachycardia in elderly patients. The skin may be warm and pink with evidence of diaphoresis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Albuterol appears to be relatively benign in animals, similar to human. Agitation, vomiting, and lethargy may be seen. In rats the oral LD_{50} was more than 2000 mg kg^{-1}; and inhalation LC_{50} could not be determined.

Human

A review of albuterol overdoses revealed that up to 20 times the oral daily dose produced no deaths. The effects of albuterol overdose are usually mild and benign, although they can be prolonged. Cardiovascular effects are usually limited to a sinus tachycardia and widened pulse pressure. Although there may be a drop in diastolic pressure, the systolic pressure is maintained by increased cardiac output from the trachycardia. Transient hypokalemia can result, caused by a shift of extracellular potassium to the intracellular space with total body stores of potassium generally remaining normal. A transient metabolic acidosis can be seen due to increased lactate production. Restlessness, agitation, tremors apprehension, dizzines, nausea, vomiting, and dilated pupils are common in albuterol overdose.

Chronic Toxicity (or Exposure)

Human

Continued dependence of salbutamol tablets taken in high doses (30–40 tablets daily and 48–64 mg day^{-1}) has lead to symptoms of toxic psychosis in one elderly woman and paranoid psychosis in a 52-year-old man. Up to 60–90 100 μg inhalations of salbutamol daily has been used by asthmatics who increased doses because they 'needed it' and wanted to 'feel good'. Long-term tolerance develops to bronchodilator action, tremor, tachycardia, prolongation of QT_c interval, hyperglycemia, hypokalemia, and the vasodilator response.

Clinical Management of Overdose

Albuterol overdoses rarely require treatment beyond gastrointestinal decontamination. Children have survived overdoses as large as 100 mg and adults have survived doses up to 240 mg without serious complications. Activated charcoal effectively adsorbs albuterol. The hypokalemia produced reflects a transient shift in potassium location rather than a true deficit of potassium; external replacement therapy is rarely necessary but can be added to intravenous fluids to support the heart if electrocardiographic changes are noted. A conservative approach to tachycardia is recommended since arrhythmias beyond an increase in rate have not occurred with overdose. Support of blood pressure and control of tachycardia are major therapeutic interventions.

See also: Kidney.

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Alcoholic Beverages and Alcoholism

Kartik Shankar and Harihara M Mehendale

Alcohol abuse may be the number one health issue in the United States with annual cost estimates as high as US$ 185 billion a year. Forty four percent of the adult US population are current drinkers having consumed at least 12 drinks in the past year. Although most people who drink do it safely, ~14 million Americans (7.4% of the population) meet the diagnostic criteria for alcohol abuse or alcoholism. More than one-half of American adults have a close family member who has or has had alcoholism. Almost 2.7 million violent crimes and 16 000 traffic crashes can be directly linked to alcohol. Alcohol consumption has consequences for the health and well-being of those who drink and, by extension, the lives of those around them. Because alcoholism

affects many aspects of our society, clearly alcoholism has enormous social implications including burden on social and health services.

In the United States, a 'drink' is considered to be 0.5 ounces (oz) or 15 g of alcohol, which is equivalent to 12 oz of beer, 5 oz of wine, or 1.5 oz of 80 proof distilled spirits. According to the Dietary Guidelines for Americans, jointly issued by the US Department of Agriculture and the US Department of Health and Human Services, moderate drinking is no more than two standard drinks per day for men and no more than one per day for women. Moderate drinking may be defined as drinking that does not generally cause problems, either for the drinker or for society. The term is often confused with 'social drinking', which refers to drinking patterns that are accepted by the society in which they occur. However, social drinking is not necessarily free of problems. The National Institute on Alcohol Abuse and Alcoholism further recommends that people aged 65 and older limit their consumption of alcohol to one drink per day. Alcoholism, also known as 'alcohol dependence', is a disease that includes four symptoms: (1) *Craving*: a strong need, or compulsion, to drink; (2) *Loss of control*: the inability to limit one's drinking on any given occasion; (3) *Physical dependence*: withdrawal symptoms, such as nausea, sweating, shakiness, and anxiety, occur when alcohol use is stopped after a period of heavy drinking; and (4) *Tolerance*: the need to drink greater amounts of alcohol in order to 'get high'.

Health Effects of Alcohol Abuse

Effects of Alcohol on the Liver

There is no question that alcohol abuse contributes significantly to liver-related morbidity and mortality in the United States. Long-term alcohol use is the leading cause of illness and death from liver disease. There are three phases of alcohol-induced liver damage, alcoholic fatty liver, which is usually reversible with abstinence; alcoholic hepatitis or inflammation; and alcoholic cirrhosis or scarring of the liver. Patients with both alcoholic cirrhosis and hepatitis have a death rate of more than 60% over a 4-year period. The prognosis is bleaker than the outlook for many types of cancers. As many as 900 000 people in the United States suffer from cirrhosis and some 26 000 of these die each year. The risk for liver disease is related to how much a person drinks: the risk is low at levels of alcohol consumption but steeply increases with higher levels of consumption. Because effects of alcohol are dose-related and because of the steepness at which the adverse effects are observed, moderation is emphasized in social or occasional drinking. Gender also plays a role in the development of alcohol-induced liver damage. Some evidence indicates that women are more susceptible to the cumulative effects of alcohol on the liver.

Cancer and Alcohol Abuse

Alcohol has been linked to a number of cancers, including cancers of the head and neck, digestive tract, and breast. Alcohol is clearly established as a cause of cancer of various tissues in the airway and digestive tract, including the mouth, pharynx, larynx, and esophagus. Research suggests that the risk of cancers is associated with both the concentration of alcohol and number of drinks consumed. Alcohol acts synergistically with tobacco to dramatically increase the risk of cancers that is above that of alcohol or tobacco alone. An increased risk of stomach cancer among alcohol drinkers has been identified in several but not the majority of studies. The link between alcohol use and chronic gastritis is clear, although the progression from chronic gastritis to neoplasia is less well understood and involves factors in addition to alcohol. Only weak positive association between alcohol use and cancers of the colon, rectum, and breast exists.

Cardiovascular Health and Alcohol Use

Cardiovascular diseases account for more deaths among Americans than any other group of diseases. Of all causes of death, coronary heart disease (CHD) is the leading cause of death among Americans. Several large prospective studies throughout the world suggest a reduced risk of CHD with alcohol use over a wide range of consumption levels. However, in these studies the apparent protective effects of alcohol against CHD were realized at low to moderate levels of alcohol (ranging from one to two drinks per week to one to two drinks per day). However, the risk increased at drinking levels above five drinks a day for men and two drinks a day for women. Both the type of alcoholic beverage consumed and the pattern of drinking (small amounts everyday versus large amounts on only one or two days a week) influence protection against CHD. The relationship between alcohol consumption and stroke risk suggests that heavy drinking increases the risk of stroke, especially in women. However, evidence suggesting that moderate level of alcohol consumption protects from stroke is at best equivocal. In addition, it appears that a high level of alcohol consumption increases blood pressure, a critical risk factor for stroke.

Alcohol and the Skeleton

An association between alcohol intake and accidental injury is well established. The risk of falling is tripled in those having a blood alcohol concentration (BAC) of 0.1–0.15% and 60 times higher in those with a BAC of 0.16% or higher, compared with those whose BAC is 0.1% or lower. Beyond the risks of falling, however, emerging evidence suggests alcoholics may also suffer from a generalized skeletal fragility, leading to alcohol-induced osteopenia. Although the degree to which alcohol contributes to the osteopenia in the general population is not clear, but data from experimental animal studies suggest that alcohol can disrupt the tightly coupled processes of bone formation and resorption.

Fetal Alcohol Syndrome

Fetal alcohol syndrome (FAS) is a set of birth defects caused by maternal consumption of alcohol during pregnancy. FAS is considered the most common preventable cause of mental retardation. The annual cost of FAS according to the 10th Special Report to the US Congress on Alcohol and Health estimated the annual cost of FAS in 1998 to be $2.8 billion.

See also: Ethanol; Fetal Alcohol Syndrome.

Further Reading

Mann RE, Smart RG, and Govoni R (2003) The epidemiology of alcoholic liver disease. *Alcohol Research & Health* 27(3): 209–219.

McIntosh C and Chick J (2004) Alcohol and the nervous system. *Journal of Neurology, Neurosurgery & Psychiatry* Sep: 75 Suppl 3(iii): 16–21.

Room R, Babor T, and Rehm J (2005) Alcohol and public health. *Lancet* Feb 5: 365(9458): 519–530.

Relevant Website

http://www.niaaa.nih.gov – *10th Special Report to the US Congress on Alcohol and Health*. US Department of Health and Human Services.

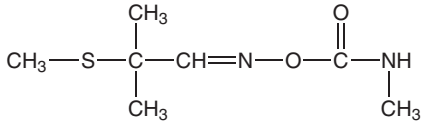
Alcoholism *See* Alcoholic Beverages and Alcoholism.

Alcohols *See* Alcoholic Beverages and Alcoholism; Allyl Alcohol; Benzyl Alcohol; Ethanol.

Aldicarb

Paul R Harp

- Chemical Abstracts Service Registry Number: CAS 116-06-3
- Synonyms: 2-Methyl-2(methylthio)-propionaldehyde *O*-(methylcarbamoyl)oxime; Aldecarb; Aldicarbe; Temik; AI3-27093; ENT 27093; OMS 771; NCI 08640; SHA 098301; UC 21149; RCRA Waste Number P070
- Chemical/Pharmaceutical/Other Class: *N*-Methylcarbamate insecticide
- Chemical Structure:



Uses

Aldicarb is a soil-applied systemic insecticide used to control a wide variety of insects, mites, and nematodes. Major uses include ornamentals, cotton, and some fruit and vegetable crops. Aldicarb is the most acutely toxic insecticide currently registered for use in the United States and is classified as a restricted-use agent that can only be applied by or under supervision of a certified applicator. Certain uses have been voluntarily canceled due to potential for groundwater contamination. Aldicarb is only available as a granular mix to help reduce the hazards associated with handling the product.

Exposure Routes and Pathways

The most common exposure routes are dermal (during processing, packaging, or application) and oral (through consumption of products containing aldicarb residues). Illegal applications to melons and cucumbers have resulted in consumer poisonings. Exposure may also occur through unprotected handling of treated plants or soil. Although inhalation of fine particles and dusts of aldicarb has been reduced through improvements in applicator design, inhalation can still represent a significant route of exposure if equipment that grinds the granules is used during application.

Toxicokinetics

Aldicarb is readily absorbed from all routes of exposure. Oxidation reactions rapidly convert aldicarb to aldicarb sulfoxide, of which a small portion may then be slowly oxidized to aldicarb sulfone. Both the parent compound and its oxidized metabolites can be converted to their respective oximes and nitriles, which may ultimately be converted to aldehydes, acids, and alcohols. Animal studies have indicated aldicarb and its metabolites are distributed to many different tissues but no evidence of accumulation has been found. In the various tissues examined, aldicarb residues were not detected more than 5 days after exposure. The presence of aldicarb in fetal tissue indicates placental transfer in pregnant rats. Various aldicarb metabolites have been found in the milk of cows acutely treated with aldicarb.

Animal studies have indicated the major route of excretion to be urinary with at least 80% of the original dose generally eliminated within 24 h. Aldicarb is excreted primarily as aldicarb sulfoxide and sulfoxide oxime; the parent compound is excreted only in trace amounts. Biliary metabolites have been shown to undergo resorption and urinary excretion.

Mechanism of Toxicity

Aldicarb binds and inhibits acetylcholinesterase, the enzyme responsible for metabolizing the neurotransmitter acetylcholine and terminating its action at cholinergic synapses. Exposure to aldicarb results in synaptic accumulation of acetylcholine in both the central and peripheral nervous systems and hyperstimulation of muscarinic and nicotinic receptors leading to 'cholinergic crisis'. Aldicarb sulfoxide is a more potent inhibitor of acetylcholinesterase than the parent compound. In contrast to the organophosphate anticholinesterases, acetylcholinesterase inhibition by *N*-methylcarbamates is reversible with fairly rapid reactivation occurring through spontaneous decarbamoylation or via hydrolysis of the carbamate.

Acute and Short-Term Toxicity (or Exposure)

Animal

Signs of acute exposure in laboratory animals are similar to those described for humans and recovery from nonlethal exposures occurs rapidly. LD_{50} values for acute exposure in rats are 0.46–1.23 mg kg^{-1} (oral) and 3.2 to >10 mg kg^{-1} (dermal).

Human

The acute effects of aldicarb exposure are due to cholinergic overstimulation and may include the SLUDGE syndrome (salivation, lacrimation, urination, diarrhea, gastrointestinal cramping, and emesis), respiratory depression, bronchospasms, increased bronchial secretions, pulmonary edema, blurred vision, miosis, headache, tremors, muscle fasciculations, convulsions, mental confusion, coma, and death due to respiratory failure. Recovery from nonlethal exposures occurs very rapidly, usually within a few hours.

Chronic Toxicity (or Exposure)

Animal/Human

Researchers have examined the possible effects of aldicarb on the induction of peripheral neuropathies. Currently, insufficient evidence exists to indicate any significant long-term health risk associated with aldicarb exposure. The US Environmental Protection Agency's (EPA) Office of Pesticide Programs has classified aldicarb as group E – evidence of noncarcinogenicity for humans.

Clinical Management

Persons providing medical assistance should avoid contact with contaminated clothing. Contaminated clothing should be removed, bagged, and discarded. Exposed dermal areas should be cleaned thoroughly with soap and water. Exposed eyes should be flushed with generous amounts of clean water for at least 15 min. If necessary, use an endotracheal tube to maintain a clear airway, aspirate any secretions, and provide oxygen via mechanical ventilation.

If the patient is asymptomatic and can be treated soon after exposure, activated charcoal may be used to reduce absorption from the gastrointestinal tract. If potentially life-threatening quantities have been ingested, gastric lavage should be considered if it can be conducted within ~1 h of exposure. Charcoal and/or catharsis are contraindicated in presence of severe vomiting or diarrhea. Muscarinic effects (i.e., SLUDGE) may be reduced by intravenous or intramuscular administration of atropine. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenobarbital may be helpful for recurrent seizures. Pralidoxime is indicated in cases of mixed exposure to both carbamates and organophosphorus compounds but is contraindicated in cases of carbamate-only exposure. Furosemide may be useful for pulmonary edema that continues after full atropinization. Metabolite analysis of a urine sample may allow confirmation of the intoxicating agent.

Ecotoxicology

Aldicarb is highly water soluble and soil application of this insecticide has the potential to result in runoff or leaching of the insecticide or active metabolites and contamination of surface or groundwater. Aldicarb is acutely toxic to bees, birds, and fish. Species-specific rates of bioactivation may influence the sensitivity of a particular organism to this insecticide.

Exposure Standards and Guidelines

The acceptable daily intake for aldicarb is $0.003\,mg\,kg^{-1}\,day^{-1}$. The reference dose for aldicarb is $0.001\,mg\,kg^{-1}\,day^{-1}$, but is currently being reassessed by the US EPA.

See also: Carbamate Pesticides; Cholinesterase Inhibition; Neurotoxicity; Pesticides; Pollution, Water.

Relevant Websites

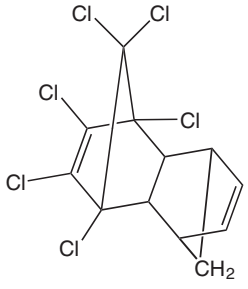
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.
http://www.inchem.org – Environmental Health Criteria 121: Aldicarb–International Programme on Chemical Safety.

Aldosterone *See* Corticosteroids.

Aldrin

Benny L Blaylock

- Chemical Abstracts Service Registry Number: CAS 309-00-2
- Synonyms: 1,2,3,4,10,10-Hexachloro-1,4,4α,5,8,8α-hexahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene; Aldrec; Aldrex; Drinox; Octalene; Seedrin; Compound 118
- Chemical/Pharmaceutical/Other Class: Organochlorine insecticide
- Chemical Formula: $C_{12}H_8Cl_6$
- Chemical Structure:



Uses

Although not currently manufactured for use in the United States, aldrin is used as an insecticide.

Exposure Routes and Pathways

Aldrin is absorbed from the gastrointestinal tract, the respiratory tract, and through the skin.

Toxicokinetics

The most important exposure routes are oral and dermal. Aldrin is readily absorbed through the gastrointestinal tract via the hepatic portal vein and through the skin.

Epoxidation by cytochromes P450 of aldrin to dieldrin occurs in the liver and, to a lesser extent, in the lungs. In mammals, two major metabolism routes of dieldrin seem to be predominant: (1) direct oxidation by cytochrome oxidases, resulting in 9-hydroxydieldrin, and (2) the opening of the epoxide ring by epoxide hydrases, resulting in 6,7-*trans*-dihydroxydihydroaldrin. Like other organochlorine insecticides, adipose tissue is the major storage tissue followed by liver, brain, and blood. The water-soluble metabolites of aldrin detoxification are excreted primarily in the feces via the bile and, to a lesser extent, in the urine. Dieldrin is also found in mothers' milk and can be excreted via that mechanism.

Mechanism of Toxicity

Aldrin and dieldrin characteristically stimulate the central nervous system (CNS) causing hyperexcitation and generalized seizures. Both *in vitro* experiments using rat brain membranes and intravenous or intraperitoneal administration of aldrin and dieldrin to rats have shown that these agents are capable of blocking the activity of GABA by blocking the influx of chloride through the $GABA_A$ receptor–ionophore complex.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal toxicity with aldrin is similar to dieldrin. Convulsion and incoordination are frequently observed. Acute symptoms observed in ducks, pheasants, and bobwhite quail following acute oral LD_{50} exposure were ataxia, low carriage, nictitating membrane closed for long periods, fluffed feathers, tremors, phonation, violent wing-beat convulsions, seizures, and opisthotonos. Death occurred 0.5 h to 10 days posttreatment. Weight losses occurred among survivors of higher levels. Gross autopsies revealed occasional liver adhesions to parietal peritoneum.

Human

The toxicity of aldrin is essentially that of dieldrin and similar to other cyclodiene insecticides. The CNS is the primary target with convulsions as the major symptom. Patients may also experience nausea, vomiting, hyperexcitability, and coma. Onset of symptoms may be between 20 min and 12 h after ingestion and include malaise, headache, nausea, vomiting, dizziness, and tremors. This may progress to clonic and tonic convulsions, sometimes without premonitory symptoms. Convulsive episodes may alternate with periods of severe central nervous depression. A 3-year-old girl who was stricken 5 min after eating a cooked meal contaminated with aldrin and who died 12 h later was thought to have consumed 120 mg of aldrin, resulting in a dosage of $\sim 8.2\,mg\,kg^{-1}$. A 23-year-old man who intentionally drank aldrin at a dose of $25.6\,mg\,kg^{-1}$ was very seriously poisoned, although eventually he recovered completely.

Chronic Toxicity (or Exposure)

Animal

Aldrin administered daily to 30- and 90-day-old Wistar rats at 8 or 11 ppm in diet showed an alteration in antibody production when exposed to *Escherichia coli* at 60 days. Functional disorders of thymus and adrenal glands and in protein synthesis were also noted. Younger rats were more strongly affected than older rats. Cats fed aldrin at $1\,mg\,kg^{-1}\,day^{-1}$ or made to inhale $0.1\,\mu g\,l^{-1}$ of air had marked lowering of conditioned reflexes and of unconditioned food and orientation reflexes, which required up to 8 days to return to normal. Groups of 12 male and 12 female Osborne–Mendel rats were fed diets containing 0, 0.5, 2, 10, 50, 100, or 150 ppm recrystallized aldrin for 2 years. Considering together groups given 0.5, 2, or 10 ppm (i.e., the groups showing survival rates at 2 years comparable to those of controls), the number of tumor-bearing animals was 25/60 compared with 3/17 controls. Among treated animals, 12 developed lymphomas, 13 had mammary tumors (malignant in four rats), two had fibrosarcomas, and three had tumors at other sites. The three tumor-bearing control rats had, respectively, a pulmonary lymphoma, a benign mammary tumor, and a tumor at another site.

Human

Among workers who have been engaged in the manufacture, handling, and spraying of aldrin, only acute effects such as eye, skin, or respiratory irritation were reported, particularly following exposures to dusty formulations of the compound. High worker exposure was associated with induction of liver microsomal enzymes and the ability of some highly exposed workers to increase their drug metabolizing capacity. Frank liver injury or injury to other human organs has not been reported in the United States, Canadian, and western European literature. Aldrin is not classifiable as to its carcinogenicity to humans by International Agency for Research on Cancer.

Clinical Management

Treatment is symptomatic. Activated charcoal as a slurry has been reported to absorb aldrin and increase its rate of excretion after oral exposure. Emesis is not recommended due to potential CNS depression or seizures. Diazepam or phenobarbital is used when anticonvulsant therapy is necessary.

Environmental Fate

Aldrin binds strongly to soil particles and is very resistant to leaching into groundwater. Volatilization is an important mechanism of loss from the soil. Due to its persistent nature and hydrophobicity, aldrin is known to bioconcentrate, mainly as its conversion products. As aldrin is readily and rapidly converted to dieldrin in the environment, its fate is closely linked to that of dieldrin. Aldrin is readily metabolized to dieldrin in both animals and plants, and therefore aldrin residues are rarely present in animals and then only in very small amounts. Residues of aldrin have been detected in fish in Egypt; the average concentration was $8.8\,\mu g\,kg^{-1}$, and a maximum concentration of $54.27\,\mu g\,kg^{-1}$. Aldrin has

low phytotoxicity, with plants affected only by extremely high application rates.

Ecotoxicology

The acute toxicity of aldrin to avian species varies in the range of $6.6\,mg\,kg^{-1}$ for bobwhite quail to $520\,mg\,kg^{-1}$ for mallard ducks. Aldrin-treated rice is thought to have been the cause of deaths of waterfowl, shorebirds, and passerines along the Texas Gulf Coast, both by direct poisoning by ingestion of aldrin-treated rice and indirectly by consuming organisms contaminated with aldrin. Residues of aldrin were detected in all samples of bird casualties, eggs, scavengers, predators, fish, frogs, invertebrates, and soil.

The toxicity of aldrin to aquatic organisms is quite variable, with aquatic insects being the most sensitive group of invertebrates. The 96 h LC_{50} values range from 1 to $200\,\mu g\,l^{-1}$ for insects and from 2.2 to $53\,\mu g\,l^{-1}$ for fish.

Other Hazards

Aldrin is corrosive to metals, owing to the slow formation of hydrogen chloride during storage. It is also noncombustible as the substance itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.0001\,mg\,kg^{-1}\,day^{-1}$.
- Reference dose is $0.03\,mg\,kg^{-1}\,day^{-1}$.
- Permissible exposure limit is $0.25\,mg\,m^{-3}$ (8 h).

See also: Charcoal; Cyclodienes; Diazepam; Dieldrin.

Further Reading

Jorgenson JL (2001) Aldrin and dieldrin: A review of research on their production, environmental deposition and fate, bioaccumulation, toxicology, and epidemiology in the United States. *Envionmental Health Perspectives* 109(suppl 1): 113–139.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Aldrin.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Aldrin.
http://www.osha-slc.gov – US Department of Labor, Occupational Safety and Health Administration.

Algae

Keiko Okamoto and Lora E Fleming

Toxins discussed in this section are produced by microscopic, simple aquatic organisms classified as algae. Algae are photoautotrophic, which means they obtain energy and nourishment from light. Included in algae are organisms also commonly referred to as phytoplankton, dinoflagellates, and diatoms. Some of these tiny organisms produce very potent toxins. This section focuses on the following algal toxins that are fairly well characterized in terms of adverse effects known to occur in humans and laboratory animals: azaspiracids, brevetoxins, ciguatoxins, maitotoxins, domoic acid, okadaic (or okadeic) acid, saxitoxins and other cyanobacterial toxins. Azaspiracids, brevetoxins, ciguatoxins, and okadaic acid are all classified chemically as polyether toxins. Domoic acid is an excitatory neurotoxic amino acid.

Commonly used synonyms for these sources and types of toxicity include: azaspiracid shellfish poisoning (AZP) caused by five azaspiracid analogs; neurotoxic shellfish poisoning (NSP) caused by 10 known brevetoxins; ciguatera fish poisoning (CFP or Ciguatera) caused by more than 10 ciguatoxin congeners and maitotoxins; amnesic shellfish poisoning (ASP) caused by one or more of three domoic acid derivatives; paralytic shellfish poisoning (PSP) caused by at least 20 derivatives of saxitoxins; diarrheic shellfish poisoning (DSP) caused by okadaic acid, and other toxins too numerous to detail in this brief overview (e.g., six derivatives of dinophysistoxin, four pectenotoxins (polyether lactones), and yessotoxins (including two sulfate esters which resemble brevetoxins); and finally, red tides; harmful algal blooms (HAB), dinoflagellate blooms, and phycotoxins.

Exposure Routes and Pathways

A major route of human exposure to algal toxins is through the consumption of contaminated seafood products. The consumption of contaminated clams, mussels, scallops, oysters, and other shellfish causes shellfish-associated diseases (ASP, AZP, DSP, NSP,

PSP). Consuming contaminated large reef fish, like barracuda and grouper, causes CFP. Consumption of puffer fish with saxitoxin through shellfish feeding has resulted in cases of PSP.

Inhalation exposure of airborne toxins is also known to occur. For example, the *Karenia brevis* organism that produces brevetoxin is relatively fragile and easily broken apart, particularly in wave action along beaches, thus releasing the toxin. During an active nearshore red tide, the water and aerosols of contaminated salt spray will contain the toxins and organism fragments both in the droplets and attached to salt particles. These airborne particulates can cause respiratory irritation in humans on or near beach areas, and also be carried inland depending on wind and other environmental conditions. The use of particle filter masks or retreat to an air-conditioned environment may provide protection from toxicity.

Ciguatera, caused by ingested ciguatoxins and maitotoxins, can reportedly be sexually transmitted. There are also reports of acute health effects of ciguatera toxin in the fetus and newborn child exposed through placental and breast milk transmission from the mother.

Humans can also be exposed to cyanobacteria and their toxins through direct skin contact or by drinking contaminated water. Other possible routes of exposure include inhalation of contaminated aerosols, consumption of contaminated food, and even through dialysis. Therefore, occupational exposures for fisherman, watermen, and scientists, as well as recreational exposures for the general public, are all possible.

Toxicokinetics

The fate and metabolism of algal toxins is unclear and understudied; however, it is known that the absorption of both lipophilic and hydrophilic algal toxins occurs rapidly from the gastrointestinal and respiratory tracts. For example, to evaluate brevetoxin toxicokinetics from acute inhalation exposure up to 7 days, 12-week-old male F344/Crl BR rats were exposed to a single dose of 6.6 $\mu g\,kg^{-1}$ the brevetoxin PbTx-3 through intratracheal instillation. More than 80% of PbTx-3 was rapidly cleared from the lung and distributed by the blood throughout the body, particularly the skeletal muscle, intestines, and liver with low but constant amounts present in blood, brain, and fat. Approximately 20% of the toxin was retained in the lung, liver, and kidneys for up to 7 days. Absorption of many of the cyanobacterial toxins occurs rapidly from the gastrointestinal tract. The greatest concentrations are found in the liver; some are found in the kidney and remain detectable for up to 24 h.

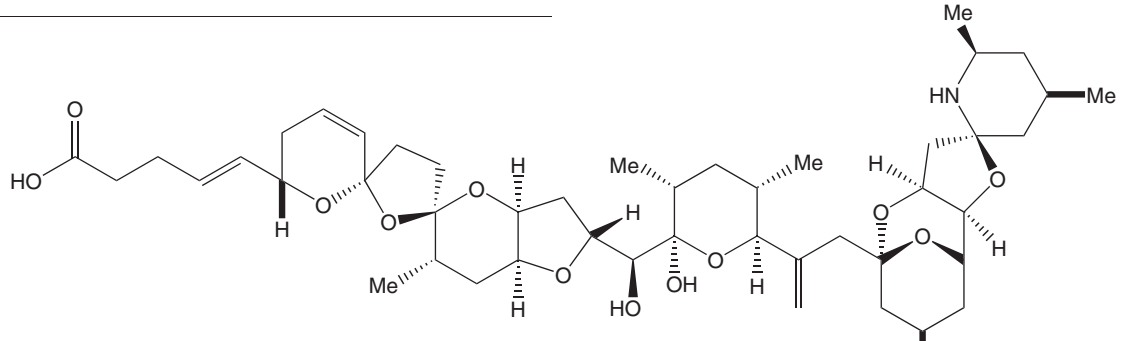
Acute and Chronic Toxicity and Mechanisms of Action

In general terms, people suffering from signs and symptoms of illnesses associated with eating seafood contaminated with algal toxins typically present the acute onset of gastrointestinal symptoms within minutes to 24 h. Victims may also exhibit a wide range of signs and symptoms involving many organ systems, including respiratory (difficulty breathing), peripheral nervous system (numbness and tingling), central nervous system (hallucinations and memory loss), and cardiovascular system (fluctuating blood pressure and cardiac arrythmias). These signs and symptoms, depending on the particular disease, may last from hours to months.

Chronic algal toxin exposure remains mostly unstudied, although some limited information about specific toxins is included in the descriptions that follow. On the other hand, exactly how some of these toxins affect cells and tissues (mechanism of action) has received considerable attention from researchers.

Azaspiracids

Azaspiracids (produced by *Protoperidinium*) are a relatively new class of polyether toxins. Consequently, little information is known yet about the AZP mechanism of toxicity except that it appears to be hepatotoxic.



Azaspiracid

The first human acute intoxications attributed to newly described azaspiracid poisoning (AZP) occurred in the Netherlands after consumption of contaminated shellfish. The symptoms were similar to the nausea, vomiting, severe diarrhea, and stomach cramps of DSP. AZP in humans has been reported throughout Europe since 1995, and azaspiracids have been found in shellfish harvested in Spain, France, and northern Europe.

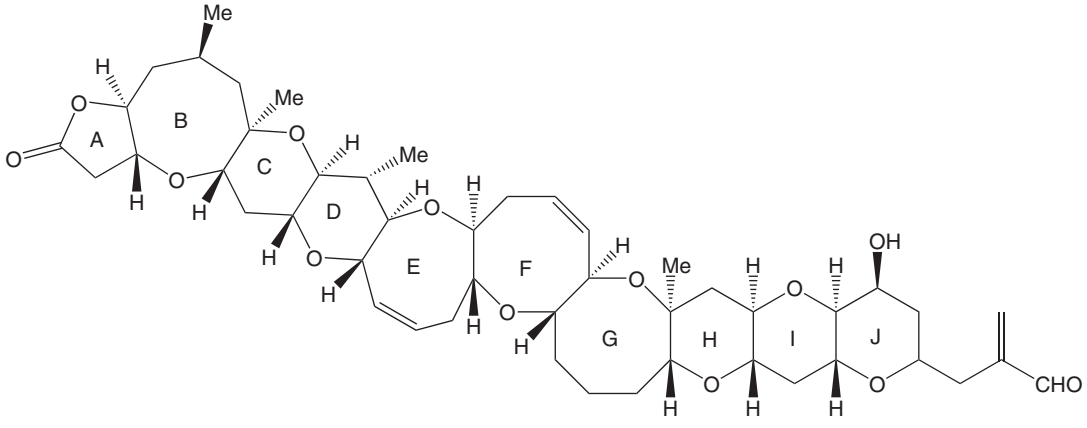
Chronic effects observed in mice after oral administration of azaspiracid were interstitial pneumonia, shortened villi in the stomach and small intestine, fatty changes in the liver, and necrosis of lymphocytes in the thymus and spleen.

Brevetoxins

Several brevetoxins (synonyms: PbTx-1, PbTx-2, PbTx-3, PbTx-4, PbTx-5, PbTx-6, PbTx-7, PbTx-8, PbTx-9) are produced by *Karenia brevis,* formerly known as *Gymnodinium breve* and *Ptychodiscus brevis*. Both brevetoxins and ciguatoxins (see below) open voltage-dependent sodium channels in cell membranes, leading to uncontrolled sodium influx into the nerve cells and striated muscle cells. Brevetoxins and ciguatoxins cause biphasic cardiovascular response with hypotension and bradycardia followed by hypertension and tachycardia. The respiratory arrest induced by a lethal dose results mainly from depression of the central respiratory center. Although evidence suggests that brevetoxins affect mammalian cortical synaptosomes and neuromuscular preparations, the majority of toxic effects associated with brevetoxins predominantly appear to result from the substantial and persistent depolarization of nerve membranes. In the lung, brevetoxin appears to be a potent respiratory toxin involving both cholinergic and histamine-related mechanisms.

Fish, birds, and mammals are all susceptible to brevetoxins. In Japanese medaka fish (*Oryzias latipes*), brevetoxins induce embryonic toxicity and developmental abnormalities. The fish are killed apparently through lack of muscle coordination and paralysis, convulsions, and death by respiratory failure. In the mosquito fish (*Gambusia affinis*) bioassay, the lethal dose (LD_{50}) is reported at $0.011\,\mu g\,l^{-1}$. Exposed birds die acutely with neurologic and hematologic effects. Brevetoxins were implicated in the deaths of manatees in Florida during a widespread bloom of *G. breve*. At necropsy, the animals did not appear to be unhealthy, and they had recently fed. High levels of brevetoxin were found by histochemical stain in cells throughout the body, particularly macrophages. The mouse LD_{50} is $0.17\,mg\,kg^{-1}$ body weight intraperitoneally, $0.094\,mg\,kg^{-1}$ body weight intravenously, and $0.520\,mg\,kg^{-1}$ body weight orally.

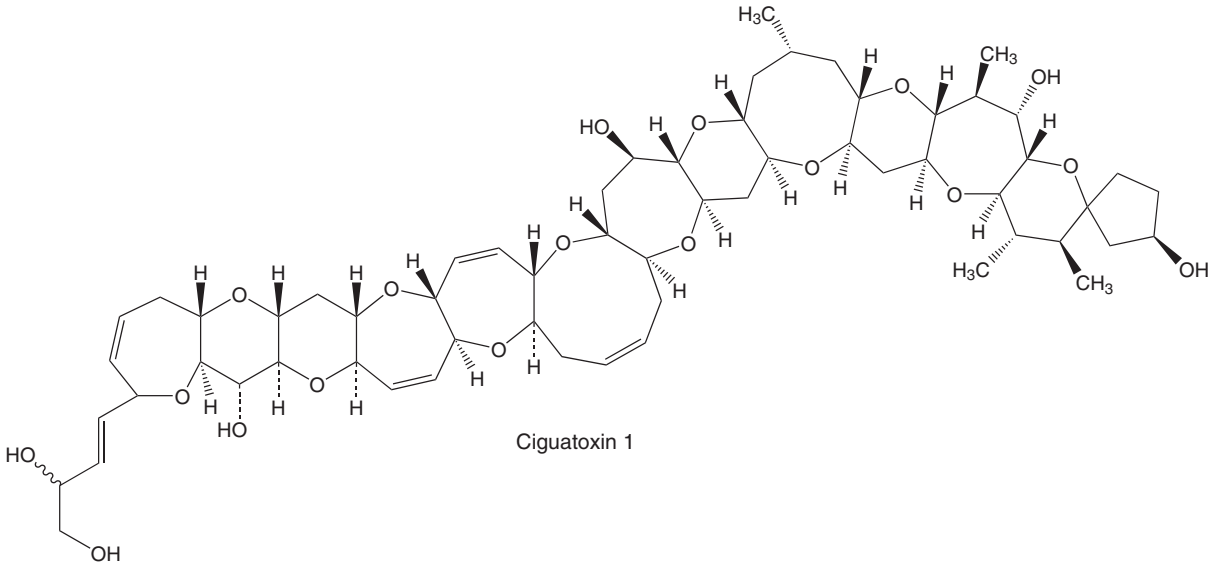
The two forms of brevetoxin-associated clinical effects first characterized in Florida are (1) an acute gastroenteritis with neurologic symptoms following ingestion of contaminated shellfish (aka neurotoxic shellfish poisoning (NSP)), and (2) an apparently reversible upper respiratory syndrome (conjunctival irritation, copious catarrhal exudates, rhinorrhea, nonproductive cough, and bronchoconstriction) following inhalation of contaminated aerosols. Recovery is reportedly complete in few days, although persons with chronic pulmonary disease such as asthma may experience more severe and prolonged respiratory effects. In addition, skin and eye irritation by environmental exposures among people living or visiting Florida during *K. brevis* bloom has been reported. NSP and the respiratory irritation associated with aerosolized brevetoxins have both been reported along the Gulf of Mexico as well as far north as North Carolina; similar brevetoxin-associated syndromes have been reported in New Zealand.



Brevetoxin A

Ciguatoxins

Ciguatoxins are produced by *Gambierdiscus toxicus*. Lipid-soluble ciguatoxins and brevetoxins have immunologic cross reactivity, and thus have similar epitopic sites and mechanisms of action, as described in the previous section.



The LD_{50} in mice for ciguatoxin CTX-1 is $0.25\,mg\,kg^{-1}$ body weight when injected intraperitoneally. Ciguatoxins are reported to induce developmental toxicity in Japanese medaka fish (*O. latipes)*.

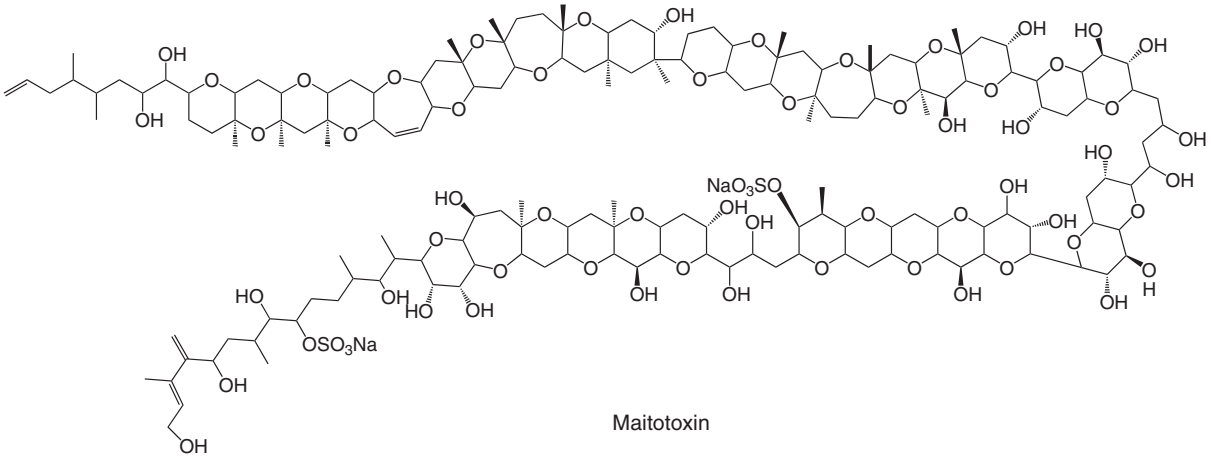
Ciguatoxin fish poisoning caused by ciguatoxins (and also by maitotoxins, see below) is the most commonly reported marine toxin disease in the world. It presents primarily as an acute neurologic disease manifested by a constellation of gastrointestinal (diarrhea, abdominal cramps, vomiting), and cardiovascular (arrhythmias, heart block) signs and symptoms within a few hours of contaminated fish ingestion, followed by neurologic (paresthesias, pain in the teeth, pain on urination, blurred vision, temperature reversal) within hours to days. Reportedly neurologic symptoms may precede the gastrointestinal symptoms in Pacific CFP. Acute fatality, usually due to respiratory failure, circulatory collapse or arrhythmias is reported. Lethality is usually seen with ingestion of the most toxic parts of fish (liver, viscera, roe). The minimal lethal dose for a person weighing 165 lbs is less than $1\,\mu g\,kg^{-1}$. Those surviving ciguatera intoxication, especially in the Caribbean, suffer for weeks to months with debilitating neurologic symptoms, including profound weakness, temperature sensation changes, pain, and numbness in the extremities. CFP outbreaks typically occur in a circumglobal belt extending approximately from latitude 35°N to 34°S, which includes Hawaii, the South Pacific including Australia, the Caribbean, and the Indo-Pacific, although the transport of contaminated fish and tourism have led to cases of CFP in both North American and Northern Europe.

Chronic ciguatera can present as a psychiatric disorder of general malaise, depression, headaches, muscular aches, and peculiar feelings in extremities for several weeks to months. This may be due to prolonged debilitating paresthesias ranging from extreme fatigue to pain in the joints and changes in temperature sensation that can last from weeks to months, and possibly to years. It is reported anecdotally that those with chronic symptoms seem to have recurrences of their symptoms with the ingestion of fish (regardless of type), ethanol, caffeine, and nuts up to 3–6 months from initial ingestion of ciguatera.

Maitotoxins

Maitotoxin precursors are also produced by *G. toxicus*, *Prorocentrum* spp., *Ostereopsis* spp., *Coolia monotis*, *Thecadinium* sp., and *Amphidinium carterae*.

In smooth muscle and skeletal muscle exposed *in vitro*, maitotoxins cause calcium ion-dependent contraction. Water-soluble maitotoxins increase the calcium ion influx through the excitable membrane. These toxins possess a specific calcium-dependent action, which causes a release of norepinephrine from rat pheochromocytoma cells. This action occurs in



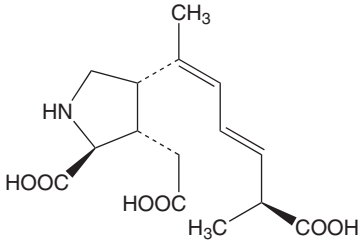
Maitotoxin

the absence of sodium ions and in the presence of tetrodotoxin, precluding the participation of sodium channels. Maitotoxin appears to exert its effects on endogenous membrane calcium channels.

Maitotoxin is lethal in mice at a dose of $0.15\,\mu g\,kg^{-1}$ body weight intraperitoneally.

Domoic Acid

Domoic acid (CAS 14277-97-5, $C_{15}H_{21}NO_6$) is produced by *Nitzchia pungens*. The toxin accumulates in the hepatopancreas of mussels, scallops, and other filter-feeding shellfish. Heat-stable neurotoxic domoic acid is similar in structure to the excitatory dicarboxylic amino acid, kainic acid, and has an antagonistic effect at the glutamate receptor. Domoic acid acts as a potent excitatory neurotransmitter, and it binds to excitatory amino acid receptors in the central nervous system in invertebrates.



Domoic acid

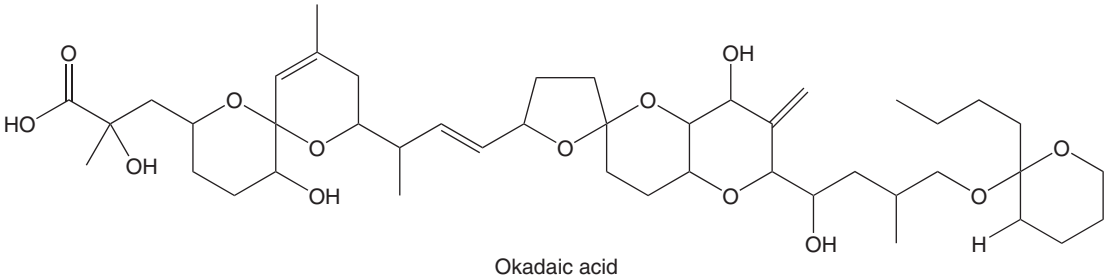
In 1998, domoic acid toxicity was reported in California sea lions. Predominantly neurological signs were observed, which included severe seizures that resulted in opisthotonus (spasm in which the head, neck and back are arched backward), then death. Domoic acid has also been implicated in the deaths of marine mammals and birds in the Pacific Northwest of the US coast. The mouse LD_{50} of domoic acid is $3.6\,mg\,kg^{-1}$ when injected intraperitoneally.

In humans, the acute symptoms of ASP caused by domoic acid include vomiting, abdominal cramps, diarrhea, severe headache, and loss of short-term memory. In some cases, confusion, memory loss, disorientation, and even coma are reported. In addition, seizures and myoclonus are observed acutely. Permanent neurologic sequelae, especially cognitive dysfunction, were reportedly most likely in persons who developed neurologic illness within 48 h, males, in older patients (>60 years), and in younger persons with pre-existing illnesses such as diabetes, chronic renal disease, and hypertension with a history of transient ischemic attacks. The first human cases of ASP were identified after an outbreak in Prince Edward Island, Canada; since then, there have been cases of ASP in marine mammals and birds in the Pacific Northwest of the United States and Canada.

Although no long-term follow-up has been done with ASP victims, the short-term memory loss associated with ASP appeared to be permanent.

Okadaic Acid

Okadaic acid (or okadeic acid, CAS 78111-17-8, $C_{44}H_{68}O_{13}$) is produced by dinoflagelates *Dinophysis* sp. and *Prorocentrum lima*. Lipophilic okadaic acid is a potent inhibitor of protein phosphorylase phosphatase-1 and -2A in the cytosol of the mammalian cells that dephosphorylates serine and threonine. Okadaic acid is thought to induce diarrhea by stimulating the phosphorylation of proteins that controls sodium secretion by intestinal cells, or by increasing phosphorylation of elements that regulate permeability to solutes, resulting in a passive loss of fluids. Okadaic acid also acts through the variation in cellular concentration of the calcium second messenger; it strongly increases the L-type inward calcium current in isolated guinea pig cardiac myocytes.



Okadaic acid

Diarrheic shellfish poisoning caused by okadaic acid is a gastrointestinal illness without chronic sequellae. There is no evidence of neurotoxicity and no fatal cases have ever been reported. Diarrhea is the most commonly reported symptom, closely followed by nausea and vomiting with onset 30 min to 12 h from ingestion of contaminated shellfish. Complete clinical recovery is seen even in severe cases within 3 days. DSP has been reported predominantly in Japan and Europe. The toxin has also been isolated from *P. lima* cultures from the Gulf of California, Mexico. The incidence of DSP in this location as a result of this is unknown.

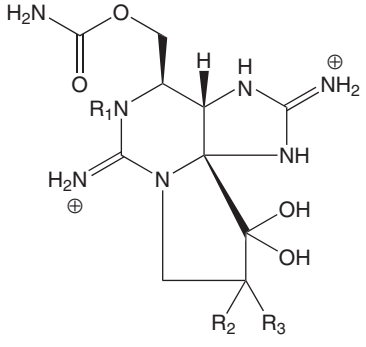
Okadaic acid is a promoter for skin tumors in mice. It also increases gonadal tumors in shellfish and is hepatoxic in other animals. In humans, okadaic acid induces apoptosis and has been linked to gastrointestinal cancer, both the evidence for this is not very strong.

Saxitoxins

Saxitoxins (see structure for Saxitoxin or SXT, CAS 35523-89-8, $C_{10}H_{17}N_7O_4$) are produced by *Alexandrium* spp. (*Gonyaulax*) and several of the cyanobacteria. Heat-stable neurotoxic saxitoxins have a relaxant action on vascular smooth muscle. They inhibit the temporary permeability of sodium ion influx by binding 1:1 with high affinity to a specific receptor site on the outside surface of the membrane in close proximity to the external orifice of the sodium channel. In fact, neurophysiologic studies using saxitoxin as a probe helped to show that sodium and potassium act independently with separate membrane channels. By preventing sodium ions from passing through the membranes of nerve cells, saxitoxins interfere with the transmission of signals along the nerves. A widespread blockage of the sodium ion channels prevents impulse generation in peripheral nerves and skeletal muscles. Saxitoxins have a direct effect on skeletal muscle by blocking the muscle action potential without depolarizing cells; they abolish peripheral nerve conduction with no curare-like action at the neuromuscular junction.

Typical neurologic effects induced by saxitoxins are nervousness, ataxia, convulsions, and paralysis. The paralysis of respiratory muscles can lead to the death of mice within a few minutes. The mouse LD_{50} is 3–9 $\mu g\,kg^{-1}$ intraperitoneally, and 263 $\mu g\,kg^{-1}$ orally. Death occurs within minutes as a result of respiratory failure.

Paralytic shellfish poisoning caused by saxitoxins presents with both gastrointestinal and neurologic symptoms. Five to 30 min after consumption of contaminated mollusks, there is a slight perioral tingling progressing to numbness that spreads to the face and neck. Other symptoms include headache, dizziness, nausea, vomiting, rapid pain, and anuria. In severe cases, there is the onset of severe



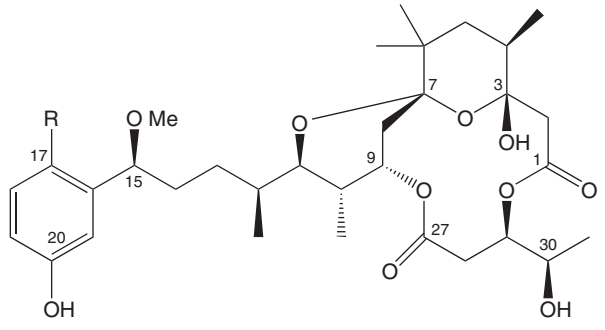
STX	R_1	R_2	R_3
STX	H	H	H
GTX-II	H	H	OSO_3^-
GTX-III	H	OSO_3^-	H
NeoSTX	OH	H	H
GTX-I	OH	H	OSO_3^-
GTX-IV	OH	OSO_3^-	H

Saxitoxin = STX, where R_1 = H, R_2 = H, R_3 = H

incoordination of the extremities and respiratory difficulty. In addition, there are medullary disturbances evidenced by difficulty swallowing, sense of throat constriction, speech incoherence, or complete loss of speech, as well as brain stem dysfunction. Access to emergency medical services in acute cases is crucial to prognosis. In very severe cases, within 2–12 h, there is complete paralysis and death from respiratory failure in the absence of ventilatory support. After 12 h, regardless of severity, victims start to recover gradually and are without any residual symptoms within a few days, although long-term studies of possible chronic effects have never been performed. The oral dose in humans for death is 1–4 mg (5000–20 000 mouse units) depending upon the age and physical condition of the patient. Historically, PSP has occurred in North America (the Pacific Northwest and the Northeast) and Europe. More recently, PSP has been reported in Japan, Malaysia, the Philippines, Indonesia, Latin America, and China.

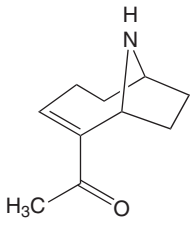
Cyanobacteria Toxins

Cyanobacteria toxins (sometimes referred to as blue green algal toxins) are represented in this entry by Aplysiatoxins, which are toxic to the skin, and anatoxin a (CAS 64285-06-9, $C_{10}H_{15}NO$) and anatoxin a (S) (very fast death factor), which are neurotoxins. Saxitoxin, discussed earlier, and neosaxitoxin are both neurotoxins that may also be classified as cyanobacterial toxins. A large variety of other toxins is produced by cyanobacteria, but is not as well documented. These include: lyngbyatoxin (dermatotoxic); cyclic peptides; predominantly microcystins, nodularins, and cylindrospermopsin (hepatotoxins); endotoxins; and other substances as yet undescribed, including additional tumor promoters.

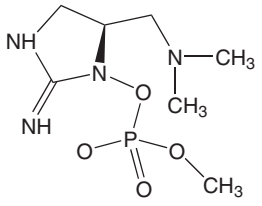


R = Br Aplysiatoxin
R = H Debromoaplysiatoxin

Aplysiatoxin a



Anatoxin a



Anatoxin a (S)

There are at least 12 different species of cyanobacteria that have been shown to produce toxins, often several different toxins per species. The main toxin-producing species include *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis raciborskii*, *Gloeotrichia*, *Hapalosiphon*, *Lyngbia*, *Nodularia*, *Nostoc*, *Oscillatoria*, *Schizothrix*, *Spirulina*, *Synechocystis*, and *Microcystis*.

Dermatotoxins associated with some cyanobacteria algal blooms are potent tumor promoters and protein kinase C activators. The neurotoxin, anatoxin a, acts like the neurotransmitter acetylcholine except that it cannot be degraded by acetylcholinesterase; anatoxin a (S) is a natural organophosphate, binding to the acetylcholinesterase enzymes. Hepatotoxins (e.g., microcystin, cylindrospermpsin and nodularins) are particularly toxic to the liver in part due to selective transport mechanisms that concentrate these toxins from the gut and blood into the liver cells; they damage the liver by deranging the cytoskeletal architecture of the hepatocytes. Microcystin is also believed to cause damage to cell DNA by the activation of endonucleases. Cylindrospermopsin is a protein synthesis inhibitor resulting in widespread necrosis of the tissues of many organs. Microcystins and nodularins are protein phosphatase inhibitors, besides being potent tumor promoters in animals (similar to the carcinogen, okadaic acid). Microcystins cause liver necrosis leading to death within hours to days.

Toxic blooms of cyanobacteria with associated animal poisonings have been reported in all continents except Antarctica. There have been frequent reports of thirsty domestic animals and wildlife consuming freshwater contaminated with toxic cyanobacterial

algal blooms, and dying within minutes to days from acute neurotoxicity and/or hepatotoxicity. Mammals and birds appear to be more susceptible to cyanobacterial algal toxins than aquatic invertebrates and fish, with some species variability. Experimentally, acute high-dose administration of microcystin can lead to death from hepatoencephalopathy within hours.

Prolonged morbidity and mortality have been reported in animals exposed to cyanobacterial algae in the wild. Chronic administration of sublethal amounts of *Microcystis* (a cyanobacterial algae which produces microcystin) extracts in drinking water to mice resulted in increased mortality with chronic active liver disease, even at fairly low doses and in relatively short time periods in the laboratory. Studies in mice have also shown that some cyanobacterial algal toxins cause precancerous damage to both the liver and the bowel. In the laboratory experimental animals, teratogenic activity has been demonstrated with oral administration of *Microcystis* extracts; ~10% of otherwise normal neonatal mice had small brains with extensive hippocampal neuronal damage.

There are individual case reports of persons exposed through swimming to cyanobacterial algal blooms with skin irritation and allergic reactions (both dermatologic and respiratory) with continued positive reaction on skin testing. In particular, urticaria (hives), blistering, and even deep desquamation of skin in sensitive areas like the lips and under swimsuits have been reported, especially with *Lyngbya majuscula* in tropical areas. Consumption of or swimming in cyanobacterial toxin-contaminated waters has also yielded increased case reports of gastrointestinal symptoms, especially diarrhea. One severe outbreak in Brazil was associated with lethality from hepatotoxicity in dialysis patients exposed to water contaminated with microcystins; another outbreak in Australia was also associated with lethality from hepatorenal syndrome in children and adults exposed to contaminated drinking water. In addition to gastrointestinal and dermatologic symptoms, eye irritation, asthma, and 'hay fever symptoms' have been reported repeatedly with exposure to contaminated recreational water exposure in the United States, Canada, UK, and Australia.

The chronic effects of exposure to small quantities of cyanobacterial algal toxins are still under study. In the mid-1980s, studies were done in China, where people were drinking untreated water contaminated with cyanobacterial algal toxins. It was found that drinking contaminated pond and ditch water was associated with high rates of liver cancer. When the quality of drinking water sources was improved in these areas, the rate of liver cancer decreased. How many cases of liver cancer can be attributed to cyanobacterial algal toxins in the United States (where drinking water is currently of higher quality) remains unknown.

Clinical Management

Very little clinical research has been conducted to determine effective treatments. Medical care is primarily supportive.

Medical treatment of CFP has been to a large extent symptomatic; a variety of agents, including vitamins, antihistamines, anticholinesterases, steroids, and tricyclic antidepressants, have been tried with limited results. If given within 3 days of exposure, i.v. mannitol (1 mg kg^{-1} given rapidly over 1 h) has been demonstrated in a single blinded control trial to resolve acute symptoms and prevent chronic symptoms, although repeated administrations may be necessary if symptoms return; a more recent clinical trial did not find an effect, however this trial included subjects treated long after the initial 3 day window. Gut emptying and decontamination with charcoal has been recommended, although often the severe ongoing vomiting and diarrhea prevents this. Atropine is indicated for bradycardia, and dopamine or calcium gluconate for shock. It is recommended that opiates and barbituarates be avoided since they may cause hypotension, and opiates may interact with maitotoxins. Amitriptyline (25–75 mg bid) and similar medications do seem to have some success in relieving the symptoms of chronic ciguatera such as fatigue and paresthesias. It is possible that nifedipine may be appropriate as a calcium channel blocker to counteract the effects of maitotoxins. Anecdotal food avoidance as mentioned above is also recommended. In addition, there is no immunity to these illnesses, and recurrences of actual ciguatera in the same individual appear to be worse than the initial illnesses. A rapid, accurate diagnosis and treatment of CFP within the first 72 h after exposure may be critical in preventing some of the neurologic symptoms that might otherwise become chronic and debilitating.

The treatment of DSP caused by okadaic acid is symptomatic and supportive. In general, hospitalization is not necessary; fluid and electrolytes can usually be replaced orally.

Supportive measures are the basis of treatment for PSP that is caused by saxitoxins, especially ventilatory support in severe cases. In animals, artificial respiration is the most effective treatment. Up to 75% of severely affected persons die within 12 h without supportive treatment. When the ingestion of contaminated food is recent, gut decontamination by the gastric lavage and administration of activated

charcoal or dilute bicarbonate solution is recommended. Care must be taken concerning aspiration with the neurologically compromised patient.

In general, the only treatment available for exposure to cyanobacterial algal toxins is supportive medical treatment after complete removal from exposure. If the exposure was oral, administration of activated carbon to decrease gut absorption may be efficacious if given within hours of exposure. Based on past outbreaks, monitoring of volume, electrolytes, liver and kidney function should all be considered in the case of acute gastroenteritis associated with some of the cyanobacterial algal toxins.

Exposure Standards and Guidelines

Global seafood safety standards have not been established. In the United States, US Food and Drug Administration (FDA) enacted the Hazard Analysis and Critical Control Points (HACCP) program of 1997. The FDA has established action levels in suspected seafood for the toxins causing some of the shellfish poisonings (see **Table 1**). When an action level is reached, the HACCP plan must be followed to prevent unsafe products from reaching the consumer.

Table 1 US FDA action levels in seafood for the toxins associated with shellfish poisonings

Shellfish poisoning	*US FDA action level*
NSP	20 mouse units $100\,g^{-1}$ shellfish brevetoxin-2 equivalent
ASP	20 ppm domoic acid; 30 ppm domoic acid in viscera of Dungeness crab (*Cancer magister*)
DSP	0.2 ppm okadaic acid plus 35-methyl okadaic acid (diphysistoxin-1)
PSP	0.8 ppm saxitoxin equivalent[a]

[a] The amount of total PSP toxins equivalent in toxicity to 0.8 ppm saxitoxin.

Source: Backer LC, Schurz-Rogers H, Fleming LE, Kirkpatrick B, and Benson J (2004) Toxins in food. In: Dabrowski W (ed.) *Phycotoxins in Marine Seafood,* Ch. 7. Boca Raton, FL: CRC Press.

See also: Ciguatoxin; Saxitoxin.

Further Reading

Backer LC, Fleming LE, Rowan A, and Baden D (2003) Epidemiology and public health of human illness associated with harmful marine phytoplankton. In: Hallegraeff GM, Anderson DM, and Cembella AD (eds.) *UNESCO Manual on Harmful Marine Algae*, pp. 725–750. Geneva, Switzerland: UNESCO/WHO.

Blythe DG, Hack E, Washington G, and Fleming LE (2001) The medical management of seafood poisoning. In: Hui YH, Kits D, and Stanfield PS (eds.) *Seafood and Environmental Toxins*, pp. 311–319. New York, NY: Dekker.

Fleming LE, Backer L, and Rowan A (2002) The epidemiology of human illnesses associated with harmful algal blooms. In: Baden D and Adams D (eds.) *Neurotoxicology Handbook*, pp. 363–381. Totowa, NJ: Humana Press Inc.

Kirkpatrick B, Fleming LE, Squicciarini D, *et al.* (2004) Literature review of Florida Red Tide: Implications for human health. *Harmful Algae* 3(2): 99–111.

Relevant Websites

http://www.mote.org – National Institution of Environmental Health Services. Red Tide Toxin, Health Effects and Exposure Study.

http://cbr-rbc.nrc-cnrc.gc.ca/issha/New_ISSHA/Images/index.htm – International Society for the Study of Harmful Algae.

http://www.rsmas.miami.edu – University of Miami. Marine and Fresh Water Biomedical Sciences Center.

http://www.whoi.edu – US Fish, Shellfish and Wildlife Affected by Toxic or Harmful Microalgal Species. Woods Hole Oceanographic Institution, The Harmful Algae Page.

Alkalies

Sanjay Chanda and Harihara M Mehendale

- Synonyms: Bases; Strong alkalies
- Chemical/Pharmaceutical/Other Class: Inorganic alkalies (e.g., sodium hydroxide, potassium hydroxide, and sodium hypochlorite)

Uses

Depends on the specific alkali. Alkalies are primarily used as cleaning agents, bleaches, and unslaked lime.

Exposure Routes and Pathways

Skin contact, ingestion, and inhalation are the most common exposure pathways.

Toxicokinetics

The toxicokinetics varies depending on the type of alkali.

Mechanism of Toxicity

Alkalies cause toxicity by liquefaction necrosis, meaning that the alkali destroys the cell membrane and cell integrity and thereby causes cell lysis.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity of alkalies in animals is the same as that in humans.

Human

Alkalies can burn skin, mucous membrane, and eyes almost immediately on contact. However, the absence of burns, irritation, erythema, or other such signs in the oral or circumoral area does not necessarily indicate that esophageal injury does not exist. Inhalation of the fumes may cause pulmonary edema or pneumonitis.

Chronic Toxicity (or Exposure)

Animal

The toxicity of alkalies in animals is the same as that in humans.

Human

Burns that at the time of injury appear to be mild can sometimes go on to cause opacification, vascularization, ulceration, or perforation.

Clinical Management

Exposure should be terminated as soon as possible by removing the victim to fresh air. The skin, eyes, and mouth should be washed with copious amounts of water. A 20–30 min wash may be necessary to neutralize and remove all residual traces of the contaminant. Contaminated clothing and jewelry should be removed and isolated. Treatment may require instillation of a local anesthetic to treat the blepharospasm (spasmodic winking from involuntary contraction of the orbicular muscle of the eyelids). Oral ingestion requires immediate dilution therapy with water or milk. Antidotes such as vinegar or lemon juice are absolutely contraindicated. Emesis should be avoided in case of ingestion.

Environmental Fate

In the case of a solid alkali spill on soil, groundwater pollution will occur if precipitation occurs prior to clean up. Precipitation will dissolve some of the solid and create an aqueous solution of that alkali which then would be able to infiltrate the soil. However, prediction of the concentration and properties of the solution produced would be difficult.

Exposure Standards and Guidelines

The guidelines given below are for sodium hydroxide (common alkali):

- Occupational Safety and Health Administration standards: permissible exposure limit: 8 h time-weighted average is $2\,mg\,m^{-3}$.
- Threshold limit values: ceiling limit is $2\,mg\,m^{-3}$.
- National Institute for Occupational Safety and Health recommendations: recommended exposure limit is $2\,mg\,m^{-3}$.

See also: Potassium; Sodium.

Further Reading

Klaassen CD, Amdur MO, and Doull J (eds.) (1995) Casarett and Doull's Toxicology. *The Basic Science of Poisons*, 5th edn. New York: McGraw-Hill.

NIOSH (1975) Criteria Document: Sodium Hydroxide. DHEW. NIOSH, pp. 76–105.

Alkyl Halides

Swarupa G Kulkarni and Harihara M Mehendale

- Representative Compounds: Methyl bromide; Methyl chloride; Methyl iodide; Dichloromethane; Tetrachloroethane; Carbon tetrachloride; Trichloroethene; Trichloroethylene; A number of fluorinated hydrocarbons (e.g., Freons)
- Synonyms: Halogenated hydrocarbons; Haloalkanes
- Chemical Formula: $R(X)_n$, where R is a hydrocarbon alkyl group and X is a halogen. One or more halogens may be present in one compound

Uses

Many halogenated hydrocarbons have important commercial applications. Alkyl halides are important intermediates in synthesis, as solvents in the laboratory and industry, and as dry cleaning fluids. They also find use as anesthetics and refrigerants. For example, trichloroethene is a common dry cleaning solvent. The fluorinated hydrocarbons (Freons) are used as refrigerants, industrial solvents, fire extinguishers, local anesthetics, and glass chillers, but mainly as propellants in aerosol products. Methyl bromide, methyl chloride, and methyl iodide are used as refrigerants in chemical synthesis and as fumigants. Methyl bromide is used with carbon tetrachloride in fire extinguishers. Methyl chloroform is used as a solvent for cleaning, degreasing, and in paint removers. Dichloromethane is used in paint removers and as an industrial solvent. Tetrachloroethane is used as a solvent in industry and occurs as a contaminant in other chlorinated hydrocarbons. It is occasionally present in household cleaners. Carbon tetrachloride is used as a solvent and intermediate in many industrial processes.

Exposure Routes and Pathways

Inhalation and dermal and ocular contact are common routes of exposure.

Toxicokinetics

Fluorocarbon compounds are lipid soluble and, thus, generally well absorbed through the lung. Absorption after ingestion is much lower than after inhalation. Most of the fluorinated hydrocarbons are immediately absorbed.

There is a significant accumulation of fluorocarbons in the brain, liver, and lungs compared to blood levels, signifying a tissue distribution of fluorocarbons similar to that of chloroform. Fluorocarbons are concentrated in body fat where they are slowly released into blood at a concentration that should not cause any risk of cardiac sensitization.

Fluorocarbons are excreted by the lungs and the parent compound is eliminated in about 15 min.

Acute and Short-Term Toxicity (or Exposure)

Animal

Deliberate ocular exposure in rabbits to liquid Freon 12 produced effects related to the duration of exposure. Severe corneal damage with opacity occurred following exposure for 30 s. In dogs, inhalation of fluorinated hydrocarbon vapors causes bradycardia followed by deterioration to ventricular fibrillation in some animals.

Human

Freons are very toxic when inhaled in high concentrations and/or for extended periods. Inhalation of fluorinated hydrocarbons such as those caused by leaking air conditioners or refrigerators usually results in transient eye, nose, and throat irritation. Palpitations and lightheadedness are also seen. Headache was a common complaint, reported in 71% of 31 workers exposed to bromotrifluoromethane in one incident. Inhalation of halides at sufficient concentrations associated with deliberate abuse, or spills or industrial use occurring in poorly ventilated areas, has been associated with ventricular arrhythmias, pulmonary edema, and sudden death. Fluorinated hydrocarbons are believed to cause arrhythmias by sensitizing the myocardium to endogenous catecholamines. Freon solvents are degreasers. Dermal contact with fluorinated hydrocarbons may result in defatting, irritation, or contact dermatitis. Severe frostbite was reported as a rare effect of severe Freon exposure. Mucosal necrosis and perforation of the stomach developed in one patient after ingesting a small amount of trichlorofluoromethane. Fluorocarbons containing bromine are more toxic than the corresponding chlorine compounds. There is a significant interpatient variation following exposure to fluorocarbons and it is difficult to predict symptoms following exposure. Compounds like dibromochloropropane, in which occupational exposure has affected male fertility, have now been removed from the market. Following acute exposure to methyl bromide, chloride, or iodide, nausea and vomiting, blurred vision, vertigo, weakness or paralysis, oliguria or anuria, drowsiness, confusion, hyperactivity, coma, convulsions, and pulmonary edema are noted. Pulmonary edema and bronchial pneumonia are most often the cause of death. Skin contact causes irritation and vesiculation.

Methyl chloroform and dichloromethane are central nervous system (CNS) depressants. Methyl chloroform sensitizes the myocardium to catecholamine-induced arrhythmias. Following exposure to tetrachloroethane, irritation of the eyes and nose, followed by headache and nausea, is observed. Cyanosis and CNS depression progressing to coma may appear after 1–4 h.

Chronic Toxicity (or Exposure)

Animal

Some of the chlorinated hydrocarbon solvents, such as methylene chloride and chloroform, have caused

cancer in several species of experimental animals and are suspect human carcinogens.

Human

A syndrome of impaired psychomotor speed, impaired memory, and learning, has been described in workers with chronic occupational exposure to fluorinated hydrocarbons. Skin irritation and defatting dermatitis upon prolonged or repeated contact with the skin to trichloromonofluoromethane have been reported. An excess of CNS symptoms was seen in a group of workers chronically exposed to trichloromonofluromethane. Repeated exposure to methyl bromide, methyl chloride, and methyl iodide will cause blurring of vision, numbness of the extremities, confusion, hallucinations, somnolence, fainting attacks, and bronchospasm. Chronic toxicity has not been reported with dichloromethane. Headache, tremor, dizziness, peripheral paresthesia, and anesthesia have been reported after chronic inhalation or skin exposure to tetrachlorethane. National Institute of Occupational Safety and Health (NIOSH) recommends that methyl chloride, methyl bromide, and methyl iodide be considered as potential occupational carcinogens and that methyl chloride be considered a potential occupational teratogen.

Clinical Management

This management is intended for use in the absence of a specific treatment protocol for a product or a chemical. Symptomatic and supportive care is the primary therapy. The general approach to a poisoned patient is to first assess the vital signs of the patient followed by assessing the route of administration for potential toxicity. Measures to prevent further absorption of the compound may be useful. Victims of inhalation exposure should be moved from the toxic environment and administered 100% humidified supplemental oxygen with assisted ventilation as required. Exposed individuals should have a careful and thorough medical examination performed to look for abnormalities. Patients with fluorohydrocarbon poisoning should not be given epinephrine or similar drugs because of the tendency of fluorohydrocarbon to induce cardiac arrhythmia, including ventricular fibrillation. Monitoring including complete blood count, urine analysis, and liver and kidney function tests is suggested for patients with significant exposure. Activated charcoal or gastric lavage may be indicated to prevent further absorption. Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persist after 15 min of irrigation, an ophthalmologic examination should be performed.

Miscellaneous

Alkyl halides are practically insoluble in water. They are miscible in all proportions with liquid hydrocarbons and are, in general, good solvents for many organic substances. Most of the common organic halides are liquids. Like alkanes, halogen compounds are insoluble in and inert to cold concentrated sulfuric acid. In a series of alkyl halides, the boiling point rises with an increase in molecular weight due to the presence of either a heavier halogen atom or a larger alkyl group. Bromides boil at temperatures distinctly higher than the corresponding chlorides, and iodides are higher boiling than the bromides. Increase in the halogen content decreases their flammability. In contact with an open flame or a very hot surface fluorocarbons may decompose into highly irritant and toxic gases such as chlorine, hydrogen fluoride, or chloride and even phosgene. Alkyl halides can be prepared by addition of the halogen or hydrogen halides to alkenes, as well as by substitution of a halogen for hydrogen in an alkane. The most important method of preparing alkyl halides is by reaction between an alcohol and a hydrogen halide.

See also: Carbon Tetrachloride; Catecholamines; Chloroform; Freons; Methyl Bromide; Methylene Chloride.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Alkyl Halides.

Allergenicity Testing *See* Toxicity Testing, Sensitization.

Allyl Alcohol

Sharmilee P Sawant and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 107-18-6
- Synonyms: 2-Propenol; 2-Propen-1-ol; Vinyl carbinol
- Chemical Structure: $CH_2 = CHCH_2OH$

Uses

Allyl alcohol is used as an industrial solvent, herbicide, and fungicide.

Background Information

Allyl alcohol is a clear liquid boiling at 96°C. It is highly toxic and hazardous to the environment. It requires special attention to handling procedures. It is synthesized by the hydrolysis of allyl chloride or isomerization of propylene oxide. It is used as a starting material in making various polymers, pharmaceuticals, pesticides, and other allyl compounds.

Exposure Routes and Pathways

The substance can be absorbed into the body by inhalation of its vapor, dermal contact, and by ingestion.

Toxicokinetics

Allyl alcohol is metabolized via two alternative oxidative pathways leading to the formation of acrolein or the epoxide 'glycidol'. The epoxide may then be converted to glycerol by epoxide hydrolase. The conversion of allyl alcohol to acrolein is mediated by alcohol dehydrogenase (ADH), which may then be further oxidized to acrylic acid by NAD- or NADP-dependent enzymes in the liver cytosol or microsomes or to glycidaldehyde by a microsomal enzyme with subsequent conversion to glyceraldehyde by epoxide hydrolase. Alternatively, acrolein may react directly both enzymatically and nonenzymatically to form stable adducts with glutathione or other low molecular weight thiol compounds prior to excretion in the urine as mercapturate. Both glycidol and glycidaldehyde are substrates for lung and liver cytosolic glutathione-*S*-transferases.

Mechanism of Toxicity

Allyl alcohol is inactive *per se* and its toxic effect is mediated by its ADH oxidation to form acrolein, which is responsible for the hepatotoxic action. The toxicity of the alcohol (or its metabolite acrolein) is dependent on the concentration of glutathione (GSH). After severe depletion of GSH, the reactive metabolite of allyl alcohol can bind to essential sulfhydryl groups in the cellular macromolecules, leading to structural and functional changes in cellular molecules, which can be responsible for cell death. In this case, the appearance of lipid peroxidation could be merely the consequence of cell death.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure to allyl alcohol causes liver and kidney damage. Allyl alcohol is classified as a periportal hepatotoxicant since it selectively damages the periportal region of the liver. Studies have shown that in adult rats, allyl alcohol produces a moderate to marked periportal necrosis with attendant inflammation, hemorrhage, and also decreases hepatic cytochrome P-450, benzphentamine *N*-demethylation, and ethoxyresorufin *O*-deethylation activities by ~30%. In immature rats, it lowered both cytochrome P-450 activity (30%) and ethoxyresorufin *O*-deethylation (75%). Benzphetamine *N*-demethylation was not significantly affected in immature rats. Intraperitoneal administration of 1.5 mmol kg^{-1} of allyl alcohol to starved Swiss albino mice causes the development of hemolysis in ~50% of the animals. Other toxic effects include renal necrosis, pulmonary edema, and central nervous system effects at higher dose levels.

Human

The most important adverse effects of occupational exposure to allyl alcohol are upper respiratory tract irritation and burning of the eyes. The substance may cause effects on the muscles, resulting in local spasm and aching. The appearance of these effects may be delayed after exposure.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to allyl alcohol can cause liver and kidney damage.

Human

Long-term exposure may lead to liver or kidney damage.

Clinical Management

Exposure should be terminated as soon as possible by removal of the patient to fresh air. Skin, eyes, and mouth should be washed with copious amounts of water. Contaminated clothings should be removed. A mild soap solution may be used for washing the skin, but should not be placed into the eye. Dilution with water may be effective if small amounts are swallowed until additional medical attention is available.

Ecotoxicology

The substance is very toxic to aquatic organisms.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration (OSHA) general industry permissible exposure limit: 2 ppm, 5 mg m^{-3} time-weighted average (TWA) (skin).

The National Institute for Occupational Health and Safety (NIOSH) recommended exposure limit: 2 ppm TWA, 4 ppm short-term exposure limit (skin).

See also: Acrolein; Allyl Formate; Liver.

Further Reading

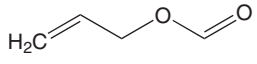
Atzori L, Dore M, and Congiu L (1989) Aspects of allyl alcohol toxicity. *Drug Metabolism and Drug Interaction* 7: 295–319.

Jaeschke H, Kleinwaechter C, and Wendel A (1987) The role of acrolein in allyl alcohol-induced lipid peroxidation and liver cell damage in mice. *Biochemical Pharmacology* 36: 51–70.

Allyl Formate

Sharmilee P Sawant and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 1838-59-1
- Synonyms: Formic acid; 2-Propenyl ester; Formic acid; Allyl ester; Allyl alcohol; Formate
- Chemical Structure:



Uses

It is used as a solvent in spray lacquers, enamels, varnishes, and latex paints and as an ingredient in paint thinners and strippers, varnish removers, and herbicides. It is also used in liquid soaps, cosmetics, industrial and household cleaners, and dry-cleaning compounds.

Exposure Routes and Pathways

The substance can be absorbed into the body by inhalation and dermal contact and by ingestion.

Toxicokinetics

Allyl formate is rapidly cleaved *in vivo* by nonspecific esterases to allyl alcohol. Allyl alcohol is metabolized via two alternative oxidative pathways leading to the formation of acrolein or the epoxide 'glycidol'. The epoxide may then be converted to glycerol by epoxide hydrolase. The conversion of allyl alcohol to acrolein is mediated by alcohol dehydrogenase, which may then be further oxidized to acrylic acid by NAD- or NADP-dependent enzymes in the liver cytosol or microsomes or to glycidaldehyde by a microsomal enzyme with subsequent conversion to glyceraldehyde by epoxide hydrolase. Alternatively, acrolein may react directly both enzymatically and nonenzymatically to form stable adducts with glutathione or other low molecular weight thiol compounds prior to excretion in the urine as mercapturate.

Mechanism of Toxicity

Allyl formate is cleaved by nonspecific esterases to allyl alcohol, which is then oxidized by alcohol dehydrogenases to the reactive acrolein, which is responsible for the hepatotoxic action. The toxicity of allyl alcohol via its metabolite acrolein is dependent on the concentration of glutathione (GSH). After depletion of GSH, the reactive metabolite of allyl alcohol can bind to essential sulfhydryl groups in the cellular macromolecules, leading to structural and functional modifications that can be responsible for hepatic injury. Appearance of lipid peroxidation signals events that follow toxication mechanisms initiated by acrolein, and subsequent and continued lipid peroxidation could be merely the consequence of cell death.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure to allyl formate causes liver and kidney damage. Allyl formate is classified as a periportal hepatotoxicant since it selectively damages the periportal region of the liver in rodents.

Human

The most important adverse effect of occupational exposure to allyl formate is upper respiratory tract irritation.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to allyl formate can cause liver and kidney damage.

Human

Long-term exposure may lead to liver or kidney damage.

Clinical Management

Exposure should be terminated as soon as possible by removal of the patient to fresh air. Skin, eyes, and mouth should be washed with copious amounts of water. Contaminated clothing should be removed. A mild soap solution may be used for washing the skin, but should not be placed into the eye. Dilution with water may be effective if small amounts are swallowed before medical attention is sought.

Ecotoxicology

The substance is very toxic to aquatic organisms.

See also: Acrolein; Allyl Alcohol.

Further Reading

Droy BF, Davis ME, and Hinton DE (1989) Mechanism of allyl formate-induced hepatotoxicity in rainbow trout. *Toxicology and Applied Pharmacology* 98: 313–324.

Rees KR and Tarlow MJ (1967) The hepatotoxic action of allyl formate. *Biochemistry Journal* 104: 757–761.

Aluminum

Abbi Heilig

- Chemical Abstracts Service Registry Number: CAS 7429-90-5
- Synonyms: Aluminum; Molten; Metana; Aluminum powder; Pyrophoric
- Chemical/Pharmaceutical/Other Class: Metals

Uses

Aluminum can be used in several different ways, either alone or compounded, and in a variety of forms, including powder. Aluminum is frequently used in food packaging, and also in utensils and electrical conductors. Aluminum compounds are widely used in industry, in the form of alums in water treatment and alumina in abrasives and furnace linings. However, aluminum is used alone very rarely since it is such a soft metal. It is often combined with other metals to create a stronger, more durable metal. These combinations are called aluminum alloys. Aluminum alloys are used extensively in aircraft. Aluminum and aluminum salts can also be found in many consumer products such as antiperspirants, food additives, antacids, astringents, and buffered aspirins. Powdered aluminum is used to make explosives and fireworks.

Background Information

Although aluminum was one of the last metals to be commercialized, it has been recognized for centuries. Aluminum was first recognized by the Romans as an astringent substance, and they called it 'alum'. By the Middle Ages it was manufactured as 'alum stone', a subsulfate of alumina and potash. In 1825, Hans C Oersted was able to isolate a few drops of the raw material, and then by 1886 it had patents from both Charles Martin Hall of the United States and Paul-Louis-Toussaint Heroult of France. Aluminum was commercialized in industry by the end of the nineteenth century.

Exposure Routes and Pathways

Aluminum is the most abundant metal, and the third most abundant element in the earth's crust. Human exposure to this metal is common and unavoidable. However, intake is relatively low because aluminum

is highly insoluble in many of its naturally occurring forms. Humans are always exposed to some form of aluminum by eating food, drinking water, ingestion of aluminum containing medicinal products, or just breathing air. The average human intake is estimated to be 30–50 mg day^{-1}. This intake comes primarily from foods, drinking water, and pharmaceuticals. Food additives can contain aluminum; due to certain additives, processed cheese and cornbread are two major contributors to high aluminum exposures in the American diet. Some common over-the-counter medications such as antacids and buffered aspirin contain aluminum, and this can increase the daily intake significantly.

There has been concern about the exposures resulting from leaching of aluminum from cookware and beverage cans; however, aluminum beverage cans are usually coated with a polymer to minimize such leaching. Leaching from aluminum cookware becomes potentially significant only when cooking highly basic or acidic foods, for example, in one study, tomato sauce cooked in aluminum pans was found to accumulate 3–6 mg aluminum per 100 g serving.

Aluminum is absorbed from the soil by many plants that humans consume. The amount that a person would inhale depends on where they reside, and aluminum levels are much higher in industrial and urban areas. Another route of exposure is through skin contact with soil, water, and with aluminum metal.

Toxicokinetics

Less than 1% of that taken into the body orally is absorbed from the gastrointestinal tract. Aluminum can increase the absorption of other chemicals such as fluoride, calcium, iron, and phosphates. Most of the aluminum absorbed into the body will eventually end up in the bones or lungs. Aluminum that is not absorbed by the bones or lungs is excreted by the kidneys.

Mechanism of Toxicity

Aluminum binds diatomic phosphates and possibly depletes phosphate, which can lead to osteomalacia. High aluminum serum values and high aluminum concentration in the bone interfere with the function of vitamin D. The incorporation of aluminum in the bone may interfere with deposition of calcium; the subsequent increase of calcium in the blood may inhibit release of parathyroid hormones by the parathyroid gland. The mechanism by which aluminum concentrates in the brain is not known; it may interfere with the blood brain barrier.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acutely, aluminum itself has minimal systemic toxicity. Overall, animals become weaker and less active due to exposure.

Human

Aluminum has not been shown to alter the immune system in humans exposed by the oral or inhalation routes. Skin sensitization may occur.

Chronic Toxicity (or Exposure)

Animal

Cats and rabbits are aluminum sensitive, and have showed neurotoxic effects from aluminum. There is no evidence that aluminum exposure will affect reproduction.

Toxicity of aluminum in animals differs from humans because animals are much more sensitive to high exposures. Monkeys on a low calcium, high aluminum diet showed neurological disease similar to those of amyotrophic lateral sclerosis and Parkinsonism. Rats and hamsters showed signs of lung damage after breathing large amounts of aluminum dust. Death often occurred after the inhalation of air highly concentrated with the chemical.

Human

Fibrosis of the lung may occur through inhalation of aluminum dust particles. Aluminum has been associated with encephalopathy, bone disease, and anemia related to dialysis. It has also been thought that aluminum may be a cofactor in the etiopathogenesis of some neurodegenerative diseases, including Alzheimer's disease (AD). Direct evidence, however, cannot link the two together. Aluminum toxicity has been well recognized in patients with renal failure. Also, an increased concentration of aluminum in infant formulas and in solutions for home parenteral nutrition has been associated with neurological consequences and metabolic bone loss.

Clinical Management

Aluminum overload has very few treatment options. Besides awareness of consumption and environment, the chelating agent, deferoxamine, is used for treatment.

Ecotoxicology

Aluminum occurs naturally in soil, water, and air. It is redistributed or moved by natural and human activities. High levels in the environment can be caused by the mining and processing of its ores and by the production of aluminum metal, alloys, and compounds. Small amounts of aluminum are released into the environment from coal-fired power plants and incinerators. Virtually all food, water, and air contain some aluminum, which nature is well adapted to handle.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA) in the United States have the following airborne exposure limits:

- *ACGIH threshold limit value*: Aluminum oxide: $10\,mg\,m^{-3}$ (time-weighted average, TWA) inhalable (total) particulate matter containing no asbestos and $<1\%$ crystalline silica, A4. Soluble salts as Al: $2\,mg\,m^{-3}$ (TWA).
- *OSHA permissible exposure limit*: Alpha alumina (aluminum oxide): $15\,mg\,m^{-3}$ total dust, $5\,mg\,m^{-3}$ respirable fraction. Aluminum as metal: $15\,mg\,m^{-3}$ total dust, $5\,mg\,m^{-3}$ respirable fraction.

See also: Metallothionein; Metals.

Further Reading

Becaria A, Campbell A, and Bondy SC (2002) Aluminum as a toxicant. *Toxicology and Industrial Health* 18(7): 309–320.

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, 5th edn., vol. 2, pp. 354–406. New York: Wiley.

Relevant Websites

http://www.inchem.org – Aluminum (Environmental Health Criteria).

http://www.aluminum.org – The (US) Aluminum Association website.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Aluminum.

Aluminum Phosphide

Christopher H Day

- Chemical Abstracts Service Registry Number: CAS 20859-73-8
- Synonyms: Al-Phos; Aluminum monophosphide; Aluminum phosphide; Celphide; Celphine; Celphos; Delicia; Delicia gastoxin; Detia; Fumitoxin; Phostoxin; Quickphos; Weevilcide
- Chemical/Pharmaceutical/Other Class: Phosphide fumigant
- Chemical Formula: AlP
- Chemical Structure: Al : P

Uses

The primary use for aluminum phosphide is as a fumigant to control insects and rodents in both nonfood and food crops in indoor environments. It is also used in the control of rodents outdoors via application to their burrows or in grain storage areas. Aluminum phosphide is formulated in solid form only, and is available for use as a tablet, pellet, or dust, and in porous bags or blister packs.

Exposure Routes and Pathways

Aluminum phosphide is usually formulated as dark gray or dark yellow crystals that have an odor similar to decaying fish or garlic. Exposure can occur to aluminum phosphide via the oral route, but because it is a solid material, dermal absorption of aluminum phosphide is unlikely. Aluminum phosphide is highly reactive with water, such that any contact with moisture will result in decomposition to phosphine gas. Phosphine gas is colorless, flammable, and explosive at room temperature. Therefore, the primary exposure route is via inhalation and absorption by the lungs. Exposure is also possible through the ingestion of commodities, such as grains and nut meats, treated with aluminum phosphide; these foods may contain residues of phosphine gas. Residues of phosphine gas in treated commodities are expected to be <0.004 ppm (limit of detection in several studies) following aeration.

Toxicokinetics

Phosphine gas is rapidly absorbed through the lungs following inhalation. Following ingestion of aluminum phosphide, phosphine gas is generated which is

then readily transferred to the bloodstream. The wide array of target organs that are affected following exposure, suggests that phosphine is effectively distributed throughout the body. However, there is a paucity of information in the literature with respect to the metabolism and elimination of phosphine. There is some evidence that unexpired phosphine may be metabolized to phosphates, hypophosphite, and phosphite.

Mechanism of Toxicity

Phosphine is known to disrupt protein synthesis and enzymatic activity, particularly in lung and heart cell mitochondria. This can lead to a blockage of the mitochondrial electron transport chain. Phosphine may cause denaturing of various enzymes involved in cellular respiration and metabolism, and may be responsible for denaturing of the oxyhemoglobin molecule.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure to elevated levels of phosphine gas by animals can result in lethargy, shallow breathing, immobility, agitation, ataxia, convulsions, seizures, and death. The 4 h inhalation LC_{50} for phosphine gas in rats has been reported at 15 mg m^{-3} (11 ppm), although a more recent study in which no mortality was noted in male and female rats exposed to a one-time 6 h exposure level of 15 mg m^{-3} suggests that the 4 h inhalation LC_{50} for rodents may exceed 15 mg m^{-3}.

Human

Acute oral exposure in humans has resulted in pulmonary edema, cardiovascular electrocardiographic abnormalities, tachycardia, hypotension, and transient atrial fibrillation following inadvertent or voluntary ingestion. These effects are likely caused by the formation and subsequent toxicity of phosphine gas liberated in the stomach following contact with water. Other adverse effects that have been noted in humans following accidental or suicide-related intake of aluminum phosphide include gastrointestinal effects such as abdominal pain and vomiting, hepatic effects such as hyperemia, hepatic dysfunction, renal effects such as profound proteinuria, anuria, renal failure, and neurological effects such as restlessness and loss of consciousness. As discussed previously, these adverse effects are likely the result of the toxicity of phosphine gas, which is produced from the decomposition of aluminum phosphide in the presence of water.

Chronic Toxicity (or Exposure)

Animal

In chronic studies with rats, exposure to aluminum phosphide fumigated chow (4.5 mg m^{-3} phosphine) resulted in decreases in food intake, body weight, hemoglobin, red blood cells, hematocrit, and in increases in platelet counts. Following a 4 week recovery period in many of the exposed rats symptoms were absent, suggesting apparent reversibility. Neither aluminum phosphide nor phosphine gas exhibit carcinogenic, reproductive, or developmental effects in animals.

Human

There is evidence that long-term phosphine exposure by individuals involved in the application of pesticides resulted in chromosome damage. Chronic exposure to very low levels of phosphine may result in altered motor, visual, and speech skills. Neither aluminum phosphide nor phosphine gas exhibit carcinogenicity in humans, or result in reproductive or developmental effects. Although definitive evidence is lacking, it is assumed that phospine is an *in vivo* inhibitor of oxidative phosphorylation.

In Vitro Toxicity Data

Phosphine has been reported as negative for induction of reverse gene mutations up to cytotoxic doses in the Ames assay (*Salmonella typhimurium*). Increased chromosomal aberrations were reported in Chinese hamster ovary (CHO) cells exposed to 2500 and 5000 ppm of phosphine without activation with the S9 fraction. Chromosomal aberrations in CHOs were also reported in cells tested with S9 activation at 2500 ppm, but not 5000 ppm.

Clinical Management

If the victim has ingested aluminum phosphide, emesis should not be induced. Phosphine gas will be produced in the stomach when aluminum phosphide contacts the resident gastric fluids. A slurry of activated charcoal may be administered at 1 g charcoal per kg body weight. Any victim who has ingested aluminum phosphide should be immediately transported to a medical facility for treatment and monitoring. Rescuers need to be aware of any solid phosphide contamination on the victim's clothing, skin, or hair which will produce phosphine following contact with water, as well as any vomitus which could off-gas phosphine.

Environmental Fate

As discussed previously, once exposed to water, even high ambient humidity, aluminum phosphide will generate phosphine gas. Therefore, atmospheric dissipation is expected to be the primary fate process for phosphine. In addition to phosphine being generated from the reaction of aluminum phosphide with water, the other reaction product is aluminum hydroxide, a common constituent of clay. If the liberated phosphine (PH_3) burns it will produce phosphorus pentoxide (P_2O_5), which when exposed to water will form orthophosphoric acid (H_3PO_4).

Ecotoxicology

Very limited ecotoxicological data are available on the effects of phosphine, while no data were found for the effects of aluminum phosphide on wildlife. One study reported that turkeys and chickens exposed to phosphine gas at concentrations of 211 and 224 mg m^{-3} for 74 and 59 min, respectively, exhibited dyspnea, organ swelling, convulsions, and death. These types of effects are unlikely in the unconfined atmospheric conditions that most birds and wildlife are exposed to in nature. However, if misapplied or disposed of incorrectly, phosphine gas liberated from the decomposition of aluminum phosphide could represent a significant hazard to nontarget wildlife exposed to the gas in burrows or other confined spaces.

Under most circumstances, exposure to aquatic organisms would be unlikely due to the limited use pattern of aluminum phosphide in terrestrial environments. Two studies of aquatic toxicity are available in the literature. Both studies are acute tests with fish, the snakehead catfish and the rainbow trout. The reported values of LC_{50} are 0.10 and 0.0041 mg l^{-1} for the snakehead catfish and rainbow trout, respectively. These results indicate that phosphine is highly toxic to these fish species.

Although there are no data for other bird or fish species, it is possible that other members of these taxa may be similarly sensitive to the effects of phosphine due to an anticipated similar mode of action.

No chronic ecotoxicity data could be located for aluminum phosphide or phosphine in the available literature.

Other Hazards

Any individual previously exposed to aluminum phosphide should check with their doctor before taking vitamins that contain phosphorus supplements.

Table 1 Summary of exposure standards and guidelines for phosphine

Agency	*Standards and guidelines (ppm)*	*Averaging time*
US EPA	RfC (0.0002)	24 h a day for a lifetime
OSHA	PEL (0.3)	8 h a day over working lifetime
ACGIH	TLV – TWA (0.3)	8 h a day over working lifetime
ACGIH	ERPG-2 (0.5)	1 h
NIOSH	IDLH (50)	NA

US EPA, United States Environmental Protection Agency; OSHA, Occupational Safety and Health Administration; NIOSH, National Institute for Occupational Safety and Health; ACGIH, American Conference of Governmental Industrial Hygienists; RfC, reference concentration; PEL, permissible exposure limit; TLV, threshold limit value; TWA, time-weighted average; ERPG-2, Emergency Response Planning Guideline; IDLH, immediately dangerous to life or health; NA, not applicable.

Exposure Standards and Guidelines

Several agencies have established exposure standards or guidelines for aluminum phosphide as phosphine (summarized in **Table 1**). Generally, a standard or guideline represents the concentration that if met, will prevent an adverse effect from occurring at low exposure doses, and will therefore necessarily prevent the occurrence of more serious effects that are known to occur at higher doses. The chronic reference concentration (RfC) of 0.0002 ppm (0.0003 mg m^{-3}) for phosphine was set to prevent decreases in body weight in the general population over a lifetime of exposure. The American Conference of Governmental Industrial Hygienists threshold limit value of 0.3 ppm was set to prevent irritation and adverse effects to the central nervous system and gastrointestinal tract in workers exposed 8 h day^{-1} throughout their working lifetime.

See also: Phosphine.

Relevant Websites

http://www.epa.gov – US Environmental Protection Agency (2004) Integrated Risk Information System (IRIS) Files: Aluminum Phosphide and Phosphine.

http://www.inchem.org – International Programme on Chemical Safety (IPCS) (1988) *Phosphine and Selected Metal Phosphides*. Environmental Health Criteria 73.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Aluminum Phosphide.

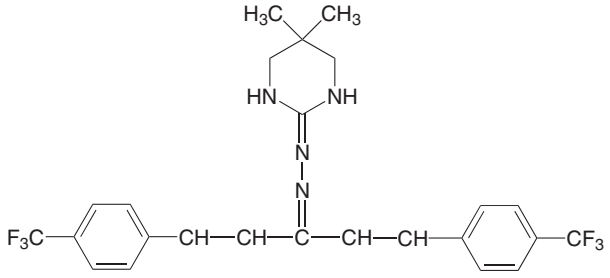
Amanitin, α- *See* Mushrooms, Cyclopeptide.

Amdro

Jamaluddin Shaikh

This article is a revision of the previous print edition article by Sushmita M Chanda, volume 1, pp. 49–50, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 67485-29-4
- Synonyms: Combat; Maxforce; Pyramdron; Aminohydrazone; Hydramethylnon
- Chemical/Pharmaceutical/Other Class: Trifluoromethyl amidinohydrazone
- Chemical Structure:



Uses

Amdro is used as an insecticide mainly for ants and cockroaches.

Exposure Routes and Pathways

Ingestion is the primary route of exposure. Dermal exposure is also possible.

Toxicokinetics

Amdro is poorly absorbed by the oral route but is well absorbed through the skin. Amdro is poorly metabolized in the body with more than 95% being excreted in the feces in the unchanged form. Elimination in rats dosed orally with amdro is 72% of the dose in 24 h and 92% of the dose by 9 days.

Mechanism of Toxicity

Amdro is a slowly activating stomach poison. The exact mechanism of toxicity is unclear.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in male and female rats was $817\,mg\,kg^{-1}$ and the acute dermal LD_{50} was $1502\,mg\,kg^{-1}$. In rabbits the LD_{50} was $>2000\,mg\,kg^{-1}$ and the acute (4 h) inhalation LC_{50} in rats was $2.9\,mg\,l^{-1}$. Amdro is not a dermal irritant or a skin sensitizer but is a mild eye irritant.

Human

Children who ingest small amounts of amdro have symptoms of diarrhea. A case study showed that one-half pound of amdro ingested by an adult diabetic patient produced no specific symptoms except diarrhea.

Chronic Toxicity (or Exposure)

Animal

A 6-month feeding study in dogs reported increased incidence of soft stools, mucoid stools, and diarrhea at a lowest-observed-effect level of $3.0\,mg\,kg^{-1}\,day^{-1}$. Based on an increase in lung adenomas and lung adenomas/carcinomas in female mice, amdro has been classified as a possible human carcinogen by the Environmental Protection Agency. Amdro is not a neurodevelopmental toxicant and is not teratogenic in either rats or rabbits. Amdro affects male reproductive function. Testicular atrophy was reported in an 18-month mouse feeding study, a two-generation reproduction study in rats, and a 91-day oral dosing study in dogs.. In a two-generation rat reproduction study, there was no evidence of systemic toxicity, nor was there any evidence of direct toxicity in the offspring. The reproductive no-observed-effect level ($1.66\,mg\,kg^{-1}\,day^{-1}$ for males) was based on histopathology in the testes. At $5.05\,mg\,kg^{-1}\,day^{-1}$, male reproductive performance (lower pregnancy,

reduced gestation weight gain, smaller litters) was decreased.

Human

Little is known regarding chronic effects of amdro in humans.

In Vitro Toxicity Data

Amdro was negative in *Salmonella typhimurium/Escherichia coli* reverse gene mutation assays, *Schizosaccharomyces pombe* P1 forward gene mutation assay, *in vitro* Chinese hamster ovary chromosome aberration, and *Saccharomyces cerevisiae* D4 mitotic gene conversion assay.

Clinical Management

The exposed area should be thoroughly washed with soap and water. If pain or irritation continues, a physician should be consulted. Eyes should be washed with copious amounts of room-temperature water for 15 min in cases of eye contamination. If irritation, pain, swelling, lacrimation, or photophobia persists after 15 min of irrigation, medical attention is necessary. Emesis is necessary only when large amounts (greater than 28 g of bait per kilogram) are ingested. In such cases, ipecac may be used for inducing emesis. Activated charcoal slurry with or without saline cathartic or sorbitol may also be administered. Basic respiratory and cardiovascular function support should be utilized.

Environmental Fate

Amdro is rapidly degraded in the environment by photolysis and more slowly by hydrolysis. The approximate half-life is 1 h in direct sunlight.

Ecotoxicology

Amdro is practically nontoxic to birds. The oral LD_{50} values in mallard duck and bobwhite quail are 2510 and 1825 mg kg^{-1}, respectively. Amdro is moderately to highly toxic to fish. The LC_{50} (4-day) values for amdro in rainbow trout, channel fish, and bluegill sunfish are 160, 100, and 1700 μg l^{-1}, respectively. Amdro is relatively nontoxic to honey bees.

Exposure Standards and Guidelines

The reference dose for amdro is 0.01 mg kg^{-1} day^{-1}.

See also: Pesticides.

Relevant Websites

http://pmep.cce.cornell.edu – Cornell University.
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Ames Test

Robin C Guy

This article is a revision of the previous print edition article by Ann D Mitchell, volume 1, pp. 56–57, © 1998, Elsevier Inc.

The Ames test is a testing system which employs a collection of genetically modified strains of bacteria in *in vitro* system to ascertain a chemical's potential to cause genetic mutations. During the mid-1960s, the test was developed by Bruce Ames of the University of California at Berkeley as a quick and inexpensive way to detect possible carcinogens and mutagens. The testing system methodology has evolved over the years and has been used extensively in regulatory and research roles, currently being the most widely performed *in vitro* test for mutagenesis. The Ames test is a part of a comprehensive battery of *in vitro* and *in vivo* studies and epidemiologic surveys employed to define the frequency or extent of genetic mutation that can be caused by a chemical and thus its potential to cause cancer, inheritable mutations or some types of degenerative diseases. The Ames test is rapid, sensitive, inexpensive, and relatively easy to conduct in a microbiology laboratory and can be used on a wide variety of materials, including volatile compounds.

Scientific Basis

The Ames test allows one to test the ability of a substance to interfere with DNA, which has the information necessary for expression of specific proteins. This information is encoded by the sequence of base pairs in the DNA molecule, with triplets of base pairs (mRNA codons) encoding for a specific amino acid in the sequence of a protein. Ames' system focuses on the fact that mutations in oncogenes and tumor suppressor genes of somatic cells can be involved in tumor formation.

Ames developed strains of bacteria that had carefully selected lethal mutations. In a test system the bacteria could survive only when its mutation had been corrected by experiencing another mutation caused by the tested material. This correction could be accomplished by causing a 'point mutation' or 'frameshift mutations'. Point mutations are base-pair substitutions, that is, a base change in DNA of at least one DNA base pair. In a reverse mutation test, this change in base pairs may occur at the site of the original mutation, or at a secondary site in the bacterial genome. Frameshift mutations are the addition or deletion of one or more base pairs in the DNA. Since amino acids are encoded by triplets of base pairs in sequence, any addition or deletion of 1 or 2 base pairs will dramatically alter the expressed protein from that point on. The Ames system employs strains of *Salmonella typhimurium* and *Escherichia coli* that require amino acids (histidine or tryptophan, respectively) to detect such reverse point and frameshift mutations. The reverse mutation allows the *S. typhimurium* or *E. coli* strains to restore the functional capability of the bacteria to be able to synthesize the specific amino acid on their own, independent of amino acid content in the medium.

Test Methodology

Testing Tools: Microbial Strains

Cultures of carefully established and karyotyped cell lines and cell strains have been developed for the Ames system. Understanding the molecular details of a strain's mutation, its sensitivity and the mechanism of repair of that mutation provides the basis to detect a mutagen and understand its mechanism of activity at the molecular level. Reliable results from the test require use of carefully developed testing strains, a disciplined testing protocol, and unbiased application of interpretative rules to the testing results. Controversy over use of the Ames test and interpretations of results from this and other microbial testing systems has sometimes resulted from use of poorly understood strains, poor testing protocols, or biased interpretative logic.

The most commonly used tester strains are *S. typhimurium* TA1535, TA1537 (or TA97 or 97a), TA98, and TA100 and either TA102 or *E. Coli* WP_2 *uvrA*. The latter two are used to detect oxidizing mutagens, cross-linking agents, and hydrazines. Other strains may be used as long as there are historical data that will support the findings. The Ames assay is considered a reverse mutation assay, as the tester strains originally used all had mutations that altered their survival in growth media: a 'reverse' mutation could enable growth by reversing the effects of the original 'forward' mutation. The *S. typhimurium* strains all have a mutation on their histidine operon as follows:

- TA1535 (hisG46),
- TA1537 (hisC3076),
- TA98 (hisD3052), and
- TA100 (hisG46).

The histidine mutation prevents the *S. typhimurium* strains from synthesizing histidine, and, therefore, prevents the growth of the cell in histidine-deficient medium. All of the *S. typhimurium* strains have additional mutations of the rfa and uvrB genes. The rfa gene has a loss of one of the enzymes responsible for the synthesis of part of the lipopolysaccharide layer of the cell wall, which increases the cell's permeability to certain chemical classes. The uvrB gene contains a deletion that causes a deficit in the DNA excision-repair system, which causes increased sensitivity to certain chemicals. Strains TA98 and TA100 also have an R-factor, in this case the pKM101 plasmid, which further causes increased sensitivity to certain chemicals. TA1537 and TA98 are reverted back to their original histidine-independent state by frameshift mutations. TA1535 is reverted back by base-pair mutations, while TA100 is reverted back by both frameshift and base-pair mutations. The tryptophan mutation prevents the *E. coli* WP_2 uvrA strain from synthesizing tryptophan. A revertant can occur by a base-pair change.

Testing Strategies

As the Ames test is an *in vitro* test, testing protocol has been developed to address the issues of metabolic changes to the test substance (activation), calibration of the response (positive and negative controls), and toxicity to the testing organism. These are critical elements of the test and can greatly influence the test results and subsequent conclusions drawn from the study. As such, it is important to give attention to the testing strategies and the documentation of study results from within a testing laboratory and between testing laboratories over time.

Activation Systems to Account for Metabolic Activity Many mutagens are activated by biotransformation pathways in the body. Therefore, testing of such chemicals in an *in vitro* system must include some metabolic activation system to mimic the biotransformation systems of *in vivo* testing. The inclusion of such a metabolic activating system, usually an exogenous source of metabolic enzymes, in the assay allows evaluation of mutagenic potential of both direct-acting mutagens and those requiring

metabolic activation. Therefore, a typical assay determines the mutagenic potential of a chemical in the absence and presence of the metabolic activating system.

The most common activating system is the S9 enzymes method, a liver preparation (supernatant from 9000*g* centrifugation) from rodents treated with enzyme-inducing agents such as Aroclor 1254. The testing is conducted with and without the enzyme preparation, each with its own control conditions, as described below. Under both conditions, a negative (solvent) and an appropriate positive control (known mutagen) should be tested concurrently.

Control Procedures The testing methodology must be calibrated to assess the degree of response of the test substance in the context of the testing methodology parameters. Substances which are known not to be active in the testing system are employed as a sentinel for false positive results. These negative controls should not elicit a positive response from the testing system being conducted simultaneously for the test substances. Likewise, positive controls are simultaneously tested and should provide a predictable positive response.

Negative (solvent) and positive controls must be utilized for a valid study. An historical database must be maintained for these results. Positive control concentrations must be documented, as different solvents and concentrations are required for different strains and metabolic activation conditions. Examples of positive control substances include:

- Absence of exogenous metabolic activation:
  - sodium azide (CAS 26628-22-8) for TAl535 and TAl00;
  - 2-nitrofluorene (CAS 607-57-8) for TA98, TA1538;
  - 9-aminoacridine or ICR 191 (CAS 90-45-9, 17070-45-0) for TA1537, TA97, and TA97a;
  - mitomycin C (CAS 50-07-7) for TA102 and WP_2 *uvrA*; and
  - 4-nitroquinoline (CAS 56-57-5) for WP_2 *uvrA*.
- Presence of exogenous metabolic activation:
  - 2-aminoanthracene (CAS 613-13-8);
  - cyclophosphamide (monohydrate) (CAS 50-18-0, 6055-19-2);
  - 9,10-dimethylanthracene (CAS #781-43-1);
  - 7,12-dimethylbenzanthracene (CAS #57-97-6); and
  - benzo(a)pyrene (CAS #50-32-8).

A third control is the 'vehicle control'. It tests the potency of the materials present in the testing cocktail in addition to the testing material which is the object of study. The test material may be solubilized in solvents such as sterile water, dimethylsulfoxide, or ethanol. Vehicle controls are tested using only the solubilizing material subjected to exactly the same protocols as those samples with the test material.

Cytotoxicity The microbes may not survive in the presence of the testing material due to nongenetic cell toxicity. If the cell does not survive and undergo cell division, it cannot express any reverse mutation, even if the test material was indeed a mutagen. In considering the meaning of the lack of response in the test system, one must consider the influence of cytotoxicity. Lack of response could mean a lack of mutagenic potency or the presence of cellular toxicity. To test for cytotoxicity, a dose range-finding study is conducted using suspensions of bacterial cells exposed to approximately five concentrations of the test material in the presence and in the absence of an exogenous metabolic activation system. Cytotoxicity is usually determined after 24–48 h of incubation. If no toxicity was observed, concentrations of up to 50 µl or 5000 µg per plate should be used. Triplicate plates per concentration per strain, with and without metabolic activation, are standard. Negative and positive controls are used as appropriate.

Methodology and Protocols (Overview) The Ames test consists of a preliminary dose range-finding phase and the final mutagenicity phase. For the dose range-finding phase each strain, with and without S9, is plated onto a single plate with nine to ten concentrations of test material.

The two most popular methods are the plate incorporation method and the preincubation method. In the plate incorporation method, tester cell suspensions are mixed with an overlay (top) agar and plated immediately onto minimal medium (bottom agar). In the preincubation method, the cell suspension mixture is incubated and then mixed with a top agar before plating onto minimal medium. For both techniques, after 2 or 3 days of incubation, normal sized, revertant colonies are counted and compared to the number of spontaneous revertant colonies on solvent control plates. The Environmental Protection Agency recommends some materials be tested using the preincubation method, namely, classes that include short-chain aliphatic nitrosamines, divalent metals, aldehydes, azo dyes and diazo compounds, pyrollizidine alkyloids, alkyl compounds and nitro compounds. Certain modifications of the methods need to be incorporated for specific types of test articles.

Interpretation and Evaluation

To ensure that the results of an assay are valid, specific criteria for interpretation of the results have evolved with experience with the tests over time among many laboratories. Both positive and negative (solvent) control values should reasonably be within the normal historical data for the laboratory. Tester strains must be identified and have a characteristic number of spontaneous revertants per plate for the vehicle controls. A minimum of three noncytotoxic concentrations needs to be evaluated.

Once the data are available for analyses, evaluation of the results follows. There are several criteria for determining a positive result, such as a concentration-related increase over the range tested and/or a reproducible increase at one or more concentrations in the number of revertant colonies per plate in at least one strain, with or without metabolic activation system. The concentration-related increase would be three times the mean concurrent vehicle control value for strains TA1535, TA1537, and TA1538 and two times the mean vehicle control value for TA98, TA100, and WP_2 uvrA. Biological relevance of the results should be considered first. Results achieved at levels of excessive cytotoxicity (50% or more) are discarded.

A negative result is when the above response criteria are not met, and the test material is thereby considered not mutagenic.

Many compounds that are positive in the Ames test are mammalian carcinogens and a huge database exists establishing that correlation. However, there is not an exact correlation between the chemical's positive response in the Ames test and carcinogenicity. Even with the addition of a metabolic activation system, this prokaryotic system cannot replicate a mammalian cell *in vivo*. Correlation may be dependent on chemical class. Care should be taken to avoid conditions that would lead to results not reflecting authentic mutagenicity. As in mammalian *in vitro* systems, positive results that do not reflect authentic mutagenicity may arise from a variety of possible changes, including pH, osmolality (very high concentrations of test article), or high levels of cytotoxicity.

The Ames test is a sensitive predictor of mutagenicity in mammals, but it should not be used in a vacuum. The Ames test should be used as part of a battery of *in vitro* and *in vivo* tests for predicting the genetic toxicity potential of test materials.

The Ames test is recommended by the International Conference on Harmonisation Guidelines as part of a standard genetic toxicology battery. The other assays include the mouse lymphoma and micronucleus tests. This bacterial mutation test may not be appropriate for the evaluation of certain classes of chemicals, for example highly bactericidal compounds (e.g., certain antibiotics), any compounds that may interfere with cell division or replication, and possibly some peptides. In such cases, mammalian mutation tests may be more appropriate.

See also: Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Genetic Toxicology; Good Laboratory Practices (GLP); International Conference on Harmonisation; Micronucleus Assay; Mitomycin C; Mouse Lymphoma Assay; Redbook; Toxicity Testing, Mutagenicity.

Further Reading

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Gatehouse D, Haworth S, Cebula T, *et al.* (1994) Recommendations for the performance of bacterial mutation assays. *Mutation Research* 312: 217–233.

Kier LD, Brusick DJ, Auletta AE, *et al.* (1986) The Salmonella typhimurium/mammalian microsomal assay: A report of the US Environmental Protection Agency Gene-Tox Program. *Mutation Research* 168: 69–240.

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Relevant Websites

http://www.epa.gov – US Environmental Protection Agency. The website includes Harmonized/870 Health Effects Test Guidelines.

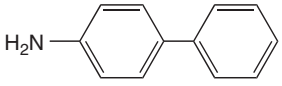
http://www.fda.gov – US Food and Drug Administration.

http://www.bruceames.com – Website of Bruce Ames.

Aminobiphenyl, 4-

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 92-67-1
- Synonyms: 4-ADP; 4-biphenylamine; 4-Aminodiphenyl; Aminobiphenyl; *p*-Phenylaniline; *p*-Xenylamine; Xenylamine
- Chemical/Pharmaceutical/Other Class: Aromatic amine
- Chemical Formula: $C_{12}H_{11}N$
- Chemical Structure:



Uses

4-Aminobiphenyl is used in research as a cancer-causing agent.

Background Information

Production and use of 4-aminobiphenyl has become very limited because of its known carcinogenic effects; however, 4-aminobiphenyl is also found in tobacco smoke.

Exposure Routes and Pathways

Because 4-aminobiphenyl is found in tobacco smoke, one of the major routes of exposure for the general population is the passive and active inhalation of tobacco smoke. Laboratory personnel working with 4-aminobiphenyl without adequate personal protection may also be exposed occupationally by the dermal or inhalation route.

Toxicokinetics

4-Aminobiphenyl is converted to its active metabolite, *N*-hydroxy-4-aminobiphenyl, in the liver and bladder. In the liver, 4-aminobiphenyl is subjected to *N*-hydroxylation and *N*-glucuronidation to produce *N*-glucuronide-4-aminobiphenyl. This metabolite accumulates in the urine in the bladder where, under acidic pH conditions, it is hydrolyzed to its active metabolite. 4-Aminobiphenyl can also be activated directly in the bladder mucosa.

Mechanism of Toxicity

4-Aminobiphenyl is one of a number of chemicals that cause methemoglobinemia, or conversion of hemoglobin to methemoglobin, which reduces the ability of the blood to carry oxygen to tissues. In addition, the active metabolite (see above) is believed to produce cancer through its reaction with cellular DNA. In animal studies, the observed incidence of 4-aminobiphenyl adducts with bladder epithelium DNA correlated well with the observed bladder tumor incidence.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} has been reported to be $500\,mg\,kg^{-1}$ in rats and $25\,mg\,kg^{-1}$ in dogs. The main target organ of toxicity is the bladder.

Human

Acute overexposure is known to produce methemoglobinemia and urinary tract damage. Signs and symptoms of overexposure include a bluish tint of the skin and mucous membranes as well as a burning sensation in the urinary tract and bloody urine.

Chronic Toxicity (or Exposure)

Animal

Chronic administration has produced tumors in bladder, mammary gland, gastrointestinal tract, and liver of exposed animals.

Human

Chronic occupational exposure has been shown to produce bladder damage and cancer. Signs and symptoms of bladder damage may include painful urination and the presence of blood and pus in the urine. The incidence of bladder tumors in workers occupationally exposed to 4-aminobiphenyl was reported to range from 11% to 17% of the exposed population.

Environmental Fate

4-Aminobiphenyl may be released into the environment during its production and use as a rubber antioxidant and dye intermediate; however, sources suggest that it was no longer in significant production since the early 1970s. It is easily oxidizable and probably also undergoes photolysis but there is little actual data on these processes. If released on land it will adsorb moderately to soil, probably binding to

humic materials and undergoing redox reactions. In water it will adsorb to sediment, and probably undergo photolysis and oxidation. Oxidation by alkoxy radicals, which are photochemically produced in eutrophic waters, has an estimated half-life of 14 days. 4-Aminobiphenyl is biodegradable and biodegradation may well occur in both soil and water but there are no rates available for soil or natural waters. It has a low potential for bioconcentration. In the atmosphere, degradation should occur due to direct photolysis, oxidation by ambient oxygen, and also photochemically produced hydroxyl radicals (estimated half-life 6.9 h in the vapor phase).

Other Hazards

Flammability is low to moderate when exposed to heat, flames (sparks), or powerful oxidizers. 4-Aminobiphenyl autoignites only at 842°F. When strongly heated, 4-aminobiphenyl emits toxic fumes.

Exposure Standards and Guidelines

The primary exposures to this compound are occupational. There is sufficient epidemiological and animal toxicological data to classify 4-aminobiphenyl as a human carcinogen. Therefore, special precautions must be taken when working with 4-aminobiphenyl. Personnel handling 4-aminobiphenyl must follow industrial hygiene and health protection requirements for handling potentially carcinogenic substances. At a minimum 4-aminobiphenyl exposures should be minimized through the use of engineering controls, work practices, and personal protective equipment, including impervious and disposable gowns and gloves as well as eye and respiratory protection. In addition, working areas and working instruments must be specially designed for handling potentially harmful substances. Although ambient air exposures are currently unlikely except for accidental releases, this compound is listed as a hazardous air pollutant by the Clean Air Act.

See also: Carcinogenesis; Clean Air Act (CAA), US; Tobacco Smoke.

Further Reading

Klaassen CD (2001) *Casarett & Doull's Toxicology, The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.

Sax NI and Lewis RJ (1989) *Dangerous Properties of Industrial Materials*, 7th edn. New York: Van Nostrand Reinhold.

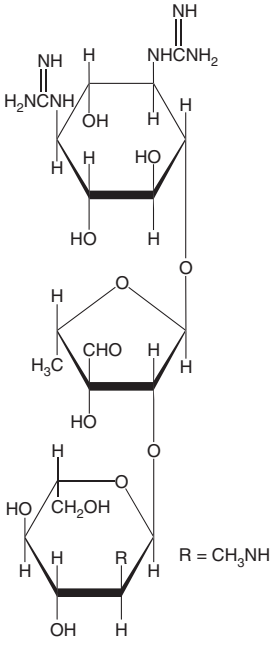
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for 4-Aminobiphenyl.

Aminoglycosides

Abraham Dalu

- Representative Compounds: Amikacin; Gentamicin; Kanamycin; Neomycin; Netilmicin; Paromomycin; Streptomycin; Tobramycin
- Chemical Abstracts Service Registry Number: CAS 57-92-1 (streptomycin)
- Chemical/Pharmaceutical/Other Class: Antimicrobial agents. These drugs contain amino sugars in glycoside linkage
- Chemical Formula: $C_{21}H_{39}N_7O_{12}$ (streptomycin)
- Chemical Structure: Aminoglycosides are antimicrobial agents with dissimilar structures; it is impossible to represent aminoglycosides with a single general structure. The following is the structure of streptomycin:



Uses

Aminoglycosides are a group of potent antibiotics primarily used to treat certain infections caused by aerobic, Gram-negative bacteria. They are used in the treatment of severe infections of the abdomen and urinary tract, complicated skin, bone, or soft tissue infection, severe pelvic inflammatory disease, bacteremia (bacteria in the blood), ocular infections (topical), inflammation of ear (topical), neonatal sepsis, and endocarditis. In general, gentamicin, tobramycin, and amikacin are used in similar circumstances, often interchangeably. Of these, gentamicin is the aminoglycoside used most often because of its low cost and reliable activity against Gram-negative aerobes. Tobramycin may be the aminoglycoside of choice for use against *Pseudomonas aeruginosa* and *Enterobacter* species because of its greater *in vitro* activity. Amikacin is used against bacteria that are resistant to other aminoglycosides, since its chemical structure makes it less susceptible to inactivating enzymes. Aminoglycosides are also effective against mycobacteria, the bacteria responsible for tuberculosis.

Aminoglycosides are ineffective against anaerobic bacteria (bacteria that cannot grow in the presence of oxygen), viruses, and fungi. Only one aminoglycoside, paromomycin, is used against parasitic infection. In addition, some of the aminoglycosides have been widely used for preparation of the bowel for surgery and as adjunct to the therapy of hepatic coma. Aminoglycosides are also used to enhance bactericidal activity of betalactam drugs for the treatment of serious infections.

Background Information

Aminoglycosides are hydrophilic sugars that possess amino and hydroxyl functionalities. They are polycationic species at physiological pH, meaning they bind to negatively charged molecules such as DNA and RNA. Since their introduction into clinical use and despite the advent of newer agents (carbapenems, monobactams, and fluoroquinolones), aminoglycoside antibiotics continue to play an important role in the treatment of severe infections, particularly those due to aerobic, Gram-negative bacilli. Several factors account for their durability and continued clinical usefulness: therapeutic efficacy, synergy with the β-lactam antibiotics, low rate of development of true resistance, and low drug cost. Their main drawback has been the occurrence of (reversible) nephrotoxicity and ototoxicity in a significant number of patients (5–25%).

The first aminoglycoside, streptomycin, was isolated from *Streptomyces griseus* in 1943; neomycin was isolated from *Streptomyces fradiae*. This antibiotic was very effective against tuberculosis. However, one of the main drawbacks to streptomycin is its toxicity, especially to cells in the inner and middle ear and the kidney. Furthermore, some strains of tuberculosis are resistant to treatment with streptomycin. Therefore, medical researchers have put considerable effort into identifying other antibiotics with streptomycin's efficacy, but without its toxicity. Gentamicin, isolated from Micromonospora in 1963, was a breakthrough in the treatment of Gram-negative bacillary infections, including those caused by *Pseudomonas aeruginosa*. Other aminoglycosides were subsequently developed, including amikacin (Amikin), netilmicin (Netromycin), and tobramycin (Nebcin), which are all currently available for systemic use in the United States.

These bacteria can be identified by their reaction to Gram's stain. In Gram's staining, a film of material containing the possible bacteria is placed on a glass slide and dried. The slide is stained with crystal violet for 1 min, cleaned off with water, and then placed into a solution of Gram's iodine solution for 1 min. The iodine solution is rinsed off and the slide is immersed in 95% ethyl alcohol. The slide is then stained again with reddish carbolfuchsin or safranine for 30 s, rinsed in water, dried, and examined. Gram-positive bacteria retain the violet purple stain. Gram-negative bacteria accept the red stain.

Exposure Routes and Pathways

Ingestion is the most common route for both accidental and intentional exposures to aminoglycosides. Dermal route of exposure is also possible, especially with some of the chronic topical applications of aminoglycosides such as 1% neomycin.

Toxicokinetics

Aminoglycosides are poorly absorbed from the gastrointestinal or respiratory tract. The extent of absorption varies with a specific agent, ranging from as low as 0.2% to as high as 9%. Protein binding of aminoglycoside is from as low as 0–3% to as high as 11% depending on the agents. The volume of distribution for aminoglycosides ranges from 0.16 to $0.34\,l\,kg^{-1}$. Greater than 90% of aminoglycosides are excreted unchanged through the kidney. After parenteral administration, aminoglycosides are primarily distributed within the extracellular fluid. Thus, the presence of disease states or iatrogenic situations that alter fluid balance may necessitate dosage

modifications. When used parenterally, adequate drug concentrations are typically found in bone, synovial fluid, and peritoneal fluid. Penetration of biologic membranes is poor because of the drug's polar structure, and intracellular concentrations are usually low, with the exception of the proximal renal tubule. Endotracheal administration results in higher bronchial levels compared with systemic administration, but differences in clinical outcome have not been consistent.

Following parenteral administration of an aminoglycoside, subtherapeutic concentrations are usually found in the cerebrospinal fluid, vitreous fluid, prostate, and brain. Aminoglycosides are rapidly excreted by glomerular filtration, resulting in a plasma half-life of therapeutic doses ranging from 1.5 to 3.2 h in a patient with 'normal' renal function to 30–60 h in patients with impaired kidney function. The half-life of aminoglycosides in the renal cortex is $\sim$100 h, so repetitive dosing may result in renal accumulation and toxicity.

Mechanism of Toxicity

The mechanism of toxicity for aminoglycosides has not been fully explained and is therefore unclear. It is known that the drug attaches to a bacterial cell wall and is drawn into the cell via channels made up of a protein, porin. Once inside the cell, the aminoglycoside attaches to the 30S bacterial ribosomes. Ribosomes are the intracellular structures responsible for manufacturing proteins. This attachment either inhibits protein biosynthesis or causes the cell to produce abnormal, ineffective proteins. The bacterial cell cannot survive with this impediment. This explanation, however, does not account for the potent bactericidal properties of these agents, since other antibiotics that inhibit the synthesis of proteins (such as tetracycline) are not bactericidal. Recent experimental studies show that the initial site of action is the outer bacterial membrane. The cationic antibiotic molecules create fissures in the outer cell membrane, resulting in leakage of intracellular contents and enhanced antibiotic uptake. This rapid action at the outer membrane probably accounts for most of the bactericidal activity.

Energy is needed for aminoglycoside uptake into the bacterial cell. Anaerobes have less energy available for this uptake, so aminoglycosides are less active against anaerobic bacteria (bacteria that cannot grow in the presence of oxygen), viruses, and fungi. And only one aminoglycoside, paromomycin, is used against parasitic infection. Like all other antibiotics, aminoglycosides are not effective against influenza, the common cold, or other viral infections.

Acute and Short-Term Toxicity (or Exposure)

Animal

Several investigators have assessed the toxic effects of high doses of aminoglycosides in animals. Studies with dogs, rabbits, rats, and guinea pigs treated with doses ranging from 7.5 to 120 $\mathrm{mg\,kg^{-1}\,day^{-1}}$ in single and divided doses for 10–29 days suggest that less frequent glycoside administration is associated with less nephrotoxicity as assessed by serum creatinine levels, the glomerular filtration rate, and histopathology. A single study in rats assessing ototoxicity based on cochlear histology reported a lack of toxicity regardless of administration frequency.

Human

Because the body does not metabolize aminoglycosides, aminoglycoside activity is unchanged by induction or inhibition of metabolic enzymes, such as those in the cytochrome P450 system. Certain medications may increase the risk of renal toxicity with aminoglycoside use (e.g., use of diuretics, radiographic contrast exposure effective circulating volume depletion, ACE inhibitors, nonsteroidal anti-inflammatory drugs, other nephrotoxic medications, concomitant use of amphotericin, and cisplatin).

All aminoglycosides have a downside: they can cause ototoxicity. In the case of systemic gentamicin, ototoxicity appears to be primarily related to the duration of treatment, especially when the treatment course exceeds 10–14 days. It is also important to realize that gentamicin-induced ototoxicity tends to be primarily vestibular, although cochleotoxicity is seen as well. Overdoses may result in renal damage or ototoxicity (deafness and vertigo), and rarely neuromuscular blockade and hypersensitivity reactions depending on the dose and duration. Nephrotoxicity receives the most attention, perhaps because of easier documentation of reduced renal function, but it is usually reversible. Nephrotoxicity results from renal cortical accumulation resulting in tubular cell degeneration and sloughing. Examination of urine sediment may reveal dark-brown, fine, or granulated casts consistent with acute tubular necrosis but not specific for aminoglycoside renal toxicity. Although serum creatinine levels are frequently monitored during aminoglycoside use, an elevation of serum creatinine is more likely to reflect glomerular damage rather than tubular damage. In most clinical trials of aminoglycosides, however, nephrotoxicity has been defined by an elevation of serum creatinine. Periodic monitoring of serum creatinine concentrations may alert the clinician to renal toxicity. Retinopathy, visual loss, and

conjunctival necrosis have been also associated with this class of antibacterial agent. Irreversible damage of the auditory and vestibular functions of the eighth cranial nerve can occur but this is thought to be related to dose and duration of treatment.

Chronic Toxicity (or Exposure)

Human

Chronic topical application of 1% neomycin to a large wound precipitated severe hearing loss in an adult within 3 weeks following application. Serious toxicity is a major limitation to the usefulness of the aminoglycosides, and all drugs in this class share the same spectrum of toxicity.

Clinical Management

With overdose of aminoglycosides, the first effort is mobilized in supporting respiratory and cardiovascular functions. For oral ingestion, treatment focuses on preventing absorption with emesis and/or activated charcoal if appropriate. Since overdoses of aminoglycosides are also associated with renal damage, maintaining urine output with intravenous fluids is recommended if necessary.

See also: Sensory Organs.

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Aminopyridine, 4-

David Roane

- Chemical Abstracts Service Registry Number: CAS 504-24-5
- Synonyms: 4-AP; 4-Pyridinamine; Pyridin-4-amine; Avitrol; Fampridine™
- Chemical/Pharmaceutical/Other Class: Aminopyridine pesticide; K^+ channel blocker
- Chemical Formula: $C_5H_6N_2$

Uses

4-Aminopyridine is used broadly as an avicide. It is classified as a restricted use pesticide by the US Environmental Protection Agency. The compound is also being developed for clinical human application in the treatment of certain nerve conduction disorders, and it is used in experimental laboratory settings as an *in vitro* antagonist of voltage sensitive K^+ channels.

Exposure Routes and Pathways

As an avicide, 4-aminopyridine is impregnated into grain, set out as bait, and elicits toxicity to all grain-consuming bird (and other) species. Oral exposure can occur as with misuse of impregnated grain. Any grain-consuming organism, including livestock species, is at risk of accidental ingestion. Human exposure is also possible in industrial and manufacturing settings, and in circumstances where individuals (e.g., applicators) may be exposed during avicide use. 4-Aminopyridine can be absorbed through the skin. In clinical settings, patients are deliberately exposed to the compound for its therapeutic effects.

Toxicokinetics

The parent compound is active. Approximately 90% of ingested amount is excreted unchanged in the urine. 4-Aminopyridine is not thought to accumulate in the body.

Mechanism of Toxicity

The mechanism of 4-aminopyridine toxicity is due to the direct actions of the compound as a blocker of voltage-sensitive K^+ channels. At toxic doses, the chemical disrupts normal action potential conduction and nonselectively enhances neurotransmitter release. Because 4-aminopyridine blocks voltage-sensitive K^+ channels on neurons, it has the capacity to enhance action potential conduction in demyelinated tissue with therapeutically beneficial results. Toward this end, a compound (fampridine) is in development

as a therapeutic agent in the treatment of multiple sclerosis. The compound is also being investigated for its ability to improve neuronal signaling in patients with partial spinal cord injuries.

Acute and Short-Term Toxicity (or Exposure)

Animal

4-Aminopyridine is highly toxic to mammalian species. The compound is absorbed through both the skin and gastrointestinal tract. The LD_{50} for dermal exposure in rabbits is $326\,mg\,kg^{-1}$. The oral LD_{50} in rats is $\sim 20\,mg\,kg^{-1}$. The commercially available, technical grade 4-aminopyridine contained in the avicide product, Avitrol, has a reported oral LD_{50} of $28.7\,mg\,kg^{-1}$ in rats and $3\,mg\,kg^{-1}$ in dogs. 4-Aminoyridine is an eye irritant.

Human

Reported toxicities are of an acute nature. The signs and symptoms in humans include paresthesia, sweating, dizziness, ataxia, tremors, tachycardia, hypertension, and convulsions. Because 4-aminopyridine is a nervous system stimulant, it has been suggested that individuals with a history of convulsive disorders may be at increased risk. Convulsions are reported to be responsive to benzodiazepines. 4-Aminopyridine has been reported to cause severe poisoning in adult humans at dosages of less than 60 mg. The dosages used for therapeutic purposes in human trials are $20–30\,mg\,day^{-1}$ in divided doses over extended periods. In one reported study, six patients were given 24 mg intravenously without experiencing serious side effects.

Chronic Toxicity (or Exposure)

Animal

Long-term exposure may affect liver and nervous system functions. Long-term dietary exposures increased brain weights.

Human

Scarce data exist on the long-term effects of low-dose exposure to 4-aminopyridine.

Environmental Fate

4-Aminopyridine is adsorbed to soil particles and is moderately persistent in the environment. It has been reported to be slowly metabolized by soil microorganisms. The rate of disappearance varies with the organic content of soils and the disappearance halftime has been reported to range from 3 to 32 months. Movement from upper soil layers is thought to be minimal due to strong soil adsorption and the compound is not expected to represent a significant threat to groundwater.

Ecotoxicology

Bird mortality is reported to be low because toxicant-induced behavioral responses occur at sublethal doses, that is, behavioral changes in small numbers of birds act as a repellant to the remainder of the flocks. The reported LD_{50} across species ranged from $3\,mg\,kg^{-1}$ in crows to $15\,mg\,kg^{-1}$ in Bob White quail. 4-Aminopyridine is moderately toxic to fish. The LC_{50} ranged from 2.4 to $4\,mg\,l^{-1}$ in channel catfish. The LC_{50} in bluegill was $3.2–3.4\,mg\,l^{-1}$.

Exposure Standards and Guidelines

The reference dose is $0.000\,02\,mg\,kg^{-1}\,day^{-1}$.

See also: Pesticides.

Further Reading

Smith KJ, Felts PA, and John GR (2000) Effects of 4-aminopyridine on demyelinated axons, synapses and muscle tension. *Brain* 123: 171–184.

US Environmental Protection Agency (1980) *Pesticide Registration Standard: 4-Aminopyridine: Avitrol*. Washington, DC: Office of Pesticides and Toxic Substances.

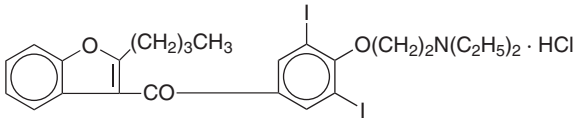
Relevant Websites

http://www.acorda.com – Acorda Therapeutics.
http://pmep.cce.cornell.edu – Cornell University.

Amiodarone

Elizabeth J Scharman

- Chemical Abstracts Service Registry Numbers: CAS 1951-25-3; CAS 19774-82-4 (hydrochloride)
- Synonyms: Amiodarone hydrochloride; Cordarone®
- Chemical/Pharmaceutical/Other Class: Class III antiarrhythmic agent, an iodinated benzofuran-derivative antiarrhythmic
- Chemical Formula: $C_{25}H_{29}I_2NO_3$
- Chemical Structure:



Uses

Amiodarone is indicated for the suppression and prevention of documented life-threatening, recurrent, ventricular tachycardia or fibrillation when other agents have failed. Amiodarone is also used in the management of supraventricular tachyarrhythmias including paroxysmal atrial fibrillation and atrial flutter, ectopic or multifocal atrial tachycardia, junctional tachycardia, and paroxysmal reentrant supraventricular tachycardia when other agents have failed to suppress or prevent their recurrence. Amiodarone has also been used to treat wide-complex tachycardia of uncertain mechanism.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to amiodarone although few cases exist in the literature. Amiodarone is available in an oral dosage form and a parenteral dosage form for intravenous administration.

Toxicokinetics

Amiodarone is slowly absorbed with an average bioavailabilty of 50% (range, 22–86%). Food has been shown to increase the rate and extent of absorption. Peak plasma concentrations are seen within 3–7 h (range, 2–12 h); however, the onset of action is not seen for at least 2–3 days and usually not until 1–3 weeks. This delay occurs even if a loading dose is given. Maximal responses may not occur until up to 5 months after starting therapy. Amiodarone is extensively metabolized to a major metabolite *N*-desethylamiodarone, which is active. The volume of distribution is $65.8\,l\,kg^{-1}$ (range $18.3–147.4\,l\,kg^{-1}$). The drug is found in adipose tissue and many organs, especially the liver, lung, spleen, and skeletal muscle. Concentrations of the drug in bile may be 50 times greater than concentrations in plasma. During chronic therapy, the metabolite appears in the same tissues with the exception of adipose tissue, which primarily contains amiodarone. Approximately 96% is protein bound. The drug can cross the placenta and is distributed into breast milk in concentrations exceeding that of maternal plasma. The therapeutic range is $1–2.5\,\mu g\,ml^{-1}$. Elimination of amiodarone is at least biphasic. With single-dose administration, the half-life is 25 days (range 9–47 days). Following chronic administration, the terminal elimination half-life in the majority of patients is 40–55 days (range 26–107 days). Amiodarone may undergo enterohepatic recirculation. Almost the entire drug is excreted in the feces.

Mechanism of Toxicity

Amiodarone is primarily a class III antiarrhythmic agent but does display activity in each of the four Vaughn–Williams antiarrhythmic classes. The drug delays repolarization via prolongation of the action potential duration and effective refractory period, decreases AV conduction, depresses sinus node and junctional automaticity, acts as a noncompetitive α- and β-adrenergic inhibitor, and slows automaticity of Purkinje fibers.

It is unknown whether thyroid dysfunction (hypothyroidism or hyperthyroidism) is a result of the amiodarone, the iodine contained in the amiodarone, or another mechanism. The production of amiodarone–phospholipid complexes within organs has been proposed as the mechanism for some of this drug's adverse effects. The mechanism of the pulmonary toxicity seen following chronic use is also uncertain but is the result of a hypersensitivity reaction in some.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in dogs is more than $3\,g\,kg^{-1}$.

Human

Determination of toxicity is based on observation as there is no milligram per kilogram toxic dose established. Symptoms may include nausea, bradycardia,

heart block, hypotension, and QT prolongation leading to torsades de pointes. The onset of toxicity may be substantially delayed for up to 3 days.

Chronic Toxicity (or Exposure)

Animal

Chronic carcinogenicity studies in rats demonstrate significant increases in thyroid tumors. The effects are dose related and have been demonstrated at doses as low as $5\,mg\,kg^{-1}$. Daily doses of $90\,mg\,kg^{-1}$ in pregnant rats showed reduced fertility. Doses of $25\,mg\,kg^{-1}\,day^{-1}$ in rabbits showed no change in fertility or adverse effects on the fetus but doses of $75\,mg\,kg^{-1}\,day^{-1}$ caused abortions in 90% of test animals.

Human

Chronic therapy with amiodarone has been associated with pulmonary interstitial pneumonitis/alveolitis, hypersensitivity pneumonitis, and pulmonary fibrosis; fatalities have resulted. Other side effects include: elevated liver function tests, worsening of arrhythmias, onset of new arrhythmias, fatigue, tremor, involuntary movements, dizziness, paresthesias, difficulty in walking, hypothyroidism, hyperthyroidism, nausea, vomiting, constipation, anorexia, corneal microdeposits, and photosensitivity. The skin may develop a blue-gray color, especially in areas exposed to the sun.

In Vitro Toxicity Data

Amiodarone has not been shown to be mutagenic in Ames, micronucleus, and lysogenic tests.

Clinical Management

Amiodarone is adsorbed to activated charcoal. Dialysis will not enhance elimination. Standard supportive therapies, such as vasopressors, should be utilized as clinically indicated. Patients may require prolonged observation due to the delay in the development of adverse effects.

See also: Iodine.

Further Reading

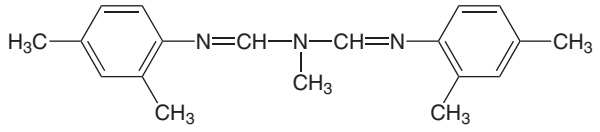
Burns KEA, Piliotis E, and Garcia BM (2000) Amiodarone pulmonary, neuromuscular and ophthalmological toxicity. *Canadian Respiratory Journal* 7: 193–197.

Olshansky B (1997) Amiodarone-induced pulmonary toxicity. *New England Journal of Medicine* 337: 1814.

Amitraz

Jamaluddin Shaikh

- Chemical Abstracts Service Registry Number: CAS 33089-61-1
- Synonyms: Aazdieno; Acadrex; Acarac; Amitraze; Ectodex; Triatox; Ovasyn; Ovidrex; Triazid
- Chemical/Pharmaceutical/Other Class: Formamidine insecticide and acaricide
- Chemical Structure:



Uses

Amitraz is used to control insects on pears, cotton, and livestock including cattle and hogs. It is used to control red spider mites, leaf miners, scale insects, and aphids. On cotton it is used to control bollworms, white fly, and leaf worms. Amitraz is effective against ticks, mites, and lice on animals.

Exposure Routes and Pathways

Amitraz is a straw-colored crystalline, odorless product. The oral route is a common exposure pathway. Amitraz is poorly absorbed by the dermal route.

Toxicokinetics

Peak plasma levels occur ~1 h after dosing. Liver, kidney, and muscle have highest residues ~0.75–1.5 h after oral dosing. Amitraz is rapidly metabolized and excreted, mainly in the urine. Metabolism of amitraz is similar among many species by hydrolysis to *N*-(2,4-dimethylphenyl)-*N*′-methyl formamide and 2,4-dimethyl formanilide, leading to production of 4-amino-3-methylbenzoic acid. This metabolite is rapidly conjugated and excreted. After repeated dosing in rats highest residues of amitraz

are found in thyroid and adrenal glands, liver, skin, spleen, and eyes.

Mechanism of Toxicity

Acute oral administration of amitraz causes central nervous system (CNS) depression. The toxic effects of amitraz are possibly from α2-adrenoreceptor agonist action. Chronic exposure of amitraz results in CNS depression, increases blood glucose levels, and produces hypothermia.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for amitraz in rats is 523–800 mg kg^{-1}. The oral LD_{50} is higher for mice (> 1600 mg kg^{-1}). The dermal LD_{50} is greater than 1600 mg kg^{-1} for rats. The inhalation LC_{50} (6 h) of amitraz for rats is 65 mg l^{-1} of air.

Human

One of the predominant signs after oral ingestion of amitraz is CNS depression. The other clinical signs reported with amitraz poisoning in humans are drowsiness, vomiting, miosis followed by mydriasis, bradycardia, hypotension, hyperglycemia, and respiratory failure. All clinical signs appear 30 min to 4 h after poisoning.

Chronic Toxicity (or Exposure)

Animal

Amitraz is not teratogenic in rats. Dogs appear most sensitive to subchronic amitraz exposures, with CNS depression being predominant. A chronic (2 year) feeding study reported that rats receiving 50 mg kg^{-1} day^{-1} and dogs receiving 0.25 mg kg^{-1} day^{-1} amitraz did not exhibit signs of overt toxicity. Chronic exposure may lead to bladder irritation, heat intolerance, and loss of muscle tone. Amitraz is not carcinogenic in rats but it does cause tumors (in the lungs, liver, and lymph nodes) in female mice.

Human

Little is known regarding chronic effects of amitraz in humans.

In Vitro Toxicity Data

Amitraz is negative in a number of mutagenesis assays and did not cause damage to DNA.

Clinical Management

With dermal exposure, areas exposed to amitraz should be washed with soap and water. Eyes should be washed with copious amounts of clean water for 15 min. Ophthalmologic consultation is required in case of persistent irritation. Gastric lavage is indicated immediately in acute oral overdose. The amitraz-poisoned patient is also treated with atropine and activated charcoal. Intubation and assisted ventilation may be required as supportive care.

Environmental Fate

Amitraz degrades in the environment to *N*-(2,4-dimethylphenyl)-*N*′-methyl formamidine. Amitraz has low potential of leaching in soils. Its half-life in soil has been reported to be less than 1 day.

Ecotoxicology

Amitraz is moderately toxic to fish. The LC_{50} (3 days) is 1.3 mg l^{-1} for bluegill sunfish and 3.2–4.2 mg l^{-1} for harlequin fish. It is slightly toxic to birds. The dietary LC_{50} (8 days) values for mallard ducks and Japanese quail are 7000 and 1800 mg kg^{-1}, respectively. Amitraz may affect reproduction in birds. It is relatively nontoxic to bees. In bees, the LD_{50} by ingestion and direct spraying are 12 μg per bee and 3.6 mg l^{-1}, respectively.

Exposure Standards and Guidelines

The oral reference dose for amitraz is 2.5 μg kg^{-1} day^{-1}.

See also: Pesticides.

Further Reading

Iyer P (2001) Developmental and reproductive toxicology of pesticides. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 375–423. San Diego, CA: Academic Press.

Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

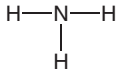
Amitroptyline *See* Tricyclic Antidepressants.

Ammonia

Ralph J Parod

This article is a revision of the previous print edition article by Edward Kerfoot, volume 1, pp. 60–61, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7664-41-7
- Synonyms: Anhydrous ammonia; Ammonia gas; Liquid ammonia; R 717
- Chemical/Pharmaceutical/Other Class: Nitrogen family
- Chemical Structure:



Uses

Commercial production of ammonia is primarily via a modified Haber–Bosch process in which atmospheric nitrogen is combined with hydrogen obtained from natural gas, a process termed nitrogen fixation. About 80% of the commercially produced ammonia is used in fertilizers with the remainder used in a variety of applications such as plastics, synthetic fibers and resins, pharmaceuticals, explosives, refrigeration, and household cleaners. Ammonia is a naturally occurring compound that is a key component of the global nitrogen cycle and all living organisms. Nitrogen fixation by current industrial processes approximates that produced naturally by biological processes and lightening strikes.

Exposure Routes and Pathways

Ammonia is a gas under normal environmental conditions; thus, human exposures typically occur via inhalation and dermal contact. Oral exposures are also possible as ammonia has high water solubility.

Toxicokinetics

Unionized ammonia can freely diffuse through tissue cells but forms ammonium hydroxide upon contact with tissue water. Dissolved ammonia and the less permeable ammonium ion exist in a dynamic equilibrium that serves to retard the absorption of ammonia into the circulation depending on the complexity of the intervening tissues. During short-term ($\leqslant$2 min) exposures to $\leqslant$500 ppm ammonia, most (83–92%) of the inspired ammonia is retained within the upper airways. This absorption process may be adaptive or saturable because most of the ammonia inspired during longer-term exposures (10–27 min) is exhaled with $\sim$4–30% being retained within the upper airways and available for systemic absorption. Ammonia or ammonium ion is well-absorbed by the gastrointestinal tract. Almost 100% of the ammonia produced endogenously in the human digestive tract ($60\,mg\,kg^{-1}\,day^{-1}$) is absorbed and metabolized in the liver to urea and glutamine. The brain can also convert ammonia to glutamine. Due to first-pass metabolism in the liver, little ammonia from the gut reaches the systemic circulation, and toxicologically significant amounts of ammonia in blood ($>1\,\mu g\,ml^{-1}$) probably occur only in severe disease states where the metabolism of ammonia by the liver and the excretion of metabolites by the kidney are compromised. It is unlikely that a significant amount of the ammonia contacting the skin is absorbed. Ammonia that reaches the circulation is distributed throughout the body where it can be used in protein synthesis or as a buffer. Most of the absorbed ammonia is excreted in the urine as urea, with minimal amounts excreted in the feces or expired air.

Mechanism of Toxicity

The primary immediate effect of ammonia exposure is burns to the skin, eyes, and respiratory tract. Ammonia dissolves in tissue water and forms ammonium hydroxide that breaks down cellular proteins, saponifies cell membrane lipids resulting in cell disruption and death, and initiates an inflammatory response that further damages surrounding tissues.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ammonia is an irritant gas that can cause severe local effects in the absence of systemic toxicity. No signs of toxicity were observed in rats continuously exposed to 58 ppm ammonia for 114 days; no abnormal changes

in the lung were noted after gross and microscopic examination. Ciliary activity in the trachea of rabbits was impaired at 100 ppm. At 300 ppm ammonia, the respiratory rate of mice and rats was depressed by 50%. Continuous exposure of four animal species to 677 ppm ammonia for 90 days resulted in extensive focal and diffuse inflammation of the pulmonary interstitium (all species), marked eye irritation with some corneal opacities (rabbit, dog), and fatalities (rat, guinea pig). The 1 h LC_{50} values in the mouse and rat were ~4500 and 9500 ppm, respectively. No genotoxicity data in laboratory animals are available.

Human

Ammonia has an odor threshold ranging from 1 to 5 ppm. Exposures between 20 and 25 ppm can cause complaints and discomfort in some workers unaccustomed to ammonia exposure but have little effect on pulmonary function or odor sensitivity. Concentrations of 100 ppm caused definite irritation of the respiratory tract and eyes, and exposures at 250 ppm ammonia are bearable for 30–60 min. Severe irritation of the respiratory tract, skin, and eyes has been observed following ammonia exposures ranging from 400 to 700 ppm. Exposure to 2500–4500 ppm ammonia can be fatal within 30 min. Immediate fatalities appear to be the result of airway obstruction, particularly laryngeal edema and glottic spasm, while infections and other secondary complications appear to be the cause of fatality among those who survive for several days to weeks.

Chronic Toxicity (or Exposure)

No data in animals and humans are available.

In Vitro Toxicity Data

Data on the *in vitro* mutagenicity and clastogenicity of ammonia are limited and equivocal.

Clinical Management

Exposures by inhalation should be monitored for respiratory tract irritation, upper airway obstruction, bronchitis, or pneumonitis. Humidified supplemental 100% oxygen should be administered to help soothe bronchial irritation. Oxygen, in combination with intubation and mechanical ventilation, may be required in severe cases. Exposed eyes and skin should be irrigated immediately with copious amounts of water; eyes should be washed for at least 30 min or until the eye reached neutral pH as tested in the conjunctival sac. If eye irritation, pain, swelling, lacrimation, or photophobia persist, the patient should be seen in a health care facility.

Environmental Fate

In the atmosphere, ammonia is estimated to have a half-life of several days. The primary fate process is reaction of ammonia with acid air pollutants and removal of the resulting ammonium compounds by dry or wet deposition. Rain washout and reaction with photochemically produced hydroxyl radicals are also expected to contribute to the atmospheric fate of vapor-phase ammonia. In water and soil, ammonia will volatilize to the atmosphere and be removed by microbial processes, by adsorption to sediment and soil matrices as well as by plant uptake.

Ecotoxicology

The 96 h LC_{50} values for a variety of fish species range between 0.1 and 5 $mg\,l^{-1}$. For a variety of aquatic invertebrates, the 48 h LC_{50} values range between 1 and 190 $mg\,l^{-1}$.

Exposure Standards and Guidelines

International occupational exposure limits (OELs) generally range between 20 and 25 ppm as an 8 h time-weighted average (TWA). The American Conference of Governmental Industrial Hygienists has established an 8 h TWA OEL for ammonia of 25 ppm with a 15 min excursion limit of 35 ppm. The National Institute of Occupational Safety and Health indicates that 300 ppm ammonia is immediately dangerous to life or health.

See also: Respiratory Tract.

Further Reading

CARB (1999) Air toxics emissions data collected in the Air Toxics Hot Spots Program CEIDARS Database as of January 29.

US Environmental Protection Agency (1985) Ambient Water Quality Criteria for Ammonia (EPA 440/5-85-001), January.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Ammonia.

Ammonium Nitrate

Prathibha S Rao

- Chemical Abstracts Service Registry Number: CAS 6484-52-2
- Synonyms: Ammonium saltpeter; German saltpeter; Norway saltpeter; Nitrate d'ammonium; Nitrate of ammonium; Herco prills; Merco prills; Varioform I; AN
- Chemical/Pharmaceutical/Other Class: Nitrate
- Chemical Formula: NH_4NO_3

Uses

Ammonium nitrate is used commonly in fertilizers; in pyrotechniques, herbicides, and insecticides, as well in the manufacture of nitrous oxide. It is used as an absorbent for nitrogen oxides, an ingredient of freezing mixtures, an oxidizer in rocket propellants, and a nutrient for yeast and antibiotics. It is also used in explosives (especially as an oil mixture) for blasting rocks and in types of mining.

Nitrates and nitrites are used to cure meats and to develop the characteristic flavor and pink color, to prevent rancidity, and to prevent growth of *Clostridium botulinum* spores in or on meats.

Background Information

Ammonium nitrate is found as colorless or white to gray crystals or odorless beads with a molecular weight of 80.06 and specific gravity of $1.725\,g\,cm^{-1}$. It has a melting point of 169.5°C and boils at 210°C with evolution of nitrous oxide. It forms chloramines on chlorination and is incompatible with acetic acid; acetic anhydride, hexamethylene tetramine acetate, and nitric acid mixture; ammonia; aluminum, calcium nitrate, and formamide mixture; metals; alkali metals; and combustible agents.

Exposure Routes and Pathways

Common exposure pathways are via products in which ammonium nitrate is used. Nitrates are also found in water from soils, rocks, decomposing organic matter, and in vegetables like beets, radish, lettuce, celery, and spinach. It is also found in secretions like saliva and formed in the mouth and gut due to bacterial action.

Toxicokinetics

Nitrates are well absorbed from the gastrointestinal tract producing peak blood levels only 40 min after ingestion. They may also be absorbed through abraded or damaged skin. Nitrates are converted to nitrites by various bacteria in the stomach and intestines of animals and humans. Approximately 14–31% of nitrate is excreted via the kidneys. The mean renal clearance for nitrates is $\approx 26\,ml\,min^{-1}$. About 40% is excreted as nitrites in the urine. It is also recycled through the saliva.

Mechanism of Toxicity

Nitrate and nitrites can combine with secondarys amines to form dimethylnitrosamines, which are acutely toxic and cause centrilobular necrosis, fibrous occlusion of central veins, and pleural and peritoneal hemorrhages in animals. In the body nitrates are converted to nitrites, which can oxidize hemoglobin to methemoglobin and lead to cyanosis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methemoglobinemia, which can lead to anoxia and death in extreme cases, is the primary acute toxic effect of oral exposure to inorganic nitrates in all animals tested. Ruminant animals are most susceptible. This effect depends on a number of factors including the conversion of nitrates to nitrites, the ability of the various animals to enzymatically reduce methemoglobin, the amount of vitamins A, C, D, and E in the diet, and the nutritional state of the animal. Acute nitrate toxicity in cattle has been reported following the ingestion of water containing 500 ppm or more nitrate or feed containing 5000 ppm or more nitrates. With acute poisoning the signs of poisoning are observed and the animal is in critical condition. Nitrate is rapidly converted to nitrite in the rumen and is immediately absorbed in large amounts into the bloodstream. Animals can die within a few hours of initial ingestion of a high nitrate feed.

If cattle are fed once a day, maximum methemoglobin levels occur ~8 h after feeding. When cattle are fed twice daily, maximum levels occur 4–5 h after feeding. The once-a-day feeding program results in higher total methemoglobin levels than twice-a-day feeding. With once-a-day feeding, a

larger quantity of feed is consumed at once and a greater amount of nitrate is released from the feed in a short period of time.

Signs of acute poisoning in cattle are: increased heart rate, muscle tremors, vomiting, weakness, blue-gray mucous membranes, excess saliva and tear production, depression, labored or violent breathing, staggering gait, frequent urination, low body temperature, disorientation, and an inability to get up. Animals are often found in a lying position after a short struggle. In most cases of acute poisoning, animals are found dead before any signs of toxicity are observed.

Human

Ammonium nitrate is irritating to the eyes, nose, throat, and mucous membranes. Inhalation of this compound can cause severe lung congestion, coughing, difficulty in breathing, and increased acid urine. Exposure to large amounts can cause systemic acidosis and abnormal hemoglobin. It is considered to have low toxicity since it causes readily reversible tissue changes that disappear when exposure stops.

In the body nitrates are converted to nitrites, which can oxidize hemoglobin to methemoglobin and lead to cyanosis. They also cause unconsciousness, dizziness, fatigue, shortness of breath, nausea, and vomiting. The skin is warm and sweaty and later becomes cold due to vasodilation. It causes coronary blood vessel contraction, bradycardia, atrial fibrillation, cardiac ischemia, headache, convulsions, and diarrhea.

Nitrate transferred through breast milk causes methemoglobinemia in the infant. Infants are more predisposed to nitrate-related toxicity than adults due to decreased ability to secrete gastric acid, higher levels of fetal hemoglobin, and diminished enzymatic capability to reduce methemoglobin to hemoglobin.

No data are available on the teratogenicity or mutagenicity of ammonium nitrate.

Chronic Toxicity (or Exposure)

Animal

Chronic nitrate toxicity is a form of nitrate poisoning where the clinical signs of the disease are not observed. It is more common to see a reduction in weight gain, lower milk production, depressed appetite, and a greater susceptibility to infections. These production-related problems or losses are not often recognized and will occur when nitrate levels are at 0.5–1.0% of the daily feed consumption.

Chronic nitrate poisoning can cause abortions to occur within the first 100 days of pregnancy because nitrates interfere with the implantation of the egg in the uterus. When implantation does not occur, the fetus dies and is reabsorbed. During the first trimester of pregnancy, no obvious signs of an abortion are seen. Reproductive problems may also occur due to a nitrate or nitrite-induced hormone imbalance, but most are usually not recognized as feed related.

Calves affected by nitrate poisoning during the last three months of gestation are usually born one to four weeks premature, and most appear normal but die within 18–24 h of birth. Surviving newborn calves that are affected by nitrate poisoning may have convulsions and seizures.

Human

Chronic ingestion of $5\,mg\,kg^{-1}\,day^{-1}$ is considered unacceptable. Common findings associated with nitrate poisoning include unconsciousness, dizziness, fatigue, shortness of breath, nausea, vomiting, coma, cyanosis, dyspnea, and pallor.

Clinical Management

Absorption should be prevented by dilution with 4–8 ounces of milk or water or by gastric lavage in patients who are comatose or at a risk of convulsing. Charcoal or saline cathartic may also be given. Emesis may be induced if initiated within 30 min of ingestion. Methylene blue is used to treat methemoglobinemia. Diazepam is administered (maximum rate $5\,mg\,min^{-1}$) to control seizures. Recurrent seizures are controlled by phenytoin or phenobarbital. An EKG should be monitored while administering phenytoin. Dopamine or norepinephrine is administered to control hypotension.

Ecotoxicology

Upon decomposition ammonium nitrate will release ammonium ions. Ammonia is a toxic hazard to fish and maybe harmful to animals on direct ingestion. Ammonium nitrate is nonpersistent and noncumulative when applied using normal agriculture practices. It is not listed as a marine pollutant.

Exposure Standards and Guidelines

The nitrate limit in drinking water was established as a safeguard against infantile acquired methemoglobinemia. EPA's maximum contaminant level (MCL) for nitrates is 10 ppm. The MCL for nitrites is 1 ppm.

See also: Nitrous Oxide.

Further Reading

Heindel JJ, Chapin RE, Gulati DK, *et al.* (1994) Assessment of the reproductive and developmental toxicity of pesticide/fertilizer mixtures based on confirmed pesticide contamination in California and Iowa groundwater. *Fundamental and Applied Toxicology* 22: 605–621.

National Academy of Sciences (1981) The health effects of nitrite, nitrate and *N*-nitroso compounds. Washington: National Academy Press.

US Environmental Protection Agency (1985) Health effects criteria document for nitrate/nitrite. Washington: US Environmental Protection Agency, Office of Drinking Water, Criteria and Standards Division.

US Environmental Protection Agency (1987) Nitrate/nitrite health advisory. Washington: US Environmental Protection Agency, Office of Drinking Water.

US Environmental Protection Agency (1990) National pesticide survey: Summary results of pesticides in drinking water wells. Washington: US Environmental Protection Agency, Office of Pesticides and Toxic Substances.

Yang R (1993). NTP technical report on the toxicity studies of pesticide/fertilizer mixtures administered in drinking water to F344/N rats and B6C3F1 mice. Toxicity Report Series 36: 1-G3.

Relevant Websites

http://risk.lsd.ornl.gov – The Risk Assessment Information System.

http://www.1.agric.gov.ab.ca – Agriculture, Food and Rural Development, Government of Alberta, Canada.

Ammonium Perchlorate

Joan Strawson

This article is a previous print edition
article by Prathiba S Rao and Harihara M Mehendale,
volume 1, pp. 62–63, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7790-98-9
- Synonyms: Ammonium perchlorate; Perchloric acid; Ammonium salt; UN 0402; UN 1442
- Chemical Formula: NH_4ClO_4
- Chemical Structure: ClO_4–H_4N

Uses

Ammonium perchlorate is an explosive agent used as a component of fireworks, flash powders, explosives, smokeless jet, and rocket propellants. It is also used in oxidizing, engraving, or etching compounds, and as a reagent in analytical chemistry. Historically, perchlorate salts have been used therapeutically to treat hyperthyroid disorders, including Graves' disease. More recently, perchlorate has been used (alone or in combination with other antithyroid drugs) to treat amiodarone-induced thyrotoxicosis, a condition in which thyroid abnormality results from excess iodine when the iodine-containing drug amiodarone is given to control cardiac arrhythmia. Perchlorate also occurs naturally in nitrate-rich mineral deposits used in fertilizers.

Exposure Routes and Pathways

Perchlorate salts dissolve readily in water and are easily absorbed from the gastrointestinal tract. However, because of its high charge, the perchlorate anion does not penetrate the skin readily. Uptake of inorganic ions such as perchlorate through the skin is typically less than 10%, and frequently less than 1%. Exposure via inhalation of fumes or vapors is considered negligible because the vapor pressure of perchlorate salts and acids is low at room temperatures. The risk from exposure to particles would depend on the particle size distribution. Thus, the ingestion route is the major concern for the risk posed by the perchlorate contamination and is the focus of this characterization.

Toxicokinetics

Perchlorate appears to be well absorbed from the gastrointestinal tract. Perchlorate appears to be eliminated rapidly, primarily in the urine ($>90\%$), and virtually unchanged from both rats and humans. Half-lives have been reported for the rat ranging from <8 to 20 h. Perchlorate has been detected in the urine within 10–15 min of oral dosing and peak plasma levels occur within 3 h.

Mechanism of Toxicity

The perchlorate anion is a tetrahedron with four oxygen molecules at the corners and a chlorine molecule at the center. Perchlorate, with a partial molal ionic volume of 44.5, has a similar ionic size as iodide with a partial molal ionic volume of 36.7 at 25°C. Because of its chemical properties, perchlorate is a competitive inhibitor of the process by which iodide circulating in the blood is actively transported into thyroid follicular cells. The site of this inhibition is

the sodium–iodide symporter, a membrane protein located adjacent to the capillaries supplying blood to the thyroid. Inhibition of iodine uptake is the basis for the current and former pharmacological uses of perchlorate, and the likely precursor of potentially adverse effects. Subsequent events include decreases in serum thyroid hormone T4 (and thyroid hormone T3), leading to the potential for altered neurodevelopment in either dams or fetuses/neonates, and increases in serum thyroid stimulating hormone (TSH), leading to the potential for thyroid hyperplasia and tumors. The repeated observation of thyroid effects such as alterations of hormones, increased thyroid weight, and alterations of thyroid histopathology (including tumors) from a large number of rat studies on perchlorate provide supporting evidence for the proposed mode of action, and confirms that the perturbation of thyroid hormone level is the primary biological effect of perchlorate.

Acute and Short-Term Toxicity (or Exposure)

The systemic toxicity of perchlorate appears to be directly related to its action as a competitive inhibitor of iodide, and the symptoms tend to be similar to those of iodine deficiency.

Animal

Oral LD_{50} values for ammonium perchlorate range from 750 to 1900 mg kg^{-1} for rabbits, from 1900 to 2000 mg kg^{-1} for mice, and from 3500 to 4200 mg kg^{-1} for rats. Drinking water studies in rodents demonstrate that the thyroid is the only target organ for perchlorate toxicity. Following drinking water exposure of 4 and 14 days, decreased serum levels of the T3 and T4 as well as increased levels of TSH were observed. Following 14 days of exposure, increases in thyroid weight and histopathological changes in thyroid including hypertrophy and hyperplasia were observed in rats at doses greater than 1 mg kg^{-1} day^{-1}. No developmental effects were observed in either rats or rabbits exposed to ammonium perchlorate in drinking water during gestation.

Human

No case reports of acute poisoning in humans have been reported. Two clinical studies have evaluated the effects of ammonium perchlorate in drinking water at doses up to 0.5 mg kg^{-1} day^{-1} in healthy adult volunteers. The threshold for inhibition of iodine uptake in humans appears to be about 0.006 mg kg^{-1} day^{-1}. Doses of 0.5 mg kg^{-1} day^{-1} resulted in iodine uptake inhibition of ~70%. However, at this dose, no effect was observed on thyroid hormone levels or on any other parameter evaluated.

Chronic Toxicity (or Exposure)

Animal

In 90 day toxicity studies, only thyroid effects were observed in rats and mice following exposure to ammonium perchlorate in drinking water. As with shorter-term studies, changes in the serum levels of T3, T4, and TSH were observed, followed by increased thyroid weight and thyroid histopathological changes at doses greater than 1 mg kg^{-1} day^{-1}. These changes were reversible following cessation of exposure. No effects were observed on any other target organ. No reproductive effects in either males or females were observed in a two-generation study of rats exposed to ammonium perchlorate in drinking water. Two-year carcinogenicity bioassays demonstrated thyroid tumors in rats.

Human

Since perchlorate has become a public health issue, several human studies have been published, including several epidemiological studies and two occupational studies. The epidemiology studies have examined thyroid endpoints, including congenital hypothyroidism and T4 and TSH levels, in neonates born in areas known to have perchlorate in the public water supply, compared with infants born in areas without perchlorate in the public water supply. Another study in the United States has compared the prevalence of thyroid disease in Medicaid users in counties with perchlorate exposure through drinking water compared to Medicaid users in counties without perchlorate exposure. All studies, except one, showed that perchlorate had no effect on thyroid parameters. The remaining study found that infants in counties with perchlorate in drinking water had elevated TSH levels when measured by an analysis of variance on the log-transformed TSH values ($P = 0.017$), but not when measured by *t*-tests for each day of birth separately. Another study tested the hypothesis that perchlorate in drinking water suppresses thyroid function in 9784 newborns and 162 school-aged children. The study was conducted in Northern Chile, which has naturally occurring perchlorate in the drinking water, and compared populations in three different cities that had perchlorate concentrations of 100–120 μg l^{-1}, 5–7 μg l^{-1}, and nondetectable (<4 μg l^{-1}), respectively. The thyroid parameters measured in the newborn or school-age children were comparable among the three cities.

The occupational studies evaluated the thyroid function of workers in perchlorate production facilities. No effect on thyroid function was observed in workers after a single shift, or after a working lifetime. Lifetime exposures were up to 0.5 mg kg^{-1} day^{-1}.

In Vitro Toxicity Data

Ammonium perchlorate was tested in a battery of genotoxicity tests and found to be negative in all tests, including a reverse mutation assay in *Salmonella typhimurium* and the L5178Y/$TK^{+/-}$ mouse lymphoma assay. In addition, ammonium perchlorate was tested *in vivo* in a mouse micronucleus assay at doses up to 1000 mg kg^{-1} for 3 days, and found to be negative. Based on these data, perchlorate does not appear to be mutagenic or clastogenic, and genotoxicity does not appear to be a mode of action for perchlorate.

Clinical Management

The victim should be removed to fresh air and monitored for respiratory distress. Early intravenous administration of corticosteroids is recommended to prevent or treat noncardiogenic pulmonary edema. Inhalation of sympathomimetic agents is used to treat bronchospasm and wheezing. Absorption can be prevented by dilution with 4–8 oz ($\sim$118–237 ml) of milk or water. Absorption can also be prevented by gastric lavage in patients who are comatose or at the risk of convulsing. Charcoal, saline, or other cathartics can also be used. Cathartics should be avoided in patients with ileus or impaired renal function.

Environmental Fate

Ammonium perchlorate's manufacture and use in a variety of explosives, as well as its presence in some nitrate fertilizers, has resulted in its release to the environment. Perchlorate dust can be suspended in the air and inhaled by individuals working in perchlorate manufacturing facilities. In addition, open detonation of explosive materials or open burning of perchlorate-containing materials can result in the release of perchlorate in air. Perchlorate may also be found in soil, particularly where perchlorate-containing fertilizer has been applied, or where perchlorate-containing water is used for irrigation. Because perchlorate is highly soluble, it is not expected to concentrate in soil. Due in part to improved analytical methods, perchlorate has been detected in surface water and groundwater near various facilities that have manufactured and tested solid rocket fuels, most notably in California, Nevada, and Utah. Perchlorate has been measured in the public drinking water supply in several areas in California and in Lake Mead in Nevada.

Ecotoxicology

Perchlorate is concentrated by plants, especially in the leaves, that are irrigated with water-containing perchlorate. Perchlorate has been detected in vegetation and wildlife (aquatic insects, fish, frogs, mammals) near an ammunition plant historically associated with perchlorate-containing rocket propellants. There has been limited testing of perchlorate in aquatic species, soil invertebrates, and amphibians. Results in aquatic species are inconclusive because of the lack of cation controls. Perchlorate appears to inhibit thyroid activity and alter gonadal differentiation in amphibians at concentrations found in surface water.

Exposure Standards and Guidelines

Currently, there are no US federal or international occupational or environmental standards for ammonium perchlorate. California has a public health goal (PHG) for perchlorate of 6 ppb.

See also: Iodine; Levothyroxine; Perchlorate.

Further Reading

Strawson J, Zhao Q, and Dourson M (2004) Reference dose for perchlorate based on thyroid hormone change in pregnant women as the critical effect. *Regulatory Toxicology and Pharmacology* 39: 44–65.

Relevant Websites

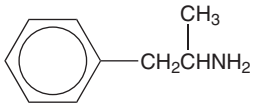
http://www.oehha.org – California Office of Environmental Health Hazard Assessment.
http://cfpub.epa.gov – US Environmental Protection Agency, National Center for Environmental Assessment.

Amobarbital *See* Barbiturates, Short-Acting.

Amphetamine

Michael Wahl

- Chemical Abstracts Service Registry Number: CAS 300-62-9
- Synonyms: 1-Phenyl-2-aminopropane; Phenylisopropylamine. In drug abuse, the word 'amphetamine' can also refer to a number of related compounds such as methamphetamine and other analogs with similar activity (e.g., meth, speed, wire, cross-tops, ice, dexies, black beauties, and hearts).
- Chemical/Pharmaceutical/Other Class: Central nervous system stimulant
- Chemical Formula: $C_9H_{13}N$
- Chemical Structure:



Uses

Amphetamine is used in the treatment of attention-deficit hyperactivity disorder and narcolepsy. It is also a drug of abuse.

Exposure Routes and Pathways

Oral and intravenous uses are probably the most common routes of exposure. Amphetamines can also be used via nasal insufflation and smoking. Peak concentrations after oral ingestion range from 1 to 4 h, depending on the specific amphetamine. Some pharmaceutical preparations are sustained or delayed release products, with lower absorption rates. More than 50% of a dose undergoes hepatic metabolism, and ~30% is excreted unchanged in urine. The amount of unmetabolized drug recovered in urine is greater with acidic urine pH. The apparent volume of distribution is 3–5 l kg^{-1} and also varies by specific drug. The half-life ranges from 8 to 15 h. Analytical methods used should distinguish the specific compound present since other compounds are structurally similar and may cross-react with antiamphetamine antibodies.

Toxicokinetics

Amphetamines are generally well absorbed from the gastrointestinal tract in therapeutic doses. Several commercially available amphetamines are formulated as sustained or delayed release products. Peak serum levels are expected within 30 min after intravenous injection and within 2–3 h after ingestion of immediate release products. In overdose and with exposure to sustained release products, delays in absorption are expected.

Amphetamines are widely distributed (generally several liters per kilogram) with low protein binding. These agents are generally extensively metabolized. Many metabolites have amphetamine activity. Elimination can vary greatly. Some amphetamines are primarily renally eliminated with the rate of elimination dependent upon the urine pH (e.g., amphetamine). Others have less than 1% of the parent compound renally excreted (e.g., methylphenidate). Half-lives vary as well with IV methylphenidate at 1–2 h and chlorphentermine at ~5 days.

Mechanism of Toxicity

The effects of amphetamines are due to the increase of neurotransmitters norepinephrine, serotonin, and dopamine in central synapses. This increase is from increased release and reuptake blockade of catecholamines. Amphetamines may also inhibit monoamine oxidase. These mechanisms combine to produce the sympathomimetic and central nervous system (CNS) effects seen with amphetamine abuse.

Acute and Short-Term Toxicity (or Exposure)

Animal

Effects in animals mimic those seen in humans. Expected signs and symptoms include hypertension, tachycardia, seizures, and hyperthermia.

Human

Toxicity primarily involves the CNS and cardiovascular system. CNS effects include increased alertness, restlessness, decreased appetite, irritability, stereotyped repetitive behavior, and insomnia with low doses. With larger exposures confusion, panic reactions, aggressive behavior, hallucinations, seizures, delirium, coma, and death can occur. Intracranial bleeding can result from untreated hypertension. Trauma is common secondary to the changes in behavior and decreased judgment. Frequent use results in fatigue, paranoia, and depression. Cardiovascular effects include increased heart rate and blood pressure, chest pain, myocardial ischemia or

infarction, dysrhythmias, cardiovascular collapse, and death. Other effects include increased temperature, rhabdomyolysis, increased respiratory rate, flushing, sweating, and dilated pupils.

Chronic Toxicity (or Exposure)

Animal

Animal models describe changes in behavior with toxicity and withdrawal. Chronic dosing of animals leads to stereotypic, compulsive behaviors of searching and examining in higher animals, sniffing and biting movements in lower animals. There has been no increased carcinogenic activity in rats and mice fed varying doses of amphetamine over studies as long as 2 years.

Human

Chronic use can result in psychosis and cardiomyopathy.

In Vitro Toxicity Data

Several amphetamines have been shown to have monaminergic neurotoxic properties. Recent studies of PC12 dopaminergic cells have shown increased activity of capsase-3 and mitochondrial cytochrome *c* release. These findings suggest that amphetamines (particularly substituted amphetamines) may induce apoptosis, possibly via a mitochondrial pathway.

Clinical Management

After assessment of airway, breathing, and circulation with necessary supportive care, decontamination of the gastrointestinal tract should be undertaken for substantial recent ingestions. Determination of specific toxic doses is difficult in chronic users of amphetamines due to the development of tolerance. Oxygen and benzodiazepines should be administered as needed for agitation, shortness of breath, or chest pain. Increased blood pressure can be managed with benzodiazepines or vasodilators. Benzodiazepines may be necessary for agitated or combative patients. Benzodiazepines, cooling, and rehydration are standard treatments for patients with increased temperature and rhabdomyolysis.

See also: Benzodiazepines; Catecholamines; Methylenedioxymethamphetamine.

Further Reading

Baselt C and Cravey RH (1989) *Disposition of Toxic Drugs and Chemicals in Man*, 3rd edn., pp. 49–52. Chicago: Year Book Medical Publishers.

Kraemer T and Maurer HH (2002) Toxicokinetics of amphetamines: Metabolism and toxicokinetic data of designer drugs, amphetamine, methamphetamine and their *N*-alkyl derivatives. *Therapeutic Drug Monitoring* 24(2): 277–289.

McCann UD and Ricaurte GA (2004) Amphetamine neurotoxicity: Accomplishments and remaining challenges. *Neuroscience and Biobehavioral Review* 27(8): 821–826.

Amphibians

Prathibha S Rao

Background Information

The class of amphibia contains ~2600 species and is divided into anura, frogs.

Mechanism of Toxicity

The chemical compositions of amphibian toxins are highly diversified. Amphibians secrete substances to prevent desiccation, control the growth of microorganisms on skin, and discourage predators. These secretions have cytotoxic and/or hemolyzing effects.

Toads

The toxins from Bufo species of toads are venom complexes that have a distinct cardioactive digitalis-like action. Toxic signs include profuse salivation with pulmonary edema, cardiac arrhythmia, hypertension, and prostration. Convulsions and death due to cardiac arrest may occur as early as 15 min after exposure to the toxin. Susceptible populations include children and pet dogs or cats playing with toads.

Salamanders

Tetrodotoxin and additional toxic components are found associated with this group. Toxic effects are noted at 10 mg kg^{-1} body weight. Toxic signs include tingling of the oral cavity with salivation, muscle weakness, motor incoordination, skin numbness,

vomiting, diarrhea, and generalized paralysis with convulsions and death in severe cases.

Clinical Management

The victim's mouth should be washed out with copious amounts of water, atropine should be administered to control salivation. Barbiturates are used to control convulsions; calcium gluconate may be used to control some physiologic effects. Phenoxybenzamine and propranolol have been used experimentally to block α- and β-adrenergic receptors. Life-support therapy may be used to maintain respiration and other vital functions.

See also: Tetrodotoxin.

Further Reading

Daly JW (2004) Marine toxins and nonmarine toxins: Convergence or symbiotic organisms? *Journal of Natural Products* 67: 1211–1215.

Karalliedde L (1995) Animal toxins. *British Journal of Anaesthesia* 74: 319–327.

Relevant Websites

http://tolweb.org – The Tree of Life Web Project
http://www.livingunderworld.org.

Amaranth *See* Red Dye No. 2.

Amyl Nitrite

Michael Wahl

This article is a revision of the previous print edition article by Prathibha S Rao and Harihara M Mehendale, volume 1, pp. 65–66, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 110-46-3
- Chemical/Pharmaceutical/Other Class: Vasodilator
- Chemical Formula: $C_5H_{11}NO_2$
- Chemical Structure:

$$CH_3–CH–(CH)_3–CH_2–CH_2–O–NO$$

Uses

Amyl nitrite is a vasodilator that acts by relaxing vascular smooth muscle. It is also a component of the Taylor Cyanide Antidote Kit.

Exposure Routes and Pathways

Inhalation is the most common route of exposure.

Toxicokinetics

Amyl nitrite is a volatile liquid and is rapidly absorbed from the lungs. It is rapidly hydrolyzed to nitrite ion and the corresponding alcohol. About 60% of the ion is metabolized by the body. About 40% of the nitrites are excreted unchanged in the urine.

Mechanism of Toxicity

Nitrites bind to hemoglobin causing oxidation of hemoglobin to methemoglobin, which is unable to transport oxygen. When methemoglobinemia exceeds 10–15%, cyanosis may become apparent.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure of amyl nitrite in some animals may result in increases in intraocular pressure.

Human

Amyl nitrite causes methemoglobinemia, unconsciousness, dizziness, fatigue, shortness of breath, nausea, and vomiting. The skin is initially warm and sweaty and later becomes cold due to vasodilation. It causes coronary blood vessel contraction, bradycardia, atrial fibrillation, cardiac ischemia, headache, convulsions, and diarrhea.

Nitrite transferred through breast milk can cause methemoglobinemia in infants. Infants are at greater risk for the development of nitrite-related toxicity than adults due to their decreased ability to secrete gastric acid, higher levels of fatal hemoglobin, and diminished enzymatic capability to reduce methemoglobin to hemoglobin. Nitrites can combine with secondary amines to form dimethylnitrosamines, which are acutely toxic to the liver and cause

centrilobular necrosis, fibrous occlusion of central veins, and pleural and peritoneal hemorrhages in animals.

Chronic Toxicity (or Exposure)

Human

Chronic ingestion of $5\,mg\,kg^{-1}\,day^{-1}$ is considered unacceptable. Common findings associated with nitrite poisoning include unconsciousness, dizziness, fatigue, shortness of breath, nausea, vomiting, coma, cyanosis, dyspnea, and pallor.

Clinical Management

For substantial recent ingestions, activated charcoal may be considered in the emergency department. Methylene blue may be used to treat patients that develop significant methemoglobinemia. Benzodiazepines may be used to control seizures. Recurrent seizures may require use of phenobarbital. Dopamine or norepinephrine can be administered to control hypotension. Aggressive supportive therapy should be instituted for all symptomatic patients.

See also: Benzodiazepines; Cyanide; Liver.

Further Reading

Edwards RJ and Ujma J (1995) Extreme methemoglobinemia secondary to recreational use of amyl nitrite. *Journal of Accident and Emergency Medicine* 12: 138–142.

Haverkos HW and Dougherty J (1988) Health hazards of nitrite inhalants. *American Journal of Medicine* 84: 479–482.

Anabolic Steroids

Sharmilee P Sawant and Harihara M Mehendale

Introduction

The synthetic substances related to the male sex hormones (androgens) are called 'anabolic steroids'. The principal effects of these substances are to promote the growth of skeletal muscle (anabolic effects) and the development of male sexual characteristics (androgenic effects).

Developed in the late 1930s, anabolic steroids were primarily used to treat hypogonadism, a condition in which the testes do not produce sufficient testosterone for normal growth, development, and sexual functioning. These compounds are used medically to treat delayed puberty, some types of impotence, and wasting of the body caused by HIV infection or other diseases. Some of the commonly used/abused anabolic steroids are: Anadrol (oxymetholone), Oxandrin (oxandrolone), Dianabol (methandrostenolone), Deco-Durabolin (nandrolone decanoate), and Depa-Testosterone (testosterone cypionate).

During the 1930s, experiments in laboratory animals revealed that anabolic steroids facilitate the growth of skeletal muscles. This discovery resulted in anabolic steroids being used by athletes, particularly bodybuilders and weightlifters. Steroid abuse has become so widespread in athletics that it is almost assumed that leading contestants use either classic anabolic steroids or new designer drugs. The outcome of sports contests is therefore most often affected by the use/abuse of anabolic steroids.

Since their discovery, more than 100 different anabolic steroids have been developed. A prescription is required for the legal use of these compounds in the United States, though supplements such as dehydroepiandrosterone and androstenedione (street name 'Andro') can be purchased legally without a prescription through many commercial sources including health food stores. They are often referred to as dietary supplements, although they are not food products. They are often taken because the user believes they have anabolic effects. In 2003, the Food and Drug Administration (FDA) became aware of a substance called tetrahydrogestrinone (THG), which is illegally used by athletes to improve their performance. Based on its analysis of this product, FDA has determined that THG is an unapproved new drug. As such, it cannot be legally marketed without FDA approval. A number of so-called 'designer drugs' are emerging and are used for performance enhancement, with THG being one example. Even as regulating agencies such as FDA and sports authorities develop ways of detecting and monitoring drug use/abuse, new designer drugs appear before ways of detecting them and monitoring can be discovered.

It is believed that steroidal supplements get converted into testosterone (male sex hormone) or a similar compound in the body. Whether such conversion produces sufficient quantities of testosterone to promote muscle growth or whether the supplements

themselves promote muscle growth is unknown. Little is known about the side effects of steroidal supplements, but if large quantities of these compounds substantially increase testosterone levels in the body, they also are likely to produce the same side effects as anabolic steroids.

Anabolic Steroids Abuse

A driving motivation to abusing steroids is to improve their performance in sports. Steroid abuse is very high among competitive bodybuilders. The incidence of abuse probably varies among other athletes, depending on the specific sport. People suffering from behavioral syndrome (muscle dysmorphia), in which they have a distorted image of his or her body, use steroids to increase muscle size and/or reduce body fat. Men with this condition perceive themselves as small and weak, even if they are large and muscular. Similarly, women with the syndrome perceive themselves as fat and flabby, even though they are actually lean and muscular.

Some people who abuse steroids to boost muscle size have experienced physical or sexual abuse. They attempt to increase their muscle size to protect themselves. In one series of interviews with male weightlifters, 25% of steroid abusers reported memories of childhood physical or sexual abuse, as compared with none of the nonabusers. In a study of women weightlifters, twice as many of those who had been raped reported using anabolic steroids and/or another purported muscle-building drugs and markedly increased bodybuilding activities after the attack, compared to those who had not been raped. They believed that being bigger and stronger would discourage further attacks because men would find them either intimidating or unattractive.

Finally, some adolescents abuse steroids as part of a pattern of high-risk behaviors. These adolescents also take risks such as drinking and driving, carrying a gun, not wearing a helmet on a motorcycle, and abusing other illicit drugs.

While conditions such as muscle dysmorphia, a history of physical or sexual abuse, or engaging in high-risk behaviors may increase steroid abuse, researchers concur that most steroid abusers are psychologically normal when they start abusing the drugs.

Human Toxicity

Anabolic steroid abuse has been associated with a wide range of adverse side effects. Health consequences associated with anabolic steroid abuse include:

- *Disruption of hormonal system:* In boys and men, reduced sperm production, shrinking of the testicles, impotence, and irreversible breast enlargement are found to occur. In girls and women, decreased body fat and breast size, deepening of the voice, growth of excessive body hair, loss of scalp hair, changes in or cessation of the menstrual cycle, and clitoral enlargement are commonly found.
- *Musculoskeletal system effects:* Premature and permanent retardation of growth among adolescents of both sexes.
- *Cardiovascular effects:* Heart attacks and strokes.
- *Liver diseases:* Potentially fatal cysts and liver cancer.
- *Skin disorders:* Acne and cysts.
- *Infections:* In steroid abusers who use parentral routes for drug administrations, HIV/AIDS, hepatitis B and C, and infective endocarditis, a potentially fatal inflammation of the inner lining of the heart.
- *Behavioral effects:* Aggressive behavior, particularly when high doses are taken. Depression, mood swings, fatigue, restlessness, and loss of appetite when steroid abuse is stopped.

Addiction

A high percentage of steroid abusers become addicted to the drugs, as evidenced by their continuous use of steroids in spite of physical problems, negative effects on social relations, or nervousness and irritability. Also, they spend lot of time and money obtaining the drugs and experience withdrawal symptoms such as mood swings, fatigue, restlessness, loss of appetite, insomnia, reduced sex drive, and the desire to take more steroids. The most dangerous of the withdrawal symptoms is depression, because it sometimes leads to suicide attempts. If left untreated, some depressive symptoms associated with anabolic steroid withdrawal have been known to persist for a year or more after the abuser stops taking the drugs.

Early measures to prevent steroid abuse depend on the strategy of drug testing and educating students about the adverse effects of anabolic steroids. A few school districts test for abuse of illicit drugs, including steroids, and studies are currently under way to determine whether such testing reduces drug abuse.

Research on steroid educational programs has shown that simply teaching students about steroid's adverse effects does not convince them that they personally can be adversely affected. Nor does such instruction discourage young people from taking

steroids in the future. Presenting both the risks and benefits of anabolic steroid use is more effective in convincing adolescents about steroid's negative effects, apparently because the students find a balanced approach more credible and less biased, according to the researchers. However, the balanced approach still does not deter adolescents from abusing steroids.

A more sophisticated approach has shown promise for preventing steroid abuse among players on high school sports teams. In the Athletes Training and Learning to Avoid Steroids (ATLAS) program, developed for male football players, coaches and team leaders discuss the potential effects of anabolic steroids and other illicit drugs on immediate sports performance, and they teach how to refuse offers of drugs. They also discuss how strength training and proper nutrition can help adolescents build their bodies without the use of steroids. Later, special trainers teach the players proper weightlifting techniques. An ongoing series of studies has shown that this multicomponent, team-centered approach reduces new steroid abuse by 50%. A program designed for adolescent girls on sports teams, patterned after the program designed for boys, is currently being tested.

Clinical Management

Current knowledge is based largely on the experiences of a small number of physicians who have worked with patients undergoing steroid withdrawal. The physicians have found that supportive therapy is sufficient in some cases. Patients are educated about what they may experience during withdrawal and are evaluated for suicidal thoughts.

If symptoms are severe or prolonged, medications or hospitalization may be needed.

Some medications that have been used for treating steroid withdrawal restore the hormonal system after its disruption by steroid abuse. Other medications target specific withdrawal symptoms; for example, antidepressants to treat depression, and analgesics for head-aches and muscle and joint pains.

Some patients require assistance beyond simple treatment of withdrawal symptoms and are treated with behavioral therapies.

See also: Androgens; Drugs of Abuse.

Further Reading

Brower KJ (1997) Withdrawal from anabolic steroids. *Current Therapy in Endocrinology and Metabolism* 6: 338–343.

Fact sheets on anabolic steroids can be ordered free, by calling NIDA (National Institute of Drug Abuse) Infofax at 1-888-NIH-NIDA (1-888-644-6432).

Goldberg L, MacKinnon DP, Elliot D, *et al.* (2000) The adolescents training and learning to avoid steroids program: Preventing drug use and promoting health behaviors. *Archives of Pediatric and Adolescent Medicine* 154: 332–338.

Porcerelli JH and Sandler BA (1998) Anabolic–androgenic steroid abuse and psychopathology. *Psychiatric Clinics of North America* 21: 829–833.

Shahidi NT (2001) A review of the chemistry, biological action, and clinical applications of anabolic–androgenic steroids. *Clinical Therapeutics* 23(9): 1355–1390.

Relevant Website

http://www.steroidabuse.org – NIDA Steroid Abuse Website.

Analytical Toxicology

Shayne C Gad

Analytical toxicology is the use of qualitative and quantitative chemical and physical techniques used in sample preparation, separation, assay calibration, detection and identification, and quantification for the purposes of toxicological research and testing. Examples of the objectives of such analysis include:

- Determining the levels of exposure to potential toxicants via air, water, or food.
- Verifying exposure levels to doses for animals in experimental studies.
- Determining levels of xenobiotics and their metabolites in animal studies.
- Screening blood and urine for the presence of illicit drugs or their metabolites.
- Measuring levels of endogenous compounds and molecules to evaluate organ function and damage (clinical chemistry).
- Identifying metabolites and macromolecular adjuncts to identify mechanisms of action.

The diagnosis and treatment of health problems induced by chemical substances and the closely allied

field of therapeutic drug monitoring rely on analytic toxicology, and advances in the field have added both power and problems to toxicology, dual gifts of increases in sensitivity and specificity. Although the analytes are present in matrices similar to those seen in forensic toxicology, the results must be reported rapidly to be of use to clinicians in treating patients. This requirement of a rapid turnaround time limits the number of chemicals that can be measured because methods, equipment, and personnel must all be available for an instant response to toxicological emergencies. Investigations for an 'unknown' drug or poison are usually carried out on specimens of urine (30 ml for qualitative tests) and blood (10 ml for quantitative tests). No preservatives should be added to urine specimens and blood samples should be heparinized.

Occupational and regulatory toxicology requires analytic procedures for implementation or monitoring. In occupational toxicology, the analytical methods used to monitor threshold limit values and other means of estimating the exposure of workers to toxic hazards may utilize simple, nonspecific, but economical screening devices. However, to determine the actual exposure of a worker, it is necessary to analyze blood, urine, breath, or another specimen by employing methods similar to those used in clinical or forensic toxicology. For regulatory purposes, a variety of matrices (e.g., food, water, and air) must be examined for extremely small quantities of analytes. Frequently, this requires the use of sophisticated methodology with extreme sensitivity. Both of these applications of analytical toxicology impinge on forensic toxicology because an injury or occupational disease in a worker can result in a legal proceeding.

Other applications of analytical toxicology occur frequently during the course of experimental studies. Confirmation of the concentration of dosing solutions and monitoring of their stability often can be accomplished with the use of simple analytical techniques. The bioavailability of a dose may vary with the route of administration and the vehicle used. Blood concentrations can be monitored as a means of establishing this important parameter. In addition, an important feature in the study of any toxic substance is the characterization of its metabolites as well as the distribution of the parent drug, together with its metabolites, to various tissues. This requires sensitive, specific, and valid analytical procedures. Similar analytic studies can be conducted within a temporal framework to gain an understanding of the dynamics of the absorption, distribution, metabolism, and excretion of toxic chemicals.

Analysis of Common Toxic Substances

Analytical toxicology is intimately involved in many aspects of experimental and applied toxicology. Since toxic substances include all chemical types and because their measurement may require the examination of biological or nonbiological matrices, the scope of analytical toxicology is broad. Nevertheless, a systematic approach and a reliance on the practical experience of generations of forensic toxicologists can be used in conjunction with the sophisticated tools of analytical chemistry to provide the data needed to understand the hazards of toxic substances more completely. "All substances are poisons: There is none which is not a poison." Analytical toxicology potentially encompasses all chemical substances. Forensic toxicologists learned long ago that when the nature of a suspected poison is unknown, a systematic, standardized approach must be used to identify the presence of most common toxic substances. An approach that has stood the test of time was first suggested by Chapuis in 1873 in *Elements de Toxicologie*. It is based on the origin or nature of the toxic agent. Such a categorization can be characterized as follows:

1. gases,
2. volatile substances,
3. corrosives,
4. metals,
5. anions and nonmetals,
6. nonvolatile organic substances, and
7. miscellaneous.

In addition to considering the descriptive classification of the substance, one must determine the method for separating a toxic agent from the matrix in which it is embedded. The matrix is generally a biological specimen such as a body fluid or a solid tissue. The agent of interest may exist in the matrix in a simple solution or may be bound to protein and other cellular constituents. The challenge here is to separate the toxic agent in sufficient purity and quantity to permit it to be characterized and quantified. At times, the parent compound is no longer present in large enough amounts to be separated. In such cases, known metabolites may indirectly provide measure of the parent substance. With other substances, interaction of the poison with tissue components may require the isolation or characterization of a protein adduct. Methods for separation have long provided a great challenge to analytical toxicologists. Only recently have methods become available which permit direct measurement of some analytes without prior separation from the matrix.

The following sections provide a closer look at analytical toxicological issues related to substance class.

Gases

Gases are most simply measured by means of gas chromatography. Some gases are extremely labile, and the specimen must be collected and preserved at temperatures as low as that of liquid nitrogen. Generally, the gas is carefully liberated by incubating the specimen at a predetermined temperature in a closed container. The gas, freed from the matrix, collects over the specimen's 'headspace', where it can be sampled and injected into the gas chromatograph. Other gases, such as carbon monoxide, interact with protein, or the adduct can be measured independently, as in the case of carboxyhemoglobin.

Volatile Substances

Volatile substances are generally liquids of a variety of chemical types. Gas–liquid chromatography is the simplest approach for simultaneous separation and quantitation in many cases. The simple alcohols can be measured by injecting a diluted body fluid directly onto the column of the chromatograph. A more common approach is to use the headspace technique, as is done for gases, after incubating the specimen at an elevated temperature.

Corrosives

Corrosives include mineral acids and bases. Many corrosives consist of ions that are normal tissue constituents. Clinical chemical techniques can be applied to detect these ions when they are in great excess over normal concentrations. Because these ions are normal constituents, the corrosive effects at the site of contact of the chemical, together with other changes in blood chemistry values, can confirm the ingestion of a corrosive substance.

Metals

Metals are encountered frequently as occupational and environmental hazards. Elegant analytic methods are available for most metals even when they are present at extremely low concentrations. Classical separation procedures involve destruction of the organic matrix by chemical or thermal oxidation. This leaves the metal to be identified and quantified in the inorganic residue. Unfortunately, this prevents a determination of the metal in the oxidation state or in combination with other elements, as it existed when the metal compound was absorbed. For example, the toxic effects of metallic mercury, mercurous ion, mercuric ion, and dimethyl mercury are all different. Analytical methods must be selected which determine the relative amount of each form present to yield optimal analytical results.

Toxic Anions and Nonmetals

Toxic anions and nonmetals are a difficult group for analysis. Some anions can be trapped in combination with a stable cation, after which the organic matrix can be destroyed, as with metals. Others can be separated from the bulk of the matrix by dialysis, after they are detected by colorimetric or chromatopathic procedures. Still others are detected and measured by ion-specific electrodes. There are no standard approaches for this group, and other than phosphorus, they are rarely encountered in an uncombined form.

Nonvolatile Organic Substances

Nonvolatile organic substances constitute the largest group of substances which must be considered by analytical toxicologists. This group includes drugs, both prescribed and illegal, pesticides, natural products, pollutants, and industrial compounds. These substances are solids of liquids with high boiling points. Thus, separation procedures generally rely on differential extractions, either liquid–liquid or solid–solid in nature. These extractions often are not efficient, and recovery of the toxic substance from the sample matrix may be poor. When the nature of the toxic substance is known, immunoassay procedures are useful because they allow a toxicologist to avoid using separation procedures. Such compounds can be classified as organic strong acids, organic weak acids, organic bases, organic neutral compounds, or organic amphoteric compounds.

Miscellaneous

Finally, a miscellaneous category must be included to cover the large number of toxic agents that cannot be detected by the routine application of the methods described previously. Venoms and other toxic mixtures of proteins or uncharacterized constituents fall into this class. Frequently, if antibodies can be grown against the active constituent, immunoassay may be the most practical means of detecting and measuring these highly potent and difficult to isolate substances. Unfortunately, unless highly specific monoclonal antibodies are used, the analytic procedure may not be acceptable for forensic purposes. Frequently, specific analytic procedures must be developed for each

analyte of this type. At times, biological endpoints are utilized to semiquantify the concentration of the isolated product.

Analytical Techniques

Due to increased levels of sensitivity of analytical techniques and a range of legal requirements (including Good Laboratory Practices and issues in potential litigation), particular care must be taken in collecting and handling samples to both avoid contamination and maintain a chain of custody of samples and sample records. There are a vast variety of techniques now employed in analysis, as outlined below.

- Chromatography
  - Thin layer, gas, high-performance liquid (HPLC)
- Mass spectrophotomology
- Photometry/spectroscopy
- Spectrophotomology (ultraviolet, infrared, and visible light)
  - Flame photometry, atomic absorption, nuclear magnetic resonance (NMR) spectroscopy, electron spin resonance (ESR) spectrophotometry, Raman spectroscopy
- Immunoassays
  - Radioimmunoassay (RIA), enzyme immunoassay (EIZ), fluorescent immunoassay (FIA)
- Isotopic labeling
  - Positron emission tomography (PET)
- Magnetic resonance imaging (MRI)

Newer and more complex material analysis techniques are:

- Atomic absorption spectroscopy
- Auger electron spectroscopy
- Controlled potential coulometry
- Crystallographic texture measurement
- Electrogravimetry
- Electrometric titration
- Electron probe X-ray microanalysis
- Elemental and functional group analysis
- Extended X-ray absorption fine structure
- Ferromagnetic resonance
- Field ion microscopy
- High temperature combustion
- Image analysis
- Inert gas fusion
- Inductively coupled plasma
- Ion chromatography
- Low energy electron diffraction
- Low energy ion scattering spectroscopy
- Mass spectrometry
- Molecular fluorescence spectrometry
- Mossbauer spectroscopy
- Neutron activation analysis
- Neutron diffraction
- Optical emission spectroscopy
- Optical metallography membrane electrodes
- Particle induced X-ray emission potentiometric
- Radial distribution function analysis
- Radio analysis
- Rutherford backscattering spectroscopy
- Scanning electron microscopy
- Secondary ion mass spectroscopy
- Single crystal X-ray diffraction
- Small angle X-ray and neutron scattering
- Spark source mass spectrometry
- Transmission electron microscopy
- Voltametry
- Wet analytical chemistry
- X-ray diffraction residual stress techniques
- X-ray photoelectron spectroscopy
- X-ray powder diffraction
- X-ray spectrometry
- X-ray topography

See also: International Society of Exposure Analysis; Microarray Analysis.

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Ancient Warfare and Toxicology

Adrienne Mayor

In antiquity, natural toxins were exploited to make poison weapons to wage the earliest forms of biological and chemical warfare. A wide range of substances, from toxic plants and venomous insects and reptiles to infectious agents and noxious chemicals, were weaponized in ancient Europe, the Mediterranean, North Africa, the Middle East, Central Asia, India, China, and in the Americas. Evidence for the concept and practice of toxic warfare can be traced back thousands of years. For example, cuneiform tablets from about 1200 BC record that the Hittites of Asia Minor deliberately drove plague victims into enemy territory.

Such practices did not require a scientific understanding of toxicology, epidemiology, and chemistry, or depend on advanced technology, but were based on centuries of observation and experimentation with easily available toxic materials. Strategies based on insidiously attacking an opponent's biological vulnerabilities with poison agents could give an advantage when facing troops superior in numbers, courage, skill, or technology. Yet the use of toxic weapons also entailed practical and ethical dilemmas in antiquity.

The first poison projectiles were probably first devised for hunting and then turned toward war. The bow and arrow was a highly effective delivery system for toxins at an early date, since a mere scratch from a treated point could be fatal.

Toxic Weapons in Mythology

The concept of poisoned projectiles is embedded in the ancient Greek language, since the word for 'poison', *toxicon*, derived from *toxon*, the word for 'arrow'. Greek mythology offers further evidence of the antiquity of the concept. The great hero of myth, Hercules, for example, invented biological weaponry when he dipped his arrows in the venom of the Hydra monster, a many-headed serpent. Homer's *Iliad*, an oral epic first written down in the eighth century BC, contains indirect allusions to the use of toxic projectiles in the legendary Trojan War. Homer's descriptions of black (rather than red) blood oozing from wounds, battlefield doctors sucking out poisons, and never-healing wounds, are all hallmarks of snake venom poisoning. In his poem the *Odyssey*, Homer clearly describes the Greek hero Odysseus smearing lethal plant juices on arrows intended for enemies. According to myth, Odysseus himself died from a wound inflicted by a spear tipped with the toxic spine of a marbled stingray, a common species in the Mediterranean. Notably, spears tipped with stingray spines are used by natives in South America.

The epic poem recounting the legendary history of Rome, the *Aeneid* by Virgil, also refers to poisoned spears wielded by the early Romans, and poisoned weapons appear in the mythological epic of India, the *Rigveda*. Myth and legend likely reflect the original invention of biological arms in various cultures and they also offered models for the actual practice of biowar.

Plant Poisons in Warfare

About two-dozen toxic Eurasian plant species, often employed as medicines in minute dosages, were gathered to make arrow poisons or other biological weapons in the ancient world. One of the most popular plant drugs was hellebore, identified by the ancients as black hellebore (probably the Christmas rose of the buttercup Ranunculaceae family, *Helleborus niger*) and white hellebore (the lily family, *Liliaceae*). The unrelated plants are each laden with powerful chemicals that cause severe vomiting and diarrhea, muscle cramps, delirium, convulsions, asphyxia, and heart attack. Hellebore was one of the arrow drugs used by the Gauls, among other ancient groups, and it was also used to poison wells.

Another favorite biowar toxin was aconite or monkshood (also called wolfsbane). *Aconitum* (buttercup family) contains the alkaloid aconitine, a violent poison, which in high doses causes vomiting and paralyzes the nervous system, resulting in death. Aconite was employed by the archers of ancient Greece and India, and its use in warfare continued into modernity. For example, during the war between the Spanish and the Moors in 1483, Arab archers wrapped aconite-soaked cotton around their arrowheads. Nepalese Gurkhas poisoned wells with aconite in the nineteenth century, and during World War II, Nazi scientists created aconitine-treated bullets.

Henbane (*Hyoscyamine niger*), a sticky, bad-smelling weed containing the powerful narcotics hyoscyamine and scopolamine, was also collected as arrow poison in antiquity. Henbane causes violent seizures, psychosis, and death. Other plant juices used on projectiles included hemlock (*Conium maculatum*), yew (*Taxus*), rhododendron, and several species of deadly nightshade or belladonna, which causes vertigo, extreme agitation, coma, and death. The

fact that the Latin word for deadly nightshade was *dorycnion*, 'spear drug', suggests that it was smeared on weapons at a very early date, as noted by Pliny the Elder, a natural historian of the first century AD.

Snake Venom Arrow Poisons

Snake venom was another well-known arrow poison. Since snake venom is digestible, it could be safely used for hunting because the venom did not make game harmful to eat, but venom in the bloodstream of an enemy brought a painful death or a never-healing wound. Numerous poison snakes exist around the Mediterranean and in Africa and Asia. According to Greek and Roman writers, archers who steeped their arrows in serpents' venom included the Gauls, the Dacians and Dalmatians (of the Balkans), the Sarmatians of Persia (now Iran), the Getae of Thrace, Slavs, Armenians, Parthians between the Indus and Euphrates, Indians, North Africans, and the Scythian nomads of the Central Asian steppes. According to the ancient Greek geographer Strabo, the arrow poison concocted by the Soanes of the Caucasus was so noxious that its mere odor was injurious. Strabo also reported that people of what is now Kenya dipped their arrows "in the gall of serpents," while the Roman historian Silius Italicus described the snake venom arrows used by the archers of Libya, Morocco, Egypt, and Sudan. Ancient Chinese sources show that arrow poisons were also in use in China at early dates. In the Americas, Native Americans used snake, frog, and plant poisons on projectiles for hunting and warfare.

Complex recipes for envenomed arrows are recorded in Greek and Latin texts. One of the most dreaded arrow drugs was concocted by the Scythians, who combined snake venom and bacteriological agents from rotting dung, human blood, and putrefying viper carcasses bloated with feces. Even in the case of a superficial arrow wound, the toxins would begin taking effect within an hour. Envenomation accompanied by shock, necrosis, and suppuration of the wound would be followed by gangrene and tetanus and an agonizing death.

Several snake species contributed the venom used by the Scythians, including the steppe viper *Vipera ursinii renardi*, the Caucasus viper *Vipera kasnakovi*, the European adder *Vipera berus*, and the long-nosed or sand viper *Vipera ammodytes transcaucasiana*. In ancient India, one of the most feared poisons was derived from the rotting flesh and venom of the white-headed Purple Snake, described by the natural historian Aelian (third century AD). His detailed description suggests that the Purple Snake was the rare, white-headed viper discovered by modern herpetologists in the late 1880s, *Azemiops feae*.

A different snake venom tipped the arrows encountered by the army of Alexander the Great in his conquest of India in 327–325 BC. According to the historians Quintus Curtius, Diodorus of Sicily, and others, the Harmatelians (of what is now Mansura, Pakistan) had smeared their arrows and swords with an unknown snake poison. Most modern historians assume the Harmatelians used cobra poison, but the ancient historians' detailed description of the gruesome deaths suffered by Alexander's men points to the deadly Russell's viper. Even the slightly wounded went immediately numb and experienced stabbing pain and wracking convulsions. Their skin became cold and livid and they vomited bile. Black froth exuded from the wounds and then purple-green gangrene spread rapidly, followed by death. Death from cobra venom is relatively painless, from respiratory paralysis, but the Russell's viper causes numbness, vomiting, severe pain, black blood, gangrene, and death – as described by Alexander's historians.

Poisoning Water and Food

Tainting water and food was another ancient biological tactic. A legendary Greek account set in about 1000 BC tells how King Cnopus conquered Erythrae (in what is now Turkey) by drugging a bull and tricking the enemy into eating the poisoned meat. The earliest historically documented case of poisoning drinking water occurred in Greece in about 590 BC, during the First Sacred War. Athens and allied city-states made war on the strongly fortified city of Kirrha, which controlled the road to Delphi, the site of the famous Oracle of Apollo. According to several ancient Greek historians, Kirrha had offended the god and was therefore to be totally destroyed. During the siege, the league of allies gathered a great quantity of hellebore and placed it in the water pipes supplying Kirrha. The soldiers guarding Kirrha's walls – and the entire population – fell violently ill and the allies easily overran the city and wiped out combatants and civilians alike. After the war, Athens and her allies had second thoughts and agreed among themselves not to interfere with water supplies should they ever find themselves at war with each other.

Roman commanders also poisoned wells. Manius Aquillius, for example, ended a long-drawn-out war to quell insurrections in the Roman province of Asia Minor in 129 BC, by pouring poison into the springs supplying the rebelling cities. According to the Roman historian Florus, however, his victory was

dishonorable because of the resort to underhanded biological tactics.

Carthaginian generals, such as Himilco and Maharbal, overcame enemies in North Africa by tainting wine with mandrake, a heavily narcotic root of the deadly nightshade. In Europe, the Celts were known to drug their foes' food and wine with plant poisons. In North America, Native Americans poisoned enemy drinking water with rotting animal skins. In ancient India, numerous recipes for poisoning enemy food and water are given in the *Arthashastra*, a warfare manual dating to the fourth century BC, written by Kautilya, the advisor of King Chandragupta.

In 65 BC, naturally occurring toxic honey was used against the army of the Roman general Pompey during the war against King Mithridates VI of Pontus. In the Black Sea region, Mithridates' allies of set out tempting honeycombs along the Romans' route and hid. The honey was made by wild bees that gathered nectar from rhododendron blossoms, which contain devastating neurotoxins. As the legionnaires succumbed to the sweet treat, collapsing with vertigo, vomiting, and diarrhea, the enemy arrived to slaughter 1000 of Pompey's men.

Stinging Insects and Biting Snakes

Stinging insects such as wasps, deadly vipers, and scorpions could also be drafted for war. Perhaps as early as Neolithic times, hives filled with furious bees were thrown at enemies, who were driven into chaos by the painful stings; later, catapults were used to hurl beehives. The ancient Maya of Central Mexico created ingenious boobytraps to repel besiegers on their fortress walls, consisting of dummy warriors whose gourd heads were filled with hornets.

In the second century BC, the Carthaginian general Hannibal devised a plan of filling clay pots with live vipers during a naval battle in which he was outnumbered by ships commanded by Pergamum, a city on the coast of Turkey. The enemy sailors were routed when the catapulted pots smashed on their ships' decks, releasing masses of snakes.

At the fortified city of Hatra (Iraq), in AD 198–199, besieging Roman legions led by the emperor Septimius Severus were forced to retreat after the Hatreni defended their walls with insect bombs. The people of Hatra had packed terracotta pots with scorpions (arthropods), assassin bugs, and other poisonous insects from the surrounding desert. The historian Herodian wrote that as the insects rained down on the Romans scaling the walls, they "fell into the men's eyes and exposed parts of their bodies, digging in, biting, and stinging the soldiers, causing severe injuries." The terror effect would be impressive, no matter how many men were actually stung. Scorpion stings inject a complex combination of toxins, causing intense pain, great agitation and thirst, muscle spasms, convulsions, slow pulse, irregular breathing, and torturous death. Assassin bugs, predatory, bloodsucking insects with sharp beaks, inflict an extremely painful bite and inject a lethal nerve poison that liquefies tissues. It is possible that *Paederus* beetles were also collected by the Hatreni. Pederin, the virulent poison secreted by the predatory Staphylinidae (rove) beetles was well known in ancient India and China. One of the most powerful animal toxins in the world, pederin is a blistering agent on the skin and eyes, and in the bloodstream its toxicity is more potent than cobra venom.

Contagion as a Weapon

Many historians have considered the Mongols' ploy of catapulting of bubonic plague victims over the walls at Kaffa on the Black Sea in 1346 to mark the beginning of biological warfare. But an empirical understanding of contagion developed much earlier in history. In Mesopotamia in 1770 BC, for example, cuneiform tablets warned that disease could be spread by fomites, infectious pathogens on clothing, bedding, other items. Legends about King Solomon suggested that he hid plague in sealed jars in the Temple of Jerusalem to infect Babylonian and Roman invaders. During the Peloponnesian War (fourth century BC), the Athenians suspected that the Spartans had spread plague (apparently smallpox) by poisoning their wells. In the first century BC, King Mithridates was forced to withdraw from his siege of a city near the Black Sea after corpses thrown out in the area fatally infected his troops. In ancient India, Kautilya's *Arthashastra* suggested ways of infecting enemies with illnesses such as fevers, wasting lung disease, and rabies.

In Roman times, historians such as Seneca and Dio Cassius deplored "man-made pestilence," the malicious transmission of plagues by saboteurs who pricked victims with infected needles during the reigns of Domitian and Commodus in the first and second centuries AD. The Great Plague of AD 165–180, probably smallpox, was spread from Babylon (modern Iraq) to Syria, Italy, and Germany by Roman soldiers returning from the war to control Mesopotamia. According to historians of the era, the epidemic began when some Roman soldiers looted a treasure chest in an enemy temple in Babylonia. The implication of the historical accounts, that the chest was boobytrapped with plague-laden items, is plausible. The local population would have had some immunity to the epidemic while the invading Roman

army would have been vulnerable. At the very least, the reports demonstrate that the notion of deliberately spreading epidemics among the enemy was widely contemplated by that time.

Toxic Aerosols and Incendiaries

Asphyxiating clouds of smoke, dust, and gases were effective chemical weapons in antiquity. One of the earliest documented examples of toxic aerosols occurred during the Peloponnesian War in 429 BC, when Sparta besieged the city of Plataia, an ally of Athens. As reported by the historian Thucydides, the Spartans created a massive fire next to Plataia's city walls, and fueled the conflagration with liberal quantities of resinous pinetree sap and sulfur. The combination of pitch and sulfur created clouds of toxic sulfur dioxide gas, fumes that can be fatal when inhaled in large amounts. A few years later, in 424 BC, the Spartans' allies the Boiotians invented a 'flame-throwing' machine to propel noxious smoke from charcoal, resin, and sulfur used against the walled city of Delium.

The Greek strategist Aeneas the Tactician, writing in 360 BC, suggested the use of incendiaries made with pitch, hemp, and sulfur. Roman historians tell how burning chicken feathers created irritating, choking fumes propelled by bellows into siege tunnels.

In 80 BC, the Roman general Sertorius deployed choking clouds of dust to defeat the Characitani of Spain, who had taken refuge in inaccessible caves. The fine white soil in the area consisted of limestone and gypsum. Sertorius ordered his soldiers to pile great heaps of the powder in front of the caves. When the wind was right, the Romans stirred up the dust and raised great clouds of caustic lime powder, a severe irritant to the eyes and lungs. The Characitani surrendered.

A similar dust was used in China to quell an armed peasant revolt in AD 178, when 'lime chariots' equipped with bellows blew limestone powder into the crowds. The powdered lime interacts with the moist membranes of the eyes, nose, and throat with corrosive, burning effect, blinding and suffocating the victims.

In the Middle East, where petroleum is abundant, naphtha (the volatile and toxic light fraction of oil) was ignited and poured on attackers. The ancient Indians and Chinese added 'fire chemicals' to their incendiaries, explosive saltpeter or nitrite salts, a key ingredient of gunpowder, and they also mixed a great variety of plant, animal, and mineral poisons, such as arsenic and lead, in smoke and fire bombs. In the New World and in India, the seeds of toxic plants and hot peppers were burned to rout attackers.

Practical and Ethical Issues

The toxicity of plants, venoms, and other poisons used in armaments posed perils to those who wielded them, and the mythology and the history of poison weapons is rife with examples of accidental self-injury and unintended collateral damage. The use of windborne toxins also involved 'blowback' problems, as acknowledged by Kautilya in his *Arthashastra*. He cautioned that protective salves and other remedies must be applied before deploying poisonous smokes. Toxic weapons are notoriously difficult to control and often resulted in the destruction of noncombatants as well as soldiers, especially in siege situations.

The use of poisons in warfare led to a search for antidotes. Ancient sources list hundreds of substances believed to counteract specific weaponized poisons, from rust filings to poultices made from medicinal plants. It was also believed that one could become invulnerable to toxins by ingesting minute amounts of various poisons over time. King Mithridates VI of Pontus (d. 63 BC) was an early experimenter in creating a 'universal antidote', later known as *mithridatium* and ingested by Roman emperors such as Nero and Marcus Aurelius to gain immunity to poisoning.

The use of toxic weapons was surrounded by ambivalence in antiquity, although there were few rules of war governing their use. Weapons that delivered hidden poisons to make an enemy defenseless or experience excessive suffering aroused moral criticism in many cultures, even as their use was rationalized in numerous recorded instances. Ancient Greeks considered poisoned projectiles a cowardly weapon, for example, yet their most admired heroes, Hercules and Odysseus, resorted to such arms, and well poisoning and toxic aerosols were used in historical Greek conflicts. Poisoned arrows and tainting water and food supplies were deplored by many Romans, yet their generals occasionally turned to such strategies. The Hindu *Laws of Manu* (dating to ~500 BC) recommended spoiling the enemy's food and water but forbade the use of poisoned arrows. In the same era, Kautilya's *Arthashastra* extolled the advantages of poisoning projectiles, food, and water and asphyxiating foes with chemical and disease-laden clouds of smoke. Notably, Kautilya stressed the deterrent effect of publicizing the horrid ingredients of one's toxic arsenal, a strategy also embraced by the Scythians and others in broadcasting their recipes for poison arrows. Sun Tzu's *Art of War* (~500 BC) praised deceptive terror strategies based on fire and Chinese treatises give myriad recipes for toxic aerosols and incendiaries. On the other hand, humanitarian codes of war in China (~450–200 BC) forbade ruses of war and harming noncombatants.

Self-defense was often a rationale for the use of toxic weapons. Besieged cities and desperate populations overcome by overwhelming invaders turned to biological weapons as a last resort. Some commanders used poisons in frustration to break stalemates or long sieges. Other situations, such as holy wars, quelling rebellions, and fighting people considered 'barbarians', encouraged the indiscriminate use of bio-weapons against entire populations. The threat of horrifying toxic weapons could discourage would-be attackers or bring quick capitulation. Some commanders had no compunctions about using any weapons at hand, and in some cultures poison arrows were the customary weapons in both hunting and warfare.

The scope of human ingenuity in weaponizing natural forces in antiquity is impressive, and many of the ancient examples anticipated, in substance or principle, almost every basic form of biological and chemical weapon known today, from spreading plague to poisoning water. For example, asphyxiating smokes were precursors of mustard and other toxic gases first used in World War I. Red-hot sand catapulted onto Alexander the Great's men in the fourth century BC is analogous to modern thermite bombs of World War II. The burning, adhering effects of ancient petroleum incendiaries were reproduced in the modern invention of napalm so notorious during the Vietnam War. Even the advanced stench and noise weapons, the so-called calmatives in mists or water supplies, and top-secret insect and animal-based weapons being developed by Pentagon scientists all have antecedents in the ancient world. Nor are the dangers of self-injury and disposing of poison weapons anything new. The ancient myth of the Hydra with its ever-proliferating heads is a fitting symbol of the dilemmas of creating toxic arms. Faced with the problem of disposing of the immortal central head of the Hydra, Hercules buried it deep in the ground and placed a huge boulder as a marker over the spot. A similar geological solution is used today to dispose of toxic and nuclear weapons material, with burial deep underground in the deserts of New Mexico and Nevada, necessitating warnings to future generations about the perils of biochemical agents. A model for avoiding the proliferation of toxic weaponry is also found in Greek myth. The archer who inherited Hercules' Hydra-venom arrows had experienced grievous injury from the arrows himself, before he deployed them against the Trojans. After the Trojan War, he dedicated the poison arrows to a temple of Apollo, the god of healing, rather than passing them on to the next generation of warriors.

See also: Animals, Poisonous and Venomous; Chemical Warfare Delivery Systems; Chemical Warfare During WW1; Plants, Poisonous; Toxicology in the Arts, Culture, and Imagination.

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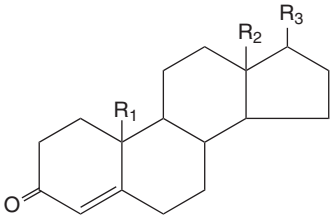
Androgens

Prathibha S Rao and Harihara M Mehendale

- SYNONYMS: Male sex hormones
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Androgens are natural and synthetic congeners of the steroid class of compounds
- CHEMICAL STRUCTURE:



	R_1	R_2	R_3
1. Testosterone	H	H	OH
2. Nandrolone decanoate	H	CH_3	$O-C(=O)-C_9H_{10}$
3. Testosterone propionate	CH_3	CH_3	$O-C(=O)-CH_2CH_3$
4. Ethylestrenol	H	CH_3	$CH(OH)-CH_2CH_3$

Uses

Therapeutic indications for androgens are deficient endocrine functions of the testes, such as hypogonadism,

treatment of refractory anemias in men and women, and hereditary angioneurotic edema. Testosterone has been known to have a palliative effect in some cases of breast cancer and in osteoporosis.

Toxicokinetics

Injected as an oil, androgens are so quickly absorbed, metabolized, and excreted that the effect is very small. Esters of testosterone are more slowly absorbed and are more effective. The majority of the androgens is inactivated primarily in the liver and involves oxidation of the hydroxy groups and reduction of the steroid ring. Alkylation at the 17-position retards hepatic metabolism and hence is effective orally.

About 90% of the androgens are excreted in the urine; 6% appear in the feces after undergoing enterohepatic circulation. Small amounts are also excreted as soluble glucuronide and sulfate conjugates. Many of the synthetic androgens have a longer half-life. Unaltered compounds are excreted in the urine and feces.

Acute and Short-Term Toxicity (or Exposure)

Animal

Six adult male baboons received weekly intramuscular injections of 200 mg testosterone enanthate (equivalent to $8\,mg\,kg^{-1}$ body weight) for up to 28 weeks, while two control animals received weekly injections of the vehicle only. Quantitative increases in the weight and volume of both prostatic lobes were seen after 15 weeks of treatment, and by week 28 there was an increase in stromal tissue with papillary ingrowth or invagination of glandular epithelium in the caudal lobe of the prostate. The serum concentrations of testosterone and dihydrotestosterone were significantly elevated, from 10 and 2–3 ng/ml to 30–40 and 5–6 ng/ml, respectively. The androstenedione concentrations were increased by three to four times and that of estradiol from 20 to 80–90 pg/ml. From this study, it was concluded that these steroids play a direct role in inducing early benign prostate hypertrophy in baboons and that their observations were similar to those in human benign prostate hypertrophy.

Human

Androgens may have a virilizing effect in women. The undesirable manifestations include acne, growth of facial hair, and coarsening of the voice. Profound virilization and serious disturbances in the growth and osseous development can occur when androgens are given to children. The capacity of androgens to enhance epiphyseal closure in children may persist for several months after discontinuation of the drug. All androgens should be used with great care in children. Androgens should not be used during pregnancy since they cross the placenta and cause masculinization of the female fetus. Feminizing effects, particularly gynecomastia, can occur in men who receive androgens. The feminizing effects are particularly severe in children and men with liver disease.

Water retention due to sodium chloride (salt) is a common manifestation that leads to weight gain. Edema is also found in patients with cardiac heart failure, renal insufficiency, liver cirrhosis, and hypoproteinemia. When large doses are used to treat neoplastic diseases, compounds with 17-alkyl substitutions can cause cholestatic hepatitis; at high doses, jaundice is the most common clinical feature with accumulation of bile in the bile capillaries. Jaundice usually develops after 2–5 months of therapy. It can be detected by increases in plasma aspartate aminotransferase and alkaline phosphatase.

Obstructive sleep apnea (OSA) causes a mild lowering of blood testosterone concentrations that is rectified by effective continuous positive airway pressure (CPAP) treatment. Although testosterone treatment has precipitated OSA and has potential adverse effects on sleep in older men, the prevalence of OSA precipitated by testosterone treatment remains unclear. It appears to be a rare idiosyncratic reaction among younger hypogonadal men but the risk may be higher among older men as the background prevalence of OSA rises steeply with age. Hence, screening for OSA by asking about daytime sleepiness and partner reports of loud and irregular snoring, especially among overweight men with large collar size, is wise for older men starting testosterone treatment although not routinely required for young men with classical hypogonadism.

Chronic Toxicity (or Exposure)

Animal

The effects of subcutaneously injected or implanted testosterone and its esters have been reviewed extensively. The working group convened by the International Agency for Research on Cancer (IARC) concluded that: "There is sufficient evidence for the carcinogenicity of testosterone in experimental animals. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard testosterone as if it presented a carcinogenic risk to

humans." The relevance of animal models to human prostate disorders has been reviewed. Besides humans, dogs are the only animals that develop prostatic cancer and benign prostatic hyperplasia at a high frequency. In this model, long-term treatment with androgens and estrogens is required to produce hyperplasia, although such synergism is not observed in other species. ACI rats spontaneously develop histologically evident prostatic cancer, which does not progress to clinically relevant disease when pharmacologically relevant amounts of exogenous androgen are administered. Prostate cancer has been induced only in the Noble and Lobund–Wistar strains of rat.

The role of hormones, including androgens, in the development of mammary neoplasia in rodents and their relevance to human risk assessment has been reviewed. Endogenous androgens are necessary for mammary development in rodents, and it was noted that rodent models mimic some but not all the complex external and endogenous factors involved in initiation, promotion, and progression of carcinogenesis. Tumor type and incidence are influenced by the age, reproductive history, and the endocrine milieu of the host at the time of exposure. The spontaneous incidence of tumors differs in different strains of rats and mice. In rats, most spontaneous neoplasias, with the exception of leukemia, occur in endocrine organs or organs under endocrine control. Russo and Russo concluded that mechanism-based toxicology is not yet sufficient for human risk assessment, and the approach should be coupled to and validated by traditional long-term bioassays.

Fischer 344 rats were given 3,2′-dimethyl-4-aminobiphenyl (a prostate carcinogen) at 50 mg kg^{-1} body weight 10 times at 2-week intervals, and then, from week 20, testosterone propionate and/or diethylstilbestrol by subcutaneous silastic implant for 40 weeks, as seven cycles of 30-day treatment and 10-day withdrawal. Intermittent administration of testosterone resulted in suppression of the development of ventral prostate adenocarcinomas and slight (nonsignificant) increases in the incidences of invasive carcinomas of the lateral prostate and seminal vesicles. Diethylstilbestrol completely suppressed tumorigenesis, and the combination with testosterone propionate inhibited prostate tumor development.

Hydroxyprogesterone caproate was given intramuscularly every other week at an average dose of 13 mg to 19 female rabbits, and testosterone ethanate was given intramuscularly every other week at an average dose of 15 mg to 21 animals; both treatments were given for up to 763 days. Rabbits treated with progesterone developed numerous endometrial cysts, sometimes associated with atypical hyperplasia; active mammary secretion was also seen. Treatment with testosterone induced two adenomatous polyps of the endometrium in one animal, but no other noteworthy endometrial changes were found and one control animal developed similar polyps. Neither significantly altered other tissues such as the ovary, adrenal, thyroid, or pituitary gland. No precancerous endometrial changes or cancers were found.

Human

With prolonged treatment, as in long-term use of androgens in mammary carcinoma, male pattern baldness, excessive body hair, prominent musculature, and hypertrophy of the clitoris may develop and may be irreversible. Patients receiving the 17α-alkyl substituted androgens may develop hepatic adenocarcinoma, the complications may be more common in people with Fanconi's anemia.

Clinical Management

Edema due to salt retention is generally treated with diuretics targeted at increased sodium excretion.

Environmental Fate

Hormones excreted in animal waste have been measured in surface and groundwater associated with manure that is applied to the land surface. Limited studies have been done on the fate and transport of androgenic hormones in soils. There were weak correlations of sorption with soil particle size, organic matter, and specific surface area. Testosterone was the dominant compound present in the soil column effluents, although it was found that testosterone degraded more readily than 17-β-estradiol, it appeared to have a greater potential to migrate in the soil because it was not as strongly sorbed.

Ecotoxicology

The EDMAR program investigated evidence of changes associated with endocrine disruption in marine life and, if so, the possible causes and potential impacts. It followed on from work that demonstrated that flounder in some UK estuaries had changes consistent with endocrine disruption. Male flounder from some industrialized estuaries showed strong vitellogenin induction. Caught sand gobies exhibited no vitellogenin induction or intersex, but feminization of secondary sexual characteristics was observed in male gobies in some estuaries. Viviparous blennies in some estuaries showed induction of vitellogenin, and incidence of intersex. Toxicity identification and

evaluation (TIE) procedures deployed on the Tyne and Tees estuaries identified three natural (steroidal) and two industrial (surfactant and phthalate) estrogenic compounds as possible causes of the observed effects.

A study utilizing fathead minnows was conducted to study the differences in the reproductive biology between groups of minnows from a stream directly below the effluent outfall from a feedlot, from a stream that receives runoff from an agricultural field with disbursed cattle, and from noncontaminated areas upstream from the two previous sample areas. The size, sex hormone levels and gonads of the sampled fish were tested for the effects of trenbolone-β, an active synthetic anabolic steroid. The female fish near the contaminated areas were found to have higher levels of androgens in their systems and smaller distances between internal organs than those from upstream. Similarly, male minnows had smaller testicles and closer internal organs than those from noncontaminated waters. No pathology was apparent in the ovaries or testicles of the fish collected in the contaminated water.

Other Hazards

For men and women, the use of male steroids (androgens) – either the hormone testosterone or the synthetic anabolic steroids – may also increase the risk of coronary artery disease. These drugs lower high-density lipoprotein (HDL) (the good) cholesterol levels, increase low-density lipoprotein (LDL) (the bad) cholesterol levels, and cause high blood pressure. All of these effects may contribute to having a heart attack at an early age or to having a stroke. What effects the use of anabolic steroids early in life has later in life are unclear.

Although mind-altering drugs typically are those that have potential for abuse, several other drugs that do not alter the mind (or do so only occasionally) are often taken without medical need, even when doing so endangers the quality of life or health and safety of the user. Using a drug this way is considered drug abuse. People who stop abusing any of these drugs do not experience withdrawal symptoms, but they may experience medical problems when the drug is discontinued abruptly (problems that are usually preventable if discontinuation is supervised by a doctor). Anabolic steroids are very similar to the hormone testosterone. They have many physical effects on the body, including muscle growth and increased strength as well as increased energy level. Thus, anabolic steroids are often abused to gain a competitive edge in sports. Users are often athletes, typically football players, wrestlers, or weight lifters, and almost all users are male. Many side effects are associated with the abuse of anabolic steroids. Very high doses of anabolic steroids may cause erratic mood swings, irrational behavior, and increased aggressiveness (often called steroid rage). Anabolic steroids can damage the liver and cause jaundice. Regular use of any amount also tends to increase body hair. Acne commonly gets worse with anabolic steroid use and is one of the few side effects for which an adolescent may visit a doctor. Laboratory tests can measure anabolic steroid breakdown products in the urine.

Up to 6% of boys in high school, including a number of nonathletes, have used anabolic steroids at least once. A particular problem with anabolic steroid use in adolescents is early closure of the growth plates at the ends of bones, resulting in permanent short stature. Other side effects are common to both adolescents and adults.

See also: Endocrine System; Reproductive System, Female; Reproductive System, Male; Toxicity Testing, Reproductive.

Further Reading

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Anesthetic Agents

Jeffrey W Allen

Anesthetic agents are a diverse class of chemicals which are extremely important in modern medicine. They are generally used to produce a loss of sensation to all stimuli, either in a specific anatomical area, or a total loss of consciousness. Anesthetics differ from analgesics in that analgesics such as aspirin, acetaminophen, ibuprofen, or morphine act to decrease pain, but not other sensations. Anesthetics can be broadly categorized into two general classes, local anesthetics and general anesthetics. These classes are independent as far as indication, chemical class, routes of administration, and toxicity, and thus will be considered separately. It will be noted when one compound within a class differs from the others.

Local Anesthetics

- Prototypical Compounds: Lidocaine; Mepivacaine; Bupivacaine; Procaine; Tetracaine; Prilocaine; Benzocaine; Cocaine
- Chemical Abstracts Service Registry Numbers:
  Amides
  Lidocaine: HCl (CAS 6108-05-0); Mepivacaine HCl (CAS 1722-62-9); Bupivacaine HCl (CAS 14252-80-3); Procaine HCl (CAS 51-05-8).
  Esters
  Tetracaine: HCl (CAS 136-47-0); Prilocaine HCl (CAS 1786-81-8); Benzocaine (CAS 94-09-7)
- Synonyms:
  Amides
  Lidocaine: 2-(Diethylamino)-2′,6′-Acetoxylidide; Lida-Mantle; Xilina; Xllina; 2-(diethylamino)-*N*-(2,6-dimethylphenyl)-Acetamide; Xyloneural; Cappicaine; α-(Diethylamino)-2,6-acetoxylidide; Duncaine; Gravocain; Isicaina; Isicaine; Leostesin; Lignocaine; Maricaine; Xycaine; Xylestesin; Xylocain; Xylocaine; Xylocitin; Xylotox; 2-(Diethylamino)-2′,6′-acetoxylidide; Diethylaminoaceto-2,6-xylidide; α-Diethylamino-2,6-dimethylacetanilide; α-Diethylaminoaceto-2,6-xylidide
  Mepivacaine: Carbocaine hydrochloride; *N*-(2,6-dimethylphenyl)-1-methyl-2-piperidinecarboxamide monohydrochloride
  Bupivacaine: Marcaine; 1-Butyl-*n*-(2,6-dimethylphenyl)-2-piperidine carboxamide
  Procaine: Novocain hydrochloride; Ethocaine; Paracain; Allocaine; 2-(Diethylamino)ethyl-4-aminobenzoate hydrochloride; 4-Aminobenzoic acid 2-(diethylamino)-ethyl ester hydrochloride; *p*-Aminobenzoyldiethylaminoethanol hydrochloride; Alocaine; Aminocaine; Anesthol; Anestil; Atoxicocaine; 4-Aminobenzoic acid, 2-(diethylamino)ethyl ester, monohydrochloride; Bernacaine; Cetain; Eugerase; Irocaine; Isocaine-Asid; Isocaine-Heisler; Jenacaine; Kerocaine; Medaject; Naucaine; Neocaine; Novocaine; Planocaine; Scurocaine; Sevicaine; Sycaine; Syncaine; Topocaine
  Esters
  Tetracaine: Amethocaine hydrochloride; 2-dimethylaminoethyl 4-*n*-butylaminobenzoate hydrochloride; 4-(Butylamino)benzoic acid 2-(dimethylamino)ethyl ester hydrochloride; Anethaine; Butethanol; *p*-butylaminobenzoyl-2-dimethylaminoethanol hydrochloride; Pontocaine hydrochloride; Tonexol; 4-(butylamino) Benzoic acid, 2-(dimethylamino)ethyl ester, monohydrochloride; Tetracainhydrochlorid; Tetracaina, clorhidrato; Tétracaine, chlorhydrate
  Prilocaine: Propitocaine HCl; Xylonest; *n*-(2-Methylphenyl)-2-(propylamino)-propanamide hydrochloride; 2-(Propylamino)-*o*-propionotoluidide hydrochloride; *n*-(α-Propylaminopropionyl)-*o*-toluidine hydrochloride; α-Propylamino-2′-methylpropionanilide hydrochloride
  Benzocaine: Anesthesin; Parathesin; Auralgan Otic; Ethyl aminobenzoate
- Chemical Formula
  Amides
  Lidocaine: $C_{14}H_{22}N_2O \cdot HCl$; Mepivacaine: $C_{15}H_{22}N_2O \cdot HCl$; Bupivacaine: $C_{18}H_{28}N_2O \cdot HCl$; Procaine: $C_{13}H_{20}N_2O_2 \cdot HCl$

Esters
Tetracaine: $C_{15}H_{24}N_2O_2 \cdot HCl$; Prilocaine: $C_{13}H_{20}N_2O \cdot HCl$; Benzocaine: $C_9H_{11}NO_2$

Uses

Local anesthetics are generally a hydrophobic aromatic ring separated from a hydrophilic tertiary or secondary amine linked by an ester or amide as seen in **Figure 1**. The more hydrophobic (nonpolar or lipid-soluble) the molecule, the more potent, and more toxic, are the compounds. They all act by binding to a hydrophobic site in the intracellular region of voltage-gated Na^+ channels on nerve fibers, blocking Na^+ entry and preventing local membrane depolarization. This action prevents the spread and generation of action potential in these previously excitable membranes. As this blockade is frequency- and voltage-dependent, an active nerve is more susceptible to the effects of local anesthetics than resting nerves. In addition, small unmyelinated nerves, the C and Aδ fibers which carry pain, and pain and temperature messages, respectively, are more sensitive to local anesthetics than the larger myelinated Aβ, Aγ, Aα, and B nerves that carry touch, pressure, proprioceptive, and motor information. Thus a highly active nociceptive (pain-transmitting) neuron's activity would be blocked before neighboring motor and pressure-sensitive nerves. It is this ability to obtain a relative differential sensory and motor block that has given these compounds their widespread clinical usefulness.

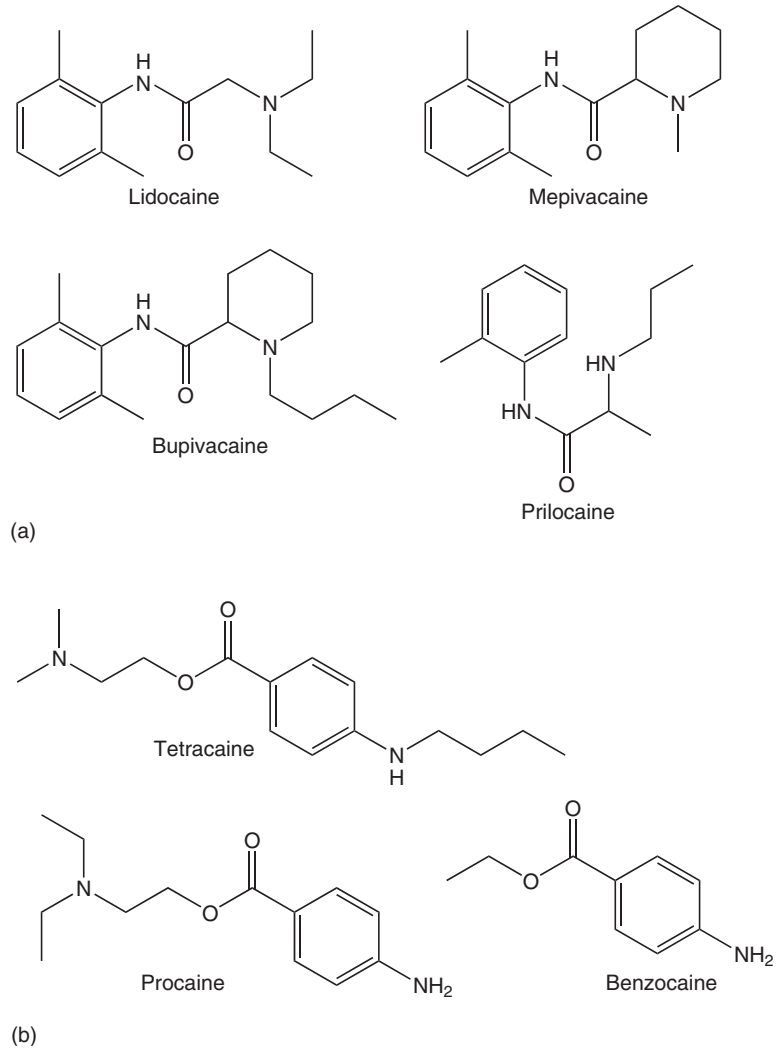

Figure 1 Chemical structures for (a) the amide-linked local anesthetics and (b) the ester-linked local anesthetics.

Exposure Routes and Pathways

Local anesthetics, particularly benzocaine and lidocaine, are found in a number of topical mixtures for treatment of minor superficial pain. These may be found in both prescription and over-the-counter formulations. An adhesive patch that contains 5% lidocaine is available by prescription. This has been successfully used for treatment of focal tactile hyperalgesia, such as that seen in post-herpetic neuralgia. A eutectic mixture of 2.5% lidocaine and 2.5% prilocaine (EMLA) provides anesthesia for superficial procedures including venipuncture and some skin graft harvesting, but requires placement of an occlusive dressing and $\sim$60 min to reach full efficacy. Local anesthetics are commonly administered by focal infiltration to provide temporary loss of sensation for minor invasive procedures such as closure of a laceration with sutures. Mepivacaine is not effective for topical or infiltration anesthesia. If anesthesia is desired in a larger region, local anesthetics can be injected directly adjacent to major nerves or nerve roots such as is often performed for dental procedures and minor surgical procedures, especially on extremities, producing a nerve block. In addition, regional anesthesia in the lower leg and foot or lower arm and hand can be performed by administering intravenous lidocaine or prilocaine in a region that has been isolated from the general circulation by compressive exsanguination and application of a tourniquet. This is commonly known as a Bier's block. Injections into the spinal space, either epidurally or intrathecally are often used for peri- and postoperative pain, in addition to neuropathic pain states. With chronic pain states, delivery of local anesthetics with or without the addition of opiates via an implanted permanent intrathecal catheter and subcutaneous pump can provide a high degree of pain relief in selected patients. Low dose, intravenous lidocaine infusions have also been effective in treating some neuropathic pain states. Lidocaine can be used in the treatment of ventricular tachycardia or fibrillation. As will be discussed below, cardiovascular effects are an important toxicological consideration of lidocaine.

Toxicokinetics

Local anesthetics are readily absorbed and distributed. They cross the intact placenta and blood–brain barrier. Recommended maximum dosages, distribution, and elimination kinetics for three of the most commonly prescribed local anesthetics are presented in **Table 1**.

Approximately 50–70% of an injected dose of amide local anesthetic (lidocaine, mepivacaine, bupivacaine) is taken up by the hepatic system and undergoes *N*-dealkylation and hydrolysis. An important caveat is that prilocaine has a hydrolytic first step which produces *o*-toluidine metabolites that can produce methemoglobinemia. Significant methemoglobinemia has been seen in patients using ELMA. Amide local anesthetics are highly protein-bound in plasma (55–95%) with α_1-acid glycoprotein being the primary protein.

Ester-containing anesthetics such as cocaine, benzocaine, and tetracaine are extensively hydrolyzed by plasma esterases in addition to a contribution from hepatic esterases.

The majority of local anesthetics and their metabolites are excreted renally. The ester anesthetics display very little ($<2\%$) excretion of unmetabolized drug while amide anesthetics can range for 10–16%. Up to 80% of lidocaine and 40% of mepivacaine and their metabolites are found in the urine of normal patients.

Local anesthetics are weak bases and are usually made as HCl salts which are soluble and stable in water. The pK_a of the compound determines when the ionized and unionized forms are equal (see **Table 1** for values of pK_a and percentage ionization at pH 7.4). The time of onset of the block is related to diffusion of the anesthetic into the nerve fiber, which occurs only in the unionized, or non-protonated form. Sodium bicarbonate is often added ($1\,\text{mEq}\,(10\,\text{ml})^{-1}$ lidocaine or $0.1\,\text{mEq}\,(10\,\text{ml})^{-1}$

Table 1 Pharmacokinetic, physiochemical characteristics, and maximal recommended doses of some commonly used amide local anesthetics

	Distribution half-life (min)		*Elimination half-life (h)*	*Time of onset (min)*	*pK_a*	*Percent non-protonated at pH 7.4*	*Maximum dose (mg kg^{-1})*
	α	β	γ				
Lidocaine	1	9.6	1.6	2–4	7.7	25	4
Mepivacaine	0.7	7.2	1.9	2–4	7.6	40	4
Bupivacaine	2.7	28	3.5	5–8	8.1	18	2

Note: The local anesthetics with a pK_a closer to physiological pH of 7.4 have a higher percentage of molecules in the unionized form and have a faster time of onset.

bupivacaine) to raise the pH of the solution. This pushes the ionization state of the local anesthetic from the protonated to the non-protonated state, thus increasing the diffusion of the local anesthetic into the cell and decreasing the time of onset of the block. The addition of bicarbonate does not have an effect on the quality or duration of the block, only the time of onset. It may also make the injection more comfortable for the patient. Once inside the fiber the anesthetic is ionized and trapped in the intracellular space.

Acute and Short-Term Toxicity (or Exposure)

The toxicity of local anesthetics is related to their potency which is directly related to their hydrophobicity. The more hydrophobic drugs such as bupivacaine produce toxicities at concentrations lower than the less potent anesthetics such as lidocaine and mepivacaine.

The two toxicological consequences of greatest concern following acute exposure of local anesthetics are the central nervous and cardiovascular systems. Local anesthetics are able to cross the blood–brain barrier and enter the central nervous system (CNS). Local anesthetic toxicity can be characterized by three phases. In the initial phase signs and symptoms such as lightheadedness, tinnitus, confusion and euphoria or dysphoria, or circumferential numbness are reported by the patient. In the second or excitation phase, clonic–tonic seizures are seen, and in the final or depressive phase unconsciousness, generalized CNS depression followed by respiratory depression and arrest can be present. This seemingly paradoxical initial increase in CNS activity including convulsions can be explained in that small inhibitory interneurons appear to have the greatest to blockade.

Following the appearance of CNS effects cardiovascular effects are often noted. Just as local anesthetics block conduction in peripheral nerves, they can also block Na^+ channels in the myocardium producing decreased action potential duration, rate of depolarization, and refractory period. At very high levels, they may block the sinoatrial and atrioventricular nodes. These agents also have a direct negative ionotropic action and produce a bi-phasic dose-related increase then decrease in vascular resistance. In addition, epidural and intrathecal administration can produce a sympathetic block, removing the sympathetic tone and producing profound hypotension. At equianalgesic doses, bupivacaine is more cardiotoxic than lidocaine.

Because of bupivacaine's longer half-life and greater tendency toward sensory than motor block it is commonly used epidurally during labor. This is in contrast to mepivacaine, which is not used in obstetrics due to increased toxicity in neonates. This toxicity is due to ion trapping because of the lower pH of the neonatal blood compared with that of the mother and the higher pK_a of mepivacaine. The decreased plasma protein in neonates also makes them more susceptible to amide local anesthetic toxicity.

Allergic reactions, including anaphylaxis, have been reported to local anesthetics. This is much more common for amide than ester-containing anesthetics. Local tissue, especially nerve fiber, toxicity can occur. This was most dramatically noted with the advent of microbore intrathecal catheters used to inject high-concentration (5%) lidocaine. It is thought this resulted in high local tissue concentrations and produced a number of cases of cauda equine syndrome and radiculopathy. As a result of these incidents, microbore catheters were removed from the market in 1993.

When local anesthetics are to be administered spinally or for regional blocks they should be preservative-free saline preparations as the neurotoxicity of preservatives and excipiants generally have not been systematically studied.

The invasive procedures used to deliver local anesthetics carry their own risk that must be evaluated in addition to any toxicity due to direct or indirect actions of the drugs.

Chronic Toxicity (or Exposure)

Animal No mutagenic or carcinogenic potential was found in preclinical testing of lidocaine or prilocaine. The 2,6-xylidine metabolite of lidocaine did display carcinogenicity in a 2 year oral toxicity study in rats 60, but not 30 times the single administration dose. The procaine metabolite *o*-toluidine has been carcinogenic in mice at 60–960 and 60–320 times standard dosing levels.

No data have been published concerning pre-clinical carcinogenic or reproductive studies for mepivacaine and no carcinogenic studies have been published for bupivacaine. Decreased pup survival in rats and embryocidal effect in rabbits have been observed when bupivacaine was administered to these species in doses comparable to nine and five times, respectively, the maximum recommended daily human dose.

Human As noted above in acute and subchronic effects, local anesthetics can produce an allergic syndrome that can manifest as urticaria in some patients. This nearly always occurs with amide anesthetics so changing to amine anesthetics greatly reduces this side effect.

There are no reports suggesting mutagenic, carcinogenic, or teratogenic potential of local anesthetics in humans.

In Vitro Toxicity Data

Mutagenesis tests of 2,6-xylidine have proved equivocal. *o*-Toluidine has displayed positive results in DNA repair assays and phage induction assays. The parent compounds displayed no mutagenicity. The mutagenesis potential has not been evaluated in the majority of local anesthetics.

Clinical Management

Reports of signs and symptoms by patients such as noted in the Acute Toxicity section should be regarded as the onset of toxicity. Administration of anesthetics should be halted and proper supportive care initiated. Benzodiazepines can be given prophylactically to prevent or decrease the expected seizure activity. If seizures have begun, then benzodiazepines or fast-acting barbiturates such as pentothal can be given intravenously.

General Anesthetics

General anesthetics are either liquids, which are delivered in their gaseous forms via inhalation using carrier gases of oxygen and air or oxygen and nitrous oxide, or they are relatively lipid-soluble compounds which are given intravenously. They all share a common property of producing a loss of awareness and recall. In addition, they have a rapid onset and also a rapid recovery following discontinuation. They also share the feature of relatively small therapeutic indexes of approximately two to four between effective dosing and cardiopulmonary arrest, making them among the most potentially dangerous drugs that are commonly used in patients.

Inhalational anesthetics are volatile organic hydrocarbons or ethers which are liquid at room temperature (**Figure 2**). Due to their high vapor pressure it is possible to volatilize these agents in a stream of gas, and using highly accurate calibrated vaporizers, to consistently deliver concentrations generally between 0.25% and 6% over a range of temperatures and gas flow rates. Their potency is directly correlated to their gas–oil partition index (Meyer–Overton correlation) with greater oil partitioning providing greater potency. It should be noted that there is a cut-off phenomenon in which lipid-soluble molecules with carbon chains longer than 10 molecules no longer have the increased anesthetic potency as would be predicted. The potency of inhalational anesthetics is measured in terms of minimal alveolar concentration (MAC). A dose of 1 MAC is defined as the concentration at which 50% of patients do not respond with movement to surgical stimulation. By using additional anesthetic or analgesic agents, the commonly used doses range from 0.5 to 2 MAC, which would produce sufficient anesthesia in a majority of patients. Unlike prior generations of volatile anesthetics such as diethyl ether and cyclopropane, the current agents are nonflammable, and unlike chloroform, are relatively nontoxic. Halothane has been the gold standard for evaluating new inhalation agents since its introduction in 1956, but it is now largely being replaced with newer less toxic agents that undergo less biotransformation.

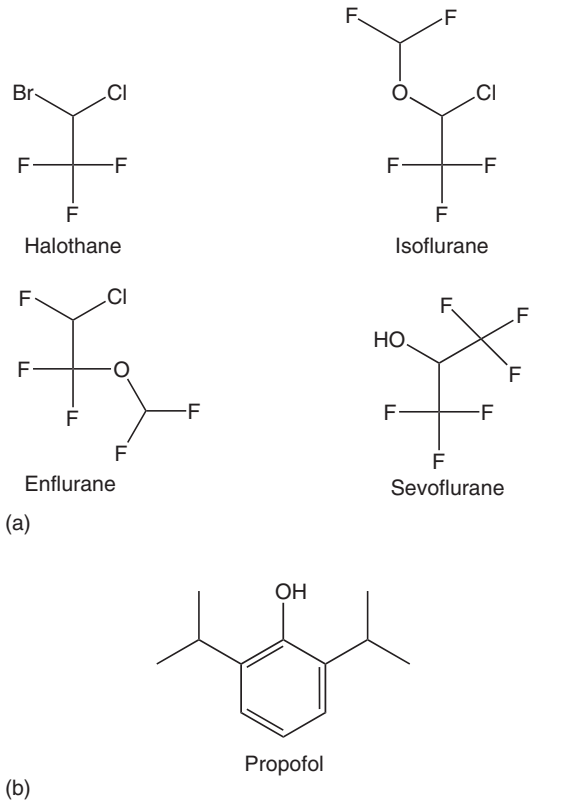

Figure 2 Chemical structures for (a) inhalational anesthetics and (b) the intravenous anesthetic propofol.

Intravenous anesthetics are also relatively lipid-soluble, which helps account for their rapid onset. This high degree of lipid solubility allows them to rapidly cross the blood–brain barrier and partition into the brain. Barbiturates such as thiopental and methohexitol and the nonbarbiturates etomidate and propofol are often used to induce anesthesia, but only propofol is commonly used today as a general anesthetic by continuous infusion, thus only propofol will be discussed.

- Prototypical Compounds: *Inhalational agents*: Halothane; Isoflurane; Enflurane, Sevoflurane. *Intravenous agents*: Propofol
- Chemical Abstracts Service Registry Numbers:
  Inhalational
  Halothane: (CAS 151-67-7); Isoflurane (CAS 26675-46-7); Enflurane (CAS 13838-16-9); Sevoflurane (CAS 28523-86-6).
  Intravenous
  Propofol: (CAS 218-206-6)
- Synonyms
  Inhalational
  Halothane: Fluothane; 2-Bromo-2-chloro-1,1,1-trifluoroethane; Bromochlorotrifluoroethane; 1,1,1-Trifluoro-2,2-chlorobromoethane
  Isoflurane: Forane; 2-Chloro-2-(difluoromethoxy)-1,1,1-trifluoro ethane; 1-Chloro-2,2,2-trifluoroethyl difluoromethyl ether
  Enflurane: Ethrane; 2-Chloro-1,1,2-trifluoroethyldifluoromethyl ether; Efrane; Alyrane
  Sevoflurane: Ultane; Propane-1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy); Fluoromethyl-2,2,2-trifluoro-1-(trifluoromethyl)ethyl ether
  Intravenous
  Propofol: Diprivan; 2,6-Diisopropylphenol; Diisopropylphenol; 2,6-Bis(1-methylethyl)Phenol; Disoprofol
- Chemical Formula: *Inhalational* – Halothane: $C_2HBrClF_3$; Isoflurane: $C_3H_2ClF_5O$; Enflurane: $C_3H_2OClF_5$; Sevoflurane: $C_4H_3F_7O$. *Intravenous* –Propofol: $C_{12}\ H_{18}O$

Uses

General anesthetics are used to produce loss of awareness and recall during invasive medical procedures such as surgery and certain diagnostic or therapeutic procedures. They can also provide varying degree of muscular relaxation which is advantageous for most procedures. In addition, agents such as propofol can be used to provide either deep or light sedation during the time spent in intensive care units (ICUs) when unwanted activity would be detrimental to the recovery process. The rapid immergence from sedation with the termination of propofol administration, even after days of sedation, is especially useful in that it can allow periodic assessments of mental and neurological functions of patients during extended recovery periods.

Intravenous anesthesia does not require the elaborate equipment needed to deliver inhalational anesthetics, but it should be recognized that intravenous anesthetics cause respiratory and cardiovascular collapse as seen with inhalation agents. Thus a general anesthetic should never be administered in a setting without appropriate monitoring, resuscitation equipment, and personnel trained in their use.

Exposure Routes and Pathways

Volatile anesthetics are administered exclusively via inhalation. Propofol is administered only intravenously. Exposure by other routes would not be anticipated.

Toxicokinetics

Inhalation anesthetics: The uptake of inhalational anesthetics by the lung, diffusion into the bloodstream and partitioning into the brain are determined by a number of factors including their solubility in blood and tissue and the adequacy of respiratory and cardiovascular function. Due to centrally mediated respiratory depression, patients are often mechanically ventilated while being administered general anesthetics. Cardiovascular function is both directly and indirectly decreased by inhalational anesthetics. This direct effect is thought to be due to decreased Ca^{2+} entry into myocytes producing deceased contractile force. Heart rate is also slowed due to the loss of sympathetic tone; however, the sensitivity of the myocardium to sympathomimetics can be increased resulting in a sensitized or irritable heart that is prone to tachyarrhythmias if large amounts of epinephrine are used in the presence of volatile anesthetics. Anesthetics may possibly also decrease cardiac function by blunting baroreceptor reflexes. While they vary somewhat in the degree of cardiac depression produced, it is a trait common to all inhalational anesthetics.

Inhalational anesthetics are largely removed in the same route as they were administered, by the lungs. For halothane, up to 80% of the gas can be exhaled unchanged by the lungs. This generally occurs over the first 24 h, but some amounts may be exhaled for many days. The remaining nonexhaled are either excreted or undergo biotransformation, largely by hepatic mixed function oxidases. The oxidative and reductive metabolism of halothane is shown in detail in **Figure 3**. Trifluoroacetic acid (TFA) is the major metabolite of halothane and is found in the urine with a half-life of 16 h. The consequence of this biotransformation following halothane exposure has been a driving factor for the development of newer inhalational agents and will be discussed below.

Propofol is widely used for the induction of anesthesia and often for maintenance during procedures when rapid recovery is beneficial. Pain is sometimes

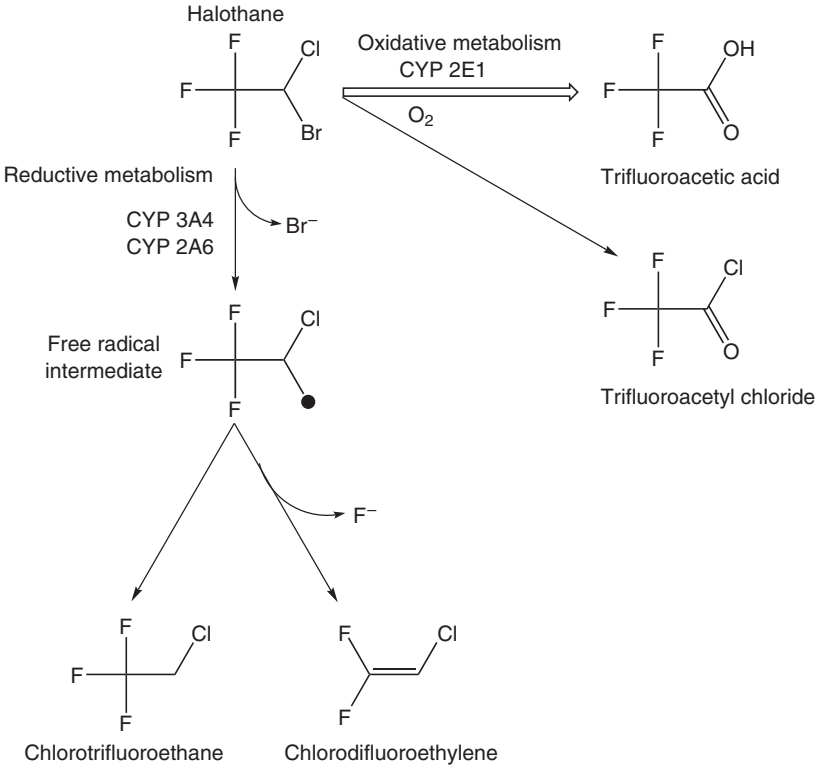

Figure 3 Halothane biotransformation. Approximately 80% of inhaled halothane is exhaled unchanged. The majority of the remaining 20% undergoes oxidative metabolism. This produces trifluoroacetic acid (TFA) as the major metabolite which is excreted in the urine, and smaller amounts of trifluoroacetyl chloride. The trifluoroacetyl chloride covalently binds to a variety hepatic proteins. Antibodies to these trifluoroacetylated proteins are thought to be a causative factor in the development of type II hepatotoxicity. Some reductive metabolism also occurs which involves the production of a free radical intermediate. It is believed that this intermediate is the hepatotoxic compound in type I hepatotoxicity. The metabolites chlorotrifluoroethane (CTF) and chlorodifluoroethylene (CDF) are exhaled and can serve as markers for reductive metabolism.

reported with propofol infusion and often a local anesthetic is added to the solution during its use for induction. Unlike inhalational anesthetics, propofol has little direct cardiovascular depressant effects. Decreases in blood pressure of up to 30% can be seen, but this is likely due to a decreased peripheral vascular resistance and is often reversed by stimulation such as endotracheal intubation. Because propofol is poorly soluble in water ($\sim 0.01\,mg\,ml^{-1}$), it is formulated for injection as an emulsion of $10\,mg\,ml^{-1}$ propofol, $100\,mg\,ml^{-1}$ soybean oil, $22.5\,mg\,ml^{-1}$ glycerol, $12\,mg\,ml^{-1}$ egg lethicin, and 0.005% EDTA. It has a molecular weight of 178.24 and pK_a is 11. The octanol–water partition coefficient for propofol is 6761:1 at a pH of 6–8.5. Propofol is chiefly eliminated by hepatic conjugation to inactive metabolites which are excreted by the kidney. A glucuronide conjugate accounts for $\sim$50% of the administered dose.

Acute and Short-Term Toxicity (or Exposure)

Inhalation anesthetics: Hepatic injury is a concern following halothane exposure due to its large amount of biotransformation which is illustrated in **Figure 3**. Two major types of hepatotoxicity are associated with halothane administration. They appear to be unrelated and are termed type I (mild) and type II (fulminant). Type I hepatotoxicity is benign, self-limiting, and relatively common with incidence rates as high as 25–30%. Type I is marked by mild, transient increases in serum transaminase and glutathione S-transferase concentrations and by altered postoperative drug metabolism. Type I probably results from reductive (anaerobic) biotransformation of halothane rather than the normal oxidative pathway. It does not occur following administration of other volatile anesthetics because they are metabolized to a lesser degree and do not undergo this reductive metabolism.

In contrast, type II hepatotoxicity is associated with massive centrilobular liver cell necrosis that can lead to fulminant liver failure. Type II hepatotoxicity is characterized by fever, jaundice, and very high serum transaminase levels. It may be immune-mediated and is thought to occur in genetically predisposed individuals. The incidence of type II hepatotoxicity is $\sim$1:35 000 with one exposure to halothane and

increases to 1:3700 on second exposure. Type II hepatotoxicity is initiated by oxidative halothane metabolism (see **Figure 3**) to an intermediate acyl halide compound, trifluoroacetyl chloride, which produces covalent triflouroacetylation of proteins in the hepatic endoplasmic reticulum. Antibodies against these trifluoroacetylated proteins are thought to be responsible for the autoimmune destruction of the lever. Approximately 20% of halothane is oxidatively metabolized, compared with only 2% of enflurane and 0.2% of isoflurane as described in **Table 2**. The occurrence of type II hepatotoxicity after enflurane or isoflurane administration is extremely rare.

A second rare, but potentially fatal, condition associated with acute inhalational anesthetic exposure is malignant hypothermia. This is an autosomal dominant disease in which there is excessive sarcoplasmic release of intracellular Ca^{2+} in skeletal muscles during exposure to inhalational anesthetics. This produces a hypermetabolic state that is manifested as increased muscle rigidity and contracture, tachycardia and metabolic acidosis. Extreme hyperthermia is also present. There are currently a number of worldwide registries for tracking this disease and an *ex vivo* testing paradigm exists to determine a potentially susceptible persons phenotype.

Propofol: Propofol has been relatively free of acute side effects other than those associated with its mechanism of action. Continuous infusions lasting greater than 10 days in ICUs have demonstrated no significant apparent toxicities. Propofol is not recommended for obstetrics, including cesarean section deliveries. It crosses the placenta, and as with other general anesthetic agents, may be associated with neonatal depression. Propofol is not recommended for use in nursing mothers because it is excreted in human milk, and the effects of oral absorption of small amounts of propofol in newborn and infants are not known.

Chronic Toxicity (or Exposure)

Animal *Inhalation anesthetics*: An 18 months inhalational carcinogenicity study of halothane at 0.05% in the mouse revealed no evidence of anesthetic-related carcinogenicity. This concentration is equivalent to 24 h of 1% halothane. Some studies have shown halothane to be teratogenic, embryotoxic, and fetotoxic in the mouse, rat, hamster, and rabbit at subanesthetic and/or anesthetic concentrations. Reproduction studies of halothane (10 ppm) and nitrous oxide in the rat caused decreased fertility. This trace concentration corresponds to 1/1000 the human maintenance dose. Studies of isoflurane have not demonstrated these effects.

Propofol: Animal carcinogenicity studies have not been performed with propofol. *In vivo* animal tests failed to show any potential for mutagenicity by propofol. Studies in rats revealed no impairment of fertility. Reproduction studies have been performed in rats and rabbits at intravenous doses of $15\,mg\,kg^{-1}\,day^{-1}$ (approximately equivalent to the recommended human induction dose on a $mg\,m^{-2}$ basis) and have revealed no evidence of impaired fertility or harm to the fetus due to propofol. Propofol, however, causes maternal death in rats and rabbits and decreases pup survival during the lactating period in dams treated with $15\,mg\,kg^{-1}\,day^{-1}$ (approximately equivalent to the recommended human induction dose on a $mg\,m^{-2}$ basis). The pharmacological activity of the drug (anesthesia) on the mother is thought to be responsible for the adverse effects seen in the offspring.

Human *Inhalation anesthetics*: Occupational exposure to inhalational anesthetics, especially halothane, produces an increase in miscarriage rate in individuals. There are no reports of inhalational anesthetic-related carcinogenesis, teratology, or mutagenesis. A recent analysis suggests anesthesiologists, who presumably have the highest exposure levels, have mortality rates equivalent to that seen in nonexposed physicians, such as internists. To date, no definitive long-term detrimental effects have been noted with chronic low-level inhalational anesthetic exposure in humans.

Propofol: There are no adequate and well-controlled studies in pregnant women. Although animal reproduction studies are not always predictive of

Table 2 Physical constants and anesthetic values for the four most commonly used inhalational anesthetics

	MAC (%)	*MAC awake (%)*[a]	*Boiling point (°C)*	*SVP (mmHg)*[b]	*Partition coefficients (37°C)*			*Biotransformation (%)*
					Oil:gas	*Blood:gas*	*Brain:blood*	
Halothane	0.75	0.41	50.2	243	225	2.2	2.9	20
Isoflurane	1.2	0.4	48.5	238	98	1.4	2.6	0.2
Enflurane	1.6	0.4	56.5	175	98	1.9	1.4	2.4
Sevoflurane	2.0	0.6	58.5	160	53	0.45	1.7	3–5

[a] MAC awake is the concentration where responses to verbal commands are lost, this is also usually the point of amnesia and loss of awareness.

[b] Vapor pressure at standard conditions of 20°C.

human responses, this drug should be used during pregnancy only if clearly needed.

In Vitro Toxicity Data

Mutagenesis testing of halothane revealed both positive and negative results. In the rat, 1 year exposure to trace concentrations of halothane (1 and 10 ppm) and nitrous oxide produced chromosomal damage to spermatogonia cells and bone marrow cells. Negative mutagenesis tests included Ames bacterial assay, Chinese hamster lung fibroblast assay, sister chromatid exchange in Chinese hamster ovary cells, and human leukocyte culture assay.

Propofol: Tests for mutagenicity included the Ames mutation test, gene mutation/gene conversion using *Saccharomyces cerevisiae*, *in vitro* cytogenetic studies in Chinese hamsters, and a mouse micronucleus test. None of these have shown mutagenic potential.

Clinical Management

Decreased respiratory and cardiovascular function due to general anesthetics must be carefully monitored and appropriate actions taken when necessary. Given the short time of induction and recovery for general anesthetics, discontinuation of drug administration may quickly resolve the depressant effect.

Malignant hyperthermia is a rapidly progressing life-threatening condition. On suspected diagnosis, all anesthetic drugs are stopped and 100% oxygen is given. Intravenous dantrolene, an inhibitor of sarcoplasmic Ca^{2+} release, should be administered and the patient should be cooled as rapidly as possible with ice.

Environmental Fate

Like the conventional hydrochlorofluorocarbon refrigerants, inhalation anesthetics are known to oxidize in the atmosphere. As in human oxidative metabolism, atmospheric transformation produces TFA and trifluoroacetyl chloride. TFA appears to be very resistant to degradation by nonbiological physicochemical and photochemical processes due to its light-absorption properties. TFA salts are highly soluble and will not precipitate from solution at concentrations expected in the environment. Thus, the stability and solubility of TFA suggest that it will tend to remain dissolved in water. The bulk of existing data suggests that TFA is resistant to biodegradation in natural environments. However, certain bacterial strains maintained in the laboratory have been shown to degrade TFA with release of carbon dioxide. The fate of the fluorine atoms in this process is unknown.

Tests have generally shown that mammals, fish, and crustaceans are resistant to TFA at concentrations many thousands of times higher than expected in the environment. Because TFA has very low affinity for lipids there is no potential for passive accumulation in fatty tissues, even after long exposure at low levels. The amount of TFA contributed to the environment by use of inhalational anesthetics is thought to be minor compared to those that are released by industrial and manufacturing processes.

Exposure Standards and Guidelines

In the past, concerns have been raised about potential health risks of exposure to airborne anesthetic agents in clinical settings where scavenging devices have either been inadequate, malfunctioned, or not been installed. Although there is no clear evidence of significant effects among occupational groups studied thus far, as was noted above, there is biological plausibility for concerns about adverse neurological, reproductive, and developmental risks. Therefore, the Occupational Safety and Health Administration provides guidelines for minimizing workplace exposures to fugitive and waste anesthetic gases. In addition, both the National Fire Protection Association (NFPA) and the American Society for Testing and Materials (ASTM) specify consensus standards, codes, and performance requirements for equipment (e.g., ventilators, medical gas systems, and gas-scavenging equipment).

The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned halothane a threshold limit value of 50 ppm ($404\,mg\,m^{-3}$) as a time-weighted average for a normal 8 h workday and a 40 h workweek. This is $\sim$1.5 times the odor threshold of 33 ppm for halothane.

The National Institute for Occupational Safety and Health (NIOSH) has established a recommended exposure limit for halothane as a waste anesthetic gas of 2 ppm ($16.2\,mg\,m^{-3}$) as a 60 min ceiling limit that should not be exceeded during any part of the workday.

See also: Benzodiazepines; Cocaine; Lidocaine; Procainamide.

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Relevant Websites

http://www.osha.gov – Occupational Safety & Health Administration (OSHA). This United States government agency sets and enforces standards for workplace safety and health, including exposure levels of inhalational anesthetics.

http://www.astm.org – American Society for Testing and Materials. The agency sets and provides standardization for equipment such as inhalational anesthetic vaporizers.

http://www.niehs.nih.gov – National Institute of Environmental Health Sciences that examines the human effects of environmental contaminates such as metabolites of inhalational anesthetics.

Aneuploidy

David A Eastmond

Aneuploidy is a condition in which the chromosome number of a cell or individual differs from a multiple of the haploid complement for that species. In common terms, an aneuploid cell will have one or more chromosomes in addition to or less than, what is normal for that cell type, such as a human somatic cell having 45 or 47 chromosomes rather than 46, the normal diploid number. Similarly, an aneuploid human germ cell would possess $\leqslant 22$ or $\geqslant 24$ chromosomes rather than the haploid chromosome number of 23. In cytogenetics, aneuploidy is considered one type of numerical chromosome aberration. The other type of aberration is polyploidy, where the chromosome number of a cell is increased by a multiple of the haploid complement for the species. (For example, a human cell having 69 or 92 chromosomes rather than the diploid 46 would be considered polyploid.) Aneuploid cells may be further described as hyperploid – having additional chromosomes, hypoploid – possessing fewer chromosomes, or as having trisomy – possessing three copies of one chromosome, or monosomy – with a single copy of a chromosome. In some cases, researchers have expanded the definition of aneuploidy to include partial or segmental aneuploidies, conditions resulting from structural rearrangements where portions of chromosomes have been added to or lost from a cell. In this article, aneuploidy will be used based on its original and more widely accepted definition, which involves the loss or gain of entire chromosomes.

Aneuploidy occurring in germ cells and early embryos is a major cause of morbidity and mortality in humans. It is associated with infertility, pregnancy loss, congenital malformations, and mental retardation. Congenital aneuploidy involving autosomal chromosomes affects $\sim 0.15\%$ of all live births, and aneuploidy involving the sex chromosomes affects another 0.175%. In addition, the frequency of chromosomal abnormalities is much higher among pregnancies that terminate at birth ($\sim 5\%$) or during gestation ($\sim 50\%$ in fetuses dying between weeks 8 and 11 of gestation). In most cases, these abnormalities are believed to have contributed to the embryonic and fetal deaths. Overall, chromosome abnormalities are estimated to be responsible for $\sim 30\%$ of lost pregnancies, with aneuploidy accounting for $\sim 75\%$ of the total. As a consequence, it has been estimated that $\sim 13\,000$ aneuploid babies will be born each year in the United States and that another 150 000–200 000 chromosomally abnormal embryos will be spontaneously aborted. Among the surviving offspring, the most common type of congenital aneuploidy is Down's syndrome, which results from trisomy of chromosome 21 and occurs in ~ 1 in 800 newborns (0.13%). Similarly, Klinefelter's syndrome (XXY), YY males (XYY), triple X females (XXX), and Turner's syndrome (XO) are congenital aneuploidies of the sex chromosomes that individually affect ~ 0.05–0.005% of live births. Because most of these individuals are infertile and exhibit developmental abnormalities, aneuploidy is responsible for a significant portion of the recognized cases of infertility, congenital malformations, and mental retardation. Indeed, aneuploidy has been reported to be the leading genetic cause of mental retardation in the United States.

Nonrandom patterns of numerical aberrations are frequently observed in cancer cells, implicating aneuploidy in carcinogenesis. Associations between

aneuploidy and neoplastic development have also been observed in patients with congenital and familial predispositions for cancer, as well as in patients with cancers resulting from chemical exposures. Similar results have been observed in animals and cellular systems in which the nonrandom gain or loss of specific chromosomes has been associated with tumorigenesis or neoplastic transformation. These patterns have been seen in tumors occurring spontaneously as well as those induced by chemical, radiation, or viral agents.

While in some tumors, chromosomal changes appear to be a secondary effect related to cell proliferation or genomic instability, a growing body of molecular and cytogenetic evidence indicates that the induction of aneuploidy plays an important role in neoplastic transformation. This has perhaps been best characterized in the case of retinoblastoma where it has been shown that the loss of the allele containing the functional Rb tumor suppressor gene frequently occurred through a mechanism involving nondisjunction of chromosome 13. Similarly, alterations in gene dosage resulting from aneuploidy are believed to contribute to the development of many other cancers.

Mechanistically there are many ways by which aneuploidy can occur. Almost any process that interferes with mitosis or meiosis during cell division can affect chromosome segregation and result in aneuploidy. In germ cells, aneuploidy appears to originate, in part, from aberrant meiotic recombination, premature separation of sister chromatids, and possibly altered DNA methylation. During carcinogenesis, aneuploidy has been reported to result from mechanisms including spontaneous errors of mitosis, chemical interference with the mitotic spindle, viral integration resulting in chromosomal instability, as well as mutations affecting the kinetochore, the centrosome or other cellular structures and organelles.

The ability of chemicals to interfere with proper chromosome segregation has been an area of considerable concern within genetic toxicology. Many chemical and physical agents including those used as pesticides, pharmaceuticals, consumer products, and industrial chemicals have been shown to induce aneuploidy *in vitro* and/or *in vivo*. Indeed, drugs such as vincristine sulfate and griseofulvin are used specifically because of their ability to induce aneuploidy, which gives them cytostatic or cytotoxic properties. In spite of the common use of aneugenic chemicals, the extent to which aneuploidy induced by these agents contributes to cancer and reproductive dysfunction in the general population remains uncertain. However, due to the clear involvement of aneuploidy in carcinogenesis and in adverse reproductive outcomes, there continues to be concern about the safety of aneuploidy-inducing agents.

Many different assays have been developed to detect aneuploidy, all of which have significant limitations. The conventional approach has been to count the number of chromosomes in metaphase preparations of dividing cells. Unfortunately, this restricts the detection to actively dividing cells, which may not be present in the tissue of interest. In addition, this technique is laborious and prone to technical artifacts, such as chromosome loss during metaphase preparation. The micronucleus assay, particularly as modified with antibodies or probes to detect centromere-containing micronuclei, has emerged as a simple way to detect aneugenic agents. While valuable, this assay is only able to detect chromosome loss and breakage, and may not detect agents that specifically induce nondysjunction or chromosome gain. Other techniques involving fluorescence *in situ* hybridization with DNA probes allow chromosome gains to be detected in many tissues but are relatively insensitive unless multiple probes and other modifications are used. As a result, efforts continue to develop assays or combinations of assays that will allow the efficient detection of aneugenic agents.

See also: Carcinogenesis; Chromosome Aberrations; Dominant Lethal Tests; Genetic Toxicology; *In Vitro* Test; *In Vivo* Test; Molecular Toxicology–Recombinant DNA Technology; Sister Chromatid Exchanges; Toxicity Testing, Mutagenicity.

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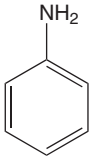
Angiotensin-Converting Enzyme Inhibitors

See ACE Inhibitors.

Aniline

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 62-53-3
- Synonyms: Phenylamine; Aminobenzene; Blue oil
- Chemical/Pharmaceutical/Other Class: Aromatic amine
- Chemical Formula: C_6H_7N
- Chemical Structure:



Uses

Intermediate in dyestuff production and in the manufacture of pharmaceuticals, photographic developers, shoe polish, resins, varnish, perfumes, and organic chemicals.

Exposure Routes and Pathways

Exposure is primarily by dermal and inhalation routes.

Toxicokinetics

Aniline is rapidly absorbed by the skin, lungs, and the gastrointestinal tract of experimental animals. After intravenous injection of radiolabeled aniline to rats, radioactivity is distributed throughout the body; highest concentrations were found in blood, liver, kidney, urinary bladder, and the gastrointestinal tract. The major urinary metabolites in various animal species tested are *o*-, *p*-amino-phenol, and their conjugates. *p*-Aminophenyl- and *p*-acetylaminophenylmercapturic acids are also excreted in rats and rabbits. *N*-Hydroxylation of aniline by liver microsomes from several species has been observed *in vitro*. The formation of phenylhydroxylamine from aniline appears to be the reactive metabolite responsible for its toxic activity.

Mechanism of Toxicity

The formation of phenylhydroxylamine from aniline appears to be the reactive metabolite responsible for its toxic activity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Moderate skin and severe eye irritant in rabbits; reproductive toxin in mice. Rat LC_{Lo} 250 ppm h^{-1}; dermal LD_{50} 1400 mg kg^{-1}; mouse oral LD_{50} 464 mg kg^{-1}. Aniline is a mutagen, that is, it is positive in the *in vivo* mouse micronucleus and sister chromatid exchange assays. DNA strand breakage was induced in the livers and kidneys of rats exposed *in vivo*.

Human

Human acute LD_{Lo} 350 mg kg^{-1}. Systemic exposure leads to methemoglobin formation, and metabolic formation of aniline from a number of drugs leads to methemoglobinemia associated with their use. Normal systemic levels should be less than 1 mg l^{-1}. Toxic oil syndrome (TOS) is a multisystemic disease

that occurred in epidemic proportions in Spain in 1981 caused by the ingestion of rapeseed oil denatured with aniline. It was one of the largest intoxication epidemics ever recorded. This oil had been illegally sold as olive oil, and many aniline-derived oil components have been identified in the oil. The pathologic findings in TOS showed primary endothelial injury, with cell proliferation and perivascular inflammatory infiltrates, and an immunological mechanism has been directly implicated in this illness.

Chronic Toxicity (or Exposure)

Animal

Can cause methemoglobin formation, and liver and endocrine effects. Causes kidney, urethra, bladder, and hematologic neoplasia. For example, aniline administered to rats for 5, 10, or 20 days resulted in splenic congestion, increased hematopoiesis and hemosiderosis, and bone marrow hyperplasia, and the dietary intake of aniline hydrochloride by rats for 104 weeks at levels of 10, 30, or 100 mg kg^{-1} diet is associated with an increased incidence of primary splenic sarcomas. Several species of animals exposed to 5 ppm of aniline vapour daily for 6 months resulted in no effects other than a slight increase in methemoglobin in the blood of rats. Repeated subcutaneous injections of 1.25 mg aniline in lard produced no tumours in mice that survived 2 years, and no tumours were observed after 15 months in mice given eight subcutaneous injections of aniline (5 mg in olive oil), or after 12 months in mice given 13 subcutaneous injections of aniline hydrochloride.

Human

The World Health Organization, International Agency for Research on Cancer, has evaluated the data for aniline and has placed aniline in its group 3 'classification of carcinogenicity', that is, aniline is not classifiable as to its carcinogenicity to humans.

In Vitro Toxicity Data

Although aniline per se is not mutagenic in the Ames *Salmonella typhimurium* assay system, the urine of rats that received aniline orally was mutagenic for *S. typhimurium* when the assay was performed in the presence of liver microsomes from PCB-induced rats. Aniline is positive in the *in vitro* sister chromatid exchange and mouse lymphoma assays. Further, results for cytogenetic effects in Chinese hamster ovary cells were positive for both chromosome aberrations and sister chromatid exchanges.

Clinical Management

Methemoglobin levels should be managed and/or reduced with suitable agents such as methylene blue.

Ecotoxicology

Toxic to aquatic organisms; for example, there was an inhibiting effect of 20–40 ppm aniline on the pigmentation of *Xenopus laevis* embryos, and of a concentration as low as 1 ppm on the body size of the young toads. Investigation of the death of pine trees in the United States found air pollution from aniline as the most likely causal agent for the needle necrosis and needle abscission.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists has established an 8 h time-weighted average of 2 ppm. The US Occupational Safety and Health Administration (OSHA) permissible exposure limit is 5 ppm.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Genetic Toxicology.

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Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Aniline.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Aniline.

Animal Models

Shayne C Gad

Introduction

The use of animals in experimental medicine, pharmacological study, and toxicological assessment is a well-established and essential practice. Whether serving as a source of isolated cells or tissues, a disease model, or as a prediction for drug or other xenobiotic action in humans, experiments in animals have provided the necessary building blocks that permitted the explosive growth of medical and biological knowledge in the latter half of the twentieth century. Animal experiments also have served rather successfully as identifiers of potential hazards to and toxicity in humans for synthetic chemicals with many intended uses.

Animals have been used as models for centuries to predict what chemicals and environmental factors would do to humans. The earliest uses of experimental animals are lost in prehistory, and much of what is recorded in early history about toxicology testing indicates that humans were the test subjects. The earliest clear description of the use of animals in the scientific study of the effects of environmental agents appears to be by Priestley (1792) in his study of gases. The first systematic use of animals for the screening of a wide variety of agents was published by Orfila (1814) and was described by Dubois and Geiling (1959) in their historical review. This work consisted of dosing test animals with known quantities of agents (poisons or drugs) and included the careful recording of the resulting clinical signs and gross necropsy observations. The use of animals as predictors of potential ill effects has grown since that time.

Current Animal Studies

The current regulatory required use of animal models in acute testing began by using them as a form of instrument to detect undesired contaminants. For example, canaries were used by miners to detect the presence of carbon monoxide – a case in which an animal model is more sensitive than humans. By 1907, the US Food and Drug Administration started to protect the public by the use of a voluntary testing program for new coal tar colors in foods. This was replaced by a mandatory program of testing in 1938, and such animal testing programs mandated by regulations have continued to expand until recently.

The knowledge gained by experimentation on animals has undoubtedly increased the quality of our lives, an observation that most reasonable people would find difficult to dispute, and has also benefited animals. As is the case with many tools, animals have sometimes been used inappropriately. These unfortunate instances have helped fuel an increasingly vituperative animal rights movement. This movement has encouraged a measure of critical self-appraisal on the part of scientists concerning the issues of the care and usage of animals. The Society of Toxicology, for example, has established an Animals in Research Committee, and has published guidelines for the use of animals in research and testing. In general, the purpose of this committee is to foster thinking on the four 'Rs' of animal-based research: reduction, refinement, research into replacements, and responsible use.

The media commonly carry reports that state that most (if not all) animal testing and research is not predictive of what will happen in humans and, therefore, such testing is unwarranted. Many of the animal rights groups also present this argument at every opportunity and reinforce it with examples that entail seemingly great suffering in animals but which add nothing to the health, safety, and welfare of society. This is held to be especially the case for safety testing and research in toxicology. Animal rights activists try to 'prove' this point by presenting examples of failure; for example, thalidomide may be presented as an example without pointing out that, in the case of thalidomide, there was lack of adequate testing (or of interpretation of existing test results) prior to marketing. In light of the essential nature of animal research and testing in toxicology, this is equivalent to seeking to functionally disarm us as scientists. Our primary responsibility (the fourth 'R') is to provide the information to protect people and the environment, and without animal models we cannot discharge this responsibility.

When confronted with this argument, all too many toxicologists cannot respond with examples to the contrary. Indeed, many may not even fully understand the argument at all. Also, very few are familiar enough with some of the history of toxicity testing to be able to counter with examples where it has not only accurately predicted a potential hazard to humans but also where research has directly benefited both humans and animals. There are, however, many such examples. Demonstrating the actual benefit of toxicology testing and research with examples that directly relate to the everyday lives of most people and not esoteric, basic research findings (which are the most exciting and interesting products to most scientists) is not an easy task. Examples that can be

seen to affect neighbors, relatives, and selves on a daily basis would be the most effective. The problem is that toxicology is, in a sense, a negative science. The things we find and discover are usually adverse. Also, if the applied end of our science works correctly, then the results are things that do not happen (and therefore are not seen).

If we correctly identify toxic agents (using animals and other predictive model systems) in advance of a product or agent being introduced into the marketplace or environment, then generally it will not be introduced (or it will be removed) and society will not see death, rashes, renal and hepatic diseases, cancer, or birth defects (for example). Also, as these things already occur at some level in the population, it would seem that seeing less of them would be hard to firmly tie to the results of toxicity testing that rely on animals. In addition, the fact that animals are predictive models for humans is controversial.

Origins of Predictive Animal Testing

The actual record of evidence for the predictive value of animal studies and how they have benefited man and domestic animals will be reviewed in the following. However, the negative image needs to be rebutted. First, it must be remembered that predictive animal testing in toxicology, as we now know it, arose largely out of three historical events.

The 'Lash Lure' Case

Early in the 1930s, an untested eyelash dye containing i-pheylenediamine (Lash Lure) was brought onto the market in the United States. This product (as well as a number of similar products) rapidly demonstrated that it could sensitize the external ocular structures, leading to corneal ulceration with loss of vision and at least one fatality.

The Elixir of Sulfanilamide Case

In 1937, an elixir of sulfanilamide dissolved in ethylene glycol was introduced into the marketplace. One hundred and seven people died as a result of ethylene glycol toxicity. The public response to these two tragedies helped prompt US Congress to pass the Federal Food, Drug, and Cosmetic Act of 1938 (FD&C Act). This law mandated the premarket testing of drugs for safety in experimental animals. It is a fact that since the imposition of animal testing as a result of these two cases, no similar occurrence has happened even though society uses many more consumer products and pharmaceuticals today than during the 1930s.

Thalidomide

The use of thalidomide, a sedative-hypnotic agent, led to some 10 000 deformed children being born in Europe. This in turn led directly to the 1962 revision of the FD&C Act, requiring more stringent testing. Current testing procedures (or even those at the time in the United States, where the drug was never approved for human use) would have identified the hazard and prevented this tragedy. In fact, tragedies like this have not occurred in Europe or the United States except when the results of animal tests have been ignored. **Table 1** presents an overview of cases in which animal data predicted adverse effects in humans.

Birth defects, for example, have occurred with isotretinoin (Accutane) where developmental toxicity had been clearly established in animals and presented on labeling, but the drug has continued to be used by potentially pregnant women.

Choosing an Animal Model

Choosing the appropriate animal model for a given problem is sometimes guesswork and often a matter of convenience. One often uses a species with which

Table 1 Animal models that predicted adverse effects of xenobiotics in humans

Agent	*Effect*	*Animal species*	*In human*
Thalidomide	Phocomelia	Rat	No/yes
Accutane	Developmental toxicity of CNS (neural tube defects)	Rat, rabbit, dog, primate	Yes
AZT	Bone marrow depression	Dog, rat, monkey	Yes
Valproic acid	Cleft palate	Rat, mouse, rabbit	Yes
Cyclosporine	Nephropathy, reversible immune response suppression (essential aid to organ transplantation)	Rat, monkey	Yes
Benoxaprofen (Oraflex)	Hepatotoxicity,	No	Yes
	photosensitivity	Guinea pig	Yes
Zomepirac (Zomax)	Anaphylactic shock	No	Yes
MPTP	Parkinsonism	Monkey	Yes
Cyclophosphamide	Hemorrhagic cystitis	Rat, dog	Yes
Mercury	Encephalopathy	Rat, monkey	Yes
Diethylene glycol	Nephropathy	Rat, dog	Yes
Razoxin	Myelomonocytic leukemia	Mouse	Yes

one is most familiar, with little consideration as to whether the chosen species is actually the most appropriate for the problem at hand. For example, the rat is probably a poor model for studying the chronic toxicity of any new nonsteroidal antiinflammatory drug (NSAID) because the acute gastrointestinal toxicity will probably mask any other toxic effects. The guinea pig is less sensitive to most NSAIDs than the rat and closer in sensitivity to humans and thus would be a more appropriate species for investigating the chronic (nongastrointestinal) toxicity of an NSAID. This practice of not rationally choosing an appropriate species for an experiment undoubtedly results in questionable science. This alone should be considered a waste of animals and resources. It results also in additional, and sometimes duplicative, experiments.

Research into replacements for test animals, such as cellular cultures, organs harvested from slaughterhouses, *in silico* (computer) modeling, and physical/chemical systems, has been extensive. While each of these has their utility, they will not replace animals for the foreseeable future. Some degree of animal use will continue, and the future is bright for the ongoing development, refinement, and usage of animal models, for example, building on the quite recent development of transgenic and knockout models for research on various diseases to go along with xenograft and other types of animal models.

See also: Analytical Toxicology; *In Vitro* Test; *In Vivo* Test; Society of Toxicology; Thalidomide; Toxicity Testing, Alternatives; Toxicity Testing, Aquatic; Toxicity Testing, Behavioral; Toxicity Testing, Carcinogenesis; Toxicity Testing, Dermal; Toxicity Testing, Developmental; Toxicity Testing, Inhalation; Toxicity Testing, Irritation; Toxicity Testing, Modeling.

Further Reading

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Relevant Websites

http://dels.nas.edu – US National Academy of Sciences, Institute of Laboratory Animal Research (ILAR). Animal Models & Strains Search Engine.

http://www.toxicology.org – Society of Toxicology, Animals in Research.

Animal Testing Alternatives *See* Toxicity Testing, Alternatives.

Animals, Poisonous and Venomous

Teresa Dodd-Butera and Molly Broderick

This article provides an overview of some of the more commonly encountered terrestrial animals that produce toxins, often referred to as venoms, with a focus on spiders, snakes, and other reptiles.

Arthropods

Phylum Arthropoda is the largest phylum in the animal kingdom. Most of the species are nontoxic. However, Class Arachnida contains spiders. Arachnidism means envenomation from a spider. Most spiders are venomous; however, the black widow, brown recluse, and hobo spiders are responsible for a significant number of toxicity events in humans, so these will be discussed in more detail.

Black Widow Spider

Lactrodectus mactans (lactrodectism is produced by a bite from the female spider). The female is larger than the male. It is noted for a black color that is shiny, with a rounded abdomen and a red hourglass mark on the ventral surface. The black widow spider produces neurotoxic venom. Alpha latrotoxin is the protein of the neurotoxin.

Exposure is usually through a painful bite, although the bite may occasionally go unnoticed until symptoms develop. The mechanism of action of black widow spider venom involves binding of the gangliosides and glycoproteins of the motor end plate in the neuromuscular junction, which affects the opening of sodium channels and the release of acetylcholine (Ach) and norepinephrine. This results in excessive stimulation and allows for penetration and circulation of the venom into the lymphatic system.

Red spots, a slight local reaction, and a 'target lesion' may appear at the site. Symptoms may occur 15 min to several hours after envenomation. Depending on the severity, symptoms may last for several days. Milder symptoms may last for 1–2 weeks. Skeletal muscle cramps are noteworthy for envenomations and may produce a tightness and pain in the chest and abdomen. 'Facies lactrodectismica' is characterized by unique facial sweating and grimacing. Other symptoms may include nausea, tremor, weakness, joint pain, seizures, hypertension, hyperreflexia, extreme restlessness, and priapism (rare). Death has occurred due to seizures and respiratory difficulty. Sensitive populations include the very old and the very young, pregnant women, and those with chronic illnesses. Medical attention should be sought if a bite occurs, particularly in members of these high-risk groups.

Treatment is primarily symptomatic and supportive for a black widow spider bite. Hypertension and severe pain may be present. Pain control may require over-the-counter antiinflammatory agents, or may be severe enough to warrant parenteral opioids. Diazepam may be used for sedation and as an anxiolytic. Effective results have been achieved using lactrodectus antivenin; however, anaphylaxis and serum sickness have been reported. The antivenin may be needed if severe pain and hypertension are refractory to treatment. However, caution must be exercised in individuals allergic to horse serum products. Monitoring the site of the wound for proper healing and tetanus prophylaxis are also important in treatment of these envenomations. Patients should be instructed on using precaution and prevention measures to avoid further envenomations. These may include avoidance of a particular area where the black widow spiders are, or in some instances, careful spraying of areas with creosote may be necessary. Wearing protective clothing may also prevent further bites, if high-risk areas cannot be avoided.

Brown Recluse Spider

Loxosceles reclusa; Violin or Fiddleback Spider (loxoscelism is a systemic syndrome due to the bite of a female brown recluse spider). This is a small reddish brown spider with a violin-shaped mark on the dorsum surface of the cephalothorax. It is generally found in the southern United States. Exposure is through a bite, which may go unnoticed. Spiders may be found in woodpiles and basements.

Loxosceles venom contains hyaluronidase, alkaline phosphatase, 5-ribonucleotide phosphohydrolase, and sphingomyelinase D. Sphingomyelinase D is a component of the cytotoxic venom, with a MW of 32 000 Da. Sphingomyelinase causes release of choline and *N*-acylsphingosine phosphate from the red blood cell membrane, which stimulates platelet aggregation and dermonecrosis.

Initially, a local lesion and swelling may appear at the site of the bite. A blister may appear with worsening pain. Ulceration with delayed healing may occur. Systemic symptoms manifest as high fever, weakness, nausea, vomiting, arthralgias, jaundice, abnormal bleeding, and rashes. Left untreated, life-threatening reactions may progress from hemolytic anemia, thrombocytopenia, and disseminated intravascular coagulation (DIC). Hemolysis may lead to shock, renal failure, and death (rare). Multiple organs may be affected including lungs, heart, pancreas, and liver.

Most of these spider bites do not result in serious toxicity and are managed without medical intervention. However, when symptoms do occur, treatment of both the wound and systemic toxicity are required. Keeping the bite area clean is necessary to avoid secondary infection and further tissue damage. There is no proven preventive measure to avoid dermonecrosis; and surgical excision may delay wound healing. However, surgery may be indicated with abscess formation. Corrective surgery, in severe cases, may be needed for skin grafts or debridement. Systemic reactions require hospitalization in order to provide aggressive supportive care for multiple organ involvement. Patients who present with mild symptoms immediately after a bite with a suspicion of *Loxosceles*, may be monitored as outpatients with adequate medical follow-up. Young children, the elderly, and chronically ill patients need to be monitored carefully, after envenomation from these spiders. Prevention is difficult, but shaking out items carefully and avoiding areas where *Loxosceles* reside may be helpful.

Hobo Spider

Tegenaria agrestis (hobo spiders) are found in Europe and the Pacific Northwestern United States. *T. agrestis* is brown with gray markings and $\sim$10 mm in length. Hobo spiders are found in woodpiles, basements, and moist areas.

Envenomation from a painless bite by *T. agrestis* may result in necrosis, similar to *L. reclusa*. Unlike the previous spiders discussed, male Hobo spiders are more venomous than females. Local symptoms of blistering may occur after envenomation. The blister may rupture and create a necrotic ulcer. Scarring and healing range from 1 month to 3 years. Systemic symptoms may include headache, nausea, intractable

vomiting, profuse diarrhea, weakness, impaired vision, memory loss, pancytopenia, and death.

Treatment is symptomatic and supportive for wound care (similar to loxoscelism) and hematologic complications. Surgical graft repair for severe ulcerative lesions may be warranted.

Scorpions

Scorpion stings occur most commonly in the southwestern United States. There is a range of symptoms which may occur, but children under 6 are at higher risk for mortality. Poisonous scorpions in the United States are *Centruroides sculpturatus* (exilicauda) and *Centruroides gertschii*. Toxin from a sting consists of phospholipase, acetylcholinesterase, hyaluronidase, serotonin, and neurotoxins. Venom of the *Centruroides* genus may cause neurotoxicity. Sodium channels are affected with prolonged action potentials. There are also scorpions outside of the United States that may cause hemorrhaging to a victim. Symptoms may include pain, numbness, restlessness, shaking movements, blurred vision, slurred speech, and respiratory collapse. Local wound care is the most common type of treatment required, but severe toxicity requires hospitalization. Therapy is symptomatic and supportive, dependent on the effects from the specific scorpion toxin. Prevention strategies include shaking out shoes, sleeping bags, and tents, and careful attention when in an area of scorpions, particularly at night.

Hymenoptera: Bees, Wasps, Hornets, and Yellow Jackets

Bites and stings from this subclass may cause toxic and allergic reactions, in numbers greater than those from poisonous snakes. Hymenoptera venom is a combination of biogenic amines, phospholipase, hyaluronidase, and contains other various substances depending on the particular species. Symptoms usually involve local swelling and pain without a systemic reaction. Swelling of the upper airway is a hazard, but is a rare occurrence with one sting. The danger arises when multiple stings occur to the victim, and large numbers have been fatal. An anaphylactic reaction may occur shortly after a sting, in sensitive individuals. Fatalities can occur within minutes. Treatment is symptomatic and supportive for life support and care of the local area of the bite, depending on the severity of the symptoms. Avoidance, if possible, and emergency epinephrine kits for sensitive individuals can be helpful prevention measures.

Snakes

Snakes belong to the phylum Chordata, Class Reptilia. Two major families of venomous snakes are Crotalidae and Elapidae.

Crotalids

Three genera of crotalids are *Crotalus* (Rattlesnake), *Sistrurus* (Massasauguas or pigmy rattlesnakes), and *Agkistrodon* (Copperhead and Cottonmouth). Crotalids ('Pit Vipers') have triangular heads, elliptical pupils, a single row of subcaudal scales behind the anal plate, and facial pits which serve as heat sensors. Crotalids have hinged front fangs ~ 2 cm in length, which are curved and hollowed. Rattlesnakes usually have a rattle – keratin scales at the end of the tail that produce a rattling sound when rubbed together. Venom glands are located posterior to the eyes and connected to fangs by venom ducts. Identifiable characteristics of copperheads are the rust-colored heads, and a white buccal cavity is noteworthy of cottonmouths or 'water moccasins'.

Envenomation from a crotalid bite leaves one or more puncture wounds with a potential for progressive edema and ecchymosis. Crotalid venom contains a mixture of proteins, lipids, and metals. The venom forms fibrin polymers, which are susceptible to normal fibrinolysis and phagocytosis. It is represented by falling fibrinogen levels. Copperhead venom has a weak effect on this series of events in coagulation, resulting in lower morbidity after envenomation.

Initial pain at the site of the bite may be followed with a 'metallic sensation' in the mouth. Victims may become weak, and experience nausea, diarrhea, diaphoresis, and chills. Edema may begin around the bite area or may be delayed. Observation of the site for edema is a clue as to whether or not a 'dry bite' has occurred; that is, that no venom was injected into the site. Envenomation is most serious if venom is injected directly into joints, muscles, or veins. Hemorrhagic blisters and tissue destruction are possible. Neurotoxicity from rattlesnakes (but generally not from cottonmouths or copperheads) may be manifested as fasciculations, which are fine continuous contractions. In some cases, systemic neurotoxicity may involve respiratory failure. In the most serious cases, massive envenomation may lead to serious bleeding, hypotension, shock, multiple organ failure, and a high incidence of mortality.

Despite popular belief, crotalid envenomation does not generally result in life-threatening symptoms. Maintaining a patent airway, intravenous access, clinical observation of edema and the bite area, adequate laboratory work, and the use of antivenin

when necessary are the essentials of treatment in snakebite envenomation. Antivenin should only be used in moderate to severe envenomations, usually within 8 h postenvenomation. This is an equine-derived product and thus skin testing for sensitivity is usually performed after the decision that antivenin is necessary has been made. Serum sickness may occur from the antivenin. Hospital monitoring, wound care, and patient follow-up are important for the recovery of these patients.

Elapidae

This family includes coral snakes, cobras, mambas, and kraits. In the United States, Elapidae are responsible for 1–2% of poisonous snakebites. The incidence of envenomations is greater in some other parts of the world. Examples of coral snakes commonly found in the United States are the eastern coral snake, the Sonoran coral snake, and the Texas coral snake. Coral snakes are smaller than pit vipers. They do not have facial pits, and the head is rounded, as are the pupils. Fangs are $\sim$2 mm and fixed to the jaw. Coral snakes are also more brightly colored, with bands of black and red, separated by yellow and white bands. Coral snakes are timid, nocturnal creatures.

Envenomation from a coral snake exerts minimal local pain, and appears as rows of teeth marks. Victims may report that the snake was 'chewing' on the bite site and had to be forcibly removed. Coral snake venom is composed of peptides and enzymes that have not all been identified, but which exert neurotoxicity rather than cytotoxicity.

Minimal local pain may be present initially; however, systemic toxicity may be delayed for several hours. Nausea, vomiting, weakness, dizziness, and numbness have been reported. Drowsiness or euphoria may occur. Central and peripheral nervous system effects and paralysis may be quite serious, but do not always occur. Life support measures should be instituted, as necessary, with stabilization of vital signs, and evaluation of pulmonary and neurological symptoms. Antivenin may be given to a patient, based on history and circumstances of the bite. Skin testing for sensitivity to horse serum is performed when that decision is made. Patients who are treated with the antivenin need to be monitored for serum sickness. In addition, monitoring the patient for respiratory symptoms, wound, and skin infections should continue. Victims may take weeks to recover, if paralysis has occurred. Long-term prognosis is generally good for these patients.

Other Reptiles

Venomous Lizards

The Gila monster (*Heloderma suspectum*) and the beaded lizard (*Heloderma horridum*), Order Squamata, are found in desert areas; for example, in the southwestern United States and Mexico. They are large, timid, nocturnal creatures. Beaded lizard bites are rarely encountered. In fact, bites of both types of lizards are uncommon; however, Gila monsters are known for their tenacity when they do bite. They may need to be forcibly disengaged from their victims. Gila monster venom contains enzymes, hyaluronidase, phospholipase A, and serotonin, in addition to other toxins. Envenomation does not always occur with bites. Symptoms following envenomation include pain, swelling, and possible anaphylactic reactions. Symptomatic and supportive care for the victim is the treatment plan.

See also: Diazepam; Marine Organisms; Scorpions; Snakes.

Further Reading

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Relevant Website

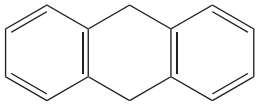
http://www.calpoison.org

Antagonism *See* Chemical Interactions.

Anthracene

Prathibha S Rao

- Chemical Abstracts Service Registry Number: CAS 120-12-7
- Synonyms: Anthracin; Paranaphthalene; Green oil; Tetra oil N2G
- Chemical/Pharmaceutical/Other Class: Polycyclic aromatic hydrocarbon
- Chemical Structure:



Background Information

Anthracene is a solid white to yellow crystal, has a weak aromatic odor, and sinks in water. Its characteristics are boiling point, 342°C; melting point, 218°C; molecular weight, 178.22; density/specific gravity, 1.25 at 27 and 4°C; octanol–water coefficient, 4.45. It is soluble in absolute alcohol and organic solvents. Maximum absorption occurs at 218 nm.

Exposure Routes and Pathways

Inhalation is the primary exposure pathway. Natural occurring sources include a high boiling fraction of coal tar, consisting of anthracene, phenanthrene, and other solid hydrocarbons as well as acridine. Other sources include volcanoes and forest fires. Artificial sources include exhaust from motor vehicles and other gasoline and diesel engines; cigarette, marijuana, and cigar smoke; emissions from coal-, oil-, and wood-burning stoves, furnaces and power plants; smoke and soot. Air pollution sources include coke oven emissions, space heating installation burning, emissions from typical European gasoline engines, dielectric in the manufacture of battery electrodes, and electric arc furnace electrodes; felt, roof, and paper manufacturing; and alumina reduction.

Toxicokinetics

Polycyclic aromatic hydrocarbons were detected in human fat and liver and their average concentrations were 1100 and 380 ppt, respectively. Anthracene was found at high levels in the liver and fat. When administered orally to animals 70–80% of the dose is excreted unchanged in the feces but metabolites present in rat urine include *N*-acetyl-*S*-(1,2-dihydro-2-hydroxy-1-anthryl)-cysteine and conjugates of *trans*-1,2-dihydroanthracene-1,2-diol and 1,2-dihydroxyanthracene. The cysteine conjugate is decomposed by mineral acids to yield 1-anthrylmercapturic acid, 1- and 2-anthrols, and anthracene. Rats metabolize anthracene into *trans*-9,10-dihydroanthracene-9,10-diol, which gives rise to anthrone and several hydroxylated metabolites.

Acute and Short-Term Toxicity (or Exposure)

Human

Anthracene is photosensitizing. It can cause acute dermatitis with symptoms of burning, itching, and edema, which are more pronounced in the exposed bare skin regions. Other symptoms are lacrimation, photophobia, edema of the eyelids, and conjunctival hyperemia. The acute symptoms disappear within several days after cessation of contact. Systemic effects of industrial anthracene manifest themselves by headache, nausea, loss of appetite, slow reactions, and adynamia.

Chronic Toxicity (or Exposure)

Animal

Anthracene showed no mutagenic activity in *Salmonella thyphimurium* TA100 and TA98 with and without addition of rat liver microsomes (S9) and no carcinogenic activity in Swiss albino mice. A significant increase in the formation of nonneoplastic melanotic tumors was observed among first- and second-generation progeny of *Drosophila melanogaster* that had been exposed chronically as larvae to low concentrations of anthracene.

Human

Chronic exposure may lead to inflammation of the gastrointestinal tract, patchy areas of increased yellow–brown pigment changes, loss of skin pigment, thinning or patchy thickening of skin, skin warts, skin cancer, and pimples. Repeated breathing of 'fumes', especially from heated anthracene, may cause a chronic bronchitis with cough and phlegm. Repeated exposure of male scrotum can cause skin thinning and increased skin pigmentation.

No occupational exposure limits have been established for anthracene. However, safe work practices should always be followed.

Clinical Management

No specific treatments have been prescribed. The patient should be moved to fresh air in case of respiratory distress.

See also: Coke Oven Emissions; Polycyclic Aromatic Hydrocarbons (PAHs).

Relevant Websites

http://www.speclab.com – Spectrum Laboratories Inc.
http://www.state.nj.us – State of New Jersey.
http://www.1.nature.nps.gov – National Park Service Nature & Science.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Anthracene.
http://rais.ornl.gov – The Risk Assessment Information System.

Anthrax

Kartik Shankar and Harihara M Mehendale

Epidemiology

Anthrax is a zoonotic disease with worldwide distribution. Anthrax is caused by *Bacillus anthracis*, a gram-positive, spore-forming, rod-shaped bacterium that primarily infects herbivores such as cattle and deer. The earliest known description of anthrax is found in the Book of *Genesis*, in which the fifth plague is said to have killed Egyptian cattle. Further, there are numerous descriptions of anthrax in animals and humans in Hindu, Greek, and Roman literature. Between 20 000 and 100 000 cases of anthrax have been estimated to occur worldwide. Human anthrax is most common in enzootic areas in developing countries, among people who work with livestock, eat undercooked meat from infected animals, or work in establishments where wool, goatskins, and pelts are stored and processed. West Africa is the most affected part in the world. Anthrax is also a significant problem in other parts of Africa, Central America, Spain, Greece, Turkey, and the Middle East. In economically advanced countries, where animal anthrax is controlled, incidence in humans is rare. Further infections have been dramatically reduced by the vaccination of high-risk individuals and improvements in industrial hygiene. Incidence in the United States declined to less than one case per year till the recent biological terrorism attacks in the fall of 2001.

Microbiology

B. anthracis is nonmotile, catalase positive, nonhemolytic on blood agar, and frequently occurs in long chains. Chains of virulent forms are surrounded by a capsule. Sporulation occurs in soil and on culture media but not in living tissue. Spores are highly resistant to UV light, high-temperature extremes, high pH, drying, high salinity levels, and routine methods of disinfection.

Mechanism of Toxicity

Anthrax toxin is composed of three proteins: protective antigen (PA; 83 kDa), lethal factor (LF; 90 kDa), and edema factor (EF; 89 kDa). Individually, none of the three proteins are toxic but interact synergistically with at least one of the others. PA and LF (called LeTx) can cause lethal shock in experimental animals, and a mixture of PA and EF (edema toxin, EdTx) induces edema at the site of injection. Since two discrete units of the toxin are required for its action, the term binary toxin has been used to this and other bacterial toxins. Anthrax is unique from other binary toxins in that the binary moieties (EF and LF) interact only after being secreted from the bacteria. Further, EF and LF enter the cell via a single PA protein. Assembly of the three toxin proteins is initiated when PA binds to a proteinaceous cellular receptor and is activated by a member of the furin family of cellular proteases. The exact mechanisms of internalization of the toxin moieties are subject of scientific enquiry. Inside the cellular cytoplasm, EF (a calcium and calmodulin-dependent adenylate cyclase) causes a dramatic increase in intracellular cAMP concentrations and LF acts proteolytically to cleave certain MAPK kinases.

Clinical Forms of Anthrax

Anthrax mainly occurs in three forms: cutaneous, inhalation, and gastrointestinal. Exposure to *B. anthracis* most likely in an occupational setting is the cause of cutaneous anthrax. The incubation period varies from 1 to 12 days. In most cases, the disease remains localized to the skin lesion. Major diagnostic characteristic is the development of edema around the

lesion. The fatality rate is 20% without and less than 1% with antibiotic treatment. Inhalation anthrax is the most lethal form of anthrax resulting from inhalation of pathogenic endospores. The US Department of Defense estimates that the lethal dose in humans is ~8000–10 000 spores. The illness is biphasic after exposure to large numbers of spores. The first phase is characterized by a 'flu-like' illness with nonproductive cough. After several days of apparent improvement there is a sudden onset of rapidly progressive respiratory failure, acute dyspnea, circulatory collapse, and pleural effusion. The mortality rate is very high, is spite of supportive care and antibiotics, generally within 24 h of the onset of the second stage due to toxemia and suffocation. Gastrointestinal anthrax, although rare, occurs after an incubation period of 1–7 days following ingestions of *B. anthracis* via contaminated food or drink. Mortality rates are estimated to be between 25% and 60% unless treatment is begun early enough. Severe abdominal pain, fever, nausea, vomiting, and bloody diarrhea are manifested during the disease. Death occurs due to toxemia and shock.

Clinical Management

Prompt clinical diagnosis and treatment with effective antimicrobial drugs is necessary for successful treatment of anthrax. Although *B. anthracis* is susceptible to penicillin *in vitro* it is not used as monotherapy. Several historical strains produce an inducible β-lactamase and are resistant to penicillin. Ciprofloxacin (400 mg intravenously twice daily) and possibly other quinolones or doxycycline (200 mg intravenously twice daily) should be used as initial therapy. The duration of treatment for inhalation anthrax should be 60 days. Corticosteroid therapy should be considered for patients with inhalation anthrax associated with meningitis or severe edema. Supportive therapy should be initiated to prevent shock, fluid and electrolyte imbalance, and loss of airway patency.

Potential for Use as a Biological Weapon

Anthrax is classified as a category A biological weapon (most dangerous) along with smallpox, plague, *Clostridium botulinum* toxins, filovoviruses, etc. *B. anthracis* has several biological, technical, and virulence characteristics that make it an attractive bioweapon. These include easy procurement from a variety of sources and relative ease to grow, process, and store. The World Health Organization estimates that 50 kg of weapon-grade anthrax spores released by an aircraft over an urban population of 5 million would result in 250 000 cases of mainly inhalation anthrax.

See also: Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents; Botulinum Toxin; Chemical Warfare Agents.

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Anticholinergics

Swarupa G Kulkarni and Harihara M Mehendale

- SYNONYMS: Parasympatholytics; Cholinergic blockers; Sympatholytics; Antispasmodics

Uses

Anticholinergics have a wide range of therapeutic uses: prior to anesthesia, as a prophylactic for preventing motion sickness, in symptomatic control of Parkinson's disease, in abnormal slowing of the heart in poisoning with organophosphates and other cholinergic drugs, and in the treatment of peptic ulcer and irritable bowel syndrome.

Since these agents act as smooth muscle relaxants they are used as antispasmodics and may be used to reduce spasms of the stomach. Antimuscarinics appear to be useful in the treatment of gastrointestinal hypersecretory states (e.g., Zollinger Ellison syndrome) and may be used in conjunction with an H_2 receptor antagonist. In addition to atropine, belladonna, and other semisynthetic derivatives, a number of other synthetic compounds are also used in gastrointestinal disorders. These compounds consist of a large blocking group linked by a short chain to a strongly basic tertiary or quaternary group. Synthetic drugs used as antispasmodics or antisecretory agents in gastrointestinal disorders include oxyphenonium bromide, isopropamide iodide, mepenzolate bromide, and dicyclomine.

Antimuscarinics are potent bronchodilators and are used in the treatment of chronic bronchitis and asthma.

Toxicokinetics

Antimuscarinics having a quaternary ammonium group are incompletely absorbed from the gut since these are completely ionized. The tertiary amine antimuscarinics are readily absorbed from the gut. The presence of food may reduce absorption. Quaternary ammonium antimuscarinics exhibit poor lipid solubility, do not cross the blood–brain barrier, and thus exhibit minimal central nervous system (CNS) effects. Also due to their poor lipid solubility they do not penetrate the eye and are unlikely to appear in the milk. Atropine and other tertiary amines are capable of crossing the CNS. Atropine is capable of crossing the placenta and has been stated to distribute into milk in small quantities. It is oxidized primarily in the liver. Atropine is apparently metabolized in the liver to tropic acid, tropine, and possibly esters of tropic acid and glucuronide conjugate.

Antimuscarinics are mainly eliminated in urine as unchanged drug and its metabolites. Following oral administration substantial amounts of antimuscarinics may be eliminated in feces as unabsorbed drug.

Mechanism of Toxicity

Antimuscarinics competitively inhibit the action of acetylcholine or other cholinergic stimuli at the muscarinic receptor. At usual doses these have little or no effect on the cholinergic stimuli at nicotinic receptors. Autonomic ganglia, where cholinergic transmission involves nicotinic receptors, produce a partial cholinergic block at relatively high doses. Receptors at various sites are not equally sensitive to inhibitory effects of antimuscarinics. Atropine acts by competitive antagonism at the receptor sites of the effector organs. It may also inhibit responses to histamine, serotonin, and norepinephrine and may block transmission at the autonomic ganglia and the skeletal neuroeffector junction.

Acute and Short-Term Toxicity (or Exposure)

Human

Single, 10 mg oral doses of atropine have produced signs of acute toxicity in adults. Children are more susceptible than adults to the toxic effects of atropine. Deaths have been reported in children following ingestion of 10 mg of atropine.

Acute overdosage with antimuscarinics produces both peripheral and CNS symptomatology. The quaternary ammonium compounds do not readily penetrate the CNS and thus exhibit minimal central effects even at toxic doses. Patients with anticholinergic toxicity will typically show peripheral symptoms including dry mouth, thirst, fixed dilated pupils, flushed face, fever, hot, dry, red skin, urinary retention, hyperthermia, hypotension, tachycardia, and increased respiratory rate. In addition to tachycardia, cardiac manifestations may include EKG abnormalities similar to those produced by quinidine. Speech and swallowing may be impaired in association with blurred vision. Other peripheral signs and symptoms may include nausea and vomiting.

In large doses, atropine induces stimulation of the CNS, which in humans is characterized by overactive coordinated movements, hallucinations, and delirium. After the stimulation has lasted for some time, depression sets in and may proceed to complete paralysis of the CNS, which is fatal through cessation of respiration. In infants, particularly those ingesting antihistamines, paradoxical excitement may occur subsequently followed by a more characteristic CNS depression. CNS manifestations may resemble acute psychosis characterized by incoherence, confusion, hallucinations, delusions, paranoia, and abnormal motor behavior.

In severe overdosage, CNS depression, circulatory collapse, and hypotension may occur. Coma and skeletal muscle paralysis may also occur followed by death due to respiratory failure. Acute overdosage with quaternary ammonium antimuscarinics may produce a curariform neuromuscular block and ganglionic blockade manifested as respiratory paralysis.

Clinical Management

Immediate treatment should include instituting emesis, with syrup of ipecac or gastric lavage, followed by administration of activated charcoal and saline cathartics if the patient is not comatose. Induced emesis may be ineffective in ingestion of antihistaminics related to phenothiazines or in massive ingestion. The use of physostigmine should generally be reserved for treatment of patients with extreme delirium or agitation. Physostigmine in a dose of 0.5–2 mg administered intravenously, which can be repeated every 30 min as needed, may be used to alleviate symptoms like confusion, agitation, or coma. Other cholinergic antagonists have not been useful since they do not cross the blood–brain barrier. Measures such as forced diuresis and dialysis have not yet been shown to be effective.

Fluid therapy and other standard treatments of shock should be administered as needed.

Miscellaneous

As a class, anticholinergics include the antihistamines, atropine and homatropine; anti-Parkinsonian agents like benzotropine, procyclidine, and trihexyphenidyl; the antimuscarinics of which atropine is the prototype; and antispasmodics like dicyclomine and oxybutymin. Most antimuscarinics are aminoalcohols or their derivatives (usually esters or ethers), aminoamides, or other amines. Antimuscarinics can be divided into two groups. These are the naturally occurring alkaloids and their semisynthetic derivatives like atropine, homatropine, scopolamine, and hyoscyamine and the synthetic amine compounds such as anisotropine, dicyclomine, and ipratropium.

See also: Cholinesterase Inhibition; Gastrointestinal System; Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates.

Further Reading

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Scarpero HM and Dmochowski RR (2003) Muscarinic receptors: What we know. *Current Urology Reports* 4: 421–428.

Relevant Website

http://bnf.org – British National Formulary: Antimuscarinic.

Antimony

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst, Shirley B Radding, and Kathryn A Wurzel, volume 1, pp. 76–77, © 1998, Elsevier Inc.

- Representative Compounds: SbH_3 (Stibine); Antimony trioxide
- Chemical Abstracts Service Registry Number: CAS 7440-36-0
- Synonym: Stiblum
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Sb^{3+}; Sb^{5+}

Uses

Antimony is used in white metal, which is any of a group of alloys having relatively low melting points. White metal usually contains tin, lead, or antimony as the chief component (e.g., the alloys Britannia and Babbitt). Antimony is used as a hardening alloy for lead, especially in storage batteries and cables, bearing metal, type metal, solder, collapsible tubes and foil, sheet and pipe, semiconductor technology, and pyrotechnics. It is also used in thermoelectric piles, and for blackening iron or coatings. Antimony-containing compounds are used in materials for refrigerators, air conditioners, aerosol sprays, paints, and flameproofing agents. Approximately half of the antimony used in the United States is recovered from lead-based battery scrap. Antimony is also used medicinally (e.g., antimony potassium tartrate as an emetic and antimony as an antiparasitic agent).

Exposure Routes and Pathways

The emission of antimony into the human environment appears to be the result of human activity, with the emission of antimony trioxide being the most significant source. Antimony trioxide is emitted as a result of coal burning, or with fly ash when antimony-containing ores are smelted. In addition, medicines containing antimony are administered orally. Antimony is present in food and drinking water is present mostly in the low μg/kg-wet weight range or less, including vegetables grown on Sb-contaminated soils. Daily oral uptake of Sb ranges from 10 to $70\,\mu g\,day^{-1}$ and appears to be significantly higher than exposure by inhalation.

Toxicokinetics

Normally, antimony is absorbed slowly when ingested or administered orally. Many antimony compounds are gastrointestinal irritants. The emetic antimony potassium tartrate is easily absorbed and, within 24 h, 50% is excreted in the urine (hamsters). Antimony can concentrate in lung tissue, the thyroid gland, the adrenal glands, the kidneys, and the liver. The trivalent compounds of antimony concentrate in the red blood cells and liver and the pentavalent compounds concentrate in the blood plasma. Both forms are excreted in feces and urine, but generally,

more trivalent compounds are excreted in urine and more pentavalent compounds in feces.

Presumably by reacting with the sulfhydryl groups, antimony can inhibit oxidative and phosphorylating enzymes like monoamine oxidase, succinoxidase, pyruvate brain oxidase, and phosphoftuctokinase. Inhibition of these enzymes can alter activities such as glucose metabolism and nerve transmission. Ten percent of the trivalent form is excreted by the kidney in 24 h; 50–60% of the pentavelent form is found in the urine within 24 h.

Mechanism of Toxicity

The toxicity of Sb is a function of the water solubility and the oxidation state of the Sb species under consideration. Antimony toxicity often parallels that of arsenic, although antimony salts are less readily absorbed than arsenic. It is presumed that antimony, like arsenic, complexes with sulfhydryl groups of essential enzymes and other proteins. By analogy, antimony can uncouple oxidative phosphorylation, which would inhibit the production of energy necessary for cellular functions. Antimony's trivalent compounds are more toxic than its pentavalent compounds.

Acute and Short-Term Toxicity (or Exposure)

Animal

The rat oral LD_{50} is $100\,mg\,kg^{-1}$. Antimony administered intravenously to experimental animals resulted in abnormal electrocardiograms.

Human

Accidental poisonings can result in acute toxicity, which produces vomiting and diarrhea. Most information regarding antimony toxicity has been obtained from industrial exposures. Occupational exposures usually occur through inhalation of dusts containing antimony compounds. Workers exposed to antimony trisulfide (used as a pigment and in match production) at concentrations greater than $3.0\,mg\,m^{-3}$ experienced heart complications and died. In addition, a temporary skin rash, called 'antimony spots', can occur in persons chronically exposed to antimony in the workplace. Inhalation of antimony hydride (stibine gas) can lead to hemolytic anemia, renal failure, and hematuria. Stibine gas is produced when antimony alloys are treated with acids.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to a dose level of $4.2\,mg\,m^{-3}$ airborne antimony trioxide dust for 1 year were reported to develop lung tumors; at a dose level of $1.6\,mg\,m^{-3}$, lung tumors were not found. Guinea pigs exposed to airborne antimony trioxide developed interstitial pneumonia. Oral feeding of antimony to rats does not induce an excess of tumors or teratogenesis. In rats it is a tumorigen, and high levels cause decreased red blood cell counts, hematocrit, hemoglobin levels, and plasma protein concentrations.

Human

Inhalation of antimony compounds produces different effects at different concentrations. Chronic inhalation of low concentrations causes rhinitis and irritation of the trachea. At high concentrations, acute pulmonary edema occurs, and bronchitis may occur (the bronchitis may lead to emphysema). Inhaled antimony concentrates in lung tissue; as a result, pneumoconiosis with obstructive lung disease has been recorded. Antimony is a suspected human carcinogen.

In Vitro Toxicity Data

The compounds $SbCl_3$ and $SbCl_5$ were reported to be genotoxic in the rec-assay with *B. subtilis*. Sb(III)acetate enhanced the Simian-Adenovirus-7-mediated transformation of SHE-cells, and enhanced rates of chromosomal breaks in human leukocytes were reported after treatment with potassium antimony tartarate (APT). $SbCl_3$ did not induce DNA/protein-crosslinks in V79-cells and peripheral human lymphocytes.

Clinical Management

The oil-soluble BAL (British anti-Lewisite; 2,3-dimercaptopropanol) administered intramuscularly appears to be the antidote of choice for antimony poisoning. The antidotal action of BAL depends on its ability to prevent or break the union between antimony and vital enzymes.

Ecotoxicology

Antimony is highly toxic to amphibians and zooplankton.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA) in the United States have the following airborne exposure limits:

- OSHA Standard: Permissible exposure limit – 8 h time-weighted average (TWA) = $0.5\,mg\,m^{-3}$.
- ACGIH threshold limit value: 8 h TWA = $0.5\,mg\,m^{-3}$ (antimony and compounds, as Sb). ACGIH classifies antimony as a suspected human carcinogen.

In the United States, antimony is listed as a Clean Air Act hazardous air pollutant generally known or suspected to cause serious health problems. Antimony and its compounds are listed as Clean Water Act toxic pollutants, subject to effluent limitations. The Federal Drinking Water Standards is $6\,\mu g\,l^{-1}$.

See also: Antimony Trioxide; Metals.

Further Reading

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.

Winship KA (1987) Toxicity of antimony and its compounds. *Adverse Drug Reactions and Acute Poisoning Reviews* 2: 67–90.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Antimony.

Antimony Trioxide

Shayne C Gad

Txcl.00081

- Chemical Abstracts Service Registry Number: CAS 1309-64-6
- Synonyms: Antimony white; Antimony oxide; Antox; Thermogrand B; ATO
- Chemical/Pharmaceutical/Other Class: Metal oxide
- Chemical Formula: Sb_2O_3

Uses

Antimony trioxide is used in flameproofing of textiles, paper, and plastics; as paint pigments, ceramic opacifier, catalyst, staining iron and copper; and as a mordant.

Exposure Routes and Pathways

Inhalation and oral routes from pottery glaze. The emission of antimony into the human environment appears to be the result of human activity, with the emission of antimony trioxide being the most significant source. Antimony trioxide is emitted as a result of coal burning or with fly ash when antimony-containing ores are smelted.

Toxicokinetics

Antimony trioxide is poorly absorbed orally and through the lungs. Trivalent antimony readily leaves the plasma but remains in the circulation bound to erythrocytes excreted in the bile after conjugation with glutathione.

Mechanism of Toxicity

The toxicity of antimony is a function of the water solubility and the oxidation state of the antimony species under consideration. It can react with red cell membrane and interfere with hemoglobin function. It has high affinity for sulfhydryl groups.

Acute and Short-Term Toxicity (or Exposure)

Animal

Antimony trioxide is a mild primary eye irritant in rabbits. Rat oral $LD_{50} > 34\,g\,kg^{-1}$. Mouse intraperitoneal $LD_{50} = 172\,mg\,kg^{-1}$. The *in vivo* genotoxicity of antimony trioxide was studied using single and repeat dose mouse bone marrow micronucleus tests, and the rat liver unscheduled DNA synthesis assay. All three studies were negative. In contrast, chromosomal damage by antimony trioxide was reported in mouse bone marrow cells after repeat dosing but not after single dosing. This discrepancy with respect to repeat dosing may be explained by the 'not specified

purity' and much higher systemic toxicity of the antimony trioxide sample used in one study; for this reason and because of the poor water solubility of antimony trioxide, it has been concluded that antimony trioxide was not genotoxic *in vivo*.

Human

Eye and respiratory irritation occur due to antimony exposure. Normal human serum levels of antimony should be from 0.05 to 0.50 mg dl^{-1}.

Chronic Toxicity (or Exposure)

Animal

Antimony trioxide is a mutagen in bacteria and human lymphocytes. Chronic exposure leads to reproductive and developmental effects. There was one experimental lifetime study of antimony trioxide with rats; however, this study was reported to have had many methodological shortcomings.

Human

The International Agency for Research on Cancer evaluated antimony trioxide and concluded that antimony trioxide was possibly carcinogenic to humans (group 2b) on the basis of the inhalation study in rats.

In Vitro Toxicity Data

Antimony trioxide was genotoxic in a number of older bacterial mutation assays but not in more recent studies. Positive results were observed with antimony trioxide in the *in vitro* cytogenetic assay with human lymphocytes and the sister chromatid exchange assay with V79-cells, but not in the L5178Y mutation assay.

Clinical Management

The oil-soluble BAL (British antilewisite; 2,3-dimercaptopropanol) administered intramuscularly appears to be the antidote of choice for antimony poisoning. The antidotal action of BAL depends on its ability to prevent or break the union between antimony and vital enzymes.

Environmental Fate

Antimony does not bioaccumulate so exposure to naturally occurring antimony through food is very small.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA) in the United States have the following airborne exposure limits:

- OSHA standard: permissible exposure limit (PEL): 8 h time-weighted average (TWA): 0.5 mg m^{-3}.
- ACGIH threshold limit value: 8 h TWA: 0.5 mg m^{-3} (antimony and compounds, as Sb). ACGIH classifies antimony as a suspected human carcinogen.

In the United States, antimony is listed as a Clean Air Act hazardous air pollutant generally known or suspected to cause serious health problems. Antimony and its compounds are listed as Clean Water Act toxic pollutants, subject to effluent limitations. The Federal Drinking Water Standard is 6 μg l^{-1}.

See also: Antimony; Metals.

Further Reading

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Anxiolytics

Swarupa G Kulkarni and Harihara M Mehendale

- SYNONYMS: Minor tranquilizers; Antianxiety drugs; Sedative-hypnotics; Benzodiazepines (BZDs)

Uses

Anxiolytics are used for preoperative relief of anxiety, for conscious sedation, as hypnotics in the treatment of insomnia, for short-term relief of symptoms of anxiety, or for the management of anxiety disorders. Benzodiazepines (BZDs) are also used for the

management of agitation associated with alcohol withdrawal, for their anticonvulsant properties, and as skeletal muscle relaxants. BZDs are preferred over barbiturates since these are less likely to produce tolerance and physical dependence and are remarkably safe in large suicidal doses.

Toxicokinetics

The BZDs meprobamate and buspirone are well absorbed from the gut. Plasma concentration of the BZDs and their metabolites exhibits considerable interpatient variation. Onset and duration of action varies depending on the BZD and the route of administration. BZDs are widely distributed into body tissues and cross the blood–brain barrier. Generally, BZDs and their metabolites cross the placenta. The concentration of diazepam in fetal circulation has been reported to be equal to or greater than the maternal plasma concentration. The drugs and their metabolites are distributed into milk. BZDs and their metabolites are highly bound to plasma proteins. Meprobamate is uniformly distributed throughout the body and is 20% bound to plasma proteins. It is capable of crossing the placenta and is distributed in milk. Buspirone is extensively distributed and is 95% bound to plasma proteins, mainly albumin. These are metabolized in the liver and may undergo conjugation. Meprobamate is metabolized to form the 2β-hydroxymeprobamate and glucosyluronide and glucuronide conjugate of meprobamate. Buspirone is metabolized in the liver, mainly via oxidation, to form the hydroxylated metabolites, which may further undergo conjugation. BZD metabolites are excreted principally in urine. Meprobamate is excreted mainly via the urine. Buspirone is excreted principally in the urine and to a lesser extent in feces.

Mechanism of Toxicity

The advantage of using BZDs is that they have a larger therapeutic index. The exact sites and mode of action of the BZDs have not been elucidated. However, their effects seem to be mediated through the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Allosteric interaction of central BZD receptors with $GABA_A$ receptors and subsequent opening of chloride channels are involved in eliciting the central nervous system (CNS) effects of the drugs. These drugs appear to act at the limbic, thalamic, and hypothalamic levels of the CNS. In usual doses, BZDs appear to have very little effect on the autonomic nervous system, respiration, or the cardiovascular system. These do not produce extrapyramidal side effects or interfere with the autonomic nervous system function. The mechanism of action of meprobamate is unknown. The mechanism of action of buspirone probably involves several neurotransmitter systems.

Acute and Short-Term Toxicity (or Exposure)

Human

The BZDs have a low order of toxicity unless ingested with other CNS depressants. Deep coma is rare. The BZDs have been known to cause dose-dependent adverse CNS effects. BZD overdosage may result in somnolence, impaired coordination, slurred speech, confusion, coma, and diminished reflexes. Hypotension, seizures, respiratory depression, and apnea may also occur. Although cardiac arrest has been reported, death from overdosage of BZDs in the absence of concurrent ingestion of alcohol and other CNS depressants is rare.

BZDs should be avoided during the first trimester and at delivery. Malformation and CNS dysfunction have been described in infants born of mothers using BZDs during pregnancy. Both animal data and human epidemiological studies suggest that BZDs are teratogens.

Severe anaphylactic reactions following intravenous administration of diazepam have been reported. Meprobamate causes toxicity similar to that of a barbiturate overdosage. Death may result from respiratory failure or hypotension. Limited information is available about the acute toxicity of buspirone. Effects are merely extensions of pharmacological effects. Nausea, vomiting, dizziness, drowsiness, miosis, and gastric distention may be seen.

Chronic Toxicity (or Exposure)

Human

Tolerance and psychologic and physical dependence may occur following prolonged use of BZDs. Such effects may occur following short-term use of BZDs particularly at high doses. Drowsiness, ataxia, slurred speech, and vertigo may be seen on dependence. Withdrawal symptoms, including anxiety, agitation, tension, dysphoria, anorexia, insomnia, sweating, blurred vision, irritability, tremors, and hallucinations, may be seen. Milder withdrawal symptoms such as insomnia have also been reported. Since some BZDs and their metabolites have long elimination half-lives, withdrawal symptoms may not occur until several days after the drug has been discontinued.

Meprobamate causes physical dependence similar to that seen with barbiturate dependence. No physical dependence on buspirone administration has been seen.

Clinical Management

Emesis is not recommended following an overdosage of BZD because of the potential of CNS depression. Gastric lavage soon after ingestion and activated charcoal/cathartic may be administered. Pulse, respiration, and blood pressure should be monitored and the patient should be closely observed. Intravenous fluids should be administered and adequate airway maintained. Hypotension may be controlled, if necessary, by intravenous administration of norepinephrine or metaraminol. Although some manufacturers recommend use of caffeine and sodium benzoate to combat CNS depression, most authorities believe that caffeine and other analeptic agents should not be used. Flumazenil (BZD antagonist) may be used in treatment. Flumazenil is an adjunct to and not a substitute for appropriate supportive and symptomatic therapy. Flumazenil (0.2–3 mg) intravenously in 0.2 to 0.3 mg increments for BZD overdose in adults and 0.2–1 mg intravenously in 0.2 to 0.3 mg increments for reversal of BZD sedation in adults may be used. Gradual dosage tapering is required. Occasionally temporary reinstitution of BZD therapy to suppress withdrawal symptoms may be necessary. Initial withdrawal symptoms may be managed with phenobarbitone or diazepam, followed by decreasing the dose by $\sim$10% per day of the initial dose required to control symptoms. Treatment of BZD physical dependence consists of cautious and gradual withdrawal of the drug using a dosage tapering schedule. In the case of meprobamate toxicity general supportive therapy should be maintained. Forced diuresis may be beneficial. In the case of withdrawal symptoms, the patient may be stabilized on phenobarbitone, which is then withdrawn over 10–14 days. No specific antidote is available for the treatment of an overdosage of buspirone and treatment involves symptomatic and supportive care.

Miscellaneous

This class of compounds includes the BZDs like diazepam (Valium) and oxazepam (Serax), chlordiazepoxide (Librium), meprobamate (carbamate derivative), and related compounds, and buspirone (aryl piperazine derivative), which is an anxioselective drug. A miscellaneous group of drugs includes certain antihistaminic and anticholinergic drugs that are difficult to classify (e.g., hydroxyzine and buclizine).

See also: Barbiturates, Long-Acting; Barbiturates, Short-Acting; Neurotoxicity.

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Relevant Website

http://bnf.org – British National Formulary: 4.1 Hypnotics and anxiolytics.

Apoptosis

Sidhartha D Ray and Harihara M Mehendale

Definition

Apoptosis, a form of cell death (Ap oh' tosis or A 'pop tosis: Greek 'apo', meaning leaf; 'ptosis', meaning falling off), is a genetically self-orchestrated naturally occurring cell death process that is associated with the course of development and induced during pathological situations for the overall benefit of the organism. In contrast, necrosis (necroh' sis), another form of cell death, typically affects groups of contiguous cells, and an inflammatory reaction usually develops in the adjacent viable tissue in response to the released cellular debris.

Introduction

Cell death is a necessary event in the life of a multicellular organism. Cells predominantly die via

apoptosis or necrosis. Since apoptosis is a form of tightly regulated genetically controlled self-orchestrated cell death, it is often referred to as programmed cell death (PCD). In contrast, necrosis is termed unprogrammed cell death since it occurs accidentally in an unplanned manner. However, occasionally, incomplete execution of biochemical cascade leads to the expression of morphological features of both apoptosis and necrosis yielding to a third type of death, also called apocrosis or aponecrosis. Apocrotic cells do not deliberately bypass the common biochemical machinery shared by both apoptosis and necrosis and are morphologically distinguishable from both apoptosis and necrosis (display signs of both apoptosis and necrosis; molecular mechanism discussed below in a separate section). The term 'apoptosis' appeared in the 1970s, but the phenomenon had been known long before. Apparently PCD was discovered by C. Vogt in the middle of the nineteenth century through observations on the morphology of dying cells during metamorphosis of amphibians. By 1885, there were publications unequivocally diagramming apoptosis, and simultaneously several researchers had noted the death of metamorphosing tissues in insects.

When apoptosis was first described over three decades ago, the terminologies describing these changes (councilman or acidophillic bodies in liver diseases; civatte bodies in lichen planus; tingible bodies in lymphoid germinal centers; basophilic or Benirschke granules characteristic of premenstrual endometrial glands) were already in the perusal of scientists who coined, defined and differentiated 'apoptosis' from 'necrosis' based on changes in cellular ultrastructures. Naming of the process evolved from 'shrinkage necrosis' or 'coagulative necrosis' (early) exclusively on morphologic grounds to 'apoptosis' (later) when it was found to play a role in regulating tissue morphogenesis and tissue size. In contrast, historically, the only form of cell death known to human kind for centuries used to be 'necrosis'. From mechanistic standpoint, necrotic cells pass through a reversible phase, often followed with explosive rapidity, by irreversible changes resulting from the insulting stimuli.

What is Cell Death?

Generally cell death or loss of cell viability can be defined as irreversible failure of vital cellular functions coupled with irreparable structural damage. Therefore, cell death is considered a near equilibrium terminal end-stage, which can be induced by a variety of physiological or nonphysiological perturbations (e.g., ischemia, hypoxia, drugs and chemicals, immune reactions, infectious agents, and high temperature or radiation), including a variety of disease states or disorders such as infectious, immunological, iatrogenic, idiopathic, or neoplastic. Paradoxically, it is now well established that exposure to any such perturbation(s) to a cell population either *in vivo* or *in vitro* could be either apoptogenic (apoptosis-inducing) or necrogenic (necrosis-inducing). For several reasons the idea that physiological cell death was medically important and biologically interesting did not capture the attention of researchers until fairly recently. This is partly because majority of life scientists focused researching on programmed cell life, mechanisms of cell injury and mechanisms of protection of cell life, rather than cell death. Surprisingly, everyone has a new sense of the importance of apoptosis or PCD, and necrosis or unprogrammed cell death. Interestingly, cell death literature also include several other terminologies, such as secondary necrosis and oncotic necrosis, which are yet to be substantiated with biochemical and morphological criteria to distinguish from necrosis.

Importance of Apoptosis or Programmed Cell Death

Positive implications of cell death are currently the subject of intense debate and considerable research activity. This interest stems, in part, from the potential for understanding oncogenesis and the possibility of exploiting the cell death program for therapeutic purposes. For example, inhibition of cell death might contribute to oncogenesis by promoting cell survival instead of death. Likewise, triggering cell death might provide the means for eliminating unwanted cells such as tumor cells. Apoptosis has been affirmatively identified as an important mechanism in both development and homeostasis. Removal of superfluous, infected, transformed or damaged cells by activation of an intrinsic suicide program is achieved via apoptosis. One form of apoptosis involves death and subsequent withdrawal (shrinkage) from the surrounding tissues so as to allow phagocytosis by neighboring cells. The other form is characterized by maintenance of intact cell membranes during the suicide process in order to allow practically any type of cell to engulf the apoptotic bodies or fragments of dying cell. These two suicidal modes circumvent release of degraded dead cell debris and bypass the emergence of a local inflammatory reaction. Beneficial roles of this process begin early on during prenatal life and continue until death.

Biological Significance of Apoptosis

Cell death by apoptosis has been reported in plants, nematodes, insects, fish, birds, amphibians, and

mammals. Examples of elimination of transitory organs and tissues via apoptosis include phylogenetic vestiges (pronephros and mesonephros in higher vertebrates), anuran tails and gills and larval organs of holometabolous insects. Regression of the tadpole tail during amphibian metamorphosis serves as one of the prime examples of PCD during early development, and perhaps is only rivaled by the cataclysms of insect metamorphosis. Tadpole tail fin collapse is followed by degradation of tail muscle, a spectacular event recorded by classical pathobiologists. Another classic example is vertebrate limb bud development. If PCD fails, in formation of the digits, digits remain joined by soft tissue. Formation of heart loops during vertebrate development is another biological architecture by apoptosis. The sloughing off of the inner lining of the uterus (the endometrium) at the start of menstruation occurs by apoptosis. Depletion of cells in spinal ganglia occurs during development of the chick embryo, and there is precise chronological and spatial control over this process. Enormous numbers of cells are deleted especially in the nervous system by apoptosis to give rise to the final configuration of the brain. In mammals, the secondary palate separating oral and nasal cavities develops by growth, rotation and fusion of left and right palatal shelves. Decreased proliferation, increased adhesiveness coupled with PCD of medial edge epithelial cells engineer this fusion of the shelves. The reproductive organs of vertebrates show stunning changes during sexual differentiation and maturation which involve massive PCD. Despite the fact that both male and female embryos have the same reproductive rudiments, the Wolffian duct differentiates into the epididymis and vas deferens, whilst the Mullerian duct regresses in the male and the opposite occurs in the female. The Mullerian duct differentiates into the uterus in mammals or the oviduct and shell gland in avian species. These events are hormone-mediated but cell removal occurs by apoptosis. All these observations indicate that 'apoptosis' or PCD is an equal and opposite force to mitosis. In certain tissues the cells survive until the organism dies while other cells are continually produced in self-renewing tissues, and then differentiate to perform specific functions, and eventually die. Noted examples of these events are the life cycles of skin cells (keratinocytes) and hemopoietic cells (cells contained in the blood such as lymphocytes, leukocytes, monocytes, and erythrocytes).

True importance of this unique cell death process was unknown until a recent upsurge in interest to unravel the mechanisms underlying this process. After three decades of research, it is now well understood that apoptosis is a very tightly regulated, energy-dependent, genetically programmed, and evolutionarily conserved self-destruction process through which cells undergo organized suicide for beneficial purposes. The idea that life requires death seems paradoxical, but cell suicide is essential for an animal to survive. It is very interesting that PCD can be affirmatively predicted in certain cells of the famous nematode *Caenorhabditis elegans*. *Caenorhabditis elegans* matures as an adult hermaphrodite with 1090 cells of which 131 undergo PCD. These PCD-designated cells at various locations automatically trigger suicidal death program at different but precisely defined times. The wide range of cells that can undergo apoptosis in micro (well controlled *in vitro* models) and macro (invertebrates, vertebrates, and plants: *in vivo* models) environments suggests that practically each and every type of cell is potentially capable of executing terms and conditions of apoptosis. For example, without selective destruction of 'nonself' T cells, an animal would lack immunity. Similarly, meaningful neural connections in the brain are whittled from a mass of cells. Apoptosis research, with roots in developmental and cell biology, genetics, and immunology, embraces this long-ignored natural law. Apoptosis as part of normal development is a strategy to select certain cells for survival, sculpting a tissue's specificity. Interestingly, the fetal thymus allows only specific population of T cells (with 'self-antigen' surface markers) to complete development. A cell whose DNA is damaged by ultraviolet radiation in sunlight is either repaired or jettisoned via apoptosis-assisted peeling. Apoptosis is the mechanism by which natural killer (NK) cells nonspecifically kill virally infected cells, stimulating the infected cell to undergo unscrupulous PCD. In multicellular organisms, such beneficial functions include maintenance of optimal tissue growth and development, and physiologic activity (by a balance between proliferation, growth arrest, and PCD). Apoptotic cells, upon completion of their mission, execute a suicidal program and form apoptotic bodies which are rapidly removed by a variety of resident and nonresident scavenger cells. Phagocytic scavengers, such as macrophages, have specialized receptors that recognize phosphatidylserine (PTS) moieties on the surface of apoptotic bodies and carry out their disposal job in an organized manner without eliciting an inflammatory response. While PTS is actively transported from the outer to the inner leaflet of the plasma membrane by ATP-dependent aminophospholipid translocase, the implication of a scramblase that moves phospholipids bidirectionally across the membrane is still debated. Cyt *c* released from mitochondria selectively peroxidizes PTS. Peroxidized PTS inhibits the ATP-dependent aminophospholipid translocase, allowing

oxidized-PTS and phosphatidylethanolamine (PTE) to be exposed also on the extracellular layer of cell membrane. Unlike necrosis, apoptotic cells do not release any enzyme; however, activation of a tissue enzyme transglutaminase has been reported in several systems. Massive cellular demise by apoptosis generally does not lead to organ dysfunction as opposed to necrotic cell death. In contrast, necrosis typically affects groups of contiguous cells, and an inflammatory reaction usually develops in the adjacent viable tissue in response to the released cellar debris.

Stages of Apoptosis

The cellular machinery of apoptotic death turns out to be as intrinsic to the cell as mitosis. Apoptotic process is executed in an organized fashion and usually solicits participation of all the intracellular organelles. The time to onset of apoptosis after a lethal stimulus is variable (minutes to hours) but the changes are extremely rapid. In apoptosis, coordinated changes occur in the nucleus, the cytoplasm, and at the cell surface. *In vivo*, apoptosis usually affects single cells or small groups of cells in an asynchronous fashion and the entire process can be primarily divided into four distinct stages: (1) cell shrinkage, (2) nuclear condensation and fragmentation, (3) cellular fragmentation and formation of apoptotic bodies, and (4) apoptotic body or debris clearance by phagocytosis. The major sequence of events that are associated with apoptosis and necrosis are presented in **Table 1**.

Step I: Cellular Shrinkage

The earliest changes observed include the loss of cell junctions and other specialized plasma membrane

Table 1 Sequence of cellular events associated with apoptosis contrasted with those associated with necrosis

Characteristic	*Apoptosis*	*Necrosis*
Distribution	Affects individual cells scattered throughout the tissue	Affects massive and contiguous cells
Adhesion between cells and to basement membrane	Lost early	Lost but late
Cellular morphology	(a) Chromatin condensation (karyorrhexis) followed by margination as large crescents to the periphery of the nuclear membrane; fragmentation in large masses (convolution). See **Figure 1b**	(a) Irregular clumping of chromatin, pyknosis or karyolysis, nucleolysis occasionally precedes collapse of nuclear membrane; cells occasionally maintain their boundaries with some or no organelles. See **Figure 1c**
	(b) Loss of cell volume (cytoplasmic compaction)	(b) Very early swelling of cell, ballooning occurs frequently
Damage to organelles (e.g., mitochondrion)	Late (organelles mostly retain integrity), occasionally organellar swelling and bleb formation on cell surface appear very late (organelles found in blebs)	Very early swelling of organelles; cells disintegrate and lyse, appear chaotic, form blebs early (organelles are not found in blebs)
DNA breakdown pattern	Internucleosomal cleavage (ladder-like pattern on agarose gel)	Random or irregular damage (appears as a smear on gel)
Release of lysosomal enzymes	Absent	Present
Duration of biochemical and morphological changes	Minutes to hours	Hours to days
Ultimate outcome	Forms apoptotic bodies, occasionally containing intact organelles	Swelling, disintegration, dissolution
Cell removal	Usually phagocytosis by all types of resident and nonresident cells	Usually cells are not removed
Inflammation	Absent	Present
Energy requirement and overall regulation	Strictly energy-dependent, very tightly regulated, signaling-dependent, can easily be delayed but can be inhibited with difficulty	Energy and signaling-independent, occasionally energy-dependent, can be blocked prior to irreversible changes (e.g., plasma membrane leakage)
Genomic control	Strictly dependent	Usually independent
Scar formation	Absent	Present
Cellular osmotic regulation	Intact	Lost leading to cell swelling

structures such as microvilli. In intact organs or tissues, a withdrawal mode from the surrounding sets in. Typically, the cytoplasm begins to shrink following the cleavage of lamins and actin filaments, and in some instances cytoplasm becomes hypertrophied.

Step II: Nuclear or Chromatin Condensation

This is the most noticeable distinguishing feature of apoptosis, which shows classic stereotypical changes. This stage goes through very complex biochemical and molecular changes. At this stage, chromatin condenses (coalesces; generation of pyknotic nucleus), fragments in an orderly fashion into one or more large (or small) masses, and migrates toward the periphery of the nuclear membrane (in many cases the nuclei of apoptotic cells take on a 'horse-shoe' like appearance). As the process continues the nucleus breaks into several fragments. Under an electron microscope, these fragments appear very dense and dark in the near-total absence or loss of volume regulation of other organelles such as the mitochondria. The contraction of cytoplasmic volume is apparently associated with loss of intracellular fluid and ions. Photomicrographs of normal (panel a), apoptotic (panel b), and necrotic (panel c) liver cells are presented in **Figure 1a–c**.

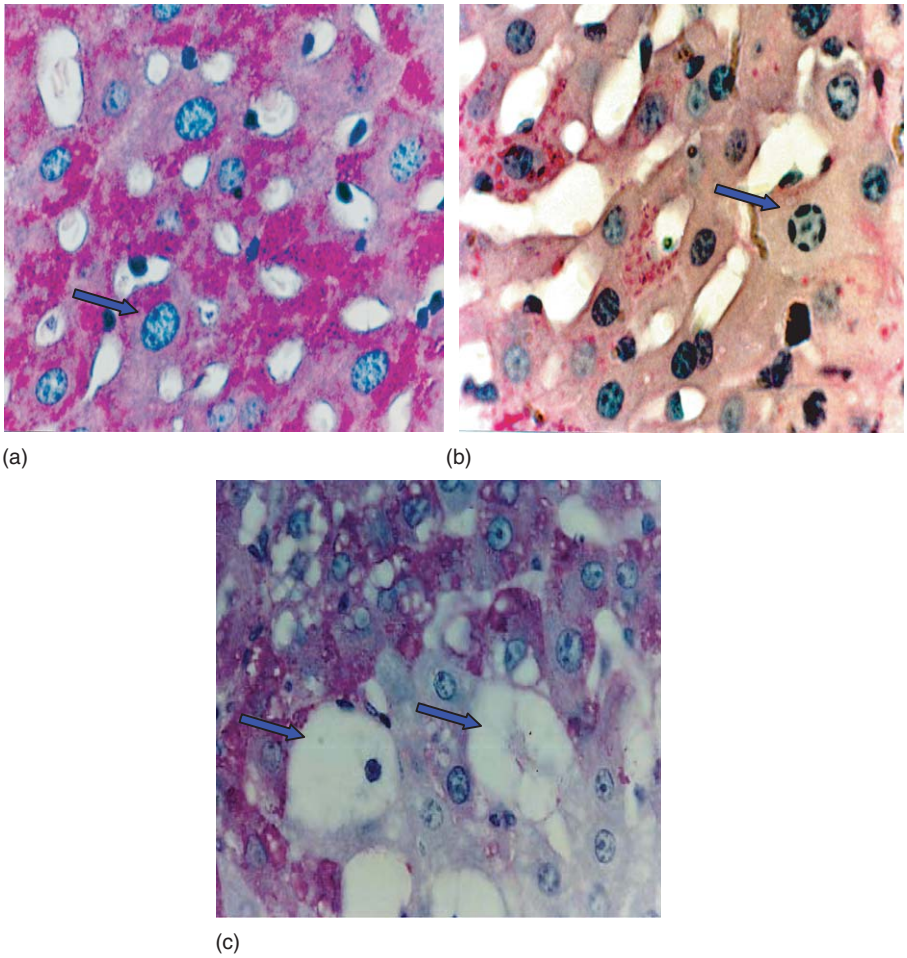


Figure 1 Light photomicrographs (PAS-stained; × 1000) of liver sections showing architecture of a normal hepatocyte with a normal nucleus (panel a: see arrow), apoptotic hepatocyte with an apoptotic nucleus (panel b: see arrow; in the vicinity of normal, damaged- and glycogen-depleted hepatocytes), and abnormally ballooned necrotic hepatocytes with necrotic changes (see panel c: a liver cell with a nucleus with disintegrated cytoplasm, and another liver cell without a nucleus with disintegrated cytoplasm; both arrows indicate necrotic cells). Liver injury and apoptosis was induced by a single hepatotoxic dose of acetaminophen (500 mg kg^{-1}, i.p.). (Reproduced with permission from Ray SD and Jena N (2000) A hepatotoxic dose of acetaminophen modulates expression of BCL-2, BCL-X(L), and BCL-X(S) during apoptotic and necrotic death of mouse liver cells *in vivo*, *Archives of Toxicology* **73**: 594–606; © Springer-Verlag. Ray, *Proceedings of the Society of Free Radical Research*, 2004.)

Step III: Cellular Fragmentation or Formation of Apoptotic Bodies

Cells committed to apoptosis continue to shrink, packaging themselves into a form that allows for easy engulfment by all types of cells. The cell transiently adopts a deeply convoluted outline and shows extensive surface blebbing. In order to promote their phagocytosis by macrophages, apoptotic cells often elicit biochemical changes on the plasma membrane surface that appeal to the macrophage response. One such change is the translocation of PTS from the inner leaflet of the cell to the outer surface. Lysophosphatidylcholine generated as a result of caspase-3-mediated activation of the Ca^{2+}-independent phospholipase-A_2 renders cells vulnerable to phagocytosis. Membrane changes can often be observed morphologically through the appearance of membrane blebs or blisters which often appear toward the end of the apoptotic process. Subsequently, the cell breaks up into several membrane-bound smooth-surfaced 'apoptotic bodies' that contain a variety of tightly compacted organelles and some nuclear fragments. Under the microscope, appearance of apoptotic bodies is a common feature used by trained pathologists to identify apoptosis in any tissue.

Step IV: Phagocytosis of Apoptotic Cells or Bodies

Apoptotic bodies show a great diversity in size, and shape, and there is no limit to the number of apoptotic bodies formed from one cell. Apoptotic bodies are typically phagocytosed by 'professional phagocytes' (macrophages) or neighboring cells serving as 'semiprofessional' phagocytes (glomerular mesangial cells, Kupffer cells, or liver cells). These phagocytic cells are responsible for removing apoptotic cells from tissues in a clean and tidy fashion that avoids many of the problems associated with necrotic cell death. Although professionally trained engulfing cells are typically members of the reticuloendothelial system (mononuclear phagocytes such as macrophages, phagocytes, etc.), any other normal or abnormal cell capable of phagocytosis may participate in cell clearance process. The endocytosed apoptotic debris is rapidly degraded by a series of enzymes within lysosomes, and the adjacent cells move around or if necessary, proliferate to replace the gap created by the just-deleted apoptotic cell.

Mechanisms of Apoptosis

Now, it has become clear that the regulatory mechanisms controlling apoptosis are as fundamental, and as complex, as those regulating cell proliferations. Based on the observations of the past three decades, apoptotic response of cells may depend upon a multitude of intrinsic and extrinsic factors, for example, (1) the stimulus (e.g., physical, chemical, or biological); (2) cellular defense mechanisms (intracellular redox status; oxidant/antioxidant balance); (3) signal transduction pathways (receptors involved); (4) level of expression of relevant pro and anti-apoptotic genes, and (5) intrinsic cellular susceptibility to apoptosis (half-life of a cell). However, the overall process appears to go through three distinct phases at the molecular level: an induction phase, an effecter phase, and a degradation phase. The induction phase depends on death-inducing signals to stimulate pro-apoptotic signal transduction cascades. These death-inducing signals include oxidative stress typically produced during drug metabolism by reactive oxygen or nitrogen species (ROS, RNS), or their hybrids (peroxynitrite), overactivation of Ca^{2+}-dependent pathways, and modulation of gene expression (Bcl-2 family proteins such as Bcl-XL, Bax, and Bad). In phase 2, the effector phase, the cell becomes committed to death by the action of a key regulator, which arguably is the mitochondrion. At this phase mitochondrial permeability transition (MPT) pores are formed by several mechanisms resulting in excessive leakage and flooding of the cytosol with Cyt *c* from the mitochondrion. The last phase, a degradation phase, involves both cytoplasmic and nuclear events. In the cytoplasm, a complex cascade of protein-cleaving enzymes called caspases is activated. In the nucleus, the chromatin condenses, the nuclear envelope breaks down, and the DNA undergoes orderly fragmentation. Finally, the cell is fragmented into apoptotic bodies, phophatidylserine on the membranes is recognized, and apoptotic bodies are cleared as described above.

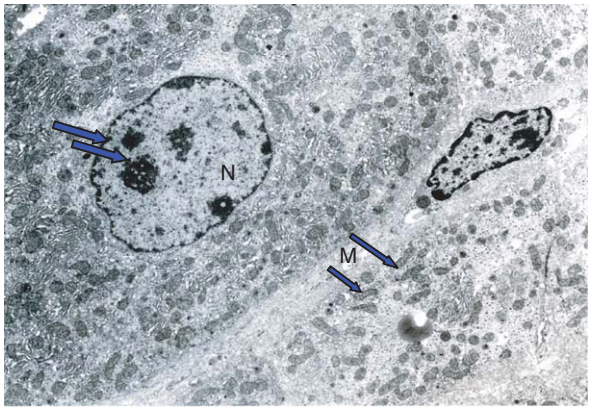
Biochemical and Molecular Events That Trigger Apoptosis

Prolonged cellular stresses, such as oxidative stress and DNA damage, if not defended against, can trigger the apoptotic pathway. Biochemical markers such as DNA fragmentation have been used to identify apoptotic cells. Unfortunately, cells dying by necrosis, a traumatic form of cell death that is accompanied by cell swelling and lysis also exhibit DNA fragmentation. In addition, cells with the characteristic morphologic appearance of apoptosis, occasionally, may not show evidence of DNA fragmentation. In this context, it is worth mentioning that induction of apoptosis in enucleated cells has also been reported. Cells exhibiting evidence of DNA damage and oxidative stress may, however, recover

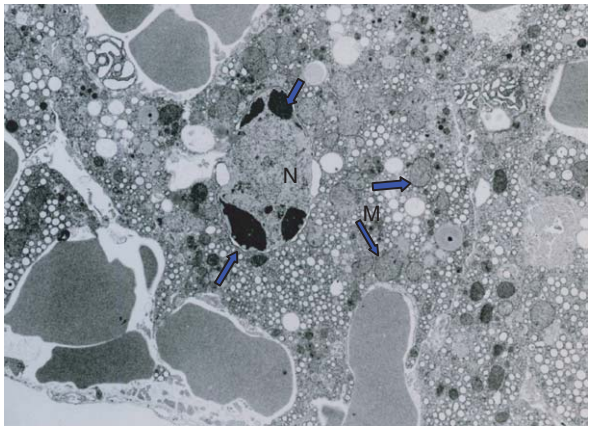
from those stresses, provided they are equipped with an adequate DNA repair system and an adequate level of stress-response proteins and/or antioxidant defenses.

One of the hallmarks of apoptosis is the cleavage of chromosomal DNA into nucleosomal units. The degradation of DNA in the nuclei of apoptotic cells is accomplished in a number of ways following activation of caspases (a family of cystein proteases). The fragmentation of DNA into nucleosomal units – as seen in DNA laddering assays – is caused by an enzyme known as caspase-activated DNase (CAD). Normally CAD exists as an inactive complex with ICAD (inhibitor of CAD, also known as DNA fragmentation factor 45 or DFF45). During apoptosis, ICAD is cleaved by caspases, including caspase 3, to release CAD. Active CAD migrates into the nucleus and cleaves DNA. Since CAD is a DNase with a high specific activity (comparable to or higher than DNase I and DNase II) rapid fragmentation of the nuclear DNA follows. Formerly, CAD was thought to be a Ca^{2+}/Mg^{2+}-dependent nuclear enzyme (endonuclease), a master operator of this beneficial form of death. Therefore, xenobiotics that are known to induce intracellular Ca^{2+}-dysregulation were classified as possible apoptosis inducers. The other viable pathway through which genomic fragmentation is achieved is via lamins, a family of intra-nuclear proteins. Lamins (there are two forms, Lamin-A and Lamin-B) maintain the shape of the nucleus and mediate interactions between chromatin and the nuclear membrane. Degradation of lamins by caspase 6 may result in the chromatin condensation and nuclear fragmentation commonly observed in apoptotic cells (see **Figure 2**).

Since mitochondrial morphology remains intact throughout apoptosis, until recently, mitochondria were not assumed to be critical players in the effecter phase of apoptosis. But accumulating evidence indicates that mitochondria exhibit major functional roles and structural changes that serve to regulate apoptosis. Data from diverse models systems conclude that: (1) collapse of mitochondrial inner transmembrane potential ($\Delta\Psi_m$), (2) mitochondrial proteins that serve as rate-limiting factors for the activation of endonucleases and caspases, (3) modulation of expression of anti-apoptotic protein, Bcl-2, is localized to the mitochondrial inner membrane, and (4) release of cytochrome *c* (cyt *c*) into the cytosol, may be of paramount importance to apoptosis regulation. Some recent reports claim the ability of this organelle to release procaspase-3 (inactive form of caspase-3) and zymogens of caspase-2 and caspase-9. Intriguingly, mitochondrial oxidative stress can also release Cyt *c* and apoptosis-inducing factor (AIF).



(a)



(b)

Figure 2 Electron photomicrographs ($\times$ 8000) of mouse liver sections showing ultrastructural details of a normal hepatocyte (panel a) and an apoptotic hepatocyte (panel b). Normal hepatocyte shows classic features of an intact nucleus with intact chromatin (see arrow) and other organelles (N, nucleus; M, mitochondria). Apoptotic liver cell shows chromatin condensation, fragmentation and migration of the heterochromatin to the nuclear periphery (N, nucleus; M, mitochondria), typical features of apoptotic changes (see **Table 1** for more details). Liver injury and apoptosis was induced by a single hepatotoxic dose of acetaminophen (500 mg kg^{-1}, i.p.). (Reproduced from Ray SD, Mumaw VR, Raje RR, and Fariss MW (1996) Protection of acetaminophen-induced hepatocellular apoptosis and necrosis by cholesterge hemisuccinate pretreatment. *The Journal of Pharmacology and Experimental Therapeutics* **279**(3): 1470–1483, with permission from ASPET.)

Cytochrome *c* serves as the key regulator of apoptosis because once it is released from the intermembrane space, the cell is irreversibly committed to death. Either apoptosis occurs through the caspase-mediated process described above, or the cell undergoes a necrosis-like death due to the collapse of electron transport. Release of Cyt *c* interrupts the transfer of electrons between respiratory chain complexes III and IV, resulting in the generation of deleterious radical species (oxidative stress) and the

cessation of ATP synthesis. Cyt *c* in its holo form (i.e., attached with its heme group), associates with Apaf-1 (a molecule associated with Bcl-2), caspase-9, and ATP to form a complex called an 'apoptosome'. This apoptosome proteolytically activates caspase-3, which leads to the activation of the caspase cascade and the degradation phase of apoptosis. Interestingly, these proteins-Bcl-2, Apaf-1, caspase-9, Cyt *c*, and caspase-3 serve as the mammalian equivalent of the apoptosome (Ced-9, Ced-4, Ced-3) in *C. elegans*. Key to all these events is 'how Cyt *c* leaks out from mitochondria?' It has been suggested that a mitochondrial outer membrane protein, voltage-dependent anion channel 2 (VDAC2), interacts with BAK (Bcl-2 antagonist, a killer pro-apoptotic protein) to keep this potentially lethal apoptotic effector under control. When the death signal is received, products of the activation cascade – such as apoptosis promoters tBID, BIM, or BAD – displace VDAC2. Subsequently BAK and BAX are activated, and the mitochondrial outer membrane becomes permeable (MPT pores). This results in the release of caspase activators, including Cyt *c*. The whole process requires energy and a cell machinery not too damaged. If the cell damage is between certain levels, the cell can start the earliest events of apoptosis and then continue with necrosis (features of apocrosis or aponecrosis).

Oxidative stresses caused by the ROS/RNS family members are powerful necrogens. For example, the highly reactive hydroxyl radical is believed to be one of the most potent inducers of oxidative damage during necrotic cell death. In apoptosis, radicals still play a major role by determining the cellular redox status, and many forms of apoptosis involve ROS, RNS, and peroxynitrite (a potent anion oxidant generated by the reaction of nitric oxide with superoxide). Oxidants like superoxide and hydrogen peroxide can act as proapoptotic stimuli by changing the cellular redox status. And in most systems, antioxidants have been shown to be antiapoptotic. In fact there is overwhelming evidence to support antiapoptotic effect of natural (pycnogenol, epigallocatechin gallate, grape seed proanthocyanidins, quercetin, resveratrol, etc.) and synthetic antioxidants (*N*-acetyl cysteine, butylated hydroxytoluene). Oxidants indirectly induce apoptosis by changing cellular redox potentials, depleting reduced glutathione, and decreasing reducing equivalents such as NADH and NADPH. Similarly, agents that deplete intracellular glutathione render cells more vulnerable to oxidative stress-induced apoptosis. Redox potential of a cell is as critical as maintaining intracellular ion homeostasis or genomic integrity. An oxidative shift by ROS may altogether modify the nature of the stimulatory signal resulting in alteration of the direction of the program (apoptosis/necrosis or proliferation). Interestingly, spontaneous redox changes facilitate the formation of MPT pores, leading to the subsequent release of Cyt *c*. These MPT pores possess several redox-sensitive sites, including one in equilibrium with mitochondrial matrix glutathione, and one directly activated by oxidants. Antioxidants typically detoxify these noxious free radicals, antagonize their damaging influence, and protect cells. However, low levels of oxidants may provoke cells to proliferate, whereas mild oxidative stress conditions counteract apoptotic stimuli. Low cellular levels of superoxide and hydrogen peroxide are continually being produced from the mitochondrial respiratory chain and electron transport chains in the endoplasmic reticulum and nuclear membranes. In addition, low levels of H_2O_2 are produced as a by-product of the activity of γ-glutamyl transpeptidase (GGT). This enzyme is responsible for metabolizing extracellular reduced glutathione. Several studies suggest that the low levels of H_2O_2 generated by GGT protect cells against apoptosis and help maintain cell proliferation.

The free radical gas nitric oxide ($NO^{\bullet}$) is an important regulator of mitochondrial function, cell signaling, and gene expression. Production of $NO^{\bullet}$ can be a common denominator during xenobiotic metabolism. Exogenous application of high levels of $NO^{\bullet}$ donating compounds (sodium nitroprusside) are known to induce $NO^{\bullet}$-mediated apoptosis. Investigators believe the mechanism may involve an upregulation of ceramide levels by activating sphingomyelinases while concomitantly inhibiting ceramidases. In contrast, at physiological levels $NO^{\bullet}$ prevents apoptosis by interfering with the activation of the caspase cascade. In one experiment, proinflammatory cytokines were used to activate inducible nitric oxide synthase (iNOS), resulting in full protection for endothelial cells undergoing UV-A radiation. The mechanism involves $NO^{\bullet}$-mediated increases in Bcl-2 expression with a concomitant decrease in the expression of antiapoptotic Bax protein. Moreover, *in vitro* and *in vivo* experiments have shown that $NO^{\bullet}$ inhibits caspase-3 by S-nitrosation of the enzyme. Associated with inhibiting caspase 3, $NO^{\bullet}$ suppresses the self-amplification feed forward loop of apoptosis by inhibiting Bcl-2 cleavage and Cyt *c* release. However, at high levels oxidants can induce both apoptosis and necrosis.

Inactivation of DNA repair enzymes can also turn on apoptosis. A fascinating apoptotic process can result from stress or toxicity that culminates in genomic damage in the cell nucleus, triggered by a nuclear enzyme poly (ADP-ribose) polymerase

(PARP). This enzyme is instrumental in maintaining genomic integrity. Massive activation of PARP can deplete the cell of energy-providing molecules (such as NAD), an event that sends signals from the nucleus for the mitochondrion to start the apoptotic process. PARP was the first protein identified as a substrate for caspases. PARP is involved in repair of DNA damage and functions by catalyzing the synthesis of poly (ADP-ribose) and by binding to DNA strand breaks and modifying nuclear proteins. The ability of PARP to repair DNA damage is prevented following cleavage of PARP by caspase-3. Similarly, inactivation of enzymes involved in cell replication can also trigger apoptosis. DNA topoisomerase II is a nuclear enzyme essential for DNA replication and repair. Caspases can inactivate this enzyme leading to DNA damage. Overall malfunctioning of DNA repair enzymes may propagate signals for PCD.

Other biochemical determinants pivotal to apoptosis include intracellular deregulation of cAMP and ceramide. Ceramides are known stimuli of apoptosis and are released by activation of an acidic and/or neutral sphingomyelinase. Both enzymes are activated by the Fas receptor. Fas-mediated apoptosis can be partially inhibited by direct inhibition of acidic sphingomyelinase using the drug imipramine. Fas-triggering of early caspases (caspase-8/may be caspase-1) stimulate directly or indirectly the acidic sphinomyelinase resulting in the release of ceramide. The sphingomyelin pathway, initiated by hydrolysis of the phospholipid sphingomyelin in the cell membrane to generate the second messenger ceramide, is thought to mediate apoptosis in response to tumor necrosis factor-α (TNF-α), to FAS ligand and to X-rays. Generation of ceramide (hydrolysis product of sphingomyelin) through the sphingomyelin pathway results in the induction of apoptosis. At least two specific intracellular targets for ceramide have been identified: (1) a membrane ceramide-activated protein kinase and (2) a cytoplasmic ceramide-activated protein phosphatase. Ceramide promotes the formation and release of oligonucleosomal DNA fragments, produces corresponding loss of integrity of bulk DNA fragments, and elicits the expression of classical morphology of apoptosis.

Participation of Ca^{2+} in a well-known capacity (as activators) comes from studies during phospholipid-dependent protein kinase (protein kinase C: PKC) activation in intracellular signaling processes. One of the pathways of the transmembrane signaling system operates through the activation of PKC, whereas the other signal system involves receptor-mediated activation of cAMP-dependent protein kinase. Information concerning a role for PKC in apoptosis mostly comes from studies with phorbol esters (TPA: 12-*O*-tetradecanoyl-phorbol 13 acetate). However, recent reports suggest, depending upon the experimental system, PKC may either enhance or retard the apoptotic process. Protein kinase B (PKB or Akt is a PKC family member) is a serine/threonine kinase, that prevents apoptosis of neurons.

Genetic Control of Apoptosis

The balance between the withdrawal of positive signals (signals needed for continued survival) and the receipt of negative signals (signals needed to commit cellular suicide) may propel apoptosis in a particular direction. The continued survival of most cells requires that they receive continuous stimulation from other cells and, for many, continued adhesion to the surface on which they are growing. Some examples of positive signals are interleukin-2 (IL-2 is a cytokine), an essential factor for the mitosis of lymphocytes. Negative signals could be growth factor deprivation, redox imbalance, radiations (UV light, X-rays), chemotherapeutic drugs, and most importantly molecules that bind to specific receptors on the cell surface and signal the cell to begin the apoptosis program. The fundamental apoptosis signaling pathway and the involved proteins have been highly conserved throughout evolution. The basic apoptotic machinery mapped out in mammalian system mimics the genetic screens discovered in the nematode *Caenorhabditis elegans* (Ced-9, Ced-4, and Ced-3). Among these, anti-apoptotic molecule Ced-9 negatively regulates the activity of the proapoptotic molecule Ced-4 which in turn activates Ced-3. Bcl-2, Apaf-1, and the caspase protease family (see **Table 2**) have been identified as mammalian homologs of Ced-9, Ced-4, and Ced-3. Another breakthrough in apoptosis research was the discovery of a family of receptors that can specifically trigger apoptosis. This growing subfamily of death receptors (and death ligands) belongs to the TNF/NGF-receptor superfamily and is characterized by the presence of extracellular cysteine-rich domains. The death receptors have an intracellular death domain (DD), which couples receptors to the apoptosis-inducing machinery. Most noted members of this family are CD95 (APO-1/FAS), tumor necrosis factor receptor 1 (TNF-R1, 55 kDa protein), Death Receptor-3 (DR3; TNF receptor family member also known as Apo-3, WSL-1, TRAMP or LARD), lymphocyte-associated receptor of death (LARD), TNF-related apoptosis-inducing ligand (TRAIL-R1; has five members) and TRAIL-R2 (death receptor-5), TNF receptor I-associated death domain (TRADD) is somehow related to TRAIL-R1. Overexpression of TRADD leads to two major TNF-induced responses,

Table 2 List of the main caspases and some of their substrates

Caspase	*Common name*	*Substrate(s)*	*Function*
Caspase-1	ICE (Ced-3 homolog)	ILs, preinterleukin-1β, interleukin-18, Lamins	Processing of ILs (inflammation). Can also induce apoptosis depending on isoform and if overexpressed
Caspase-2	Ich-1 (human), Nedd2 (rat, mouse)	Golgin-160, Lamins (?)	Apoptosis (activity suppressed by serum deprivation)
Caspase-3	CPP32, Yama, apopain	PARP, SREBs, ICAD Gelsolin, Caspase-6, Caspase-7, Caspase-9, DNA-PK, MDM2, Gas2, Fodrin, β-Catenin, Lamins, NuMA, FAK, $p21^{Waf1}$, HnRNP proteins, Topoisomerase I, Calpastatin, Presenelin2	Apoptosis
Caspase-4	Ich-2, $ICE_{rel}II$	Caspase-1	Inflammation/apoptosis (note: this could be the human form of mouse caspase-11). Related to human caspase-5 and caspase-1
Caspase-5	$ICE_{rel}III$, TY	?	Inflammation/apoptosis (related to human caspase-4 and caspase-1)
Caspase-6	Mch2	PARP, Lamins, NuMA, FAK, Caspase-3, Keratin-18	Apoptosis
Caspase-7	Mch3, ICE-LAP3, CMH-1	PARP, Gas2, SREB1, EMAP II, FAK, Calpastatin, $p21^{Waf1}$	Apoptosis (activity blocked by cIAP1 and cIAP2). Similar in structure and substrate specificity to caspase-3
Caspase-8	FLICE, MACH, Mch5	Caspase-3, caspase-4, caspase-6, caspase-7, caspase-9, caspase-10, caspase-13, PARP, Bid	Apoptosis (death receptors)
Caspase-9	Apaf-3, ICE-LAP6, Mch6	Caspase-3, pro-caspase-9, caspase-7, PARP	Apoptosis
Caspase-10	FLICE-2, Mch4	Caspase-3, caspase-4, caspase-6, caspase-7, caspase-8, caspase-9	Apoptosis (death receptors)
Caspase-11	Ich-3, ICE-B	?	Murine caspase similar to human caspase-4. Belongs to the same family as caspase-3 of enzymes. May be involved in inflammation and apoptosis
Caspase-12	ICE-C	?	Involved in mediating apoptosis following ER stress. Related to mouse caspase-1 and caspase-11 and human caspase-4 and caspase-5
Caspase-13	ERICE (FLICE activatable caspase)	?	Member of the ICE family of caspases that include caspase-1 and caspases-4, -5 and -11. Involved in inflammation
Caspase-14	MICE		Cysteinyl aspartic acid-protease-14, also known as MICE. Overexpression of MICE induces apoptosis
Granzyme A	Granzyme A		A serine protease located in the granules of cytotoxic T cells and NK cells that are involved in the induction of target cell apoptosis (*Journal of Immunology* 1988, 141: 3471–3477)
Granzyme B	Granzyme B	Can activate caspases -3, -7, -8, and -10 (a chemical substrate 7-amino-4-methylcoumarin used for evaluation)	A serine protease located in the granules of cytotoxic T cells and NK cells that is involved in the induction of target cell apoptosis

apoptosis, and activation of NF-kβ. The death ligands have co-evolved as a death ligand family, corresponding to death receptors. Activation of death receptors is controlled in many cases by the inducible *de novo* expression of the respective death ligands such as CD95L, TNF, or TRAIL. Besides DD, there are also death effector domains (DEDs) involved downstream of the process.

Classic examples of death activators include: (1) TNF-α that binds to the TNF receptor; (2) lymphotoxin (also known as TNF-β) that binds to the TNF receptor, and (3) Fas ligand (FasL), a molecule that binds to a cell-surface receptor named Fas (also called CD95). Fas and the TNF receptor are integral membrane proteins with their receptor domains exposed at the surface of the cell. Binding of the complementary death activator (FasL and TNF, respectively) transmits a signal to the cytoplasm that leads to activation of caspase 8. Caspase 8 (like caspase 9) initiates a cascade of caspase activation leading to phagocytosis of the cell. When cytotoxic T cells recognize (bind to) their target, they produce more FasL at their surface. This binds with the Fas on the surface of the target cell leading to its death by apoptosis. The early steps in apoptosis are reversible – at least in *C. elegans*. In some cases, final destruction of the cell is guaranteed only invagination and digestion by a phagocyte.

Neurons, and perhaps other cells, have another way to self-destruct and that is independent of caspase activation but dependent on a factor known as apoptosis-inducing factor (AIF). AIF is a protein that is normally located in the intermembrane space of mitochondria. When the cell receives a death-inducing signal, AIF is released from the mitochondria, migrates into the nucleus, binds to DNA, which ultimately triggers the destruction of the DNA and cell death. The other straightforward mechanism is executed by caspases (mentioned at several places in this chapter), which are normally suppressed by inhibitor of apoptosis (IAP) proteins. When a cell receives an apoptotic stimulus, IAP activity is relieved after second mitochondria-derived activator of caspases (SMAC, also called DIABLO), a mitochondrial protein, is released into the cytosol in order for the proper execution of the death program. Activation-induced cell death (AICD) involves over expression of receptors for pro-apoptotic ligands (e.g., negative selection of T lymphocytes).

Bcl-2 Family of Proteins Regulate Apoptosis

The concept of active cell death or PCD being a genetically encoded process has stimulated an intense search for the genes involved in the cell-death program schemes. The first anti-apoptotic gene to be clearly identified in humans was Bcl-2 (B-cell lymphoma-leukemia-2 gene), cloned from the breakpoint of the 14:18 translocation found in the majority of follicular lymphomas. The discovery of Bcl-2 oncogene helped stimulate recognition of the concept that gene products which modulate the susceptibility of certain cell types to apoptosis, may play an important role in the process leading to malignant transformation. This is primarily due to the ability of survival of cells in inappropriate physiological situations. In both pathological as well as physiological conditions, the Bcl-2 gene has emerged as a critical regulator of apoptosis. Expression of the Bcl-2 protein directly prevents apoptosis by enhancing cellular antioxidant capacity possibly through scavenging reactive oxygen radicals, or indirectly by counteracting oxidative stress. The other potential mechanisms of this gene include a role in regulation of intracellular calcium ion, nuclear transport, and control of signal transduction pathways. Bcl-2 is no longer a single entity but one member of a growing multi-gene family. Included in this family are: Bcl-X (Bcl-XL and Bcl-XS), myeloid cell leukemia 1 (Mcl-1, Mcl-1 is a mitochondrial protein that enhances cell viability under apoptotic conditions), Bax (Bcl-2 associated $\times$ protein; pro-apoptotic; has four forms: α, β, γ, ω), A1, Bag (Bcl-2 associated athanogene-1; Bag is a Bcl-2-binding protein which provides protection from apoptotic cell death), Bak (Bcl-2 antagonist/killer, pro-apoptotic), Bad (Bad is a heterodimeric partner for Bcl-XL and Bcl-2 that displaces Bax and promotes cell death), Bcl-w (promotes cell survival), and Ced-9. The transcripts of bcl-X are alternatively spliced to form bcl-XL (long form; antiapoptotic property) and bcl-XS (short form; proapoptotic).

But how does the Bcl-2 family regulate apoptosis? Bcl-2 and Bcl-X_L prevent MPT pore opening in isolated mitochondria. Since Bcl-2 can prevent MPT pore opening in a system without Cyt *c*, this indicates that Bcl-2 can directly regulate MPT pores. However, Bcl-2 can also maintain mitochondrial $\Delta\Psi_m$ (membrane potential) under conditions that do not allow permeability transitions. This suggests that Bcl-2 regulates $\Delta\Psi_m$ rather than MPT pores through enhancing H^+ efflux in the presence of stimuli that collapse $\Delta\Psi_m$. Second, Bcl-2 inhibits the release of Cyt *c*. Third, Bcl-2 attenuates the MPT-pore promoting effects of atractyloside (an activating ligand of the adenine nucleotide translocator, ANT) and Bax. Moreover, Bcl-2 homologs can form ion channels or pores in artificial membranes. In fact, they have a crystal structure similar to colicins and the b-subunit of diphtheria toxin. The diphtheria b-subunit translocates the A-subunit across membranes; likewise

Bcl-2 and Bcl-X_L can potentially translocate molecules across membranes. Bcl-2 and Bcl-X_L may interact physically or functionally with the MPT pore or with non-MPT pore proteins that control volume regulation of the matrix. In several experimental paradigms, Bcl-2 further reduces the cellular redox potential. Moreover, free radical-induced cell death is accompanied by lipid peroxidation, which is attenuated with Bcl-2 overexpression. More research is needed to determine whether intracellular redox status is a key regulator in apoptosis-signaling pathways.

p53, The Guardian of Genome

Considerable focus has fallen upon another gene, known as the guardian of the genome, associated with this suicidal process – p53. This suppressor gene codes for a 53 kDa protein that helps organisms cope with DNA damage by either stalling cell division or inducing cell death. The p53 tumor suppressor limits cellular proliferation by inducing cell cycle arrest and apoptosis in response to cellular stresses such as DNA damage, hypoxia, and oncogene activation. Many apoptosis-related genes that are transcriptionally regulated by p53 have been identified. These are candidates for implementing p53 effector functions. In response to oncogene activation, p53 mediates apoptosis through a linear pathway involving bax transactivation, Bax translocation from the cytosol to membranes, Cyt *c* release from mitochondria, and caspase-9 activation, followed by the activation of caspase-3, -6, and -7. P53-mediated apoptosis can be blocked at multiple death checkpoints, by inhibiting p53 activity directly, by Bcl-2 family members regulating mitochondrial function, by blocking caspase-9 activation, and by caspase inhibitors. Understanding the mechanisms by which p53 induces apoptosis, and the reasons why cell death is bypassed in transformed cells, is of fundamental importance in cancer research, and has great implications in the design of anticancer therapeutics. For example, the genotoxic anti-cancer drugs such as etoposide and γ-radiation generate damage in chromosomal DNA. The signal seems to be transferred to mitochondria in a p53-dependent manner by as yet an identified mechanism. This releases Cyt *c* from mitochondria, and activates caspase 9 as described in this article.

Inappropriate Regulation of Apoptosis May Head to Cancer and Autoimmune Diseases

Aberrant regulation of apoptosis contributes to well-known pathologies such as autoimmune diseases, cancer, and viral infections. As discussed above, p53 serves as a checkpoint for cell cycle and apoptotic cell death. While massive apoptotic death may signal cell proliferation, uncontrolled cell division may lead to cancer. There are a number of proteins that regulate and control the cell cycle. They dictate the cell when is the proper time to grow and divide, and they stop the cell when the time is not right. The main families involved in cell cycle are the cyclins (especially cyclin D and cyclin E and cyclin A), the cyclin-dependent kinases (especially CDK4, CDK6, and CDK2), the CDK inhibitors (especially p16, p21, and p27), and the tumor suppressor genes (especially Rb and p53). There are two pathways which regulate (the Rb pathway and the p53 pathway) this complex event. One of the main clinical interests of cell cycle control is cancer. Therefore, research in understanding cell cycle control has many implications for cancer, especially for the development of therapeutics. A major breakthrough of the twenty-first century in the field of medicine may be select induction of PCD in cancerous cells in *in vivo* system including humans.

Another oncogene which was long thought to play a critical role in life and death processes of cells is *C-myc*. The *C-myc* gene is the cellular homolog of the viral oncogene v-myc, and it plays a prominent role in carcinogenesis. *C-myc* gene encodes nuclear phosphoprotein of $\sim$60 kDa size, a member of helix-loop-helix family of transcription factors abundant in proliferating cells. *C-myc* and *C-fos* (and cofactors *c-max* and *c-myb*) expressions appear transiently during castration-induced and postlactation breast regression-induced apoptotic death of prostate and mammary cells, respectively. Deregulated expression of *C-myc* not only promotes proliferation, but also can either induce or sensitize cells to apoptosis. Inappropriate expression of c-myc under conditions which inhibit growth and downregulate endogenous *C-myc* expression, including serum deprivation and exposure to cytotoxic agents including the anticancer agents vinblastine, etoposide, Ara-C, and nocodazole, usually results in PCD in many different cell types. Moreover, inappropriate *C-myc* expression is associated with an apoptotic response elicited by induction of differentiation. The proto-oncogene *C-myc* encodes a transcription factor *C-myc*, which is of great importance in controlling cell growth and vitality. The quantity of *C-myc* is carefully controlled by many mechanisms, and its actions to induce and repress genes modulated by interactions with other regulatory proteins. Understanding the kinetic and quantitative relationships that determine how and what genes *C-myc* regulates is essential to understanding how *C-myc* is involved in apoptosis.

Execution of apoptosis in mammalian cells requires the coordinated action of several aspartate-specific cysteine proteases, called caspases, which are responsible for the cleavage of key enzymatic and structural

substrates, resulting in the systematic and orderly disassembly of the dying cell. The caspases exist within the cell as inactive pro-forms or zymogens. zVAD-FMK, Benzyloxy-valine-alanine-aspartate-*O*-methyl-fluoromethylketone, is a well-known synthetic caspase inhibitor. These zymogens can be cleaved to form active enzymes following the induction of apoptosis. The finding that the product of the Ced-3 gene (*Caenorhabditis elegans*) is strongly related to mammalian interleukin1β-converting enzyme (ICE), together with the observation that overexpression of ICE induces apoptosis, prompted an intensive search for new family members, that has led to the identification of at least 14 related caspases. These proteins are characterized by an absolute specificity for Asp in the cleavage site's P1 position, and they all contain a conserved QACXG (where X is R, Q, or G) pentapeptide motif in the catalytic site. Phylogenetic analysis of the caspases revealed that they can be grouped into three subfamilies: an ICE subfamily, comprising caspases -1, -4, and -5 (ICE, TX, and TY, respectively), a CED-3/CPP32 subfamily, comprising caspases -3, -6, -7, -8, -9, and -10 (CPP32, Mch2, Mch3, FLICE, Mch6, Mch4), and an Ich-1/Nedd-2 subfamily. Accumulating evidence indicates that members of the ICE subfamily predominantly play a role in inflammation, whereas members of the CPP32 subfamily are largely involved in apoptosis. Induction of apoptosis via death receptors results in the activation of an initiator caspase such as caspase 8 or caspase 10. These caspases can then activate other caspases in a cascade. This cascade eventually leads to the activation of the effector caspases such as caspase 3 and caspase 6. These caspases are responsible for the cleavage of the key cellular proteins that leads to the typical morphological changes observed in cells undergoing apoptosis.

The interaction of toxicants and apoptosis is complex and the mechanisms of interaction are likely to differ among different toxicants. The apoptosis-inducing potential of a variety of drugs, chemicals, and carcinogens has been intensively investigated from a mechanistic standpoint. Decades of research have focused on the ability of carcinogens to induce cell transformation, however, although it is not entirely true, many investigators share the notion that carcinogens antagonize (or alter) or mutate the apoptotic pathway and improve the chances of cell survival. On the contrary, many carcinogens are powerful inducers of apoptosis. These issues have been addressed at great length during recent years. A short list of classic apoptogens has been provided in **Table 3**. Treatment of cells with certain pharmacologic agents may not result in either classic apoptosis or classic necrosis. The formation of micronuclei, aberrant mitoses, a mitotic arrest and other cellular perturbations can result in cell death that may be nonapoptotic/non-necrotic in nature (called apocrotic, aponecrotic, or oncotic necrosis; mechanism described elsewhere in this chapter).

Clinical Relevance

Overactivation of apoptosis causes tissue damage. For example, administration of Fas ligand, exposure to γ-irradiation, or treatment with a high dose of glucocorticoid kills the animals by causing massive apoptosis in the liver or thymus. Hepatitis, insulitis, graft-versus-host disease, and allergic encephalitis are due to the excessive apoptosis by Fas ligand

Table 3 Examples of interactions of drugs and toxicants with apoptosis

Drugs and chemicals	*Physical insults and free radicals*	*Microbes*	*Cytokines*	*Withdrawal from trophic factors*
Acetaminophen, ethanol, chloroform, CCl_4, furosemide, dimethylnitrosamine, doxorubicin, chemotherapeutic agents, glucocorticoids, glutamate, calcium, azide, hydrogen peroxide, propanolol, TCDD, okadaic acid, lead nitrate, vincristin, vinblastin, TPA, PMA, BAP, PAHs	Neutrons, X-rays, β-rays, γ-rays, UV-radiation, heat shock quinones $O_2^{\bullet}$, $NO^{\bullet}$, $OH^{\bullet}$	HIV-1, Sindbis Baculo virus, influenza virus-A, human papilloma virus, Reo virus, Epstein–Barr virus, *Escherichia* spp., *Yersinia* spp., *Salmonella* spp., *Propionibacterium* spp., fungal toxins (ochratoxin A and fumonisins)	TNF-α, TGF-β, some ILs	Glucose, growth factors (interleukin-2, interleukin-3, interleukin-10, interleukin-13, granulocyte-macrophage colony stimulating factor, granulocyte stimulating factor, fibroblast growth factor, transforming growth factor β1, neurotrophic factor), hormones (estrogen, androgen, progesterone, ACTH)

expressed on cytotoxic lymphocytes. Apoptotic cells are detected in the brain of ischemia or Alzheimer patients, suggesting that apoptosis is at least in part responsible for the disease manifestification of these patients. A proper dose of anti-cancer drugs or γ-irradiation can kill cancer cells by activating the apoptotic death program in the target cells. Some cancer cells are resistant to these drugs by an unknown mechanism. It is hoped that elucidation of the molecular mechanisms of apoptosis leads to development of an efficient cancer therapy.

A disadvantage to the organism of the mechanism that necessitates signaling to prevent apoptosis is that its failure by mutation can lead to the survival of unwanted cells, which – paradoxically – can lead to death of the organism itself. On the other hand, an opportunity is presented by such a mechanism to allow investigators to devise means for targeting unwanted cells for destruction. Prostate cancer is an example. The survival of prostate cells is dependent upon androgens; androgen depletion leads to a reduction in cell number by apoptosis. Recently, the dependence of prostate cells on androgens to avoid cell death has been exploited therapeutically by the use of androgen ablation to invoke apoptosis in prostate cancer cells and prolong survival in men with prostate cancer.

Another noteworthy example is nonvirally produced cancer cells perplexing host tissues to bypass apoptosis. Some B-cell leukemias and lymphomas express high levels of Bcl-2, thus blocking apoptotic signals they may receive. The high levels result from a translocation of the *BCL-2* gene into an enhancer region for antibody production. Melanoma (the most dangerous type of skin cancer) cells avoid apoptosis by inhibiting the expression of the gene encoding Apaf-1. Some cancer cells, especially lung and colon cancer cells, secrete elevated levels of a soluble 'decoy' molecule that binds to FasL, plugging it up so it cannot bind Fas. Thus, cytotoxic T cells (CTL) cannot kill the cancer cells. Other cancer cells express high levels of FasL, and can kill any cytotoxic T cells (CTL) that try to kill them because CTL also express Fas (but are protected from their own FasL). The hallmark of AIDS (acquired immunodeficiency syndrome) is the decline in the number of the patient's $CD4^+$ T cells. What causes the disappearance of $CD4^+$ T cells is a mystery! All T cells, both infected and uninfected, express Fas. Expression of a HIV gene (called *Nef*) in a HIV-infected cell causes the cell to express high levels of FasL at its surface while preventing an interaction with its own Fas from causing it to self-destruct. However, when the infected T cell encounters an uninfected one (e.g., in a lymph node), the interaction of FasL with Fas on the uninfected cell kills it by apoptosis. Exploration of a similar interaction may yield to new possibilities of preventing graft rejection and other chemotherapies.

Tools for Apoptosis Detection

There are several methods available for the detection of apoptosis *in vivo* and *in vitro*. *In vitro* methods may include but not limited to the following: (1) flow cytometry, (2) fluorescence microscopy, (3) DNA fragmentation assay (quantitatively by spectrophotometry or spectroflurometry, and qualitatively by agarose gel electrophoresis and single cell gel assay), (4) brightfield and electron microscopy, (5) Tdt-Utp Nick End Labeling assay (TUNEL), (6) Western blot analysis for gene expression, and (7) DNA microarray or apoptosis gene array analysis. *In vivo* methods may include but not limited to the following: (1) Brightfield microscopy (a minimum of X1000), (2) fluorescence microscopy, (3) DNA fragmentation assay (quantitatively by spectrophotometry or spectroflurometry, and qualitatively by agarose gel electrophoresis), (4) electron microscopy, (5) TUNEL assay on tissue sections, (6) Western blot analysis for gene expression, (7) study-specific monoclonal antibodies, and (8) DNA microarray or apoptosis gene array analysis.

Summary

The benefit of a comprehensive knowledge of cell death is not easily predicted but clearly stands to be immense. So, what has propelled apoptosis into the forefront of basic research? Widespread involvement of apoptosis in diverse normal physiological and disease conditions gives rise to numerous hopes suggesting that targeting this response will lead to the development of novel therapeutic regimens. The fact that apoptosis is present in tumors suggests that its induction could be used as a therapy. The ability to modify sensitivity to apoptosis through the regulatory pathways has clear implications for the treatment of malignancy. Potential strategies fall into three categories: direct induction of apoptosis by cytotoxic agents, enhancing vulnerability to apoptosis to increase the efficacy of other therapies, and boosting the resistance of normal cells to apoptosis. Among these direct antitumor therapies targeting apoptotic modulation may prove to be much less systemically toxic than standard chemotherapy and could also be used in an adjuvant manner, to increase the susceptibility of tumors at the time they are exposed to chemotherapy.

The field apoptosis has captured worldwide attention of biomedical scientists is attested by the fact

that the 2002 Nobel Prize in Physiology and Medicine was awarded to Sydney Brenner (Great Britain), H. Robert Horvitz (US), and John E. Sulston (GB) for their discoveries concerning genetic regulation of PCD.

See also: Cell Cycle; Cell Proliferation.

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Aquatic Ecotoxicology

See Ecotoxicology, Aquatic.

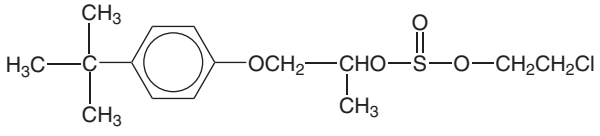
Aquatic Toxicity Testing

See Toxicity Testing, Aquatic.

Aramite

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 140-57-8
- Synonyms: 2-(*p*-Butyl phenoxy)-1-methylethyl-2-chloroethylsulfite; 2-(*p*-Butyl phenoxy) isopropyl-2-chloroethyl sulfite; Aracide; Aramit; Aratron; Ortho-mite
- Chemical Structure:



Uses

Aramite was formerly used as an antimicrobicide agent and as a miticide.

Exposure Routes and Pathways

Although exposure through oral consumption of contaminated fruits is possible, it should no longer be occurring since the use of aramite has been discontinued voluntarily on the basis of oncogenicity according to the US Environmental Protection Agency. Occupational exposure through dermal contact and inhalation of aerosols and dusts is possible.

Acute and Short-Term Toxicity (or Exposure)

Animal

A large oral dose causes central nervous system depression of long duration in laboratory mammals. The principal autopsy finding was hemorrhagic syndrome involving particularly the lung. Undiluted aramite and its concentrated solution are irritating to the skin and conjunctiva of experimental animals. Aramite has been found to give electroretinographic indications of intoxication of retinal photoreceptors when injected into mice and when applied to the eyeball.

Human

Acute exposure to aramite in an undiluted form may cause skin irritation.

Chronic Toxicity (or Exposure)

Animal

Increased incidence of liver tumors and/or neoplastic nodules in three strains of male and female rats and males of one strain of mice, and extrahepatic biliary system tumors were noted in dogs following chronic oral exposure.

Human

This compound is classified as a probable human carcinogen (classification B2) based on insufficient human data. No data are available on the number of workers who were actually or potentially exposed to aramite during its manufacture and formulation. The lowest published lethal dose/concentration in humans is $429\,mg\,kg^{-1}$.

Environmental Fate

Aramite can be released directly into the environment through its use as an acaricide; however, this use has been discontinued. If released to soil, aramite is not expected to leach. If released to water, it may sediment in water. Insufficient data are available to predict the relative importance of chemical or biological degradation processes in soil or water. If released into air, aramite is expected to be physically removed by deposition processes such as rainfall.

Miscellaneous

Aramite is a clear light-colored oil with a melting point of $-31.7^{\circ}C$ and a boiling point of $175^{\circ}C$ at 0.1 mmHg. It is noncorrosive and has a specific gravity of 1.145 at $20^{\circ}C$. It is practically insoluble in water and is miscible with many organic solvents. When heated to decomposition, it emits highly toxic fumes of chlorides and oxides of sulfur [SO(X)].

See also: Pesticides.

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Systems, National Library of Medicine. Search for Aramite.

Arsenic

Robert Kapp

This article is a revision of the previous print edition article by Arthur Furst, Shirley B Radding, and Kathryn A Wurzel, volume 1, pp. 80–82, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-38-2
- Synonyms: Arsen; Arsenic black; Arsenic-75; Arsenicals; Colloidal arsenic; Gray or grey arsenic; Metallic arsenic
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: As^{3+}; As^{5+}

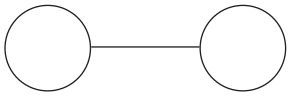
Uses

Arsenic was used as one of the earliest poisons and it is still used in some sheep dips, rat poisons, wood preservatives, weed killers, and other pesticides. It has been used in bronzing operations and is presently in use in pyrotechnics, in electronic devices, and as a laser material in converting electricity directly into coherent light. For many decades, arsenic was used medicinally to treat syphilis. Certain alloys of arsenic are used in making designer glass. The largest consumption of arsenic in the United States is in wood preservatives, especially in chrome copper arsenate (CCA), $CrO_3 \cdot CuO \cdot As_2O_5$ treated wood.

Background Information

In its elemental form, arsenic is an odorless, tasteless semimetallic compound, which appears steel gray in color, is brittle and is a crystalline solid. The symbol is As; the atomic number is 33. Arsenic compounds were mined by the early Chinese, Greek, and Egyptian

civilizations. There are many different forms of arsenic widely distributed in nature including the trivalent and pentavalent forms. It is believed that Albertus Magnus discovered the element in AD 1250. He obtained it by heating soap together with orpiment (arsenic trisulfide, As_2S_3). Arsenic is one of the elements that has an alchemical symbol, shown below (alchemy is an ancient pursuit concerned with, for instance, the transformation of other metals into gold):



Exposure Routes and Pathways

The primary exposure pathway for arsenic exposure is ingestion of water or food (including some wines). Inhalation exposure is a minor component and dermal absorption is negligible. Some food and wine exposures result from the use of arsenic-containing pesticides; however, the primary source is drinking water in places where the natural arsenic content of the water is high. Occupational exposure is generally associated with smelting industries and the manufacture of arsenic-containing compounds.

Arsenic is also still used for murder by poisoning, for which use it has a long and continuing history in literature and real life.

Toxicokinetics

Soluble forms of arsenic (such as arsenite) are readily absorbed from the gastrointestinal tract and the lungs. Less soluble compounds such as arsenic selenide, lead arsenate, and gallium arsenide are less efficiently absorbed. Absorbed arsenic is widely distributed in the body concentrating in the liver, kidneys, lungs and skin. Chronic exposure may result in hair, nail and skin accumulation, which is reflected in Mees' lines (transverse white bands across the fingernails). These lines can help estimate the time of onset of arsenic exposure based upon the rate of nail growth, which is $\sim$0.1 mm per day. In addition, arsenic tends to accumulate in the skin and can be found in sweat of exposed individuals. Metabolism of ingested arsenic compounds results in the excretion of methylated arsenic. The half-life of ingested inorganic arsenic is $\sim$10 h with up to 80% excreted in 3 days or less while the half-life of ingested organic arsenic is $\sim$30 h.

Mechanism of Toxicity

Arsenic affects mitochondrial enzymes and impairs tissue respiration. This appears to be related to the cellular toxicity of arsenic. Arsenic reacts primarily with enzymes containing two thiol groups. Some sulfhydryl-containing proteins and enzymes are functionally altered when exposed to arsenic. When arsenic accumulates in the mitochondria, the respiration mediated by the NAD-linked substrates results in a reaction between the arsenite ion and the dihydrolipoic acid cofactor, which is needed for the oxidation of the substrate. It is further shown that arsenic inhibits succinic dehydrogenase activity resulting in stimulation of ATPase activity by uncoupling oxidative phosphorylation. Studies have shown that the addition of glutathione and British anti-Lewisite (BAL) can reverse some of these arsenic changes. In addition, inhibition of mitochondrial respiration is shown to increase the production of hydrogen peroxide, which in turn, may cause the production of reactive oxidative species (ROS). ROS result in the induction of major stress protein families. Arsenical-induced oxidative stress and ROS may be critical factors in mediating DNA damage and initiation of carcinogenesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values for elemental arsenic in rats and mice are 763 and 145 mg kg^{-1}, respectively. Arsenic compounds can have vesicant effects, which means they can cause blisters, irritation, and necrosis in exposed tissues, whether internal or external.

Human

Ingestion of large concentrations ($>$70 mg) of arsenic may be fatal. Symptomology varies and may include fever, anorexia, nausea, vomiting, difficulty swallowing, cardiac arrhythmia, damage to the mucous membranes, respiratory tract distress, peripheral neuropathy, and hematopoietic effects. Sensory loss in the peripheral nervous system is the most common effect appearing 1–2 weeks postexposure. Symptoms of anemia, neuropathy and leukopenia are reversible once exposure is terminated.

Chronic Toxicity (or Exposure)

Animal

High doses of inorganic arsenic have produced various developmental malformations in animals. Animal data suggest that inorganic arsenite rather than methylated metabolites of arsenic caused the developmental abnormalities.

It has been difficult to demonstrate arsenic carcinogenicity in animal experiments. Oral and dermal administrations of arsenic trioxides and pentoxides have not resulted in carcinogenic outcomes in animal studies. Likewise, animal studies on organic arsenic compounds have been negative. Inorganic arsenic compounds induce deletion mutations and some chromosomal abnormalities, but no point mutations.

Human

Chronic exposure to arsenic may result in neuropathy in both the peripheral and central nervous system. The result is generally muscle weakness progressing from proximal to distal muscle groups. Chronic exposures produce much more gradual effects that can occur over many years and are clinically difficult to detect. Liver injury is also characteristic with chronic exposure first manifesting itself as jaundice and subsequently progression to cirrhosis and ascites. Peripheral vascular disease has been observed in some individuals exposed to high arsenic concentration in drinking water in Taiwan and Chile. These effects included Raynard's phenomenon and gangrene of the lower extremities (blackfoot disease) and are related to the cumulative dose of arsenic.

The skin also appears to be a critical target of arsenic toxicity. Dermatitis is observed with erythema followed by itching and swelling with a mottled appearance. Melanosis (abnormal pigmentation) subsequently appears at various points on the body often followed by hyperkeratosis (thickening of the skin).

Arsenic has also been shown to cross the placenta in pregnant women. Much of the arsenic was in the form of dimethyl arsenic, suggesting that there is an increase in methylation during pregnancy.

Hutchinson recognized the carcinogenic potential of arsenic long ago in 1887 when an unusual number of skin cancers occurred in patients treated with arsenicals. The International Agency for Research on Cancer, MAK, National Institute for Occupational Safety and Health, Occupational Safety and Health Administration (OSHA), National Toxicology Program (NTP), and the Environmental Protection Agency (EPA) have all classified arsenic as a human carcinogen based on sufficient evidence from epidemiological studies, that arsenic causes skin cancer and lung cancer. The hyperkeratosis that is seen in the skin of chronically exposed individuals can lead to basal cell carcinomas and/or squamous cell carcinomas. Angiosarcoma has been reported in vineyard workers chronically exposed to arsenic in drinking water, patients exposed to Fowler's solution, and agricultural workers exposed to various arsenic-containing pesticides. Lung cancers have been reported among copper smelter workers. The mode of action of arsenic carcinogenesis remains elusive.

Clinical Management

Syrup of ipecac (purging solution) and gastric lavage should be administered within 4–6 h of oral exposure to arsenic. Antidotes include 3–5 $mg\,kg^{-1}$ BAL (2,3-dimercaptopropanol) administered intramuscularly. Penicilamine has also been administered with optical neuritis as a side effect. Certain synthetic, water-soluble dimercapto compounds (DMSA – meso-2, 3-dimercaptosuccinic acid and 2,3-dimercaptopropane-1-sulfonate) have been found effective.

Environmental Fate

Arsenic is released to the atmosphere as a result of smelting of ores, incineration of arsenic containing materials and blowing of arsenic containing soils. It is usually found in air as a mixture of the trivalent and pentavalent forms. Arsenic can be transported significant distances and then will settle out or be carried to the earth's surface in rain or snow. Once arsenic reaches water from the atmosphere, runoff, discharges or other sources, it can be converted to a variety of forms; generally, arsenate is the predominant one. In addition, aquatic microorganisms can convert arsenate to arsenite and can methylate arsenic. Since arsenic is a natural component of the earth's crust, it is ubiquitous in soil. In some places, natural soil arsenic levels are high and this can lead to elevated groundwater arsenic levels.

Arsenic is found in a variety of foods, with seafood, meats, and grains generally showing the highest levels. Most of this arsenic is in the organic form and so does not pose a health risk. Arsenic is also found in plant tissues, often as a result of pesticide applications.

Exposure Standards and Guidelines

OSHA has established permissible exposure limits (PELs) for both inorganic and organic arsenic compounds. For the inorganic compounds, the PEL time-weighted average is $10\,\mu g\,m^{-3}$ and for the organic ones, it is $500\,\mu g\,m^{-3}$ ($0.05\,mg\,m^{-3}$). Although the current US EPA maximum contaminant limit (MCL) for arsenic in drinking water is $50\,mg\,l^{-1}$, the agency has recently adopted a new standard, an MCL of $10\,mg\,l^{-1}$, which will take effect on January 23, 2006.

See also: Metals; Neurotoxicity; Pollution, Soil; Pollution, Water; Sister Chromatid Exchanges; Skin.

Further Reading

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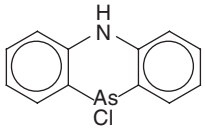
Arsenical Vomiting Agents

Harry Salem, Bryan Ballantyne, and Sidney A Katz*

Published by Elsevier Inc.

Adamsite (DM)

- Chemical Abstracts Service Registry Number: CAS 578-94-9
- Synonyms: Diphenylaminearsine; Diphenylaminochlorarsine; 10-Chloro-5,10-dihydrophenarsazine; White Cross Gas; Phenarsazine chloride
- Description: It was first synthesized in 1915 by a German chemist Weiland, and then again in 1918 by US chemist Robert Adams who named it adamsite. DM is a yellow-green, odorless crystalline solid that is not very volatile. It is insoluble in water and relatively insoluble in organic solvents
- Chemical/Pharmaceutical/Other Class: It is classified as a chemical warfare vomiting agent
- Chemical Formula: $C_{12}H_9AsClN$
- Chemical Structure:



Uses

Adamsite is used as a vomiting agent. It is considered insufficiently toxic for use in war, but too potent for control of civilian disturbances. Thus, it was banned in 1930 for use against civilians. Adamsite (DM) has found extensive use as a pesticide for treatment of wood against insects.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Exposure Routes and Pathways

Normally a solid, but upon heating DM first vaporizes and then condenses to form an aerosol. It is toxic through inhalation, ingestion, and skin contact. It irritates the eyes and respiratory tract, but not necessarily the skin.

Toxicokinetics

By any route of administration, the effects are slower in onset and longer in duration than typical riot control agents such as CS (*o*-chloro-benzylmalononitrile). Vomiting agents are irritants upon initial exposure. The slow onset for DM allows for the absorption of much more DM before a warning is perceived. The estimated threshold concentrations for irritation of the throat, lower respiratory tract, and initiation of the cough reflex are 0.38, 0.5, and 0.75 $mg\,m^{-3}$, respectively.

Mechanism of Toxicity

DM's primary action is on the upper respiratory tract, causing irritation of the nasal mucosa and nasal sinuses, burning in the throat, tightness and pain in the chest, and uncontrollable coughing and sneezing. It also causes eye irritation and burning, with tearing, blepharospasm, and injected conjunctiva.

DM is more toxic than other riot control agents; the LCt_{50} for humans has been estimated to be 11 000 $mg\,min\,m^{-3}$. The amount that is intolerable

for humans has been estimated by some to be 22 mg min m^{-3} and by others to be 150 mg min m^{-3}. The threshold for irritation in humans is about 1 mg m^{-3}, but some people have tolerated Ct exposures of 100–150 mg min m^{-3}.

This class of compounds is unique among the riot control agents because the effects do not appear immediately on exposure or seconds afterwards, but rather several minutes later.

The other characteristic of these compounds is that there may be more prolonged systemic effects, including headache, mental depression, chills, nausea, abdominal cramps, vomiting and diarrhea, which last for several hours after exposure. The Ct necessary to cause nausea and vomiting has not been established, but is estimated to be about 370 mg min m^{-3}.

DM is considered less effective as a riot control or incapacitating agent than CS and CN (chloroacetophenone), and it has been conjectured that there are greater differences in susceptibility among people to DM than to the other agents. DM, like CS, is considered to be a cholinesterase inhibitor, which may be responsible for its lacrimatory effect. DM also has a direct effect on gastric activity, but the evidence suggests that the lethal effect is respiratory related.

Toxicity (or Exposure)

Animal

Various animal species including monkeys have been exposed to DM. Following acute exposures, the animals exhibited ocular and nasal irritation, hyperactivity, salvation, labored breathing, ataxia, and convulsions.

Histopathology did not reveal any abnormalities at exposure dosages of below 500 mg min m^{-3}. At higher dosages, animals that died or were killed demonstrated hyperemia of the trachea, pulmonary congestion and edema, and pneumonia. These effects were consistent to exposure to pulmonary irritants. DM toxicity values are given in **Table 1**.

Monkeys have been exposed to varying concentrations and durations. At a Ct dosage of 2565 mg min m^{-3} only one animal responded, and that was with oral and nasal discharges and a diminished response to stimuli. A Ct of 8540 mg min m^{-3} resulted in ocular and nasal conjunctival congestion, facial erythema, and decreased responses, all of which were resolved within 24 h. Exposure to the high dosage of 28 765 mg min m^{-3} resulted in hyperactivity, copious nasal discharge, conjunctival congestion, marked respiratory distress, as well as gasping and gagging in all the exposed monkeys. Eight of these exposed monkeys died within 24 h of exposure. Necropsy of these animals revealed congestion and extremely edematous lungs. Microscopic examination revealed ulceration of the tracheobronchial tree and pulmonary edema.

Table 1 DM toxicity values

Species	*LCt_{50} (mg min m^{-3})*	*Intravenous LD_{50} (mg kg^{-1})*
Mouse	22 400	17.9
2Rats	3 700	14.1
Guinea pig	7 900	2.4

Theoretical dose calculated from respiratory volume, LCt_{50}, and estimated percent retention.

Studies were also conducted in which monkeys were exposed to low target concentrations of 100 and 300 mg m^{-3} DM for 2–60 min and 2–40 min, respectively. The signs of toxicity increased as the duration increased, characteristic of exposure to irritants. At the maximum dosage of 13 200 mg min m^{-3}, the animals exhibited nausea and vomiting, oral and nasal discharge, and conjunctival congestion. Only blinking was noted below 1296 mg min m^{-3}.

The effects of DM on the gastrointestinal tract were suggested as a possible cause of death. Dogs were dosed both intravenously and orally with lethal doses of DM, while central venous pressure, right ventricular pressure, cortical electric activity, alveolar CO_2, respiratory rate, heart rate electrocardiogram, and gastric activity were monitored. DM caused a marked elevation of both amplitude and rate of gastric activity for 15–20 min, and then returned to normal. Pretreatment with trimethobenzamide, an effective antiemetic for peripheral and centrally acting emetics, did not prevent DM gastric activity; however, pretreatment with chlorpromazine was effective. The authors concluded that DM affects the stomach directly, and that the primary cause of death following exposure to DM is its effects on the lungs.

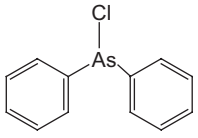
The effects of DM on the eyes and skin of rabbits were studied. DM was suspended in corn oil and instilled into the eyes of rabbits in doses of 0.1, 0.2, 0.5, 1.0, and 5.0 mg. No effect was observed at 0.1 mg, but mild conjunctivitis was observed at 0.2 mg. At 0.5 mg, mild blepharitis was also seen. Corneal opacity persisted over the 14 day observation period in rabbit eyes that were dosed with 1.0 and 5.0 mg. Corn oil suspensions of DM (100 mg ml^{-1}) were placed on the clipped backs of rabbits at doses of 1, 10, 50, 75, and 100 mg. Necrosis of the skin was observed at 10 mg and higher. The skin sensitization potential of DM in guinea pigs was negative.

Other Arsenical Vomiting Agents

The other arsenical vomiting agents include: diphenylchlorarsine (DA) and diphenylcyanoarsine (DC).

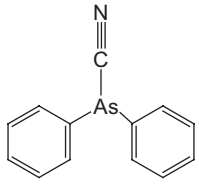
Diphenylchlorarsine (DA)

- Chemical Abstracts Service Registry Number: CAS 712-48-1
- Synonyms: Clark I
- Chemical/Pharmaceutical/Other Class: It is classified as a chemical warfare vomiting agent
- Chemical Formula: $C_{12}H_{10}AsCl$
- Chemical Structure:



Diphenylcyanoarsine (DC)

- Chemical Abstracts Service Registry Number: CAS 23525-22-6
- Synonyms: Clark II
- Chemical/Pharmaceutical/Other Class: It is classified as a chemical warfare vomiting agent
- Chemical Formula: $C_{13}H_{10}AsN$
- Chemical Structure:



See also: Arsenic; Riot Control Agents.

Relevant Websites

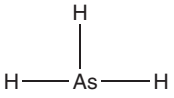
http://www.bt.cdc.gov – (US) Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – (US) National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Arsine

Felix Ayala-Fierro

- Representative Chemicals: Arsine; Monomethyl arsine; Dimethylarsine; Trimethylarsine
- Chemical Abstracts Service Registry Number: CAS 7784-42-1
- Synonyms: Arsenic hydride; Arsenic trihydride; Arsenous hydride; Hydrogen arsenide
- Chemical/Pharmaceutical/Other Class: Inorganic compounds
- Chemical Formula: AsH_3
- Chemical Structure:



Uses

Arsine is used commercially by the electronics industry for epitaxial growth of gallium arsenide and as a dopant applied to ultrapure crystals to increase electrical conductivity for silicon-based electronic devices.

Background Information

Arsine gas is a potent hemolytic agent. It was first identified in 1775 and since then recognized as one of the most potent and least inherently detectable gaseous toxicants. It can be formed when acid or base contacts inorganic arsenic and elemental metals like aluminum, zinc, and others. This makes possible the formation of arsine in the environment in such places as hazardous waste dumpsites. The current occupational exposure limit for arsine is 50 ppb; however, American Conference of Governmental Industrial Hygienists (ACGIH) placed arsine in the '2003 under study' list. In 2004, it listed arsine in the 'notice of intended changes' list, with a proposed (trial value) of 5 ppb.

Exposure Routes and Pathways

Arsine is highly poisonous, colorless, nonirritating, flammable, and with slight garlic odor at levels 10-fold greater than Occupational Safety and Health Administration (OSHA)/ACGIH limit values. Human exposure occurs via inhalation. Since arsine is used in the semiconductor industry, human exposure to arsine can occur from accidental release of the gas during these manufacturing processes or from accidental generation

from arsenic-contaminated substances, such as wastes from mining, smelting, refining, soldering, galvanizing, painting, and during application of herbicides. The reaction occurs when caustic or acidic solutions are used to clean arsenic-containing material in the presence of metals such as zinc or aluminum. The continuous inhalation of 250 ppm ($800\,mg\,m^{-3}$) is fatal, although lower levels (10 ppm) for longer time periods (up to 2 h) can be also lethal.

Toxicokinetics

Arsine gas is quickly absorbed through the lungs. Since arsenic in arsine is the fully reduced form of arsenic ($-$III), it will be oxidized in the presence of oxygen. Arsine may oxidize in several ways. One is to produce superoxide and arsenous acid (As(III)) as illustrated in this equation: $AsH_3 + 6O_2 + 2H_2O \rightarrow 6O_2^- + 6H^+ + HAsO_2$. The standard reduction potential, E'_0, for this reaction at physiological pH is $+0.31$ V. From the lungs arsine rapidly diffuses and dissolves in the blood, where it produces hemolysis. If dissolved in the plasma, it is possible that arsine reacts with water in the plasma (low probability) as depicted in this reaction: $AsH_3 + 2H_2O \rightarrow HAsO_2 + 3H_2$; $E_0 = +0.189$ V. In blood, it binds to hemoglobin. This reaction results in heme oxidation and may probably happen in a series of two electron steps: As($-$III) to As($-$I) to As($+$I) to As($+$III). As arsine oxidizes, oxygen, water, or biological molecules could be the electron acceptors in cell systems. Blood distributes arsine, hemolytic products, and arsine metabolites to other tissues, such as the liver and kidney. The mechanism of toxicity in these organs is not completely known. The resulting arsenicals from arsine exposure are eliminated in urine.

Mechanism of Toxicity

Hemolysis constitutes the main effect for arsine toxicity, a process which requires oxygen, hemoglobin containing the reduced iron, and access to the heme–ligand binding. This effect is due to a loss of membrane integrity that is characterized by a loss of intracellular potassium and influx of extracellular sodium (reversible) and calcium (irreversible). The influx of calcium is responsible for the morphologic changes within the erythrocyte. The mechanism of toxicity seems to be via oxidation of key sulfhydryl-containing ion gradients (transport pathways) across the cell membrane altering ion permeability. A single transport pathway has not been identified. In liver and kidney, arsine is oxidized to very small amounts of arsenite and arsenate. Liver toxicity seems to be caused by a similar mechanism as in blood; that is, disruption of the cell membrane, a process that may be related in part to an unknown metabolite and may involve hemoproteins. In the kidney, arsine produces early toxicity on endothelial cells from glomerular capillaries and peritubular microvessels, causing compromised filtration and edema. At a later point the presence of insoluble hemolytic products along with arsine metabolites would lead to oliguric renal failure.

Acute and Short-Term Toxicity (or Exposure)

Animal

The 10 min median lethal concentrations (LC_{50}s) reported in the literature for rats and rabbits are 120–210 and 200–300 ppm, respectively. The lethal effect of arsine is dependent on exposure concentration and duration. The rat LC_{50} at 0.5, 1 and 4-h exposures is 240, 178, and 45 ppm, respectively. Female rats have slightly greater mortality than males. The effects in animals include dyspnea, hematuria, dark material around the head or anogenital area, and pallor of ears and eyes. During necropsy the animals showed red, yellow, or orange fluid in the bladder, stomach, or intestine, and discoloration of the kidneys, lungs, and liver. Most of the available data come from experiments in rats; however, some authors state that the rat is not a suitable model for arsine toxicity because of differences in arsenic methylation and excretion compared to humans.

Human

The human literature on arsine primarily consists of case reports; therefore, data on arsine effects versus concentration in air do not exist. At the time of exposure there is no discomfort due to the nonirritating characteristics of arsine. Concentrations of arsine as low as 3–10 ppmv (parts per million volume) have been associated with symptoms. Within 24 h these symptoms include headaches, dizziness, weakness, dyspnea, abdominal cramping, nausea, vomiting, and hematuria (dark red urine). After 2 weeks of exposure renal effects are evident (morphological and functional changes) due to a direct arsine effect and the presence of a massive quantity of hemoglobin in the nephron. In severe cases oliguric renal failure is the final effect. Other effects normally reported include abnormalities of the nervous system (central and peripheral), cardiovascular (electrocardiographic changes due in part to electrolyte disturbances), pulmonary (edema), and immune system. Arsine is oxidized to inorganic arsenicals, which results in elevated levels of arsenic in the body and eventually elevated urinary excretion.

Chronic Toxicity (or Exposure)

Animal

The main chronic toxic effect in animals exposed to arsine for 28–90 days is in the hematopoietic system, including a decrease in packed erythrocyte volume and a peripheral erythrocyte regenerative response. The reproductive and developmental toxicity has not been completely studied. Rats exposed to 2.5 ppm arsine 6 h day^{-1} on gestation days 6–15 exhibited an increase in fetal body weight.

Human

The main chronic effect of arsine exposure (low dose) in humans is anemia. Repeated exposure to arsine may also damage the kidneys, liver, heart, and nervous system. There is a concern about the formation of carcinogenic arsenic from long-term exposure to arsine.

In Vitro Toxicity Data

In vitro toxicity studies in the rat indicate that arsine toxicity is tissue-specific. Red blood cells are very susceptible to arsine toxicity, followed by the primary hepatocytes and renal cortical epithelial cells. In blood arsine is the only factor responsible for hemolysis whereas in other tissues it is only responsible for the early signs of toxicity. At later points the toxicity effect is a combination of many other factors, including formation of inorganic arsenicals and hemolysate (kidney toxicity).

Clinical Management

In acute exposure prompt medical attention is critical. The victim should be immediately removed to fresh air and away from the source of exposure. Oxygen should be provided if there is a respiratory distress. Initial therapy should be directed at stopping the ongoing hemolysis by performing exchange transfusion. Currently there is no other treatment to decrease arsine hemolysis; however, studies *in vitro* have shown that some dithiol chelators (meso-2,3-dimercaptosuccinic acid, DMSA; 2,3-dimercapto-1-propanesulfonic acid, DMPS; and 2,3-butanedithiol) are effective (see Further Reading). This should be followed by aims to restore renal function or compensate for lost renal function (hemodialysis). This process does not remove any formed arsenic from the exposed body. Administration of dimercaprol (British Anti-Lewisite, BAL) has no effect on arsine hemolysis, but it lowers blood arsenic levels resulting from arsine exposure. The use of chelators must be carefully evaluated due to potential side effects. Other treatment that may be considered includes urine alkalinization (pH > 7.5 using sodium bicarbonate), monitoring of serum electrolytes, hemoglobin, and creatinine, and supportive care to improve oxygenation of the body.

Environmental Fate

Arsine accidentally released in air or water will be rapidly diluted and oxidized to other arsenicals. The final oxidized state would be arsenate (thermodynamically more stable). A small percentage would remain in water whereas the rest would be distributed along the sediment zones.

Ecotoxicology

There is no aquatic toxicity information available for arsine. However, one predicts arsine oxidation to small quantities of inorganic arsenicals in water.

Other Hazards

Arsine and arsine gas/air mixtures are flammable and explosive. The explosive limits (% by volume in air) are 4.5% and 78% for lower and upper, respectively. The gas is heavier than air and may ignite at distant ignition sources and flash back. Poisonous gases are produced during a fire. Arsine is incompatible with oxidants and oxidizing agents.

Exposure Standards and Guidelines

Arsine was placed under revision for the 2003 ACGIH threshold limit values (TLVs) because

Table 1 Summary of exposure criteria for arsine

Agency	*Criteria*		*Averaging time*
ACGIH	TLV – TWA	0.05 ppm	8 h/40 h week
NIOSH	IDLH	3 ppm	NA
NIOSH	Ceiling	0.002 mg m^{-3}	15 min sampling period
OSHA	PEL (TWA)	0.05 ppm (0.2 mg m^{-3})	8 h/40 h week

Conversion: 1 ppm = 3.19 mg m^{-3}.
OSHA, Occupational Safety and Health Administration; NIOSH, National Institute of Occupational Safety and Health; ACGIH, American Conference of Governmental Industrial Hygienists; IARC, International Agency for Research on Cancer; NTP, National Toxicology Program; TLV – TWA, threshold limit value – time-weighted average; IDLH, immediately dangerous to life or health; PEL, permissible exposure limit.

OSHA, National Toxicology Program (NTP), and International Agency for Research on Cancer (IARC) classify its metabolites (arsenic and arsenic inorganic compounds) as human carcinogens. The 2002 ACGIH proposal requested a new arsine TLV – TWA of 3 ppb and a designation as A1 confirmed human carcinogen. However, in 2004 ACGIH listed arsine in the 'notice of intended changes', with a proposed (trial value) of 5 ppb and a designation A4 not classifiable as a human carcinogen. Arsine is also reported in the List of Highly Hazardous Chemicals, Toxics, and Reactives (Mandatory), OSHA 1910.119 App A. This list contains all chemicals which present a potential for a catastrophic event if present at or above the threshold quantity (TP). The TP for arsine is 100 lbs. The current exposure standards and guidelines are summarized in **Table 1**.

See also: Arsenic; Arsenical Vomiting Agents.

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Arts and Crafts Materials and Processes

Angelique Dosh

Introduction/Background

Art and craft materials include those substances that are used to create works of visual or graphic art. Art processes include activities such as painting, printmaking, photography, pottery, and sculpting. The Consumer Product Safety Commission (CPSC) has defined two main categories of art materials. The first category includes products that become a component of the work of art such as crayons, paint, clay, ink, etc. The second includes products that are closely associated with the creation of art such as solvents, brush cleaners, mold making materials, and photo developing chemicals. Many of these products are composed of or contain substances that have the potential to cause both acute and chronic health hazards. Users may be exposed through the skin, via inhalation (volatile or particulates) or by accidental/incidental ingestion. Engaging in art processes may pose certain health risks, particularly in children. Use of these materials may pose a greater risk to children for several reasons: first, because of their age and size (smaller body mass), they may be more susceptible to the effects of a particular toxicant than adults; second, they may have a greater likelihood of ingestion because of behavior patterns (such as putting their hands in their mouths, not washing hands, or not washing hands thoroughly before eating); and lastly the widespread use of art materials in schools, which leads to more frequent exposures. Proper labeling of art materials is required by law to notify consumers about the risks associated with them.

Toxicity of Art Materials

Paint

Paints often contain solvents such as toluene, xylene, halogenated aromatic hydrocarbons, and methylene chloride, as well as heavy metals in their pigments including: chromium yellow, lemon yellow (barium chromate), vermilion red (cadmium and mercuric sulfides), and flake white (lead). Both acute and chronic exposures to toluene and xylene are associated with neurotoxicity and can also damage the liver and kidneys.

Toluene and xylene have inhalation reference concentrations (RfCs) of 0.4 and 0.1 $mg\,m^{-3}$, respectively. The RfC is an estimate of a daily inhalation exposure of the human population that is likely to be without an appreciable risk of harmful effects over a

lifetime. Methylene chloride is a liver toxicant with a no-observed-adverse-effect level (NOAEL) of $5.85\,mg\,kg^{-1}\ day^{-1}$ (rat). The NOAEL is the highest concentration of a substance that has been shown through testing to cause no-adverse-observed health effects in humans or animals. Barium and chromium are confirmed human carcinogens and cadmium is a probable carcinogen. Mercury and lead are highly neurotoxic and their effects on humans have been well documented.

Ceramics

Clay used in ceramics is usually composed of powdered aluminum silicates. It has been found that long-term exposures from inhaling silica dust can result in silicosis. Potters may handle dry clay and respirable dust may accumulate in areas where clay is routinely used. Ceramic glazing components usually consist of silica and a flux. The flux that is often used in ceramics may include heavy metals such as lead or barium. Glazing components are mixed with water and then brushed onto a piece of pottery prior to firing it. Exposure to the fumes from firing pottery with lead glazes may result in lead poisoning, particularly among children. Glazing components may also contain metal oxides such as arsenic, beryllium, cadmium, chromium, and nickel for color. Arsenic, nickel (dust), and chromium(VI) are all classified as known human carcinogens and beryllium and cadmium are probable human carcinogens.

Printmaking

Printmaking (lithography, intaglio, photoetching, relief printing, and screen printing) involves printing or transferring images onto various media (metal plates, wood, etc.). Pigments that are often used in creating the images include cadmium, zinc chromate, lead chromate, and strontium. Acids such as hydrochloric, acetic, hydrofluoric, tannic, phosphoric, and nitric, which are all corrosive, are used to etch the plates. Toluene, xylene, trichloroethylene, and kerosene are often used to clean printmaking equipment.

Other Art Materials

There are many other art materials that contain potentially hazardous substances. For example: magic markers often contain volatile organic compounds; glues and adhesives can contain solvents such as toluene, methyl ethyl ketone, acetone, and hexane; and instant papier-mâché and some modeling compounds may contain asbestos. In addition, many products pose an inhalation hazard due to the fine powders they are composed of, or the dusts they generate. Examples of these are powdered dyes, wood, and fibers from materials used in spinning and weaving.

Legislation

Prior to the early 1980s, the Federal Hazardous Substances Act (FHSA) was the only regulation governing art materials. The FHSA required consumer products (which included art materials) to be tested only for acute toxicity and labeled according to their associated hazard. Those products which are considered acutely hazardous must have warning labels with the following information: name and address of manufacturer, packer, distributor, or seller; common or chemical name of the substance; the signal word 'DANGER' for extremely flammable, corrosive, or highly toxic substances; the signal word 'WARNING' or 'CAUTION' on other hazardous substances; a statement of major hazards; precautionary measures; first-aid instructions; the word 'POISON' for highly toxic substances; special handling and storage instructions; the statement 'Keep out of reach of children'.

During the early 1980s, a group which included art material manufacturers, of artist organization representatives, and health experts came together to develop what is now the American Society of Testing and Materials (ASTM) D-4236: Standard Practice for Labeling Art Materials for Chronic Health Hazards. This was a voluntary standard, which to comply with, the formulation of an art material would need to be evaluated by a toxicologist for potential chronic hazards and then labeled accordingly.

On November 18, 1988 the Labeling of Hazardous Art Materials Act (Public Law 100-695, also known as LHAMA) was passed by Congress and signed into law by the President. This law, which is enforced by the CPSC, amended the FHSA to require manufacturers of art and craft materials to determine if their products pose any chronic health hazards and identify those hazards. If a chronic hazard exists, appropriate warning labels must be placed on the product including the ingredients causing the hazard, and directions for safe use.

Benefits to Consumers

When art materials are labeled with appropriate warning statements, consumers are made aware of the hazards associated with them, if any exist. Labeling is especially helpful for parents so that they are able to determine if an art material is suitable for their children to use. If an art material is not labeled

with the statement, 'Conforms to ASTM D-4236', the consumer may want to obtain additional information on product ingredients and potential risks associated with its use.

See also: Cadmium; Chromium; Consumer Product Safety Commission; Lead; Methylene Chloride; Toluene; Xylene.

Further Reading

California Office of Environmental and Health Hazard Assessment. Guidelines for the Safe Use of Art and Craft Materials. California Office of Environmental and Health Hazard Assessment (OEHHA), December 2003.

Consumer Product Safety Commission. Law Requires Review and Labeling of Art Materials Including Children's Art and Drawing Products. Consumer Product Safety Commission – CPSC Document #5016.

Arts and Toxicology *See* Toxicology in the Arts, Culture, and Imagination.

Arum

Susan M Stejskal

This article is a revision of the previous print edition article by Rita Mrvos, volume 1, p. 82, © 1998, Elsevier Inc.

Uses

Historically, some of the plants in the Arum family have been used to treat respiratory illnesses due to some properties as an expectorant, to treat dermal corns due to its irritant properties, and, after repeated processing, as a starch for thickening foods.

Background Information

The name Arisaema is derived from the Greek word Aris (arum) and Haema (blood). Arum constitutes a large family of plants that includes ~2000 species and includes members from the Arum, Arisaema, Calla, Dieffenbachia, and Philodendron genera. Common names included in this family include calla lilies, dumb cane, jack-in-the-pulpit, taro, and skunk cabbage.

Exposure Routes and Pathways

Routes of exposure include accidental ingestion and dermal contact.

Mechanism of Toxicity

Calcium oxalate crystals are commonly found in all members of this family. It is postulated that needle-like calcium oxalate monohydrate crystals with grooved ends are located within specialized cells known as idioblasts. Inside each idioblast is a bundle of raphides, which are ejected from the plant when pressure such as squeezing is applied. These raphides are then embedded in the skin or mucous membranes. There are also other proteolytic enzymes found in various species.

Acute and Short-Term Toxicity (or Exposure)

Animal

Feline ingestion of philodendron results in central nervous system excitability, seizures, renal failure, and encephalitis.

Human

Symptoms include skin irritation consisting of erythema and vesiculation. Ingestion of plant material may result in local mouth or throat irritation and resultant swelling. Salivation and dysphagia may also be present. Systemic effects are extremely rare. Ocular exposure will result in pain and photophobia, followed by eyelid edema and corneal disturbances such as abrasions and chemosis.

Clinical Management

Treatment for dermal exposure should include irrigation of the contaminated area followed by cool compresses. Treatment for oral exposures should consist of removing any plant material from the oral cavity and administering cool liquids. Significant toxicity is rare. Irrigation should be performed in instances of ocular contamination. Further care should be symptomatic and supportive.

See Also: Oxalates.

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http://www.botanical.com – Botanical.com. A Modern Herbal. Mrs. M. Grieve. Botanical: Arum maculatum. Family: N.O. Araceae.
http://earthnotes.tripod.com – Earthnotes Herb Library.

Asbestos

Xuannga Mahini

- Chemical Abstracts Service Registry Number: CAS 1332-21-4 (Other Registry Numbers: CAS 12413-45-5; CAS 77641-59-9)
- Synonyms: Asbestos fiber; Actinolite; Amianthus; Amosite; Amphibole; Anthophylite; Ascarite; Chrysotile; Crocidolite; Fibrous Grunerite; Serpentine; Tremolite
- Chemical/Pharmaceutical/Other Class: Mineral fibers

Uses

Asbestos is a generic name that refers to a group of six naturally occurring fibrous silicate minerals (actinolite, amosite, anthophylite, chrysotile, crocidolite, and tremolite). Asbestos minerals are characterized by fibers or bundles of fine single crystal fibrils. Chrysotile (curved, flexible fibers that can be woven) belongs to the serpentine family, while all others (straight, brittle fibers) belong to the amphibole family. It should be noted that serpentine and amphibole minerals also occur in nonfibrous form and are not asbestos. Chrysotile, known as white asbestos, is the predominant commercial form of asbestos (99% in the United States); amphiboles are of minor commercial importance. Because of its insensitivity to heat and chemical attack, asbestos is widely used in textiles, electrical and sound insulation, ceiling and floor tiles, dry wall, roof shingles, reinforced cement, industrial water filters, gaskets, and automotive brake and clutch linings. Consumptions of asbestos in the United States have been declining for two decades. Roofing products, gaskets, and friction products will continue to be the only significant domestic markets for asbestos in the foreseeable future. Only chrysotile is presently used for manufacturing in the United States. Ninety-four percent of chrysotile consumed was grade 7, a short 3 μm fiber.

A particle visible under phase contrast microscopy (PCM) is counted as a fiber if it has a length greater than 5 μm, a diameter less than 3 μm, and length/diameter ratio greater than 3:1, equivalent to one fiber per millimeter ($f\,ml^{-1}$). Based on the 1984 NRC suggestion, both 1 PCM $f\,ml^{-1}$ and 60 transmission electron microscopy (TEM) $f\,ml^{-1}$ are approximately equal to a mass concentration of 30 $\mu g\,m^{-3}$.

Exposure Routes and Pathways

Epidemiological studies of asbestos-exposed workers and supporting animal studies indicate that inhalation of asbestos is the principal route of exposure of public health concern. Other routes of exposure to asbestos include incidental ingestion or dermal exposure. Asbestos fibers may be ingested through food or drink or by swallowing of inhaled asbestos cleared from the lungs.

Toxicokinetics

The most common route of entry into the body is by inhalation. When asbestos is inhaled, larger fibers (10–20 μm in length) tend to be filtered out in the upper airways or collide with the walls of the conducting airway walls in the lungs where they are captured in the respiratory mucous. These fibers are then removed by cilia of the tracheobrocheal tree and are swallowed. Asbestos fibers less than 10 μm in length may eventually reach the alveoli. In autopsy lung specimens, asbestos fibers of 5–200 μm have been found in alveoli. Very small fibers may be engulfed by alveolar macrophages and transported to lymph nodes. In human autopsies, asbestos fibers have also been found in the thoracic diaphragm and chest wall. Once deposited in alveoli, asbestos fibers remain permanently embedded as asbestos bodies (ferruginous bodies) and are not excreted. Thus, asbestos fibers build up in lung tissues over time, but

some fibers, particularly chrysotile fibers, can be removed or degraded in the lung with time.

Asbestos fibers may be deposited in the gastrointestinal tract via ingestion. There is no systemic absorption of asbestos fibers and they do not appear to stimulate an inflammatory reaction or any other adverse effect in the gastrointestinal tract. Nearly all ingested asbestos fibers pass along the intestines within a few days and are excreted in the feces. Asbestos fibers may penetrate the skin but are not absorbed and metabolized in the body.

Mechanism of Toxicity

Asbestos produces its toxic effects by direct contact with lung tissue or by stimulating an acute or chronic inflammatory reaction in the tissue (via active oxygen mechanism or other cell-mediated mechanisms). The important determinants of asbestos toxicity are fiber size, fiber durability, and iron content.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, mesothelioma developed in two rats exposed to high concentrations of amosite or crocidolite for only 1 day. These data are not extensive enough to define the dose–response but data indicate that short-term exposures should not be regarded.

Human

It has been noted that workers exposed to asbestos for 1–12 months had an increased risk of developing lung cancer a number of years later. There is some evidence that acute oral exposure may induce precursor lesions of colon cancer.

Chronic Toxicity (or Exposure)

Animal

Animals exposed to asbestos over a long period of time can develop lung tumors (adenomas, Aden carcinomas, and squamous cell carcinomas) and mesothelioma. Animals given very high doses of asbestos in food did not get significantly increased fatal cancers compared to the control group, although some extra nonfatal tumors did occur in the intestines of rats in one study. A few studies in rats have reported some alterations in cells of the gastrointestinal tract after chronic exposure to chrysotile.

Human

Chronic inhalation of asbestos produces a disease called asbestosis, which is characterized by interstitial fibrosis of lung parenchyma. All types of asbestos fibers can cause asbestosis, but crocidolite is most potent. The first symptoms of asbestosis are dyspnea with exertion and reduced exercise tolerance. Lung function abnormalities can include decreases in vital capacity, residual volume, functional residual capacity, and lung compliance. The disease can progress to massive pulmonary fibrosis. In these cases, the diffuse fibrosis and contraction of lung tissue causes constriction of the pulmonary vasculature, leading to pulmonary hypertension, which may lead to death. Asbestos can cause a fibrous pleuritis in which the pleural membrane thickens to encase the lung in a rigid fibrous capsule. There is formation of pleural plaques. Radiologic evidence of asbestos-induced lung damage is not present at least until 5 years after exposure. The most important physical sign is the presence of high-pitched fine crepitations (crackles) at full inspiration, which persist after coughing. As the total lung volume is decreased, especially the forced vital capacity, blood flows to the lungs may also decrease, and this causes the heart to enlarge. Asbestosis is a serious disease and can eventually lead to disability or death in people exposed to high amounts of asbestos over a long period. However, asbestosis is not usually of concern to people exposed to low levels of asbestos.

Chronic exposure to asbestos can also cause lung cancer, bronchogenic carcinoma, and mesothelioma (cancer of the thin membrane that surrounds the lung and other internal organs). The latency period for lung cancer is 20–30 years. Symptoms of lung cancer may include chest pain, chronic cough, hemoptysis, and decreased exercise tolerance. Mesothelioma is another malignant disease associated with asbestos. The latency period is 35–40 years. Asbestos is the only known cause of this tumor. The first symptoms of mesothelioma are those associated with pleural irritation such as cough and chest pain. Lung cancer is usually fatal, while mesothelioma is almost always fatal, often within a few months of diagnosis. For lung cancer, the magnitude of risks appear to be a complex function of a number of parameters, the most important factors are: (1) the level and duration of exposure; (2) the time since exposure occurred; (3) the age at which exposure occurred; (4) the cigarette smoking history; and (5) the type and size distribution of the asbestos fibers. There is some evidence from animal studies that asbestos-induced cancer stems from regions in the lung with advanced fibrosis (asbestosis); however, lung cancer caused by chrysotile was also produced at fiber concentrations that did not lead to detectable fibrosis. With respect to lung cancer, some studies have indicated that the interaction between asbestos and smoking is greater

than additive. Asbestos may increase the risk of cancer at other sites, but the evidence is not strong. Significant effects on other tissues have not been detected.

There is some evidence that chronic oral exposure to asbestos may lead to an increased incidence risk of gastrointestinal tumors. However, the health effects from ingesting asbestos are unclear. Although some groups of people who have been exposed to asbestos fibers in drinking water have higher-than-average death rates from cancer of the esophagus, stomach, and intestines, it is very difficult to determine whether this is caused by asbestos or by other causes. Handling asbestos without gloves can cause corns (asbestos warts), which are areas of thickened skin surrounding implanted fibers.

In Vitro Toxicity Data

Studies of exposed asbestos workers, residentially exposed Turkish villagers, mesothelioma patients, and lung cancer patients suggest that asbestos is genotoxic. The number of chromosomal aberrations and the rate of sister chromatid exchange were significantly elevated in the peripheral blood lymphocytes of the exposed individuals compared with the nonexposed control group. Tests of asbestos for gene mutations have been mixed, in both *in vivo* and *in vitro* toxicity data.

Clinical Management

A chest X-ray cannot detect the asbestos fibers themselves, but it can detect early signs of lung disease caused by asbestos from relatively heavy exposure. The most reliable test for asbestos exposure is the detection of microscopic asbestos fibers in pieces of lung tissue removed by surgery (invasive test). The use of biological markers, such as tissue polypeptide antigen, may play a useful role in the early detection of mesothelioma in individuals at risk.

There is no effective treatment for asbestosis. The only preventive measure is to keep asbestos fibers out of the lungs. Once asbestosis has started, further inhalation of asbestos fibers causes acute inflammatory reactions, which can worsen the disease. At the time of diagnosis, lung cancer is usually too advanced for successful treatment. Long-term survival rates are low following surgery and treatment with chemotherapeutic agents or radiation. There is no effective treatment for mesothelioma, and death usually occurs within 1 year after diagnosis. Early detection, radiation therapy, and chemotherapy may prolong survival.

Environmental Fate

Asbestos minerals are widespread in the environment. Asbestos fibers are chemically inert – they do not evaporate, dissolve, or undergo significant degradation in the environment. They enter the air and water from wearing down or disturbance of natural deposits or manufactured asbestos products. Small diameter fibers can remain suspended in the air and water for a long time and be carried long distances by wind or water. Asbestos fibers are not able to move through soil and are generally not broken down to other compounds in the environment. Chrysotile may undergo some dissolution in the aquatic environment, especially at low pH.

Other Hazards

Transplacental transfer of asbestos may occur, but this has not been linked with any adverse reproductive outcomes in humans. Results of animal studies do not indicate that exposure to asbestos is likely to result in birth defects. A recent study of brake workers, who are typically exposed to short chrysotile fibers, indicates that brake work does not increase the risk of mesothelioma. The results add to the evidence that fiber type and size are important determinants of mesothelioma risk. Along with asbestos, Simian virus 40 (SV40), a DNA monkey virus, has recently been implicated in the etiology of mesothelioma. It was proposed that SV40 and asbestos are possibly cocarcinogens.

Exposure Standards and Guidelines

Asbestos is considered by the US Department of Health and Human Services (DHHS), the US Environmental Protection Agency (EPA), and International Agency for Research on Cancer to be a known human carcinogen. EPA established a ban on new uses of asbestos on July 12, 1989. EPA also established regulations that require school systems to conduct asbestos inspection and abatement. They also regulate the release of asbestos from building demolition/renovation and management of waste containing asbestos. The 2000 American Conference of Governmental Industrial Hygienists threshold limit values, time-weighted average (8 h exposure), for asbestos is $0.1\,\mathrm{f\,ml^{-1}}$. The National Institute for Occupational Safety and Health recommended exposure limit and Occupational Safety and Health Administration permissible exposure limit (8 h exposure) is also $0.1\,\mathrm{f\,ml^{-1}}$. EPA has proposed a maximum contaminant level of 7 million fibers (greater than or equal to 10 μm) per liter in drinking water.

US Food and Drug Administration currently regulates the use of asbestos in preparation of drugs and restricts the use of asbestos in food-packaging materials.

Miscellaneous

Asbestos fibers do not have any detectable odor or taste.

See also: Carcinogenesis; Respiratory Tract.

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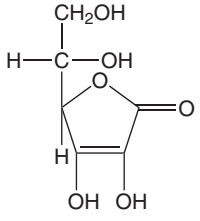
Ascorbic Acid

John Sanseverino

This article is a revision of the previous print edition article by Dennise L Kurta, volume 1, pp. 84–85, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 50-81-7
- Synonyms: Vitamin C; Acidum; Antisorbutic vitamin; Ascurbicum; Cevitamic acid; 2,3-Didehydro-L-threo-hexono-1,4-lactone; E300; L-Ascorbic acid; L-Xyloascorbic acid; L-3-Ketothreohexuronic acid lactone
- Chemical/Pharmaceutical/Other Class: Organic acid; Vitamin
- Chemical Formula: $C_6H_8O_6$
- Chemical Structure:



Uses

Ascorbic acid, a water-soluble vitamin widely distributed in the plant and animal kingdoms, is used for the growth and repair of bodily tissues. It is essential for the formation of collagen, skin, tendons, ligaments, and blood vessels as well as wound repair, and the repair and maintenance of cartilage, bones, and teeth. Deficiencies in ascorbic acid lead to dry and splitting hair, gingivitis and bleeding gums, dry skin, decreased wound healing, easy bruising, nosebleeds, weakened tooth enamel, swollen and painful joints, anemia, decreased ability to ward off infection, and possibly weight gain due to a slowed metabolism. Ascorbic acid is used as a nutritional supplement during deficiency states (scurvy). Ascorbic acid needs may increase during chronic illness, infection, trauma, pregnancy, and lactation. It has also been used as a urinary tract acidifier and purportedly is a cure for the common cold. Ascorbic acid and α-tocopherol may alleviate arsenic-induced alterations in mitochondria. It may also have a protective role in lead toxicity; its derivative ascorbyl palmitate may alleviate gastric disease (gastritis, duodenal ulcer, and carcinoma) by direct action on *Helicobacter pyroli*; and it may relieve symptoms of aflatoxin B1 toxicity.

Background Information

Ascorbic acid, or vitamin C, was discovered after scientists had searched for centuries for a cure for the disease known as scurvy. The name ascorbic acid comes from word 'anti-scurvy' acid, because it was known to dramatically cure this disease. This disease was caused by a serious deficiency of vitamin C, and it caused its victim's small blood vessels to rupture, bones to weaken, and joints to swell, among other symptoms. These symptoms were due to the fact that without a source of vitamin C one developed severe problems concerning the body's connective tissues, which is found in bones, skin, muscles, teeth, blood vessels, and cartilage. This disease would eventually lead to death if it went untreated, and was not uncommon, especially during the winter months of the year. The disease often plagues armies, explorers, and crusaders, since these men's diets normally consisted of biscuits and salted meat that could easily be stored and kept unspoiled on a ship.

Exposure Routes and Pathways

Routes of exposure are oral, intravenous, intramuscular, and subcutaneous. Dietary sources of ascorbic acid include citrus fruits, tomatoes, potatoes, cantaloupe, raw peppers, and green leafy vegetables.

Toxicokinetics

Ascorbic acid is readily absorbed from the gastrointestinal tract; however, absorption may be delayed with large doses. It is metabolized hepatically, reversibly oxidized to dehydroascorbic acid, and metabolized to inactive ascorbate-2-sulfate and oxalic acid. Protein binding is 25%, and ascorbic acid is widely distributed into body tissue. It is renally excreted. Elimination increases with higher doses. Vitamin C is an antioxidant, which means that it quenches free radicals that can damage organs, tissues, and cells. Free radicals are believed to be one of the causes of the degenerative changes seen with aging, but it is not yet known whether consumption of additional antioxidants like vitamin C can help.

Mechanism of Toxicity

Metabolism of ascorbic acid can lead to deposition of oxalate crystals in kidney tissue. Reduction of carcinogenic Cr(VI) by ascorbic acid generates ascorbate–Cr(III)–DNA cross-links that have been linked to mutagenicity and the formation of DNA lesions. Uranyl acetate–ascorbate has also been shown to nick plasmid DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity is not expected; however, the oral LD_{50} dose for rats is $11.9\,g\,kg^{-1}$ body weight.

Human

Toxicity is unlikely following acute ingestions of even 100 times the recommended daily allowance. The most common manifestations of vitamin C toxicity are kidney stones, and in very rare circumstances, anemia (caused by interference with vitamin B_{12} absorption).

Chronic Toxicity (or Exposure)

Human

Chronic megadoses of vitamin C may precipitate formation of calcium oxalate renal stones, oxalate nephropathy, and renal failure. The amount required to cause this is variable from 2 to $8\,g\,day^{-1}$. Bone oxalate deposits have also been reported. Esophageal and dental erosion are possible with tablet ingestion. Heinz body hemolytic anemia has been seen in premature infants.

Clinical Management

Acute ingestions seldom require treatment. Dilution is recommended to reduce the risk of esophageal and gastrointestinal irritations. During chronic excessive use, patients should be instructed to discontinue the supplement and observe for signs of rebound scurvy. Any toxic symptom should be treated symptomatically.

Exposure Standards and Guidelines

Recommended dietary allowances are defined as the levels of intake of essential nutrients that, on the basis of scientific knowledge, the Food and Nutrition Board judges to be adequate to meet the known nutrient needs of practically all healthy persons. Vitamin C should be consumed every day, since it is not a fat-soluble vitamin and cannot be stored for later use. Specific recommendations for each vitamin depend on age, gender, and other factors (such as pregnancy). There has been much debate regarding the use of vitamin C in cancer and heart disease prevention. Although the evidence is mixed regarding a definitive benefit of vitamin C in this regard, it is still encouraged that individuals maintain adequate intake. On the other hand, the majority of current evidence does not support vitamin C's role in the prevention or treatment of the common cold.

See also: Dietary Supplements; Vitamin A; Vitamin D; Vitamin E.

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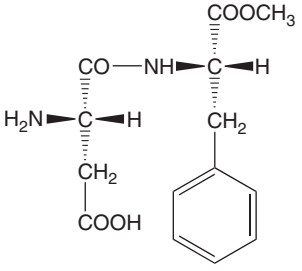
Aspartame

Robin C Guy

This article is a revision of the previous print edition article by Arvind K Agarwal, volume 1, pp. 85–86, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 22839-47-0
- Synonyms: L-α-aspartyl-L-phenylalanine 1-methyl ester; NutraSweet
- Chemical/Pharmaceutical/Other Class: Dipeptide methyl ester
- Chemical Formula: $C_{14}H_{18}N_2O_5$
- Chemical Structure:



Uses

Aspartame is a synthetic sweetener commonly used in soft drinks and many foods. It is a high-intensity sweetener and a flavor enhancer.

Background Information

More than 20 years have elapsed since aspartame was approved by regulatory agencies as a sweetener and flavor enhancer. The safety of aspartame and its metabolic constituents was established through extensive toxicology studies in laboratory animals, using doses much higher than people could possibly consume. The safety profile was further confirmed in studies of several human subpopulations, including healthy infants, children, adolescents, and adults, and in obese individuals and diabetics. Further, the studies included individuals heterozygous for the genetic disease phenylketonuria (PKU) who have a decreased ability to metabolize the essential amino acid, phenylalanine. In total, prior to marketing, the safety of the high-intensity sweetener aspartame for its intended uses as a sweetener and flavor enhancer was demonstrated by the results of over 100 scientific studies in animals and humans.

Aspartame has been a noteworthy example of a high-profile chemical with many years of risk perception issues. The scientific issues continued to be raised after approval, including concern for theoretical toxicity from aspartame's metabolic components. The metabolic components include the amino acids, aspartate and phenylalanine, and methanol, even though dietary exposure to these components is much greater than from aspartame. In the postmarketing period, the safety of aspartame was further evaluated through extensive monitoring of intake, postmarketing surveillance of anecdotal reports of alleged health effects, and additional research to evaluate these anecdotal reports and other scientific issues.

The results of the extensive intake evaluations in the United States and in other countries demonstrated that intakes which were well below the acceptable daily intakes (ADIs) set by the Food and Drug Administration (FDA) and regulatory bodies in other countries, as well as the Joint FAO/WHO Expert Committee on Food Additives (JFECFA). The studies have also included evaluations of possible associations between aspartame and headaches, seizures, behavior, cognition, and mood as well as allergic-type reactions and use by potentially sensitive subpopulations, has continued after approval. Evaluation of the anecdotal reports of adverse health effects were the first ones done for a food additive, and revealed that the reported effects were generally mild and also common in the general population,

and that there was no consistent or unique pattern of symptoms that could be causally linked to consumption of aspartame. Finally, the results of the extensive scientific research done to evaluate these allegations did not show a causal relationship between aspartame and adverse effects. Recent reviews have stated that when all the research on aspartame, including evaluations in both the pre- and postmarketing periods, is examined as a whole, it is clear that aspartame is safe, and there are no unresolved questions regarding its safety under conditions of intended use.

Exposure Routes and Pathways

The mode of aspartame exposure is oral.

Toxicokinetics

Aspartame is hydrolyzed entirely in the gastrointestinal tract to its constituent amino acids, aspartate and phenylalanine, and methanol. These are absorbed by the body and utilized via the same metabolic pathways as when these same constituents are derived from common foods; they are found in common foods in much larger quantities than from aspartame in foods or beverages.

Mechanism of Toxicity

Individuals with the rare, genetic disease PKU cannot properly metabolize phenylalanine. These individuals are placed on special low-phenylalanine diets to control their blood phenylalanine concentrations, and need to be aware that aspartame is a source of phenylalanine.

Acute and Short-Term Toxicity (or Exposure)

Animal

The bone marrow cells isolated from mice exposed to blends of aspartame and acesulfame-K via gavage were analyzed for chromosome aberrations, and the results show that aspartame in combination with acesulfame-K is not genotoxic. In a study examining the effect of aspartame on the cytogenetic effects of dioxydin and cyclophosphan, aspartame was found to possess antimutagenic properties in relation to chromosome aberration counts in the bone marrow cells of mice. The antimutagenic activity of aspartame was manifested more when it was injected for 5 days before the administration of a mutagen, while joint administration of aspartame with the mutagens did not change the clastogenic effect of dioxydin and cyclophosphan.

No micronuclei were formed in bone marrow cells isolated from mice (rats) exposed to an acute dose of aspartame. Aspartame is not toxic, as tested in acute animal studies in mice, rats, and rabbits.

Human

Aspartame is not toxic when administered in acute doses. When humans were administered aspartame at dosages up to 200 mg kg^{-1} body weight as a single bolus dose, the blood concentrations of aspartic acid, phenylalanine, and methanol were well below levels considered potentially harmful. The toxic effects of methanol in humans are due to accumulation of its metabolite, formate, and the blood formate concentrations did not increase after this high dose of aspartame (equal to the amount in ~28 l of beverage with aspartame consumed at once or ~65–70 times the amount of aspartame people consume daily at the 90th percentile). Urinary excretion of formate increased significantly in samples collected 0–4 and 4–8 h after aspartame ingestion. Therefore, the rate of formate formation did not exceed the rate of formate excretion, even after this very large bolus dose. Studies have also shown that when aspartame is consumed at levels within the ADI-limit of 40 mg kg^{-1} body weight, there is no significant risk for an aspartate-induced neurotoxic effect in the brain. Further, the available behavioral studies in humans with acute dosing found no adverse effects.

Chronic Toxicity (or Exposure)

Animal

Aspartame is not toxic, carcinogenic, mutagenic, or teratogenic, and has no effect on reproduction.

Human

As noted above, the available evidence suggests that consumption of aspartame by normal humans is safe and is not associated with serious adverse health effects. Specific chronic studies included studies up to 27 weeks duration in healthy adults, children, and adolescents, obese subjects, individuals with diabetes, and individuals heterozygous for PKU. The results of these studies show that there was no accumulation of plasma aspartate, phenylalanine, or methanol in humans following long-term exposure.

Further, a 6 month study in healthy adult volunteers aged 18–62 years used 75 mg kg^{-1} body weight per day of aspartame or placebo (provided as three divided doses daily), approximately the same amount of aspartame per day as 10 l of a soft drink

sweetened with 100% aspartame. There was no accumulation of blood or plasma aspartate, phenylalanine, methanol, or formate over the course of the study. In addition, urinary formate excretion did not increase, indicating no significant increase of formate formation. There were no adverse experiences and no effects on physical examinations, including vital signs, electrocardiograms, ophthalmologic examinations, or biochemical parameters after aspartame compared to placebo.

In Vitro Toxicity Data

The nonnutritive sweeteners acesulfame-K, aspartame, cyclamate, saccharin, and sucralose were tested for DNA damaging activity in the rat hepatocyte/DNA repair assay using hepatocytes from rats. The results found no evidence of genotoxic potential.

Exposure Standards and Guidelines

Based on the lack of toxicity observed in animal studies, a no-observed-effect level of at least $4000\,mg\,kg^{-1}$ body weight per day was established by the JECFA, the Scientific Committee on Food, and the Health Protection Branch of Health and Welfare Canada. As a result, an ADI of $40\,mg\,kg^{-1}$ body weight was set by these agencies. The (US) FDA set the ADI at $50\,mg\,kg^{-1}$ body weight based on both animal and human studies. Further, it is the position of The American Dietetic Association that consumers can safely enjoy a range of nutritive and nonnutritive sweeteners, including aspartame, when consumed in a diet that is guided by current (US) federal nutrition recommendations, such as the Dietary Guidelines for Americans and the Dietary References Intakes, as well as individual health goals.

Aspartame has also been considered by other bodies including the UK Committee on Toxicity and the European Commission's Health and Consumer Protection Directorate-General's Scientific Committee on Food (SCF). In December, 2002, the SCF concluded that, on the basis of its review of all the data in animals and humans available to date, there is no evidence to suggest that there is a need to revise the outcome of the earlier risk assessment or the $40\,mg\,kg^{-1}$ body weight ADI previously established for aspartame.

See also: Food Additives; Food and Drug Administration, US; Redbook.

Further Reading

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Relevant Website

http://europa.eu.int – European Commission. Health and Consumer Protection Directorate-General. Scientific Committee on Food (SCF). 2002. Opinion of the Scientific Committee on Food. Update on the Safety of Aspartame. December 10, 2002.

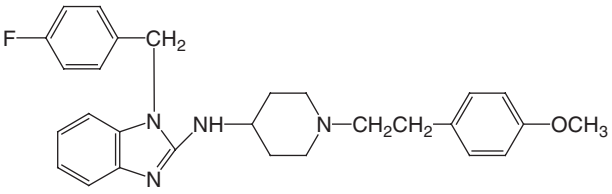
Aspirin *See* Acetylsalicylic Acid.

Astemizole

Michael D Reed

This article is a revision of the previous print edition article by Carla M Goetz, volume 1, pp. 86–87, © 1998, Elsevier Inc.

- Synonyms: Hismanal
- Chemical/Pharmaceutical/Other Class: Antihistamine; Nonsedating antihistamine; H-1 receptor antagonist
- Chemical Formula: NyNHOCH
- Chemical Structure:



Uses

Astemizole is indicated for the symptomatic relief of seasonal allergic rhinitis and chronic idiopathic urticaria. The drug is used in any medical condition in which histamine-1 (H-1) receptor antagonism is beneficial.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to astemizole.

Toxicokinetics

The drug is available (has been removed from the US market due to cardiotoxicity) as a tablet formulation. Astemizole is rapidly and very well absorbed after oral administration; reaching peak plasma concentrations within 1 h (0.5–1 h) of administration. Concurrent administration with food will decrease the rate but not the overall extent of intestinal absorption. The drug is ~97% bound to plasma protein and is extensively distributed within the body; astemizole V_d is $\sim 250\,l\,kg^{-1}$ in adults.

Astemizole undergoes extensive first-pass hepatic metabolism via cytochrome P450 (CYP 450) enzymes (primarily 3A4) to three primary, active, metabolites, desmethylastemizole, norastemizole, and 6-hydroxydesmethylastemizole. The major metabolites of astemizole, desmethylastemizole and norastemizole, possess antihistaminic activity approaching that of the parent compound. The $t_{1/2}$ of astemizole is ~1.1 days whereas the estimated $t_{1/2}$ for desmethylastemizole is ~9.5 days. Drugs that interact, that is, stimulate or antagonize CYP 3A4 activity, will modulate astemizole systemic exposure and toxic potential.

Mechanism of Toxicity

The primary and life-threatening toxicity associated with astemizole administration is cardiotoxicity; torsades de pointes arrhythmia. The exact mechanism(s) of the cardiotoxic effects of astemizole is not well understood though is believed to be via a similar, and possibly identical pathway as observed with terfenidine. Both astemizole and its primary desmethylastemizole metabolite appear to inhibit cardiac delayed potassium rectifier (Ik) channels in a manner similar to terfenidine. The Ikr (delayed potassium rectifier – rapid acting) channel is the potassium channel involved in repolarization of cardiac cells. Blockade of the cardiac Ikr channel produces a depressed peak in the voltage and a decrease in potassium cellular outflow predisposing the myocardium too early after repolarization, which, when sizeable, results in dysrhythmia and most notably torsades de pointes. Further, the drug's remaining two metabolites, norastemizole and 6-hydroxyastemizole, can also inhibit Ikr channels but to a much lesser extent than the parent astemizole or desmethylastemizole. Nevertheless, the additive or synergistic inhibition of Ikr channel activity under conditions of high dose astemizole administration, administration of excessive astemizole doses in patients with hepatic and/or renal dysfunction leading to accumulation of astemizole and/or its metabolites, and in the case of overdose, all predispose the patient to a very high likelihood of astemizole-induced cardiotoxicity.

Acute and Short-Term Toxicity (or Exposure)

Human

Unlike traditional, first-generation (e.g., diphenhydramine, hydroxyzine) antihistamines, astemizole is considered 'nonsedating' and lacks the anticholinergic properties noted with first-generation H-1 receptor antagonists. Patients presenting with astemizole overdose are usually fully awake or only slightly sedated. Serious cardiac effects, including prolongation of the QT interval, arrhythmias (i.e., ventricular tachycardia, torsades de pointes, ventricular fibrillation, and heart block), arrest, hypotension, palpitations, syncope, dizziness, and death have

been described in patients receiving astemizole. As noted above, these cardiotoxic effects are usually associated with higher than recommended doses and/or increased plasma concentrations of the drug and its active metabolites. Although rarely reported, cardiotoxic effects may occur at the recommended dose and at doses two or three times the recommended dose (10 mg daily). Concomitant administration of drugs known to inhibit CYP 3A4 activity including the azole antifungals (e.g., itraconazole, ketoconazole, fluconazole) and macrolide antibiotics (e.g., erythromycin, clarithromycin), cimetidine, metronidazole, certain selective serotonin re-uptake inhibitors (SSRIs), and anti-retroviral drugs substantially increase the risk of astemizole-induced cardiotoxicity.

Chronic Toxicity (or Exposure)

Animal

Varying effects were seen in a rat model of teratogenicity. At lower doses (50 times the recommended human dose), no toxicity was observed in the mothers or pups. At higher doses of 100 times the recommended human dose, toxic effects were noted on the unborn rat pups as well as in the mothers.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Activated charcoal will adsorb astemizole following oral ingestion. The effectiveness of activated charcoal and/or gastric lavage will depend upon the time these therapies are instituted after the ingestion, as the drug is rapidly absorbed from the intestine. Further, the effectiveness of multidose (e.g., q4 h, etc.) activated charcoal would appear limited considering the drug's extensive V_d ($250\,l\,kg^{-1}$) and high protein binding (97%). Close EKG monitoring should be instituted immediately upon presentation to a healthcare facility, including equipped ambulances and medical helicopters, and continued for a minimum of 24 h.

See also: Cimetidine; Diphenhydramine; Erythromycin.

Further Reading

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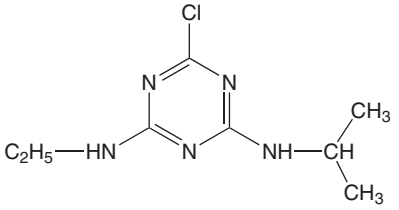
Atrazine

Jing Liu

- Chemical Abstract Service Registry Number: CAS 1912-24-9
- Synonyms: Atrasol; Atranex; Atratol; Gesaprim; Primatol; Crisazine; 2-Chloro-4-ethylamino-6-isopopylamine-*s*-triazine
- Chemical/Pharmaceutical/Other Class: Triazine herbicide
- Chemical Formula: $C_8H_{14}ClN_5$
- Chemical Structure:



Uses

For decades, atrazine has been the most heavily used herbicide in the United States. Atrazine is used for selective and nonselective weed control in various field crops and industrial applications.

Exposure Routes and Pathways

The ocular and dermal routes are the primary exposure pathways. Ingestion and inhalation of atrazine are other possible routes of exposure. In all cases, atrazine falls into toxicity category III or IV.

Toxicokinetics

Atrazine has the potential to be absorbed through the gastrointestinal tract, through the intact skin, and by inhalation. The percentage absorbed

through dermal application increased with time and decreased with dose. However, the majority (65–95%) of atrazine applied on the skin was recovered in the water used for washing or was found associated with the skin at the site of exposure. Once absorbed, it follows first-order distribution kinetics and undergoes *N*-dealkylation and dechlorination of the triazine ring. The highest level of atrazine is noted in the red blood cell followed by lungs, liver, spleen, and kidneys. The half-life of atrazine in the tissues is ~31–39 h, indicating that atrazine does not bioaccumulate. Urinary excretion is the major route of elimination in mammals. A small amount is also excreted in the feces. The major metabolite in both urine and feces is diaminochlorotriazine.

Mechanism of Toxicity

The triazine herbicides are selective inhibitors of the Hill reaction in plant photosynthesis. In mammals, atrazine disrupts luteinizing hormone and prolactin secretion through direct action on the hypothalamus–pituitary axis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Atrazine has low acute toxicity in mammals. The oral LD_{50} in rats is ~2 g kg^{-1}. The dermal LD_{50} and inhalation LC_{50} (1 h) values in rats are ~3 g kg^{-1} and 700 mg m^{-3}, respectively. The oral LD_{50} values in mice and rabbits are ~1.8–4.0 g kg^{-1} and 750 mg kg^{-1}, respectively. Atrazine was negative in primary skin irritation and dermal sensitization tests. Rats exposed to high dosages of atrazine showed changes in arousal and motor function, dyspnea, hypothermia, and spasms. With lethal oral dosages, death occurred rapidly (within 12–24 h).

A 90 day subchronic oral study in rat and 21 day dermal study in rabbit provided no-observed-adverse-effect levels (NOAELs) of 3.3 and 100 mg kg^{-1} day^{-1}, respectively. Prenatal developmental toxicity study in female Sprague–Dawley rats exposed to atrazine during gestation day 6 through day 15 demonstrated maternal and developmental NOAELs of 25 mg kg^{-1} day^{-1}.

Using both Long–Evans and Sprague–Dawley female rats, atrazine was found to disrupt the hypothalamic control of pituitary-ovarian function as indicated by alteration in luteinizing hormone and prolactin serum levels. Females treated with atrazine (75, 150, and 300 mg kg^{-1} day^{-1} for 21 days by gavage) showed irregular cycles and repetitive pseudopregnancies. Maternal exposure to atrazine during lactation may result in prostatitis in adult male offspring due to atrazine's suppressive effect on suckling-induced prolactin release.

Human

There have been only 65 recorded cases of human poisonings among occupationally exposed workers during 1966–81 in the United States. One death was reported following extensive dermal exposure. Dermal exposure to atrazine can cause skin rash, erythema, blisters, and edema. Ocular irritation, chest pains, and a feeling of tightness in the chest, nausea, and dizziness have also been reported after dermal, oral, or inhalation exposures.

Chronic Toxicity (or Exposure)

Animal

About 40% of rats died with signs of respiratory distress and paralysis of the limbs following oral administration of 20 mg kg^{-1} day^{-1} atrazine for 6 months. Structural and chemical changes were noticed in various organs including heart, liver, ovaries, etc. Dogs treated with 33.65 mg kg^{-1} day^{-1} of atrazine in the diet for 52 weeks showed various treatment-related cardiac changes including EKG alterations, moderate to severe atrial dilation, and enlarged hearts. Histopathology revealed cardiac myolysis and focal atrophy. The NOAEL for atrazine in dogs of both sexes was established at 4.97 mg kg^{-1} day^{-1}.

When CD-1 mice of both sexes were treated with atrazine in the diet at dose levels of 10–3000 ppm daily for 91 weeks, no treatment-related increase in tumor incidence was noted when compared to controls. Neither male and female Fischer 344 rats nor male Sprague–Dawley rats given atrazine at a maximum tolerated dose in the diet for 24 months exhibited any increase in the incidence of tumors of any type. However, mammary tumors were observed in female Sprague–Dawley rats after 24 months of dietary administration of high levels of atrazine. The differences in response to the carcinogenic effect of high levels of atrazine observed in mice versus rats and male versus female Sprague–Dawley rats is because of differences in endocrine control mechanisms affecting reproductive senescence and the development of the mammary tumors during aging.

Based on evidence derived from a large array of assays such as bacterial reverse mutation test, mammalian bone marrow chromosome aberration test, dominant lethal assay, and UDS assay, atrazine was concluded to lack mutagenic potential.

Human

The carcinogenic effect of high doses of atrazine noted in female Sprague–Dawley rats is a strain-, sex-, and tissue-specific response that may not have biological relevance to humans due to the differences in the endocrine control of reproductive senescence.

In Vitro Toxicity Data

Atrazine was found to strongly potentiate arsenic trioxide-induced cytotoxicity and transcriptional activation of stress genes in transformed human hepatocytes, while atrazine itself did not show any significant effects.

Clinical Management

Treatment is symptomatic.

Environmental Fate

Atrazine is highly persistent in the environment due to its resistance to abiotic hydrolysis (stable at pHs 5, 7, and 9) and to direct aqueous photolysis (stable under sunlight at pH 7). Moreover, the compound has a limited volatilization potential and is only moderately susceptible to aerobic biodegradation, which is the main route of dissipation of atrazine. A colder climate makes atrazine even more persistent in the environment. Atrazine does not get adsorbed to soil particles strongly and therefore has a relatively high potential to contaminate ground and surface waters despite its moderate solubility in water.

Ecotoxicology

Atrazine, with acute oral LD_{50} values of $>900\,mg\,kg^{-1}$, is practically nontoxic to birds. The compound is slightly toxic to aquatic animals. Rainbow trout and midge, the most sensitive freshwater species tested, have 96 and 48 h LC_{50} values of 5.3 and $0.72\,mg\,l^{-1}$, respectively. The most sensitive marine animals tested were the spot fish (*Leiostomus xanthurus*) with a 96 h LC_{50} value of $8.5\,mg\,l^{-1}$ and the copepod (*Acartia tonsa*) with a 96 h LC_{50} value of $88\,\mu g\,l^{-1}$. Atrazine is not toxic to bees (oral LD_{50} $>97\,\mu g$ per bee).

Exposure Standards and Guidelines

Oral RfD (reference dose) is $0.035\,mg\,kg^{-1}\,day^{-1}$. ACGIH TLV – TWA for atrazine is $5\,mg\,m^{-3}$.

See also: Pesticides; Pollution, Water.

Further Reading

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Relevant Websites

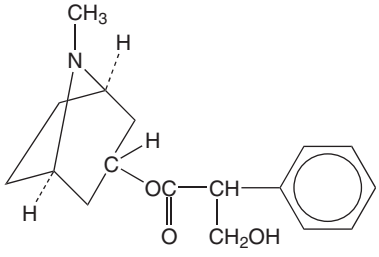
http://ace.orst.edu – Extension Toxicology Network.
http://www.epa.gov – US Environmental Protection Agency.

Atropine

Amanda Lofton

This article is a revision of the previous print edition article by Bridget Flaherty, volume 1, pp. 87–88, © 1998, Elsevier Inc.

- Synonyms: AtroPen auto injector; Atropine sulfate injection; Atropisol; Atrosulf-1; Ocu-tropine; Ocean-A/S; Sal-tropine
- Chemical/Pharmaceutical/Other Class: Anti-muscarinic agent; Anticholinergic agent
- Chemical Formula: $C_{17}H_{23}NO_3$
- Chemical Structure:



Uses

Atropine is used in the management of sinus bradycardia with hemodynamic instability and in the treatment of peptic ulcer disease, irritable bowel syndrome, urinary incontinence, and organophosphate and carbamate poisoning. It is also present in ophthalmic preparations to induce mydriasis and cyclopegia. Atropine is often administered preoperatively to decrease secretions.

Background Information

Atropine is the racemic mixture of L- and D-hyoscyamine and possesses 50% of the antimuscarinic potency of L-hyoscyamine. Atropine is derived from components of the Belladonna plant and is also present in other plants from the Solanaceae family. Women in ancient times often dripped the plant's juices into their eyes, causing mydriasis and thereby enhancing their beauty. In Italian, Belladonna translates to 'beautiful lady'. In the United States, the atropine autoinjector has been in use since 1973 for the treatment of exposures to chemical warfare nerve agents and insecticides.

Exposure Routes and Pathways

Ingestion is the most frequent route of exposure. Exposure can also occur following instillation of eye solutions and via subcutaneous, intramuscular, intravenous, and inhalation routes. Accidental overdosage may occur when atropine is administered for the treatment of organophosphate or carbamate insecticide poisoning.

Toxicokinetics

In therapeutic doses, atropine is well absorbed. In toxic doses, absorption may be prolonged secondary to decreased gastric motility. Atropine is $\sim$18% bound to plasma protein and its volume of distribution ranges from 2 to 4 l kg^{-1}. Atropine is metabolized in the liver to tropic acid, tropine, esters of tropic acid, and glucuronide conjugates. Elimination follows first-order kinetics. Approximately 30–60% is excreted unchanged in the urine. Drug clearance is dependent on glomerular filtration. The elimination half-life is 2–3 h in adults but may be longer in children.

Mechanism of Toxicity

Atropine competitively antagonizes acetylcholine at the neuroreceptor site. Atropine prevents acetylcholine from exhibiting its usual action but does not decrease acetylcholine production. Cardiac muscle, smooth muscle, and the central nervous system are most affected by the antagonism of acetylcholine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals are at risk for anticholinergic poisoning from atropine. Toxicity is similar to that in humans. Gastrointestinal decontamination and supportive care should be employed.

There is interspecies variability and variability based on route of exposure to atropine. The rat LD_{50} oral is 500 mg kg^{-1}; the LD_{50} IP is 280 mg kg^{-1}, and the LD_{50} IV is 73 mg kg^{-1}.

Human

Overdosage of atropine results in signs and symptoms consistent with the anticholinergic toxidrome. Signs and symptoms have been reported following the ingestion of as few as four to five drops of 4% ocular atropine solution. Patients exhibit warm, flushed, and dry skin as a result of peripheral vasodilitation. Mydriasis occurs due to antagonism of acetylcholine in the muscles of the iris. Urinary retention, thirst, delirium, hallucinations, and decreased bowel sounds may occur. Tachycardia with ensuing hypertension can appear secondary to vagal blockade. The anticholinergic toxidrome may be delayed and can occur in cycles. Severe intoxications may progress to seizures, coma, and arrhythmias.

Chronic Toxicity (or Exposure)

Animal

A juvenile pygmy sperm whale (*Kogia breviceps*) was treated with several doses of atropine to relieve symptoms of pyloric stenosis. The animal developed signs and symptoms of anticholinergic toxicity including hyperexcitability, ascending weakness, vomiting, and aspiration of seawater. Symptoms resolved after administration of physostigmine.

Human

Chronic ingestion of greater than therapeutic amounts of atropine may produce symptoms of the anticholinergic toxidrome.

Clinical Management

Basic and advanced life support measures should be utilized as necessary for atropine exposure. Gastric decontamination procedures should be employed based on the patient's history and current symptomatology. Activated charcoal can be given to adsorb atropine. The mainstay of treatment is supportive care. Physostigmine, a cholinesterase inhibitor, can be given to patients to reverse signs and symptoms of

the anticholinergic toxidrome. However, the administration of physostigmine may be contraindicated in the patient who has also been exposed to a tricyclic antidepressant, or another agent known to cause QRS interval widening on the EKG. Extracorporeal elimination measures are ineffective.

See also: Anticholinergics; Carbamate Pesticides; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates; Poisoning Emergencies in Humans.

Further Reading

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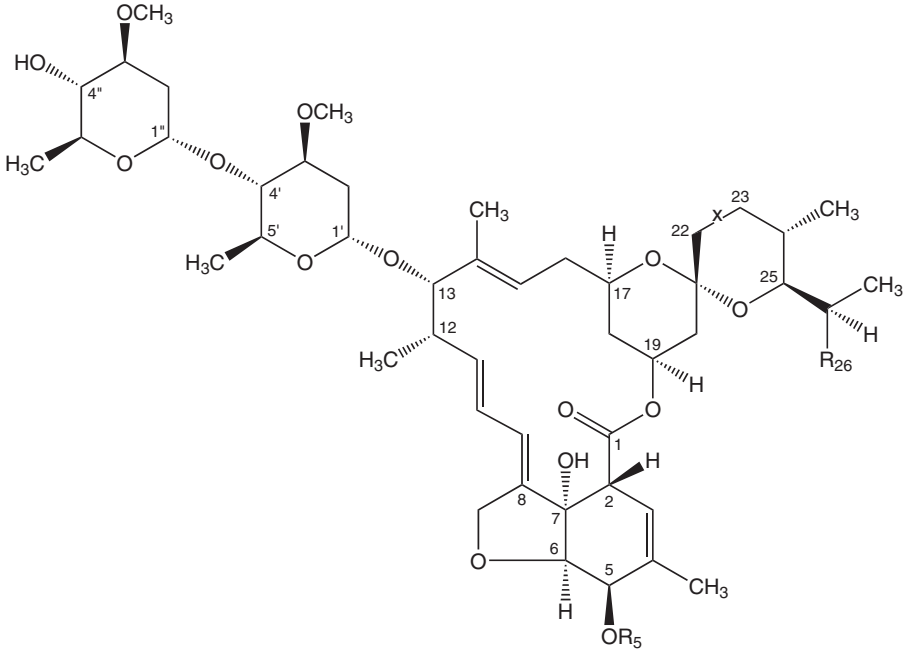
Avermectins

Katherine K Williamson

This article is a revision of the previous print edition article by Arvind K Agarwal, volume 1, pp. 89–90, © 1998, Elsevier Inc.

- Chemical/Pharmaceutical/Other Class: Avermectins are a group of chemically related natural and semisynthetic macrocyclic lactones (macrolide endectocides) produced from the fermentation products of *Streptomyces avermitilis*. The mechanism of action of macrocyclic lactones was long believed to be due to an increased release of GABA. However, recent information indicates that avermectins produce flaccid paralysis of parasites via selective, high-affinity binding to glutamate-gated chloride channels in invertebrate neural and muscle cells. These compounds also interfere with the reproductive cycle of nematodes and arthropods through a poorly understood process.
- Chemical Structure:



Components A: $R_5 = CH_3$ Components a: $R_{26} = C_2H_5$ Components 1: X = –CH═CH–

Components B: $R_5 = H$ Components b: $R_{26} = CH_3$ Components 2: X = –CH_2═$\overset{\overset{OH}{\vdots}}{C}$H–

Uses

Avermectins have a broad spectrum of activity against arthropods, endoparasites, and ectoparasites. However, avermectins have no significant anticestodal (i.e., efficacy against tapeworms), antifungal, or antimicrobial activity. Currently available avermectins include abamectin, selamectin, eprinomectin, doramectin, and ivermectin. Avermectins do not readily cross the blood–brain barrier of mammals, which accounts for the wide margin of safety associated with these products. The recommended antiparasitic dose of ivermectin in cattle and horses is $0.2\,mg\,kg^{-1}$. The dose used to prevent heartworm infection in dogs is $0.006\,mg\,kg^{-1}$ and to treat intestinal parasites is $0.2–0.4\,mg\,kg^{-1}$. The wide range in dosage recommendations between and within a given species demonstrates the wide therapeutic index of ivermectin. Avermectins (most commonly ivermectin) are routinely used as antiparasitics in humans and domestic animals. They are also used in horticulture and agronomy as pesticides and are useful in combating fire ants (abamectin).

Ivermectin is the most widely used and studied member of the avermectin family. It is a semisynthetic derivative of avermectin B1. Ivermectin is produced as an off-white photolabile powder which is very lipophilic, hydrophobic, and poorly soluble in water. Ivermectin has a very broad spectrum of activity against adult, larval, and microfilarial stages of nematodes and arthropods. It is approved for a wide variety of uses in a number of species including humans, domestic and wild ruminants, horses, swine, dogs, and cats. In humans, ivermectin is used in the treatment of *Strongyloides stercoralis*, microfilarial stages of *Onchocerca volvulus* and *Wuchereria bancrofti*, as well as *Ascaris lumbricoides*, *Trichuris trichiura*, and *Enterobius vermicularis* parasitisms. The list of susceptible organisms for which ivermectin is used in domestic animals is extensive and includes gastrointestinal nematodes, ticks, lice, mites, cattle grubs, and lungworms. Ivermectin is perhaps most commonly used in the prevention of heartworm (*Dirofilaria immitis*) infection in dogs and cats. It is safe for use in pregnant animals and in animals as young as 4 weeks of age. Care should be taken to properly dose animals less than 6 months of age as they are more sensitive to acute toxicity following overdose.

Exposure Routes and Pathways

Accidental exposure via skin contact, inhalation, ingestion, or injection stab are all possible.

Toxicokinetics

The pharmacokinetics of ivermectin depends greatly upon the species, formulation of the product, and route of administration. The half-life following a single intravenous dose in cattle is 2.8 days whereas in dogs it is approximately 1.7 days. In ruminants receiving subcutaneous ivermectin the half-life increases to 8 days. In humans, the plasma half-life of ivermectin is 16 h. The volume of distribution of ivermectin ranges from $4.6\,l\,kg^{-1}$ in sheep to $1.9\,l\,kg^{-1}$ in cattle. Oral bioavailability is 95% in simple stomached species and 25–33% in ruminants; as a result, ruminants often receive an injectable or topical formulation. In horses the bioavailability of ivermectin is greatly increased when an aqueous micelle formulation is administered as compared to a paste. Ivermectin is metabolized in the liver through oxidative pathways, 98% is excreted in the feces and the remainder in the urine. In lactating females however, up to 5% of the original dose may be excreted in the milk. Residues may remain in the liver of food animal species for up to 14 days following administration; therefore, labeled withdrawal times should be closely followed.

Mechanism of Toxicity

In humans, the mechanism of toxicity following accidental exposure to veterinary products is poorly understood but may include the penetration of ivermectin into the central nervous system (CNS). Humans undergoing treatment with ivermectin may suffer from anaphylactic-type reactions as microfilaria die off. Toxicity in animals is usually associated with extreme overdosing. Purebred and mixed breed Collies and Australian Shepherds exhibit greater distribution of ivermectin into the CNS resulting in ataxia, tremor, and often death. Use of ivermectin should be avoided in these animals.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ivermectin has an extremely wide margin of safety in all species (with the exception of Collie and Australian Shepherd dogs). The LD_{50} in mice is $25–40\,mg\,kg^{-1}$. In ruminants, swine, dogs, and horses ivermectin is generally considered to have a $10\times$ safety margin. The LD_{50} for dogs has been estimated at $80\,mg\,kg^{-1}$. The margin narrows slightly to $7\times$ label dose in cats. Although extremely rare at labeled doses, toxic reactions may result from

extreme overdose resulting in CNS exposure. Signs of ivermectin overdose include mydriasis, depression, ataxia, tremors, and occasionally death. Much more commonly, toxicity is a result of anaphylaxis and tissue damage associated with the death of the parasite. As stated earlier, use of ivermectin is not advised in Collie and Australian Shepherd dogs due to an increased penetration of the compound through the blood–brain barrier (avermectin derivatives such as milbemycin oxime are safe to use in these animals). Young animals may be more sensitive than adults to avermectin toxicity.

Human

Acute toxicity in humans leads to a variety of clinical signs including rash, edema, headache, dizziness, nausea, vomiting, diarrhea, seizure, dyspnea, ataxia, paresthesia, abdominal pain, and urticaria. Toxicity following treatment with ivermectin is often the result of a hypersensitivity reaction known as the Mazzotti reaction. Signs of the Mazzotti reaction include fever, pruritus, arthralgia, myalgia, postural hypotension, edema, lymphadenopathy, gastrointestinal upset, sore throat, and headaches.

Chronic Toxicity (or Exposure)

Animal

Toxicity may be increased with repeated lower doses as opposed to a single high dose of ivermectin. Dogs given oral doses of 0.5 or 1 mg kg^{-1} day^{-1} for 1 year showed pupillary dilation, weight loss, lethargy, tremors, and recumbency. Rats fed avermectin for 2 years at 0.75, 1.5, or 2 mg kg^{-1} day^{-1} exhibited body weight gains significantly higher than the controls and some tremors in the high-dose group. Male mice fed 8 mg kg^{-1} day^{-1} for 94 weeks showed dermatitis while females exhibited tremors and weight loss. Reproductive toxicity was noted at 0.4 mg kg^{-1} day^{-1} in rats. Avermectins are not considered carcinogenic.

Human

Little is known about the chronic toxicity of avermectins in humans.

In Vitro Toxicity Data

Ivermectin was negative in Ames mutagenesis assays.

Clinical Management

Accidental exposure – supportive care, emesis, and gastric lavage. Treatment toxicity – analgesics and antihistamines.

Environmental Fate

Avermectins and the breakdown products are nearly insoluble in water and bind strongly to soil. Thus they have little mobility and are unlikely to leach into groundwater. Avermectins are rapidly degraded in soil, sensitive to rapid photodegradation. When applied to the soil surface, its soil half-life was about 1 week. Under dark, aerobic conditions, the soil half-life is somewhat extended (2 weeks to 2 months). Microbial degradation also contributes to rapid loss from soils. Avermectins are also rapidly degraded in water (half-life $\sim$12–24 h), principally due to photodegradation.

Ecotoxicology

Avermectins are relatively nontoxic to birds. The LD_{50} for avermectin in Bobwhite quail is 2 g kg^{-1}. Mallard ducks appear more sensitive than quail to the acute toxicity of avermectin. However, avermectin caused no reproductive problems in mallards fed dietary doses of 3, 6, or 12 ppm for 18 weeks. Avermectin is highly toxic to fish and aquatic invertebrates. The 96 h LC_{50} in rainbow trout, bluegill, sheepshead minnow, catfish, and carp was 3.2, 9.6, 15, 24, and 42 ppb, respectively. The 48 h LC_{50} in Daphnia was 0.34 ppb. Avermectin did not bioaccumulate in bluegill sunfish exposed for 28 days. Avermectin is highly toxic to bees, with a 24 h LC_{50} of 2 ng per bee and an oral LD_{50} of 9 ng per bee. The 28 days LC_{50} for avermectin in earthworms was 28 ppm.

See also: LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Selamectin; Veterinary Toxicology.

Further Reading

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Richard Adams H (ed.) (2001) *Veterinary Pharmacology and Therapeutics*, 8th edn., pp. 964–967, 1029. Ames, IA: Iowa State University Press.

Relevant Website

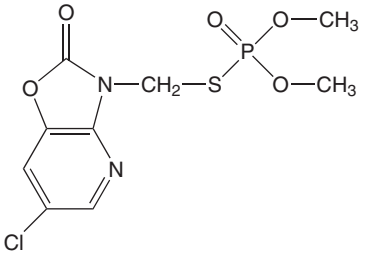
http://pmep.cce.cornell.edu – Extension Toxicology Network, Cornell University.

Avian Ecotoxicology *See* Ecotoxicology, Avian.

Azamethiphos

Jason R Richardson

- Chemical Abstracts Service Registry Number: CAS 35575-96-3
- Synonyms: *S*-(6-Chlorooxazolo(4,5-*b*)pyridine-2(3*H*)-on-3-ylmethyl)-*O*,*O*-dimethyl phosphorothioate; Ciba-Geigy 18809; Snip; Alfacron 10
- Chemical/Pharmaceutical/Other Class: Synthetic organophosphorus insecticide in the phosphorothiolate class
- Chemical Formula: $C_9H_{10}ClN_2O_5PS$
- Chemical Structure:



Uses

Azamethiphos is used as a pesticide spray for control of flies and cockroaches primarily in Europe, as it is not available for use in the United States. It has been used in commercial aquaculture to control external parasites (sea lice) in salmon. In addition, locally procured granular azamethiphos was used as a fly bait by US troops in the first Gulf War.

Exposure Routes and Pathways

Dermal, oral, and inhalation routes are all primary exposure pathways.

Toxicokinetics

Azamethiphos is well absorbed following oral administration to rats but much less effectively by the dermal route. Unlike many other organophosphorus insecticides, azamethiphos does not undergo bioactivation through the P450 monooxygenase pathway, as it is already in its active oxon form. Following oral administration, azamethiphos is rapidly excreted, primarily in the urine, with the major metabolite being 2-amino-3-hydroxy-5-chloropyridine, which is then conjugated to glucuronic or sulfuric acid for excretion. Hepatic and serum carboxylesterases have also been suggested to detoxify azamethiphos.

Mechanism of Toxicity

Similar to other organophosphorus insecticides, azamethiphos elicits toxicity through the inhibition of acetylcholinesterase.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral toxicity of azamethiphos is low to moderate, with rat oral LD_{50} values of 1040–1180 mg kg^{-1}. Azamethiphos is much less toxic when applied dermally, with LD_{50} values greater than 2150 mg kg^{-1}. Azamethiphos is much more toxic to birds, with an acute oral LD_{50} of 91 mg kg^{-1} in quail. Skin sensitization was observed in guinea pigs administered azamethiphos.

Human

No human toxicity data are available for azamethiphos. For experimental animals, toxicity is low for a single oral dose. Prolonged skin exposure may cause skin irritation. Ocular contact may cause eye irritation and pain.

Chronic Toxicity (or Exposure)

Animal

Azamethiphos has not been found to be carcinogenic, teratogenic, or to result in reproductive toxicity in rodent studies. The no-observed-adverse-effect level established in a 52 week study with beagles fed azamethiphos was 2.7–2.9 mg kg^{-1} day^{-1} in males and females, respectively. Azamethiphos did not cause delayed neuropathy in hens given two LD_{50} dosages 21 days apart.

Human

Cholinesterase inhibition may persist for a period of days to weeks. Therefore, repeated exposure to azamethiphos over a period of time may result in the accumulation of enzyme inhibition and onset of acute toxicity. Azamethiphos does not appear to be capable of eliciting organophosphate-induced delayed neuropathy. Likewise, azamethiphos does not appear to be carcinogenic.

In Vitro Toxicity Data

Azamethiphos has been reported to be mutagenic in several *in vitro* assays. However, it was negative in follow-up *in vivo* mutagenicity assays.

Clinical Management

For dermal contact, hands and exposed skin should be washed immediately. For ocular exposure, eyes should be flushed with clean water for a period of 15–20 min. If irritation develops and persists from either dermal or ocular exposure, the victim should seek medical attention.

In the case of inhalation exposure, the victim should be moved to fresh air and medical attention sought immediately. Artificial ventilation is indicated in the case of diminished respiratory function.

If exposure is through ingestion, the victim should seek medical help immediately. Emesis should not be induced. Initial management involves establishment of adequate ventilation and maintenance of adequate respiratory function. Activated charcoal therapy may be used to retard absorption from the gastrointestinal tract. Atropine sulfate alone, or in combination with pralidoxime chloride, can be administered as an antidote. Atropine is initially administered intravenously at a dosage of 1–2 $mg\,kg^{-1}$ every 5–10 min until cholinergic signs decrease. Pralidoxime is preferably administered by slow intravenous infusion at a maximum rate of 8–10 $mg\,kg^{-1}\,h^{-1}$ until full recovery. Seizure activity may be treated with anticonvulsants such as diazepam.

Environmental Fate

Azamethiphos degrades rapidly in seawater and does not bioaccumulate. A study evaluated movement of azamethiphos after application in salmon aquaculture. Dye was added to aid tracking of plumes, and samples were analyzed for azamethiphos content as well as toxicity to a small crustacean. The results suggested that azamethiphos used under recommended conditions posed little contamination or nontarget toxicity potential.

Ecotoxicology

Azamethiphos is toxic to several aquatic species. Biotransformation of azamethiphos in salmon is similar to that in rats, with formation of 2-amino-3-hydroxy-5-chloropyridine and conjugation to glucuronic or sulfuric acid.

Exposure Standards and Guidelines

The accepted daily intake for azamethiphos is 0.025 $mg\,kg^{-1}\,day^{-1}$. As this pesticide is not used in the United States, no reference dose is available.

See also: Carboxylesterases; Cholinesterase Inhibition; Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphates.

Relevant Websites

http://www.ec.gc.ca – Environment Canada.
http://www.scotland.gov.uk – Scottish Executive Publications.

Azathioprine

Eric M Silberhorn

- Chemical Abstracts Service Registry Number: CAS 446-86-6
- Synonyms: 6[(1-Methyl-4-nitro-1*H*-imidazol-5-yl) thio]-1*H*-purine; 6-(1-Methyl-4-nitro-5-imidazolyl) mercatopurine; Azamune; Azanin; Azothioprine; Imuran; Imurek; Imurel; Zytrim
- Chemical/Pharmaceutical/Other Class: Immunosuppressive antimetabolite; Disease modifying antirheumatic drug (DMARD)
- Chemical Formula: $C_9H_7N_7O_2S$

Uses

Azathioprine is an immunosuppressive and antiproliferative agent used in the treatment of several

different indications and conditions. It is effective as an adjuvant for protection against rejection of human organ transplants and in the treatment of immune-mediated and inflammatory diseases (e.g., inflammatory bowel disease including Crohn's disease, severe rheumatoid arthritis, chronic active hepatitis, acute leukemia). It has also been found to be an effective steroid sparing agent.

Background Information

Azathioprine was first introduced in 1961 and helped make allogenic kidney transplantation possible. It was originally designed as a prodrug of 6-mercaptopurine (6-MP), which had previously been found to produce remissions in acute childhood leukemia as a result of its immunosuppressive properties.

Exposure Routes and Pathways

Exposure as a therapeutic drug is oral, intravenous (IV), or colonic depending on the indication. IV administration is not as common as it once was because it does not reduce response time. The adult therapeutic dose for organ transplant is typically 3–5 $mg\,kg^{-1}\,day^{-1}$ for several days, then reduced to ~1–3 $mg\,kg^{-1}\,day^{-1}$ for maintenance. For inflammatory bowel disease, the adult dosage ranges from 1.5 to 3.0 $mg\,kg^{-1}\,day^{-1}$.

Toxicokinetics

In healthy individuals, anywhere from 16% to 50% of the ingested dose of azathioprine is absorbed. This percentage may be significantly less in individuals with bowel problems such as Crohn's disease. Maximum blood levels of azathioprine peak within 1–2 h after administration and rapidly drop as it is converted to 6-MP. The plasma half-life of azathioprine is ~12–15 min, while that for 6-MP is from 30 min to 4 h. Other metabolites have much longer half-lives. Binding of azathioprine to plasma proteins is low, a maximum of 30%.

Approximately 88% of azathioprine is converted to 6-MP, presumably in the liver. Conversion takes place nonenzymatically by sulfhydryl-containing compounds such as glutathione, which cause the imidazole group to split off, but also may occur enzymatically. 6-MP is further metabolized enzymatically to (1) the active compounds 6-thioguanine nucleotides (6-TGN) and 6-metilmercaptopurinic ribonucleotides (6-MMPR), or (2) inactive metabolites 6-thiouric acid (6-TU; the major urinary metabolite) and 6-methylmercaptopurine (6-MMP). Enzymes resulting in inactivation include xanthine oxidase (conversion to 6-TU) and thiopurine methyltransferase (TPMT; conversion to 6-MMP).

Up to 50% of the dose is excreted in the urine within 24 h of administration; however, only a small amount (<10%) of azathioprine is excreted unchanged. A further 12% of the dose is excreted unchanged in the feces.

Mechanism of Toxicity

Azathioprine is classified as an antiproliferative and immunosuppressive agent. Primarily through its metabolites, azathioprine antagonizes purine metabolism and may inhibit synthesis of DNA, RNA, and proteins. It may also interfere with cellular metabolism and inhibit mitosis. Following exposure to nucleophiles (e.g., glutathione, cysteine), azathioprine is cleaved nonenzymatically to 6-MP, an analogue of hypoxanthine. This conversion is believed to contribute to many, but not all, of the pharmacological and toxicological effects of azathioprine. The toxicity of azathioprine/6-MP has been attributed to at least three different mechanisms including the following:

1. Azathioprine/6-MP are a source of thioguanine nucleotides, which incorporate into DNA, yielding abnormal DNA that, in turn, interferes with the function of DNA polymerases, ligases, and endonucleases.
2. Aathioprine/6-MP are catalyzed to inhibitors of enzymes that are important in *de novo* purine synthesis.
3. Azathioprine and 6-MP promote rapid cell death by apoptosis and produce additional changes in B lymphocytes that favor apoptotic processes in those cells.

A feature of the pharmacologic action of azathioprine is its delayed onset, which may take 8–12 weeks to become apparent, possibly due to the slow accumulation of 6-TGN within the cells. The same is not necessarily true for the toxic effects of azathioprine, some of which may occur at any time during treatment (e.g., bone marrow suppression). Azathioprine appears to be a more potent immunosuppressive agent than does 6-MP itself, which may reflect differences in the pharmacodynamics and pharmacokinetics of the two compounds, as well as the relative abundance of different metabolites which are formed after their administration. Studies with hepatocytes have found that azathioprine toxicity involves depletion of reduced glutathione leading to mitochondrial injury with profound depletion of ATP and cell death by necrosis. Cell death was

prevented by potent antioxidants, glycine, and blocking the mitochondrial permeability transition pore.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} in the rat after oral administration is $535\,mg\,kg^{-1}$. Oral toxicity to the mouse is less, with an LD_{50} of $1389\,mg\,kg^{-1}$. The rat LD_{50} after intraperitoneal administration is $300\,mg\,kg^{-1}$, while that for the mouse is $272\,mg\,kg^{-1}$. In dogs, a dose of $10\,mg\,kg^{-1}$ for 10 days produced death from agranulocytosis. In general, animal studies have shown the hemopoietic system to be particularly sensitive to the effects of azathioprine with depression of granulopoiesis, magakaryocytes and, as a result, platelet formation. Dogs are very susceptible to hepatotoxicity, which is reversible, when given doses of $5\,mg\,kg^{-1}$ daily. The lymphatic system is also affected in monkeys, with atrophy of the lymphoid tissue at levels as low as $1\,mg\,kg^{-1}\,day^{-1}$.

Human

Signs of acute overdose include vomiting, diarrhea, and bone marrow depression in the form of mild leucopenia (in 2–3 days).

Chronic Toxicity (or Exposure)

Animal

There is limited evidence of carcinogenicity of azathioprine in experimental animals. Studies with rats produced squamous cell carcinomas of the ear duct after administration at $150\,mg\,kg^{-1}$ in the diet for 52 weeks and lymphomas of the thymus gland under other conditions. In mice, intraperitoneal injections at $40\,mg\,kg^{-1}$ in 1–4-day-old offspring produced leukemia and long-term studies produced lymphoma and hemangiodothelioma of the uterus.

Animals studies have show adverse effects of azathioprine (and 6-MP) on different stages of embryonic and fetal development. These include cleft palate, open eye, and skeletal anomalies in the offspring of mice injected intraperitoneally during gestation with 4–13 times the human therapeutic dose of azathioprine. Similar findings have been found in the offspring of rabbits injected with doses equivalent to two to six times those used in humans, although no malformations were observed in rat fetuses at the same doses. Doses within the human dose range did not produce anomalies in mice or rat offspring, although increased frequencies of fetal loss and growth retardation were observed.

Human

The toxic side effects of azathioprine and 6-MP may be classified into two categories: (1) idiosyncratic or allergic, and (2) direct toxicity. The first group includes pancreatitis, hepatitis, rash, pain in the joints, and other symptoms that are not dose-dependent. Direct toxicity results in increased susceptibility to infection, hematological toxicity (e.g., leucopenia), and increased development of tumors. Overall, side effects that necessitate stopping azathioprine/6-MP treatment occur in 10–25% of patients. Among the most frequent secondary effects are infections (7.4%), pancreatitis (3.3%), hematological toxicity (2–5%), and cutaneous allergic reactions (2%). Other less frequent side effects include fever, nausea, vomiting, diarrhea, headaches, and pellagra. There are also reports of long-term azathioprine treatment resulting in chronic liver disease (hepatitis) and portal hypertension. Most side effects improve or resolve with dose reduction or withdrawal of the drug.

Of the toxic side effects, a major concern among clinicians is for dose-dependent bone marrow suppression (myelotoxicity), which occurs in 2–4.6% of patients and can be fatal if not addressed properly. Study data suggest that a high incidence of secondary acute myeloid leukemia or brain cancer is correlated with low TPMT activity and high 6-TGN levels in children under immunosuppressive therapy. TPMT activity is subject to wide interindividual and interethnic variability due to TPMT gene polymorphism. In the Caucasian population, $\sim$0.3% of all individuals have no TPMT activity and 11% have intermediate activity, leading some to advocate additional monitoring of this activity in patients to help prevent unnecessary bone marrow toxicity from azathioprine treatment.

Azathioprine is classified as carcinogenic to humans (group 1) by the International Agency for Research on Cancer and known to be a human carcinogen by the National Toxicology Program based on sufficient evidence in humans. Several studies, including two large prospective epidemiological studies, have shown that renal transplant patients are at increased risk for several types of cancers as a result of azathioprine treatment. Cancers produced by azathioprine include non-Hodgkin's lymphoma, squamous cell cancers of the skin, hepatobilliary carcinomas, and mesenchymal tumors. Patients who have received azathioprine treatment for other conditions, including rheumatoid arthritis, systemic lupus and other collagen disorders, inflammatory bowel disease, and certain skin and renal diseases

have also been studied. Some of these same malignancies have been found in these patients, although to a lesser extent than in renal transplant patients. However, two recent large studies in patients with inflammatory bowel disease have not confirmed the increase in neoplasia with extended azathioprine treatment, so the picture is not entirely clear and may depend on a multitude of factors.

Recent studies have indicated that exposure of pregnant women to azathioprine may adversely affect the human embryo and fetus. The US Food and Drug Administration classifies azathioprine in category D, which indicates positive evidence of risk. Effects associated with antenatal exposure to azathioprine include spontaneous abortions and prematurity (40–52%), intrauterine growth reduction (19–40%), and low birth weight. The frequencies of prematurity and fetal growth retardation appear to be increased in pregnancies of renal transplant recipients treated with azathioprine, particularly if the woman requires a high dose therapy or has reduced renal function.

Exposure to azathioprine during pregnancy is associated with a slight increase in the frequency of congenital malformations, which varied from 0% to 11.8% in a total of 27 different clinical studies of infants of renal transplant patients. This compares with a background incidence of 3–5% in children born in developed countries. Malformations include microencephaly, hydrocephalus, anencephaly, hypospadias, malformed hand and face, polydactyly, cleft palate, and congenital heart disease. The incidence of malformations has also been shown to increase in the offspring of fathers who took azathioprine/6-MP within 3 months before conception. In contrast, the evidence is much weaker, or absent, for malformations caused by treatment with azathioprine during pregnancy for other conditions such as rheumatoid arthritis and inflammatory bowel disease.

Prenatal exposure to azathioprine/6-MP during the second trimester has been associated with chromosomal abnormalities in offspring, including chromatid breaks, deletions and extra fragments, translocations, and bridging fusions. The significance of these aberrations is unknown at this time.

In Vitro Toxicity Data

Azathioprine gave positive results in the Ames *Salmonella typhimurium* mutagenicity assay, both with and without metabolic activation, in the TA100 strain. Negative results were found for the TA98 strain under similar test conditions. Azathioprine has been shown to induce chromosomal aberrations in human and rabbit lymphocytes *in vitro*, however, it did not induce sister chromatid exchanges in either human lymphocytes or Chinese hamster bone marrow cells.

The presence of azathioprine affected the development of 9.5–11.5-day-old rat embryos cultured *in vitro* during organogenesis, producing alterations of the brain, caudal trunk, the heart and forelimb regions, and vesicular structures.

Clinical Management

There is no antidote for azathioprine toxicity. Treatment for an overdose entails ipecac within 30 min or lavage within 1 h, followed by activated charcoal. Side effects may be minimized with adequate monitoring of peripheral blood count and liver enzymes. Asymptomatic leucopenia, as well as most other side effects, may be treated with dose reduction or drug cessation (and changing to 6-MP); however, a life-threatening leucopenic episode may require administration of granulocyte colony-stimulating factor as well as other supportive care.

Other Hazards

Xanthine oxidase, an enzyme involved in the catabolism of metabolites of azathioprine, is blocked by allopurinol. If azathioprine and allopurinol are used in the same patient, the dose of azathioprine should be reduced to 25–33% of the usual dose, although it is best not to use these two drugs together.

See also: Blood; Carcinogenesis; Liver.

Further Reading

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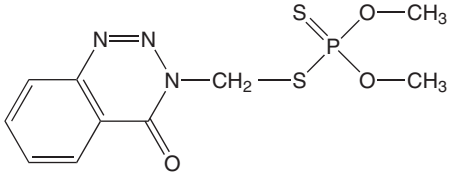
Azinphos-Methyl

Subramanya Karanth

This article is a revision of the previous print edition article by Thuc Pham, volume 1, pp. 90–92. © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 86-50-0
- Synonyms: Chrysthyon; Gusathion; Gusathion-M; Guthion; Methyl Guthion; Metiltrizotion
- Chemical/Pharmaceutical/Other Class: Organophosphorus (phosphorodithioate) insecticide
- Chemical Formula: $C_{10}H_{12}N_3O_3PS_2$
- Chemical Structure:



Uses

Azinphos-methyl is a broad-spectrum nonsystemic insecticide and acaricide commonly used on a number of fruit, vegetable, and nut crops. It is not used in residential and public health pest control.

Exposure Routes and Pathways

Dermal, inhalation, and ingestion are primary routes of exposure for azinphos-methyl.

Toxicokinetics

Azinphos-methyl is readily absorbed and distributed throughout the body following exposure. Mixed-function oxidase-mediated oxidative desulfuration of the parent compound produces the active metabolite azinphos methyloxon. Other major metabolites include dimethylphosphorothioic and dimethylphosphoric acids and desmethyl azinphosmethyl.

Mechanism of Toxicity

Azinphos-methyl requires bioactivation for its action. The parent compound is activated to the potent 'oxon' by microsomal mixed-function oxidase enzymes, which in turn elicits toxicity by inhibiting acetylcholinesterase in synapse and neuromuscular junctions. AChE inhibition leads to overstimulation of cholinergic receptors on postsynaptic neurons, muscle cells, and/or end-organs and consequent signs and symptoms of cholinergic toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity studies in laboratory animals have shown that azinphos-methyl is highly toxic to mammals. Oral and dermal LD_{50} values in laboratory rats are 4–16 and 88–220 mg kg^{-1}, respectively.

Human

Because of its high acute toxicity, low doses of azinphos-methyl ($\sim$1.5 mg day^{-1}) can lead to severe poisoning. Most common signs and symptoms of acute poisoning include salivation, excessive sweating, stomach pain, vomiting, and diarrhea. Inhalation of dust or aerosol containing azinphos-methyl can lead to wheezing, tearing of the eyes, blurred vision, and tightness in the chest. Eye contact with concentrated solutions of azinphos-methyl can be life threatening.

Chronic Toxicity (or Exposure)

Animal

Laboratory rats can tolerate a dietary dose of 0.5 mg kg^{-1} day^{-1} for 2 months without any adverse effects. Repeated long-term exposure to azinphos-methyl can lead to memory loss and irritability. While it is not mutagenic, the carcinogenic

potential of azinphos-methyl is not clearly understood due to lack of sufficient data.

Human

Acetylcholinesterase inhibition caused by azinphos-methyl can persist for a long time (2–6 weeks). Repeated chronic exposure may therefore result in prolonged acetylcholinesterase inhibition that may lead to flu-like illnesses.

Clinical Management

General decontamination procedures should be immediately initiated in case of azinphos-methyl exposure. For skin decontamination, the exposed area should be washed with plenty of water or soap and shampoo can be used during showering. The eyes are flushed with water repeatedly for several minutes. The contaminated clothing is removed and the airway cleared. In case of ingestion, vomiting should be induced. Atropine treatment should be initiated immediately to counteract muscarinic effects. Atropine (adults and children >12 years: 2–4 mg; children <12 years: 0.05–0.1 mg) treatment should be repeated every 15 min until oral and bronchial secretions are controlled and atropinization is achieved. The duration and dosage of atropine treatment is slowly reduced as the condition of the patient improves. Pralidoxime (2-PAM) should be administered slowly at the recommended dosage (adults and children >12 years: 1–2 g; children <12 years: 20–50 mg by IV infusion in 100 ml saline at $\sim 0.2\,\mathrm{g\,min^{-1}}$). This dosage can be repeated every 1–2 h intervals initially and at 10–12 h intervals later depending on the condition of the patient.

Environmental Fate

Persistence in the soil is generally low (half-life under aerobic and anaerobic conditions is 21 and 68 days, respectively). In sterile soil, the half-life is almost 1 year. Azinphos-methyl adsorbs strongly to soil particles and has low solubility in water. Biodegradation and evaporation are the primary routes of elimination from soil but azinphos-methyl is also degraded by ultraviolet light. Degradation is more rapid at higher temperatures. Azinphos-methyl has a short half-life in surface waters (2 days). Hydrolysis is more prominent under alkaline conditions but the compound is relatively stable in water below pH 10. The half-life on crops is 3–5 days under normal conditions.

Ecotoxicology

Azinphos methyl is highly toxic to fish and other aquatic organisms and moderately toxic to birds.

Exposure Standards and Guidelines

The chronic reference dosage is $0.0015\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ and the acceptable daily intake is $0.005\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.

See also: Acetylcholine; Biotransformation; Neurotoxicity; Organophosphates; Pesticides.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

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Bacillus cereus

Lee R Shugart

This article is a revision of the previous print edition article by Vittoria Werth, volume 1, p. 93, © 1998, Elsevier Inc.

Description

Bacillus cereus is a gram-positive, facultatively aerobic sporeformer whose spores do not swell the sporangium. These characteristics, along with specific biochemical features, are used to differentiate *B. cereus* from other species of the genus *Bacillus* (i.e., *B. thuringoemsos* and *B. anthracis*). The organism is widely distributed in nature and in food. It is commonly found in soil, milk, cereals, starches, herbs, spices, and other dried food stuffs.

Mechanism of Toxicity

B. cereus can cause two distinct types of food-borne intoxicants (as opposed to infections): (1) an emetic (vomiting) illness with a short incubation time of a few hours; and (2) a diarrheal illness with an incubation time of 8–6 h. The emetic form is caused by a preformed heat-stable enterotoxin of molecular weight less than 5000 Da. The long incubation form of the illness is mediated by a heat-labile enterotoxin of molecular weight of ~50 000 Da, which activates intestinal enzymes and causes intestinal fluid secretion.

Nature of Disease

B. cereus food poisoning occurs year-round without any particular geographic distribution and all people are believed to be susceptible. The emetic type of food poisoning is most often associated with rice products that have been cooked and then held at warm temperatures for several hours; other starchy foods such as potato, pasta, and cheese products have also been implicated. The emetic form is characterized by nausea and vomiting with 0.5–6 h after consumption of contaminated foods, symptoms that parallel those of *Staphylococcus aureus* food poisoning. The diarrheal type of food poisoning is frequently associated with foods (meats, milk, vegetables, and fish) after cooking (i.e., prepared food held above room temperature for a prolonged period). The onset of watery diarrhea, abdominal cramps, and pain occurs 6–15 h after consumption of contaminated foods. Nausea may accompany diarrhea, but vomiting rarely does. These symptoms resemble food poisoning caused by *Clostridium perfringens*. Nonanthrax *Bacillus* species are occasionally implicated in local infections especially those involving the eye. *B. cereus* is one of the most destructive organisms to infect the eye and can cause conjunctivitis, keratitis, iridocyclitis, dacryocystitis, orbital abscess, and panophthalmitis.

Control

B. cereus bacteria are common and widespread. Preventing contamination of food with spores is virtually impossible and because *B. cereus* are naturally present in some soil, their presence on fresh produce is not rare. Treatment of produce with chlorinated water reduces populations of microorganisms but cannot eliminate them. Effective prevention and control measures depend on inhibiting spore germination and preventing growth of vegetative cells in cooked, ready-to-eat foods. Freshly cooked food eaten hot, immediately after cooking is safe. Temperatures under 100°C will allow for the survival of some *Bacillus* spores, thus steaming under pressure, thorough roasting, frying, and grilling are most likely to destroy cells and spores.

See also: Clostridium perfringens; Staphylococcus aureus.

Further Reading

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Salyers AA and Whitt DD (1994) *Bacterial Pathogenesis: A Molecular Approach*. Washington, DC: American Society for Microbiology.

Relevant Website

http://vm.cfsan.fda.gov – *Bacillus cereus* and other *Bacillus* spp. (from the US Food and Drug Administration).

Bacillus thuringiensis

Eric M Silberhorn

- SYNONYMS: Bt; Acrobe; B 401; Bactimos; Bactospeine; Berliner (variety kurstaki); Biotrol 4K; Certan (variety aizawai); Dipel; Foray; Gnatrol; Javelin; Leptox; Novabac; Teknar (variety israelensis); Thuricide; Vectobac; Victory
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Bacterium

Uses

Bacillus thuringiensis (Bt) is a microbial insecticide.

Background Information

Bt is a group of strains or isolates of naturally occurring soil bacteria that are used to control insect pests on agricultural crops, stored food crops, ornamental plants, in bodies of water, and around homes. Different strains of Bt have specific toxicity to particular types of insects depending on the specific crystalline protein (delta-endotoxin) that they produce. For example, Bt *aizawai* (Bta) is effective against wax moth larvae; Bt *israelensis* (Bti) is effective against mosquitoes, blackflies, and some midges; Bt *kurstaki* (Btk) controls various types of lepidopterous species including the gypsy moth and cabbage looper; and Bt *san diego* is effective against certain beetle species and the boll weevil. Bt is considered an almost ideal agent for pest management because of its combination of insecticidal specificity and lack of toxicity to humans and nontarget organisms. Most Bt-based insecticides are formulated mixtures of delta-endotoxin crystals and Bt spores. The Bt spores synergize the toxicity of the crystalline proteins.

Exposure Routes and Pathways

The most likely routes of exposure to Bt for the general public are oral, dermal, and inhalation.

In addition to these routes of exposure, accidental parenteral or ocular exposures may occur in workers that apply Bt in the field.

Mechanism of Toxicity

Bt is ineffective against adult insects and must be eaten by feeding larvae in order to be toxic. During sporulation, Bt produces a parasporal inclusion body referred to as a crystal, which is made up of proteins also known as delta-endotoxins. When eaten, the delta-endotoxins (crystalline proteins) produced by Bt dissolve and act as poisons in the target species, paralyzing cells in the midgut, interfering with normal digestion, and triggering the insect to stop feeding and eventually die. The time until death may range from a few hours to several weeks depending on the insect species and the amount of Bt ingested. Gut paralysis is caused by toxins that bind to specific receptors present on the membranes of epithelial midgut cells. These toxins are formed during proteolytical processing of solubilized Bt protein crystals (the protoxin). Once membrane-bound, the toxin induces formation of pores or open channels in the midgut epithelial cell membrane, which causes paralysis and cell death, and later death of the larvae.

Acute and Short-Term Toxicity (or Exposure)

Animal

To date, numerous laboratory studies have been conducted on the infectivity and toxicity of Bt isolates and these studies have demonstrated that the isolates of Bt used in commercial products are safe. In several acute oral toxicity/pathogenicity studies, no adverse effects, infectivity, or pathogenicity has been observed in laboratory animals at doses up to 4.7×10^{11} spores kg^{-1}. In acute pulmonary toxicity studies, no adverse toxic effects have been seen at doses up to 2.6×10^{7} spores kg^{-1}. Similarly, Bt is nontoxic and not pathogenic in acute studies that administered Bt intraperitoneal to mice at dose levels below 10^{8} colony forming units (cfu) per animal. Repeated oral exposures for 21 days did not produce mortality or changes in weight gain in rats (1.2×10^{11} cfu) or mice (4.7×10^{10} cfu). Dermal exposures of several different Bt strains at levels up to 2500 mg kg^{-1} were not toxic or pathogenic to rabbits, but did produce mild irritation in some cases.

Human

A short-term study with human volunteers has not demonstrated any adverse health effects of Bt. Eight subjects ingested 1 g of Bt formulation (3×10^{9} spores g^{-1} of powder) daily for 5 days. Five of these volunteers also inhaled 100 mg of the Bt powder daily for 5 days. No adverse effects were observed in comprehensive medical examinations conducted before or after (including 4–5 weeks after)

the exposures, and clinical chemistry data were also negative.

Due to its mode of action, acute exposures to Bt via routes other than oral ingestion are not expected to produce toxicity. However, contact with Bt formulations in high enough concentrations may still potentially cause irritation of the skin, eyes, and respiratory tract due to the physical nature of these materials. For example, dermatitis was reported by one worker after contact with Bt solution.

Chronic Toxicity (or Exposure)

Animal

In rats, no toxicity or infectivity was associated with dietary exposure to Bt at a level of $4\,g\,kg^{-1}\,day^{-1}$ for 3 months. Sheep exposed repeatedly to commercial Bt formulations for 60 days showed no clinically significant effects. Rats fed a Bt product for 2 years in the diet at $8400\,mg\,kg^{-1}\,day^{-1}$ experienced a decrease in body weight gain (females only) during weeks 10–104 of the study, but no other significant effects.

Human

Bt microbial products have a long history (greater than 40 years) of safe use. Reports of serious adverse effects in humans from the use of these products are rare and none were considered to be casually related to Bt itself. Two detailed epidemiology studies have been carried out on the exposure of humans to Bt. In a Canadian study on ground spray operators in a control program for the gypsy moth, researchers found that workers without protective clothing developed minor irritations of the skin, eyes, and respiratory tract, but no serious health problems. Symptoms were reported at two to three times the rate for the control group. These symptoms were transient and frequently occurred during the beginning of a spray run and when Bt spray concentrations were increased. Mean exposure values ranged from 3.0×10^3 to 5.9×10^6 Bt spores m^{-3} of sampled air. The exposure rates for the spray operators were up to 500 times greater than that estimated for the general population. In a passive surveillance study conducted in Oregon during 1985–86, there was only one health complaint that could be attributed to Bt: dermatitis and eye irritation in a spray operator who was splashed in the face and eyes with a spray solution. More recently, the presence of specific IgE and IgG antibodies has been demonstrated in farm workers who picked vegetables treated with Bt products. The incidence of antibodies was higher in workers in the high exposure group; however, the significance of these finding is unknown as there was no increase in the incidence of asthma or other occupationally related clinical diseases in these workers.

Environmental Fate

Bt is moderately persistent in soil with a half-life of ~4 months. It is rapidly inactivated in soils that have a pH below 5.1. Bt is relatively short-lived on foliage due to rapid photodegradation. Its half-life under normal sunlight conditions is 3.8 h. In general, Bt loses 50% of its insecticide activity in 1–3 days after spraying.

Ecotoxicology

To date based on extensive laboratory and field data, there is little evidence that commercial Bt formulations cause any significant ecological impacts when used for insect control. Bt strains are classified as practically nontoxic to birds based on acute toxicity studies conducted for the US Environmental Protection Agency as part of the pesticide registration process. In general, field studies have not shown effects on bird populations after aerial spraying of Bt formulations, although effects on avian reproductive parameters (e.g., nesting attempts, fledgling success) have been measured in two instances. Field studies have also not shown any significant adverse effects of Bt products on mammals or plants exposed at typical application rates. Some Bt strains are highly toxic to certain aquatic invertebrates such as *Dipteran* (mosquitoes and blackflies) larvae; however, most aquatic species are quite tolerant to the effects of Bt. Field monitoring studies after application of Bt for control of the spruce budworm found no measurable effects on a wide variety of aquatic insects and similar results have been found in other studies. Bt is practically nontoxic to fish with acute LC_{50} values greater than $8.7 \times 10^9\,cfu\,l^{-1}$ for bluegill sunfish, rainbow trout, and the sheepshead minnow. There has been no documented evidence of fishes killed as a result of the many forestry, agriculture, and urban Bt spraying programs conducted in Canada and the United States over the past 30 years or more. In contrast, temporary reductions in populations of nontarget Lepidoptera and some other susceptible insects have been documented in the field after aerial applications of Bt. Because of the lack of persistence of Bt in the environment, the effects are primarily limited to the period of use.

See also: Genetically Engineered Foods; Pesticides.

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Joung K-B and Côté J-C (2000) A review of the environmental impacts of the microbial insecticide *Bacillus thuringiensis*. Technical Bulletin No. 29. Horticulture Research and Development Centre, Agriculture and Agri-Food Canada.

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Siegel JP (2001) The mammalian safety of *Bacillus thuringiensis*-based insecticides. *Journal of Invertebrate Pathology* 77: 13–21.

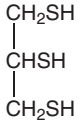
US Environmental Protection Agency (1998) *Reregistration Eligibility Decision (RED) Bacillus thuringiensis*. Washington, DC: Office of Prevention, Pesticides and Toxic Substances.

BAL (British Antilewisite)

Sharmilee P Sawant and Harihara M Mehendale

This article is a revision of the previous print edition article by Arvind K Agarwal, volume 1, pp. 93–94, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 59-52-9
- Synonyms: 2,3-Dimercaptopropanol; Dimercaprol; Dicaptol; Sulfactin; 1,2-Dithioglycerol; Dimercaptol; 2,3-Dimercaptopropan-1-ol; USAF ME-1
- Chemical Structure:



Uses

British antilewisite (BAL) is a chelating agent used as an antidote for treatment of metal poisoning, especially arsenic (organic and inorganic), gold salts, and mercury. BAL is more effective when given soon after toxic exposure because it is more effective in preventing inhibition of sulfhydryl enzymes than in reactivating them.

Background Information

Dimercaprol is a synthetic therapeutic substance developed during World War II as an antidote against the vesicant arsenic war gases (lewisite). The first experiments were based on the fact that arsenic products react with SH radicals. Among all the compounds originally tested, BAL was the most effective and the least toxic. In 1951, BAL was used by a renowned neurologist, Derek Denny-Brown, to treat patients suffering with Wilson's disease (hepatolentricular degeneration), which results from excessive copper accumulation, especially in the brain and liver. The intrinsic toxicity of BAL later led to the development of its water-soluble and less toxic derivatives dimercaptosuccinic acid and dimercaptopropanesulfonic acid.

Exposure Routes and Pathways

BAL is given only by deep intramuscular (i.m.) injection (never intravenous (i.v.) or subcutaneous (s.c.)). Oral ingestion is only accidental or intentional. Dimercaprol can be applied to the skin to heal local effects caused by arsenic vesicant substances.

Toxicokinetics

Peak concentrations in blood are obtained in about 30–60 min after intramuscular injection of dimercaprol. It is readily absorbed through the skin after topical application. Because it is a lipophilic drug, dimercaprol rapidly penetrates the intracellular spaces. The highest concentrations are found in the liver, kidneys, brain, and small intestine. BAL's biological half-life is short and metabolic degradation and renal excretion is complete within 6–24 h according to animal studies. The renal excretion is most often cited as its major elimination route but there appears to be a significant contribution from its conjugation with glucuronic acid. The major portion of the drug is excreted rapidly in the urine, and part of it is eliminated in the feces (via bile). The dimercaprol–metal complexes dissociate rapidly in the body, especially in an acid internal medium; alkalinization of the urine may prevent this dissociation and protect the kidneys from metal and BAL nephrotoxicity.

If the BAL–metal complex is oxidized, the metal is released and can exert its toxic effect again; therefore, the dosage of dimercaprol must be high enough to ensure the excess of free BAL in body fluids until the metal is completely excreted.

Mechanism of Toxicity

BAL is believed to compete with tissue sulfhydryl groups and interferes with cellular respiration. It also competes with metallic cofactors of metabolic enzyme systems and increases capillary permeability.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} in rabbits and rats is in the range of 0.6–1.0 mmol kg^{-1} by i.m., i.p., or s.c. absorption. In another study, the LD_{50} in rats after i.m. injection was 105 mg kg^{-1}. In animals, a lethal dose of dithiols causes convulsions and severe spasm of the abdominal muscles shortly before death occurs. Sublethal injection of dimercaprol to animals results in lacrimation, edema of the conjunctiva, salivation, and vomiting. With increasing doses, they develop ataxia, analgesia, tachypnea, and hyper-excitability. Nystagmus and muscle tremor develop; tonic and clonic convulsions occur at the final stages. Death occurs during coma. The most important acute toxic effect of dimercaprol is cardiovascular depression as judged by a fall in systemic and pulmonary artery pressure following i.v. injection in cats.

Human

A common side effect of BAL is an increase in systolic and diastolic arterial pressures with tachycardia. About 50% of patients who receive high therapeutic doses (4–5 mg kg^{-1}) have minor reactions: nausea, vomiting, fatigue, restlessness, apprehension, headache, burning sensation of the mouth, throat, and eyes, lacrimation, salivation, tingling of extremities, a feeling of constriction in the chest muscle, diffuse pain, and muscle spasm. Large doses may cause convulsions and coma. There may be pain at the injection site. BAL may cause hemolytic anemia in individuals with a glucose-6-phosphate dehydrogenase (G6PD) deficiency. When applied locally to skin, it produces redness and swelling. It is an irritant to eyes and mucous membrane.

Chronic Toxicity (or Exposure)

Animal

Very few chronic toxicity studies have been reported. After repeated local applications in animals, sensitization dermatitis may develop. Chronic parenteral administration increases the white blood cell count by 30%.

Human

Long-term exposure of BAL is unnecessary. There are no reports on the long-term toxic effects of BAL.

Clinical Management

There is no specific treatment, but symptomatic measures can be taken to improve the clinical course. Dimercaprol is stopped immediately if adverse reactions are observed. No antidote is available. If there has been dermal exposure, the skin should be washed with a nonirritating soap and water. If the eyes have been exposed, they must be irrigated with tap water. If ingested, activated charcoal must be given. Convulsions should be treated as usual with benzodiazepines and barbiturates. If cardiovascular collapse develops, fluids should be given according to the patient's hydroelectrolytic balance. Dopamine can be used, if necessary. Bicarbonate solution is useful, not only to correct acidosis but also to increase renal elimination of BAL–metal complexes, but also in preventing their dissociation and decreasing their toxicity. Some symptoms can be relieved by administration of an antihistamine. Alkalinization of urine may prevent kidney damage.

Miscellaneous

BAL should be administered carefully and under strict clinical control in patients with hypertension, hepatic, or renal impairment, and G6PD deficiency.

See also: Arsenic; Mercury; Metals.

Further Reading

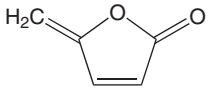
Vilensky JA and Redman K (2003) British anti-lewisite (dimercaprol): An amazing history. *Annals of Emergency Medicine* 41: 378–383.

Waters LL and Stock C (1945) Bal (anti-lewisite). *Science* 102: 601–606.

Baneberry

Rebeca Gracia

- Chemical Name: 4-Methylenebut-2-en-4-olide
- Representative Chemical: Protoanemonin
- Synonyms: *Actaea pachypoda* (white baneberry); *Actaea rubra* (red baneberry); *Actaea spicata* (grape wort, herb-Christopher); *Actaea erythrocarpa*; Doll's eyes; Cohosh; Snakeberry; Coral berry; Toadroot; Bugbane
- Chemical/Pharmaceutical/Other Class: *Actaea* species, of the order Ranunculaceae; Gastrointestinal irritants
- Chemical Formula: $C_5H_4O_2$
- Chemical Structure:



Uses

Topical compress for arthritis or frostbite, antispasmotic, anti-infective.

Background Information

Baneberry is a tall perennial herb that grows in most woodlands throughout the United States and Canada. It has large compound leaves, small white flowers, and either red or white berries (depending on the region).

There is substantial overlap between baneberry and black cohosh, a plant from the same order and more significant toxicity. Black cohosh is most likely to be associated with the pungent plant properties purported to be used as an antivenom and vermin repellant.

Exposure Routes and Pathways

The leaves, berries, and small white flowers may be ingested or applied dermally.

Toxicokinetics

Protoanemonin is released through enzymatic cleavage when the plant is crushed but is poorly absorbed due to low solubility. Large amounts may cause systemic toxicity. Ingestion of six or more berries may result in toxicity. Protoanemonin is excreted primarily by the kidneys.

Mechanism of Toxicity

The toxic effects of baneberry result from the irritant and vesicant effect of protoanemonin on mucous membranes.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxic effects in animals are similar to those in humans after ingestion of baneberry. Reports describe seizure activity and paralysis in livestock that ingest large amounts of the fresh plant. Protoanemonin is rapidly polymerized to the inactive anemonin if dried.

Human

The ingestion of the berries of the plant results in an initial burning sensation, increased salivation, and mucosal irritation, resulting in oral ulcerations. This is followed by acute stomach cramps and vomiting within 30 min. Dizziness, headache, and delirium were noted 1 h after ingestion. The symptoms usually disappear 3 h after ingestion. Prolonged dermal contact with the juice of berries or leaves may result in severe burning and skin irritation. The irritant properties persist even as protoanemonin is excreted, resulting in inflammation of the urinary tract, hematuria, and dysuria.

Clinical Management

Treatment of baneberry exposure is supportive. Careful evaluation and management of fluid and electrolytes is required. Gastric decontamination is effective if performed within the first hour after exposure. Tissue damage in the oral area from the effect of protoanemonin should be assessed before any gastrointestinal decontamination is performed. Monitoring renal output is necessary.

See also: Plants, Poisonous.

Relevant Website

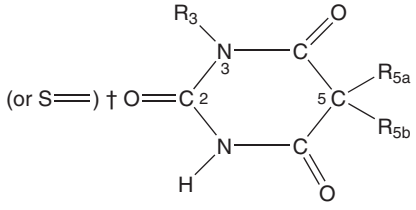
http://www.botanical.com – The electronic version of 'A Modern Herbal' by Maud Grieve.

Barbiturates, Long-Acting

Alexander B Baer and Christopher P Holstege

This article is a revision of the previous print edition article by Gregory P Wedin, volume 1, pp. 95–97, © 1998, Elsevier Inc.

- Representative Chemicals: Barbital (CAS 57-44-3); Mephobarbital (CAS 115-38-8); Phenobarbital (CAS 50-06-6)
- Synonyms:
  - Barbituratess – Courage pills; Downers; F-40s; Goof balls; Gorilla pills; Mexican yellows; Pink ladies
  - Barbital – Diethylbarbituric acid; Diethylmalonyl urea; Barbitone; DEBA
  - Mephobarbital – Methylphenobarbital; Mebaral
  - Phenobarbital – Phenylethylmalonylurea; Barbenyl; Barbiphenyl; Dormiral; Phenylbarbital; 5-Ethyl-5-phenylbarbituric acid; Solfoton
- Chemical/Pharmaceutical/Other Class: Barbituric acid derivative
- Chemical Structure:



Uses

The long-acting barbiturates are used for insomnia, anxiety, psychosis, preoperative sedation, and control of seizures. Long-acting barbiturates are utilized as drugs of abuse. This abuse peaked in the 1970s, but has since declined with the increased use of other sedatives.

Exposure Routes and Pathways

The most common route of exposure to the long-acting barbiturates is ingestion of oral dosage forms. Phenobarbital is also available for parenteral administration.

Toxicokinetics

Approximately 50–90% of the long-acting barbiturates are slowly absorbed from the gastrointestinal tract. Absorption is more rapid when ingested on an empty stomach and in the presence of alcohol. The onset of action varies: 30–60 min for mephobarbital and 8–12 h for oral phenobarbital. Mephobarbital is primarily metabolized by *N*-demethylation to form phenobarbital. Phenobarbital is metabolized by the hepatic microsomal enzyme system to an inactive metabolite. Long-acting barbiturates induce the hepatic microsomal enzyme system, especially CYP3A. This can lead to numerous drug interactions. Long-acting barbiturates, compared to short-acting barbiturates, are less lipid soluble, accumulate more slowly in tissue, are excreted more readily by the kidney as active drug, and have an elimination half-life longer than 40 h. The long-acting barbiturates are extensively distributed to all body tissues and fluids with highest concentrations achieved in the brain, liver, and kidneys. The apparent volume of distribution for phenobarbital is 0.5–1.01 kg^{-1}. Approximately 20–45% is bound to plasma proteins. A minimal amount of mephobarbital is eliminated unchanged in the urine. Phenobarbital has a long elimination half-life of ~2–6 days. Approximately 25% of a dose is eliminated unchanged in the urine with the remainder eliminated as inactive metabolites. The pK_a of phenobarbital (7.24) is similar to physiologic pH. As a result, the elimination of unchanged drug is significantly influenced by changes in the urine pH. Alkalinization of the urine can enhance the elimination of phenobarbital. This is referred to as ion trapping.

Mechanism of Toxicity

Barbiturates bind to specific sites on γ-aminobutyric acid (GABA)-sensitive ion channels found within the central nervous system (CNS). By binding to these sites, barbiturates allow an influx of chloride into cell membranes and, subsequently, hyperpolarize the postsynaptic neuron. GABA is the major inhibitory neurotransmitter in the CNS. Barbiturates enhance GABA-mediated chloride currents by binding to the GABA-A receptor–ionophore complex and increasing the duration of ionophore opening. At high doses, barbiturates stimulate GABA-A receptors directly in the absence of GABA. Barbiturates also block glutamate (excitatory neurotransmitter) receptors in the CNS. The CNS is particularly sensitive to the effect of barbiturates; however, with intoxication, the cardiovascular system and other peripheral functions are also depressed.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may be affected by the long-acting barbiturates much in the same way as humans. Lethargy,

coma, shallow respirations, incoordination, and depressed reflexes may occur. Standard supportive measures should be employed.

Human

Doses of 8 mg kg^{-1} or greater of phenobarbital will likely cause signs and symptoms of toxicity. The estimated potentially fatal dose in nondependent adults is 6–10 g. Overdose will produce CNS depression ranging from drowsiness to profound coma with a suppressed electroencephalogram. There have been reports of patients who achieved full neurological recovery after having isoelectric electroencephalograms for several days. Therefore, it is important to obtain drug levels on these patients before declaration of anoxic brain death. Patients who overdose with long-acting barbiturates may be comatose for several days. Severe intoxication may result in cardiovascular depression and vasodilation leading to hypotension, cardiovascular collapse, and cardiac arrest. Apnea and respiratory arrest also may occur. Depression of the gastrointestinal tract may cause an ileus. Horizontal gaze nystagmus may be seen. Comatose patients may develop bullous skin lesions primarily, but not always, over areas of pressure that are commonly called 'barb burns'. Barbiturate plasma concentrations aid in diagnosis and help determine whether to institute methods to enhance elimination. Barbiturate plasma concentrations are not accurate for predicting the duration or severity of toxicity.

Chronic Toxicity (or Exposure)

Animal

Phenobarbital is considered a carcinogen in experimental animals. Mice treated while pregnant developed a dose-related increase in the numbers of pups born with cleft-palate (0.6% of fetus in the 50 mg kg^{-1} diet vs. 3.9% in the 150 mg kg^{-1} diet).

Human

Chronic use of high doses of the long-acting barbiturates may produce psychological and physical dependence. Abrupt discontinuation of therapy may result in withdrawal signs and symptoms. Mild withdrawal may include weakness, anxiety, muscle twitching, insomnia, nausea, and vomiting. Severe withdrawal may consist of hallucinations, autonomic instability, delirium, and seizures. Unlike opioid withdrawal, long-acting barbiturate withdrawal may be life threatening. Barbiturates induce hepatic microsomal enzymes and can increase the metabolism of certain drugs, like acetaminophen, to their toxic metabolites potentially increasing the risk for adverse effects in polydrug overdoses.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Gastrointestinal decontamination procedures should be used as appropriate based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used to adsorb the long-acting barbiturates. Multiple-dose activated charcoal therapy (every 2–6 h for 24–48 h) enhances the nonrenal elimination of phenobarbital, but has not been shown to improve outcomes. It may be effective with other long-acting barbiturates. The patient's level of consciousness and vital signs should be monitored closely. Obtunded patients with reduced gag reflex should be intubated to reduce the risk of aspiration. Respiratory support including oxygen and ventilation should be provided as needed. There is no antidote for the long-acting barbiturates. If hypotension occurs it should be treated with standard measures including intravenous fluids, Trendelenburg positioning, and vasopressor support by intravenous infusion in refractory cases. Urine alkalinization may enhance elimination. Hemodialysis is effective for removing long-acting barbiturates but should be reserved for severe cases when standard supportive measures are inadequate. In chronic use of long-acting barbiturates, a gradual taper rather than abrupt discontinuation of barbiturates is appropriate to prevent the occurrence of life-threatening withdrawal.

See also: Anxiolytics; Neurotoxicity; Poisoning Emergencies in Humans.

Further Reading

Goodman JM, Bischel MD, and Wagers PW (1976) Barbiturate intoxication: Morbidity and mortality. *Western Journal of Medicine* 124: 179–186.

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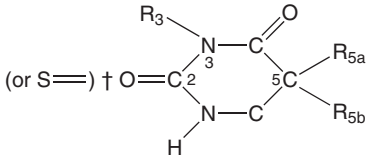
Barbiturates, Short-Acting

Alexander B Baer and Christopher P Holstege

This article is a revision of the previous print edition article by Gregory P Wedin, volume 1, pp. 97–99, © 1998, Elsevier Inc.

- Representative Chemicals: Amobarbital; Aprobarbital; Butabarbital sodium; Cyclobarbital; Heptabarbital; Hexobarbital; Methohexital sodium; Pentobarbital; Secobarbital sodium; Talbutal; Thiamylal; Thiopental sodium
- Chemical Abstracts Service Registry Number: CAS 309-36-4 (methohexital sodium)
- Synonyms:
  - Amobarbital – Amytal; $C_{11}H_{18}N_2O_3$; 5-Ethyl-5-(3-methylbutyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Aprobarbital – Alurate; $C_{10}H_{14}N_2O_3$; 5-(1-Methylethyl)-5-(2-propenyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Butalbital – Sandoptal; $C_{11}H_{16}N_2O_3$; 5-(2-Methylpropyl)-5-(2-propenyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Butabarbital – Butisol; Butolan; Sarisol; $C_{10}H_{15}N_2NaO_3$; 5-Ethyl-5-(1-methylpropyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione sodium salt
  - Cyclobarbital – $C_{12}H_{16}N_2O_3$; 5-(1-Cyclohexen-1-yl)-5-ethyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Heptabarbital – $C_{13}H_{18}N_2O_3$; 5-(1-Cyclohepten-1-yl)-5-ethyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Hexobarbital – $C_{12}H_{16}N_2O_3$; 5-(1-Cyclohexen-1-yl)-1,5-dimethyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Methohexital – Brevital; Compound 25398; $C_{14}H_{17}N_2NaO_3$; 1-Methyl-5-(1-methyl-2-pentynyl)-5-(2-propenyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione sodium salt
  - Pentobarbital – Nembutal; $C_{11}H_{18}N_2O_3$; 5-Ethyl-5-(1-methylbutyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Secobarbital – Seconal; $C_{12}H_{17}N_2NaO_3$; 5-(1-Methylbutyl)-5-(2-propenyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione sodium salt
  - Talbutal – Lotusate; $C_{11}H_{16}N_2O_3$; 5-(1-Methylpropyl)-5-(2-propenyl)-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione
  - Thiamylal – Surital; $C_{12}H_{18}N_2O_2S$; dihydro-5-(1-Methylbutyl)-5-(2-propenyl)-2-thioxo-4,6-(1*H*,5*H*)-pyrimidinetrione
  - Thiopental – Pentothal; $C_{11}H_{17}N_2NaO_2S$; 5-Ethyldihydro-5-(1-methylbutyl)-2-thioxo-4,6-(1*H*,5*H*)-pyrimidinetrione sodium salt
- Chemical/Pharmaceutical/Other Class: Barbituric acid derivative
- Chemical Structure:



Uses

The short-acting barbiturates are used for short-term treatment of insomnia, anxiety, psychosis, preoperative sedation, control of seizures, and anesthetics. Short-acting barbiturates are also used as drugs of abuse. Barbiturate use has dramatically decreased since the 1970s with the introduction of benzodiazepines.

Exposure Routes and Pathways

The most common route of exposure to the short-acting barbiturates is ingestion of oral dosage forms. Several of these agents are also available for parenteral administration (intramuscular or intravenous) and have been used as rectal suppositories.

Toxicokinetics

The short-acting barbiturates are rapidly and completely absorbed from the gastrointestinal tract. The sodium salts are absorbed more rapidly than the acids by all routes. Absorption is more rapid when ingested on an empty stomach and also in the presence of alcohol. The onset of action varies from 10 to 30 min. The short-acting barbiturates are all extensively metabolized by the hepatic microsomal enzyme system. The short-acting barbiturates are rapidly distributed to all body tissues and fluids with the highest concentrations achieved in the brain, liver, and kidneys. The apparent volume of distribution ranges from 0.6 to 1.9 l kg^{-1}. Inactive metabolites of the short-acting barbiturates are eliminated in the urine. Only aprobarbital, which is less lipid soluble, has a significant fraction (13–24%) that is eliminated unchanged in the urine. The elimination half-life ranges from 1 to 48 h.

Mechanism of Toxicity

These barbiturates are known to decrease the excitability of postsynaptic membranes by binding to the γ-aminobutyric acid (GABA) receptor and increasing the duration of time these chloride channels are open. Enhanced GABA receptor-mediated chloride conductance also occurs through a stronger receptor affinity for other ligands like GABA and benzodiazepines in the presence of some barbiturates. The central nervous system (CNS) is particularly sensitive to these effects, but with intoxication the cardiac and vascular smooth muscle tone can also be depressed.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may be affected by the short-acting barbiturates much in the same way as humans. Lethargy, coma, respiratory depression, ataxia, hypothermia, and hypotension have been described in poisoning. Some short-acting barbiturates are utilized as veterinary euthanasia agents. The treatment of overdose is similar to that in humans.

Human

There is a broad spectrum of signs and symptoms associated with acute short-acting barbiturate toxicity. Lethargy, ataxia, nystagmus, diplopia, amnesia, slurred speech, confusion, hypotonia, hypotension, hypothermia, hypoglycemia, coma, respiratory depression, and death have been reported. Comatose patients may develop erythematous or hemorrhagic bullous skin lesions primarily over areas of pressure (e.g., elbows and knees). These lesions are commonly referred to as 'barb burns'. Doses of 3–5 mg kg^{-1} of most short-acting barbiturates will cause toxicity in children. The estimated potentially fatal dose in nondependent adults is 3–6 g.

Hypersensitivity to barbiturates can result in a life-threatening syndrome called the Drug, Rash with Eosinophilia and Systemic Symptoms (DRESS) Syndrome with a mortality of 10%. In persons developing hypersensitivity to barbiturates, there is a potential of cross-sensitivity with other aromatic antiepileptics, such as phenytoin and carbamazepine.

Chronic Toxicity (or Exposure)

Animal

Dogs fed amobarbital chronically developed CNS depression, slowed reaction times, and incoordination.

Human

Tolerance and physical dependence may develop in persons who chronically use short-acting barbiturates. Abrupt discontinuation of chronic barbiturate therapy may result in a withdrawal syndrome consisting of anxiety, agitation, insomnia, tremors, headache, myalgias, nausea, vomiting, diaphoresis, hyperpyrexia, psychosis, seizures, and death. Chronic barbiturates use may result in induction of the hepatic microsomal enzyme system.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Activated charcoal can be used to adsorb the short-acting barbiturate drug as long as the patient's airway is protected. The patient's level of consciousness and vital signs should be monitored closely. Obtunded patients with reduced gag reflex should be intubated. Respiratory support including oxygen and ventilation should be provided as needed. If hypotension occurs, it should be treated with standard measures including intravenous fluids, Trendelenburg positioning, and dopamine by intravenous infusion. There is no antidote for the short-acting barbiturates. Forced alkaline diuresis is of no value for the short-acting barbiturates. Major complications associated with barbiturate intoxication include anoxic brain injury, aspiration pneumonia, rhabdomyolysis, and compartment syndrome. The occurrence of withdrawal signs and symptoms indicates the need to reinstitute barbiturate or substitute alternative benzodiazepine therapy and gradually reduce the dose until discontinued.

See also: Anxiolytics; Neurotoxicity; Poisoning Emergencies in Humans.

Further Reading

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Barium

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst, Shirley B Radding, and Kathyrn A Wurzel, volume 1, pp. 99–100, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-39-3
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Ba^{2+}

Uses

Barium is found in various alloys, paints, soap, paper, photographic chemicals, explosives, and rubber, and is used in the manufacture of ceramics and glass. Some of its compounds are used as mordents in fabric dying and in the preparation of phosphors. One major use is in a slurry of ground barite ($ZnS + BaSO_4$) for gas and oil drilling. Barium fluorosilicate has been used as an insecticide and some barium compounds are used as rodenticides. Medicinally, barium sulfate, being very sparingly soluble, is used as a radiopaque contrast material for X-ray diagnostic purposes, and other medical imaging uses.

Background Information

Sir Humphrey Davy discovered barium in 1808.

Exposure Routes and Pathways

Exposure pathways for barium primarily consist of ingestion (e.g., food and water) and inhalation. Barium is relatively abundant in nature; hence, most food contains small amounts of barium. Brazil nuts have very high barium concentrations (from 3 to 4000 ppm). It is also found in drinking water from natural deposits in certain regions. Barium is also detected in the air of most cities.

Toxicokinetics

Soluble barium compounds are absorbed by the lungs and gastrointestinal tract and small amounts are accumulated in the skeleton. The highest concentration of barium in the body is present in the lungs. Although some barium is excreted in the urine, it is reabsorbed by the renal tubules. It is primarily excreted in feces.

Mechanism of Toxicity

Ingestion of toxic doses of barium affects the muscles, especially the heart. Barium has a digitalis-type effect on the heart. Ventricular fibrillation and slowed pulse rate are noted. This may be related to barium's tendency to displace potassium; the resulting potassium deficiency causes muscle weakness.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} for rats is $630\,mg\,kg^{-1}$ for barium carbonate, $118\,mg\,kg^{-1}$ for barium chloride, and $921\,mg\,kg^{-1}$ for barium acetate.

Human

The toxicity of barium is related to the solubility of the compound. Barium sulfate, being very sparingly soluble, is relatively nontoxic. Soluble barium salts are toxic by ingestion (e.g., acetate, chloride, nitrate, sulfide, as well as carbonate and hydroxide compounds). Ingestion results in nausea, vomiting, stomach pains, and diarrhea. Severe gastrointestinal irritation is followed by muscle twitching and then a flaccid muscular paralysis. Barium can activate catecholamines, resulting in muscle twitching and other nervous system effects. Ingestion of barium compounds can lead to gastroenteritis, hypokalemia, hypertension, cardiac arrhythmias, and skeletal muscle paralysis. Potassium infusion is used clinically to reverse many of the toxic effects, but cannot reverse the hypertensive response. Barium released during welding can decrease plasma potassium levels.

Soluble compounds also irritate skin, eyes, and mucous membranes and can be absorbed following inhalation. Barium carbonate dust is a bronchial irritant. Barium oxide dust is a dermal and nasal irritant.

The estimated lethal dosage of the rodenticide barium carbonate in humans is $\sim 70\,mg\,kg^{-1}$. The LD_{50} for barium chloride is estimated at $\sim 14\,mg\,kg^{-1}$, and the LD_{Lo} is $\sim 0.8\,g$. Convulsions and death from cardiac and respiratory failures can occur. Survival for more than 24 h is usually followed by complete recovery. Direct aspiration of a large amount of barium into the airway resulted in tachycardia, rapid breathing, fever, and low oxygen saturation. A family eating fish accidentally battered with barium carbonate developed nausea, vomiting, diarrhea, and abdominal pain within minutes and the parents also

developed ventricular tachycardia, flaccid paralysis of the extremities, dyspnea (mother), and respiratory failure (father). Patients were treated symptomatically and all fully recovered.

Chronic Toxicity (or Exposure)

Animal

In guinea pigs, barium caused various changes in the blood and pathological changes in bone marrow, spleen, and liver. Cardiovascular effects are evident in rats after long-term exposures. Ultrastructural changes in the kidney glomeruli were noted in rats consuming barium ($1\,g\,l^{-1}$) in the drinking water for 36 weeks. Increased kidney weights were noted in female rats consuming barium (2500 ppm) in the drinking water for 15 months.

Human

Inhalation of insoluble sulfate and oxide, as dusts, produces a pneumoconiosis called baritosis, which is a relatively benign condition that is usually reversible with cessation of exposure. Cardiovascular effects are also of concern after long-term exposure in humans.

Clinical Management

Addition of sodium sulfate as a lavage solution may precipitate the very insoluble barium sulfate. As potassium deficiency occurs in acute poisoning, serum potassium and cardiac rhythm must be monitored closely. Administration of intravenous potassium appears beneficial. As renal failure is also a concern, urinary output also must be monitored closely.

Environmental Fate

Barium is a highly reactive metal that occurs naturally only in a combined state. The element is released to environmental media by both natural processes and anthropogenic sources.

Barium is released primarily to the atmosphere as a result of industrial emissions during the mining, refining, and production of barium and barium chemicals, fossil fuel combustion, and entrainment of soil and rock dust into the air. In addition, coal ash, containing widely variable amounts of barium, is also a source of airborne barium particulates. Most barium released to the environment from industrial sources is in forms that do not become widely dispersed. In the atmosphere, barium is likely to be present in particulate form. Although chemical reactions may cause changes in speciation of barium in air, the main mechanisms for the removal of barium compounds from the atmosphere are likely to be wet and dry depositions.

In aquatic media, barium is likely to precipitate out of solution as an insoluble salt (i.e., as $BaSO_4$ or $BaCO_3$). Waterborne barium may also adsorb to suspended particulate matter. Precipitation of barium sulfate salts is accelerated when rivers enter the ocean because of the high sulfate content in the ocean. Sedimentation of suspended solids removes a large portion of the barium content from surface water. Barium in sediments is found largely in the form of barium sulfate (barite). Coarse silt sediment in a turbulent environment will often grind and cleave the barium sulfate from the sediment particles leaving a buildup of dense barites. Estimated soil–water distribution coefficients (K_d) (i.e., the ratio of the quantity of barium absorbed per gram of sorbent to the concentration of barium remaining in solution at equilibrium) range from 200 to 2800 for sediments and sandy loam soils.

As pH levels increase above 9.3 and in the presence of carbonate, barium carbonate becomes the dominant species. Barium carbonate also exhibits fast precipitation kinetics and very low solubility and in alkaline environments limits the soluble barium concentration. Barium forms salts of low solubility with arsenate, chromate, fluoride, oxalate, and phosphate ions. The chloride, hydroxide, and nitrate of barium are water soluble and are frequently detected in aqueous environments.

Ecotoxicology

The uptake of barium by fish and marine organisms is an important elimination mechanism. Barium levels in seawater range from 2 to $63\,mg\,l^{-1}$ with a mean concentration of $\sim 13\,mg\,l^{-1}$. Barium was found to bioconcentrate in marine plants by a factor of 1000 times the level present in the water. Bioconcentration factors in marine animals, plankton, and in brown algae of 100, 120, and 260, respectively, have been reported. Relatively little information is available on the effects of barium compounds in aquatic organisms. Barium carbonate was practically nontoxic to fish (96 h LC_{50} in *Gambusia* was $>10\,g\,kg^{-1}$).

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value – time-weighted average is $0.5\,mg\,ml^{-1}$ for soluble barium compounds and $10\,mg\,ml^{-1}$ for barium sulfate. The permissible exposure limit is $0.5\,mg\,m^{-3}$ for barium in soluble compounds. The reference dose for barium is $0.07\,mg\,kg^{-1}\,day^{-1}$ and the tolerable daily intake (The Netherlands) is $0.02\,mg\,kg^{-1}\,day^{-1}$.

See also: Metals.

Further Reading

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.

Relevant Websites

http://risk.lsd.ornl.gov – Risk Assessment Information System.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Barium.

Bases *See* Alkalies.

Batrachotoxin

John P Dumbacher

- Representative Chemicals: Batrachotoxin; Homobatrachotoxin; Batrachotoxinin A; and several other batrachotoxinin A congeners
- Chemical Abstracts Service Registry Number: CAS 23509-16-2 (Batrachotoxin)
- Synonyms: *Phyllobates* toxin; *Pitohui* toxin; *Ifrita* toxin; poison dart frog toxin
- Chemical/Pharmaceutical/Other Class: Steroidal alkaloid neurotoxin
- Chemical Formulas:
  - Batrachotoxin: $C_{31}H_{42}N_2O_6$
  - Homobatrachotoxin: $C_{32}H_{44}N_2O_6$
  - Batrachotoxinin-A: $C_{24}H_{35}NO_5$

Background Information

Batrachotoxins are a class of steroidal alkaloid neurotoxins found in Colombian poison dart frogs of the genus *Phyllobates* (family Dendrobatidae). The frogs have special skin glands that store and secrete the toxins, and these glands are most densely packed on the back behind the head. Evidence suggests that the frogs acquire the toxins from a dietary source; however, no potential source of these frog poisons has been identified. Interestingly, of all of the so-called poison dart frogs, only three species of *Phyllobates* were actually used by Native Americans for poisoning dart tips, and the major toxic element responsible for poisoning are the batrachotoxins. More recently, identical toxins were found in New Guinean birds in the genus *Pitohui* (family Pachycephalidae) and *Ifrita* (family Cinclosomatidae). The toxins are most concentrated in the skins and feathers of birds.

Several naturally occurring batrachotoxins have been identified from frog and bird extracts. The most common are batrachotoxin and homobatrachotoxin, which contain a pyrrole moiety. These occur in frogs in roughly equal proportions, and have an LD_{50} in mice of $\sim$2–3 $\mu g\,kg^{-1}$ (subcutaneous injection). Toxicity via other routes has not been well studied. The pyrrole can be manipulated in nature and in the lab to give the non-pyrrole form, called batrachotoxinin-A, which is $\sim$1/500th as toxic as batrachotoxin or homobatrachotoxin. Several other congeners have been identified in nature, but the pharmacology of many of these remains unstudied.

Exposure Routes and Pathways

From *Phyllobates* frogs, exposure occurs through ingesting skin and flesh of the frogs. Toxin quantities can be high enough to make even handling these frogs dangerous, so presumably some absorption can occur through skin. Exposure to the toxins may occur by subcutaneous injection, such as a puncture from a poisoned dart tip. From birds, exposure can occur by eating flesh, however even handling the birds can cause 'allergic' reactions such as itchy eyes, runny nose, sneezing, and tingling around buccal membranes. These reactions are believed to be caused by powder or tiny feather fragments released from toxic feathers. Batrachotoxins are lipid soluble and soluble in a variety of organic solvents such as methanol, chloroform, and ethanol.

Toxicokinetics

Batrachotoxin can be absorbed through skin as well as from the gastrointestinal tract. Effects can occur within 10 min and can last for several hours to more than a day.

Mechanism of Toxicity

Batrachotoxins bind specifically to voltage-gated sodium channels in nerve and muscle membranes.

Once activated, bound batrachotoxins stabilize the channel in its open conformation. This allows sodium ions to flow freely across the membrane, and depolarize it, causing local tingling, irritation, and numbness, and in higher concentrations convulsions, paralysis, and cardiac or pulmonary failure. Because a relatively small proportion of activated channels can depolarize the membrane, batrachotoxins are highly toxic. Batrachotoxins bind strongly to sodium channel proteins, so binding is often referred to as 'irreversible', although light exposure (resulting in local tingling or numbness) generally subsides within a few minutes to 24 h.

Acute and Short-Term Toxicity (or Exposure)

Human

Very little is known about toxicity of batrachotoxins in humans. If it is assumed that human and mouse toxicity are equivalent (at $\sim 2.5\,\mu g\,kg^{-1}$ subcutaneously), then a median lethal dose for a 68 kg human would be $\sim 170\,\mu g$ of batrachotoxin. Other studies show that mice are less susceptible to neurotoxins than humans, so another estimate can be based upon toxicity relationships of batrachotoxin to aconitine, digitoxin, and strychnine and their toxicity in humans. Using these relationships, it is expected that a dose as small as 2–10 μg of purified batrachotoxin injected subcutaneously may be lethal to humans. Likewise, ingested amounts of as little as 120–500 μg are expected to be lethal. These are certainly rough estimates, and few, if any, human poisonings have been reported in medical literature. However, purified toxins as well as frog skin secretions should be handled with extreme care.

Human Use of Frog Secretions Containing Batrachotoxins

Very small amounts of frog secretions from *Phyllobates terribilis*, *P. bicolor*, and *P. aurotanea* can be used to poison dart tips, which are reportedly effective at immobilizing a variety of animals including jaguar, bear, deer, and humans. A single *P. bicolor* or *P. terribilis* can effectively poison 20–30 darts.

Human Knowledge of *Pitohui* and *Ifrita* Birds Containing Batrachotoxins

In New Guinea, traditional hunters are aware that *Pitohui* and *Ifrita* birds carry neurotoxins. Local names for these birds often reflect the fact that they are bitter or contain burning chemicals. Toxins in these birds are more diffuse than in the frogs, but even a single feather, if tasted, can cause an acute burning sensation that may last for several minutes to hours. Handling the birds can cause allergy-like reactions such as itchy watery eyes, running nose, and sneezing. To the author's knowledge, there have been no human deaths or serious poisonings due to bird ingestion, as it is generally recognized as inedible, and an unpleasant burning sensation sets in before much of the toxin is eaten. No anthropologists have reported local New Guineans using the toxins to immobilize prey.

In Vitro Toxicity Data

Batrachotoxin is an important research tool because of its action of holding voltage-gated sodium channels open as well as its specific effects at other ligand-binding sites. It was previously used commonly in channel and ligand research. There are no commercially available stocks of batrachotoxins, however, and work in Colombia is currently difficult or impossible, so these chemicals are used less frequently in research.

Clinical Management

No antidote is available.

See also: Animals, Poisonous and Venomous.

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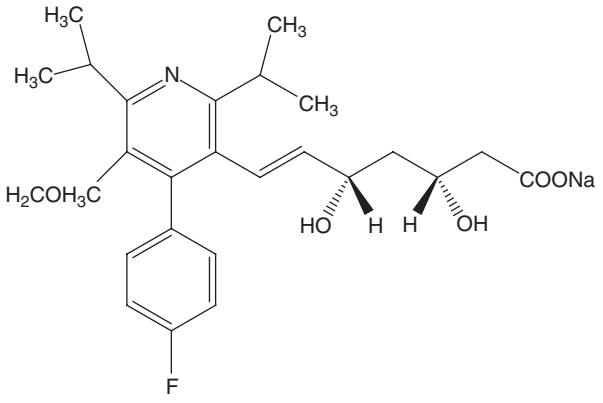
Baycol

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 143201-11-0
- Synonym: Cerivastatin sodium
- Chemical/Pharmaceutical/Other Class: Statins; HMG-CoA inhibitors
- Chemical Formula: $C_{26}H_{33}FNO_5Na$
- Chemical Structure:



Uses

Baycol was indicated as an adjunct to diet to reduce elevated total-cholesterol, low-density lipoprotein cholesterol (LDL-C), apo B, and triglycerides (TG) and to increase high-density lipoprotein cholesterol (HDL-C) levels in patients with primary hypercholesterolemia and mixed dyslipidemia (Fredrickson types IIa and IIb) when the response to dietary restriction of saturated fat and cholesterol and other nonpharmacological measures alone had been inadequate. Therapy with lipid altering drugs should bea component of multiple risk factor intervention in those patients at significantly high risk for atherosclerotic vascular disease due to hypercholesterolemia.

Background Information

Baycol was removed from the market in 2001 because of an unacceptable level of risk of adverse effects.

Toxicokinetics

Absorption: The mean absolute bioavailability of cerivastatin following a 0.2 mg tablet oral dose is 60% (range 39–101%). In general, the coefficient of variation (based on the intersubject variability) for both systemic exposure (area under the curve, AUC) and C_{max} is in the 20–40% range. The bioavailability of cerivastatin sodium tablets is equivalent to that of a solution of cerivastatin sodium. No unchanged cerivastatin is excreted in feces. Cerivastatin exhibits linear kinetics over the dose range of 0.2–0.8 mg daily. In male and female patients at steady state, the mean maximum concentrations (C_{max}) following evening cerivastatin tablet doses of 0.2, 0.3, 0.4, and 0.8 mg are 2.8, 5.1, 6.2, and 12.7 $\mu g l^{-1}$, respectively. AUC values are also dose-proportional over this dose range and the mean time to maximum concentration (t_{max}) is $\sim$2 h for all dose strengths. Following oral administration, the terminal elimination half-life ($t_{1/2}$) for cerivastatin is 2–4 h. Steady-state plasma concentrations show no evidence of cerivastatin accumulation following administration of up to 0.8 mg daily.

Results from an overnight pharmacokinetic evaluation following single-dose administration of cerivastatin with the evening meal or 4 h after the evening meal showed that administration of cerivastatin with the evening meal did not significantly alter either AUC or C_{max} compared to dosing the drug 4 h after the evening meal. In patients given 0.2 mg cerivastatin sodium once daily for 4 weeks, either at mealtime or at bedtime, there were no differences in the lipid-lowering effects of cerivastatin. Both regimens of 0.2 mg once daily were slightly more efficacious than 0.1 mg twice daily.

The volume of distribution (V_d) is calculated to be 0.31 kg^{-1}. More than 99% of the circulating drug is

bound to plasma proteins (80% to albumin). Binding is reversible and independent of drug concentration up to $100\,mg\,l^{-1}$.

Mechanism of Toxicity

Cerivastatin is a competitive inhibitor of HIVIG-CoA reductase, which is responsible for the conver sion of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) to mevalonate, a precursor of sterols, including cholesterol. The inhibition of cholesterol biosynthesis by cerivastatin reduces the level of cholesterol in hepatic cells, which stimulates the synthesis of LDL receptors.

Clinical Pharmacology

Cholesterol and triglycerides circulate as part of lipoprotein complexes throughout the bloodstream. These complexes can be separated via ultracentrifugation into HDL, intermediate-density lipoprotein (IDL), LDL, and very-low-density lipoprotein (VLDL) fractions. In the liver, cholesterol and TG are synthesized, incorporated into VLDL, and released into the plasma for delivery to peripheral tissues.

Chronic Toxicity (or Exposure)

Animal

Chronic administration of cerivastatin to rodent and nonrodent species demonstrated the principal toxicologic targets and effects observed with other HMG-CoA reductase inhibitors: hemorrhage and edema in multiple organs and tissues including the central nervous system (CNS) (dogs); cataracts (dogs); degeneration of muscle fibers (dogs, rats, and mice); hyperkeratosis in the nonglandular stomach (rats and mice, this organ has no human equivalent); liver lesions (dogs, rats, and mice). CNS lesions in the dog were found at a dose of $0.1\,mg\,kg^{-1}\,day^{-1}$. This dose resulted in plasma levels of cerivastatin (C_{max} measured as free drug) that were $\sim$17 times higher than the mean values in humans taking $0.8\,mg\,day^{-1}$. No CNS lesions were observed after chronic treatment with cerivastatin for up to 2 years in the mouse (at up to six times human C_{max} free drug levels) and rat (in the range of human C_{max} free drug levels).

A 2 year carcinogenicity study was conducted in rats with dietary administration resulting in average daily doses of cerivastatin of 0.007, 0.034, or $0.158\,mg\,kg^{-1}$. The high dosage level corresponded to plasma free drug levels (AUC) of approximately two times those in humans following a 0.8 mg oral dose. Tumor incidences of treated rats were comparable to controls in all treatment groups. In a 2 year carcinogenicity study conducted in mice with dietary administration resulting in average daily doses of cerivastatin of 0.4, 1.8, 9.1, or $55\,mg\,kg^{-1}$, hepatocellular adenomas were significantly increased in male and female mice at $\geqslant 9.1\,mg\,kg^{-1}$ (AUC free values about three times the human value at $0.8\,mg\,day^{-1}$). Hepatocellular carcinomas were significantly increased in male mice at $1.8\,mg\,day^{-1}$ (AUC free values in the range of human exposure at $0.8\,mg\,day^{-1}$).

In a combined male and female rat fertility study, cerivastatin had no adverse effects on fertility or reproductive performance at doses up to $0.1\,mg\,kg^{-1}\,day^{-1}$ (in the range of human C_{max} free drug levels). At a dose of $0.3\,mg\,kg^{-1}\,day^{-1}$ (about three times the human C_{max} free drug levels), the length of gestation was marginally prolonged, stillbirths were increased, and the survival rate up to day 4 postpartum was decreased. In the fetuses (F1), a marginal reduction in fetal weight and delay in bone development was observed. In the mating of the F1 generation, there were a reduced number of female rats that littered.

In the testicles of dogs treated chronically with cerivastatin at a dose of $0.008\,mg\,kg^{-1}\,day^{-1}$ (in the range of human C_{max} free drug levels), atrophy, vacuolization of the germinal epithelium, spermatidic giant cells, and focal oligospermia were observed. In another 1 year study in dogs treated with $0.1\,mg\,kg^{-1}\,day^{-1}$ ($\sim$17-fold the human exposure at doses of 0.8 mg based on C_{max} free), ejaculate volume was small and libido was decreased. Semen analysis revealed an increased number of morphologically altered spermatozoa indicating disturbances of epididymal sperm maturation that was reversible when drug administration was discontinued.

Cerivastatin caused a significant increase in incomplete ossification of the lumbar center of the vertebrae in rats at an oral dose of $0.72\,mg\,kg^{-1}$. Cerivastatin did not cause any anomalies or malformations in rabbits at oral doses up to $0.7\,mg\,kg^{-1}$. These doses resulted in plasma levels about six times the human exposure (C_{max} free) for rats and three times the human exposure for rabbits (C_{max} free) at a human dose of 0.8 mg. Cerivastatin crossed the placenta and was found in fetal liver, gastrointestinal tract, and kidneys when pregnant rats were given a single oral dose of $2\,mg\,kg^{-1}$.

Human

Because of possible adverse effects on liver function, caution was advised when Baycol (cerivastatin sodium

tablets) was administered to patients with a history of liver disease or heavy alcohol ingestion.

In Vitro Toxicity Data

No evidence of genotoxicity was observed *in vitro* with or without metabolic activation in the following assays: microbial mutagen tests using mutant strains of *Salmonella typhimurium* or *Escherichia coli*, Chinese hamster ovary forward mutation assay, unscheduled DNA synthesis in rat primary hepatocytes, chromosome aberrations in Chinese hamster ovary cells, and spindle inhibition in human lymphocytes. In addition, there was no evidence of genotoxicity *in vivo* in a mouse micronucleus test; there was equivocal evidence of mutagenicity in a mouse domi nant lethal test.

Market Removal for Safety

Baycol was removed from the market place in 2001 due to concerns about its safety. The primary adverse effects were:

Liver enzymes: HMG-CoA reductase inhibitors have been associated with biochemical abnormalities of liver function. These abnormalities usually occurred within the first six months of treatment, usually resolved after discontinuation of the drug, and were not associated with cholestasis.

Skeletal muscle: Cases of rhabdomyolysis (muscle fiber breakdown), leading to acute renal failure secondary to myoglobinuria, have been reported with cerivastatin and other drugs in this class. Myopathy, defined as muscle aching or muscle weakness, associated with increases in plasma creatine kinase values to greater than 10 times the upper limit of normal, was seen in 0.4% of patients in US cerivastatin clini cal trials.

Endocrine function: HMG-CoA reductase inhibitors interfere with cholesterol synthesis and lower cholesterol levels and, as such, might theoretically blunt adrenal or gonadal steroid hormone production.

Safety in pregnant women has not been established.

See also: Food and Drug Administration, US; Kidney.

Further Reading

Dipiro JT (2005) *Pharmacotherapy*, 6th edn. New York: McGraw-Hill.

Walsh PM (2003) *Physicians' Desk Reference*, 58th edn. Montvale, NJ: Thomson PDR.

Relevant Website

http://www.fda.gov – Baycol Information (from the US Food and Drug Administration).

BCNU (Bischloroethyl Nitrosourea)

Madhusudan G Soni

- Chemical Abstracts Service Registry Number: CAS 154-93-8
- Synonyms: Carmustine; *N*,*N*-bis(2-Chloroethyl)-*N*-nitrosourea; BiCNU; Carmubris; Nitrumon
- Chemical/Pharmaceutical/Other Class: Alkylating agent

Uses

Bischloroethyl nitrosourea (BCNU) has been used in human medicine as an antineoplastic agent (alone or in combination with other agents) in the treatment of Hodgkin's lymphoma, multiple myeloma, and in primary or metastatic brain tumors.

Exposure Routes and Pathways

Intravenous injection is the most common route of exposure. Doses range from 100 to 250 mg m^{-2} body surface for courses of 2 or 3 days.

Toxicokinetics

In animal experiments, BCNU is rapidly absorbed, following different routes of ingestion. A few minutes after administration, no unchanged BCNU can be detected in plasma. BCNU undergoes spontaneous decomposition under physiological conditions to release both alkylating and carbamoylating entities. In addition to chemical decomposition, BCNU may be denitrosated enzymatically via hepatic microsomal enzyme oxidation system to its corresponding urea. BCNU is rapidly distributed to most tissues including brain and cerebrospinal fluid. The volume

of distribution is $\sim 0.18\,l\,kg^{-1}$. Approximately 80% of the drug appears in the urine within 24 h as degradation products. Approximately 10% of the ingested BCNU is removed by respiratory excretion and 1% in feces. BCNU is reported to have a biological half-life of less than 20 min.

Mechanism of Toxicity

It is generally assumed that BCNU exerts its cyto toxicity through the liberation of alkylating and carbamoylating moieties. An alkylating entity, particularly chloroethyl carbonium ion, is strongly electrophilic and can alkylate a variety of biomolecules, including the purine and pyrimidine bases of DNA. The interstrand cross-linking is generally associated with the cytotoxicity of BCNU. The carbomylation of lysine residues of protein can inactivate certain DNA repair enzymes thus interfering with repair processes.

Acute and Short-Term Toxicity (or Exposure)

Animal

In dogs, high doses of BCNU resulted in severe bone marrow hypoplasia with delayed, reversible thrombocytopenia. The other major toxicities observed were cardiopulmonary (pulmonary edema, myocardial infarction, and pericardial hemorrhage), intestinal mucosal damage with hemorrhage, renal toxicity, and delayed hepatotoxicity. Similar toxicity was seen in monkeys except that cardiopulmonary toxicity did not occur. In rats, initially well-tolerated doses may cause death later. There is sufficient evidence for the carcinogenicity of BCNU in rats. BCNU is embryo- and fetolethal in rats and rabbits at doses nontoxic to the mother and can induce a variety of teratogenic effects in rats.

Human

Various cytotoxic effects of BCNU in humans are reported. The drug is not a vesicant, but local burning pain has been reported after intravenous administration. Nausea and vomiting occur ~ 2 h after injection. Flushing of the skin and conjunctiva, central nervous system toxicity, esophagitis, diarrhea, interstitial pulmonary fibrosis, and renal and hepatic toxicities have been reported. Hepatotoxicity and pulmonary toxicity may be dose limiting. Although bone marrow suppression is observed, this drug characteristically causes an unusually delayed onset of leukopenia and thrombocytopenia. The nadir of the leukocyte and platelet counts may not reach normal levels until 6 weeks after treatment.

Clinical signs associated with BCNU-induced pulmonary toxicity in humans are dyspnea, tachypnea, and a dry hacking cough. The incidence of these symptoms is between 20% and 30% and mortality varies from 24% to 80%. The onset of symptoms is usually within 3 years of treatment. There is a linear relationship between total dose received and pulmonary toxicity at doses $> 1000\,mg\,m^{-2}$, with 50% of patients developing pulmonary toxicity at total cumulative doses of $1500\,mg\,m^{-2}$.

Chronic Toxicity (or Exposure)

Animal

Administration of BCNU three times a week for six months, followed by 12 months observation, to Swiss mice at intraperitoneal doses of 2.5 and $5.0\,mg\,kg^{-1}$ and to SD rats at dose of $1.5\,mg\,kg^{-1}$ resulted in increases in tumor incidence in all treated animals, predominantly subcutaneous and lung neoplasms.

Human

In a long-term study of patients receiving BCNU in childhood and early adolescence (1–16 years), delayed onset pulmonary fibrosis occurring up to 17 years after treatment has been reported. Pulmonary toxicity characterized by pulmonary infiltrates and/or fibrosis has been reported to occur from 9 days to 43 months after treatment with BCNU. Most of these patients were receiving prolonged therapy with total doses of BCNU greater than $1400\,mg\,m^{-2}$. However, there have been reports of pulmonary fibrosis in patients receiving lower total doses. The occurrence of acute leukemia and bone marrow dysplasias have been reported in patients following long-term BCNU therapy. Renal abnormalities consisting of progressive azotemia, decrease in kidney size, and renal failure have been reported in patients who received large cumulative doses after prolonged therapy with BCNU.

In Vitro Toxicity Data

BCNU was mutagenic *in vitro* (Ames assay, human lymphoblast HGPRT assay) and clastogenic *in vitro* (V79 hamster cell micronucleus assay).

Clinical Management

Most of the adverse reactions of BCNU are reversible if detected early. When such effects or reactions do occur, the drug should be reduced in dosage or discontinued and appropriate corrective measures should be taken according to the clinical judgment

of the physician. Blood counts should be monitored weekly for at least 6 weeks after the dose. Baseline pulmonary function studies, hepatic functional tests, and periodic renal functional tests should be monitored. No proven antidotes have been established for BCNU overdosage.

Environmental Fate

There is no information available on the environmental fate of BCNU. However, one can predict that as a very reactive agent, BCNU spontaneously decomposes.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Toxicity Testing, Mutagenicity.

Further Reading

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Carter SK, Schabel FM, Broder LE, and Johnston TP (1972) 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and other nitrosoureas in cancer treatment: A review. *Advances in Cancer Research* 16: 273–332.

DeVita VT, Carbone PP, Owens AH, *et al.* (1965) Clinical trials with 1,3-bis(2-chloroethyl)-1-nitrosourea, NSC-409962. *Cancer Research* 25: 1876–1881.

Bee *See* Hymenoptera.

Behavioral Toxicity Testing *See* Toxicity Testing, Behavioral.

Behavioral Toxicology

Deborah A Cory-Slechta

Introduction

Behavioral toxicology is that scientific discipline which studies the effects of therapeutic drugs and other chemicals on behavior, the ultimate output of the nervous system, and also seeks to determine how such effects are caused. The impetus for such studies has come from multiple sources. Both human and experimental animal studies have been carried out to assess the behavioral consequences arising from exposures to chemicals used in the workplace as well as those dispersed in the environment. These efforts have been important in determining safe exposure and risk levels, as well as in furthering our understanding of these chemicals. A second force behind many such studies has been the need to screen newly synthesized chemicals for any potential adverse behavioral effects before their introduction into use, efforts which are obviously carried out only in experimental laboratory contexts.

Human behavior is, of course, extremely diverse and complex, composed of numerous different functions, any or all of which might be perturbed by exposure to a toxicant. Thus, understanding how a chemical affects human behavior may require a determination of its effects across these different behavioral functions. Furthermore, some human behaviors require an integration of several different behavioral functions. If we think about learning in a classroom, for example, in addition to cognitive functions, sensory functions are needed to process the information presented, and motor functions are required for executing the correct response. Thus, in the event that a chemical is suspected to produce effects on cognitive functions, the possibility that such effects, instead, result indirectly from changes in sensory or motor functions must always be considered.

The entire range of behavioral functions and the tests designed to evaluate them cannot be presented here. This entry first presents the types of methods that comprise the test batteries used in screening newly developed chemicals for behavioral toxicity. While screening batteries are extremely useful in providing a preliminary assessment of adverse behavioral effects, they are less useful for elaborating the actual nature of the behavioral deficits or for yielding an understanding of their underlying behavioral and neurobiological mechanisms. For such purposes, more specific tests of various behavioral functions are utilized. Such higher-order tests, in particular those related to sensory, motor, and cognitive functions, are subsequently presented in this entry and are followed by some discussion of the testing methods utilized in experimental animals to determine adverse behavioral effects of chemicals during the course of development as well as some of the test methods unique to human populations.

Screening Batteries

Because a newly developed chemical may have effects on any of the numerous behavioral functions that comprise a behavioral repertoire, screening batteries must necessarily assess a wide variety of functions with sufficient sensitivity to suggest potential behavioral toxicity even in a single behavioral domain. These screening batteries are typically executed in studies using rats and mice and generally consist of two components: a functional observational battery (FOB) and a measure of motor activity (see below). FOBs include an array of measures, generally of unlearned or instinctive behaviors, designed to detect any indications of gross changes in reflexes and in gross motor or sensory function. Most FOBs are relatively easy to implement since there is typically no behavioral training or sophisticated equipment required for any of the behavioral measures utilized as they are carried out and scored by an experienced observer. An FOB may include measures of general integrity, such as any signs of convulsions, palpebral closure, lacrimation, piloerection, salivation, and vocalizations. In addition, assessments of sensory capability, based on measures such as response to a finger snap or a tail-pinch, righting reflex, and assessments of motor function, as evaluated by the posture or gait of the animal, catalepsy, hindlimb foot splay, forelimb and hindlimb grip strength, and the time to begin ambulating, may be included. Finally, any signs of arousal or stress can be measured, such as ease of removal and handling, the animal's response to touch or approach, and urination and defecation. In addition, certain physiological responses, including body temperature and body weight, are measured. These evaluations are sometimes carried out in two different environments: a familiar one, such as the animal's home cage, and an unfamiliar flat surface of some type. This series of measures can be made relatively rapidly on each animal, consistent with the goal of screening of new compounds across a wide range of doses. In the event that behavioral activity of the chemical is indicated in such a screening test, more advanced and specific behavioral procedures would be required to delineate the precise nature of the behavioral impairment.

One question that has arisen with respect to the use of FOBs is whether changes in only one or two of the numerous measures made are really indicative of neurotoxicity. For example, how is a change in two seemingly unrelated measures interpreted (e.g., vocalizations and hindlimb grip strength). One answer that has been suggested is that neurotoxicity would be indicated by similar changes occurring within a single behavioral domain. Thus, changes in both forelimb and hindlimb grip strength would be indicative of altered motor function. Some have contended that if the toxicant under test produces body weight changes, then any changes also observed in the FOB may simply be due to 'sickness syndrome' or general malaise of the animal, not neurotoxicity. This is not necessarily a valid conclusion, however, since body weight changes may occur totally independently of any observed FOB effects. In fact, FOB changes are often reported in the absence of any body weight changes.

Motor Function

Motor function is a critical component of human behavior because it embodies the ultimate execution of a behavioral response. The feats of highly skilled athletes provide one example of incredibly refined motor performance, but even everyday functions such as walking or driving to work depend on adequate motor capabilities. Motor behavior is not a unitary behavioral function, but rather one with many different components. Various motor responses entail such aspects as strength, coordination and endurance, precision and duration, frequency of occurrence, and for ambulation, gait and balance as well. Measurement of these different aspects of motor function obviously requires different procedures. As is the case for measurement of virtually all behavioral functions, the paradigms for assessing motor capabilities range from simple assessments to more complex technologies. The former provide easily implemented but generally less specific and selective measures of function and the more advanced procedures provide specific measures of motor function as distinct from changes in sensory or motivational processes but may require some training of the experimental subject.

Motor Activity Levels

Motor activity measures the frequency of occurrence of integrated movements and/or ambulation of the organism over some designated period of time, a behavior that generally occurs at some baseline level in mammalian species and which may be altered by exposure to a toxicant. A measure of motor activity is typically one component of a screening battery, and most studies of motor activity are carried out using rodents. Generally, the animals are placed on a horizontal surface, which could be square, rectangular, or even a maze such as a T-shaped apparatus, and the number of defined movements over a specified time period are recorded. In nonautomated versions, movements are typically recorded by an

observer who, it is hoped, has no information with respect to the treatment condition (toxicant-exposed or control) of the subject which might bias the recording of data. In most automated versions as are typically used now, the movements of the animal either interrupt a light beam or trip a switch which then records a count.

Measurements of motor activity have been used to evaluate the potential central nervous system (CNS) effects of a wide variety of drugs and toxicants. One of the advantages of such measures of motor function is that no training is required of the subject. In addition, measures of motor activity can be made repeatedly across time so that the time course, including the onset and reversibility of toxicant effects, can be determined. In these types of repeated measurement experiments, moreover, an animal can serve as its own control, meaning that the experimenter looks for a change in the animal's normal pattern of motor activity after receiving the toxicant compared to the pattern observed before the treatment.

The experimenter must be cognizant of the fact that different devices for measuring motor activity may measure markedly different aspects of motor function. For example, in devices such as a figure-eight maze, an animal may rear up on its hindlimbs in front of a light beam. This response will break the beam of light and be counted as a response. In contrast, in devices like the open field shown in **Figure 1**, the investigator tallies the number of squares in a rectangular field entered by the animal with all four paws, and a rearing response might not be counted. Furthermore, beam height may differ in different devices and thus capture different aspects of behavior. Such differences can preclude the direct comparisons of various studies of the effects of a toxicant on motor activity and also underscore the importance of precise specifications of the response(s) being recorded in any given study with a particular device. Failure to do so may result in seemingly inconsistent results. Other influences must also be considered in the interpretation of changes in motor activity. For example, motor activity levels are known to be influenced by a variety of nonmotoric variables, such as time of day at which testing is carried out (rodents are nocturnal and show greater activity levels during dark hours), room lighting, and odors. As this list indicates, changes in sensory capabilities (perceived difference in the room odors or lighting) or circadian (nocturnal) rhythms could influence measures of motor activity independently of any direct toxicant-induced changes.

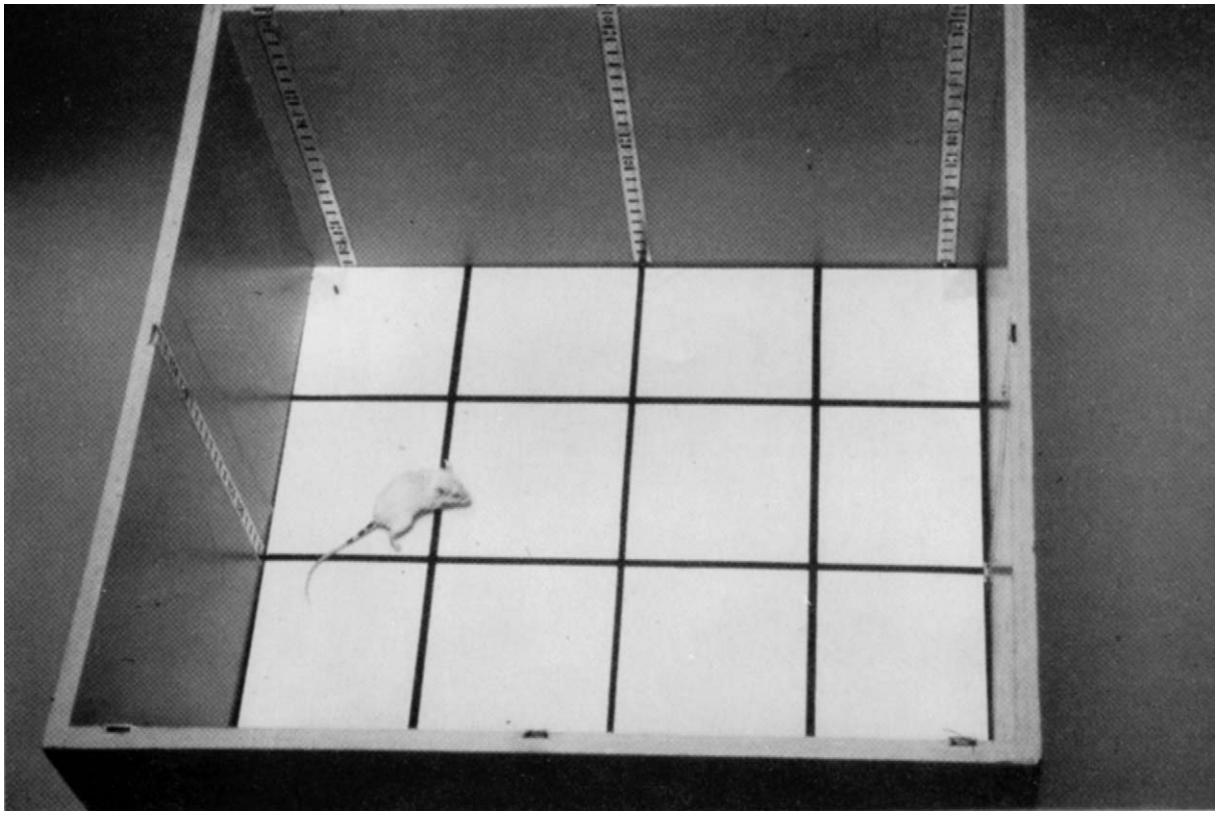

Figure 1 Open field apparatus in which the number of squares entered by the mouse or rat is counted over some fixed period of time. (Reproduced from Cory-Slechta DA (1989) Behavioral measures of neurotoxicity. *Neurotoxicology* 10: 271–296, with permission.)

Motor activity may be an insensitive measure of toxicant-induced changes when it relies on relatively gross measures such as total counts per unit time. For example, in the open field test mentioned previously and shown in **Figure 1**, the total number of squares entered into by the animal over a period of time is measured. However, the same total number may be achieved through very different patterns of behavior. For example, the organism might show an initial period of rapid movement followed by immobility or, alternatively, cycles of high activity followed by low activity or, finally, even a continuous but moderate rate of ambulation. All three could lead to the same total number of squares entered, but the disparate patterns suggest differences in behavior that are not being captured.

Strength, Coordination, and Endurance

Weakness and fatigue are common complaints resulting from exposures to a number of different chemicals. Both simple and more complex approaches to measuring these facets of motor function are available. A simple and commonly used procedure that has the advantage of not requiring any specific training of the animal is the rotarod device shown in **Figure 2**. A rat or mouse is placed on a rotating cylinder, the speed of which can be manipulated, and the time the animal remains on the rotating device before falling onto the plate below is recorded. Falling off more quickly may be an indication of changes in coordination and/or endurance. As with motor activity, time spent on the rotarod can be measured repeatedly, and a stable baseline performance can be generated across experimental sessions against which the impact of toxicants may be compared.

The difficulties with such an approach are also evident in **Figure 2**. Mice frequently attempt to scramble up the dividers; some attempt to run backwards. Others begin to jump off the device and will not remain on the device regardless of being repeatedly placed back on the rotarod. As these examples indicate, the rotarod device thus measures aspects of behavior in addition to coordination and endurance which must obviously be considered in interpreting such data. In other words, one cannot necessarily be certain that decreased time spent on a rotarod after toxicant treatment necessarily reflects changes in endurance and coordination or, for

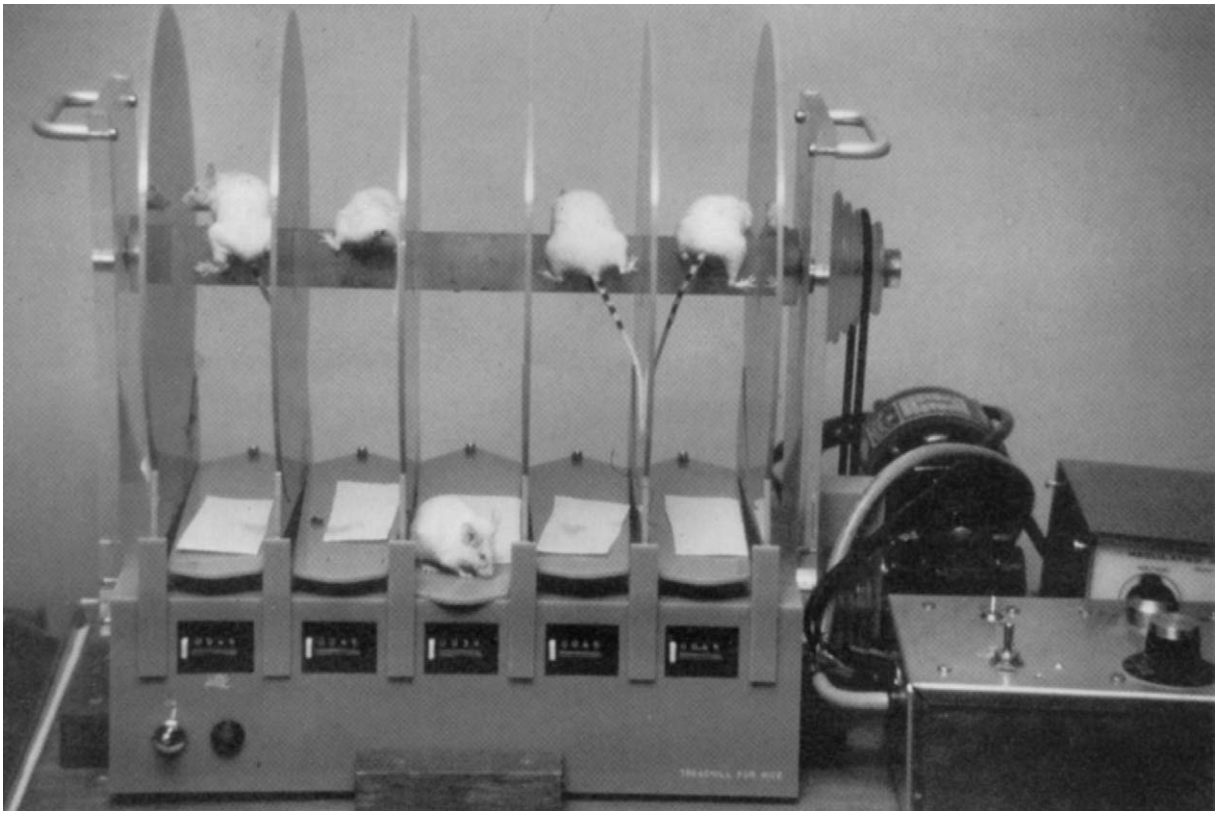

Figure 2 Illustration of the rotarod apparatus for mice. Each mouse is placed on the rotating cylinder; speed of revolution can be manipulated and time on rotarod typically constitutes the dependent variable of interest. (Reproduced from Cory-Slechta DA (1989) Behavioral measures of neurotoxicity. *Neurotoxicology* 10: 271–296, with permission.)

example, whether it could reflect, for example, increased distraction.

More advanced techniques that rely on learned behavior of animals (i.e., operant behavior) can provide controls for such nonmotoric behavioral factors and thus provide a more specific indication of changes in endurance and coordination. Rats can be trained to depress a lever with a specified amount of force in order to obtain a reward, for example, food delivery. The amount of force required to depress the lever can then be successively increased until the maximal force that can be exerted is reached. In addition, the force that the animal can sustain over time can also be measured as an indication of endurance. The ability to manipulate reward conditions facilitates the ability to differentiate motoric impairments from motivational deficits.

Gait and Balance

Walking, running, and many other motor responses depend on intact gait and balance, and such functions may be particularly vulnerable to chemicals that affect the peripheral nervous system. One simple procedure that has been devised to assess postural dysfunction is known as hindlimb splay. In this procedure, the hindpaws of a mouse or rat are dipped in ink and the animal is then dropped from a fixed height onto a piece of paper below as can be seen in **Figure 3**. An increase in the distance between the hindlimbs upon landing is indicative of damage to the peripheral nervous system with consequent effects on gait and ambulation. This approach is simple in that the rodent does not have to be specifically trained for the task, and this measurement can be made repeatedly across time without extensive equipment requirements so that time to onset and recovery of a toxicant's effects can be followed. However, hindlimb splay may not be a totally specific measure of altered motor function. Sensory disturbances, for example, might alter landing foot distance as well.

A more advanced type of approach, an automated hindlimb movement detection apparatus, is shown in **Figure 4**. In this scheme, a TV–microprocessor system is utilized to record the placement of a rat's hindpaws as it traverses from one rung to the next in a running wheel analogous to those offered in pet stores for rodents. Computer analysis of the recording provides a measurement of both quantitative and temporal characteristics of stepping, such as correct small steps and large steps, missteps, and the temporal parameters of these movements. Thus, an experimenter can measure with great precision how different parameters of gait differ before and after exposure to a toxicant. The animal need not be explicitly trained, and this approach provides a relatively specific measure of motor function *per se*. Procedures for measuring bodily sway in children have also been used in behavioral toxicology studies. In these procedures, the child stands on foam or on a hard surface under conditions of either eyes opened or closed, and the extent of the sway of his or her body is measured utilizing strain gauges.

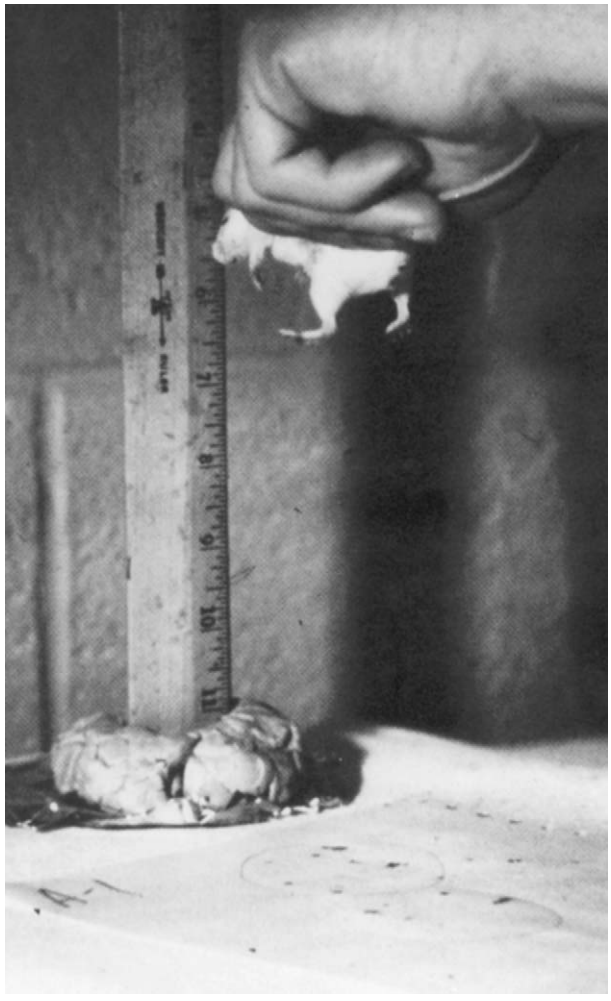

Figure 3 Illustration of the hind-limb splay procedure: the hind paws of the mouse are dipped in ink, the animal is then dropped from a fixed height onto a piece of paper and the distance between some parameter of the hindlimbs is measured. (Reproduced from Cory-Slechta DA (1989) Behavioral measures of neurotoxicity. *Neurotoxicology* 10: 271–296, with permission.)

Sensory Function

A wide range of sensory functions provide us with information about the environment. These functions include our abilities to hear, see, smell, and detect

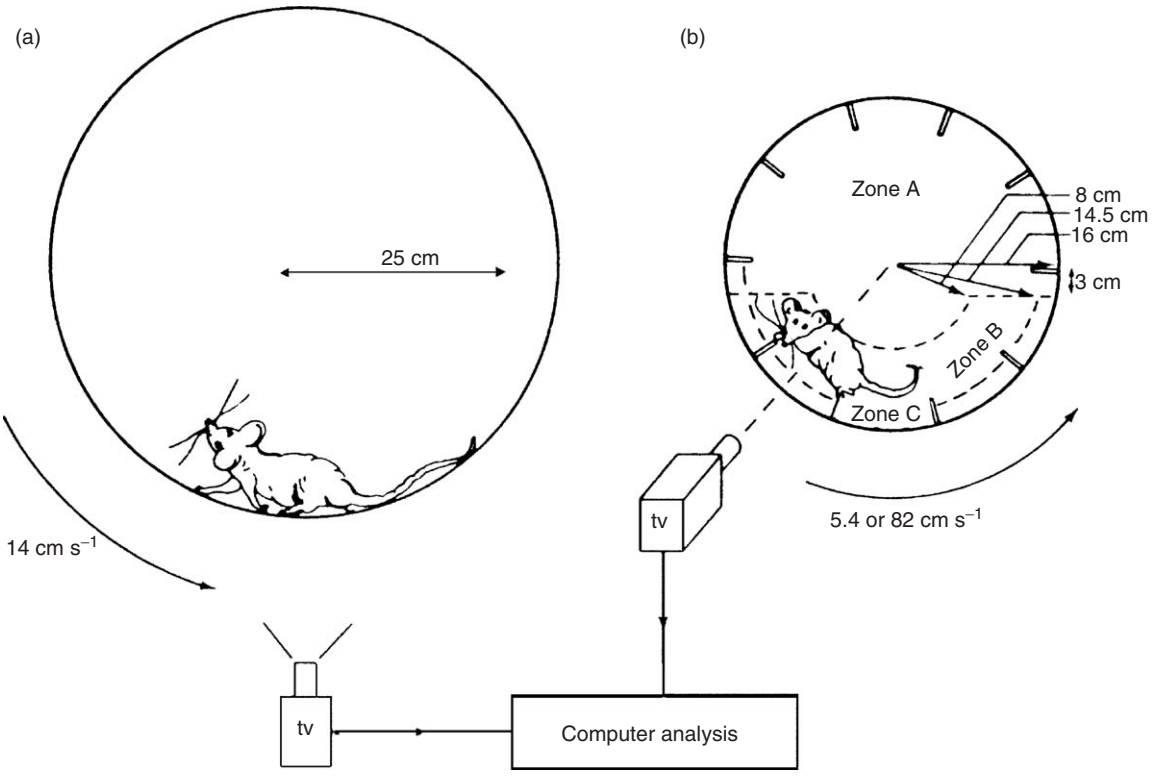

Figure 4 Automated hind-limb movement apparatus. (a) The camera can register the movements of the dyed soles of the paws of the rat from below. (b) The wheel with a transparent front facing the axially mounted color TV camera. (Reproduced from Tanger HJ, Vanwersch RAP, and Wolthius OL (1984) Automated quantitative analysis of coordinated locomotor behaviour in rats. *Journal of Neuroscience Methods* 10: 237–245, with permission from Elsevier.)

movement, vibration, and pain. Deficits in sensory function sometimes constitute some of the earliest or even the most pronounced manifestations of chemical exposures that affect the nervous system. As with the measurement of motor function, both simple and more advanced procedures are available to measure sensory function. Since almost all sensory procedures require the subject to make motor responses to indicate whether it has detected some sensory stimulus, changes in motor capabilities could conceivably be measured instead of sensory changes. Thus, while the simple procedures do not require any training of the subject, the experimenter must recognize that any changes measured may be due to changes in sensory function or motor function or both. The more complex techniques not only require training of the subject before it is possible to measure sensory function, but they also offer the possibility of differentiating the contributions of motor abnormalities from sensory changes. The more advanced procedures can also be used across species, including rats, nonhuman primates, and humans, thus alleviating some of the questions that arise with respect to the extrapolation of findings across species.

In most procedures used to evaluate sensory function, a sensory stimulus is presented to the subject, and a response by the subject, either learned or unlearned depending on the specific procedure, then indicates whether the subject has detected that stimulus. The stimulus may vary from one presentation to the next in its important dimensions such as frequency and intensity, yielding a complete profile of sensory capabilities for that specific sensory modality. For example, in measuring hearing, tones differing in their loudness and pitch are used so that hearing along the entire spectrum is measured.

A clinical neurological examination often includes components designed to measure sensory function, but relatively speaking, these tend to be less sensitive; thus, subtle changes in sensory function might not be detected. One of the simpler experimental procedures used to test sensory function is referred to as reflex modification and is based on unlearned reflexes, in particular the startle reflex. A stimulus such as a loud noise can elicit a startle response (i.e., a startle reflex). It is also known that a stimulus presented prior to the presentation of that loud noise (a prestimulus) can measurably decrease the magnitude of the startle response. Thus, a prestimulus is detectable if it decreases the magnitude of the subsequent startle response. The prestimulus can be varied in intensity and frequency dimensions during a testing session to

produce a complete profile of sensory changes in a particular modality. For example, across the trials of a test session, the intensity and frequency of an auditory stimulus can be modified, and a threshold (e.g., the intensity for a given tone frequency that inhibits startle on 50% of its presentations) can be determined for each tone frequency, generating a classical audiogram. The advantages of reflex modification include its utility across different stimulus modalities (e.g., visual, auditory, and proprioceptive), its utility across species, and the absence of any requirement for training subjects. This approach has already been successful in revealing auditory impairments resulting from exposures to neurotoxic compounds such as trimethyltin and PCBs.

One factor that must be considered in reflex modification procedures is that a less pronounced startle response could result from alterations in motor function *per se* rather than deficits in the subject's ability to detect the prestimulus. For this reason, it is imperative that some trials be interspersed throughout each test session in which no prestimulus is presented, only the startle stimulus. This allows the experimenter to determine whether the magnitude of the startle response remains constant after a chemical has been administered. If so, then any changes in the amplitude of the startle response during prestimulus trials necessarily reflect altered sensory function. Another caution regarding this procedure is that the startle reflex itself may diminish over time. Thus, the number of trials in an experimental session must be carefully controlled.

The more advanced methods for the measurement of chemical-induced changes in sensory function are termed operant psychophysical procedures. These methods have been used in almost identical forms across a range of sensory modalities and in numerous species, including rodents (rats and guinea-pigs), chinchillas, pigeons, nonhuman primates, and humans. **Figure 5** depicts an example of both a human and a nonhuman primate being tested for sensitivity to a vibratory stimulus presented to the hand using operant psychophysical methods. Here the subject is typically required to make a specified response within

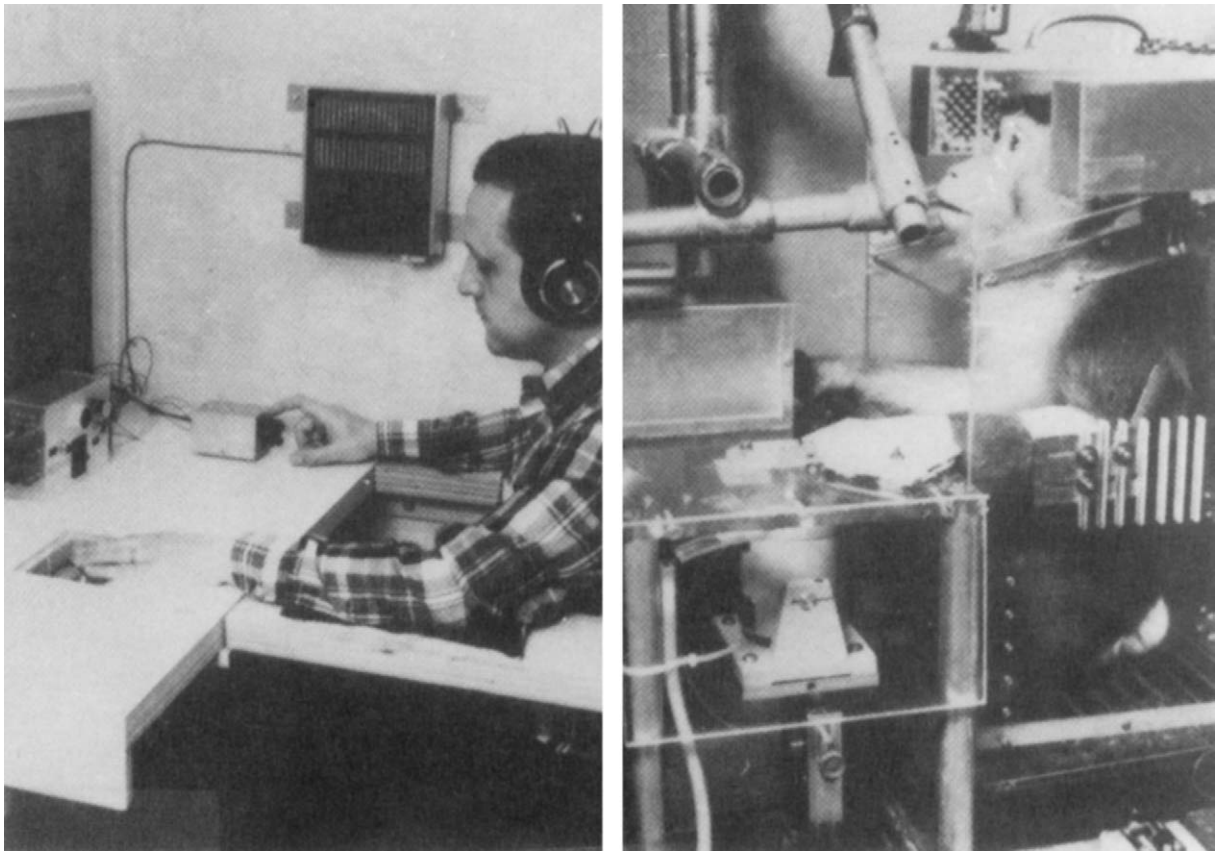

Figure 5 Photograph of a human and nonhuman primate working on the vibration sensitivity paradigm. In each case, the left hand of the subject is placed atop a device that delivers the vibratory stimulus, while the right hand holds down a telegraph key to be released when the subject detects the vibratory stimulus. A spout at the level of the monkey's mouth delivers a squirt of fruit juice for a correct response. (Reproduced from Maurissen JPJ (1979) Effects of toxicants on the somatosensory system. *Neurotoxicology and Teratology* 1(Suppl.): 22–31, with permission from Elsevier.)

some designated period of time to signify that a stimulus presentation was detected. Experimental training of the subject is required before any sensory capabilities can be precisely gauged. In **Figure 5**, the subjects were required to hold down a key when a tone sounded. If they detected a vibratory stimulus delivered to the fingertips during the tone delivery, they released the key and received a reward. To determine how much subjects were simply guessing as to whether a vibratory stimulus was presented, some trials involved no vibratory stimulus presentation. On those trials, the subjects were rewarded for releasing the key only after the tone ended, to indicate that they had detected no vibratory stimulus. As with measures of sensory function such as reflex modification, the various parameters of the sensory modality being evaluated are varied from trial to trial (e.g., intensity, magnitude, and frequency) allowing a determination of that specific sensory function along its significant dimensions. Since changes in sensory function may sometimes be quite selective (e.g., hearing loss for high frequency tones but not low frequency tones), the ability to map sensory changes along the entire spectrum of its significant dimensions is an important component of these methods.

There are several different variations of the methods by which stimuli are presented in the operant psychophysical procedures. In the method of constant stimuli, the subject is presented with several different values (e.g., intensities) of the stimulus in a random sequence or order across trials. The proportion of stimulus presentations detected at each intensity is then calculated, and the value yielding a 50% detection response is deemed the threshold. The method of limits presents a series of stimulus intensities which begin either well above or well below the presumed threshold value. The stimulus value is then either progressively decreased or increased, respectively, until a change in the subject's ability to report the stimulus presentation occurs. The intensity of the stimulus at which this change in detectability occurs is designated as the threshold. In the up-and-down, staircase, or titration method of stimulus presentation, the threshold is continuously tracked by raising or lowering the stimulus intensity depending on whether the subject correctly detected the stimulus. If the subject fails to detect the stimulus, presumably because it is below the threshold for detection, the intensity is raised on the next trial; if the stimulus was detected (i.e., was above threshold), the intensity is then lowered on the next trial. In this fashion, the stimulus intensity can be titrated around the threshold value of the subject.

One of the advantages of operant psychophysical procedures over methods such as reflex modification is that stimulus presentation and subsequent responses occur on a continuous or response-dependent basis. In other words, a response of the subject is recorded, and the next stimulus is presented. In the reflex modification procedure, stimuli are presented during trials which are experimenter initiated and which are separated by specified time interval. The continuous procedures permit the experimenter to measure the rate of responding over time and the time required to respond following stimulus presentations (latency). These measures provide the experimenter with information as to any possible motor dysfunction or motivational problems that the subject may experience as a result of chemical administration which could contribute to behavioral changes in operant psychophysical procedures. Motor dysfunction might increase the latency to respond following stimulus presentations, while an unmotivated subject might be expected to show periods of nonresponding. Armed with this information, the experimenter can proceed to determine which behavioral changes result from true sensory loss.

Cognitive Function

One of the major concerns aroused by exposures to chemicals that affect the nervous system is their potential to adversely impact cognitive functions such as learning and memory. Such a concern certainly has precedent. Lead exposure at high levels can leave children with permanent mental retardation. Recently, it has been demonstrated that even very low levels of lead exposure (i.e., environmental exposures) can produce subtle changes in cognitive processes. Pesticides are known to exert pronounced effects on cholinergic neurotransmitter systems, the very system that has been repeatedly implicated as a causative factor in Alzheimer's disease.

Learning

Learning might be defined simply as an enduring change in behavior that results from experience with changes in environmental events. As a topic of long historical interest in psychology and neuroscience, there are numerous different methods that have been applied to the study of learning ranging from the relatively simple to the more complex and advanced paradigms. Methods for assessing learning involve the processing of sensory stimuli, the execution of motor responses, and a motivated subject. Difficulties in distinguishing the contributions of sensory, motor, and motivational deficits from learning deficits are frequently encountered when using relatively simple learning paradigms. Some of the more complex

procedures are designed to specifically differentiate such functions from learning and thus allow the experimenter to determine whether the chemical has specific effects on learning *per se* as distinct from changes in sensory or motor function, motivational levels, or other nonspecific behavioral alterations.

Many of the earliest studies of learning utilized rats as experimental subjects and required them to run mazes of various shapes and sorts, generally from a start box to a goal box where some type of food reward (reinforcement) was available. For example, in a T-maze, named because of its shape, the subject is reinforced for running from the start box at the base of the T to that arm of the T which has been designated as the correct arm and contains the goal box where food is located. The designation of which arm (stimulus) is correct may be based on side (the right side is correct), color (the arm painted black), or some other stimulus feature. Choosing the wrong arm at the choice point means no reinforcement. After entering an arm and either being reinforced or not, the animal is removed, and after a period of time (the intertrial interval) the animal is placed back in the goal box and another trial initiated. Learning under such conditions is typically measured as the number of trials required to reach a specified accuracy level or until behavior reaches a stable level of accuracy, at which point it is stated that the subject has learned to 'discriminate' between the correct and incorrect arm. The experimenter may compare two groups of rats in such an experiment: one treated with a chemical and one not treated, with the latter serving as a control group indicating 'normal' performance under the particular experimental conditions.

More complex versions of mazes soon emerged in response to the need for more difficult tasks because the T-maze was a relatively simple problem for a rodent to solve and thus not always adequately sensitive to effects of drugs or chemicals. Moreover, once the animal learned which was the correct arm, learning is no longer being measured, only the performance of an already learned response. Two different approaches were offered to circumvent these limitations. One was the construction of more complicated mazes, such as the Hebbs–William maze, which is actually a series of mazes. The correct route to the goal box in this device can be modified as needed by moving the various arms and boundaries into new configurations and thus requiring the subject to learn a new problem, allowing a repeated assessment of learning.

A second approach is embodied in reversal learning. Using this approach, the correct and incorrect arms (stimuli) in the maze are reversed after the subject initially learns which is correct. For example, after the rat learns to run to the right arm of the maze with 90% accuracy, the discrimination is reversed, such that the left arm of the maze is now the rewarded arm. After criterion accuracy is achieved following this reversal, the designation of correct and incorrect stimuli may be reversed again, allowing the repeated measurement of learning over time. Eventually, however, this scheme is also learned by the subject, a phenomenon known as 'learning to learn', such that it comes to learn each successive reversal problem with maximal efficiency (i.e., after only one or two trials).

All maze procedures have limitations that must be considered when interpreting data obtained with these methods. One related particularly to their use with rodents is that subjects leave an odor trail in the maze that can influence the behavior of rodents subsequently tested in the maze. While the experimenter can clean the maze between subjects, it must also be noted that the rodent's sense of smell is much more sensitive than humans, making it difficult to be certain that indeed no odors are still present. Another potential problem is that these procedures obviously require interactions between the experimenter and the subject during the course of testing because the rat must be constantly retrieved from the arms and replaced in the start box. This raises the distinct possibility of both subject and experimenter bias, unless the experiment can be carried out by an individual with no knowledge of any treatment (e.g., exposure to drug or chemical) of any subjects.

Another limitation of simple maze methods for assessing learning is that they do not selectively measure learning. While a decrease in the speed of reaching a 90% accuracy level may be observed in response to a chemical treatment, it may not necessarily be due to alterations in cognitive processing, since changes in either motor performance or sensory capabilities may impact performance in the maze, altering learning independently of any real cognitive changes. Impaired motor function may increase the time taken to reach the goal box; delayed reward is known to impair learning. Further sensory deficits may cause the subject to be unable to utilize the environmental stimuli that normally guide its path to the goal box. Motivational changes (e.g., if the reward becomes less appealing) may clearly retard the rate at which learning occurs. Changes in motor, sensory, and motivational functions as potential contributors to the observed effect may then have to be ruled out in separate additional experiments.

The water maze is a method increasingly being used in behavioral studies for evaluating learning. Used typically with rodents, the subject is placed in a large tub of water made opaque by the addition of a

substance such as nonfat milk powder. Since most laboratory rodents do not prefer water, the reward is escaping from the water by locating a platform submerged just below the surface of the water which is not visible to the subject. Learning is measured as a decrease in the time to locate the hidden platform across successive trials; a learning deficit is suggested by a slower decrease in that time requirement or a greater number of trials to reliably locate the platform. The procedure is relatively simple and imposes no food restriction on the subject. However, despite its ostensible simplicity, it suffers from many of the same limitations as nonwater-based mazes. First, the procedure is not fully automated and thus requires subject–experimenter interaction which can introduce bias into the results. Furthermore, since it is a relatively simple problem, the maze may be learned rapidly and, thus, it is of little utility for experiments aimed at understanding the time course of a chemical's effects on learning. This problem can be alleviated to some extent by moving the platform to a new location each time the subject has mastered the previous location. Although one might suspect that odor trails would not be a factor in a water maze, it has indeed been shown that odors are present and can be utilized by other subjects later placed in the maze. In addition, water temperature plays an important role in this task since age-related deficits in learning in the maze can be alleviated by warming up old rats between trials.

Finally, a rat that requires a greater number of trials or exhibits a slower decrease in the time to locate the platform following chemical treatment is not necessarily exhibiting a learning deficit. Changes in motor capabilities may affect swimming performance and thus lengthen the time it takes the subject to swim to the submerged platform, even if it knows the location of the platform. It is known that subjects rely on environmental cues to find the platform; thus, changes in sensory capabilities could mean an inability of the subject to detect the necessary environmental cues, a deficit which would also increase the length of time the subject required to reach the platform. These alternative explanations of any deficits in swimming time must be ruled out by additional experiments before one can reasonably conclude that a cognitive impairment is present. One way to achieve this is to have the same subjects, in other circumstances, simply be required to swim to a platform, the location of which never changed. This response would also require intact motor and sensory capabilities in performing the same response but no learning since the platform location remains constant. The observation of treatment effects when the platform is moved around, in the absence of any treatment effects when the platform remains fixed, would provide support for an interpretation that the chemical induced learning deficits.

Another maze procedure frequently utilized to evaluate learning (and memory) with rodents is the radial-arm maze. The device itself consists of a central circular area from which eight arms radiate outward like the spokes of a wheel. At the end of each of the eight arms is some type of reinforcer, usually a food pellet. In essence, the subject has access to eight reinforcement deliveries, one in each of the eight arms, and the accuracy and speed (efficiency) with which the subject learns to retrieve all eight reinforcer deliveries is measured. Obviously, under these conditions, the most efficient performance is to obtain all eight reinforcements without revisiting an arm from which the food has already been obtained. The measure of learning is the number of trials required for the subject to reach some specified level of efficiency in the maze. One way of further increasing task difficulty is to provide reinforcement only in a specified number of the arms (e.g., four of the eight) and to change which of the arms provides reinforcement over time or trials. The radial-arm maze obviously presents a more difficult problem to the subject than the T-maze or other simple mazes, but the possibility of interference from motor or sensory deficits produced by chemical treatment still remains. Thus, an increase in the number of trials to reach efficient performance is not necessarily indicative of a learning deficit with this method. By measuring the time of entry into each arm, investigators can begin to get some indication of whether changes in overall activity levels are affecting performance.

In addition to mazes, learning can be measured in Skinner boxes, also known as operant chambers, and these types of approaches have been used across a variety of species, notably rodents, pigeons, nonhuman primates, and humans. In such chambers are some type of response device, speakers, and/or lights for presentation of auditory or visual stimuli, respectively, as well as some type of reinforcement delivery device. An operant chamber configured for a rat is shown in **Figure 6**. Discrimination paradigms are among the simplest measures of learning in operant chambers. In such procedures, a response is reinforced in the presence of one stimulus, the 'correct' stimulus, but not in the presence of another stimulus. Accuracy is defined as the percentage of the total responses that occur in association with the correct stimulus, and learning can be assessed as the number of experimental sessions required by the subject to achieve a criterion level of accuracy.

One distinct advantage of discrimination paradigms in the operant chamber is that behavior can

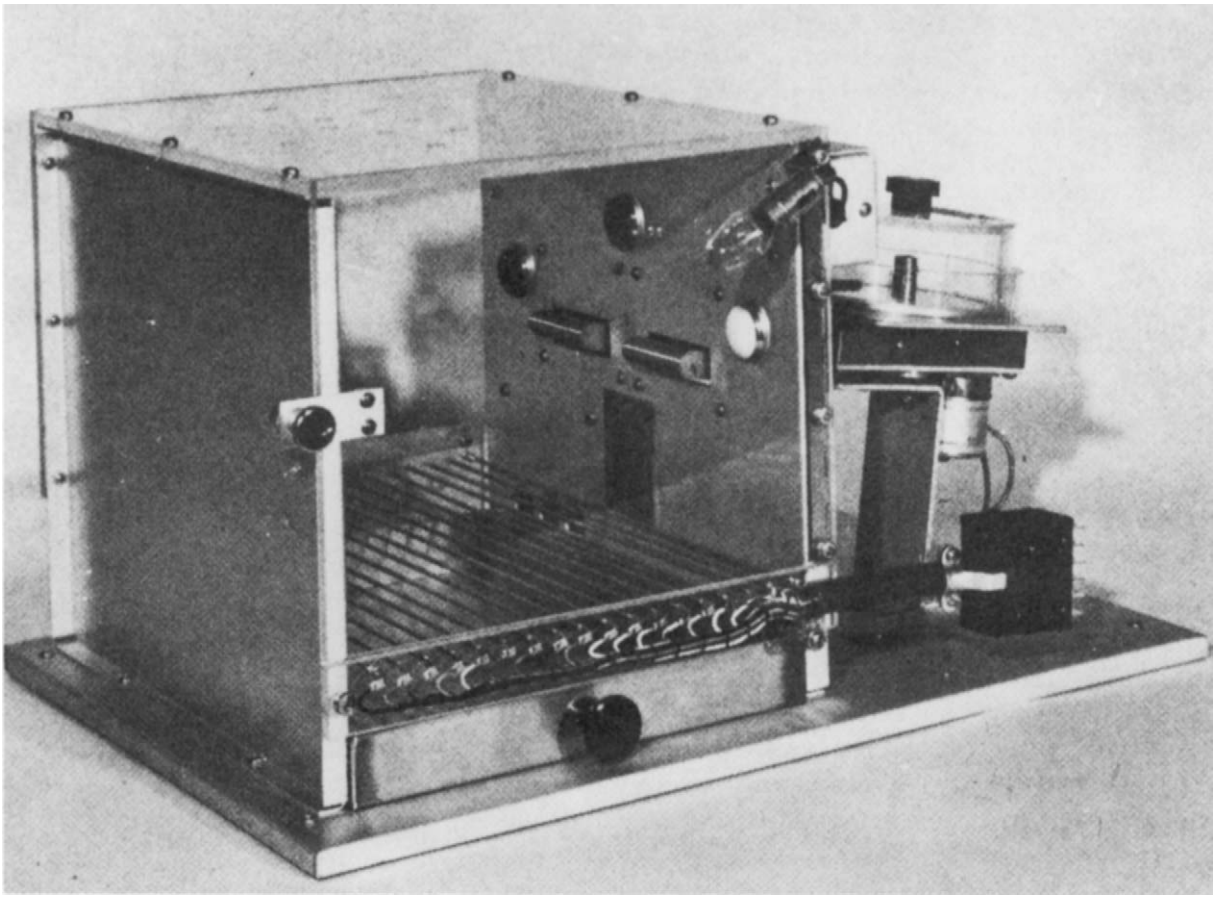

Figure 6 An operant chamber or Skinner box for a rodent. The front wall (right) shows two response levers, below which is situated a food pellet trough. Food pellets are dispensed from a feeder located behind the front wall into the pellet trough. Above and to the left of the levers are two keys which can be illuminated with various colors and used for external or environmental stimuli. Above and to the right of the rightmost lever is a speaker through which auditory stimuli can be projected and used as external (environmental) stimuli. (Reproduced from Reynolds GS (1968) *A Primer of Operant Conditioning.* Glenview. IL: Scott Foresman.)

occur at any time (i.e., the frequency with which it occurs is not constrained by trials as necessitated by the requirement of moving the animal from the goal box back to the start box in most maze-based methods). When responding can occur at any time, the rate or frequency of responding over time can be measured and used to gauge the possibility that motor deficits or motivational insufficiencies may contribute to any observed changes in learning accuracy. A decrease or slow-down in rate of responding would suggest such possibilities.

Another advantage of operant chamber-based procedures is the enormous flexibility they provide for behavioral assessment and the ease with which they can be carried out in these devices. For example, conditional discrimination problems, which are more difficult discrimination tasks, can be easily implemented in operant chambers. Matching to sample is one such method. In this task, the subject first makes a designated response to indicate that it is attending to a sample stimulus that is presented for a short period of time. Subsequently, a sample stimulus is presented for a short period of time. This is followed, after an interval of time, by the presentation of two or more stimuli, and reinforcement is contingent upon a response to the stimulus that matches the sample stimulus. The accuracy and speed with which the subject learns to match the sample stimulus is, of course, the measure of learning. Such tasks can be used with different species by simply increasing or decreasing the number of choice stimuli or the similarity of the stimuli appropriately. Because the procedure includes an initiating response on the part of the subject, a measure of rate of responding is possible, again providing information on potential motoric or motivational contributions to any deficits observed in matching accuracy.

Even the more complex matching to sample discrimination problems are eventually mastered by the subject, in which case discrimination reversals may be implemented in which the stimuli associated with

reinforcement and nonreinforcement are repeatedly switched. Eventually, however, the subjects will learn the reversal concept as well, such that they come to solve each reversal problem with maximal efficiency; that is, on the basis of only one or two responses (e.g., which stimulus is correct today?).

One of the most advanced methods for the assessment of learning is the repeated learning paradigm, also called repeated acquisition, sequence acquisition, or response sequence learning. It specifically addresses the limitations discussed previously. This method actually originated for the measurement of learning in human subjects and has since been adapted for a variety of species. In repeated learning, the subject must make a sequence of responses for reinforcement, and the correct sequence changes with each successive experimental test session. Because the procedure thus requires subjects to learn a new sequence of responses each day, learning can be measured repeatedly across time. A high rate of errors is typically evident during the early part of each test session, as the subject begins to learn the correct sequence for the specific session. The error rate gradually declines as the session progresses, and reinforcers for completing the correct sequence of responses occur at an increasing rate. The ability to measure learning repeatedly across time with this task provides the basis for the measurement of the time course of a chemical's effects (i.e., the time to onset of any learning disabilities and their potential reversibility). This is a particular advantage in situations where chemical exposure occurs in a chronic fashion.

The control for changes in sensory, motor, motivational, or other nonspecific behavioral changes as potential contributors to apparent chemical-induced learning impairments comes when the repeated acquisition task is run in conjunction with a 'performance' task in what is known as a multiple schedule format. The performance component also requires the subject to emit a sequence of responses for reward, but in this case, the sequence of responses stays constant across time. Thus, the subject simply performs an already learned response sequence. In the multiple schedule format, the repeated learning and performance components are presented alternately during the course of the experimental session, with a transition between them occurring either on the basis of time or on the number of reinforcers the subject has earned (e.g., after 15 min or 30 food deliveries switch from repeated learning to performance). Thus, during some portions of the test session, the subject is responding on the repeated acquisition task, while at other times during the session, the performance baseline is operative. Typically, different environmental stimuli, such as different colored lights, are used to indicate to the subject whether the performance or the repeated learning component is in effect.

Both the repeated learning and the performance tasks require intact motor and sensory capabilities, as well as appropriately motivated subjects. Learning *per se* is only required during the repeated acquisition task; the performance task simply requires completion of an already learned response sequence. Thus, if a toxicant or treatment has selective effects on learning *per se*, impairments in accuracy should only be evident during the repeated learning components of the session. If these changes arise, however, as a result of nonspecific behavioral changes (i.e., from sensory, motor, or motivational impairments), then accuracy impairments would be expected in both the repeated learning and performance components of the session since both require these behavioral capabilities. The elegance of this technique derives not only from its ability to distinguish learning effects from other types of behavioral changes but also from its ability to do so in the same subject during the same test session.

Behavior of a normal rat under these conditions is depicted in the top of **Figure 7**. In this diagram, the top tracing shows correct responses, which cumulate vertically; time is represented horizontally. P indicates the performance components of the session, whereas A indicates the repeated learning components. This 1 h behavioral test session began with a performance component and was followed by the repeated learning component, once again by the performance component, and finally by the repeated learning component. Illumination of lights in the operant chamber signaled to the subject that the performance component was operative, while turning out the lights signaled the repeated learning component was in effect. Each short pip mark in the top tracing indicates where the rat earned a food delivery for correctly completing the sequence of three responses required by the schedule. The bottom tracing shows the concurrent errors that occurred.

As **Figure 7** shows, this well-trained rat exhibited a relatively high level of accuracy during the first performance component, earning a steady rate of food rewards and making few errors. The switch to the repeated learning component is accompanied by an increase in errors and a decline in the number of food rewards earned, as the subject begins to learn the correct sequence of three responses for this specific session. Behavior during the second performance component is again composed of a steady rate of food rewards and the occurrence of relatively few errors. The second presentation of the repeated learning component is marked by both a gradual increase in the rate at which food rewards were earned and a

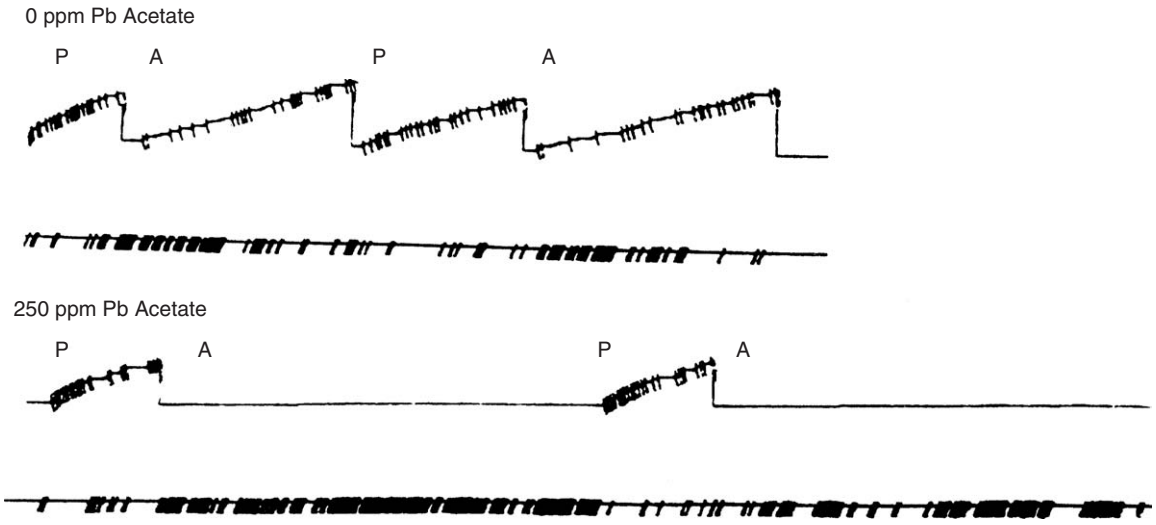

Figure 7 Behavior of a control rat (top) and a rat exposed to 250 ppm lead acetate in drinking water from weaning (bottom) on a multiple repeated acquisition (A; repeated learning) and performance (P) schedule of reinforcement. The top line of each record shows correct responses cumulating vertically with pips indicating food delivery for the completion of the correct sequence of responses; the bottom line shows errors. Time is represented horizontally. (Reproduced from Cohn and Cory-Slechta, unpublished data, with permission.)

decrease in the number of errors relative to levels occurring in the first presentation of the repeated learning component, consistent with a gradual learning of the correct sequence for this session.

The bottom set of tracings shows behavior under the same conditions for a rat that has been exposed from weaning to a relatively low level of lead in drinking water. It shows, in a rather dramatic fashion, a selective effect of lead on learning processes *per se*, as distinct from nonspecific behavioral changes. Specifically, behavior during both presentations of the performance component is unimpaired in that a substantial rate of food deliveries and a minimal rate of errors is evidenced. In contrast, there is no evidence of learning during either presentation of the repeated learning component of the schedule, in that virtually no food deliveries were obtained and a very high rate of errors was sustained. In fact, the rat continued to make errors throughout the entire time in the repeated learning components. Thus, in this case, the effects of lead on accuracy were restricted to the repeated learning component of the schedule. These impairments could not have resulted from deficits in motor or sensory function, or in appropriate motivation, since behavior in the performance components, which also required such functions, was perfectly normal.

Although an effect of a toxicant on behavior during the repeated learning but not during the performance component of a multiple schedule is strong evidence of a chemical's selective effects on cognitive functions, there are other factors that should be taken into consideration. Some investigators subscribe to the idea that a selective effect of a chemical on learning means that its effects should be evident across a variety of learning tasks. While this notion has some validity, it should not be considered a necessary condition since, as has already been described, all learning paradigms are not equal. The extent to which different learning tasks selectively measure learning *per se*, as distinct from sensory, motor, or motivational influences, clearly differs, as does the possible 'contamination' of the learning measure by changes in other behavioral properties. This is not to diminish the importance of these other types of behavioral effects, be they sensory or motor, for example, since such processes are clearly essential for integrated behavioral function, including cognitive functioning. Another important consideration is that the ability to detect effects of a chemical upon learning may depend to a large extent on the degree of task difficulty. It is well established that learning tasks that are relatively easy (i.e., those resulting in relatively high levels of accuracy) will be less sensitive to disruption either by drugs or by toxicants than are tasks of greater difficulty.

Memory

Memory, or remembering, is behavioral recall (i.e., the preservation of learned behavior over time). A distinction is often made between what is referred to as short-term or working memory, occurring over relatively short delay periods, and long-term or reference memory, considered more permanent memory. Obviously, the temporal parameters associated

with what is designated as short- and long-term memory are species dependent.

The measurement of memory is typically based on the persistence of a previously learned response following some time delay; differences in recall accuracy are compared before and after delay intervals. Typically, the longer the delay, the greater the decrease in accuracy. An impairment of memory by a chemical accelerates the rate at which accuracy decreases with increasing delay values.

Both simple and more advanced techniques are available to evaluate memory. Again, however, many of the ostensibly simple tasks cannot differentiate memory deficits *per se* from deficits produced by changes in other behavioral functions, be they motor or sensory functions, or in level of motivation. For example, an inability to execute the response as efficiently (motor impairment) may in essence mean that the delay interval for the subject is functionally longer, thus indirectly impairing accuracy. Alternatively, a treatment which somehow increased the speed of responding could cause the subject to respond before adequately evaluating stimulus options, and thus decrease accuracy independently of a real change in remembering. Here, again, the more advanced methods include the capabilities for differentiating real effects of a chemical upon remembering from those caused by other behavioral consequences of the exposure.

One widely used simple measure of memory is passive avoidance. In this task, the subject, most often a rodent, is placed in a chamber that has two quite distinct compartments. The subject receives a shock in the compartment it prefers (spends most time in), engendering an association between the shock and the distinctive characteristics of that compartment. At some later time (i.e., after some delay interval), the subject is placed back into this two-compartment chamber, and memory is evaluated on the basis of the time (referred to as latency) that elapses before the subject steps back into the side of the chamber in which it previously received shock. The contention is that the longer the subject waits to enter that compartment, the better it remembers the shock it received there.

While changes in latency on this task are produced by a variety of drugs and chemical treatments, the interpretation of these changes can be problematic. If, for example, the chemical causes hyperactivity, the subject might reenter the shocked compartment sooner even if it does remember its association with shock. If the treatment disrupts sensory capabilities, altering perceived distinctions between the compartments, this too may result in a more rapid reentry into the compartment in which the subject had previously been shocked. If the administration of a chemical causes a sedative effect in the subject, rendering it less mobile, the time to reenter the shocked compartment may be increased relative to that seen in nontreated controls, but this would not be considered facilitation of memory. Again, such possible alternative interpretations must necessarily be worked out in additional experiments or with additional manipulations. Depending upon the experimental design, chemical treatments could alter shock sensitivity and thereby modify performance.

The more advanced procedures for memory evaluation not only require more extensive training of the subject, but they also control for some of the possible confounds mentioned previously. There are two general types of more advanced procedures for the assessment of memory. One uses the previous responding of the subject as the event to be remembered, such as in the delayed alternation paradigm. In this procedure, the subject has access to at least two response manipulanda and is required to alternate its responses on the two for reward after some delay interval ends. That is, a response on manipulanda A initiates a delay interval, after which a response on B produces reward. This event initiates another delay interval, after which a return to manipulanda A produces reward. Typically a series of delay intervals are tested in each session, with the length of the delay interval varied randomly across the trials of a session and the specific lengths of the delays appropriate to species. Responses during the delay start the delay over again, thus increasing the time to reward. On this task, then, the subject has to remember which response manipulanda it responded on before the delay interval started in order to respond correctly after the delay. Typical behavior observed under these conditions is a decrease in remembering (accuracy) as the length of the delay interval increases. A chemically induced impairment of memory would then be manifest as a more pronounced decrease in accuracy as delay length increases than is observed under nontreatment (control) conditions.

Critical to the interpretation of any memory-related deficits with the delayed alternation task is the inclusion of a zero-second or no-delay condition. The no-delay condition requires no memory, as there is no delay. Therefore, if a treatment is impairing accuracy under the no-delay condition as well as at the various delay intervals, it is likely that the effects are due to changes in behavioral processes other than remembering. The pattern of change consistent with a selective memory impairment of a toxicant, then, is one composed of no change in accuracy at the zero-second delay but a more pronounced decrease in

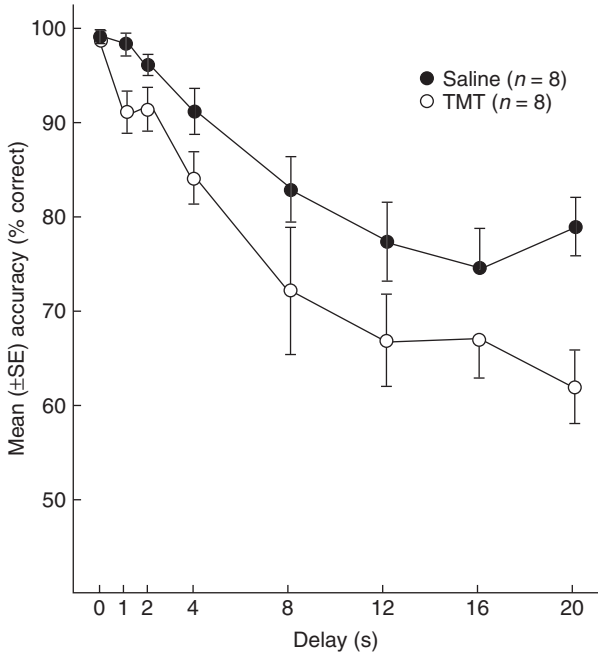

Figure 8 Effects of 7 mg kg^{-1} trimethyltin on delayed alternation performance. Lower accuracy values were evident in TMT-treated rats at all delay values, but no impairment was seen in the zero-second delay condition, consistent with a specific effect on memory function. (Reproduced from Bushnell PJ (1988) Effects of delay, intertrial interval, delay behavior and trimethyltin on spatial delayed response in rats. *Neurotoxicology and Teratology* 10: 237–244, with permission from Elsevier.)

accuracy with increasing delay values relative to nontreated control subjects.

An example of an apparently selective impairment of memory independently of changes in other behavioral processes is shown in **Figure 8**. As can be seen, the accuracy level of a group of nontreated normal rats (solid circles) declines as the delay value increases, as expected. Corresponding data for a group of rats treated with the organic metal, trimethyltin, are shown in the open circles. In this group, accuracy was unaffected at the zero-second delay but decreased more rapidly than did that of normal rats as delay value lengthened.

Other methods for measurement of memory function rely on explicit discrimination tasks. The matching to sample task described earlier is one example. In this paradigm, a sample stimulus is presented briefly to the subject. The subject must then pick the sample stimulus when subsequently presented with multiple stimulus options (i.e., the subject must match the sample). When delay intervals are imposed between the presentation of the sample stimulus and the subsequent presentation of multiple stimuli, the task becomes a memory task. In this case, the subject must remember the sample stimulus in order to perform correctly. As in the delayed alternation procedure, delay intervals of various lengths are used, including the no-delay or zero-delay condition, and a delay function similar to that shown in **Figure 8** is expected. Many of the caveats mentioned with respect to interpreting memory effects in the delayed alternation task likewise apply to the delayed matching to sample paradigm. Separation of a chemical effect arising directly from changes in memory processes rather than from changes in motor, sensory, or motivational functions depends on the inclusion of a no-delay condition. Furthermore, as with learning paradigms, the contention that if a chemical produces a true memory deficit it will be observed across different memory tasks must be tempered by the fact that not all memory paradigms produce an equally selective measure of memory.

Schedule-Controlled Operant Behavior

Learned voluntary behavior is a function of the consequences that follow it. If a response is followed by a reinforcing stimulus, the rate of that response subsequently increases; if followed by a punishing stimulus, or by the absence of a reinforcing stimulus, the rate of responding subsequently decreases. In addition to determining the subsequent frequency of that response, these consequence stimuli will also determine the intensity and temporal pattern with which that response will be emitted in the future.

In the real world, consequence stimuli do not necessarily follow every occurrence of the response. In fact, typically, consequences follow the response on an intermittent basis. Paychecks, for example, are typically distributed on a weekly, biweekly, or even monthly basis, not after each instance of work-related activity that occurs. The pianist plays the entire piece of music before the audience applauds. This strategy of intermittent reinforcement of responding actually provides greater behavioral efficiency and economy as well as greater response strength and persistence than does continuous reinforcement. A response that has been reinforced after every occurrence declines much more rapidly when reinforcement is withheld (extinction) than does one that has been reinforced on an intermittent schedule.

The term schedule of reinforcement refers to the nature of the rules governing the allocation of consequences for a particular response. Behavioral performance controlled by a schedule of reinforcement is referred to as schedule-controlled operant behavior. These schedules of reinforcement are critical because they govern the rate and pattern of responding in time which underlie other behavioral functions. For example, the rate of learning may well be influenced by the underlying schedule of reinforcement. If reinforcement of the correct response during

a learning task is too infrequent, the task may not be adequately learned or not learned at all. Likewise, remembering that response, as in a memory task, may depend on the extent to which it was sufficiently reinforced to begin with.

Consequence stimuli can occur on the basis of time elapsing or on the basis of the number of responses that have occurred or both. In the human environment, schedules of reinforcement exhibit a remarkable complexity. For the purposes of understanding how these various reinforcement schedules or payoff schemes control the frequency and the pattern of behavior in time, simpler versions were initially studied in a laboratory context. As the understanding of simple reinforcement schedules evolved, increasingly complex schedules that more closely mimicked the human environment were elaborated and examined in laboratory experiments.

One of the important aspects of schedule-controlled behavior that deserves note is the remarkable similarity of behavior patterns generated by these schedules across a wide variety of species, even when type of response and type of consequence stimuli differ – a phenomenon of obvious importance for the issue of cross-species extrapolation because it shows the similarity and contiguity of such behavioral process across species.

Simple Schedules of Reinforcement

There are four simple schedules of reinforcement: the fixed interval (FI) and the variable interval (VI), both of which are temporally based reinforcement schedules, and the fixed ratio (FR) and the variable ratio (VR) schedules, both of which are response-based schedules. The FI and the VI schedules both stipulate that a certain amount of time must elapse from the occurrence of a previously reinforced response before a response will again produce reinforcement. On the FI schedule, that time interval remains constant and the parameter value of the schedule indicates the length of that temporal interval (e.g., FI 1 min means that the first response occurring at least 1 min after the preceding reinforced response will result in reinforcement). On the VI schedule, the length of the interval varies from one interval to the next with the parameter value of the schedule indicating the average of the different interval lengths. For example, on a VI 1 min schedule, the average time between reinforcement opportunities is 1 min, but each interval may be either longer or shorter. Responses during the interval have no specific consequence attached to them on either the FI or VI schedules.

Because of the differences in the way in which they schedule reinforcement, the FI and VI control quite different rates of responding (responses per unit time) and patterns of responding, as can be seen in **Figure 9**. The FI schedule generates a characteristic 'scallop' pattern of responding, which engenders pausing, that is, little or no responding immediately after reinforcement delivery (indicated by the short pip marks), followed by a gradual increase in the rate of responding as the time of reinforcement availability again approaches. In the human environment, studying for an examination has features that are characteristic of FI performance: little or no responding early in the semester but a gradual increase as the time of the examination approaches. While one

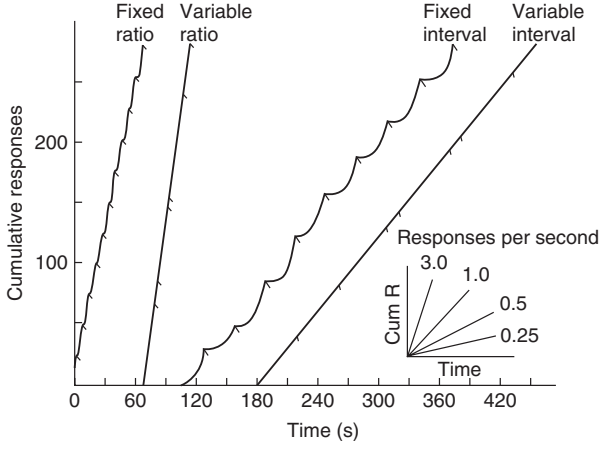

Figure 9 Schematic cumulative records of performance on the fixed ratio (FR), variable ratio (VR), fixed interval (FI), and variable interval (VI) schedules of reinforcement. Responses are cumulated vertically over time. Each downward deflection of the pen represents reinforcement delivery; horizontal lines indicate pausing. (Reproduced from Seiden LS and Dykstra LA (1977) *Psychopharmacology: A Biochemical and Behavioral Approach*. New York, NY: Van Nostrand Reinhold.)

might expect that the performance under such conditions would be characterized by a single response as soon as the interval ends, such a pattern would require the subject to have perfect timing capabilities. Responding at a very rapid rate as the end of the interval approaches ensures that reinforcement delivery will occur with minimal delay as soon as it is available.

The pattern of responding on the VI schedule differs from that on the FI (**Figure 9**) in that no pausing occurs after reinforcement delivery. Instead, the subject continues to respond at a steady and relatively uniform rate over time. The absence of pausing on the VI schedule is thought to reflect the lack of predictability of reinforcement. On the VI schedule, reinforcement may be available immediately after a previous reinforcement delivery since the interval length varies. Thus, pausing after reinforcement could result in a reduction in the rate or number of reinforcement deliveries. One example of VI-maintained behavior sometimes cited is that of getting a busy signal when calling someone on the telephone. The caller continues to redial and is eventually reinforced by a ringing sound on the other end. The persistent redialing reflects the variable length or interval of telephone conversations and, therefore, the unpredictability of when the line will no longer be busy.

In the other two simple reinforcement schedules, reinforcement availability is based on the number of occurrences of the designated response. On an FR schedule, the completion of the number of responses specified by the schedule parameter value is required for each reinforcement delivery. An FR 100 schedule, then, requires 100 occurrences of the designated response for reinforcement delivery. The classic examples of FR schedules are the piecework systems that operated in factories early in US history, where workers were paid for each piece or unit they produced. The FR schedule generates its own characteristic behavior pattern which consists of a pause or period of no responding after each reinforcement delivery, followed by an abrupt transition to a very rapid rate of responding – a pattern known as 'break and run' and shown in **Figure 9**.

A VR schedule also requires the occurrence of a designated number of responses for reinforcement delivery, but the response requirement varies from one reinforcement delivery to the next in an unpredictable fashion. The parameter value of the schedule indicates the average response requirement. Thus, on a VR 100 schedule, the average number of responses required for reinforcement is 100, but the actual number varies from one reinforcement delivery to the next. Perhaps the most obvious example of behavior maintained by a VR schedule is that of gambling. A slot machine may pay off on the average once every 100 plays, but the number of plays between payoffs varies in an unpredictably way; thus, one play that results in a payoff may follow immediately after a preceding payoff or may follow only after a large number of subsequent plays. The VR schedule maintains the highest rates of responding of the four simple schedules (**Figure 9**). In essence, it is characterized by a continuous high rate of responding without pausing after reinforcement deliveries.

Like the VI, the pattern of responding on the VR schedule reflects the lack of predictability of reinforcement availability. Since reinforcement availability may always be imminent, pausing would delay reinforcement. The high rates characteristic of VR and FR schedules are thought to be due to the ratio basis of reinforcement in that the faster the response requirement is completed, the faster reinforcement is available. Increases in rates of responding on interval-based schedules such as the FI and VI cannot accelerate the availability of reinforcement; one must still wait for the time interval to end.

Complex Schedules of Reinforcement

As mentioned previously, the complexity of reinforcement schedules encountered in the human environment is much greater than those embodied in the simple schedules studied in the laboratory. Combinations and variants of the simple schedules of reinforcement produce greater approximations of this complexity. One such example is a multiple schedule of reinforcement, in which component schedules alternate over the course of a behavioral test session. On a multiple FI–FR schedule, for example, the session could begin with an FI schedule in effect and would be indicated to the subject by some explicit stimulus (e.g., illumination of a red light). After some specified period of time elapsed or after the delivery of designated number of reinforcers on the FI schedule, the red light would change to a green light, and the schedule would switch to an FR. The FR schedule component would then remain in effect until a designated time had elapsed or a specified number of reinforcers had been delivered, and would be followed by a switch back to the FI component, and so on. After training on this schedule, patterns of behavior characteristic of each schedule component emerge; thus, during the FI component, a scalloped pattern of responding is maintained, whereas during the FR component break-and-run performance is exhibited. In addition, after experience on the schedule, the colored light stimuli associated with each schedule component come to exert strong control over

behavior, such that performance appropriate to the schedule occurs immediately upon switching the color of the light. That is, these stimuli serve as discriminative stimuli signaling the schedule in effect.

This arrangement allows the experimenter to measure two very different types of schedule-controlled performances in the same subject during the same test session, making it a highly efficient experimental paradigm. This permits a determination as to whether a chemical may have selective effects on certain schedules (e.g., change FI performance without affecting the FR). If the compound being evaluated affects the control of the stimulus lights over responding, it might be manifest as a delay in transition to schedule-appropriate behavior whenever the light colors switched.

A mixed schedule of reinforcement is identical to a multiple schedule of reinforcement, except that there are no external stimuli provided to the subject to indicate that the operative reinforcement schedule has switched. Thus, the only indication to the subject as to 'what pays off' is the feedback it receives from its own behavior. This minimizes the extent of stimulus control over behavior relative to that of a comparable multiple schedule of reinforcement.

A chained schedule of reinforcement, like a multiple schedule of reinforcement, also has different external stimuli associated with each component of the schedule, but it requires the completion of a sequence of components for reinforcement delivery. Thus, on a chained FI–FR schedule, a red light may signal that the FI component is in effect. Completion of the FI with the first response after the interval ends produces the external stimulus (e.g., green light) associated with the FR component. Completion of the response requirement during the FR component then produces reinforcement, and the chain subsequently begins over – a course which continues throughout the behavioral session. A tandem schedule is identical to a chained schedule, but like the mixed schedule, it provides no external stimuli to signal which component schedule is in effect.

The schedule which probably most resembles those operative in the human environment is known as a concurrent schedule of reinforcement. In the real world, we are routinely faced with a multitude of simultaneously operative schedule options with various schedule conditions and consequences, and we must make choices among them. The foraging (food seeking) environment of many species likewise provides such concurrent options with differential probabilities of reinforcement among which species must make choices. Concurrent schedules provide an experimental analog of this facet of the environment and require the subject to make choices among component reinforcement schedules and reinforcers. For example, in an operant chamber such as shown in **Figure 6**, different response manipulanda might be associated with different but simultaneously available reinforcement schedule options, perhaps associated with different reinforcing events as well. In some cases, once the subject chooses one option, the alternative schedule options are no longer available for some period of time. Others allow subjects to switch back and forth between schedule options. These types of schedules allow experimenters to ask questions about how much behavior the subject is willing to emit for specified reinforcers, preferences for reinforcers and response patterns, relative magnitude of reinforcement and allocation of behavior depending on effort and reinforcement availability.

Measurement of Schedule-Controlled Behavior

The universal measure of schedule-controlled behavior is the rate of responding, which is simply the total number of responses divided by total time. While this is a useful measure of behavior, it provides no indication of other aspects of schedule-controlled behavior, such as the extent of pausing or the patterns of behavior over time. For such purposes, a more fine-grained analysis or microanalysis of performance must be undertaken.

One such measure, applicable to both FR and FI schedules, is postreinforcement pause (PRP) time, which is simply measured as the time from reinforcement delivery until the first response occurs in the next interval (FI) or ratio (FR). For the FI schedule, one may be interested in the extent to which the scalloped pattern of performance occurs as an indication of the extent to which responding is controlled by the contingencies operative on the schedule. For this, one of two measures is utilized: the index of curvature or the quarter life. Index of curvature simply utilizes a mathematical formula to indicate how the observed scallop deviates from a straight line that would be generated by a constant rate of responding throughout the interval. Quarter life measures the time it takes for the first 25% of responses in the interval to occur.

Another measure of schedule-controlled behavior is that provided by the distribution of the times between successive responses or interresponse times (IRTs). These can be generated as a frequency distribution and have been shown to be important targets of chemical exposure. For example, lead exposure appears to affect primarily the very short IRTs on FI schedules. Many different drugs from a variety of different classes have been shown to increase the frequency of long IRTs and to decrease the frequency of

short IRTs on an FI schedule – a phenomenon known as 'rate-dependency' and which results in a more uniform and less scalloped pattern of responding. Rates of responding can also be calculated on schedules of reinforcement after the PRP or the IRTs longer than some designated time (pauses) have been subtracted out. This results in a 'truer' rate of responding and is known as running rate.

Behavioral Teratology

Behavioral teratology, or neurobehavioral teratology, is often referred to separately from behavioral toxicology. Behavioral teratology focuses on the behavioral impact of toxic exposures occurring prenatally or during early development. In some cases, these studies may only track the consequences of chemical exposures into early postnatal life, but in others effects may be studied well into the juvenile and even adult stages of the life cycle. Because the possibility has been raised that developmental exposures may accelerate the processes of aging, some studies are now beginning to follow subjects throughout the lifespan. Behavioral teratology studies typically include a series of tasks designed to evaluate multiple behavioral functions. Consequently, such experiments may include assessment of the development of various reflexes and developmental landmarks (e.g., eye opening), performance on a functional battery (FOB), motor activity, sensory capabilities, learning, and even schedule-controlled operant behavior. In addition, some such experiments may include evaluation of behaviors deemed 'species specific' (i.e., behaviors that are innate and unique to that species), such as the ontogeny of aggression, play, or vocalization in rodents.

In cases in which outcome is followed through maturity, many of the behavioral paradigms that have already been described are utilized. Assessment of behavioral changes early in life, however, may require modification of such procedures and even the development of specialized behavioral preparations. One example of such a specialized preparation which concurrently measures sensory and motor capabilities (though not independently) is that known as 'homing behavior', a behavior utilized by rodent pups to locate the nest should they wander. In this procedure, a rat pup is placed in the center of a rectangular apparatus, one side of which contains clean bedding material, whereas the other side contains bedding material from the home nest with its scent familiar to the pup. The time taken for the pup to orient to or to reach the home cage bedding is then measured. Such a test is deemed apical because it requires the integration of both motor and sensory capabilities.

There are certain issues uniquely related to behavioral teratology studies that require special consideration. One is that of toxicant effects on the dam (mother). Since the behavior of the dam may ultimately influence behavior and development of the offspring, great care must be taken to determine whether any observed effects of a chemical in the offspring are direct effects of the toxicant itself or whether they arise indirectly as a result of the effect of the compound on the dam's behavior. This is typically done by using a variety of fostering procedures. A cross-fostering procedure distributes the pups of treated dams to dams that are treatment free, in which case there should be no chemical-induced changes in maternal behavior.

There are also issues related to statistical analyses of the data that are unique to behavioral teratology. The offspring of a given litter are not considered as individual subjects since, as members of a litter, they have all experienced factors of the fetal environment which may be unique to their dam. This means that the total number of subjects in a treatment group is really equivalent to the total number of litters represented in that group, a factor which can change the degrees of freedom in the statistical analyses.

Human Testing

Behavioral toxicological studies in humans have focused primarily on adults occupationally exposed to chemicals and children exposed to toxicants environmentally. There is frequently a good deal of overlap in the specific behavioral functions evaluated in each case, although the tests utilized must be age appropriate. However, studies in children also often include measurements of developmental profiles and landmarks which are not relevant to studies of occupationally exposed adults. In adults, in contrast, assessment of exposure-related symptomatology is possible. Both types of studies also generally assess a broad variety of behavioral functions and may include tests of motor function, sensory capabilities, complex or cognitive behaviors, attentional processes, and vigilance, usually in the context of a standardized test or test battery. In the past, many such functions would be evaluated as part of a neurological or clinical examination. However, it has become increasingly clear that such examinations, meant to diagnose disease or brain damage, are neither sufficiently sensitive nor quantitative for purposes of detecting subtle effects of toxicants and ultimately for setting standards of exposure.

The test batteries commonly used in human studies have come primarily from the field of clinical neuropsychology, in which human testing has predominated. Behavioral measures such as are utilized

in experimental animal studies were, in the past, rarely included in human studies. This has changed, however, and will likely increase even more in the future given the advantages of utilizing the same tests across species. In part the overall emphasis on broad testing of behavioral functions in human studies has been driven by the lack of any information on the behavioral properties of many of these chemicals as well as by the need to establish dose–effect and dose–response relationships.

Many of the same issues raised with respect to experimental animal studies also apply to human testing and to the choices of particular tests to be utilized. There are numerous tests that can be utilized for measurement of behavioral functions in humans, and questions remain as to the correct choice. One consideration related to the various tests is deemed validity and refers to the degree to which the test actually measures the behavioral function that it was designed to measure. For example, does a test of memory really evaluate memory function? In addition, how specifically does the test measure that function? The related issue was raised in experimental animal studies in which the possibility that changes in motor, sensory, motivational processes, etc. might contaminate a measure of memory function, and appropriate controls were included in the more advanced procedures to evaluate those possibilities.

Another important issue relates to the reliability of the test. That is, how reproducible or consistent are the test results across multiple administrations? Inadequate reliability almost guarantees that a subtle toxicant effect will not be detected against a background of scores of broad individual variability that will be present in any normal population. An issue that has not received adequate attention is the sensitivity of these tests to detect toxicant effects, a factor that is of particular importance if the test results are used in the context of setting exposure standards. If a particular test indicates effects of lead, for example, at a blood lead concentration of $40\,\mu g\,dl^{-1}$, one may wonder whether this represents the bottom limits of sensitivity of the test or the actual blood lead value at which such effects occur. In other words, could the test have detected effects at even lower levels of exposure if it had been more sensitive? A deficiency in test sensitivity could mean that exposure standards will be set at levels that are too high and will not protect the exposed populations.

A related question of relevance, particularly to tests of achievement such as the so-called intelligence tests, is standardization. This refers to the population from which the normative scores for the test were collected. This issue is often raised in the interpretation of intelligence tests for populations that are culturally and socially distinct from the populations of white middle-class English-speaking children from which normative scores for such tests have typically been derived.

Developmental Assessments

As mentioned previously, several unique considerations affect the assessment of toxicant-induced behavioral changes in children. One such consideration is the rapid development that children undergo from birth through even the preschool and early school stages. Moreover, this development is marked by wide individual differences in the rate at which it occurs and, for some facets of behavior, gender-related differences as well. An additional difficulty is that many of the behavioral processes that are of particular interest, such as complex cognitive behavior, are more difficult to evaluate at a young age. While it seems clear that children certainly have both learning and memory capabilities even from birth, assessment of such changes has typically relied on tests which may require language or motor skills well beyond the capabilities represented by these early stages of development.

Because of this rapid change in the behavioral repertoire over the course of early development, the tests that are utilized in studies of children tend to differ at different ages. One test frequently utilized in the first few days after birth is the Brazelton Neonatal Behavioral Assessment Scale, which is composed of two subscales. The first taps a range of behavioral items such as habituation and responsiveness to environmental stimuli. The second primarily measures a variety of unconditioned reflexes. While the Brazelton scale is obviously limited in the extent to which it can tap cognitive functions, or define specific behavioral deficits, its utility in detecting drug-induced changes has been established.

A recently developed technique for infant assessment is embodied in the Fagan Test of Infant Intelligence, which assesses visual recognition memory. In this test, an infant faces a display with two screens. On one screen, a visual stimulus is presented for a specified period of time. Subsequently, that visual stimulus is projected on one screen and, at the same time, another visual stimulus is projected onto another screen. An observer records the amount of time the infant spends gazing at each screen. Normal infants look away from the visual stimulus which they have already seen and spend more time gazing at the novel stimulus, a trait which has been shown to correlate with higher scores later in development on the Stanford–Binet intelligence test.

A widely used test at a slightly later stage of development is the Bayley Scales of Infant Development, appropriate to children from 2 to 30 months of

age. The test is composed of three subscales: motor, mental, and behavioral. Each is arranged with respect to chronological development. One of the advantages of this test is the ability to carry out repeated testing over the normed age range.

As children reach preschool and school age, the number of test choices available increases. For example, the McCarthy Scales of Children's Abilities provides an analog of an intelligence test score by combining the scores from its five subscales into a general cognitive index score. Its applicability extends from children aged 2.5–8.5 years. Like the Bayley Scales, it too allows for repeated measurement over time, which is a particular advantage for longitudinal studies; utilization of the same test instrument over time, given appropriate reliability of the instrument, provides greater assurance of the continuity and of the onset or disappearance of an effect than does the use of different instruments at different ages.

Various intelligence tests are available for preschool age children, such as the Weschler Preschool and Primary Scale of Intelligence (WPPSI). The advantage of this particular instrument is that it represents an extension of the well-standardized and widely used Weschler Intelligence Scale for Children (revised; WISC-R). The WISC-R is an intelligence test for children of 6 years of age or older; the WPPSI extends this age range to include children of ages 4–6.5 years. In addition, both rely on the same two subscales, verbal and performance, to measure a variety of behavioral functions, thus providing a type of continuity from the preschool to the school-aged child for repeated assessment of behavioral function.

One of the major concerns with developmental and intelligence tests such as the WISC-R and others is to be able to rule out contributions from numerous sociodemographic and other variables known to covary with intelligence test score. Variables which may potentially modulate intelligence include birth weight, length of gestation, maternal age, birth order, parental education, parental IQ, socioeconomic status, and quality of the home environment. Appropriate statistical controls or subject matching must be undertaken to evaluate the contributions of these variables to outcome measures.

While these developmental and intelligence tests may clearly be important to the determination of the levels and conditions of exposures to a toxicant associated with adverse behavioral function, they are less useful, as noted previously, in providing a precise delineation of the behavioral functions actually affected by a chemical. Measures such as intelligence test scores are global measures in that they rely on the integration of all behavioral functions. Even performances on subscales of these tests are jointly dependent on integrative motor, sensory, and cognitive functions. Thus, even a preferential deficit on a verbal scale, which is clearly geared toward cognitive function, may not provide a precise understanding of the nature of the behavioral deficit.

To achieve a true understanding of the behavioral processes affected by a chemical will necessarily require direct measurement of those specific functions, much as is done in the experimental animal studies described previously. Some neuropsychologists have recognized this problem and have begun to employ measures of specific behavioral functions such as learning, memory, sustained attention, and abstract thinking in an attempt to determine the source of the deficits in global intelligence test scores produced by lead exposure. An alternative approach is to utilize many of the behavioral tasks already employed in experimental animal studies – tasks which are designed to evaluate specific functions and which have already been widely used across species, including humans, in other research contexts. The repeated learning paradigm actually originated in studies using human subjects and was later adapted for nonhuman primates and rodents. Procedures such as delayed matching to sample and operant psychophysical procedures have also been used across species with appropriate parametric modifications. These types of paradigms may play a more significant role in future developmental studies of children because they provide direct and specific measures of behavioral functions that are more difficult to differentiate in standardized tests.

Adult Assessments

Assessments of behavioral toxicity in adults frequently occur in the context of occupational exposures to chemicals. Like studies carried out in school-aged children, these evaluations have relied largely on standardized tests, including intelligence tests. They also tend to employ a broad variety of tests so that numerous behavioral functions can be tested, particularly when the effects of a toxicant are ill-defined. As such, the same considerations must be taken into account with respect to the choice of tests utilized. These include validity, reliability, and sensitivity, as well as standardization issues related to the population from which test norms were derived.

One distinction between many of the studies of behavioral toxicity in children and adults is that while the former have tended to be primarily longitudinal in nature, following the effects of toxicant exposures to children across the course of development, most of the occupational exposure studies are cross-sectional studies that encompass only a single time point of measurement of behavioral function.

This no doubt reflects the added difficulties of carrying out studies in the workplace, where it may be more difficult to obtain appropriate amounts of the subjects' time for behavioral evaluation and where resistance to such experiments may be encountered from either the employer or the employee.

One of the most common inclusions in these test batteries that have been utilized in studies of occupational behavioral toxicity is the Weschler Adult Intelligence Scale (WAIS), which is actually a battery of tests subsumed under verbal and performance subscales which, combined, provide a full-scale intelligence test score. The series of verbal tests includes information (general information questions), comprehension (interpretation test), arithmetic, similarities (between nouns), digit span (repeating sequences of digits), and vocabulary. The performance tests include digit symbol (associating digits with symbols), picture completion, block design (duplicating block patterns), picture arrangement, and object assembly. Because of the obvious overlap of behavioral functions in some of these subtests, and the consequent global and nonspecific nature of any change detected in full-scale intelligence test scores, some investigators have opted to use only selected tests from the battery to provide a shortened version of the WAIS for occupational behavioral toxicity studies.

Two different test batteries, the World Health Organization (WHO) Neurobehavioral Core Test Battery and the Neurobehavioral Evaluation System (NES), are currently the most widely used test batteries in occupational behavioral toxicology studies. Both include components of the WAIS described previously in addition to other psychometric tests of behavioral function. The WHO Neurobehavioral Core Test Battery is a pencil and paper-administered test battery, whereas the NES is a computerized test battery that has been translated into several languages and in fact presents a more extensive set of tests than does the WHO in that it includes tests of psychomotor performance, cognition, memory and learning, and perceptual ability and affect.

Memory dysfunction has been a frequent complaint in populations of workers exposed to various neurotoxicants and is tapped by several different tests used in a human testing context. One of the most widely used for this purpose is the digit span that constitutes one of the WAIS subtests. As indicated earlier, this test requires the subject to recall a series of digits, and the length of the list is successively increased contingent upon the subject correctly recalling the members of the list. In some cases, words or letters are utilized instead of digits. As is the case with experimental animal studies, more complex versions of these tests have been devised and implemented. In procedures such as continuous recognition memory or memory scanning, subjects may be shown a list of digits or letters and then shown, after a delay interval, a longer list of various digits or letters and asked to recall those that were on the original list. Analogies to such tests are embodied in procedures such as the Benton Visual Recognition Test, which requires a subject to reproduce a drawing or geometrical design.

Paired-associates learning is also frequently used in a memory context in occupational exposure test batteries as well as in studies with children. In these paradigms, a list of paired words is read to the subject, who must then recall the second member of the pair when the first is read after a delay. The task can be made relatively simple by using pairs which have some type of obvious relationship or made more difficult by having pairs with no apparent relationship. The test can be used in a memory context by including a delay between the experimenter's reading of the list and the subject recalling the second member of each pair. In addition, the task can be used in a repeated learning context, much as the repeated acquisition paradigm described earlier, by using new lists of paired-associates after the subject masters the initial list. This particular approach has a long history of use with human subjects and has been found to be sensitive to toxicants such as lead.

Measures of vigilance, attention, or distractibility are also frequently included in assessments of occupational behavioral toxicology. These range from very simple procedures, such as reaction time, to more complex tasks, such as simulated cockpit or tracking tasks. Even reaction time can be varied from a very simple to a highly complex procedure. In a simple reaction time task, the subject is typically presented with some type of screen on which a single visual stimulus will appear at intermittent and unpredictable intervals. The subject must respond on the single response manipulanda as soon as the stimulus appears. Complex reaction time presents the subject with multiple stimuli as well as multiple response options. For example, there may be four different stimuli, each of which is presented at random and unpredictable intervals. The appropriate response depends on which of the stimuli is presented, and the subject is asked to respond on the appropriate manipulanda as quickly as possible after detecting the stimulus. Thus, the more complex reaction time task involves not only attending to the screen to detect the stimulus presentation but also making a decision as to the correct manipulanda and then executing the response. Obviously, the number of options can be modified to fit the experimental situation.

One of the important parameters of the reaction time task is the rate at which the attention of the subject deteriorates, such that reaction time is slowed down or even that the subject misses stimulus presentations entirely. This rate of deterioration of performance will depend on many factors, one of which is the rate at which stimuli are presented to the subject and another being the length of the session during which reaction time is measured. While one might intuitively think that the slower the rate of presentation, the more rapid the rate of deterioration of performance, in fact sometimes the opposite is true. A very rapid rate of presentation of stimuli can render the subject exhausted and less alert or less motivated. With respect to session length, one typically expects to see a gradual decrement in performance as the session progresses, such that an adequately long session must be implemented to catch this function. Finally, a critical variable in reaction time studies is the prominence of the stimuli used. In fact, this parameter can be manipulated to change the sensitivity and difficulty of the task.

Reaction time tasks are by no means limited to the presentation of discrete visual stimuli. Other variants have included those in which the subject must respond to a stimulus that is different from a continuously presented array of stimuli. The so-called clock test is one example. In this procedure, the subject is instructed to respond when the hand of a clock ticks off 2 s at once rather than the typical 1 s tick; the 2 s tick is an infrequent and unpredictable occurrence. In other situations, a continuous presentation of letters or numbers may be presented and the subject instructed to respond to one particular letter or number whenever it appears.

Pursuit and tracking tests represent even more complex versions of vigilance assays. In these kinds of tasks, subjects must continuously monitor a stimulus which drifts off a home position on the dial. The situation can be made quite complex, as in flight simulators in which there may be multiple dials which must be continuously monitored and returned to the home position, with drift occurring at varying rates on each dial across time. The various vigilance tasks described previously have a long experimental history and have been shown to be sensitive to a wide variety of influences, including fatigue and various drugs and chemical exposures.

The kinds of vigilance procedures described obviously require reasonably intact motor function and are often interpreted with that in mind. However, these techniques also depend on sensory processes. In fact, assuming intact motor functions, vigilance tasks such as those described can be adapted to provide some indication of sensory function changes by modifying the saliency (intensity) of the sensory stimuli used in the paradigm. However, more direct approaches to the evaluation of sensory function following occupational or environmental exposures to toxicants are provided by the types of operant psychophysical procedures elaborated previously. In fact, psychophysical procedures were developed using human subjects and only later adapted for various species of experimental animals. The psychophysical procedures clearly provide more direct and straightforward assessments of sensory detection capabilities in the absence of confounding changes in a subject's motor capabilities or motivation to respond.

Assessments of motor function are often included in the neuropsychological test batteries utilized in occupational exposure studies. Typically, these tend to be relatively simple measures of motor capabilities, probably for two reasons. The first is that the inclusion of vigilance tasks such as those described previously depends on motor coordination in addition to sensory capabilities; therefore, toxicant-induced changes in such performances may already be indicative of motor impairment. This can then be pursued by inclusion of some additional and more direct assessments of motor function in the battery. The second reason relates to logistical reasons and practicalities. Test batteries such as the WHO Neurobehavioral Core Test Battery and the NES are typically taken to the site where measurements of subjects are to be made. Thus, portability is a major consideration, and more complex assessments of motor function would incur greater equipment needs. Since the purpose of these batteries is generally to screen for adverse effects, studies providing more precise delineations of affected functions can be pursued at a later time.

Simple tests of motor function utilized are generally those such as finger tapping in which subjects are asked to tap a key or a button at as rapid a rate as possible for a designated period of time. The subjects may be asked to carry out this task with the preferred hand as well as with the alternate hand. In some cases, toe tapping has been used in addition to finger tapping. Other batteries have relied on the tests of manual dexterity that are frequently used in screening prospective applicants for some types of factory work jobs. One of the most frequently used of such tests is the Santa Ana test, which requires the subject to remove pegs from a hole and to reinsert them into the hole after turning them 180°. The measurement of interest in this case is the number of pegs that are successfully rotated within the specified time interval. The Purdue Pegboard test is likewise used in this capacity. It requires the proper orientation and placement of

pins in a series of holes. Such tests have indeed successfully defined subjects occupationally exposed to chemicals from those nonexposed.

One final common inclusion in many studies of occupational behavioral toxicology and in some test batteries is assessments of symptoms experienced by those exposed to chemicals. While this might be perceived as an ostensibly simple procedure, it entails numerous potential confounds. These evaluations are typically administered via questionnaires. Items for the questionnaire must be carefully constructed with respect to not only the choices of items but also the wording of the text and the manner in which the response is recorded. Clearly, the motivation of the subject in answering the questions must be considered. One problem can arise when the list of symptoms includes only those that are associated with the toxicant of concern. It is necessary to include symptoms that are not associated with the particular toxicant under evaluation so that some assessment of the tendency of the subject to respond positively to all symptoms can be evaluated. Several such evaluations of subjective and mood states are available. The most widely used is the Profile of Mood States (POMS), which consists of 65 adjectives of various moods that the subject answers according to a 5-point rating scale. The POMS has been used extensively in the evaluation of the acute effects of CNS drugs and toxicants.

Acknowledgments

Supported by NIEHS Grants ES05903, ES05017, and ES01247.

See also: Multiple Chemical Sensitivities; Occupational Toxicology; Pesticides; Pollution, Air Indoor; Psychological Indices of Toxicity; Sensory Organs; Sick Building Syndrome.

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Belladonna Alkaloids

Madhusudan G Soni

- Representative Compounds: Atropine; Scopolamine; Homatropine
- Chemical/Pharmaceutical/Other Class: Naturally occurring antimuscarinic drugs

Uses

Belladonna alkaloids are used in clinical medicine for their ability to block the effects of parasympathetic nerve stimulation.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposure to belladonna alkaloids. They are available in oral and tincture forms.

Toxicokinetics

The belladonna alkaloids are absorbed rapidly from the gastrointestinal tract. They also enter the circulation when applied locally to the mucosal surfaces of the body. Absorption from intact skin is limited. Belladonna alkaloids are metabolized mainly in liver to glucuronide conjugates after metabolic hydroxylation of the aromatic ring. The volumes of distribution for important belladonna alkaloids, atropine, and scopolamine, are ~ 1.7 and $1.4\,l\,kg^{-1}$, respectively. Elimination of belladonna alkaloids is rapid. About half of the atropine is excreted unchanged in urine. Traces of atropine are found in various secretions including breast milk. The elimination half-life for intravenously injected atropine ranges from 1.9 to 4.3 h.

Mechanism of Toxicity

Toxic doses of belladonna alkaloids prominently lead to central excitement. The earliest symptoms of

atropine toxicity are due to blocking of acetylcholine receptor sites on cells of organs innervated by the craniosacral division of the visceral effector nervous system. Scopalamine differs from atropine intoxication in that the cardiac rate is rarely increased and cerebral excitement is of short duration. Although the mechanism of action of these compounds is well studied the mechanism of toxicity is not entirely understood.

Acute and Chronic Toxicity (or Exposure)

Animal

Cats, dogs, and birds are sensitive to belladonna alkaloid toxicity; horses and oxen less so; and pigs, goats, and sheep are comparatively resistant to the alkaloids. Parenteral administration of lethal doses of atropine to young rabbits produces two distinctly different types of deaths. About half of the animals died promptly in a convulsive state, perhaps comparable to the commonly encountered clinical syndrome of central excitement, but a smaller group suffered delayed deaths in ~ 2 weeks with endarteritis obliterans in the distal portion of the injected limb. Chronic belladonna alkaloid or atropine poisoning has evidently not been encountered as a clinical entity, but the parenteral administration of large doses of atropine ($16\,mg\,kg^{-1}$ daily) for periods of 1–3 weeks produces in young puppies a syndrome clinically similar to advanced fibrocystic disease of the pancreas.

Human

The deliberate or accidental ingestion of belladonna alkaloids is a major cause of toxicity in humans. The most dangerous and spectacular manifestation of poisoning arises from the intense excitation of the central nervous system (CNS). Infants and young children are especially susceptible to the toxic effects of atropinic drugs. In adults, delirium or toxic psychoses without undue peripheral manifestations have been reported after instillation of atropine eye drops. Transdermal preparation of scopolamine has been reported to cause toxic psychoses, especially in children and in the elderly. Serious intoxication may occur in children who ingest berries or seeds containing belladonna alkaloids. In case of full-blown poisoning, the syndrome may last 48 h or longer. Depression and circulatory collapse are evident only in cases of severe intoxication; the blood pressure declines, respiration becomes inadequate, and death due to respiratory failure may follow after a period of paralysis and coma.

Clinical Management

The diagnosis is suggested by the widespread paralysis of organs innervated by parasympathetic nerves. Intramuscular injection of physostigmine may be used for confirmation. If the typical salivation, sweating, and intestinal hyperactivity do not occur after physostigmine injection, intoxication with atropine or a related agent is almost certain. Measures to limit intestinal absorption should be initiated without delay if the poison has been taken orally. The most effective antagonist to the CNS manifestation is physostigmine salicylate in doses of 0.5–2.5 mg by any parenteral route. Because physostigmine is metabolized fast, repeated doses may be needed. If marked excitement is present and more specific treatment is not available, diazepam is the most suitable agent for sedation and for the control of convulsions. Large doses should be avoided. Artificial respiration may be necessary. Ice bags and alcohol sponges help to reduce fever, especially in children.

See also: Cholinesterase Inhibition; Neurotoxicity; Poisoning Emergencies in Humans.

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Relevant Website

http://www.emea.eu.int – European Medicines Agency.

Benchmark Dose

Qiyu Jay Zhao

Introduction

Until recently, safe doses for noncancer effects have been derived directly from toxicology study doses such as no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs). The NOAEL represents the highest experimental dose for which no adverse health effects are observed, while the LOAEL represents the lowest dose for which the adverse health effect is observed. Dividing the study NOAEL or LOAEL by a series of uncertainty factors yields a dose that is generally considered safe. A recent alternative to using the NOAEL or LOAEL in this calculation is the use of a benchmark dose (BMD) to serve as the starting point for deriving safe doses.

BMD Definition

As defined by US EPA: a BMD is "a statistical lower confidence limit for a dose that produces a predetermined change in response rate of an adverse effect…compared to background." The BMD modeling is conducted by fitting a flexible mathematical model to the observed data, and the dose corresponding to the level of predetermined change (i.e., benchmark response (BMR)), is determined from the model. A common model fitting approach is to use maximum likelihood methods, and the resulting central estimated dose for the BMR is the BMD (see **Figure 1**). To replace a NOAEL in the computation of the safe dose, a lower confidence limit (usually 95%) on the BMD is often used instead of the BMD to account for statistical uncertainties in the study. This lower bound value is commonly referred to as the BMDL.

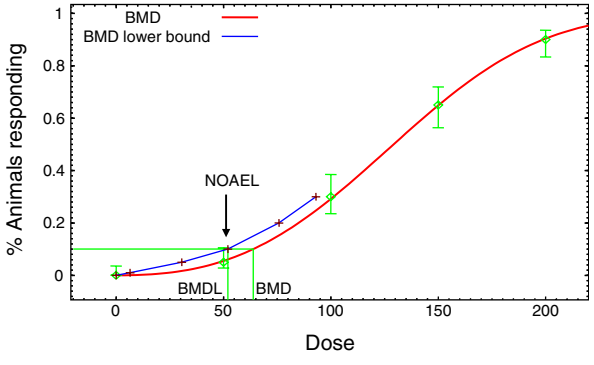

Figure 1 Example of BMD modeling results.

Characteristics of the BMD Approach

The BMD approach was developed to better define the point of departure in the computation of the safe dose in order to overcome the shortcomings of using NOAELs or LOAELs. The traditional NOAEL approach has several limitations: (1) it is limited to one of the doses in the study, making it dependent on study design; (2) it does not account for variability in the estimate of the dose–response; (3) it does not account for the slope of the dose–response curve; and (4) it cannot be applied when there is no NOAEL, except through the application of an uncertainty factor when a LOAEL is used.

In comparison with the NOAEL approach, the BMD approach provides four major advantages. First, the BMD is derived based on data from the entire dose–response curve for the critical effect, rather than only from the single dose (e.g., NOAEL). Therefore, the BMD reflects the slope of the dose–response curve. Second, the BMD approach treats sample size appropriately when the lower confidence limit on the BMD (i.e., BMDL) is used. For example, the smaller the sample, the larger the uncertainty associated with the BMD estimates and the lower the confidence limits (all else being equal). Therefore, data with lower statistical power will result in lower BMDLs (making their use health protective), and better experiments with more statistical power are 'rewarded' with higher BMDLs. Third, the BMDL is not constrained to be one of the experimental doses, and calculation of the BMDL allows for estimation of a NOAEL surrogate when only a LOAEL is available. In addition, the dose-independent BMDL also facilitates comparison of toxicity potencies across chemicals or endpoints. Fourth, the BMD approach can be useful when the dose spacing in a study is such that the LOAEL is much larger than the NOAEL. Thus, any good study can be used, even in the absence of a NOAEL, as long as sufficient and appropriate dose–response data are provided so that the dose corresponding to the BMR can be estimated.

Data Requirements and Parameter Selection

A number of dose–response models have been developed for BMD analyses. The form of the model used and the necessary inputs for the modeling depend on the type of data to be modeled. For quantal data (e.g., histopathology incidence data), the incidence of the effect of interest and the total size of the group are needed, and for continuous data (e.g., liver enzyme activity), the group size, mean, and a measure of variability (i.e., standard deviation or standard error) are required.

To estimate the BMD and BMDL, it is necessary to define a desired BMR. For quantal data, the BMR is defined as an incidence change from the estimated control. Usually, a 10% extra risk $\{[P(\text{dose}) - P(0)]/P(0)\}$ is used to define effective doses for comparing potencies across chemicals or endpoints. This response level is used because it is at or near the limit of sensitivity in most chronic bioassays. If a study has greater than usual sensitivity, then a lower BMR (5% or even 1%) can be used. For continuous data, BMR is defined as a percentage change from the estimated control mean, or as a change of a certain number of standard deviations from the control. If there is a minimal level of change in the endpoint (e.g., liver enzyme activity) that is generally considered to be biologically significant, then that amount of change can be used as the BMR. In the absence of endpoint-specific data to determine the appropriate level of response as adverse, a change in the mean equal to one control standard deviation can be used. This default approach is used because when values beyond the 98th–99th percentile of control animals are considered abnormal, a dose that causes a shift in the average of 1 standard deviation results in approximately an excess risk of 10% of the animals in the abnormal range. Whenever, a BMR is chosen based on biological considerations, US Environmental Protection Agency recommends to present the resulting BMDL with the BMDL estimated for the default BMR.

To make the BMD from continuous data comparable to the BMD from quantal data, the BMR for continuous data can also be expressed as incidence data. To do this, individual animal data are categorized based on a predetermined cutoff value (e.g., a $>10\%$ change in organ weight). This incidence data could then be modeled as a quantal endpoint, with the BMR expressed in terms of an incidence change from the control. This approach is not optimal, since some information would be lost in this data categorization. A better way to convert the continuous BMR to a quantal BMR is to use a 'hybrid' approach, which uses all of the information contained in the original observations. The hybrid approach fits continuous models to the continuous data. Based on the probability information in the continuous dose–response curve and a cutoff value for defining adverse response, a BMD for a specified quantal BMR (e.g., 10%) can be calculated. This result can be compared directly to other BMDs estimated from quantal data. A limitation for using 'hybrid' approach is that it requires definition of a background incidence of abnormality, or the specification of a level of response that can be considered the cutoff point between normal and abnormal responses. The selection of the cutoff point is often difficult.

BMD Model Evaluation

Although the BMD offers several advantages over the NOAEL, it can only be used in cases where available data are suitable for modeling. A good BMD model software should provide statistics for assessing model fit, including measures of global and local data fit. Guidance is available from US EPA to evaluate the statistical fit of various models.

Although statistical evaluation is critical, the use of scientific judgment remains essential when conducting dose–response modeling. An ideal data set should provide information on the shape of the dose–response curve, especially at the region close to the BMR. When this occurs BMD estimates from various models should provide similar results as long as these models provide a comparable data fit (i.e., BMD estimates are not model dependent). On the contrary, the BMD model is of limited utility if the dose spacing is such that there is no information on the shape of the dose–response curve, such as when there is 0% response in the control group, and very high (e.g., over 80%) response in the low-dose group. In some cases, however, applicable models might diverge with respect to BMD estimates. In such cases, it is necessary to analyze the data and determine whether there is a reason to prefer certain models, such as there is an underlying biological basis for choosing a dose–response shape in the region of the BMR or one of the models fits the data better in the 10% response region. For some data sets, 'plateauing' or nonmonotonicity of the response rates may occur in the high-dose region. If such plateauing drives the model fit, resulting in poor fit in the low-dose region, it may be appropriate to consider excluding the high dose(s) from the modeling. These examples are just a few of the many qualitative considerations needed to select an appropriate modeling result.

US EPA has developed a benchmark dose software package that provides various models for quantal, continuous, and nested data (developmental toxicity study results). The software can be downloaded from US EPA's website (see section Relevant Websites) free of charge, and it is frequently updated. This website also provides support documentation, including a software user's manual and a guidance document on the interpretation and use of BMD modeling.

See also: Risk Assessment, Human Health.

Further Reading

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Benomyl

Jamaluddin Shaikh

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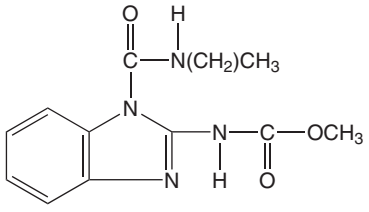
- Chemical Abstracts Service Registry Number: CAS 17804-35-2
- Synonyms: Agrocit; Fundazole; Benomyl 50W; Benlate; Benlate T; Methyl 1-(butylcarbamoyl)-2-benzimidazolylcarbamate
- Chemical/Pharmaceutical/Other Class: Benzimidazole fungicide
- Chemical Structure:



Uses

Benomyl is a protective and eradicant fungicide that is especially effective against a wide range of fungi affecting fruits, nuts, vegetables, turf, and field crops. The manufacturer (DuPont Crop Protection) ceased all manufacturing and sales of benomyl on December 31, 2001.

Exposure Routes and Pathways

Benomyl is formulated as a powder (Benlate and Benlate T). Dermal and oral routes are the most common exposure pathways.

Toxicokinetics

Benomyl is poorly absorbed as it is degraded in the gastrointestinal tract. Blood levels after oral administration are only one-tenth of those found after intraperitoneal injection in rats. Benomyl is hydroxylated and/or methylated, then conjugated, and promptly excreted in the urine. There is minimal or no tissue storage of benomyl or metabolites based on a 2 year feeding study in rats and dogs. In rats, the major metabolites of benomyl are converted to sulfate and/or glucuronide conjugates and excreted in urine (78%) and feces (8.7%). In mice, rabbits, and sheep, 44–71% of benomyl metabolites are found in urine and ~21–46% in feces.

Mechanism of Toxicity

Benomyl is a microtubule-disrupting agent in fungi. This agent may cause chromosomal aberrations (e.g., aneuploidy). There is very little evidence of benomyl toxicity in mammals, however. Benomyl itself does not have any direct effect on acetylcholinesterase. Under certain conditions, however, benomyl breaks down to produce carbendazim and butyl isocyanate, of which the isocyanate is an irreversible inhibitor of acetylcholinesterase with comparable potency to some active organophosphorus inhibitors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Benomyl has very low acute toxicity in laboratory animals. The oral LD_{50} value in rats is greater than $10\,g\,kg^{-1}$.

Human

Benomyl is a potential mild skin, eye, and respiratory tract irritant. Systemic poisoning is rare. Contact dermatitis has been reported in occupationally exposed workers. The skin lesions, which consisted of redness and edema, occurred on the back of hands, forearms, and in other places not covered by clothing. These

lesions generally clear within 3 weeks. Hyperpigmentation and photosensitization have also been reported.

Chronic Toxicity (or Exposure)

Animal

Rats given benomyl (150 mg kg^{-1} day^{-1} in the diet) for 2 years showed no signs of toxicity. Dogs receiving 150 mg kg^{-1} day^{-1} benomyl in their diet for 90 days showed no overt signs of toxicity but evidence of alterations in liver function. More severe liver changes and cirrhosis were noted after 2 years of dosing in dogs at this dosage. Liver tumors were noted in both male and female mice in long-term studies using from 40 to 400 mg kg^{-1} day^{-1} benomyl. In a 2 year study, however, rats given up to 2500 mg kg^{-1} day^{-1} of benomyl showed no significant signs of toxicity.

Human

Little is known regarding chronic effects of benomyl in humans.

In Vitro Toxicity Data

Benomyl induced aromatase activity in a human ovarian tumor cell line. Benomyl does not cause mutations or structural chromosomal aberrations in somatic or germ cells. Benomyl does not interact directly with DNA in mammalian or nonmammalian systems. Benomyl causes chromosomal aberrations (aneuploidy and/or polyploidy) both *in vitro* and *in vivo* due to disruption of microtubules. Benomyl is not clastogenic, however.

Clinical Management

Dermal decontamination should be accomplished by repeated washing with soap. Leather clothing can absorb benomyl; any contaminated leather clothing should therefore be discarded. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min. Emesis can be induced in cases of recent ingestion. In such cases, ipecac can be used to induce emesis. Emesis is not encouraged if the patient is comatose or convulsing. Activated charcoal slurry with or without saline cathartic and sorbitol may be used.

Environmental Fate

Benomyl binds strongly to soil and does not leach to any substantial degree. Its half-life after turf or soil application is 3–6 and 6–12 months, respectively. It did not accumulate from year to year with repeated applications, however. Benomyl completely degrades to carbendazim within several hours in nonalkaline water. The half-life of carbendazim is 2 months.

Ecotoxicology

Benomyl is moderately toxic to birds. The LC_{50} (5 day) in bobwhite quail and mallard ducks is $>10\,000$ ppm. The benomyl LD_{50} value in redwing blackbirds is 100 mg kg^{-1}. It is highly toxic to fish. The order of susceptibility to benomyl for various fish species is catfish < bluegill < rainbow trout < goldfish. A single application of benomyl reduces some soil dwelling organisms. Multiple applications at low concentration over a long time is very lethal to earthworm. The LC_{50} (7 day) in earthworms is 1.7 mg l^{-1} and the LC_{50} (14 day) is 0.4 mg l^{-1}. Benomyl is relatively nontoxic to bees.

Exposure Standards and Guidelines

The reference dose is 50 μg kg^{-1} day^{-1}, the acceptable daily intake is 20 μg kg^{-1} day^{-1}, and the permissible exposure limit is 5 mg m^{-3} (8 h).

See also: Chromosome Aberrations; Pesticides.

Further Reading

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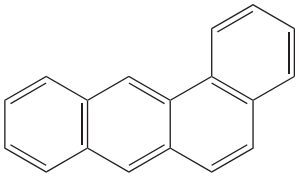
Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Benz[*a*]anthracene

Madhusudan G Soni

- Chemical Abstracts Service Registry Number: CAS 56-55-3
- Synonyms: 1,2-Benzanthracene; 2,3-Benzphenanthrene; 2,3-Benzophenanthrene; Tetraphene; Naphthanthracene; Benzanthrene; BA
- Chemical/Pharmaceutical/Other Class: Polycyclic aromatic hydrocarbon
- Chemical Structure:



Uses

There is no commercial production or known use of this compound.

Exposure Routes and Pathways

Human exposure to benz[*a*]anthracene occurs primarily through smoking of tobacco, inhalation of polluted air, and by ingestion of food and water contaminated by combustion effluents.

Toxicokinetics

Like benzo[*a*]pyrene, benz[*a*]anthracene may cross the gastrointestinal lining, pulmonary endothelium, or percutaneous barriers. Benz[*a*]anthracene is biotransformed to five dihydrodiols and a number of phenolic metabolites by P450 mixed-function oxidases. Detectable levels of benz[*a*]anthracene can be observed in most internal organs from minutes to hours after administration. Regardless of route of administration, once metabolized, hepatobiliary excretion and elimination through feces is the major route.

Mechanism of Toxicity

The arrangement of the aromatic rings in the benz[*a*] anthracene molecule gives it a "bay region" often correlated with carcinogenic properties. In general, the bay-region polycyclic aromatic hydrocarbons and some of their metabolites are known to react with cellular macromolecules, including DNA, which may account for both their toxicity and carcinogenicity. Benz[*a*]anthracene has been proposed to exert toxic effects through irreversible (covalent) binding of its electrophilic metabolites to nucleophilic sites within biological molecules. The species thought to be responsible for the genotoxic effects of benz[*a*]anthracene are diol epoxides. The genotoxic effects are due to the reactions of hard electrophiles derived from the diol epoxides with DNA. The ease of production of these hard electrophiles is related to the extent of delocalization of positive charge formed during the formation of benzyl carbonium ion intermediate.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal studies suggest that exposure to bay-region polycyclic aromatic hydrocarbons can damage the hematopoietic system leading to progressive anemia as well as agranulocytosis. Subcutaneous injection of 5 mg benz[*a*]anthracene daily to rats from the first day of pregnancy resulted in fetal death and resorption. Benz[*a*]anthracene has been shown to induce benzo[*a*]pyrene hydroxylase activity in the rat placenta.

Human

Direct evidence of acute toxicity resulting from oral exposure of humans to benz[*a*]anthracene was not found in the published literature.

Chonic Toxicity (or Exposure)

Animal

Benz[*a*]anthracene has been shown to be carcinogenic to experimental animals. Benz[*a*]anthracene given by several routes of administration has proven to be carcinogenic in mouse. Following repeated administration to young mice, it produced hepatomas and lung adenomas.

Human

No case reports or epidemiological studies on the significance of benz[*a*]anthracene exposure to humans are available. However, coal tar and other materials which are known to be carcinogenic in humans may contain benz[*a*]anthracene.

In Vitro Toxicity Data

Benz[*a*]anthracene was mutagenic to *Salmonella typhimurium* in the presence of an exogenous metabolic system. It was also mutagenic to mammalian cells *in vitro* in the presence of an exogenous metabolic system. In cultured mammalian cells, benz[*a*]anthracene induced unscheduled DNA synthesis and morphological transformation.

Exposure Standards and Guidelines

Benz[*a*]anthracene alone is not regulated; however, all polycyclic aromatic hydrocarbons or volatile coal tar products together are regulated. The World Health Organization has established $0.2\,\mu g\,l^{-1}$ as the limit for aromatic hydrocarbons in a domestic water supply. The US Occupational Safety and Health Administration limit in workplace air (coal tar volatiles) is $0.2\,mg\,m^{-3}$. The US Environmental Protection Agency weight-of-evidence classification for benz[*a*]anthracene is B2, a probable human carcinogen, for both oral and inhalation exposure based on adequate animal evidence and no human evidence.

Environmental Fate

As benz[*a*]anthracene is a universal product of combustion of organic matter, it is released into air and water and is associated with particulate matter. Biodegradation of benz[*a*]anthracene will occur very slowly with a half-life of approximately a year. Benz[*a*]anthracene will bioconcentrate in aquatic organisms. In the atmosphere, benz[*a*]anthracene is found both as the free vapor and adsorbed to particulate matter.

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

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Benzene

Stephen R Clough

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- Chemical Abstracts Service Registry Number: CAS 71-43-2
- Synonyms: Cyclohexatriene; Benzol; Coal naphtha; Benzole; Phenyl hydride
- Chemical/Pharmaceutical/Other Class: Aromatic hydrocarbon
- Chemical Formula: C_6H_6
- Chemical Structure:

Uses

The toxicological properties of benzene, particularly its ability to adversely affect blood forming elements in bone marrow and the subsequent classification of benzene as a human carcinogen, have substantially reduced its industrial use. Benzene, however, is still a minor component of some petroleum products such as gasoline and diesel fuel. In the past, benzene was used as a solvent for oils, resins, rubber, varnishes, lacquers, and waxes; as a chemical intermediate in the manufacture of pharmaceuticals, adhesives, and coatings; and as a solvent for dyes and inks. Its current use is primarily limited to the production of synthetic organic chemicals and plastics. Products in which benzene is used as a raw material include polystyrene plastics, polyester resins, synthetic rubber, phenol, nylon, aniline, detergents, and chlorobenzenes.

Exposure Routes and Pathways

Human exposure to benzene may occur as a result of exposure to petroleum products. Occupational exposures are now, however, very limited because the hazards to human health are known and measures are taken to protect against inhalation or skin exposures. Benzene is volatile at room temperatures so, for

the general population, pumping gasoline and the subsequent inhalation of gas fumes represent the primary source and route of exposure, respectively. A lesser number of people may be inadvertently exposed as a result of petroleum spills contaminating groundwater. Residents using private wells containing contaminated groundwater may be exposed orally by drinking the water or by inhalation after benzene volatilizes from groundwater into air during showering or migrates from soil gas into basement air. If exposure in the workplace did occur it would mainly occur via inhalation and dermal contact. Oral exposure can occur from accidental or intentional consumption of benzene-containing products.

Toxicokinetics

Benzene is lipid soluble and highly volatile at room temperature. As such, benzene readily crosses the alveolar membranes and is taken up by circulating blood in pulmonary vessels. The lung also serves as an excretion pathway for unmetabolized benzene, particularly following acute exposures. Benzene can also be readily absorbed from the gastrointestinal tract and from intact skin. Circulating benzene is preferentially taken up by lipid-rich tissues such as adipose and nervous tissue. Benzene has also been detected in the bone marrow, liver, kidneys, lungs, and spleen.

The human liver can metabolize benzene through a number of metabolic pathways. The major endproducts of benzene metabolism include phenol (hydroxybenzene), catechol (1,2-dihydroxybenzene), and quinol (1,4-dihydroxybenzene). These metabolic products are subsequently conjugated with inorganic sulfate and glucuronic acid in various degrees before being excreted in the urine. A small fraction of the catechol derived from benzene metabolism is oxidized to hydroxyhydroquinol or transformed to mucuronic acids.

Mechanism of Toxicity

Benzene can be irritating to mucus membranes. Dermal exposures defat the skin's keratin layer and can result in erythema, vesiculation, and dry, scaly dermatitis. Acute exposures to high concentrations can produce pulmonary irritation and edema, and gastrointestinal irritation (if consumed). Chronic exposure to benzene produces bone marrow depression. Experimental evidence indicates that benzene's bone marrow toxicity is mediated by one or more of its metabolites. For example, inhibition of benzene metabolism by administration of toluene or partial hepatectomy protects bone marrow against benzene damage, and benzene metabolites, such as 1,2-dihydroxybenzene (catechol), 1,4-dihydroxybenzene (quinol), and 1,2,4-trihydroxybenzene(hydroxyhydroquinol), have been shown to inhibit cell mitosis.

Acute and Short-Term Toxicity (or Exposure)

Animal

The literature on the toxicological properties of benzene in laboratory animals is extensive. Benzene can cause severe eye irritation and moderate skin irritation. When given orally, benzene is moderately toxic. The oral LD_{50} in rats and mice is 3400 and 4700 mg kg^{-1}, respectively. The median lethal dose through inhalation has been evaluated in rats, mice, dogs, and cats. In these laboratory species, the LC_{50} ranges from 31 887 in mice to 170 000 mg m^{-3} in cats.

Human

The literature on the toxicity of benzene in humans is extensive. The acute effects of benzene exposure generally differ markedly from the chronic effects. Acute exposure to high doses of benzene in air (at concentrations in excess of 3000 ppm) causes symptoms typical of organic solvent intoxication. Symptoms may progress from excitation, euphoria, headache, and vertigo, in mild cases, to central nervous system depression, confusion, seizures, coma, and death from respiratory failure in severe cases. The rate of recovery depends on the initial exposure time and concentration, but, following severe intoxication, the symptoms may persist for weeks.

Chronic Toxicity (or Exposure)

Animal

The effects of lifetime exposure to benzene have also been evaluated in laboratory animals. Chromosomal abnormalities in bone marrow cells have been reported to appear in rats, rabbits, mice, and amphibians as a consequence of experimental benzene exposure.

Human

The major toxicological manifestation of chronic benzene exposure in humans is bone marrow depression. Clinical manifestations include anemia, leucopenia, and thrombocytopenia. In severe cases, bone marrow aplasia develops. Later stages of toxicity are manifested by pancytopenia and aplastic anemia. Death may result from aplastic anemia or from leukemia. The US Environmental Protection Agency (EPA) and International Agency for Research on Cancer classify benzene as a known human carcinogen.

This classification was given to benzene in view of strong epidemiological and experimental evidence.

Clinical Management

The victim should be removed from the contaminated atmosphere. Contaminated clothing should be removed and the affected area should be washed with soap and water. Supportive treatment should be provided. In cases of ingestion, vomiting should not be induced. Benzene or organic solvents containing benzene can cause acute hemorrhagic pneumonitis if aspirated into the lungs. Activated charcoal can be given to minimize absorption from the gastrointestinal tract. Charcoal can be given in a slurry or mixed with sorbitol or a saline cathartic. The recommended doses of activated charcoal are 30–100 g for adults, 15–30 g for children, and 1 or 2 g kg^{-1} for infants. The indicated doses can be prepared in a slurry by mixing charcoal in a diluent at a rate of 10 g charcoal per 80 ml of diluent.

Environmental Fate

Benzene has a short half-life in surface water because it is so volatile. Detection of benzene in natural waters would therefore only be seen in areas adjacent to grossly contaminated waste sites. It would also tend not to bioaccumulate into fish tissue or biomagnify up the food chain.

Ecotoxicology

The US EPA ECOTOX database reports that *Ceriodaphnia* and *Daphnia* species are the most sensitive freshwater organisms following acute (48 h) exposure to benzene, with respective EC_{50} values of 130 and 400 ppb. Most organisms, however, can tolerate acute concentrations higher than this (in the 1–10 mg l^{-1} range). Following chronic exposures (4–7 day exposures), fish are relatively unaffected at concentrations up to 5 mg l^{-1} (at higher concentrations fish start to show adverse narcotic effects).

Exposure Standards and Guidelines

The odor threshold for benzene is 30 ppm, but the current American Conference of Governmental Industrial Hygienists Threshold Limit Value considered safe for occupational exposure (8 h day) is below that threshold at 0.5 ppm. The Occupational Safety and Health Administration permissible exposure limit (PEL) is 1 ppm, with a short-term exposure limit (STEL) of 5 ppm. The National Institute for Occupational Safety and Health recommends an exposure limit (recommended exposure limit) of 0.1 ppm with a STEL of 1 ppm.

See also: Blood; Carcinogenesis; Neurotoxicity; Pollution, Water; Respiratory Tract; Skin.

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Zhang L, Eastmond DA, and Smith MT (2002) The nature of chromosomal aberrations detected in humans exposed to benzene. *Critical Reviews in Toxicology* 32(1): 1–42.

Relevant Website

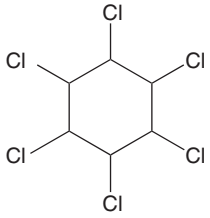
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Benzene.

Benzene Hexachloride, Mixed Isomers

Madhusudan G Soni

- Chemical Abstracts Service Registry Number: CAS 608-73-1
- Preferred Name: BHC (technical-grade BHC is a mixture of eight isomers)
- Synonyms: HCCH; HCH; 1,2,3,4,5,6-Hexachlorocyclohexane; Hexachlor; Hexachloran; Benzahex; Benzex; Hexator; Kotol; Lindane
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbon. Technical-grade BHC consists of 65–70% α-BHC, 6–8% β-BHC, 12–15% γ-BHC, and ~10% of other isomers and compounds

- Chemical Structure: Isomers differ on the spatial positions of the chlorine atoms on the boat and chair forms



Uses

The only known use of benzene hexachloride (BHC) is as an insecticide.

Exposure Routes and Pathways

More than 90% of BHC intake in humans originates from food.

Toxicokinetics

BHC is absorbed through all portals including the intact skin. Different isomers of BHC are reported to be absorbed rapidly from the gastrointestinal tract and transferred exclusively to blood. Metabolism of BHC mainly takes place in the liver by four enzymatic reactions. Dehydrogenation, dechlorination, and hydroxylation are via P450 mixed-function oxidases, whereas dehydrochlorination is carried out by cytosolic enzymes. The end products of biotransformation are di-, tri-, tetra-, penta-, and hexachloro compounds. Within a few hours of uptake, BHC is distributed to all organs and tissues. The highest concentrations are found in adipose tissues and skin. In a long-term high-level BHC feeding study, it was shown that adipose tissue retains more α-isomer than β- and γ-isomers. BHC is excreted rapidly in urine and feces after metabolic degradation. The excreted metabolites are either free or conjugated forms of glucuronic or sulfuric acids of *N*-acetyl cysteine.

Mechanism of Toxicity

BHC produces a variety of neurological effects in insects and mammals. However, at both levels of the nervous system (peripheral and central), the mechanism of toxic action of BHC is poorly understood. Central nervous system stimulation appears to be due to blockade of the effects of γ-aminobutyric acid. *In vitro* BHC isomers are reported to increase the calcium uptake of isolated rat brain synaptosomes. In addition, γ-isomer of BHC has been shown to inhibit the uptake of chloride ions at inhibitory synapses in the brain, and it is this mode of action that is now widely considered to account primarily for the convulsant activity of this insecticide. The results of studies on initiation–promotion, on mode of action, and on mutagenicity indicate that tumorigenic effects of BHC in mice result from nongenetic mechanisms.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity of BHC has been investigated in numerous studies in a variety of species and strains via different routes. Signs of acute poisoning in rats include diarrhea, hypothermia, epistaxis, and convulsions; death is due to respiratory failure. Animal studies suggest a decreased ability to reproduce when fed moderate to high levels of BHC.

Human

Clinical signs of intoxication can appear from a few minutes to some hours after BHC ingestion. Ingestion of large (unspecified) doses of BHC has led to muscle and kidney necrosis and in one case to pancreatitis. The symptoms of poisoning include nausea, restlessness, headache, vomiting, tremor, ataxia, and tonic–clonic convulsion. Digestive tract inflammation, hemorrhage, coma, and death have also been reported after poisoning.

Chronic Toxicity (or Exposure)

Animal

Feeding of BHC (10–1600 mg kg^{-1} diet) for life span to rats resulted in decreased body weight and an increase in mortality at 800 mg kg^{-1} and above. Fatty degeneration and focal necrosis of the liver were observed at higher doses. Chronic nephritis with glomerular fibrosis and hyaline deposits was seen in rats fed 800 mg kg^{-1} diet BHC.

Human

Chronic liver damage (cirrhosis and chronic hepatitis) were observed in liver biopsies from eight workers heavily exposed to BHC, DDT, or both for periods ranging from 5 to 13 years. Several case reports indicate a relationship between exposure to BHC and the occurrence of aplastic anemia. It is not clear if BHC affects the ability of people to reproduce or if it causes birth defects in humans.

In Vitro Toxicity Data

γ-BHC did not induce unscheduled DNA synthesis in human cells *in vitro* and did not induce micronuclei or chromosomal aberrations in cultured rodent cells. It induced DNA strand breaks but not unscheduled DNA synthesis. β-BHC was not mutagenic to yeast, but the γ-BHC isomer induced gene conversion. Neither γ- nor β-BHC were mutagenic to bacteria, and did not cause DNA damage in bacteria.

Clinical Management

Gastric decontamination by lavage and saline cathartics should be carried out. Oil laxatives should not be used because they promote BHC absorption. Pentabarbital or phenobarbital in adequate amounts or calcium gluconate intravenously in conjunction with anticonvulsants may be used in the control of convulsions.

Exposure Standards and Guidelines

The exposure limit for γ-BHC in most countries is $0.5\,\mathrm{mg\,m^{-3}}$. The US Food and Agricultural Organization/World Health Organization acceptable daily intake of γ-BHC is $0.008\,\mathrm{mg\,kg^{-1}}$ body weight. The US Environmental Protection Agency has set a limit in drinking water of 0.2 ppb of BHC in water.

Environmental Fate

Residues of BHC on eight types of soils were found to be decreased by 40–80% per year. When sprayed on the surface, the half-life of BHC was 4–6 weeks with 90% gone in 30–40 weeks. The typical half-life for BHC was 400 days. BHC can be washed off and into the soil, especially when humus content is low. BHC is very stable in water. It will disappear from the water by adsorption on sediment, biological breakdown by microflora and fauna, and adsorption by fish through gills, skin, and food.

See also: Pesticides.

Further Reading

Agency for Toxic Substances and Disease Registry (ATSDR) (1999) *Toxicological Profile for Alpha-, Beta-, Gamma-, and Delta-Hexachlorocyclohexanes*. Update (Final Report), p. 313. Atlanta, GA: ATSDR, Public Health Service, US Department of Health and Human Services.

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Relevant Website

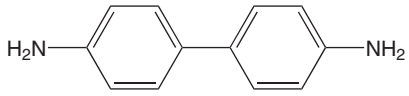
http://www.inchem.org – International Program on Chemical Safety, Hexachlorocyclohexane (Mixed Isomers).

Benzenedicarboxylic Acid, 1-2 *See* Phthalate Ester Plasticizers.

Benzidine

C Vaman Rao

- Chemical Abstracts Service Registry Number: CAS 92-87-5
- Synonyms: *p*-Diaminodiphenyl; 4,4′-Diaminobiphenyl; 4,4′-Diaminodiphenyl; [1,1′-Biphenyl]-4,4′-diamine
- Chemical/Pharmaceutical/Other Class: Benzidine is a diamine, manufactured synthetic aromatic hydrocarbon with two benzene rings covalently bonded to one another (1,1), substituted by amino group at 4,4′. It is a crystalline (sandy or sugar-like) solid that may be grayish-yellow, white, or reddish-gray in color. In the environment, benzidine is found in either its 'free' state (as an organic base) or as a salt (benzidine dihydrochloride or benzidine sulfate).
- Chemical Formula: $C_{12}H_{12}N_2$
- Chemical Structure:



Uses

Benzidine is used as an intermediate in the production of azo dyes, sulfur dyes, fast color salts, naphthol, and other dye compounds. However, it has not been

marketed or sold in the United States since the mid-1970s and US dye companies no longer manufacture benzidine-based dyes. However, a small amount of benzidine may still be manufactured or imported for scientific research in the United States, but in some countries it is still being manufactured. To date, more than 250 benzidine-based dyes have been reported. These dyes are primarily used for dyeing textiles, paper, and leather products.

Exposure Routes and Pathways

The primary routes of potential human exposure to benzidine are inhalation, ingestion, and dermal contact. The general population is not likely to be exposed to benzidine through contaminated air, water, soil, or food. People living near a hazardous waste site are likely to get exposed to benzidine through contaminated drinking water or by breathing contaminated air or by swallowing contaminated dust and soil. Benzidine can also enter the body by passing through the skin through contaminated clothing and gloves. People working at or near a hazardous waste site may get exposed to benzidine in a similar manner.

Toxicokinetics

Benzidine is rapidly absorbed through the skin in solid and vapor form. It is also quickly absorbed through the lungs on inhalation and from the gastrointestinal tract on consuming contaminated water and food. Generally, it will take only few hours for most of the benzidine to get into the body through the lungs and intestine. Breathing, eating, or drinking benzidine-based dyes may also expose a person to benzidine because the intestinal bacteria can break down these dyes into benzidine. It is a lipophilic substance, hence easily stored in fat tissues, and it firmly binds to cell receptors. Benzidine is metabolized to aromatic amine by intestinal microflora or liver azoreductase. The liver is the chief organ of metabolism where benzidine is converted to more reactive, toxic, and mutagenic (carcinogenic) *N*-hydroxyarylamides and *N*-hydroxylamine is considered to be a proximate carcinogen. *N*-Hydroxylamides are converted to the ultimate carcinogens through conjugation with sulfuric, acetic, or glucuronic acids. *N*-Acetoxyarylamines are also produced as metabolites and are highly reactive mutagens and carcinogens. Glutathione transferase plays an important role in the elimination of reactive metabolites of benzidine. Sulfonation, carboxylation, deamination, or substitution of an ethyl alcohol or an acetyl group for the hydrogen in the amino groups leads to a decrease in mutagenicity of benzidine metabolites as well as to easy elimination, primarily through urine and feces.

Mechanism of Toxicity

Benzidine is metabolized to highly toxic, reactive metabolites, such as *N*-hydroxyarylamides and *N*-hydroxyarylamines, which act as procarcinogens and are more mutagenic than parent compounds. The metabolites act as DNA adducts and bind to cell receptors. The metabolites on conjugation with sulfuric, acetic, and glucuronic acids form ultimate carcinogens. Acetylated benzidine metabolites such as *N*-acetoxyarylamines are known to cause bladder cancer in dye industry workers.

Chronic Toxicity (or Exposure)

Animal

There is sufficient evidence from animal studies that benzidine is a carcinogen. When administered in the diet, benzidine induced bladder cancer in dogs, multiple mammary carcinomas in rats and liver cell tumors in hamsters of both sexes. When administered by the subcutaneous route to mice of both sexes, it induced malignant tumors of the Zymbal gland (ear) and hepatocellular carcinoma; hepatomas, malignant tumors of the Zymbal gland, and local sarcomas in male rats; and malignant tumors of the Zymbal gland, mammary adenocarcinomas, and amyloid leukemia in female rats. When administered by intraperitoneal injection, benzidine induced Zymbal gland adenomas and carcinomas and malignant mammary tumors in female rats. The lethal dose in dogs is $400\,mg\,kg^{-1}$ by the subcutaneous route and $200\,mg\,kg^{-1}$ by the oral route. Dyes made from benzidine, such as Direct Blue 6, Direct Black 38, and Direct Brown 95 have been shown to cause cancer in animals. The Department of Health and Human Services (DHHS) has determined that Direct Black 38 and Direct Blue 6 cause cancer in animals, and the International Agency for Research on Cancer (IARC) has also determined that Direct Black 38, Direct Blue 6, and Direct Brown 95 cause cancer in animals.

Human

Benzidine can cause cancer in humans. This has been shown in studies of workers who were exposed for many years to levels much higher than the general population would be. An IARC study on dye industry workers reported that there is a direct correlation between the incidence of bladder cancer in the occupationally benzidine-exposed workers and the incidence of this cancer decreasing in workers after reduction in occupational exposure. Some evidences indicate that dyes made from benzidine, such as Direct Blue 6, Direct Black 38, and Direct Brown 95 may cause cancer in humans. Benzidine poisoning

causes vomiting, nausea, hemolysis, liver and kidney damage, and hematuria (bloody urine). Benzidine is considered to be acutely toxic to humans by ingestion, with an estimated oral lethal dose of between 50 and 500 mg kg^{-1} for a 70 kg person. Symptoms of acute ingestion exposure include cyanosis, headache, mental confusion, nausea, and vertigo. Dermal exposure may cause skin rashes and irritation.

Clinical Management

There is no antidote for benzidine poisoning. Since it produces reactive metabolites, administration of free radical scavengers would alleviate the toxicity. A complex of benzidine metabolites with copper and hydrochloride is known to decrease its mutagenic effects.

Environmental Fate

Industries release benzidine into the environment in the form of liquid waste and sludges. Benzidine may also be released into the environment due to spillage during transport. In air, benzidine is found bound to suspended particles or as a vapor, which may be brought back to the earth's surface by rain or gravity. Very small amount of benzidine dissolves in water at moderate environmental temperatures. When released into waterways, it sinks to the bottom and becomes part of the bottom sludge. In soil, most benzidine is strongly attached to soil particles, so it does not easily leach into underground water from the waste dumps.

Benzidine is slowly destroyed in the environment by light, certain other chemicals and microorganisms. Accumulation in the food chain has not been recorded so far but it is documented that water life may take up and store very small amounts of benzidine.

Further Reading

Rosenman KD and Reilly MJ (2004) Cancer mortality and incidence among a cohort of benzidene and dichlorobenzidene dye manufacturing workers. *American Journal of Industrial Medicine*. 46(5): 505–512.

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Relevant Websites

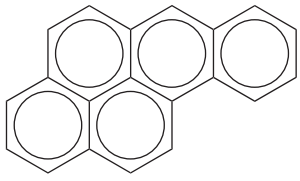
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Benzidene.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Benzidene.

Benzo(*a*)pyrene

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 50-32-8
- Synonyms: BAP; B(*a*)P; BP; 3,4-Benzopyrene; 6,7-Benzopyrene; 3,4-Benzpyrene; 3,4-Benz(*a*)-pyrene
- Chemical/Pharmaceutical/Other Class: Polycyclic aromatic hydrocarbon
- Chemical Formula: $C_{20}H_{12}$
- Chemical Structure:



Uses

In research, benzo(*a*)pyrene (BP) is used extensively as a positive control in a variety of laboratory mutagenicity and carcinogenicity short-term tests. It is not produced commercially in the United States.

Exposure Routes and Pathways

The primary routes of exposure to BP are inhalation and ingestion.

Toxicokinetics

Polycyclic aromatic hydrocarbons (PAHs) are absorbed following ingestion, inhalation, and dermal exposure. Following absorption, PAHs enter the lymph and then the bloodstream. BP is readily absorbed from the intestinal tract and tends to localize primarily in body fat and fatty tissues such as the breast. Disappearance of BP from blood and liver of

rats following a single intravenous injection is very rapid, having a half-life in blood of less than 5 min and a half-life in liver of 10 min. In blood and liver, the initial rapid elimination phase is followed by a slower disappearance phase, lasting 6 h or more. A rapid equilibrium is established between BP in blood and that in liver. The fast disappearance of the compound from blood is due to metabolism and distribution in tissues. BP is known to cross the placenta in mice and rats. ^{14}C metabolites were secreted into the bile of rats within 7 min of receiving an intravenous dose of ^{14}C BP. Pretreatment of animals with this carcinogen enhanced biliary secretion of ^{14}C radiolabel. PAHs are primarily metabolized enzymatically in the liver and kidneys. Additional sites of PAH metabolism include the adrenal glands, testes, thyroid, lungs, skin, and sebaceous glands. PAHs are metabolized by aryl hydrocarbon hydroxylase. The ultimate carcinogen of CYP450 metabolism of BP is 7,8-dihydro-7,8-diol-9,10-epoxide. The predominant metabolites of BP in mammals are 3- and 9-hydroxy BP, BP-1,6-quinone and BP-3,6-quinone, BP-4,5-dihydrodiol, BP-7,8-dihydrodiol, and BP-9,10-dihydrodiol. Human liver microsomal fractions were characterized for differences in the metabolism of BP. Pronounced interindividual differences in the composition of microsomal proteins in the molecular weight range of 49 000–60 000 were found. Large variation among human liver microsomal samples was also seen in BP metabolism. The results indicate the presence of seven or eight different forms of CYP450 in human liver microsomes and interindividual variations seen in metabolism may partly be explained by variations in the distribution of these isozymes.

Acute and Short-Term Toxicity (or Exposure)

Animal

Mild hepatotoxicity and nephrotoxicity have been observed in rats exposed to PAHs. Intraperitoneal administration of BP to rats produced an immediate and sustained reduction in growth rate of young rats. A single topical exposure of BP in acetone increased the mitotic rate of epidermal cells. Single oral administration of 100 mg BP to 50-day-old Sprague–Dawley rats produced mammary tumors. Single intraperitoneal administration of 10 mg BP produced two mammary and two uterine carcinomas among 10 Wistar rats within 1 year.

Human

In general, PAHs have a low order of acute toxicity in humans. BP may cause skin irritation with rash, redness, and/or a burning sensation. Exposure to sunlight and the chemical together can increase these effects. BP can irritate and/or burn the eyes on contact.

Chronic Toxicity (or Exposure)

Animal

In rats chronically fed PAHs, agranulocytosis, anemia, leukopenia, and pancytopenia have been observed. There is sufficient evidence suggesting that BP is carcinogenic to experimental animals. Exposure to BP caused a dose-dependent increase in the pulmonary tumor burden of mice administered B16F10 melanoma cells intravenously 1 day after the last of a 14 day exposure to BP. Biweekly administration of BP in oil by stomach tube produced papillomas of the stomach in hamsters. Biweekly painting with 0.3% solution of BP in benzene for 400 days produced one carcinoma and 10 papillomas among 10 rabbits. A possible causal relation between BP/diribonucleoside adduct formation and papilloma formation in Sencar mice was found. Among rats fed 1 mg BP per gram of diet during pregnancy many resorptions and dead fetuses were observed but only one malformed fetus was noted from seven litters. BP has been shown to be embryotoxic and teratogenic in mice. A reduction in fertility in male and female offspring was observed in mice following exposure *in utero*.

Human

Long-term health effects can occur at some time after exposure to BP and can last for months or years. BP is a probable carcinogen in humans. There is some evidence that it causes skin, lung, and bladder cancer in humans and animals. BP has caused cancer in the offspring of animals exposed to the substance during pregnancy. Many scientists believe that there is no safe level of exposure to a carcinogen. Cancer is the most significant toxicity associated with PAHs. The first occupational cancer described was that of scrotal cancer in chimney sweeps exposed to PAHs in soot and ash. Studies have noted increased lung cancer and a suggestion of increased gastrointestinal cancer incidence in the coal carbonization and coal gasification industries. BP has been observed to produce epithelial hyperplasia and inhibition of connective tissue growth on human fetal lung cultures. Since tobacco smoke contains BP, smoking may increase the risk of lung cancer with exposure to BP. BP on the skin in the presence of sunlight and/or ultraviolet light also increases the risk of skin cancer. Persistent nodules diagnosed as squamous epithelioma developed in a man who had been exposed to BP

for 3 weeks while carrying out an experiment on mice. BP may damage the developing fetus. There is some evidence that BP may affect the sperm and the testes. BP may be transferred to nursing infants through mother's milk. Repeated exposure to substances that contain BP can cause skin changes such as thickening, darkening, and pimples. Later skin changes include loss of color, reddish areas, thinning of the skin, and warts. Bronchitis may result from repeated exposure to BP-containing mixtures. Coke oven workers exposed to BP had significantly depressed levels of IgG and IgA compared to cold-rolling mill workers.

Clinical Management

Because of the low acute toxicity associated with PAHs, induced emesis is not recommended. Activated charcoal/cathartic may be used. On inhalation exposure, the patient should be moved to fresh air and monitored for respiratory distress. If cough or difficulty in breathing develop, evaluation for respiratory tract irritation, bronchitis, or pneumonitis should be performed. Humidified supplemental oxygen (100%) should be administered with assisted ventilation as required. On ocular exposure, the eyes should be irrigated for at least 15 min with tepid water. On dermal exposure, the affected area should be washed thoroughly with soap and water. Patients developing dermal hypersensitivity reactions may require treatment with systemic or topical corticosteroids or antihistamines. Treatment of gastric, lung, or skin cancer is no different from that for the same cell type.

Environmental Fate

Released BP is moderately persistent in the environment. It readily binds to soils and should not leach to groundwater, though it has been detected in some groundwater. If released into water, it will adsorb strongly to sediments and particulate matter. In most waters and sediments it will resist breakdown by microbes and reactive chemicals. BP is expected to bioconcentrate in aquatic organisms that cannot metabolize it, including plankton, oysters, and some fish.

Exposure Standards and Guidelines

A class I, type B biological safety hood should be used when working with BP in a laboratory. The following work practices are recommended: (1) Contaminated clothing should be removed immediately and laundered by individuals who have been informed of the hazards of exposure to BP. (2) Eye wash fountains should be provided for emergency use. Emergency shower facilities should be available if there is a possibility of skin exposure. On skin contact, affected skin should be washed immediately to remove the chemical. (3) Eating, smoking, or drinking should be prohibited where BP is handled. (4) Protective clothing (suits, gloves, footwear, and headgear) should be donned before work. Workers in industries that produce coal or coal tar products and those who tar road surfaces and roofs are at maximum risk.

Under Resource Conservation and Recovery Act (RCRA), BP must be managed as a hazardous waste according to federal and/or state regulations. The US Environmental Protection Agency (EPA) federal drinking water standard is $0.2\,\mathrm{mg\,l^{-1}}$. The National Institute for Occupational Safety and Health occupational exposure recommendations are $0.1\,\mathrm{mg\,m^{-3}}$ for cyclohexane extractable fraction and $0.1\,\mathrm{mg\,m^{-3}}$, 10 h time-weighted average for coal tar products.

The maximum contaminant level for BP has been set to 0.2 ppb by the EPA.

Miscellaneous

BP has a faint aromatic odor. It has a boiling point of greater than 360°C at 760 mmHg and a melting point of 179–179.3°C. It has a specific gravity of 1.351. Crystals of BP may be monoclinic or orthorhombic.

On contact with strong oxidizers, BP may cause fire or explosion. BP is light labile and is oxidized by chromic acid and by ozone.

BP is found in fossil fuels and occurs in products of incomplete combustion. It is present in charcoal, chimney sweepings, and coal tar.

See also: Immune System; Occupational Toxicology; Pollution, Water; Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

Miller AP and Ramos KS (2001) Impact of cellular metabolism on the biological effects of benzo(*a*)pyrene and related hydrocarbons. *Drug Metabolism Reviews* 33: 1–35.

Relevant Websites

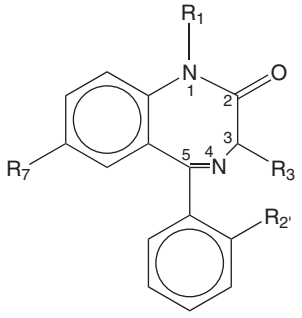
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Benzo(*a*)pyrene.
http://www.inchem.org – Benzo(*a*)pyrene (1983) IARC Summary and Evaluation, vol. 32.

Benzodiazepines

Christopher P Holstege

This article is a revision of the previous print edition article by Gregory P Wedin, volume 1, pp. 139–141, © 1998, Elsevier Inc.

- Representative Chemicals: Alprazolam; Chlordiazepoxide; Clonazepam; Clorazepate dipotassium; Clorazepate monopotassium; Diazepam; Estazolam; Flunitrazepam; Flurazepam; Halazepam; Lorazepam; Midazolam; Nitrazepam; Oxazepam; Prazepam; Quazepam; Temazepam; Triazolam
- Chemical Abstracts Service Registry Numbers: CAS 28981-97-7 (alprazolam); CAS 439-14-5 (diazepam); CAS: 604-75-1 (oxazepam)
- Synonyms:
  - Alprazolam – Xanax; $C_{17}H_{13}ClN_4$; 8-Chloro-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
  - Chlordiazepoxide – Librium; $C_{16}H_{14}ClN_3O$; 7-Chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine-4-oxide
  - Clonazepam – Klonopin, Rivotril, Clonapam; $C_{15}H_{10}ClN_3O_3$; 5-(2-Chlorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepin-2-one
  - Clorazepate dipotassium – Tranxene; ClorazeCaps; ClorazeTabs; $C_{16}H_{11}ClK_2N_2O_4$
  - Clorazepate monopotassium – Azene; $C_{16}H_{11}ClKN_2O_4$
  - Diazepam – Valium, Vivol, E-Pam; $C_{16}H_{13}ClN_2O$; 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one
  - Estazolam – ProSom; $C_{16}H_{11}ClN_4$; 8-Chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
  - Flunitrazepam – Rohypnol; $C_{16}H_{12}FN_3O_3$; 5-(2-Fluorophenyl)-1,3-dihydro-1-methyl-7-nitro-2*H*-1,4-benzodizepin-2-one
  - Flurazepam – Dalmane, Somnol, Som Pam; $C_{12}H_{23}ClFN_3O$; 7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepine-2-one
  - Halazepam – Paxipam; $C_{17}H_{12}ClF_3N_2O$; 7-Chloro-1,3-dihydro-5-phenyl-1-(2,2,2-trifluoroethyl)-2*H*-1,4-benzodiazepin-2-one
  - Lorazepam – Ativan; $C_{15}H_{10}ClN_2O_2$; 7-Chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one
  - Midazolam – Versed; $C_{18}H_{13}ClFN_3$; 8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidadazo[1,5-*a*][1,4]benzodiazepine
  - Nitrazepam – Mogadon; $C_{15}H_{11}N_3O_3$; 1,3-Dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one
  - Oxazepam – Serax; $C_{15}H_{11}ClN_2O_2$; 7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one
  - Prazepam – Verstran; $C_{19}H_{17}ClN_2O$; 7-Chloro-1-(cyclopropylmethyl)-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one
  - Quazepam – Doral; $C_{17}H_{11}ClF_4N_2S$; 7-Chloro-5-(2-fluorophenyl)-1,3-dihydro-1-(2,2,2-trifluoroethyl)-2*H*-1,4-benzodiazepine-2-thione
  - Temazepam – Restoril; 3-Hydroxydiazepam; $C_6H_{13}ClN_2O_2$; 7,-Chloro-l,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one
  - Triazolam – Halcion; $C_{17}H_{12}C_{l2}N_4$; 8-Chloro-6-(2-chlorophenyl)-1-methyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine
- Chemical/Pharmaceutical/Other Class: 5-Aryl-1,4-benzodiazepines
- Chemical Structure:



Uses

The benzodiazepines are primarily administered for their sedative–hypnotic effects. Benzodiazepines are commonly used as anxiolytics, muscle relaxants, anticonvulsants, and to treat alcohol withdrawal, insomnia, and agitation. They are administered preoperatively for their anterograde amnesia effects and are combined frequently with other medications for conscious sedation before procedures. They are also utilized as drugs of abuse.

Exposure Routes and Pathways

The most common route of exposure to the benzodiazepines is ingestion of oral dosage forms. Several of these agents are also available for parenteral administration (intramuscular or intravenous). Diazepam may be administered through an

endotracheal tube; aerosolized diazepam is under investigation.

Toxicokinetics

The benzodiazepines are generally well absorbed from the gastrointestinal tract. The time to peak concentration of the benzodiazepines ranges from 0.5 to 6 h after ingestion. The benzodiazepines are all extensively metabolized by microsomal enzyme systems in the liver. The metabolites of many benzodiazepines are pharmacologically active and are biotransformed much more slowly than the parent compounds. The benzodiazepines that are not biotransformed to active metabolites include clonazepam, estazolam, lorazepam, nitrazepam, oxazepam, temazepam, and triazolam. The benzodiazepines and their active metabolites are widely distributed into body tissues and readily cross the blood–brain barrier and placenta. All are highly bound to plasma proteins. The elimination half-lives of the benzodiazepines range from 1 to 70 h at therapeutic doses. The half-lives of active metabolites, however, may be as long as 120 h. These metabolites are ultimately conjugated, largely with glucuronic acid, to inactive compounds that are excreted primarily in the urine.

Mechanism of Toxicity

Benzodiazepines exert their action by potentiating the activity of γ-aminobutyric acid (GABA). GABA is the major inhibitory neurotransmitter in the central nervous system (CNS). They bind to a specific receptor on the GABA receptor complex, which facilitates the binding of GABA to its specific receptor site. Benzodiazepine binding causes increased frequency of opening of the chloride channel. Chloride channel opening results in membrane hyperpolarization, thereby inhibiting cellular excitation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may be affected by the benzodiazepines much in the same way as humans. Lethargy, coma, shallow respirations, incoordination, and depressed reflexes may occur. Dogs may show a contradictory response (CNS excitement) following exposure. Standard supportive measures should be employed.

Human

There is a broad spectrum of signs and symptoms associated with acute benzodiazepine toxicity. Lethargy, ataxia, nystagmus, diplopia, amnesia, slurred speech, confusion, hypotonia, hypotension, hypothermia, coma, respiratory depression, and death have been reported. Rarely, paradoxical excitation may occur at lower doses. Toxic doses for each agent have not been clearly established. When large doses of lorazepam have been infused chronically, there are multiple reports of the development of a syndrome consisting of a hyperosmolar state with metabolic acidosis and cardiovascular compromise. This syndrome has been attributed to propylene glycol, the diluent in lorazepam.

Chronic Toxicity (or Exposure)

Animal

Chronic dosing in pregnant rats has resulted in increased rates of cleft palate formation as well as decrease in serum thyroxine levels. Prenatal exposure to benzodiazepines in rats describes learning and memory deficits in pups as well as absence of usual startle responses.

Human

Tolerance and physical dependence may develop in persons who chronically use benzodiazepines. Abrupt discontinuation of chronic benzodiazepine therapy may result in a withdrawal syndrome consisting of anxiety, agitation, insomnia, tremors, headache, and myalgias. In more severe cases nausea, vomiting, diaphoresis, hyperpyrexia, psychosis, seizures, and death may occur.

In Vitro Toxicity Data

In vitro studies examining the effects of diazepam in the immune system have shown mixed results. Several cultured cell models have demonstrated diazepam-induced inhibition of cell proliferation.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Gastrointestinal decontamination procedures should be used as appropriate based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used to adsorb the benzodiazepines. The patient's level of consciousness and vital signs should be monitored closely. Obtunded patients with reduced gag reflex should be intubated to prevent pulmonary aspiration. Respiratory support, including oxygen and ventilation, should be provided as needed. If hypotension occurs it should be treated with standard

measures including intravenous fluids, Trendelenburg positioning, and dopamine by intravenous infusion. Forced diuresis, hemoperfusion, and hemodialysis are of no value in benzodiazepine toxicity. If withdrawal signs and symptoms develop, treatment should focus on either benzodiazepine or phenobarbital therapy with a gradual dose reduction.

Flumazenil (Romazicon) is a benzodiazepine antagonist that can reverse the CNS depressant effects of these agents. It should be used with caution in acute intentional benzodiazepine overdoses. Because acute benzodiazepine overdoses generally result in only mild toxicity, it has limited clinical utility in this setting. Flumazenil's use in the acute benzodiazepine intoxicated patient may lead to an unnecessarily long observation period after fumazenil's infusion. This observation is necessary to be certain that reoccurrence of benzodiazepine toxic effects do not occur after flumazenil is metabolized. Flumazenil must be used with caution in mixed drug overdoses as seizures can develop, particularly if tricyclic antidepressants have been coingested. Also, it can induce potentially serious benzodiazepine withdrawal in dependent patients.

See also: Diazepam; Levothyroxine; Propylene Glycol.

Further Reading

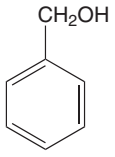
Finkle BS, McCloskey KL, and Goodman LS (1979) Diazepam and drug-associated deaths. *Journal of the American Medical Association* 242: 429–434.

Gaudreault P, Guay J, and Thivierge RL (1991) Benzodiazepine poisoning: Clinical and pharmacological considerations and treatment. *Drug Safety* 6: 247–265.

Benzyl Alcohol

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 100-51-6
- Synonyms: Benzene carbinol; Benzene methanol; Benzoyl alcohol; Phenyl carbinol; Phenyl methanol; Hydroxymethyl benzene; Hydroxy toluene
- Chemical/Pharmaceutical/Other Class: Alcohol
- Chemical Formula: $C_6H_5CH_2OH$
- Chemical Structure:



Uses

Benzyl alcohol is primarily used as a solvent and an antimicrobial preservative, but it has also found use as an antiseptic and local anesthetic. It is also used as a raw material of various esters, used in the soap, perfume, and flavor industries. Acceptable daily intakes were established at $5\,mg\,kg^{-1}$ for benzyl alcohol by the World Health Organization. In 1998, benzyl alcohol was reported by the US Food and Drug Administration as being used in 322 cosmetic formulations, belonging to 43 cosmetic categories.

Toxicokinetics

Body tissue possibly takes up benzyl alcohol rapidly and releases it slowly into the bloodstream. Rabbits when given 1 g (subcutaneously) of benzyl alcohol eliminated 300–400 mg of hippuric acid within 24 h. Rabbits eliminated 65.7% of a dose of 0.4 g of benzyl alcohol as hippuric acid in the urine. The plasma half-life of benzyl alcohol administered as a 2.5% solution in saline was found to be ~1.5 h in dogs injected intravenously at doses of 52 and $105\,mg\,kg^{-1}$.

Benzyl alcohol is oxidized by the liver alcohol dehydrogenase. Humans readily oxidize benzyl alcohol to benzoic acid, which, after conjugating with glycine, is rapidly eliminated as hippuric acid in the urine. Within 6 h after taking 1.5 g of benzyl alcohol orally, human subjects eliminated 75–85% of the dose in urine as hippuric acid. Benzyl alcohol yields benzaldehyde in rabbits and phenol in guinea pigs. If the dose is sufficiently high to allow the rate of formation of benzoic acid to exceed that of hippuric acid some of the benzoic acid is excreted as benzoylglucuronide.

Mechanism of Toxicity

Benzyl alcohol is oxidized by the liver to benzoic acid, and then conjugated with glycine to form hippuric acid. Metabolic acidosis can be explained by a direct effect of benzoic acid and/or secondary lactic acid production through depression of cellular metabolism. Benzyl alcohol is a weak local anesthetic with disinfectant properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

When injected into chickens, benzyl alcohol produced birth defects of the central nervous system (CNS) and skeleton. Doses of 0.2 ml kg^{-1} or more to dogs by stomach tube induced emesis and defecation. This was apparently due to irritation of the gastric mucosa. Diuresis was more pronounced in the rabbit than in the dog after administration of benzyl alcohol by various routes. Mice suffered respiratory stimulation, respiratory and muscular paralysis, convulsions, and CNS depression following a subcutaneous injection. A decrease in arterial blood pressure of rabbits, cats, and dogs was seen following intravenous injection of benzyl alcohol. No such decrease in arterial blood pressure was noted following oral administration to dogs. Benzyl alcohol displayed antiarrhythmic, antifibrillatory effects when injected intravenously into dogs and rats with spontaneous and drug-induced arrhythmias. Instillation of pure benzyl alcohol into rabbit conjunctival sac produces corneal necrosis, which is resolved after several weeks. The undiluted material when applied to depilated skin of guinea pigs for a period of 24 h caused moderately strong primary irritation, and there was evidence of systemic symptoms with death from applications of less than 5 ml kg^{-1}.

Because its primary effects are expected to be irritation and perhaps mild CNS depression, benzyl alcohol is in class I for general toxicity (may cause reversible effects which are generally not life-threatening). From the one study in chickens, it is in class B for reproductive hazard (few effects in animals but no human data). Some differences between control and benzyl alcohol-treated populations were noted in one reproductive toxicity study using mice, but these were limited to lower maternal body weights and decreased mean litter weights. Another study also noted that fetal weight was decreased compared to controls, but a third study showed no differences between control and benzyl alcohol-treated groups. The actual human reproductive hazard is unknown.

Human

High doses of benzyl alcohol cause nausea, vomiting, diarrhea, CNS, depression, and vertigo. Dilute solutions (1%) produce local anesthesia and slight irritation when instilled into the eye. Pure benzyl alcohol produces corneal necrosis. Following acute exposure lethargy, seizures, intraventricular hemorrhage, and neurological sequelae (cerebral palsy, developmental delay) have been seen in neonates with parenteral benzyl alcohol toxicity. Metabolic acidosis was a common finding with parenteral toxicity in neonates. Thrombocytopenia was a delayed feature of parenteral toxicity in neonates. Deaths associated with intravenous or endotracheal administration of benzyl alcohol-containing solutions in neonates were preceded by symptoms of respiratory distress progressing to gasping respirations, metabolic acidosis, CNS depression, hypotension, renal failure, and occasionally seizures and intracranial hemorrhage. Thrombocytopenia was a delayed feature of parenteral toxicity in neonates. Severe striated keratopathy, progressing to chronic edema of cornea, was noted following intraocular use of a sodium chloride solution containing 2% benzyl alcohol.

Chronic Toxicity (or Exposure)

Animal

There was no evidence of carcinogenic activity of benzyl alcohol for male or female F344/N rats dosed with 200 or 400 mg kg^{-1}. There was no evidence of carcinogenic activity of benzyl alcohol for male or female B6C3F1 mice dosed with 100 or 200 mg kg^{-1} for 2 years.

Human

Chronic exposure to benzyl alcohol would presumably produce effects similar to those from acute exposure. No other industrial illness is known from benzyl alcohol. No reproductive effects on humans are known.

In Vitro Toxicity Data

Benzyl alcohol was not mutagenic when tested by the preincubation protocol in the presence or absence of exogenous metabolic activation in the *Salmonella* assay. A significant increase in chromosomal aberrations was observed after exposure to benzyl alcohol in the presence, but not absence of S9.

Clinical Management

Treatment is supportive following exposure. The victim should be monitored for CNS and respiratory depression, metabolic acidosis, and hypotension. Ipecac-induced emesis is not recommended. On ocular exposure, the eyes should be irrigated for at least 15 min with tepid water. On dermal exposure, the exposed area should be washed with soap and water. If irritation, pain, swelling, lacrimation, or

photophobia persists, the victim should be seen in a health care facility.

Environmental Fate

When released into the soil, benzyl alcohol is expected to leach into groundwater. When released into the soil, this material may evaporate to a moderate extent and biodegrade to a moderate extent. When released into the water, benzyl alcohol is not expected to evaporate significantly and may evaporate to a moderate extent. It has an estimated bioconcentration factor of less than 100 and is not expected to significantly bioaccumulate. When released into air, benzyl alcohol is expected to have a half-life between 1 and 10 days and may be removed from the atmosphere to a moderate extent by wet deposition.

Exposure Standards and Guidelines

The odor threshold for benzyl alcohol is 5.5 ppm.

Miscellaneous

Benzyl alcohol is a water-white liquid with a faint aromatic odor and a sharp burning taste. It has a molecular weight of 108.13 and a specific gravity of 1.045. Aqueous solution of benzyl alcohol is neutral. Benzyl alcohol decomposes to benzaldehyde slowly when exposed to air. For this reason, storage tanks should be blanketed with nitrogen. Benzyl alcohol is incompatible with oxidizing agents. Problems may occur when polystyrene syringes are used with certain types of drug products containing benzyl alcohol since these agents can extract and dissolve the plastic. At times the rubber tip may release a constituent to the drug product.

See also: Fragrances and Perfumes; Respiratory Tract.

Further Reading

CIR (2001) Cosmetic Ingredient Review Expert Panel, final report on the safety assessment of benzyl alcohol, benzoic acid and sodium benzoate. *International Journal of Toxicology* 20(Suppl. 3): 23–50.

Nair B (2001) Final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate. *International Journal of Toxicology* 20(Suppl. 3): 23–50.

NTP (1989) Toxicology and Carcinogenesis Studies of Benzyl Alcohol (CAS No. 100-51-6) in F344/N Rats and B6C3F1 Mice (Gavage Studies). National Toxicology Program Technical Report Series 343, pp. 1–158.

Relevant Website

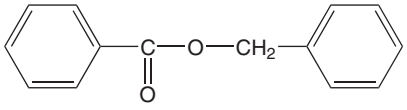
http://www.intox.org – Chem Info. Chemical Profiles Created by CCOHS.

Benzyl Benzoate

Jamaluddin Shaikh

This article is a revision of the previous print edition article by Sushmita M Chanda, volume 1, pp. 143–144, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 120-51-4
- Synonyms: Ascabin; Ascabiol; Ascarbin; Benzylate; Scabanca; Tenutex; Vanzoate; Venzoate; Benzoic acid phenylmethyl ester; Benzy alcohol benzoic ester
- Chemical/Pharmaceutical/Other Class: Benzoic acid phenylmethyl ester
- Chemical Structure:



Uses

Benzyl benzoate is used as an acaricide, scabicide, and pediculicide in veterinary hospitals and as a repellent for chiggers, ticks, and mosquitoes.

Exposure Routes and Pathways

Dermal exposure is the most common route of exposure. Benzyl benzoate occurs naturally in balsams of Peru and Tolu and other essential oils. It is also available in liquid, emulsion, and lotion dosage forms.

Toxicokinetics

Benzyl benzoate is rapidly absorbed from the stomach. It is rapidly hydrolyzed to benzoic acid and benzyl alcohol, which is subsequently hydrolyzed to benzoic acid. Benzoic acid is conjugated with glycine to give benzoylglycine or hippuric acid and with glucuronic acid to give benzoylglucuronic acid. The

conjugates are rapidly eliminated in urine in varying ratios depending on species and dose.

Mechanism of Toxicity

Benzyl benzoate is a local irritant.

Acute and Short-Term Toxicity (or Exposure)

Animal

Benzyl benzoate has low acute toxicity in laboratory animals. The oral LD_{50} value for rats is greater than $1\,g\,kg^{-1}$. If applied too frequently or to a large area, it can induce systemic signs of toxicity including salivation, piloerection, muscular incoordination, tremors, progressive paralysis of hindlimbs, prostration, violent convulsions, dyspnea, and death. Cats are especially susceptible to such toxicity. In contrast, dogs are highly resistant to acute benzyl benzoate toxicity. When given in large doses to laboratory animals, benzyl benzoate can cause hyperexcitation, incoordination, ataxia, convulsions, and respiratory paralysis.

Human

Benzyl benzoate is a slightly toxic compound when used topically. It may cause slight allergenic responses, which may disappear after the end of exposure. If used as an acaricide, it may cause peristalsis of the intestine, diarrhea, intestinal colic, enterospasm, pylorospasm, spastic constipation, contraction of the seminal vesicles, hypertension, and bronchospasms.

Chronic Toxicity (or Exposure)

Human

Relatively little is known about the chronic effects of benzyl benzoate. Contact dermatitis may occur with repeated use (blistering, crusting, oozing, reddening, or scaling of skin).

In Vitro Toxicity Data

Benzyl benzoate was negative in an *in vitro* screen for estrogenic stimulation.

Clinical Management

Basic life-support measures for respiratory and cardiovascular functions should be utilized. Dermal decontamination should be accomplished by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min.

Ecotoxicology

Benzyl benzoate is toxic to some aquatic organisms. The LC_{50} (4 day) was $4.5\,mg\,l^{-1}$ in fish.

See also: Pesticides.

Relevant Website

http://www.inchem.org – International Programme on Chemical Safety.

Beryllium

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst, Shirley B Radding, and Kathyrn A Wurzel, volume 1, pp. 144–145, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-41-7
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Be^{2+}

Uses

Beryllium is an important industrial metal because of its material properties, that is, it is lighter than aluminum and six times stronger than steel. Often alloyed with other metals such as copper, beryllium is a key component of materials used in the aerospace and electronics industries. Beryllium has a small neutron cross-section, which makes it useful in the production of nuclear weapons and in sealed neutron sources. Specifically, beryllium is used in nuclear reactors as a neutron reflector or moderator, and in the aerospace industry in inertial guidance systems; beryllium alloys (consisting of copper or aluminum) are also used in structural material. Beryllium oxide is used as an additive in glass, ceramics, and plastics and as a catalyst in organic reactions. In the past, beryllium was widely used in the manufacture of fluorescent lights and neon signs. Alloyed with copper, aluminum, or nickel, beryllium imparts excellent electrical and thermal conductivity.

Background Information

Beryllium was discovered as an element in 1798. Its use in metallurgy and electrical components were largely developed in the 1920s.

Exposure Routes and Pathways

The primary exposure pathway for beryllium is inhalation. Inhalation, ingestion, and dermal contact are possible exposure pathways in workplace settings.

Exposure to small amounts of beryllium occurs with ingestion of some foods and drinking water. Beryllium enters the air, water, and soil as a result of natural and human activities. Emissions from burning coal and oil increase beryllium levels in air. Beryllium enters waterways from the wearing away of rocks and soil. Most of the man-made beryllium that enters waterways comes when industry dumps waste water and when beryllium dust in the air from industrial activities settles over water. Beryllium, as a chemical component, occurs naturally in soil; however, disposal of coal ash, incinerator ash, and industrial wastes may increase the concentration of beryllium in soil. In air, beryllium compounds are present mostly as fine dust particles. The dust eventually settles over land and water.

Toxicokinetics

Beryllium is not well absorbed by any route; oral absorption of beryllium is less than 0.01% and probably only occurs in the acidic stomach environment. About half of inhaled beryllium is cleared in $\sim$2 weeks; the remainder is cleared slowly and the residual becomes fixed in the lung (granulomata). The half-life of beryllium in rat blood is $\sim$3 h. Beryllium is distributed to all tissues. High doses generally go to the liver and then are gradually transferred to the bone. Most beryllium concentrates in the skeleton. Beryllium is excreted in the urine; however, the fraction of administered dose excreted in urine is variable.

Mechanism of Toxicity

Beryllium compromises the immune system. Enzymes catalyzed by magnesium or calcium can be inhibited by beryllium; succinic dehydrogenase is activated. Beryllium exposure leads to a deficiency in lung carbon monoxide diffusing capacity. Hypercalcemia (excess of calcium in the blood) can occur.

Acute and Short-Term Toxicity (or Exposure)

Animal

The pulmonary effects of inhaled beryllium have been evaluated in a variety of laboratory animal species. Monkeys, for example, exposed to relatively high concentrations of beryllium compounds developed symptoms and histopathological findings consistent with acute beryllium disease.

Human

The major toxicological effects of beryllium are on the lung. Acute exposure to soluble beryllium compounds (e.g., fluoride, an intermediate in the ore extraction process) irritates the entire respiratory tract, may produce acute chemical pneumonitis, and can result in fatal pulmonary edema. Hypersensitivity, which appears to be mediated by the immune system, may also occur following exposure. This means that future exposure to beryllium may produce health effects at concentrations lower than those generally associated with the effect (the individual becomes much more sensitive to beryllium).

The acute disease in humans is also marked by conjunctivitis, nasopharyngitis, tracheobronchitis, and dermatitis.

Chronic Toxicity (or Exposure)

Animal

Although beryllium produces cancer in more than one animal species (lung cancer in rats and monkeys; osteogenic sarcoma in rabbits), it does not appear to be teratogenic.

Human

Chronic exposure to insoluble beryllium compounds, particularly the oxide, leads to berylliosis (a chronic granulomatous disease), which begins with a cough and chest pains. In most cases, these symptoms soon lead to pulmonary dysfunction. The latency period ranges from months to 25 years. Diagnosis based on clinical, radiographic, and lung function evidence has been found to be difficult.

Other effects of beryllium exposure include enlargement of the heart (which can lead to congestive heart failure), enlargement of the liver, and kidney stones. Finger 'clubbing' is often seen with berylliosis.

Skin lesions are the most common industrial exposure symptom. Three distinct skin lesions have been noted following exposure to beryllium: dermatitis, ulceration, and granulomas. There appears to be an immunological component to chronic beryllium disease, including the dermal responses.

Although available information from epidemiological studies is insufficient to confirm human carcinogenesis, the data strongly suggest beryllium is associated with cancer in humans, and it is categorized

as a B1 (probable human carcinogen) by the US Environmental Protection Agency.

In Vitro Toxicity Data

In vitro studies indicate beryllium will induce morphological transformations in mammalian cells, but beryllium is not mutagenic in bacterial systems.

Clinical Management

Treatment of the acute disease includes bed rest, oxygen therapy, mechanical ventilation when needed, and corticosteroids. Chelation has been used to treat beryllium toxicity; however, no one agent is recommended over another. Aurin tricarboxylic acid has been used to protect primates from beryllium overdose, but human trials have not been conducted.

Ecotoxicology

Fish do not accumulate beryllium from water into their bodies to any great extent. A major portion of beryllium in soil does not dissolve in water but remains bound to soil, so it is not very likely to move deeper into the ground and enter groundwater. In the environment, chemical reactions can change the water-soluble beryllium compounds into insoluble forms. In some cases, water-insoluble beryllium compounds can change to soluble forms. Exposure to water-soluble beryllium compounds in the environment, in general, will pose a greater threat to human health than water-insoluble forms.

No evidence was found to substantiate that biomethylation or any other environmental process results in the volatilization of beryllium into the atmosphere from water or soil.

Beryllium is extremely toxic to warm water fish in soft water. The degree of toxicity decreases with increasing water hardness. Bioconcentration of beryllium in fish to high levels is not likely due to the low uptake of beryllium from water by aquatic animals. A measured bioconcentration factor (BCF) of 19 was reported for beryllium in bluegill fish. Other investigators have reported a BCF of 100 for freshwater and marine plants, invertebrates, and fish. Chemicals with BCFs <1000 will not bioaccumulate significantly in aquatic organisms. It is possible that bottom-feeding crustaceans, such as clams and oysters, could accumulate beryllium from sediment and show higher bioconcentration than freshwater fish. No evidence for significant biomagnification of beryllium within food chains was found.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, 8 h time-weighted average is $0.002\,\mathrm{mg\,m^{-3}}$ for beryllium and beryllium compounds and ACGIH classifies beryllium as a suspected human carcinogen.

See also: Metals; Respiratory Tract.

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Beta Blockers

Michael Wahl

- Representative Chemicals: Acebutolol; Atenolol; Betaxolol; Bisoprolol; Carteolol; Esmolol; Labetalol; Metoprolol; Nadolol; Penbutolol; Pindolol; Propranolol; Sotalol; Timolol
- Synonyms: Sectral (CAS 37517-30-9); Tenormin (CAS 29122-68-7); Kerlone (CAS 63659-19-8); Zebeta (CAS 66722-44-9); Cartrol (CAS 51781-21-6); Brevibloc (CAS 81161-17-3); Normodyne; Trandate (CAS 32780-64-6); Lopressor (CAS 37350-58-6); Corgard (CAS 42200-33-9); Levatol (CAS 38363-32-5); Visken (CAS 13523-86-9); Inderal (CAS 318-98-9); Betapace (CAS 959-24-0); Blocadren (CAS 26921-17-5)
- Chemical/Pharmaceutical/Other Class: Beta adrenergic blockers

Uses

Beta blockers are used in the treatment of hypertension, angina pectoris, supraventricular arrhythmias, supraventricular tachycardia, sinus tachycardia, ventricular tachycardia, myocardial infarction, pheochromocytoma, migraine headache, and essential tumor.

Exposure Routes and Pathways

Ingestion is the most common route for both accidental and intentional exposures to the beta blockers. Esmolol, labetalol, metoprolol, and propranolol are all available for parenteral administration; therefore, toxicity can occur via this route. Beta blockers are also administered as ocular medications and systemic toxicity can occur following administration by this route.

Toxicokinetics

The extent of absorption varies widely from 30% (nadolol) to 100% (labetalol, betaxolol). The rate of absorption is rapid for nonsustained release preparations. Sustained release preparations are more slowly absorbed and can have delayed and prolonged clinical effects following poisoning/overdose. The degree of protein binding has a wide range from 0% (sotalol) to 98% (penbutolol). Most of the beta blockers have significant hepatic metabolism (e.g., at least 50%). Atenolol, nadolol, and sotalol are principally excreted unchanged in the urine. Absolute bioavailability is often limited by significant first-pass metabolism. Esmolol is metabolized by esterases in the cytosol of red blood cells. Both renal and fecal eliminations occur. Elimination half-life ranges from 0.15 h (esmolol) to 24 h (nadolol).

Mechanism of Toxicity

The toxicities of the beta blockers are directly related to their pharmacologic effects. These agents block the effects of catecholamines such as epinephrine and norepinephrine on the beta-1 and beta-2 receptors. Beta-1 receptors are located in the heart, kidneys, and eyes. Toxicity is most often due to antagonism of the cardiac beta-1 receptors.

Acute and Short-Term Toxicity (or Exposure)

Human

Cardiac beta-1 stimulation results in increases in sinoatrial rate, myocardial contractility, and increased atrial, atrioventricular node, and ventricular conduction velocity. Beta blockers decrease heart rate, contractility, and conduction. Beta-2 receptors are found in the bronchioles, vasculature, intestines, uterus, pancreas, adipose tissue, and the liver. Stimulation of bronchial and vascular beta-2 receptors causes smooth muscle relaxation with resultant bronchial dilation and vasodilation. Blocking beta-2 receptors can cause contraction of bronchial smooth muscle and result in emergence of worsening asthma in asthmatic patients.

Chronic Toxicity (or Exposure)

Animal

No evidence of carcinogenicity has been documented in rats at doses of atenolol up to 300 mg kg^{-1} day^{-1}. Doses of up to 200 mg kg^{-1} day^{-1} have not shown decreased fertility in rats. However, dose related fetal resorptions were noted at doses of greater than 50 mg kg^{-1} day^{-1}. Chronic propranolol dosing at 100 mg kg^{-1} to newborn rats resulted in decreased weight gain and growth. The effects were reversible once the propranolol was discontinued.

Human

The primary clinical effects observed in beta blocker toxicity are cardiovascular in nature. Direct cardiac effects include bradycardia (sinus, atrioventricular node, and ventricular), all degrees of atrioventricular block, bundle branch blocks, and asystole. Ventricular arrhythmias may occur secondary to bradycardia. Torsades de pointes has been associated with chronic toxicity from sotalol. Hypotension occurs and is due to decreased cardiac output and/or vasodilation. Central nervous system effects of these drugs including lethargy, coma, and seizures are secondary to the cardiovascular toxicities. Seizures and coma may be secondary to hypoglycemia. Bronchospasm can occur secondary to beta-2 blockade. Hypoglycemia and hyperkalemia can occur.

In Vitro Toxicity Data

Ames testing of propranolol by different laboratories has demonstrated equivocal results.

Clinical Management

Advanced life-support measures should be instituted as deemed appropriate. A baseline 12-lead electrocardiogram should be obtained. Continuous cardiac and blood pressure monitoring should be initiated. Gastric decontamination procedures should be initiated based on the history of the ingestion and the patient's neurologic status. Consider charcoal, up to 1 gm kg^{-1} for recent ingestions. Whole bowel irrigation may be useful following ingestions of sustained release preparations. Bradyarrhythmias and conduction

disturbances should be managed with atropine and a pacemaker. Isoproterenol can be effective in increasing heart rate and contractility, but should be used with caution due to its arrhythmogenic and vasodilatory potential. Ventricular arrhythmias should be managed with class IB antiarrhythmics (e.g., lidocaine) and overdrive pacing. Class IA and IC antiarrhythmics should be avoided due to their potential to interfere with conduction. Hypotension should be managed initially with normal saline solution. If decreased cardiac output is responsible for hypotension, dobutamine, amrinone, or isoproterenol can be used. Glucagon has been effective in increasing myocardial contractility in beta blocker toxicity. Glucagon stimulates production of cyclic adenosine monophosphate, which enhances contraction. Initial intravenous doses of 50–100 $\mu g\,kg^{-1}$ have been used. These are followed by infusions of 70 $\mu g\,kg^{-1}\,h^{-1}$. If cardiogenic shock is resistant to traditional measures, use of insulin and glucose (to maintain euglycemia) has been successful in small numbers of patients as well as in animal models of beta blocker toxicity. For patients who fail all other therapies, an intra-aortic balloon pump and cardiopulmonary bypass should be considered. If systemic vascular resistance is low, vasopressors such as dopamine and norepinephrine should be administered. Hemodialysis or hemoperfusion may be effective in removing acebutolol, atenolol, nadolol, and sotalol.

See also: ACE Inhibitors; Calcium Channel Blockers; Cardiovascular System.

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Bhopal

Pallavi B Limaye and Harihara M Mehendale

Background Information

Bhopal, the capital city of the state of Madhya Pradesh in central India is the site of possibly the greatest industrial disaster in history till date. On the night of December 2, 1984, a disastrous accident at Union Carbide plant led to a massive leakage of methyl isocyanate (MIC) gas and other related by-products. MIC was used in production of carbaryl, an insecticide. The available evidence suggests that inadvertent seepage of water into the storage tank containing over 40 metric tons of MIC led to a violent exothermic reaction resulting in emission of MIC and a number of other toxic decomposition by-products that could not be contained by safety valves. The exact nature and the constituents of the gas mixture are not known. This Union Carbide plant was shut down after the accident.

Estimated Total Release of MIC and Estimated Individual Exposure

It has been estimated that ~27 tons of MIC escaped from the plant in a period of 1–2 h. Due to lack of planning, air monitoring for MIC was not possible, nor was it subsequently attempted. The Central Water and Air Pollution Control Board, India estimated MIC concentration to be ~27 ppm, which is ~1400 times that of the US Occupational Safety and Health Administration workplace standard of 0.02 ppm calculated over an 8 h work day. The established limit for immediate danger to life or health for MIC is 3 ppm. The American Industrial Hygiene Association's Emergency Response Planning Guideline, level 2 (ERPG-2) limit, defined as maximum airborne concentration below which it is believed that nearly all persons could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair their abilities to take protective action is determined to be 0.5 ppm. This indicates that the MIC concentration released after the accident was ~50 times that of the accepted limit.

Mechanism of Toxicity

Before the accident not much was known about MIC toxicity. Even today, mechanisms of MIC-induced toxicity are not clearly understood. It is known that MIC is a corrosive agent for the eyes, respiratory tract, and skin. Acute exposure to high vapor concentrations may cause severe pulmonary edema and injury to the alveolar walls of the lung, severe

corneal damage, and death. MIC may cross the placenta and enter a developing fetus.

Acute and Short-Term Toxicity (or Exposure)

Animal

The Indian Council of Agricultural Research's report indicates that a large number of cattle (~4000), as well as dogs, cats, and birds were killed due to exposure to the toxic gases released from the Bhopal plant.

Human

The gas leak had devastating effects on the exposed population. Over 200 000 residents (that comprised about one-fourth of the total population of city of Bhopal) were exposed to MIC and other related toxic gases released from the plant. Most of these residents were from the poor class and were living in the immediate surroundings of the Union carbide plant. The human mortality is estimated to be between 2500 and 5000 from this accident. Respiratory failure due to MIC inhalation was the principal cause of death. MIC caused bronchial necrosis and pulmonary edema. Within the first 24 h after the accident, ~90 000 patients were admitted in local hospitals and clinics with multiple symptoms of respiratory distress, breathlessness, choking, cough, chest pain, and hemoptysis. Acute ophthalmic effects were also reported with severe eye irritation and watering of the eyes.

Reproductive and gynecological effects were evaluated by retrospective cohort studies. In an epidemiological survey conducted nine months after the accident, revealed that 43% of 865 pregnancies amongst exposed women suffered fetal loss, as compared to 6–10% among the general Bhopal population. The spontaneous abortion rate was highest among those exposed during their first trimester. A study conducted by Shilotri NP and coworkers after 105–110 days of the accident showed a higher incidence of abnormal uterine bleeding and abnormal Pap smears amongst exposed women in the childbearing age.

Few immunological toxicity studies of MIC have been reported. A study of humoral and cell mediated immunity, in exposed subjects two months after exposure, found that cell-mediated immunity was suppressed, and that MIC-specific antibodies persisted for several months after the accident.

Long-Term Health Effects

Human

Till this date, Bhopal accident has claimed more than 6000 lives, and ~50 000 survivors are estimated to be suffering from long-term health effects that are termed as 'Bhopal syndrome' due to lack of information on the exact constituents of the gas cloud. The Indian Council for Medical Research established a field office called as Bhopal Gas Disaster Research Centre (BGDRC) immediately after the accident. In addition, International Medical Commission on Bhopal (IMCB) was established in 1993 comprising 15 professionals from 12 different countries. BGDRC and IMCB have reported that after 15 years of exposure, the affected population is still suffering from multisystemic toxicities. The major long-term health effects observed are shortness of breath, chest pain, muscle/bone pain, asthma, increased spontaneous abortions, and certain psychological problems. A randomized retrospective cohort study undertaken 10 years after the exposure by Cullinan *et al.* indicates the presence of persistent small airways obstruction. The lung examination carried out among the survivors several months later exhibited presence of obliterative bronchiolitis and interstitial fibrosis. Thirty-nine percent of 783 patients examined showed ventilatory impairment.

A recent study published in *Journal of the American Medical Association* in October of 2003 indicates that even the second generation of the exposed population is adversely affected. According to this study, significant growth retardation has been observed in boys who were either exposed to the gases as toddlers or born to exposed parents. Interestingly, no significant effects have been observed in girls.

Prevention and Management Measures

To prevent and manage such disasters in the future, international guidelines such as the United Nations Environment Program Awareness and Preparedness for Emergencies at the Local Level and Organization for Economic Cooperation and Development Guiding Principles for Chemical Accident Prevention, Preparedness and Response have been established. Few of the important recommendations proposed by these committees are to institute Local Emergency Planning Committees that will make the community aware of the dangerous substances used locally by industries and also try to prepare the local medical personnel, emergency first responders, and municipal administrators for the management of unexpected toxic substance release into local community by an industry. These measures have been effectively undertaken in every city and township in the United States today.

Litigations

The Indian Government filed a lawsuit against the Union Carbide, which was settled out of court. The Union Carbide paid $470 million in compensation.

See also: Carbaryl; Methyl Isocyanate.

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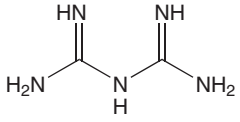
Biguanides

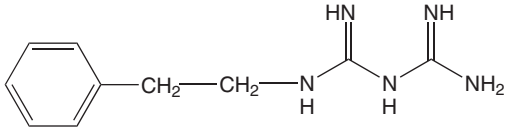
C Vaman Rao

- Chemical Abstracts Service Registry Numbers: CAS 5188-42-1 (chromate salt); CAS 6272-66-8 (dinitrate salt)
- Synonyms: Guanyl guanide; Diguanide; Amidinoguanidine; Phenformin; Metformin; Buformin; Glucophage (brand name)
- Chemical/Pharmaceutical/Other Class: Synthetic aliphatic amino alkanes with a large number of amino substituted groups
- Chemical Structures:

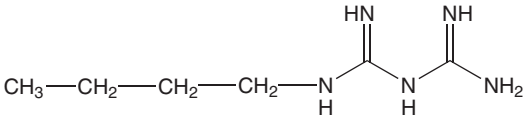


Biguanide

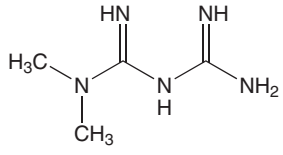
Modified form of biguanides:



Phenformin (β-Phenethyl biguanide)



Metformin (n-butylbiguanide)



Buformin (1,1-dimethyl biguanide)

Uses

Biguanides are used as an oral drug for the management of mild to moderately severe, noninsulin-dependent (type II) diabetes mellitus in obese patients who are usually above 40 years of age. It is important that for the administration of this drug the disease should have adult onset.

Polymeric biguanides were originally developed as a presurgery antimicrobial scrub and in 1977 it was introduced in the market for treating pools and spas as a disinfectant under the trade name Baquacil. It has Environmental Protection Agency (EPA) approval as the only nonhalogen sanitizer of pools and spas. The biguanide itself is combined with algecides and hydrogen peroxide for periodic oxidation of pools and spas. Biguanides are incompatible with chlorine, ozone, detergents, and ionizers, but are compatible with water ion balancing chemicals. Biguanides reduce the surface tension of water, which gives it a smoother feeling. They are stable in sunlight and temperature. At recommended concentrations when used in pools and spas, biguanides do not irritate the skin or eyes and do not corrode the pool equipment.

Exposure Routes and Pathways

The exposure to this drug is through oral route and absorption through the gastrointestinal tract.

Toxicokinetics

Phenformin is ~50% absorbed from the gastrointestinal tract. Its protein binding ability is very poor, which is ~20%. Phenformin is distributed throughout the major organs and it is mainly metabolized in the liver by hydroxylation. On hydroxylation, it produces *N-P*-hydroxy-*B*-phenyl-ethyl biguanide as

a metabolite. About 66% of the biguanide is excreted unchanged and the remaining 33% as a metabolite. Phenformin's half-life in the plasma is 7–15 h versus metformin's 1.5 h and buformin's 4–6 h. Metformin and buformin are excreted largely in an unchanged manner. The renal clearance of buformin, metformin, and phenformin are 393, 440, and 42–262 ml min^{-1}, respectively.

Biguanides are known to show interaction with furesemide, nifedipine, and cationic drugs.

Mechanism of Toxicity

A modification of the basic biguanide structure results in difference in potency, metabolism, excretion, and probably toxicity. The drug has a two-fold mechanism of action: it enhances the peripheral muscle glucose uptake and utilization, and inhibits glucose release from the liver. Biguanides induce an increase in peripheral gluconeogenesis and a decrease in intestinal absorption of glucose, vitamin B_{12}, and bile acids. Biguanides do not usually decrease blood sugar in a normal individual unless ethanol or another hypoglycemic agent is simultaneously administered or there is severe hepatic insufficiency. Biguanides are known to cause decreased absorption of vitamin B_{12} and folic acid. In the medical registry, it has been advised to avoid biguanide treatment to patients having hepatic insufficiency, renal insufficiency, peripheral vascular disease, and coronary diseases.

Phenformin generally lowers the blood sugar level in diabetics and nutritionally starved patients. Phenformin appears initially to produce a gastric mucosal irritability, which may predispose a person to a number of gastrointestinal symptoms, including gastric hemorrhage. Phenformin may act on the cell membrane to decrease oxidative phosphorylation, produce tissue anoxia, increase peripheral glucose uptake (Pasteur effect), and lead to lactic acidosis (accumulation of lactic acid) by inhibition of lactic acid metabolism.

Chronic Toxicity (or Exposure)

Animal

No systematic animal study has been reported to date.

Human

Biguanides are known to cause vomiting, nausea, abdominal cramps, gastrointestinal intolerance, anorexia, epigastric fullness, photosensitivity, dyspepsia, and dysgeusia (metallic taste), confusion, and lethargy.

Phenformin is the only biguanide to have been marketed in the United States and removed from the market by the US Food and Drug Administration (FDA) in 1977 because of its association with the development of lactic acidosis, a metabolic aberration that results in mortality in 50–75% of cases. Ethanol intake before the administration of phenformin therapeutic doses or excessive dose appears to predispose the patient to the development of lactic acidosis with a serious outcome. Phenformin and its other relative biguanides are still sold in European and other countries worldwide.

Daily therapeutic doses recommended for humans for three different biguanides are as follows: buformin, 100 mg; metformin, 500 mg ($\times 3$) and 850 mg ($\times 2$); phenformin, 25 mg.

Clinical Management

Biguanide toxicity is primarily managed by supportive means. Its elimination from the body could be enhanced by dialysis or enhancing excessive urination.

Environmental Fate

There is no official report on the environmental fate of biguanides.

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Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents

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Introduction and Classification

Toxins are toxic chemicals that can be elaborated by a biological organism. The word 'toxin' is often loosely used to mean poison but should be reserved for its more restricted definition; toxicant is a better synonym for poison. Several of the less complex toxins can now be synthesized in the laboratory or produced by other organisms following gene insertion, but synthetic toxins identical to their naturally occurring counterparts are still by definition toxins. Related terms include phycotoxins (toxins from algae), mycotoxins (fungal toxins), phytotoxins (plant toxins), and venoms (toxins from animals, especially vertebrates). Endotoxins are lipopolysaccharide toxins in the cell walls of certain gram-negative bacteria, and enterotoxins are toxins, such as cholera toxin, that damage intestinal mucosal cells. An exotoxin is a toxin that an organism releases into the environment. The actual toxin secreted by cells has in some cases been altered from the protoxin initially formed within the cells. Toxins usually do not perform crucial metabolic functions within their organisms of origin but act as offensive or defensive reactions to other organisms.

More than 400 toxins are known. They may be grouped according to size: low-molecular weight (LMW) toxins, which may be either peptides or nonpeptide organic compounds such as domoic acid, weigh less than 1 kDa and, if peptides, have no more than ~10 amino acids; heavier (larger) toxins are called protein toxins. Classification by organism of origin leads to the division of toxins into bacterial, algal, fungal, plant, marine dinoflagellate, marine soft coral, arthropod, molluscan, and vertebrate toxins. Toxins of similar chemical structure can be grouped together. Pathophysiologically, toxins comprise at least three major groups depending upon their toxicodynamics, or mechanisms of action. Neurotoxins, which affect neurotransmission, include botulinum toxin (which blocks the release of acetylcholine from cholinergic neurons), anatoxin, saxitoxin, and many animal venoms, some of which act presynaptically and others of which act postsynaptically. Membrane-damaging toxins include ricin, microcystin (which is also a hepatotoxin), certain venoms (such as the hemolytic snake venoms), and the trichothecene mycotoxins. Superantigen toxins such as staphylococcal enterotoxin B, toxic shock syndrome toxin-1, and streptococcal pyrogenic exotoxins exert pronounced systemic effects by activating the immune system in a nonspecific way.

Bioregulators are potent low-molecular peptides and proteins that modulate a wide variety of physiological processes such as inflammation, blood clotting, and neurotransmission. Unlike most toxins, they have definite roles in the normal physiology of their hosts. Bioregulators are not normally considered poisons but at toxicological doses may produce dramatic effects on blood pressure, body temperature, and other physiological parameters.

As chemicals produced by biological organisms, toxins and bioregulators occupy a zone that lies between chemical and biological agents and overlaps them to some extent. Saxitoxin and ricin are listed as chemical agents in the Chemical Weapons Convention, and toxins are listed separately from biological agents in the Biological and Toxin Weapons Convention (BTWC). The usual practice is to group toxins with biological agents. This is natural and appropriate from the perspectives of production, storage, and treaty issues, since toxins are generally produced by and often stored near their biological agents of origin. However, from a clinical standpoint, both toxins and bioregulators resemble other chemicals in that they do not replicate inside their hosts, are not transmissible, and are amenable to a chemical-based approach to clinical management. The term mid-spectrum agents (or mid-spectrum chemical warfare agents) has been proposed to refer to toxins and bioregulators along with synthetic viruses and genocidal agents produced by recent advances in biotechnology. **Table 1** displays one classification scheme for these compounds; the agents that are underlined will receive particular attention in this entry. Agents discussed as separate entries in this encyclopedia are also so indicated.

History

For millennia, indigenous South Americans deliberately used plant-derived arrow poisons such as curare and also toxins from poison-dart frogs, although these preparations were used mainly for hunting; similar toxins were used in Africa. The military use

*The conclusions and opinions expressed in this document are those of the author and do not necessarily reflect the official position of the United States Government, the Department of Defense, the United States Army Medical Research Institute of Chemical Defense, or the Uniformed Services University of the Health Sciences.

Table 1 Toxins and other mid-spectrum agents relevant to warfare and terrorism: a classification scheme

TOXINS
Bacterial toxins
Phycotoxins (algal toxins)
Botulinum toxin (CDC Category A)
Epsilon toxin from *Clostridium perfringens* (CDC Category B)
Staphylococcal enterotoxin B (SEB) (CDC Category B)
Diphtheria toxin
Tetanus toxin
Shigatoxin (veratoxin)
Mycotoxins (fungal toxins)
Aflatoxins
Ergot alkaloids (historical)
Trichothecene mycotoxins
  Stachybotrotoxins, including satratoxin H
  T-2 mycotoxins
Marine toxins
Phycotoxins (algal toxins)
  Algal toxins (blue-green algal) toxins
    Anatoxin-A (AnTx-a)
    Microcystins and nodularins
    Saxitoxins (STX), causing paralytic shellfish poisoning (PSP)
  Diatom toxin
    Domoic acid, causing amnesic shellfish poisoning (ASP)
  Dinoflagellate toxins
    Brevetoxins (PbTx), causing neurotoxic shellfish poisoning (NSP)
    Ciguatoxins (CTX) and maitotoxins (MTX), causing ciguateric fish poisoning (CFP)
    Diarrheic shellfish toxins (DST), causing diarrheic shellfish poisoning (DSP)
     Okadaic acid
    Palytoxin (concentrated by corals)
Conotoxins (from cone snails)
Scombrotoxins (mainly histamine)
Tetrodotoxin (TTX)
Phytotoxins (plant toxins)
(numerous alkaloids, including curare)
Type 2 ribosomal-inhibitory-protein (RIP) toxins
  Ricin (CDC Category B)
  Abrin
  Eranthis hyemalis lectin (EHL) from winter aconite
  Modeccin
  Viscumin
  Volkensin
Venoms from land animals
Invertebrate toxins, mostly from arthropods
Vertebrate toxins
  Amphibian toxins, including batrachotoxin
  Snake and lizard venoms
  Bird toxins (mainly batrachotoxin)

BIOREGULATORS
Cytokines
Early-phase proinflammatory cytokines (endogenous pyrogens)
  Interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α)
  IL-6
  IL-18
  Interferon gamma (IFN-γ)
Chemokines
  IL-8

Table 1 Continued

Eicosanoids (prostanoids and leukotrienes)
Prostaglandin D_2 (PGD_2), leukotrienes C_4 (LTC_4), LTD_4, LTE_4
LTB_4
Neurotransmitters and hormones
Catecholamines (e.g., epinephrine, norepinephrine, serotonin, dopamine)
Amino acid neurotransmitters (e.g., glutamate, aspartate, glycine, and γ-aminobutyric acid, or GABA)
Neuropeptides
  Neuropeptide Y
  Opioids (endorphins and enkephalins)
  Tachykinins
    Neurokinins A and B
    Substance P
Insulin
Vasopressin
Cholecystokinin
Somatostatin
Neurotensin
Bombesin
Vasoactive plasma proteases
Kallikreins and bradykinins
Tissue factor and thrombin

SYNTHETIC VIRUSES
Poliovirus
Other viruses identical to their natural counterparts
Genetically modified or combined synthetic viruses

GENOCIDAL AGENTS
Toxins, bioregulators, synthetic viruses, or traditional agents modified to enhance virulence
Toxins, bioregulators, synthetic viruses, or traditional agents modified to target specific genotypes

of toxins dates from at least the sixth century BC, when Assyrian soldiers poisoned enemy wells with ergot-contaminated rye. The ancient Greeks, for whom *toxikon* meant 'arrow poison', tipped arrows with aconite, and this practice continued into medieval Europe and persisted into the seventeenth century in Spain and Portugal. Japanese scientists in the infamous Unit 731 investigated tetrodotoxin during World War II, and suspicions have surfaced that the bomb used in the assassination of Reinhard Heydrich in Czechoslovakia in 1942 contained botulinum toxin. After World War II, ricin saw use as an injectable assassination weapon. More recently, Iraq had a weapons program that included the development of botulinum toxin, epsilon toxin from *Clostridium perfringens*, and aflatoxin. Militia groups in the United States and terrorist groups throughout the world have used ricin for political purposes. Toxins could be used on the battlefield or by terrorists to generate large numbers of military or civilian casualties as a mass-casualty weapon (MCW), to spread panic, for assassinations, or, in the case of toxins that damage crops, to create damage to an economy.

Concepts Relevant to Military or Terrorist Use

Toxicity

Toxins include several of the most acutely toxic chemicals known. For example, botulinum toxin is generally conceded to be the most lethal poison in existence. However, agent toxicity by itself is only a beginning variable that is subsequently modified by environmental and host factors. Because most toxins are far less toxic than botulinum toxin, tons of those toxins may be needed to cover a desired area on the battlefield. Many groups of toxins can thus be assumed to be at low risk of use as mass casualty weapons, or MCWs (but not necessarily as tools of assassins or for terrorist use in buildings), simply because of their relatively low toxicities. Others can be excluded on the basis of difficulties with isolation, synthesis (in general, chemical synthesis of only selected LMW toxins is currently feasible), production, storage, or dissemination; and still others degrade too rapidly in the environment. In general, the highest toxicities (lowest LD_{50} values, or lethal doses for 50% of a group) are associated with high-molecular weight (HMW) bacterial toxins such as botulinum toxin (MW 150 000 Da; LD_{50} 0.001 $\mu g\,kg^{-1}$), tetanus toxins (MW 150 000 Da; LD_{50} 0.002 $\mu g\,kg^{-1}$), and shigatoxin (MW 55 000 Da; LD_{50} 0.002 $\mu g\,kg^{-1}$). On the other end of the scale, aconitine (MW 647 Da; LD_{50} 100 $\mu g\,kg^{-1}$) is of the same order of toxicity as the nerve agent sarin; and T-2 mycotoxin (MW 466 Da; LD_{50} 1210 $\mu g\,kg^{-1}$) is approximately as toxic as the vesicating chemical agent sulfur mustard. Nonetheless, exceptions do occur; and the potency of a given toxin depends not only upon its size and structure but also upon its formulation (e.g., particle size in an aerosol) and, notably for most toxins, upon the route of entry.

States in the Environment and Routes of Entry

Although toxins do not volatilize as do many (but not all) chemical agents, this difference is moot as long as aerosolized toxin is capable of being inhaled. The preferred mode of dissemination for toxins used as MCWs would usually be the generation of appropriately sized particles in an aerosol, and the most pertinent route of exposure would be inhalation during the initial dispersion of agent. Primary aerosolization and inhalation have been conclusively demonstrated to be practical for botulinum toxin, staphylococcal enterotoxin B, ricin, T-2 mycotoxin, aflatoxins, brevetoxins (naturally, from wave-induced sprays of algal blooms), domoic acid, saxitoxin, tetrodotoxin, and others. Once an agent has settled onto environmental surfaces, its ability to reaerosolize largely determines its likelihood for subsequent inhalation. This property varies from toxin to toxin and from formulation to formulation and also depends upon environmental conditions such as humidity and temperature. Secondary aerosolization is presumed to be negligible for many toxins, but this assertion has not been rigorously demonstrated for all of these compounds. Many toxins are stable for long periods on environmental surfaces and in water; trichothecene mycotoxins and palytoxin are particularly persistent, but even botulinum toxin can remain in nonmoving water and in food for weeks. Personnel decontamination can usually be accomplished by gentle flushing using water with or without soap.

Most toxins are neither absorbed through intact skin nor dermally active, but there are exceptions, to include T-2 mycotoxin, lyngbyatoxin, and other blue-green algal toxins (cyanobacterial toxins). Ricin and abrin are among toxins that may incite an allergic contact response. Certain toxins and certain bioregulators, especially if heavily glycosylated, can survive ingestion to be absorbed from the gastrointestinal tract, and parenteral absorption of injected ricin was responsible for the death of Bulgarian dissident Georgi Markov in London in 1978 and was suspected in several other assassinations. This kind of attack, depending upon surreptitious employment of an umbrella modified to inject a ricin-filled pellet, would appear to be more suited to isolated assassinations than to use as a mass casualty weapon, but saxitoxin, which is particularly stable even under high temperatures, has been considered for coating bullets.

Threat Estimates

Risk assessment of possible use of toxins and related mid-spectrum agents on the battlefield or as terror weapons is an inexact science at best. Even if a toxin or a bioregulator is extremely potent, it may not be easily weaponizable, it may not survive long in the environment (particularly for bioregulators), or it may be so rare that the resources required to isolate it from natural sources or (in the case of LMW compounds) to synthesize it would be prohibitive. However, a determined individual, terrorist cell, or state with available time, personnel, equipment, and natural product may overcome these limitations, particularly if the goal is to use a little-known agent that will be low on the list of differential diagnoses for the effects produced. The rapid development of new techniques in biotechnology, toxicogenomics, and proteomics may also help to open the door to the

production, storage, and dissemination of exotic mid-spectrum agents. The Centers for Disease Control and Prevention (CDC) in the United States has established three threat categories for biological agents and toxins; botulinum toxin appears in category A (the highest-threat group), and ricin, epsilon toxin from *C. perfringens*, and staphylococcal enterotoxin B (SEB) all reside in category B. Abrin does not appear on the list, perhaps because it is less readily available than ricin. However, its toxicity is almost an order of magnitude higher than that of ricin. T-2 mycotoxin is asserted to have been used in Southeast Asia from 1975 to 1981, and despite continuing controversy regarding these claims, its potential for use appears to be significant despite its absence from the CDC list. Iraq stockpiled aflatoxin at one point for possible use, and saxitoxin, domoic acid, and tetrodotoxin have been mentioned as toxins capable of weaponization as MCWs. It seems safe to assert that although the most likely toxins to be used in warfare or terrorism include botulinum toxin, ricin, staphylococcal enterotoxin B, and perhaps trichothecene mycotoxins, all of which have been seriously investigated for military use, several other candidates exist and should not be excluded from consideration. The likelihood of development and use of bioregulators, synthetic viruses, and genocidal agents is even less predictable but should be expected to rise with new advances in biotechnology. Space prohibits discussion of each of the agents in **Table 1**, but a brief overview of each of the underlined toxins in **Table 1** will be presented.

Representative Agents

Botulinum Toxin

Chemical Abstracts Service Registry Number: CAS 93384-43-1. Botulinum toxins comprise a series of seven related protein neurotoxins that prevent fusion of synaptic vesicles with the presynaptic membrane and thus prevent release of acetylcholine. Exposure in a battlefield or terrorist setting would most likely be to inhaled aerosolized toxin. The clinical presentation is that of classical botulism, with descending skeletal muscle weakness (with an intact sensorium) progressing to respiratory paralysis. A toxoid vaccine is available for prophylaxis, and a pentavalent toxoid can be used following exposure; its effectiveness wanes rapidly, however, after the end of the clinically asymptomatic latent period. Because treatment is supportive and intensive (involving long-term ventilatory support), the use of botulinum toxin has the potential to overwhelm medical resources especially at forward echelons of care.

Ricin

Chemical Abstracts Service Registry Number: CAS 9009-86-3. Ricin, easily extracted from the castor bean plant (*R. communis*), is a globular glycoprotein membrane-damaging toxin with an A chain and a B chain separated by a disulfide bond. The A chain binds to the 28S unit of ribosomes to impair protein synthesis. The clinical presentation is very much dependent upon the route of entry: ingestion produces predominantly gastrointestinal effects, inhalation causes airway necrosis and damage to alveolar-capillary membranes leading to diffuse necrotizing pneumonitis and pulmonary edema, and parenteral exposure (from injection or from contamination of wounds) generally spares the respiratory tract but leads to necrosis of lymph nodes, gastrointestinal mucosa, the liver, the kidneys, and the spleen and to disseminated intravascular coagulation. Local cutaneous reactions and absorption may also follow contact with intact skin. The results of active prophylaxis with toxoid have been encouraging in animal studies, but treatment in humans remains empirical and supportive.

Abrin

Chemical Abstracts Service Registry Number: CAS 1393-62-0. Abrin is a toxalbumin similar in structure, absorption, and mechanism of action to ricin but is found not in castor beans but rather in jequirity beans. No reports of its use as a battlefield or terrorist agent exist, but in mice it is 75 times more potent than ricin. No specific treatment is available. Both ricin and abrin are type 2 ribosomal inhibitory proteins (RIPs): the other potent toxins in this class are *Eranthis hyemalis* lectin (EHL) from winter aconite, modeccin and volkensin from African succulents, and viscumin from mistletoe.

Epsilon Toxin from *Clostridium perfringens*

Clostridium perfringens has at least six serotypes and produces over 20 toxins. Epsilon toxin, along with alpha, beta, and iota toxins, is dermonecrotic and lethal. It is produced by some strains of type B and especially type D as a protoxin that is then converted to an active, mature, heat-labile toxin. The resulting toxin binds to cell membranes and forms a membrane complex that promotes the efflux of intracellular potassium. Because the usual route of entry is the gastrointestinal tract, the resulting pathology is an increase in intestinal permeability that enhances absorption of more toxin and ensures systemic toxemia. In animals, increased vascular permeability leads to enterotoxemia, 'pulpy kidney', altered hepatic function, and cerebral edema and necrosis.

Aerosolized alpha toxin from *C. perfringens* causes serious pulmonary damage with vascular leakage, hemolysis, thrombocytopenia, and liver damage and could easily be lethal, but the effects in humans of epsilon toxin, especially from inhalation, are unclear. However, the Iraqi biological agent program included the study not only of *Bacillus anthracis* and *Clostridium botulinum* but also of *C. perfringens*, including its epsilon toxin. Theoretically, this toxin could be genetically combined with another agent to increase the absorption of both. Animal toxoids exist but have not been evaluated for safety or efficacy in humans.

Staphylococcal Enterotoxin B (SEB)

Chemical Abstracts Service Registry Number: CAS 11100-45-1. SEB, the toxin that after ingestion causes sudden-onset staphylococcal food poisoning, is one of seven enterotoxins elaborated by *Staphylococcus aureus*. It is resistant to both heat and freezing. As a superantigen toxin, its mechanism of action involves binding to receptors for T-cell antigens and to major histocompatibility complex class II molecules, bypassing normal routes for antigen recognition and leading to antigen-nonspecific activation of the immune system and a massive release of bioregulatory cytokines to include not only histamine and leukotrienes (responsible for the intestinal response) but also interferon gamma, interleukin-6, and tumor necrosis factor alpha (responsible for systemic effects). Inhalation of aerosolized SEB leads to incapacitating respiratory signs and symptoms, although deaths at high doses may occur from pulmonary edema. Inadvertently swallowed toxin may also produce nausea and vomiting.

In a military or a terrorist setting, SEB could be added to unguarded food or water or could be disseminated by aerosol. The resulting incapacitation may be a desirable goal either on the battlefield or for terrorism. Human trials of a pre-exposure toxoid and of post-exposure passive immunization are underway but have not yet led to approved products.

T-2 Mycotoxin

Chemical Abstracts Service Registry Number: CAS 21259-20-1. T-2 mycotoxin is a trichothecene toxin, so-called because of two particular chemical moieties in its structure. Many otherwise unrelated groups of fungi produce a rich variety of trichothecene mycotoxins, each with its own toxicological profile. T-2 mycotoxin has been associated with disease in animals and, in the 1930s in the Soviet Union, with a largely gastrointestinal condition called alimentary toxic aleukia, a chronic intoxication from repeated consumption of contaminated bread. This toxin was also found in autopsy specimens from one of the Khmer Rouge casualties associated with the yellow, green, red, or white smoke that came to be called yellow rain in Laos and Cambodia (now Kampuchea) in the 1970s. Whether the T-2 toxin acted in concert with other mycotoxins found in the victim and to what extent it was responsible for the observed results remain matters of controversy, even though laboratory exposures to the toxin created similar cutaneous, ocular, and systemic effects. The toxicity of T-2 mycotoxin, which is also one of the few toxins capable of creating small vesicles on the skin after direct contact, is roughly comparable to that of the chemical agent sulfur mustard, and relatively large quantities would be needed to cause casualties over a large area. The cytotoxicity of T-2 toxin is thought to be related to lipid peroxidation of plasma membranes, inhibition of electron (proton) transport in mitochondria, and especially RNA inhibition and consequent disruption of protein synthesis in ribosomes. Treatment is supportive, supplemented with steroids.

Aflatoxin

Chemical Abstracts Service Registry Number: CAS 1402-68-2. Aflatoxins are toxic, immunosuppressive, mutagenic, and carcinogenic mycotoxins produced by the mold *Aspergillus flavus* and commonly contaminating cereals, oilseeds, tree nuts, and spices. They are quite resistant to dry heat but gradually deteriorate under conditions of moist heat. They are also inactivated by food additives such as sodium bisulfite. Aflatoxin was first recognized as a toxin for animals following a severe outbreak of 'Turkey X' disease in the United Kingdom in 1960. Since that time, outbreaks of human disease have been reported, including one from contaminated maize in Kenya during May 2004; the case fatality rate for this outbreak approached 50% in one of the affected districts. Iraq is known to have included aflatoxin in its arsenal; it is unclear whether this toxin was intended to be used to cause acute effects or to cause cancer years later in survivors (or both) is unclear. In the body, cytochrome P450 converts the toxin (usually after ingestion) to an epoxide that reacts with RNA and DNA, inhibits protein and DNA synthesis in the liver and bone marrow, and can lead to mutations and eventually cancer. The acute clinical manifestations are protean and include vomiting, abdominal pain, gastrointestinal hemorrhage, fatty change of the liver, pulmonary edema, convulsions, and cerebral edema; chronic effects include liver cancer. Treatment is supportive.

Domoic Acid

Chemical Abstracts Service Registry Number: CAS 14277-97-5. Domoic acid, a glutamic acid analog that is resistant to temperature extremes, is an excitatory neurotoxin produced by a diatom and concentrated in shellfish. Ingestion leads to amnesic shellfish poisoning, which can also include seizures. Its relevance to use in warfare and terrorism, apart from its being unfamiliar to most disaster-response personnel, is that it is also easily absorbed by inhalation and across mucous membranes. No specific antitoxin is available, and treatment is supportive.

Saxitoxin (STX)

Chemical Abstracts Service Registry Number: CAS 35523-89-8. Saxitoxin, a heat-stable neurotoxin produced by blue-green algae, is associated with paralytic shellfish poisoning. It leads to weakness and paralysis by blocking sodium channels in neurons. It is a potential agent for use on the battlefield or in terrorism because of its increased potency via inhalation, its fast onset and progression, and its proposed use for coating projectiles such as bullets. No toxoid or antitoxin is available.

Tetrodotoxin (TTX)

Chemical Abstracts Service Registry Number: CAS 4368-28-9. Tetrodotoxin, a neurotoxin produced by several species of starfish, crabs, salt-water fish, octopi, newts, and salamanders, blocks sodium channels within neurons. In a terrorist scenario, tetrodotoxin could be inhaled as an aerosol or ingested in contaminated food or water. Mortality may reach 50%.

Bioregulators

All of the bioregulators listed in **Table 1** are potentially weaponizable, although not all with present-day technology. Their attractiveness as weapons of assassination or to produce mass casualties is tied to several possible advantages. They are not on most standard lists of agents to be expected in warfare or terrorism, they are easily purchased (partly because they are used extensively in research), they are rapid in onset (making them useful assassination agents) but relatively nonspecific in their clinical effects (thus not arousing suspicion), and no vaccines are available against them. However, the costs of production or purchase may be high, they may not be available in large enough quantities to be effective, and neither their aerosolizability nor their environmental persistence has been characterized thoroughly. Nevertheless, since enteral absorption is significant for many of these compounds, they could also be added to foodstuffs.

Synthetic Viruses

Simple viruses such as the polio virus, consisting of a single strand of RNA, have already been successfully assembled using commercially available reagents. Larger and more complex viruses will undoubtedly be synthesized in the near future, and the relative ease with which this can be done, and the possibility of designing and testing novel viral structures not found in nature, could lead to large quantities of completely new agents. Since these compounds can be synthesized in a laboratory setting but can then replicate within hosts, they are prototypical mid-spectrum agents.

Genotoxic Agents

The ability to synthesize viral genomes is part of the burgeoning development of biotechnology, which uses high-speed data processing, microarrays, and the new sciences of genomics and proteomics to alter genetic code and to affect the expression of that code. Bioengineered viruses and other organisms could be targeted toward individuals or populations with specific genotypes. Toxicogenomics could be used in a similar way for chemical agents and for mid-spectrum agents such as toxins and bioregulators.

Summary

Toxins, which are chemical poisons produced by living organisms, and bioregulators, which are LMW molecules involved in physiological processes within the body, are biological in origin but are noninfectious and nonreplicating. As mid-spectrum agents, they occupy a position between and overlap the traditional dichotomy of mass-casualty agents as chemical versus biological agents. This part of the spectrum may also be said to include synthetic viruses and genotoxic agents. All of the mid-spectrum agents have potential for use in small-scale or large-scale operations against military forces, civilians, or both. An appreciation of the unique position of these agents and of the threat that they pose and a heightened level of suspicion for their use are necessary in order to recognize their use and institute appropriate preventive and treatment measures.

See also: Aflatoxin; Algae; Botulinum Toxin; Castor Bean; Ciguatoxin; *Clostridium perfringens*; Marine Organisms;

Mold; Mycotoxins; Ricin and Other Toxalbumins; Saxitoxin; Scombroid; *Staphylococcus aureus*; Tetrodotoxin.

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Biocides

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The word ‘biocide’ encompasses a broad class of chemical agents and literally means an agent that destroys life. The United States Environmental Protection Agency defines the term ‘biocide’ as follows:

> A diverse group of poisonous substances including preservatives, insecticides, disinfectants, and pesticides used for the control of organisms that are harmful to human or animal health or that cause damage to natural or manufactured products.

This broad definition includes terms and topics covered in this encyclopedia and other literature, including pesticides, which encompasses herbicides, insecticides, miticides, rodenticides, algaecides, etc. Biocides have sometimes been considered a subcategory of pesticides. When the term is used in this context, it refers specifically to the control or destruction (killing) of microorganisms, typically in nonagricultural applications. Biocides as nonagricultural pesticides encompass a wide range of applications, including disinfectants and sanitizers, preservatives and microbicides, antifouling products, wood preservatives, and structural treatments.

Biocides are used widely in industry. There are at least three main classes of industrial chemical biocides. The first class includes the oxidizing and bleaching agents, such as chlorine dioxide, hydrogen peroxide, and sodium hypochlorite. The oxidizing action may directly kill bacteria or fungi or weaken the cell walls so that they are more susceptible to other classes of biocides (see below). Sodium hypochlorite (like all hypochlorites) is a salt of hypochlorous acid. In solution, it splits into the sodium cation (Na^+) and the hypochlorite anion (ClO^-). The oxidizing power of the latter causes the bleaching and disinfecting effect. Chemicals with oxidizing and bleaching properties have been under scrutiny in recent years. This is largely because of the toxicity of reaction by-products, particularly chlorine and its derivatives. There is a high probability of the formation of toxic gases (chloramine gas) and mutagenic and/or carcinogenic halogen-containing organic substances (e.g., trihalomethanes) during water treatment activities and when these chlorine-containing compounds are released into the environment. As a result, there has been an increase in the use of oxygen, hydrogen peroxide, and other oxygenated compounds in bleaching applications, and a sharp decline in the demand for chlorine and the hypochlorites.

A second class of industrial chemical biocides involves highly toxic organic chemicals. Subclasses of toxic biocides include thiazoles, thiocyanates, isothiazolins, cyanobutane, dithiocarbamate, thione, and bromo-compounds. As the names imply, many of the toxic biocides contain sulfur (‘thio’-).

A third class of industrial chemical biocides consists of agents with the ability to inhibit biological film formation, also called ‘surfactants’. The term surfactant originates from the phrase surface active agent. Surfactants fall into four broad categories: anionic (e.g., soaps, alkyl benzenesulfonates, alkyl sulfonates, alkyl phosphates), cationic (e.g., quaternary ammonium salts), nonionic (e.g., alkyl polyglycosides, alcohol ethoxylates, alkylphenol ethoxylates), and zwitterionic.

See also: Consumer Products; Organochlorine Insecticides; Pesticides.

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Biocompatibility

Samantha E Gad

The biological evaluation of medical devices is performed to determine the potential toxicity resulting from contact of the component materials of the device with the body. The device materials should not produce adverse local or systemic effects, be carcinogenic, or produce adverse reproductive and developmental effects, either directly or through the release of their material constituents. Systemic testing must ensure that the benefits of the final product will outweigh any potential risks produced by device materials.

In 1986, the US Food and Drug Administration (FDA), Health and Welfare Canada, and Health and the United Kingdom (UK) Social Services issued the 'Tripartite Biocompatibility Guidance' #G87-1 for Medical Devices. This guidance was used by the FDA and device manufacturers until July 1, 1995, when the new blue book memorandum #G95-1 entitled *Use of International Standard ISO-10993, Biological Evaluation of Medical Devices Part-1: Evaluation and Testing*, became effective by direction of the ODE (Office of Device Evaluation). The 'ANSI/AAMI/ISO' Standard 10993 *Biological Evaluation of Medical Devices* is accepted in Europe and Asia and is also referred to as ISO 10993/EN 30993. Also, since 1995, medical devices marketed and sold within the European Union (EU) have been required to comply with safety assessment requirements stated in EU Medical Devices Directive 93/42/EEC. The European Committee for Standardization (CEN) is now in the process of adopting ISO 10993. Japanese procedures for sample preparation and testing are slightly different from either United States Pharmacopoeia (USP) or ISO tests. The unofficial translation of the Japanese toxicological testing requirements is available as *Guidelines for Basic Biological Tests of Medical Materials and Devices*. In addition, FDA has recognized standard developed by the USP and by the American Society for Testing and Materials (ASTM).

For use in the United States, the blue book memorandum includes an FDA-modified matrix designating the type of testing required for various medical devices and also a flowchart entitled 'Biocompatibility Flow Chart for the Selection of Toxicity Tests for 510(k)s'. The matrix also consists of two tables: Table 1 – Initial Evaluation Tests for Consideration; and Table 2 – Supplementary Evaluation Tests for Consideration. In general, the agency does not have a list of approved materials.

An ISO standard, it should be noted, is a document that undergoes periodic review and is subject to revision. Recently, the FDA, more specifically the Center for Devices and Radiological Health (CDRH), has been considering the use of international consensus standards for the toxicological evaluation of medical devices.

FDA notes that the ISO standard acknowledges certain kinds of discrepancies. It states "due to diversity of medical devices, it is recognized that not all test identified in a category will be necessary and practical for any given device. It is indispensable for testing that each device shall be considered on its own merits: additional tests not indicated in the table may be necessary." It is necessary to consider the properties of device materials and the nature degree, frequency, and duration of exposure to the body when determining appropriate tests for a particular device. Material found to be safe for one intended use in a device might not be in a device intended for another use. The final assessment must be not only made for all components but more importantly on the finished product. Generally, the tests include: acute, subchronic, and chronic toxicities; irritation to the skin, eyes, and mucosal surfaces; sensitization; hemocompatibility; genotoxicity; carcinogenicity; and effects on reproduction

including developmental effects. Depending on the characteristic and intended uses of the device these tests may not be necessary or sufficient. Neurotoxicity and immunotoxicity, among other tests, may be necessary for some devices. The specific clinical application and the materials used in the manufacture of a device determine which tests are appropriate. Some materials that have been well characterized both chemically and physically in published literature and which have a long history of safe use may prove unnecessary to complete all tests if substantial equivalence to marketed products under 510(k) is shown. In this case the manufacturer must document the use of a particular material in a legally marketed predicate device or a legally marketed device with comparable patient exposure.

The FDA has made several modifications to the tests required by Part 1 of the ISO 10993 standard for the category of surface devices that permanently contact mucosal membranes. The ISO does not require acute, subchronic, or chronic implantation tests as does FDA. FDA requires irritation, systemic toxicity, acute, subchronic, and chronic tests for external communicating devices, tissue/bone/dentin with prolonged and permanent contact. Device manufacturers are advised to consider tests to detect chemical components of device materials that may be pyrogenic. This matrix is a framework and not a checklist and it is stressed by the FDA that necessary safety testing will be decided on a case-by-case basis.

ISO-10993 includes the following sections:

1. Guidance on Selection of Tests
2. Animal Welfare Requirements
3. Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicity
4. Selection of Tests for Interactions with Blood
5. Tests for Cytotoxicity – *In Vitro* Methods
6. Tests for Local Effects after Implantation
7. Ethylene Oxide Sterilization Residuals
8. Clinical Investigation of Medical Devices
9. Degradation of Materials Related to Biological Testing
10. Test for Irritation and Sensitization
11. Test for Systemic Toxicity
12. Sample Preparation and Reference Materials
13. Identification and Quantification of Degradation Products from Polymers
14. Identification and Quantification of Degradation Products from Ceramics
15. Identification and Quantification of Degradation Products from Coated and Uncoated Metals and Alloys
16. Toxicokinetic Study Design for Degradation Products and Leachables
17. Establishment of Allowable Limits for Leachable Substances

The following tests are recommended:

- cytotoxicity,
- acute systemic toxicity,
- sensitization,
- genotoxicity,
- skin irritation,
- implantation,
- intracutaneous reactivity, and
- hemocompatibility.

Subchronic and Chronic toxicities and also carcinogenicity may be appropriate.

When designing a medical device it is important to first select appropriate materials and then a sterilization method before searching for relative information on the materials and beginning testing. It is advisable to test individual components of a device prior to testing the complete device in case one component has toxic properties. Premarket Approval (PMA) applicants often use another party's product or facility in the manufacture or their device. Many manufacturers keep data on qualified materials used in their products. This information regarding the product is pertinent to its review; the third party may choose to submit confidential information directly to the FDA in a device master file. This is not a marketing application and additional testing or information may be necessary.

It may be necessary to repeat biocompatibility tests when modifying a device based on the changes made (see the flow chart given in the Appendix for conditions necessary in order not to have to repeat testing). If available, clinical data can be used to satisfy some biological effects categories from the ISO 10993-1 test selection matrix. Medical device toxicity problems are most often caused by leachable or extractable toxins. Extracts of materials are often tested for biocompatibility. Section 17 of 10993 entitled *Establishment of Allowable Limits of Leachable Substances*, gives guidance on the use of analytical data (e.g., extraction studies) to reduce biocompatibility test requirements. The extraction media should be comprised of a series of media with various polarities to capture results found in different solubilities. The most common extraction media include physiological saline, vegetable oil, dimethylsulfoxide, and ethanol; other less common ones include polyethylene glycol or aqueous dilutions of ethanol. The temperature at which the

extraction should be carried out varies throughout various guidelines, but it is generally recommended that extraction be performed at approximate body temperature for 72 h. For *in vitro* cytotoxicity testing, complete cell-culture medium is most commonly utilized, with extraction performed at 37°C for 24 h. Inexpensive nonanimal studies such as cytotoxicity and hemocompatibility tests can be used to screen device materials.

Biological control tests are recommended to determine sources of possible contamination and to ensure safety of the final product. Microbiological tests to determine the status of the final product (e.g., sterility, bacteria, contaminants, and microbial count limits) are necessary. Devices should be tested for endotoxins as cell wall lipopolysaccharides (from gram-negative bacteria) may be present even after sterilization. Assessment of nonspecific toxicological effects should be performed by intravenous injection of device eluate in mice. It has also become more common since the general acceptance of ISO 10993 that device materials be more rigorously characterized analytically and also that more extensive genotoxicity testing be performed.

The following are special considerations that must be considered when testing devices and their component materials for safety.

Color additives: A color additive is a dye, pigment, or other substance, whether derived from a vegetable, animal, mineral, or other source, which imparts a color when added to a food, drug, cosmetic, or the human body. The US Food, Drug and Cosmetic (FD&C) Act states devices containing a color additive are considered unsafe, and therefore adulterated, unless one is in effect listing the color additive for such use. The FD&C Act limits applicability of these color additives for devices that directly contact the body for a significant period of time (undefined by FDA). Manufacturers of devices should choose a color additive listed for use in foods, drugs, or cosmetics as a starting point but keep in mind that these may not be appropriate for devices. The color listing regulation may permit the use of the color additives or may place limitations on its use; PMA applicants must demonstrate their safety. Color additives listed for use in medical devices are provided in 21 CFR 73 (Color additives exempt from batch certification) and 21 CFR 74 (Color additives subject to batch certification).

Combination products: A combination product is a product consisting of two or more regulated components (drug/biologic/device, etc.) that are combined as a single entity or is a product labeled for use with a separate device or biologic where both are required to achieve the intended use, indication, or effectiveness. Intercenter agreements have been made within FDA to review and oversee these categories. More information can be found at FDA website for the CBER (Center for Biologics Evaluation and Research) and CDRH (Center for Devices and Radiological Health) Intercenter agreement, and the CDER (Center for Drug Evaluation and Research) and CDRH Intercenter agreement.

In vitro diagnostic (IVD) products: These are medical devices which analyze human body fluids, such as blood or urine, to provide information for the diagnosis, prevention, or treatment of a disease. Classification for these devices can be found under 21 CFR 862, 21 CFR 86?, and 21 CFR 866.

Radiation emitting products: Electronic product radiation means any ionizing or nonionizing electromagnetic or particulate radiation, or any sonic, infrasonic, or ultrasonic wave, which is emitted from an electronic production of the operation of an electronic circuit in such product. If a medical device emits electronic product radiation, additional requirements apply through the Radiation Control for Health and Safety Act (RCHSA). Additional information concerning radiation-emitting products can be found at the FDA website.

Software: If a device contains software, the PMA submission must include documentation of software testing appropriate to the level of risk of the device.

The FDA recognizes certain consensus standards of conformance when making regulatory decisions. In addition, sterility assurance is necessary, and FDA validated method for sterilization should be used and included in the PMA.

When positive biocompatibility results are reported, development discontinuation is not the only option. First it should be confirmed that no mistakes were made in the testing laboratory, including the testing of the proper article and formulation. In addition, it should be made certain that the article was properly manufactured, cleaned, stored, and tested (e.g., the extractant used, the testing conditions, and the procedure). Finally, reproducibility of positive biocompatibility results should be confirmed. In a certain situation where the possible benefits outweigh the risks, or when quality of life is a factor, a level of toxicity may be acceptable.

Appendix

Biocompatibility Testing Flow Chart

Material Characterization/Risk Assessment

Flow Chart for the Selection of Toxicity Tests for 510(k)s

Category		*Examples (not exclusive)*	*Contact duration* **(A) Limited** *≤ 24 h* **(B) Prolonged** *24 h to 30 days* **(C) Permanent** *> 30 days*	*Biological effect*									
				Initial evaluation tests								*Other*	
				Cytotoxicity	*Sensitization*	*Irritation*	*Systemic Tox*	*Subchronic Tox*	*Genotoxicity*	*Implantation*	*Hemocompatibility*	*Chronic Tox*	*Carcinogenicity*
Surface devices	Skin	Devices that contact intact skin surfaces only (electrodes, external prostheses, fixation tapes, compression bandages)	A	X	X	X							
			B	X	X	X							
			C	X	X	X							
	Mucous membrane	Devices communicating with intact mucosal membranes (contact lenses, urinary catheters, intraintestinal devices, endotracheal tubes)	A	X	X	X							
			B	X	X	X	F	F		F			
			C	X	X	X	F	X	X	F		F	
	Breached or compromised surfaces	Devices that contact breached or otherwise compromised external body surfaces (wound dressings, healing devices, occlusive patches)	A	X	X	X	F						
			B	X	X	X	F	F		F			
			C	X	X	X	F	X	X	F		F	
External communicating devices	Blood path indirect	Devices that contact the blood path at one point and serve as a conduit for entry into the vascular system (solution administration sets, extension sets, blood administration sets)	A	X	X	X	X				X		
			B	X	X	X	X	F			X		
			C	X	X	F	X	X	X	F	X	X	X
	Tissue/bone/dentin communicating	Devices communicating with tissue (includes fluids and subcutaneous spaces), bone, and pulp/dentin system (lacroscopes, arthroscopes, draining systems, dental cements, dental filling materials, skin staples, surgical instruments)	A	X	X	X	F						
			B	X	X	F	F	F	X	X			
			C	X	X	F	F	F	X	X		F	X

continued

Category		*Examples (not exclusive)*	*Contact duration* **(A) Limited** *⩽24 h* **(B) Prolonged** *24 h to 30 days* **(C) Permanent** *>30 days*	*Biological effect*									
				Initial evaluation tests								*Other*	
				Cytotoxicity	*Sensitization*	*Irritation*	*Systemic Tox*	*Subchronic Tox*	*Genotoxicity*	*Implantation*	*Hemocompatibility*	*Chronic Tox*	*Carcinogenicity*
	Circulating blood	Devices that contact circulating blood (intravascular catheters, temporary pacemaker electrodes, oxygenators, dialyzers, hemodsorbents, and immunoadsorbents)	A	X	X	X	X		F[1]		X		
			B	X	X	X	X	F	X	F	X		
			C	X	X	X	X	X	X	F	X	X	X
Implant devices	Tissue/bone	Devices principally contacting bone, tissue and tissue fluid (orthopedic pins, plates, replacement joints, pacemakers, drug supply devices, neuromuscular sensors and stimulators, replacement tendons, breast implants, ligation clips)	A	X	X	X	F						
			B	X	X	F	F	F	X	X			
			C	X	X	F	F	F	X	X		X	X
	Blood	Devices principally contacting blood (pacemaker electrodes, heart valves, vascular grafts and stents, internal drug delivery catheters, and ventricular assist devices)	A	X	X	X	X			X	X		
			B	X	X	X	X	F	X	X	X		
			C	X	X	X	X	X	X	X	X	X	X

ISO Recommended Initial Tests

Noncontact devices: These are devices that do not contact the patient's body directly or indirectly (*in vitro* diagnostic devices). Regulatory agencies rarely require biocompatibility testing for these devices.

X: ISO evaluation tests for consideration.

F: Additional Tests which the FDA may require.

[1]For all devices used in extracorporeal circuits.

In addition to these tests, pyrogenicity, reproductive and developmental, and biodegradation should be considered depending on the nature and intended use of the device.

See also: Foreign Body Response; Implant Studies.

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Bioinformatics

Kartik Shankar and Harihara M Mehendale

Advances in the fields of cellular and molecular biology coupled with the technological advances in genome and proteome scale investigations have led to an explosive increase in biological information. An efficient way to organize and utilize these data has become a necessity. Bioinformatics is the field that utilizes computer science and information technology to organize, view, and mine biological information. Bioinformatics till recently has been organization of large genome-scale data in public and private databases. Databases allow the storage and management of these data sets. A biological database is a large, organized body of persistent data, usually associated with computerized software designed to update, query, and retrieve components of data stored within the system. For example, the National Center for Biotechnology Information maintains several large databases containing genome information, protein sequences, transcription factors, promoters, and single nucleotide polymorphisms. However, in the 'postgenomic' era the emphasis of bioinformatics will be on data mining and knowledge-discovery. Consequently, bioinformatics is increasingly being integrated with bench-based science as hypotheses generated *in silico* are tested *in vitro* and *in vivo*. Some publicly available databases and bioinformatic software for several biomedical researches are listed in the 'Relevant websites' section. It should be noted that an extensive summary of the tools available for the interested researcher is not provided in the 'Relevant websites' section, but only a primer to the more popular bioinformatic tools.

See also: Genomics, Toxicogenomics; Microarray Analysis; Proteomics.

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http://www.ncbi.nlm.nih.gov – Database/software – dbEST; Application – Database of expressed sequence tags.

http://www.ncbi.nlm.nih.gov – Database/software – Unigene database; Application – Database of expressed sequence tags.

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http://www.gene-regulation.com – Database/software – TRANSFAC; Application – Transcription factor database.

http://wwwmgs.bionet.nsc.ru – Database/software – Transcription Regulatory Regions Database (TRRD); Application – Transcription factor database.

http://www.genomatix.de – Database/software – MatInspector; Application – Transcription factor binding site search engine.

http://www.ebi.ac.uk – Database/software – ClustalW; Application – Multiple sequence alignment program.

Biological Exposure Index

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Human biological exposure indices are guidance levels of determinants for assessing worker dose from occupational exposures. They differ from other occupational exposure limits (OELs) for chemicals, which typically are measured in air, in that their determinants are measured in biological materials from the workers. BEIs consider the dose that has entered a worker's body by all routes. Thus, these measurements can provide more complete estimates of exposure, especially for chemicals that may be absorbed by routes other than inhalation and when inhalation rates are altered because, for example, of increased work rates.

Defining Biological Exposure Indices

Most BEIs are defined as concentrations of determinants or biomarkers anticipated in biological specimens collected from healthy workers whose exposure to certain chemicals by all routes is equivalent to that of workers with inhalation only exposure at the OEL. Others measure reversible effects on the body, and still others are those that are below the concentrations associated with health effects. However, other definitions are common. For example, the German biological tolerance values (BAT) can be defined as rates of excretion of the chemical or its metabolites, or the maximum possible deviation from the norm of biological parameters induced by these substances in exposed humans. BEIs for some chemicals use other criteria, such as direct comparison with a measurable toxic effect, like carboxyhemoglobin in blood for carbon monoxide.

The most commonly used Biological Exposure Indices are known by the abbreviation, BEI®, which is a trademark of the American Conference of Governmental Industrial Hygienists (ACGIH®). Like most, ACGIH defines BEIs as guidance values for assessing human biological monitoring results. ACGIH indicates that most of its BEIs are bioequivalent to its airborne OELs, the threshold limit values (TLVs®): a "BEI generally indicates a concentration below which nearly all workers should not experience adverse health effects." It also asserts that BEIs should not be used as measures of adverse health effects or for diagnosing occupational illness. In addition to the ACGIH BEIs and German BATs, other sources of BEGs include the Finnish Institute of Occupational Health, United Kingdom Health and Safety Executive, Italian Society of Reference Values, and the Japan Society for Occupational Health.

The determinant for a biological exposure index can be the chemical itself, one or more metabolites, or a characteristic biochemical change induced by the chemical. The specimen used for biological monitoring usually is urine, blood, or exhaled air. For example, the BEI for trichloroethylene includes four determinants:

- a metabolite, trichloroacetic acid, in urine;
- another metabolite, trichloroethanol, in blood;
- the parent compound, trichloroethylene, in blood; and
- trichloroethylene in end-exhaled breath.

The latter two determinants are recommended as confirmatory tests to document exposure to trichloroethylene, since other chemicals also can be metabolized to trichloroacetic acid and trichloroethanol.

Basis for Biological Exposure Indices

While most BEIs are based on overall exposures equivalent to inhalation exposures at an OEL, several provide the basis for the corresponding airborne OEL. For example, airborne OELs for carbon monoxide, acetylcholinesterase inhibitors, certain solvents like hexane, and most heavy metals represent inhalation exposures that are expected to cause measurable biological concentrations or changes that available data indicated should be safe for most workers. For substances with low potential for inhalation exposure that are readily absorbed through the skin, there is likely to be little correlation between airborne concentrations and measurement of biological determinants. BEIs for these substances are based on the relationships between health effects and the biological concentrations of the determinants.

While most BEIs are quantitative, data sometimes support only a screening-type guideline that is nonquantitative or semiquantitative. Such guidelines typically are used for substances on which there are good qualitative data on human exposure and the biological determinant concentration, but poor quantitative data relating exposure to the determinant. They most commonly are used for substances that cause chronic, systemic health effects when absorbed through the skin. Nonquantitative determinants are useful especially for substances, like 4,4′-methylene bis(2-chloroaniline) (MBOCA), that meet these criteria and

for which there is a long lag time from exposure to health outcome and low or no background level of the determinant in the unexposed population. While there are several good methods to measure either MBOCA or its metabolites in urine, none of these measurements relates well enough to exposure or risk of health effects to determine a quantitative biological exposure index. So, for example, the ACGIH BEI for MBOCA is a nonquantitative guideline for total MBOCA in urine.

Applying Biological Exposure Indices

In addition to providing comprehensive estimates of recent exposures, in many cases biological monitoring can allow health professionals to do one or more of the following:

- measure body burden of a chemical;
- supplement air monitoring to document exposures;
- detect small exposures;
- distinguish nonoccupational exposures;
- identify unknown or undiscovered exposures, especially from noninhalation sources such as dermal absorption or ingestion;
- examine effectiveness of engineering controls, work practices, and personal protective equipment;
- follow trends of exposure over time;
- reconstruct past exposures; and
- enhance individual or group risk assessments.

As is the case for all types of OELs, credible BEIs are explained and supported by documents that critically review the scientific criteria on which they are based and often provide practical information for their application. These documentations generally describe the following types of pertinent information:

- scientific rationale;
- sampling and analytical methods;
- quality control measures;
- issues related to specimen collection and storage;
- potential for confounding exposures;
- typical background concentrations of the determinant;
- quality of the relevant database;
- other limitations; and
- research needs.

Like all occupational exposure values, BEIs should be used by knowledgeable health professionals who understand their bases and how they are intended to be applied.

See also: American Conference of Governmental Industrial Hygienists; Occupational Toxicology; Occupational Exposure Limits.

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Biomarkers, Environmental

Lee R Shugart

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Introduction

Ecotoxicology is a relatively new scientific discipline and is a branch of toxicology that studies the subtle toxic effects that pollution exerts on living organisms by investigating the fate and effects of chemicals and natural substances on ecosystems. The term ecotoxicology merges the fields of ecology and toxicology. A major difficulty with the merging of these two fields is that each focuses on a different organizational level. Toxicology is concerned with adverse effects of chemicals on living organisms, whereas ecology is focused at the population, community, or even ecosystem level. Ecotoxicology is sometimes used synonymously with environmental toxicology; however, the latter also encompasses the effects of environmental pollution on humans.

There are many tests available to the ecotoxicologist to measure toxicity of chemicals but the problem becomes one of the extrapolation of data from a few species to many and from laboratory or

limited field tests to effects on natural communities and ecosystems. Although pollution can produce stress at the ecosystem level, the response observed is latent and so far removed from the initial event of exposure at the organismal level that causality is almost impossible to establish.

Biological Markers

Definition/Classification

The use of biological responses (biological markers or biomarkers) in organisms exposed to toxic substances is an approach that may help resolve the problems of causality. Chemicals and physical agents are known to elicit measurable and characteristic biological responses in exposed organisms and such evidence can provide a link between exposure and effect.

The definition of biomarker used here is 'a biological response to a chemical(s) that gives a measure of exposure and sometimes also of toxic effect in an organism'. Biological responses measured above the organism level are considered as 'ecological indicators'. Thus, biomarkers are any of a series of biochemical or molecular responses to compounds that have entered an organism, reached sites of toxic action, and are exerting an effect on the organism. In this context, the organism functions as an integrator of exposure, accounting for abiotic and physiological factors that modulate the dose of toxicant taken up from the environment. Because of the commonality of biochemical and cellular structure and function among all living organisms, biomarkers are potentially applicable over a broad range of species and across most ecosystem types.

Four classes of biomarkers have been proposed: exposure, effect, exposure/effect, and latent effect. The scientific journal *Biomarkers* makes the following division: biological markers of disease and of response, exposure, and susceptibility to drugs and other chemicals. The latter category, susceptibility, is considered to include genetic factors and changes in biological receptors, which alter the susceptibility of an organism to exposure to a chemical substance. These subdivisions seem artificial since all biomarkers are, by definition, biomarkers of exposure. Whether they are also biomarkers of effect depends on the state of our scientific knowledge.

Specific and Nonspecific Biomarkers

The specificity of biomarkers to chemicals varies greatly. Both specific and nonspecific biomarkers have their place in environmental assessment. A nonspecific biomarker can tell one that a pollutant is present in a meaningful concentration but does not tell one as to which chemical is present. Based on this information a more detailed chemical investigation can be justified. In contrast, a specific biomarker tells one about which chemical is present, but gives no information on the presence of other chemicals.

Criteria for Evaluating Biomarkers

A list of criteria for evaluating biomarkers that should be given consideration are

1. *Biological specificity*. It is important to know which classes of organisms the biomarker may be used on. The inhibition of the enzyme acetyl choline esterase (AChE) by organophosphates and carbamates can be applied throughout the animal kingdom whereas the induction of vitellogenin is confined to those vertebrates that lay eggs.
2. *Clarity of interpretation*. How clear cut is the endpoint as an indicator of exposure to anthropogenic stress? Can the endpoints be clearly distinguished from natural stresses? It is valuable to know the mechanism of response to the chemical in assessing this point.
3. *Time of responses*. The temporal expression of different biomarkers can vary widely from nearly instantaneous to years. Depending on the type of study, slow or rapid manifestation maybe desirable.
4. *Permanence of response*. Similarly, it is important to know how long the response lasts. If it is transient, it may readily be missed. The inhibition of AChE, especially in blood, is a transient response and thus it is necessary to know when the exposure occurred to assess the importance of the degree of inhibition. In contrast, the inhibition the enzyme amino levulinic acid dehydratase by lead is only slowly reversed.
5. *Reliability*. This can be considered under two headings: (1) environmental influences that modulate the organism's response to a chemical, and (2) inherent variation in the biological response to a given exposure. It is important to know the extent of all variations in order to have a reliable biomarker.
6. *Methodological considerations*. Important considerations here are precision (analytical reproducibility of the method), cost, and ease of the analysis. Although many reliable assays have been developed there is a need for standardization, along the lines used in analytical chemistry, so that results from different laboratories are comparable.

7. *Relative sensitivity*. It is important that the biomarker be sensitive when compared to other endpoints, such as mortality or reproductive impairment, and it is important to know the relative sensitivity of this comparison.
8. *Validation in the field*. For a biomarker to be useful in environmental assessment, it must be validated in the field. Organisms in the field are subjected to a wide range of variables that are usually accounted for or controlled in laboratory experimentation.
9. *Linkage to higher-level effects*. A biomarker is more useful if there is clear linkage to effects at higher levels of organization. Studies on invertebrates have been particularly fruitful as population changes occur more rapidly than in higher species.

Biological Monitoring

At the present time there is a strong drive to clean up the environment; however, since costs increase rapidly with the degree of cleanup, there is a pressing need to have a practical, defensible strategy that provides information for establishing both priorities for environmental restoration and endpoints for regulatory compliance. The study of biological responses in living organisms to contaminants in their environment (i.e., biological monitoring) is an informative, cost-effective, and logical complement to chemical monitoring of toxicants of many environmental monitoring programs.

Ecological Application

In order to predict and avoid unacceptable health risks associated with environmental pollution such as disease in humans, mass mortality, and loss of commercially or ecologically important species, an understanding of the critical cellular events between exposure at the organismal level and effects expressed at higher levels of biological organization must be established. In this context, it is often necessary to regress along the conceptual sequence of responses to toxicant exposure and select appropriate biological responses that are causally related to, or predictive of, longer term effects. The biochemical and physiological consequences of toxicant exposure of most concern to ecotoxicologists are those that might affect reproductive health. There is a need to relate biomarker responses to changes in Darwinian fitness parameters in individuals so that population and other effects at higher levels can be predicted. In this regard there is considerable evidence that some toxicants may potentiate any number of measurable biological mechanisms (i.e., mutational events in embryonic tissue, impaired growth, and change in genetic diversity) that eventually manifested this toxicity at the population level.

Concept of Meaningful Exposure

Biomarkers have the advantage over chemical analysis in that they can demonstrate whether or not an organism is meaningfully exposed. For some classes of persistent organic chemicals, especially the organochlorines, detection limits are now down to parts per trillion. Thus, in almost all samples these man-made chemicals can be detected, but the physiological significance is rarely known.

With biomarkers it is possible to determine if the physiology of the organism is significantly different from normal. If it is, then the organism is considered to be meaningfully exposed. Equally important, if the physiology is not significantly different, then the organism is considered not to be meaningfully exposed even though the chemical(s) can be detected. The ability to determine whether or not an organism is meaningfully exposed is important in making the decision whether regulatory action should be taken and also in making the decision whether or not remedial action has been successful. The following criteria need to be met before the concept of meaningful exposure can be used. These are

1. Data must be available on what is normality for each biomarker. Because of the diversity of species involved, this is a good deal more complex than in the case where biochemical levels are used in the diagnosis of human health. Obviously, it is impossible to have data on all species. While there is a great deal of data available, there is a need for a centralized database to collect, verify, and validate this baseline information.
2. To adequately assess the impact of the major classes of chemicals of concern, biomarkers are needed that indicated the status of the important functions of the organism. While we have not reached this point yet, the rate of progress toward the goal is encouraging.

See also: Biomarkers, Human Health; Chemicals of Environmental Concern; Ecotoxicology.

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Biomarkers, Human Health

Rogene F Henderson

Introduction

In the field of environmental health research, it is often difficult to determine if exposures to a specific chemical or to a chemical mixture have induced an adverse health effect. In the 1980s, a promising field of research, focused on 'biological markers' (or 'biomarkers'), began to develop as an aid for linking toxicant exposures with potential health effects. A biological marker is defined by the National Research Council (NRC) of the National Academy of Sciences (NAS) as an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism. Biomarkers are always found within an organism and may be used to demonstrate the relationship between exposure, internal dose, dose to target organ, biologically effective dose, initial biological effects, and induced adverse health effects (**Figure 1**).

In the absence of biomarkers, epidemiology studies have relied on external indicators of exposure (area or personal monitors, questionnaires, histories) to establish associations between exposures and subsequent disease development. Such tools are not highly accurate, and most studies address only exposures to populations, not the dose received by individuals. New molecular techniques, such as measurements of chemical adducts formed by exogenous compounds with macromolecules in the body, have provided the tools for assessing the dose received by an individual; the same molecular techniques also provide information on the initial changes induced by the biologically effective dose, changes that may eventually lead to disease. These new biological markers take into account individual variability in processing of potential toxicants and show promise for providing more accurate information for the assessment of the effect of exposures to noxious agents on the induction of adverse health effects in individuals.

The most useful biomarkers are those that are chemical-specific, and: (1) quantitatively reflect the degree of the prior or ongoing exposure; or (2) quantitatively reflect, or predict, later developing disease.

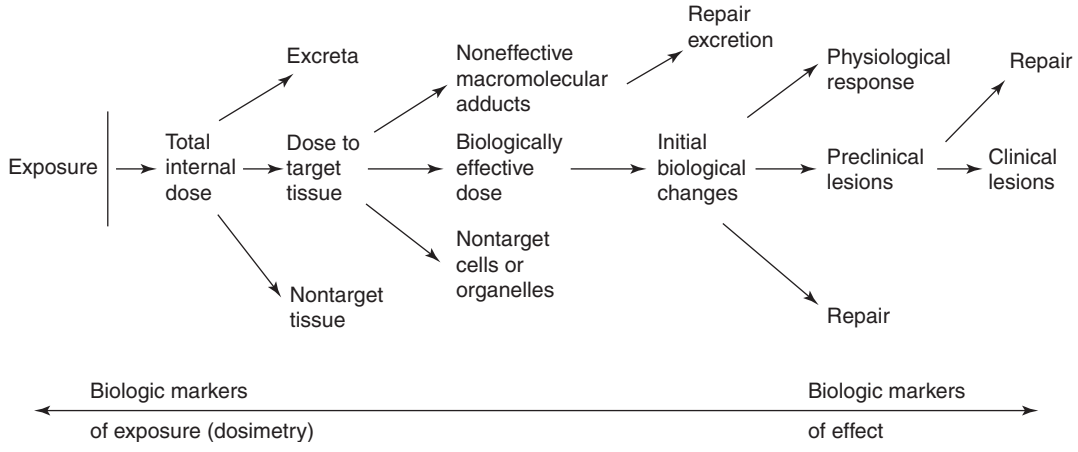

Figure 1 Biomarkers for risk assessment. Toward the left are biomarkers of exposure (dosimetry); most of these markers represent values obtained from toxicokinetic studies. Toward the right are biological markers of effect; many of these markers are standard signs and symptoms familiar to clinicians. The goal of biomarker research is to obtain more information on the link between biologically effective doses and the early, initial biological changes that can lead to disease; such values will come from studies on the mechanism of disease induction.

Ideally, the biomarker of the extent of exposure could also be used to predict the health outcome, but it is rare that sufficient information is available to make such predictions. Quantitation is needed when establishing regulations governing allowable exposures that are protective of health. If it is only important to determine if an exposure has occurred, the presence of a biological marker specific for the chemical of concern may be all that is needed. However, for the purposes of risk assessment – that is, determining the potential for a given exposure to an exogenous substance to cause adverse health effects – quantitation of the amount of biomarker present is required.

There are also practical limitations to the selection and use of biomarkers in human studies. The biomarker should be measurable in a relatively available tissue or fluid; for example, urine and breath. Sampling blood is an invasive process and so is more difficult to perform although it is done routinely. However, sampling liver tissues from humans for DNA adducts is much too invasive and would not be performed except at the time of autopsy. In addition, the assays for the marker of interest should not be so expensive that the cost of a study using the marker is prohibitive. Finally, the marker must be validated for its accuracy in quantitatively reflecting either exposure or health outcome. Otherwise, the results of the biomarker assays cannot be interpreted.

Biomarkers are generally divided into three categories: markers of exposure, markers of effects, and markers of susceptibility. Each of these types of biomarkers is described below, along with how they may be used in risk assessment.

Biomarkers of Exposure

As stated previously, the NRC/NAS has defined biological markers as exogenous substances or their metabolites or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism. Biomarkers of exposure are measures of internal substances, and thus reflect various manifestations of the internal dose that results from exposures. Markers of interest include those that provide measures of the: (1) total internal dose (such as blood, urine, or breath levels of a chemical); (2) dose to a target organ (which may be in the form of macromolecular adducts formed between the chemical or its metabolite and the organ tissue); or (3) biologically effective dose (which can only be measured if the mechanism of disease induction is known in sufficient detail to suggest what entities might represent the biological effect). An example of the last type of marker is a specific DNA adduct known to lead to a disease.

Some of these biomarkers are the same parameters measured in classical pharmacokinetic (or toxicokinetic) studies such as blood, urine, or breath levels of a substance. Other biomarkers have become available only recently due to the development of new techniques that allow detection, and sometimes quantitation of macromolecular adducts formed from the interaction of the chemical of interest or its metabolite with blood proteins or tissue DNA. Even if the biomarkers of exposure are not thought to lead to disease, such biomarkers can be useful if they can be linked quantitatively to other biomarkers that do lead to disease. For example, knowledge of the quantitative relationship between levels of hemoglobin adducts formed during exposure to a specific chemical (example of an adduct formed with a blood protein and an adduct that is not known to lead to disease) and levels of liver DNA adducts (example of an adduct formed in the target tissue and a biomarker of the biologically effective dose) from the same exposure to a liver carcinogen, could lead to the use of the more available blood adducts (vs. liver tissue adducts) as predictors of the biologically effective dose.

Strategies for Use of Biologic Markers of Exposure to Assess Prior Exposures

Many commonly measured pharmacokinetic values can be used as biomarkers of exposure. Examples include parent compound or metabolites in exhaled breath, blood, or urine and macromolecular adducts or their degradation products that appear in urine. To make quantitative assessments of the relationship of such markers to prior exposures, it is necessary to determine the rate of formation and removal (clearance) of the marker. From this information it is possible to predict the steady-state concentrations of the marker following various exposure scenarios. In addition, with information on the rate of formation and removal of a marker and knowledge of the factors that influence those rates (such as gender, dose, repeated exposures, route of exposure, rate of exposure), a mathematical model that describes the concentration of the marker under different exposure conditions can be developed. While the concentration of the marker cannot be used to identify a unique exposure scenario, the marker can indicate the types of exposure regimens that would produce the measured level of the biomarker.

From a practical viewpoint, human populations cannot be used to determine the rate of formation and clearance of markers and the influence of various factors on those rates. Therefore, most toxicokinetic studies are conducted in animal models. From detailed studies in animals, mathematical models are

derived based on the animal toxicokinetic data, animal physiological data, and the physical/chemical properties of the compounds of interest (such as partition coefficients). The models, which are often referred to as 'physiologically based pharmacokinetic models', can then be modified for use in making predictions for humans by substituting human physiological data into the model and using the results of metabolic rate studies conducted with human tissues *in vitro*. The validity of such modified models must then be verified by limited studies in humans.

A second strategy for the use of biomarkers in establishing prior exposures is to make use of a battery of biomarkers with differing half-lives. Some biomarkers of exposure have half-lives of minutes or hours (volatile parent compound in exhaled breath, some blood or urinary metabolites); other biomarkers may be present for days or weeks (some DNA adducts, blood albumin adducts); while others may accumulate over longer periods of time due to longer half-lives (blood hemoglobin adducts, some DNA adducts, products of DNA repair in the urine). There are also differences in the fraction of the internal dose of a chemical that is converted to each type of biomarker. In general, some markers with shorter half-lives, such as urinary metabolites, represent large fractions of the internal dose, while macromolecular adducts, many of which have longer half-lives, represent only a small fraction of the dose. By combining knowledge of the half-lives of markers and the amount of marker formed relative to the total dose, it is possible to obtain more information about a prior exposure using a battery of biomarkers rather than by using a single biomarker. For example, if multiple markers of a single chemical are determined in an individual, it should be possible to distinguish between someone who has had a recent exposure, someone who is receiving an ongoing exposure, and someone who was exposed repeatedly in the past but has had no recent exposures. If someone has had only a recent single exposure to a chemical, the shorter half-life, more abundant biomarkers in the form of urinary metabolites should be readily detectable, but there should be very little of the longer half-life, less abundant DNA adducts present. If the person has had an ongoing exposure for many years to the same chemical, there should be relatively high amounts of both the urinary metabolites and the DNA adducts. If the person was exposed some time ago but not recently, then only the longer-lived DNA adducts or hemoglobin adducts may be detectable.

Biomarkers of Effect

The NRC/NAS defines a biomarker of effect as any change in a biological system that is qualitatively or quantitatively predictive of health impairment or potential impairment resulting from exposure. While a distinction is made between biomarkers of exposure and biomarkers of effect, in practice, the two areas overlap. For example, DNA adducts may be biomarkers of exposure, but if they occur at specific sites known to induce mutations leading to cancer, the adducts also may be biomarkers of effect.

Many types of responses may be made to toxicants. Some of the induced effects may be merely physiological responses that are not deleterious. Other responses may be deleterious, but are quickly repaired. But some responses represent the earliest indicators of a change that, if persistent, can lead to an adverse health effect. The persistence and amplification of such a response leads to a clinical disease state. The most useful biomarker of an effect is one that is definitive for a specific adverse health effect, is quantitatively predictable for health outcome, and occurs early enough in the process that its detection allows intervention in the disease process. Signs and symptoms that occur in later stages of a disease process are the tools of clinical medicine; the goal of scientists working on biomarkers is to discover preclinical markers of the early stages of a disease process when intervention is still possible. Examples of early markers of a disease process might be a preneoplastic, proliferative lesion, an increase in a cytokine that is associated with a fibrotic process or the under- or over-expression of a gene known to be associated with a disease process.

New genomic techniques have been developed that can be used to measure the responses of the total gene array of a tissue in one assay. These responses can be considered biomarkers of either exposure or of effect. The challenge now is to assemble and analyze the tremendous amount of data made available by this new field of toxicogenomics and to interpret the meaning of the responses. In the future, it is hoped that the responses can be used to elucidate the pathways involved in the early stages of disease development.

To relate biomarkers of exposure to health outcomes, it is necessary to know which markers can be associated with the disease outcome and the degree of that association. That is, given the presence of a certain level of a biomarker, what is the probability of contracting a disease? This query is certain to be made by participants in any occupational or general population study in which biomarkers are assayed. Currently, very little information is available on which to base an answer. The inability to use biological markers of either exposure or effect to predict health outcome represents a major gap in knowledge and decreases the potential usefulness of the markers. What are needed are valid markers of risk.

Strategies for Improving the Ability to Link Biomarkers of Effect to Disease Outcome

Perhaps the most fruitful area of research for identifying biomarkers of exposure that can be linked to disease outcome is the study of mechanisms of disease induction. It is not possible to define a marker of a 'biologically effective dose' unless the mechanism by which the biological effect is induced is known. Likewise, the earliest biological events that lead to a disease cannot be determined unless the mechanism of disease induction is understood. Mechanistic studies should help to link the biologic markers represented by traditional toxicokinetic measurements and the biologic markers represented by traditional clinical markers of disease.

In addition to knowledge on the mechanism of disease induction, it is necessary to define the quantitative relationship between the level of the marker and the probability of progression to an adverse health effect. To accomplish this, pharmacodynamic or toxicodynamic modeling describing the kinetics of disease development, similar to the toxicokinetic modeling used to describe the kinetics of internal dosimetry, is required. For example, to use chemical-specific DNA adducts to predict cancer induction, the following pieces of information are required. First the various DNA adducts formed by the chemical must be identified. Then the biological half-lives of each adduct (How long will they be present before they are repaired?) and the mutagenic potential of each adduct (How much harm will the adducts cause if they are present?) must be determined. If adducts are formed that have relatively long half-lives and high mutagenic potential, it is possible to determine if the mutations induced by the adduct in *in vitro* studies are present in tumors induced by the chemical. Once enough is known about the disease induction to form a hypothesis for the process, intervention studies, in which the proposed path to disease is blocked, can be used to validate the path as the active disease generating process. Finally, toxicodynamic models can be generated that describe the quantitative relationship between adduct levels and cancer induction. Such models require knowledge of the cellular dynamics involved in tumor formation.

Biomarkers of Susceptibility

Indicators of individual or population differences that influence the response to environmental agents are called 'biomarkers of susceptibility'. These indicators might include such characteristics as an enhanced metabolic capacity for converting a chemical to its reactive, more toxic metabolite; an enhanced capacity to detoxicate reactive metabolites; or differences in number of receptor sites that are critical for a specific response. An example is the inherited deficiency in the enzyme, α-1-antitrypsin, which is associated with an increased susceptibility to development of emphysema. The new assays developed by researchers in the field of toxicogenomics allow detection of genetic polymorphisms that can affect susceptibility to pollutant exposures. Such markers can be quite valuable in providing information that can contribute to protection of susceptible populations. Knowledge of the mechanisms of susceptibility can also be important in designing therapy for a disease. However, the use of such markers is fraught with legal and ethical problems, because identification of persons with enhanced susceptibility to adverse health effects from exposure to chemicals could lead to discrimination against those persons in obtaining jobs and insurance.

Uses of Biomarkers

As mentioned in the beginning of this section, a major potential use for biological markers is to link environmental exposures causally and quantitatively to health effects in individuals or populations. If the whole chain of events illustrated in **Figure 1** can be defined in a quantitative fashion for a single toxicant, it may be possible to regulate the exposure to such a toxicant and to prevent adverse health effects with a reasonable degree of certainty. This would avoid over- or under-estimation of the risk from such a toxicant. In practice, this use of biomarkers is still in its infancy because of insufficient data to fill out all the information illustrated in **Figure 1**. Strategies for improving the use of biomarkers to quantitate the ability of environmental agents to produce adverse health effects have been discussed.

Another practical use for biomarkers is to detect and quantitate prior or ongoing exposures to specific chemicals; biomarkers have been successfully used in biological monitoring programs in industry but have only recently been used to monitor environmental exposures. Medical researchers are seeking biomarkers that can be employed to: (1) detect early stages of a disease to enhance successful intervention; (2) determine the effectiveness of intervention strategies; and (3) detect cells at risk from a toxicant. Finally, research is ongoing, particularly in the field of genetics, to find inherited biomarkers of susceptibility that can be used for the detection and protection of sensitive populations.

See also: Analytical Toxicology; Biomarkers, Environmental; Carcinogen–DNA Adduct Formation and DNA Repair;

Epidemiology; Mechanisms of Toxicity; Medical Surveillance; Molecular Toxicology–Recombinant DNA Technology; Pharmacokinetic Models; Pharmacokinetics/Toxicokinetics; Risk Assessment, Human Health.

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Biomonitoring

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Environmental surveillance is the systematic collection of samples of air, water, soil, sediment, and living organisms to determine the magnitude, persistence, and severity of environmental contamination. Biomonitoring is the biotic component of environmental surveillance, in which living organisms, including humans, are used to assess environmental (or occupational, in humans) contaminant exposure and effects. Ecological applications of biomonitoring include: (1) ecological risk assessments; (2) determining the efficacy of pollution remediation ('cleanup'); (3) assessing the effects or persistence of 'environmental disasters' such as chemical/oil spills or accidental releases; and (4) assessing compliance with environmental regulations. In fact, any industry, company, or facility that discharges effluent (wastewater) into a natural body of water ('receiving water') must have a National Pollutant Discharge and Elimination System (NPDES) permit, as required by the Clean Water Act. Biomonitoring of the effluent and receiving waters is a mandatory requirement of all NPDES permit holders. Components of ecological biomonitoring may include: (1) collection of contaminated media for laboratory toxicity testing; (2) *in situ* exposures of organisms at contaminated sites; or (3) field collections and surveys of organisms from contaminated sites.

Contaminated media that are collected for laboratory toxicity tests may include contaminated water, soil, or sediment (the 'mud' at the bottom of lakes, rivers, streams, bays, etc.), or extracts of these media. Organisms used for conducting soil toxicity tests may include worms, insects, plants, or bacteria. In aquatic systems, effluent water is collected from discharge pipes and tested in whole effluent tests. The effluent is diluted with noncontaminated water in the laboratory to provide dose–response curves. Subchronic or acute toxicity tests are usually carried out with fish, crustaceans, or algae. Such tests may also be carried out on the receiving water. Sediment may also be collected from contaminated sites, and toxicity tests carried by placing freshwater or saltwater worms, clams, or sediment-dwelling crustaceans in contaminated sediment. Other soil/sediment toxicity tests include elutrate and pore water toxicity tests. Elutrate is an aqueous extract made by vigorously mixing soil or sediment with water, and removing solid material by centrifugation or filtration. Pore water is extracted from wet sediment by centrifugation at high speeds to collapse the sediment and squeeze out the water between sediment particles. Toxicity tests are then conducted with the elutrate or pore water. Besides toxicity endpoints such as survival, growth, reproduction, and seed germination, bioaccumulation of chemicals from the soil or sediment may also be measured.

Environmental contaminants are present as complex mixtures, so that if toxicity is found, procedures known as 'toxicity identification evaluation' may be carried out. This procedure identifies toxic components by systematically treating the effluent, elutrate, or pore water to remove various fractions – hydrophobic ('fat soluble') chemicals, metals, acids, volatile compounds, etc. – and retesting the toxicity after each extraction. Loss of toxicity after an extraction implicates the chemical that was extracted. This is confirmed by chemical analysis and toxicity tests on

the extracted fraction and the pure chemicals therein. After the toxic chemical has been identified and industrial processes have been modified to remove it or reduce its concentration to nontoxic levels, follow-up 'toxicity reduction evaluation' procedures are carried out to confirm that the toxicants have been removed or abated.

In contrast to the aforementioned toxicity tests, *in situ* toxicity tests involve exposing organisms to contaminants on-site. This provides for more environmental realism, but there is also less control over confounding variables that may affect toxicity (spatial or temporal variation in temperature, sunlight, nutrients, pH, etc.), or other factors that may disturb or disrupt the test (animals, winds, floods, vandalism, etc.). For these tests, animals may be placed in mesh cages or corralled by impermeable barriers, such as wood, metal, or plastic sheets, at various locations throughout the contaminated zone. Plants may be planted in plots of contaminated soils. Toxicity endpoints may include survival, sublethal effects, or accumulation of contaminants in body tissues. For these tests, organisms are also placed in less contaminated sites for comparison.

An alternative to *in situ* exposures or toxicity tests is to conduct field surveys, in which organisms that are indigenous to the contaminated sites are collected and analyzed. Endpoints that are examined may be concentrations of chemicals in living organisms ('body burden'), sublethal responses, ecological effects, abundance of indicator species, or biotic indices. Indicator species are those whose abundance or presence/absence is indicative of pollution stress. Examples of indicator species include (1) aquatic tubificid worms, which thrive in streams that are impacted by sewage effluent; (2) certain midge fly (Chrionomid) larvae tolerant of low dissolved oxygen in the water; and (3) many darter fish species, which are intolerant of pollution or heavy silt loads in the water. Biotic indices (e.g., the Index of Biotic Integrity) use a variety of metrics to calculate a score that can be used to compare the degree of pollution in different streams or rivers. Such metrics include species diversity, abundance of indicator species or pollution tolerant/sensitive species, prevalence of gross injuries (tumors, lesions, deformities, etc.), and relative abundance of organisms at different trophic levels (e.g., carnivores, herbivores, omnivores). Similarly, the 'EPT Index' uses the relative abundance of pollution-intolerant mayfly (Ephemeroptera), stonefly (Plecoptera), and caddisfly (Tricoptera) larvae as a measure of the relative degree of pollution in streams and rivers.

However, there are a variety of 'confounding variables' such as temperature, season, and water or soil chemistry, and other disparities between sites that have nothing to do with degree of contamination, but may affect relative differences between contaminated and reference (noncontaminated) sites. Therefore, in a variety of 'Criteria for Establishing Causality' have been developed in order to aid in distinguishing between natural variation and pollution impacts. Also, biomonitoring efforts that integrate a variety of different endpoints are also useful in differentiating between natural variation and effects of environmental contamination. For instance, sediment toxicologists often use a 'Sediment Quality Triad' to assess contaminant effects. This triad includes chemical analysis of pollutants, sediment toxicity tests, and determination of the diversity of aquatic invertebrate species in contaminated sediments.

Other recently developed biomonitoring technologies involve the use of biosensors or genetically engineered organisms (GEOs). Biosensors consist of both biotic and electronic sensing components enclosed in a chamber. The electronic sensing components include electrodes, video cameras, or fiber optic sensors. Examples of biotic components are isolated cellular constituents (enzymes, DNA, etc.), bacteria and other microbes, cultured cells, or multicellular organisms (fish, plants, clams, or other invertebrates, etc.). The concept behind biosensors is that a biotic response – for example, a change in enzyme activity, DNA integrity, metabolism, or animal behavior – is converted into an electronic signal that can be remotely recorded to detect release of environmental contaminants in real (or near real) time. Biosensors can be stationed at field sites, with the signal relayed to a recorder via electric cables or radio transmissions. Alternatively, air or water can be continuously collected from field stations, and organisms can then be exposed to these media in mobile or stationary labs located on site.

GEOs that have been developed for biomonitoring include microbes, cultured cells, fish, plants, rodents, and invertebrates. Some of these can produce colored, fluorescent, or luminescent ('glowing') substances when exposed to specific contaminants. Such changes can be detected visually with the naked eye or, if tissue sections, isolated cells, or small organisms are used, with the aid of a microscope. Alternatively, electronic devices such as spectrophotometers, colorimeters, fluorometers, or luminometers can be used to quantify the signal in whole cells, tissue sections, or tissue homogenates. GEOs can be used in the laboratory or as part of a biosensor. Other GEOs have been developed that have bacterial or viral DNA inserted into their genome. These genetic markers can be isolated from the DNA of the GEO and analyzed for mutations or other DNA damage.

Other endpoints often used in biomonitoring are 'biomarkers', which can be defined as alterations of physiological, cellular, biochemical, or molecular structures or processes that are indicative of contaminant exposure and effects. Examples of such biomarkers include histopathology, induction of contaminant detoxification enzymes, induction of enzymes that repair molecular or cellular lesions, damage to biological macromolecules (proteins, lipids), DNA damage (genotoxicity), inhibition of endogenous enzyme activity, patterns of gene expression, and metabolites of xenobiotic chemicals or endogenous compounds. Biomarkers of exposure – which include such endpoints as contaminant metabolites in body fluids – are unambiguous and specific indicators that an organism has been exposed to xenobiotic compounds. However, the consequences of such biomarker responses to overall health of the organism may be unclear. Biomarkers of effects (e.g., histopathology, gross lesions) are unequivocal indicators of contaminant-induced harm to the organism, but the causative agent may not be implicated. In reality, however, most biomarkers can be classified on a gradient between two extremes. Some biomarkers are specific indicators of exposure or effects of individual chemicals, others may be indicative of a class of chemicals (e.g., heavy metals, aromatic hydrocarbons, organophosphate pesticides), while still others are responsive to a wide array of chemicals. Recent advances in molecular techniques and gene expression assays, as well as genome sequencing projects, have contributed great opportunities for discovery and interpretation of biomarkers, as well as provided insight as to the mechanism of action of single chemicals and complex mixtures. Studies that use a large suite of biomarkers are also useful in discriminating between natural variation and pollution impacts, particularly if complex mixtures of pollutants are present, especially when used in combination with chemical analysis, ecological indicators, and other sublethal effects.

Biomonitoring for human health hazard surveillance typically involves collection of hair, expired air, bodily fluids (blood, urine, saliva, breast milk, semen), feces, epithelial scrapings, exfoliated cells, or, less frequently, tissue biopsies from people known or suspected of being exposed to potential chemical, physical, or biological hazards. These samples are analyzed for toxic chemicals, their metabolites, or biomarker responses. The most commonly used biomarkers in humans are those relating to DNA and chromosomal damage, because these effects contribute to cancer risk. Besides biomarkers of chemical exposure and effects, there are also human biomarkers of tumor formation and susceptibility to toxic chemicals or cancer. Biomarkers of tumor formation are proteins, metabolites, or malformed cells in biological samples that are indicative or highly suggestive that a tumor has formed in the body. Such biomarkers may be used by clinicians as early warning indicators and noninvasive methods for assessing carcinogenesis. Biomarkers of susceptibility include genetic markers or enzymatic activities for chemical detoxification and DNA repair systems in the body. These biomarkers indicate relative risk of effects from toxic chemical exposures in individual members of the population. Similar to ecological biomonitoring studies, human health biomonitoring may involve collection of environmental media (air, water, soil, food) or chemicals extracted from such media for determination of effects in human cell or tissue cultures and animal models of human health risk (rodents, rabbits, primates, etc.).

Human health biomonitoring may also use animal surrogates in the environment to assess potential health hazards to humans – the proverbial 'canary in the coal mine'. For example, chemical and biomarker analysis of bodily fluids or tissue biopsies from family pets, especially dogs, are sometimes used to assess potential chemical exposure and effects in children. This is because dogs often accompany children in the outdoor environment, and both have a tendency to (accidentally or intentionally) consume environmental media such as soil and surface water. There has also been an increasing trend to use native animals as sentinel species, that is, fish, wildlife, or invertebrates that are indicators of possible human health risks from environmental hazards. For example, increased incidences of tumors or endocrine disruption in fish may indicate the presence of compounds in the water that may cause cancer or reproductive dysfunction in humans. Concern has also been raised over the increased incidence of deformities in frogs, because these may indicate an increased level of chemicals in the environment, which can cause birth defects in humans.

Human health biomonitoring using biomarkers and chemical analyses are used in the following applications: (1) Health surveillance of persons who are known to have high occupational or environmental exposures to potentially toxic chemicals. This may include those who work with chemicals, radioactive materials, or biohazards as part of their occupation. Examples include factory workers, chemical industry employees, farmers, health care professionals, nuclear plant employees, and veterans of the Gulf War I. This may also consist of those who are involuntarily exposed to such hazards in their everyday surroundings. Some examples are people living near land fills, factories, hazardous waste sites, or environmental catastrophes such as the Chernobyl

nuclear plant explosion, chemical spills, and other accidental releases of hazardous materials. (2) Human health risk assessment of environmental or occupational health hazards. (3) Epidemiological studies of occurrence or potential for environmentally induced diseases. Such studies that use molecular biomarkers are termed 'molecular epidemiology' studies. (4) Assessment of potential health effects of certain behaviors such as smoking and alcohol consumption.

See also: Biomarkers, Environmental; Biomarkers, Human Health; Ecotoxicology; Environmental Health; Environmental Toxicology.

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Bioremediation

Lee R Shugart

Worldwide, the introduction of a wide variety of anthropogenic chemicals into waters and soils has caused a growing concern about the consequences of such practices. Public awareness concerning the vulnerability of the environment to pollution has only been heightened by major incidents such as the Union Carbide (DOW) Bhopal and the Seveso disasters, the Three Mile Island and the Chernobyl accidents, and the Amoco Cadiz and the *Exxon Valdez* oil spills.

Environmental pollutants are defined as chemicals of natural or synthetic origin that are released by various human activities into the environment where they have an undesirable effect on the environment and/or on humans. Heavy metal compounds are found as environmental contaminants. Organic compounds are the most common and range from slightly water-soluble organics such as aromatic and halogenated hydrocarbon solvents to hydrophobic organics such as polychlorinated biphenyls (PCBs) and aliphatic hydrocarbons. Organic solvents degrade only slowly, if at all, once they enter groundwater. Hydrocarbon compounds such as fuels, lubricating oils and creosote contain toxic components. Naphthalene and its methyl-substituted derivatives are some of the most acutely toxic, water-soluble components of crude oils. As the molecular size of hydrocarbons increases, their lipophilicty, environmental persistence and mutagenicity also increase. Many chemicals represent classes of molecules not previously investigated, some have no close structural analogs in nature, and there are those that were intentionally developed to be resistant to microbial attack and to persist in nature.

Over 80% of industrial wastes, much of which can be classified as hazardous, are disposed of in landfills. Many chemicals enter the environment directly as a result of accidents, spills, or leakage from industrial facilities and waste disposal sites. In the past, contaminated wastes were buried, burned, or chemically treated in place. These treatments are costly, have limited effectiveness, and are difficult to regulate. Landfill and *in situ* fixation do not destroy waste, and landfilling only changes the place of residence delaying future liability. Contamination of the environment has placed many of our vulnerable resources (e.g., groundwater, wet lands, fisheries, and agricultural lands) at risk.

The release by humans, intentionally or otherwise, of chemicals and other pollutants into the environment has forced government, industry, and the public to come to grips with the undesirable consequences to the environment and to human health. As a result, the US Federal Government enacted laws to ameliorate these problems and included the Safe Drinking Water Acts of 1974, the Resources Conservation Recovery Act of 1976 (RCRA), the Clean Water of 1977, the Comprehensive, Environmental Response, Compensation and Liability of 1980 (CERCLA), and the Superfund Amendments and Reauthorization Act of 1986 (SARA). These laws focus on problems associated with the cleanup of disposal sites and spills of toxic substances and also with the need to reduce the volume and toxicity of waste as well as to develop safe and effective alternatives for waste disposal. In this context, the biotechnology industry has embraced bioremediation as a safe approach for these problems. Bioremediation is the enhanced microbiological treatment of unwanted chemicals of natural or synthetic origin released by human activities. An old technology once primarily used in wastewater treatment, today bioremediation is routinely applied to a wide variety of environmentally contaminated sites. Bioremediation techniques are versatile and can be used for raw materials before processing, pipeline wastes, decontamination of soils and surface groundwater, and the cleanup of dumpsites. Its salient features include attractive economics, undisturbed environment, destroyed contaminants, and eliminated liability.

Natural attenuation in contaminated environments is accomplished by biochemical degradation, evaporation, adsorption, metabolism, and transformation by microorganisms. Microorganisms such as bacteria, actinomycetes, and fungi are capable of degrading a wide range of organic compounds via biodegradation. Indigenous microbial populations, those occurring naturally, are the chief agents involved in the metabolism of chemicals in waters and soils. Metabolism may result in mineralization, which is the complete biodegradation of an organic molecule to inorganic compounds. Microorganisms can also transform hazardous organic compounds into innocuous or less toxic organic metabolic products a process that may be promoted by cometabolism (i.e., growth of the microorganism on another substrate while the organic molecule is degraded coincidentally). Heterotrophic bacterial and fungi are responsible for most of the chemical transformations. Many aerobic bacterial found in soil and water can metabolize petroleum hydrocarbons, converting them to carbon dioxide and water. Anaerobic bacteria are important for the biodegradation of chlorinated pesticides and halogenated organics (e.g., trichloroethylene and pentachlorophenol).

Degradation in contaminated soil and water may be affect by environmental constraints; therefore, treatment generally consists of optimizing conditions of pH, temperature, soil moisture and oxygen content, and nutrient concentrations necessary for the stimulation of the growth of the desired microorganisms. If the locally occurring organisms are not effective for the given set of contaminants, inoculation with microbial isolates that have been selectively adapted or genetically altered to degrade these compounds can be used. For example, recombinant PCB-degrading microorganisms with improved stability and survivability in mixed populations of soil microorganisms have been developed. Use of microbes for bioremediation is not limited to the detoxification of organic compounds; selected microbes have been used to reduce the toxic cations of heavy metals to the less toxic and much less soluble elemental forms.

As versatile as bioremediation may seem, there are certain complex xenobiotic chemicals which have proven resistant to microbial degradation. Nevertheless, the state-of-the-art in bioremediation of inorganic and recalcitrant organic contaminants is rapidly advancing.

See also: Chemicals of Environmental Concern; Environmental Toxicology; Hazardous Waste.

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Biotransformation

Tanya C McCarthy and Christopher J Sinal

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Introduction

Biotransformation refers to the process by which lipophilic (fat-soluble), xenobiotic (foreign), or endobiotic (endogenous) chemicals are converted in the body by enzymatic reactions to products that are more hydrophilic (water-soluble). In this context, metabolism and metabolic transformation are synonymous with biotransformation. A xenobiotic is a relatively small (molecular weight <1000), nonnutrient chemical that is foreign to the species where metabolism occurs.

The major purpose of biotransformation is to chemically modify (metabolize) poorly excretable lipophilic compounds to more hydrophilic chemicals that are readily excreted in urine and/or bile. Without metabolism, lipophilic xenobiotics accumulate in biota, increasing the potential for toxicity. Examples of such compounds are highly halogenated polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (TCDD and dioxins) that occur as tissue residues in humans. On the contrary, biotransformation is normally not required for xenobiotics with high water solubility because of rapid excretion in urine.

Two or more sequential enzymatic reactions are routinely required to convert lipophilic chemicals to metabolites that are efficiently excreted. R.T. Williams, a pioneer in biotransformation studies, classified these pathways as phase I (oxidation, reduction, and hydrolysis reactions) and phase II (conjugation reactions; **Table 1**). Normally, a phase I reaction precedes its phase II counterpart, but some compounds contain functional groups that are sites for direct conjugation (e.g., $-$OH, $-$COOH, and $-NH_2$). Frequently, the biological activity of a chemical decreases (termed 'detoxication') during metabolism but this is not always the case. Both phase I and phase II reactions can function in 'toxication' or metabolic activation processes as well, and this is a fundamental mechanism for the formation of many chemical toxicants. Multiple classes of toxic compounds, including polycyclic aromatic hydrocarbon-derived carcinogens and mutagens, are formed by cytochrome P450-dependent oxidative metabolism, the most common toxication pathway.

The highest concentration of xenobiotic metabolizing enzymes is routinely found in liver, but epithelial cells of extrahepatic tissues, such as the lung, kidney, intestine, placenta, and eye, also have activity. Relative to liver, extrahepatic tissues do not normally play a major quantitative role in the biotransformation of foreign compounds, including drugs. Extrahepatic organs, however, can be extremely important in the metabolic activation of xenobiotics and resultant target organ toxicity because the ratio of activation to detoxication enzyme activity is frequently higher in these cells than in hepatocytes (i.e., bioactivation predominates over detoxication and results in the formation of concentrations of active metabolites that overwhelm the capacity of detoxication pathways). The contribution of intestinal flora to the *in vivo* metabolism of xenobiotics can also be significant, especially for chemicals that require anaerobic (oxygen-deficient) reduction as a quantitatively important pathway.

Table 1 Classification of major biotransformation pathways

Classification	*Enzymes*
Phase I	
Oxidation	Cytochrome P450
	Flavin-containing monooxygenase
	Alcohol dehydrogenase
	Aldehyde dehydrogenase
	Monoamine oxidase
	H_2O_2-dependent peroxidase
Reduction	Cytochrome P450
	NADPH-P450 reductase
	Carbonyl reductase
Hydrolysis	Epoxide hydrolase
	Carboxylesterase/amidase
Phase II	
Conjugation	UDP-glucuronosyltransferase
	Sulfotransferase
	Glutathione *S*-transferase
	Mercapturic acid biosynthesis
	Cysteine conjugate β-lyase/thiomethylase
	N-Acetyltransferase
	N-Methyltransferase
	O-Methyltransferase

Oxidation Reactions

Oxidation is the most common metabolic reaction for lipophilic xenobiotic and endobiotic compounds, in part because most mammalian tissues are well oxygenated.

Cytochrome P450 Monooxygenase System

The cytochrome P450-dependent monooxygenase system is concentrated in the endoplasmic reticulum

of cells and is referred to as a microsomal enzyme system. This P450 system is composed of multiple forms or isozymes of P450 belonging, in humans, to at least 18 distinct gene families as well as the flavoprotein, NADPH-P450 reductase. This monooxygenase system has been termed a 'universal' oxidase because it catalyzes the oxidation of a multitude of lipophilic compounds including both xenobiotics (antioxidants, carcinogens, drugs, environmental pollutants, food additives, hydrocarbons, and pesticides) and endobiotics (bile acids, cholesterol, eicosanoids, fatty acids, lipid hydroperoxides, retinoids, and steroid hormones).

With several classes of xenobiotic substrates, including chemical carcinogens such as benzo(*a*)pyrene or the mycotoxin, aflatoxin B1, some metabolites are more toxic than the parent chemical, a process termed toxication. Endogenous compounds can also be bioactivated by P450 to metabolites with greater biological activity . For example, arachidonic acid is metabolized to four isomeric epoxyeicosatrienoic acids which have potent physiological and/or pathobiological effects in multiple tissues and cell types. Consequently, the P450 system is extremely important in toxicology (toxication and detoxication of both endogenous and exogenous substances), pharmacology (rate-limiting step in the metabolism of many drugs, drug–drug interactions, and individual qualitative and quantitative differences in drug metabolism due to genetic differences), and physiology (formation and metabolism of endobiotics that function as intercellular and/or intracellular messengers).

The multiple forms of P450 vary in their substrate selectivity and level of expression in different tissues and cell types. In lung, for example, the highest concentrations of P450 are normally found in (epithelial) Clara and alveolar type II cells but lower amounts occur in ciliated, goblet, and vascular endothelial cells as well as alveolar macrophages. The selective modulation (relative increase or decrease in concentration) of P450 isozymes in a single tissue or cell type can have pronounced effects on the metabolism of both endogenous and exogenous substances and on chemical-mediated target organ and/or cell toxicity by altering the balance between toxication and detoxication reactions.

The overall oxidation of a substrate, RH, by P450 is summarized in **Figure 1**, in which reduced nicotinamide-adenine dinucleotide phosphate (NADPH) is shown as the required cofactor.

$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

Figure 1 Overall reaction that occurs during the cytochrome P450-dependent oxidation of a substrate, RH.

Some of the important reactions catalyzed by the P450 monooxygenase system include aliphatic hydroxylation, aromatic hydroxylation, epoxidation, heteroatom (*N*-, *O*-, and *S*-)dealkylation, nitrogen oxidation, oxidative deamination, oxidative dehalogenation, oxidative denitrification, and oxidative desulfuration. Most of these reactions result from the initial oxidation of a carbon atom, another reason that P450 is so important in the oxidative biotransformation of lipophilic chemicals. Some P450-catalyzed oxidation reactions are illustrated in **Table 2**.

The microsomal P450 system is most highly concentrated in the liver, but it is also present in many extrahepatic tissues including the lung, kidney, placenta, small intestine, skin, adrenal, testis, ovary, eye, pancreas, mammary gland, aorta wall, brain, nasal epithelial membrane, colon, salivary gland, prostate, heart, lymph node, spleen, thymus, and thyroid. A second P450 monooxygenase system, localized to mitochondria of steroid-metabolizing tissues (adrenal, ovary, and testis), is primarily involved in the oxidative biosynthesis of endogenous steroids such as cholecalciferol, cortisone, and deoxycorticosterone. In contrast to the 'universal' oxidase properties of the microsomal system, the mitochondrial P450 tem has a much higher degree of substrate specificity.

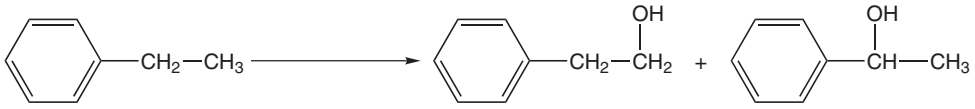
Flavin-Containing Monooxygenases

There is also a P450-independent monooxygenase enzyme family, termed the flavin-containing monooxygenases (FMOs); that is, localized in the endoplasmic reticulum of virtually all nucleated mammalian cells. Six distinct genes encoding FMOs have been identified in the human genome. These enzymes contain the coenzyme flavin adenine dinucleotide (FAD) and, similar to the P450 system, also require NADPH as a cofactor. A major difference between the FMOs and P450 is that the former do not oxidize carbon atoms. However, FMOs do oxidize many nitrogen-, sulfur-, selenium-, and phosphorus-containing xenobiotics (**Table 3**).

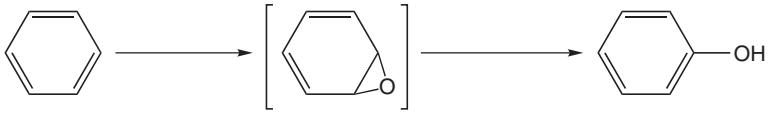
Since there are many drugs and environmental pollutants that contain sulfur, it is of considerable interest that FMO preferentially catalyzes the oxidation of sulfur in compounds containing both nitrogen and sulfur. Thus, FMO is an important enzyme system for the oxidation of selected classes of xenobiotics, and its spectrum complements that of the P450 system because the latter prefers oxidation of carbon atoms. Other ways in which FMO enzymes differ from many microsomal P450 isozymes include their

Table 2 Examples of important reactions catalyzed by the microsomal P450 monooxygenase system

Aliphatic hydroxylation

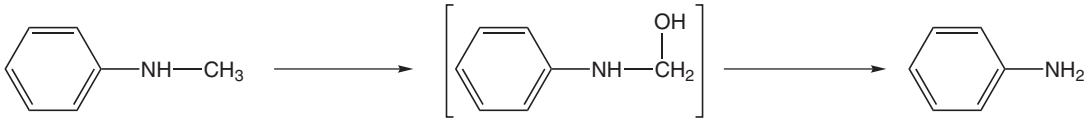


Aromatic hydroxylation

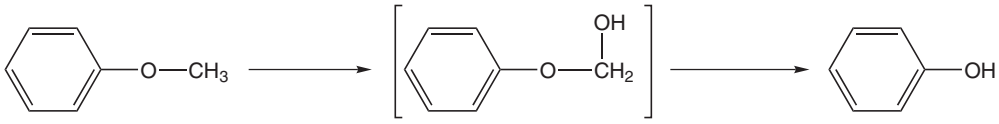


Epoxidation

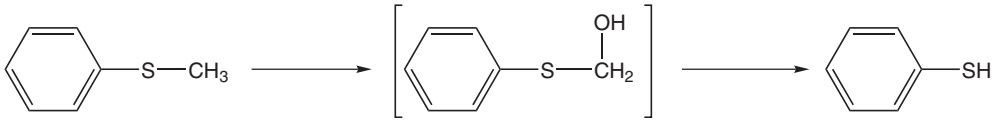
N-Dealkylation



O-Dealkylation

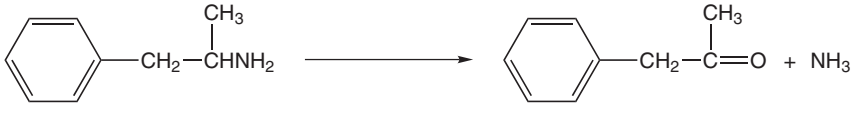


S-Dealkylation



Nitrogen oxidation

Oxidative deamination



Oxidative dehalogenation

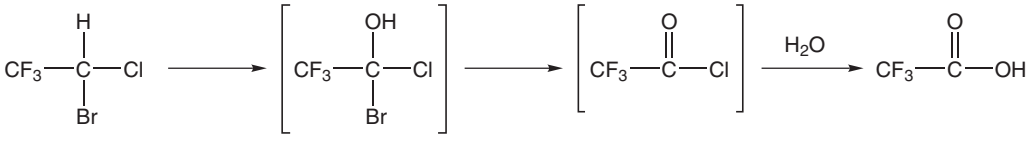
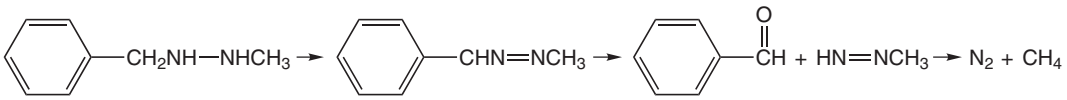


Table 2 Continued

Oxidative denitrification



Oxidative desulfuration

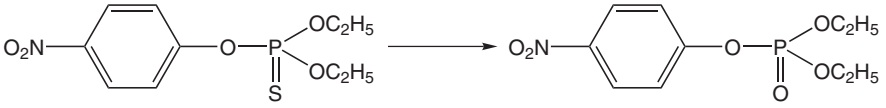
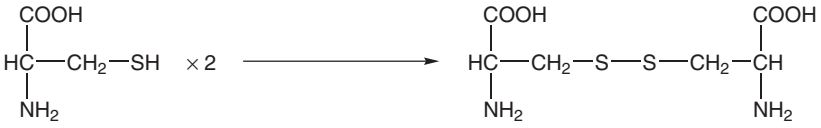


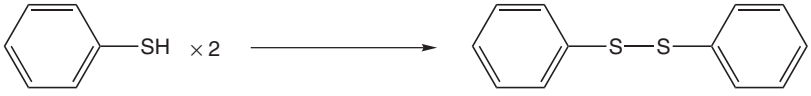
Table 3 Examples of important reactions catalyzed by microsomal flavin-dependent monooxygenases

Tertiary amine oxidation

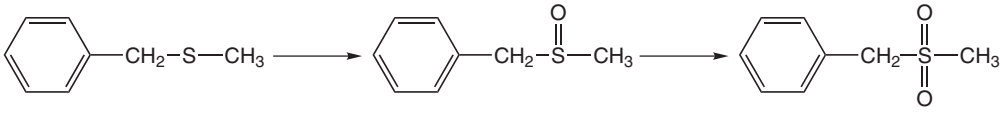
Alkyldisulfide formation



Aryldisulfide formation



Thioether oxidation



Phosphorus oxidation

apparent lack of induction (increased enzyme concentration) or repression (decreased enzyme concentration) by environmental factors and their more limited role in metabolic activation. Consequently, the P450 system is of greater significance in chemical toxicology.

Alcohol and Aldehyde Dehydrogenases

An extremely important metabolic pathway for alcohols and aldehydes is oxidation to aldehydes and ketones and to carboxylic acids, respectively. Mammalian liver alcohol dehydrogenases are a family

of zinc-containing, cytosolic NAD^+-dependent enzymes that catalyze the oxidation of primary and secondary aliphatic, arylalkyl, and cyclic alcohols. Aromatic alcohols (phenols), however, are not substrates for these enzymes. Alcohol dehydrogenases are widely distributed in mammalian tissues, with the highest concentrations occurring in the liver. As shown in **Figure 2**, alcohol dehydrogenases also catalyze the reverse reaction – reduction of aldehydes to primary alcohols in the presence of reduced nicotinamide adenine dinucleotide (NADH).

However, the *in vivo* reduction of aldehydes by this enzyme is not normally a quantitatively important reaction because aldehydes are rapidly oxidized to their corresponding carboxylic acid derivatives by aldehyde dehydrogenase. Alcohol dehydrogenase is a very important enzyme for the metabolism of ethanol.

Aldehyde dehydrogenases are also widely distributed in mammalian tissues, with the highest concentration in the liver. Both aliphatic and aromatic aldehydes are readily oxidized to carboxylic acids by this enzyme in the presence of NAD^+, the required cofactor (**Figure 3**).

Although this is a reversible reaction *in vitro*, the carboxylic acids formed are either converted rapidly to their ester glucuronide derivatives (a phase II reaction catalyzed by UDP-glucuronosyltransferase; see below) or, if polar enough, are excreted unchanged. Consequently, the reverse reaction is generally not of significance *in vivo*.

Monoamine Oxidases

The monoamine oxidases are localized in the outer membrane of the mitochondria of cells and are widely distributed in most mammalian tissues, with exceptions being the erythrocyte and plasma. This enzyme system catalyzes the oxidative deamination of a wide variety of xenobiotic and endobiotic (e.g., neurotransmitter) monoamines (**Figure 4**).

Monoamine oxidases are flavoproteins that contain one molecule of FAD per molecule. There are two major types of monoamine oxidase (A and B), whose relative concentration varies in tissues of the same species. In general, the A form of the enzyme is more active with endogenous neurotransmitter amines (serotonin, norepinephrine, and epinephrine), whereas the B form is more active toward xenobiotic amines such as 2-phenethylamine.

$$CH_3CH_2OH + NAD^+ \rightleftharpoons CH_3CHO + H^+ + NADH$$

Figure 2 Oxidation of ethanol and reduction of acetaldehyde by alcohol dehydrogenase and the appropriate form of NAD^+.

$$CH_3CHO + NAD^+ \rightleftharpoons CH_3COOH + H^+ + NADH$$

Figure 3 Oxidation of acetaldehyde and reduction of acetic acid by aldehyde dehydrogenase and the appropriate form of NAD^+.

H_2O_2-Dependent Peroxidases

Easily oxidized phenols and arylamines are excellent substrates for peroxidase-catalyzed one-electron oxidation reactions. These reactions are very important in toxicology because of the reactivity and toxicity of the free radicals (molecules with a highly reactive unpaired electron) formed. A well-studied example of this type is the cooxidation of xenobiotics catalyzed by the hydroperoxidase activity of prostaglandin H synthase. This enzyme, which converts arachidonic acid to prostaglandin (PG) H_2, has two distinct enzyme sites: cyclooxygenase, which oxidizes arachidonic acid to PGG_2, and hydroperoxidase, which reduces PGG_2 to PGH_2. PGG_2 reduction requires the donation of single electrons that can come from a xenobiotic and result in its conversion to a free radical. Many chemicals that are oxidized to toxic products, including acetaminophen, 2-aminofluorene, diethylstilbestrol, benzo(*a*)pyrene 7,8-dihydrodiol, and 4-phenetidine, are bioactivated to free radicals during reduction of PGG_2 to PGH_2 (**Figure 5**).

Prostaglandin H synthase activity is high in several extrahepatic sites that are targets for chemical-mediated toxicity but which contain very low amounts of P450 monooxygenase activity. These include skin, kidney medulla, lung of certain species, and platelets. It is now generally accepted that prostaglandin H synthase hydroperoxidase activity is important for the metabolic activation of amines and

$$RCH_2NH_2 + O_2 + H_2O \longrightarrow RCHO + NH_3 + H_2O_2$$

Figure 4 Oxidation of a substituted methylamine by monoamine oxidase. *R* can be an alkyl (CH_3^-) or aryl ($C_6H_5^-$) substituent.

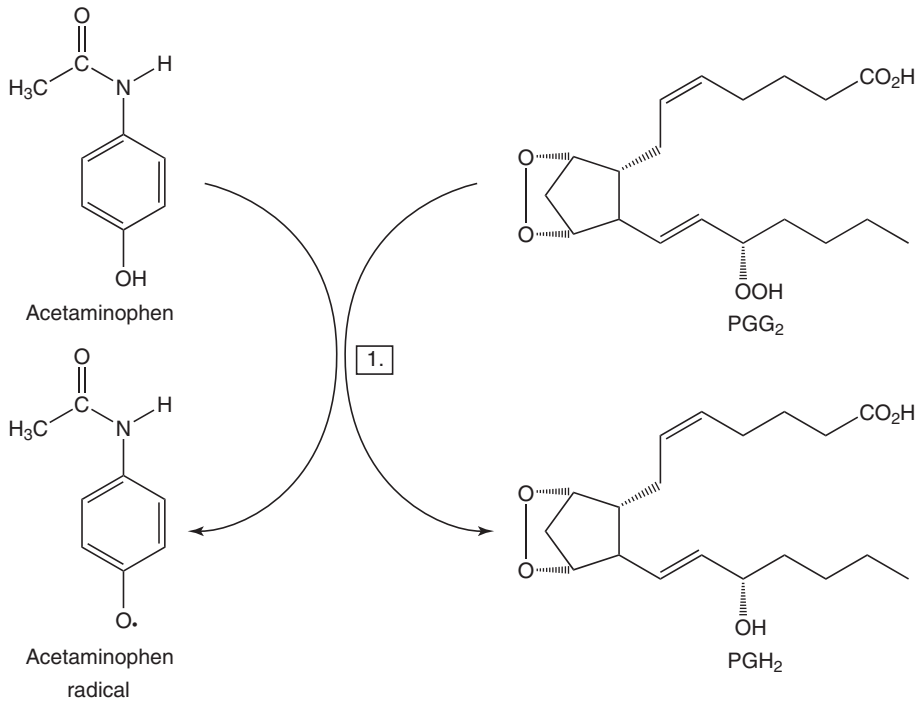


Figure 5 Conversion of acetaminophen to its reactive free radical by co-oxidation mediated by the hydroperoxidase activity of prostaglandin H synthase-catalyzed reduction of prostaglandin G_2 (PGG_2) to prostaglandin H_2 (PGH_2).

phenols, some of which are converted to potent mutagens and carcinogens, particularly in cells deficient in P450 monooxygenase activity but high in prostaglandin synthesis activity.

Other peroxidases are also involved in bioactivation of easily oxidized compounds. Oxyhemoglobin in erythrocytes can oxidize arylamines to products that cause methemoglobinemia; chloroperoxidase and myeloperoxidase of activated polymorphonuclear leukocytes and macrophages bioactivate certain drugs including various sulfonamides by *N*-oxidation to reactive nitroso products that contribute to adverse drug reactions; and diethylstilbestrol, a transplacental carcinogen, is oxidized by estrogen-inducible peroxidases in the reproductive tract.

These few examples emphasize that H_2O_2-dependent peroxidases can activate aromatic alcohols (phenols) and aromatic amines to reactive free radicals, which are often very toxic.

Reduction Reactions

Several functional groups, including nitro, azo, tertiary amine *N*-oxide, aldehyde, ketone, sulfoxide, and alkyl polyhalide, are reduced by mammals *in vivo*. Toxic free radicals are often formed as intermediates during reduction. Although some of these reactions, or more accurately the initial sequence of the reactions, occur under aerobic conditions *in vitro*, anaerobic conditions are generally required for the complete reduction of xenobiotics. Those reactions that go to completion *in vivo* are either reductions of carbonyl groups or are catalyzed by the intestinal microflora. Reduction that occurs anaerobically is of much less toxicological concern due to the decreased formation of toxic oxygen-free radicals.

Cytochrome P450-Dependent Reactions

Under aerobic and anaerobic conditions, several reduction reactions can be catalyzed by the intact P450 monooxygenase system or only by its flavoprotein component, NADPH-P450 reductase.

In addition to being oxidatively metabolized, many polyhalogenated alkanes are converted by a P450-dependent, one-electron reduction pathway to a free radical intermediate and inorganic halide. The best studied example of this reaction is the reduction of carbon tetrachloride (CCl_4) to chloroform ($CHCl_3$), which occurs *in vitro* under aerobic or anaerobic conditions and *in vivo*. The trichloromethyl radical formed (CCl_3) is believed to be a major contributor to CCl_4-mediated hepatotoxicity. Halothane, trichlorofluoromethane, hexachloroethane, pentachloroethane, and DDT are other halogenated compounds that are substrates for this P450-dependent reductive pathway.

Several other classes of xenobiotics are also efficiently reduced by the P450 monooxygenase system

under anaerobic conditions. These include tertiary amine *N*-oxides (converted to tertiary amines), hydroxylamines (primary amines), and hydrazo derivatives (primary amines).

Flavoprotein-Dependent Reactions

The first step of the NADPH-dependent reduction of aromatic nitro and azo compounds by hepatic microsomes is catalyzed by NADPH-P450 reductase and results in the formation of a free radical. In the presence of oxygen these radicals are rapidly reoxidized to the parent aromatic nitro or azo compound, concomitant with the generation of the superoxide anion radical. This futile cycling explains the toxicity of compounds, such as paraquat (**Figure 6**) or nitrofurantoin, which generate toxic superoxide under conditions in which little or no metabolism of the compound is detected. NADPH-P450 reductase is widely distributed in mammals and, consequently, these potentially toxic reactions occur in different tissues and subcellular organelles. Easily reduced compounds are readily reduced by NADPH-P450 reductase. Compounds that are more difficult to reduce, such as carbon tetrachloride, require the intact P450 monooxygenase system as a source of electrons for reduction.

Carbonyl Reductases

As mentioned previously, both alcohol and aldehyde dehydrogenases can function as reductases in the presence of NAD^+. In addition, there are a number of other carbonyl reductases that are $NADP^+$-dependent. Aldehyde reductases and carbonyl reductases are localized in the cytosol of cells, have a broad substrate specificity, have low molecular weights, and are widely distributed in extrahepatic tissues. In general, aldehyde reductases reduce only aldehydes, whereas carbonyl reductases reduce both aldehydes and ketones. Reduction of ketones can be an important metabolic pathway *in vivo*.

Hydrolysis Reactions

When certain xenobiotics, including esters and amides, are administered to animals they are hydrolyzed. Hydrolysis reactions are important for the sequential metabolism of chemicals converted to epoxides by the P450 system. These reactions are classified as phase I because they free up functional groups (e.g., $-COOH$, $-NH_2$, $-OH$, $-SH$, and $-SO_3H$) that are important sites for conjugation (phase II) reactions.

Epoxide Hydrolase

Epoxide hydrolases catalyze the hydration of epoxides to *trans*-dihydrodiols and are very important enzymes in toxication–detoxication processes. Unsaturated aliphatic and aromatic hydrocarbons are converted to epoxides (alkene and arene oxides, respectively) by P450 monooxygenase activity. Some of these electrophilic epoxides react covalently with macromolecules, such as proteins, RNA, and DNA, resulting ultimately in acute or chronic toxicity, including necrosis, mutagenesis, carcinogenesis, and teratogenesis. In most cases, the diols produced by epoxide hydrolase are much less toxic than the epoxide substrate. With some polycyclic aromatic hydrocarbons, however, the diols are precursors for potent carcinogenic and mutagenic products. For example,

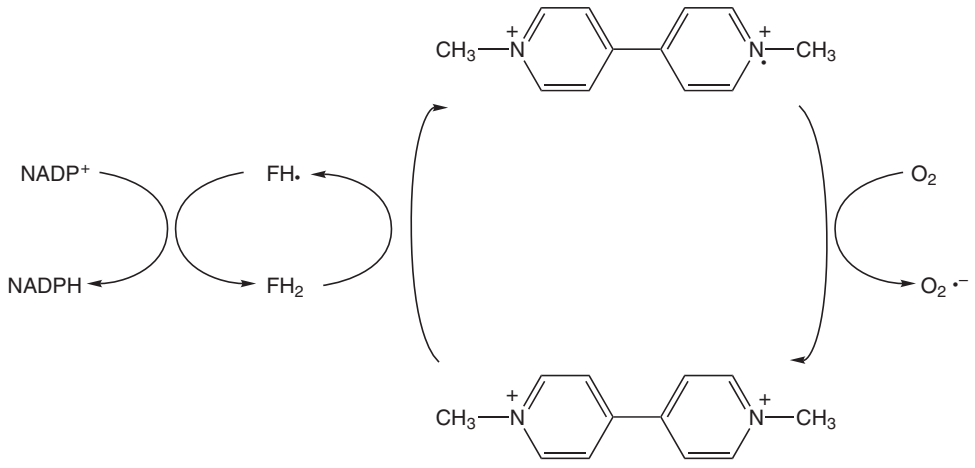


Figure 6 Futile cycle due to reaction of paraquat cation radical with molecular oxygen to generate superoxide radicals, with subsequent regeneration of paraquat. Cycle will operate as long as NADPH required as co-factor for P450 reductase is present.

benzo(*a*)pyrene 7,8-dihydrodiol, formed enzymatically from benzo(*a*)pyrene 7,8-oxide (**Figure 7**), is converted to the highly toxic benzo(*a*)pyrene 7,8-dihydrodiol-9,10-oxide by the P450 system or by cooxidation by prostaglandin H synthase.

There are two distinct types of epoxide hydrolases, both widely distributed in mammalian tissues. One type is localized primarily in the endoplasmic reticulum, the second in the cytosol. The microsomal and cytosolic enzymes have different properties, including substrate selectivities. Several inducers of xenobiotic metabolizing enzymes, including phenobarbital, planar PCB congeners, and *trans*-stilbene oxide, selectively increase (induce) microsomal, but not cytosolic, epoxide hydrolase activity.

Carboxylesterases/Amidases

The term carboxylesterase refers to a wide variety of enzymes with both esterase and amidase activity. They cleave carboxylesters, carboxylamides, and carboxylthioesters, producing a carboxylic acid and an alcohol or phenol (**Figure 8**), amine, or mercaptan, respectively. There are many different esterases, some of which are important for the hydrolysis and detoxication of toxic organophosphate esters. In general, esterases are present in almost all mammalian tissues, occur as multiple isozymes, and are concentrated in the liver. The esterase activity present in plasma is normally due to the release of these enzymes from liver.

Ester or amide cleavage can result in detoxication or metabolic activation, depending on the biological and chemical properties of the acids, alcohols, or amines released during hydrolysis. For example, hydroxamic acid hydrolysis has been implicated in the formation of proximate mutagens. The functional groups that become available for reaction during hydrolysis normally undergo phase II metabolism, as discussed below.

Conjugation Reactions

Most phase II reactions markedly increase the water solubility of xenobiotics and facilitate excretion of the chemical. Exceptions are acetylation and methylation reactions.

UDP-Glucuronosyltransferases

The most common phase II reaction is the synthesis of glucuronic acid derivatives (β-D-glucuronides) of lipophilic xenobiotics and endobiotics. Alcohols, phenols, carboxylic acids, mercaptans, primary and secondary aliphatic amines, and carbamates are converted to their β-glucuronide derivatives by UDP-glucuronosyltransferases (UDP-GT). Sixteen distinct human isozymes of UDP-GT have been identified, nine of which are encoded by a single gene. In common with the P450 monooxygenase system, UDP-GT is a microsomal enzyme, is present at highest concentrations in the liver, is expressed in many extrahepatic

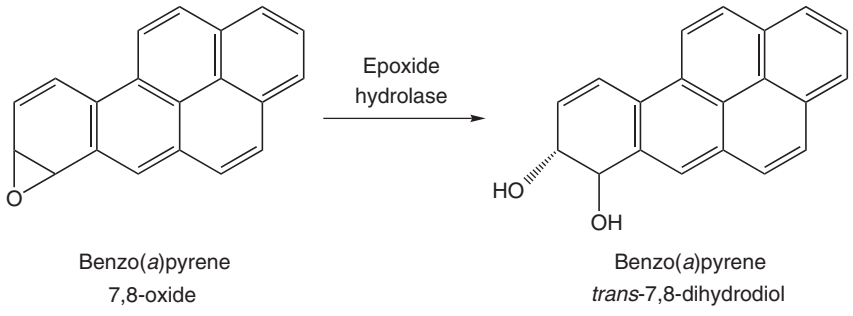


Figure 7 Conversion of benzo(*a*)pyrene 7,8-oxide to benzo(*a*)pyrene *trans*-7,8-dihydrodiol by microsomal epoxide hydrolase.

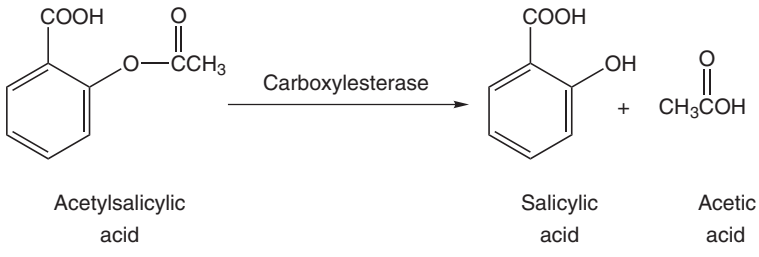


Figure 8 Hydrolysis of acetylsalicylic acid (aspirin) to acetic acid and salicylic acid (a phenolic acid) by carboxylesterase activity.

tissues, and is induced by exposure to different classes of compounds known to modulate P450, including phenobarbital, polycyclic aromatic hydrocarbons, planar PCB congeners, and dioxins.

UDP-GT catalyzes the translocation of glucuronic acid to a substrate from the cosubstrate UDP-α-D-glucuronic acid (UDPGA) as shown in **Figure 9**. The resulting glucuronide conjugates are excreted largely in the bile and can be hydrolyzed to their aglycone by β-glucuronidase of the intestinal microflora. The deconjugated chemical (i.e., the aglycone) can be reabsorbed and the cycle repeated. This process is called enterohepatic circulation and accounts for the prolonged excretion of some xenobiotics that are readily glucuronidated.

Certain β-glucuronides are electrophilic in nature and may also function in toxication processes. Covalent binding of the aglycone portions of several carboxylic acid (ester) glucuronides is known to occur to nucleophilic sites on serum albumin via transacylation reactions, for example.

Sulfotransferases

Another very common phase II reaction for phenols is conjugation with sulfate to form sulfate esters (**Figure 10**). Other substrates for this pathway include alcohols, primary and secondary amines, hydroxylamines, and sulfhydryl compounds such as thiophenols. These reactions are catalyzed by a family of cytosolic enzymes, the sulfotransferases, which require 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as the cofactor.

The sulfotransferases have been divided into several groups as a result of substrate specificity determinations with purified enzymes and molecular biology studies; aryl sulfotransferases are active toward phenols, hydroxylamines, tyrosine esters, and catecholamines; alcohol sulfotransferases are active toward primary and secondary steroid alcohols; and amine sulfotransferases are active toward arylamines.

A few sulfate esters are chemically reactive and alkylate nucleophilic sites on macromolecules. This electrophilic characteristic implicates these conjugates as ultimate chemical toxicants.

Phenols, quantitatively important P450-derived metabolites of aromatic hydrocarbons, are substrates for both UDP-GT and sulfotransferases. Generally, glucuronide metabolites predominate after administration of a phenol or phenol precursor to mammals because sulfate formation is a high-affinity, low-capacity (due to sulfate depletion) system, whereas glucuronidation is a low-affinity, high-capacity system.

Glutathione *S*-Transferases

The glutathione (L-γ-glutamyl-L-cysteinylglycine; GSH) *S*-transferases (GSTs) are a multigene family of dimeric

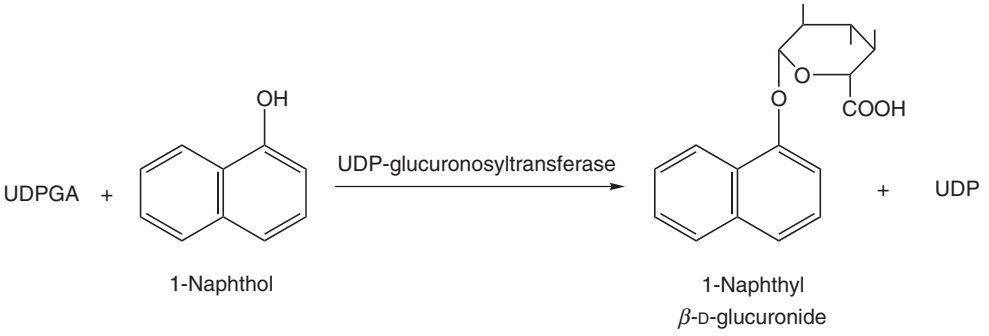


Figure 9 Conversion of 1-naphthol to its corresponding β-D-glucuronide.

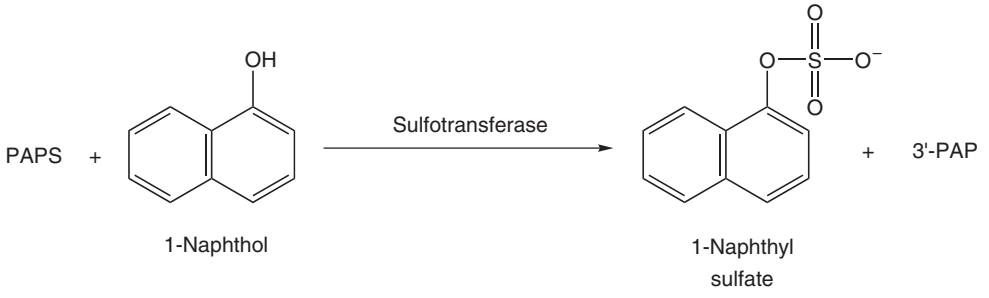


Figure 10 Conversion of 1-naphthol to 1-naphthyl sulfate by sulfotransferases.

proteins found at relatively high concentrations in the cytosolic fraction of mammalian liver, as well as in a wide variety of extrahepatic tissues. Some GST isozymes are also localized in microsomes and within the mitochondrial matrix of the liver, at much lower concentrations than the cytosolic enzymes. A wide variety of potentially toxic, electrophilic compounds (**Figure 11**) are converted to *S*-substituted GSH adducts by this family of enzymes. These include aromatic compounds containing good leaving groups (halogen, sulfate, sulfonate, phosphate, and nitro). Halogens are readily displaced from aromatic compounds as long as they are activated by the presence of electron-withdrawing groups (e.g., nitro). Strained three-membered rings, such as alkene and arene oxides, and four-membered lactones are readily cleaved by GSTs. The major factor in the transferase-catalyzed reaction of these substrates with GSH is the electrophilicity of the carbon atom where the thiol attacks. Since electrophilic chemicals are frequently very toxic the importance of the GSTs in detoxication cannot be overstated.

GSTs also catalyze a number of reactions in which an *S*-substituted GSH adduct is not formed or in which this adduct is oxidized glutathione. Examples of these reactions include the release of nitrate from nitrate esters and the release of cyanide from thiocyanates. Some GSTs also have glutathione peroxidase activity.

Although catalysis by GSTs is almost always associated with detoxication, a few substrates (e.g., the ethylene dihalides) are bioactivated to more toxic products by this pathway. Recent studies have also shown that glutathione conjugates are selectively accumulated in epithelial cells of the kidney where they are hydrolyzed. Those releasing metabolites that can undergo oxidation–reduction cycling result in cell-specific renal toxicity.

Mercapturic Acid Biosynthesis

A large variety of compounds, mostly xenobiotics, are excreted in urine as *S*-substituted *N*-acetylcysteines, also called mercapturic acids (**Figure 12**). The initial enzymatic reaction in their formation is catalyzed by the GSH *S*-transferases, as described previously. Subsequently, the glutamic acid residue is removed by γ-glutamyltranspeptidase, an enzyme with very high activity in the kidney. Next, the glycine moiety is removed by dipeptidases, which have cysteinylglycinase activity. The resulting *S*-substituted cysteine is converted to the corresponding mercapturic acid by *N*-acetyltransferase activity (see below).

Although mercapturic acids are normally the major thioether products of lipophilic xenobiotics found in urine of mammals, small amounts of the corresponding *S*-cysteine conjugates are also frequently excreted. All four thioether products formed during mercapturic acid biosynthesis are routinely excreted in bile.

Cysteine Conjugate β-Lyase/ Thiomethylation

In addition to being acetylated to mercapturic acids, some *S*-substituted cysteine conjugates are also hydrolyzed. The key enzyme in this reaction

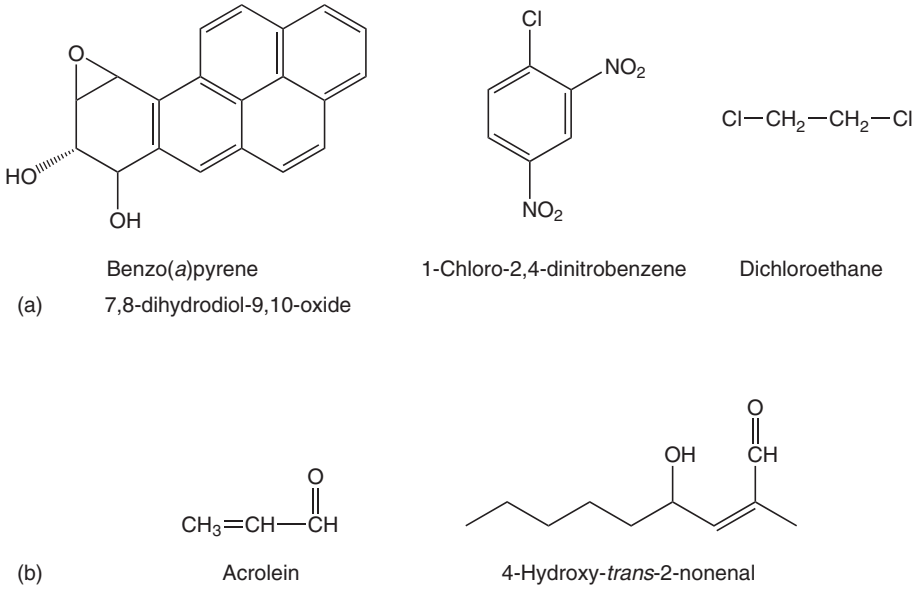


Figure 11 Structures of some common substrates of the glutathione *S*-transferases.

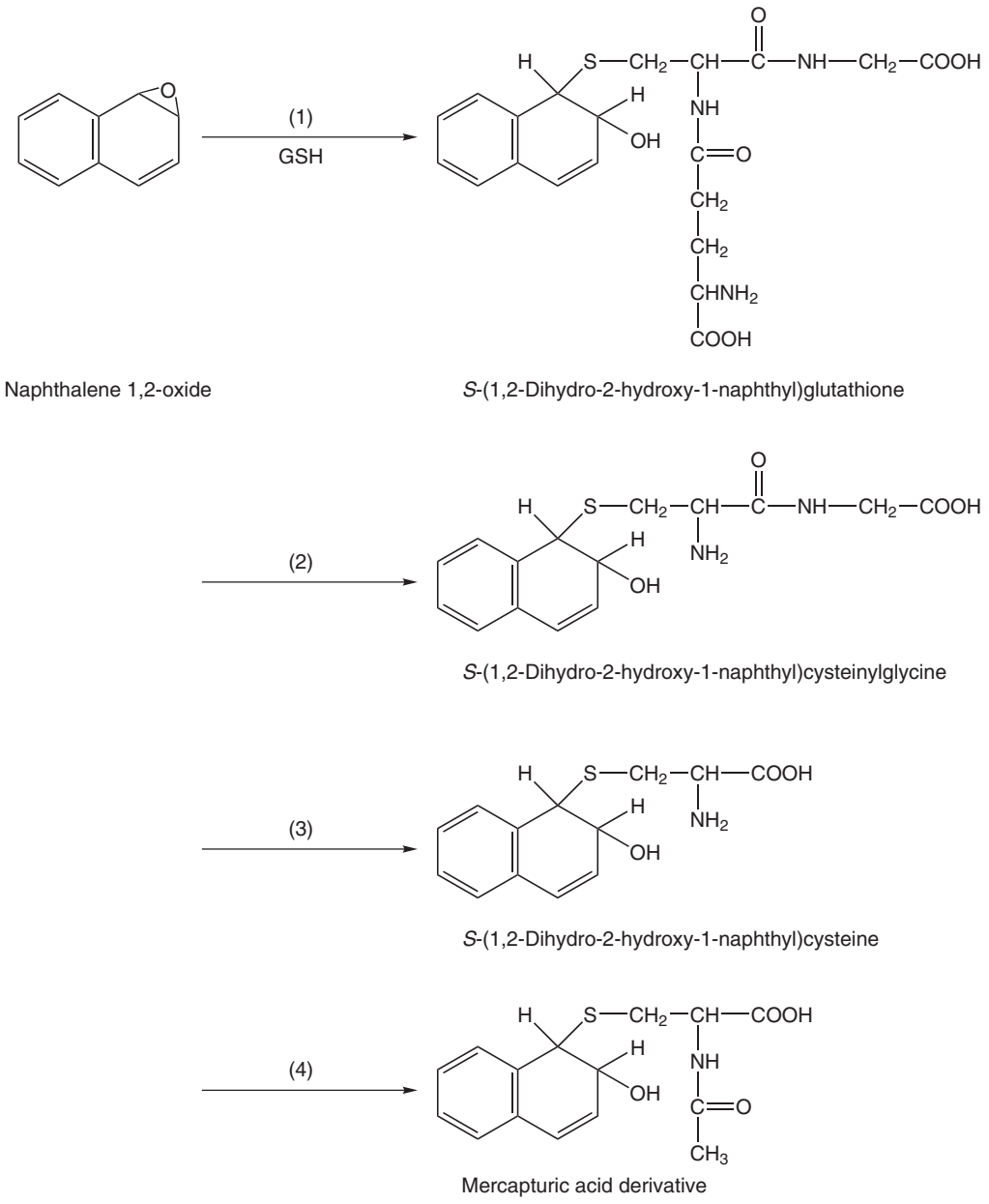


Figure 12 Mercapturic acid biosynthesis from a naphthalene 1,2-oxide. Only one of the isomers resulting from reaction of GSH with the arene oxide is shown. (1) glutathione *S*-transferase, (2) γ-glutamyltranspeptidase, (3) cysteinylglycinase activity (dipeptidases), and (4) *N*-acetyltransferase.

sequence is cysteine conjugate β-lyase, which cleaves the cysteine adduct to a free thiol, ammonia, and pyruvate (**Figure 13**).

This enzyme is present in the cytosolic fraction of rat liver and kidney and also in the microflora of the gut. Because thiols may be toxic and are more lipophilic than their cysteine conjugate precursors, β-lyase is generally a toxication pathway.

Thiols formed by mammalian or bacterial β-lyase *in vivo* are substrates for *S*-methyltransferase (**Figure 14**), an enzyme widely distributed in mammalian tissues.

This pathway accounts for the thiomethyl metabolites formed from several classes of xenobiotics. Thiomethyl metabolites can be further oxidized by the microsomal flavin-containing monooxygenases to their corresponding sulfoxide and sulfone derivatives.

Acyl-CoA:Amino Acid *N*-Acyltransferases

Several types of xenobiotic carboxylic acids (aromatic, heteroaromatic, arylacetic, and aryloxyacetic) are conjugated with a variety of endogenous amino

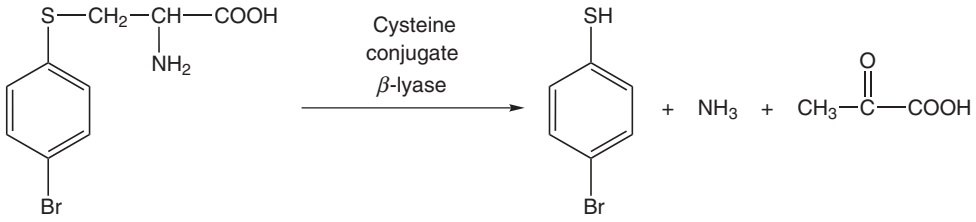


Figure 13 Hydrolysis of *S*-4-bromophenyl-L-cysteine by cysteine conjugate β-lyase.

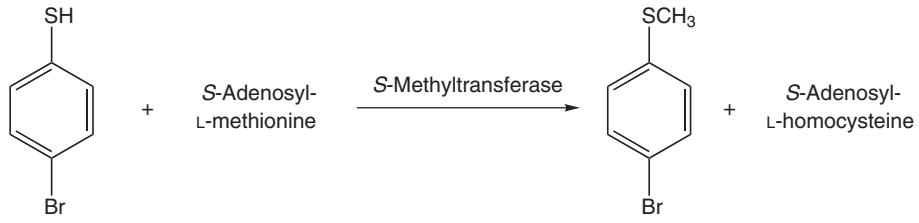


Figure 14 *S*-Methylation of 4-bromothiophenol by *S*-methyltransferase.

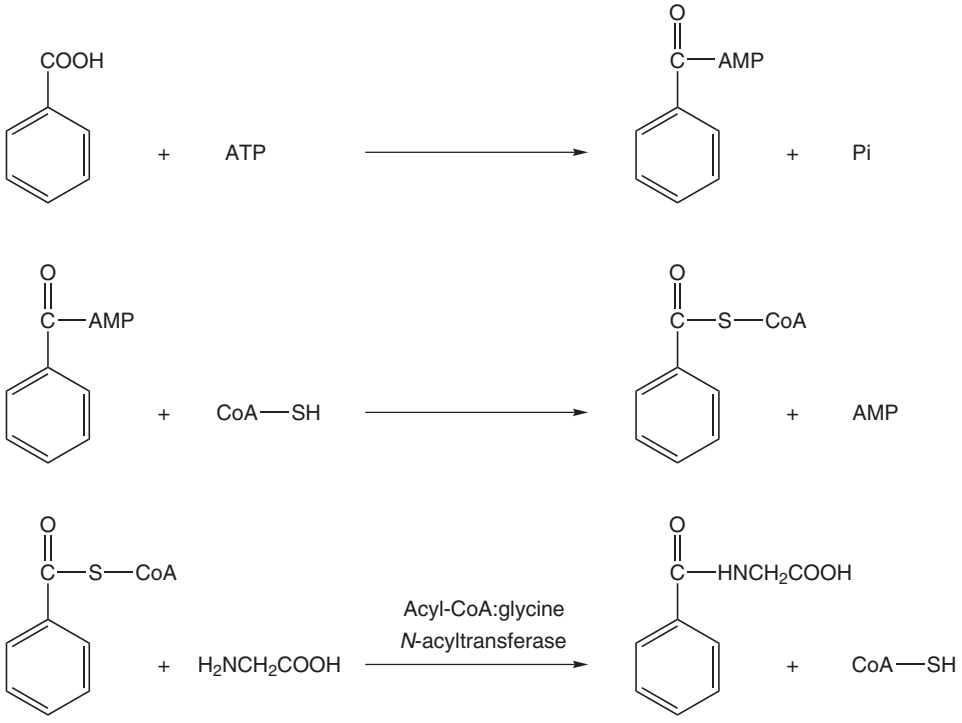


Figure 15 Metabolism of benzoic acid via its acetyl CoA derivative to hippuric acid (benzoylglycine).

acids, including glycine, glutamine, or taurine, prior to excretion in mammals. An amide (peptide) bond is formed between the carboxylic acid group and the α-amino group of the amino acid during conjugation. The reactions involved in the conversion of a carboxylic acid (e.g., benzoic acid) to its glycine derivative (hippuric acid) are illustrated in **Figure 15**.

Conversion of the carboxylic acid to its CoA ester derivative is the rate-limiting step. The enzyme that catalyzes the final reaction, acyl-CoA:amino acid *N*-acyltransferase, is localized in the mitochondria of the kidney and liver. The amino acid substrate selectivity, which varies from species to species, resides in the specific *N*-acyltransferase that

catalyzes this reaction. In most mammalian species conjugation with glycine predominates.

N-Acetyltransferases

Acetylation of xenobiotic primary amine groups is a common metabolic pathway, whereas acetylation of xenobiotic hydroxyl and sulfhydryl groups is not. Primary aliphatic and aromatic amines, sulfonamides, hydrazines, and hydrazides are readily *N*-acetylated *in vivo*, and the reaction is catalyzed by various acetyl CoA:*N*-acetyltransferases, commonly called *N*-acetyltransferases, as shown in **Figure 16**.

This family of enzymes is cytosolic and is widely distributed in a variety of mammalian tissues. There are also enzymes that hydrolyze *N*-substituted acetamides (i.e., amidases, as described previously) and the extent to which free versus acetylated amines are present *in vivo* depends on the relative rates of the acetylation and deacetylation reactions, on the physical and chemical properties of the two products, and whether or not the amine is metabolized by competing pathways. Some acetylated hydroxamic acids are chemically reactive and appear to be ultimate carcinogens.

N- and *O*-Methyltransferases

S-adenosyl-L-methionine (SAM)-dependent methylation was briefly discussed under Thiomethylation (see **Figure 14**). Other functional groups that are methylated by this mechanism include aliphatic and aromatic amines, *N*-heterocyclics, monophenols, and polyphenols. The most important enzymes involved in these methylation reactions with xenobiotics are catechol *O*-methyltransferase, histamine *N*-methyltransferase, and indolethylamine *N*-methyltransferase – each catalyzes the transfer of a methyl group from SAM to phenolic or amine substrates (*O*- and *N*-methyltransferases, respectively). Methylation is not a quantitatively important metabolic pathway for xenobiotics, but it is an important pathway in the intermediary metabolism of both *N*- and *O*-containing catechol and amine endobiotics.

Regulation of Biotransformation

The biotransformation and elimination of numerous potentially toxic xenobiotic compounds requires the concerted function of phase I and phase II enzymes. As such, exposure to elevated concentrations of xenobiotics can lead to the coordinate induction of genes encoding these enzymes. This inducibility is mediated by ligand-activated transcription factors that serve as sensors of intracellular xenobiotic concentration. Upon binding with xenobiotic compounds, these receptors interact with the regulatory region of target genes and increase the rate of gene transcription. Ultimately, this leads to an increase in the amount of phase I and phase II enzymes and the rate of biotransformation of the xenobiotic substrate. This process is self-limiting as the induction in metabolism ultimately leads to a decrease in the intracellular concentration of xenobic and thus, induction of the target gene. Examples of ligand-activated transcription factors that activate biotransformation include: the aryl hydrocarbon receptor that is activated by polycyclic aromatic hydrocarbons such as the procarcinogen benzo(*a*)pyrene; the pregnane X receptor and the constitutive androstane receptor that are activated by a large group of structurally diverse xenobiotic and endobiotic compounds; and, the peroxisome proliferator-activated receptor-alpha that is activated by a number of herbicides, industrial solvents, and plasticizers.

Summary

A number of enzyme systems have evolved in animals and plants which effectively convert lipophilic xenobiotics to more polar compounds that are efficiently excreted. Phase I enzymes, responsible for oxidation, reduction, and/or hydrolysis, are integrated with phase II or conjugation enzymes for reactions of both types and are normally required for the formation of products polar enough to be readily excreted. The intracellular level of these enzymes, and thus, the capacity for biotransformation, increases in a coordinate fashion in response to exposure to xenobiotic compounds. This response is

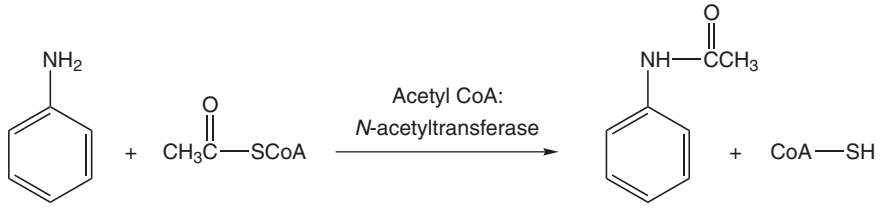


Figure 16 Acetylation of aniline by acetyl CoA:*N*-acetyltransferase activity.

achieved through changes in gene expression that are mediated by a number of ligand-activated transcription factors that serve as intracellular sensors of xenobiotic concentration. While the primary role of biotransformation is the elimination of potentially toxic xenobiotics, toxic metabolites can also be formed, primarily but not exclusively, during oxidation. When the concentration of these reactive metabolites exceeds the capacity of detoxication systems, acute (necrosis) or chronic (mutagenesis, carcinogenesis, and teratogenesis) toxicity can occur. Thus, anything that results in the reduced biotransformation of a toxic xenobiotic to an inactive metabolite, or alternatively, that increases the conversion of a relatively harmless xenobiotic to a reactive metabolite(s) increases the probability that a toxic response will occur.

See also: Carboxylesterases; Glutathione; Kidney; Liver; Pharmacokinetics/Toxicokinetics.

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Bis-Chloromethyl Ether *See* Chloromethyl Ether, Bis-.

Bismuth

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst and Shirley B Radding, volume 1, p. 174, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-69-9
- Chemical/Pharmaceutical/Other Class: Metal
- Chemical Formula: Bi^{3+}

Uses

Several bismuth compounds have been used medicinally. Some are used for gastrointestinal distress (pepto-bismol contains bismuth subsalicylate), others are used as salves and, in rare cases, for treatment of parasites. In the past, bismuth has also been used to treat syphilis and malaria.

Commercially, bismuth is also used in the manufacture of permanent magnets, semiconductors, and thermoelectric materials; as a catalyst in making acrylonitrile; and as an additive to improve the machinability of steels and other metals.

Background Information

Like water, the solid form is less dense than the liquid.

Exposure Routes and Pathways

The primary exposure pathway for bismuth is from medicinal preparations that are administered orally or intramuscularly. For the general population the total daily intake via food is ~5–20 μg, with much smaller amounts contributed by air and water. The cosmetic use of bismuth compounds still continues to be fairly widespread.

Toxicokinetics

Bismuth compounds are considered to be poorly to moderately absorbed following inhalation, topical application or ingestion. Gastrointestinal absorption depends on the water solubility of bismuth salts. Citrate enhances intestinal absorption. Absorbed bismuth is distributed throughout the soft tissues and bone. The biological half-life for whole-body retention is ~ 5 days but intranuclear inclusions containing bismuth seem to remain for years in the kidney of patients treated with bismuth compounds. Peak plasma bismuth concentrations were noted within one hour of consuming colloidal bismuth subcitrate, with none being detected by 4 h. Bismuth can accumulate, however, with repeated colloidal bismuth subcitrate exposures. Bismuth binds to plasma proteins and concentrates in the kidneys, the liver (to a lesser extent), and the skin. Bismuth can displace bound lead, thus increasing the concentration of lead in the circulatory system. The urine is the major route of excretion. For some bismuth compounds, elimination may be equal between urine and feces.

Mechanism of Toxicity

The mechanism by which bismuth produces toxicity has not been identified. Interaction with thiol compounds has been proposed as a primary mechanism.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral rat LD_{50} for bismuth metal is $5\,g\,kg^{-1}$. Insoluble salts, for example, bismuth nitrate and bismuth trioxide, also have reported oral LD_{50} values in rats of $4–5\,g\,kg^{-1}$.

Human

Oral human LD_{Lo} is equal to $221\,mg\,kg^{-1}$. Adverse acute reactions to bismuth include acute renal failure following ingestion of excessive concentrations. Bismuth can cause nausea, vomiting, and abdominal pain within hours of exposure. Muscle cramps and weakness, blurred vision, and hyperreflexia may be exhibited. Liver transaminase activities may be elevated.

Chronic Toxicity (or Exposure)

Animal

In animals, bismuth interferes with the metabolism of copper and zinc, induces metallothionein, and can alter heme biosynthesis in the liver and kidney. Bismuth has not been found to be carcinogenic in animal models. Bismuth subnitrate can decrease Leydig cell density and plasma testosterone levels in rats.

Human

High-level exposure causes renal failure with degeneration and necrosis of the epithelium of the renal proximal tubules, fatty changes and necrosis of the liver, reversible dysfunction of the nervous system, skin eruptions, gingivitis and pigmentation of the gums and intestine. Effects in humans also include reversible neurotoxic and sometimes fatal encephalopathy and bone weakness. Symptoms of bismuth poisoning include fever, weakness, pain similar to rheumatism, and diarrhea. Certain people display a rash. Bismuth salts may cause contact sensitivity. The bone and brain may also be targets for toxicity.

In Vitro Toxicity Data

Bismuth subsalicylate (pepto-bismol) was negative in the Ames assay at concentrations up to 0.67 mg per plate.

Clinical Management

There does not appear to be an antidote of choice for bismuth toxicity in humans. Gastric lavage can be used within 1 h of exposure. Replace fluids and electrolytes. Monitor renal and liver function for several days and treat failure conventionally. The newer chelating agents, meso-2,3-dimercaptosuccinic acid and D,L-2,3-dimercapto-propane-l-sufonic acid, are being investigated experimentally as antidotes for bismuth toxicity, and the latter has been shown to be effective. In mice, D-penicillamine has proven effective.

Environmental Fate

Bismuth may potentially lead to contamination through leaching of shotgun pellets.

Ecotoxicology

Little information is available on the ecotoxicity of bismuth or bismuth compounds.

Exposure Standards and Guidelines

The permissible exposure limit (PEL), threshold limit value (TLV), and the recommended exposure limit for bismuth metal have not been established. The PEL for bismuth trioxide is $15\,mg\,m^{-3}$. The PEL for

bismuth subsalicylate is 15 mg mg^{-3}, and the TLV is 10 mg m^{-3}.

See also: Metallothionein; Metals.

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Relevant Websites

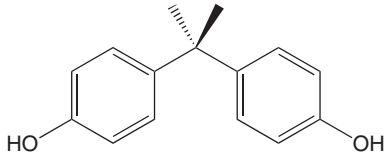
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Bismuth.

http://www.intox.org – International Programme on Chemical Safety.

Bisphenol A

Alan L Blankenship and Katie Coady

- Chemical Abstracts Service Registry Number: CAS 80-05-7
- Synonyms: 4,4′-Isopropylidenediphenol; 4,4′-Dihydroxy diphenyldimethylmethane; *p*,*p*′-Dihydroxydiphenyldimethylmethane; 4,4′-BPA; Bis (4-hydroxyphenyl)dimethylmethane; Bisphenol; DIAN; 2,2-Bis(4-hydroxyphenyl)propane; Bis(4-hydroxyphenylpropane; 4,4′-Bis-phenol a; *p*,*p*′-Dihydroxydiphenylpropane; 2,2-(4,4-dihydroxydiphenyl)propane; 4,4′-Dihydroxdiphenylpropane; 4,4′-Dihydroxydiphenyl-2,2-propane; 4,4′-Dihydroxy-2,2-diphenylpropane; Dimethylmethylene-*p*,*p*′-diphenol; *β*-di-*p*-Hydroxyphenylpropane; Dimethyl bis(*p*-hydroxyphenyl)methane; Diphenylolpropane; 2,2-di(4-Phenylol)propane; *p*,*p*′-Isopropylidenebisphenol; 4,4′-Dimethylmethylenediphenol; Phenol, 4,4′-(1-methylethylidene)bis-; 2,2-Bis(4,4′-hydroxyphenyl)propane
- Chemical/Pharmaceutical/Other Class: Phenolic
- Chemical Structure:



Uses

The estimated worldwide production of Bisphenol A (BPA) was 2.8 million tons in 2002. Approximately 90% of all BPA is used as an intermediate in the production of epoxy resins and polycarbonate plastics. Epoxy resins are used as food-contact surface coatings for cans, metal jar lids, coatings and finishes, automobile parts, adhesives, aerospace applications, and as a coating for polyvinyl chloride (PVC) water pipe walls. Polycarbonate plastics are hard plastics used to make numerous products, such as eyeglass lenses, water bottles, and consumer electronics. Some, but not all, dental sealants contain BPA. Additionally, BPA is a component of some specialty applications, such as flame-retardants, and as an antioxidant and stabilizer in the production of PVC and other plastics.

Background Information

In 1905, the synthesis of BPA, via the combination of acetone and phenol, was first reported by Thomas Zincke of the University of Marburg, Germany. One method of production is by the condensation of 2 mol of phenol with 1 mol of acetone while bubbling hydrogen chloride through the mixture. In 1953, the polycarbonate plastic manufacturing process was described using BPA as the starting material. Commercial production of polycarbonates began in 1957 in the United States and in 1958 in Europe. Because of the widespread use of BPA in polycarbonate plastics and epoxy resins, the manufacturing of BPA is expected to continue to increase in the future.

Exposure Routes and Pathways

The most probable routes of human exposure to BPA are inhalation and dermal contact of workers involved in the manufacture, use, transport or packaging of this compound. Potential exposure to BPA can also be expected through oral intake, since BPA is

largely used in resins and in food-can linings, from where it has the potential to leach into foods. Potential oral exposure to BPA is also possible through its use as a sealant in dentistry. However, studies have shown that the potential for exposure through such pathways is very low. The primary sources of environmental release of BPA are expected to be effluents and emissions from facilities which manufacture epoxy, polycarbonate, and polysulfone resins.

Toxicokinetics

Several studies have demonstrated the rapid clearance of BPA from blood following oral administration to adult rats. The principal metabolite of BPA in the rat is BPA-monoglucuronide (BPA-glucuronide). There appears to be route and dose-dependent differences in the pharmacokinetics of BPA. BPA administered by the oral route has reduced bioavailability and greater metabolism when compared with the subcutaneous route of exposure. This finding is consistent with the role of the liver in the first-pass metabolism of BPA through the oral exposure route. In order to evaluate the ontogeny of glucuronyl transferases (GT), the enzyme responsible for glucuronidation of BPA, a study was designed in which ^{14}C-BPA was administered via gavage at 1 or 10 mg kg^{-1} body weight (bw) to rats at postnatal day (PND) 4, PND 7, PND 21, or to 11-week-old adult rats (10 mg kg^{-1} dose only). Age dependency for the elimination of BPA-glucuronide was observed with more rapid elimination of BPA-glucuronide from the plasma of neonates ($t_{1/2}$: 4.4–9.8 h) when compared with adult animals ($t_{1/2}$: 10.8–22.5 h), likely due to reduced microflora β-glucuronidase activity in neonates and thus, an absence of enterohepatic recirculation. Nearly complete metabolism of BPA to BPA-glucuronide (94–100% of the plasma radioactivity) was observed at a dose of 1 mg kg^{-1}. Unlike the parent BPA, BPA-glucuronide is not a ligand for the estrogen receptor and it does not induce estrogenic activity in MCF-7 cells.

Studies indicate that BPA does not accumulate in body fat or sex organs of either male or female test animals, but is excreted in both rats and humans via the urine and feces. When administered as a single dose by gavage (800 mg kg^{-1} bw) to male rats, 28% of the ^{14}C-labeled BPA was excreted in the urine (primarily as glucosamide) and 56% in the feces (20% as free BPA, 20% as a hydroxylated BPA, and the rest as an unidentified conjugate). No ^{14}C-labeled residues were detected in animals killed after 8 days. In another rat study conducted with a lower dose (10 mg BPA kg^{-1} bw), 81.3% and 16.0% of the administered dose was excreted in feces and urine, respectively, in males, whereas 71.7% and 24.1% of the administered dose was excreted in feces and urine, respectively, in females. In BPA-dosed human volunteers, BPA was cleared from human blood and urine with a half-life of less than 6 h and the applied dose was completely recovered in the urine in the glucuronide form.

Mechanism of Toxicity

Investigations with the MCF-7 human breast cancer cell line showed that BPA binds to the estrogen receptor with a relative potency that is ~3–4 orders of magnitude less than that of 17-β-estradiol. BPA elicits estrogenic effects (e.g., increased cell proliferation) at concentrations at or above 2 μg l^{-1}. In addition to acting as a weak estrogen mimic, BPA also competitively inhibits estrogen from binding to the estrogen receptor. Studies have also indicated that BPA can act as an anti-androgen, blocking the androgen receptor-mediated effects of dihydrotestosterone in biological systems.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of BPA is relatively low. At 1–3 h after ingestion of high doses of BPA, animals exhibited atony and profuse diarrhea. Published LD_{50} values for laboratory mammals include 4150 mg kg^{-1} bw (male F344 rat, oral), 3300 mg kg^{-1} bw(female F344 rat, oral), 5280 mg kg^{-1} bw (male B6C3F1 mouse, oral), 4100 mg kg^{-1} bw (female B6C3F1 mouse, oral), 150 mg kg^{-1} bw (mouse, intraperitoneal), and 2230 mg kg^{-1} bw (rabbit, oral), and 4000 mg kg^{-1} (guinea pig, oral).

Human

One clinical report describes photoallergic contact dermatitis to BPA, with subsequent persistent light reactivity, in a group of eight outdoor workers.

Chronic Toxicity (or Exposure)

Animal

There are considerable data on the subchronic and chronic toxicity of BPA in laboratory animals. For decades, BPA has been shown to produce weak but consistent responses in uterotrophic assays. The focus of these investigations has typically been evaluation of the potential reproductive and developmental effects of BPA, due to its ability to modulate estrogen

receptor-mediated responses. Many end points are not consistently observed across studies. Some of this variability may result from differences in the conditions, design, and other test-specific variables of the toxicity tests. For example, since phytoestrogens are abundant in most laboratory animal feeds (such as found in soy and alfalfa) and are known to modulate estrogen receptor-mediated responses, phytoestrogens may be confounding factors as a result of the feed selection.

In a developmental toxicity test, CD rats were exposed to 0, 160, 320, or 640 mg BPA kg^{-1} day^{-1} and CD-1 mice were exposed to 0, 500, 750, 1000, or 1250 mg BPA kg^{-1} day^{-1} by daily dosing via gastric intubation on gestational days 6–15. Timed-pregnant dams were sacrificed 1 day prior to parturition. The uterine contents and fetuses were examined. In mice, some maternal mortality and an increase in relative maternal liver weight was observed at all BPA doses, reaching 18% at the high dose. At the highest dose (1250 mg BPA kg^{-1} day^{-1}), there was also a significant increase in the percentage of resorptions per litter, and reductions in gravid uterine weight and average fetal body weight. In rats, there were significant reductions in maternal weight gain during gestation, weight gain corrected for gravid uterine weight, and weight gain during treatment at all BPA doses. However, there were no observed effects of BPA on gravid uterine weight, average fetal body weight per litter, the percentage of resorptions per litter, or percentage fetuses malformed per litter. In summary, BPA treatment at maternally toxic dose levels during organogenesis produced fetal toxicity in mice but not in rats and did not alter fetal morphologic development in either species.

In a two-generation reproductive toxicity test conducted through the National Toxicology Program, CD-1 mice were exposed to 0%, 0.25%, 0.5%, and 1.0% BPA in feed to produce estimated daily intakes of 0, 437, 875, and 1750 mg BPA kg^{-1} day^{-1}. There was a 5–9% decrease in the number of litters/pair at the two highest doses and the number of live pups/litter was reduced by 20% at the 0.5% BPA dose and by 48% at the highest dose. The second generation did not appear more sensitive than the first to the reproductive toxicity of BPA.

There is some controversy over the possibility of low dose effects of BPA. Researchers who have published results suggesting that there is a low dose effect of BPA assert that the dose–response relationship is 'nonmonotonic', which means that health effects may only be observed at low doses while much higher doses result in no effects. However, these studies are weakened by lack of reproducibility and statistical robustness. Furthermore, there is considerable evidence to support a classical dose response for the effects of BPA. For example, based on pharmacokinetic studies, low doses of ^{14}C-BPA (via oral exposure) result in nearly complete metabolism of BPA to BPA-glucuronide (94–100% of the plasma radioactivity) at a dose of 1 mg kg^{-1}. In addition, there is a general lack of effects of low doses of BPA as noted above in chronic, multigenerational studies that focus on sensitive reproductive and developmental end points. For example, in a three-generation study, male and female Sprague–Dawley rats were fed a diet containing BPA at approximate dietary intakes of 0, 0.001, 0.02, 0.3, 5, 50, or 500 mg kg^{-1} bw day^{-1}. Exposures were continued until adulthood of the third-generation offspring. Analysis of the data for all of these end points for the parental and three offspring generations revealed no evidence of a low-dose effect of BPA for any of the reproductive and developmental end points including parental growth rate, food intake, reproductive performance, sperm production and motility, gross and histopathology, organ weights, litter size, pup survival and growth, and anogenital distance. This study clearly demonstrated the absence of low-dose effects of BPA.

In toxicity tests to evaluate the potential carcinogenicity of BPA, male and female F344 rats were exposed to 0, 1000, or 2000 mg BPA kg^{-1} (in feed) for 103 weeks and male and female B6C3F1 mice were exposed to 0, 5000, or 10 000 mg BPA kg^{-1} (in feed) for 103 weeks. Under the conditions of this bioassay, there was no evidence that BPA was carcinogenic for F344 rats or B6C3F1 mice of either sex.

Human

There are insufficient data to characterize chronic toxicity or exposure in humans.

In Vitro Toxicity Data

BPA, at levels greater than 2 $\mu g\,l^{-1}$, was found to be estrogenic in cultured human mammary cancer cells (MCF-7). Some of the reported effects of BPA in MCF-7 cells include induction of progesterone receptors in MCF-7 cells at a potency of 5000 times less than 17-β-estradiol, increased rate of cell proliferation, and competition with estradiol for estrogen receptor binding sites.

Environmental Fate

The primary sources of BPA to the environment are likely effluents and emissions from facilities that either manufacture or utilize BPA in large quantities.

If released to acclimated water, biodegradation would be the dominant fate process (half-life 2.5–4 days). BPA may adsorb extensively to suspended solids and sediments (K_{oc} values range from 314 to 1524), and it may photolyze in the presence of sunlight. BPA is not expected to bioaccumulate significantly in aquatic organisms (BCF 5–68), volatilize, or undergo chemical hydrolysis.

Ecotoxicology

Due to the potential for release to aquatic environments, considerable work has been done on evaluating the aquatic toxicity of BPA. Acute toxicity (96 h LC_{50} values) for freshwater organisms ranged from 4.6 mg l^{-1} for the fathead minnow (*Pimephales promelas*), to 9.4 mg l^{-1} for the Atlantic silverside (*Menidia menidia*).

BPA is considered an endocrine disruptor chemical and, in chronic studies, induces production of vitellogenin in male fathead minnows (*P. promelas*) at concentrations of 640 and 1280 µg l^{-1} after 43 days and 160 µg l^{-1} after 71 days. Induction of vitellogenin is a process normally occurring only in female fish in response to estrogenic hormones during the reproductive cycle.

Overall, chronic toxicity values of BPA for freshwater organisms ranged from 160 µg l^{-1} for the fathead minnow (*P. promelas*; based on egg hatchability) to 11 000 µg l^{-1} for rainbow trout (*Oncorhynchus mykiss*; based on growth), Typically, chronic effects on survival, growth, and reproductive end points only occur at concentrations of BPA greater than 160 µg l^{-1}. Published no-observed-effect concentrations in aquatic organisms range from 16 to 3640 µg l^{-1}. Taken together with concentrations of BPA in typical surface water samples that are less than 1 µg l^{-1}, potential risks to aquatic organisms are very low.

Exposure Standards and Guidelines

The Draft EPA water quality criterion for BPA is 5.9 µg l^{-1}. The State of Florida's drinking water guideline is 350 µg l^{-1}.

See also: Acetone; Phenol; Vinyl Chloride.

Further Reading

Tyl RW, Myers CB, Marr MC, *et al.* (2002) Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague–Dawley rats. *Toxicological Sciences* 68: 121–146.

Black Widow Spider *See* Spider, Black Widow.

Bleach

Julie Weber

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- Representative Chemical: Sodium hypochlorite
- Chemical Abstracts Service Registry Number: CAS 7681-52-9 (sodium hypochlorite)
- Synonyms: Household laundry bleach (Purex®, Clorox®, and Dazzle®); Commercial laundry bleach; Caustic soda bleach; Dakin's solution; Modified Dakin's solution; Sodium hypochlorite pentahydrate; Surgical chlorinated soda solution
- Chemical/Pharmaceutical/Other Class: Hypochlorites and related agents
- Chemical Formula: NaHClO

Uses

Sodium hypochlorite is used in household laundry bleach, disinfectant and cleaning products, toilet sanitizers, deodorizers, for water purification, and as antiseptics. Regular household laundry bleaches are ~5.25% sodium hypochlorite in water with an adjusted pH of 10.8–11.4. 'Ultra' formulations are slightly more concentrated and contain 6–8% sodium hypochlorite. Commercial laundry bleaches contain 15% sodium hypochlorite at a pH slightly over 11.

Exposure Routes and Pathways

Ingestion is the most common route of exposure to sodium hypochlorite. Other modes of exposure are inhalation, dermal, ocular, and inadvertent injection.

Mechanism of Toxicity

The toxicity of hypochlorite arises from its corrosive activity on skin and mucous membranes. Corrosive burns may occur immediately upon exposure to concentrated bleach products. Most of this corrosiveness stems from the oxidizing potency of the hypochlorite itself, a capacity that is measured in terms of 'available chlorine'. The alkalinity of some preparations may contribute substantially to the tissue injury and mucosal erosion. Sodium hypochlorite when combined with an acid or ammonia may produce chlorine or chloramine gas, respectively. An inhalation exposure to these gases may result in irritation to mucous membranes and the respiratory tract, which may manifest itself as a chemically induced pneumonitis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Emesis is likely to be spontaneous. Clinical signs may include salivation, emesis, abdominal pain and tenderness, hematemesis, and bleached hair. Rats given 5–15 ml kg^{-1} of an alkaline (pH 12.0) solution containing 4.5% sodium hypochlorite died within 1–3 h from severe local damage to the esophagus and stomach.

Human

The resulting symptoms from an exposure to sodium hypochlorite and related compounds may range from mildly irritating to corrosive depending on the volume/amount of the exposure, duration of contact, and pH and viscosity of the product. Small accidental ingestion of household bleach, containing 4–6% sodium hypochlorite, usually causes nothing more serious than orogastric irritation characterized by nausea, spontaneous emesis, sore throat, and abdominal pain. Very large ingestions, usually intentional, have caused fatal hypernatremia and hyperchloremic acidosis, and significant gastric injury. Sodium hypochlorite solutions stronger than 10% or powders may result in corrosive burns of the mouth, hypopharynx, and stomach. Prolonged dermal contact can result in irritation or burn. Inadvertent injection into the surrounding tissue during dentistry use of Dakin's solution (0.5–5% sodium hypochlorite) has resulted in severe acute pain and burning sensation accompanied by immediate edema of the surrounding area. Delayed effects can include tissue necrosis, paresthesia, and secondary infection. Household bleach has been advocated as a disinfectant for syringes and needles of IV drug users. There are few reports on the effects of inadvertent or intentional IV injection. Case reports of symptoms have included erythema at the injection site, vomiting, chest pain, bradycardia, and hypotension. Ocular exposure may result in irritation, lacrimation with a burning discomfort. Superficial disturbance of the corneal epithelium may occur, which recovers completely within 2 days. Eyelid edema has been reported, but is more common after exposure to chloramine gas. Inhalation of liberated chlorine or chloramine gas may cause respiratory tract irritation, cough, substernal chest discomfort and tightness, hoarseness, dyspnea, and wheezing. Chemical pneumonitis, acute respiratory distress syndrome, and hypoxia have developed in severe prolonged exposures.

Chronic Toxicity (or Exposure)

Animal

Bleach should not be considered a carcinogen in experimental animals. Exposure of the esophagus of rabbits and dogs to typical household bleach resulted only in minor lesions. Rats fed water with high bleach concentrations demonstrated decreased weight gain, but no other untoward signs or symptoms.

Human

Most data indicate that low-dose hypochlorite solutions (e.g., those seen in typical municipal drinking water) do not directly contribute to the development of cancer.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Treatment is generally symptomatic and supportive. Gastrointestinal evacuation procedures are generally unnecessary. If the patient is alert and able to swallow, milk or water should be immediately offered, stopping if vomiting occurs during administration. Administration of an acidic substance to neutralize sodium hypochlorite is contraindicated.

For inhalation exposure, the patient should be removed from fumes into fresh air. Respirations should be established along with the creation of an artificial airway if necessary. If cough or difficulty in breathing develops and is not relieved by the fresh air, the patient should be evaluated for respiratory irritation, bronchitis, or pneumonitis in a health care facility.

For ocular exposures to sodium hypochlorite and related agents, contact lenses should be removed if present. The eye(s) should be immediately irrigated with tap water or normal saline for at least 15 min. If ocular irrigation is delayed, the potential for injury is

greater and the patient may have to be evaluated in a health care facility.

For dermal exposures, contaminated clothing should be immediately removed and the exposed skin should be flooded with water. The skin should be gently washed with soap and water.

See also: Chlorine; Coniine.

Further Reading

Ehrich DG, Brian JD Jr, and Walker WA (1993) Sodium hypochlorite accident: Inadvertent injection into the maxillary sinus. *Journal of Endodontics* 19(4): 180–182.

Jakobsson SW, Rajs J, Jonsson JA, *et al.* (1991) Poisoning with sodium hypochlorite solution: Report of a fatal case, supplemented with an experimental and clinico-epidemiological study. *American Journal of Forensic Medicine and Pathology* 12: 320–327.

Blister Agents/Vesicants

Harry Salem and Frederick R Sidell*

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Background

Blister agents, also known as vesicants, are cytotoxic alkylating compounds. They are exemplified by chemicals collectively known as 'mustard' or 'mustard gas' (military designator: H). Other blister agents are sulfur mustard (HD), nitrogen mustard (HN), lewisite (L; an arsenic-containing vesicant), and phosgene oxime (CX; a halogenated oxime that is very different in properties and toxicity from the other agents). Mustard vapor injury is a particular threat in hot climates. In addition, humidity or moisture in a hot environment enhances damage to the skin.

Examples of vesicant or blister agents with military designators in parentheses:

1. mustard or mustard gas (H);
2. sulfur mustard (HD), characterized by delayed action;
3. sulfur mustard with Agent T (HT) – the latter is bis-2-(2)chloroethylthioethyl ether, similar to HD in structure;
4. nitrogen mustard (HN);
5. lewisite (L), similar to sulfur mustard in action, except that immediate effects occur within minutes;
6. mixture of mustard and lewisite (HL) – the combination of sulfur mustard (37%) with lewisite (63%) gives it a garlic odor;
7. phenyldichloroarsine (PD) – like lewisite, it is an organic dichloroarsine; and
8. phosgene oxime (CX), a pulmonary toxin with vesicant effects.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Mechanism of Toxicity

The action on cell components results in inhibition of cellular division (mitosis) with decreased tissue respiration that leads to cell death. It produces eye, airway, and skin and mucous membrane injury that can be fatal. Systemic effects with extensive exposures include bone marrow inhibition with a drop in the white blood cell count and gastrointestinal tract damage.

Mustard Gas (H)

Mustard gas was first used in chemical warfare during World War I in 1917 and more recently during the Iran–Iraq War (1984–88). The term mustard gas refers to several chemicals. Most commonly, it means sulfur mustard (HD), which is reviewed below. The word gas used in the context of mustard gas is not accurate since mustard gas is not a true gas, but rather a liquid. Mustard gas is stored as a liquid, and is not likely to change into a gas immediately if released at ordinary temperatures. As a liquid it is colorless and odorless when pure, but brown with a slight garlic smell when mixed with other chemicals. It dissolves easily in fats and petroleum products. It dissolves slowly in water, where it turns rapidly into less toxic chemicals. Therefore, drinking, cooking, bathing, and swimming in mustard gas-contaminated water are activities unlikely to lead to significant exposure. However, the chemicals produced in water may cause skin or eye irritation, rather than the characteristic blisters. Should mustard gas be released, it will stay in the air or on the ground for ~30–50 h with the full potential for toxic effects.

Mustard gas is a greater threat in hot and humid climates as a blister agent. Should unprotected exposure occur, mustard gas enters the body quickly either through the breathing of vapors or through skin contact with liquid or vapors. It can pass through clothing to get onto and through the skin. Subsequently, it enters the circulation and causes systemic effects at higher doses. Mustard gas and the

other chemicals it can change into in the body, leave the body through the urine within a few weeks. Initially, there is no pain, and exposure from the development of symptoms may not be apparent until the next day. At the point of contact, mustard gas can cause skin blisters to occur within several days. The extent of the blisters is determined by the amount and area of exposure, and this will determine the course of the immediate illness from mustard gas. Those areas which are exposed, and which are sweaty, are the most affected. Exposure to the eyes and the natural tears will increase susceptibility to tearing and blinking. Cough and bronchitis can result if vapors are inhaled. Large exposures can cause death in the near term.

The long-term consequences from mustard gas exposure, particularly at low doses, are unknown. However, a one-time high-dose exposure can result in chronic and recurrent lung and eye problems. Mustard gas is also a known carcinogen, and can cause lung cancer later in life. The ability to cause birth defects in the children of exposed adults is not presently known; however, it has the potential to be teratogenic.

Sulfur Mustard (HD)

Sulfur mustard is the distilled or purified form of mustard gas. Its properties and toxicities are similar to those of mustard gas. If spilled, sulfur mustard evaporates into air where it decomposes. It can persist within soil with its blister-forming activity intact for many years, particularly in colder climates. Because of its relative insolubility, it generally does not contaminate groundwater.

Unprotected exposure leads to symptoms that are delayed and occur within hours. Erythema and blistering occur 2–24 h after exposure to skin. With exposure to eyes, tearing, itching, burning, accompanied by a gritty feeling in the eyes, can occur within 4–24 h. With more significant exposure to the eyes, there may be conjunctivitis with eyelid swelling in 3–6 h. Severe eye exposure leads to marked eyelid swelling, corneal opacity, and eye pain, all occurring in 1–2 h. Airway toxicity can occur in 12–24 h, and this is marked by rhinorrhea, sneezing, coughing, nosebleeds, and hoarseness. Severe inhalation injury is marked by productive cough and shortness of breath. There can be variable gastrointestinal symptoms with ingestion.

Sulfur Mustard with Agent T (HT)

The toxicities and properties of HT are similar to those of mustard gas or sulfur mustard.

Nitrogen Mustard (HN)

Nitrogen mustard is composed of three similar compounds (HN-1, HN-2, and HN-3). The color of the liquid can range from pale yellow to dark, and the smell can be either odorless or like that of herring fish. Nitrogen mustard can persist in soil. HN-1 is less persistent, with one-fifth the blistering potency of sulfur mustard. HN-3 is more persistent and is equal to sulfur mustard in blistering potential. When nitrogen mustard is vaporized in air, it degrades in a matter of hours. Its properties and toxicity are similar to those of the agents mentioned above, and it is a strong blister agent. Eye irritation and skin erythema occur sooner upon exposure to nitrogen mustard than with sulfur mustard. Eye lesions also appear to be more severe with nitrogen mustard. It is known to cause cancer years later.

Lewisite (L)

Lewisite (L) contains arsenic and is a potent blister agent. Chlorovinyldichloroarsine is another name for lewisite, and like phenyldichloroarsine (PO), ethyldichloroarsine, and methyldichloroarsine, it is an organic dichloroarsine. When purified, lewisite and the organic dichloroarsines are colorless, odorless oily liquids, but when produced with impurities, they have a fruity or geranium-like odor. As liquids, they can penetrate rubber and most fabrics, and are more dangerous as liquids than as vapor.

Little is known about lewisite's stability in the environment, but it can react with water in a manner whereby its volatility and most of its blistering potency are lost. As a potent blister agent, it has irritant effects on the eyes and respiratory system, and has similar toxicities to the other blister agents mentioned above (except that it exhibits less bone marrow suppression). Similar to its dichloroarsine cousins and phosgene oxime, but unlike the mustard vesicants, it can cause pain at the time of initial contact. There is often no erythema around the vesicles as with other mustard agents.

Mustard and Lewisite Mixture (HL)

Mixing lewisite (L) with sulfur mustard (HO) at the concentrations of 63% L and 37% HL produces a liquid with a low freezing point that provides a more effective weapon in colder climates at higher altitudes. It has a garlic odor and is effectively insoluble in water. It persists on the ground from 1 to 2 days under average weather conditions when splashed, and can remain 1 week or more under very cold conditions before dissipating. Along with its blistering properties, it is also cytotoxic to the hematopoietic or blood-forming cells in the bone marrow. Other

mixtures like mustard and phenyldichloroarsine act in a similar manner.

Phenyldichloroarsine (PO) and Other Dichloroarsines

Phenyldichlorarsine, ethyldichloroarsine, and methyldichloroarsine have similar properties and toxicities as lewisite. They may be mixed with sulfur mustard similarly as can be done with lewisite and mustard mixtures, and this can confuse the diagnosis between either an arsenical or a mustard injury.

Phosgene Oxime (CX)

Phosgene oxime or dichlorformoxime is a nettle gas or urticant. These act as irritants to the skin and mucous membrane and, like arsenicals but unlike mustards, cause pain on immediate skin contact. Severe pain is noted. Very low doses cause lacrimation. It is a liquid or colorless solid and has a disagreeable odor. Unlike mustards and arsenicals, it is readily soluble in water.

Diagnosis

Diagnosis of a blister agent injury, without obvious overt contamination, requires a high level of suspicion when eye, skin, and respiratory signs and symptoms become evident. The first effects of blister agent exposure are eye and airway irritation. Conjunctivitis can occur after 1 h at a concentration of a blister agent that is barely perceptible by odor. Mild exposure results in tearing and the sensation of eye grit in 4–12 h. Severe eye lesions may occur within 2 h on heavy exposure. Lewisite and the dichloroarsines can cause gray scarring of the cornea at the point of contact. Severe lacrimation can occur with low doses of phosgene oxime.

Skin damage may not be immediately evident because the first effects may be painless until deeper skin layers are involved and blisters appear. However, the diagnosis of a chemical skin injury is readily made when the fluid-filled skin blisters appear and are recognized. There is a 1–12 h (or more) latent period, during which skin burning and itching may occur. Erythema or skin redness appears on exposed skin after 2–48 h. In darker-skinned individuals, sulfur mustard lesions may turn coal black in the face, neck, axilla, groin, and genitalia areas. Most American survivors from World War I had scrotal and perianal burns, because of increased moisture and ambient temperature in these areas. This redness (or darkness) is followed by coalescing blisters on a red base. At this stage, any vesicant on contaminated patients may still pose a hazard to other individuals coming in contact with them, so care needs to be taken in decontamination. Lewisite (L) and phosgene oxime (CX) differ in that pain may be immediately noted on contact, but areas of erythema may recede without blister formation. Lewisite and the dichloroarsines cause a more opaque blister fluid than the mustards. They also lead to deeper injury to the connective tissue, which can include muscle and vasculature, with more inflammation. Phosgene oxime causes immediate pain and skin necrosis at the site of contact. In 30 s, the contact area becomes blanched and is surrounded by a ring of erythema. A wheal then occurs in 30 min, and this area turns brown within 1 day. An eschar forms and sloughs off within 1–2 weeks.

Healing and resorption of uninfected blisters occur in 1–3 weeks for all vesicants. Broken blisters must be protected to minimize chances of infection and subsequent scarring of denuded skin. There is no useful medical test to determine if there has been mustard gas exposure.

Respiratory symptoms can include fever, dyspnea, ronchi with moist rales. Chest X-rays can reveal pulmonary edema. Changes consistent with chemical pneumonitis may appear after the first 24 h. Lewisite and the other organic dichloroarsines do not cause a significant respiratory injury from vapor concentrations found in the field. Skin pain usually occurs on immediate cutaneous contact, and that is a signal to wear a mask to prevent further respiratory and eye injury.

Treatment

Treatment follows decontamination of the patient, after donning protective gear. The various agents may vary in their ability to generate local and systemic pathology; however, the general treatment principles remain the same for all vesicants except for the availability of British Antilewisite (BAL) for dichloroarsine exposure.

Mild eye lesions require little treatment other than flushing with water immediately. Slow running water is applied as one tilts the head from side to side, pulling the eyelids apart. Steroid and antibiotic ointment can be applied to the eye.

Sterile petroleum jelly between the eyelids can provide lubrication, as would boric acid 5%. With eyelid edema, which occurs with more severe injuries, the eyelids may be gently opened to provide reassurance to the patient that one is not blind. Pain can be controlled by oral or parenteral narcotics. Photophobia can be eased by placing the patient in a darkened room, and by providing sunglasses or eyeshades. Do not cover the eyes with bandages. Atropine sulfate ointment should be instilled in each eye

to obtain good mydriasis in all cases where there are corneal erosions, iritis, cyclitis, or marked photophobia or miosis. Blepharospasm, or eyelid spasm, is treated with atropine sulfate solution 1% applied 3 times a day. To prevent infection, a few drops of sodium sulfacetamide 15% should be instilled every 4 h. Another antibacterial ophthalmic preparation may be substituted. The eye must not be bandaged and the lids must be kept separated. The patient should be seen by an ophthalmologist as soon as possible.

Treatment of skin lesions also follows decontamination and removal of clothes. Decontamination should be completed within 15 min after exposure to minimize any systemic effects. Contaminated hair should be shaved off. The decontaminating solutions should be washed off within 3–4 min to prevent additional skin injury. Sodium hypochlorite (5%) or liquid household bleach can be used. If erythema is already present, soap and water are preferred. Blisters should be left intact, but if broken, should be debrided to prevent secondary infection. Cleansing with tap water or saline and the application of dressings is done when needed. Silver sulfadiazine or mafenide acetate can be applied and the wounds treated as burn wounds. Infected skin wounds require antibiotics as appropriate.

In cases of lewisite skin injury, dimercaprol (BAL) ointment should be used on contaminated skin where blisters have not yet formed. Sometimes BAL itself causes irritation with stinging and itching with wheal formation, but this should resolve 1 h after application. Frequent BAL ointment application does cause a mild dermatitis, so it cannot be used as a protective barrier on skin not contaminated by dichloroarsines. Because of the deeper injury with dichloroarsines as with lewisite, wounds may heal more slowly and skin grafting may be required in the future.

Systemic treatment with parenteral antilewisite is considered when there is (1) greater than 5% area of skin contamination (1 ft^2) which results in immediate skin blanching or erythema within 30 min after exposure or (2) a burn the size of the palm (1% of skin area) which was not decontaminated within the first 15 min after exposure. There are two types of parenteral BAL therapies which can be used. One involves applying BAL ointment liberally onto the skin (after removing any other protective ointment first), and allowing that area to remain covered. The other parenteral method is to give an intramuscular injection of 10% BAL in oil into the buttocks (without injecting into a blood vessel). The dose given of 10% BAL in oil is 0.5 ml per 25 lb body weight, up to a maximum of 4.0 ml for those individuals who weigh 200 lb or over. Intramuscular injection of BAL in oil (10%) should be repeated every 4 h for a total of four doses at alternate sites on the buttocks. In severe cases, the frequency can be shortened between the first and second doses by 2 h. For severe cases, one injection can be given per day for 3–4 days. One should be aware of symptoms which occur with BAL injections, which may last 30 min but do not indicate that therapy should be stopped. These symptoms include tightening of the throat, chest pressure, lip burning, lacrimation, eye redness, mouth dryness, aching muscles, abdominal pain, tenderness and increased muscle tone at the injection site, anxiety, nausea, vomiting, and transient increased blood pressure.

Inhalation of vapors from mustard or arsenical vesicants can result in laryngeal and tracheobronchial mucosal injury. Mild injury with hoarseness and sore throat requires either no treatment or mist inhalation. Moderate exposures result in hyperemia and necrosis of the bronchial epithelium, and require hospitalization to prevent secondary infection. Antibiotics are used in an appropriate manner. Pneumonia was the usual cause of death from mustard agents during the preantibiotic World War I era. Severe injuries cause tracheobronchial tree casts from pseudomembrane formation. Hypoxia can occur, but subsequent bronchitis and pneumonia from infection were the chief causes of pulmonary-related deaths in World War I (2% mortality). Pulmonary resuscitation is required if breathing stops. Mustard–lewisite (HL) mixtures can cause pleural effusion in severe cases. Blister agents at extensive exposure can have systemic toxicity that affects not only the lungs, but also the bone marrow, lymph nodes, spleen, and endocrine systems. In these cases, complete blood counts with monitoring of granulocytes, red cells, and platelets should be performed routinely. If granulocyte depletion occurs, isolation and antibiotic prophylaxis may be necessary. Many past fatalities were due to the combination of pneumonia and bone marrow failure. Anemia and thrombocytopenia should be treated as the situation dictates. If local effects remain mild, systemic effects are not likely to be significant. In severe cases of lewisite or dichloroarsenine respiratory injury, dyspnea with frothy sputum indicating pulmonary edema indicated that intramuscular BAL is necessary.

If ingestion of blistering agent occurs, do not induce vomiting. Milk can be given to drink to mitigate damage. Giving 0.4–0.8 mg atropine subcutaneously can help in reducing systemic or local gastrointestinal activity. Morphine can be given intravenously for intestinal pain with close monitoring for shock. Fluid resuscitation for vomiting and diarrhea will require intravenous saline. Sedatives may be necessary.

Prognosis

With eye injury, temporary blindness occurs, but permanent blindness is rare with vapor exposure. The patient should receive this reassurance except for the severest eye injury. Blindness is more likely to occur when liquid mustard is directly splashed into the eye. With mild eye injury, recovery occurs in 1–2 weeks. More severe involvement with corneal erosions as detected by fluorescein staining can take 2–3 months of hospital care before recovery occurs. Corneal involvement beyond erosions with opacification and ulceration (less than 0.1% of mustard casualties in World War I) takes several months for recovery, and then late relapses can still occur. In these cases blindness may ensue. Eye injuries are more severe with nitrogen mustard than with sulfur mustard. The iris is frequently discolored and atrophied with nitrogen mustard exposure.

With mild blister formation, healing occurs with little scarring, but it may take months to heal while remaining painful during this time. When secondary infection occurs or in more extensive blistering, scarring can be more severe. Itching may persist after healing. Hypopigmentation or hyperpigmentation can occur as with any healing process. Deeper burns with lewisite and the dichlorarsines have similar outcomes as second- or third-degree thermal burns. Repeated exposures over time to mustards or arsenicals such as dichloroarsines can cause sensitization. Delayed healing beyond 2 months occurs with skin lesions caused by phosgene oxime.

A single low-dose exposure to mustard vapor with laryngeal and tracheobronchial mucosal effects may not lead to significant injury once healed. A cough may persist 1 month or longer. Hoarseness usually lasts only 1–2 weeks. However, repeated or chronic low-dose exposure can lead to progressive pulmonary fibrosis, chronic bronchitis, and bronchiectasis.

See also: Lewisite; Mustard Gas; Nitrogen Mustard; Phosgene Oxime.

Relevant Websites

http://www.bt.cdc.gov – (US) Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – (US) National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Blood

Gary R Krieger and Scott D Philips

Introduction

Hematology is the study of pathophysiology of the cellular elements and coagulation proteins in the blood. Physicians who specialize in this field are referred to as hematologists. Hematology has several new tools available to detect abnormalities in numbers or dysfunctions. Hematologists diagnose and treat both benign and malignant blood disorders. Primary hematologic diseases are uncommon, while secondary hematologic conditions occur frequently. Thus the inquiring physician must extensively question the patients for all forms of medications, herbals, folk-remedies, occupations and family history to name a few areas that require an in-depth history.

The formed elements of the blood – red blood cells (RBCs or erythrocytes), white blood cells (myeloids), immunocytes (T and B cells), platelets (thrombocytes), and their diseases – have traditionally been the main focus of hematology. However, with the advent of increasingly sophisticated molecular investigatory tools, such as recombinant DNA technology, the appreciation of the scope and inherent complexity of the blood-forming organ has dramatically increased. The bone marrow and its formed elements can be considered as a complex organ with a total mass that is over twice as large as the liver. The cells produced by this organ provide several critical functions such as the transport of oxygen (RBCs), hemostasis (platelets), and host resistance (immunocytes and white blood cells). Generally, each step in the intricate sequence required to produce the formed elements is vulnerable to adverse effects from a wide variety of chemicals and drugs. This entry will present a basic overview of normal bone marrow function, followed by a discussion of some of the abnormal physiologic effects that can be produced by exposure to various common drugs and chemicals.

Bone Marrow Structure and Function

In the normal adult, the marrow is found in the central hollow segment of bones. Hematopoiesis, or the production of the formed blood elements, occurs in the bone marrow. However, in the adult, it is largely restricted to scattered clusters of hemopoietic cells in the proximal epiphyses of the long bones, skull, vertebrae, pelvis, ribs, and sternum. The hematopoietic

picture in adults is quite different from that seen in either prenatal or childhood time periods. Within the first 1–5 prenatal months, the liver and spleen act as the hematopoietic organs. By the fifth prenatal month, the marrow achieves sufficient maturity to assume the dominant role in hematopoiesis. During childhood, there are high demands on the bone marrow system to produce large quantities of the formed elements; however, with increasing chronological maturity, there is less demand on the bone marrow system and the total output of the bone marrow significantly declines.

In addition to hematopoietic cells, there are other separate and distinct cells that support and augment marrow activities. Among these cells are fibroblasts, fat cells, and reticuloendothelial and endosteal cells. In aggregate, these cells are known as the bone marrow stroma. Occasionally, the term hematopoietic microenvironment is also employed to differentiate these cells and supporting structure from the stem and progenitor cells. These cells are the focus of the next section, which presents an overview of the basic physiology of the blood-forming elements.

Hematopoiesis

In the average adult, between 200 and 400 billion blood cells are destroyed and replaced each day. This enormous turnover implies that new cells are constantly formed rather than simply released from a central storage area that contains all the cells necessary for an individual's lifetime. Hemopoiesis is the key concept that has been used to explain how the body can provide a lifetime worth of formed blood elements. Hemopoiesis is a process of cell amplification and differentiation in which a few stem cells give rise to increasingly more developed or differentiated progenitor cells, which in turn give rise to the formed blood elements. The earliest cell is known as the pluripotent stem cell or PSC. PSCs are uniquely responsible for the production of the formed elements throughout the lifetime of a human. Relatively few PSCs are required since, as these cells undergo mitosis or cell division, one replacement stem cell and one committed or daughter cell are produced. This daughter cell subsequently develops and proliferates into the various formed elements. Hence, the PSCs are considered to be self-renewing because of their ability to reproduce themselves. **Figure 1** presents the overall organization and development of the bone marrow cells. This structure is quite hierarchical and resembles a company organization chart with a single chief executive officer presiding over separate divisions, which in turn develop other specialized departments or functions. Not surprisingly, each step in the organization requires both a series of growth factors and interactions with the hematopoietic microenvironment to promote and control the development of each cell type. The stimulatory or growth factors are known as poietins or colony stimulating factors (CSFs). CSFs can either be lineage-specific, i.e., they act on specific cell lines, or direct acting on multipotential progenitors and stem cells. Examples of lineage-specific CSFs include (1) erythropoietin, which stimulates production of erythrocytes or RBCs; and (2) interleukin-7, which induces the growth of B- and T-lymphocyte progenitors. Direct-acting CSFs include interleukins 2–6, which act on a variety of cell lines.

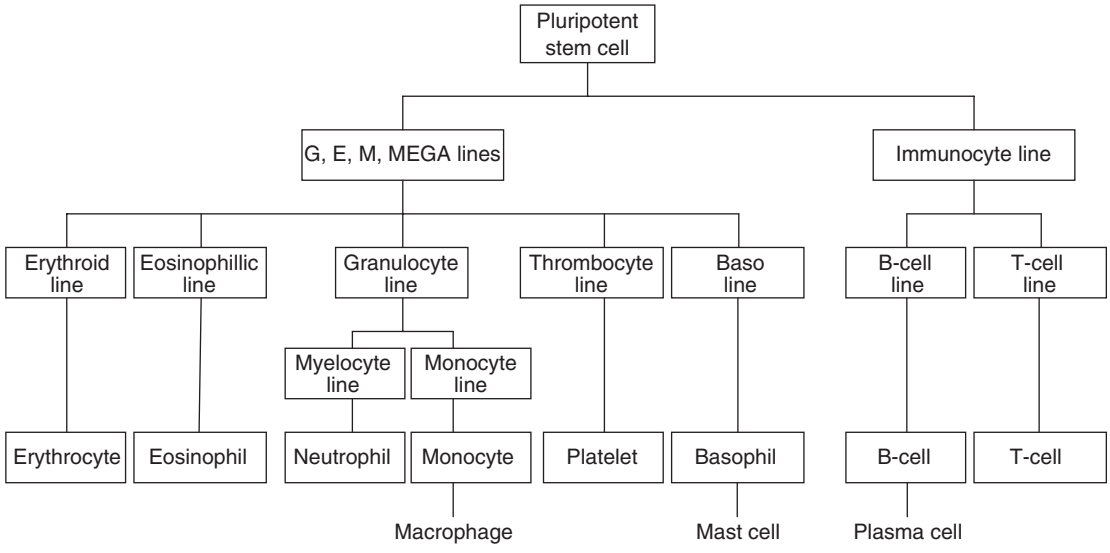

Figure 1 Bone marrow cell organization. G, Granulocyte; E, Erythrocyte; M, Monocyte; and MEGA, Megakaryocyte.

Formed Elements – Erythrocytes, Myeloids, and Thrombocytes

Erythrocytes

The RBC is a biconcave disk with a diameter of ~8 μm and a lifespan in the circulation of ~120 days. Due to its unique shape, the RBC is twice as thick at the edges (2.4 μm) as at its center. The explanation for this specialized geometry is not fully known; however, this shape tends to minimize intracellular diffusional distance and allows for easier passage through small blood vessels. The critical function of the RBC is transportation and delivery of oxygen to peripheral tissues. Approximately 30% of the wet weight of the RBC is composed of hemoglobin, the essential protein which is integral to the oxygen/carbon dioxide transport and delivery system. Hemoglobin is also capable of transporting nitric oxide (NO). NO is a unique gas that can affect the ability of blood vessels to expand or contract in addition to having a role in learning and memory.

The mature RBC is formed through a series of cell divisions that progressively increase the amount of hemoglobin in the cytoplasm. Following the last division, a special cell known as a reticulocyte is formed. The reticulocyte stays in the bone marrow for 2 or 3 days before being released into the general circulation, where over a period of 24 h it undergoes a series of transformations that results in the appearance of a mature RBC. The reticulocyte is easy to identify in laboratory tests and the reticulocyte count or index is an important parameter that can provide information about marrow function. The reticulocyte index is equal to the reticulocyte percentage multiplied by the ratio of the patient's hematocrit (packed cell volume) to a normal hematocrit.

Hemoglobin

Hemoglobin, in the normal adult, is a protein whose main function is to transport oxygen from the lungs to tissues and to transport carbon dioxide from tissues to the lung. The hemoglobin molecule contains four separate folded peptide chains, which form a hydrophobic or water 'repelling' pocket around a heme group. The heme group is composed of a central iron atom complexed to four nitrogen atoms. Oxygen is capable of reversibly binding to the heme unit in a process known as oxygenation. The interactions among the subunits in a hemoglobin molecule are known as cooperativity. There are well-described regulators of the affinity of hemoglobin for oxygen that provide a control mechanism. The S-shaped graph of this oxyhemoglobin relationship is known as the oxyhemoglobin dissociation curve and represents the relationship between the partial pressure of oxygen (Po_2) in mm of mercury (Hg) and the oxygen content per 100 ml of blood (**Figure 2**).

The shape of this relationship is very important since it can be moved to the right, i.e., decreased affinity of hemoglobin for oxygen producing oxygen unloading, or to the left, i.e., increased affinity. These changes are produced by a variety of intracellular cofactors: hydrogen ion (pH), carbon dioxide, and the RBC enzyme 2,3-biphosphoglycerate (BPG). Molecules of 2,3-BPG bind to hemoglobin and decrease the affinity of the molecule for oxygen. This causes enhanced oxygen release, or unloading, and is frequently seen in situations in which the body responds to conditions of low oxygen supply. There are a wide variety of potential diseases and toxic exposures that can impact oxygenation and cooperativity and these will be discussed in subsequent sections.

Anemia

There are many other events that can produce a significant reduction in the RBC mass and a subsequent decrease in the oxygen-carrying capacity of the blood. Normally, the blood volume is maintained at a relatively constant level; hence, any process or event that causes a reduction in either RBCs or hemoglobin produces a condition known as an anemia. Anemias can also shift the oxyhemoglobin dissociation curve

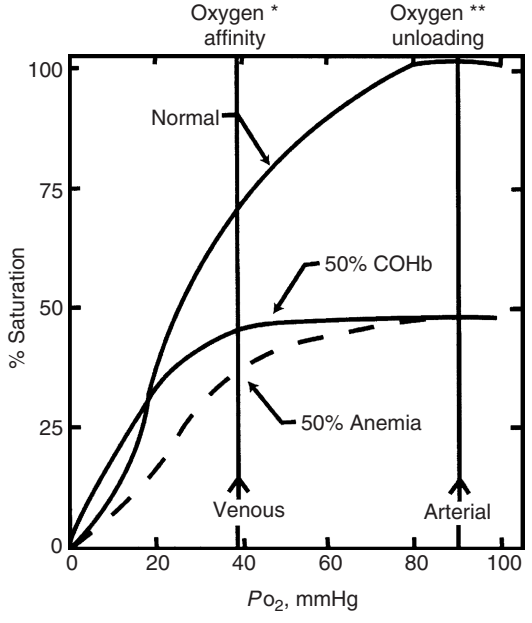

Figure 2 Oxyhemoglobin dissociation curve. *Modifiers of oxygen affinity – increase in plasma pH, decrease in temperature, decrease in 2,3-BPG. **Modifiers of oxygen unloading – decrease in plasma pH, increase in temperature, increase in 2,3-BPG.

as the body attempts to compensate for reduced oxygen-carrying capacity. In general, the etiology of anemias falls into three general categories: (1) acute or chronic blood loss from any source, (2) underproduction associated with a decreased reticulocyte count, and (3) hemolysis or destruction of RBCs associated with an increased reticulocyte count. There are a variety of laboratory tests that are useful for the evaluation of anemia; however, three of the most critical are the measurement of RBC size and shape (known as RBC indices), examination of the peripheral blood smear, and bone marrow examination. Each of these tests reveals information that can provide clues which lead to the etiology of the anemia. In later sections, some examples of toxins (e.g., carbon monoxide, hydrogen sulfide, and hydrogen cyanide) which cause anemias by altering the binding affinity for hemoglobin and oxygen will be presented.

In general, the amount of oxygen delivered to a given organ or tissue is directly related to three variables: (1) blood flow or cardiac output, (2) hemoglobin concentration, and (3) the difference in oxygen content (saturation) between arterial and venous blood. For example, the cardiac output can significantly increase in order to maintain adequate oxygenation of vital organs such as the brain and kidney at the expense of the smooth muscle. Similarly, erythropoiesis can be stimulated by erythropoietin so that the overall hemoglobin levels increase. Finally, as illustrated in **Figure 2**, oxygen unloading or delivery can be augmented by a right shift in the oxygen dissociation curve facilitated by the RBC enzyme 2,3-BPG.

The converse to anemia is known as polycythemia or erythrocytosis, which is an increase above normal in the circulating quantity of RBCs. Not surprisingly, this increase in total RBCs is usually associated with a corresponding increase in hemoglobin. There are numerous causes of polycythemia such as response to high altitude, pulmonary disease, steroids (both androgenic and glucocorticoid), stress, and smoking.

Myeloids

The myeloids or leukocytes are a highly complex and sophisticated group of cells that are primarily involved in host resistance and inflammatory response against both foreign organisms and material (e.g., chemicals and toxins). For simplicity, the leukocytes can be divided into two major groups: (1) immunocytes and (2) phagocytes. The general organizational structure and normal values are shown in **Figure 1**. These cells are thought to arise from a common PSC in the bone marrow; hence, any toxin that affects the PSC will have a potentially disastrous impact on the body's ability to respond to challenges from an external agent or foreign substance.

The immunocytes are all involved in specific types of immune response that are generally divided into two types: (1) cell-mediated, i.e., specifically sensitized T cells (derived from the thymus) which are associated with graft rejection, resistance to certain viruses, bacteria, fungi, and protozoa, and delayed-type hypersensitivity; and (2) humoral-mediated, i.e., B cells (bursa equivalent) which produce specific antibodies after the body is exposed to a specific antigen.

The phagocytes are so named because their major function is to engulf or ingest foreign organisms or material. The phagocytes include the three granulocytes known as neutrophils (54–62%), eosinophils (1–3%), and basophils (<1%) and the monocytes (3–7%). Monocytes circulate in the blood for several days until they migrate into the reticuloendothelial tissues (liver, spleen, and bone marrow), where they are known as macrophages. Macrophages are not only involved in inflammatory responses but also have a major role in the destruction and removal of old RBCs and other plasma proteins, including hemoglobin.

The phagocytes act by engulfing the foreign material/agent and produce a respiratory burst. The respiratory burst involves the production of hydrogen peroxide and other highly reactive chemicals that attack the ingested material. An inflammatory response is quite commonly produced in this situation. Glucocorticoids (steroids such as prednisone) tend to decrease the numbers of granulocytes that will be involved in an inflammatory reaction. This effect accounts for beneficial impact of these drugs when an anti-inflammatory result is desired; however, there is also an increased susceptibility to infections that has been well documented.

There are several key terms and definitions that are given to absolute decreases or increases in the numbers of leukocytes. A fall in the total granulocyte count below $3000\,mm^{-3}$ is known as granulocytopenia. Granulocytopenia is commonly associated with chemically induced bone marrow damage; however, ionizing radiation and a myriad of drugs can also produce this effect. Finally, a particularly severe form of bone marrow failure is known as aplastic anemia. Aplastic anemia is diagnosed when at least two different marrow cell lines are severely depressed as demonstrated by (1) granulocytes $<500\,mm^{-3}$, (2) platelets $<20\,000\,mm^{-3}$, (3) reticulocyte count <1%, or (4) a bone marrow biopsy demonstrating <25% cellularity.

Granulocytosis is the opposite phenomenon of decreased cellularity and refers to elevated counts over $10\,000\,mm^{-3}$. Stress, drugs, and some bacterial

toxins can produce short-term granulocytosis; however, chemical exposure is not typically associated with mild, elevated counts. Leukemias are associated with counts over 30 000 mm^{-3} and have been associated with certain chemical exposures. This association will be presented in further detail in a subsequent section.

Thrombocytes

Platelets are produced by the fragmentation of megakaryocytes, the largest cell type in the bone marrow. Approximately one-third of the platelets are taken up by the spleen, while the other two-thirds freely circulate for 7–10 days until they are taken up by phagocytic cells. A normal platelet count is between 150 000 and 450 000 mm^{-3}. The normal platelet count is quite variable and can be affected by an individual's nutritional state or, in females, by the menstrual cycle.

Platelets are the rapid reaction troops in the situation of accidental blood loss associated with damaged blood vessels that expose collagen fibers. Normally, platelets are nonsticky; however, they rapidly and easily adhere or aggregate to exposed collagen fibers where they undergo a series of reactions that results in the formation of a thick mass known as a platelet plug. This plug acts to quickly stop bleeding; however, it must usually be reinforced by help from the clotting system so that vascular integrity is maintained. Platelet reactions are highly sensitive and vulnerable to substances that interfere with the aggregation reaction. For example, aspirin acts in a unique fashion to inhibit the aggregation reaction and has become a useful drug in the prevention of heart attacks and strokes caused by small platelet plugs.

Any disorder or agent that injures the stem cells or prevents their proliferation can drastically affect the absolute platelet count. The minimal platelet count necessary for initial hemostasis is $\sim$50 000 mm^{-3}. If the platelet count falls below 20 000 mm^{-3}, a condition known as thrombocytopenia exists and the affected organism is extremely vulnerable to spontaneous bleeding episodes. Usually, thrombocytopenia due to marrow failure is also associated with reduced leukocyte and red blood cell production since chemicals or disorders that affect the megakaryocytes also impact other stem cells. This is typically determined by examining a peripheral smear of the blood or by a hematologist's bone marrow aspiration.

The opposite phenomenon, elevated platelet count or thrombocytosis, is diagnosed when counts are greater than 400 000 mm^{-3}. There are many causes of thrombocytosis, including primary (e.g., essential thrombocytosis (ET)) and secondary (e.g., response to inflammation, acute bleeding, iron deficiency, or cancers). In ET, there are colonies of megakaryocytes in the absence of any known stimulus.

Toxic Agents and Responses

Carbon Monoxide

Carbon monoxide (CO) is an odorless, tasteless, and colorless gas that is rapidly absorbed by the lungs and attaches to hemoglobin with an affinity that is 250 times greater than oxygen. Due to this extreme differential, as CO concentrations increase, the number of available sites on the hemoglobin molecule for oxygen decreases. Normally, this reaction would cause oxygen to be more freely released so that adequate tissue oxygenation can be maintained. This would typically produce a right shift of the oxyhemoglobin dissociation curve; however, with increasing exposure to CO and formation of carboxyhemoglobin (COHgb), there is a change in the oxyhemoglobin complex which produces a left shift in the oxygen dissociation curve (**Figure 2**). The overall effect is decreased tissue oxygenation, anaerobic metabolism, and lactic acid formation.

Exposure to CO results in a wide variety of potential adverse effects, particularly in individuals who have pre-existing cardiac or lung disease. Infants, the elderly, and the developing fetus are particularly vulnerable since they have less capacity to tolerate cardiovascular compromise. An additional problem is the delayed neurological and neuropsychiatric effects that have been documented after some significant exposures. The incidence of delayed neurotoxicity is between 2% and 30%.

CO poisoning is usually diagnosed by measuring the presence of (COHgb) in blood. Nonsmokers have COHgb levels of $<1\%$, whereas smokers have levels of 5–10%. Unfortunately, the measured COHgb level does not always correlate with clinical findings and symptoms; therefore, the clinician should always have a high index of suspicion and aggressively evaluate and treat exposed patients. Treatment consists of removal from the source and administration of 100% oxygen and any other basic life-support measures required. In certain circumstances, i.e., COHgb levels over 25%, the use of hyperbaric oxygen is indicated.

Hydrogen Cyanide and Hydrogen Sulfide

Both hydrogen cyanide (HCN) and hydrogen sulfide (H_2S) are metabolic poisons that act in relatively similar mechanistic ways. At the cellular level, the major energy source is adenosine triphosphate (ATP).

ATP is primarily produced through a process known as oxidative phosphorylation, which involves the transfer of electrons to substances known as cytochromes. The cytochrome system can be viewed as a 'bucket brigade' that moves critical electrons in an orderly fashion so that cellular respiration is maintained. As electrons are transferred, energy is released and used to generate ATP and water. Oxygen is the final electron acceptor in the cytochrome system and can be severely affected by metabolic toxins like HCN and H_2S. These toxins ultimately act by blocking electron transfer to molecular oxygen. This blockade produces a rise in peripheral tissue partial pressure of oxygen and a decrease in the unloading gradient for oxyhemoglobin. The net effect is the production of both high levels of oxyhemoglobin in venous return blood and significant levels of lactic acid. At high exposure concentrations, cardiopulmonary compromise is rapidly produced and death ensues.

The treatment of either HCN or H_2S toxicity is based on the use of chemicals that interrupt the binding of these materials to the cytochrome oxidase system. Sodium nitrate and amyl nitrate are both used as antidotes. These substances act by overwhelming the RBC with oxidant stress and producing a somewhat less toxic material known as methemoglobin (MetHgb). MetHgb serves as a source of circulating ferric iron (Fe^{3+}), which preferentially competes for binding by cyanide or sulfide and causes the cyanide or sulfide to dissociate from the cytochrome system and move into blood in a form complexed to methemoglobin in RBCs. This less toxic material is further detoxified by the use of another drug, sodium thiosulfate, which further enhances the conversion of cyanide to the less toxic thiocyanate. The situation with H_2S is somewhat more complex since the second step use of sodium thiosulfate is not typically recommended; however, vigorous use of 100% oxygen therapy is appropriate for treating exposure to both HCN and H_2S.

Methemoglobin

At the molecular level, the transport of oxygen in the body is highly dependent on the maintenance of intracellular Hgb in a chemical condition known as the reduced state, or Fe^{2+}. When hemoglobin is oxidized, the Fe^{3+} state, it is known as MetHgb and is unable to bind oxygen. A small amount, $<1\%$, of MetHgb is always found in normal RBCs. MetHgb can be chemically reduced by an enzyme system so that the body maintains adequate levels of Fe^{2+}. If MetHgb exceeds 10% of the total hemoglobin, then clinically observable changes such as dusky complexion can be detected in the affected individual. As MetHgb levels reach 35%, symptoms such as headache, fatigue, and shortness of breath are common. MetHgb levels over 80% are usually fatal.

There are many causes of MetHgb, including both hereditary and acquired. Drugs and toxins, such as nitrates, nitrites, nitroglycerine, aniline dyes, and sulfonamides, are associated with the production of MetHgb in certain situations. Toxic levels of MetHgb can be treated with a compound known as methylene blue, which acts to rapidly reduce the level of circulating MetHgb.

Leukemia

The leukemias are a diverse group of hematologic malignancies that arise from the malignant transformation of hematopoietic cells. These cells develop in the bone marrow and lymphoid tissue and ultimately interfere with normal cell development and immunity. Leukemias are generally divided into two groups, myeloid and lymphoid. In addition, leukemias can be further subdivided by their natural history into acute or chronic forms. The leukemias represent 3% of all malignancies and $\sim$24 000 new cases a year develop in the United States.

The etiology of leukemia in most cases is unknown, although a combination of genetic and environmental factors is probably important. The most important environmental factors are drugs, radiation, and chemical exposures to a few selected substances. The most common form of leukemia associated with either chemicals or drugs is the acute nonlymphatic leukemias (ANLL), which are also referred to as acute myeloid leukemias (AML). In ANLL, large numbers of immature hematopoietic cells develop and replace the normal cells. These abnormal cells are released into the circulation and can easily be seen on peripheral blood smears. Since these cells are quite immature, the blood does not contain adequate numbers of normally functioning mature RBCs, leukocytes, and thrombocytes. AML is an aggressive and rapidly fatal disease unless appropriate therapy is begun.

The role of chemical exposure and development of ANLL has been quite controversial. This controversy is partially due to the problems associated with accurately and appropriately classifying the various leukemias. Since the mid-1980s, the nomenclature of the ANLL subtypes was established by the French–American–British Cooperative group also known as FAB. Older studies in the literature that do not use this classification scheme present a serious problem since there was a tendency to lump different

categories together in order to achieve sufficient statistical power for epidemiological analysis. Nevertheless, there does appear to be sufficient evidence to link ANLL with certain exposures to benzene.

The association between benzene exposure and leukemia has been made since the late nineteenth century; however, the dose–response relationship and mechanistic explanation have been quite contentious. The most reliable evidence associating chronic benzene exposure with AML was presented in a retrospective NIOSH study of rubber hydrochloride workers in Akron, OH, from 1940 to 1949. Unfortunately, the mechanism of how benzene exposure leads to the development of AML is not known. The two most frequently discussed potential mechanisms of toxicity involve either a point mutation or a chromosomal deletion. The latter is considered more likely since neither benzene nor its metabolites are mutagenic or teratogenic.

See also: Benzene; Carbon Monoxide; Cardiovascular System; Distribution; Hydrogen Sulfide; Immune System; Kidney; Liver.

Further Reading

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Blue-Green Algae *See* Algae.

Boric Acid

Michael Wahl

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- Chemical Abstracts Service Registry Number: CAS 10043-35-3
- Synonyms: Boracic acid; Orthoboric acid; Borofax; Three elephant; NCI-C36417
- Chemical Formula: H_3BO_3

Uses

Boric acid is used as a fireproofing agent for wood, as a preservative, and as an antiseptic. It is used in the manufacture of glass, pottery, enamels, glazes, cosmetics, cements, porcelain, leather, carpets, hats, soaps, artificial gems, and in tanning, printing, dyeing, painting, and photography. It is a constituent of nickling baths and electric condensers, and it is used for impregnating wicks and hardening steel. In laboratory procedures, boric acid is used in the preparation of buffer solutions.

Boric acid is also used as a fungicide and as an insecticide powder. Domestic use may include its application as an insecticide for crawling insects such as roaches. In medicine, it has been used as a disinfectant and is a constituent of baby powders, antiseptics, diaper rash ointments, eye washes, gargles, and a variety of other consumer products for its mild antiseptic property.

Background Information

Boric acid exists in natural deposits as a mineral, sassolite. It is also found in hot mineral water sources. The minerals are extracted with sulfuric acid and crystalline boric acid is separated.

Exposure Routes and Pathways

Accidental ingestion and subcutaneous routes are the primary exposure pathways. The maximum workplace concentration is $10\,mg\,m^{-3}$. The maximum concentration in water used in fisheries is $0.1\,mg\,l^{-1}$.

Toxicokinetics

Water emulsifying and hydrophobic ointments containing boric acid liberate only small amounts within 24 h compared with a near total liberation from a

jelly. Boric acid is readily absorbed from the gastrointestinal tract, mucous membranes, and abraded skin. Boric acid is excreted unchanged in urine with ~50% excreted in the first 12 h and the remainder excreted over a period of a few days. The half-life of boric acid given orally is estimated to be 21 h. The fatal dose of boric acid is estimated to be ~20 g in an adult and ~5 or 6 g in an infant.

Mechanism of Toxicity

The exact mechanism of toxicity is not known. Boric acid can inhibit production of adenosine triphosphate, a cellular form of energy.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals have demonstrated similar toxic effects to those seen in humans.

Human

Acute boric acid poisoning is extremely rare. Symptoms in extremely large doses will be similar to those seen in chronic overexposure (see below).

Chronic Toxicity (or Exposure)

Animal

Dogs and rats were able to tolerate boric acid doses of up to 350 ppm for 2 years. Larger doses of boric acid (1750 ppm) over a period of time have been shown to cause testicular damage and sterility in rats and dogs.

Human

Toxicity may occur after ingestion, injection, application to damaged skin (e.g., abrasion, burns, or diaper rash), lavage, or enema. Severe systemic toxicity is most likely to occur from repeated dermal application to damaged skin; this has been reported mainly in the treatment of diaper rash in young children. Symptoms include nausea, vomiting, bloody diarrhea, severe colic, and abdominal pain. There may be restlessness, delirium, headache, tremors, and generalized convulsions usually followed by weakness and coma. There is fever and tachypnea followed by Cheyne–Stokes-type respirations and respiratory arrest.

Changes on the skin include an erythematous skin eruption, with papules or vesicles appearing between the fingers and on the back of the hands initially and eventually becoming generalized enough to give a 'boiled lobster' appearance. The skin lesions may undergo bullous formation, desquamation, excoriation, and sloughing. Hypothermia often occurs.

Renal injury can occur, usually in the form of renal tubular necrosis, and can be demonstrated by the presence of oliguria, albuminuria, and eventually anuria. Signs of meningeal irritation, oliguria, and circulatory collapse may be followed by death within 5 days. Infants and young children are more susceptible to boric acid intoxication. Low levels of boric acid ingestion may lead to dry skin and mucous membranes, followed by the appearance of a red tongue, patchy alopecia, cracked lips, and conjunctivitis. Infertility among men is possible.

No major toxicological distinctions between boric acid and its salts are recognized in human beings.

In Vitro Toxicity Data

No mutagenic effects have been seen in *Salmonella typhii* strains TA98 and TA100 via the preincubation method.

Clinical Management

There is no specific antidote. Supportive care should be instituted for all patients with history of serious boric acid exposure. Substantial recent ingestions may benefit from administration of activated charcoal. Fluid and electrolyte balance, correction of acid/base disturbance, and control of seizures are essential to therapy. Hemodialysis has been successfully used to treat acute boric acid poisoning. Sodium bicarbonate may be used for any metabolic acidosis.

See also: Cosmetics and Personal Care Products; Federal Insecticide, Fungicide, and Rodenticide Act, US; Nickel and Nickel Compounds.

Further Reading

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Boron

William S Utley

- Chemical Abstracts Service Registry Number: CAS 7440-42-8

Uses

Boron is used as a reinforcing material for composites. It is used in the nuclear industry as a neutron absorber. Boron is used to harden metals and used as an oxygen scavenger for copper and other metals. Amorphous boron is used in pyrotechnic flares to produce a green color. Used as a catalyst in olefin polymerization and alcohol dehydration. The principal consumption pattern in the United States for boron is for the production of glass products with minor usage in the production of soaps and detergents.

Exposure Routes and Pathways

Ingestion and inhalation are the primary routes of exposure. Boron can be found in dusts, water, and in fruits and vegetables. Dermal absorption will not be a factor unless the dermal barrier is compromised.

Toxicokinetics

Absorption

Boron is well absorbed via the gastrointestinal tract. Systemic toxicity is more likely to result from multiple exposures rather than from single acute exposures.

Distribution

Boron is distributed fairly rapidly (30 min to 3 h) to all tissues of the body.

Elimination

The apparent half-life of elimination is 5–10 h. The primary route of elimination is via the kidneys.

Mechanism of Toxicity

Boron is concentrated in the kidneys during excretion, making the kidneys a prime target organ for boron toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Gastrointestinal and pulmonary disorders have been reported in lambs grazing in pastures containing high boron content in the soil. High exposures to boron (1000–2000 ppm) for 90 days have caused oligospermia and testicular atrophy in rodents. Exposure to boron in pregnant rats has led to central nervous system abnormalities in offspring. It is interesting to note that the dog appears to be twice as sensitive to the toxic effects of boron as rodents. Animal studies also indicate that high levels of boron may affect the testis.

Human

Single acute exposures to boron are well tolerated. Reversible irritation to the respiratory tract and mucosal membranes may be seen initially but these are expected to resolve themselves. Chronic exposures can lead to anorexia, weight loss, vomiting, mild diarrhea, erythematus rash, alopecia, convulsions, anemia, and kidney damage. Both vomitus and feces will be blue-green.

Clinical Management

General life support should be maintained. Symptoms should be treated and decontamination undertaken if necessary. Emesis may be indicated in instances where the patient has recently ingested a significant quantity.

Environmental Fate

Boron in the form of various oxides is removed from the atmosphere by precipitation and deposition fairly rapidly (a half-life measured in days). Whether via atmospheric deposition, precipitation, or weathering of boron-containing rocks, boron can be expected to migrate to the water column where it will hydrolysis to its weak acid form. Once in the water column the only significant factor that will affect its fate is possible adsorption to soils and sediment. The adsorption process is not well predicted, and will need to be determined for each sediment type being considered.

Exposure Standards and Guidelines

The oral reference dose for boron is 0.09 mg kg^{-1} day^{-1} and is based on testicular atrophy and

spermatogenic effect. An ambient water quality criterion of 750 ppb boron in water has been suggested based on long-term irrigation of sensitive crops. The Federal drinking water guideline for boron is 600 ppb.

Miscellaneous

Powdered boron has pyrotechnical properties and can spontaneously ignite in the air.

Further Reading

World Health Organization/International Programme on Chemical Safety (1998) Environmental Health Criteria 204. Boron, pp. 1–10.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Boron.

Botulinum Toxin

Fermin Barrueto Jr.

- Chemical Abstracts Service Registry Number: CAS 93384-43-1
- Synonyms: *Clostridium botulinum*; Foodborne (classic) botulism; Infant botulism; Wound botulism; Unclassified botulism
- Chemical/Pharmaceutical/Other Class: Foodborne toxins

Uses

The use of botulinum toxin has been increasing in cosmetic dermatology, muscle rigidity/spasticity syndromes, hyperhidrosis, some types of chronic pain syndromes, and headaches. It has the unfortunate distinction of being the most potent toxin that exists and is a weapon of mass destruction.

Exposure Routes and Pathways

Ingestion is the primary exposure pathway for botulism. Wound botulism occurs when the bacterium encounters devitalized human tissue, synthesizes toxin, and thus causes disease. Intestinal (adult and infant types) botulism involves ingestion of spores or the live bacterium and, due to impaired intrinsic defenses, the gastrointestinal tract becomes colonized with *Clostridium botulinum*. In infant type, the mucosal surface of the intestines is susceptible to colonization due to multiple factors including decreased acidity of the stomach and lack of bile of acids, which are natural barriers. In adult-type intestinal botulism, patients have had surgical vagotomy/Billroth procedures and/or medical treatment for peptic ulcer disease, making them susceptible to colonization of the toxin producing bacterium. Foodborne botulism usually results from exposure to canned foods that are inadequately sterilized during cooking and canning. Occasional larger outbreaks occur following ingestion of contaminated food at restaurants or from commercial sources. A variety of preserved foods have been implicated, including string beans, corn, garlic, seafood, pork, and beef.

Infant botulism is the most common form of the illness. This occurs in children less than 1 year old before the gastric mucosa becomes an acidic environment. Most cases occur from 1 week to 11 months of age, with a peak incidence at 2–4 months of age. Breast-feeding, feeding of honey or corn syrup, decreased frequency of bowel movements, and living in a rural area have been implicated as sources of *C. botulinum* spores that cause infant botulism. For this reason it has been recommended to avoid feeding honey to any child 12 months of age or younger. Types A or B botulinum toxin, and rarely type F, have been responsible for all infant cases. Inhalation through aerosolization, though rare, is thought to be the method in which botulinum toxin would be dispersed in a biological attack.

Mechanism of Toxicity

Despite the ubiquitous nature of botulinum spores, the incidence of disease is low. For optimal growth, *C. botulinum* requires a low acidic environment (generally pH >4.5), a temperature of at least 10°C, an anaerobic environment, and a lack of competition from other bacteria. While most toxins are destroyed by boiling, only pressure cooking to 240°F will ensure destruction of spores.

Three classified types of botulism (foodborne, intestinal-infant type, and wound) result from infection

with *C. botulinum* organisms whereas, in a biological attack, aerosolization of the neurotoxin itself would be used. *C. botulinum* is a strictly anaerobic, spore-forming, gram-positive rod that elaborates a potent exotoxin. Botulinum toxin is a protein that consists of a heavy chain and a light chain, bridged by a disulfide bond. The light chain, a zinc-dependent endopeptidase, is pharmacologically active and responsible for both the therapeutic and toxic effects. The heavy chain facilitates endocytosis of the toxin into the cell.

There are seven distinct antigenic types of botulinum toxin, assigned letters A–G. Guided by the heavy chain, the toxin enters a neuron in the peripheral nervous system. The light chain of the toxin binds the SNARE proteins (Synaptobrevin, SNAP-25, Syntaxin) that normally facilitate exocytosis of acetylcholine-containing vesicles. The endopeptidase site on the light chain then cleaves a portion of the SNARE proteins rendering the complex inactive, thereby blocking acetylcholine release. By weight, botulinum toxin is the most potent natural poison in the world where 7 pg of toxin is sufficient to kill a 70 kg adult if administered intravenously. While there are seven immunologically distinct toxins (A–G), the majority of poisonings in humans are caused by three toxins: A, B, and occasionally E.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cattle seem to be particularly sensitive to botulinum toxin. The estimated LD_{50} for lactating cows is $0.388\,ng\,kg^{-1}$, $\sim$13 times more sensitive to botulinum toxin type C than are mice.

Human

Initially, the toxin affects the bulbar musculature and patients typically present with any combination of signs and symptoms such as diploplia, dysphagia, dysarthria, and ptosis. Although the dose of the toxin may affect the rate of progression of the paralysis, all patients will develop multiple cranial nerve palsies with progression to a symmetrical descending flaccid paralysis with loss of deep tendon reflexes. Despite the neurologic findings, patients should maintain a normal mental status until the paralysis affects the muscles of respiration and results in respiratory failure and hypoxia. Once affected, a muscle remains paralyzed for several weeks, which is the time required for resynthesis of the SNARE proteins that were destroyed by the botulinum toxin.

Chronic Toxicity (or Exposure)

Human

Botulinum toxin type A is approved for use in humans for the treatment of strabismus, blepharospasm associated with dystonia, head position and neck pain associated with cervical dystonia (a movement disorder characterized by involuntary muscle contractions), as well as for the temporary improvement in the appearance of moderate to severe glabellar lines in adult men and women 65 years or younger. Clinical trials have noted few adverse effects associated with use of botulinum toxin type A for these conditions.

In Vitro Toxicity Data

Recent studies have demonstrated that secretory vesicle proteins, synaptotagmins I and II, mediate the entry of botulinum toxin type B into PC12 cells and function as protein receptors for the toxin. These findings were not demonstrated with botulinum toxins A or E.

Clinical Management

Proper supportive care and administration of antitoxin are the mainstays of current therapy. Patients who present with respiratory failure will need full ventilatory support; however, there will be a subgroup of patients who present early without obvious signs of respiratory muscle paralysis. The negative inspiratory force, pulse oximetry, and gag reflex of these patients should be evaluated serially to determine the degree of respiratory muscle weakness and likelihood of impending respiratory failure.

The current antitoxin is a trivalent equine-derived antibody that is only available from the Centers for Disease Control through local and state health departments. It contains types A, B, and E, which are the most common natural foodborne causes of botulism. Since the antitoxin prevents the progression of paralysis but will not reverse existing paralysis, its administration should not be delayed pending definitive laboratory confirmation of the diagnosis. This is critical as patients who progress to respiratory failure and become mechanically ventilated will be exposed to all the risks of the intensive care unit setting, including barotrauma, pneumonia, and sepsis, for several weeks to months.

The antitoxin itself carries some risk during administration since it is an equine-derived whole antibody. It can cause anaphylaxis, uriticaria, serum sickness, and other hypersensitivity reactions.

See also: Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents.

Further Reading

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Bovine Spongiform Encephalopathy (Mad Cow Disease)

Todd Canedy

Background

Bovine spongiform encephalopathy (BSE) is a chronic, degenerative disease affecting the central nervous system of cattle. BSE was first reported in the United Kingdom in November 1986. BSE belongs to a family of diseases known as transmissible spongiform encephalopathies, or TSEs. Though the source of the transmissible agent is yet to be fully characterized, there are common characteristics of all TSEs including a prolonged incubation period of months to years, a progressive debilitating neurological illness that is always fatal, pathological changes appear to be limited to the central nervous system and include vacuolation and astrocytosis, and the transmissible agent elicits no detectable specific immune response in the host. Furthermore, detergent treated extracts of brain tissue from animals or humans affected by these diseases reveal the presence of scrapie associated fibrils under electron microscopic examination.

Due to the lack of detectable specific immune responses, development of a preclinical live animal diagnostic test has been unsuccessful. There is no treatment for BSE, and affected cattle will die, usually within 2 weeks to 6 months from the onset of clinical symptoms.

Signs of Infection

The clinical signs of BSE begin to appear after a prolonged incubation period from 2 to 8 years. Symptoms include a change in temperament, such as nervousness or aggression, abnormal body posture, incoordination, difficulty in rising, decreased milk production, and/or the loss of body condition despite continued appetite.

Causes of Infection

The causative agent of BSE, like other TSEs, is yet to be fully characterized. Several theories currently under study include:

1. An unconventional virus.
2. A prion or abnormal partially proteinase K-resistant protein, devoid of nucleic acid, capable of causing normal prion protein in the host to change form into an abnormal protein.
3. A virino or 'incomplete' virus composed of naked nucleic acid protected by a host protein.

The BSE agent is smaller than most viral particles and is highly resistant to heat, ultraviolet light, ionizing radiation, and common disinfectants that normally render viruses and bacteria inactive. It causes no detectable immune or inflammatory response in the host, and has not been observed microscopically.

Detection of BSE

There is no test to detect the disease in a live animal. There are two methods currently available for the postmortem diagnosis of BSE:

1. A microscopic examination of the brain tissue to identify characteristic changes.
2. Use of immunohistochemistry, immunoblotting, and enzyme-linked immunosorbent assay to detect the partially proteinase resistant form of the prion (PrP^{res}) protein.

Transmission of BSE in Cattle

There is no evidence supporting the causal spread of BSE among cattle, that is, through contact with infected animals. Infection is thought to occur through feed containing meat-and-bone meal derived from infected animals. This feeding method has historically had widespread use, but is currently highly restricted in the United States. There is an occurrence of BSE in otherwise unexposed offspring of BSE-affected cattle. However, the study did not ascertain whether the symptoms were the result of genetic factors or true transmission from mother to offspring. The study concluded that the epidemic under study could not have been sustained through maternal transmission alone.

Extent of Epidemic

As of June, 2003, there have been no cases of BSE reported in the United States, and only one known case has been reported in Canada, from a cow imported from Europe. The United Kingdom, where the disease was first identified, claims some 95% of all BSE cases. Worldwide, more than 185 000 cattle have tested positive for BSE since the disease was first diagnosed. Other countries affected include Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Israel, Italy, Japan, Luxembourg, Liechtenstein, the Netherlands, Northern Ireland, Poland, Portugal, Slovakia, Slovenia, Spain, and Switzerland.

BSE in Humans

BSE is not transmissible to humans. However, there appears to be a strong connection between BSE and a variation of Creutzfeldt–Jakob disease, known as variant Creutzfeldt–Jakob disease (vCJD), another disease grouped with other TSEs. Evidence to date indicates that there has never been a case of vCJD transmission from person to person, but rather it is thought to spread from the consumption of cattle products contaminated with BSE. BSE and vCJD share many characteristics, to the point of being nearly indistinguishable from each other. Clinical studies have shown that mice inoculated with BSE showed the same pattern of incubation time, clinical signs, and brain lesions as mice inoculated with tissues from patients with vCJD. This provides evidence that BSE and vCJD are of the same 'strain'. Furthermore, these two diseases were not similar to other TSEs such as sporadic CJD and known scrapies strains.

See also: Food and Drug Administration, US; Neurotoxicity.

Relevant Websites

http://www.organicconsumers.org – US Continues to Violate World Health Organization Guidelines for BSE. January 23, 2004, Michael Greger, MD, for the Organic Consumers Association.

http://www.cdc.gov – CDC. Update 2002: Bovine Spongiform Encephalopathy and Variant Creutzfeldt–Jakob Disease. National Center for Infectious Diseases.

http://www.who.int – Bovine Spongiform Encephalopathy (BSE) (From the World Health Organization).

http://www.fda.gov – Bovine Spongiform Encephalopathy (BSE) (From the US Food and Drug Administration).

www.aphis.usda.gov – Animal and Plant Health Inspection Service.

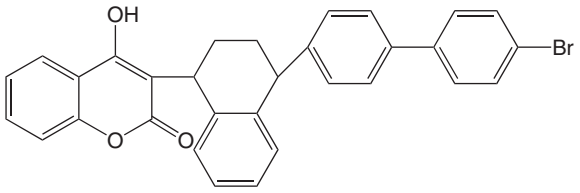
Brodifacoum

Henry A Spiller

This article is a revision of the previous print edition article by Michael J Hodgman, volume 1, pp. 185–187, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 56073-10-0
- Synonyms: PP 581; WBA 8119; 3-[3-(4-Bromo [1,1-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2*H*-1-benzopyran-2-one; Talon G; Ratac; Havac
- Chemical/Pharmaceutical/Other Class: A long-acting 4-hydroxycoumarin derivative; one of the superwarfarins
- Chemical Formula: $C_{31}H_{23}BrO_3$

- Chemical Structure:



Uses

Brodifacoum is used as a rodenticide (commonly 0.005% by weight).

Exposure Routes and Pathways

The most common route of exposure is oral. Transcutaneous and inhalation exposures have been implicated in workers involved in the manufacture of brodifacoum and pesticide operators.

Toxicokinetics

The metabolic fate of brodifacoum in humans is not well understood. Brodifacoum is much more lipid soluble than warfarin, resulting in a larger volume of distribution. There is extensive hepatic sequestration and prolonged high liver concentrations in the rat. Brodifacoum may also undergo enterohepatic recirculation in the rat. Based on the limited data available, the elimination half-life of brodifacoum in humans ranges from 16 to 36 days. There is considerable species variation. The apparent elimination half-life in dogs is 120 days. Inducers of the cytochrome P450 system have been reported to reduce the half-life of brodifacoum in animals.

Mechanism of Toxicity

Brodifacoum, like other hydroxycoumarins, interferes with the production of vitamin K-dependent coagulation factors. Vitamin K is a cofactor for the carboxylation of specific glutamic acid groups in coagulation factors II (prothrombin), VII, IX, and X. During this step, vitamin K is oxidized to vitamin K 2,3-epoxide. The regeneration of vitamin K by vitamin K 2,3-epoxide reductase is prevented by brodifacoum. As a result, dysfunctional decarboxycoagulation factors are produced and coagulation is impaired. Brodifacoum is over 100 times more potent than warfarin on a molar basis in rats.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity has been described in dogs, cats, horses, cows, pigs, sheep, goats, rats, mice, rabbits, voles, possums, Australian marsupials, chickens, ducks, and hedgehogs. Toxicity is expected in mammals, marsupial, and avian species. Owls died of hemorrhaging after feeding on rats killed with brodifacoum. Signs of poisoning occur after a latent period of 12 h to several days and may include: bruising easily with occasional nose or gum bleeds; blood in stools or urine; excessive bleeding from minor cuts or abrasions; labored breathing; pale mouth and cold gums; anorexia; and general weakness. You may also see lethargy, weakness, and lack of muscular coordination. Prolonged bleeding may occur from any small wounds and extensive bruising and subcutaneous hemorrhage.

Human

Depletion of preformed, circulating coagulation factors must occur before any anticoagulant effects are apparent. Typically, there is a delay of 24–36 h following ingestion before any effect is evident by measurement of the prothrombin time (PT). Significant toxicity from brodifacoum may be the result of large, one-time intentional ingestions. However, generally, repeated exposures over time are more likely to produce clinical toxicity. Single, small accidental ingestions in children are usually benign. Bleeding may occur virtually anywhere although cutaneous, mucosal, urinary, and gastrointestinal bleeding would be expected to be most common. Fatal intracerebral hemorrhage has been reported. Poisoning due to brodifacoum has led to prolonged periods of anticoagulation, often weeks and in some cases up to 6 months or longer. The clinical effect of brodifacoum is best monitored by following the PT and International Normalized Ratio (INR). Serum brodifacoum levels can be measured to confirm exposure, although there are no data to correlate serum levels and extent of toxicity. Factor activity can be assayed. An elevated serum ratio of vitamin K epoxide to vitamin K is further evidence of the presence of vitamin K reductase inhibition.

Chronic Toxicity (or Exposure)

Animal

Repeated exposures over time can lead to prolonged anticoagulation and appears to require a lower total

dose than acute exposure. Clinical effects are similar to those seen in acute exposures.

Human

Repeated exposures over time can lead to prolonged anticoagulation and appears to require a lower total dose than acute exposure. Clinical effects are similar to acute exposure.

In Vitro Toxicity Data

Ames *Salmonella* tests for genotoxicity have not demonstrated mutagenic effects. However, use of brodifacoum concentrations of $50\,g\,ml^{-1}$ in cultures of human lymphocytes did show mitotic activity, but no chromosomal aberrations.

Clinical Management

Animal

Treatment in animals is as for humans. The dose of vitamin K recommended for dogs and cats is 2.5–$5.0\,mg\,kg^{-1}\,day^{-1}$ for up to 4 weeks with monitoring of coagulation parameters.

Human

For acute, single-dose ingestions, activated charcoal may be administered. Induced emesis should be avoided in the anticoagulated individual. In large acute ingestions the PT should be determined at 24–48 h postingestion to assess the potential for toxicity. In the patient with clinical evidence of significant anticoagulation, extreme caution should be exercised with any invasive procedure. The airway should be protected if compromised by bleeding or hematoma formation. Volume resuscitation should be provided as indicated by clinical status. With active, uncontrolled, or life-threatening hemorrhage, fresh frozen plasma will provide preformed coagulation factors. Vitamin K_1 (phytonadion) is a specific antidote for brodifacoum toxicity. Pharmacologic doses of vitamin K allow the production of functional coagulation factors despite the presence of brodifacoum. The dose and route depend on the clinical setting. For rapid reversal, 5–25 mg should be administered intravenously no faster than $1\,mg\,mm^{-1}$. In children, doses of $0.6\,mg\,kg^{-1}$ have been recommended in warfarin poisoning, and larger doses may be necessary with brodifacoum. Clinical effects may be seen within hours. The response and duration of a single dose is variable and depends on the severity of the intoxication. Repeat doses will be necessary. In the less emergent setting, vitamin K may be given subcutaneously or orally. The doses needed to maintain adequate coagulation status may be quite large; in some cases, doses of $100\,mg\,day^{-1}$ or more orally have been reported, although typical doses are in the range of $25\,mg\,day^{-1}$. Titrate the daily dose by monitoring response to changes in the PT and INR. Oral vitamin K therapy may be necessary for weeks to months. Serial monitoring of the PT should be used to help guide therapy. Factor activity analysis may also be of use in assessing the adequacy of therapy. (*Note*: Anaphylaxis has been reported with intravenous vitamin K. Vitamin K_3 (menadione) is not an effective therapy.) Phenobarbital, $100–180\,mg\,day^{-1}$, has been administered to adults in an attempt to induce liver microsomal enzymes and hasten metabolism of brodifacoum, but its efficacy has not been proven. Administration of phenobarbital to an adult poisoned with chlorophacinone (another long-acting hydroxcoumarin derivative) resulted in a decrease in the apparent elimination half-life from 22.8 to 5.9 days.

Environmental Fate

In an aerobic soil, the half-life of brodifacoum is 14 days. If released into water, brodifacoum is expected to adsorb to suspended solids and sediment. Volatilization from water surfaces is not expected to be an important fate. The potential for bioconcentration in aquatic organisms is high. Brodifacoum is stable to hydrolysis in the environment. Brodifacoum is degraded by UV light when in solution.

See also: Coumarins; Warfarin.

Further Reading

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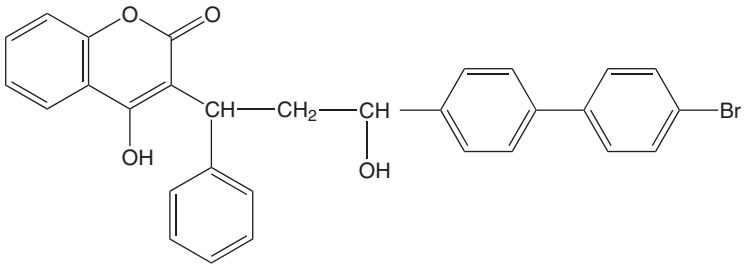
Bromadiolone

K S Rao

- Chemical Abstracts Service Registry Number: CAS 28772-56-7
- Synonyms: 3-[3-(4′-Bromo[1,1′-biphenyl]-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2*H*-1-benzopyran-2-one
- Chemical/Pharmaceutical/Other Class: Rodenticide
- Chemical Formula: $C_{30}H_{23}BrO_4$
- Chemical Structure:



Uses

Bromadiolone is a rodent control agent for rats and mice in and around buildings, inside transport vehicles, and inside sewers. It is formulated as meal bait, paraffinized pellets, rat and mouse bait ready-to-use place packs, and paraffin blocks (all formulations contain 0.005% active ingredient). Baits and bait packs are placed at 15 ft intervals for rats and 8 ft intervals for mice. The maximum rates of application are 16 ounces of bait for 15 ft intervals for controlling commensal rats and 2 ounces of bait for 8 ft intervals for house mice. According to labels, all baits are to be placed out of the reach of children, pets, domestic animals, and nontarget wildlife; or tamper-resistant bait stations may be used.

Exposure Routes and Pathways

Bromadiolone is a nonfood use pesticide. Therefore, it is unlikely that there will be any exposure through food sources or residues in ground or surface water. At this time, some products containing bromadiolone are intended primarily for homeowner use, and others are intended primarily for occupational use.

The Environmental Protection Agency (EPA) has determined that there is a potential exposure to applicators or other handlers during typical use patterns associated with bromadiolone. Specifically, the Agency is concerned about potential dermal and inhalation exposures of handlers during the loading and application of bromadiolone at bait stations.

Based on the use patterns, it is possible that applicators may experience dermal exposure during bait station loading. Some inhalation exposure may be associated with meal baits or pellets.

The EPA has determined that there is a potential for exposure to consumers and others following applications of bromadiolone, particularly in residences. The EPA has concerns about possible post-application exposures if (1) baits are not placed out of reach of children or are not placed in tamper-resistant bait stations, as specified in the labeling; (2) baits are available to homeowners in packages that are not tamper resistant and could be accessible to children; or (3) baits are brightly colored or packaged in a way in which they could be appealing to children or mistaken by children as food or candy.

Toxicokinetics

Bromadiolone is absorbed rapidly and effectively by the oral route but less effectively by the dermal route. Oral administration of bromadiolone results in substantial retention of the chemical in the liver for an extended period of time. The half-life for the decline of liver bromadiolone concentration was calculated to be 63 days.

Mechanism of Toxicity

Bromadiolone toxicity is a function of its anticoagulant properties. In an antidotal treatment study, groups of male Crl:CD rats (10 per dose) were exposed to bromadiolone baited pellets (0.005% a.i.) for 24, 48, or 78 h. The estimated mean total bromadiolone

doses were 5.69, 9.76, and 15.63 mg kg^{-1} for the 24, 48, and 72 h groups, respectively. At the end of the exposure period, the first five surviving rats of each group were given vitamin K_1 at 5 mg kg^{-1}. Initially, a loading dose was given subcutaneously and, subsequently, vitamin K_1 was administered daily by gavage for 13 days. The survivors were sacrificed at 8–10 days after discontinuing the vitamin K_1 treatment. The animals in each exposure group which did not receive vitamin K_1 died. The deaths frequently occurred within 3–4 days of the beginning of the study. The clinical and gross pathology findings indicated hemorrhage-related toxicity in all test-article treated animals. The death rates in vitamin K_1-treated animals were 1/5, 2/5, and 5/5 in the 24, 48, and 72 h exposure groups, respectively. With vitamin K_1 treatment, the clinical findings (hemorrhagic-related toxicity) were resolved by the fifth day of the antidote treatment, and the decrease in body weight observed during the bromadiolone treatment was also restored in the surviving animals. At the second week of the study, the prothrombin times of the vitamin K_1-treated animals were essentially comparable to those of the control group. However, for the 48 h exposure group, the prothrombin time was slightly decreased relative to that of the control group. The results demonstrate that vitamin K_1 treatment, as employed in this study, can restore the clotting process of an animal that is exposed to bromadiolone below an estimated total dose of 15.63 mg kg^{-1} body weight during a 72 h period. However, the antidotal treatment may not completely prevent death (i.e., all the rats in the 72 h exposure groups died despite vitamin K_1 treatment) when rats are exposed to bromadiolone even at the lowest exposure dose (5.69 mg kg^{-1}) in this study.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral LD_{50} of bromadiolone is 10 mg kg^{-1}. It is slightly irritating to eyes and is not a skin sensitizer. It does not induce acute delayed neurotoxicity in hens.

Human

No case reports of toxicity have been directly attributed to bromadiolone. However, based on its mechanism of action as an anticoagulant, it is expected that excessive human exposure of bromadiolone is likely to produce epistaxis, bleeding of gums, pallor, and sometimes petechial hemorrhage leading to hematomas around the joints and on the buttocks, and ultimately blood in urine and feces. Exposures to very high concentrations of bromadiolone would be expected to cause paralysis due to cerebral hemorrhage, leading to hemorrhagic shock and death.

Chronic Toxicity (or Exposure)

Animal

Subchronic oral administration of bromadiolone to dogs caused signs of loose, bloody stools following 15 μg kg^{-1} dosing. Five days following 100 μg kg^{-1} dosing, animals also showed signs of hypothermia, respiratory difficulties, pale mucosa, drowsiness, atonia, bloody urine, hematomas, and external hemorrhage. Both mid- and high-dose dogs had increased prothrombin time and hematuria. Histological examination showed that in high-dose groups, four out of four male or female dogs had hemorrhage, congestion, and/or edema of the spleen, kidneys, lungs, urinary bladder, small intestine, liver, thyroid, and skin. Based upon the clinical and hematological findings, the lowest-observed-effect level (LOEL) for subchronic toxicity of bromadiolone is 15 μg kg^{-1}; the no-observed-effect level (NOEL) is 10 μg kg^{-1}. No developmental toxicity was observed in rats and rabbits. In addition, bromadiolone did not induce any mutagenic effects.

Clinical Management

The principal diagnostic test for excessive exposure of bromadiolone is markedly reduced prothromibin activity, and therapy is directed at correcting this by the administration of vitamin K_1.

Environmental Fate

Bromadiolone is readily metabolized in aerobic soil ($t = 14$ days) and is generally immobile except in soils low in organic matter and clay, such as sand. Bromadiolone was stable to hydrolysis in pH 5, 7, and 9 buffer solutions. Although the parent compound is not persistent and is essentially immobile except in soils low in organic matter and clay, two of the major degradates identified in the aerobic soil metabolism study are persistent. Bromadiolone can leach in soils low in organic matter and clay; leaching was observed in a soil column (silt loam) with 0.5% organic matter and 3.2% clay. Since bromadiolone is applied as a food bait (pellets, place packs, or paraffinized blocks), leaching is expected to be minimal. Bioaccumulation factors of 160× and 1658× were

obtained for edible and nonedible tissues in bluegill sunfish, respectively.

Ecotoxicology

Bromadiolone is highly toxic to birds with an LC_{50} of 37 ppm in Northern Bobwhite. The results of the 96 h bluegill sunfish and rainbow trout acute toxicity studies indicate that bromadiolone is moderately toxic to fish with an LC_{50} of 3 ppm. Bromadiolone is considered moderately to highly toxic to freshwater invertebrates on an acute basis with an EC_{50} in the range of 0.1–10 ppm. The EPA believes that there is a high risk of secondary poisoning to mammals that feed on poisoned rodents in rural and suburban areas.

See also: Federal Insecticide, Fungicide, and Rodenticide Act, US; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Pesticides.

Further Reading

World Health Organization (1995) *Environmental Health Criteria No. 175, Anticoagulant Rodenticides*. Geneva: UNEP/ILO/WHO.

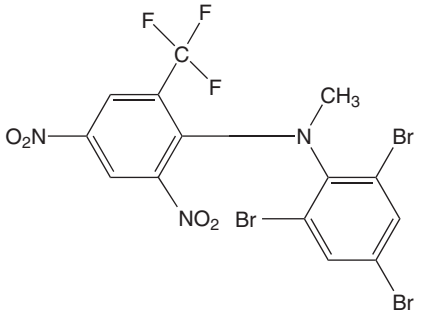
Relevant Website

http://www.epa.gov – US Environmental Protection Agency (1998) *Registration Eligibility Decision (RED): Rodenticide Cluster*. Washington, DC: Office of Prevention, Pesticides and Toxic Substances.

Bromethalin

Eric M Silberhorn

- Chemical Abstracts Service Registry Number: CAS 63333-35-7
- Synonyms: α,α,α-Trifluoro-*N*-methyl-4,6-dinitro-*N*-(2,4,6-tribromophenyl)-*o*-toluidine; *N*-Methyl-2,4-dinitro-*N*-(2,4,6-tribromophenyl)-6-trifluoromethyl)benzenamine
- Chemical/Pharmaceutical/Other Class: Acute rodenticide
- Chemical Formula: $C_{14}H_7Br_3F_3N_3O_4$
- Chemical Structure:



Uses

Single-dose rodenticide: This chemical is used in commercially available rodenticide baits (usually pellets or meal) for the control of rats and mice both indoors and outdoors. In the United States, federal regulations require that bromethalin rodenticide products be applied only in locations out of reach of children, pets, domestic animals, and nontarget wildlife, or in tamper-resistant bait stations. Bait stations must be resistant to destruction by dogs and by children under 6 years of age, and must be used in a manner that prevents children from reaching into bait compartments and obtaining bait.

Background Information

The concentration of bromethalin in rodent baits is 0.005% or 0.01%. It is effective against rodents that are resistant to anticoagulant rodenticides and does not induce bait shyness. Anorexia and neurological effects occur after an effective dose has been consumed.

Exposure Routes and Pathways

The primary exposure route to bromethalin is through accidental ingestion of commercially available rodenticide products that are used for rodent control. Children and pets are most likely to be accidentally exposed. Bait applicators may also be exposed through dermal contact.

Toxicokinetics

Information on toxicokinetics is largely derived from studies in the rat. After ingestion, bromethalin is rapidly absorbed with a peak plasma concentration occurring in ~4 h. The primary route of metabolism in the rat is through *N*-demethylation to desmethylbromethalin. Elimination is quite slow, with a plasma half-life for bromethalin of ~6 days.

Excretion occurs mainly in the bile, and enterohepatic circulation is suspected.

Mechanism of Toxicity

Bromethalin is an uncoupler of oxidative phosphorylation in mitochondria in cells of the central nervous system. Uncoupling leads to a decreased cellular ATP production and failure of the Na^+, K^+-ATPase pumps, which in turn leads to sodium retention and a loss of ability to maintain osmotic control. The outcome is a buildup of cerebrospinal fluid and vacuolization of myelin. The resulting edema and high intracranial pressures cause damage to nerve axons, inhibiting neural transmission, and leads to paralysis, convulsions, and, ultimately, death.

Acute and Short-Term Toxicity (or Exposure)

Animal

Symptoms of poisoning include ataxia, tremors, convulsions, prostration, and hind-limb paralysis. A single dose in excess of the LD_{50} will cause death within 8–12 h and is typically preceded by one to three episodes of clonic convulsions with death usually due to respiratory arrest. Oral LD_{50} values for pure bromethalin range from a low of $1.8\,mg\,kg^{-1}$ in the cat to $\sim 13\,mg\,kg^{-1}$ in rabbits. (*Note*: the LD_{50} values for rodenticide products containing bromethalin are 10 000 or more times higher on a milligram per kilogram basis because of the very small concentrations of bromethalin used in these products, typically 0.005% or 0.01%.) The guinea pig is highly tolerant of bromethalin with an oral LD_{50} in excess of $1000\,mg\,kg^{-1}$. The high LD_{50} is believed to be related to the fact that guinea pigs do not effect *N*-demethylation and therefore do not produce the highly toxic desmethylbromethalin metabolite (their oral LD_{50} for desmethylbromethalin is $7.5\,mg\,kg^{-1}$, which is similar to that for bromethalin in other species).

The dermal LD_{50} in rabbits is $2000\,mg\,kg^{-1}$. The inhalation LC_{50} for the rat is $0.024\,mg\,l^{-}$. Bromethalin causes slight irritation of the eye in rabbits but is not an irritant of the skin in this species, and does not cause dermal sensitization in the guinea pig. In addition, bromethalin does not cause acute delayed neurotoxicity in the hen.

Human

Reports of toxicity in humans have not been widely documented. The very low concentrations of bromethalin (0.005% or 0.01%) in rodenticide products help reduce the potential for toxicity due to accidental ingestion or direct contact. Children are potentially most at risk because of their small body size. Irritation of the eye or skin is not expected due to dermal contact based on studies in rodents. Expected signs and symptoms of oral exposure include headache, confusion, personality change, tremors, convulsive seizures, and respiratory distress.

Chronic Toxicity (or Exposure)

Animal

Multiple low (sublethal) doses of bromethalin yield hind leg weakness and loss of tactile sensation in rodents. Histopathology of the brain and spinal cord of these animals reveals spongy degeneration of the white matter (intramyelenic edema). The 90 day no-observed-effect level (NOEL) for dogs and rats is reported to be $0.025\,mg\,kg^{-1}\,day^{-1}$. The NOEL for developmental toxicity in both rats and rabbits is $0.5\,mg\,kg^{-1}\,day^{-1}$ when given by gavage during gestation. No compound-related external, visceral, or skeletal effects in treated fetuses were observed compared to controls on either a litter or fetal basis.

Human

Bromethalin is an acute rodenticide and most effects are expected to be short term in nature unless exposures continue over a long period. Symptoms of chronic exposure could include those associated with cerebral edema such as neuromuscular disorders.

In Vitro Toxicity Data

No data of this type are available for bromethalin.

Clinical Management

No specific antidote is available. If ingested, absorption should be limited by either emesis or gastric lavage. Sublethal symptoms, if present, would result in cerebral edema and should be treated accordingly through administrations of an osmotic diuretic and corticosteroid. Treatment regimens for dogs and cats exposed to bromethalin includes emesis (in nonsymptomatic animals only) and multiple doses of activated charcoal.

Environmental Fate

Bromethalin is stable to hydrolysis over the pH range of 5–9 when incubated in the dark for up to 30 days. Data from an anaerobic soil metabolism study indicate that bromethalin is relatively stable to

microbial/chemical degradation in the soil, with a calculated half-life for the parent compound of 178 days.

Ecotoxicology

Nontarget animals most at risk from exposure to rodenticides containing bromethalin include pets such as dogs and cats that may accidentally ingest baits used in and around homes. Assuming ~21 g of bait per pack and a bromethalin concentration of 0.01% in the bait, a typical 5 kg dog would need to consume five to six packages of bait to reach toxic levels, while a cat would need to consume only about one or two packages.

Bromethalin in its pure form is highly to very highly toxic to birds via oral ingestion (acute single-dose oral $LD_{50} = 4.6–11.0\,mg\,kg^{-1}$) and moderately to highly toxic via a 5 day dietary exposure (LC_{50} = 210–620 ppm in diet).

Bromethalin is classified as very highly toxic to aquatic organisms, although because this compound has an extremely low solubility in water, very little, if any, exposure of aquatic organisms is anticipated through use of rodenticides containing this compound. The acute 48 h EC_{50} for the water flea, *Daphnia magna,* is $\sim 2.0–5.1\,\mu g\,l^{-1}$ (ppb). Acute 96 h LC_{50} values for bluegill sunfish and rainbow trout are 598 and $38\,\mu g\,l^{-1}$, respectively.

Other Hazards

Rodenticide products containing bromethalin are not flammable or reactive as formulated.

Exposure Standards and Guidelines

There are no occupational exposure guidelines for this compound.

Miscellaneous

Use of rubber gloves is recommended when handling rodent baits containing this compound.

See also: Pesticides.

Further Reading

Dorman DC (2001) Bromethalin. In: Peterson ME and Talcott PA (eds.) *Small Animal Toxicology*, pp. 435–444. Philadelphia, PA: Sanders.

US Environmental Protection Agency (1998) Reregistration Eligibility Decision (RED) Rodenticide Cluster. EPA738-R-98-007. Office of Prevention, Pesticides and Toxic Substances, Washington, DC.

Van Lier RBL and Cherry LD (1988) The toxicity and mechanism of action of bromethalin: A new single feeding rodenticide. *Fundamentals of Applied Toxicology* 11: 664–672.

Bromine

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 7726-95-6
- Synonyms: Brom; Brome; Broom; Dibromine
- Chemical/Pharmaceutical/Other Class: Antiseptic

Uses

Bromine was formerly used medicinally as a topical antiseptic. It is used in gold extraction, in the bleaching of fibers and silk, in shrink-proofing of wool, in photography, and in the manufacture of bromine compounds, military gas, antiknock compound (ethylene bromide), and fire extinguishing fluid.

Background Information

Bromine is a naturally occurring element that can be found in many inorganic substances. Through industrial revolution, organic bromines in the environment at high concentrations have been introduced. These are not natural and can cause serious harm to human health and the environment. Humans can absorb organic bromines through the skin, with food, and by inhalation. Organic bromines are widely used as sprays to kill insects and other unwanted pests. They are not only poisonous to the animals that they are used against, but also to larger animals. In many cases they are poisonous to humans too. The most important health effects that can be caused by bromine-containing organic contaminants are malfunctioning of the nervous system and disturbances in genetic material. However, organic bromines can also cause damage to organs such as liver, kidneys, and lungs and can cause stomach and gastrointestinal

malfunctioning. These bromines can damage the nervous system and the thyroid gland. Some forms of organic bromines, such as ethylene bromide, can even cause cancer.

Exposure Routes and Pathways

Inhalation, ingestion, and eye and skin contact are the most common routes of exposure. Bromine may be absorbed through skin.

Toxicokinetics

Bromine has cumulative properties and is deposited in tissues as bromides, displacing other halogens.

Mechanism of Toxicity

Bromine causes toxicity as bromides by displacing other halogens from the body.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity in animals is similar to that in humans. The LC_{50} value in mice by inhalation exposure is 750 ppm/9 months.

Human

The respiratory system, eyes, and central nervous system are the points of attack. Bromine is extremely irritating to skin, eyes, and mucous membranes of the upper respiratory tract. Severe burns of the eye may result from liquid or concentrated vapor exposure. Liquid bromine splashed on skin may cause vesicles, blisters, and slow-healing ulcers. Inhalation of bromine is corrosive to the mucous membranes of the nasopharynx and upper respiratory tract, producing brownish discoloration of the tongue and buccal mucosa, a characteristic odor of breath, edema and spasm of the glottis, asthmatic bronchitis, and possibly pulmonary edema, which may be delayed until several hours after exposure. A measles-like rash may occur. Exposure to high concentrations of bromine may lead to death due to choking caused by edema of glottis and pulmonary edema. Exposure to low concentrations results in cough, copious mucus secretion, nose bleeding, respiratory difficulty, vertigo, and headache. Usually, these symptoms are followed by nausea, diarrhea, abdominal distress, hoarseness, and asthmatic-type respiratory difficulty.

Chronic Toxicity (or Exposure)

Animal

Pulmonary edema, pneumonia, diarrhea, and rashes may be delayed complications of severe exposures.

Human

Chronic exposure may cause acne-like skin lesions and neurotoxicity.

Clinical Management

Exposure should be terminated as soon as possible by removing the victim to fresh air. The eyes and mouth should be washed with copious amounts of water. A 15–20 min wash may be necessary. Skin should be washed with soap. Contaminated clothing and jewelry should be removed and isolated. Contact lenses should be removed from the eyes to avoid prolonged contact of the chemical with the area. When the chemical has been swallowed, large quantities of milk should be given; if milk is not available, water should be given. Emetics should not be given. If breathing has stopped, artificial respiration should be given. If breathing is difficult, oxygen should be given.

Environmental Fate

The average content of bromine in soils of grasslands, orchards, and upland crop fields were 10-fold higher than those recorded from overseas. The average content in these soils was higher than those recorded in forest soil of the basins. The average values reported in the leaves of plants were 12 ppm. The contents of iodine and bromine in the forest soil, plants, and rainwater were generally higher in coastal than in the inland areas.

Ecotoxicology

Organic bromines are often applied as disinfecting and protecting agents, due to their damaging effects on microorganisms. When they are applied in greenhouses and on farmland they can easily rinse off to surface water, which has very negative health effects on daphnia, fishes, lobsters, and algae. Organic bromines are also damaging to mammals, especially when they accumulate in the bodies of their preys. The most important effects on animals are nerve and DNA damage, which can enhance the chances of development of cancer. The uptake of organic bromine takes place through food, breathing, and the skin. Organic bromines are not very biodegradable; they are decomposed to inorganic bromines. At high doses these can damage the nervous system. It has occurred in the past

that organic bromines ended up in the food of cattle. Thousands of cows and pigs had to be killed in order to prevent contagion of humans. The cattle suffered from symptoms such as liver damage, loss of sight, depletion of growth, decrease of immunity, decreasing milk production, sterility, and malformed children.

Exposure Standards and Guidelines

The acceptable daily intake is $1\,mg\,kg^{-1}$ body weight and the threshold limit value is 0.1 ppm.

See also: Ecotoxicology; Peyote.

Further Reading

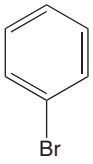
Shannon M (1998) Bromine and iodine compounds. In: Haddad L, Shannon M, and Winchester J (eds.) *Clinical Management of Poisoning and Drug Overdose*, 3rd edn., pp. 803–812. Philadelphia: Saunders.

Seiler HG, Sigel H, and Sigel A (1988) *Handbook on the Toxicity of Inorganic Compounds*, p. 150. New York: Dekker.

Bromobenzene

William S Utley

- Chemical Abstracts Service Registry Number: CAS 108-86-1
- Synonyms: Monobromobenzene; Phenyl bromide
- Chemical Formula: C_6H_5Br
- Chemical Structure:



Uses

Bromobenzene is used as an industrial solvent, in organic synthesis, and as an additive to motor oils.

Exposure Routes and Pathways

The primary routes of exposure are via inhalation, skin, and accidental ingestion.

Toxicokinetics

Bromobenzene is excreted as the free and sulfate or mercapturic conjugates of the catechol derivatives. Initially, bromobenzene may concentrate in the adipose tissues. Bromobenzene concentrations can be 300 times higher in the adipose tissues in the first 3 h of exposure. Bromobenzene is rapidly excreted in the urine; one report indicated that 85% of the bromobenzene may be excreted in the urine in the first 24 h.

Mechanism of Toxicity

Bromobenzene is believed to be relatively inert, requiring metabolic activation to express toxicity to the liver and the kidney. Liver toxicity is believed to result from activation of bromobenzene to a reactive epoxide by the cytochrome P450 system. The reactive epoxides are primarily detoxified by glutathione transferase and epoxide hydratase. The involvement of glutathione transferase explains in part the decrease in glutathione levels observed following exposure to bromobenzene. It is interesting to note that in animal experiments, sulfhydrol-containing compounds such as cysteine or methionine partially prevented bromobenzene-induced hepatic necrosis.

It has been suggested that the enterhepatobiliary cycle plays a role in the hepatic necrosis observed in bromobenzene toxicity. This is supported by the experimental findings that bromobenzene-induced hepatic necrosis can be prevented by the administration of cholestyramine.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral route of exposure in dogs to high concentrations leads to vomiting, diarrhea, and death. The major histopathological findings were centrilobular hepatic necrosis. The necrosis included the central hepatic veins and their respective tributaries. Bromobenzene was not found to be mutagenic in the Ames assay.

Human

Bromobenzene is known to be irritating to the skin and is suspected of being irritating to the eyes and

respiratory tract. The probable lethal dose is between one teaspoon and one ounce for a 154 lb person. Bromobenzene is directly irritating to the skin and can act as an anesthetic when inhaled in high concentrations.

Clinical Management

General life support should be maintained. Symptoms should be treated and decontamination undertaken if necessary. Treatment is generally symptomatic and supportive.

Environmental Fate

Bromobenzene poorly degrades and is expected to be persistent in the environment. The low water solubility and moderate K_{oc} (268) suggest that when released to the water, bromobenzene will tend to migrate to soils. Bromobenzene has a low to moderate chance of bioconcentrating (measured bioconcentration factors (BCFs) of 8.8–190). Bromobenzene exhibits enough of a vapor pressure (4.18 mmHg at 25°C) that it can be expected to volatilize from soils and surface water.

Ecotoxicology

Bromobenzene is moderately toxic to aquatic organisms (acute LC_{50} to fathead minnow of 5.6 mg l^{-1}).

Exposure Standards and Guidelines

Bromobenzene is regulated under section 111 of the Clean Air Act. Bromobenzene is a DOT and IMDGC Marine pollutant. European Union classifies bromobenzene as dangerous to the environment and a skin irritant: Xi, N: R-10, R-38, R-51/53.

Further Reading

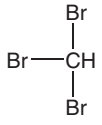
Bambal RB and Hanzlik RP (1995) *Chemical Research in Toxicology* 8(5): 729–735.

Heijen WH, Slitt AL, van Bladeren PJ, *et al.* (2004) Bromobenzene-induced hepatoxicity at the transcriptome level. *Toxicological Sciences* 79(2): 411–422.

Bromoform

William S Utley

- Chemical Abstracts Service Registry Number: CAS 75-25-2
- Synonym: Tribromomethane
- Chemical Formula: $CHBr_3$
- Chemical Structure:



Uses

Bromoform is used as a chemical intermediate in the synthesis of organic chemicals and pharmaceuticals. It is used as an ingredient in fire-resistant chemicals and as an industrial solvent in liquid-solvent extractions. Bromoform is used in polymer reactions and in the vulcanization process for rubber. Bromoform is also used for medicinal purposes as a sedative, antitussive, and antiseptic.

Exposure Routes and Pathways

Ingestion is the most common form of accidental and intentional exposure. This occurs primarily via drinking bromonated water. In the past, inhalation was a more common route of exposure during anesthesia. However, due to the associated toxicities, bromoform is no longer popular as an anesthetic. Inhalation can still be a significant route of exposure via volatilization of household or workplace water. Dermal absorption is possible but not likely to be a significant route of exposure in intact skin.

Toxicokinetics

Absorption

Bromoform is readily absorbed from the gastrointestinal tract following ingestion and from the lungs following inhalation. Significant absorption may occur through abraded skin or open wounds.

Biotransformation

Bromoform is metabolized in the liver by the mixed function oxidase system (cytochrome P450) to carbon monoxide and bromide. Inorganic bromide has

been observed in tissues and urine following administration of bromoform.

Distribution

Bromoform readily distributes through the tissues of the body.

Elimination

Bromoform and its metabolites are rapidly expelled out of the body through exhalation. It has been shown that the majority of bromoform (50–90%) is expelled in an 8 h period. As such, bromoform does not tend to build up in the body.

Mechanism of Toxicity

The wide spectrum of toxic effects elicited by bromoform suggests multiple mechanisms of actions. Bromoform is an irritant, capable of directly irritating mucosal membranes when directly exposed. Chronic exposure to bromoform may also cause the defatting of the skin leading to drying and cracking. Bromoform is capable of dissolving in phospholipid membranes giving it the ability to produce anesthetic effects when given in high concentrations.

The toxic effects on the liver and kidneys may be mediated by reactive intermediates produced by the hepatic P450 oxidative metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

Bromoform was noted to be more toxic to the liver and more irritating than chloroform when given via inhalation. Bromoform was noted to produce decreased liver functions and pathologic changes to both the liver and kidneys by both oral and inhalation routes in rodents. Single oral doses to rodents produced sedation, ataxia, piloerection, and prostration. Undiluted bromoform caused moderate irritation to the eyes of rabbits, which recovered in 1–2 days.

Bromoform tested positive for the induction of sex-linked recessive lethal mutations in *Drosophila* at a dose of 3000 ppm when administered to males by feeding.

Human

The odor has been described as 'chloroform-like' and the taste has been described as 'sweet'. Bromoform can be toxic by all routes of exposure. People appear to be able to detect bromoform at very low concentrations in liquid (0.3 ppm). Symptoms of acute exposure appear to be primarily one of sedation.

Chronic Toxicity (or Exposure)

Animal

In a 2 year gavage study conducted by the National Toxicology Program, rats and mice were given bromoform 5 days a week for 103 weeks. Dose-related lethargy and nonneoplastic changes to the liver (mixed cell foci and fatty changes) were noted. These observations were noted at the lowest dose tested 100 mg kg^{-1} (lowest-observed-adverse-effect level).

In Vitro Toxicology Data

Bromoform is mutagenic in the Ames assay.

Clinical Management

Medical surveillance may be indicated in persons with predisposing skin, liver, kidney, or respiratory conditions. General life-support should be maintained, symptoms treated and decontamination considered if necessary. Treatment is generally symptomatic and supportive. The patient should be monitored for delayed liver and kidney damage. If central nervous system depression occurs, EKG and vital signs should be monitored carefully. Patients who exhibit dermal hypersensitivity may require systemic or topical antihistamines or corticosteroids.

Environmental Fate

Bromoform poorly degrades and is expected to be persistent in the environment. The low K_{oc} (24) suggests bromoform will be highly mobile in soils. Bromoform has a low probability of bioconcentrating (measured bioconcentration factor (BCF) of 4). Bromobenzene exhibits enough of a vapor pressure (44.4 mmHg at 25°C) to be expected to volatilize from soils and surface water, where it will undergo photochemical oxidations with a half-life of 142 days.

Ecotoxicology

With the exception of the eastern oyster (48 h LC_{50} 1 mg l^{-1}), most aquatic species are relatively immune to the acute effects of bromoform expressing LC_{50} values in the range of 1600–29 000 mg l^{-1}.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit and the American Conference of Governmental Industrial Hygienists threshold limit value is 0.5 ppm (with a notation for skin absorption). As a waste, bromoform is considered a toxic waste (number U225). The US Environmental Protection Agency recommends that drinking water levels for bromoform should not be more than 0.7 ppm. Integrated Risk Information System (IRIS) has assigned an oral reference dose of $0.02\,mg\,kg^{-1}\,day^{-1}$ for bromoform.

Bromoform is regulated under Section 112 of the Clean Air Act and under Section 307 of the Clean Water Act. The CERCLA reportable quantity for a spill (RQ) is 100 pounds. Bromoform is reportable under EPCRA SARA 313. Transport is regulated under DOT and IMDG as a marine pollutant. Bromoform is regulated as a California Proposition 65 carcinogen with a no significant risk level of $64\,\mu g\,day^{-1}$. Bromoform is regulated in the European Union as toxic by inhalation, dangerous to the environment, and irritating to the skin and eyes (T, N: R-23, R-36/38, R-51/53). International Agency for Research on Cancer has classified bromoform as a class 3 carcinogen (not classifiable as a human carcinogen based on inadequate human data and limited animal data). IRIS has classified bromoform as a B2 (probable human carcinogen based on sufficient animal evidence).

See also: Chloroform.

Further Reading

International Toxicity Estimates for Risk (ITER) from Toxicological Excellence for Risk Assessment (available through the US National Library of Medicine's TOXNET system).

Relevant Websites

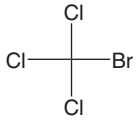
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Bromoform.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Bromoform.

Bromotrichloromethane

Midhun C Korrapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 75-62-7
- Synonyms: EINECS 200-886-0; Carbon bromotrichloride; Trichlorobromomethane
- Chemical/Pharmaceutical/Other Class: Not a member of any pharmaceutical class
- Chemical Formula: $CBrCl_3$
- Chemical Structure:



Uses

Although bromotrichloromethane is no longer produced in the United States, it is still used in organic syntheses of various compounds.

Background Information

Bromotrichloromethane is a colorless nonflammable liquid. It is sparingly soluble in water and readily evaporates when exposed to air.

Exposure Routes and Pathways

It is used in organic syntheses and may result in its release into the environment directly to the atmosphere. The most common exposure route to bromotrichloromethane is through inhalation or dermal contact in workplaces, where it is produced or used.

Toxicokinetics

Bromotrichloromethane is readily absorbed from the lungs and rapidly reaches equilibrium with levels in blood and expired air approximately proportional to the exposure concentration. At high concentrations, kinetic processes like metabolism or excretion may become saturated, limiting the rate of uptake. It is metabolized by conjugation with glutathione to yield

S-methylglutathione, *S*-methyl cysteine, and other sulfur-containing compounds.

Mechanism of Toxicity

With the loss of bromide ion, mediated by cytochrome P450 enzyme in the liver, the trichlorocarbon free radical is responsible for lipid peroxidation, which is the predominant mechanism of hepatotoxicity:

$$BrCCl_3 \xrightarrow{P450} Br\bullet + \bullet CCl_3$$

It is also known to cause cerebellar degeneration in rodents. It is cytotoxic to the sperm in the testes at the time of exposure. Renal tumors are induced in male mice due to depletion of glutathione, increased lipid peroxidation, and DNA lesions.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation of bromotrichloromethane by rats increased total lipids in liver and stimulated hepatic lipid peroxidation. After intragastric administration, liver steatosis was observed. Rats injected with 0.26 mmol bromotrichloromethane died after massive accumulation of neutral lipids and necrosis of the liver.

Human

Bromotrichloromethane causes irritation and reddening of eyes. Prolonged or repeated exposure may cause cataract and severe, permanent damage to the eyes. Bromotrichloromethane causes rash, blistering, and allergic reactions upon dermal contact; it may also cause nasal, gastrointestinal, and lung irritation.

Chronic Toxicity (or Exposure)

Animal

Bromotrichloromethane, when tested for its mutagenic activity, gave positive results for the Ames test.

Human

Rated D (not classifiable as human carcinogen) in Environmental Protection Agency's IRIS Database.

In Vitro Toxicity Data

In the *in vitro* test with FAF-cells of Chinese hamsters only bromochloromethane produced an increase of the sister chromatid exchange frequency.

Clinical Management

Administration of vitamin E and cadmium acetate were shown to be protective against bromotrichloromethane toxicity by free radical scavenging and chelating properties of vitamin E and cadmium acetate, respectively.

Environmental Fate

Terrestrial Fate

Bromotrichloromethane is expected to have low mobility in soil. The potential for volatilization of bromotrichloromethane from dry soil surfaces may exist based on a measured vapor pressure of 39 mmHg. Based upon the highly halogenated structure of bromotrichloromethane, biodegradation in soil is expected to be slow.

Aquatic Fate

Bromotrichloromethane is expected to adsorb to suspended solids and sediment in water. Volatilization from water surfaces is expected. The potential for bioconcentration in aquatic organisms is moderate. Based upon the highly halogenated structure of bromotrichloromethane, biodegradation in water is expected to be slow.

Atmospheric Fate

According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere, bromotrichloromethane, which has a measured vapor pressure of 39 mm Hg at 25°C, is expected to exist solely as a vapor in the ambient atmosphere. Based on bromotrichloromethane's structural similarity to bromotrifluoromethane, it is expected to slowly degrade in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for bromotrichloromethane's reaction in air is estimated to be greater than 44 years. Photolysis may occur based on bromotrichloromethane's structural similarity to other halogenated methane compounds but not at an environmentally relevant rate.

Ecotoxicology

Bioconcentration of bromotrichloromethane in aquatic organisms is moderate. Aquatic toxic effects to aquatic organisms are not reported as such.

Exposure Standards and Guidelines

According to Occupational Safety and Health Administration, the threshold limit value – time-weighted

average (TLV – TWA) limit for bromotrichloromethane is 8 h time-weighted average (TWA) 200 ppm. Excursions in worker exposure levels may exceed three times the TLV – TWA for no more than a total of 30 min during a work day, and under no circumstances should they exceed five times the TLV – TWA.

See also: Ames Test; Carcinogen Classification Schemes; Vitamin E.

Further Reading

Burk RF and Lane JM (1979) Ethane production and liver necrosis in rats after administration of drugs and other chemicals. *Toxicology and Applied Pharmacology* 50: 467.

Lowrey K (1981) Destruction of liver microsomal calcium pump activity by carbon tetrachloride and bromotrichloromethane. *Biochemical Pharmacology* 30: 135.

Brown Recluse Spider *See* Spider, Brown Recluse.

BSE *See* Bovine Spongiform Encephalopathy (Mad Cow Disease).

Buckthorn

Christopher P Holstege

This article is a revision of the previous print edition article by Lanita B Meyers, volume 1, pp. 190–191, © 1998, Elsevier Inc.

- SYNONYMS: Rhamnaceae family; *Rhamnus frangula*; Alder buckthorn; Frangulin; Trollidora; Coyotillo; Wild cherry; Purging buckthorn; Arrow wood; Berry alder; Black dogwood; Cascara; Senna, Hart's thorn; May thorn; Persian berry; Rhine berry; Common buckthorn

Uses

Buckthorn and its pharmaceutical derivatives (senna, cascara) are most commonly taken as laxatives. Other purported but unsubstantiated uses include treating skin disorders, ulcers, cardiovascular disease, and cancer.

Background Information

Buckthorn is a shrubby tree that grows 6–12 ft tall. The leaves (up to 6 cm) are simple, ovate elliptic, with serrate margins. Some of the branches end in short thorns. The flowers have four small petals and grow solitary or in clusters from the leaf axis. They are replaced later by a berry that is green at first, turns red, and then turns black at maturity. The berry contains up to four seeds. This ornamental shrub is often found as hedges throughout the eastern United States. The shrubs are also grown along gullies in the southwestern United States and northern and central Mexico. Other species of this plant are found throughout the northern temperate zones.

Exposure Routes and Pathways

The most common route of exposure is ingestion of any part of the plant. The seeds are the most poisonous part of the plant. Laxatives (senna, cascara) are derived from these plants and are taken either orally or rectally.

Toxicokinetics

Buckthorn contains anthraquinone glycosides that are poorly absorbed after ingestion. Anthraquinone glycosides undergo hydrolysis by colonic bacteria into senna and cascara. Bowel movements occur within 6–12 h of ingestion of senna and cascara laxatives. Fresh plant ingestions are associated with much more rapid symptom onset. After ingestion and hydrolysis, the anthraquinones are eliminated by the kidneys, in the feces, and in the bile. Senna and cascara are not significantly excreted in breast milk and subsequently do not cause toxicity in breastfeeding infants.

Mechanism of Toxicity

Anthraquinone glycosides exhibit their effect by increasing the tone of the smooth muscle wall in the

large intestine. A direct action is exhibited on the intestinal mucosa, increasing the colonic motility and colonic transit and inhibiting water and electrolyte secretion. These agents may also act directly on the intramural nerves of the colon and have stool-softening properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals that have ingested anthraquinone-containing plants are reported to have developed signs and symptoms similar to those of humans.

Human

Buckthorn poisonings are rare. Determination of a toxic dose is difficult due to various species containing different concentrations of anthraquinones. Mild intoxications may result in nausea, vomiting, diarrhea, abdominal cramps, and possible palpitations. Severe poisoning has been reported from use of the bark as an abortifacient. In severe cases, kidney damage, oliguria, proteinuria, gastrointestinal hemorrhage, seizures, dyspnea, and fluid depletion can result. Ingestion of seeds or fruit of the *K. humboltiana* species can also produce neurotoxic symptoms after a latent period of 1–4 weeks. A diffuse segmental demyelination has been reported, causing a rapidly ascending motor neuropathy with minimal sensory findings similar to Guillain–Barre syndrome. This may progress for a month or longer and can lead to respiratory failure.

Chronic Toxicity (or Exposure)

Human

Electrolyte abnormalities and dehydration may occur with chronic ingestion of these agents. Chronic ingestion of *K. humboltiana* has been reported to cause a progressive, symmetrical polyneuropathy that resulted in flaccid quadriplegia and respiratory insufficiency. A slow but progressive improvement to an almost complete functional recovery occurred with some persistent reflex deficits. Finger clubbing has been reported with abuse of senna and is reversible with discontinuation of the drug.

Clinical Management

Basic and advanced life-support measures should be utilized. Activated charcoal without a cathartic may be used in early decontamination. Most ingestions are self-limiting. Treatment after decontamination is symptomatic and supportive. Monitoring of fluids and electrolytes is recommended for symptomatic patients. If a significant ingestion of anthraquinones does occur, Borntrager's reaction may occur (red color is seen in alkaline urine and a yellow-brown color in acid urine). No other specific laboratory tests are available to assist in diagnosis and treatment.

See also: Charcoal; Gastrointestinal System; Plants, Poisonous.

Relevant Website

http://www.cbif.gc.ca – Canadian Poisonous Plants Information System; search for Alder Buckthorn.

Busulfan

Matthew Janes

- Chemical Abstracts Service Registry Number: CAS 55-98-1
- Synonyms: Preferred: 1,4-Butanediol dimethanesulfonate; Myleran®. Also 1,4-Bis- or di(methane-sulfonoxyl)butane; Tetramethylene ester of methanesulfonic acid; Busulphan; C.B.2041; Mablin; Myeloleukon; Mielevcin; Misulban; Myelosan; Mitosan; Sulfabutin; Tetramethylene bis(methanesulfonate); 1,4-Bis(methanesulfonoxy) butane; 1,4-Bis(methanesulfonyloxy)butane
- Chemical/Pharmaceutical/Other Class: Chemotherapeutic agent, bifunctional alkylating agent
- Chemical Formula: $CH_3SO_2O(CH_2)_4OSO_2CH_3$

Uses

Busulfan has been used extensively as a chemotherapeutic agent in the treatment of a variety of cancers. Busulfan is clinically successful for treating leukemia, solid tumors, and correcting hemoglobinopathies, and other inborn errors of the immune system. It is

currently used primarily in the treatment for myeloproliferative disorders such as chronic myelogenous leukemia (CML), polycythemia vera, and essential thrombocythemia. Busulfan is also an effective and sufficiently safe drug used as a conditioning regimen for autologous or allogeneic bone marrow transplantations (BMTs) in efforts to induce organ transplant tolerance.

Background Information

First investigations of busulfan's antitumor characteristics were noted in CMLs in 1953 by Galton. Active development of the compound was pursued by GlaxoSmithKline, launching Myleran in 1954 for the treatment of CML in the United States. Following its development in the United States, Myleran was recommended for approval in Europe in 1983. Busulfan has become a widely adopted therapy. The drug was developed as a substitute for total body irradiation preceding BMT. Preparative regimens were first studied in a rat myelocytic leukemia model and then moved into clinical trials. Doses of busulfan in combination with cyclophosphamide or other drugs have been shown to be effective as a preparative regimen for patients undergoing allogeneic or autologous BMT. There are reports of more than 500 trials involving more than 15 000 patients receiving BMTs in the literature. Complete response rates from various leukemia patients described in literature have exceeded 50% in busulfan-based regimens. Regimen-related mortality is described to be lower then antileukemia benefits in the aggregate for acute and chronic myeloid leukemia as well as acute lymphocytic leukemia.

Recent clinical studies have been focusing on a major drawback of high-dose busulfan regimens. Oral formulation is the major route of uptake for the drug. Erratic and unpredictable absorption of the compound from the gastrointestinal tract have resulted in large inter-/intrapatient variations in the busulfan plasma concentrations. Pharmacokinetic studies have verified great interpatient variations due to age, underlying diseases, and drug–drug interactions. Busulfan bioavailability shows great variation in children to adults. Investigators have reported hepatic veno-occlusive disease (VOD) as a common complication associated with this regimen. VOD occurs in over half of bone marrow transplant patients, and although approximately half of all cases resolve, the mortality rate can be over 90% in severe cases. Acute central nervous system toxicity (grand mal seizures), pulmonary complications, and VOD are associated with high systemic exposure expressed as area under the plasma concentration–time curve (AUC). The aim of current clinical investigations lies in the variable toxicodynamic effects of busulfan.

Exposure Routes and Pathways

Exposure to this compound therapeutically is by the oral route. Since variation in the AUC for oral busulfan results in substantial risk of over- or undertreatment with risk of toxicity or relapse, the use of an intravenous (IV) formulation has been studied. IV formulation reduces this variability by eliminating variability in absorption. Busulfan is a small, highly lipophilic molecule that easily passes the blood–brain barrier. The typical dosage level (tablet form) is 4–8 mg daily. The recommended intravenous dose given prior to bone marrow transplant is $0.8\,\mathrm{mg\,kg^{-1}}$ body weight given as a 2 h infusion every 6 h for 4 days.

Toxicokinetics

Toxicokinetic studies have shown that high-dose busulfan toxicities are age dependent, and directly correlated with AUC. Pharmaco-/toxicokinetic-guided dose adjustments are given to patients receiving IV formulations. Targeted AUC of 1250 µmol min ($\pm 20\%$) are monitored in an attempt to optimize the antitumor effect and minimize serious toxicity. AUC values are currently being studied in correlation with patient outcomes, including survival time, gastrointestinal toxicity, mucositis, hepatic toxicity, and acute graft-versus-host disease, to effectively define an optimal therapeutic window. Radioactively labeled busulfan studies have been carried out to quantitate toxicokinetics. Gas liquid chromatography analysis suggests that busulfan doses of 2–6 mg are well absorbed and the data can be extrapolated to a zero-order absorption, one compartment open model. The mean half-life of elimination was 2.57 h in humans. Patients receiving high-dose administration ($1\,\mathrm{mg\,kg^{-1}}$ orally every 6 h for 4 days) show variability in mean steady-state plasma concentrations after dosing, with unpredictable elimination half-life. Variable absorption kinetics suggests oral formulations are very difficult to effectively evaluate for clinical activity.

Mechanism of Toxicity

Busulfan is a bifunctional alkylating agent. It consists of two methanesulfonate groups attached at opposite ends of a four-carbon alkyl chain. Alkylating agents form covalent DNA interstrand cross-links that inhibit DNA synthesis. Toxicity of busulfan's alkylation of intercellular nucleophiles is associated with its

biological activity. The N-position of guanine and other DNA sites tend to be the main site of alkylation and the release of the methylsulfonate group. Its mechanism of action is still not fully understood.

Induced changes in biological parameters of the cell cycle have been assayed in various doses and exposure times, all indicating cytotoxic effects to rapidly proliferating tissues, in particular to the cells of the granulopoietic lineage of the bone marrow. In all studies busulfan toxicities occur in an AUC-dependent manner inducing apoptosis. *In vitro* studies show decrease in proliferation and colony formation and arrest of cell cycle in G2 phase. The development of apoptosis occurred secondarily to the interruption of other vital metabolic pathways that still remain to be characterized. Typical chemotherapeutic-induced apoptotic effects are observed in *in vitro* studies.

Acute and Short-Term Toxicity (or Exposure)

Busulfan is a potent cytotoxic drug. Early in development of the compound, *in vivo* experiments indicated that busulfan caused severe depression in the bone marrow. The most prevalent acute toxic effects associated with busulfan in animals are severe pancytopenia from bone marrow failure. Associated *in vivo* experiments show bone marrow aplasia, stromal cell damage, immunosuppression (impaired T-lymphocyte function), and pronounced adverse effects on reproductive glands, germ cells, and fertility in animals (lowest effective dose tested was $2\,mg\,kg^{-1}$).

Chronic Toxicity (or Exposure)

Busulfan is mutagenic in mice and, possibly, in humans. Chronic toxicity in animals is manifested by carcinogenic, teratogenic, mutagenic, and reproductive effects. Busulfan is known to be a human carcinogen based on sufficient evidence of carcinogenicity in humans. Patients receiving busulfan have developed leukemia as well as cytological and hematological abnormalities. Human studies show severe side effects associated with bone marrow failure resulting in severe pancytopenia.

In Vitro Toxicity Data

Busulfan has been shown to induce gene mutations and chromosomal damage in bacteria, fungi, plant species, *Drosophila*, and in animal cell lines in culture. Busulfan does not require S9 activation in *in vitro* toxicity assays.

Clinical Management

The difficulty in determining dose delivered with oral administration of high-dose busulfan in preparative regimens for hematopoietic stem cell transplantation results in lethal toxicity due to overdosing and increased potential for relapse with recurrent disease. Oral pharmacokinetic studies ineffectively determine proper AUC for reliable establishment for a proper therapeutic dose. Studies with IV formulations have demonstrated that all patients are evaluable. With the development of a limited sampling strategy to analyze proper AUC over intermittent time periods, improved patient risk profiles for busulfan have been implemented in clinical practice.

Environmental Fate

Busulfan is a white powder that is soluble in acetone. The powder is insoluble in water and readily hydrolyzes in water. Any environmental hazards that pose threats to the environment remain unknown. Busulfan is a pharmaceutical and is used in relatively small amounts; therefore, so far it has been of little regulatory concern to the US Environmental Protection Agency. The potential threat of busulfan exposure is limited to workers involved in its manufacture, to patients receiving this agent as a chemotherapeutic regime, and to hospital personnel administering the drug to patients.

Miscellaneous

The risks of taking the medicine must be weighed against the good it will do. Precautions should be taken if therapy is prescribed during pregnancy and breastfeeding. Children and older adults are particularly susceptible to side effects and require closer monitoring during therapy. Busulfan will temporarily lower the number of white blood cells in the blood, increasing the chances of getting an infection. It can also lower the number of platelets, which are necessary for proper blood clotting. A physician or drug index should be consulted for other common side effects, including allergies.

See also: Cancer Chemotherapeutic Agents; Immune System.

Further Reading

Bishop JB and Wassom JS (1986) Toxicological review of busulfan (Myleran). *Mutation Research* 168: 15–45.

Buggia I, Locatelli F, Regazzi MB, and Zecca M (1994) Busulfan. *The Annals of Pharmacotherapy* 28: 1055–1062.
Galton DGA (1953) Myleran in chronic myeloid leukemia: Results of the treatment. *Lancet* 1: 208–213.
Vaughan WP, Carey D, Perry S, Westfall AO, and Salzman DE (2002) A limited sampling strategy for pharmocokinetic therapy with intravenous busulfan. *Biology of Blood and Marrow Transplantation* 8: 619–624.

Relevant Website

http://www.rxmed.com – RxMed: Pharmaceutical Information – Myleran.

Butadiene, 1,3-

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 106-99-0
- Synonyms: Biethylene; Butadiene; Divinyl; Erythrene; Pyrrolylene; Vinylethylene
- Chemical Formula: C_4H_6
- Chemical/Pharmaceutical/Other Class: Hydrocarbon
- Chemical Structure: $H_2C=CH–CH=CH_2$

Uses

Butadiene is used as a chemical intermediate and as a polymer component in the synthetic rubber industry, the latter accounting for ~75% of the butadiene produced. Styrene–butadiene rubber, polybutadiene rubber, adiponitrile, styrene–butadiene latex, acrylonitrile–butadiene–styrene resins, and nitrile rubber are used in the manufacture of tires, nylon products, plastic bottles and food wraps, molded rubber goods, latex adhesives, carpet backing and pads, shoe soles, and medical devices.

Exposure Routes and Pathways

Butadiene is a gas under normal environmental conditions. In the workplace, the most significant route of exposure to butadiene is inhalation during its production and use. Potential exposures to butadiene are likely to be limited to the industrial setting as the residual butadiene monomer content in consumer products is low and unlikely to pose a significant health threat to the general public. Butadiene is also produced during the combustion of organic matter. Significant amounts of butadiene are released to the environment from both natural and anthropogenic sources such as forest fires, gasoline and diesel engine exhaust, and wood space heating. It is also a component of cigarette smoke.

Toxicokinetics

Butadiene appears to be readily absorbed through the respiratory tract. Dermal absorption is anticipated to be limited due to the volatility of liquid butadiene. Although limited, available data indicate that the uptake of butadiene at comparable exposure levels is greatest in the mouse (5- to 100-fold more than rat), with progressively lesser amounts by the rat and monkey (4- to 14-fold less than rat).

Metabolism of butadiene is qualitatively similar across species, although there are quantitative differences in the amounts of metabolites formed. Butadiene is rapidly metabolized via enzyme systems located in the liver, lung, nasal mucosa, and possibly bone marrow. Initially, butadiene is converted to the reactive metabolite 1,2-epoxy-3-butene (EB) by cytochrome P450 monooxygenase, an enzyme that also metabolizes EB to another reactive metabolite 1,2:3,4-diepoxybutane (DEB). EB and DEB, which are thought to cause the DNA damage necessary for the butadiene-induced tumorigenesis, are further metabolized (inactivated) by conjugation with glutathione via glutathione *S*-transferase and by hydrolysis via epoxide hydrolase. Glutathione conjugation predominates in mice followed by rats and then humans; conversely, hydrolysis via epoxide hydrolase predominates in humans followed by rats and then mice. The activation/inactivation profiles of these three enzyme systems are species specific. In comparably exposed rodents, tissue levels of EB and DEB in mice can be several fold and 100-fold higher, respectively, than those in rats.

Once absorbed, butadiene is rapidly distributed throughout the body. A tissue distribution study in rats indicates that the highest concentrations of butadiene are located in peripheral fat; lower concentrations are observed in the liver, brain, spleen, and kidney.

Based on a radiolabel study in mice, most of the absorbed butadiene is exhaled as the parent compound, with a lesser amount exhaled as CO_2. Smaller amounts of butadiene and/or its metabolites are detected in urine and feces, with most of the label being eliminated from the carcass within 65 h. This is

consistent with another study in rats and mice that showed that the bulk of their butadiene body burden (77–99%) is eliminated with a half-life of 2–10 h.

Mechanism of Toxicity

The mechanism by which butadiene exerts acute toxicity is unknown. The carcinogenic potential of butadiene in rodents is thought to reflect the metabolism of butadiene to DNA-reactive metabolites resulting in genetic alterations in protooncogenes and/or tumor suppressor genes. Mechanistic data suggest that the higher carcinogenic potency of butadiene in mice versus rats is primarily due to the higher body burden of DEB in mice. This is supported by the observations that carcinogenicity tests with EB were equivocal while DEB was carcinogenic in mice and rats when administered via dermal or subcutaneous exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute inhalation studies have shown that butadiene exhibits low toxicity in animals. Butadiene is a relatively weak central nervous system (CNS) depressant. Mice exposed to butadiene for 6–12 min exhibited excitement and narcosis (200 000 ppm), light narcosis (150 000 ppm), and no effects (100 000 ppm). Deep anesthesia was produced in rabbits exposed to 200 000–250 000 ppm butadiene for 8–10 min; death due to respiratory paralysis occurred within 25–35 min at 250 000 ppm. The LC_{50} in rats exposed to butadiene for 4 h is 128 000 ppm; the LC_{50} in mice exposed to butadiene for 4 h is 117 000 ppm. The acute oral LD_{50} values for butadiene in rats and mice are 5480 and 3210 mg kg^{-1}, respectively. Butadiene is mutagenic and clastogenic in rodents with mice being more sensitive to genetic damage than rats. Both EB and DEB are mutagenic and/or clastogenic *in vivo* with little consistent evidence of interspecies differences. These *in vivo* genotoxicity data suggest that the interspecies differences in butadiene-induced toxicity are related to quantitative differences in the formation of reactive metabolites.

Human

Exposures of industrial workers to butadiene concentrations of 2000–8000 ppm have been reported to cause eye, skin, and nasal irritation. High butadiene levels may cause CNS depression as evidenced by blurred vision, drowsiness, fatigue, bradycardia, and hypotension. The mildly aromatic odor of butadiene, which can be detected at $\sim$1 ppm, serves as a good warning aid. Dermal contact with liquid butadiene may produce frostbite due to cooling caused by the rapid evaporation of butadiene from the skin.

Chronic Toxicity (or Exposure)

Animal

Lifetime studies in rats and mice indicate that the inhalation of butadiene increases the incidence of tumors at various sites, with mice being significantly more susceptible to the tumorigenic effect of butadiene than rats. In mice, tumors were induced at lifetime exposures of 6.25–1250 ppm or in as little as 13 weeks at 625 ppm. In rats, tumors were observed primarily at 8000 ppm and typically only in organs where tumors develop spontaneously. A similar species difference was noted in noncarcinogenic effects on reproductive organs. In mice, ovarian atrophy was induced in a dose-dependent fashion at lifetime exposures $\geqslant$6.25 or at 1000 ppm after a 13 week exposure. Degenerative changes in the testes of mice were observed only after lifetime exposures to butadiene at $\geqslant$200 ppm. No effects on the reproductive organs were seen in rats receiving a lifetime exposure up to 8000 ppm butadiene. Based on limited available data, there is no conclusive evidence that butadiene is fetotoxic or teratogenic at concentrations below those toxic to the mother.

Human

It is not known if butadiene itself poses a carcinogenic risk in humans. A large well-conducted epidemiological study reported an increase in mortality from leukemia among workers in the styrene–butadiene rubber industry and that the increase was associated with cumulative butadiene exposure. The risk of leukemia remained but was attenuated after controlling for exposures to styrene and other potential confounding agents. However, these results were not consistent with those of two smaller studies of adequate statistical power in butadiene monomer workers. In a recent study, air exposure as well as biomarkers of exposure and genetic toxicity were evaluated in workers occupationally exposed to low levels of butadiene monomer. Air exposures and hemoglobin adducts were well correlated, but no correlation existed with any genetic effect biomarkers. Thus, while there may be an increased risk of cancer in the styrene–butadiene industry, it is unclear as to what portion of the risk is related to butadiene alone.

In Vitro Toxicity Data

Butadiene was mutagenic with metabolic activation in the Salmonella reverese mutation assay, while

mutagenic responses in the mouse lymphoma assay were equivocal. In cultured mammalian cells, butadiene dissolved in ethanol was clastogenic while gaseous butadiene was not. Both EB and DEB are mutagenic and clastogenic in the absence of exogenous metabolic activation.

Clinical Management

The primary toxicity of butadiene is CNS depression at high concentrations. Treatment involves removal from exposure and support of respiratory function.

Environmental Fate

Butadiene is a gas under normal environmental conditions with limited water solubility (735 mg l^{-1} at 25°C). Butadiene released to the atmosphere will remain there with very small amounts being distributed to water and soil. In air, butadiene will be removed by reaction with photochemically produced hydroxyl radicals (0.24–1.9 day half-life), nitrate radicals, and ozone. When released to water, butadiene will be removed by volatilization to air (Henry's law constant of 7460 Pa m^3 mol^{-1}), biodegradation (aerobic half-life of 7–28 days), and reaction with singlet oxygen. Based on its estimated organic-carbon partition coefficient (K_{oc} of 72–228), butadiene will not exhibit significant adsorption to soil. Due to volatilization to air and degradation in soil, butadiene is not expected to leach to groundwater. Similarly, it is not expected to bind significantly to or suspended particulate matter. As butadiene is readily metabolized, it is not expected to pose a significant bioaccumulation hazard.

Ecotoxicology

Butadiene exhibited a 24 h LC_{50} of 71.5 mg l^{-1} in an estuary fish species; estimated LC_{50} values for a variety of freshwater fish ranged from 21.4 to 49.8 mg l^{-1}. A 96 h EC_{50} (immobilization) of 24.8 mg l^{-1} was reported in an aquatic invertebrate (*Daphnia magna*).

Other Hazards

Butadiene is a highly flammable gas at standard temperature and pressure. In air, butadiene will form explosive peroxides that are sensitive to shock or heating above 27°C and will explode upon contact with aluminum tetrahydroborate. Butadiene has lower and upper explosive limits of 2% and 11.5% by volume in air, respectively.

Exposure Standards and Guidelines

International occupational exposure limits (OEL) for butadiene generally range from 0.5 to 15 ppm as an 8 h time-weighted average (TWA), with 2 ppm being the TWA OEL established by the American Conference of Governmental Industrial Hygienists (ACGIH). The US Occupational Safety and Health Administration lists a permissible exposure limit of 1 ppm for butadiene (TWA) with a 15 min short-term exposure limit of 5 ppm. The National Institute of Occupational Safety and Health indicates 2000 ppm butadiene is immediately dangerous to life or health. Butadiene is classified as probably carcinogenic to humans (group 2A) by the International Agency for Research on Cancer and as known to be a human carcinogen by the US National Toxicology Program.

See also: Carcinogen Classification Schemes; Styrene.

Further Reading

Hughes K, Meek ME, Walker M, and Beauchamp R (2003) 1,3-Butadiene: Exposure estimation, hazard characterization, and exposure–response analysis. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews* 6(1): 55–83.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Butadiene.

Butane

Michael A Kamrin

- Chemical Abstracts Service Registry Number: CAS 106-97-8
- Synonyms: *n*-Butane; Butyl hydride; Methylethylmethane; Liquefied petroleum gas
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon
- Chemical Formula: $CH_3(CH_2)_2CH_3$

Uses

Butane is used as a fuel in lighters, small blow torches, and camping stoves. It is also used in calibrating instruments and as a food additive. In addition, it is a raw material for organic synthesis.

Exposure Routes and Pathways

Since butane is a gas, the major routes of exposure are inhalation and contact with skin and eyes. It is a widely used substance of abuse by inhalation.

Mechanism of Toxicity

Gaseous butane acts as a simple asphyxiant, which means that it causes toxicity by displacing oxygen and preventing it from reaching important tissues and organs. In its liquid state, it causes frostbite due to rapid cooling on evaporation.

Acute and Short-Term Toxicity (or Exposure)

Animal

An LC_{50} of $658\,g\,m^{-3}$ has been established in rats for a 4 h inhalation exposure.

Human

Because of its asphyxiant properties, high doses of inhaled butane can affect the central nervous system and lead to a variety of symptoms. These include euphoria, excitation, vomiting, confusion, hallucinations, drowsiness, and coma. Skin contact with liquid butane can cause frostbite.

Clinical Management

The affected person should be removed from exposure and provided fresh air. Symptomatic and supportive treatment should be administered. This may include support of both the cardiovascular and respiratory systems.

Environmental Fate

Butane is relatively nonpersistent in the environment and has a low leaching potential. It is moderately volatile from water and it does not bioaccumulate.

Other Hazards

Butane poses severe fire and explosion hazards. It should be stored and used distant from any ignition sources.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists time-weighted average (TWA) for butane is 800 ppm ($1900\,mg\,m^{-3}$) and the TWA for liquefied petroleum gas is 1000 ppm ($1800\,mg\,m^{-3}$).

See also: Drugs of Abuse.

Further Reading

International Program on Chemical Safety (1998) *Butane*. INCHEM Poisons Information Monograph 945, Geneva, Switzerland.

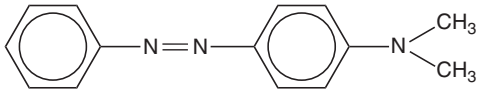
Relevant Website

http://www.inchem.org – Chemical Safety Information from Intergovernmental Organization.

Butter Yellow

Kashyap N Thakore

- Chemical Abstracts Service Registry Number: CAS 60-11-7
- Synonyms: *p*-Dimethylaminoazobenzene (DAB); *N*,*N*-Dimethyl-4-(phenylazo) benzenamine; Methyl yellow; C.I. solvent yellow 2; C.I. 11020
- Chemical/Pharmaceutical/Other Class: Food dye
- Chemical Formula: $C_{14}H_{15}N_3$
- Chemical Structure:



Uses

Early in last century, butter yellow was largely used as a food coloring. It is also used for the determination

of free HCl in gastric juice, spot test identification of peroxidized fats, as a pH indicator, and as a laboratory reagent.

Exposure Routes and Pathways

Inhalation is the most common route of exposure. When heated to decompose, it emits toxic fumes of nitrous oxides.

Toxicokinetics

Butter yellow may be rapidly absorbed by various routes including ingestion, inhalation, and dermal contact. Biotransformation involves reduction (catalyzed by at least two types of cytochrome P450) and cleavage of the azo group, demethylation, ring hydroxylation, *N*-hydroxylation, *N*-acetylation, and *O*-conjugation of metabolites in liver. The metabolites can bind to proteins and nucleic acids. When [^{14}C-dimethyl]-aminoazobenzene was fed to rats, most of the radioactivity was found in expired carbon dioxide. Urine of rats administered butter yellow contained 50–60% of it in the form of sulfates or glucuronides of *N*-acetylated metabolites.

Mechanism of Toxicity

It is metabolized *in vivo* to a reactive form that covalently binds to cellular macromolecules, such as proteins and DNA, to cause toxicity. Agents that prevent these bindings can decrease the toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Butter yellow is poisonous by the intravenous route. It is moderately toxic by the oral, intraperitoneal, intramuscular, and subcutaneous routes. It is an antihypertensive agent.

Human

The only occupational health observation in humans was of contact dermatitis in factory workers handling butter yellow. The target organs for toxicity are skin, liver, and bladder. Potential symptoms of overexposure are enlarged liver, hepatic and renal dysfunction, contact dermatitis, coughing, wheezing, difficulty in breathing, bloody sputum, bronchial secretions, frequent urination, hematuria, and dysuria.

Chronic Toxicity (or Exposure)

Animal

It shows mutagenic properties after activation. It is carcinogenic by various routes in the rat and mouse (liver carcinoma). By the oral route, it causes carcinoma of the bladder and lungs. Its carcinogenic action is influenced by diet. It is shown to be teratogenic. The LD_{50} in the rat is $200\,mg\,kg^{-1}$ orally and $230\,mg\,kg^{-1}$ intraperitoneally. The LD_{50} in the mouse is $300\,mg\,kg^{-1}$ orally and $230\,mg\,kg^{-1}$ intraperitoneally.

Human

Butter yellow can also cause adverse reproductive effects.

In the United States, Occupational Safety and Health Administration lists butter yellow as a suspected human carcinogen. Human mutation data are also reported.

Workers exposed to butter yellow should wear personal protective equipment and their work should be carried out only in restricted areas. Technical measures should prevent any contact with the skin and mucous membranes. After use, clothing and equipment should be placed in an impervious container for decontamination or disposal. Preemployment and periodic medical examination should focus on liver function.

In Vitro Toxicity Data

Butter yellow is active in inducing unscheduled DNA synthesis in Hela human cervical cancer cells. It was mutagenic in *Salmonella typhimurium* TA100 and TA98.

Clinical Management

In case of contact, the eyes and skin should be flushed with water for 15–20 min. For inhalation exposure, the victim should be moved to fresh air. Oxygen and artificial respiration should be administered, if necessary. If the patient is in cardiac arrest, cardiopulmonary resuscitation should be given. Life-support measures should be continued until medical assistance has arrived. In the case of an unconscious or convulsing person, liquids should not be administered and vomiting should not be induced.

Environmental Fate

It may bind to the soil. It may bioconcentrate in aquatic organisms, adsorb to sediment, and may be subject to direct photolysis.

See also: Food Additives.

Relevant Websites

http://ntp.niehs.nih.gov – National Toxicology Program (NTP) (2002) 10th *Report on Carcinogens*. Research Triangle Park, NC: US Department of Health and Human Services, Public Health Service.

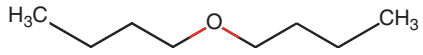
http://www.cdc.gov/niosh – National Institute for Occupational Safety and Health (NIOSH) (2003) *Pocket Guide to Chemical Hazards*, Cincinnati, OH.

http://www.iarc.fr – IARC (1987) Monographs on the Evaluations of Carcinogenic Risks to Humans: Complete List of Agents, Mixtures and Exposures Evaluated and their classification; *para*-Dimethylaminoazobenzene; Suppl. 7; p. 62.

Butyl Ether

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 142-96-1
- Synonyms: Dibutyl ether; 1-Butoxybutane, dibutyl oxide, di-*n*-butyl ether, *n*-dibutyl ether
- Chemical/Pharmaceutical/Other Class: Ether
- Chemical Formula: $C_8H_{18}O$
- Chemical Structure:



Uses

Butyl ether is used mainly as a solvent for organic materials such as resins, oils, hydrocarbons, esters, gums, and alkaloids. It is also used as an extracting agent in metal separation and as a reacting medium in organic synthesis processes. It is a solvent commonly found in teaching, research, and analytical laboratories.

Exposure Routes and Pathways

Exposure to butyl ether can occur through inhalation of vapor or mist, dermal contact, or oral ingestion of liquid dibutyl ether. Oral ingestion of dibutyl ether has been practiced to produce an 'alcoholic' euphoria. Occupational exposure to dibutyl ether may occur through inhalation and dermal contact with this compound at workplaces where dibutyl ether is produced or used. The general population may be exposed to dibutyl ether through the use of consumer products, such as latex paints, containing this compound.

Toxicokinetics

Dibutyl ether is rapidly adsorbed and eliminated from the body. Dibutyl ether can cause irritation to the skin, mucous membranes, eyes, and respiratory and gastrointestinal tracts. Systemically, dibutyl ether causes central nervous system (CNS) depression and transient liver changes.

Mechanism of Toxicity

Butyl ether has the ability to dissolve lipids. As a result, it may cause irritation and pain upon contact with eyes and nose mucosa. It also causes dermal irritation and dermatitis upon contact with the skin. Damage caused by butyl ether appears to be scattered loss of epithelial cells due to dissolution of phospholipid cell membranes. At the CNS level, butyl ether, like other volatile organic solvents, depresses the CNS by dissolving in the cell lipid membrane and disrupting the lipid matrix. These effects are known as membrane fluidization. At the molecular level, membrane fluidization disrupts solute gradient homeostasis that is essential for cell function.

Acute and Short-Term Toxicity (or Exposure)

Animal

Butyl ether is moderately toxic by the oral route. The oral LD_{50} in rats has been reported to range from 3200 to 7400 mg kg^{-1}. The inhalation LC_{50} in rats has been found to be 4000 ppm in air for 4 h. The skin LD_{50} in rabbits is 10 000 mg kg^{-1}.

Human

Signs and symptoms of excessive exposure to dibutyl ether resemble those of ethanol intoxication except that symptoms are seen shortly after exposure and the effects are short lived. Typical symptoms include dizziness, giddiness, headache, euphoria, and CNS depression.

Chronic Toxicity (or Exposure)

In humans, chronic, repeated dermal exposure may cause dermal irritation, defatting of skin, and

dermatitis. Excessive consumption of dibutyl ether as an intoxicating agent has been reported to produce ether jags, respiratory depression, and death.

Clinical Management

Given the CNS and respiratory depression properties of dibutyl ether, treatment is directed at maintaining respiration and treating irritation at the site of exposure. Patient should be monitored for respiratory distress and apnea, hyperglycemia, as well as hepatic and renal dysfunction.

Environmental Fate

Dibutyl ether's production and use as an extracting agent and as a solvent may result in its release to the environment through various waste streams. If released to air, a vapor pressure of 6.0 mmHg at 25°C indicates dibutyl ether will exist solely as a vapor in the ambient atmosphere. Vapor-phase dibutyl ether will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 13 h. Direct photolysis is not expected to be an important removal process since aliphatic ethers do not absorb light in the environmental spectrum. If released to soil, dibutyl ether is expected to have high mobility based upon an estimated K_{oc} of 51. Volatilization from moist soil surfaces may be an important fate process based upon a Henry's law constant of 6.0×10^{-3} atm m^3 mol^{-1}. Dibutyl ether is expected to volatilize from dry soil surfaces based upon its vapor pressure. If released into water, dibutyl ether is not expected to adsorb to suspended solids and sediment in water based on its K_{oc}. Aqueous screening studies indicate biodegradation may be an important fate process in both soil and water; 16% BODT was observed over a period of 5 days using acclimated microbial cultures and dibutyl ether reached 3–4% of its theoretical BOD over 4 weeks using an activated sludge seed. Volatilization from water surfaces is expected to occur based on this compound's estimated Henry's law constant. Estimated volatilization half-lives for a model river and model lake are 3.5 h and 4.6 days, respectively. Bioconcentration factors (BCFs) ranging from 30 to 114 in carp suggest that bioconcentration in aquatic organisms is moderate to high. Dibutyl ether is not expected to undergo hydrolysis in the environment due to the lack of hydrolyzable functional groups.

See also: Ethanol.

Further Reading

Sax NI and Lewis RJ (eds.) (1989) *Dangerous Properties of Industrial Materials*, 7th edn. New York: Van Nostrand Reinhold.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Butyl Ether.

Butyl Nitrite

Kashyap N Thakore

- Chemical Abstracts Service Registry Number: CAS 544-16-1
- Synonyms: NBN; NCI-C56553; Nitrous acid-*n*-butyl ester
- Chemical Formula: $C_4H_9NO_2$
- Chemical Structure: $CH_3CH_2CH_2CH_2ONO$

Uses

Butyl nitrite is used in the manufacture of rare earth azides. It is also used as a recreational drug (for vasodilatation).

Exposure Routes and Pathways

Butyl nitrite is a poison by ingestion and intraperitoneal routes. It is mildly toxic by inhalation. When heated to decompose, it emits toxic fumes of nitrogen oxides.

Toxicokinetics

Butyl nitrite is ineffective by ingestion because it is degraded in the gastrointestinal tract. A 44% uptake of butyl nitrite was observed when rats were atmospherically exposed for 5 min periods. It is very rapidly transformed in the body. The likely products of butyl nitrite *in vivo* might be butyl alcohol, methemoglobin, nitrite ion, nitrate ion, nitrosothiols, and possibly other nitroso compounds. Butyl nitrite

is also very rapidly distributed to various parts of the body such as muscles and vascular and circulating systems. The metabolites bind to hemoglobin, glutathione, and other plasma proteins. Metabolites such as nitrite ions can be eliminated in exhaled air.

Mechanism of Toxicity

Following exposure, butyl nitrite causes rapid *S*-nitrosyl glutathione formation, then a concomitant decrease in protein thiols, followed by a marked adenosine triphosphate depletion. It also causes lipid peroxidation. It produces methemoglobinemia in which oxidized hemoglobin has no oxygen carrying capacity. Also in the clinical state of methemoglobinemia, the unaltered hemoglobin shows an increased affinity for oxygen resulting in symptoms of tissue hypoxia. Cyanosis occurs when methemoglobin levels are greater than 10%. Levels above 70% are potentially lethal.

Acute and Short-Term Toxicity (or Exposure)

Animal

The formation of butyl alcohols from butyl nitrite in experimental mice produced hepatotoxicity. The oral LD_{50} is 83 mg kg^{-1} in rats and 171 mg kg^{-1} in mice; the intraperitoneal LD_{50} is 158 mg kg^{-1} in mice. The LC_{50} is 420 ppm/4 h in rats and 567 ppm/1 h in mice.

Human

Butyl nitrite is harmful if swallowed, inhaled, or absorbed through skin. It causes irritation of eyes, skin, mucous membranes, and the upper respiratory tract. Overexposure by ingestion can cause methemoglobinemia–carboxyhemoglobinemia, lowered blood pressure by vasodilatation, headache, pulse throbbing, and weakness. It can also cause behavioral changes such as altered sleep time, excitement, change in motor activity, ataxia, and rigidity. It also causes dyspnea, cyanosis, and changes in liver and kidneys. It is immunosuppressive for human lymphocytes *in vitro*.

Workers exposed to butyl nitrite should wear personal protective equipment and their work should be carried out only in restricted areas. Clothing and equipment after use should be placed in an impervious container for decontamination or disposal. Technical measures should prevent any contact with the skin and mucous membranes.

Chronic Toxicity (or Exposure)

It has not been tested for cancer, reproductive, and other long-term effects. It can initiate tumors via *in vivo* formation of *N*-nitroso compounds from butyl nitrite following exposure.

Clinical Management

In case of contact, affected eyes and skin should be flushed with water for 15–20 min. If inhaled, the affected person should be moved to fresh air. If necessary, oxygen and artificial respiration should be administered. If patient is in cardiac arrest, cardiopulmonary resuscitation should be administered. Life-support measures should be continued until medical assistance has arrived. Liquids should not be administered and vomiting should not be induced in an unconscious or convulsing person.

See also: Lipid Peroxidation.

Relevant Website

http://www.drugabuse.gov – National Institute on Drug Abuse (NIDA) (1988) Health Hazards of Nitrite Inhalants, Research Monograph Series 83, pp. 27–39.

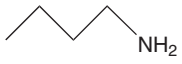
Butylamines

Janice McKee

- Representative Chemicals: *n*-Butylamine; *s*-Butylamine; *t*-Butylamine
- Chemical Abstracts Services Registry Numbers:
  - *n*-Butylamine (CAS 109-73-9)
  - *s*-Butylamine (CAS 13952-8-6)
  - *t*-Butylamine (CAS 75-64-9)
- Synonyms:
  - *n*-Butylamine: 1-Aminobutane; 1-Butanamine; Aminobutane; Butyl amine; Monobutylamine; mono-*n*-Butylamine; *n*-Butylamine; Norralamine; Tutane;
  - *s*-Butylamine: 2-Butylamine; 2-Butanamine; 2-Aminobutane; Frucote; Deccotane; 1-Methylpropane; 1-Methylpropylamine;

- *t*-Butylamine: Isobutylamine; 2-Methyl-2-propanamine; 2-Aminoisobutane; 2-Amino-2-methyl-propane; 1,1-Dimethylethylamine; 2-Methyl-2-aminopropane; Trimethylaminomethane

- Chemical/Pharmaceutical/Other Class: Amine
- Chemical Formulas:
  - *n*-Butylamine: $CH_3CH_2CH_2CH_2NH_2$
  - *s*-Butylamine: $CH_3CH_2CH(NH_2)CH_3$
  - *t*-Butylamine: $(CH_3)_2CHCH_2NH_2$
- Chemical Structure:



Uses

Butylamines are used for many purposes, including as intermediates in the manufacture of textiles, plastics, dyes and tanning agents, corrosion inhibitors, lubricating oil additives, antioxidants, fungicides, herbicides, rubber products, and emulsifying agents. They are also used in pharmaceuticals, photographic materials, and as flavors in seafood and chocolate. In addition, they are reportedly used in alcoholic beverages, ice cream, candy, baked goods, etc., and can occur naturally in some plants and foods.

Exposure Routes and Pathways

Butylamines are primary irritants and may be encountered as vapor, liquid, or as components of mixtures. They may cause damage at the point of contact (i.e., skin, eyes, lungs, and gastrointestinal tract) and also may be absorbed into the body through the intact skin, when inhaled or ingested.

Occupational exposure may occur through inhalation and dermal contact where these chemicals are produced or used. Most exposures to the general population are through various foods, from water, and from inhalation of ambient air.

Toxicokinetics

Butylamines are well absorbed from the gut and respiratory tract. Butylamines are expected to be readily metabolized, and the metabolic pathway is similar to that of other lower amines. Amines may be metabolized by monoamine oxidase and diamine oxidase (histaminase).

Mechanism of Toxicity

Butylamines are strong alkalis and potent skin, eye, and mucous membrane irritants. Contact may cause minor irritation to severe tissue damage. Butylamines may be neutralized (strong alkali to a weak acid/base) by hydrochloride, for example, in the stomach. Cellular changes may include hyperplasia, squamous metaplasia, and necrosis. Amines may cause a selective blockade of lysosomal degradation of protein. Exposure to pregnant animals has been shown to harm the fetus. The mechanism of fetotoxicity may be free radical production, metabolic acidosis, and lysosomotrophy.

Acute and Short-Term Toxicity (or Exposure)

Butylamines are primary irritants. Direct contact with liquid or sufficiently high concentrations of vapor may cause severe irritation, blistering, burns, and tissue necrosis. Contact with the eye may result in loss of vision. Ingestion of *n*-butylamine at sufficiently high concentrations may cause irritation to the mouth, throat, and gastrointestinal tract and may cause nausea, vomiting, and possibly death. Skin absorption may cause damage at the site of contact, as well as nausea, vomiting, and shock. Central nervous system effects have been observed after exposure.

Animal

Ingestion of butylamines has been shown to affect the reproductive process and cause harm to the fetus. Reported effects include increased early postimplantation losses, reduced fetal and placental weight, retarded skeletal development, and malformations.

Inhalation exposure produced maternal effects, but no developmental or fetoxicity effects were reported. Rats have been administered *n*-butylamine hydrochloride by gavage on days 6–15 postcoitum (sperm-positive = day 0), or inhaled *n*-butylamine (whole-body exposure), 6 h day^{-1} on days 6–19 postcoitum. Oral *n*-butylamine HCl 1000 mg kg^{-1} reduced maternal feed consumption, increased early postimplantation losses, reduced fetal and placental weight, retarded skeletal development, and produced malformations; 100 mg kg^{-1} was the no-observed-adverse-effect level (NOAEL) for prenatal developmental toxicity. Inhaled *n*-butylamine produced concentration-dependent nasal epithelial hyperplasia and squamous metaplasia, inflammation, and necrosis; the maternal NOAEL was less than 17 ppm. There were no treatment-related signs of embryo fetotoxicity; particularly, no effects on fetal morphology. The developmental NOAEL was 152 ppm.

Severe skin irritation with necrosis has been reported after dermal contact in the guinea pig. The LD_{50} by dermal exposure in rabbits was reported to be

850 mg kg^{-1} for *n*-butylamine and 2500 mg kg^{-1} for *s*-butylamine.

Butylamines are severely damaging to the eye when directly applied; however, the vapor is only mildly irritating to the eyes. At 3000–5000 ppm, *n*-butylamine produces an irritant response, labored breathing, and pulmonary edema, with death following in a matter of minutes or hours. An inhalation LC_{50} for *n*-butylamine was reported to be 800 mg m^{-3} for 4 h in mice.

At near lethal concentrations of *n*-butylamine administered orally, rats and rabbits exhibited increased reflex excitability, increased pulse and respiration, dyspnea, convulsions, cyanosis, and coma. The LD_{50} in rats was reported to be 147 mg kg^{-1} for *s*-butylamine and 78 mg kg^{-1} for *t*-butylamine. The oral LD_{50} in rats was reported to be 366 mg kg^{-1} for *n*-butylamine, with death due to pulmonary edema. Prior to death, the rats exhibited sedation, ataxia, nasal discharge, gasping, salivation, and convulsions.

Human

The principal hazard of concentrated *n*-butylamine to human health is its capacity to produce severe burns of the skin and eyes, as well as respiratory tract irritation (the maximal effect being pulmonary edema). Harm may occur due to direct contact with liquid or vapor at sufficiently high concentrations. Signs of toxicity include tissue damage at the site of contact, sedation, ataxia, nasal discharge, gasping, and salivation, followed by convulsions and death at very high doses. A minimum lethal human dose of *n*-butylamine has not been defined.

Vapors may be irritating to the respiratory tract at concentrations greater than 5 ppm. Workers with daily exposures from 5 to 10 ppm may complain of nose, throat, and eye irritation, and headaches. Concentrations greater than 25 ppm are difficult to tolerate for even short periods of time and concentrations greater 300 ppm may immediately threaten life. Exposure to *n*-butylamine vapors may result in erythema, particularly about the face. The face and neck may become florid within 3 h after exposure, and desquamation of the facial skin may follow in 3 days. A burning, itching sensation accompanies these symptoms. Exposure to vapors may induce allergic asthma.

Chronic Toxicity (or Exposure)

Animal

Data reviewed indicate that chronic effects may be related to the changes related to irritation. Chronic exposure to skin and mucous membranes at sufficiently high levels may cause inflammation, hyperplasia, metaplasia, and necrosis. No effects were reported as related to *s*-butylamine exposure in a 2 year feeding study in rats and dogs exposed to 2500 ppm in the diet.

Human

Generally, workers exposed to 1–5 ppm do not report symptoms. Individuals with chronic respiratory, skin, or eye disease are at increased risk from *n*-butylamine exposure. Chronic exposure to irritating levels of butylamines can cause symptoms and/or increase the severity of symptoms in people with preexisting conditions.

In Vitro Toxicity Data

Butylamine was not shown to be mutagenic in *Salmonella* in tests reviewed. Millimolar concentrations of various primary aliphatic monoamines have been shown to cause the release of lysosomal beta-glucuronidase in cultured mouse peritoneal macrophages. In selected culture systems exposed to butylamine, lysosomal enzymes were selectively released.

Clinical Management

Degree of injury should be considered when determining initial treatment. Exposed skin and eyes should be immediately irrigated with copious amounts of tepid water. The extent of damage to the eye may not be fully evident until 48–72 h after exposure.

After inhalation exposure, the victim should be moved to fresh air and monitored for respiratory distress. Also, 100% humidified supplemental oxygen with assisted ventilation should be administered as required. If coughing or breathing difficulties are noted, the patient should be evaluated for irritation, bronchitis, or pneumonitis, including chest X-rays and determination of blood gasses. If pulmonary edema is present, positive end expiratory pressure ventilation and steroids should be considered. For ingestion exposures, copious amounts of water should be given to dilute stomach contents. Because of the potential for gastrointestinal tract irritation or burns, do not induce emesis. Significant esophageal or gastrointestinal tract irritation or burns may occur following ingestion. The possible benefit of early removal of some ingested material by cautious gastric lavage must be weighed against potential complications of bleeding or perforation.

Environmental Fate

n-Butylamine's production and use as a chemical intermediate, as well as its presence in animal waste,

may result in its release to the environment through various waste streams. When released into the soil, *n*-butylamine is expected to leach into groundwater. When released into the soil, it may biodegrade and evaporate to a moderate extent. When released into water, it may biodegrade to a moderate extent and is expected to quickly evaporate. *n*-Butylamine is not expected to significantly bioaccumulate. It has an estimated bioconcentration factor (BCF) of less than 100. When released into the water, it is expected to have a half-life between 1 and 10 days. When released into the air, it is expected to be readily degraded by reaction with photochemically produced hydroxyl radicals, is expected to have a half-life between 1 and 10 days, and is expected to be readily removed from the atmosphere by wet deposition. If released to air, a vapor pressure of 92.9 mmHg at 25°C indicates *n*-butylamine will exist solely as a vapor in the ambient atmosphere.

Other Hazards

n-Butylamine has an almost unlimited shelf-life in unopened, original containers if protected from heat and properly stored in a protected storage area. It is neither explosive nor spontaneously flammable in air. However, it is flammable, malodorous and corrosive, and may corrode some metals in the presence of water.

Contact with strong acids may cause spattering and may corrode some metals in the presence of water. Liquid butylamine will attack some forms of plastics, rubber, and coatings. Some forms react violently with water. Contact with strong oxidizers may cause fires and even explosions under the right conditions. Toxic oxides of nitrogen may form in fire. Vapors may travel to source of ignition and flash back. Most vapors are heavier than air. Vapors can present an explosion hazard indoors or in enclosed spaces at high enough concentrations. Some butylamines may polymerize explosively when heated.

One should always refer to the Material Safety Data Sheet for detailed information on handling and disposal.

Exposure Standards and Guidelines

Butylamines have numerous occupational exposure standards and guidelines. *n*-Butylamine occupational exposure criteria include the following:

- USA: Occupational Safety and Health Administration permissible exposure limit ceiling value of 5 ppm ($15\ mg\ m^{-3}$).
- USA: National Institute for Occupational Safety and Health values include a recommended exposure limit of 5 ppm as a 15 min ceiling value, and an immediately dangerous to life or health value of 300 ppm.
- USA: American Conference of Governmental Industrial Hygienists ceiling limit of 5 ppm, with a skin notation.
- Australia: 5 ppm, peak limitation, with a skin notation.
- Federal Republic of Germany: 5 ppm, short-term level 25 ppm, 30 min, two times per shift.
- Sweden: 5 ppm ceiling limit, with a skin notation.
- United Kingdom: 5 ppm, 10 min, short-term exposure limit 15 ppm, with a skin notation.

Miscellaneous

n-Butylamine may exist as a liquid or vapor. The liquid is clear and colorless. Its odor is described as 'fish-like' and 'ammonia-like'. Odor may be detected at concentrations slightly less than 1 ppm, is noticeable at 2 ppm, moderately strong at 2–5 ppm, and strong at 5–10 ppm.

n-Butylamine occurs naturally in some foods. These include: kale (7 ppm); pickles; cucumbers in aromatic vinegar (0.6 ppm); cucumbers pickled with mustard (5.3 ppm); Tilsiter cheese (3.7 ppm); brown bread (1.1 ppm); mulberry leaves; fish and seafood. *n*-Butylamine has been identified as a volatile component of boiled beef. Butylamines have been reported to be a component of animal waste, perhaps from decomposition of manure.

See also: Corrosives; Respiratory Tract; Skin.

Further Reading

Clansky KB (ed.) *Suspect Chemicals Sourcebook: A Guide to Industrial Chemicals Covered Under Major Federal Regulatory and Advisory Programs*, section 3, p. 44. Burlington, CA: Roytech Publications.

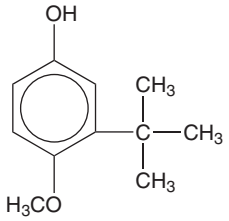
Clayton GD and Clayton FE (eds.) (1993–1994) *Patty's Industrial Hygiene and Toxicology*, vols. 2A, 2B, 2C, 2D, 2E, 2F: *Toxicology*, 4th edn. New York: Wiley.

FAO and WHO (1981) Pesticide Residues in Food. In: Lewis RJ Sr and Tatken RL (eds.) *Registry of Toxic Effects of Chemical Substances*. DHEW (NIOSH) Publication No. 79-100. EO 2975000. Cincinnati, OH: National Institute for Occupational Safety and Health.

Butylated Hydroxyanisole

Kashyap N Thakore

- Chemical Abstracts Service Registry Number: CAS 25013-16-5
- Synonyms: (1,1-Dimethylethyl)-4-methoxyphenol; 2(3)-*t*-Butyl-4-hydroxyanisole; BHA; Anthracine 12
- Chemical Formula: $C_{11}H_{16}O_2$
- Chemical Structure:



Uses

Butylated hydroxyanisole (BHA) is an antioxidant and preservative, especially in foods, cosmetics, and pharmaceuticals, and also in rubber and petroleum products.

Exposure Routes and Pathways

There is a widespread human exposure to BHA by ingestion and skin application. When heated to decompose, it emits acrid and irritating fumes and causes inhalation exposure.

Toxicokinetics

In experimental animals and in humans, BHA is absorbed rapidly after oral administration. The major metabolic pathways are conjugation (phase II) reactions, oxidative metabolism (*O*-demethylation) being relatively unimportant. BHA is metabolized to main metabolites, 4-*O*-conjugates, *O*-sulfates, and *O*-glucuronides. In dogs, oxidative metabolism is more important. It also induces both phase I and phase II drug metabolizing enzyme mRNA, protein activity, and hepatic and intestinal glutathione *S*-transferases.

BHA is distributed to various organs such as liver, lungs, and gastrointestinal tract. The metabolites are rapidly excreted through urine with little evidence of long-term tissue storage. When human volunteers were given a single oral dose of ^{14}C-labeled BHA ($\sim$0.5 mg kg^{-1} body weight), 60–70% of the radioactivity was excreted in the urine within 2 days and 80–86.5% by day 11. After administration of a single dose of 1000 mg BHA to New Zealand White rabbits, 46% of the dose was excreted in the urine as glucuronides, 9% as etherial sulfates, and 6% as free phenols. Excretion of glucuronides was inversely dose dependent.

Mechanism of Toxicity

The metabolites can bind to cellular macromolecules, such as proteins and DNA, to cause toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the oral LD_{50} is 2000 mg kg^{-1} and the intraperitoneal LD_{50} is 881 mg kg^{-1}. The oral LD_{50} is 1100 mg kg^{-1} in mice and 2100 mg kg^{-1} in rabbits.

Human

BHA is harmful if swallowed, inhaled, or absorbed through skin. It is irritating to the eyes, skin, mucous membranes, and upper respiratory tract. Prolonged or repeated exposure may cause allergic reactions in certain sensitive individuals. The target organs for toxicity are liver, lungs, and forestomach.

Chronic Toxicity (or Exposure)

Animal

BHA induces benign and malignant tumors of the forestomach in rats and hamsters by administration through diet. It is toxic to the reproductive system and embryo in rats but not toxic to rabbits, pigs, or rhesus monkeys.

Human

BHA may cause cancer.

Workers exposed to BHA should wear personal protective equipment and take measures to prevent any contact with the skin and mucous membranes.

Approximately 50 countries reportedly permit the use of BHA as a food additive. BHA is classified as Generally Recognized as Safe by the US Food and Drug Administration, when the total content of antioxidants represents not more than 0.02% w/w of the total fat or oil content of the food. It is also permitted at maximum levels of 0.001–0.02% in other specific products.

In Vitro Toxicity Data

It is not mutagenic to *Salmonella typhimurium*, *Drosophila melanogaster*, or Chinese hamster cells *in vitro* and does not cause chromosomal effects.

Clinical Management

In case of contact, eyes and skin should be flushed with water for 15–20 min. In the case of inhalation exposure, the victim should be moved to fresh air. If necessary, oxygen and artificial respiration should be administered. Cardiopulmonary resuscitation should be administered, if the patient is in cardiac arrest. Life-supporting measures should be continued until medical assistance has arrived. An unconscious or convulsing person should not be given liquids or induced to vomit.

Environmental Fate

It has low soil mobility and volatilizes slowly from water. It may bioconcentrate in aquatic organisms, adsorb to sediment, and may be subject to direct photolysis.

Exposure Standards and Guidelines

BHA or its residues are exempted from the requirement of a tolerance when used as an antioxidant in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest. BHA used as a chemical preservative in food for human consumption, in animal drugs, feeds, and related products is generally recognized as safe when used in accordance with good manufacturing or feeding practice.

See also: Food Additives.

Relevant Websites

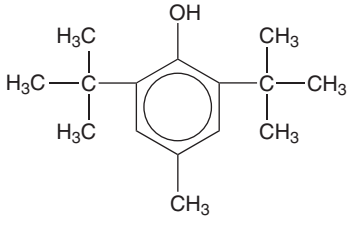
http://ntp.niehs.nih.gov – National Toxicology Program (NTP) (2002) *10th Report on Carcinogens*, Research Triangle, NC: US Department of Health and Human Services, Public Health Service.

http://www.iarc.fr – IARC (1986) Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation, vol. 40, p. 123.

Butylated Hydroxytoluene

Kashyap N Thakore

- Chemical Abstracts Service Registry Number: CAS 128-37-0
- Synonyms: 2,6-Bis(1,1-dimethylethyl)-4-methylphenol; 2,6-Di-*t*-butyl-*p*-cresol; BHT; Anthracine 8
- Chemical Structure:



Uses

Butylated hydroxytoluene (BHT) is an antioxidant for food, animal feed, petroleum products, synthetic rubbers, plastics, animal and vegetable oils, and soaps. It is also used as an antiskinning agent in paints and inks.

Exposure Routes and Pathways

Ingestion is the most common route of exposure in addition to inhalation and skin absorption. BHT is combustible when exposed to heat or flame and can emit acrid smoke and fumes.

Toxicokinetics

In BALB/c mice, 40% of an intragastric dose of BHT was taken by the tissues within 30 min by males, whereas only 10% was absorbed in females. Oxidative metabolism (phase I reactions) mediated by the microsomal monooxygenase system is the major route for degradation; oxidation of the ring methyl group predominates in rat, rabbit, and monkey and oxidation of the *t*-butyl groups in man. The predominant metabolic pathway involves oxidation of the 4-methyl group. The major metabolites are 3,5-di-*t*-butyl-4-hydroxybenzoic acid, both free and as a glucuronide, and *S*-(3,5-di-*t*-butyl-4-hydroxybenzyl)-*N*-acetylcysteine. Moreover, BHT-quinone methide (2,6-di-*t*-butyl-4-methylene-2,5-cyclohexadienone), a reactive metabolite, has been identified in the liver and bile of rats. Metabolites produced in

mice are similar to those produced in rats, except that the major biotransformation in mice was by oxidation of *t*-methyl groups.

Accumulation of BHT is greatest in tissues. In male and female BALB/c mice, a single intragastric dose was widely distributed to various tissues within 30 min, primarily to the small intestine, stomach, liver, kidneys, and lungs. Enterohepatic circulation of BHT has been reported in rats. BHT is also converted by cytochrome P450 monooxygenases to a chemically reactive metabolite – possibly BHT-quinone methide, which forms BHT–glutathione by nonenzymatic conjugation with glutathione.

BHT is cleared less rapidly from most species, enterohepatic circulation being partly responsible for the delay. The major metabolites of BHT in rat urine are 3,5-di-*t*-butyl-4-hydroxybenzoic acid (BHT acid; III), both free (90% of the dose) and as a glucuronide (15%), and *S*-(3,5-di-*t*-butyl-4-hydroxybenzyl)-*N*-acetylcysteine. The ester glucuronide and mercapturic acid were major metabolites in rat bile, while free BHT acid was the main component in the feces. In addition, 1,2-bis(3,5-di-*t*-butyl-4-hydroxyphenyl) ethane has been identified in rat bile. In BALB/c mice, ~75% of a single oral dose was excreted in the urine during the first 24 h; this was followed by a slower phase during which an additional 10% was excreted over the next 4 days. The total amount found in the feces was less than 1%. Female rats have greater urinary excretion of BHT than male rats, whereas male BALB/c mice excreted BHT more rapidly than females.

Mechanism of Toxicity

The metabolites can bind to cellular macromolecules, such as proteins and DNA, to cause toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, BHT is poisonous by intraperitoneal and intravenous routes and moderately toxic by ingestion. The oral LD_{50} is $890\,mg\,kg^{-1}$ in rats and $1040\,mg\,kg^{-1}$ in mice. In mice, the intraperitoneal LD_{50} is $138\,mg\,kg^{-1}$ and the subcutaneous LD_{50} is $650\,mg\,kg^{-1}$. In the guinea pig the oral LD_{50} is $10\,700\,mg\,kg^{-1}$.

Human

BHT is harmful if swallowed, inhaled, or absorbed through skin. It causes irritation of the eyes, skin, mucous membranes, and upper respiratory tract. Prolonged or repeated contact can damage the eyes and cause nausea, dizziness, and headache.

Chronic Toxicity (or Exposure)

Animal

BHT has produced reproductive effects in animal experiments. It is a questionable carcinogen based on experimental carcinogenic and neoplastigenic data. It induces liver tumors in long-term experiments.

Human

BHT is a possible carcinogen with the target organ being the lungs. It does not represent a relevant mutagenic/genotoxic risk to humans.

Approximately 40 countries reportedly permit the use of BHT as a direct or indirect food additive. BHT was approved and classified as 'Generally Recognized as Safe' by the US Food and Drug Administration. Regulated food products could contain a combined total of up to 0.02% BHT and butylated hydroxyanisole, based on the fat content of the food. It is also permitted at maximum levels of 0.001–0.01% in other specific products.

Workers exposed to BHT should wear personal protective equipment and take measures to prevent any contact with the skin and mucous membranes. The American Conference of Governmental Industrial Hygienists recommends that occupational exposure to airborne BHT not exceed $10\,mg\,m^{-3}$ (threshold limit value) as an 8 h time-weighted average or $20\,mg\,m^{-3}$.

In Vitro Toxicity Data

In vitro studies on bacterial, yeast, and various mammalian cell lines, and primary hepatocytes demonstrate the absence of interactions with or damage to DNA.

Clinical Management

In case of contact, eyes and skin should be flushed with water for 15–20 min. In the case of inhalation exposure, the victim should be moved to fresh air. If necessary, oxygen and artificial respiration should be administered. Cardiopulmonary resuscitation should be administered if the patient is in cardiac arrest. Life-supporting measures should be continued until medical assistance has arrived. An unconscious or convulsing person should not be given liquids or induced to vomit.

Exposure Standards and Guidelines

BHT and its residues are exempted from the requirement of a tolerance when used as an antioxidant in accordance with good agricultural practice as inert (or occasionally active) ingredients in pesticide formulation applied to animals. BHT used as a chemical preservative in food for human consumption, in animal drugs, feeds, and related products is generally recognized as safe when used in accordance with good manufacturing or feeding practice.

See also: Food Additives; Respiratory Tract.

Relevant Websites

http://www.cdc.gov/niosh – NIOSH (2003) Pocket Guide to Chemical Hazards. Cincinnati, OH: National Institute for Occupational Safety and Health.

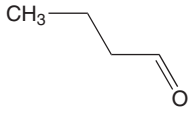
http://www.iarc.fr – IARC (1986) Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation, vol. 40, p. 161.

Butyraldehyde, *n*-

Sanjay Chanda and Harihara M Mehendale

This article is a revision of the previous print edition article by Michael J Brabec, volume 1, pp. 201–202, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 123-72-8
- Synonyms: Butyraldehyde; Butal; Butaldehyde; Butyl aldehyde; *n*-Butyl aldehyde; Butyral; *n*-Butyraldehyde; Butyric aldehyde; Butyrylaldehyde; *n*-Butanal; Butanaldehyde
- Chemical/Pharmaceutical/Other Class: Aliphatic aldehyde
- Chemical Structure:



Uses

Butanal is used in the manufacture of rubber accelerators, synthetic resins, solvents, and plasticizers. *n*-Butyraldehyde is used as an intermediate in the manufacturing of plasticizers, alcohols, solvents, and polymers (such as 2-ethylhexanol, *n*-butanol, trimethylolpropane, *n*-butyric acid, polyvinyl butyral, methyl amyl ketone). It is also used as an intermediate to make pharmaceuticals, agrochemicals, antioxidants, rubber accelerators, textile auxiliaries, perfumery, and flavors. It has no therapeutic use at the present time.

Background Information

n-Butyraldehyde is a clear, mobile, flammable, liquid with a pungent odor. It is miscible with all common solvents, for example, alcohols, ketones, aldehydes, ethers, glycols, and aromatic and aliphatic hydrocarbons, but is only sparingly soluble in water.

Exposure Routes and Pathways

Butanal is a liquid at room temperature, with a relatively low vapor pressure. Limited contact could occur by exposure to butanal vapors. Butanal has appreciable solubility in water; therefore, exposure would be expected to be primarily through ingestion of or through skin contact with the compound or a solution of the compound.

Toxicokinetics

Butanal is readily metabolized to carbon dioxide by conversion to butyryl CoA and subsequent metabolism via the pathways of short-chain fatty acid oxidation. Detoxication by reaction with glutathione also occurs. Clearance is rapid and complete.

Mechanism of Toxicity

Butanal does not possess high acute toxicity but is a potent irritant of the skin, eyes, and upper respiratory tract. The mechanism of toxicity probably involves direct reaction between the active aldehyde group and cellular components.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} value for rat is $5.9\,g\,kg^{-1}$, whereas the LC_{50} is 60 000 ppm (30 min exposure). Acute exposures to butanal vapors induce inflammation of the alveolar and bronchial regions of the lung, with death due to pulmonary edema. Severe irritation of

the eyes and nose are noted. Relatively high levels of butanal in the drinking water of mice for 50 days produced abnormal sperm morphology. Exposure of rodents to low concentrations of butanal allowed rapid recovery after exposure is ceased.

Human

Butanal has low acute toxicity. Exposure to a large dose may have a temporary narcotic effect. Exposure to low concentrations of butanal vapors produces irritation of the eyes, nose, and throat. The compound has an unpleasant odor. Impurities (butyric acid) may be present that make the smell even more objectionable. Health effects attributed to chronic exposure to low doses of butanal vapors have not been described. Dermatitis may be expected after prolonged and repeated exposures to solutions containing butanal.

Chronic Toxicity (or Exposure)

Animal

Not a recognized carcinogen.

Human

Not a recognized carcinogen.

Clinical Management

Support should be given to the patient until butanal has been cleared from the body, which occurs in a relatively short time. Recovery is uneventful.

Environmental Fate

Terrestrial Fate

The primary degradation process in soil is expected to be biodegradation. A number of biological screening studies have demonstrated that butyraldehyde is readily biodegradable. Butyraldehyde's vapor pressure of 111.4 mmHg at 25°C indicates that it will evaporate rapidly from surfaces.

Aquatic Fate

The major environmental fate processes for butyraldehyde in water are biodegradation and volatilization. A number of biological screening studies have demonstrated that butyraldehyde is readily biodegradable. Volatilization half-lives of 9 h and 4.1 days have been estimated for a model river (1 m deep) and an environmental pond, respectively. Aquatic hydrolysis, adsorption to sediment, and bioconcentration are not expected to be important fate processes.

Atmospheric Fate

In excess of 99% of the butyraldehyde present in the atmosphere will occur in the vapor phase, although a small fraction has been shown to occur in the particulate aerosol. Vapor-phase butyraldehyde will degrade relatively rapidly in an average ambient atmosphere by reaction with photochemically produced hydroxyl radicals (estimated half-life of 16.4 h). Direct photolysis may also be a major degradation process. During intense smog-pollution episodes, the natural formation rate of butyraldehyde can exceed the degradation rate. The detection of butyraldehyde in cloud and fog water indicates that physical removal from air can occur through wet deposition.

Exposure Standards and Guidelines

Workplace environmental exposure level (WEEL): 8 h time-weighted average (25 ppm).

See also: Butyric Acid; Pollution, Soil; Pollution, Water.

Relevant Website

http://www.epa.gov – Butyraldehyde Fact Sheet (from the US EPA's OPPT) (EPA 749-F-95-005a), 1994.

Butyric Acid

James Deyo

- Chemical Abstracts Service Registry Number: CAS 107-92-6
- Synonyms: Butanoic acid; Acide butyrique; *n*-Butanoic acid; *n*-Butyric acid; Butanic acid; Ethylacetic acid; Acido butirico; 1-Propanecarboxylic acid; Propylformic acid; Kyselina maselna; Buttersaeure; RTECS ES5425000; UN2820; FEMA number 2221
- Chemical/Pharmaceutical/Other Class: Industrial intermediate; Food additive
- Chemical Structure: $HOCH(=O)–CH_2–CH_2–CH_3$

Uses

Most butyric acid is consumed in the manufacture of cellulose acetate butyrate (CAB) plastics. CAB sheets are used for thermoformed sign faces, blister packaging, and goggles and face shields, while molded CAB is used to make pen barrels, eyeglass frames, and screwdriver handles. CAB is a component in acrylic enamel for automotive original equipment manufacturing coatings. Some butyric acid is used to make butyroperoxides and herbicides. It is also used as an intermediate for pharmaceuticals, emulsifiers, and disinfectants, as a leather tanning agent, and a sweetening agent in gasoline. It is used in the synthesis of butyrate ester perfumes and in the manufacture of esters, some of which serve as the bases of artificial flavoring ingredients of certain liquors, soda-water syrups, candies. Another use is as a food additive in butter, cheese, butterscotch, caramel, fruit and nut flavors (butyric acid is a (US) Food and Drug Administration generally considered as safe (GRAS) material). Butyric acid is also used in the preservation of high moisture wheat grains to prevent fungal deterioration.

Exposure Routes and Pathways

Exposure to butyric acid may occur by inhalation, dermal contact, or ingestion.

Toxicokinetics

Butyric acid is rapidly metabolized in the liver to acetic acid and ketone bodies (acetone, acetoacetate, beta-hydroxybutyrate). In humans, the butyric acid elimination curve can be divided into two parts corresponding to two half-lives: for the first (0.5 min), the slope suggests an accelerated excretion; for the second (13.7 min), a slow plateau is observed.

Mechanism of Toxicity

The most probable mechanism of toxicity is the formation of an acid proteinate following exposures to high concentrations. Such complexes result in an inhibition of protein function and disruption of cellular homeostasis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Data in laboratory animals suggest that *n*-butyric acid is only slightly acutely toxic with an oral LD_{50} value in rats ranging from 2940 to 8790 mg kg^{-1}, an inhalation LC_{50} of >40 mg l^{-1} in rabbits, and a dermal LD_{50} value in rabbits of 530 mg kg^{-1}. Butyric acid is a moderately strong irritant to skin and may induce severe eye irritation.

Human

Acute exposure to butyric acid would be anticipated to induce irritation or burns following contact with skin or eyes, or if inhaled. Such effects will occur in a concentration-dependent manner.

Chronic Toxicity (or Exposure)

Animal

Repeated inhalation or oral exposures to moderate to high doses of *n*-butyl acetate and *n*-butanol are well tolerated. These aforementioned molecules are readily and rapidly metabolized to *n*-butyric acid. The no-observed-effect level (NOEL) for repeated dose oral exposure to *n*-butanol was 125 mg kg^{-1} day^{-1}. In a 90 day inhalation study in rats with *n*-butyl acetate a NOEL of 500 ppm was reported for systemic effects, and a NOEL of 3000 ppm (highest dose tested) was reported for postexposure neurotoxicity based on functional observational battery endpoints, quantitative motor activity, neuropathy, and scheduled-controlled operant behavior endpoints. Results of inhalation studies conducted on *n*-butanol and *n*-butyl acetate were negative for inducing reproductive and developmental toxicity. The NOEL for female reproductive toxicity was 6000 ppm with *n*-butanol and 1500 ppm for *n*-butyl acetate. In a 90 day repeated-dose inhalation toxicity study with butyl acetate the NOEL for male reproductive toxicity was 3000 ppm. For developmental toxicity, a NOEL of 3500 ppm was observed with *n*-butanol and a NOEL of 1500 ppm (the highest exposure tested) was seen in both rats and rabbits following exposure to *n*-butyl acetate.

Human

Butyric acid is generally recognized as safe (GRAS) as a food additive for chronic consumption when used in accordance with good manufacturing practice. Chronic exposure also occurs through endogenous production as *n*-butyric acid is an important metabolite in the breakdown of carbohydrates, fats, and proteins and is produced in the human colon by fermentation. *n*-Butyric acid is present in butter as an ester to the extent of 4–5%.

In Vitro Toxicity Data

Data indicate butyric acid is not genotoxic. Negative results were observed in assays assessing for both mutations (Ames test) and chromosomal aberrations.

Clinical Management

Exposure should be terminated as soon as possible by the removal of the victim to fresh air. The skin, eyes, and mouth should be washed with copious amounts of water. A 15 to 20 min wash may be necessary to neutralize and remove all residual traces of the contaminant. Contaminated clothing and jewelry should be removed. Contact lenses should be removed from the eyes to avoid prolonged contact of the acid with the area. A mild soap solution may be used for washing the skin and as an aid to neutralize the acid, but should not be placed into the eyes. No cream, ointment, or dressing should be applied to the affected area. If a large quantity has been swallowed, then gastric lavage should be considered. Dilution with water may be the solution for small quantities swallowed. The victim should be kept quiet and normal body temperature should be maintained.

Environmental Fate

n-Butyric acid is not environmentally persistent or likely to bioaccumulate.

Ecotoxicology

The LC_{50} (48 h) value for fish (*Oryzias latipes*) is 90 mg l^{-1}; the EC_{50} (24 h) value for *Daphnia magna* is 1950 mg l^{-1}; and the EC_3 (3% or greater reduction in growth; 8 day) for *Scenedesmus quadricauda* is 2600 mg l^{-1} *Microcystis aeruginosa* 318 mg l^{-1}.

Other Hazards

Forms carbon dioxide and carbon monoxide during combustion.

Exposure Standards and Guidelines

None established.

See also: Food Additives; Generally Recognized as Safe (GRAS).

Relevant Website

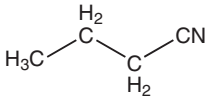
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Butyric Acid.

Butyronitrile

Carey N Pope

This article is a revision of the previous print edition article by Rhonda S Berger and Wendy Khune, volume 1, p. 203, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 109-74-0
- Synonyms: Butanenitrile; Butyric acid nitrile; Cyanopropane; Propyl cyanide; *n*-Butyronitrile; 1-Butyronitrile; 1-Cyanopropane
- Chemical/Pharmaceutical/Other Class: Alkyl nitrile
- Chemical Formula: C_4H_7N
- Chemical Structure: $CH_3CH_2CH_2C{\equiv}N$



Uses

Butyronitrile is an industrial solvent.

Exposure Routes and Pathways

Dermal, oral, and inhalation routes are all primary exposure pathways.

Toxicokinetics

Toxicokinetic data are available only for propionitrile. When administered as a ^{14}C radioisotope, 92.5% of the compound was recovered. The majority was eliminated in air or urine within 24 h. About 27% was recovered as volatile organic material within 0.5 h of gavage exposure. By 3 h, either carbon dioxide or cyanide exhalation was estimated at 38–49% of the total. At 24 h, the total ^{14}C recovery in the urine was 0.76–5.83%. A small amount (<2%) was found in liver and kidneys at 72 h after dosing. It was concluded that propionitrile is rapidly absorbed from the gastrointestinal tract and eliminated through expired air as the parent compound, CO_2, or cyanide.

Mechanism of Toxicity

The acute toxicity of butyronitriles is thought to be due to release of cyanide through metabolism of the parent compound. Signs of acute butyronitrile intoxication including dyspnea, ataxia, and convulsions are similar to those noted with acute cyanide intoxication. The onset and duration suggests that these nitriles require metabolism to elicit toxicity. Cyanide and thiocyanate have both been found in urine and

blood after butyronitrile exposure. Butyronitrile toxicity is antagonized by sodium thiosulfate and sodium nitrite and blockade of hepatic metabolism. All of these support the hypothesis that cyanide is the ultimate toxicant following butyronitrile exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values in rats for all butyronitriles are from 40 to 270 mg kg^{-1}. Inhalation LD_{50} values (1–4 h exposure) were from 1000 to 1465 ppm. Dermal LD_{50} values of *n*-butyronitrile and isobutyronitrile in rabbits were 239–389 mg kg^{-1}. Butyronitrile is a mild eye and skin irritant.

Human

Butyronitrile exposure may cause dizziness, dysnpea, nausea, vomiting, weakness, confusion, and unconsciousness.

Chronic Toxicity (or Exposure)

Animal

Butyronitriles do not elicit reproductive toxicity. In a developmental toxicity study, rats exposed to 50, 100, or 200 ppm butyronitrile for 6 h a day during gestation showed no teratogenic effects but did exhibit decreased fetal weights at the highest dosage. Little is known regarding toxicity of prolonged exposures to butyronitrile. Repeated exposures to propionitrile led to neurotoxicity (ataxia, tremors, convulsions) at high dosage levels. Dyspnea, nasal and ocular discharge, increased salivation, reduced motor activity, and alopecia were observed. Significant reduction in red blood cells and hemoglobin and increases in spleen weights were also noted.

Human

Little is known regarding chronic effects of butyronitrile exposure in humans.

In Vitro Toxicity Data

Butyronitriles were negative in *in vitro* mutagenicity and cytogenicity assays.

Clinical Management

For mild signs of intoxication (nausea, dizziness, drowsiness) with blood cyanide concentrations <2 mg l^{-1}, oxygen and bed rest should be given. With more severe intoxication exhibiting short-lived periods of unconsciousness, convulsions, vomiting, and/or cyanosis and with blood cyanide concentrations of 2–3 mg l^{-1}, l00% oxygen should be provided for not more than 24 h and observation should be made in an intensive care area. Fifty milliliters of 25% sodium thiosulfate solution (1.5 g) should be given intravenously over 10 min.

Environmental Fate

Butyronitriles undergo microbial degradation. Butyronitrile does not significantly hydrolyze at environmentally relevant pHs.

Ecotoxicology

Concentrations near 100 mg l^{-1} (96 h static) of both isobutyronitrile and *n*-butyronitrile were without effect on fathead minnows. Daphnia treated with 94.3 mg l^{-1} isobutyronitrile showed no abnormal behavior or movement changes. The same concentration of *n*-butyronitrile resulted in 1/20 daphnids becoming immobile at 48 h, but this was not considered treatment related. According to the Environmental Protection Agency assessment criteria, these values correspond to a 'low concern level'.

Exposure Standards and Guidelines

The 10 h time-weighted average for butyronitrile is 8 ppm. The proposed ERPG-3 (maximum air concentration below which all individuals could be exposed for up to 1 h without developing life-threatening health effects) for butyronitriles is 100 ppm (280 mg m^{-3}) and the ERPG-2 (maximum air concentration below which nearly all individuals could be exposed for up to 1 h without developing irreversible or serious health effects or symptoms that could impair an individual's ability to take protective action) is 30 ppm (84 mg m^{-3}).

See also: Cyanide; Neurotoxicity.

Further Reading

National Institute for Occupational Safety and Health (1978) *NIOSH Criteria for a Recommended Standard, Occupational Exposure to Nitriles* (DHEW Publication No. 78-212). Washington, DC: Government Printing Office.

Relevant Website

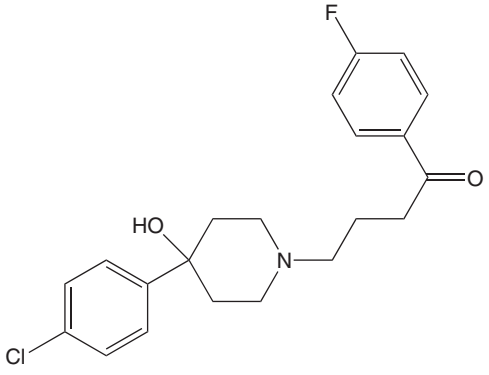
http://www.epa.gov – US Environmental Protection Agency.

Butyrophenones

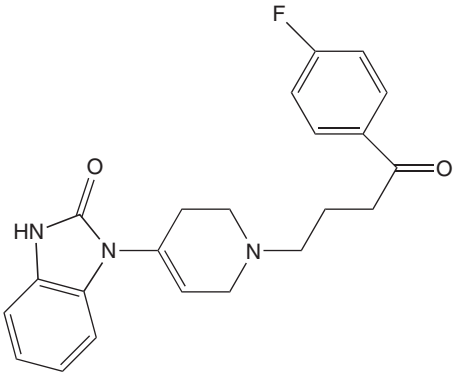
Jaya Chilakapati and Harihara M Mehendale

This article is a revision of the previous print edition article by Douglas J Borys, volume 1, pp. 203–205, © 1998, Elsevier Inc.

- Representative Compounds: Haloperidol; Droperidol
- Chemical Abstracts Service Registry Numbers: CAS 495-40-9; CAS 52-86-8 (haloperidol); CAS 548-73-2 (droperidol)
- Synonyms: Haloperidol-haldol; 4-(4-(*p*-Chlorophenyl)-4-hydroxypiperidino)-4′-fluorobutyrophenone; Droperidol-dehydrobenzperidol; Inapsine; 1-(1-(3-*p*-Fluorobenzoylprpyl)-1,2,3,6-tetrahydropyrid-4-yl)-2-benzimidazolinone
- Chemical/Pharmaceutical/Other Class: Neuroleptics; Antipsychotics; Major tranquilizers
- Chemical Formula: $C_{10}H_{12}O$
- Chemical Structures:



Haloperidol



Droperidol

Uses

Butyrophenones are used to treat psychoses including schizophrenia, organic psychosis, paranoid syndrome, acute idiopathic psychotic illnesses, and the manic phase of manic depressive illness. Other uses include treatment of aggressive behavior, delirium, acute anxiety, nausea and vomiting, pain, organic brain syndrome, and Tourette's syndrome.

Exposure Routes and Pathways

Haloperidol is available both in an injectable form and in oral dosage form. The principal exposure pathway is intentional ingestion by adults or accidental ingestion by small children. Pharmacists, physicians, and nurses dispensing or administering haloperidol could be exposed through dermal contact. Droperidol is available only as an injectable drug. The most common route of exposure is an accidental injection.

Toxicokinetics

Haloperidol is well absorbed orally with a bioavailability of 60–65% due to first-pass hepatic metabolism. It has a reversible oxidation/reduction metabolic pathway: it is metabolized via reduction to reduced haloperidol, which is biologically inactive. Both agents are rapidly absorbed after intramuscular injection, peaking within 10 min. Butyrophenones are metabolized in the liver to inactive metabolites. Concentrations of butyrophenones are found in the liver, central nervous system, and throughout the body. Haloperidol is 92% protein bound. Haloperidol is 15% eliminated through the bile. The elimination half-life is 14–41 h. The half-life of droperidol is 2 h; 10% is recovered unchanged in the urine.

Mechanism of Toxicity

Butyrophenones work primarily by blocking dopamine-mediated synaptic neurotransmission by binding to dopamine receptors. In addition to significant antidopaminergic action, butyrophenones also possess anticholinergic, α-adrenergic blockade, and quinidine-like effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Signs of toxicity reported in animals have included sedation, dullness, photosensitivity, weakness, anorexia, fever, icterus, colic, anemia, and hemoglobinuria. Treatment consists of gastric decontamination and aggressive supportive care.

Human

Clinical signs of toxicity most commonly include extrapyramidal effects, somnolence, coma, respiratory

depression, cardiac dysrhythmias, hypotension, and sedation. Neuroleptic malignant syndrome has been reported after therapeutic use and acute intoxication. The most commonly reported dystonic reactions include akathesias, stiff neck, stiff or protruding tongue, and tremor. Children appear to be more sensitive than adults to the extrapyramidal effects of butyrophenones with facial grimacing and oculogyric crisis noted. Anticholinergic effects, including dry mouth, blurred vision, and tachycardia, may occur. Other cardiac effects include prolonged QT interval and mild hypotension. Hypokalemia has also been noted. Since haloperidol may lower the seizure threshold, the drug should be used with caution in patients receiving anticonvulsant agents and in those with a history of seizures of electroencephalographic abnormalities. Possible sequelae include neuroleptic malignant syndrome and acute renal failure. Adverse reactions following therapeutic use include sedation, dysphoria, anorexia, nausea, vomiting, constipation, diarrhea, and dyspepsia.

Chronic Toxicity (or Exposure)

Animal

Rats chronically treated with haloperidol (1.5 mg kg^{-1} ip) significantly developed vacuous chewing movements and tongue protrusions.

Human

Chronic poisoning by ingestion may induce neurological syndromes, the most severe of which are parkinsonism, akathisia, and tardive dyskinesia, a syndrome which is characterized by rhythmical, involuntary movements of the tongue, face, mouth, or jaw (e.g., protrusion of tongue, puffing of cheeks, puckering of mouth, chewing movements). Sometimes these may be accompanied by involuntary movements of extremities.

Clinical Management

All basic and advanced life-support measures should be implemented. Gastric decontamination should be performed. Butyrophenones are readily absorbed by activated charcoal. Aggressive supportive care should be instituted. Dystonic reactions respond well to intravenous benztropine or diphenhydramine. Oral therapy with diphenhydramine or benztropine should be continued for 2 days to prevent recurrence of the dystonic reaction. For patients suffering from neuroleptic malignant syndrome, a potentially fatal condition associated with the administration of antipsychotic drugs, dantrolene sodium, and bromocriptine have been used in conjunction with cooling and other supportive measures. Arrhythmias should be treated with lidocaine or phenytoin. Diazepam is the drug of choice for seizures; phenytoin is used to prevent recurrence. Hemodialysis and hemoperfusion have not been shown to be effective.

Environmental Fate

If released to air, haloperidol will exist primarily in the particulate phase; physical removal from air will occur through wet and dry deposition processes.

See also: Anxiolytics; Neurotoxicity

Further Reading

Richards JR and Schneir AB (2003) Droperidol in the emergency department: Is it safe? *The Journal of Emergency Medicine* 24(4): 441–447.

BZ

Harry Salem*

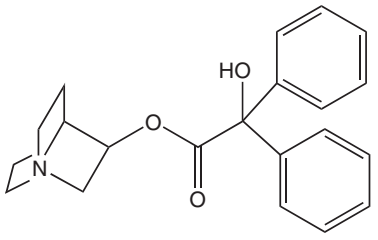
Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 6581-06-2
- Synonyms: TNB; α-Hydroxy-α-phenylbenzeneacetic acid; 1-Azabicyclo[2,2,2]octan-3-yl ester; Agent 15; 3-QNB; QNB; Agent buzz
- Description: BZ is a glycolate anticholinergic chemical related to atropine, scopolamine, and hyoscyamine. It is odorless, nonirritating, and is stable in most solvents. It has a half-life of 3–4 weeks in moist air and is extremely persistent in soil and water as well as on most surfaces. Agent 15, believed to have been stockpiled in Iraq, is speculated either to be identical to BZ or a closely

*The views of the author do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

related derivative, and has similar physicochemical properties as BZ. BZ has a slow onset and a long duration of action

- Chemical/Pharmaceutical/Other Class: Potent anticholinergic psychomimetic that produces incapacitation and is considered a hallucinogenic chemical warfare agent
- Chemical Formula: $C_{21}H_{23}NO_3$
- Chemical Structure:



Uses

BZ is considered an incapacitating chemical warfare agent. It is a nonlethal glycolate anticholinergic psychomimetic that produces incapacitation and is hallucinogenic.

Exposure Routes and Pathways

Exposure is through inhalation of aerosolized solid or BZ dissolved in a solvent such as propylene glycol, dimethyl sulfoxide, and other solvents. Exposure can also occur through the skin and via the gastrointestinal tract.

Toxicokinetics

BZ is a competitive inhibitor of muscarinic receptors associated with the parasympathetic nervous system that innervate the eyes, heart, respiratory system, skin, gastrointestinal tract, and bladder. The sweat glands, innervated by the sympathetic nervous system, are also modulated by muscarinic receptors. By any route of exposure, the onset of action is approximately 1 h, with peak effects occurring 8 h postexposure. Signs and symptoms gradually subside over 2–4 days. Most of the absorbed BZ is excreted via the kidney.

Mechanism of Toxicity

BZ acts by blocking the action of acetylcholine on the central and peripheral nervous systems. It is a tertiary amine and crosses the blood–brain barrier. BZ on acute exposure increases both heart and respiratory rates, dilates the pupils, and causes paralysis of the eye muscles necessary for near focusing. It also causes dry mouth and skin, elevates body temperature, impairs coordination, and causes flushing of the skin, hallucinations, stupor, forgetfulness, and confusion. Within 15 min to 4 h following exposure, the principal effects are dizziness, involuntary muscle movements, near vision difficulty, and total incapacitation. From 6 to 10 h after exposure, the effects are psychotropic and full recovery is expected after 4 days.

The peripheral nervous system effects are considered as under-stimulation of the end organs. This decreased stimulation of eccrine and apocrine sweat glands in the skin results in dry skin and a dry mouth, and is considered 'dry as a bone'. The reduction in the ability to dispel heat by evaporative cooling decreases sweating, and the compensatory cutaneous vasodilation causes the skin to become warm or 'hot as a hare' and 'red as a beet'. This is similar to the atropine flush. The decreased heat loss also results in an increased core temperature.

The peripheral effects described above usually precede the central nervous system effects and have been summarized by the mnemonic 'dry as a bone, hot as hares, red as a beet, and blind as a bat'.

The central nervous system effects of BZ and agent 15 result in dose-dependent 'mad as a hatter' mental changes. These effects fluctuate between a conscious state and delirium that ranges from drowsiness to coma. Disorientation, decreased social restraints, inappropriate behavior, and decreased short-term memory are common. Speech becomes slurred and indistinct.

The human estimated incapacitation ICt_{50} is reported to be 100 mg min m^{-3} and the LCt_{50} to be 200 000 mg min m^{-3}.

Acute and Short-Term Toxicity (or Exposure)

Animal

Species	*LCt_{50} (mg min m^{-3})*	*IV LD_{50} (mg kg^{-1})*
Mouse	12 000	14.1
Rats	64 000	14.0
Guinea pig	123 000	10.0
Rabbits	32 000	10.0
Dogs	25 000	9.6

See also: Atropine; Chemical Warfare Agents.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

C

Cadmium

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst, Shirley B Radding, and Kathryn A Wurzel, volume 1, pp. 207–209, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-43-9
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Cd^{2+}

Uses

Cadmium is primarily used for electroplating and galvanizing other metals because it is relatively resistant to corrosion. It is also used in electrical contacts, in soldering alloys, in nickel–cadmium storage batteries, in television phosphors, and as a stabilizer for polyvinyl chloride. Given its brilliant orange color, it has been used extensively as a pigment in paints, plasters, and plastics. Cadmium is also a by-product of zinc, lead, and copper mining and smelting.

Exposure Routes and Pathways

Due to the wide use of cadmium-based products, cadmium is widely distributed in the environment. The cadmium content in soil and water has been increasing as a result of disposal of cadmium-contaminated waste and the use of cadmium-containing fertilizers (particularly on cereal crops). Commercial sludge, contaminated with cadmium, has been used to fertilize agricultural fields. Cadmium concentrations in urban air are quite low, because of regulation of industrial air emissions. Lead and zinc smelters and waste incineration account for the majority of cadmium present in ambient air.

Ingestion and inhalation are the primary routes of exposure to cadmium. Dermal contact is not a significant route of exposure. Exposure to cadmium via foodstuffs is common since plants and animals accumulate cadmium from soil or water, especially fish and crustaceans. Cigarette smoke is a major source of cadmium exposure via inhalation.

Toxicokinetics

Absorption of cadmium in the gastrointestinal tract is ~4–7% in adults; absorption is probably higher in children. Diets low in calcium, iron, and protein enhance cadmium absorption. Zinc is an antagonist to cadmium (decreases cadmium absorption). Cadmium absorption by the lungs is dependent on particle size and the solubility of the cadmium compound, but is generally between 15% and 30%. Dermal absorption of cadmium is insignificant.

Cadmium is a classic cumulative poison that accumulates in the kidneys over a lifetime. It is transported in the blood by erythrocytes and by albumin, and it is stored mainly in the liver and kidneys as metallothionein (50–75% of the body burden). Cadmium binds to many proteins at the sulfate and carbonyl sites. The half-life of cadmium in these two organs may be as long as 30 years. The correlation between years of exposure and blood levels does not appear to be significant. Cadmium also accumulates in the bones and the placenta of pregnant women.

Urine is the most important excretion mechanism in humans. Urine concentration of cadmium increases with age and following kidney damage. Cadmium found on examination of hair is generally due to external contamination rather than internal absorption and distribution to the hair.

Mechanism of Toxicity

Cadmium inhibits plasma membrane calcium channels and Ca^{2+}-ATPases. It also inhibits repair of DNA damaged by various chemicals, an effect which is believed to be associated with the induction of tumors. Although cadmium forms a metallothionein, the preformed cadmium metallothionein is nephrotoxic (toxic to the kidneys); it is suggested that effects occur when, at some stage in the kidney, the cadmium is dissociated from the metallothionein. In *Itai-Itai* disease (see 'Chronic Toxicity, Human' section), patients were found to have chromosome abnormalities.

Cadmium has an affinity for sulfhydryl groups and hence, can inhibit enzymes; however, cells treated

with cadmium showed proliferation of peroxisomes, which contain catalase, an enzyme. It appears that cadmium at first inhibits catalase activity and then, after a time, enhances that activity. In addition, cadmium inhibits enzymes involved in gluconeogenesis (the generation of glycogen for energy production from noncarbohydrate precursors). It also inhibits oxidative phosphorylation (energy production) and depresses trypsin inhibitor capacity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cardiac effects (electrical and biochemical changes in the myocardium) were observed in rats exposed to cadmium in drinking water.

Human

Acute toxicity may result from ingestion of relatively high concentrations of cadmium from contaminated food or beverages (e.g., $16\,mg\,l^{-1}$ cadmium in a beverage). Cadmium exhibits local irritant effects on the gastrointestinal tract such as nausea, vomiting, diarrhea, abdominal pain, and a choking sensation. The effects of acute toxicity are apparent immediately.

Inhalation of cadmium fumes produces local irritant effects and may result in chemical pneumonitis and pulmonary edema, possibly resulting in death.

Chronic Toxicity (or Exposure)

Animal

Animal studies have shown cadmium to be a teratogen and a reproductive toxin; however, the results of mutagenesis experiments are equivocal. Cadmium produced local sarcomas in a number of rodent species when the metal, sulfide, oxide, or salts were administered subcutaneously. Intramuscular injection of cadmium powder and cadmium sulfate also produced local sarcomas. Injection of cadmium chloride into the ventral prostate resulted in a low incidence of prostatic carcinoma. Exposure via inhalation of cadmium chloride produced a dose-dependent increase in lung carcinomas in rats.

Human

Chronic exposure to cadmium from any route will have adverse effects on the heart, lungs, bones, gonads, and especially, the kidneys. The principal long-term effects of low-level cadmium exposure are generally chronic obstructive pulmonary disease, emphysema, and chronic renal tubular disease. Cardiovascular and skeletal effects are also possible. The initial symptoms of chronic inhalation exposure are those associated with metal fume fever (e.g., fever, headache, chest pain, sore throat, coughing, and rhinitis). Metal fume fever is most often associated with inhalation of zinc oxide but may occur following exposure to other metals such as cadmium. Although inconclusive, there is evidence that the cadmium burden in the body can lead to hypertension.

Since cadmium can displace zinc, cadmium accumulation in the testes can suppress testicular function. Evidence obtained in the past several years appears to relate cadmium to prostate cancer in young men who work with cadmium. Additional investigation (such as epidemiological studies with a larger cohort) needs to be performed to investigate this apparent association of cadmium with prostate cancer.

Skeletal changes due to cadmium accumulation are probably related to calcium loss, which can be influenced by diet and hormonal status. These skeletal changes include osteomalacia (softening of bone resulting from loss of minerals) and pseudofractures. In Japan, people who ate fish contaminated with cadmium experienced skeletal changes, especially in their backs. This very painful effect was called the '*Itai-Itai*' ('ouch-ouch') disease. Postmenopausal women with low calcium and vitamin D intake were apparently most susceptible.

Since the kidneys are the main depot for cadmium, they are of greatest concern for cadmium toxicity. Cadmium interferes with the proximal tubule's reabsorption function. This leads to abnormal actions of uric acid, calcium, and phosphorus. Amino aciduria (amino acids in the urine) and glucosuria (glucose in the urine) result; in later stages, proteinuria (protein in the urine) results. When this happens, it is assumed that there is a marked decrease in glomerular filtration. Long-term exposure to cadmium leads to anemia, which may result from cadmium interfering with iron absorption.

Cadmium metallothionein has also been studied extensively. This metalloprotein is high in the amino acid cysteine ($\sim 30\%$) and is devoid of aromatic amino acids. Metallothionein itself may function to help detoxify cadmium. For some experimental tumors, cadmium appears to be anticarcinogenic (e.g., it reduces the induction of tumors).

Clinical Management

For treatment of oral poisoning, administration of syrup of ipecac is indicated, followed by gastric

lavage. The chelating agent calcium EDTA (calcium disodium salt of ethylenediaminetetraacetic acid) is indicated for acute exposure if administered shortly after cadmium exposure before new metallothionein is synthesized. BAL (British antilewisite; 2,3-dimercaptopropanol) is contraindicated as it may enhance kidney toxicity. Newer dimercapto compounds dimercaptosuccinic acid (DMSA) and dimercaptopropane sulfonate (DMPS) are being evaluated as are derivatives of dithiocarbamates. Delayed pulmonary edema may result from inhaled cadmium dusts; therefore, supportive measures are indicated.

The apparent affinity for zinc metallothionein may someday be found to be useful as an antidote for cadmium toxicity. Antagonists to cadmium toxicity include a pretreatment with selenium and zinc. It is believed that this pretreatment allows cadmium to displace zinc in the zinc metallothionein.

Environmental Fate

As indicated in the Exposure section, cadmium is widely distributed in the environment from a variety of natural and anthropogenic sources. Cadmium emitted into the air is often found bound to small particulates and can travel with these particulates over long distances. As a result, cadmium can remain in the atmosphere for long periods of time until it is deposited by gravitational settling or in rain and snow. Cadmium tends to be more mobile in water than other heavy metals although it will complex with humic substances and can precipitate out under certain conditions. Cadmium can bioaccumulate in aquatic organisms; the degree of accumulation is associated with the pH and humic content of the water. It can also bioaccumulate in plants and in the animals that feed on these plants; for example, cattle and wildlife. However, terrestrial bioaccumulation is much lower than that in water and cadmium concentrations at the top of the terrestrial food chain are not much higher than those at the lower end of the chain.

Exposure Standards and Guidelines

American Conference of Governmental Industrial Hygienists (ACGIH) lists cadmium as a suspected human carcinogen. The ACGIH threshold limit value – time-weighted average (TLV – TWA) is $0.01\,mg\,m^{-3}$ for elemental cadmium and inorganic compounds as total dust/particulate. The ACGIH TLV – TWA for the respirable fraction of cadmium particulate is $0.002\,mg\,m^{-3}$.

See also: Cardiovascular System; Kidney; Metallothionein; Metals; Pollution, Air; Pollution, Soil; Pollution, Water; Respiratory Tract; Sensory Organs.

Further Reading

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Satarug S, Baker JR, Urbenjapol S, *et al.* (2003) A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicological Letters* 137(1–2): 65–83.

Verougstraete V, Lison D, and Hotz P (2003) Cadmium, lung and prostate cancer: A systematic review of recent epidemiological data. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews* 6(3): 227–255.

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Relevant Website

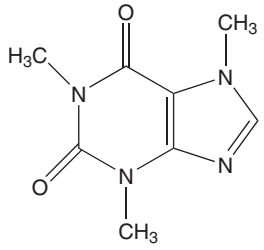
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Cadmium.

Caffeine

Christopher P Holstege

This article is a revision of the previous print edition article by Michael J Hodgman, volume 1, pp. 209–211, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 58-08-2
- Synonyms: 1,3,7-Trimethylxanthine; Guaranine; Methyltheobromine; Thein
- Chemical/Pharmaceutical/Other Class: Methylxanthine
- Chemical Structure:



Uses

Caffeine is used as a central nervous system (CNS) stimulant, anorexiant, diuretic, and in a number of analgesic and cold medication compounds. It is also used in the treatment of spinal headaches and has been used as a respiratory stimulant in preterm infants.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Caffeine is consumed in wide variety of beverages, such as coffee, tea, and soda. It is found alone or in combination with other pharmaceutical products. It is also available for injection.

Toxicokinetics

Caffeine is rapidly absorbed after an oral dose, with peak levels reached within 1–2 h at therapeutic doses. Onset of clinical effects occurs within 60 min. In adults, caffeine is extensively metabolized by the liver primarily by *N*-demethylation. It is excreted in the urine primarily as 1-methyluric acid and 1-methylxanthine. Theophylline (1,3-dimethylxanthine) is a minor product of caffeine metabolism in adults ($<1\%$). After massive caffeine overdoses, serum levels of theophylline are measurable. The elimination half-life of caffeine is 3–6 h at therapeutic doses. The half-life is shorter in smokers and is prolonged by oral contraceptives, cimetidine, late pregnancy, and in overdose. The half-life of caffeine is much longer in infants and does not approximate that seen in adults until 6 months of age. The half-life of caffeine may exceed 100 h in preterm infants. Only 1–10% of caffeine appears unchanged in the urine in adults. Neonates may excrete up to 85% of caffeine unchanged.

Mechanism of Toxicity

Caffeine can have profound effects on the cardiovascular system. At least four mechanisms have been proposed for the pro-arrhythmic potential of caffeine in overdose. First, caffeine increases circulating catecholamines. Second, caffeine inhibits phosphodiesterase. Increased circulating catecholamines after caffeine overdose increase β1-receptor stimulation. Stimulation of β1-receptors increases intracellular cAMP by G protein stimulation of adenylate cyclase. The activity of cAMP is prolonged due to its decreased metabolism as phosphodiesterase is inhibited by caffeine. Subsequently, β1-receptor effects are exaggerated and tachydysrhythmias are induced. Third, caffeine increases myocardial intracellular calcium. Caffeine both induces release of calcium from the sarcoplasmic reticulum and blocks calcium's reuptake into the sarcoplasmic reticulum. This resulting increase in cytosolic calcium may provoke dysrhythmias. Fourth, caffeine blocks cardiac adensosine receptors, which have been shown to be antiarrhythmic.

The hypotension that has been noted with overdoses of caffeine is primarily due to two mechanisms. First, caffeine-induced tachydysrhythmias lead to inadequate filling of the heart and subsequent decrease in cardiac output. Second, caffeine augments β2-effects and causes subsequent vasodilation with resulting hypotension.

Caffeine in overdose also acts as a nonselective antagonist of neuronal adenosine receptors that may lead to seizures. Caffeine is also a mild diuretic and it stimulates gastric acid secretion, respiration, and lipolysis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity in animals is similar to that found in humans. Dehydration and hyperthermia may occur.

Human

Acute toxicity manifests primarily in the CNS, cardiovascular system, and gastrointestinal system. CNS signs include restlessness, tremor, nervousness, headache, insomnia, tinnitus, confusion, delirium, psychosis, and seizures. Cardiac manifestations of overdose include sinus tachycardia, various dysrhythmias, asystole, and cardiovascular collapse. Other findings include tachypnea, nausea, vomiting, hematemesis, diarrhea, and fever. Case reports also include rhabdomyolysis and pulmonary edema. Laboratory findings include metabolic acidosis, respiratory alkalosis, ketosis, hypokalemia, and hyperglycemia. The estimated lethal dose in adults is 150–200 mg kg^{-1}, whereas doses of 10–15 mg kg^{-1} may produce early signs of toxicity. Serum levels greater than 30 mg ml^{-1} have been associated with adverse symptoms. Levels exceeding 80 mg ml^{-1} have been associated with death, although levels as high as 405 mg ml^{-1} have been reported in survivors.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies in rats do not seem to produce increased levels of anxiety compared with

controls. When added to the diets of overweight, diabetic rats, caffeine produced slight increases in heart rate and blood pressure, but more profound changes in the kidney. Proteinuria increased dramatically and creatinine clearance was reduced compared to matched controls.

Human

No definite association has been demonstrated between habitual caffeine use and hypertension, myocardial infarction, carcinogenicity, or teratogenicity. Abrupt cessation of chronic caffeine ingestion may cause withdrawal headaches.

In Vitro Toxicity Data

Caffeine has been found to be weakly mutagenic in some nonmammalian animal models. It is not been found to be mutagenic in Ames *Salmonella* assays.

Clinical Management

After assessment of airway, breathing, and circulation with necessary supportive care, decontamination of the gastrointestinal tract should be undertaken for substantial recent ingestions. The patient should be placed on continuous cardiac monitoring with pulse oximetry. The initial treatment of hypotension consists of intravenous fluids. If hypotension persists, then pressors may be considered. Frequent neurological checks should be made. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered, but vomiting may make retention difficult. Beta-blocking agents have been used to treat caffeine tachydysrhythmias; however, one report described cardiovascular collapse following β-blocker administration. Standard therapy for seizures should be employed. Monitoring should be performed for fluid and electrolyte imbalances.

Various techniques to enhance elimination of caffeine have been reported in the literature. Multidose activated charcoal has been advocated to both prevent further absorption of drug and enhance elimination by gut dialysis. Hemodialysis has been reported in the literature for the treatment of caffeine toxicity. The mean plasma protein binding of caffeine (36%), the molecular size (194), and the volume of distribution ($0.6–0.8\,l\,kg^{-1}$) make hemodialysis a possible modality to enhance elimination. There have also been cases of severe caffeine toxicity treated with peritoneal dialysis, but this modality is less efficient at drug clearance than hemodialysis.

See also: Catecholamines; Theophylline.

Further Reading

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Nawrot P, Jordon S, Eastwood J, Rotstein J, Hugenholtz A, and Feeley M (2003) Effects of Caffeine on human health. *Food Additives and Contaminants* 20(1): 1–30.

Calcium Channel Blockers

Shayne C Gad

- Representative Compounds: Amlodipine; Bepridil; Diltiazem; Felodipine; Isradipine; Nicardipine; Nifedipine; Nimodipine; Verapamil
- Chemical Abstracts Service Registry Numbers: Norvasc (CAS 88150-42-9); Vascor (CAS 74764-40-2); Cardizem (CAS 42399-417); Plendil (CAS 72509-76-3); DynaCirc (CAS 75695-93-1); Cardene (CAS 55985-32-5); Procardia (CAS 21829-25-4); Nimotop (CAS 66085-59-4); Isoptin (CAS 15211-4)
- Chemical/Pharmaceutical/Other Class: Antiarythmics

Uses

Calcium channel blockers are used in the management of angina pectoris, hypertension, supraventricular arrhythmias, and subarachnoid hemorrhage.

Exposure Routes and Pathways

Ingestion is the most common route for both accidental and intentional exposures. Verapamil and diltiazem

are both available for parenteral administration, and toxicity can occur via the parenteral route.

Toxicokinetics

Following oral administration, absorption is rapid and almost complete (80–100%), but the ultimate bioavailability is limited and variable (15–94%) following oral administration due to significant first-pass metabolism in the liver. Protein binding is high and ranges from 70% to 99%. Volumes of distribution for some calcium channel blockers are as follows: verapamil, 5 l kg^{-1}; diltiazem, 3.1 l kg^{-1}; nifedipine, 0.78 l kg^{-1}; and nicardipine, 1.1 l kg^{-1}. Extensive hepatic metabolism occurs. Only small amounts (0–10%) are excreted unchanged in the urine. Elimination half-life ranges from 1 h (nimodipine) to 50 h (amlodipine).

Mechanism of Toxicity

The pharmacologic and toxicologic mechanisms of the calcium channel blockers are complex. They include interference with electrical conduction through the atrioventricular node, decreased myocardial contractility, and direct vasodilation. Calcium channel blockers also interfere with pancreatic release of insulin.

The interference with electrical conduction through the atrioventricular node is caused by interference with the influx of calcium in phase II of the action potential and manifest by bradycardia, lengthening of the PR interval, QRS widening, and QTc prolongation.

Decreased myocardial contractility is due to calcium influx into the cell, which results in increased release of calcium from the sarcoplasmic reticulum. The overall effect of this calcium influx and release is the bridging of actin and myosin and subsequent myocardial contraction. The negative inotropic effect of the calcium blockers is due to interference with this process.

Vasoconstriction occurs when calcium activates vascular myosin kinase, which in turn allows for phosphorylation of myosin and subsequent bridging with actin. Administration of calcium channel blockers will interfere with this process and produce vasodilation.

Acute and Short-Term Toxicity (or Exposure)

Human

The clinical effects of the calcium channel blockers are primarily cardiovascular in nature. Due to their interference with conduction, they can cause a variety of dysrrhythmias including sinus bradycardia, all degrees of atrioventricular block, junctional rhythms, pulseless electrical activity, and asystole. The negative inotropic effects of the calcium channel blockers cause significant decreases in cardiac output. Profound hypotension is observed following calcium channel blocker poisoning due to their vasodilatory properties. Renal failure secondary to decreased perfusion may be seen. The neurologic toxicities of the calcium channel blockers are most likely secondary to their cardiovascular effects. The most common neurologic effects are lethargy and coma. Neurologic deterioration can be rapid. Some patients with significant hypotension may have intact neurologic examinations initially. Seizure activity has also been observed in calcium channel blocker toxicity. The most common metabolic effects that occur in calcium channel blocker toxicity are metabolic acidosis, hyperglycemia, and hypokalemia. Hyperkalemia has also been reported.

Clinical Management

Advanced supportive care is a primary component of patient management. Emergent intubation and assisted ventilation are often necessary in these patients. Pulse oximetry should be utilized to assess respiratory status. Extensive cardiovascular monitoring is also necessary. Arterial blood gases, serum electrolytes, and glucose measurements should be obtained. Serum concentrations of specific calcium channel blockers are difficult to obtain and have limited clinical utility. Syrup of ipecac-induced emesis is contraindicated due to the rapid decreases in level of consciousness that may occur as well as emesis-induced vasovagal effects. Gastric lavage and activated charcoal can be used if warranted by the history of the ingestion and the patient's neurologic status. Whole bowel irrigation along with activated charcoal should be utilized in ingestions involving sustained-release products. Calcium salts are often administered as antidotes for calcium channel blocker toxicity although they have been used with limited success. Calcium chloride is preferred over calcium gluconate since it contains more elemental calcium on a milligram-per-milligram basis. Doses of up to 4 g of calcium have been recommended in this setting. Glucagon, which has been used in β-adrenergic blocker toxicity, has been recommended in calcium blocker toxicity. This agent has positive inotropic properties due to activation of cyclic adenosine monophosphate. It has limited beneficial effects in calcium channel blocker toxicity. Control of heart rate and rhythm present a significant challenge in this patient population. Transcutaneous pacemakers should be utilized to stabilize rate and enhance

atrioventricular conduction. A vagolytic, atropine, has also been used to increase heart rate. It has limited effect since it primarily affects the sinoatrial node. The negative inotropic effects of these agents must also be treated aggressively. Positive inotropic agents, such as dopamine, dobutamine, amrinone, and isoproterenol, can be utilized to increase contractility. Isoproterenol should be used with caution due to its vasodilatory properties. Vasopressors, such as dopamine, epinephrine, and norepinephrine, may be effective. Cardiopulmonary bypass has been used experimentally to treat patients with calcium channel blocker toxicity who do not respond to traditional therapy. Sodium bicarbonate should be administered to treat acidosis.

Seizure activity should be initially treated with benzodiazepines. If benzodiazepines are not effective, phenytoin and barbiturates can be administered. Insulin replacement may be necessary to correct hyperglycemia.

See also: Cardiovascular System.

Further Reading

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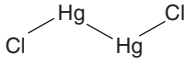
Eisenberg MJ, *et al.* (2004) Calcium channel blockers: An update. *American Journal of Medicine* 116(1): 35–43.

Calomel

Kashyap N Thakore

- Chemical Abstracts Service Registry Number: CAS 7546-30-7
- Synonyms: Mercurous chloride; Mercury(I) chloride; Mercury monochloride
- Chemical/Pharmaceutical/Other Class: Heavy metals
- Chemical Formula: Hg_2Cl_2
- Chemical Structure:



Uses

Calomel is used as a laboratory reagent, as a fungicide, and as a depolarizer in dry batteries.

Exposure Routes and Pathways

The primary routes of entry are ocular and dermal contact, inhalation, and ingestion. Calomel is found in environmental and occupational settings, such as in mercury mining operations, battery plants, paints and dyes, photography, perfumes and cosmetics, and chemical laboratories. It is poisonous by ingestion through food and intraperitoneal routes. Calomel is moderately toxic by skin contact. When heated to decompose, it emits very toxic fumes of Cl^- and Hg.

Toxicokinetics

After inhalation, ~70–80% of metallic vapor is retained and absorbed. Little is taken up in the gastrointestinal tract, and less than 10% is absorbed. In the body, it is oxidized to mercuric mercury, which binds to reduced sulfhydryl groups. The kidney is the main depository following exposure to both metallic and mercuric mercury. In addition to other organs, it passes into the brain and fetus.

The metabolite is eliminated mainly in urine and feces; it is also excreted in milk. In humans, inorganic mercury compounds have two elimination half-lives: one lasts for days or weeks and the other much longer.

Mechanism of Toxicity

Calomel can generate reactive oxygen species and deplete glutathione levels. Both genotoxic and nongenotoxic mechanisms may contribute to renal carcinogenic effect of mercury.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, intense exposure causes lung damage, intestinal and renal tubular necrosis, immunosuppression, and possible cytogenetic effects. The oral LD_{50} is $210\,mg\,kg^{-1}$ in rats and $180\,mg\,kg^{-1}$ in mice. The intraperitoneal LD_{50} is $10\,mg\,kg^{-1}$ in mice.

Human

Calomel is harmful and may be fatal, if swallowed or inhaled. When swallowed, it causes central nervous

system depression; when inhaled, it causes tightness and pain in the chest, coughing, and breathing difficulties. Ocular and dermal exposures cause irritation of the eyes and skin. In cases of chronic exposure, mercury builds up in the brain, liver, and kidneys and causes headache, shakes, loose teeth, loss of appetite, skin ulceration, and impaired memory. Mercury concentration in urine, blood, and plasma is useful for biological monitoring.

The recommended health-based limits are $0.05\,mg\,m^{-3}$ for occupational exposure, $50\,\mu g\,g^{-1}$ creatinine in urine for long-term occupational exposure to mercury vapors, and $1\,\mu g\,l^{-1}$ for exposure by drinking water (WHO report, 1980).

Chronic Toxicity (or Exposure)

Animal

There is limited evidence for carcinogenicity. Calomel causes renal adenoma and adenocarcinoma in male mice and female rats.

Human

There is inadequate evidence for carcinogenicity.

Clinical Management

In case of contact, eyes and skin should be flushed with water for 15–20 min. If inhaled, the victim should be removed to fresh air. If necessary, oxygen and artificial respiration should be administered. If the patient is in cardiac arrest, cardiopulmonary resuscitation should be provided. These life-supporting measures should be continued until medical assistance has arrived. An unconscious or convulsing person should not be given liquids or induced to vomit.

Other Hazards

It is not flammable.

See also: Mercury; Metals.

Further Reading

International Agency for Research on Cancer (IARC) (1993) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Beryllium, Cadmium, Mercury and Exposures in the Glass Manufacturing Industry*, vol. 58, p. 239.

US Environmental Protection Agency (US EPA) (1997) Mercury Study Report to Congress: Health Effects of Mercury and Mercury Compounds, vol. V.

Relevant Websites

http://www.iarc.fr – International Agency for Research on Cancer.

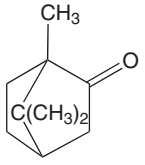
http://www.epa.gov – US Environmental Protection Agency.

Camphor

Fermin Barrueto Jr.

This article is a revision of the previous print edition article by Bonnie S Dean, volume 1, pp. 213–214, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: CAS 464-48-2 and CAS 464-49-3 (optical isomers); CAS 21368-68-3 (racemic mixture)
- Synonyms: Campho-phenique; Musterole; Ben-Gay children's vaporizing rub; Vicks Vaporub; Vicks Vaposteam; Heet; Sloan's Liniment; Camphorated oil; Camphor spirits
- Chemical/Pharmaceutical/Other Class: Cyclic ketone of the hydroaromatic terpene group
- Chemical Formula: $C_{10}H_{16}O$
- Chemical Structure:



Uses

Camphor is employed externally as a rubefacient, mild analgesic, antipruritic, and counterirritant in commercially available products that contain 1.0–10% camphor. It is currently produced synthetically. It has a characteristic odor and a pungent aromatic taste.

Exposure Routes and Pathways

Ingestion is the most common route of both intentional and unintentional exposure to camphor.

Ocular exposures may also occur as can transdermal exposure.

Toxicokinetics

Camphor in liquid form is rapidly absorbed through the skin, mucous membranes, and gastrointestinal tract. Symptoms may appear within 5–90 min following ingestion. The absorption is highly dependent on the presence of food and other chemicals that may influence the rate of camphor absorption. Camphor is metabolized to a campherol, which is conjugated with glucuronic acid in the liver. It is unclear whether camphor toxicity is attributed to the parent compound, a metabolite, or both. Camphor-related metabolites are fat soluble. Thus, significant concentrations may accumulate in fat tissue. Camphor is distributed widely in all tissues. Measurable serum levels are apparent within minutes after ingestion of $\sim$0.5–1.0 g. The volume of distribution is $\sim$2–4 l kg^{-1}. The glucuronide form is excreted in the urine. The half-life of a 200 mg dose is known to be 167 mm.

Mechanism of Toxicity

Its action has been postulated to be intraneuronal on the oxidation cycle at a phase above the cytochrome *b* level of the cytochrome oxidase system though its precise mechanism has not been elucidated. It is primarily a neurotoxin with a chemical structure that allows for easy penetration of the blood–brain barrier.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal toxicity corresponds to human toxicity.

Human

Upon ingestion, an initial burning sensation may be noted in the mouth and throat. Spontaneous nausea and vomiting may occur within minutes of ingestion. Confusion, vertigo, restlessness, delirium, hallucinations, tremors, and convulsions are all directly related to the central nervous system involvement and may be predictors of serious toxicity. More severe intoxications may result in hepatic failure. Death may be caused by respiratory depression or may follow status epilepticus. Camphor should be considered an eye irritant.

Chronic Toxicity (or Exposure)

Animal

Chronic camphor dosing in a mouse model has lead to development of neuronal necrosis.

Human

The chronic ingestion of camphor may produce similar toxicity but in a more insidious fashion. Liver failure is a more pronounced clinical manifestation.

In Vitro Toxicity Data

Camphor inhibited catecholamine secretion from bovine adrenal chromaffin cells.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be used for substantial recent ingestions. Activated charcoal is marginally effective in adsorbing camphor. Oils, alcohols, and other lipophilic substances enhance intestinal absorption and are contraindicated. Ocular exposures necessitate flushing with a gentle system of tepid water for a minimum of 15 min. If signs of irritation persist, an ophthalmology consultation is required. The seizure activity is often singular and self-limiting and responsive to benzodiazepines.

See also: Benzodiazepines; Catecholamines; Charcoal.

Further Reading

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Cancer *See* Carcinogenesis.

Cancer Chemotherapeutic Agents

David S Fischer

Cancer is a general term used to describe 100 or more malignant neoplasms that invade other tissues and may metastasize to distant sites and then grow there. The defining characteristic of the cancer cell is uncontrolled proliferation and multiple genetic alterations. A tumor is a circumscribed noninflammatory growth arising from existing tissue but growing independently of the normal rate or structural development of such tissue and serving no physiological function. It may be malignant or benign. The benign tumor does not invade or metastasize.

A chemotherapy drug is a chemical agent used to treat diseases. The term may be applied to a drug used to treat infection, but more frequently is used to refer to drugs used to treat cancer. The term, cancer chemotherapy, is used by some to include biological agents that are used to treat cancer while others prefer to use the more specific terms biotherapy, cancer biotherapy, or biologic therapy of cancer.

Historical Development

For several centuries, the only useful treatment for tumors was surgical removal. With the development of cellular and tissue pathology in the mid-nineteenth century, malignant tumors could be identified without demonstrating distant metastases, and malignancies of the blood were identified and called leukemia. In 1865, Lissauer, a German physician, used potassium arsenite (Fowler's solution) by chance and found that it restored to health two near moribund patients with chronic myeloid leukemia. This was the first chemical agent effective in the treatment of a malignant disease and it continued to be used for 70 years. Recently, arsenic trioxide has been used as an effective drug for treating acute promyelocytic leukemia (APL).

After Roentgen discovered X-rays in 1895, they were used for many medical purposes and were particularly effective in shrinking Hodgkin's disease tumors and the enlarged spleens of chronic leukemias with a resultant drop in their high white cell counts, results similar to those produced by potassium arsenite. Paul Ehrlich used organic arsenicals in his search for a 'magic bullet' to cure syphilis. Other investigators were frustrated by their inability to find effective agents to treat cancers because they did not understand the biology of cancer and the search was largely abandoned for many decades.

Modern Chemotherapy

The development of effective antibacterial agents, for example, sulfanilamide and penicillin in the 1930s, aroused interest in chemical and biological agents in the treatment of cancer. During World War II, a number of investigators studied the effects of chemical warfare agents that might be used by adversaries. Nitrogen mustard, then known by the wartime code name HN2, was extensively studied in the laboratory and in mice and rabbits before the first near moribund patient with lymphoma was treated in early December 1942 at the New Haven Hospital affiliated with the Yale School of Medicine.

The treatment resulted in a dramatic regression of disease and the era of cancer chemotherapy began. Several books relate the story that the use of nitrogen mustard as a chemotherapeutic agent was suggested by the serendipitous finding of marrow and lymphoid hypoplasia in seamen exposed to mustard gas following the sinking of a ship in Bari Harbor, Italy, containing chemical warfare agents. That event is well documented but it occurred on December 2, 1943, one year after the Yale human trials. This is an interesting story but there is no direct connection.

Nitrogen mustard will hereafter be referred to by its generic name, mechlorethamine, and generic names will be used for all drugs. Trade names for some are listed in Appendix 1.

As a therapeutic agent, mechlorethamine has many toxic effects. Acutely, it causes nausea and vomiting, skin blistering, and ulceration. After a week or two, it causes leukopenia, lymphopenia, anemia, thrombocytopenia, diarrhea, oral ulcers, and hyperuricemia. It can cause sterility and after a few years, leukemia. The most susceptible tissues are those with renewable cell populations, bone marrow, lymphoid tissues, and gastrointestinal (GI) epithelium. The therapeutic dose of mechlorethamine and most of the cytotoxic chemotherapy drugs is very close to the toxic dose. The therapeutic index (ratio of beneficial effect to toxic effect) is small.

Both the benefits and toxicities of mechlorethamine stimulated a worldwide search for new antineoplastic agents. In the United States, the National Cancer Institute (NCI) had been established in 1937 and was already empirically studying plant extracts for anticancer activity. In 1955, the NCI established the Cancer Chemotherapy National Service Center to systematically screen drugs *in vitro* and *in vivo*. NSC numbers were assigned to each new drug screened and the number now exceeds 720 000. Over the past half century, a growing understanding

of the biology and metabolism of proliferating cells has led to the development of about 100 active anticancer drugs that have been FDA approved and marketed, and many more are in the pipeline. The groups have similar or related toxicities. The mechanism of action that is successful in injuring or eliminating the cancer cell is usually the same mechanism of action that injures or destroys the normal cell leading to the adverse effects that we call toxicity. Of course, drugs in the same group also have some dissimilar and unique toxicities. The goal is to develop drugs that are able to differentially damage or kill neoplastic cells and spare benign cells. Penicillin is effective because it destroys the cell wall of plants. Bacteria are plants and are susceptible to destruction when the plant wall is injured sufficiently. Animals have a cell membrane but no cell wall and therefore penicillin has minimal toxicity in those humans who are not allergic to it. The goal in cancer chemotherapy is to develop similarly targeted precision drugs and a few new drugs appear to fill that role.

Cell Kinetics and the Cell Cycle

The rate of growth of a tumor is a reflection of the proportion of actively dividing cells (the growth fraction), the length of the cell cycle (doubling time), and the rate of cell loss. Acute leukemias, some lymphomas, germ cell tumors, Wilms' tumor, neuroblastoma, and choriocarcinoma are characterized by a rapid growth fraction as demonstrated by tritiated thymidine uptake and turnover studies. Most solid cancers are not characterized by rapid growth. For example, breast, lung, and colon cancer cells may take up to 100 days to double their population. The growth and division of normal and neoplastic cells occur in a sequence of events called the cell cycle. The cell cycle is divided into several different phases (**Figure 1**). Many of the antineoplastic drugs have been and many continue to be classified based on whether their activity is cell cycle specific or nonspecific. Alkylating agents are nonspecific. Antimetabolites, vinca alkaloids, taxanes, podophyllotoxins, and a few others are specific (**Table 1**).

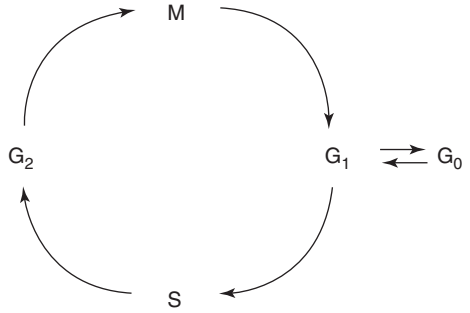

Figure 1 Phases of the cell cycle. G_0, resting phase (nonproliferation of cells): G_1, pre-DNA synthetic phase (12 h to a few days). S, DNA synthesis (usually 2–4 h); G_2, post-DNA synthesis (2–4 h; cells are tetraphoid in this stage); M, mitosis (1–2 h).

Synthesis of ribonucleic acid (RNA) and protein occurs during the G_1 phase. When cells are in G_1 for prolonged periods of time, they are often said to be in a resting phase, referred to as G_0. Synthesis of deoxyribonucleic acid (DNA) occurs during the S phase. During G_2, DNA synthesis halts, and RNA and protein synthesis continue. The final steps of chromosome replication and segregation occur during the mitotic or M phase. The cell undergoes cell division and produces two daughter cells. The rate of RNA and protein synthesis slows during this phase as the genetic material is transferred into the daughter cells. Also located within the cell cycle of normal cells are check points. These are biochemically designated areas that can be activated during the cell cycle process. They prevent the cell from moving forward from one phase to the next if adverse genetic conditions have occurred in the previous phase. Many cancer cells have lost these check points. Drugs that exert their cytotoxic effects during a specific phase of the cell cycle (i.e., phase-specific agents) are usually not effective against cells that are predominantly in a dormant phase (G_0). In contrast, nonphase-specific agents are theoretically more likely to be effective against a tumor population that is not in a state of rapid division. The antineoplastic drugs can best be studied in groups related either to their mechanism of action or by their source of origin (**Table 2**).

Table 1 Cell-cycle-phase-specific drugs

S phase-dependent	*M phase-dependent*
Antimetabolites	Vinca alkaloids[a]
Capecitabine	Vinblastine
Cytarabine	Vincristine
Doxorubicin	Vinorelbine
Fludarabine	Podophyllotoxins
Floxuridine	Etoposide
Gemcitabine	Teniposide
Hydroxyurea	Taxanes
Mercaptopurine	Docetaxel
Methotrexate	Paclitaxel
Pemetrexed	G_2 phase-dependent
	Bleomycin
Procarbazine	Irinotecan
Thioguanine	Mitoxantrone
	Topotecan
	G_1 phase-dependent
	Asparaginase

[a] Have greatest effect in S phase and possibly late G_2 phase; cell blockade or death, however, occurs in early mitosis.

Table 2 Classification of anticancer drugs by mechanism of action or derivation

Alkylating agents
Antimetabolites
Natural products
Hormonal agents
Biotherapeutic agents
Miscellaneous agents

Table 3 Alkylating agents

Nitrogen mustards
Chlorambucil
Cyclophosphamide
Estramustine
Ifosfamide
Mechlorethamine
Melphalan
Aziridine
Thiotepa
Alkyl sulfonate
Busulfan
Nitrosoureas
Carmustine
Lomustine
Streptozocin
Platinum complexes
Carboplatin
Cisplatin
Oxaliplatin
Nonclassical alkylators
Altretamine
Dacarbazine
Procarbazine
Temozolomide

The Alkylating Agents

Alkylating agents are highly reactive compounds that easily attach to DNA and cellular proteins. The primary mode of action for most alkylating drugs is via cross-linking of DNA strands. They can be classified as either monofunctional alkylating agents, implying reactions with only one strand of DNA, or bifunctional alkylating agents, which cross-link two strands of DNA. Replication of DNA and transcription of RNA are prevented by these cross-links.

Many alkylating agents have been developed (**Table 3**). Although these drugs have similar mechanisms of action, there are major differences in spectrum of activity, pharmacokinetic parameters, and toxicity. Alkylating agents play a significant role in the treatment of lymphoma, Hodgkin's disease, breast cancer, multiple myeloma, and other malignancies. In addition to conventional chemotherapy, the linear dose–response curve of alkylating agents expands their role for incorporation into transplant regimens.

The major clinical toxicities of most of the alkylating agents are similar to those of mechloramine, primarily bone marrow depression (including anemia, leukopenia, and thrombocytopenia) and nausea and vomiting. Individual drugs have additional toxicities. Chlorambucil, mechlorethamine, melphalan, and procarbazine can cause gonadal dysfunction and occasionally, late leukemias. Busulfan, carmustine, chlorambucil, and lomustine can cause pulmonary fibrosis. Cyclophosphamide and ifosfamide can cause hemorrhagic cystitis and in a small percent of patients, bladder cancer. Cisplatin, carmustine, lomustine, and streptozocin can cause renal damage. Carboplatin and cisplatin can cause ototoxicity and peripheral neuropathy. Procarbazine is a weak monoamine oxidase inhibitor. It can cause hypertensive reactions if used concurrently with sympathomimetic agents, tricyclic antidepressants, foods with high tyramine content, and with the narcotic meperidine.

Antimetabolites

The interest in antibacterial chemotherapy and its mechanisms of action had direct consequences for antineoplastic drug development. After sulfanilamide was found to be an antimetabolite of paraminobenzoic acid, an essential growth factor for streptococci, the group at Lederle Laboratories synthesized antimetabolites of folic acid – first aminopterin and later amethopterin now known generically as methotrexate. In 1948, Farber and the Harvard Childrens Hospital group used these antimetabolites to palliate acute lymphoblastic leukemia in children. This led to further studies of anticancer drugs based on the biochemistry and metabolism of cancer cells. In 1954, Hitchings and Elion at the Burroughs Wellcome laboratories developed the antipurine drugs, 6-mercaptopurine and 6-thioguanine for leukemias. In 1957, Heidelberger and his group at the McArdle Institute at the University of Wisconsin introduced the first antipurine, 5-fluorouracil for GI tumors. Additional antimetabolites have been developed (**Table 4**).

Antimetabolites interfere with the synthesis of DNA, RNA, and ultimately proteins. They exert their effects largely in the synthetic (S) phase of the cell cycle. Some antimetabolites are structural analogs of normal metabolites essential for cell growth and replication. This property allows some of them to be incorporated into DNA and/or RNA so that a false message is transmitted. Other antimetabolites inhibit enzymes that are necessary for the synthesis of essential compounds. The action and toxicity of the antimetabolites are significantly

Table 4 Antimetabolites

Folate analogs
Methotrexate
Pemetrexed
Trimetrexate
Purine analogs
Cladribine
Fludarabine
Mercaptopurine
Pentostatin
Thioguanine
Pyrimidine analogs
Capecitabine
Cytarabine
Floxuridine
Fluorouracil
Gemcitabine
Ribonucleotide reductase inhibitor
Hydroxyurea

Table 5 Natural products

Camptothecin analogs
Irinotecan
Topotecan
Epipodophyllotoxins
Etoposide
Teniposide
Antitumor antibiotics
Bleomycin
Dactinomycin
Daunorubicin
Doxorubicin
Epirubicin
Idarubicin
Mitomycin
Mitoxantrone
Valrubicin
Microtubule agents
Docetaxel
Paclitaxel
Vinblastine
Vincristine
Vinorelbine
Enzymes
Asparaginase
Pegasparaginase
Metals
Arsenic trioxide
Gallium nitrate

modified by the duration of exposure as well as the dose. Prolonged infusions or prolongation of absorption by pegylation or incorporation into liposomes can change both the response and the toxicity. Since this is a large subject, it will suffice to note here that some of the anticancer antibiotics and biotherapy drugs are also available in pegylated or liposomal forms.

The toxicity of antimetabolites is, as expected, due to their incorporation into the metabolism of normal cells, which is nearly identical to that of the malignant cells that they were designed to injure. The normal cells injured most severely are the rapidly proliferating cells of the bone marrow, the lymphoid system, and the GI epithelium. Thus, the common toxicities are bone marrow depression, nausea and vomiting, diarrhea, and mucositis. Cytarabine and pentostatin can cause conjunctivitis. Capecitabine and prolonged use of fluorouracil or cytarabine can cause cerebellar ataxia and the hand-foot syndrome, that is, palmar-plantar erythrodysesthesia or acral erythema. Pentostatin and high-dose methotrexate can cause renal toxicity.

Natural Products

The natural products may be divided into six primary groups (**Table 5**): campothecin analogs, epipodophyllotoxins, antitumor antibiotics, microtubule agents, enzymes, and metals. The first three act primarily on the topoisomerases. Topoisomerases are enzymes that break and reseal DNA strands. The plant alkaloid campothecin and its analogs (topotecan and irinotecan) are nonclassic enzyme inhibitors of topoisomerase I. These agents are no longer referred to as inhibitors but are instead classified as topoisomerase I targeting agents or topoisomerase I poisons. The epipodophyllotoxins (etoposide and teniposide) and the antitumor antibiotics (dactinomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, and valrubicin) are inhibitors of topoisomerase II. The drugs form a stable complex by binding to DNA and topoisomerase enzymes, resulting in DNA damage that interferes with replication and transcription.

Mitotic Inhibitors

A group of mitotic inhibitors (vinblastine, vincristine, and vinorelbine) exert their cytotoxic effects by binding to tubulin. This inhibits formation of microtubules, causing metaphase arrest. Their mechanism of action and metabolism are similar, but the antitumor spectrum, dose and clinical toxicities of vinblastine, vincristine, and vinorelbine are very different. Paclitaxel and docetaxel are also mitotic inhibitors. However, they differ from the vinca alkaloids by enhancing microtubule formation. As a result, a stable and nonfunctional microtubule is produced.

The major toxicities of these four groups are bone marrow depression, nausea and vomiting, mucositis, and diarrhea. Daunorubicin, doxorubicin, epirubicin, idarubicin, and to a lesser extent, mitoxantrone, cause cardiac toxicity. Mitomycin and bleomycin cause

pulmonary fibrosis. Paclitaxel and vincristine cause peripheral neuropathy, and paclitaxel (or its vehicle) can cause anaphylaxis. Dactinomycin, daunorubicin, docetaxel, doxorubicin, epirubicin, etoposide, idarubicin, mitoxantrone, mitomycin, paclitaxel, teniposide, and vinblastine all cause alopecia to varying degrees. Etoposide and topotecan can cause leukemia. Other individual unique toxicities will be noted in Appendix 2.

Enzymes

L-Asparaginase is an enzyme product that acts primarily by inhibiting protein synthesis by depriving tumor cells of the amino acid asparagine. Cells that have the ability to form their own asparagine, such as many normal cells, are not affected by L-asparaginase.

L-Asparaginase is a foreign protein, is antigenic, and can cause serious hypersensitivity reactions. Included in this category are the long-acting pegylated asparaginase and Erwinia-derived asparaginase, both similar in mechanism to L-asparaginase.

Metals

Of the two metals used in cancer chemotherapy, only one is significant. At one time, gallium nitrate was used for the treatment of hypercalcemia and bladder cancer, but it causes nausea, vomiting, and renal toxicity and has been largely replaced by superior drugs. Arsenic trioxide has been available for a century and was sometimes used instead of potassium arsenite for treatment of chronic myeloid leukemia. Both arsenicals were abandoned for this purpose after superior agents became available. Arsenic trioxide was recently reintroduced into cancer chemotherapy by the Chinese. Its efficacy for inducing remissions in APL has been confirmed in Europe and the United States. Its major toxicities are nausea, vomiting, abdominal pain, diarrhea, pruritis, headache, dermatitis, hyperpigmentation, some skin exfoliation, and some bone marrow depression. 'Retinoic acid syndrome' (RAS) occurs in ~30% of patients treated and is characterized by high fever, dyspnea, respiratory distress, pulmonary infiltrates, and pericardial and/or pleural effusions. Some patients have required intubation and mechanical ventilation. Initiation of corticosteroid treatment at the first sign of dyspnea is advised and then maintained until symptoms resolve.

Hormonal Agents

The palliation of breast and prostate cancer by means of endocrine manipulation is an effective and relatively nontoxic therapy. Toward the end of the nineteenth century, it was noted that the ovaries influenced mammary physiology. In 1896, Beatson, an English surgeon, removed the ovaries in some premenopausal women with breast cancer and reported striking palliation in a few. This was the first use of cancer therapy that involved hormonal manipulation although the term hormone and the concept of a humoral regulator were not developed until 1902. Subsequent studies of oophorectomy showed temporary improvement in one-third of premenopausal patients and it is still used in some selected patients although it causes a prompt menopause with all its side effects.

In 1941, Huggins, Stevens, and Hodges showed that bilateral orchiectomy could lead to shrinkage of prostatic cancer and its metastases and relieved the pain of bone metastases in many patients. This approach is still used, although less frequently since the availability of medical alternatives. As expected, orchiectomy leads to impotence, loss of libido, gynecomastia, softening of the skin and beard, fatigue, loss of muscle tone, changes in personality, decreased bone mineral density, and hot flashes. In recent years, long-acting gonadotropin-releasing hormone (GnRH) analogs also known as leuteinizing-hormone releasing hormone (LHRH) analogs alone or in combination with androgen antagonists, have offered an alternative therapy with equal efficacy and more control and reversibility of the side effects with intermittent therapy. These drugs are listed in **Table 6** and their individual toxicities in Appendix 2.

Hormonal management of breast cancer used to depend on androgens, estrogens, and progestins. In recent years, they have been largely replaced by estrogen antagonists, aromatase inhibitors, and LHRH analogs. These new groups of agents have the side effects one would expect from estrogen deprivation, such as hot flashes, decreased energy, a variable decrease in bone mineral density, variable nausea, and in some cases an increased incidence of thromboembolic phenomena.

Corticosteroids are widely used throughout medical practice. In cancer therapy, prednisone and dexamethasone are the most frequently used. They have a lytic effect on lymphoma and myeloma cells, reduce the edema associated with brain metastases, reduce immunological and allergic reactions and exert an antiemetic effect alone and with 5-HT_3 blockers. The many side effects of corticosteroids are often the consequence of the desired effect on the disease process being treated also impacting the normal tissues adversely. These toxicities are well known as they are seen throughout clinical medicine.

Table 6 Hormonal agents

Androgens
Fluoxymesterone
Testosterone
Androgen antagonists
Bicalutamide
Flutamide
Ketoconazole
Nilutamide
Aromatase inhibitors
Aminoglutethimide
Anastrazole
Exemestane
Letrozole
Corticosteroids
Dexamethasone
Prednisone
Estrogens
Diethylstilbesterol
Estradiol
Estrogen antagonists
Fulvestrant
Raloxifene
Tamoxifen
Toremifene
Luteinizing hormone-releasing hormone (LHRH) analogs
Abarelix
Goserelin
Leuprolide
Triptorelin pamoate
Progestins
Medroxyprogesterone acetate
Megestrol acetate

Biotherapeutic Agents

The immune system is responsible for protecting the body from bacteria, viruses, and cancer. Early work with nonspecific stimulators of the immune system failed to demonstrate any reliable benefit. More recent investigations of immunological responses have increased our knowledge of tumor biology and coupled with recombinant DNA technology have led to the development of the biologic response modifiers and monoclonal antibody targeting agents that are effective as targeted cancer treatment options (**Table 7**). More treatment options are in the pipeline.

Interferons were originally isolated from human leukocytes as antiviral agents, but the interferon alfa-2 that we use today in cancer therapy is a recombinant product. It is used primarily in the treatment of Hairy Cell leukemia, the Kaposi sarcoma of AIDS, melanoma, and renal cell carcinoma. The major toxicity is a flu-like syndrome with fever, chills, rigors, and myalgias. Longer term toxicities include profound fatigue, confusion, neurologic side effects, and depression, sometimes severe enough to lead to suicide.

Table 7 Biotherapeutic agents

Interferon alfa-2
Interleukin-2 (aldesleukin)
Monoclonal antibodies
Alemtuzumab
Bevacizumab
Cetuximab
Gemtuzumab
Ibritumomab tiuxetan
Rituximab
Tositumomab
Trastuzumab

Interleukins are a family of cytokines, substances secreted by T-cells (lymphocytes), monocytes, macrophages, and other cells. Recombinant IL-2, known generically as aldesleukin, is effective in the therapy of a small percent of patients with renal cell carcinoma and melanoma, sometimes with very gratifying results. Its toxicity is dose-, route-, and time-dependent. At its worst, high-dose intravenous prolonged infusions cause fever, fluid retention, hypotension, respiratory distress, capillary-leak syndrome, suppression of hematopoiesis, nephrotoxicity, and hepatotoxicity.

Monoclonal antibodies were made possible by the development of the hybridoma methodology in 1986. Monoclonal antibodies are classified and named based on their derivation. Murine monoclonal antibodies having the suffix ending 'momab,' are cleared quickly from the body, and have a greater chance of inducing a HAMA reaction (human antimouse antibody). Chimeric antibodies are a human-mouse antibody mixture; they possess the suffix ending 'imab' and are more efficient and effective at destroying cells via CDC (complement-dependent cytotoxicity) and ADCC (antibody-dependent cell-mediated antibody). Chimeric antibodies circulate longer in the human body and are less likely to invoke a HAMA reaction. Humanized monoclonal antibodies possess the suffix ending 'umab' and are not likely to invoke a HAMA reaction.

Monoclonal antibody therapy is based on the ability to target markers and bind to cell membrane antigens with great specificity. Many times the enhanced specificity demonstrated toward the tumor antigens allows normal cells to be protected against harmful effects, unlike conventional chemotherapy. There are several mechanisms by which monoclonal antibodies destroy or prevent further replication of malignant cells. Some monoclonal antibodies utilize tumor immunology and components of the host natural defense mechanism to exert their desired effect. For example, monoclonal antibodies can utilize tumor effector cells to promote tumor cell lysis or have the ability to directly modulate tumor function.

Conjugated monoclonal antibodies can be used as carriers of toxic therapy, such as radionuclides, (e.g., yttrium-90 ibritumomab tiuxetan and iodine-131 tositumomab), cytotoxic drugs, or cell toxins to specific cell targets. They are also being employed to create tumor vaccines by stimulating a host antibody reaction causing the production of anti-idiotype antibodies.

In the last 2–5 years, selected monoclonal antibodies have become a routine part of care for certain malignancies. Rituximab, a chimeric monoclonal antibody used against CD 20 positive B-cell non-Hodgkin's lymphoma, is now utilized in combination with the CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone). Trastuzumab, a humanized monoclonal antibody, is a weekly maintenance therapy for HER2neu-positive metastatic breast cancer patients.

A common toxicity of monoclonal antibodies that react with antigen is the potential to produce a side effect referred to as an infusion-related symptom complex. The probability of this reaction occurring increases in patients with a large tumor burden. This reaction is generally observed with the first or second dose of the monoclonal antibody, however, it is important to note that mild to severe latent reactions have occurred. The symptom complex is characterized by one or more of the following: fever, chills, rigors, dyspnea, bronchospasm, headache, hypotension, rash, nausea, throat tightness, flushing, and urticaria. This reaction can range from very mild symptoms to a severe and/or fatal reaction. It is vital to assess each patient on an individual basis due to the variability of reactions. The management of infusion-related reactions begins with stopping the infusion, assessing the patient, and administering hypersensitivity medications as needed (e.g., diphenhydramine, meperidine, H2 blockers, corticosteroids, and epinephrine). Once patient symptoms have resolved, many patients can have the infusion restarted at a slower rate, under clinical observation.

Patients need to be aware of the side effects that are likely to occur, and they must be informed about what they can do to prevent or minimize the severity of these side effects. They also need to know that virtually all of the side effects are reversible, most subsiding within a few days after stopping treatment. The severity of symptoms varies from patient to patient. The lack of acute side effects is not usually predictive of adverse effects that may occur after weeks to months of treatment. Many of the side effects are subjective (i.e., fatigue, bone pain) and accurate documentation requires frequent communication and the cooperation of the patient and healthcare provider.

Miscellaneous Agents

Several agents are difficult to classify (**Table 8**).

Denileukin diftitox is a fusion protein that combines portions of the IL-2 molecule with the diphtheria toxin to destroy cells with the IL-2 receptor by inhibition of protein synthesis. It is used primarily in cutaneous T-cell lymphoma (CTCL) in patients whose disease expresses the CD 25 component of the IL-2 receptor. Its major toxicity is hypersensitivity reactions and the vascular leak syndrome.

Mitotane is an adrenal cytotoxic agent for the treatment of adrenocortical cancer. It has been suggested that it damages the mitochondria of adrenocortical cells. The major toxicity is nausea and vomiting and central nervous system effects like lethargy, somnolence, dizziness, and vertigo.

Octreotide is a long-acting somatostatin analog that inhibits the secretion of serotonin, vasoactive intestinal peptide, gastrin, motilin, insulin, glucagons, secretin, and pancreatic polypeptide. It is used for the control of symptoms in patients with carcinoid and vasoactive intestinal peptide-secreting tumors (VIPomas). Its major toxicity is nausea and vomiting.

Retinoids are differentiation agents related to or derivative of vitamin A. They bind to a cellular protein that facilitates their transfer from the cytoplasm to the nucleus where they are believed to increase DNA, RNA, and protein synthesis and to affect cellular mitosis.

Alitretinoin is dispensed as a gel, which is applied topically to treat the skin lesions of Kaposi's sarcoma secondary to AIDS. Except for mild skin irritation and a rash, it has no significant toxicity.

Betarotene is used in the treatment of refractory CTCL and the treatment of AIDS-related Kaposi's sarcoma. It may cause headache, rash, bone marrow depression, and photosensitivity.

Isotretinoin is widely used for the treatment of severe disfiguring acne. It is being evaluated for the

Table 8 Miscellaneous agents

Denileukin diftitox
Mitotane
Octreotide
Retinoids
Alitretinoin
Bexarotene
Isotretinoin
Tretinoin
Thalidomide
Tyrosine kinase inhibitors
Gefitinib
Imatinib

treatment of head and neck cancer, CTCL, and, neuroblastoma and as a prevention agent for myelodysplastic syndromes. Isotretinoin is teratogenic, and fetal abnormalities can result if used during pregnancy, particularly in the first trimester. Its toxicities include bone pain, myalgia, arthralgia, nausea, vomiting, headache, cheilitis, and elevated serum lipids. Although depression is uncommon, it has been associated with suicides, especially in teenage patients receiving it for the treatment of acne.

Tretinoin is better known as all-transretinoic acid or ATRA. It is a derivative of vitamin A and binds to a chromosomal receptor that is near the chromosomal lesion that is associated with APL. Differentiation of APL cells occurs after administration of tretinoin and remissions occur but the treatment is not curative and must be followed with cytotoxic chemotherapy for consolidation. Tretinoin is teratogenic and should not be used during pregnancy. General toxicity can also be severe and includes headache, xerosis, pruritis, arthralgia, myalgia, cheilitis, hypertriglyceridemia, and RAS which was described in relation to arsenic trioxide. It may be that the APL contributes to the drug effect in causing RAS. In either case, corticosteroid therapy with dexamethasone can control it.

Tyrosine kinase inhibitors are targeted to interfere with a crucial metabolic pathway that is more vulnerable in certain tumor cells than in normal cells.

Imatinib mesylate inhibits the Bcr-Abl tyrosine kinase and can induce apoptosis (programmed cell death) and inhibit further proliferation of the cell lines that are positive for Bcr-Abl. These cell lines are prominent in Philadelphia chromosome-positive chronic myeloid leukemia and in GIST (gastrointestinal stromal tumors). In many cases the initial responses have been very good. Toxicity consists mainly of nausea, vomiting, diarrhea, abdominal pain, skin rash, neutropenia, fluid retention, arthralgia, fatigue, fever, muscle cramps, myalgia, and muscle pain. In most cases therapy has not been interrupted due to the symptoms.

Gefitnib is a signal transduction inhibitor that is thought to exert its antitumor effects primarily by preventing activation of tyrosine kinase, which is necessary for the function of epidermal growth factor. Gefitnib exerts its primary antineoplastic effect by inhibiting EGFR. The EGFR is located in varying amounts in tumor cells of the colon, lung, head, and neck. Gefitinib is FDA approved for the treatment of nonsmall cell lung cancer, and is being investigated for activity in other malignancies. The major side effects are diarrhea and skin rash.

Thalidomide is best known as a drug that caused an international medical disaster. In 1957 it was marketed in Europe as a hypnotic, particularly for use by pregnant women. After a short period, it became apparent there was an increased incidence of a relatively rare birth defect, phocomelia, in which the hands and feet are attached close to the body resembling flipper of a seal or develop only as limb buds with no digits. It soon reached epidemic proportions, and retrospective epidemiologic research firmly established the causative agent to be thalidomide taken early in the course of pregnancy. Thalidomide was not licensed in the United States and was withdrawn from the European market in 1961. There were some cases in the United States in children born to women on investigational studies. In 1962, the Food Drug and Cosmetic Act was amended to give the FDA more authority in requiring evidence of both efficacy and relative safety before marketing new drugs.

In 1998, the FDA approved the marketing of thalidomide for erythema nodosum leprosy. Subsequently it has demonstrated activity against multiple myeloma, myelodysplastic syndrome, AIDS wasting syndrome, melanoma, and renal cell carcinoma.

To prevent severe birth defects and possible death of the newborn child, when thalidomide is used in women of child-bearing age or in sexually active men (due to levels of thalidomide in semen) adherence to strict guidelines adopted by the FDA is required. The specific guidelines fall under the term 'S.T.E.P.S. program' or 'System for Thalidomide Education and Prescribing Safety'. All prescribers (physicians) and distributors (pharmacists, etc.) must register and adhere to these guidelines. Other toxicities include headache, dizziness, rash, pruritis, drowsiness, somnolence, peripheral neuropathy, leucopenia, and venous thrombosis.

Combination Cancer Chemotherapy

Just as combination antibiotic chemotherapy has been found to be more efficacious in the treatment of tuberculosis and serious Gram-negative sepsis, as compared to single antibiotics, in a similar fashion, combination anticancer chemotherapy has been achieving better results than single agents in many of the tumors tested. Possible exceptions include some of the more sensitive neoplasms such as gestational trophoblastic tumors and African Burkitt's lymphoma where a single agent is often curative. Still, combination regimens seem to have higher response rates and longer durations of disease-free survival in many instances when compared to single agents.

It is best to select drugs with different mechanisms of cell destruction. One can combine an alkylating agent to kill cells in G_0 or any other phase of the

cycle, an antimetabolite to kill rapidly developing tumors in M phase, and a corticosteroid or other hormone to control cell growth without definitive cell kill. These agents with differing mechanisms reduce the chances of cell resistance.

While combination chemotherapy and high-dose therapy (with or without stem cell transplantation) can increase cancer response rates, they generally increase toxicity significantly and sometimes in unanticipated ways when drugs interact with each other. Hence, the toxicity of each combination chemotherapy protocol and each high-dose therapy protocol must be considered individually.

Management of Organ System Toxicity

It has been previously emphasized that cancer chemotherapy involves a process of differential and selective toxicity. Agents are used that injure neoplastic cells and normal cells and the goal is to damage the neoplastic cells irreversibly and allow the normal cells and tissues to recover sooner. In addition, it is important to ameliorate the unpleasant side effects and to support the patient. Discussion of a few major toxicities is in order.

Bone Marrow Suppression

All elements of the bone marrow are injured by cytotoxic drugs.

Neutrophils are depressed first because they renew their population every day. Neutropenia is defined as an absolute neutrophil count (ANC) 500 cells μl^{-1}. Patients with an ANC of less than 100 cells μl^{-1} or those with prolonged neutropenia (more than 7 days) are at significantly high risk for serious infection. That risk can be reduced with prophylactic antibiotics and the administration of colony-stimulating factors (CSFs). Current evidence does not support the routine use of CSFs (filgrastim, pegfilgrastim, and sargramostim) in afebrile neutropenic patients unless the patient is at high risk because of bone marrow compromise or comorbidity, for example, previous radiation to large areas of bone marrow, recurrent febrile neutropenia with similar dose chemotherapy, extensive prior chemotherapy, or active tissue infection. The exceptions to this guideline include administration of trimethoprim-sulfamethoxazole for immunosuppressed patients at risk for Pneumocystis carinii pneumonitis, and antifungal therapy (with fluconazole) and antiviral therapy (with acyclovir or gancyclovir) for prophylaxis of patients undergoing allogeneic stem cell transplantation. The development of fever (a single temperature of 101°F or 38.3°C or persistent temperature greater than or equal to 100.4°F or 38°C) in a neutropenic patient represents an urgent clinical problem requiring a prompt infectious agent assessment and intervention with appropriate antibiotics. Leukocyte transfusions are seldom, if ever, indicted.

Thrombocytopenia (platelet count of less than 10 000 μl^{-1} is a frequent consequence of cytotoxic chemotherapy. A moderate risk of bleeding exists when the platelet count falls to less than 50 000 μl^{-1} and a major risk is associated with platelet counts less than 10 000 μl^{-1}. Adequate coagulation can be further compromised by drugs that interfere with platelet function, like aspirin, nonsteroidal anti-inflammatory drugs, ginkgo biloba, and anticoagulants like warfarin and heparin. Platelet transfusions can reduce or eliminate fatal consequences in patients at high risk because of thrombocytopenia. Generally accepted guidelines for platelet transfusions are summarized in **Table 9**. An infrequently used approach is to stimulate the production of

Table 9 Summary highlights of ASCO's clinical practice guidelines for platelet transfusions[a]

Indication	*Guideline*
Platelet product	Use random donor pooled platelets unless histocompatible platelets are needed, then use single donor platelets
Prophylactic platelet transfusion: acute leukemia and hematopoietic cell transplant	A threshold of 10 000 μl^{-1} is recommended for asymptomatic patients. Transfusions at levels above this threshold are indicated for patients with complicating clinical conditions
Prophylactic transfusions: solid tumors	A threshold of 20 000 μl^{-1} is recommended for patients with bladder cancer receiving aggressive therapy and those with necrotic tumors. For all others, a threshold of 10 000 μl^{-1} is recommended
Surgical or invasion procedures	A platelet count of 40 000–50 000 μl^{-1} is deemed sufficiently safe to perform invasive procedures in the absence of coagulation problems
Prevention of alloimmunization with leukoreduced blood products	Recommended for patients with AML from time of diagnosis; consider for all other patients

[a] Data from: Shiffer CA, Anderson KC, Bennet CL, *et al.* (2001) Platelet transfusion for patients with cancer: Clinical practice guidelines of the American Society of Clinical Oncology. *Journal of Clinical Oncology* 19: 1519–1538.

platelets before administering chemotherapy by the administration of oprelvekin, a recombinant IL-11. This drug stimulates megakaryocytopoeisis and thrombopoiesis and platelet increases are observed 5–9 days after initiation of treatment.

Anemia is associated with cancer and may be multifactorial. It may be due to bleeding, hemolysis, or bone marrow suppression secondary to the malignancy or it may be due to chemotherapy. Treatment for an acute need is generally by red cell transfusion. For chronic anemia in patients due to cancer chemotherapy who are not hemolyzing and not iron deficient, epoietin alfa or the longer-acting darbepoietin alfa, can raise hemoglobin levels and relieve some of the fatigue of malignancy.

Nausea, Vomiting, and Antiemetic Therapy

There are three patterns of nausea and vomiting associated with chemotherapy: acute, delayed, and anticipatory. Acute occurs within the first 24 h of treatment, delayed occurs or is a continuation beyond 24 h, and anticipatory is the experience of nausea or vomiting before receiving another chemotherapy treatment. It is a conditioned or learned response to previous effects from therapy. It may be prevented by minimizing the adverse effects of the first and subsequent treatments. The incidence and severity of nausea and vomiting are related to the emetogenic potential of the drug (**Table 10**), dose, route of administration, schedule, infusion rate, time of day drug is given, patient characteristics, and combination of drugs. It is easier to prevent nausea and vomiting than to treat. Hence, antiemetics are given shortly before chemotherapy administration. In general, one should use aggressive antiemetic therapy for chemotherapy naïve patients, give an adequate duration of coverage for the predicted risk period and select the appropriate agents and dosing according to the emetic potential of the chemotherapy. While **Table 10** is a good guide, combination chemotherapy will frequently move the potential antiemetic effect higher, that is, one group to the left.

Therapy for nausea and vomiting is directed at blocking the effect on the chemoreceptor trigger zone of the brain and the receptors in the GI tract. For low-risk emetogenic chemotherapy, dexamethasone, metoclopromide, or prochlorperazine are most useful. A psychotropic agent like lorazepam may be helpful if one suspects a degree of apprehension. There are other antiemetics available (e.g., butyrophenones and the cannabinoids), but they are of low therapeutic efficacy and are not recommended as first line therapy. For moderate or high-risk emetogenic therapy, a 5-HT_3 antagonist (dolasetron, ganisetron, ondansetron, and palonosetron) with dexamethasone is recommended. For delayed emesis due to moderately emetogenic chemotherapy, a single dose of the longer-acting palonosetron (with dexamethasone) may be more effective than the other 5-HT_3 inhibitors. For both acute and delayed emesis due to highly emetogenic drugs, aprepitant plus a 5-HT_3 inhibitor plus dexamethasone is the current treatment of choice. For breakthrough emesis despite optimal prophylactic pretreatment, an agent from another pharmaceutical class may be added and antiemetic doses increased.

Renal and Bladder Toxicity

Major risk factors for renal toxicity in cancer patients include nephrotoxic chemotherapy drugs, age, nutritional status, concurrent use of other nephrotoxic drugs (e.g., aminoglycoside antibiotics), and preexisting renal dysfunction. Drugs with a high risk for renal toxicity include cisplatin, ifosfamide,

Table 10 Emetic potential of chemotherapy drugs as single agents

Very high (> 90%)	*High (60–90%)*	*Moderate (30–60%)*	*Low (10–30%)*
Carmustine[a]	Azacitidine	Altretamine	Cytarabine
Cisplatin	Carboplatin	Daunorubicin	Docetaxel
Cyclophosphamide[a]	Carmustine	Doxorubicin	Etoposide
Cytarabine[a]	Cyclophosphamide	Epirubicin	5-Fluorouracil
Mechlorethamine	Dacarbazine	Idarubicin	Gemcitabline
Melphalan[a]	Dactinomycin	Ifosfamide	Irinotecan
Streptozocin	Lomustine	Mitomycin	Paclitaxel
		Mitoxantrone	
		Oxaliplatin	Thiotepa
		Plicamycin	Topotecan
		Procarbazine	

[a] High dose.

methotrexate (high dose), mitomycin, and streptozocin. Carboplatin is significantly less nephrotoxic than cisplatin, but if administered in high doses (e.g., in stem cell transplantation), or given with other nephrotoxic drugs, it has the potential to contribute to renal damage. Before using a renal toxic chemotherapy agent, renal function should be evaluated with a serum creatinine or creatinine clearance as a guide to the need for dose reduction or omission.

Hemorrhagic cystitis and an increased incidence of bladder cancer are associated with use of ifosfamide and cyclophosphamide. Contact of the bladder wall with their toxic metabolites, primarily acrolein, produces mucosal erythema, inflammation, ulceration, necrosis, diffuse small-vessel hemorrhage, oozing, and a reduced bladder capacity. Symptoms include hematuria (microscopic or gross) and dysuria. The uroprotective agent 2-mercaptoethane sulfonate sodium (mesna) acts by binding to acrolein to result in a nontoxic thioether. The use of adequate mesna and hydration with ifosfamide or high-dose cyclophosphamide significantly reduces the incidence of bladder toxicity.

Cardiopulmonary Toxicity

Chemotherapy drugs can directly or indirectly cause: acute pneumonitis (bleomycin, carmustine, gemcitabine, methotrexate, mitomycin, procarbazine, and vinca alkaloids); pulmonary fibrosis (bleomycin, carmustine, cyclophosphamide, methotrexate, and mitomycin); hypersensitivity pneumonitis (bleomycin, methotrexate, and procarbazine); noncardiogeneic pulmonary edema (cytarabine, cyclophosphamide, methotrexate, mitomycin, and teniposide). Docetaxel is associated with fluid retention, which may result in pulmonary edema or pleural effusion. Some of these conditions respond to corticosteroid therapy but some cases of pulmonary fibrosis are fatal.

Cardiomyopathy is the most common chemotherapy-associated cardiac toxicity. Myocardial ischemia, pericarditis, arrhythmias, miscellaneous electrocardiogram (ECG) changes, and angina occur much less frequently. The anthracyclines (daunorubicin, doxorubicin, epirubicin, and idarubicin) have the highest consistent risk for cardiomyopathy, which is cumulative dose related. There is evidence that high-dose cyclophosphamide, mitoxantrone, and fluorouracil also pose an increased risk of cardiac damage. The concurrent use of traztuzumab with an anthracycline and cyclophosphamide is associated with a risk of cardiac dysfunction, but the consequences of sequential use are not yet known.

Management of chemotherapy-induced cardiac dysfunction is conventional therapy for heart failure. Because of the limited value of this intervention in the face of existing cardiac disease, prevention of cardiac toxicity is important. This can be done by limiting the cumulative total dose, giving it more slowly, and using dexrazoxane, an intracellular iron-chelating agent that prevents iron from combining with anthracyclines to form free oxygen radicals. Dexrazoxane is initiated after two-thirds of the cumulative toxic dose is administered (i.e., at $300\,\mathrm{mg\,m^{-2}}$ for doxorubicin). Long-term follow-up is indicated because congestive heart failure may develop several years after therapy is completed.

Dermatological and Neurological Toxicity

Chemotherapy drugs can cause a variety of dermatological conditions including rashes, pruritis, swelling, hyperkeratosis, urticaria, exfoliation, photosensitivity, flushing, nail changes, and pigmentation. Extravasation of some agents, especially carmustine, dactinomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, mechlorethamine, mitomycin, and the vinca alkaloids, can lead to tissue necrosis, ulceration, and sloughing. To reduce the incidence of extravasation, central venous catheters are frequently used to administer these drugs.

Encephalopathy, peripheral neuropathy, cerebellar syndromes, autonomic neuropathy, and cranial nerve toxicity represent the range of neurological complications associated with cancer chemotherapy. Dose, route of administration, age of the patient, hepatic and renal function, prior and/or concomitant use of other neurotoxic drugs, and the concurrent use of cranial or CNS radiotherapy can each influence the incidence rate and severity of neurologic symptoms associated with selected chemotherapy drugs.

The management of the dermatological and neurological toxicities secondary to chemotherapy drugs is essentially the same as those due to other causes. Tables of drugs and their specific subtypes of these toxicities and fuller discussions of them are available in the references listed as further reading. They also include discussions of other toxicities including mucositis, diarrhea, constipation, hypercalcemia, headache, depression, anxiety, fatigue, anorexia, weight loss, impotence, sterility, premature menopause, pregnancy risks, and teratogenicity.

Appendix 1 Drugs of choice for cancer – partial list of brand names

Accutane – isotretinoin
Adriamycin[a] – doxorubicin
Adrucil[a] – fluorouracil
Alimta – pemetrexed
Alkeran – melphalan
Arimidex – anastrozole
Aromasin – exemestane
Avastin – bevacizumab
Bexxar – tositumomab
BiCNU – carmustine
Blenoxane[a] – bleomycin
Caelyx – liposomal doxirubicin
Campath – alemtuzumab
Camptosar – irinotecan
Casodex – bicalutamide
CeeNU – lomustine
Cerubidine[a] – daunorubicin
Cosmegen – dactinomycin
Cytadren – aminoglutethimide
Cytosar[a]-U–cytarabine
Cytoxan[a] – cyclophosphamide
Daunoxome – liposomal daunorubicin
Depo-Provera – medroxyprogesterone acetate
Doxil – liposomal doxorubicin
DTIC[a] – *Dome*-dacarbazine
Ellence – epirubicin
Eloxatin – oxaliplatin
Elspar – asparaginase
Emcyt – *estramustine phosphate sodium*
Erbitux – cetuximab
Eulexin – flutamide
Fareston – toremifene
Faslodex – fulvestrant
Femara – letrozole
Fludara – fludarabine
FUDR – floxuridine
Ganite – gallium nitrate
Gemzar – gemcitabine
Gleevec – imatinib
Gliadel – carmustine wafer
Halotestin – fluoxymesterone
Herceptin – trastuzumab
Hexalen – altretamine
Hycamtin – topotecan
Hydrea[a] – hydroxyurea
Idamycin – idarubicin
Ifex – ifosfamide
Intron-A[b] – interferon alfa-2b
Iressa – gefitinib
Kidrolase – asparaginase
Leukeran – chlorambucil
Leustatin – cladribine
Lupron[a] – leuprolide acetate
Lysodren – mitotane
Matulane – procarbazine
Megace[a] – megestrol acetate
Mesnex – mesna
Mustargen – mechlorethamine
Mutamycin[a] – mitomycin
Myleran – busulfan
Mylotarg – gemtuzumab
Natulan – procarbazine
Navelbine – vinorelbine tartrate
Neosar[a] – cyclophosphamide
Neutrexin – trimetrexate
Nilandron – nilutamide
Nipent – pentostatin
Nizoral[a] – ketoconazole
Nolvadex[a] – tamoxifen citrate
Novantrone – mitoxantrone
Oncaspar – pegaspargase
Oncovin[a] – vincristine sulfate
Ontak – denileukin diftitox
Panretin – alitretinoin
Paraplatin – carboplatin
Pharmorubicin – epirubicin
Platinol[a] – cisplatin
Proleukin – interleukin-2 (aldesleukin)
Provera[a] – medroxyprogesterone *acetate*
Purinethol – mercaptopurine
Rituxan – rituximab
Roferon-A – interferon alfa-2a
Rubex[a] – doxorubicin
Sandostatin – octreotide
Stilphostrol – diethylstilbestrol
Tamofen – tamoxifen
Tarabine[a] – cytarabine
Targretin – bexarotene
Taxol – paclitaxel
Taxotere – docetaxel
Tegison – etretinate
Temodar – temozolomide
Thalomid – Thalidomide
TheraCys – live BCG
Thioplex – thiotepa
TiceBCG – live BCG
Tomudex[b] – raltitrexed
Trelstar – triptorelin
Trisenox – arsenic trioxide
Uromitexan – mesna
Velban[a] – vinblastine
Velbe[a] – vinblastine
VePesid[a] – etoposide
Vesanoid – tretinoin
Vincasar[a] – vincristine
Vumon – teniposide
Wellcovorin[a] – leucovorin
Xeloda – capecitabine
Zanosar – streptozocin
Zevalin – ibritumomab tiuxetan
Zoladex – goserelin

[a]Also available generically.
[b]Available in the USA at this time for investigational use only.
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Appendix 2 Drugs of choice for cancer – toxicity of some anticancer drugs

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Alemtuzumab (*Campath* – Berlex)	Infusion reactions (fever, rigors, fatigue, musculoskeletal pain, dyspnea, hypotension, urticaria); autoimmune hemolytic anemia	**Bone marrow depression**
Alitretinoin (*Panretin* – Ligand Pharmaceuticals)	Erythema; rash; pruritus	Continued application may result in worsening of acute symptoms and also edema and vesiculation
Altretamine (hexamethymelamine; *Hexalen* – MGI Pharma)	Nausea with vomiting	**Bone marrow depression**; CNS depression; peripheral neuropathy; visual hallucinations; ataxia; tremors; alopecia; rash
Anastrozole (*Arimidex* – AstraZeneca)	Nausea; diarrhea; hot flashes; headache	Asthenia; pain (bone pain, back pain); dyspnea; peripheral edema; rash
Arsenic trioxide (*Trisenox* – Cell Therapeutics)	"Retinoic acid-like syndrome" (fever, dyspnea, pulmonary infiltrates, pleural effusions, peripheral edema, hypotension); fatigue; musculoskeletal pain; prolongation of QT interval and cardiac arrhythmias; hyperglycemia	Peripheral neuropathy; dysesthesias; rash; alopecia; renal toxocity; myelosuppression

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Asparaginase (Elspar S – Merck; *Kidrolase* in Canada)	Nausea and vomiting; fever; chills; headache; hypersensitivity, anaphylaxis; abdominal pain; hyperglycemia leading to coma	CNS depression or hyperexcitability; acute hemorrhagic pancreatitis; coagulation defects, thrombosis; renal damage; hepatic damage
Bevacizumab (Avastin-Genentech)	**Allergic reactions**	**Gastrointestinal perforations; hemoptysis**
Bexarotene (*Targretin* – Ligand Pharmaceuticals)	Headache; rash	Leukopenia; anemia; asthenia; hypothyroidism; hypertriglyceridemia; hypercholesterolemia; photosensitivity
Bicalutamide (*Casodex* – AstraZeneca)	Nausea; diarrhea; hot flashes; hematuria; increased aminotransferase activity	Gynecomastia; breast pain; hypersensitivity pneumonitis
Bleomycin (*Blenoxane* – Bristol-Myers Squibb Oncology, and others)	Nausea and vomiting; fever; anaphylaxis and other allergic reactions; phlebitis at injection site	**Pneumonitis and pulmonary fibrosis**; rash and hyperpigmentation; stomatitis; alopecia; Raynaud's phenomenon; hemorrhagic cystitis
Busulfan (*Myleran* – GlaxoSmithKline)	Nausea and vomiting (with high-dose therapy only); diarrhea (rare)	**Bone marrow depression**; pulmonary infiltrates and fibrosis; alopecia; gynecomastia; ovarian failure; hyperpigmentation; azoospermia; leukemia; chromosome aberrations; cataracts; hepatitis; seizures and veno-occlusive disease with high doses; secondary malignancy with prolonged use
Capecitabine (*Xeloda* – Roche)	Nausea and vomiting	**Palmar-plantar erythrodysesthesia (hand-foot syndrome)**; diarrhea; stomatitis; dermatitis; bone marrow depression; hyperbilirubinemia; ocular irritation and corneal deposits
Carboplatin (*Paraplatin* – Bristol-Myers Squibb Oncology)	Nausea and vomiting; hypersensitivity, anaphylaxis	**Bone marrow depression**; peripheral neuropathy; hearing loss; transient cortical blindness; hemolytic anemia
Carmustine (BCNU; BiCNU – Bristol-Myers Squibb Oncology; *Gliadel* – Guilford)	Nausea and vomiting; local phlebitis	**Bone marrow depression (cumulative) with delayed leukopenia and thrombocytopenia** (may be prolonged); pulmonary fibrosis (may be irreversible); delayed renal damage; reversible liver damage; leukemia; myocardial ischemia; veno-occlusive disease of liver after transplantation doses
Cetuximab (C225; *Erbitux* – ImClone Systems)	Rash; fever and chills	Asthenia
Chlorambucil (*Leukeran* – GlaxoSmithKline)	Nausea and vomiting	**Bone marrow depression**; pulmonary infiltrates and fibrosis; leukemia; hepatic toxicity; sterility
Cisplatin (Cis-DDP; *Platinol* – Bristol-Myers Squibb Oncology, and others)	Nausea with vomiting; diarrhea; hypersensitivity reactions	**Renal damage**; ototoxicity; bone marrow depression; hemolysis; hypomagnesemia; peripheral neuropathy; hypocalcemia; hypokalemia; Raynaud's phenomenon; sterility; hypophosphatemia; hyperuricemia; anorexia
Cladribine (2-chlorodeoxy-adenosine; 2-CdA; *Leustatin* – Ortho-Biotech)	Fever	**Bone marrow depression**; immunosuppression; peripheral neuropathy with high doses
Cyclophosphamide (*Cytoxan* – Bristol-Myers Squibb Oncology; *Neosar* – Pharmacia)	Nausea and vomiting; Type 1 (anaphylactoid) hypersensitivity; facial burning and metallic taste with IV administration; visual blurring	**Bone marrow depression**; alopecia; hemorrhagic cystitis; sterility (may be temporary); pulmonary infiltrates and fibrosis; hyponatremia; leukemia; bladder cancer; inappropriate ADH secretion; cardiac toxicity; amenorrhea
Cytarabine HCl (*Cytosar*-U – Pharmacia, and others)	Nausea with vomiting; diarrhea; anaphylaxis, sudden respiratory distress and high doses; fever	**Bone marrow depression**; conjunctivitis; megaloblastosis; oral ulceration; hepatic damage; pulmonary edema and central and peripheral neurotoxicity with high doses; rhabdomyolysis; pancreatitis when used with asparaginase; rash

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Dacarbazine (*DTIC-Dome* – Bayer)	Nausea and vomiting; diarrhea; anaphylaxis; pain on administration; phlebitis at infusion site	**Bone marrow depression**; alopecia; flu-like syndrome; renal impairment; hepatic necrosis; facial flushing; paresthesia; photosensitivity; urticarial rash
Dactinomycin (*Cosmegen* – Merck)	Nausea and vomiting; hepatic toxicity with ascites; diarrhea; severe local tissue damage and necrosis on extravasation; anaphylactoid reaction	**Stomatitis; oral ulceration; bone marrow depression**; alopecia; folliculitis; dermatitis in previously irradiated areas
Daunorubicin HCl (*Cerubidine* – Bedford, and others)	Nausea and vomiting; diarrhea; red urine (not hematuria); severe local tissue damage and necrosis on extravasation; transient ECG changes; facial flushing; anaphylactoid reaction	**Bone marrow depression**; **cardiotoxicity** (may be delayed for years); alopecia; stomatitis; anorexia; diarrhea; fever and chills; dermatitis in previously irradiated areas; skin and pigmentation; photosensitivity
Liposomal daunorubicin (*DaunoXome* – Gilead)	Less nausea and vomiting; no red urine; less local tissue damage; infusion reactions	Less cardiotoxicity; minimal alopecia
Denileukin diftitox (*Ontak* – Ligand Pharmaceuticals)	Hypersensitivity reactions (hypotension, back pain, dyspnea, rash, chest tightness, tachycardia, dysphagia); chills; fever; headache; nausea and vomiting; diarrhea; pruritus	Vascular leak syndrome (hypotension, edema, hypoalbuminemia); anemia; infection; anorexia; asthenia; increased aminotransferase activity; hypocalcemia
Diethylstilbestrol (*Stilphostrol* – Bayer)	Nausea and vomiting; abdominal cramps; headache	Gynecomastia in males; breast tenderness; loss of libido; thrombophlebitis and thromboembolism; hepatic injury; sodium retention with edema; hypertension; change in menstrual flow
Docetaxel (*Taxotere* – Aventis)	Hypersensitivity reactions; nausea and vomiting; bowel perforation (rare)	**Bone marrow depression**; peripheral neuropathy; fluid retention (including generalized edema and pleural effusions); myalgia; alopecia; mucositis; cutaneous fibrosis; onycholysis, subungual hemorrhage; increased hepatic aminotransferase activity; epiphora; phlebitis
Doxorubicin HCl (*Adriamycin* – Pharmacia, and others)	Nausea and vomiting; red urine (not hematuria); severe local tissue damage and necrosis on extravasation; diarrhea; fever; transient ECG changes; ventricular arrhythmia; anaphylactoid reaction	**Bone marrow depression; cardiotoxicity** (cumulative and may be delayed for years); alopecia; stomatitis; anorexia; conjunctivitis; acral pigmentation; dermatitis in previously irradiated areas; acral erythrodysesthesia; hyperuricemia; leukemia
Liposomal doxorubicin (*Doxil* – Alza; Caelyx – Schering in Canada)	Less nausea and vomiting; no red urine; less local tissue damage; infusion reactions	Less cardiotoxicity; minimal alopecia; palmar-plantar and acral dysesthesia; mucositis
Epirubicin (*Ellence* – Pharmacia; Pharmorubicin in Canada)	Local tissue damage; red urine (not hematuria); nausea and vomiting; ECG changes; arrhythmias; anaphyactoid reaction	**Bone marrow depression**; alopecia; paresthesias; fatigue; cardiotoxicity; leukemia
Estramustine phosphate sodium (*Emcyt* – Pharmacia)	Nausea and vomiting; diarrhea	Mild gynecomastia; increased frequency of vascular accidents; myelosuppression (uncommon); edema; dyspnea; pulmonary infiltrates and fibrosis; decreased glucose tolerance; thrombosis; hypertension
Etoposide (VP-16; *VePesid* – Bristol-Myers Squibb Oncology, and others)	Nausea and vomiting; diarrhea; fever; hypotension; hypersensitivity reactions; phlebitis at infusion site	**Bone marrow depression**; rashes; alopecia; peripheral neuropathy; mucositis and hepatic damage with high doses; leukemia
Exemestane (*Aromasin* – Pharmacia)	Hot flashes; nausea	Peripheral edema and weight gain; fatigue
Floxuridine (*FUDR* – Roche)	Nausea and vomiting; diarrhea	**Oral and gastrointestinal ulceration; bone marrow depression**; alopecia; dermatitis with hepatic infusion; jaundice; sclerosing cholangitis
Fludarabine (*Fludara* – Berlex)	Nausea and vomiting	**Bone marrow depression**; immunosuppression; CNS effects; visual disturbances; renal damage with higher doses; pulmonary infiltrates

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Fluorouracil (5-FU; *Adrucil* – Pharmacia, and others)	Nausea and vomiting; diarrhea; mucositis; hypersensitivity reaction (rare)	**Oral and GI ulcers; bone marrow depression**; diarrhea (especially with fluorouracil and leucovorin); neurological defects, usually cerebeller; cardiac arrhythmias; angina pectoris; alopecia; hyperpigmentation; **palmar-plantar erythrodysesthesia**; conjunctivitis; heart failure; seizures
Fluoxymesterone (*Halotestin* – Pharmacia)	Nausea and vomiting	Menstrual changes; gynecomastia; androgenic effects; hepatic toxicity
Flutamide (*Eulexin* – Schering; *Euflex* in Canada)	Nausea; diarrhea; hot flashes	Gynecomastia; hepatic toxicity; hypersensitivity pneumonitis
Fulvestrant (*Faslodex* – AstraZeneca)	Nausea; pain at injection site; headache; rash; hot flashes	Anorexia; anemia; dyspnea; asthenia; musculoskeletal symptoms; occasional thromboembolism
Gallium nitrate (*Ganite* – SoloPak)	Hypocalcemia; metallic taste	**Hypophosphatemia**; nephrotoxicity; anemia; optic neuritis
Gefitinib (*Iressa* – AstraZeneca)	Diarrhea; rash; dry skin; acne; nausea; vomiting; dyspnea; pruritus	Mucostis; anorexia; asthenia; interstitial pneumonitis (rarely)
Gemcitabine (*Gemzar* – Lilly)	Fatigue; nausea and vomiting; fever	**Bone marrow depression**; edema; pulmonary toxicity, anal pruritus
Gemtuzumab (*Mylotarg* – Wyeth)	Fever; chills; nausea; hypotension; hypersensitivity reactions	Bone marrow depression; increased aminotransferase activity; veno-occlusive disease
Goserelin (*Zoladex* – AstraZeneca)	Transient increase in bone pain; transient increase in tumour mass, resulting in ureteral obstruction and/or spinal cord compression in patients with metastatic prostate cancer; hot flashes	Impotence; testicular atrophy; gynecomastia; allergic reactions; peripheral edema; decreased bone mineral density
Hydroxyurea (*Hydrea* – Bristol-Myers Squibb Oncology, and others)	Nausea and vomiting; allergic reactions to tartrazine dye	**Bone marrow depression**; stomatitis; dysuria; alopecia; rare neurological disturbances; pulmonary infiltrates; secondary leukemias
Ibritumomab tiuxetan (*Zevalin* – IDEC Pharmaceuticals)	Rash; pruritus; urticaria; nausea; vomiting; fever; chills (See also Rituximab)	**Neutropenia; thrombocytopenia**; hemorrhage; anorexia
Idarubicin (*Idamycin* – Pharmacia)	Nausea and vomiting; tissue damage on extravasation	**Bone marrow depression**; alopecia; stomatitis; myocardial toxicity; diarrhea
Ifosfamide (*Ifex* – Bristol-Myers Squibb Oncology)	Nausea and vomiting; confusion; nephrotoxicity; metabolic acidosis and renal Fanconi's syndrome; cardiac toxicity with high doses	**Bone marrow depression; hemorrhagic cystitis** (prevented by concurrent mesna); alopecia; inappropriate ADH secretion; renal failure; neurotoxicity (somnolence, hallucinations, blurring of vision, coma)
Imatinab (STI-571; *Gleevec* – Novartis)	Nausea and vomiting; rash; diarrhea; muscle cramps; increased aminotransferase activity	**Bone marrow depression**, pulmonary, periorbital and pedal edema
Interferon alfa-2a (*Roferon-A* – *Roche*), alfa-2b (*Intron A* – *Schering*)	Flu-like syndrome (fever, chills, myalgias, fatigue, arthragias, headache); nausea; diarrhea; hypotension	Bone marrow depression; anorexia; neutropenia; anemia; confusion; depression; renal toxicity; possible hepatic injury; facial and peripheral edema; cardiac arrhythmias; rhabdomyolysis
Interleukin-2 (aldesleukin; *Proleukin* – Chiron)	**Fever; fluid retention; hypotension; respiratory distress**; rash; anemia; thrombocytopenia; nausea and vomiting; diarrhea; capillary leak syndrome; nephrotoxicity; myocardial toxicity; hepatotoxicity; erythema nodosum; neutrophil chemotactic defects	Neuropsychiatric disorders; hypothyroidism; nephrotic syndrome; possibly acute leukoencephalopathy; brachial plexopathy; bowel perforation
Irinotecan (*Camptosar* – Pharmacia)	Nausea and vomiting; **early diarrhea** (<24 h); fever	**Late diarrhea** (>24 h); **leukopenia**; anorexia; asthenia; alopecia; abdominal cramping and pain; thromboembolism
Isotretinoin (*Accutane* – Roche)	Fatigue; headache; nausea and vomiting; pruritus; pancreatitis; depression	**Teratogenicity**; cheilitis xerostomia; rash; conjuctivitis and eye irritation; bone and joint pain; anorexia; hypertriglyceridemia; pseudotumor cerebri; psychosis; increased aminotransferase activity

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Ketoconazole (*Nizoral* – Janssen, and others)	Nausea and vomiting	Hepatocellular toxicity; hypertension; gynecomastia, breast tenderness; impotence; nail changes; pruritus; adrenal insufficiency
Letrozole (*Femara* – Novartis)	Hot flashes; nausea and vomiting; headache	Peripheral edema and weight gain; dyspnea; fatigue; musculoskeletal pain; arthralgia; constipation; diarrhea; rare thromboembolic events
Leucovorin (*Wellcovorin* – GlaxoSmithkline, and others)	Hypersensitivity reactions; nausea; diarrhea	
Leuprolide acetate (*Lupron*, *Lupron* Depot – TAP, and others)	Transient increase in bone pain; transient increase in tumor mass, resulting in ureteral obstruction and/or spinal cord compression in patients with metastatic prostate cancer; hot flashes; hematuria	Impotence; testicular atrophy; gynecomastia; peripheral edema; allergic reactions; decreased bone mineral density
Lomustine (CCNU; *CeeNU* – Bristol-Myers Squibb Oncology)	Nausea and vomiting	**Bone marrow depression** (cumulative) with delayed leukopenia and thrombocytopenia (may be prolonged); transient elevation of transaminase activity; neurological reactions; pulmonary fibrosis; renal damage; leukemia
Mechlorethamine HCl (nitrogen mustard; *Mustargen* – Merck)	Nausea and vomiting; local reaction and phlebitis	**Bone marrow depression**; alopecia; diarrhea; oral ulcers; leukemia; amenorrhea; sterility; hyperuricemia
Megestrol acetate (*Megace* – Bristol-Myers Squibb Oncology, and others)	Nausea and vomiting; headache; weight gain	Menstrual changes; hot flashes; thrombophlebitis and thromboremolism; fluid retention; edema; impotence
Melphalan (*Alkeran* – GlaxoSmithKline)	Mild nausea; hypersensitivity reactions	**Bone marrow depression** (especially platelets); pulmonary infiltrates and fibrosis; amenorrhea; sterility; leukemia
Mercaptopurine (*Purinethol* – GlaxoSmithKline)	Nausea and vomiting; diarrhea	**Bone marrow depression**; cholestasis and rarely hepatic necrosis; oral and intestinal ulcers; pancreatitis
Mesna (Mesnex – Bristol-Myers Squibb Oncology; *Uromitexan* in Canada)	Nausea and vomiting; diarrhea; allergic reactions	
Methotrexate (MTX; *Folex* – Pharmacia, and others)	Nausea and vomiting; diarrhea; fever; hepatic necrosis; hypersensitivity reactions	**Oral and gastrointestinal ulceration**, perforation may occur; **bone marrow depression**; hepatic toxicity including cirrhosis; renal toxicity; **pulmonary infiltrates and fibrosis**; osteoporosis; conjunctivitis; alopecia; depigmentation; menstrual dysfunction; encephalopathy; infertility; lymphoma; photosensitivity
Mitomycin (*Mutamycin* – Bristol-Myers Squibb Oncology, and others)	Nausea and vomiting; tissue necrosis; fever	**Bone marrow depression** (cumulative) with delayed leukopenia and thrombocytopenia; stomatitis; alopecia; acute pulmonary toxicity; pulmonary fibrosis; cardiotoxicity; hepatotoxicity; renal toxicity; amenorrhea; hemolytic-uremic syndrome; bladder contracture (with intravesical administration)
Mitotane (*o,p′*-DDD; *Lysodren* – Bristol-Myers Squibb Oncology)	Nausea and vomiting; diarrhea	**CNS depression**; rash; visual disturbances; adrenal insufficiency; hematuria; hemorrhagic cystitis; albuminuria; hypertension; orthostatic hypotension; cataracts; prolonged bleeding time
Mitoxantrone HCl (*Novantrone* – Immunex)	Blue-green pigment in urine; blue-green sclera; nausea and vomiting; fever; phlebitis	**Bone marrow depression**; cardiotoxicity; alopecia; white hair; skin lesions; hepatic damage; renal failure; extravasation necrosis; stomatitis
Nilutamide (*Nilandron* – Aventis)	Nausea and vomiting; hot flashes; alcohol intolerance	Delayed adaptation to darkness; hepatic toxicity; gynecomastia; hypersensitivity pneumonitis
Octreotide (*Sandostatin* – Novartis)	Nausea and vomiting; diarrhea	Steatorrhea; gallstones

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Oxaliplatin (*Eloxatin* – Sanofi-Synthelabo)	Peripheral sensory neuropathy; pharyngolaryngeal dysesthesias; paresthesias; nausea and vomiting; rare cases of anaphylaxis (reduced systolic blood pressure, flushing, tachycardia, respiratory distress)	Bone marrow depression; diarrhea; persistent neuropathy
Paclitaxel (*Taxol* – Bristol-Myers Squibb Oncology)	Hypersensitivity reactions	**Bone marrow depression**; peripheral neuropathy; alopecia; arthralgias; myalgias; cardiac toxicity; mucositis; onycholysis
Pegaspargase (PEG-L-asparaginase; *Oncaspar* – Aventis)	Similar to asparaginase	Similar to asparaginase
Pemetrexed (*Alimta* – Lilly)	Diarrhea; rash	**Neutropenia**; stomatitis; thrombocytopenia
Pentostatin (2′-deoxycoformycin; *Nipent* – SuperGen)	Nausea and vomiting; rash	Nephrotoxicity; CNS depression; **bone marrow depression**; respiratory failure; hepatic toxicity; arthralgia; myalgia; photophobia; conjunctivitis
Procarbazine HCl (*Matulane* – Sigma-Tau, Natulan in Canada)	Nausea and vomiting; CNS depression; disulfiram-like effect with alcohol; adverse interactions typical of a monoamine oxidase (MAO) inhibitor	**Bone marrow depression**; stomatitis; peripheral neuropathy; pneumonitis; leukemia; rash
Raltitrexed[b] (*Tomudex* – AstraZeneca)	Vomiting; nausea; rash; diarrhea	**Leukopenia**; thrombocytopenia; mucositis; malaise; elevated liver function tests; anorexia
Rituximab (*Rituxan* – IDEC Pharmaceuticals/Genentech)	Fever; chills; nausea; vomiting; headache; myalgia; pruritus; rash; pain at sites of disease; severe hypersensitivity reactions (hypontension, bronchospasm, angioedema); cardiac arrhythmias; renal failure	Bone marrow depression; mucocutaneous reactions including Stevens-Johnson syndrome and toxic epidermal necrolysis
Streptozocin (*Zanosar* – Pharmacia)	Nausea and vomiting; local pain	**Renal damage**; hypoglycemia; hyperglycemia; liver damage; diarrhea; bone marrow depression (uncommon); fever; eosinophilia; nephrogenic diabetes insipidus
Tamoxifen citrate (*Nolvadex* – AstraZeneca, and others; Tamofen in Canada)	Hot flashes; transient increased bone or tumor pain	Thromboembolism; vaginal bleeding and discharge; hypercalcemia; thrombocytopenia; peripheral edema; headache; decreased visual acuity; cataracts; purpuric vasculitis; uterine adenocarcinoma and sarcoma
Temozolomide (*Temodar* – Schering)	Nausea and vomiting; headache	Bone marrow depression, especially thrombocytopenia and neutropenia; asthenia; fatigue
Teniposide (*Vumon* – Bristol-Myers Squibb Oncology)	Severe hypersensitivity reactions; nausea and vomiting; diarrhea; phlebitis at infusion site	**Bone marrow depression**; alpoecia; mucositis; rash; hepatic toxicity; leukemia
Thalidomide (*Thalomid* – Celgene)	Rash; pruritus; headache; dizziness; drowsiness; somnolence; constipation; rarely toxic epidermal necrolysis	Severe birth defects; peripheral neuropathy; neutropenia; venous thrombosis
Thioguanine (GlaxoSmithKline; *Lanvis* in Canada)	Occasional nausea and vomiting; diarrhea	**Bone marrow depression**; hepatic damage; stomatitis
Thiotepa (*Thioplex* – Immunex)	Nausea and vomiting; rare hypersensitivity reaction	**Bone marrow depression**; menstrual dysfunction; interference with spermatogenesis; leukemia; mucositis with high doses
Topotecan (*Hycamtin* – GlaxoSmithKline)	Nausea and vomiting; diarrhea; headache; flu-like symptoms	**Bone marrow depression**; asthenia; stomatitis; alopecia; abdominal pain
Toremifene (*Fareston* – Shire)	Hot flashes; nausea and vomiting	Vaginal bleeding and discharge; peripheral edema; dizziness; increased aminotransferase activity; hypercalcemia; thromboembolic events (rarely)
Tositumomab (*Bexxar* – Coulter)	Nausea; vomiting; pruritus; hypotension; fever; chills	**Neutropenia; thrombocytopenia**; anorexia; fatigue; myalgia; arthralgia; increased incidence of leukemia and myelodysplasia

Appendix 2 Continued

Drug	*Acute toxicity*[a]	*Delayed toxicity*[a]
Trastuzumab (*Herceptin* – Genentech)	Fever; chills; rigors; nausea and vomiting; headache; rash; hypotension; hypersensitivity reactions (hypotension, angioedema, tachycardia, respiratory distress)	Cardiac dysfunction, including congestive heart failure, particularly when combined with an anthracycline; diarrhea
Tretinoin (*Vesanoid* – Roche)	**Headache; xerosis**; pruritus; "retinoic acid syndrome" (fever, dyspnea, pulmonary infiltrates, pleural effusions, peripheral edema, hypotension); arthralgias; myalgias	**Cheilitis; teratogenicity; rashes**; leucocytosis; hypertriglyceridemia; pseudotumor cerebri; thrombophlebitis
Trimetrexate (*Neutrexin* – MedImmune Oncology)	Fever; chills; malaise; rash; pruritus; hyperpigmentation	**Bone marrow depression**; mucositis
Triptorelin (*Trelstar* Depot – Pharmacia)	Pain at injection site; tumor flare; bone pain; pain at injection site; nausea; pruritus; headache; hot flashes	Decreased libido and impotence; emotional lability; depression; gynecomastia; decreased bone mineral density
Vinblastine sulfate (*Velban* – Lilly, and others; Velbe in Canada)	Nausea and vomiting; local reaction and tissue damage with extravasation	**Bone marrow depression**; alopecia; peripheral neuropathy; stomatitis; jaw pain; muscle pain; paralytic ileus
Vincristine sulfate (*Oncovin* – Lilly, and others)	Tissue damage with extravasation	**Peripheral neuropathy**; alopecia; mild bone marrow depression; constipation; paralytic ileus; jaw pain; inappropriate ADH secretion; optic atrophy
Vinorelbine tartrate (*Navelbine* – GlaxoSmithKline)	Nausea and vomiting; injection site reactions (erythema, discoloration, phlebitis, pain)	**Bone marrow depression**; alopecia; anorexia; stomatitis; asthenia; peripheral neuropathy; constipation; myalgias

[a]Dose-limiting effects are in bold type. Cutaneous reactions (sometimes severe), hyperpigmentation, and ocular toxicity have been reported with virtually all nonhormonal anticancer drugs. For adverse interactions with other drugs, see The Medical Letter *Handbook of Adverse Drug Interactions*, 2003.
[b]Available in the United States at this time for investigational use only. (Reproduced with permission from (2003) *Treatment Guidelines*, vol. 1(7), pp. 41–52; © Medical Letter.)

See also: Androgens; Arsenic; BCNU (Bischloroethyl Nitrosourea); Carcinogenesis; Cisplatin; Corticosteroids; Cyclophosphamide; Gallium; Mitomycin C; Nitrogen Mustard; Platinum.

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Cancer Potency Factor

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In the evaluation of carcinogenicity of chemicals, data obtained from human and animal studies are analyzed for hazard identification and dose–response relationships. The results are used in combination with exposure assessment and risk characterization for the assessment of cancer risks of the chemicals to humans.

Cancer risk assessment involves a quantitative estimate of the carcinogenic activity of a carcinogen. For genotoxic carcinogens, this estimate is derived from the cancer potency of the carcinogen. Cancer potency is defined as the slope of the dose–response curve for induction of tumors, and is a function of the dose and the magnitude of response, measured as a slope. The endpoint is the cancer incidence or frequency of occurrence of cancer (tumor induction) in

the test population. Potency may also be expressed in terms of an effective dose (ED) or lowest effective dose (LED). A less commonly used approach defines potency as a function of the breadth of biological effects (i.e., number of sites or species) that can be affected by the chemical. In general, data on the incremental increase in an effect proportional to an incremental increase in dose above the background is used to establish the dose–response curve for the toxic effects of chemicals. The slope of the curve expresses extra risk per dose unit, or risk per milligram per kilogram body weight per day.

Most of the data available for determining carcinogenicity of chemicals are based on the results of animal testing. Mathematical models have been developed for the calculation of the cancer potency or prediction of cancer potency at low doses from these animal bioassay data. These are statistical models designed to determine the shape and slope of the dose–response curve taking into consideration biological plausibility and mode of action. The most common one used by regulatory agencies in the United States is the multistage model constructed based on multistage carcinogenesis and the absence of a carcinogenic threshold. This is a default model used in the absence of additional information (e.g., mode of action) that warrants the inclusion of additional parameters such as for analysis.

The equation for the multistage model is as follows, estimating the probability of developing cancer from exposures equivalent to a daily dose d:

$$P(d) = 1 - \exp - (q_0 + q_1 d + q_2 d^2 + \cdots + q_k d^k)$$

where $P(d)$ is the probability or risk of cancer, 'exp' is the natural base 'e' raised to the exponent in parentheses, d is the dose, and k, q_0, q_1,…, q_k are parameters. For the low-dose range of interest the equation reduces to approximately the linear term of the exponent, represented by q_1 (the slope of the low dose, linear portion of the curve) times the dose.

Cancer potency is taken as the upper 95% confidence limit (UCL) on the linear term q_1, which is called the cancer slope factor (CSF) or q_1^*, in units of $(\text{mg/kg-day})^{-1}$, derived using maximum likelihood estimate techniques. Cancer risks at low doses equals 'dose $\times$ q_1^*' and the dose associated with a specific risk equals 'risk/q_1^*'. The calculated risks are upper bound estimates and calculated doses are lower bound estimates. These are further summarized below.

$$\begin{aligned}\text{Cancer potency} &= 95\%\ \text{UCL of the slope of the low dose, linear portion of the dose-response curve (using multistage model)}\\ &= \text{Cancer slope factor (CSF), or } q_1^*\ (\text{mg/kg-day})^{-1}\text{, which relates dosage to the probability of an individual developing cancer}\\ \text{Cancer risk} &= \text{dose} \times q_1^*\\ \text{Dose} &= \text{risk}/q_1^*(\text{mg/kg-day})^{-1}\end{aligned}$$

The choice of models for a chemical depends on two factors: (1) the hypothesis for the mechanism of carcinogenesis for that chemical; and (2) science policy. In the absence of definitive data supporting one model or another, the more conservative model among biologically plausible models may be used, or results from a range of plausible models are presented. The choice of the low-dose extrapolation model (Weibull, probit, multistage) can have a major impact on the estimate of risk at low exposure levels, sometimes varying by orders of magnitude at the same exposure level.

The linearized multistage model is used on the basis of its biological plausibility as it assumes no threshold, and its conservatism, as it is unlikely to underestimate risk at low exposure levels to develop CSFs. The probit, logistic, and Weibull models are also used to describe quantal–response toxicity data. Although these models generally provide similar fits to data from dose–response experiments within the observable response range, they can differ appreciably when extrapolated to very low doses. The shape of the dose–response curve may be linear, sublinear, or supralinear for the logistic and Weibull models, whereas the probit model exhibits a sublinear behavior in the low-dose region regardless of the values of the model parameters.

The cancer potency factor is used to predict cancer risk. Risk can be predicted by multiplying the cancer potency factor by the daily dose, averaged over lifetime (mg/kg-day).

In using animal data to extrapolate to humans, the basic assumption is that carcinogenic effects in animal studies indicate that the agent under study can have carcinogenic potential in humans. For exposure by the oral route, the default assumption is that delivered doses are related to applied dose by a power of body weight based on similarities of mammalian physiology, anatomy, and biochemistry generally observed across species. Oral slope factors incorporate a cross-species scaling factor based on equivalence of $\text{mg/kg}^{3/4}$-day. When oral potency is derived from animal data, the equivalent human oral dose uses the default procedure to scale a daily-applied dose for a lifetime in proportion to body weight

raised to the 0.75 power ($W^{0.75}$):

$$\text{Dose, human} = \text{dose} \times (\text{BW}_{\text{human}}/\text{BW}_{\text{animal}})^{0.75}$$

or

$$\text{Cancer potency, human} = q_{\text{human}} = q_{\text{animal}} \times (\text{BW}_{\text{human}} \times \text{BW}_{\text{animal}})^{0.25}$$

For dose estimation, physiologically based pharmacokinetic (PBPK) modeling has been used as a tool for tissue dosimetry, or to obtain a better estimate of the ED than the administered dose, using model parameters measured in or estimated from experiments. The models describe the pharmacokinetic behavior of toxic chemicals and predict the dose of reactive metabolites reaching the target tissues. PBPK modeling provides mathematical descriptions of the update and disposition of chemicals based on quantitative interrelations among the biological parameters of these processes. The models represent the body as compartments and require information on such determinants as partition coefficients, blood flow, tissue volume, and metabolic parameters. In cancer risk assessment, the modeling may help to establish a relationship in terms of a cancer dose–response from animal or human data, relating exposure to ED, and estimating risk as a function of exposure. Use of PBPK modeling in cancer risk prediction requires the identification of activation pathways associated with carcinogenesis and a kinetic model (e.g., Michaelis–Menten). Such models have been used in cross-species, route and dose extrapolations. Accurate parameterization is fundamental in using PBPK models.

Similarly, unit risk estimates for lifetime exposures can be made for inhalation (risk per $\mu g\,m^{-3}$ air breathed) and drinking water (risk per $\mu g\ l^{-1}$ water). Unit risks are potencies in terms of the equation above, risk = dose $\times\ q_1^*$, and in this case

$$\text{Risk} = \text{intake} \times \text{unit risk}$$

For the oral route, US Environmental Protection Agency (EPA) calculates both a slope factor (formerly referred to as potency factor) and a unit risk. The oral slope factor expresses the risk per mg/kg-day and the unit risk is a numerically equivalent term expressed as the risk associated with a drinking water concentration of the chemical at $1\,\mu g\,l^{-1}$, based on the assumption of a 70 kg body weight and $2\,l\,day^{-1}$ of water consumption. For the inhalation route, the US EPA only expresses the risk in terms of a unit risk expressed as risk per $1\,\mu g\,m^{-3}$, or the risk associated with an air concentration of $1\,\mu g\,m^{-3}$, assuming that a 70 kg adult breathes $20\,m^3\,day^{-1}$. For estimating cancer risk from inhalation or oral exposure, toxicological data specific to the exposure route of interest are preferred. Extrapolation from one route to another may be appropriate in some but not all cases. Related parameters used in estimating risk (e.g., inhalation rate versus water consumption rate, percent absorption via inhalation versus oral intake) need to be modified as appropriate.

Oral slope factors and unit risk estimates for lifetime exposures incorporate exposure factors that are based on adult parameters and adjustments have to be made when assessing risks from less than lifetime exposures that occur, for example, during childhood.

Animal studies are conducted at dose levels much higher than the environmental levels to which humans are exposed, and the accuracy of prediction in environmental level is unknown.

Potency estimates derived from such animal studies help to characterize the dose–response relationship at the low-exposure levels to which humans are likely to be exposed and to predict the quantitative estimate of the risks that humans are likely to encounter at ambient exposures. Experimental evidence for various shapes of the dose–response curve for carcinogens showed that reliable high-dose data from human studies contain examples of superlinearity, linearity, and sublinearity. These are also seen in animal studies. But there are no data to indicate the shape of the dose–response relationship corresponding to lifetime risk of one in a million, the insignificant risk level generally used by the regulatory agencies.

The US EPA in its revised draft cancer risk assessment guidelines has revised the approach to dose–response assessment in consideration of the uncertainties in dose–response modeling for low-dose cancer risk and the increasing acceptance of a threshold dose–response relationship for some carcinogens, generally nongenotoxic carcinogens. New risk assessments are being conducted consistent with the proposed guidelines.

If sufficient data are available to support the use of a biologically based dose–response model, it may represent the most appropriate method for using the observed data to extrapolate to exposure below the observed dose range. If data are not available for a biologically based model, which is the case for the majority of chemicals studied, a 'point of departure' (POD) approach is recommended. The POD represents a dose within the range of observed data associated with a 10% extra tumor

risk. It is developed using the linearized multistage model (or the most appropriate model based on the shape of the dose–response curve) and expressed at the lower 95% confidence limit on the dose with the 10% extra risk (LED_{10}). Risks below the LED_{10} are characterized either through linear extrapolation (for chemicals believed to act via a linear dose–response relationship, or genotoxic carcinogens) or through a margin of exposure analysis (for chemicals whose dose–response relationship is likely to be threshold or nonlinear). For chemicals where data might support either a linear extrapolation or a margin of exposure approach, both analyses are to be presented. Potencies calculated are based on $0.1/LED_{10}$.

Overall, cancer risk assessment involves the four steps of hazard identification, dose–response, exposure assessment, and risk characterization. The dose–response curve established for cancer potency derivation for a chemical is based on evaluation of data on the carcinogenicity and dose–response characteristics of the chemical. The pharmacokinetics and mechanistic data evaluation (e.g., genotoxic or nongenotoxic) and a dose–response review of all adequate bioassays are conducted to determine, if target dose estimates or a dose–response model different from the default may be suggested.

Inherent in the default potency derivation are the following assumptions: that the dose–response relationship in the most sensitive species is representative of that in humans; low-dose potency estimate can be derived from using the multistage polynomial to extrapolate potency outside the range of the experimental observations; surface area scaling is used as an interspecies factor; tissue dose resulting in a tumor is proportional to the administered dose; and cancer hazard increases with the third power of age. Based on the above and tumor findings, potency is derived from data on malignant tumors, combined malignant, and benign tumors or tumors that are believed to progress to malignancy.

Most of the risk assessments are performed for decision-making such as setting exposure limits for a chemical. The human cancer potency (q_{human}) derived from oral route exposure data can be used to estimate chemical exposure or intake associated with a given level of cancer risk based on the following:

$$I\ (\text{intake } \mu\text{g day}^{-1}) = \frac{10^{-6} \times 70\ \text{kg} \times 1000\ \mu\text{g mg}^{-1}}{q_{\text{human}}\ (\text{mg/kg-day})^{-1}}$$

The intake value can then be converted to an air or drinking water equivalent for the corresponding environmental standards. It is important that cancer risk assessments be performed by trained risk assessors who have the training, experience, and understanding of the toxicological principles and the risk assessment methodologies involved.

See also: Carcinogenesis; Dose–Response Relationship; Risk Assessment, Human Health; Uncertainty Factors.

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Cannabinoids

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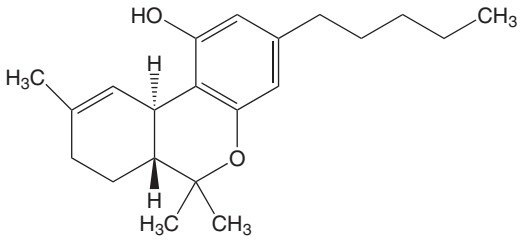
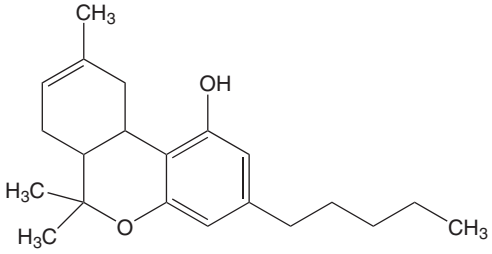
- Chemical Abstracts Service Registry Number: CAS 8063-14-7
- Chemical/Pharmaceutical/Other Class: Hallucinogenic substances
- Chemical Structures:



[Delta]^9-tetrahydrocannabinol



[Delta]^8-tetrahydrocannabinol

Representative Compounds

Cannabinoids are aryl-substituted meroterpenes unique to the plant genus *Cannabis*. The pharmacology of most of the cannabinoids is largely unknown but the most potent psychoactive agent, [Delta]^9-tetrahydrocannabinol ([Delta]^9-THC, or THC), has been isolated, synthesized, and much studied. Other plant cannabinoids include [Delta]^8-THC, cannabinol, and cannabidiol.

Sources

Cannabinoids are present in the stalks, leaves, flowers, and seeds of the plant, and also in the resin secreted by the female plant. A 'joint' made out of skunkweed, netherweed, and other potent subspecies of *Cannabis sativa* may contain ~150 mg of THC, or 300 mg if laced with hashish oil.

Uses

Delta-9-THC and some synthetic analogs are used therapeutically, for example, for nausea and vomiting produced by antineoplastic chemotherapy, analgesic, anticonvulsant for epilepsy, anti-inflammatory agent, appetite stimulant for patients with AIDS, as well as treatment for conditions such as asthma and glaucoma. Synthetic cannabinoids used therapeutically include dronabinol, nabilone, and levonamtradol.

Exposure Routes and Pathways

The usual route of administration for medical purposes is oral. The commonest way of abusing it is by inhalation.

Toxicokinetics

About 50% of the THC in a 'joint' of herbal cannabis is inhaled in the mainstream smoke; nearly all of this is absorbed through the lungs, rapidly enters the bloodstream, and reaches the brain within minutes. Effects are perceptible within seconds and fully apparent in a few minutes. Bioavailability after oral ingestion is much less; blood concentrations reached are 25–30% of those obtained by smoking the same dose, partly because of first-pass metabolism in the liver. The onset of effect is delayed (0.5–2 h) but the duration is prolonged because of continued slow absorption from the gut. Once absorbed, THC and other cannabinoids are rapidly distributed to all other tissues at rates dependent on the blood flow. Because they are extremely lipid soluble, cannabinoids accumulate in fatty tissues, reaching peak concentrations in 4–5 days. They are then slowly released back into other body compartments, including the brain. Because of the sequestration in fat, the tissue elimination half-life of THC is ~7 days, and complete elimination of a single dose may take up to 30 days. Clearly, with repeated dosage, high levels of cannabinoids can accumulate in the body and continue to reach the brain. Within the brain, THC and other cannabinoids are differentially distributed. High concentrations are reached in neocortical, limbic, sensory, and motor areas.

Cannabinoids are metabolized in the liver. A major metabolite is 11-hydroxy-THC, which is possibly more potent than THC itself and may be responsible for some of the effects of cannabis. More than 20 other metabolites are known, some of which are psychoactive and all of which have long half-lives of

several days. The metabolites are partly excreted in the urine (25%) but mainly into the gut (65%) from which they are reabsorbed, further prolonging their actions. Because of the pharmacokinetic characteristics of cannabinoids – both the sequestration in fat and the presence of active metabolites – there is a very poor relationship between plasma or urine concentrations and degree of cannabinoid-induced intoxication.

Mechanism of Toxicity

Cannabinoids exert their effect by interaction with specific endogenous cannabinoid receptors. Neuronal cannabinoid receptors are termed CB_1 receptors and have been found in rat, guinea pig, dog, monkey, pig, and human brains and peripheral nerves. A second cannabinoid receptor, the CB_2 receptor, was identified in macrophages in the spleen and is also present in other immune cells. The distribution of CB_1 receptors is very similar to that of the distribution of injected THC and includes cerebral cortex, limbic areas (including hippocampus and amygdala), basal ganglia, cerebellum, thalamus, and brainstem.

The discovery of cannabinoid receptors naturally stimulated a search for an endogenous ligand with which the receptors naturally interact. Such a substance was isolated from the pig brain. It was found to be chemically different from plant cannabinoids: it is a derivative of the fatty acid arachidonic acid (arachidonyl ethanolamide) related to the prostaglandins. This endogenous substance was named anandamide after the Sanskrit word for bliss, *ananda*. It has a high affinity for CB_1 receptors and has most of the actions of THC.

Acute and Short-Term Toxicity (or Exposure)

Animal

With THC, the oral LD_{50} in mice is $482\,mg\,kg^{-1}$, the rat oral LD_{50} is $666\,mg\,kg^{-1}$, and the intravenous LD_{50} is $29\,mg\,kg^{-1}$. Delta-9-THC and other cannabinoids with psychoactive effects in man have particularly unusual effects on the overt behavior of dogs. At dose levels that elicit blood concentrations of THC similar to those found in regular human marijuana users, THC markedly disrupts the menstrual cycle in the rhesus monkey. Naturally occurring cannabinoids, unique to the plant *Cannabis sativa* and constituting 15% of the cannabis by weight, have been implicated as immunomodulatory. Delta-9-THC has been studied to characterize its immunosuppressive properties and studies have shown that it suppresses both humoral and cell-mediated immunity in experimental animals.

Human

High levels of intoxication are associated with decreased motor coordination, muscle strength, and hand steadiness. Lethargy, sedation, poor concentration ability, slurred speech, ataxia, and an increase in reaction time may also occur. High doses of delta 9-THC can induce frank hallucinations, delusions, and paranoid feelings. Thinking becomes confused and disorganized; depersonalization and altered time sense are accentuated. Anxiety reaching panic proportions may replace euphoria, often as a result of the feeling that the drug-induced state will never end. The most consistent effects on the cardiovascular system are an increase in heart rate, an increase in systolic blood pressure while supine, decreased blood pressure while standing, and a marked reddening of the conjunctivae.

Chronic Toxicity (or Exposure)

Animal

Under the conditions of 2 year gavage studies, there was no evidence of carcinogenic activity of 1-*trans*-delta9-tetrahydrocannabinol in male or female F344/N rats administered 12.5, 25, or $50\,mg\,kg^{-1}$. There was equivocal evidence of carcinogenic activity of THC in male and female B6C3F1 mice based on the increased incidences of thyroid gland follicular cell adenomas in rats treated with 125 mg THC per kilogram.

Human

Chronic use may be associated with the induction of 'amotivational syndrome' and loss of memory. Endocrine effects have been reported following chronic use, including impairment of gonadotrophin secretion (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)), reduction in testosterone levels, and direct effect on cytochrome P-450 of the Leydig cells with inhibition of testosterone synthesis. Abrupt discontinuation of chronic THC use has resulted in a mild abstinence syndrome, consisting of agitation, apprehension, and aggressiveness, as well as tremulousness, insomnia, and diaphoresis, and the development of common migraine headaches.

Clinical Management

Activated charcoal is administered as a slurry. Depressive, hallucinatory, or psychotic reactions

should be treated by placing the patient in a quiet area and providing them with reassurance that no permanent effects will occur. Benzodiazepines are preferred drugs for treatment of extreme agitation. When psychotic phenomena predominate, haloperidol 5 mg i.m. is recommended. The patient should be kept well hydrated.

Exposure Standards and Guidelines

FDA requirements: tetrahydrocannabinol is a chemical derivative of cannabis (marihuana), named in section 502(d) of the Federal Food, Drug, and Cosmetic Act, and is thereby designated as habit forming.

See also: Benzodiazepines; Marijuana.

Further Reading

Adams IB and Martin BR (1996) Cannabis: Pharmacology and toxicology in animals and humans. *Addiction* 91: 1585–1614.

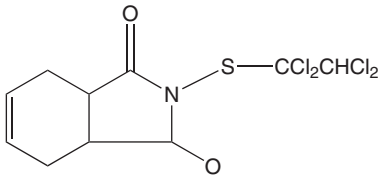
Howlett AC, Breivogel CS, Childers SR, *et al.* (2004) Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology* 47: 345–358.

Cannabis *See* Cannabinoids.

Captafol

Priya Raman

- Chemical Abstracts Service Registry Number: CAS 2425-06-1
- Synonym: 3*a*,4,7,7*a*-Tetrahydro-2-[(1,1,2,2-tetrachloroethyl)thio]-1*H*-isoindole-1,3(2*H*)-dione
- Chemical/Pharmaceutical/Other Class: Phthalimide fungicide
- Chemical Structure:



Uses

Captafol is a widely used broad-spectrum contact fungicide belonging to the class of sulfanilamides. It is effective for the control of a wide variety of fungal diseases in plants and is widely used outside the United States to control foliage and fruit diseases on apples, citrus, tomato, cranberry, sweet corn, barley, wheat, and several other plants. Captafol is also extensively used as a seed protectant in cotton, peanuts, and rice. It is also used to reduce losses from wood rot fungi in logs and wood products.

Exposure Routes and Pathways

Dermal and ocular exposures are the most common routes of exposure to captafol. Contact dermatitis has been reported after exposure to captafol. During occupational exposure, captafol has been reported to cause severe irritation of the respiratory tract, eye damage and other systemic effects. Oral ingestion of captafol is unlikely to cause acute poisoning.

Toxicokinetics

Captafol is poorly absorbed from the gastrointestinal tract. The liver and the gastrointestinal tract are the primary sites of metabolism of captafol. Captafol is eliminated via urine, feces, and expired air. The major single metabolite, tetrahydrophthalimide (THPI), was detected in blood, urine, and feces, but most of the activity in the blood and urine was in the form of more water-soluble metabolites. Following oral administration in animals, captafol is hydrolyzed to THPI and dichloroacetic acid. THPI is degraded to tetrahydrophthalimidic acid and further down to phthalic acid and ammonia.

Mechanism of Toxicity

The primary toxicity following captafol exposure probably occurs through a hypersensitivity mechanism.

Acute and Short-Term Toxicity (or Exposure)

Animal

The reported acute oral LD_{50} of captafol $6780\,mg\,kg^{-1}$ in male rats and $6330\,mg\,kg^{-1}$ in female rats. The rabbit dermal LD_{50} is reported to be $15\,400\,mg\,kg^{-1}$, showing moderate dermal irritation at 72 h with severe dermal sensitization. Another test for captafol-induced eye irritation in rabbit showed corneal opacity and iris and conjuctival irritation, all symptoms being present for 21 days. Captafol was reported to be teratogenic and to cause fetal developmental abnormalities at high (maternally toxic) doses in hamsters. Teratogenicity studies in rabbits indicated a teratogenic no-observed-effect level (NOEL) $>50\,mg\,kg^{-1}\,day^{-1}$ and a fetotoxic NOEL of $16.5\,mg\,kg^{-1}\,day^{-1}$. Captafol was, however, found to have no effect on embryonic development in rabbits and monkeys.

Human

The primary symptoms of captafol exposure reported in humans include contact dermatitis and conjunctivitis. The reaction may be severe and may include stomatitis and painful bronchitis. Persons with a skin rash following exposure to captafol were found to have systemic as well as dermal disorders. Hypertension was reported in patients with marked edema. Other findings following captafol exposure include protein and urobilinogen in the urine, depression of liver function, anemia, and depression of blood cholinesterase activity. Acute oral or dermal exposure to captafol rarely results in severe toxicity. However, due to a higher level of toxicity in animal models following intraperitoneal exposure, parenteral exposure may present a greater hazard potential. Captafol has been classified as a group 2A probable human carcinogen.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to captafol at dietary levels of 1500 and 5000 ppm demonstrated growth depression, some liver and kidney changes, as well as an increased mortality. Following exposure to 300 or $100\,mg\,kg^{-1}$ of captafol, dogs suffered frequent vomiting and diarrhea during the first 4 weeks and were observed to be slightly anemic and deficient in growth during a 2 year study. Dogs at dosages of $30\,mg\,kg^{-1}$ or greater developed both absolute and relative increases in the weights of the liver and kidney. Oral administration in mice produced a high incidence of adenocarcinomas of the small intestine, vascular tumors of the heart, and spleen and hepatocellular carcinomas. In a 2 year rat feeding study, a dose-related increased incidence of neoplastic nodules in the liver of females was reported. The US Environmental Protection Agency reported an NOEL for nononcogenic effects at 56 ppm based on a chronic toxicity study in rats. There is sufficient evidence in experimental animals for carcinogenicity of captafol.

Human

Captafol is also known to be a skin sensitizer and has been reported to cause both allergic and contact dermatitis in humans. Breakdown products may contribute to the skin irritation and sensitization associated with captafol.

Clinical Management

Exposed eyes and skin should be flushed with copious amounts of water. In case of an inhalation exposure, the patient should be monitored for respiratory distress. Artificial ventilation may be provided and symptomatic treatment may be administered as necessary.

Environmental Fate

Captafol is not persistent in the environment. Captafol is stable under ordinary environmental conditions and rapidly degrades in soil, the rate of degradation being a function of soil type and pesticide concentration. It does not leach from basic soils and is unlikely to contaminate groundwater. Captafol sprayed on most crops has a half-life of less than 5 days. Captafol and/or its metabolites and degradates are readily absorbed by roots and shoots of plants.

Ecotoxicology

Avian toxicity for captafol is low, the LD_{50} being greater than 2510 ppm. However, high levels of exposure can cause reproductive impairment. Captafol is characterized as being very highly toxic to both cold-water and warm-water fish, 96 h LC_{50} being 0.027–0.50 and $0.045–0.230\,mg\,l^{-1}$ in rainbow trout and bluegill sunfish, respectively. It is considered only moderately to very highly toxic to freshwater invertebrates. Captafol is considered nontoxic to bees.

Exposure Standards and Guidelines

Captafol is a general use pesticide with a toxicity classification of IV (relatively nontoxic). It is classified a as 'restricted use' pesticide in the United States. It is no longer sold in the United States. The Occupational Safety and Health Administration threshold limit value for captafol is reported to be $0.1\,mg\,m^{-3}$.

Miscellaneous

Captafol is a colorless to pale yellow in color with a distinct odor. It has a molecular weight of 349.1, a water solubility of 1.4 mg l^{-1} at 20°C and a melting point of 160–162°C. Some common trade names of products containing captafol include Crisfolatan, Difolatan, Difosan, Folcid, Haipen, Kenofol, Pillartan, and Sanspor.

See also: Pesticides.

Further Reading

Tamano S, Kawabe M, Sano M, Masui T, and Ito N (1993) Oral Toxicity Study of Captafol in B6C3F1Mice. *Journal of Toxicology and Environmental Health* 38(1): 69–75.

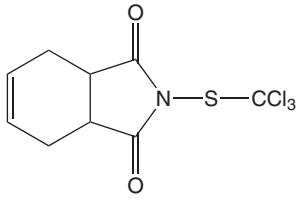
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Captafol.

Captan

Xun Song

- Chemical Abstracts Service Registry Number: CAS 133-06-2
- Synonyms: 3a,4,7,7a-Tetrahydro-2-[(trichloromethyl)thio]-1*H*-isoindole-1,3(2*H*)-dione; 1,2,3,6-Tetrahydro-*N*-(trichloromethylthio)phthalimide; Captano (Italy); Captane (France); Captex; Hexacap; Kaptan; Orthocide®; Vancide 89®
- Chemical/Pharmaceutical/Other Class: Phthalimide
- Chemical Structure:



Uses

Captan is widely used as a fungicide. The main application is for foliage protection in agriculture.

Exposure Routes and Pathways

Exposure to captan may occur through dermal, oral, or inhalation route during manufacture or application of captan, or consumption of agricultural products with captan residues.

Toxicokinetics

Captan is readily excreted after either oral or systemic dosing. Twenty-four hours after treatment in rats, ~75% of captan is eliminated in urine and 6.5% in the feces. Nearly complete elimination occurs within 36 h. There are little gender differences in biotransformation. A small portion of captan given orally was metabolized into thiozolidine-2-thione-4-carboxylic acid, a salt of dithiobis(methanesulfonic acid) and the disulfide monoxide derivative of dithiobis(methanesulfonic acid).

Mechanism of Toxicity

Liver enzymes were modulated after repeated captan exposures at relatively high dosages. Captan led to the breakdown of the inner membrane of mitochondria. *In vitro* studies showed that captan caused swelling of mitochondria in rat liver and loss of intracellular potassium in human erythrocytes. Captan inhibits mitochondrial function nonspecifically, leading to uncoupling of oxidative phosphorylation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Generally, captan has been found to have low toxicity to laboratory animals after oral dosing. Oral LD_{50} values greater than 5000 mg kg^{-1} have been reported in rats. Oral LD_{50} values of 7840 and 7000 mg kg^{-1} were reported for male and female mice. Captan was more potent by intraperitoneal administration (LD_{50} values of 462–518 and 35–40 mg kg^{-1} in rats and mice, respectively). Dermal LD_{50} values greater than 2000 mg kg^{-2} were reported in rabbits.

Human

Captan has a low acute toxicity potential. Sensitivity, e.g., dermatitis, to captan exposure has been reported. Captan is a weak eye irritant.

Chronic Toxicity (or Exposure)

Animal

Dietary captan (10 000 ppm for 54 weeks) caused marked growth depression in both male and female rats. Captan feeding at 5000 ppm for 2 years ($\sim 50\,mg\,kg^{-1}\,day^{-1}$) led to growth depression in female but not male rats. Testicular atrophy was observed at autopsy in some animals consuming 10 000 ppm captan in the diet.

At high dosages, captan increased tumorous cancer in female mice and in male rats. Captan is similar in structure to other pesticides (folpet and captafol) that also cause cancer in test species. Tumors were localized to the gastrointestinal tract and kidneys. Teratogenicity studies with rats, rabbits, hamsters, and dogs have given both negative and positive results.

Human

Evidence indicates that captan is not a teratogen in humans.

In Vitro Toxicity Data

Captan is not mutagenic in most assays.

Clinical Management

Intoxication after acute captan exposure is unlikely; treatment is symptomatic.

Environmental Fate

Captan has a low persistence in soil (half-life of 1–10 days). Captan has little mobility in soils. Captan is rapidly degraded in surface waters at neutral pH. Captan is readily taken up into plant tissues.

Ecotoxicology

Captan is practically nontoxic to birds (LD_{50} 2–$5\,g\,kg^{-1}$). High captan exposures can reduce egg production in chickens but have no effect on fertility or hatching. Captan is very highly toxic to fish. The LC_{50} (96 h) for captan was $0.056\,mg\,l^{-1}$ in cutthroat trout and Chinook salmon and $0.072\,mg\,l^{-1}$ in bluegill. The LC_{50} in *Daphnia magna* was 7–$10\,mg\,l^{-1}$. Captan has a low to moderate tendency to bioaccumulate (concentration factor = 10–1000). Captan has relatively little toxicity in bees.

Exposure Standards and Guidelines

The reference dose for captan is $0.13\,mg\,kg^{-1}\,day^{-1}$, the acceptable daily intake is $0.1\,mg\,kg^{-1}\,day^{-1}$, and the threshold limit value (8 h) is $5\,mg\,m^{-3}$.

See also: Pesticides.

Further Reading

Gordon EB (2001) Captan and folpet. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1711–1742. San Diego, CA: Academic Press.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Carbamate Pesticides*

Stephanie Padilla

Published by Elsevier Inc.

Chemical Structure and Uses

Carbamate compounds are usually subdivided into at least three main groups with respect to their structure and general use (see **Figure 1**): insecticides, herbicides, and thio- or dithiocarbamates. A variety of 'R' groups may be substituted in the molecule producing, as is the case for insecticides, a variety of alkyl or aryl esters of carbamic acid. Although technically characterized as carbamate pesticides, thio- and dithiocarbamate fungicides are not included in this

*The research described in this article has been reviewed by the National Health and Environmental Effects Research Laboratory, US EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency nor does mention of trade names and commercial products constitute endorsement or recommendation for use.

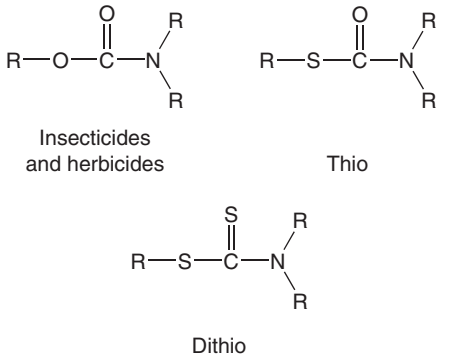


Figure 1 General formulas for carbamates.

brief overview because they have drastically different modes of action from the first group.

Background Information

Carbamate pesticides have a colorful and interesting history of discovery and development. Oral administration of calabar bean paste, which is rich in carbamate alkaloids, was used in West Africa to reveal the guilt or innocence of people accused of witchcraft. If the alleged 'witches' died after being forced to eat calabar bean paste, then they were indeed witches; if not, then they were declared innocent. Scientific investigation revealed that the active carbamate in the calabar bean was physostigmine. In fact, the synonym for physostigmine, eserine, comes from the West Africans' word for calabar bean, esere. In the mid to late 1940s, the first carbamate pesticides were synthesized in an effort to develop new insect repellents, but the insecticidal properties of this class of compound were quickly recognized and appreciated.

Exposure Routes and Pathways

Carbamates do not require hepatic activation for their toxicity. In other words, the parent compound is the active moiety. The majority of carbamate compounds are easily absorbed through mucous membranes and the respiratory and gastrointestinal tracts. Therefore, not only can carbamates be absorbed through the skin (dermal exposure) and lungs (inhalation exposure), but also through foods treated with carbamates (oral exposure). Most of the acute poisoning episodes in humans occurred via the dermal or inhalation route. Although the data are limited, the half-life of selected carbamate pesticides is short in mammals, for example, on the order of 8 h in the adult rat. In the latest NHANES survey of pesticides in the urine of Americans of various ages, there were very low levels of carbamate pesticides, with the most prevalent being carbaryl, an indication of a chronic low-level exposure to this carbamate.

Mechanism of Toxicity

Generally, carbamate compounds, like organophosphorus pesticides, exert their primary toxic action through the inhibition of acetylcholinesterase (EC 3.1.1.7), although it is well known that carbamates are inhibitors of many other esterases. The inhibition of acetylcholinesterase activity is thought to precipitate a toxic response through the short-term increase in the concentration of acetylcholine at cholinergic junctions (e.g., central nervous system, neuromuscular junction, all autonomic preganglionic and parasympathetic postganglionic synapses, and the sympathetic innervation of the adrenal and sweat glands). Carbamates interact with acetylcholinesterase in the same manner as the natural substrate, acetylcholine, except that the carbamate remains in the active site for a markedly longer period of time, thereby preventing the hydrolysis of acetylcholine and resulting in a net inhibition of the enzyme's activity. The carbamylation of the active site of acetylcholinesterase is a much more labile union than is phosphorylation by an organophosphate and does not lead to 'aging' of the enzyme as can inhibition by some organophosphorus compounds. Therefore, restoration of acetylcholinesterase activity (i.e., decarbamylation or reactivation) is highly likely with the carbamate-inhibited enzyme. That is why carbamates are often labeled 'reversible' inhibitors of acetylcholinesterase, that is, because enzyme activity is restored within hours without significant *de novo* synthesis of acetylcholinesterase. Actually, in a biochemical sense, carbamates are not 'reversible' inhibitors because the carbamate does not exit the active site intact; rather, the carbamate is hydrolyzed just as acetylcholine is hydrolyzed. Because of this unstable inhibition, great care must be taken when analyzing cholinesterase inhibition in tissues from carbamate-treated animals to prevent reactivation of the enzyme activity. Generally, carbamates do not cause peripheral neuropathy as do some organophosphorus compounds. Interestingly, carbamates may inhibit neurotoxic esterase activity (the 'first step' in the precipitation of the neuropathy), but do not 'age' (the definitive step in precipitation of the neuropathic response).

Acute and Short-Term Toxicity (or Exposure)

The overall toxicity profile of the carbamate pesticides covers a wide spectrum from virtually nontoxic to some of the most highly toxic pesticides in commercial use; carbamate LD_{50} values can range from 5000 to $1\,mg\,kg^{-1}$. More than 50 commercially available carbamate pesticides are in use today with

the highest volume usage attributed to butylate, carbofuran, methomyl, carbaryl, and benomyl. Generally, metabolites are less toxic than the parent compound, and the metabolites are commonly excreted in the urine. The general metabolic profile is basically the same in insects, plants, or animals. The first step in the catabolic scheme is usually hydrolysis to carbamic acid, but the mechanism of hydrolysis is different for *N*-methyl and *N*-dimethyl derivatives. In general, the predominant, acute effect is acetylcholinesterase inhibition, although there are reports of some carbamates causing disturbances of gonadotrophic function at relatively low doses.

Chronic Toxicity (or Exposure)

In addition to inhibition of acetylcholinesterase activity, carbamates have been reported to cause skin and eye irritation, hemopoietic alterations, degeneration of the liver, kidneys, and testes, as well as functional and histological changes in the nervous system after long-term, high-dose exposures. Moreover, some carbamates are known to produce reproductive and teratogenic effects. Fetuses of mothers dosed with a carbamate have been reported to exhibit increased mortality and decreased weight gain. Carbamates are also considered embryotoxic, and some have also been reported to be mutagenic, but they have little carcinogenic potential.

Clinical Management

Reported effects in humans have usually been confined to the expected cholinergic overstimulation. These signs and symptoms include salivation, lacrimation, diarrhea, nausea, tremors, pin-point pupils, bradycardia, tachycardia, headache, confusion, and, rarely, death. Signs and symptoms are reported within minutes of exposure and can last for hours, but because of the 'reversibility' of the inhibition, recovery is usually apparent within 24 h, depending on the severity of the dose. Metabolites in the urine or red blood cell cholinesterase activity may be used for biological monitoring (although potential for reactivation of inhibited enzyme must be carefully considered in the analysis). During the acute cholinergic crisis produced by these compounds, atropine (a muscarinic antagonist) may be used to counteract the effects, but oximes are typically contraindicated.

Environmental Fate

As a group, carbamates have a relatively low environmental persistence, in contrast to the organochlorine pesticides. Carbamates are broken down by microorganisms, plants, animals, soil, or water, and hydrolysis is accelerated by increased light intensity, temperature, and/or alkalinity. Therefore, carbamates rarely bioaccumulate in animals. The possibility exists, however, that carbamates used in agriculture may be present in groundwater, and by extension, drinking water.

Ecotoxicology

In general, carbamates are not markedly toxic to wildlife. In higher doses, however, carbamate usage has the potential to produce ecotoxicity. When applied directly to the soil, many carbamates will cause significant reduction in microflora and worms. Bees are also especially sensitive to some carbamate pesticides. Because there is very little bioaccumulation of carbamate pesticides, the threats to wildlife are usually through direct exposure after application rather than through the food chain. Note that there have been reports of wildlife morbidity and mortality even if applications of certain carbamate pesticides were made at the recommended rate.

Exposure Standards and Guidelines

For human health, carbamates are usually regulated based on their potency for inhibiting acetylcholinesterase activity in the nervous system. Within the next two years (2004–2006), carbamate pesticides in the United States will be regulated as a mixture of compounds (rather as single compounds) with a common mechanism of action under the direction of the Food Quality Protection Act.

See also: Acetylcholine; Benomyl; Carbaryl; Carbofuran; Carboxylesterases; Cholinesterase Inhibition; Dithiocarbamates; Methomyl; Organochlorine Insecticides; Organophosphates; Pesticides; Pollution, Soil.

Further Reading

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Smith GJ (1993) *Toxicology and Pesticide Use in Relation to Wildlife: Organophosphate and Carbamate Compounds*. Boca Raton, FL: CRC Press.

Relevant Websites

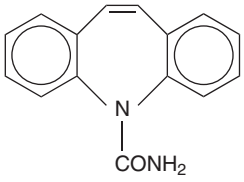
http://www.epa.gov – US Environmental Protection Agency.

http://www.inchem.org – World Health Organization (1986) *Carbamate Pesticides: A General Introduction*. Geneva: WHO.

Carbamazepine

Henry A Spiller

- Chemical Abstracts Service Registry Number: CAS 298-46-4
- Synonyms: CBZ; *5H*-Dibenz(*b*,*f*)-azepine-5-carboxamide; Tegretol
- Chemical/Pharmaceutical/Other Class: Synthetic iminostilbene derivative structurally similar to imipramine, a tricyclic antidepressant. While unrelated structurally, carbamazepine shares a similar therapeutic action with phenytoin
- Chemical Formula: $C_{15}H_{12}N_2O$
- Chemical Structure:



Uses

Carbamazepine is used in the treatment of epilepsy and trigeminal neuralgia. Unlabeled uses include treatment of postherpetic pain syndrome, neurogenic diabetes insipidus, bipolar disorder, alcohol withdrawal, and cocaine dependence.

Exposure Routes and Pathways

The exposure pathway for carbamazepine is exclusively oral (ingestion of tablets or suspension).

Toxicokinetics

Carbamazepine is slowly and incompletely absorbed during therapeutic use. With large ingestions, absorption may be delayed and unpredictable, producing peak levels from 4 to 72 h after the overdose. The absorption phase in an overdose is highly variable because of carbamazepine's poor solubility, ability to significantly decrease gut motility, and to form pharmacobezors. One of the primary metabolites of carbamazepine is carbamazepine-10,11-epoxide (CBZE), which also has anticonvulsant activity. A minor pathway results in iminostilbene formation. Further hydrolysis and conjugation produce six other known metabolites including 10,11-dihydroxycarbamazepine. Protein binding is 75% for carbamazepine and 50% for CBZE. However, the percentage of protein binding may decrease in massive overdose due to saturable binding sites. The volume of distribution is 0.8–1.9 l kg^{-1}. The hydrolyzed and conjugated metabolites are eliminated through the kidneys, with only 1.2% free carbamazepine being found in the urine. Twenty-eight percent is eliminated unchanged in the feces. Carbamazepine induces drug metabolizing enzymes so that drug half-life is reduced in chronic use. The half-life in healthy adults ranges from 18 to 65 h in a single dose to 8–17 h during chronic administration. In newborns and children, the half-life is $\sim$9 h.

Mechanism of Toxicity

Carbamazepine is both an important anticonvulsant in therapeutic doses and a powerful proconvulsant in overdose. The therapeutic anticonvulsant mechanism is primarily related to blockade of presynaptic voltage-gated sodium channels. Blockade of the sodium channels is believed to inhibit the release of synaptic glutamate and possibly other neurotransmitters. Carbamazepine is also a powerful inhibitor of the muscurinic and nicotinic acetylcholine receptors, *N*-methyl-D-aspartate (NMDA) receptors and the central nervous system (CNS) adenosine receptors. In addition, carbamazepine is structurally related to the cyclic antidepressant impramine and in massive overdose may affect cardiac sodium channels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Carbamazepine is not commonly used in animals. Limited information on toxicity exits. Tachyarrhythmias, hypotension, and seizures have been seen.

Human

The primary and common toxic event involves the CNS. Cardiac conduction delays and ventricular arrhythmias can be seen but are infrequent. Sinus tachycardia and hypotension are more commonly seen. In the few deaths directly attributable to carbamazepine toxicity ventricular dysrhythmias have been the terminal event. Coma, seizures, and respiratory depression are commonly seen in adults at levels greater than 40 μg ml^{-1} (170 mmol l^{-1}). Status epilepticus has been reported. The incidence of serious toxicity is similar in adults and children. However, serum levels are less predictive in children.

Therefore, coma, seizures, and apnea may be seen at lower serum levels than in adults. Other manifestations of neurologic toxicity are nystagmus, ataxia, choreoathetoid movements, encephalopathy, absent corneal reflexes, decreased deep tendon reflexes, urinary retention, and dystonias. A cyclic clinical course can be seen, with a waxing and waning of symptoms. This may be due to the presence of a pharmacobezor in the gut or more commonly due to a decrease in gastrointestinal motility produced by the prominent anticholinergic effects of carbamazepine.

Chronic Toxicity (or Exposure)

Animal

Male albino rats given injections of carbamazepine over 3 months demonstrated decreased prostate weight and decreased sperm motility. These changes did not affect fertility. Rats born to mothers chronically fed carbamazepine during gestation demonstrated challenges with maintaining balance and had more difficulty lifting their hind legs than controls.

Human

Idiopathic hepatotoxicity has been reported as a rare manifestation of chronic therapy and is not dose related.

In Vitro Toxicity Data

Studies of carbamazepine on rat cerebellar granule cells have shown inhibition of NMDA-stimulated calcium entry in a rapid and reversible manner. These findings occurred in therapeutic concentrations of carbamazepine, which may help explain the antiseizure activity of carbamazepine. It is believed that the toxic cerebellar effects of carbamazepine may be due to this mechanism.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be used as appropriate. Activated charcoal effectively binds carbamazepine. Multiple dose activated charcoal ($0.5\,g\,kg^{-1}$ every 4 h) has been shown to decrease the half-life of carbamazepine. Generally, supportive measures are all that is required in carbamazepine overdose. Seizures initially should be managed with diazepam or lorazepam. However, persistent seizures may require advancement to phenobarbital or pentobarbital. Ventricular arrhythmias should be managed with lidocaine. The presence of persistently high serum levels or fluctuating elevated serum levels may suggest the presence of a pharmacobezor in the gut. Removal should be attempted, in the presence of an active bowel, with whole bowel irrigation using a polyethylene glycol-electrolyte solution.

Environmental Fate

No information is currently available on breakdown in soil groundwater or surface water.

See also: Diazepam; Lidocaine; Polyethylene Glycol.

Further Reading

Bridge TA, Norton RL, and Robertson WO (1994) Pediatric carbamazepine overdoses. *Pediatric Emergency Care* 10: 260–263.

Kasarskis EJ, Kuo CS, and Berger R (1992) Carbamazepine-induced cardiac dysfunction: Characterization of two distinct clinical syndromes. *Archives of Internal Medicine* 152: 186–191.

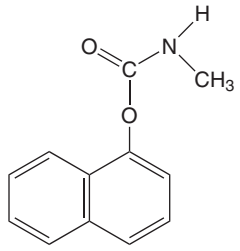
Spiller HA (2001) Management of carbamazepine overdose. *Pediatric Emergency Care* 17: 452–456.

Carbaryl

Paul R Harp

- Chemical Abstracts Service Registry Number: CAS 63-25-2
- Synonyms: 1-Naphthyl-*N*-methylcarbamate; Atoxan; Caprolin; Carbacide; Carbamine; Carbex; Carpolin; Cekubaryl; Denapon; Denopton; Devicarb; Dicarbam; Efaryl; Gamonil; Hexavin; Karbaspray; Karbatox; Karbosep; Kilex; Menaphtam; Monsur; Murvin; NAC; Panam; Pomex; Rayvon; Septene; Sevidol; Sevin; Sevinox; Tercyl; Toxan; Tricarnam; Vioxan; Vanisect; ENT 23969; UC 7744; OMS 29; SHA 056801

- Chemical/Pharmaceutical/Other Class: *N*-Methylcarbamate insecticide
- Chemical Structure:



Uses

Carbaryl is effective as both a contact and an ingested agent and is one of the most widely used broad-spectrum insecticides. Uses include control of a wide variety of pests on field crops, fruits, vegetables, nuts, ornamentals, turf, lawns, and domestic animals in agricultural, commercial, and residential environments. Liquid broadcast applications to residential lawns in the United States are to be voluntarily canceled, however. Formulations include dusts, wettable powders, granules, oils, aqueous suspensions, and baits.

Exposure Routes and Pathways

Due to extensive use, numerous types of applications, and the variety of formulations, human exposure may occur through all of the major pathways (dermal contact, ingestion, and inhalation). Dermal contact probably represents the pathway through which exposure most frequently occurs.

Toxicokinetics

The rate of dermal absorption of carbaryl in animal studies is dependent on the solvent used. Carbaryl can be hydrolyzed to 1-naphthol (the major metabolite) or hydroxylated to a naphthylmethylcarbamate, either of which may be conjugated with glucuronic acid or sulfate. Nonhydrolytic pathways also play a minor role in the biotransformation of carbaryl. In rats treated with carbaryl through oral gavage, the highest tissue levels of the pesticide were found in the liver, kidneys, and adipose tissue. Most mammals eliminate at least 75% of the original dose within 24–48 h. The route of elimination is generally urinary with small amounts of certain metabolites undergoing fecal elimination.

Mechanism of Toxicity

Carbaryl binds and inhibits acetylcholinesterase, the enzyme responsible for metabolizing the neurotransmitter acetylcholine and terminating its action at cholinergic synapses. Exposure to carbaryl results in synaptic accumulation of acetylcholine in both the central and peripheral nervous systems and hyperstimulation of muscarinic and nicotinic receptors leading to 'cholinergic crisis'. In contrast to the organophosphate anticholinesterases, acetylcholinesterase inhibition by the *N*-methylcarbamates is reversible with fairly rapid reactivation occurring through spontaneous decarbamoylation or via hydrolysis of the carbamate.

Acute and Short-Term Toxicity (or Exposure)

Animal

Signs of acute exposure in laboratory animals are similar to those described for humans and recovery from nonlethal exposures occurs rapidly. LD_{50} values for acute exposure in rats: 233–850 mg kg^{-1} (oral), >5000 mg kg^{-1} (dermal), and 0.005–0.023 mg l^{-1} (inhalation).

Human

The acute effects of exposure are due to cholinergic overstimulation and may include the SLUDGE syndrome (salivation, lacrimation, urination, diarrhea, gastrointestinal cramping, and emesis), respiratory depression, bronchospasms, increased bronchial secretions, pulmonary edema, blurred vision, miosis, headache, tremors, muscle fasciculations, convulsions, mental confusion, coma, and death (due to respiratory failure). Recovery from nonlethal exposures occurs very rapidly (usually within a few hours).

Chronic Toxicity (or Exposure)

Animal

Pigs receiving carbaryl in their diets developed incoordination, muscle contractions, and tremors cumulating in paraplegia. It is unclear if carbaryl or a metabolite, possibly unique to pigs, is responsible.

Human

Several cases of persistent neurophysiological or neurobehavioral effects have been reported following acute high-dose exposure to carbaryl. Studies of oral exposure in hogs also indicate possible neuropathological effects of carbaryl. The US Environment Protection Agency's Office of Pesticide Programs has classified carbaryl as likely to be a human carcinogen based on findings of hemangiosarcomas in mice. However, evaluation of the various

use scenarios indicated the noncancer effects of carbaryl generally presented a greater risk than did the carcinogenic potential.

In Vitro Toxicity Data

Carbaryl inhibited neurite outgrowth in N2a cells *in vitro*. Carbaryl also exhibited DNA-damaging activity in a human lymphoblastoid cell line and human liver HepG2 cells.

Clinical Management

Persons providing medical assistance should avoid contact with contaminated clothing. Contaminated clothing should be removed, bagged, and discarded. Contaminated leather garments such as shoes or gloves should be discarded. Exposed dermal areas should be cleaned thoroughly with soap and water. Exposed eyes should be flushed with copious amounts of clean water for at least 15 min. If necessary, use an endotracheal tube to maintain a clear airway, aspirate any secretions, and provide oxygen via mechanical ventilation.

If the patient is asymptomatic and can be treated soon after exposure, activated charcoal may be used to reduce absorption from the gastrointestinal tract. If potentially life-threatening quantities have been ingested, gastric lavage should be considered if it can be conducted within $\sim$1 h of exposure. Charcoal and/or catharsis are contraindicated in presence of severe vomiting or diarrhea. Muscarinic effects (i.e., SLUDGE) may be reduced by intravenous or intramuscular administration of atropine. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenobarbital may be helpful for recurrent seizures. Pralidoxime is indicated in cases of mixed exposure to both carbamates and organophosphorus compounds but is contraindicated in cases of carbamate-only exposure. Furosemide may be useful for pulmonary edema that continues after full atropinization. Metabolite analysis of a urine sample may allow confirmation of the intoxicating agent.

Environmental Fate

Carbaryl has low persistence in soil (half-life of 7–21 days). Degradation is primarily due to sunlight and microbial action. Carbaryl is bound to organic matter and can be transported in runoff. Carbaryl has been detected in groundwater. In surface water, carbaryl is degraded by hydrolysis and microbial processes. It has low volatility. The half-life in surface waters varies greatly with water pH.

Ecotoxicology

Carbaryl has low toxicity in birds. Reported LD_{50} values in mallard ducks, pheasants, quail, and pigeons were all greater than 1 g kg^{-1}. Carbaryl is only moderately toxic to aquatic organisms (LC_{50} values in rainbow trout and bluegill of 1.3 and 10 mg l^{-1}, respectively). Some accumulation of carbaryl in aquatic species can occur, for example, residues in fish were 140 times the concentration in water. Carbaryl shows less bioaccumulation in alkaline conditions. Carbaryl can be lethal to a variety of nontarget species including honey bees, earthworms, and beneficial insects.

Exposure Standards and Guidelines

The acute and chronic reference dose for carbaryl is 0.01 mg kg^{-1} day^{-1}, the acceptable daily intake is 0.01 mg kg^{-1} day^{-1}, and the permissible exposure limit is 5 mg m^{-3} (8 h).

See also: A-Esterases; Carbamate Pesticides; Cholinesterase Inhibition; Neurotoxicity; Pesticides.

Further Reading

Ecobichon DJ (2001) Toxic effects of pesticides. In: Klaassen CD (ed.) *Casarett and Doull's Toxicology*, 6th edn., pp. 763–810. New York: McGraw-Hill.

Relevant Websites

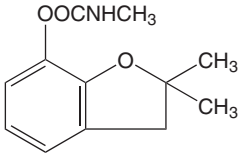
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.
http://www.infoventures.com – Carbaryl Pesticides Fact Sheet, US Forest Service.

Carbofuran

Xun Song

This article is a revision of the previous print edition article by Todd A Bartow and Paul W Ferguson, volume 1, pp. 221–222, © 1998, Elsevier Inc.

- Chemical Abstract Service Registry Number: CAS 1563-66-2
- Synonyms: 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl-*N*-methylcarbamate; Brifur®; Crisfuran®; Curaterr®; Furadan®; Pillarfuran®; Yaltox®; FMC 10242; Bay 70143; Chinufur; Niagra NIA-10242; OMS 864; NIOSH/RTECS FB 9450000; NA 2757; STCC 4921525
- Chemical/Pharmaceutical/Other Class: *N*-Methyl carbamate insecticide; Acaricide; Nematocide
- Chemical Structure:



Uses

Carbofuran is used as an agricultural insecticide on tobacco, corn, alfalfa, and other field crops.

Exposure Routes and Pathways

Exposure may occur through the oral, inhalation, and dermal routes.

Toxicokinetics

Carbofuran is well absorbed by oral and inhalation routes of exposure but poorly absorbed through intact skin. Approximately 75% of absorbed carbofuran is protein bound. Carbofuran is metabolized to yield 3-hydroxycarbofuran and 3-ketocarbofuran via oxidation, and to yield 3-hydroxy-7-phenol, 3-keto-7-phenol, and 7-phenol via hydrolysis. Most metabolites are in the form of glucuronide or sulfate conjugates, which are excreted in the urine. The half-life in the rat is 20 min for the parent compound and 64 min for the 3-hydroxycarbofuran metabolite.

Mechanism of Toxicity

Carbofuran is an inhibitor of acetylcholinesterase. Inhibition of acetylcholinesterase activity leads to an increase in acetylcholine at the nerve synapse resulting in excessive cholinergic stimulation. Following intravenous injection of $50\,\mu g\,kg^{-1}$ in rats, blood acetylcholinesterase activity was depressed by 83% within 2 min. With oral exposures, acetylcholinesterase activity was depressed by 37% within 15 min of ingestion. Recovery of acetylcholinesterase activity parallels carbofuran elimination.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values for carbofuran were 5.3–$13.2\,mg\,kg^{-1}$ in rats, $2\,mg\,kg^{-1}$ in mice, $19\,mg\,kg^{-1}$ in dogs, and $>400\,mg\,kg^{-1}$ in birds. The dermal LD_{50} values are greater than 1–$2\,g\,kg^{-1}$ in rabbits. The inhalation LC_{50} values were $85\,mg\,m^{-3}$ in rats and $52\,mg\,m^{-3}$ in dogs.

Human

Exposure to carbofuran may lead to cholinergic crisis with signs and symptoms including increased salivation, lacrimation, urinary incontinence, diarrhea, gastrointestinal cramping, and emesis (SLUDGE syndrome). The syndrome may be indistinguishable from that seen after organophosphate poisoning. Seizures, coma, diaphoresis, muscle weakness and fasciculation, bradycardia, and tachycardia may occur. Death may be due to severe bronchoconstriction and/or respiratory paralysis.

Workers in a pesticide plant in China exhibited dizziness, weakness, blurred vision, nausea and sweating, pallor, epigastric pain, vomiting, and tightness of the chest following carbofuran intoxication. Miosis was a common finding. Muscle fasciculations (gastrocnemius and orbicularis oculi) were noted in some workers. Blood cholinesterase inhibition was correlated with clinical signs. A pregnant woman was acutely poisoned by carbofuran ingestion. She recovered but the fetus died. Fetal liver, brain, and kidney all had carbofuran in concentrations similar to the maternal blood, indicating the ability of carbofuran to cross the human placenta and adversely affect the developing fetus.

Chronic Toxicity (or Exposure)

Animal

Long-term dietary exposure to carbofuran (50 ppm) led to significant decreases in cholinesterase activity in dogs and rats. No-observed-adverse-effect levels were 20 and 25 ppm in dogs and rats, respectively. High dosages ($5\,mg\,kg^{-1}\,day^{-1}$) for 2 years showed decreased body weight in rats and mice. Prolonged exposure to carbofuran can elicit signs of acute cholinergic toxicity.

Human

While prolonged exposures to carbofuran could lead to typical signs of cholinergic toxicity, recovery from cholinergic signs is rapid and residual chronic toxicity is not likely regardless of exposure.

In Vitro Toxicity Data

Carbofuran is not mutagenic in the Ames assay.

Clinical Management

Rescuers and medical personnel must take precautions to avoid becoming contaminated themselves during rescue and emergency treatment. Victims should be removed from the environment and 100% humidified supplemental oxygen should be administered with assisted ventilation as required. Patients with significant bronchorrhagia, pulmonary edema, convulsions, or coma may require endotracheal intubation and airway suctioning. Exposed skin and eyes should be flushed with copious amounts of water. Measures to decrease absorption may be beneficial soon after ingestion, but induced emesis should be avoided because of the potential for early development of coma or seizures. Atropine is antidotal for muscarinic symptoms and should be given in an initial dose of 2 mg and repeated every 15–30 min as required. The endpoint for atropinization is normalization of vital signs and drying of pulmonary secretions, not pupillary dilatation.

Administration of 2-PAM chloride (protopam and pralidoxime) is generally not recommended in carbamate poisoning since it has been shown to interfere with the efficacy of atropine. It was reported that the condition of patients suffering carbaryl-related poisoning deteriorated rapidly following the administration of 2-PAM. Seizure control with diazepam, phenobarbital, or phenytoin may be required. Cardiovascular support and intensive supportive care may be required in serious cases.

Environmental Fate

Carbofuran is soluble in water and moderately persistent in soil (half-life 30–120 days). Carbofuran is degraded by chemical, photochemical, and microbial processes. Hydrolysis is more rapid in alkaline conditions. Carbofuran breaks down in sunlight. Carbofuran has a high potential for leaching into groundwater. Carbofuran is mobile in sandy loam, silty clay, and silty loam soils. In surface water, carbofuran is subject to hydrolysis, particularly under alkaline conditions. Hydrolysis of carbofuran (half-lives) in water is 690, 8, and 1 weeks at pH values of 6, 7, and 8, respectively. As in soils, photodegradation and microbial transformation may also contribute to degradation. Carbofuran is not volatile and does not adsorb to sediment or particles.

Ecotoxicology

Carbofuran is highly toxic to birds. Carbofuran granules resemble grain seeds, thus the granular formulation can be highly toxic to birds. Predatory birds can be poisoned by prey that recently consumed carbofuran. The LD_{50} is 0.238–12 $mg\,kg^{-1}$ in a variety of bird species. Carbofuran is also highly toxic to many fish. The LC_{50} (96 h) is 0.24–0.38 $mg\,l^{-1}$ in bluegills and rainbow trout. Carbofuran has little potential for bioaccumulation. Carbofuran is toxic to bees except when used as a granular formulation.

Exposure Standards and Guidelines

The reference dose for carbofuran is $0.005\,mg\,kg^{-1}\,day^{-1}$, the acceptable daily intake is $0.002\,mg\,kg^{-1}\,day^{-1}$, and the threshold limit value (8 h) for carbofuran is $0.1\,mg\,m^{-3}$.

See also: Carbamate Pesticides; Cholinesterase Inhibition; Neurotoxicity; Pesticides.

Further Reading

Ecobichon DJ (2000) Carbamates. In: Spencer PS and Schaumburg HH (eds.) *Experimental and Clinical Neurotoxicology*, 2nd edn., pp. 289–298. New York: Oxford University Press.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Carbon Dioxide

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 124-38-9
- Synonyms: Carbon ice; Dry ice
- Chemical Formula: CO_2

Uses

Carbon dioxide is used in the synthesis of urea, for organic synthesis, and in the manufacture of dry ice, soft drinks, and fire extinguishers.

Mechanism of Toxicity

Carbon dioxide is a simple asphyxiant; that is, it causes toxicity by displacing oxygen from the breathing atmosphere primarily in enclosed spaces and results in hypoxia. It has been postulated that the cause of death in breathing high concentration of carbon dioxide is due to carbon dioxide poisoning and not hypoxia based on a study performed in dogs.

Acute and Short-Term Toxicity (or Exposure)

Animal

Extremely high concentrations (~40%) have resulted in death. At lethal concentrations effects have been seen in the central nervous system, lungs, liver, kidneys, and the myocardium in rats. Dogs exposed to 50% carbon dioxide for ~90 min or 80% for several minutes died from respiratory or cardiac failure.

Human

Carbon dioxide is a simple asphyxiant that displaces oxygen from the breathing atmosphere resulting in hypoxia. Four stages have been described (depending on the arterial oxygen saturation): (1) indifferent stage, 90% oxygen saturation; (2) compensatory stage, 82–90% oxygen saturation; (3) disturbance stage, 64–82% oxygen saturation; and (4) critical stage, 60–70% oxygen saturation or less.

Following exposure to asphyxiants, cardiovascular effects like tachycardia, arrhythmias, and ischemia are noted. Carbon dioxide exerts a direct toxic effect to the heart, resulting in diminished contractile force. It is also a vasodilator and the most potent cerebrovascular dilator known. Respiratory effects like hyperventilation, cyanosis, and pulmonary edema are also noted. Various neurologic effects like dizziness, headaches, sleepiness, and mental confusion can occur. Prolonged hypoxia may result in unconsciousness; seizures may be seen during serious cases of asphyxia. Gastrointestinal effects, like nausea and vomiting, may occur, but usually resolve within 24–48 h following termination of exposure. Decreased vision and increased intraocular pressure may be seen with inhalation of 10% carbon dioxide. Combined respiratory and metabolic acidosis was seen in a serious exposure to dry ice. The Lake Nyos disaster in August 1986 has been postulated to have resulted from the release of carbon dioxide from rising cold deep water producing a deadly cloud of gas. Cough, headache, fever, malaise, limb swelling, and unconsciousness were noted in the victims. Inhalation of carbon dioxide is teratogenic and has caused both male and female adverse reproductive effects in rodents. Increased fetal movements have been noted in humans following inhalation with 5% carbon dioxide in air.

The lowest lethal concentration (inhalation) for humans is 100 000 ppm for 1 min. Carbon dioxide concentrations of 20–30% can cause convulsions and coma within 1 min. Unconsciousness may occur when inhaling a concentration of 12% for 8–23 min. Inhalation of 6–10% causes dyspnea, headache, dizziness, sweating, and restlessness.

Chronic Toxicity (or Exposure)

Animal

Changes in body weight, nutrient metabolism, adrenal cortical activity, and blood chemistry were observed in guinea pigs following inhalation of 1.5% for up to 91 days. Rats on chronic exposure have had reversible tissue changes in the central nervous system, lungs, liver, kidneys, and muscle tissue of the heart.

Human

Carbon dioxide is an important component of the body and would not be expected to have a chronic toxicity. However, long-term exposures to levels as low as 0.5–1%, while being generally well tolerated, can alter the acid base and calcium–phosphorus balance resulting in metabolic acidosis and increased calcium deposits in soft tissues. Long-term exposures in the range of 1–2% can stress the adrenal cortex because of constant respiratory stimuli and this level of exposure is considered dangerous after several hours. Exposure to 2% for several hours produces

headache, breathing difficulty upon exertion, and deepened respiration. Fatalities have occurred with prolonged exposure to 15–30%.

Clinical Management

Victims should be moved immediately from the toxic atmosphere and receive 100% humidified supplemental oxygen with assisted ventilation as required. Patients with severe or prolonged exposure should be carefully evaluated for neurologic sequelae and provided with supportive treatment. Seizures may be controlled by administration of diazepam. If seizures cannot be controlled with diazepam or recur, phenytoin or phenobarbital should be administered. Rewarming has been indicated for frostbite. On ocular exposure, the eyes should be rinsed for at least 15 min.

Environmental Fate

The general concerns about greenhouse gases and climate changes are well known, though our ability to model the climate and the timing and magnitude of these effects is uncertain. The major greenhouse gases are carbon dioxide and methane, which together represent 92% of all US greenhouse gas emissions (carbon dioxide accounts for 82%). There is a clear trend of increasing concentrations of greenhouse gases in the atmosphere. The impact of further increases in concentrations of these gases will lead to ever-increasing warming of the climate, leading to a serious impact on human health and the environment. Many scientists believe that these impacts could include an increase in severe weather events such as hurricanes and floods, sea level rise, and increase in heat waves. These weather changes would trigger an increase in heat strokes, may cause a migration of tree and plant species, and initiate the penetration of airborne diseases in areas that do not currently experience these. Little attention has also been directed to investigating the possibility that escalating levels of carbon dioxide may serve as a selection pressure altering the genetic diversity of plant populations.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value – time-weighted average (TLV – TWA) is 5000 ppm and the ACGIH short-term exposure limit (STEL) is 30 000 ppm; the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) – TWA is 500 ppm (transitional limit) and 10 000 ppm (final rule limit), and the OSHA PEL – STEL is 30 000 ppm; the National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit is 10 000 ppm (TWA).

Miscellaneous

Carbon dioxide is a colorless and odorless gas. It has a molecular weight of 44.01 and specific gravity of 1.101 at $-37^{\circ}C$. It is incompatible with metals (e.g., aluminum peroxide, sodium peroxide, lithium peroxide, sodium, sodium carbide, titanium, and sodium–potassium alloy).

See also: Combustion Toxicology.

Further Reading

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Leaf D, Verolme HJ, and Hunt WF Jr. (2003) Overview of regulatory/policy/economic issues related to carbon dioxide. *Environment International* 29: 303–310.

Relevant Website

http://www.ccohs.ca – Health Effects of Carbon Dioxide Case from the Canadian Centre for Occupational Health and Safety.

Carbon Disulfide

Christopher H Day

This article is a revision of the previous print edition article by Linda Larsen, volume 1, pp. 223–224, © 1998, Elsevier Inc.

- Chemical Abstracts Services Registry Number: CAS 75-15-0
- Synonyms: Carbon bisulfide; Carbon sulfide; Dithiocarbonic anhydride; Sulfocarbonic anhydride
- Chemical/Pharmaceutical/Other Class: Solvent
- Chemical Formula: CS_2
- Chemical Structure: S : C : S

Uses

The primary use of carbon disulfide is in the manufacture of cellophane, carbon tetrachloride,

cellulose fibers, various dyes, and rayon. It is also used in the production of certain pesticides, paints and paint removers, enamels and enamel removers, rubber and rubber cement, tallows and waxes, preservatives, fumigants, and nematicides.

Exposure Routes and Pathways

Carbon disulfide is a liquid at room temperature, but quickly evaporates when exposed to air. The primary route of exposure to carbon disulfide is via inhalation of vapors. Exposure can also occur via ingestion, dermal, or eye contact. Most exposure occurs occupationally by individuals who come in contact with carbon disulfide under workplace conditions. Carbon disulfide is also produced under natural conditions by certain species of soil and sediment microorganisms, vegetation burned during forest and grass fires, and volcanic activity. On a global basis, it has been estimated that between 40% and 80% of carbon disulfide released into the environment is the result of these types of biogenic processes. Ambient air concentrations of carbon disulfide have been reported at $\sim$41 parts per trillion (ppt) in rural areas and 65 ppt in urban areas. The odor threshold for most people ranges from 0.02 to 0.1 ppm.

Toxicokinetics

Exposure to carbon disulfide via inhalation, ingestion, and dermal routes has been shown to result in rapid and pervasive absorbtion throughout the body. Carbon disulfide is metabolized to thiocarbamates by the liver and other target organs, such as the brain. Carbon disulfide is also metabolized by cytochrome P-450 to a short-lived oxygen intermediate. Most carbon disulfide metabolites are eliminated from the body in urine as dithiocarbamates. To a lesser extent, it is expelled from the body in the breath if it has not been metabolized. Small amounts may be eliminated from the body in saliva and sweat. Despite wide distribution throughout the body following exposure, carbon disulfide preferentially accumulates in organs such as the liver due to its lipophilicity.

Mechanism of Toxicity

The mechanisms of toxicity for carbon disulfide have not been definitively resolved. Although carbon disulfide is extensively metabolized in the human body, there is not a complete understanding of the metabolic pathways or products. However, two possible mechanisms have been forwarded to explain the neurotoxicity of carbon disulfide. One mechanism is associated with the formation of dithiocarbamates and potential derivatives, which inhibit dopamine-β-hydroxylase. The second mechanism suggested to result in neurotoxic effects associated with carbon disulfide exposure involves the formation of a form of vitamin B_6, of pyridoxamine, a dithiocarbamate derivative.

Acute and Short-Term Toxicity (or Exposure)

Animal

In general, acute studies with common test animals such as mice, rats, and rabbits indicate that carbon disulfide has low toxicity via the inhalation route, and is moderately toxic via the ingestion route.

Human

Acute toxicity observed in several early cases of inhalation exposure included a range of psychological effects (e.g., hallucinations; psychosis) following exposure to carbon disulfide concentrations ranging from approximately 1560 to 3125 mg m^{-3}. Short-term exposure to carbon disulfide vapors can cause headache, dizziness, blurred vision, disorientation, lethargy, damage to the cornea, retina, and optic nerve, and irritation of mucous membranes and the upper respiratory tract. Acute exposure to air concentrations that are well above occupational levels have been reported to cause significant neurological effects such as dyspnea, psychosis, and convulsions, and at exceedingly high concentrations (e.g., 15 625 mg m^{-3}) can result in coma and death.

Dermal exposure to high levels of carbon disulfide can result in redness and blistering of the skin, and if exposure to elevated levels continues long enough then second- and third-degree chemical burns are possible.

Chronic Toxicity (or Exposure)

Animal

Long-term exposure to carbon disulfide vapors has resulted in changes to liver enzymes in test animals.

Human

Long-term exposure to carbon disulfide via inhalation has been reported to result in neurological effects such as polyneuropathy, neurophysiologic changes, and general depression of nerve conduction velocities in humans. Cardiovascular effects that have been observed following chronic inhalation of carbon disulfide vapors by workers include generalized symptoms of heart disease, vascular

atherosclerotic changes, myocardial infarction, and increased incidents of angina.

There is evidence to suggest that carbon disulfide is a reproductive toxicant in humans. Menstrual disturbances in female workers and decreases in sperm count and libido in male workers have been reported following long-term inhalation exposure to carbon disulfide under workplace conditions.

Occupationally exposed individuals have also reported muscle aches and pain, lethargy, general fatigue, and headaches following long-term exposure to carbon disulfide vapors. Ocular effects that have been observed in people exposed in occupational settings include dot hemorrhages and microaneurysms.

Dermal exposure to fibers containing carbon disulfide by workers resulted in the development of blisters and eczema-like lesions on their hands.

In Vitro Toxicity Data

There are no studies available for carbon disulfide that provide strong evidence of genotoxicity.

Clinical Management

The victim of carbon disulfide exposure should be separated immediately from the source and placed in a fresh air environment, or provided with supplied air or oxygen as needed. Rescuers should carefully consider use of respiratory and dermal personal protective equipment due to the chemical, physical, and toxicological properties of carbon disulfide. In the case of exposure to liquid carbon disulfide, the rescuer must be aware of the potential for secondary exposure via direct contact with the victim's clothing. Once isolated from the source, the contaminated clothing should be removed and the skin flushed with water. Victims exhibiting symptoms of significant exposure, such as abnormal behavior, skin or eye irritation, or respiratory distress, should be transported to a medical facility for evaluation and monitoring.

Environmental Fate

Based on the physical and chemical properties of carbon disulfide, it is not expected to persist in the environment. Carbon disulfide has a high vapor pressure, relatively rapid oxidation rate, moderate solubility in water, and a low organic carbon partitioning coefficient (K_{oc}). Volatilization and photooxidation are the primary fate processes for carbon disulfide.

Ecotoxicology

The vast majority of carbon disulfide released into the environment is in the atmosphere. Therefore, terrestrial wildlife and birds in the vicinity of a release have the highest potential for primary exposure. Aquatic organisms would have a minimal exposure potential from an air release, but if the release is from a spill or an end-of-pipe discharge that empties into a water body, then the potential for aquatic organism exposure would be high.

Acute toxicity data are available for mammals, amphibians, fish, phytoplankton (e.g., algae), and zooplankton (e.g., daphnids). The lowest concentration reported to cause adverse effects in aquatic organisms is a 48 h LC_{50} of 2.1 mg l^{-1} for the daphnid, *Daphnia magna*. In mammalian test species, the lowest concentration reported to cause adverse effects is 690 mg m^{-3}, a 1 h LC_{50} for mice. These results suggest that carbon disulfide is moderately toxic to aquatic organisms and of low toxicity via inhalation to mammalian wildlife. No chronic ecotoxicity data could be located for carbon disulfide in the available literature.

Literature data for toxicological effects of carbon disulfide to avian or reptilian species are lacking at this time. There would be a great amount of uncertainty in attempting to quantify the potential for adverse effects to these taxa, or in the extrapolation of adverse effects from other taxa.

No experimentally derived bioconcentration or bioaccumulation factors were found in the available literature. Based on the moderate solubility and low octanol–water partitioning coefficient (K_{ow}), carbon disulfide is not expected to represent a significant

Table 1 Summary of exposure standards and guidelines for carbon disulfide

Agency	*Standards and guidelines* (ppm)	*Averaging time*
US EPA	RfC (0.2)	24 h a day for a lifetime
OSHA	PEL (20)	8 h a day over working lifetime
NIOSH	REL TWA (1)	10 h a day over working lifetime
NIOSH	IDLH (1 0 0)	NA
ACGIH	TLV TWA (10)	8 h a day over working lifetime

US EPA, United States Environmental Protection Agency; RfC, reference concentration; OSHA, Occupational Safety and Health Administration; NIOSH, National Institute for Occupational Safety and Health; ACGIH, American Conference of Governmental Industrial Hygienists; PEL, permissible exposure limit; REL, recommended exposure limit; TWA, time-weighted average; IDLH, immediately dangerous to life or health; TLV, threshold limit value; NA, not applicable.

concern to aquatic or terrestrial organisms via bioaccumulation or biomagnification.

Exposure Standards and Guidelines

Several agencies have established exposure standards or guidelines for carbon disulfide (summarized in **Table 1**). Generally, a standard or guideline represents the concentration that if met, will prevent an adverse effect from occurring at low exposure doses, and will therefore necessarily prevent the occurrence of more serious effects that are known to occur at higher doses. The chronic reference concentration (RfC) of 0.2 ppm ($0.7\,mg\,m^{-3}$) was set to prevent peripheral nervous system dysfunction in the general population over a lifetime of exposure. The ACGIH TLV of 10 ppm was set to prevent adverse effects to the cardiovascular system and central nervous system in workers exposed 8 h per day throughout their working lifetime.

See also: Hydrogen Sulfide; Neurotoxicity.

Relevant Websites

http://www.epa.gov – Website of the US Environmental Protection Agency. US Environmental Protection Agency (2004) *Integrated Risk Information System (IRIS) File: Carbon Disulfide*.

http://www.who.int – Website of the World Health Organization. World Health Organization (2002) *Concise International Chemical Assessment Document 46: Carbon Disulfide*. Geneva, Switzerland: WHO.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Carbon Disulfide.

Carbon Monoxide

Christine Stork and Deborah Anguish

This article is a revision of the previous print edition article by Daniel Steinmetz, volume 1, pp. 224–226, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 630-08-0
- Synonyms: Carbonic oxide
- Chemical/Pharmaceutical/Other Class: Inorganic compound of carbon and oxygen
- Chemical Formula: CO
- Chemical Structure: $C^{+}\equiv O^{-}$

Uses

Carbon monoxide is used in industries as a feedstock for the production of methanol, acrylates, phosgene, and ethylene. It is also used in metallurgy applications and in industrial fuels. A major source of carbon monoxide is the incomplete combustion of carbon-containing materials.

Exposure Routes and Pathways

Exposure to this colorless, odorless gas is via inhalation. Most exposures result from incomplete combustion, especially the emissions created by internal combustion engines. Other sources include the burning of wood, charcoal, or natural gas or propane for heating and cooking, and propane-powered indoor equipment such as fork lifts and ice rink resurfacers. Dermal and inhalation exposures to the paint stripper methylene chloride can cause carbon monoxide poisoning.

Toxicokinetics

Absorption of inhaled carbon monoxide occurs in the gas exchange region of the respiratory tract following inhalation. After absorption methylene chloride is metabolized in the liver to carbon monoxide. The half-life of carbon monoxide after exposure to methylene chloride can be prolonged due to continued absorption and metabolism. Most carbon monoxide binds reversibly to hemoglobin (Hb) in red blood cells; smaller amounts remain in solution or bind to cellular cytochromes. The absorption of the carbon monoxide molecule by Hb is a function of the alveolar partial pressures of carbon monoxide and oxygen, and the concentrations of carbon monoxide and oxygen in blood. Carbon monoxide's affinity for hemoglobin is 200–250 times greater than that of oxygen. Carboxyhemoglobin is completely dissociable, and carbon monoxide is liberated and eliminated through the lungs after exposure to carbon monoxide ceases. Small amounts are oxidized to carbon dioxide.

After binding to Hb to displace oxygen and form carboxyhemoglobin, carbon monoxide is transferred

rapidly throughout the body, where it produces asphyxia. The majority of the body burden exists as carboxyhemoglobin, bound to hemoglobin of red blood cells, while ~10% is present in extravascular space.

Carbon monoxide is eliminated via the lungs. Dissociation and excretion of carbon monoxide occur rapidly after cessation of exposure but slow as carboxyhemoglobin levels decrease. Cardiovascular injury can result from carboxymyoglobin formation and vasodilation from cellular effects of carbon monoxide. Clinical neurological effects and any delayed neurological sequelae can be attributed to asphyxia as well as lipid peroxidation, and hypotension, which induce ischemic-reperfusion injury.

Mechanism of Toxicity

As a result of hemoglobin's high relative affinity for carbon monoxide compared to oxygen and the resulting production of carboxyhemoglobin, decreased delivery of oxygen to tissues occurs, resulting in anemic hypoxia and metabolic and functional impairment. Carbon monoxide may also exert a toxic effect by binding to cellular cytochromes. Displacement of oxygen in the tissues ultimately results in anaerobic metabolism with subsequent buildup of metabolic acids.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals display similar toxicity to humans when exposed to carbon monoxide. Organ systems with large oxygen demands are affected initially and most profoundly.

Human

The effects of acute and chronic exposures to carbon monoxide have been well documented. The effects generally result from the hypoxic action exerted on the tissues. Among the earliest and most prominent effects are central nervous system disorders, such as headache and lightheadedness. At blood COHb levels approaching 30–40%, dizziness, incoordination, nausea, vomiting, and loss of consciousness may result. At still higher levels (>40% blood saturation), cardiovascular collapse, seizures, coma, and death may occur – usually attributed to cardiac dysrhyrhmias. Some studies have indicated that relatively small increments in carboxyhemoglobin levels may produce adverse cardiovascular effects, such as myocardial ischemia. Delayed neurological sequelae most likely involve lesions of the white matter. The absorption and elimination of carbon monoxide are slower in the fetal circulation than in the maternal circulation. Thus, the fetus may experience toxicity when the mother is at a low carbon monoxide level with no effects.

Chronic Toxicity (or Exposure)

Animal

Chronic, low-level carbon monoxide exposures produce decreased birth weights, cardiomegaly, EKG changes, and disruptions of cognitive function in several animal models. Rabbits exposed to carbon monoxide for 11 weeks demonstrated plaque formation in cardiac vessels indistinguishable from those seen from atherosclerotic heart disease.

Human

Humans are exposed to low levels of carbon monoxide every day from automobile traffic, from smoking, or being close to those who are cooking or heating with natural gas, or through occupational means. Toxicity is dose dependent. At doses that produce carboxyhemoglobin concentrations of <10%, no symptoms were evident in studies of humans, even during vigorous exercise. Higher doses produce more pronounced toxic effects. Epidemiologic evidence suggests that humans exposed to even moderate doses of carbon monoxide during pregnancy have lower birth weight children and have offspring who are at higher risk for sudden infant death syndrome.

Clinical Management

If carbon monoxide is inhaled, the victim must be removed from exposure and assisted in breathing as necessary. Methylene chloride should be washed well off the skin and the victim removed from the area to avoid continued absorption. Administration of oxygen in any carbon monoxide poisoned patient decreases recovery time significantly: The half-life of blood carboxyhemoglobin decreases from ~6 h in adults breathing air to less than 100 min when oxygen is administered. Hyperbaric oxygen accelerates the process of carboxyhemoglobin dissociation, decreasing the half-life of carbon monoxide to 23 min. Hyperbaric oxygen has recently been shown superior over normobaric oxygen therapy for preventing cognitive deficits in carbon monoxide poisoning.

See also: Blood; Combustion Toxicology; Methanol; Methylene Chloride; Phosgene.

Further Reading

Burney RE, Wu S, and Nemiroff MJ (1982) Mass carbon monoxide poisoning: Clinical effects and results of treatment in 184 victims. *Annals of Emergency Medicine* 11: 394–399.

Thom SR, Taber RL, and Mendiguren II (1995) Delayed neuropsychologic sequelae after carbon monoxide poisoning: Prevention by treatment with hyperbaric oxygen. *Annals of Emergency Medicine* 25: 474–480.

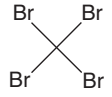
Relevant Website

http://www.inchem.org – Environmental Health Criteria (Number 213) for Carbon Monoxide (Second Edition) fron IPCS INCHEM.

Carbon Tetrabromide

Kashyap N Thakore and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 558-13-4
- Synonyms: Carbon bromide; Tetrabromide methane; Tetrabromo methane
- Chemical/Pharmaceutical/Other Class: Halomethane, oxidizing agent, solvent
- Chemical Formula: CBr_4
- Chemical Structure:



Uses

Carbon tetrabromide is used as an industrial solvent.

Exposure Routes and Pathways

The primary routes of entry are eye contact, skin contact, inhalation, and ingestion. When heated to decompose, carbon tetrabromide emits toxic fumes of Br^-.

Toxicokinetics

Carbon tetrabromide may be absorbed by dermal, inhalation, or oral routes. It is oxidatively metabolized by rat liver microsomes to electrophilic and potentially toxic metabolites. It is metabolized in the liver but causes primary effects on the kidneys. The electrophilic bromine derivatives formed can be excreted as such.

Mechanism of Toxicity

Carbon tetrabromide inhibits protein synthesis and causes lipid peroxidation, both of which may be involved in cell injury or death mediated by free radicals.

Acute and Short-Term Toxicity (or Exposure)

Animal

Carbon tetrabromide is poisonous by subcutaneous and intravenous routes and moderately toxic by ingestion. It causes kidney toxicity and is narcotic at high concentrations. LD_{50} values in mice are $298\,mg\,kg^{-1}$ (subcutaneous) and $56\,mg\,kg^{-1}$ (intravenous).

Human

Carbon tetrabromide is harmful by inhalation, ingestion, or skin absorption. It causes irritation to eyes, skin, mucous membranes, and the upper respiratory tract.

In occupational settings, technical measures should prevent any contact with the skin and mucous membranes. Workers exposed to carbon tetrabromide should wear personal protective equipment and their work should be carried out only in restricted areas. After use, clothing and equipment should be placed in an impervious container for decontamination or disposal. The American Conference of Governmental Industrial Hygienists threshold limit value for carbon tetrabromide is 0.1 ppm.

Chronic Toxicity (or Exposure)

No data are available to assess the mutagenic or genotoxic, carcinogenic, and teratogenic potential of this agent.

Clinical Management

In case of contact, eyes and skin should be flushed with water for 15–20 min. If inhaled, the victim should be removed to fresh air. If necessary, oxygen and artificial respiration should be administered. If the patient is in cardiac arrest, cardiopulmonary resuscitation should be performed. These life-supporting measures should be continued until medical assistance has arrived. An unconscious or

convulsing person should not be given liquids or induced to vomit.

Environmental Fate

Carbon tetrabromide is expected to have very high mobility in soil and volatilizes slowly from dry soil surface. Its biodegradation is expected to be slow and to exist solely as a vapor in the ambient atmosphere. It is not expected to adsorb to suspended solids and sediment in the water column. Its potential for bioconcentration in aquatic organisms is moderate.

Other Hazard

It is not flammable.

See also: Carbon Tetrachloride; Trihalomethanes.

Relevant Website

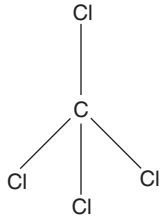
http://www.cdc.gov/niosh – NIOSH (2003) *Pocket Guide to Chemical Hazards*. Cincinnati, OH: National Institute for Occupational Safety and Health.

Carbon Tetrachloride

Thomas R Parker, Robert Howd, and Hierberto Robles

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 56-23-5
- Synonyms: Methane tetrachloride; Carbon tet; Carbon chloride; Tetrachloromethane; Perchloromethane; Tetrachlorocarbon; Carbona; Freon® 10; Halon® 104
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbon
- Chemical Structure: Tetrahedral structure with bond angles of 109.50°



Uses

The carcinogenic properties of carbon tetrachloride have caused a decline in the industrial use of this chemical. It was earlier used as a solvent for fats, oils, waxes, varnishes, lacquers, and resins and was often employed for cleaning equipment and machinery. The compound was also used as a refrigerant, as a fire extinguisher, a grain fumigant, and a dry cleaning agent; it was also used in veterinary medicine as an anthelmintic. The current use of carbon tetrachloride is limited to that of a chemical intermediate in the industrial production of a few chlorinated, organic chemicals.

Exposure Routes and Pathways

Exposure can occur via inhalation, ingestion, and dermal contact. Industrial exposures are anticipated to be the most common setting, with inhalation the most likely route.

Toxicokinetics

Due to its high lipid solubility, carbon tetrachloride is readily absorbed through inhalation and ingestion pathways, and to a lesser extent by dermal contact with the liquid form. Absorbed carbon tetrachloride tends to concentrate in body fat, liver, bone marrow, kidney, and brain. Laboratory experiments indicate that about half the absorbed dose is exhaled unchanged from the lungs. The remainder of the absorbed dose is metabolized primarily in the liver and eliminated in exhaled air and in urine and feces. Carbon tetrachloride is metabolized in the liver to a biologically active trichloromethyl radical. This radical can then undergo dimerization to hexachloroethane, reduction to chloroform, or bind to cellular macromolecules. An alternative metabolism pathway can transform carbon tetrachloride, via phosgene formation, to carbon monoxide and carbon dioxide.

Mechanism of Toxicity

Carbon tetrachloride is metabolized by cytochrome P-450 to the reactive metabolites trichloromethyl free radical and trichloromethylperoxy free radical. The trichloromethyl free radical may bind directly to cellular macromolecules such as lipids and proteins, and also to DNA, disrupting cell processes and breaking down membranes. The free radical can take part in *anaerobic* reactions, subsequently forming such toxic compounds as chloroform, hexachloroethane, and carbon monoxide. *Aerobic* biotransformation of the

•CCl_3 radical can yield trichloromethanol, a precursor to carbonyl chloride (phosgene). Since reactive metabolites are responsible for the bulk of the toxicity, tissues rich in CYP2E1, such as the liver and kidney, are the most sensitive toxicity targets for the compound. The unmetabolized fraction also produces some toxicity, and is associated with central nervous system (CNS) depression and irritation of the gastrointestinal tract.

Acute and Short-Term Toxicity (or Exposure)

Animal

Carbon tetrachloride produces systemic toxicity following short-term exposure via ingestion or inhalation. The major effects are CNS depression and hepatic and kidney damage. Symptoms of hepatic damage may appear after a delay of one or more days following acute exposure, while kidney damage develops within a few weeks. Pulmonary toxicity and respiratory disturbances have been observed in some cases. The acute oral toxicity of carbon tetrachloride to mice is relatively low, with a single dose median lethal dosage (LD_{50}) value of $13\,000\,mg\,kg^{-1}$. A rat study yielded higher acute lethality, with an LD_{50} of $\sim 8000\,mg\,kg^{-1}$.

Human

The early effect of acute carbon tetrachloride exposure by all routes is CNS depression, which can be accompanied by gastrointestinal effects such as nausea and vomiting. CNS depression can be followed by hepatic or renal injury. Hepatotoxic effects appear rapidly in humans; alterations in lipid metabolism in the liver may be observed 30 min following exposure, and histological changes within 1 h. Centrilobular necrosis, fatty degeneration, tender hepatomegaly, and jaundice are characteristic of the toxic lesions of the liver. Biological indicators of injury may include altered levels of serum enzymes such as SGOT.

The kidney is also a major target of carbon tetrachloride toxicity. The characteristic injuries observed are nephritis, nephrosis, and proteinuria. Delayed pulmonary edema and renal failure may follow hepatic damage. Renal failure is the most frequent cause of death in carbon tetrachloride poisonings.

Chronic Toxicity (or Exposure)

Animal

Chronic animal studies yielded results similar to shorter exposure durations. Rodents exposed to carbon tetrachloride in air for 6 months or longer were observed to have increased liver weights, total lipid increases, and hepatic fatty degeneration. Renal toxicity was also evident.

Chronic carbon tetrachloride exposures have produced liver tumors in several rodent species, with the tumor types including hepatocellular carcinoma and adenoma, and adrenal pheochromocytoma.

Human

Human health effects from longer-term human exposures to carbon tetrachloride generally resemble acute effects of liver and kidney damage. Consumption of alcohol and poorly controlled diabetes may increase the risk of harmful effects associated with carbon tetrachloride intoxication. The US EPA classifies carbon tetrachloride as a group B2, 'probable' human carcinogen.

In Vitro Toxicity Data

Almost all bacterial mutagenicity tests for carbon tetrachloride have been negative. Ames tests for reverse mutations using several strains of *Salmonella typhimurium*, with and without metabolic activation, were mostly negative. A weakly positive genotoxic response was reported in yeast. Negative or weak responses were observed in four studies examining unscheduled DNA synthesis.

Other studies indicate that carbon tetrachloride has the potential to form reactive intermediates that can covalently bind to DNA, which suggests genotoxicity.

Clinical Management

The victim should be removed from the contaminated environment and provided with supportive treatment. Care should be taken to maintain respiration by giving humidified oxygen through assisted ventilation, if necessary. Any contaminated clothing should be removed and the affected area should be washed with water and soap. Eyes exposed to the liquid should be irrigated with copious amounts of water. If liver and kidney damage is apparent, supportive therapy should be provided. Renal damage may be manifested by the appearance of polyuria, which might progress to oliguria and anuria. Hematuria and proteinuria may also be seen.

Environmental Fate

Carbon tetrachloride is highly volatile and does not easily break down in the environment. Most of the compound that is released to the environment accumulates in the atmosphere, where photodegradation

by shorter wavelength ultraviolet radiation appears to be the primary removal process. Absorption by the oceans and reactions with hydroxyl radical are likely lesser removal routes. The estimated half-life of atmospheric carbon tetrachloride is 30–100 years.

Ecotoxicology

Carbon tetrachloride is highly volatile and is relatively stable in the environment. Therefore, nearly all of the carbon tetrachloride produced is eventually emitted to the atmosphere. The chemical moves readily through soil and adsorbs only slightly to sediment. The estimated half-life of carbon tetrachloride in the atmosphere is 30–100 years. The hydrolysis half-life in water is estimated to be 7000 years at 25°C. Carbon tetrachloride has a low potential to bioconcentrate in animals. The logarithm of the bioconcentration factor in trout is 1.24.

Other Hazards

Carbon tetrachloride is nonflammable, and at one time was used as a fire extinguishing liquid. However, not only were the carbon tetrachloride vapors toxic, but also highly toxic phosgene gas was produced under fire conditions.

Exposure Standards and Guidelines

Cancer classifications of carbon tetrachloride by several groups are listed below:

- American Conference of Governmental Industrial Hygienists (ACGIH) Group A2 (suspected human carcinogen).
- US Environmental Protection Agency (EPA) Group B2 (probable human carcinogen).
- International Agency for Research on Cancer Group 2B (possibly carcinogenic to humans).

Drinking water standards are listed below:

- US EPA maximum contaminant level: 0.005 $mg\,l^{-1}$.
- Cal/EPA Public Health Goal: 0.005 $mg\,l^{-1}$.

Workplace standards (inhalation) are listed below

- Occupational Safety and Health Administration permissible exposure limit: 10 ppm (ceiling: 25 ppm, and 5 min maximum peak in any 4 h: 200 ppm).
- ACGIH threshold limit value – time-weighted average: 5 ppm.
- ACGIH threshold limit value – short-term exposure limit: 10 ppm.

See also: Alkyl Halides; Common Mechanism of Toxicity; Phosgene; Pollution, Soil; Pollution, Water.

Further Reading

Weber LW, Boll M, and Stampfl A (2003) Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. 33(2): 105–136.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Carbon Tetrachloride.
http://www.cdc.gov – NIOSH International Chemical Safety Cards.
http://ehp.niehs.nih.gov – NTP Tenth Report on Carcinogens (12/02).

Carbonyl Sulfide

Amy Merricle

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 463-58-1
- Synonyms: Carbon monoxide monosulfide; Carbon oxide sulfide; Carbon oxysulfide; RTECS/NIOSH FG6400000
- Chemical/Pharmaceutical/Other Class: Sulfides
- Chemical Formula: COS
- Chemical Structure: C–O–S

Uses

There are limited commercial uses of carbonyl sulfide. It is produced only in small quantities and used for small-scale experimental purposes and as a nonisolated, site-limited intermediate in the synthesis of organic sulfur compounds, thiocarbamate herbicides, and alkyl carbonates. Pesticide manufacturers

are believed to be the largest users of carbonyl sulfide. Similar to carbon disulfide, research conducted by the Australia Commonwealth Scientific and Industrial Research Organization's (CSIRO) Stored Grain Research Laboratory has shown carbonyl sulfide to be an effective soil fumigant for controlling insects on crops such as wheat, barley, oats, and peas, although carbonyl sulfide is not currently approved for this commercial use.

Exposure Routes and Pathways

Exposure occurs predominantly by the inhalation route, as most carbonyl sulfide released to the environment is released to the air. Occupational exposure to carbonyl sulfide may occur through inhalation or dermal contact during its production and use. The general population is exposed primarily from inhalation to ambient air. Carbonyl sulfide is released from natural sources such as deciduous and coniferous trees, volcanoes, salt marshes, and soils. Industrial sources of atmospheric carbonyl sulfide release include automobile exhaust, coal-fired power plants, biomass combustion, fish processing, combustion of refuse and plastics, petroleum manufacture, and manufacture of synthetic fibers, starch, and rubber. An estimated two-thirds of total carbonyl sulfide release worldwide is attributed to natural sources. Carbonyl sulfide can also be formed in the atmosphere through the chemical reaction of gas-phase carbon disulfide, and photochemically produced hydroxyl radicals. Carbonyl sulfide can be discharged to surface waters in the wastewater of viscose rayon plants. Drinking water normally does not contain carbonyl sulfide. Carbonyl sulfide is not expected to bioaccumulate in fish or other aquatic organisms; therefore, fish consumption is not considered a relevant route of exposure to this substance. A preliminary study of purgeable organic compounds in breast milk detected carbonyl sulfide in one of eight breast milk samples from nursing mothers living in urban centers in Pennsylvania, New Jersey, and Louisiana, suggesting the potential for exposure to breastfed infants. The source of the carbonyl sulfide in the breast milk sample was not discussed, and it may have been a metabolism by-product.

Toxicokinetics

Carbonyl sulfide is absorbed primarily in the lungs via the inhalation route, but can also be absorbed through the gastrointestinal tract and through the skin. Carbonyl sulfide is known to be absorbed into the blood, but transport and distribution is not fully understood. Studies of carbon disulfide metabolism using rat liver microsomes have demonstrated that carbonyl sulfide is a metabolic intermediate in the formation of carbon dioxide. Metabolism of carbonyl sulfide is mediated by the microsomal cytochrome P-450 monooxygenase system and is NADPH-dependant. Carbonyl sulfide is oxidized to atomic sulfur and carbon dioxide. The oxidative metabolism of carbonyl sulfide is a potential cause of toxicity due to the formation of highly reactive sulfur atoms. The atomic sulfur liberated in these reactions can be covalently bound to macromolecules or be oxidized to sulfate and excreted in urine. Carbonyl sulfide can be catalyzed by carbonic anhydrase to monothiocarbamate, which is spontaneously degraded to carbon dioxide and hydrogen sulfide. The hydrogen sulfide may be oxidized to sulfate or other still unknown metabolites. Monothiocarbamate can enter the urea cycle, forming thiourea, which is excreted in urine.

Mechanism of Toxicity

Toxicity from exposure to carbonyl sulfide is likely the result of the decomposition of carbonyl sulfide to carbon dioxide and hydrogen sulfide. Hydrogen sulfide inhibits respiration on the cellular level causing methemoglobinemia, which inhibits the cytochrome oxidase system causing cytotoxic anoxia. In one study, rats were treated with acetazolamide, an inhibitor of carbonic anhydrase. Test animals showed lower blood levels of hydrogen sulfide following exposure to carbonyl sulfide and exhibited decreased toxicity relative to rats that were not pretreated with acetazolamide. Hydrogen sulfide is believed to be primarily responsible for many of the reported adverse effects associated with exposure to carbonyl sulfide.

Acute and Short-Term Toxicity (or Exposure)

Animal

Exposure to carbonyl sulfide in animals produces serious nervous system effects with narcotic effects and acute respiratory failure at high concentrations. Acute inhalation exposure to carbonyl sulfide produced nervous system dysfunction and lower respiratory system irritation in rats. Rats exposed to carbonyl sulfide via inhalation for 4 h showed some central nervous system effects at 1062 and 1189 ppm. Results showed hypoactivity, lacrimation, breathing difficulties, cyanosis, bleeding from the nose, convulsions, tremors, and behavioral abnormalities, the most prominent of which, circling,

was demonstrated by approximately 50% of the 1062 ppm dose group survivors during the first 4 days postexposure. The lowest LC_{50} (95% confidence) was determined to be 1070 ppm for female rats.

As a follow-up to the LC_{50} study, a 2 week inhalation study was conducted. Results showed carbonyl sulfide toxicity for the high dose group (450 ppm), but only after at least 6 days of exposure. Females of the high exposure group weighed statistically less than controls after the second week of exposure. Signs of central nervous system dysfunction, ataxia, head tilting, circling, tremors, and convulsions were observed in 50% of the high dose group. Females in the mid (150 ppm) and the mid-high (250 ppm) dose groups had depressed red cell counts along with a slight depression in mean corpuscular volume. The authors concluded that no exposure-related effects occurred in animals exposed to carbonyl sulfide at 51 ppm for 11 days, 6 h per day.

Continuous exposure of rabbits to 50 ppm carbonyl sulfide for 1–7 weeks resulted in a slightly elevated mean serum cholesterol level, but had no significant effect on myocardial ultrastructure and did not show histopathological changes in lungs or coronary arteries.

Human

Carbonyl sulfide appears to elicit similar symptoms of poisoning as those seen from exposure to hydrogen sulfide, although produces less prominent initial warning signs, such as local irritation to the skin, eyes, and respiratory tract. Exposure to carbonyl sulfide may cause central and peripheral nervous system damage, damage to the respiratory tract, and ocular effects. Carbonyl sulfide exposure has also been associated with cardiovascular disease. Breathing high concentrations of carbonyl sulfide (greater than 1000 ppm) over a short time period may cause sudden unconsciousness, convulsions, coma, and fatal central respiratory paralysis. At low to moderately high vapor concentrations carbonyl sulfide can cause burning or redness of the eyes, painful conjunctivitis, photophobia, corneal opacity, headache, nausea, dizziness, confusion, cardiac arrhythmia, and pain and weakness in the extremities. Direct skin contact with carbonyl sulfide vapors may produce skin irritation and pain. Prolonged or repeated exposure to the skin may cause dermatitis. Gastrointestinal effects include profuse salivation, nausea, vomiting, and diarrhea. Central nervous system effects include giddiness, headache, vertigo, amnesia, confusion, and unconsciousness.

Chronic Toxicity (or Exposure)

Animal

No information was identified on the chronic reproductive, developmental, or carcinogenic effects of carbonyl sulfide in animals. However, carbonyl sulfide is the oxidation product of carbon disulfide, which has been shown by the National Institute of Health to be positive in the strain A mouse lung tumor bioassay. Significant increases in the incidence (tumor-bearing mouse) and frequency (tumors per mouse of lung adenomas) was observed in A/J mice.

Human

Chronic exposure to low concentrations of carbonyl sulfide may cause damage or irritation to the respiratory tract including symptoms of rhinitis, pharyngitis, bronchitis, and pneumonitis, and may cause pulmonary edema. Recovery depends upon the length of exposure and the dose. Residual effects during recovery may include coughing, slow pulse, and amnesia.

No information regarding the potential carcinogenicity or the developmental or reproductive toxicity of carbonyl sulfide in humans was identified. The Environmental Protection Agency has not classified carbonyl sulfide with respect to potential carcinogenicity.

In Vitro Toxicity Data

The National Toxicology Program found that carbonyl sulfide produced a weak positive response in the salmonella mutagenicity test. No further information regarding this test was identified.

Clinical Management

Following inhalation exposure, the victim should be moved to fresh air immediately. If the victim is not breathing, artificial respiration or cardiopulmonary resuscitation should be given, if necessary. If breathing is labored, the victim should be given oxygen. In case of ocular or dermal contact, the skin or eyes should be flushed with running water immediately. Soap and water may be used for washing exposed skin. If carbonyl sulfide is accidentally ingested, medical treatment should be sought immediately. Vomiting should not be induced. Further treatment is symptomatic. Rescuers must prevent exposure by wearing a self-contained breathing apparatus to rescue the victim.

Environmental Fate

Most of the releases of carbonyl sulfide to the environment are to air, where it is believed to have a long residence time. The half-life of carbonyl sulfide in the atmosphere is estimated to be ~2 years. It may be degraded in the atmosphere via a reaction with photochemically produced hydroxyl radicals or oxygen, direct photolysis, and other unknown processes related to the sulfur cycle. Sulfur dioxide, a greenhouse gas, is ultimately produced from these reactions. Carbonyl sulfide is relatively unreactive in the troposphere, but direct photolysis may occur in the stratosphere. Also, plants and soil microorganisms have been reported to remove carbonyl sulfide directly from the atmosphere. Plants are not expected to store carbonyl sulfide.

Carbonyl sulfide is extremely mobile in soils. If released to soil it will volatilize quickly to the atmosphere. It has a high solubility in water and will not readily adsorb to soil particles, sediment, or suspended organic matter. Therefore, carbonyl sulfide is expected to volatilize rapidly from soil and water or, depending upon volume, concentration, and site-specific characteristics (e.g., soil type, depth to groundwater, temperature, humidity), may be able to move rapidly through the ground and impact groundwater. Carbonyl sulfide may hydrolyzed in water to form hydrogen sulfide and carbon dioxide.

Ecotoxicology

Carbonyl sulfide is not expected to bioaccumulate in fish or other aquatic organisms. The United States Environmental Protection Agency reported that quantitative structure activity relationship estimates of acute toxicity for fish, daphnid, and algae are greater than $1000\,mg\,l^{-1}$.

Other Hazards

Carbonyl sulfide is a flammable gas, and may be explosive or spontaneously flammable in air under the right conditions. Vapors may ignite at distant ignition sources and flash back. When exposed to fire, humidity, or strong alkalis, carbonyl sulfide may form the toxic decomposition products carbon monoxide and hydrogen sulfide gas. In the presence of strong oxidizers, carbonyl sulfide presents a fire or explosion hazard. Carbonyl sulfide has a vapor density of 2.1 and is therefore heavier than air. Cylinders or tank cars containing carbonyl sulfide may rupture violently or rocket under fire conditions.

The National Fire Protection Agency (NFPA) flammable limits are as follows:

- lower – 12% by volume,
- upper – 29% by volume, and
- explosive limits is 12–29%.

Always refer to the Material Safety Data Sheet for information on proper handling and disposal.

Miscellaneous

The Clean Air Act (CAA) Amendments of 1990 list carbonyl sulfide as a hazardous air pollutant (HAP) generally known or suspected to cause serious health effects. Section 112(b) (1) of the CAA lists pollutants that are judged to be hazardous if emitted into the air. Carbonyl sulfide is included on this list. The statute calls for the identification of source categories that emit these HAPs, and the subsequent promotion of technology-based emission standards requiring compliance with maximum achievable control technology.

Section 112(r) of the CAA establishes a list of substances that, if present in a quantity in excess of a specific threshold, would require that the facility establish a risk management program to prevent chemical accidents, prepare a risk management plan, and submit the plan to the State and local emergency planning organizations. Carbonyl sulfide is regulated under CAA 112(r) because of its chemical property as a flammable gas.

Carbonyl sulfide may be regulated as a D003 hazardous waste under the Resource Conservation and Recovery Act when a solid waste containing this sulfide compound exhibits the characteristic of reactivity as stipulated in Title 40 of the Code of Federal Regulations, Section 261.23.

Carbonyl sulfide is also regulated under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Releases of substances on the CERCLA list of hazardous substances that are in excess of a specified reportable quantity must be reported to the United States Environmental Protection Agency's National Response Center. The reportable quantity for carbonyl sulfide is 100 pounds. Carbonyl sulfide is also subject to regulation under the Superfund Amendments and Reauthorization Act, Title III, Sections 311, 312, and 313, and Section 4 of the Toxic Substances Control Act.

See also: Carbon Disulfide; Hydrogen Sulfide; Pollution, Air.

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Relevant Website

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Carboxylesterases

Ramesh C Gupta

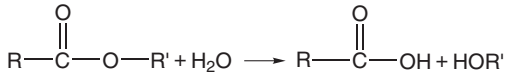
Carboxylesterases (CarbEs, EC 3.1.1.1; also known as aliesterases or tributyrinases) are a heterogeneous group of enzymes as they differ in substrate specificity. Despite the wide distribution of CarbEs in mammalian systems, most of their known substrates are foreign compounds that are not normally involved in intermediary metabolism. CarbEs hydrolyze xenobiotics containing an ester, thioester, or amide group, and thus play an important role in drug metabolism, carcinogenesis, and detoxification of many noxious chemicals present in our environment. The physiological function of CarbEs still remains obscure.

Physical, Chemical, and Biochemical Properties of CarbEs

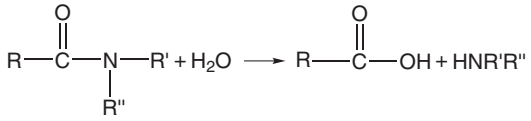
The mammalian hepatic, renal, and intestinal CarbEs consist of units with a molecular weight of ~60 000. Each unit bears one active site. The amino acid sequence around the active site of several CarbEs is Gly-Glu-Ser$^+$-Ala-Gly. The p*I* of hepatic CarbEs is usually in the range of pH 4.7–6.5 with the pH optimum in the range of pH 6–10. The behavior of hepatic microsomal and cytosolic CarbEs in *in vitro* and *in vivo* studies indicates that these enzymes are different. CarbE in hepatic microsomes consists of three isoenzymes (RH1, molecular weight 174 000, trimer, p*I* 6.0; RL1, molecular weight 61 000, monomer, p*I* 6.5; and RL2, molecular weight 61 000, monomer, p*I* 5.5), which differ considerably in terms of inducibility, substrate specificity, and immunological properties.

CarbEs can catalyze hydrolytic reactions of the following types:

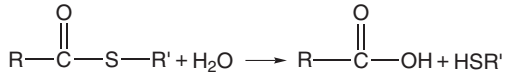
1. Carboxylester hydrolysis



2. Carboxylamide hydrolysis



3. Carboxythioester hydrolysis



The first two of these reactions are equally relevant for biotransformation process. Amides are often more stable to enzymatic hydrolysis than the corresponding esters with similar structures. For example, phenylacetate is hydrolyzed much faster than acetanilide. In addition, CarbE can hydrolyze therapeutically useful drug esters, such as chloramphenicol succinate, prednisolone succinate, procaine, and methylparaben.

Tissue Distribution of CarbEs

The activities of CarbEs have been localized and determined in almost all tissues, with the highest activity in liver. A substantial amount of the enzyme activity is present in heart, kidney, lungs, brain, skeletal muscles, testes, small intestine, pancreas, nasal mucosa, adipose tissue, and plasma. Normal values of CarbEs for some of the tissues, using tributyrin as the substrate, are given in **Table 1**. No significant variability in the

Table 1 Normal values of CarbE in different tissues of male Sprague–Dawley rats

Tissue	*CarbE activity (μmol tributyrin per gram per hour)*
Brain regions	
Cortex	68±2.6
Brainstem	68±2.3
Striatum	71±1.5
Hippocampus	72±3.1
Muscles	
Diaphragm	67±2.0
Heart	92±2.9
Liver	3014±6.0
Serum/plasma	30±2.0

Note: Values are mean ± SEM.

values of CarbEs has been found among discrete brain regions and among different fiber-dependent skeletal muscles. Studies have shown that CarbE activities of the liver, kidneys, brain, and intestinal mucosa are predominantly present in the microsomal fraction. The liver cytosolic CarbE activity is 1/20 of that present in the microsomes. The lowest CarbE activity is determined in plasma (1/70 of that in hepatic microsomes). At least some of the serum CarbE isoenzymes might originate from the liver.

Significance of CarbE Induction or Inhibition in Metabolism and Toxicity

As mentioned before, CarbEs have a limited physiological role *per se*, but their induction or inhibition by some drugs or xenobiotics can modify the metabolism and toxicity of their own or others to a great extent.

Induction of CarbEs

Oral or parenteral administration of phenobarbital can increase the cytosolic CarbE activity more than the microsomal activity, and the activity can remain elevated for 7 days after the last phenobarbital treatment. Phenobarbital treatment has no effect on the extrahepatic CarbE activity.

Parenteral administration of *p*,*p*′-DDT can augment hepatic CarbE for 14 days, probably due to the sequestration of the compound in adipose tissue. The hepatic CarbE activity can be increased up to threefold by phenobarbital and *p*,*p*′-DDT. Plasma CarbE activity is not altered by *p*,*p*′-DDT.

Hepatic and extrahepatic CarbE activities have been studied after the exposure of rats to polycyclic aromatic hydrocarbons. In dose- and time-dependent studies, benz(*a*)anthracene, benzo(*a*)pyrene, and 3-methylcholanthrene moderately induced the hepatic cytosolic and kidney microsomal CarbEs activities, while anthracene, phenanthrene, and chrysene had no effects on these enzymes. The hepatic microsomal and kidney cytosolic enzyme activities were not altered by the polycyclic aromatic hydrocarbons. The inducibility of hepatic cytosolic CarbE by the polycyclic aromatic hydrocarbons suggests that these compounds could be divided into two groups: benz(*a*)anthracene, benzo(*a*)pyrene, and 3-methylcholanthrene are moderate inducers, while anthracene, phenanthrene, and chrysene are noninducers. The commercial arochlors (i.e., polychlorinated biphenyls) increase hepatic CarbE by twofold.

From a toxicological point of view, the induction of CarbEs may protect against the toxic effects of an ester if the compound itself is directly responsible for the toxic action (e.g., the reduced toxicity of malathion or malaoxon following hexachlorobenzene exposure in rats). Conversely, the induction of CarbE may potentiate the toxic effects produced by the hydrolytic products of the compound (e.g., in the metabolism of allyl alcohol).

Inhibition of CarbEs

The most important inhibitors of CarbEs are organophosphorus insecticides (malathion, parathion, paraoxon, methyl parathion, EPN, and others), nerve agents (DFP, soman, sarin, tabun, and VX) and carbamate insecticides (carbofuran, carbaryl, aldicarb, propoxur, oxamyl, methomyl, and others). Organophosphorus toxicants inhibit CarbEs irreversibly by phosphorylation and carbamates inhibit CarbEs reversibly by carbamylation; similar to the basic mechanism (i.e., acylation of the active site):

$$\mathrm{EH} + \mathrm{AB} \underset{K_{-1}}{\overset{K_{+1}}{\leftrightarrow}} \mathrm{EHAB} \overset{K_{+2}}{\rightarrow} \mathrm{EA} + \mathrm{BH} \underset{\mathrm{H_2O}}{\overset{K_{+3}}{\rightarrow}} \mathrm{EH} + \underset{(+\mathrm{BH})}{\mathrm{AOH}}$$

where EH is the enzyme, AB is the inhibitor, EHAB is the enzyme–inhibitor complex, and EA is the acyl enzyme. In other words, organophosphates and carbamates inactivate CarbEs by rapid esterification of a serine residue in the active site. It is often followed by a slow hydrolysis of the new ester bond. Therefore, these compounds are not only inhibitors of CarbEs but also poor substrates. Other inhibitors of CarbEs include disulfiram (tetraethylthiuram disulfide) and glucocorticoids (dexamethasone).

Role of CarbEs in Organophosphate and Carbamate Poisoning

Depending on the involvement of one or more anticholinesterase agents, and their single or repeated exposure, CarbEs can have multiple roles, such as (1) a protective role by detoxifying organophosphates or

carbamates, (2) preinhibition of CarbEs as a major factor in potentiation of toxicity, and (3) role in tolerance development following repeated exposure to organophosphates.

Role of CarbEs in Detoxification of Organophosphates and Carbamates

The acute toxicity of organophosphates and carbamates is attributed to their effectiveness as inhibitors of AChE. During both acute and prolonged exposure to organophosphates and carbamates, the activities of other serine-containing esterases, such as CarbEs, are inhibited in both neuronal and non-neuronal tissues. Inhibition of CarbEs generally serves as a detoxifying mechanism by reducing the free concentration of AChE inhibitors. Low-level exposure to organophosphate or carbamate causes marked inhibition of CarbEs without inhibiting AChE, suggesting greater affinity of CarbEs than AChE to these inhibitors. CarbEs act like false targets or scavengers, which bind and thereby inactivate significant amounts of these inhibitors. In recent studies, one of the oximes, HI-6, reactivated CarbE activity, thereby providing additional protection against soman or possibly other organophosphate poisoning by acting as endogenous scavengers.

CarbEs, in addition to serving as nonspecific binding sites, can hydrolyze the carboxylester bond (esterolytic detoxification) in malathion-type organophosphates, carbamates, pyrethroids, and benzilate insecticides, and thereby reduce the free concentration of these insecticides. Therefore, CarbEs can detoxify organophosphates and carbamates by multiple mechanisms.

The diversity between the toxic effects of organophosphates, carbamates, or pyrethroids could partly be due to the existing variability in the levels of CarbE activity, which is related to species, strain, and gender differences. For example, rabbit liver CarbE is more sensitive to inhibition by malathion and isomalathion than pig liver CarbE; some strains of rats and mice have higher CarbE activity than others; and female rat plasma has higher CarbE activity than male rat plasma.

Role of CarbEs in Potentiation of Toxicity of Organophosphates or Carbamates

The toxicity of organophosphates or carbamates can be potentiated several-fold if the activity of CarbE is inhibited by pretreatment. Potentiation of malathion toxicity by EPN (*O*-ethyl-*O*-*p*-nitrophenyl phenylphosphonothionate) was reported about half a century ago. The mechanism responsible for potentiation by EPN of malathion toxicity has been explained on the basis of inhibition of the enzymatic hydrolysis of the carboxylester linkages of malathion. An impurity compound, *O*,*O*,*S*-trimethyl phosphorothioate, present in commercial formulations of malathion and phenthoate, potentiates the acute toxicity of malathion and phenthoate by inhibiting tissue CarbEs. One of the most susceptible animals is the dog.

Toxicity of organophosphates can be potentiated 15–20-fold in rats and mice by pretreatment with a metabolite of tri-*O*-cresylphosphate, CBDP (2-*O*-cresyl)-4*H*-1,3,2-benzodioxa-phosphorin-2-oxide), which is an irreversible inhibitor of CarbEs. In similar studies, tetraisopropylpyrophosphoramide (iso-OMPA), or mipafox, an organophosphate-irreversible inhibitor of CarbEs, potentiates three- to fivefold the toxicity of several OPs (soman, DFP, and methylparathion) and carbamates (carbofuran, aldicarb, propoxur, and carbaryl). Inhibition of CarbEs by CBDP, iso-OMPA, or mipafox pretreatment, particularly in plasma, liver, heart, brain, and skeletal muscles, is a major contributory factor in the potentiation of toxicity of organophosphates and carbamates. Thus, the toxicity of any drug, pesticide, or other type of agent that is normally detoxified by CarbEs, could be potentiated by pre-exposure to an organophosphorus or other carboxylesterase inhibitor.

Role of CarbEs in Organophosphate Tolerance Development

Daily exposure of rats, mice, or guinea pigs to certain organophosphates with sublethal doses can lead to severe toxicity during the first few days, but further exposure for 7–14 days can lead to development of tolerance. For example, daily dosing of DFP ($0.5\,mg\,kg^{-1}$, s.c.) produces severe anticholinesterase signs on day 5 (toxicity phase), but further administration results in tolerance development; because on day 14 rats are free of signs (tolerance phase). During tolerance, CarbE activity can recover up to 40% or more compared with the initial inhibition (day 5), suggesting renewed availability of nonspecific binding sites (CarbEs). The recovery of CarbE is probably due to *de novo* synthesis, since treatment with an inhibitor of protein synthesis, cycloheximide, abolishes tolerance development. In contrast to organophosphates, rats that were administered carbamates such as carbofuran or aldicarb daily for 3–4 weeks showed no development of tolerance to toxicity, probably due to lack of CarbE recovery. With organophosphorus toxicants, protection can be attenuated, toxicity can be potentiated, and tolerance can be abolished by preinhibition

of CarbEs with iso-OMPA, mipafox, or any other CarbE inhibitor against organophosphates.

See also: Biotransformation; Dithiocarbamates; Liver; Nerve Agents; Organophosphates; Pesticides.

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Carcinogen Classification Schemes

Michael A Kamrin

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Introduction

Several classification schemes have been developed for ranking the relative hazards to humans associated with chemicals that, by one or more criteria, may be considered to be potential carcinogens. The classification schemes are based on scientific judgments that typically take into account all the data available from *in vivo* animal bioassays, *in vitro* tests for genetic toxicity, human epidemiology, and structural relationships with other known carcinogens. Classification of a chemical as a carcinogen involves the consideration of many different factors. Classification schemes provide guidance on evaluating and weighting the available evidence and placing chemicals into defined categories that can be used to communicate the implications for risk. Factors usually taken into consideration in interpreting the results of an animal bioassay include the following:

- Adequacy of experimental design and conduct.
- Statistical significance of any increase in tumor incidence.
- Presence or absence of a dose–response relationship and correct dose selection.
- Nature of tumors (benign or malignant) and relevance of tumor type to humans.
- Historical control data (incidence and variability) for tumor type.
- Common (spontaneous) versus uncommon tumors.
- Number of organs/tissues with tumors.
- Mechanistic information.

Two commonly used classification schemes are the one developed by US Environmental Protection Agency (US EPA) and the one developed by the International Agency for Research on Cancer (IARC). The US EPA classification scheme is used as a tool for the regulation of chemicals under those laws it administers (e.g., FIFRA and TSCA) as well as by many state regulatory agencies. The IARC classification

scheme is commonly used in the European Community and is considered in certain US regulations and laws (e.g., OSHA Hazard Communication Standard). Other respected carcinogenic classification schemes include those developed by the National Institute of Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration (OSHA), National Toxicology Program (NTP), and American Conference of Government Industrial Hygienists (ACGIH). Each of these schemes is described in the following sections. It should be emphasized that these classification schemes are constantly evolving and that changes may occur over time.

US EPA Carcinogen Classifications

The US EPA carcinogen classifications are assigned to chemicals using the approach detailed in the *Guidelines for Carcinogens Risk Assessment* (51 FR 33992). The US EPA 'total-weight-of-evidence' scheme classifies potential carcinogens into five groups, A–E, that indicate the likelihood they are human carcinogens. These groups are described below.

- *Group A*: Human carcinogen – this is reserved for chemicals where there exists clear epidemiological evidence indicating an association between exposure to the chemical and cancer.
- *Group B*: Probable human carcinogen – this group is divided into two subgroups, B1 and B2. Group B1 indicates that there is 'sufficient' evidence to indicate that the material is an animal carcinogen and that there is 'limited' evidence of effects in humans. Group B2 indicates that although there is sufficient evidence in animals, the total weight of evidence for effects in humans is weaker or 'inadequate'.
- *Group C*: Possible human carcinogen – classification in this group indicates limited, often marginal evidence of carcinogenicity in animals and no evidence of any effects in humans.
- *Group D*: Not classifiable as to human carcinogenicity – this group is used for chemicals for which no data are available.
- *Group E*: Evidence of noncarcinogenicity for humans – this group is used for chemicals that show no evidence of any carcinogenicity in at least two adequately conducted animal tests with different species.

Proposed US EPA Classification Scheme

The US EPA classification scheme was published in the 1986 cancer guidelines (51 FR 33992). In April 1996, the US EPA proposed new cancer guidelines which differ substantially from the previous guidelines. The new guidelines recommend a narrative with descriptors that replace the previous letter designations. The narrative explains the kinds of evidence available and how they fit together in drawing conclusions, along with highlighting the significant issues and strengths and limitations of the data and conclusions. The descriptors have standardized definitions. The descriptors are not meant to replace an explanation of the nuances of the biological evidence but rather to summarize it. The use of descriptors within a narrative is intended to preserve the complexity (including the gray areas) that is an essential part of the hazard classification. Risk managers are instructed to consider the entire range of information included in the narrative rather than focusing simply on the descriptor.

Each category spans a wide variety of potential data sets and weights of evidence. The three proposed categories of descriptors for human carcinogenic potential are 'known/likely', 'cannot be determined', and 'not likely'.

Known/Likely

This category of descriptors is used when the available tumor effects and other key data are adequate to convincingly demonstrate carcinogenic potential for humans. It includes cases in which agents are known human carcinogens based on either epidemiologic evidence or a combination of epidemiologic and experimental evidence, demonstrating causality between human exposure and cancer; agents that should be treated as if they were known human carcinogens, based on a combination of epidemiologic data showing a plausible causal association (not demonstrating it definitively) and strong experimental evidence; and agents that are likely to produce cancer in humans due to the production or anticipated production of tumors by modes of action that are relevant or assumed to be relevant to human carcinogenicity.

Cannot Be Determined

This category of descriptors is used when available tumor effects or other key data are suggestive or conflicting or limited in quantity and, thus, are not adequate to convincingly demonstrate carcinogenic potential for humans. It includes cases in which agents' carcinogenic potential cannot be determined but there is suggestive evidence that raises concern for carcinogenic effects; agents whose carcinogenic potential cannot be determined because the existing evidence is composed of conflicting data (e.g., some

evidence is suggestive of carcinogenic effects, but other equally pertinent evidence does not confirm any concern), and agents whose carcinogenic potential cannot be determined because there are inadequate or no data to perform an assessment.

Not Likely

This category of descriptors is used when, in the absence of human data suggesting a potential for cancer effects, the experimental evidence is satisfactory for deciding that there is no basis for human hazard concern. It includes cases in which agents are not likely to be carcinogenic to humans because they have been evaluated in at least two well-conducted studies in two appropriate animal species without demonstrating carcinogenic effects; agents not likely to be carcinogenic to humans because they have been appropriately evaluated in animals and show only carcinogenic effects that have been shown not to be relevant to humans; agents not likely to be carcinogenic to humans when carcinogenicity is dose or route dependent (e.g., not likely below a certain dose range or not likely by a certain route of exposure); and agents not likely to be carcinogenic to humans based on extensive human experience that demonstrates lack of effect (e.g., phenobarbital).

In 1999, the EPA published additional draft materials relating to carcinogenicity classification. These materials provided additional guidance for evaluating weight of evidence and also proposed changing the three categories described in the 1996 document. The new categories are Carcinogenic to Humans; Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential; Data are Inadequate for an Assessment of Human Carcinogenic Potential; and Not Likely to Be Carcinogenic to Humans. None of these post-1986 guidelines have been finalized.

IARC Carcinogen Classifications

IARC is a department of the World Health Organization (WHO). The overall classification scheme developed by IARC is similar to that used by the US EPA (the US EPA scheme was initially developed based on an IARC scheme). Chemicals are classified into four groups with respect to their potential to cause cancer in humans. The classification reflects the strength of the evidence available from animal studies, epidemiology, and other relevant data. The IARC groups are outlined below.

- *Group 1*: The agent is carcinogenic to humans – this group is reserved for those chemicals or agents where there is 'sufficient evidence' of carcinogenicity in humans.
- *Group 2*: The agent is probably carcinogenic to humans – this group, like the US EPA group B, is divided into two subgroups, groups 2A and 2B, depending on the strength of the evidence available. Groups 2A and 2B indicate that the agent is 'probably' or 'possibly' carcinogenic to humans, respectively.
- *Group 3*: The agent is not classifiable as to its carcinogenicity to humans – this group is used for chemicals that do not fall into any of the other groups.
- *Group 4*: The agent is probably not carcinogenic to humans – this group is used for compounds where there exists evidence suggesting an absence of carcinogenic potential in humans.

NTP Carcinogen Classifications

The NTP is responsible for preparing *Reports on Carcinogens*. The *Reports on Carcinogens* are mandated by Public Law 95-662 and are for are informational purposes only. The listing of a substance in the annual report does not by itself establish that such a substance presents a risk to persons in their daily lives. Clause (I) in subparagraph (4) (A) of Section 301 (b) of the Public Health Service Act requires that a report be published which contains a list of all substances (1) 'which are either known to be carcinogens or may reasonably be anticipated to be carcinogens', and (2) to which a significant number of persons residing in the United States are exposed. The conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. As of the 2002 update, for the purpose of *Biennial Report on Carcinogens*, the classification scheme is outlined below.

- *Group 1*: Known to be human carcinogens – this group is reserved for those chemicals where there is sufficient evidence of carcinogenicity from studies in humans which indicate a causal relationship between exposure to the agent, substance, or mixture and human cancer.
- *Group 2*: Reasonably anticipated to be human carcinogens – this group, like the US EPA group B, is divided into two subgroups, groups 2A and 2B, depending on the strength of the evidence available.
- *Group 2A*: There is limited evidence of carcinogenicity from studies in humans which indicate that a causal interpretation is credible, but that alternative explanations, such as chance,

bias, or confounding, could not adequately be excluded.

- *Group 2B*: There is sufficient evidence of carcinogenicity from studies in experimental animals which indicate that there is an increased incidence of malignant tumors and/or combined benign and malignant tumors (a) in multiple species or at multiple tissue sites, (b) by multiple routes of exposure, or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or there is less than sufficient evidence of carcinogenicity in humans or laboratory animals. However, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous *Annual* or *Biennial Report on Carcinogens* as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

OSHA Carcinogen Classifications

The Occupational Safety and Health Act of 1970 provides the establishment of workplace standards for toxic materials or harmful physical agents

> which most adequately assures, to the extent feasible, on the basis of the best available evidence, that no employee will suffer material impairment of health or functional capacity even if such employee has regular exposure to the hazard dealt with by such standard for the period of his or her working life.

Potential occupational carcinogens regulated under OSHA are classified into two main categories based on the nature and extent of the available scientific evidence: category I potential carcinogens and category II potential carcinogens.

Category I Potential Carcinogens

A substance shall be identified, classified, and regulated as a category I potential carcinogen if, upon scientific evaluation, the secretary determines that the substance meets the definition of a potential occupational carcinogen in (1) humans, or (2) a single mammalian species in a long-term bioassay in which the results are in concordance with some other scientifically evaluated evidence of a potential carcinogenic hazard, or (3) a single mammalian species in an adequately conducted long-term bioassay, in appropriate circumstances in which the secretary determines the requirement for concordance is not necessary. Evidence of concordance is any of the following: positive results from independent testing in the same or other species, positive results in short-term tests, or induction of tumors at injection or implantation.

Category II Potential Carcinogens

A substance shall be identified, classified, and regulated as a category II potential carcinogen if, upon scientific evaluation, the secretary determines that (1) the substance meets the criteria set forth for category I, but the evidence is found by the secretary to be only 'suggestive'; or (2) the substance meets the criteria set forth for category I in a single mammalian species without evidence of concordance.

NIOSH Carcinogen Classifications

Acting under the authority of the Occupational Safety and Health Act of 1970 (Public Law 91-596), the NIOSH develops and periodically revises recommended exposure limits (RELs) for hazardous substances or conditions in the workplace. These recommendations are then published and transmitted to OSHA for use in promulgating legal standards. NIOSH may identify numerous chemicals that it believes should be treated as occupational carcinogens even though OSHA has not yet identified them as such. Generally, where OSHA has adopted the NIOSH recommendations as OSHA standards, the OSHA PELs and NIOSH RELs are equal. In cases in which the NIOSH recommendations have not been formally adopted by OSHA, the NIOSH RELs may be different from the OSHA PELs. For example, the NIOSH exposure limit for trichloroethylene (25 ppm) differs from the OSHA exposure limit (50 ppm).

The NIOSH classification scheme is one of the simplest carcinogen classification schemes; it combines all carcinogens into one category. Within this single category, NIOSH narratively describes the site of the cancer and whether the effect was seen in humans or animals. In determining carcinogenicity, NIOSH uses a classification scheme outlined in 29 CFR 1990.103, which states in part:

> Potential occupational carcinogen means any substance, or combination or mixture of substances, which causes an increased incidence of benign and/or malignant neoplasms, or a substantial decrease in the latency period between exposure and onset of neoplasms in humans or in one or more experimental mammalian species as the result of any oral, respiratory, or dermal exposure, or any other exposure which results in the induction of tumors at a site other than the site of administration. This definition also includes any substance

which is metabolized into one or more potential occupational carcinogens by mammals.

The NIOSH thresholds for carcinogens were not designed to be protective of 100% of the population. NIOSH usually recommends that occupational exposures to carcinogens be limited to the lowest feasible concentration. This perhaps is the reason that the NIOSH exposure limit for vinyl chloride is the lowest reliably detectable concentration and the OSHA exposure limit is 1 ppm.

ACGIH Carcinogen Classifications

ACGIH classifies substances associated with industrial processes that are recognized to have carcinogenic or cocarcinogenic potential. In general, the stated classification is intended to provide a practical guideline for the industrial hygiene professional to assist in control of exposures in the workplace. The classification and threshold limit values (TLVs) are not mandated by federal or state regulations, although the ACGIH classifications and values may be considered when standards are adopted by the regulatory agencies. Currently, five categories of carcinogens have been designated by the TLV Committee to recognize the qualitative differences in research results or other data. These five categories are outlined below.

- *A1*: Confirmed human carcinogen – the agent is carcinogenic to humans based on the weight of evidence from epidemiologic studies of exposed humans, and/or convincing clinical evidence in exposed humans.
- *A2*: Suspected human carcinogen – the agent is carcinogenic in experimental animals at dose levels, by route(s) of administration, at site(s), of histologic types(s), or by mechanism(s) that are considered relevant to worker exposure. Available epidemiologic studies are conflicting or insufficient to confirm an increased risk of cancer in exposed humans.
- *A3*: Animal carcinogen – the agent is carcinogenic in experimental animals at a relatively high dose, by route(s) of administration, at site(s), of histologic types(s), or by mechanism(s) that are not considered relevant to worker exposure. Available epidemiologic studies do not confirm an increased risk of cancer in exposed humans. Available evidence suggests that the agent is not likely to cause cancer in humans except under uncommon or unlikely routes or levels of exposure.
- *A4*: Not classifiable as a human carcinogen – there are inadequate data on which to classify the agent in terms of its carcinogenicity in humans and/or animals.
- *A5*: Not suspected as a human carcinogen – the agent is not suspected to be a human carcinogen on the basis of properly conducted epidemiologic studies in humans. These studies have sufficiently long follow-up, reliable exposure histories, sufficiently high dose, and adequate statistical power to conclude that exposure to the agent does not convey a significant risk of cancer to humans. Evidence suggesting a lack of carcinogenicity in experimental animals will be considered if it is supported by other relevant data.

Substances for which no human or experimental animal carcinogenic data have been reported are assigned no carcinogen designation by the ACGIH.

See also: American Conference of Governmental Industrial Hygienists; Carcinogenesis; Dose–Response Relationship; Epidemiology; Federal Insecticide, Fungicide, and Rodenticide Act, US; International Agency for Research on Cancer; Levels of Effect in Toxicological Assessment; National Institute for Occupational Safety and Health; National Toxicology Program; Occupational Safety and Health Act, US; Occupational Safety and Health Administration; Risk Assessment, Human Health; Toxic Substances Control Act, US; Toxicity Testing, Carcinogenesis.

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http://www.osha.gov – Occupational Safety and Health Administration.
http://www.acgih.org – American Conference of Governmental Industrial Hygienists.
http://www.cdc.gov – National Institute for Occupational Safety and Health.
http://www.iarc.fr – International Agency for Research on Cancer.
http://ntp-server.niehs.nih.gov – National Toxicology Program.

Carcinogen–DNA Adduct Formation and DNA Repair

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Definition

Carcinogen–DNA adducts are addition products formed by covalent binding of all or part of a carcinogen molecule to chemical moieties in DNA; adducts are formed when an activated chemical species (electrophilic, positively charged metabolite) binds covalently to negatively charged moieties in DNA.

Importance of DNA Adduct Formation in the Process of Carcinogenesis

Carcinogen–DNA adducts of exogenous genotoxic chemical carcinogens may induce errors in DNA sequence (mutations). Subsequent transcription on a damaged template may result in the formation of abnormal proteins or the absence of protein. DNA adduct formation and mutagenesis are considered to bring about changes in gene expression that produce clonal expansions of cells lacking in growth control (tumors). A substantial period of time is required for a tumor to become evident, and DNA damage is considered to be necessary but not sufficient for tumorigenesis, since other events must also take place. DNA adduct levels, measured at any point in time, reflect tissue-specific rates of damage processing that include DNA adduct formation and removal (DNA repair), DNA adduct instability, tissue turnover and other events. In experimental model systems dose–response associations have been observed for DNA adduct formation, mutagenesis, and tumorigenesis. Reductions in tumor incidences have been observed when DNA adduct levels have been lowered, either by DNA repair processes or by administration of chemopreventive agents that inhibit DNA adduct formation with no change in dose.

Biotransformation of Carcinogenic Chemicals to Species that Modify DNA

Exogenous carcinogenic chemicals that form DNA adducts can be direct acting if they are highly reactive. Examples are the nitrosoureas, some nitrosamines, ethylene oxide, and ozone. However, most are inert like the polycyclic aromatic hydrocarbons (PAHs) and require biotransformation (metabolic activation). Biotransformation consists of metabolic alteration by families of enzymes that convert a small fraction of the initial dose to highly reactive intermediate metabolites able to 'modify' (become bound to) DNA, thus accomplishing the first essential step (initiation) in the carcinogenic process. Exogenous carcinogens that require metabolic activation include some plant and fungal products (aflatoxins, ochratoxins, hydrazines), pyrolysis products from cooking (heterocyclic amines, PAHs), industrial combustion products (aromatic amines, PAHs, nitro-PAHs, benzene, vinyl chloride, nitrosamines, ethylene oxide), urban pollution contaminants (PAHs, nitro-PAHs, aromatic amines) and components of tobacco (tobacco-specific nitrosamines) and tobacco smoke (PAHs, nitrosamines, and aromatic amines). The metabolic processes that lead to DNA adduct formation for several classes of genotoxic chemical carcinogens, including the PAHs, the aromatic amines, the heterocyclic amines, some fungal products and oxyradical damage, are described briefly.

The PAHs, which include the human carcinogen benzo[*a*]pyrene (BP) (**Figure 1**), are composed of variable numbers of fused benzene rings and are chemically unreactive, as well as insoluble in water. These compounds are ubiquitous environmental contaminants found in cigarette smoke and products of partial combustion, and are produced by many industrial processes. They are metabolized to simple epoxides by cytochrome P-450, hydrated through the action of epoxide hydrolase and subjected again to epoxidation (cytochrome P-450) to form unstable dihydrodiol-epoxides. The unstable metabolites spontaneously convert to positively charged, highly reactive free radicals (carbocations, the ultimate

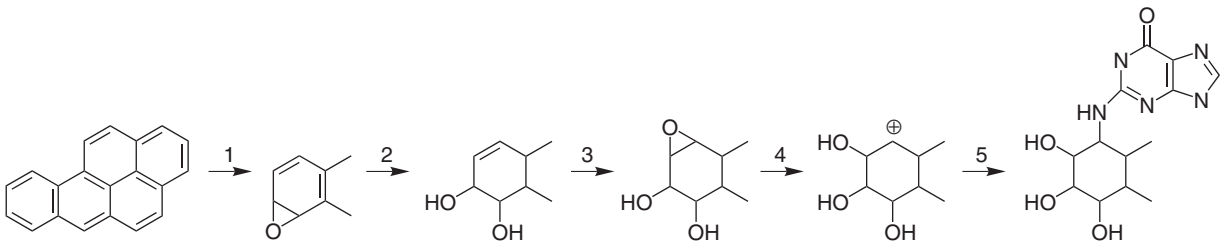

Figure 1 Metabolic activation of benzo[*a*]pyrene (a representative PAH). The parent hydrocarbons are chemically inert and require metabolic activation before they can exert their biological effects. Cytochrome P-450 enzymes (principally CYP1A1) catalyze the formation of simple arene oxides from the parent hydrocarbons (1). The arene oxides are converted to dihydrodiols by the action of epoxide hydrolase (2). The resulting dihydrodiols are further oxidized by cytochrome P-450 enzymes (principally CYP3A4) at the site of the olefinic double bond (3). Vicinal diol-epoxides are high unstable and the arene-ring opening is spontaneous yielding a highly reactive carbocation (4). The electrophilic carbocationic species can form a covalent bond with the exocyclic amino group of deoxyguanosine (5). The resulting polycyclic aromatic hydrocarbon–DNA adduct lies in the minor groove of the double helix.

carcinogenic forms), which bind covalently to DNA and protein. The metabolic scheme for BP is shown in **Figure 1** and the structure of the major DNA adduct, between BP and deoxyguanosine is shown in **Figure 2a**.

Aromatic amines are characterized by the presence of benzene rings and an exocyclic nitrogen. A prototypical aromatic amine, 4-aminobiphenyl (4-ABP), found in tobacco smoke and industrial exhaust, has been implicated in human bladder cancer. The presence of the amino group, that can be either acetylated or nonacetylated, contributes to the complexity of aromatic amine metabolism. Activation of aromatic amines proceeds by *N*-oxidation with sulfotransferase catalysis, resulting in the formation of acetylated (**Figure 2b**), and nonacetylated (**Figure 2c**), guanine adducts. **Figure 2b** and **c** shows guanine adducts of the carcinogen *N*-2-acetylaminofluorene.

Heterocyclic amines, for example, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), are formed from the pyrolysis ($>150^{\circ}C$) of amino acids, creatinine, and glucose that occurs during cooking of meat and fish. They are known as food mutagens and their metabolism, largely influenced by the amine moiety, is similar to that of the aromatic amines. They undergo cytochrome P-450-induced *N*-hydroxylation (CYP1A2). *N*-hydroxylation metabolites of some heterocyclic amines (2-amino-3-methyl-imidazole-[4,5-*f*]quinoline; IQ) can react directly with DNA, while others require further enzymic *O*-esterification. The major guanine adduct of PhIP is shown in **Figure 2d**.

Fungal mycotoxins, including aflatoxin B_1 derived from *Aspergillus flavus*, contaminate cereals, grains, and nuts, and aflatoxin B_1 ingestion is correlated with a high incidence of liver cancer in animal models and humans. Aflatoxins are heterocyclic and contain several endocyclic oxygen molecules. They are activated by simple epoxidation (cytochrome P-450) across the olefinic double bond at the 8,9-position, giving rise to a carbocation. However, some addition products with DNA are unstable and lead to nonmutagenic depurination. The major aflatoxin-guanine adduct is shown in **Figure 2e**.

Oxyradicals (reactive oxygen species), formed as a result of endogenous processes or exposure to exogenous chemicals, can cause oxidation of DNA. Two common examples of oxyradical damage found in DNA include thymine glycol and 8-hydroxydeoxyguanosine adducts (**Figure 2h**). Probably the most common endogenous oxyradical exposure is to $O_2^{\bullet -}$ (superoxide anion) and H_2O_2 (hydrogen peroxide). This occurs when O_2 is reduced for the production of energy, and although most of the electrons are contained, there is some leakage. Other endogenous sources of oxyradicals include reactions of $O_2^{\bullet -}$ with Fe^{3+} or NO to form unstable intermediates (e.g., ONOOH) that are powerful, direct-acting oxidants or that yield hydroxyl radical. The mechanism involving NO is also the basis for inflammation, and can represent a normal response to infection. Exposure to organic peroxides, catechol, hydroxyquinone and 4-nitroquinoline-*N*-oxide among others, leads to oxyradical damage. Moreover, cells can be stimulated to produce peroxisomes by treatment with certain drugs and plasticizers. The role of oxyradical DNA damage in chemical carcinogenesis is currently unclear, although the mutagenic potential of these adducts has been amply demonstrated in experimental systems.

Measurement of Carcinogen–DNA Adducts as Human Exposure Dosimeters

The promise of human DNA adduct biomonitoring is the application of a human biomarker that is directly correlated with cancer risk for cancer prevention or

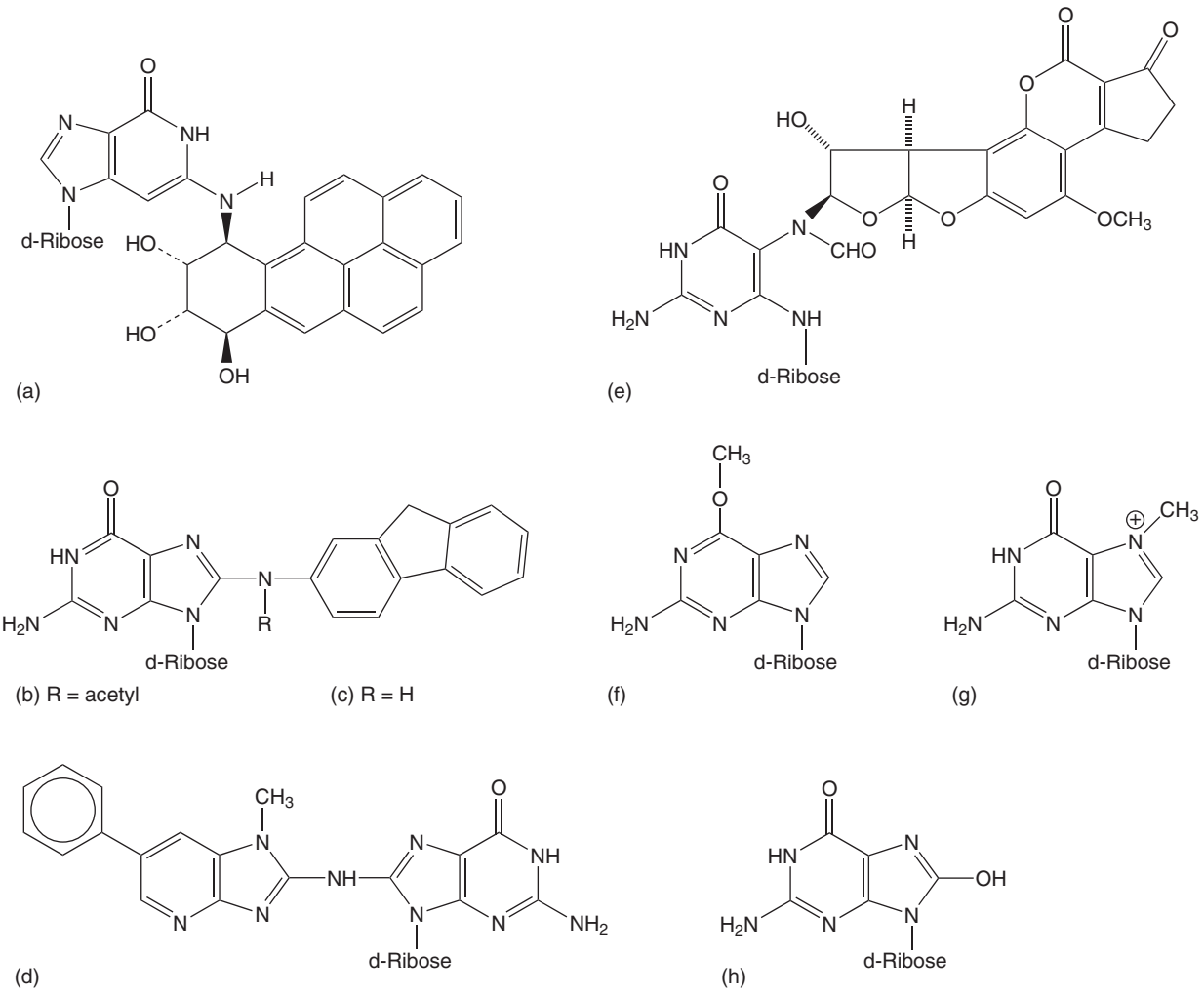

Figure 2 Molecular structures of carcinogen adducts of deoxyguanosine: (a) (7R)-N^2-(10-{7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene}-yl)-deoxyguanosine, formed when benzo[*a*]pyrene-7,8-diol 9,10-epoxide reacts with the exocyclic amino group of deoxyguanosine; (b) *N*-(deoxyguanosin-8-yl)-2-(acetylamino)fluorene, formed when *N*-hydroxylacetylaminofluorene reacts with the C8 position of the imidazole ring; (c) *N*-(deoxyguanosin-8-yl)-2-(amino)fluorene, formed when *N*-hydroxylaminofluorene reacts with the C8 position of the pyrimidine ring structure; (d) *N*-(deoxyguanosin-8-yl)-2-amino-1-methyl-6-phenylimidazo-[4,5*b*]-pyridine, formed when the *N*-hydroxylamine metabolite of 2-amino-1-methyl-6-phenylimidazo-[4,5*b*]-pyridine (PHIP), a glutamic acid pyrolysate, reacts with deoxyguanosine; (e) ring-opened form of *N*-(deoxyguanosin-7-yl)-9-hydroxyaflatoxin B_1, formed following the reaction of the 8,9-epoxide metabolite of aflatoxin B_1 at the N7 position of deoxyguanosine; (f) O^6-methyldeoxyguanosine, formed when an alkyl radical (CH_3^+), derived from an alkylating agent, reacts at the O^6-position of deoxyguanosine; (g) N7-methyldeoxyguanosine, formed when an alkyl radical (CH_3^+), derived from an alkylating agent, reacts at the N7-position of deoxyguanosine; (h) 8-hydroxydeoxyguanosine, formed through exogenous or endogenous oxy-radical damage (H_2O_2, $^{\bullet}OH$, $O_2^{\bullet -}$) at the C8 position of deoxyguanosine.

intervention. This field has expanded exponentially since the 1980s, a progress made possible by the development of highly sensitive methods for the detection of DNA adducts in human tissue. The most widely used methods include immunoassays and immunohistochemistry, ^{32}P-postlabeling, fluorescence and phosphorescence spectroscopy, and gas chromatography/mass spectrometry. Detection limits for quantitative assays are typically in the range of 1 adduct in 10^9 nucleotides. However, accelerator mass spectrometry, a highly sophisticated but less accessible method, has a detection limit of $\sim$1 adduct in 10^{12} nucleotides.

When used without preparative procedures, the most commonly used techniques are typically unable to provide quantitation of individual adducts and chemical characterization of a specific adduct. This is because humans are exposed to complex mixtures of chemical carcinogens, and human DNA will contain multiple DNA adducts induced by different xenobiotic agents. The development of preparative strategies for sample purification that can be applied

prior to the ultimate DNA adduct quantitation has made possible chemical characterization of specific DNA adducts in human tissues. The combination of preparative methods (immunoaffinity chromatography, high-performance liquid chromatography or other chromatography) with immunoassays, ^{32}P-postlabeling or synchronous fluorescence spectrometry has made possible the identification of specific DNA adduct structures. In addition, chemical derivatization approaches have facilitated the various novel permutations of gas chromatography/mass spectrometry that have become widely applied for the determination of specific human DNA adducts.

The majority of studies designed to monitor DNA adducts in human tissues fall into the category of exposure documentation and have concentrated on environmental and occupational exposures to agents for which precise dosimetry is difficult or impossible. Many studies have shown decreases in DNA adduct levels (qualitative dosimetry) in groups of subjects removed from exposure by virtue of location or season. Quantitative dosimetry for human DNA adduct formation has been established with medicinal (cisplatin, procarbazine, dacarbazine, and 8-methoxypsoralen) and dietary (aflatoxins) exposures where dosimetry can be established accurately. A major goal of carcinogen dosimetry is the application of human DNA adduct formation data within epidemiologic study designs to predict human cancer risk. This goal has been achieved in two prospective nested case–control studies and several case–control studies. In the prospective studies which involved lung cancers in smokers and liver cancers in individuals exposed to aflatoxins, relative risks for cancer were increased three- to sevenfold in individuals with elevated DNA adduct levels. In the case–control studies elevated DNA adduct levels (odds ratios 2.3–16.2) were found in the cases compared to the controls. Whereas the epidemiologic studies investigating the relationship between human DNA adduct levels and cancer risk will take many years, these early studies appear to support the data from experimental models that has shown that DNA adduct formation is necessary but not sufficient for tumorigenesis.

Biological Repair of Adduct Damage in DNA (DNA Repair)

Toxicological damage to DNA can alter its chemical structure in many different ways. Covalent addition products may be formed with activated, bulky aromatic compounds or smaller alkyl-species (**Figure 2f** and **g**). Oxyradical formation, often the by-product of normal metabolic processes, dimerization and deamination also modifies the chemical structure of DNA, and single- and double-strand breaks (DSBs) can occur. All of these types of DNA damage may lead to permanent changes in DNA sequence, and some have been associated with the development of disease (e.g., cancer, progeria, Cockayne's syndrome, retinal dystrophy, thalassemia, xeroderma pigmentosum, and birth defects) and normal aging.

A series of metabolic pathways has evolved to counteract DNA damage through removal of the lesions. Mechanisms of DNA repair are complex, generally requiring the products of several genes to act in concert to accomplish restoration of DNA structure. Cell cycle restriction point genes are responsible for conducting the whole DNA repair process. These complexes usually comprise a damage sensor, a damage eliminator, a polymerase or patch synthesizer, and a ligase. However, more than 150 genes are known to participate in DNA repair and some contribute to more than one pathway. For example, genes from multiple pathways are assembled in the BRCA-1-associated genome surveillance complex that constitutes a sensory apparatus for detection and binding to damaged DNA.

There are six general mechanisms of DNA repair: direct repair (DR), nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination repair (HRR), and nonhomologous end joining (NHEJ).

Direct DNA Repair

In contrast to the general scheme outlined above, DR employs only a single suicide enzyme. An alkyltransferase commutes an alkyl group from an alkylated base (O^6-methyldeoxyguanosine) to a cysteine residue in its own active site. Because there is no strand scission, there is also no need for patch synthesis or ligation. In this suicidal process one adduct consumes one molecule of enzyme.

Nucleotide Excision Repair and Base Excision Repair

Both of these processes require multiple enzymes acting either sequentially or, as indicated above, as molecular complexes. The hallmarks of both NER and BER are strand scission, removal of a segment of DNA containing the adducted base, 5′ to 3′-oriented DNA patch synthesis through the action of a polymerase, using the intact strand as a template, and ligation of the free ends. The distinctions between these two mechanisms are the proteins involved and the types of adducts that are repaired. Bulky adducts, like those of aromatic compounds or

malondialdehydes (the result of lipid metabolism), cause DNA distortions that are relatively large and are repaired by NER. In NER the structural distortion is recognized by products of sensory genes (e.g., *XPA*, *XPC*, or *XPE*) and excised by endonucleases (e.g., XPF, XPG, or FEN), the patch is synthesized by a polymerase (polΔ or polε) and ligated (DNA ligase I or DNA ligase III). Further, there are two types of NER, global genomic repair (GGR) and transcription-coupled repair (TCR), which are characterized by different sets of genes. The repair patch generated for TCR is limited to the transcribed strand of transcriptionally active genes and involves multiple (15–30) nucleotides. The repair patch for GGR is limited to a single nucleotide, but GGR can occur on either strand in both transcriptionally active and inactive regions of the genome.

Small adducts, like 3-methyladeneine, 5-hydroxuracil and 5-hydroxymethlyuracil, are repaired by BER. In BER a lesion is detected and removed by a glycosylase (e.g., hOgg1 or UDG) creating an apurinic site (AP). Subsequently an endonuclease degrades the damaged strand (e.g., APEI, FEN1) a patch is synthesized by a polymerase (polβ) and ligation occurs (DNA ligase I or DNA ligase III). The patch size in BER can either be short (one nucleotide) or long (two to 10 nucleotides). Short BER is polβ-dependent and ligation is accomplished by DNA ligase III, whereas long BER is associated with proliferating cell nuclear antigen (PCNA) and ligation is accomplished by DNA ligase I. Oxidative damage may be repaired by either NER or BER.

Mismatch Repair

Nucleotide mismatches occur when DNA repair processes insert an inappropriate but unmodified, conventional base opposite a noncomplementary partner. These may be transitions (purine to purine or pyrimidine to pyrimidine: G–T or A–C) or transversions (purine to pyrimidine or pyrimidine to purine: C–C, T–T, C–T; A–A, G–G, A–G). For example, in post-replication 'repair', a DNA damage tolerance mechanism that leaves a gap in response to replication on a damaged template, the polymerase always inserts an adenine in the gap. In addition, deamination of cytosine results in thymidine. Both NER and MMR feature degradation of a relatively large portion of the damaged strand, followed by 5′ to 3′ patch synthesis using the undamaged strand as a template, and ligation to complete the repair. The DNA mismatch is recognized by a repair protein complex (either MSH1–MSH2–MSH6–PMS1 or MSH1–MSH2–MSH6–PMS2) that simultaneously anchors to both the mismatch and the closest unmethylated adenine in the GATC recognition sequence. The entire sequence between the mismatch and the GATC recognition sequence is eroded, and PCNA is recruited to act as a sliding clamp and support the action of a DNA polymerase (polΔ or polε) in replication of the repair patch. A ligase (DNA ligase I) subsequently complexes with polymerase (polΔ) to complete the repair function.

Mismatch DNA repair is critical in the maintenance of a stable genome. Inheritance of mutations in genes involved in mismatch DNA repair (*MSH1, MSH2, MSH6, PMS1, PMS2*) can predispose to cancers of the brain, endometrium, ovaries, and bowel. Somatic mutations in these genes may also contribute to the mutator phenotype.

Homologous Recombination Repair

Double-strand breaks can be caused by ionizing radiation, oxidative stress and mechanical stress (e.g., when a topoisomerase encounters a bulky adduct during DNA replication). There are two distinct mechanisms for repairing DSBs, HRR and NHEJ. Several sensors of HRR that trigger the process include ATM, RAD3-related ATM, and DNA-protein kinases (Chk2). Homologous recombination involves a number of DNA repair proteins (RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54, XRCC2, XRCC3, BRCA1, and BRCA2). First, a nibrin (NBS1) complex with RAD50/MRE11 (also known as MRN complex) brings about simultaneous recision of both strands, which is thought to be 5′ to 3′. RAD51 and its paralogs (accessory proteins – RAD51B, C, D) prepare the single-stranded DNA segments for sister chromatid exchange and invasion of the homologous duplex. Polymerization occurs using the undamaged homologous duplex DNA sequences as a template. The process is completed by either through resolution of Holliday junctions by the action of an endonuclease and strand sealing by a ligase, or disengagement of the Holliday junctions, DNA pairing and gap filling in the damaged homolog. The first scenario would give an equal opportunity for crossover events and noncrossover events to occur. However, there is now evidence to suggest that HRR is more often accomplished without a crossover event, a mechanism that is more conservative in reducing genomic alterations.

Nonhomologous End-Joining

The other major repair pathway involved in DNA DSB repair is NHEJ. While this pathway brings about DSB repair, it is also involved in immunological diversification by re-ligating the products of recombinase (RAG1 and RAG2) cleavage. Unlike

HRR, NHEJ is independent of a genetic DNA sequence homolog because repair occurs without copying an undamaged template. Components of the NHEJ pathway include XRCC4, ligase IV, KU70, and DNA protein kinase. In addition, if ligation of two blunt ends cannot restore the original sequence, a deletion mutation will result. Therefore, this mechanism is sometimes referred to as 'illegitimate'. In the presence of single-stranded over-hanging segments, some degradation may occur to create a blunt end before ligation occurs (ligase IV). Alternatively, recruitment of polμ or polλ by the XRCC4-ligase IV complex may result in gap filling.

See also: Cytochrome P-450; Polycyclic Aromatic Amines; Polycyclic Aromatic Hydrocarbons (PAHs).

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Carcinogenesis

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Overview

Cancer, or neoplasia, which occurs in one of every four individuals and results in the death of one of every five individuals in the United States, is a complex disease with multiple causes. Many intrinsic and extrinsic factors influence the development of cancer. Intrinsic or host factors include age, sex, genetic constitution, immune system function, metabolism, hormone levels, and nutritional status. Extrinsic factors include substances eaten, drunk, or smoked; workplace and environmental (air, water, and soil) exposures; natural and medical radiation exposure; sexual behavior; and elements of lifestyle such as social and cultural environment, personal behavior, and habits. Intrinsic and extrinsic factors can interact with one another to influence the development of cancer. Because of the physical and emotional suffering associated with cancer and the immense cost to the nation in lost production and income and medical and research expenditures, considerable effort continues to be exerted to understand this complex disease so that strategies can be developed to decrease or prevent its occurrence. Current regulatory guidelines have been crafted to reduce the probability of developing cancer by lowering human exposure to agents identified as potentially capable of causing cancer.

During the past 40 years of cancer research, much information has been generated indicating that

cancer is a multistep, progressive disease. Support for this contention is derived from research on epidemiology and population genetics, morphological and clinical study of neoplasms, as well as experimental investigations in animals. Structural studies of biopsy and autopsy tissue samples from humans and animals, particularly experimental animal models of carcinogenesis, have provided important information about this multistep process at the phenotypic level. More recently, molecular biological analyses have confirmed the principles that neoplasms arise from the clonal expansion of a single cell and that during its evolution into a neoplastic mass, it accumulates nonlethal genetic damage, particularly in genes that regulate growth and DNA repair processes. The process of carcinogenesis may take months in experimental laboratory animals and years in humans. Identification of this process early in its evolution enhances the likelihood that intervention strategies such as surgical removal of a benign neoplasm may result in termination of the disease and clinical cure. By the time a neoplasm has progressed to the malignant stage and spread throughout the body, even heroic radiation and chemotherapy combined with surgery are unlikely to result in clinical cure. The process of carcinogenesis may be depicted schematically as in **Figure 1** with the various steps along the pathway from normalcy to malignancy characterized by morphological and/or clinical features. It is here that the disciplines of clinical oncology, molecular biology and pathology are utilized to define the location of the specific neoplasm in this progressive cascade.

Nomenclature of Cancer (Neoplasia)

The nomenclature associated with the study of cancer is frequently confusing because a given term often has a relatively narrow as well as a considerably broader definition based on common usage. Carcinogenesis, for example, is narrowly defined as the production of carcinoma but is more commonly used in the broadest possible sense to indicate generation of neoplasms which are new and typically abnormal growths, generally uncontrolled and becoming progressively more serious with time. Neoplasia, meaning 'new growth' and often used synonymously with carcinogenesis, refers to the process of development of neoplasms. Two important terms which relate to the clinical behavior and growth characteristics of neoplasms are (1) benign and (2) malignant, characteristic features of which are listed in **Table 1**. Basically, benign neoplasms are slow-growing, localized growths frequently amenable to surgical removal with a low probability of recurrence. Malignant neoplasms have a more aggressive growth, are locally invasive, sometimes metastasize (spread to distant sites), and are difficult to remove surgically.

Two terms that have both a narrow and a broad definition are (1) tumor and (2) cancer. Tumor

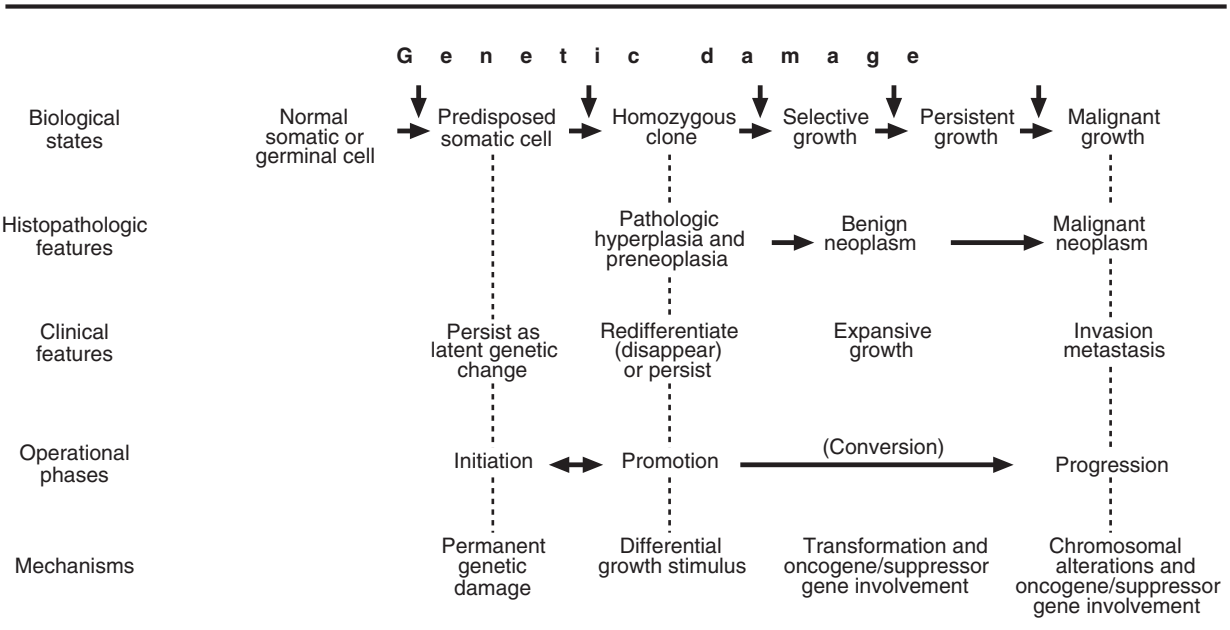

Figure 1 Process of carcinogenesis depicted schematically showing the postulate pathway in which accumulation of genetic damage leads to malignant neoplasia.

Table 1 Comparative features of benign and malignant neoplasms

Effect	*Benign*	*Malignant*
General effect on host	Little; not generally lethal	Will usually kill the host if not treated
Injury to host	Usually negligible but may compress or obstruct vital tissue	Can kill the host by destruction of vital tissue
Growth rate	Slow	Rapid (but slower than tissue repair); growth escapes normal control mechanisms
Extent of growth	Encapsulated; remains localized at site of origin	Infiltrates or invades and spreads to distant sites
Mode of growth	Typically grows by expansion and displaces surrounding tissues	Invades and destroys surrounding tissues
Microscopic features	Cells and structures formed by cells resemble normal tissues; may be encapsulated	Anaplastic, dysplastic, and pleomorphic; may be associated with hemorrhage, necrosis, and inflammation
Cytologic features	Mitoses rare; nucleus normal in staining and shape; nucleolus not conspicuous	Mitoses may be numerous and abnormal; nucleus often enlarged, irregular in shape, and hyperchromatic; nucleolus hyperchromatic and enlarged
Radiation sensitivity	Radiation sensitivity similar to that of normal tissues; rarely treated with radiation	Radiation sensitivity increased in approximate proportion to the degree of malignancy; frequently treated with radiation

broadly refers to any tissue enlargement or swelling, however it is often used synonymously with the term neoplasm. A cancer generally refers to a malignant neoplasm. Unfortunately, the layperson and the professional frequently use tumor and cancer interchangeably alike without qualifying whether it is a benign or malignant process. In other words, if it is said that an individual has a tumor, that individual may have a benign neoplasm (most often the case) but could have a malignant neoplasm if the term 'tumor' is being used loosely. If an individual is said to have a cancer, that usually means the individual has a malignant neoplasm but, here again, loose use of the term 'cancer' might include any neoplasm, including a benign one. Scientists contribute to the confusion by sometimes indicating that an agent may cause cancer, meaning either benign or malignant neoplasia. Alternatively, they may indicate that an agent is tumorigenic, which could mean that it causes tumors but frequently means that it may also cause malignant neoplasms (cancers). Common and uncritical usage of these terms is so ingrained that attempts to standardize nomenclature have been largely unsuccessful. The least ambiguous terms are 'benign neoplasm' and 'malignant neoplasm'.

Most neoplasms are classified and named based on (1) the cell or tissue of origin and (2) benign or malignant growth characteristics. There are two basic cell types from which neoplasms may originate: mesenchymal cells and epithelial cells (**Figure 2**). Mesenchymal pertains to mesenchyma (embryonic connective tissue in the mesoderm) from which adult tissues such as connective tissue, blood and lymphatic vessels, and muscles and bones are formed. Epithelial cells line the internal and external surfaces of the body and form many of the major organs such as liver and lungs. Most epithelial tissues are derived from the embryonic germ layers referred to as entoderm and ectoderm.

There are general guidelines used in naming neoplasms. A benign epithelial neoplasm originating within a glandular tissue is called an 'adenoma', having the prefix 'adeno' to designate that the origin is one of many glandular tissues and the suffix 'oma' to indicate a swelling or tissue enlargement. One or more qualifiers may be added to the name to indicate the tissue of origin and various morphological features as in hepatocellular (liver cell) adenoma, thyroid follicular (forming follicles) adenoma, or renal (kidney) tubular cell adenoma. An adenoma with morphological features resembling finger-like or warty projections would be called a papillary adenoma; with cystic spaces, a cystadenoma; with both of these features, a papillary cystadenoma. Benign mesenchymal neoplasms also utilize the 'oma' suffix in their name, as in meningioma, hemangioma, and fibroma. The prefix for mesenchymal neoplasms usually identifies the specific tissue of origin such as meninges (meningioma), blood vessels (hemangioma), or fibrous connective tissue (fibroma). Nomenclature for several benign neoplasms is presented in **Table 2**.

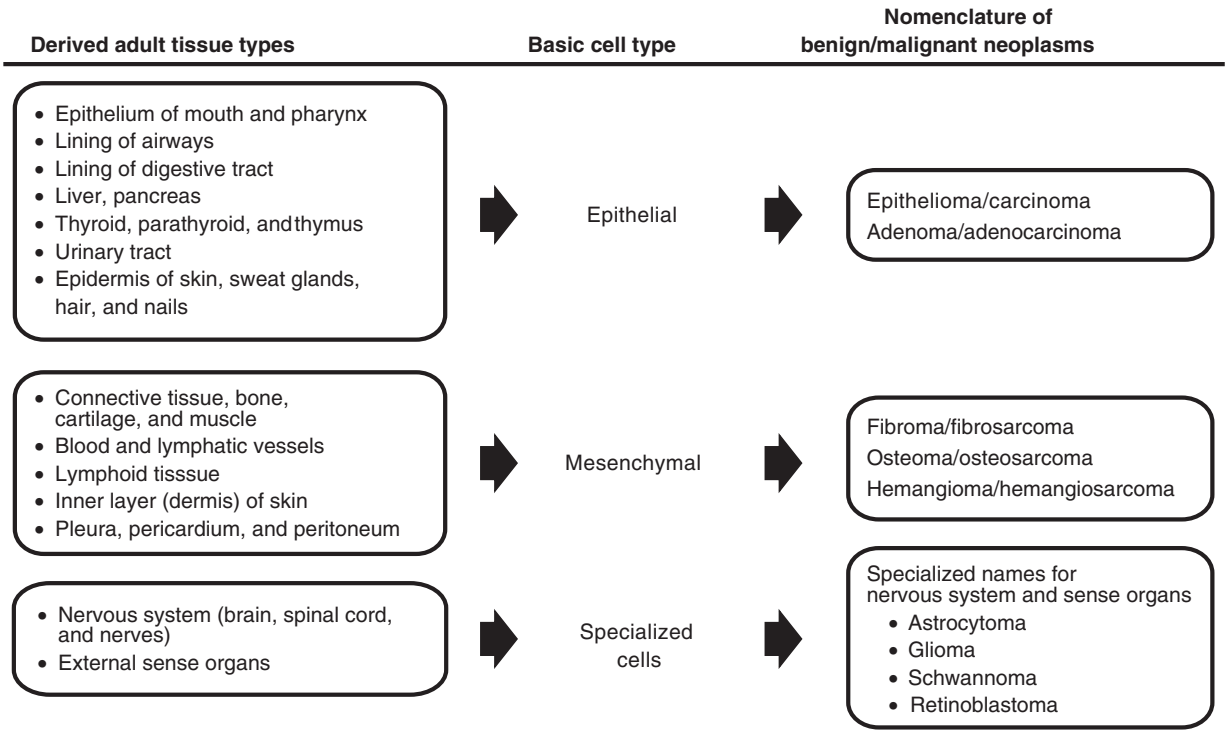

Figure 2 Tissue types associated with neoplasm names.

Malignant epithelial neoplasms are typically called 'carcinomas' and qualified by histogenetic origin. Thus, malignant skin neoplasms are called epidermal carcinomas if they arise in the superficial layers or epidermis of the skin. If they are composed predominantly of squamous cells, they are called squamous cell carcinomas; if chiefly basal cells, basal cell carcinomas. Malignant mesenchymal neoplasms are called 'sarcomas'. Examples of the latter include fibrosarcoma, a malignant neoplasm of the connective tissue; osteosarcoma, a malignant bone neoplasm; and leiomyosarcoma, a malignant neoplasm of the smooth muscle tissue. The nomenclature for several malignant neoplasms is presented in **Table 2**.

Much of the general confusion surrounding the nomenclature of neoplasms results from numerous exceptions and permutations in the general histogenetic and clinical guidelines for naming neoplasms. Many of these exceptions are deeply ingrained in traditional pathology practice, and attempts at standardization have been largely unsuccessful. Examples are thymoma, lymphoma, melanoma, and neuroblastoma – neoplasms which are generally regarded as malignant despite their benign-sounding names and should more properly be called malignant thymoma or thymic sarcoma, malignant lymphoma or lymphosarcoma, malignant melanoma or melanosarcoma, and malignant neuroblastoma, respectively. Other neoplasms are named for their physical attributes such as pheochromocytoma (dark-colored neoplasms typically arising in the adrenal medulla). In addition, some neoplasms were originally named for the person first describing the lesion, and examples such as Hodgkin's disease of lymphoid tissue and Wilms' kidney tumor have persisted to this day. Neoplasms composed of mixtures of cells are named accordingly; examples include fibroadenoma, adenosquamous carcinoma, and carcinosarcoma. To complicate matters further there are several tissue alterations that are not neoplasms but have names suggesting that they are: hamartomas (a disorganized aggregate of normal tissue components thought to represent faulty differentiation during embryonic development) and choristomas (focal collections of normal tissue found at an abnormal site such as islands of pancreatic cells in the wall of the stomach). There are also instances in which a neoplasm is histologically considered malignant but clinically benign, such as in basal cell carcinoma of the skin. In addition, localized overgrowths of normal tissue components such as skin tags and vocal cord polyps are clinically recognized as tumors but are not truly neoplastic.

For brief definitions of various terms associated with carcinogenesis, refer to the glossary at the end of this entry.

Table 2 Selected nomenclature of neoplasia

Tissue	*Benign neoplasia*	*Malignant neoplasia*
Epithelium		
Squamous	Squamous cell papilloma	Squamous cell carcinoma
Transitional	Transitional cell papilloma	Transition cell carcinoma
Glandular		
Liver cell	Hepatocellular adenoma	Hepatocellular carcinoma
Islet cell	Islet cell adenoma	Islet cell adenocarcinoma
Connective tissue		
Adult fibrous	Fibroma	Fibrosarcoma
Embryonic	Myxoma	Myxosarcoma
Cartilage	Chondroma	Chondrosarcoma
Bone	Osteoma	Osteosarcoma
Fat	Lipoma	Liposarcoma
Muscle		
Smooth	Leiomyoma	Leiomyosarcoma
Skeletal	Rhabdomyoma	Rhabdomyosarcoma
Cardiac	Rhabdomyoma	Rhabdomyosarcoma
Endothelium		
Lymph	Lymphangioma	Lymphangiosarcoma
Blood	Hemangioma	Hemangiosarcoma
Lymphoreticular		
Thymus	Not recognized	Thymoma
Lymph nodes	Not recognized	Lymphosarcoma (malignant lymphoma)
Hematopoietic		
Bone marrow	Not recognized	Leukemia
Neural tissue		
Nerve sheath	Neurilemmoma	Neurilemmosarcoma
Astrocytes	Not recognized	Astrocytoma

Note: -oma, swelling; sarc-, malignant neoplasm of mesenchymal origin; carcin-, malignant neoplasm of epithelial origin.

Tissue Changes Associated with Carcinogenesis

Quantitative – Hyperplasia and Preneoplasia

Proliferative lesions, which may be classified morphologically as hyperplasia, preneoplasia, benign neoplasia, or malignant neoplasia, represent a continuum of change with considerable overlap rather than discrete morphologic entities (**Figure 3**). The definitive classification of a given lesion as preneoplasia, benign neoplasia, or malignant neoplasia represents a judgment based on the experience of the diagnostic pathologist and familiarity with the species and tissue in question. These lesions are recognized by their microscopic appearance and effect on surrounding tissues and typically are a localized proliferation or hyperplasia of a specific cell type. Most neoplasms are believed to be derived from the clonal proliferation of a single initiated cell. Usually at some point early in the clonal expansion, the differentially proliferating cells become phenotypically distinguishable from the surrounding normal tissue. Although such lesions may not yet have sufficient characteristics to qualify as neoplasms, their recognition early in the process of carcinogenesis has led many to regard them as 'preneoplastic'.

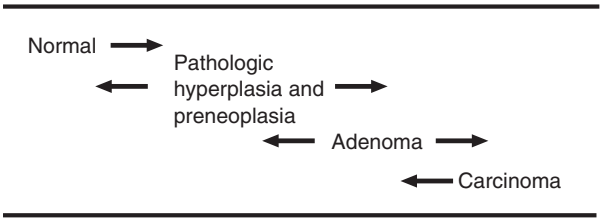

Figure 3 Morphologic continuum of carcinogenesis.

There is considerable confusion regarding the significance of hyperplasia in the neoplastic process. Hyperplasia is an increase in the number of cells per unit of tissue, typically limited in amount and terminating when the stimulus that evoked it is removed. Different cell types have varying capacities to undergo hyperplasia in response to physiological or pathological stimuli. One of the most difficult judgments, even for the experienced pathologist, is whether an observed hyperplasia is part of the process of cancer development or merely an adaptive or physiologic response not likely to progress to neoplasia. The tissue affected, whether the hyperplasia is diffuse or nodular, the age of the affected individual, the proximate cause of the hyperplastic response, and the growth pattern of the hyperplastic tissues, influences this judgment.

Preneoplasia is a form of hyperplasia (an absolute increase in the number of cells in a tissue). Although not all neoplasms exhibit a preneoplastic change recognizable by the pathologist, in those instances in which presumptive alterations are observed, their occurrence documents that there is a response to tissue insult. Examples of presumptive preneoplastic lesions are presented in **Table 3**. In those experimental models of carcinogenesis in which preneoplasia is observed, it precedes the occurrence of benign neoplasia. An important feature of preneoplastic lesions is their propensity for reversibility. In some instances a preneoplastic lesion represents the clonal expansion of a cell that has sustained genetic damage so that benign neoplasms arise within the preneoplastic lesion, presumably when one of the preneoplastic cells sustains additional genetic damage, giving it a growth advantage. In other situations, the antecedent change is a localized polyclonal cellular proliferation historically associated with subsequent development of a neoplasm in the same tissue.

A classical example is alcoholic cirrhosis, which in the case of chronic alcohol abuse, leads to multiple, polyclonal areas of liver cell hyperplasia and an increased risk for development of hepatocellular neoplasia. In both preneoplasia and certain forms of hyperplasia, the antecedent lesions typically have a higher rate of cell proliferation than the surrounding normal cells and, thus, these cells are at increased risk to sustain additional genetic damage and progress to the next stage in the carcinogenic process.

A benign neoplasm is generally a localized expansive growth that compresses adjacent normal tissue but is usually not immediately life threatening unless it physically interferes with normal function, for example, by blocking the intestinal tract or compressing vital areas in the brain. Controversy regarding the significance of benign neoplasia with respect to the development of malignancy is similar to that associated with preneoplastic lesions. A benign neoplasm, the clonal expansion of cells that have sustained some degree of genetic damage, is further along the spectrum of changes that precede the development of malignant neoplasia. In experimental carcinogenesis animal models, malignant neoplasia is not infrequently observed arising from or within a benign neoplasm. Features of benign neoplasms are listed in **Table 1**.

Malignant neoplasms are rapidly growing, locally invasive tissue proliferations that destroy surrounding tissues and are thus life threatening. They also have the malicious feature of spreading to distant sites in the body via the blood and lymphatic system. Although malignancy develops with greater frequency in association with (1) pathologic hyperplasia and preneoplasia, (2) qualitative alterations in cells, and (3) benign neoplasia than in association with normal tissues, these changes are not necessary precursors to malignancy. *In situ* carcinomas are malignant neoplasms that originate without evidence of antecedent benign tissue alteration. When precursor lesions are present prior to or concomitant with malignant neoplasia, it is probable that the malignancy is a consequence of the same or similar factors that produced the precursor lesions. Characteristics of malignant neoplasms are listed in **Table 1**.

Table 3 Examples of presumptive preneoplastic lesions

Tissue	*Presumptive preneoplastic lesion*
Mammary gland	Hyperplastic alveolar nodules
	Atypical epithelial hyperplasia
	Lobular hyperplasia
	Intraductal hyperplasia
	Hyperplastic terminal duct
Liver	Foci of cellular alteration
	Hepatocellular hyperplasia
	Oval cell proliferation
	Cholangiofibrosis
Kidney	Atypical tubular dilation
	Atypical tubular hyperplasia
Skin	Increase in dark basal keratinocytes
	Focal hyperplasia/hyperkeratosis
Pancreas	Foci of acinar cell alteration
	Hyperplastic nodules
	Atypical acinar cell nodules
Colon	Aberrant crypt foci

Qualitative – Metaplasia, Dysplasia, and Anaplasia

In addition to quantitative increases in certain cells, several qualitative cytological features help allow the morphologic classification of the spectrum of proliferative lesions that may be observed in the process of carcinogenesis. Three frequently used qualitative cytological features are metaplasia, dysplasia, and anaplasia.

Metaplasia is the reversible substitution of one type of fully differentiated cell for another within a given tissue. A classic example is the replacement of the normal ciliated columnar epithelial cells in the respiratory tract airways by squamous epithelium (**Figure 4**) in situations in which there is chronic irritation from certain components of inhaled tobacco smoke. While the squamous epithelium is believed to provide functional protection against the irritant properties of the smoke, the loss of the ciliated columnar epithelium results in reduction of the functional capacity of the lungs to clear particulates from the respiratory tract. When the irritation is removed, the squamous epithelium is replaced by normal ciliated columnar epithelium.

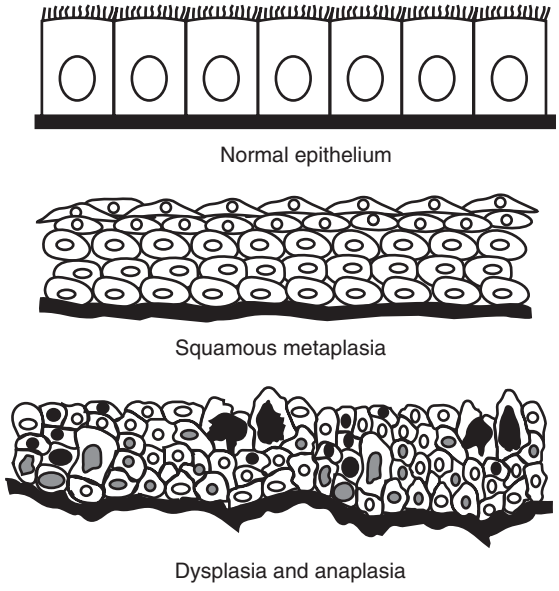

Figure 4 Qualitative changes in epithelial tissues.

Dysplasia is defined as abnormal growth of a tissue with respect to shape, size, and the organization of component cells. Normal cell-to-cell orientations are disorganized or disrupted, and the cells themselves vary in size and shape (**Figure 4**). When present, dysplasia may be associated with chronic irritation, occur with metaplasia, and be seen in neoplastic transformation. It is a change that is a hallmark of increased risk for development of neoplasia. Like metaplasia, dysplasia is a potentially reversible tissue alteration. It is also considered in some circumstances as a preneoplastic change.

Anaplasia is a qualitative alteration of cellular differentiation. Anaplastic cells are typically undifferentiated and may bear little, if any, resemblance to mature cells. This feature is considered a hallmark of malignancy.

Staging and Grading of Cancers

In human oncology the experience from collective years of observation of the outcome of many cancers has strengthened the predicitivity of histological grades and clinical staging in prognostication. The purpose of grading and staging a neoplasm is to predict its biological behavior and to help establish an appropriate therapeutic regimen. Grading is a subjective evaluation of morphologic characteristics based on the extent of cellular anaplasia and the degree of proliferation evident from microscopic evaluation. Generally, neoplasms with a high degree of anaplasia, associated specific morphologic patterns of growth, and evidence of numerous mitoses, some of which may be abnormal, are given a high grade of malignancy. Most grading schemes categorize neoplasms into one of three or four grades of increasing malignancy.

Staging of a cancer, which is independent of grading, is an index of the extent to which a cancer has spread in the body. It also provides information regarding the patient's clinical prognosis, and usually influences the choice of appropriate therapy more than grading. Criteria used for staging neoplasms include the size of the primary neoplasm, the degree to which there is invasion of surrounding normal tissues, whether the cancer has spread to local lymph nodes, and the presence of spread to distant sites in the body. Thus, it is apparent that staging will have a large influence on the therapeutic approach. A small and localized breast cancer would most likely be treated by surgical excision and possibly radiation therapy, whereas a large, infiltrative breast cancer would more likely be treated by mastectomy. If the cancer has spread to lymph nodes or distant sites, more aggressive therapy is implemented.

The ultimate fate of cells or proliferative tissue masses is influenced by the amount of sustained genetic damage. Cells with minimal DNA damage may persist in a latent form, indistinguishable from surrounding normal cells. If such a latent cell sustains additional damage even long after the initial insult, it may then progress further along the pathway to malignancy (see **Figure 1**). As additional genetic damage occurs, the altered cell population expands and eventually leads to irreversible uncontrolled growth that may or may not be corrected by aggressive medical intervention.

Molecular Basis of Cancer

Multistep Genetic Model of Carcinogenesis

Genetically, the multistage process involves the activation of growth-enhancing protooncogenes, inactivation of the recessive growth-inhibitory tumor suppressor genes as well as epigenetic events that alter gene expression and processes such as those involved in cell death, DNA repair, and methylation (**Table 4**). Cancer cells frequently contain mutations in multiple genes as well as large chromosomal abnormalities. Since their discovery $\sim$25 years ago, more than 100 protooncogenes and $\sim$15 tumor suppressor genes have been identified. Protooncogenes were first discovered in cancer-causing animal viruses that carried them. Intense study of these viruses, particularly by Varmus and Bishop in the 1970s, resulted in the discovery that endogenous animal genes had been picked up by virus ancestors and incorporated into the viral genome. Soon thereafter a number of these protooncogenes were identified in both the animal and human genome and later found to play a role in cancer development.

Table 4 Genetic and epigenetic events involved in cancer development

Proto-oncogenes (growth-enhancing)	
Growth factors	PDGF-B, FGF, sis
Growth factor receptors	EGFR, CSF
Signal transduction	*ras*, *abl*
Nuclear regulatory proteins	*myc*, *fos*
Cell cycle regulators	Cyclins and cdks
Tumor suppressor genes (growth-inhibiting)	
Cell surface molecules	TGF-βR
Regulate signal transduction	NF1
DNA repair, cell cycle	p53, Rb, BRCA1
Apoptosis genes	Bcl-2, Bcl-x, Bax, bad, Bcl-xS
DNA repair genes	HNPCC, XP
Epigenetic events	Methylation

A widely accepted multistep model of carcinogenesis proposed by Fearon and Vogelstein in 1990 serves as the framework for studies in carcinogenesis (**Figure 5**). By studying multiple benign and malignant colonic neoplasms from individuals with multiple tumors it was found that benign neoplasms harbored mutations in genes such as APC, *ras*, and p53, and that there were frequently multiple mutations per neoplasm, particularly of the malignant neoplasms. The model describes a progressive acquisition of mutations and it is believed the total accumulation of mutations (at least five to seven) rather than the order is important in the carcinogenic process. New evidence has been published to further refine this model. Recently it has been proposed that some neoplasms are dependent on the continued activation or overexpression of a particular oncogene for maintaining malignant behavior. Others have found that some neoplasms are 'hypersensitive' to the inhibitory effects of specific tumor suppressor genes. These findings suggest that the multistage process of carcinogenesis is not simply a summation of individual effects of cancer genes but that some individual cancer genes can override the others (referred to by some as the 'Achilles heel of cancer') and they offer new strategies for the prevention and therapy of cancer.

Oncogenes

Among the estimated 25 000 genes in the mammalian genome, there are ~100 genes that are classified as oncogenes because activation of these genes appears to be an essential event for the development of many, if not all, cancers. In fact, oncogenes were first discovered by studying genetic alterations in cancers. The term oncogene activation indicates a quantitative or qualitative alteration in the expression or function of the oncogene. The term oncogene is unfortunate since the unaltered (nonactivated) oncogene (usually referred to as a protooncogene) actually serves an essential function in the mammalian genome. That protooncogenes are highly conserved in evolution is evidenced by structurally and functionally similar genes in yeast, earthworms, animals, and humans. The highly conserved nature of protooncogenes is believed to be related to their essential function in normal tissue growth and differentiation. Since their normal function is to control how a tissue grows and develops, it is apparent that, if they do not function appropriately, abnormal growth and development may occur. When a primary manifestation of such abnormal growth was observed to be neoplasia, these protooncogenes were named oncogenes. This nomenclature has persisted despite the ultimate discovery that the unaltered

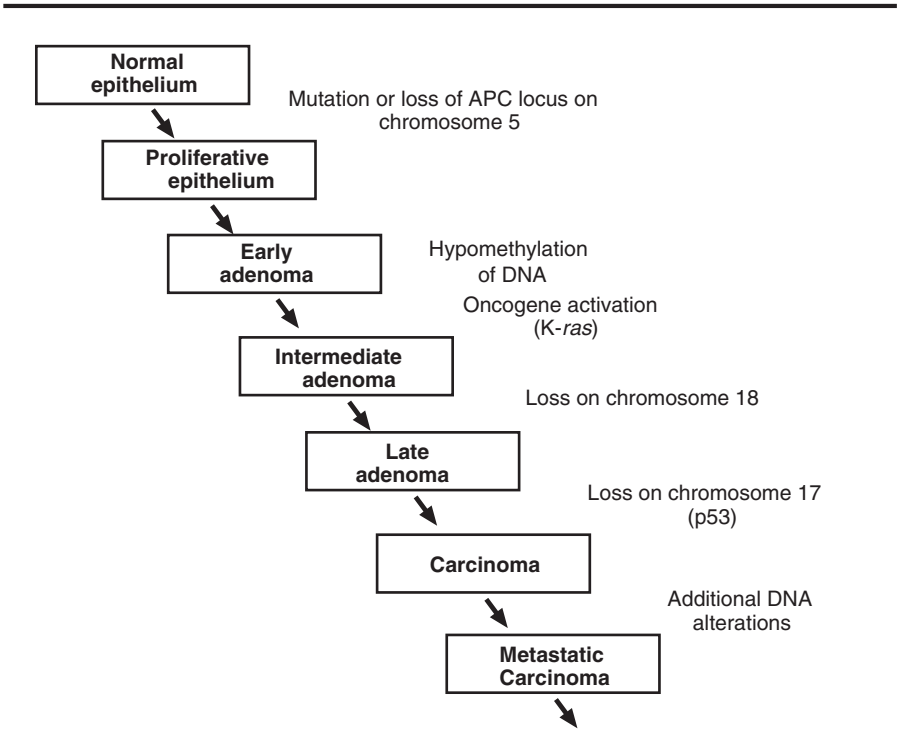

Figure 5 Multistep aspects of human colon carcinogenesis.

forms of these genes are normal components of the genome.

The appearance (phenotype) and function of a tissue is a consequence of which genes are actively producing their programmed product, typically a protein, which in turn affects the structure and function of the cells comprising a given tissue. All somatic cells in the body inherit a complete complement of maternal and paternal genes. The reason that some cells form liver and produce products such as albumin while other cells form kidney tubules that function to excrete substances from the body is a consequence of which genes are expressed in those cells. In liver cells, several critical genes that are important in kidney function are not expressed and vice versa. Specific gene expression and its effect upon tissue phenotype and function are modulated by several intrinsic and extrinsic factors (**Figure 6**). Since a primary function of many oncogenes is to control cell growth, proliferation, and differentiation, inappropriate expression of these genes would be expected to influence abnormally tissue proliferation and growth. Oncogene activation is a consequence of inappropriate or excessive expression of a proto-oncogene.

Oncogenes can be activated by several different mechanisms (e.g., retroviral transduction, chromosomal translocation, gene amplification, point mutation, promoter/enhancer insertion, or decreased methylation of promoters). Once activated an oncogene will either be inappropriately expressed (e.g., production of an altered message and protein) or overexpressed (e.g., production of too much of a normal message and protein). Either situation may contribute to the neoplastic process by influencing cellular proliferation and differentiation. Examples of activated or amplified oncogenes detected in human and animal neoplasms are listed in **Tables 5** and **6**. For some cancers the frequency of oncogene activation is relatively high, while for other cancers the activation of known oncogenes is uncommon. Identification of specific alterations in oncogenes in certain cancers represents a first step in determining the molecular basis of cancer and could eventually lead to the development of molecular intervention and therapeutic strategies. Experimental evidence indicates that oncogene activation can be an early critical event in carcinogenesis, and experimental studies with known chemical carcinogens show that they produce specific alterations in certain oncogenes reflecting the manner in which the carcinogen chemically affects DNA.

Tumor Suppressor Genes

Tumor suppressor genes, originally called anti-oncogenes, function to suppress the development of cancerous growth. While oncogenes must be activated to be effective, tumor suppressor genes must be inactivated or lost for cancer to develop. It has been shown that loss or mutation of both paternal and maternal copies, that is, in both alleles, of a

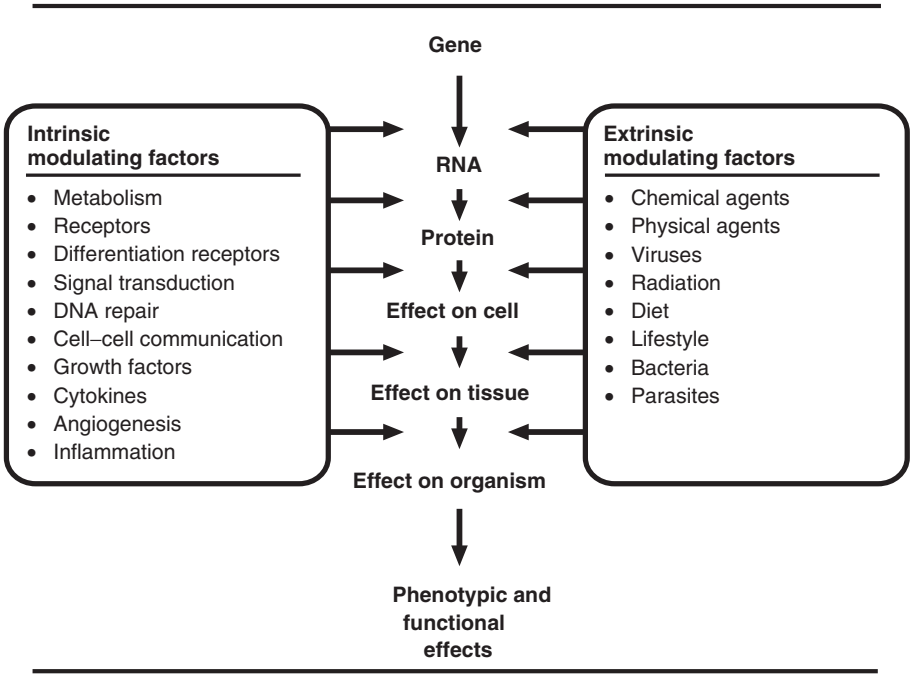

Figure 6 Intrinsic and extrinsic factors modulating specific gene expression and its effect on tissue phenotype and function.

Table 5 Examples of human neoplasms associated with activated or amplified oncogenes

Oncogene	*Type of human neoplasia*
H-RAS	Squamous cell carcinoma
	Urinary bladder carcinoma
	Lung carcinoma
	Acute myelogenous leukemia
K-RAS	Lung adenocarcinoma
	Colon carcinoma
	Ovarian carcinoma
	Gastric carcinoma
	Renal cell carcinoma
	Acute myelogenous leukemia
	Pancreatic ductal adenocarcinoma
N-RAS	Acute myelogenous leukemia
	Chronic myelogenous leukemia
ABL	Chronic myelogenous leukemia
ERBB$_2$	Breast carcinoma
	Salivary gland adenocarcinoma
MYC	Small cell carcinoma of the lung
	Burkitt's lymphoma
N-MYC	Neuroblastoma

Table 6 Examples of animals neoplasms associated with activated oncogenes

Oncogene	*Type of animal neoplasia*
H-ras	Hepatocellular adenoma and carcinoma
	Harderian gland adenoma
	Mammary carcinoma
	Skin squamous cell carcinoma
K-ras	Lung adenoma and adenocarcinoma
	Pancreatic carcinoma
	Hepatocellular carcinoma
N-ras	Leukemia
	Lymphosarcoma
Raf	Fibrosarcoma
neu (erbB$_2$)	Neuroblastoma
Abl	Lymphosarcoma
c-myc	Leukemia
	Lymphosarcoma

tumor suppressor gene must occur to ablate their effect of suppressing cancer formation. A well-known and extensively studied tumor suppressor gene is the retinoblastoma gene (*RB-1*). In hereditary retinoblastoma an affected child is born with deletions of portions of one allele of chromosome 13 containing the *RB-1* gene. If a second event leading to a loss or alteration of the remaining *RB-1* allele occurs while retinal cells are undergoing growth during development, the ocular neoplasm, retinoblastoma, frequently present in both eyes, will occur early in life. Loss or alteration of both copies of this tumor suppressor gene is sufficient to cause retinoblastoma. Although named for the disease in which it was discovered, alterations in the *RB-1* gene have been detected in breast, lung, prostate, and bone cancers.

Acquisition of Mutations

The rate of mutation has been intensely studied in the carcinogenic process. Mutations in cellular DNA can arise during normal cell replication by infidelity in DNA replication (mispairing) as well as by chromosomal deletions, amplifications, or rearrangements. Considering mispairing in nucleotide bases alone, it is estimated that spontaneous mispairing during normal cell replication can occur with a frequency of $\sim 1.4 \times 10^{-10}$ nucleotide bases per cell division. Since there are $\sim 10^{16}$ cell divisions per human lifespan and 2×10^9 nucleotide base pairs per genome, a total of 2.8×10^{15} mispairings could occur in a lifetime $((1.4 \times 10^{-10}) \times (2 \times 10^9) \times 10^{16})$. If each mispair led to a mutation that resulted in a cancer, a typical human would have billions of cancers in one average lifetime. Since such estimates of cancer frequency are clearly in excess of what is observed, it is necessary to postulate that events in addition to a single mutation are necessary for most cancers to occur and that many mispairings are repaired or fatal to the cell. There are efficient mechanisms to repair DNA damage, thereby precluding successive accumulation of critical mutations. Cell proliferation is also critical for 'fixing' DNA damage since, without production of daughter cells from a damaged mother cell, there would be no inheritance of DNA damage. The cell has relatively efficient mechanisms to repair damage provided there is time prior to cell division. If a tissue is proliferating rapidly, cell division could occur before the cell has time to mend damaged DNA. While all of the above underscore the importance of cell proliferation in carcinogenesis, neoplasia does not occur exclusively or necessarily at higher frequency in tissues that have a rapid intrinsic rate of cell proliferation. Consequently, other important mechanistic factors influence the complex process of carcinogenesis.

In 1994, Loeb *et al.* proposed that neoplastic cells likely have a higher mutation rate than normal cells ($\sim 2 \times 10^{-7}$ per gene per cell division) and thereby increase the likelihood of neoplastic cells acquiring further mutations conducive to neoplastic growth features. This is referred to as the 'mutator phenotype' (**Figure 7**). It suggests that early mutation in stability genes (i.e., DNA repair, mismatch repair, DNA replication, or chromosome maintenance) will lead to the mutator phenotype and further mutations contribute to the subsequent invasive and metastatic properties of the neoplastic growth. Others argue that the mutation rate is similar between neoplastic

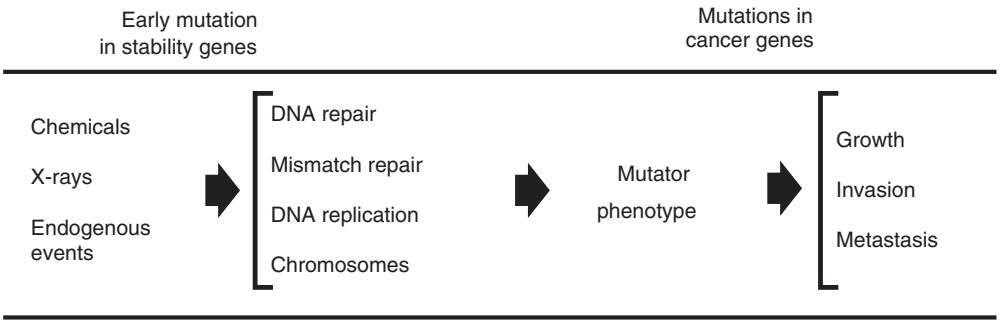

Figure 7 Mutator phenotype model.

and normal cells and that it is the higher rate of cell proliferation in neoplasms that gives them more opportunity to accumulate mutations. The healthy debates continue to feed our quest to prevent and cure the neoplastic process.

Growth Factors, Hormones, and Signal Transduction

While alterations in cellular DNA are critical in carcinogenesis, some cancer-causing agents, particularly those that are not genotoxic, play a major role in cancer development by indirectly influencing gene expression and growth control by altering signal transduction. While the pivotal role of hormones in the orchestration of tissue growth and development has been appreciated for decades, the recent discovery of polypeptide growth factors has added to our knowledge of the complex constellation of control mechanisms that affect normal cellular growth. Both hormones and growth factors bind to specific cellular receptors and thereby trigger a cascade of intracellular reactions that seem to be associated ultimately with cellular proliferation. This cascade of intracellular reactions is sometimes referred to as signal transduction, the process whereby a stimulus external to the cell triggers a cascade of intracellular biochemical reactions that ultimately lead to expression of specific genes. A simplified depiction of the interaction of hormones and growth factors with cellular signal transduction is presented in **Figure 8**. This concept is perhaps best exemplified by the process whereby a normal hormone stimulates a tissue to grow. An example is breast development and milk production in response to the hormone prolactin. In this example, prolactin binds to a specific prolactin receptor on the external surface of the cell, which, in turn, triggers a biochemical change inside the cell membrane via molecules that are attached to the external receptor and pass through the cell membrane. This in turn triggers a long chain of biochemical reactions ultimately resulting in a signal to specific genes in the cellular DNA so that they become active. The specific genes, in this example, initiate a program that causes breast cells to divide and secrete milk. The signal transduction pathways in mammalian cells are highly interactive with numerous positive (signal-sending) and negative (signal-blocking) feedback loops. An appropriate balance between the positive and negative feedback loops is necessary for the proper functional response to the initial stimulus.

Some forms of cancer development are believed to be facilitated by perturbations in one or more places in the signal transduction pathway. Thus, exposure to certain agents may potentially affect the balance of positive and negative feedback loops in the signal transduction pathway and make cells more susceptible to stimuli that promote growth. An example is the nongenotoxic skin tumor promoter, phorbol ester, which activates protein kinase C, a multifunctional element in the signal transduction pathway that mediates many critical cellular regulatory processes. Treatment of initiated mouse skin with phorbol ester activates protein kinase C, resulting in the development of benign and malignant skin neoplasms. The complexity and pivotal importance of the signal transduction pathways help explain why multiple types of agents influence carcinogenesis, why multiple steps are involved in the carcinogenic process, and why different cancers are so heterogeneous. Signal transduction involves shifts in intracellular ion fluxes for elements such as sodium, potassium, and calcium. It also often involves activation of protein kinase C, an enzyme that phosphorylates many proteins that may be important in producing a mitogenic response. Part of the signal transduction cascade involves increased expression of cyclic adenosine monophosphate, now recognized as a mitogenic signal, and increased expression of one or more cellular protooncogenes. Current research results demonstrate that increasing numbers

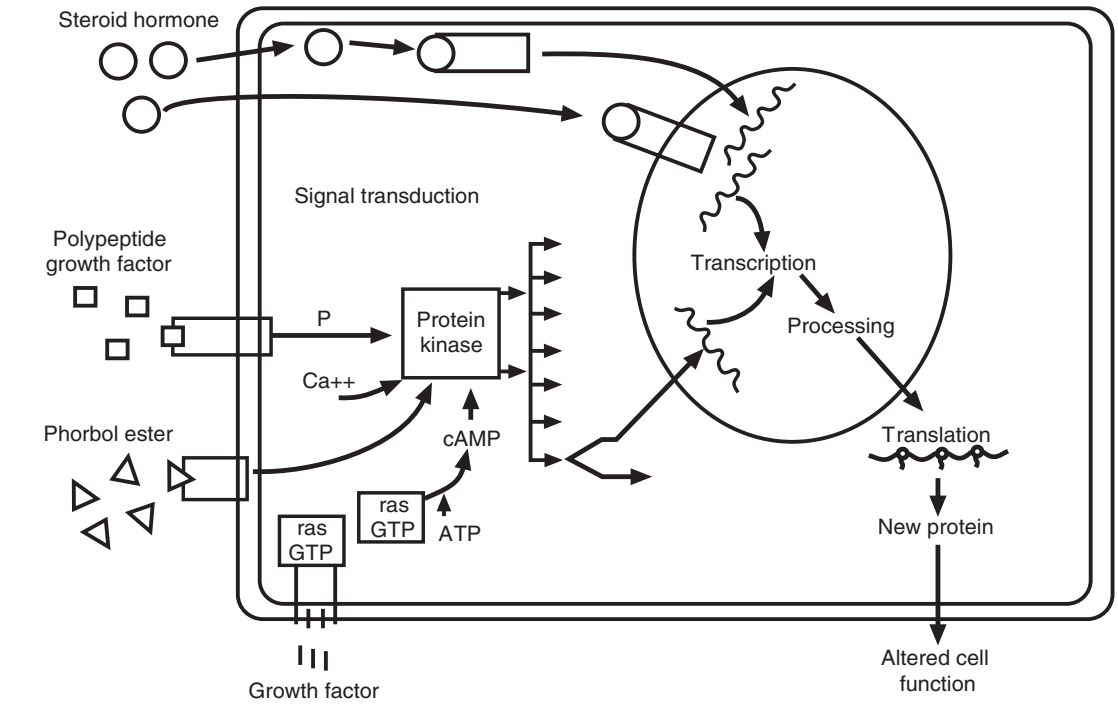

Figure 8 Simplified depiction of the interaction of hormones and growth factors with cellular signal transduction.

of protooncogenes and growth factors are integral parts of the signal transduction pathway and, when altered, influence development of cancer by subverting signal transduction.

Telomeres and Telomerase

Telomerase activation appears to be a critical component of the immortalization process in neoplastic cells and it may provide the basis for new therapeutic targets. Telomeres are specialized structures at the ends of chromosomes and telomerase is the enzyme that maintains the length of the telomeres. During each round of cell division there is a loss of a small number of nucleotides causing progressive erosion of genetic material at the end of each chromosome. As the normal cell divides the telomeres shorten and telomerase is inactive. After a certain number of divisions the shortened telomeres signal the cell to cease dividing and the cells become 'senescent' or perhaps will die by apoptosis. Germ cells and some neoplastic cells have sustained function of the telomerase enzyme which helps maintain lengthening of the telomeres and promote continued replication. Tumors having an increased telomerase activity suggest a direct effect, but it is only part of the story. For example, p53 is activated by telomerase and in the absence of p53 these cells fail to undergo apoptosis and go on to proliferate.

Heredity and Cancer: Family Cancer Syndromes

That certain cancers occur in greater frequency within families represents primary empirical evidence for susceptibility based on some hereditary element. Some genetic predispositions exist for cancers of unknown etiology, while interactions between genetic susceptibility and environmental factors are probably responsible for a large proportion of human cancers. Hereditary predispositions include DNA repair deficiencies, inability to detoxify carcinogens, and germline loss or mutations of critical genes. Examples of genetic predispositions to cancer are listed in **Table 7** and include neurofibromatosis, retinoblastoma, breast cancer, and adenomatosis of the colon. In many of these instances, one event in the carcinogenic process is believed to be an inherited germline mutation in the DNA. Another inherited anomaly, an inability to repair ultraviolet light-induced DNA damage in individuals with the condition xeroderma pigmentosum, is associated with sensitivity to sunlight and a high incidence of skin

Table 7 Examples of genetic predisposition to cancer development in humans

Genetic predisposition	*Associated cancer*
Germline deletion on chromosome 13	Retinoblastoma
	Osteosarcoma
Germline deletion on chromosome 11	Renal nephroblastoma (Wilms' tumor)
	Hepatoblastoma
	Rhabdomyosarcoma
	Adrenal carcinoma
Germline mutation in BRCA1 or BRCA2	Breast or ovarian cancer
Li–Fraumeni syndrome	Soft tissue sarcomas in children
	Breast cancer in mothers
Von Hippel Lindau disease	Hemangiomas in the brain and retina
Von Recklinghausen's disease	Fibrosarcoma
	Neuroma
	Pheochromocytoma
Familial dysplastic nevi	Malignant melanoma
Xeroderma pigmentosa – defective ability to repair damaged DNA	Cutaneous squamous cell carcinoma
Ataxia-telangiectasia	Leukemia
	Malignant lymphoma
	Stomach carcinoma
Familial adenomatous polyposis	Colon adenocarcinoma

neoplasia. However, the majority of genetic damage associated with carcinogenesis is acquired either *in utero* or from environmental and/or lifestyle factors to which individuals are exposed. Even for those individuals with a hereditary predisposition to neoplasia, additional DNA damage is necessary to lead ultimately to its development. Environmental factors that may increase the risk of cancer development in genetically predisposed individuals include exposure to radiation and agents that stimulate cellular proliferation. Experimental systems in which to study genetic susceptibility to cancer are critically needed to assess the role of gene–environmental interaction in the development of human cancer.

For some cancers in genetically predisposed individuals, the data are consistent with an association between malignant neoplasia and biallelic genetic alteration and this is supported by studies of tumor suppressor genes which prevent the development of neoplasia. Alteration or loss of a single tumor suppressor gene allele is usually insufficient to permit the development of neoplasia. In other words, the remaining functional tumor suppressor gene copy is sufficient to prevent the development of neoplasia; if it is lost or altered, however, neoplasia can develop. This situation occurs in hereditary childhood retinoblastoma, a malignant neoplasm of the retinal cells of the eye. Susceptible individuals inherit a partial loss of one copy (one allele) of chromosome 13, where the retinoblastoma tumor suppressor gene (*RB-1*) is located, and acquire an alteration or loss of the remaining *RB-1* allele during early development. The affected child subsequently develops retinoblastoma, often within the first 2 years of life.

The Immune System and Cancer

The proper functioning of the immune system is evidenced by recovery from common childhood diseases such as mumps and chicken pox. A properly functioning immune system recognizes the foreignness of the agents responsible for these diseases, responds to the infection, eliminates the foreign agents, and confers long-term immunity to subsequent infection by the same or similar agents. It has been proposed that cancer cells are recognized as foreign and that the immune system functions to eliminate such cells from the body before they are transformed into large, malignant neoplasms. This process involves elaboration of antibodies that bind to the cancer cells and activate a process whereby the cancer cells are killed. In addition, specific cells of the immune system, such as cytotoxic T lymphocytes, natural killer cells, and macrophages, have a mechanism for recognizing foreign cells and eliminating them from the body. The process of immune surveillance and removal of cancer cells is facilitated when the cancer cells express surface antigens that are recognized as foreign. Exposure to agents that depress the normal functioning of the immune system can lead indirectly to neoplasia by permitting early persistence and development of recently emergent cancer cells. Once a neoplasm has reached a critical size and growth rate, it may not be possible for even a properly functional immune system to effectively eliminate the neoplastic cells.

Operational Phases and Theoretical Aspects of Carcinogenesis

In addition to being complex, the process of carcinogenesis is typically prolonged, requiring a significant portion of the lifespan to become clinically apparent. While perturbations in cellular DNA are essential to carcinogenesis, they alone are not sufficient to cause cancer in all cases. Thus, in some experimental situations, a few minutes of exposure to a carcinogen is sufficient to result ultimately in cancer, whereas in other situations, exposure to the same carcinogen will not result in cancer unless there is additional experimental manipulation. Smokers illustrate this principle since many, but not all,

ultimately develop lung cancer. In other experimental studies, simultaneous administration of a carcinogen and a second agent may enhance, reduce, or block the carcinogenic process depending on the agent employed. These and other carcinogenesis studies have elucidated some of the mechanisms and factors that influence carcinogenesis, delimited some of the specific stages in the multistep process, and continually reminded us of the complexity of this disease process.

Multistep experimental models of carcinogenesis are useful in defining events in the neoplastic process; provide the foundations for current operational descriptions and hypotheses of the biological mechanisms of carcinogenesis (see **Figure 1**); are available for many organ systems including the skin, liver, urinary bladder, lung, intestine, mammary gland, and pancreas; and frequently are derived from studies of the effects of chemical agents on laboratory animals. The operational phases of carcinogenesis include initiation, promotion, and progression.

Table 8 Salient features of initiation and promotion of neoplasia

Initiators/initiation
- Effect is irreversible
- Only one exposure may suffice
- Multiple exposures may be additive
- Cannot identify initiated cells
- Agents are considered carcinogens
- Agents are usually mutagenic
- No measurable threshold dose
- Must be administered before the promoter
- Does not result in neoplasia unless promoter is subsequently applied
- Number of initiated cells dependent on dose of initiator

Promoters/promotion
- Nonadditive
- Agents not capable of initiation
- Modulated by diet, hormones, environment, and other factors
- Measurable threshold dose
- Measurable maximal response
- Agents not considered carcinogens but may be cocarcinogens
- Must be administered after the initiator
- Agents are usually not mutagenic
- Prolonged exposure is usually required

Initiation

During the initiation phase of chemical carcinogenesis, a chemical agent or carcinogen interacts with a cell to produce an irreversible change that may ultimately be manifested by a capacity for autonomous growth. The initiated cell appears normal, and the capacity for autonomous growth may remain latent for weeks, months, or years. Initiation implies alteration of the affected cell's DNA at one or more sites, a mutational event that is by definition hereditary. Direct-acting carcinogens interact directly with cellular DNA to produce the damage while indirect-acting carcinogens must be metabolized by the cell to produce a chemical species that interacts with cellular DNA. The majority of damaged cells have the ability to repair the damaged DNA over a period of days or weeks; however, if a cell undergoes cell division with its attendant DNA replication prior to repair of the DNA damage, the DNA alteration becomes 'fixed', is no longer reparable, and is inherited by all subsequent daughter cells. The operational phase of initiation is relatively short and may occur within hours or days. In contrast, the progression of an initiated cell to a fully malignant neoplasm is a prolonged process requiring months in animals and years in humans. Based on a large body of evidence that most initiators are mutagenic or genotoxic, a battery of short-term mutagenicity tests in bacteria and cell culture systems has evolved to identify chemicals with genotoxic properties. Once identified, such chemicals should be rigorously regulated to prevent human exposure. This approach is considered prudent because of the irreversible and hereditary nature of the changes that occur during initiation. Indeed, it is generally believed that even a single molecule of a mutagenic substance is potentially sufficient to damage DNA irreversibly. Thus, for practical purposes there is no threshold or safe level of exposure to a mutagenic agent. Salient features of initiation are listed in **Table 8**.

Initiators interact with host cellular macromolecules and nucleic acids in specific patterns. The majority of known initiators have both initiating and promoting (see below) activity and can thus induce neoplasms rapidly and in high yield when there is repeated or high-level exposure. When given at sufficiently low single doses, an initiated cell requires subsequent promotion for the development of any neoplasia. Thus, the dose of an initiator is a critical determinant of its carcinogenic potential.

Promotion

Promotion is classically considered that portion of the multistep carcinogenic process in which specific agents, known as promoters, enhance the development of neoplasms by providing initiated cells with a selective growth advantage over the surrounding normal cells. The characteristic features of promotion are listed in **Table 8**. By definition, a promoter is given at some time after chemically induced or

fortuitous initiation and the experimental doses of promoting agent are insufficient to produce cancer without prior initiation. When classical promoters are administered at sufficiently high doses and for prolonged intervals, neoplasia can occur without evidence of prior initiation. Under these conditions, a promoting agent must be considered a complete carcinogen unless fortuitous initiation from background radiation, dietary contaminants, environmental toxins, etc., is believed to have occurred. However, under experimental conditions commonly employed in short- and medium-term initiation-promotion experiments, neoplasia does not typically occur in animals that are not previously initiated.

The temporal sequence of promoter administration is critical to the operational definition of promotion. The agent must be administered after initiation and cause enhancement of the neoplastic process to be considered a promoter. If an agent is given simultaneously with an initiator and results in enhancement of development of neoplasms, it is regarded as a cocarcinogen rather than a promoter. While some promoters are cocarcinogenic (e.g., phorbol esters), not all promoters (e.g., phenobarbital and phenol) possess cocarcinogenicity and, conversely, not all cocarcinogens are promoters. Under these same conditions of simultaneous administration, a diminution in the neoplasm response is considered evidence of anticarcinogenic activity. Several rodent liver tumor promoters, which are active when administered after a variety of initiators, prevent or delay the development of liver neoplasms when added to diets along with an active carcinogen. Finally, reversing the order of administration by giving a known promoter prior to an initiator may prevent the expression of carcinogenic activity on the part of the initiator.

While upper and lower thresholds have been demonstrated experimentally for promoters, some consider that, in an absolute sense, it is statistically impossible to prove or disprove the existence of thresholds for promoters for much the same reasons that this cannot be done for initiators. One can never be certain that an apparent no-effect level would, indeed, be without effect if a sufficiently large enough number of animals were used. Promoters include agents such as drugs, plant products, and hormones that do not directly interact with host cellular DNA (are not genotoxic) but somehow influence the expression of genetic information encoded in the cellular DNA. Experimental evidence suggests that regulation of gene expression is unique to the nature of the promoting agent administered. Some promoters are believed to produce their effect by interaction with receptors in the cell membrane, cytoplasm, or nucleus (e.g., hormones, dioxin, phorbol ester, and polychlorinated biphenyls). Alternatively, promoting agents may exert their effect through their molecular orientation at cellular interfaces. Other promoters may selectively stimulate DNA synthesis and enhance cell proliferation in initiated cells, thereby giving them a selective growth advantage over surrounding normal cells.

Promoters appear to have a relatively high tissue specificity. Thus, phenobarbital functions as a promoter for rodent liver neoplasia but not urinary bladder neoplasia. Saccharin, on the other hand, promotes urinary bladder neoplasia but not liver neoplasia in the rat. Similarly, 12-*o*-tetradecanoylphorbol-13-acetate (phorbol ester) is a potent skin and forestomach neoplasm promoter in the laboratory rodent but has no appreciable activity in the liver. Other agents, such as the antioxidants 3-*t*-butyl-4-methoxyphenol and 2,6-di-*t*-butyl-4-methoxyphenol, may act as promoters in one organ and antipromoters in another and have no effect in a third organ. Thus, the practical definition of a promoter must include the designation of the susceptible tissue.

Tumor promotion may be modulated by several factors such as age, sex, diet, and hormone balance. The correlation of increased rates of breast cancer in women following a 'Western' lifestyle has implicated meat and fat consumption as playing an important role in breast cancer development. Experimental demonstration of the role of a high-fat diet in the promotion of mammary cancer in rats exposed to the mammary carcinogen dimethylbenzanthracene has been documented. Similarly, bile acids, as modulated by fat consumption, are known promoters of rat liver carcinogenesis and human colorectal cancer. Age- and sex-associated modulations in hormonal levels of estrogens, progesterone, and androgens have been implicated as potential promoters of breast cancer on the basis of epidemiological studies in humans. Experimental studies have repeatedly shown that these hormones, in addition to pituitary prolactin, serve to promote mammary cancer in rats initiated with mammary carcinogens.

Progression

Progression is that part of the multistep neoplastic process associated with the development of an initiated cell into a biologically malignant cell population. In common usage progression is frequently used to signify the stages whereby a benign proliferation becomes malignant or, alternatively, where a neoplasm develops from a low grade to a high grade of malignancy. During progression neoplasms show

increased invasiveness; develop the ability to metastasize; and then biochemical, metabolic, and morphologic characteristics are altered.

Expression of tumor cell heterogeneity, an important characteristic of tumor progression, includes production of antigenic and protein product variants, ability to elaborate angiogenesis factors, emergence of chromosomal variants, development of metastatic capability, alterations in metabolism, and a decrease in sensitivity to radiation. The development of intraneoplastic diversity may result from increasing genetic damage. Alternatively, the heterogeneity observed in tumor progression may be generated by epigenetic, regulatory mechanisms that are a part of the process of promotion. More than likely, genetic and nongenetic events subsequent to initiation operate in a nonmutually exclusive manner during progression, possibly in an ordered cascade of latter events superimposed upon earlier events.

The most plausible mechanism of progression invokes the notion that, during the process of tumor growth, there is a selection that favors enhanced growth of a subpopulation of the neoplastic cells. In support of this mechanism is increased phenotypic heterogeneity observed in malignant but not benign neoplastic proliferations. Presumably, a variety of subpopulations arises, and it is only a matter of time before the emergence of a subpopulation with more malignant biological characteristics or at least an accelerated growth advantage. This can be observed occasionally during experimental hepatocarcinogenesis when a phenotypically distinguishable carcinoma can be observed arising within an existing adenoma.

Distinction between tumor promotion and tumor progression is not readily discernible in the routine histopathologic evaluation of neoplasms and may be somewhat academic because promotion may be considered part of the process of progression. In both situations the critical event is accentuated growth. What is believed to distinguish progression from promotion is the presence of structural genomic alterations in the former and their absence in the latter. Both structural genomic changes and biochemical changes associated with tumor progression cannot be defined by conventional histopathology. Established and emerging technologies centered around histochemistry, immunocytochemistry, *in situ* hybridization, identification of activated oncogenes, loss of tumor suppressor genes, gene expression, proteomic and metabolomic profiling and discovery offer promise to distinguish various stages of progression in the evolution from benign to malignant neoplasms.

Exogenous Factors Influencing Carcinogenesis

Important exogenous factors that contribute to induction of cancer include natural and synthetic chemicals, environmental exposures to ultraviolet and medical radiation, diet and lifestyle, and infectious agents, such as viruses, parasites, and bacteria. Evidence for a causal association between exogenous factors and neoplasia is derived from studies of epidemiology, occupationally common cancers, and animal models.

Chemical and Physical Agents and Lifestyle Factors

Many chemicals that cause cancer interact directly with and alter DNA or are metabolized to chemical derivatives capable of doing so. Exposure to carcinogens can occur in certain occupational settings. Associations of human hepatic angiosarcomas with workplace exposure to vinyl chloride, pulmonary mesotheliomas with exposure to asbestos fibers, and leukemia with benzene are well-known examples. Exposure to other carcinogenic agents may occur in the diet or as a consequence of certain lifestyle practices, such as cigarette smoking associated with pulmonary cancer and high animal fat diets linked to breast and colon cancer. Strong associations have been made between exposure of light-skinned individuals to ultraviolet radiation and skin cancer. Exposure to occupational ionizing radiation, X-rays, and medical use of radioisotopes has also been associated with human neoplasia. Examples include leukemias in radiologists and atom bomb victims, lung cancer in uranium mineworkers, and thyroid and breast cancer following diagnostic or therapeutic use of radiation.

Infectious Agents and Inflammation

Viral, parasitic, and bacterial infections have been linked to cancer (**Table 9**). DNA viruses such as Epstein–Barr, hepatitis B, hepatitis C, papillomaviruses, and Kaposi sarcoma herpes virus and RNA viruses such as human T-cell leukemia virus type I and human immunodeficiency virus have been implicated in causing cancer in humans and are listed as 'known-to-cause-cancer' in humans by the International Agency for Research on Cancer (IARC). In man, the liver fluke, *Opisthorchis viverrini,* is associated with the development of cholangiocarcinomas of the liver and the blood fluke, *Schistosoma haematobium*, with carcinoma of the urinary bladder. There is evidence that chronic *Helicobacter pylori* infection of the stomach in man is not only related to

Table 9 Viruses causally related to or strongly associated with animal and human neoplasia

Virus	*Type of neoplasm*	*Species*
DNA viruses		
Myxoma	Myxoma	Rabbit
	Myxomatosis	Rabbit
Herpes	Lymphosarcoma	Chicken
		Monkey
		Rabbit
Herpes simplex 2	Cervical carcinoma	Human
Papillomaviruses	Papillomas	Cow
		Rabbit
		Horse
		Dog
Human papillomavirus	Warts	Human
	Epidermoid carcinoma	
	Cervical carcinoma	
Woodchuck hepatitis virus	Hepatocellular carcinoma	Woodchuck
Hepatitis B virus	Hepatocellular carcinoma	Human
RNA retroviruses		
Human T cell leukemia virus (HTLV-I and -II)	T cell lymphoma	Human
Avian erythroblastosis virus	Leukemia	Chicken
	Sarcoma	
Abelson leukemia virus	Leukemia	Mouse
Hervey sarcoma virus	Sarcoma	Rat
	Leukemia	
Feline sarcoma virus	Sarcoma	Cat

gastrointestinal ulcers, but also may be linked to gastric carcinoma or lymphoma development.

For oncogenic viruses, the viral or host genes generally drive the neoplastic process while with some agents there appears to be an association of chronic inflammation and nitric oxide (NO) production in the development of cancer. When DNA viruses infect cells, the viral DNA inserts itself wholly or partially into the genome of the infected cell. It appears that such integration of viral DNA into the mammalian genome is sometimes sufficient to cause neoplastic transformation of the infected cell, which is accompanied by the production of new proteins essential for the neoplastic process. RNA viruses associated with neoplasia are chiefly represented by the retroviruses. RNA viruses possess an enzyme called reverse transcriptase, which is capable of forming a DNA copy of the viral RNA when the virus infects a host cell. This DNA ultimately inserts itself into the host genome in much the same way as DNA viruses do, possibly resulting in the development of neoplasia.

The role of inflammation in cancer development is being intensely studied. There are a number of chronic inflammatory conditions, infectious and noninfectious, in man and animals associated with an increasing risk of cancer and there are many investigators examining the role of NO and oxygen radical damage to DNA or other cellular processes such as cell proliferation and apoptosis. NO induces p53, prevents apoptosis in cells such as endothelium, promotes angiogenesis, and inhibits DNA-repair activities – all processes that might provide a selective advantage to neoplastic cell growth.

Identification of Carcinogenic Agents

There are two methods utilized to identify potential human carcinogens, the most direct of which is based on retrospective epidemiological studies in human populations using existing historical records associated with known cases of neoplasia. These records include death certificates where cause of death is indicated; hospital records; responses to questionnaires that document environmental or work-associated exposure to potential carcinogenic agents; and studies of neoplasia in culturally, ethnically, or religiously distinctive human populations. Association of cigarette smoking with lung cancer and exposure to asbestos with mesotheliomas was the result of such retrospective epidemiological work. Prospective epidemiological studies identify a given population of individuals who agree to be monitored for several years to permit identification of potential carcinogenic factors associated with neoplasms which may occur.

Another method used to identify potential human carcinogens involves testing known chemicals and agents in experimental animals. Such tests have been referred to as animal bioassays and are typically conducted using rats and mice exposed to high doses of the suspect agent for a large portion (typically 2 years) of their lifespan. If such agents are observed to produce neoplasia in the experimental animals, the agent is regarded as a potential human carcinogen. In countries throughout the world, legal requirements mandate that all new chemical agents and drugs be tested in animal bioassays to determine whether they cause cancer in the test animals. Additionally, since the mid-1960s in the United States, the National Cancer Institute and currently the National Toxicology Program have collectively conducted animal bioassays on more than 500 chemical agents to assess their potential to cause cancer.

Interpretation of results from human epidemiological studies and animal bioassays to identify carcinogenic agents has often proved difficult and

controversial. Humans are rarely exposed to only one potential cancer-causing agent in their lifetime, and the amount and duration of that exposure may be difficult or impossible to quantify rigorously. Many years may intervene between exposure to a potential carcinogen and ultimate development of neoplasia, making accurate assessment of cause and effect almost impossible. Despite such limitations, epidemiological studies that clearly show an association between a given chemical exposure or lifestyle habit with an enhanced rate of a specific cancer are regarded as the most relevant method for identification of human carcinogens. While animal bioassays have proved useful for the identification of agents that can cause cancer in the laboratory rodent, they only identify an agent as potentially hazardous to human health. Additional facts and factors must be considered in classifying such an agent as a likely human carcinogen.

The current approach for assessing the scientific relevance of either epidemiological or animal bioassay results to human health risk involves a 'weight-of-evidence' procedure in which national and international panels of expert scientists from several disciplines examine all available information on the suspect agent in making their assessment. Included in this analysis are the strength of the epidemiological evidence, the dose–response curve of the animal response, comparative species metabolism and ability to extrapolate between species, likely mechanism of cancer induction for the agent in question, the genotoxicity of the agent, the amount of the agent in the environment, and the number of people potentially exposed to the agent. Based on this type of analysis, so far 88 agents have been classified as known human carcinogens by the International Agency for Research on Cancer (some of which are in **Table 10**) and 64 more agents have been designated as probable human carcinogens. The 10th US Health and Human Services *Annual Report on Carcinogens* lists 49 known human carcinogens and 174 substances that are reasonably anticipated to be human carcinogens.

Table 10 Some selected agents or mixtures for which there is sufficient evidence of carcinogenicity in humans

Organic compounds
2-Napthylamine
4-Aminobiphenyl
Aflatoxin B_1
Analgesics containing phenacetin
Azathioprine
Benzene
Benzidine
Betel quid with tobacco
Bis(chloromethyl)ether
Chlorambucil
Chlonaphazine
Chloromethyl methyl ether
Cyclophosphamide
Diethylstilbesterol
Melphalan
Methyl-CCNU
MOPP
(and other combined therapies)
Mustard gas
Myleran
Thiotepa
Tobacco products and tobacco smoke
Treosulfan
Vinyl chloride
Soots, tars, and oils
Coal tar pitches
Coal tars
Mineral oils,
untreated and mildly treated
Shale oils
Soots
Hormones
Diethylstilbesterol
Estrogens
Oral contraceptives
Metals
Arsenic compounds
Chromium compounds
Nickel and nickel compounds
Fibers
Asbestos
Erionite
Talc-containing asbestos fibers
Other
8-Methoxypsoralen + UV radiation
Viruses
Epstein-Barr
Hepatitis B & C
Papillomaviruses
T-cell leukemia virus type I
Parasites
Opisthorchis virerrini
Schistosoma haematobium

Molecular Epidemiology of Cancer

The molecular epidemiology of cancer is the study of molecular alterations, primarily mutations, in investigating the causative agents of cancer as well as identifying individual cancer risk. The possibility of identifying cancer-causing agents based on the occurrence of predictable molecular alterations that are found in the neoplasm is intriguing. It is based on the hypothesis that there are carcinogen-specific patterns of mutations that reflect direct interactions of

carcinogens with cancer genes. For example, lung and colon cancers from people who smoke tend to have a specific mutation in the *ras* oncogene or p53 tumor suppressor gene (i.e., mostly a G–T nucleotide base substitution) and that this mutation is likely due to the direct interaction of the carcinogen in smoke benzo(*a*)pyrene with DNA. Such chemical-specific mutational profiles (or 'molecular signatures') have been used to support a causal association between particular genetic events in tumors and a specific carcinogen such as neoplasms associated with exposure to radon, aflatoxin B_1, vinyl chloride, and the nitrosamines (**Tables 11** and **12**). The strongest evidence for linkage between a cancer-causing agent and a specific type of neoplasm is that of the CC–TT double base changes observed in skin neoplasms of man and animals. This mutation is consistent with the predicted UV-induced damage of dipyrimidine dimers. In liver tumors from persons living in geographic areas with a high exposure to aflatoxin B_1 there is a frequent mutation at the third nucleotide pair of codon 249 in the p53 gene, suggesting the mutation is chemical-specific and imparts a specific growth or survival advantage to the mutated liver cells.

Table 11 Molecular signatures of malignant human cancers

Exposure	*Neoplasm type*	*Predominant mutation (nucleotide base changes)*
Cigarette smoke	Lung carcinoma	K-*ras*, codons 12 and 13 (G–T)
	Colon	K-*ras*, codons 12 and 13
	Lung carcinoma	p53, multiple codons (G–T)
Radon	Lung carcinoma	p53, codon 249 (G–T)
Aflatoxin B_1	Hepatocellular carcinoma	p53, codon 249 (G–T)
Ultraviolet light	Skin carcinoma	p53, dipyrimidine sites (CC–TT)
Vinly chloride	Hepatic angiosarcoma	p53, codon 249 (A–T)
	Hepatocellular carcinoma	K-*ras*, codons 12 and 13 (G–A)

Table 12 Molecular signatures of malignant rodent cancers

Exposure	*Neoplasm type (species)*	*Predominant mutation (nucleotide base changes)*
Methylnitrosourea	Mammary carcinoma (R)	K-*ras*, codon 12 (G–C)
Aflatoxin B_1	Lung carcinomas (M)	K-*ras*, codon 12 (G–C)
Diethylnitrosamine	HCC (M)	H-*ras*, codon 61 (A–G)
Ultraviolet light	Skin carcinoma (M)	p53, dipyrimidine sites (CC–TT)
Vinly chloride	HCC (R)	H-*ras*, codon 61 (A–T)

R, rat; M, mouse. HCC, hepatocellular carcinoma.

Animal studies have confirmed that there are certainly chemical-specific mutational profiles in neoplasms; however, there are many examples where the mutational profile varies by strain (**Table 13**), species, dose, or dosing regiment. For example, diethylnitrosamine, a strong, cross-species hepatocarcinogen, will induce liver neoplasms in mice, rats, and rainbow trout, but the frequency and type of *ras* mutation in the neoplasm varies widely, and the mutations are not simply a reflection of direct DNA interaction (**Table 14**). In some studies, *in vitro* mutation assays were poor predictors of liver tumor mutation profiles in the mouse. In this complex process, carcinogens might also be influencing events such as DNA repair, oxidative DNA damage, methylation, cell death, proliferation, and/or a hypermutable state.

Molecular epidemiologic studies aimed at identifying an individual's risk of developing cancer have found that persons with germline mutations in cancer genes (i.e., BRCA1 or BRCA2) or variations (polymorphisms) of carcinogen-metabolizing enzyme activities (i.e., cytochrome P-450s or glutathione-*S*-transferases) or DNA repair capacities can be at increased risk of developing neoplasia in their

Table 13 Sensitivity to liver tumor development and H-*ras* codon 61 mutations in spontaneous hepatocellular tumors of various strains of mice

Sensitivity	*Strain*	*Frequency*		*Codon 61 mutation (normal = CAA)*		
				AAA	*CGA*	*CTA*
High	C3H	23/89	(26%)	17	3	3
Intermediate	B6C3F1	183/333	(56%)[a]	106	50	21
	CD-1	9/36	(25%)	8	1	0
Low	C57BL	5/34	(15%)[a]	0	1	4

[a] Occasional mutations in other codons of H- and K-*ras*.

Adapted from Maronpot RR, Fox T, Malarkey DE, and Goldsworthy TL (1995) Mutations in the *ras* protooncogene: Clues to etiology and molecular pathogenesis of mouse liver tumors. *Toxicology* 101: 125–156.

Table 14 Species and strain comparisons of mutational profiles induced by diethylinitrosamine (DEN)

Animal	*Frequency of ras mutations*		*Type*	*Nucleotide base substitutions*				
				C–A	*A–G*	*A–T*	*A–C*	*G–A*
CD-1 mouse	13/25	(52%)	H- and N-*ras*	12	1	0	0	0
C3H mouse	54/114	(26%)	H-*ras*	28	24	2	0	0
B6C3F1 mouse	63/239	(26%)	H-*ras*	16	32	15	0	0
C57BL mouse	2/59	(2%)	H-*ras*	0	1	0	1	0
F344 rat	0/19	(0%)	K-*ras*	0	0	0	0	0
Rainbow trout	6/7	(86%)	K-*ras*	0	0	0	0	6

Data adapted partly from Maronpot RR, Fox T, Malarkey DE, and Goldsworthy TL (1995) Mutations in the *ras* protooncogene: Clues to etiology and molecular pathogenesis of mouse liver tumors. *Toxicology* 101: 125–156.

lifetime. High-throughput analyses to examine single nucleotide polymorphisms (SNPs) are being used to search for biomarkers of cancer risk in individuals and some of this information is being used to help people take preventive measures to decrease their risk of developing cancer.

Summary and Conclusions

All of life is a balancing act of good versus evil and production versus destruction. Similar balancing factors are evident in carcinogenesis where regulatory mechanisms for tissue proliferation are balanced against those for cellular differentiation. It is well established that carcinogenesis requires the accumulation of multiple alterations in the genome of the affected (cancer) cells. At the genetic level, two opposing classes of genes, oncogenes, and tumor suppressor genes, have been implicated in the carcinogenic process. In addition, the development of cancer is influenced by host factors such as age, sex, diet, nutrition, general health status, and inherited predispositions for cancer and by complex positive and negative intracellular signaling mechanisms. Treatment of cancer is based on our understanding of the mechanistic underpinnings of the carcinogenic process and attempts to shift the balance of critical factors in favor of patient survival. The probability of developing cancer is directly proportional to the intensity, route, and duration of exposure to cancer-causing factors as well as genetic susceptibility. Public health strategies are based on the premise that reduction or prevention of exposure to cancer-causing factors will decrease the incidence of cancer.

See also: Carcinogen Classification Schemes; Carcinogen–DNA Adduct Formation and DNA Repair; Cell Proliferation; Chromosome Aberrations; Epidemiology; Immune System; International Agency for Research on Cancer; Mechanisms of Toxicity; Molecular Toxicology–Recombinant DNA Technology; Mouse Lymphoma Assay; Radiation Toxicology, Ionizing and Nonionizing; Skin; Toxicity Testing, Carcinogenesis.

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Glossary

Adenomatosis	a condition in which numerous adenomatous growths develop in a tissue.
Allele	one of the two gene pairs situated at the same location on a chromosome; one allele is inherited from the mother and the other from the father; characteristics such as being short or tall or having blue eyes versus brown eyes are determined by the expression of inherited alleles.

Anaplasia	lack of normal organizational or structural differentiation of a tissue; anaplastic cells are typically poorly differentiated.
Angiogenesis	the development of blood vessels.
Benign	a classification of anticipated biological behavior of neoplasms in which the prognosis for survival is good; benign neoplasms grow slowly, remain localized, and usually cause little harm to the host.
Biallelic damage	damage to both maternal and paternal copies of a gene.
Cancer	generally refers to a malignant neoplasm.
Carcinogenesis	the process of development of cancer or neoplasms.
Choristoma	a mass or collection of well-differentiated cells from one organ found within another organ, for example, adrenal tissue present in the lung.
Clonal	pertaining to a clone; a line of cells descended from a single cell.
Cocarcinogen	an agent that has no inherent carcinogenic activity by itself but is capable of augmenting neoplasm formation when given simultaneously with a genotoxic carcinogen.
DNA	abbreviation for deoxyribonucleic acid; the basic building block of genetic material in all organisms except RNA viruses.
Dysplasia	disordered tissue formation characterized by changes in size, shape, and orientational relationships of adult types of cells; primarily seen in epithelial cells.
Epithelial cell	cells which line the internal and external surfaces of the body and form the bulk of many of the major organs of the body; they are formed from the embryonic germ layers known as entoderm and ectoderm.
Gene	the basic biological unit of heredity which is located on a chromosome.
Genome	the total complement of genes present in the set of chromosomes characteristic of a given organism.
Genotoxic	toxic to DNA; an agent or process that interacts with cellular DNA either directly or after metabolic transformation; mutagens are genotoxic agents.
Grade (grading)	a subjective evaluation of the morphologic characteristics of a neoplasm based on the degree of anaplasia and proliferation evident from microscopic examination as a measure of biological outcome or degree of malignancy.
Growth factors	agents that contribute to and stimulate tissue growth.
Hamartoma	a localized overgrowth of differentiated cells that have an altered growth pattern in relation to the tissue in which they are found, for example, a nodule of disorganized striated muscle fibers within a normal skeletal muscle.
Hepatocarcinogenesis	the development of liver cancer.
Heterozygous	having different alleles at a specific position on a chromosome.
Histogenetic	pertaining to the origin, formation, or development of tissues from undifferentiated embryonic germ cell layers.
Homeostatic	pertaining to the natural state of equilibrium of the normal internal environment of the body; maintained by complex positive and negative feedback control mechanisms.
Homozygous	having identical alleles at a specific position on a chromosome.
Hyperplasia	a numerical increase in the number of normal-appearing cells within a tissue or organ.
Immune system	a primary defense system in the body capable of attacking and potentially destroying cancer cells; consists of lymphoid and related tissues from which cells are recruited to produce antibodies or to directly attack cancer cells.
Initiation	the first operational phase of the process of carcinogenesis during which a cell sustains a heritable alteration in DNA.
Malignant	a classification of anticipated biological behavior of neoplasms in which the prognosis for survival is poor; malignant neoplasms grow rapidly, invade, destroy tissue, and are usually fatal.
Mesenchymal cell	cells derived from embryonic mesoderm, which constitute the supporting structure of tissue such as connective tissue, blood vessels, muscles, and bones.
Metaplasia	the substitution of one type of fully differentiated cells for the fully differentiated cell type normally present in a given tissue.

Metastasize	the spreading of neoplastic cells from a primary site of origin to a distant, noncontiguous site where their growth occurs.
Mitogenic	stimulating cell proliferation or division; causes mitosis.
Mutation	a structural alteration in DNA that is hereditary and may give rise to an altered phenotype.
Neoplasia	the process of the development of neoplasms; essentially synonymous with carcinogenesis.
Neoplasm	new and typically abnormal growth which is generally uncontrolled and becomes progressively more serious with time.
Neurofibromatosis	a hereditary condition of the nervous system and other tissues of the body characterized by development of numerous neoplasms (neurofibromas) distributed over the entire body.
Nucleotide	a biochemical component of DNA that consists of a purine or pyrimidine base, a ribose or deoxyribose sugar, and a phosphate group; a basic building block of DNA.
Oncogene	a so-called cancer gene because alterations in its structure or expression are typically associated with neoplasms; an activated form of a protooncogene.
Oncogene activation	the process whereby a protooncogene is altered such that it stimulates enhanced cellular growth; several different mechanisms can lead to such activation.
Oncology	the study of neoplasia or carcinogenesis.
Phenotype	the physical appearance, biochemical makeup, and physiological behavior of an individual.
Preneoplasia	refers to the recognizable structural changes in a tissue that are sometimes antecedent to the development of neo-plasia; the presence of preneoplasia indicates an increased probability for the development of neoplasia.
Progression	an operational phase of carcinogenesis associated with the development of an initiated cell into a fully malignant neoplasm; sometimes used in a more limited sense to refer to the change from a benign neoplasm to a fully malignant neoplasm.
Promotion	an operational phase of carcinogenesis in which there is enhancement of neoplasm formation when an agent (the promoter) is administered after exposure to a genotoxic carcinogen.
Protooncogene	a normal cellular structural gene that, when activated by mutations, amplifications, rearrangements, or viral transduction, functions as an oncogene and is associated with neoplasia; regulates normal processes related to cell growth and differentiation.
Retinoblastoma	an ocular neoplasm arising from germ cells of the retina.
Retroviruses	a large group of RNA viruses.
Stage (staging)	a subjective assessment of the extent to which a neoplasm has spread in the body and, thus, an indication of the patient's clinical prognosis.
Threshold	the level of an agent below which no physiological, biochemical, or pathological effect can be measured.
Tumor	any tissue enlargement or swelling; frequently used as equivalent to a benign neoplasm.
Tumor suppressor gene	a gene that normally functions to suppress uncontrolled tissue growth.
Weight of evidence	an approach for assessing the potential carcinogenic risk of an agent by considering all available information relative to the biological action of the agent.

Carcinogenicity Toxicity Testing *See* Toxicity Testing, Carcinogenesis.

Cardiovascular System

Arthur Penn and Gleeson Murphy

Introduction

The central tenet of toxicology was stated by the sixteenth-century physician Paracelsus: "All substances are poisons. There is none that is not a poison. The right dose differentiates a poison and a remedy." The scope of this chapter includes the toxic effects of several classes of chemicals on the human cardiovascular system (CVS). These include drugs (including those commonly abused, as well as therapeutic), pesticides, other organic chemicals, metals and other inorganic chemicals, some recent FDA- and media-scrutinized compounds, and complex mixtures (e.g., cigarette smoke).

The CVS consists of the heart and the vasculature (arteries, arterioles, veins, venules, and capillaries). The focus will be on the heart and arteries as they display the bulk of characteristic toxic effects. The entry begins with a description of the normal anatomy and physiology of the heart. This is followed by a series of examples of agents that can act on ion movement, muscle function, and blood flow. The second part of the entry begins with a description of the anatomy and physiology of blood vessels followed by examples of vasculotoxic agents. A list of cardiotoxic agents and their mechanisms of toxicity is presented in **Table 1**. A listing of some vasculotoxic

Table 1 General mechanisms of cardiotoxicity; and cardiotoxicity of – key pharmaceutical agents, naturally occurring substances, and selected industrial agents

Mechanism	*Cellular perturbations*	*Organ manifestations*
General mechanisms of cardiotoxicity		
Interference with ion homeostasis		
Inhibition of Na^+/K^+ ATPase	↑ $[Ca^{2+}]_i$, ↓ Conduction velocity	Positive inotropic effect Proarrhythmic
Na^+ channel blockade	↓ Na^+ channel activity ↓ $^+$Conduction velocity	Proarrhythmic
K^+ channel blockade	↓ K^+ channel activity ↓ Repolarization ↑ Action potential duration	Proarrhythmic
Ca^{2+} channel blockade	↓ L-type Ca^{2+} channel activity ↓ Ca^{2+}-induced-Ca^{2+} release ↓ AV conduction	Negative inotropic effect Negative chronotropic effect Bradycardia
Altered coronary blood flow		
Coronary vasoconstriction or obstruction	Ischemia (ATP depletion, intracellular acidosis)	Myocardial infarction Cardiac myocyte death Cardiac remodeling
Ischemia/reperfusion injury	Oxidative stress, ↑ $[Ca^{2+}]_i$ Intracellular pH change	Cardiac myocyte death
Oxidative stress	Lipid peroxidation DNA damage Mitochondrial dysfunction, Altered $[Ca^{2+}]_i$ homeostasis	Cardiac myocyte death
Organellar dysfunction		
Sarcolemmal injury	Altered membrane integrity	Cardiac myocyte death
Sarcoplasmic reticulum dysfunction	Altered $[Ca^{2+}]_i$ homeostasis	Cardiac myocyte death
Mitochondrial injury	ATP depletion Cytochrome *c* release Altered mitochondrial $[Ca^{2+}]_i$ homeostasis	Cardiac myocyte death
Apoptosis	Cellular shrinkage Sarcolemmal blebbing Chromatin condensation Redistribution of membrane phospholipids DNA fragmentation	Cardiac myocyte death
Oncosis	Cellular swelling Sarcolemmal blebbing Chromatin clumping Mitochondrial swelling	Cardiac myocyte death

Table 1 Continued

Agents	*Cardiotoxic manifestations*	*Proposed mechanisms of cardiotoxicity*
Cardiotoxicity of key pharmaceutical agents		
Ethanol	↓ Conductivity (acute) Cardiomyopathy (chronic)	Acetaldehyde (metabolite) Altered $[Ca^{2+}]_i$ homeostasis Oxidative stress Mitochondrial injury
Antiarrhythmic drugs		
Class I (disopyramide, encainide, flecainide, lidocaine, mexiletine, moricizine, phenytoin, procainamide, propafenone, quinidine, tocainide)	↓ Conduction velocity Proarrhythmic	Na^+ channel blockade
Class II (acebutolol, esmolol, propranolol, sotalol)	Bradycardia, heart block	β-adrenergic receptor blockade
Class III (amiodarone, bretylium, dofetilide, ibutilide, quinidine, sotalol)	↑ Action potential duration QTc interval prolongation Proarrhythmic	K^+ channel blockade
Class IV (diltiazem, verapamil)	↓ AV conduction Negative inotropic effect Negative chronotropic effect Bradycardia	Ca^{2+} channel blockade
Inotropic drugs and related agents		
Cardiac glycosides (digoxin, digitoxin)	Action potential duration AV conduction Parasympathomimetic (low doses) Sympathomimetic (high doses)	Inhibition of Na^+, K^+-ATPase, ↑ $[Ca^{2+}]_i$
Ca^{2+} sensitizing agents (adibendan, levosimendan, pimobendan)	↓ Diastolic function? Proarrhythmic?	↑ Ca^{2+} sensitivity, Inhibition of phosphodiesterase
Other Ca^{2+} sensitizing agents (allopurinol, oxypurinol)	?	Inhibition of xanthine oxidase
Catecholamines (dobutamine, epinephrine, isoproterenol, norepinephrine)	Tachycardia Cardiac myocyte death	β_1-adrenergic receptor activation Coronary vasoconstriction Mitochondrial dysfunction ↑ $[Ca^{2+}]_i$ Oxidative stress Apoptosis
Bronchodilators (albuterol, bitolterol, fenoterol, formeterol, metaproterenol, pirbuterol, procaterol, salmeterol, terbutaline)	Tachycardia	Nonselective activation of β_1-adrenergic receptors
Nasal decongestants (ephedrine, ephedrine alkaloids, ma huang, phenylephrine, phenylpropanolamine, pseudoephedrine)	Tachycardia	Nonselective activation of β_1-adrenergic receptors
Appetite suppressants (amphetamines, fenfluramine, phentermine)	Tachycardia, pulmonary hypertension Valvular disease	↑ Serotonin? Na^+ channel blockade?
Antineoplastic drugs		
Anthracyclines (daunorubicin, doxorubicin, epirubicin)	Cardiomyopathy Heart failure	Altered $[Ca^{2+}]_i$ homeostasis Oxidative stress Mitochondrial injury Apoptosis
5-Fluorouracil	Proarrhythmic	Coronary vasospasm?
Cyclophosphamide	Cardiac myocyte death	4-Hydroxycyclophosphamid (metabolite) Altered ion homeostasis
Antibacterial drugs		
Aminoglycosides (amikacin, gentamicin, kanamycin, netilmicin, streptomycin, tobramycin)	Negative inotropic effect	↓ $[Ca^{2+}]_i$
Macrolides (azithromycin, clarithromycin, dirithromycin, erythromycin)	↑ Actin potential duration QTc interval prolongation Proarrhythmic	K^+ channel blockade

Table 1 Continued

Agents	*Cardiotoxic manifestations*	*Proposed mechanisms of cardiotoxicity*
Fluoroquinolones (grepafloxacin, moxifloxacin, sparfloxacin)	↑ Action potential duration QTc interval prolongation Proarrhythmic	K^+ channel blockade
Tetracycline	Negative inotropic effect	↓ $[Ca^{2+}]_i$
Chloramphenicol	Negative inotropic effect	↓ $[Ca^{2+}]_i$
Antifungal drugs		
Amphotericin B	Negative inotropic effect	Ca^{2+} channel blockage? Na^+ channel blockade? ↑ Membrane permeability?
Flucytosine	Proarrhythmic Cardiac arrest	5-fluorouracil metabolite Coronary vasospasm?
Antiviral drugs		
Nucleotide analog reverse transcriptase inhibitors (stavudine, zalcitabine, zidovudine)	Cardiomyopathy	Mitochondrial injury Inhibition of mitochondrial DNA polymerase Inhibition of mitochondrial DNA synthesis Inhibition of mitochondrial ATP synthesis
Centrally acting drugs		
Tricyclic antidepressants (amitriptyline, desipramine, doxepin, imipramine, protriptyline)	ST segment elevation QTc interval prolongation Proarrhythmic Cardiac arrest	Altered ion homeostasis Ca^{2+} channel blockade Na^+ channel blockade K^+ channel blockade
Selective serotonin reuptake inhibitors (fluoxetine)	Bradycardia, Atrial fibrillation	Ca^{2+} channel blockade Na^+ channel blockade
Phenothiazine antipsychotic drugs (chlorpromazine, thioridazine)	Anticholinergic effects Negative inotropic effect QTc interval prolongation PR interval prolongation	Ca^{2+} channel blockade?
Other antipsychotic drugs (clozapine)	Blunting of T waves ST segment depression	
General inhalational anesthetics (enflurane, desflurane, halothane, isoflurane, methoxyflurane, sevoflurane)	Negative inotropic effect Decreased cardiac output Proarrhythmic	Ca^{2+} channel blockade Altered Ca^{2+} homeostasis, β-adrenergic receptor sensitization
Other general anesthetics (propofol)	Negative inotropic effect	Ca^{2+} channel blockade Altered Ca^{2+} homeostasis, β-adrenergic receptor sensitization
Local anesthetics		
Cocaine	Sympathomimetic effects Ischemia/myocardial infarction Proarrhythmic Cardiac arrest Cardiac myocyte death	Na^+ channel blockade Coronary vasospasm, Altered Ca^{2+} homeostasis Mitochondrial injury Oxidative stress Apoptosis
Other local anesthetics (bupivacaine, etidocaine, lidocaine, procainamide)	Decreased excitability ↓ Conduction velocity Proarrhythmic	Na^+ channel blockade
Antihistamines		
(astemizole, terfenadine)	↑ Action potential duration QTc interval prolongation Proarrhythmic	K^+ channel blockade
Immunosuppressants		
(rapamycin, tacrolimus)	Cardiomyopathy Heart failure	Altered Ca^{2+} homeostasis
Miscellaneous drugs		
Cisapride	↑ Action potential duration QTc interval prolongation Proarrhythmic	K^+ channel blockade

Table 1 Continued

Agents	*Cardiotoxic manifestations*	*Proposed mechanisms of cardiotoxicity*
Methylxanthines (theophylline)	↑ Cardiac output Tachycardia Proarrhythmic	Altered Ca^{2+} homeostasis, Inhibition of phosphodiesterase
Sildenafil	?	Inhibition of phosphodiesterase
Radiocontrast agents (diatrizoatemeglumine, iohexol)	Proarrhythmic Cardiac arrest	Apoptosis?
Cardiotoxicity of naturally occurring substances		
Estrogens		
Natural estrogens (17β-estradiol, estrone, estriol)	QTc interval prolongation?	Gender differences in K^+ channel expression?
Synthetic estrogens (diethylstilbestrol, equilin, ethinyl estradiol, mestranol, quinestrol)	Cardioprotection?	Antiapoptotic effects? Antioxidant activity? ↑ Na^+, K^+-ATPase activity? Ca^{2+} channel blockade?
Nonsteroidal estrogens (bisphenol A, diethylstilbestrol, DDT, genistein)		Other mechanisms?
Progestins		
(desogestrel, hydroxyprogesterone, medroxyprogesterone, norethindrone, norethynodrel, norgestimate, norgestrel, progesterone)	Enhanced toxicity of cocaine?	Mechanisms?
Androgens		
Natural androgens (androstenedione, dehydroepiandrosterone, dihydrotestosterone, testosterone)	Myocardial infarction Cardiac hypertrophy	Mitochondrial injury? Altered Ca^{2+} homeostasis? Other mechanisms?
Synthetic androgens (boldenone, danazol, fluoxymesterone, methandrostenolone, methenolone, methyltestosterone, nandrolone, oxandrolone, oxymetholone, stanozolol)		
Glucocorticoids		
Natural glucocorticoids (corticosterone, cortisone, hydrocortisone)	Cardiac hypertrophy Cardiac fibrosis	Increased collagen expression Other mechanisms?
Synthetic glucocorticoids (e.g., dexamethasone, methylprednisolone, prednisolone, prednisone)		
Mineralocorticoids		
(aldosterone)	Cardiac fibrosis Heart failure	Increased collagen expression Other mechanisms?
Thyroid hormones		
(thyroxine, triodothyronine)	Tachycardia Positive inotropic effect Increased cardiac output Cardiac hypertrophy Proarrhythmic	Altered Ca^{2+} homeostasis
Cytokines		
Interleukin-1β	Negative inotropic effect Cardiac myocyte death	↑ Nitric oxide synthase expression Apoptosis
Interleukin-2	Negative inotropic effect	↑ Nitric oxide synthase expression
Interleukin-6	Negative inotropic effect	↑ Nitric oxide synthase expression
Interferon-γ	Cardomyopathy Proarrhythmic	↑ Nitric oxide synthase expression Altered ion homeostasis

Table 1 Continued

Agents	*Cardiotoxic manifestations*	*Proposed mechanisms of cardiotoxicity*
Tumor necrosis factor-α	Negative inotropic effect Cardiac myocyte death	↑ Nitric oxide synthase expression ↑ Sphingosine production ↓ Ca^{2-} transients Apoptosis
Cardiotoxicity of selected industrial agents		
Solvents		
Toluene (paint products)	Proarrhythmic	↓ Parasympathetic activity ↑ Adrenergic sensitivity Altered ion homeostasis
Halogenated hydrocarbons		
(carbon tetrachloride, chloroform, chloropentafluoroethane, 1,2-dibromotetra-fluoromethane, dichlorodifluoromethane, *cis*-dichloroethylene *trans*-dichloroethylene dichlortetrafluorethane, difluoroethane, ethyl bromide, ethyl chloride, fluorocarbon 502, heptafluoro-1-iodo-propane 1,2-hexafluoroethane, isopropyl chloride, methyl bromide, methyl chloride, methylene chloride, monochlorodifluoroethane monochlorodifluoromethane, octafluorcyclobutane, propyl chloride, 1,1,1-trichloroethane, trichloroethane, trichloroethylene, trichlorofluoromethane, trichloromonofluoroethylene trichlorotrifluoroethane, trifluoroiodomethane, trifluorobromomethane)	Proarrhythmic Negative inotropic effect Decreased cardiac output	↓ Parasympathetic activity ↑ Adrenergic sensitivity Altered ion homeostasis Altered coronary blood flow
Ketones		
(e.g., acetone, methyl ethyl ketone)	Proarrhythmic	↓ Parasympathetic activity ↑ Adrenergic sensitivity Altered ion homeostasis
Heavy metals		
(cadmium, cobalt, lead)	Negative inotropic effect Cardiac hypertrophy Proarrhythmic	Complex formation, Altered Ca^{2+} homeostasis
(barium, lanthanum, manganese, nickel)	Proarrhythmic	Ca^{2+} channel blockade

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therapeutic agents and related compounds is presented in **Table 2**. Neither the contents of this chapter nor the material in the tables is intended to be exhaustive or fully inclusive. The agents noted here serve as examples of cardio toxic and vasculotoxic agents. There are detailed chapters in specialty texts that discuss these and other agents more fully. A brief bibliography plus mention of some websites with excellent graphics of the CVS are presented at the end of this chapter.

The Heart

The Heart as a Pump

The mammalian heart (**Figure 1**) can generally be viewed as two side-by-side (left and right) pumps

Table 2 Vasculotoxic agents

Agents	*Sources*	*Prominent vascular effects*	*Associated diseases*
Industrial and environmental agents			
Allylamine	Synthetic precursor	Bioactivation of parent compound by amine oxidase to acrolein and hydrogen peroxide results in smooth muscle cell injury; intimal smooth muscle cell proliferation in large arteries	Atherosclerosis
β-Aminopropionitrile		Damage to vascular connective tissue; aortic lesions; atheroma formation, aneurysm	
Boron		Hemorrhage; edema; increase in microvascular permeability of the lung	Pulmonary edema
Butadiene	Synthetic precursor	Hemangiosarcomas in several organs	
Carbamylhydrazine		Tumors of pulmonary blood vessels	Cancer
Carbon disulfide	Fumigant/solvent	Microvascular effect on ocular fundus and retina; direct injury to endothelial cells; atheroma formation	Coronary vascular disease Atherosclerosis
Chlorophenoxy herbicides			Hypertension
Dimethylnitrosamine		Decreased hepatic flow; hemorrhage; necrosis	Occlusion of veins
Dinitrotoluenes	Synthetic precursor		
4-Fluoro-10-methyl-12-benzyanthracene		Pulmonary artery lesions; coronary vessel lesion	
Glycerol		Strong renal vasoconstriction	Acute renal failure
Hydrogen fluoride		Hemorrhage; edema in the lungs	Pulmonary edema
Hydrazinobenzoic acid	Constituent of *A. bisporus*		
Paraquat		Vascular damage in lungs and brain	Cerebral purpura
Polycyclic aromatic hydrocarbons	Environmental tobacco smoke		
Pyrrolidine alkaloids		Pulmonary vasculitis; damage to vascular smooth muscle cells; proliferation of endothelium and vascular connective tissue in the liver	Pulmonary hypertension; hepatic venoocclusive disease
Organophosphate pesticides			Cerebral arteriosclerosis
T-2 toxin	*Fusarium* mycotoxin		
Vinyl chloride		Portal hypertension; tumors of hepatic blood vessels	Cancer
Gases			
Auto exhaust		Hemorrhage and infarct in cerebral hemispheres; atheroma formation in aorta	Atherosclerosis
Carbon monoxide	Environmental	Damage to intimal layer; edema; atheroma formation	Atherosclerosis
Nitric oxide		Vacuolation of arteriolar endothelial cells; edema, thickening of alveolar-capillary membranes	Pulmonary edema
Oxygen		Vasoconstriction – retinal damage; increased retinal vascular permeability – edema; increased pulmonary vascular permeability – edema	Blindness in neonate; shrinking of visual field in adults; edema
Ozone		Arterial lesion in the lung	Pulmonary edema
Therapeutic agents and related compounds			
Antibiotics/antimitotics			
Cyclophosphamide		Lesions of pulmonary endothelial cells	
5-Fluorodeoxyuridine		GI tract hemorrhage; portal vein thrombosis	
Gentamicin		Long-lasting renal vasoconstriction	Renal failure
Vasoactive agents			
Amphetamine		Cerebrovascular lesions secondary to drug abuse	Disseminated arterial lesions similar to periarteritis nodosa
Dihydroergotamine		Spasm of retinal vessels	
Ergonovine		Coronary artery spasm	Angina
Ergotamine		Vasospastic phenomena with and without medial atrophy	Gangrene of the thrombosis; peripheral tissues

Table 2 Continued

Agents	*Sources*	*Prominent vascular effects*	*Associated diseases*
Epinephrine		Peripheral arterial thrombi in hyperlipemic rats	Participates in thrombogenesis
Histamine		Coronary spasm; damage to endothelial cells in hepatic portal vein	
Methysergide		Intimal proliferation; vascular occlusion of coronary arteries	Coronary artery disease
Nicotine	Tobacco	Alteration of cytoarchitecture of aortic endothelium; increase in microvilli	
Nitrites and nitrates		'Aging' of coronary arteries	Repeated vasodilation
Norepinephrine		Spasm of coronary artery; endothelial damage	
Metabolic affectors			
Alloxan		Microvascular retinopathy	Diabetes; blindness
Chloroquine		Retinopathy	
Fructose		Microvascular lesions in retina	Diabetes-like condition
Iodoacetates		Vascular changes in retina	
Anticoagulants			
Sodium warfarin: warfarin		Spinal hematoma; subdural hematoma; vasculitis	Uncontrolled bleeding; hemorrhage
Radiocontrast dyes			
Metrizamide; metrizoate		Coagulation; necrosis in celiac and renal vasculature	
Cyanoacrylate adhesives			
2-Cyano-acrylate-*n*-butyl		Granulation of arteries with fibrous masses	
Ethyl-2-cyanoacrylate		Degeneration of vascular wall with thrombosis	
Methyl-2-cyanoacrylate		Vascular necrosis	
Miscellaneous			
Aminorex fumarate		Intimal and medical thickening of pulmonary arteries	Pulmonary hypertension
Aspirin		Endothelial damage, gastric erosion obliteration of small vessels, ischemic infarcts	
Cholesterol; oxygenated		Atheroma formation; arterial damage	Atherosclerosis derivatives of cholesterol: noncholesterol steroids
Homocysteine		Increase of vascular fragility, loss of endothelium, proliferation of smooth muscle cells promotion of atheroma formation	Atherosclerosis; synthesis
Oral contraceptives		Thrombosis in cerebral and peripheral vasculature	Thromboembolic disorders
Penicillamine		Vascular lesion in connective tissue matrix of arterial wall, glomerular immune complex deposits, inhibits synthesis of vascular connective tissue	Glomerulonephritis
Talc and other silicates		Pulmonary arteriolar thrombosis, emboli	
Tetradecylsulfate Na		Sclerosis of veins	
Thromboxane A2		Extreme cerebral vasoconstriction	Cerebrovascular ischemia
Vitamin D	Dietary		

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that are joined together and pump in a simultaneous rhythm. Each pump, however, propels blood through a different pipeline circuit. The pumps each consist of two connecting chambers – an atrium and a ventricle – which contract in sequence to provide the pumping action. The ventricles, which are responsible for pumping the blood through the circuits, have thick muscular walls and are located beneath the thinner-walled atria, which function primarily as reservoirs for blood between the heart's contractions. To ensure one-way flow through the CVS, the heart is equipped with specialized valves. The atrioventricular valves prevent backflow of blood into the atria during ventricular contraction (systole), and the aortic/pulmonic (semilunar) valves prevent backflow of blood into the ventricles during

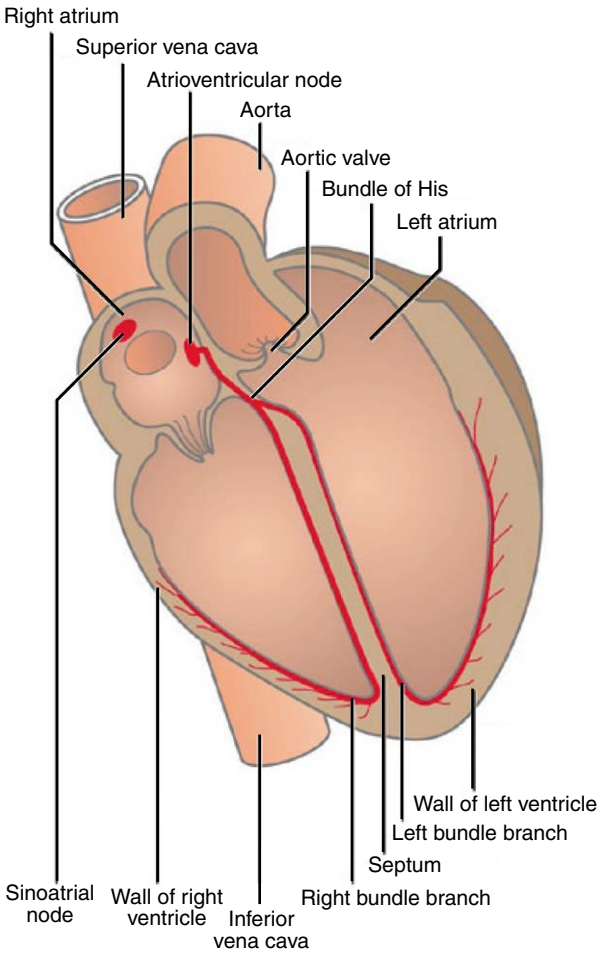

Figure 1 The conducting system of the heart. (Reprinted with permission from fleshandbones.com)

ventricular relaxation (diastole). During systole, the two ventricles develop pressure and eject blood into the pulmonary artery and aorta. At this time the atrioventricular valves are closed and the semilunar valves are open. The semilunar valves are closed and the atrioventriular valves are open during diastole. The right atrium receives blood flowing from the systemic venous system via the superior and inferior venae cavae. This blood initially passes passively through the right atrioventricular orifice into the right ventricle. An atrial contraction then propels a slight additional amount of blood into the right ventricle. A ventricular contraction closes the atrioventriular valve allowing blood now to be propelled past the pulmonic valve into the pulmonary circuit. As blood flows through the pulmonary vasculature, carbon dioxide in the venous return blood is exchanged for oxygen so that the blood pumped through the next (systemic) circuit to the rest of the body will be properly oxygenated. The left atrium receives freshly oxygenated blood from the pulmonary vasculature via the pulmonary vein. Again, blood passively traverses the atrioventricular orifice until an atrial contraction provides complete filling of the ventricle and closes the atrioventricular valve. The strong contraction of the thick-muscled left ventricle now opens the aortic valve, allowing blood to access the systemic circulation (under relatively high pressure) via the aorta. In the absence of injury and/or disease, the heart is a very efficient, durable, and reliable pump. In the 80 year life span of a person, and at a contraction rate of 72 beats per minute, a heart will beat $\sim 3\,000\,000\,000$ times. Two major features of the heart contribute to its unique characteristics: the nature of the heart muscle and the specialized electrical conduction system of the heart.

Cardiac Muscle

There are three distinct types of muscle tissue in vertebrates: striated, smooth, and cardiac. Striated, or skeletal, muscle is attached, at least at one end, to the skeleton via tendons. This muscle type is often referred to as the voluntary muscle, as it can be consciously controlled. Smooth muscle is usually arranged in sheets or layers in tubular systems, such as arteries and veins (see Blood Vessels), the gastrointestinal and respiratory tracts, and the genitourinary tracts. The activities of the smooth muscles are not under conscious control; rather they are coordinated by the autonomic (involuntary) nervous system. The cardiac muscle comprises the bulk of the heart wall proper; and small amounts are found in the superior vena cava and pulmonary vein. The cardiac muscle is not under conscious control; it has an automaticity center which responds to the autonomic nervous system when needed (see section Impulse Conduction). In the heart, cardiac muscle cells are joined in a network of fibers and are connected by gap junctions, which facilitate the conduction of electrical impulses through the cardiac muscle network. In addition to the typical cardiac myocytes, there are other cardiac muscle cells that are specialized to initiate, attenuate, or accelerate the electrical impulses for coordinated contraction of the cardiac network.

Impulse Conduction

The specialized electrical conduction system of the heart allows for the synchronous contraction of the left and right sides of the heart and the sequential contraction of the atria and ventricles (**Figure 1**). Electrical impulses most quickly arise in

the spontaneously-firing cells of the sinoatrial (SA) node commonly called the 'pacemaker'. The SA node is located at the junction of the superior vena cava and the right atrium; therefore, a wave of depolarization (see below) originating at the SA node is conducted first to the cells of the right atrium, then to the cells of both atria, finally converging on a second group of specialized cells – the cells of the atrioventricular (AV) bundle. AV bundle cells act as a conduit for the original impulse from the SA node to the AV node, which lies at the junction of the median wall of the right atrium and the septum separating the two ventricles. From the AV node, the impulse wave next passes into the ventricular conduction system – the bundle of His and Purkinje fibers – located within the ventricular septum, which allows for depolarization of ventricular muscle.

If a microelectrode is inserted into a resting muscle or nerve cell (termed 'excitable tissue'), an electrical potential difference will be recorded across the membrane of that cell. In the case of cardiac muscle cells, this resting potential is $-90\,\text{mV}$ (intracellular relative to extracellular). In other words, the cell membrane is electrically polarized with the inward facing surface of the membrane having a net negative charge with respect to the outer facing surface of the membrane. This polarity is maintained primarily by the presence of extracellular, positively charged ions and intracellular negatively charged proteins. The flux of ions through active (requiring cellular energy) and passive (concentration-driven) processes is responsible for changes in electrical potential. In the resting cardiac muscle cell, the concentration of potassium ions (K^+) is higher inside the cell than outside, while sodium ions (Na^+) are at a much higher concentration outside the cell than inside. Cellular energy is required to maintain the appropriate resting state distributions of the different ions across the cell membrane. In the case of potassium and sodium ions, there is a cell membrane pump, which requires energy derived from the hydrolysis of the terminal phosphate group from adenosine triphosphate (ATP). The associated enzyme responsible for this hydrolysis is the sodium–potassium ATPase. When an electrical stimulus is received by a cardiac muscle cell, voltage-gated channels in the cell membrane open allowing sodium to diffuse down its concentration and electrical gradient into the cell. This influx of positive charge causes the cell membrane to become 'depolarized' (i.e., to have less negative charge). As depolarization proceeds, the membrane may reach the threshold potential ($\sim -70\,\text{mV}$ for most cardiac muscle cells). Any further depolarization results in a phenomenon known as the action potential, which completely depolarizes the cell. At the peak of the action potential, the inside of the cell actually becomes positive relative to the outside ($+30\,\text{mV}$). The cell membrane then repolarizes relatively slowly and reaches the $-90\,\text{mV}$ resting potential before it can respond to another electrical impulse. The wave of depolarization moves very rapidly across the membrane of an individual cardiac muscle cell. In addition, the wave of action potentials is propagated to adjacent cells via the specialized gap junctions. This propagation allows for the complete depolarization of most cells in the network, thus initiating the contraction of the heart muscle as a group.

Cardiac muscle cells predominantly display a fast response action potential (**Figure 2**), and cells in the atria and ventricles exhibit a rapid conduction velocity due to the gap junctions. The depolarization–action potential–repolarization process is divided into five phases. Phase 0 begins when the threshold potential has been reached. At this time, many 'fast' sodium channels in the cell membrane open allowing an inrush of sodium ions to initiate the action potential. At the end of phase 0, the cell is completely depolarized. Toward the end of phase 1 and the start of phase 2, the sodium influx begins to decrease, as does the membrane potential. During the relatively long (200–300 ms) phase 2 plateau, calcium and sodium ions enter through 'slow' membrane channels. Movement of ions through these 'slow' channels only takes place after the membrane potential has dropped to $\sim -55\,\text{mV}$, that is, after the 'fast' sodium ion current has ceased. While these 'slow' inward currents occur, there is also a slow outward movement of potassium ions which keeps the plateau relatively steady. The calcium influx of phase 2 triggers the process known as excitation–contraction coupling, in which the myosin thick filaments slide past the thin actin filaments in the contractile unit of the muscle known as the sarcomere. This process requires energy and involves activation of a myosin ATPase that hydrolyzes ATP. The released energy is utilized to form cross-bridges between the actin and myosin molecules. Both the velocity and the force of contraction are dependent on the amount of calcium ions that reaches the site of contraction. Within the resting muscle cell, calcium is sequestered in a compartment called the sarcoplasmic reticulum. During the action potential, calcium and sodium ions that enter the cell cause depolarization of the sarcoplasmic reticulum membrane, resulting in the release of large amounts of calcium which are needed for effective contraction of the sarcomere. Between contractions, calcium is once again sequestered in the sarcoplasmic reticulum so that the actin–myosin interaction is not overly prolonged. During the long

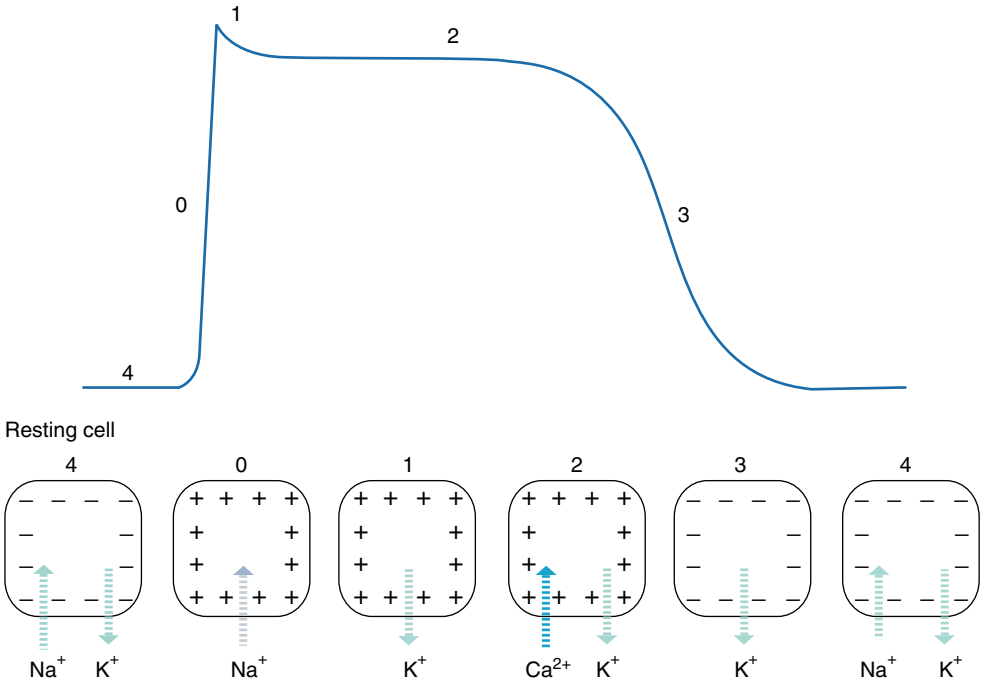

Figure 2 The principal ionic movements during the different phases of the action potential in a cardic muscle cell. (Reprinted with permission from fleshandbones.com)

duration of the plateau phase, a new action potential cannot be initiated because the 'fast' sodium channels are inactivated or refractory to further electrical stimulation. During phase 3, membrane permeability to potassium increases and the 'slow' calcium and sodium channels become inactive. The ensuing efflux of potassium ions allows for repolarization of the membrane until the normal resting potential is reached (phase 4).

In contrast, conduction velocity is slow in muscle fibers at the SA and AV nodes. Unlike the majority of cardiac muscle cells, these pacemaker cells have an unstable resting potential (~ -60 mV) due to a cell membrane alteration that allows sodium ions to leak into the cell without a concurrent potassium ion efflux. This sodium leakage reduces the membrane potential allowing even more sodium ions to move into the cell. In addition to the inward sodium movement, there is also an inward calcium flow which causes the pacemaker cells to have a more positive resting potential. Finally, the cell produces an action potential at ~ -40 mV. This phenomenon is called spontaneous diastolic depolarization. The overall effect is that pacemaker cells initiate waves of depolarization that move across the heart causing the muscle to contract. As noted previously, this phenomenon occurs ~ 72 times per minute (more or less depending on autonomic nervous system stimulation, periods of stress, or physical activity). The waves of electrical activity may be recorded in an electrocardiogram (ECG), which displays the net electrical changes relative to where the recording electrodes are placed on the surface of the body.

Intrinsic Modulators of Cardiac Activity

The heart responds constantly to hormonal and nervous system signals. Sympathetic nervous system terminals releasing norepinephrine are found in cardiac cells of the atria and ventricles. This allows for reflex regulation of heart muscle contractility. Sympathetic innervation is also present to the SA node and AV junction, where norepinephrine release acts to increase heart rate (enhanced phase 4 depolarization) and also to increase conduction velocity by reducing the AV junction impedance to conduction. Parasympathetic innervation is provided by cranial nerve 10, the vagus nerve, to the SA node and the AV junction. These fibers release acetylcholine, which slows SA node activity (decreasing the rate of phase 4 depolarization) and decreases conduction throughout the AV junction.

A practical example of normal nervous system regulation of cardiovascular activity is the processes of blood pressure regulation. Pressure receptors in the carotid sinus and aortic arch sense arterial wall

stretching. These receptors send impulses to the cardiovascular regulatory sensors in the medulla where reflex impulses are generated via the vagus nerve resulting in decreases of heart rate, peripheral vascular resistance, and thus decreased venous return. This results in a decreased blood pressure. Conversely, a decrease in blood pressure will decrease vagal stimulation in favor of sympathetic input. The sympathetic reflex is characterized by increases in heart rate, myocardial contractility, venous return, peripheral vascular resistance, and cardiac output. In addition, the sympathetic response can be produced by the release of naturally occurring catecholamines (epinephrine and norepinephrine) from the medulla of the adrenal gland.

Pathologic Changes in the Heart

The major pathologic changes that occur in the heart are associated with effects on heart rate, contractility of heart muscle, or electrical conduction. Regarding heart rate changes, an arrhythmia, as the name indicates, is a loss of rhythm and here refers to an irregularity of the heartbeat. Two of the more common forms are tachycardia, which is an abnormally rapid heart beat, and fibrillation, which is a rapid twitching of the muscle fibrils. Either of these can occur in the atria or the ventricles. Agents that alter ion levels and fluxes and thereby alter aspects of impulse transmission can produce arrhythmias. The most common site of arrhythmic impulse generation is the SA node. If depolarization after an action potential is accelerated or delayed anywhere within the heart, an aberrant action potential can be triggered and result in an arrhythmia.

Another set of pathologic changes is associated with effects on the force of contraction. The heart muscle exhibits a higher rate of oxygen consumption and a greater energy requirement than many other tissues. Thus, impaired contraction can result from interference with any of the major cycles critical for proper energy metabolism or from processes that interfere with delivery or utilization of the optimum levels of oxygen. For example, if blood flow through the coronary arteries is occluded, as occurs during atherosclerosis, there will be decreased delivery of oxygen to the heart muscle. When this occurs acutely a myocardial infarction, commonly referred to as an MI, may result leading to devitalization of a segment of the heart musculature. Even if death does not occur, there will likely be a decrease in the force or efficiency of contraction of the heart muscle. 'Recreational' use of psychoactive drugs (e.g., amphetamines, cocaine) can result in profound and sudden cardiovascular responses including increases in blood pressure and heart rate due to acute catecholamine release in response to the drugs. These effects can be life threatening in individuals with underlying, and possibly previously unknown, cardiovascular problems including coronary artery disease, high blood pressure, or cerebrovascular disease.

Cells with high energy requirements, such as heart cells, have large numbers of organelles called mitochondria, which produce and supply ATP. Enzymes are organic catalysts that interact with specific substrate molecules to help speed up chemical reactions. The ATPases are enzymes that catalyze the hydrolysis of ATP with its attendant release of energy, which is made available for cellular processes. The myosin ATPase involved in muscle contraction was mentioned above and ATPases involved in the energy-driven pumping of ions including sodium, potassium, and calcium were mentioned above and are noted again below. During oxidative metabolism of organic substrates, the process of electron transport to molecular oxygen in mitochondria is coupled to oxidative phosphorylation, which yields ATP. Some poisons and anticancer drugs, such as cyanide and doxorubicin, interfere with electron transport and/or uncouple phosphorylation. This causes a direct decrease in the amount of energy available to the heart muscle and results in reduced contractility.

As noted above, the inward calcium ion movement is vital for the contraction of the cardiac muscle. This inward movement is blocked by calcium antagonists, such as cobalt and barium, and is stimulated by catecholamines. Increased calcium influx leads to increases in the intracellular level of cyclic AMP, a compound that helps mediate numerous metabolic responses within cells. This, in turn, leads to increased availability of calcium ions for interaction with the contractile proteins. The same effect can be achieved by increased levels of free calcium ions outside of the cells or increased levels of cyclic AMP within cells, as is seen with the vasodilating drug papaverine. Another mechanism for increasing intracellular calcium levels in cardiac cells involves the cardiac glycoside drugs, for example, digitalis from the foxglove plant. This drug inhibits the ATPase that pumps sodium ions out of cells. This results in elevation of sodium ion levels inside the cell, which in turn leads to increases in intracellular calcium ion levels and therefore increased rate and strength of contraction. Toxins that increase the permeability of the cardiac muscle cell membrane to the sodium ion, for example, the marine compound, ciguatoxin or the Columbian frog poison active agent, batrachotoxin, have a similar effect. On the contrary, agents that decrease membrane permeability to sodium ions

will depress myocardial contractility. Included here are a diverse group of compounds including tetrodotoxin, from the Japanese pufferfish; the shellfish-derived poison, saxitoxin; and polyethylene glycol, the active ingredient in many antifreeze preparations. Local anesthetics such as lidocaine and procaine depress the fast inward sodium ion current, the slow inward current, and the potassium ion outward current. They tend to slow the heart rate and the force of contraction; thus, they are commonly used as anti-arrhythmic drugs.

Drugs prescribed to alleviate one set of medical problems can have striking and sometimes fatal effects on the cardiac system. Antipsychotics derived from phenothiazine, including chlorpromazine, depress myocardial contractility and cardiac output. Chlorpromazine can also impair cardiac reflex mechanisms and cause a focal myocardial necrosis. Cyclophosphamide, an anti-cancer agent, also causes myocardial necrosis as well as changes in ECG patterns. Another anticancer agent, adriamycin (doxorubicin), can produce cardiomyopathies with subsequent congestive heart failure. Severe dysrhythmias and some cases of sudden death have been reported. Overdoses of the tricyclic antidepressants, for example, amitryptaline, can result in severe cardiotoxicity, probably due to anticholinergic activity. At high doses, the antidepressant imipramine will depress contractility, lower heart rate, and depress cardiac output. Cardiac arrest may also occur. Some antibiotics, including gentamycin and neomycin, depress calcium ion uptake and therefore reduce contractility of the cardiac muscle. Although the sympathetic system transmitters, the catecholamines, are essential for maintenance of normal myocardial contractility, it has been long recognized that when administered at higher than normal levels for extended periods of time, they can lead to severe myocardial necrosis.

Profound cardiotoxic responses can result from inhalation of a number of halogenated alkanes. These are low molecular weight hydrocarbons with some or all of the hydrogen atoms being substituted by halogens, usually chlorine or fluorine. These agents depress heart rate, contractility, and electrical conduction. The effects are generally more pronounced as the number of halogen atoms increases. Some of these compounds have the additional and profound effect of sensitizing cardiac muscle cells to catecholamines. In humans without pre-existing cardiac disease, the effects of most of these compounds are reversible, although chronic exposure may cause some irreversible damage. As would be expected, the older halogenated hydrocarbon anesthetics such as halothane and enflurane had similar effects.

In contrast, low-pressure fluorocarbons, such as trichlorofluoromethane, can be particularly toxic. In most cases, the levels generally encountered in the environment are too low to have any major lasting effect and even at relatively high levels (up to 15%) fatalities are rarely recorded. However, at levels much above this, for example, over 20%, tragic results can ensue. Among people who inhale these agents from closed bags to 'get high', fatalities can result because the levels of these agents in the bags can reach 35–40%.

There are compounds that interfere with the regular activity of calcium ions in cardiac cells, either by replacing them (as is the case with a number of heavy metals) or by altering the flux of calcium ions across the cell membrane. Among metals, lanthanum, manganese, and nickel all block calcium channels in the cell membrane. Both barium and cobalt ions antagonize endogenous calcium ion levels and tend to shorten the action potential. Lead ions have multiple effects, including displacement of calcium and interference with calcium ion availability, energy metabolism, and ATP synthesis in heart muscle cells. Among organic chemicals, the opium derivative, papaverine, also blocks slow calcium ion channels. Cobra venom cardiotoxin and bacterial endotoxin both interfere with calcium ATPase activity and endotoxin also depresses calcium uptake by heart muscle cells.

Agents Causing Morphologic Changes

A number of cardiotoxic compounds have been listed to this point, including some that interfere with sodium/potassium ATPases; increase sodium or calcium influx; or depress myocardial function by replacing calcium, decreasing sodium permeability, or altering contractility. These agents produce toxic responses in the heart muscle often resulting in death, but do so without causing any major morphologic changes in the heart. Other cardiotoxic compounds produce characteristic morphologic lesions in the heart muscle. There are a few basic types of such pathologic alterations. The first is toxic myocarditis. Chemicals which produce this effect cause cell damage and, ultimately, cell death. Whether or not they produce damage acutely or chronically is generally a function of the dose of the toxic agent. The acute form is characterized by edema, that is, accumulation of excess fluid, as well as inflammatory cell responses and multiple regions of cardiac cell death. However, the inflammatory response will be attenuated or may even be absent if the toxic agent suppresses the immune system, for example, drugs given to prevent rejection of transplanted organs.

The second type of major morphologic alteration in the heart arises from a sudden insufficiency or local arrest of the blood supply to the heart that can result in necrosis of a region of the heart. This condition is called a myocardial infarction (MI). In advanced arteriosclerosis, occlusion of the major arteries supplying the heart muscle with blood can result in an MI. Even in the absence of arteriosclerosis, MIs can result, for example, from amphetamine abuse, which produces severe inflammations of critical arteries (i.e., an arteritis). Intravenous drug use can cause infective endocarditis (an inflammation of the internal lining of the heart), which can lead to vessel occlusion with an embolus, thus resulting in an infarction. Cocaine abuse can result in ventricular tachycardia (i.e., rapid heart beat) and fibrillation, MI, and sudden death. At higher doses, cocaine can increase the levels of catecholamines, ultimately resulting in increased calcium ion activity, accelerated heart beat, arrhythmias, etc. Chemicals that antagonize calcium ion movement through calcium-specific membrane channels prevent the ventricular arrhythmias induced by cocaine. Gross MI can result from toxic exposures to carbon monoxide, nitrates, ergot derivatives, and some potent anticancer drugs (see above).

Recent media attention and FDA guidelines have focused on the cardiovascular effects of appetite-suppressant drugs and nutraceuticals or herbal medicines. In 1997, the anti-obesity drugs fenfluramine and dexfenfluramine were withdrawn from the United States sales market due to convincing correlations made between drug usage and cardiac valvular abnormalities. Since then, the deleterious morphologic effects of these drugs have been described as valvular encasement and/or endocardial fibrosis. These lesions can have life-threatening consequences, including progressive aortic valvular regurgitation. The mechanism underlying the pathogenesis of these lesions remains unclear. There appears to be a correlation with increased serotonin levels in the blood and endocardial fibrosis; thus, there is speculation that these drugs may increase serotonin levels or may increase sensitivity of tissues to serotonin. In addition, the dietary supplement ephedra (ma huang), has been implicated in cases of myocardial infarction due to coronary artery vasoconstriction; thus, in 2004, the FDA issued a warning about ephedra with a proposal to ban its sale and use in the United States.

Another type of gross morphologic lesion in the heart muscle is hypersensitivity myocarditis. This is an inflammatory response that is the most common type of heart disease associated with drug use. There are five primary clinical criteria for diagnosis of this condition: (1) previous use of the drug(s) without deleterious incidents; (2) no apparent relationship between the size of the drug dose and the hypersensitivity response; (3) clinical symptoms consistent with responses to allergens or infectious disease agents; (4) independent confirmation of immunologic responses; and (5) persistence of the symptoms as long as drug use is continued. Histologically, there is infiltration of the heart muscle with numerous types of white blood cells and this is associated with local regions of lysis of the cardiac muscle cells. However, gross fibrosis and extensive regions of myocardial necrosis are usually absent. Among the drugs that have been reported to elicit this response are the antibiotics penicillin, streptomycin, ampicillin, tetracycline, and sulfadiazine.

The Blood Vessels

The second part of the CVS is composed of the blood vessels, which are an extensive series of tubular conduits of varying diameters. All but the narrowest of these vessels have a complex wall structure (see below). One major group of vessels, the arteries, distributes blood under various degrees of pressure to all parts of the body. A second major group of vessels, the veins, returns the blood to the heart. With the exception of the pulmonary artery, which brings blood from the heart to the lungs, the arteries carry blood that is more oxygenated than the blood in their venous counterparts. The large- and medium-sized arteries and veins share the same general structure, although the thicknesses of specific cell layers as well as the cell density within layers can vary considerably.

Blood flow

Despite the system's vital function of transporting blood throughout the body, it would be overly simplistic to view the vascular system as merely a series of pipes of varying diameter. When the left ventricle contracts to deliver blood to the aorta, the largest artery in the body, not only is the blood pressure generated at contraction relatively high, but it is also maintained at a moderately high pressure between contractions of the heart. If the arteries were a set of rigid pipes, the pressure in the artery system would fall to zero between contractions. The fact that this does not occur is due chiefly to the presence of numerous elastic layers (composed of the protein elastin) in the largest arteries. As the heart contracts, the blood pumped into these large arteries causes the elastin in the walls to stretch. Following contraction, the semilunar valves close (see description of valves

above) and the walls of the elastic arteries contract passively to maintain pressure within the system until the ventricles fill and contract once again. There are large, elastic arteries, which function primarily to maintain the pressure within the arterial system during diastole, the resting phase of heart contraction. There are also muscular arteries, which function primarily to distribute the blood throughout the body to organs and tissues, each of which may require different amounts of blood. To help ensure that appropriate volumes of blood are delivered on demand, the size of the lumen (the space through which the blood flows) of the muscular arteries must be regulated quickly and reliably. This is accomplished via innervation by sympathetic fibers of the autonomic nervous system.

Because capillary walls are thin (to permit diffusion) the blood that is delivered to them must be delivered under reduced pressure. This is accomplished by the arterioles, which combine relatively muscular walls with a narrow lumen. The arterial blood pressure is a function of cardiac output and the total peripheral vascular resistance, which is primarily a function of the degree of normal tension (tonus) of the smooth muscle cells in the walls of the arterioles. If this tonus increases above the normal range for extended periods of time, hypertension (high blood pressure) will result. This tonus is under the control of the autonomic nervous system and of adrenergic hormones (catecholamines).

From the capillaries, blood flows first into the narrowest members of the venous system, the collecting venules, and from there into the muscular venules, whose diameter is approximately twice larger than that of the former and whose walls contain one or two layers of smooth muscle cells. Blood then flows into progressively larger veins, first to the small and then to the medium-sized veins. Veins that are located deep within tissue tend to have thinner, less muscular walls than do superficial veins. The final sets of veins to receive blood before it is delivered back to the heart are the inferior and superior venae cavae. The outermost cellular layer in these veins is considerably thicker and the innermost layer is considerably thinner than those of the aorta, the first artery leaving the heart. Another difference between arteries and veins is that the latter have a more extensive vasa vasorum, an arterial blood supply to the vessel wall. Since venous blood is relatively poorly oxygenated, veins require supplemental oxygenation supplied by the vasa vasorum. Because venous blood is under low pressure, the vasa vasorum can penetrate closer to the innermost layer of the vein without being occluded by compressive pressures in the wall.

Pathological Changes

Approximately 90% of the pathologic alterations seen in veins are associated with one of three conditions: deep-vein thrombosis, which often appears following acute MI, thrombotic strokes and/or major surgery; varicose veins, which usually arise secondary to sustained increases of venous pressure; and superficial thrombophlebitis, which occurs in humans with varicose veins as well as in some females after pregnancy. A few venotoxic responses to exogenous (i.e., from outside the body) agents are noted below; however, the great majority of vasculotoxic agents have their effects on the arteries. Therefore, only a description of the artery wall structure is presented below along with listings and selected descriptions of agents that damage the arteries.

Artery Wall Structure

There are three principal cell coats (tunics) that have been identified in the wall of large- and medium-sized arteries (**Figure 3**). The outermost coat, the tunica adventitia, is composed of connective tissue cells plus extensive deposits of the proteins collagen and elastin. The adventitia in muscular arteries is approximately one-half the thickness of the middle coat, the media. In muscular arteries, the media is composed primarily of layers of smooth muscle cells. The principal extracellular protein component is elastin. In elastic arteries the tunica media also predominates, but in this case there are many layers of elastin with smooth muscle cells between the layers. The media is separated from the adventitia by a prominent elastic layer, the external elastic lamina. The adventitia of elastic arteries is thinner than that of muscular arteries. In large arteries a vasa vasorum will also be present. The innermost layer of the artery wall is the tunica intima, which is separated from the media by the internal elastic lamina. In photomicrographs, the inner elastic lamina appears fenestrated. This may serve as a relatively low-resistance pathway for migration of smooth muscle cells into the intima from the media, a process thought to be involved in the development of arteriosclerotic plaques. A single layer of endothelial cells (see below) borders the intima at the lumenal surface.

The media is the most heterogeneous in composition and the most variable in size of the three major coats of the artery. The predominant cell type in the media is the smooth muscle cell. Although some subtle differences both in appearance and behavior have been noted between smooth muscle cells in the intima versus those in the media, it is still not clear whether this is due to the presence of more than one

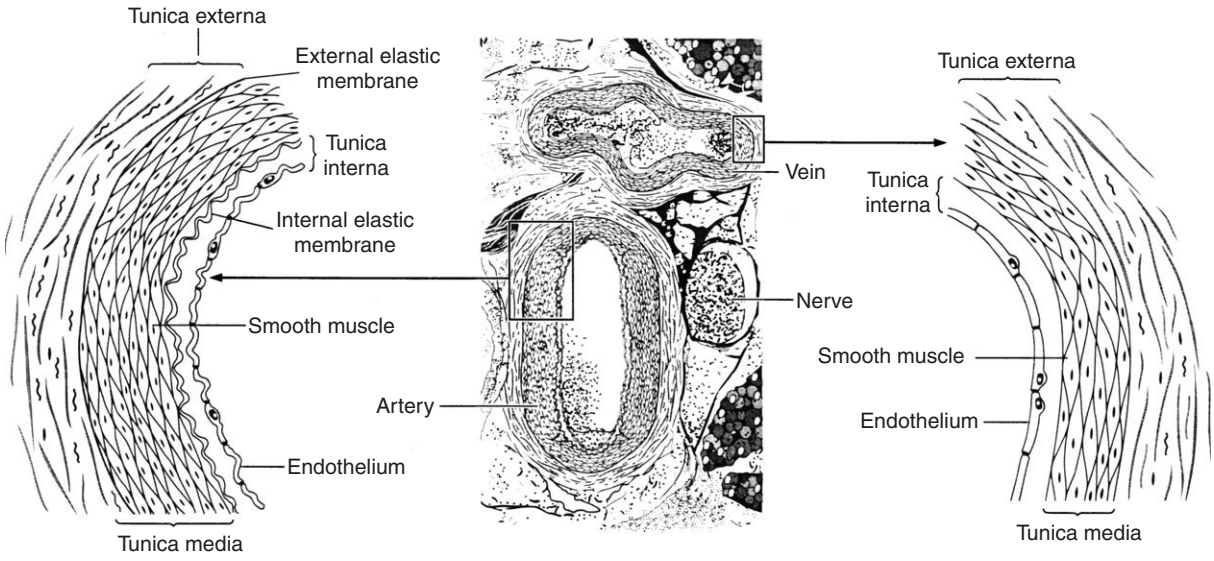

Figure 3 Comparision of typical artery and typical vein. (Reprinted with permission from Cotran R, Kumar V, and Collins T (eds.) (1999) *Robbins Pathologic Basis of Disease*, 6th edn. Philadelphia, PA: Saunders, with permission from WB Saunders.)

type of smooth muscle cell, or to differing microenvironments in these two adjacent regions of the artery wall.

Atherosclerosis is a major cause of death in most industrial societies. The characteristic lesion of this disease, the atherosclerotic plaque, is found in the intima of large- and medium-sized arteries. An additional problem with advanced plaques is that thrombus formation is likely to occur in regions of plaque rupture. The combination of the two events can lead to partial or even total occlusion of major arteries. If this occurs in one or more of the coronary arteries, a serious or even fatal MI may result. A discussion of arteriosclerosis and exogenous agents that can modulate this condition is presented below.

There exist a large variety of compounds, some of which are noted below, that evoke toxic responses within the arterial intima. These compounds are of interest not only because some humans suffer their cardiovascular effects each year but also because an understanding of the mechanism(s) whereby these agents act in living organisms may provide new insights into the complexities of the arterial intima. The existence of agents that can behave as vascular toxins should not be a primary public health concern, with the striking exception of cigarette smoke, discussed below. In fact, the extensive list of vasculotoxic agents listed in textbooks of toxicology notwithstanding, it is clear that if most deaths from heart disease and stroke were due only to those agents, then these two related conditions would quickly cease being the single greatest cause of death in the USA. As it is, there are nearly 900 000 deaths from these two diseases combined, every year. The major and largely avoidable vasculotoxic agent associated with these diseases is tobacco smoke, which is discussed in a subsequent section.

Endothelial Damage

Maintenance of the integrity of the single layer of endothelial cells that lines all of the vascular system is critical for normal vessel function. The intact endothelium is a dynamic system. It acts as a permeability barrier, preventing access of bloodborne contaminants to intimal cells. The intact endothelium also prevents adherence of white blood cells and thrombi; produces and secretes a wide range of growth regulatory molecules; and maintains vascular tone by releasing molecules that modulate dilation and constriction of blood vessels. The endothelium may even participate in its own injury on occasion. Endothelial cells are capable of oxidizing low-density lipoprotein (LDL), which is primarily responsible for transporting cholesterol through the blood to tissues. Oxidized LDL can injure the endothelium directly, produce molecules that allow specific types of white blood cells to adhere to the endothelial surface, and attract inflammatory cells to the inner surface of the artery. Presently, the prevailing view is that these events are critical to the early stages of arteriosclerotic plaque formation. Since the structural and metabolic integrity of endothelial cells is vital to normal arterial function, and since agents causing damage to endothelial cells might be present in

the blood at any time, there must be efficient processes available to repair the endothelium and maintain its integrity should it become damaged.

Blood vessels of similar anatomical structure have distinct responses to chemical stress depending on the organ system with which they are associated. This may be due to subtle differences at the cellular and subcellular levels between similar cells and/or to local responses to different stimuli, for example, due to specific hormone receptors or patterns of innervation. Consider the blood–brain barrier, which prevents many potentially toxic blood-borne agents from reaching the brain. If the metabolic status of the endothelial cells in vessels at the brain level is altered, one result can be a disruption of the tight junctions between the endothelial cells, with a resulting increase in permeability. As a result, the brain, which normally is shielded from a number of toxic agents, may now be exposed to them. Lack of oxygen or markedly reduced local blood flow (ischemia) will lead to swelling of the endothelial cells and a widening of the junctions. Chemicals, including alcohols and surfactants, that solubilize lipids, which are an important component of cell membranes, can also impair the barrier. Lead ions interact with sulfhydryl (–SH) groups that are critical to the functioning of many endothelial cell enzymes and structural proteins. Lead ions thus produce damage to the endothelial cells in blood vessels supplying the brain well before the typically recognized damage to nervous system cells is recognized. Chemicals that raise osmotic pressure, such as solutions of high salt or the alcohol, mannitol, can cause endothelial cells to shrink, thereby causing the tight junctions between the cells to separate.

The liver is the organ largely responsible for detoxification of xenobiotic (foreign biological) chemicals and partly, as a consequence, is also constantly at risk for damage by toxic chemicals. One such chemical, the carcinogen dimethylnitrosamine, first induces the proliferation of endothelial cells, followed by increased formation of vascular connective tissue, and, ultimately, total venous occlusion. Plant toxins of the pyrrolizidine alkaloid family, including monocrotaline, can produce identical effects. Monocrotaline, which enters the body in a non toxic form, is metabolized to its toxic form(s) by the liver. In addition to liver damage, this agent causes structural remodeling of blood vessels in the lung and a resultant increase in pulmonary arterial pressure. This effect is similar to the chronic pulmonary hypertension from which many people suffer.

Metals

A number of metals that cause kidney damage act on arteries supplying this organ. Elevated levels of cadmium are associated with hypertension, at least in animal studies. Cadmium has also been implicated in thickening of the wall of arterioles and deposition of fibrotic tissue in capillaries in the testes as well as the kidneys. Agents that chelate cadmium can reverse many of these effects, as can elevation of body levels of zinc. It appears that cadmium and zinc are antagonistic and that maintenance of a cadmium/zinc ratio within fairly well defined limits may be important in preventing cadmium-associated vessel wall changes.

Three other metal ions that have been implicated in damage to vessel walls are mercury, chromium, and arsenic. Mercury, which interferes with protein –SH groups, may cause vasoconstriction of preglomerular vessels in the kidney.

Arsenic, though an unlikely contributor to blood vessel damage on a worldwide level, represents a striking example of how local environmental alterations can have profound effects on a large portion of a population. On the southwest coast of Taiwan, the artesian well water consumed by the local population has high levels of arsenic and about one out of every 100 people suffer from blackfoot disease. In late stages of this disease, extremities can become gangrenous, leading to spontaneous or surgical amputation of extremities. People suffering from this disease exhibit much higher levels of both peripheral vascular disease and cardiovascular disease. The mechanism of the action of arsenic on the blood vessels remains unclear.

Primary Amines

Cardiotoxicity of primary amines (epinephrine, norepinephrine, isoproterenol) was noted earlier, and has been recognized for nearly 100 years. The vascular toxicity of these and related compounds has also recently been recognized. The effects seem to focus on medial cells of the artery wall, rather than on adventitial or endothelial cells. Early changes include loss of medial cells, mineralization, and loss of elastic fibers. Later there is a compensatory proliferation of intimal cells. The vascular toxicity of two related compounds is particularly striking. One of these compounds, allylamine, will be discussed near the end of this chapter. The second is β-aminoproprionitrile (β-APN), which is the active agent in the toxic sweet pea, *Lathyrus odoratus*. Consumption of flour derived from this plant results in lathyrism, a condition often seen in children and young

adults residing in Algeria, Ethiopia, and parts of India. Sudden death can result because of rupture of aortic aneurysms, which are ballooned and weakened segments of the artery wall. The toxicity of β-APN has been related to its inhibition of an enzyme which normally cross-links collagen and elastin in large elastic arteries, including the aorta, thereby strengthening them.

Atherosclerosis

Arteriosclerosis (literally 'artery hardening') is the general term used to describe thickening and stiffening that can occur for a variety of reasons in arteries of all sizes. From a clinical perspective, the lesion of greatest interest to cardiovascular disease is the atherosclerotic plaque (**Figure 4**). It is the principal lesion associated with human myocardial and cerebral infarction, which are the primary causes of death in the United States, Canada, Europe, and Japan. Plaque development is complex, involving processes as diverse as cell proliferation, cell death, synthesis, and deposition of a variety of extracellular macromolecules (e.g., collagens, elastin, proteoglycans), lipid accumulation and mineralization. The plaque typically appears in the arterial intima with a variety of associated cell types, including smooth muscle cells, macrophages, lymphocytes, platelets, and endothelial cells. Plaque formation has been classified both as a problem of proliferation and one of degeneration, as well as an inflammatory process, a response to injury, and a process related to benign tumor formation. In truth, there is evidence supporting each of these views.

Although in most cases atherosclerosis does not become manifest as a clinically serious condition until well into middle age or beyond, it is a disease that begins early in life. Autopsy studies on US soldiers killed during the Korean War revealed that many already had arterial deposits characteristic of the early stages of atherosclerosis. More recent studies on children through people in the third decade of life have confirmed and expanded these findings. The good news is that while there are genetic factors which may predispose an individual to develop atherosclerosis, there is considerable evidence that individual choices and lifestyle decisions can play a large role in preventing, or at least mitigating, the early onset of clinical symptoms of this disease. Further, results from a limited number of laboratory animal studies suggest that it may even be possible to reverse the clinical course of the disease.

There are three areas where lifestyle modification can have profound effects on moderating development of clinically significant atherosclerosis. In addition to exercise, the two areas most amenable to change are diet and smoking. There is strong epidemiological evidence associating elevated levels of serum cholesterol with increasing risk of atherosclerosis and subsequent heart attacks. As noted above, LDL is primarily responsible for transporting cholesterol and its esters through the bloodstream to the tissues. Oxidized LDL can damage vessel wall cells, including endothelial cells. Oxidized LDL can act as and also generate a chemoattractant, which attracts monocytes to the endothelial surface and possibly helps mediate passage of monocytes across the endothelium where they may differentiate into

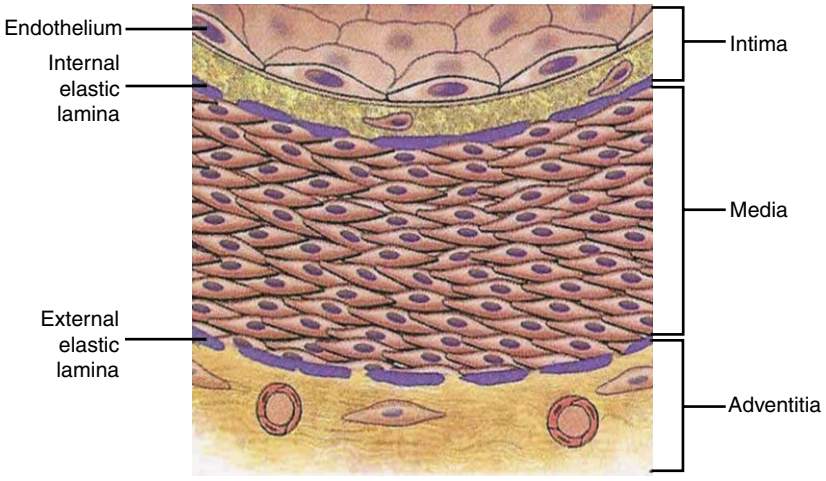

Figure 4 Diagrammatic representation of the main components of the vascular wall. (Reprinted with permission from Cotran R, Kumar V, and Collins T (eds.) (1999) *Robbins Pathologic Basis of Disease*, 6th edn. Philadelphia, PA: Saunders, with permission from WB Saunders.)

tissue macrophages. Monocyte-derived macrophages act as scavengers to help remove harmful molecules such as oxidized LDL. When normal control mechanisms are dysfunctional, macrophages filled with oxidized LDL can become foam cells, which are critical to the formation of early stage atherosclerotic plaques. Studies on experimental animals as well as humans have shown that reduction in levels of plasma cholesterol and LDL can lead to significant widening of the arterial lumen. There is evidence that probucol, a drug originally used for its plasma cholesterol-lowering capability, may function primarily as an antioxidant protecting the integrity of LDL.

Relaxation of blood vessels appears to be at least partially under the control of endothelial cells and their secreted products, especially endothelium-derived relaxation factor (EDRF). Oxidized LDL directly inhibits the endothelial cell-associated vessel relaxation. The generation of increased reactive oxygen species in association with elevated levels of blood cholesterol has also been reported. One of these reactive oxygen species, superoxide (O_2^-), may interact with vasoactive EDRF (nitric oxide) locally in the artery wall, preventing endothelial cell-dependent vasodilation. In addition, a product of the reaction of nitric oxide and superoxide, the reactive peroxynitrite, may act to stimulate lipoprotein oxidation, which, as noted above, is regarded as an early step in atherosclerotic plaque generation.

Oxidants arise from two sources. The first, which is internal, is related to various metabolic processes, including respiration, phagocytic activity to destroy bacteria- and/or virus-infected cells, and, paradoxically, attempts to detoxify foreign substances. In the process of carrying out the latter activity, toxic oxidant by-products can be produced. The second source is external. While the potential protective effects of dietary components and supplements, for example, vitamins, are still being debated, it is reasonable to conclude that decreasing exposure to oxidants from external sources would be beneficial not only in reducing chances of premature atherosclerosis, but also of other diseases, including cancer. By far the most common, avoidable, and dangerous source of external oxidants is cigarette smoke, which is considered a principal contributor to one-quarter of all heart disease cases, one-third of all cancers and $\sim$400 000 premature deaths in the United States every year. As economies of developing countries expand and as cigarette smoking becomes more popular throughout the world, health problems associated with cigarette smoking will increase rapidly.

Cigarette Smoke

Cigarette smoke is composed of active smoke, the smoke coming from the mouth end of the cigarette and breathed in by the smoker; and passive smoke (second-hand smoke; environmental tobacco smoke) which is composed mostly of the smoke coming off the burning end of the cigarette plus a small percentage of exhaled smoke. Active and passive smoke contain many constituents in common, but often in strikingly different concentrations. Among the more than 4000 different chemicals that have been identified in cigarette smoke, prominent candidates that have been considered as vasculotoxic agents include carbon monoxide and various carcinogens. In addition to interfering with transport of well-oxygenated blood, carbon monoxide may cause endothelial cell damage directly, although the mechanism is not clear. Another major class of potential vasculotoxins in cigarette smoke is the carcinogens. Most of these are found in the tar condensate fraction of cigarette smoke. Some, including benzo(*a*)pyrene, are well-known carcinogens that are found in other environmentally prominent substances including coal tar derivatives, charcoal-broiled meat and automobile exhaust. Other smoke carcinogens include the nitrosamines, some of which are tobacco-specific. Both benzo(*a*)pyrene and the parent nitrosamines require metabolic activation to become carcinogenic. The enzymes involved in these processes are members of the cytochrome P-450 system. During the course of detoxifying these agents so that they ultimately can be excreted readily, one or more toxic and possibly carcinogenic metabolites may be generated. Compounds such as benzo(*a*)pyrene induce the appearance of the cytochrome P-450 system enzymes, and smokers are constantly exposed to the P-450 inducers. Generation of endothelial cell-damaging agents during the metabolism of benzo(*a*)pyrene derived from cigarette smoke has been recently proposed , but not proved, as a mechanism to explain the initiation of atherosclerotic plaques. Oxidants derived from cigarette smoke can damage lipids, an important constituent of cell membranes, as well as cellular macromolecules, including DNA. These is no direct evidence that cigarette smoke causes damage to artery wall cell DNA in either living animals or humans; however, if such damage does occur it would provide independent support for the view that DNA alterations are characteristic of atherosclerotic plaques in animal models of the disease as well as in humans. In related experimental animal studies, the chemical allylamine caused both myocardial lesions and vascular fibrosis. Allylamine

toxicity is thought to be mediated via metabolism of this compound to the reactive aldehyde, acrolein, which is also a prominent component of cigarette smoke. Studies with cultured artery wall cells indicate that the primary arterial effect of allylamine is on the smooth muscle cells. Proliferation of intimal smooth muscle cells in response to allylamine exposure results in activation of a specific cellular DNA sequence, the H-ras oncogene, which is implicated in the development of certain forms of cancer. This lends further support to the contention that there may be molecular similarities between the development of the lesions of atherosclerosis and of cancer.

One of the problems researchers have faced in identifying specific health-threatening components of cigarette smoke is that while at moderate to high concentrations many of these agents can be toxic, in many cases the individual concentrations of these factors in cigarette smoke are likely too low to be able to account individually for the toxic and disease-promoting effects of cigarette smoke. The US Environmental Protection Agency sidestepped this problem in 1992 by declaring environmental tobacco smoke, with its thousands of components, to be a human class A carcinogen. The American Heart Association has classified environmental tobacco smoke as an environmental poison and as a major preventable cause of cardiovascular disease. Regarding environmental tobacco smoke, there have been estimates that as many as 60 000 excess heart disease deaths in the United States every year can be attributed directly to involuntary exposure to cigarette smoke. In support of this estimate, a number of laboratories have reported that inhalation of sidestream cigarette smoke accelerates arteriosclerosis in different experimental model systems of the disease. Since epidemiological and autopsy evidence strongly support the view that atherosclerosis begins as early as childhood, the experimental results with environmental tobacco smoke suggest that involuntary exposure of children to tobacco smoke may accelerate plaque development. The insidious nature of involuntary exposure to environmental tobacco smoke is further emphasized by recent findings in a mouse model of atherosclerosis. Male mice exposed only *in utero* to environmental tobacco smoke develop accelerated atherosclerosis as adults, even in the absence of a high fat diet. In the United States, where studies show that many children are less active physically and have poorer diets than children growing up a few of generations ago, involuntary exposure to second-hand smoke may well represent a major additional risk factor for the development of atherosclerosis. Fortunately, extensive epidemiologic evidence from both cancer and heart disease studies indicates that as the time since cessation of smoking increases, the chances of dying prematurely from either disease decrease. Thus, the vasculotoxic effects of cigarette smoke, both active and passive, may be largely reversible.

See also: Amphetamine; Arsenic; Batrachotoxin; Blood; Chemicals of Environmental Concern; Chromium; Cocaine; *hERG* (Human Ether-a-Go-Go Related Gene); Mercury; Tetrodotoxin; Tobacco Smoke.

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Relevant Website

http://www.fleshandbones.com – Pertains to the biomedical sciences including anatomy, physiology, pharmacology and general medicine. Among this website's features is an "Imagebank" containing color images that can be down-loaded.

Castor Bean

Brenda Swanson-Biearman

- Chemical Abstracts Service Registry Number: CAS 9009-86-3
- Synonyms: *Ricinus communis*; Castor-oil plant; Palma christi; Koll; Moy bean; Mole bean; Dog tick seeds
- Chemical/Pharmaceutical/Other Class: Toxalbumins

Uses

Ricin (castor bean) immunotoxin has been developed to attack the CD5 T-cell antigen (present in T-cell and some B-cell malignancies) as well as the interleukin-2 receptor of cancerous tumors. Neurobiological applications of ricin involve the study of brain function via lesioning. It is also used as a reagent for pepsin and trypsin, and as a commercial mole killer. Historically, it has been used as a biochemical warfare agent.

Exposure Routes and Pathways

Ingestion is the most common route, but ocular and dermatologic exposures to castor bean powder have been reported.

Toxicokinetics

The glycoproteins (ricin) are poorly absorbed from the gastrointestinal tract; however, once absorbed, they most likely follow a distribution pattern similar to that of albumin. Many cell surfaces contain receptors specific for the ricin molecules. This molecule consists of two subunits, A and B, bound by a disulfide link. When this link is broken, the B subunit binds to galactose-containing receptors in the cell wall and is transported intracellularly. The A subunit inhibits protein synthesis. The liver, spleen, adrenal cortex, and bone marrow are the primary sites of distribution. The biotransformation and elimination of toxalbumins are poorly understood. The elimination half-life in one patient was 2 days. The reported disappearance of ricin from the plasma is according to first-order kinetics when injected intravenously into mice and human cancer patients.

Mechanism of Toxicity

The principal toxicity of the toxalbumins is protein synthesis inhibition causing hemagglutination within the first hour, adrenal insufficiency, hepatic and renal failures, endothelial damage, and, in severe cases, profound capillary hemorrhage.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity of the castor bean in animals is similar to that in humans.

Human

The toxicity of the castor bean is variable due to erratic absorption patterns. Symptomatology can occur with the ingestion of one seed. The seeds are minimally toxic if the seed coat remains intact when ingested. Acutely, the toxalbumins cause an initial aggregation/sludge formation of red cells within the first hour, severe gastrointestinal lesions, retinal hemorrhages, rapid and weak pulse, and possible shock due to fluid and electrolyte loss from vomiting and diarrhea. Mild to moderate central nervous system depression may be seen. Seizures can occur, but are not common. Fever may be noted. Hepatic damage can occur in large overdoses with increases in alanine aminotransferase, total bilirubin, and aspartate aminotransferase. Elevated serum creatinine and hematuria are often seen. Unlike most toxalbumins, castor beans contain several allergens that can cause severe reactions in the hypersensitive individual. Late-phase complications (2 days postexposure) reflect the cytotoxic effects of the ricin. Patients may be asymptomatic prior to this phase, but damage to the hepatic, central nervous, renal, and adrenal systems may ensue. Laboratory radioimmunoassay is available for ricin (usually not on an emergent basis), but management must be based on symptomatology alone.

Clinical Management

No specific treatment is available for toxalbumin exposure. Aggressive gastric decontamination such as whole bowel irrigation is recommended. It is unlikely

that activated charcoal will be beneficial. Supportive care primarily consists of maintaining appropriate fluid volume and electrolyte balance.

See also: Ricin and Other Toxalbumins.

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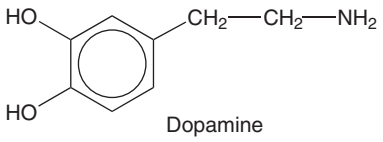
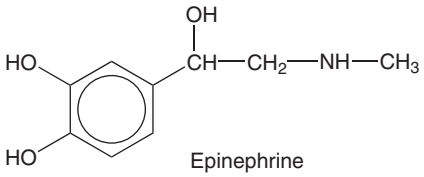
Catecholamines

Zhengwei Cai

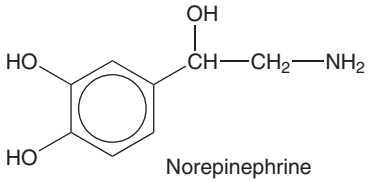
- Description: Catecholamine is the name of a group of compounds that contain a catechol nucleus (a benzene ring with two adjacent hydroxyl substituents) and an amine group. This group includes the mammalian neurotransmitters or hormones, such as dopamine, norepinephrine, and epinephrine, and nonmammalian compounds such as octopamine. Each compound has its own synonyms.
- Representative Chemical: Dopamine
- Chemical Abstracts Service Registry Number: CAS 51-61-6
- Synonyms: Pyrocatechol; 4-(2-aminoethyl)pyrocatechol; 3-hydroxytyramine; 3,4-dihydroxyphenethylamine; 4-(2-aminoethyl)-1,2-benzenediol; Dopastat; Intropin
- Chemical Formula: $C_8H_{11}NO_2$
- Representative Chemical: Epinephrine
- Chemical Abstracts Service Registry Number: CAS 51-43-4
- Synonyms: Benzyl alcohol; Adnephrine; Adrenal; Adrenalin; Adrine; Antiasthmatique; Asthma-Nefrin; Balmadren; Epifrin; Epirenamine; Glaucosan; Hemostatin; Methylaminoethanolcatechol; Renagladin; Renalina; Renostypticin; Soladren; Simplene; Supranefran; Suprarenin; Susphrine; Sympathin; Takamine; Vasoton; Vasotonin
- Chemical Formula: $C_9H_{13}NO_3$
- Representative Chemical: Norepinephrine
- Chemical Abstracts Service Registry Number: CAS 51-41-2
- Synonyms: 4-(2-Amino-1-hydroxyethyl)-1,2-benzenediol; α-(Aminomethyl)-3,4-dihydroxybenzyl alcohol; 2-Amino-1-(3,4-dihydroxyphenyl)ethanol; 1-(3,4-Dihydroxyphenyl-2-aminoethanol; Adrenor; Aktamine; Levarternol; Levonorepinephrine; Levophed; Noradrenaline; Nortrinal; Sympathin E
- Chemical Formula: $C_8H_{11}NO_3$
- Chemical/Pharmaceutical/Other Class: Catecholamines are endogenous neurotransmitters or hormones. Dopamine and norepinephrine are in the monoamine class
- Chemical Structures:



Dopamine



Epinephrine



Norepinephrine

Uses

Catecholamines are sympathomimetic drugs. Dopamine and norepinephrine are used as vasopressors (antihypotensives). Epinephrine is used as a vasoconstrictor, cardiac stimulant, or bronchodilator to counter allergic reaction, anesthesia, and cardiac arrest. It is also an antiglaucoma agent.

Background Information

Catecholamines are endogenous compounds and are synthesized in the brain, the adrenal medulla, and by some sympathetic nerve fibers. The biosynthesis of catecholamines begins with the hydroxylation of tyrosine by tyrosine hydroxylase to form L-dopa, which is decarboxylated by aromatic amino acid decarboxylase to form dopamine. Norepinephrine

is formed from dopamine by the enzyme dopamine β-hydroxylase, and epinephrine is formed from norepinephrine by enzyme phenylethanolamine *N*-methyltransferase. Dopamine is widely distributed throughout the CNS and is involved in the control of movement. Norepinephrine is an important neurotransmitter in both the CNS and the sympathetic part of the autonomic nervous system. The hormone epinephrine acts together with the sympathetic nervous system to initiate the body's quick response to stressful stimuli.

Exposure Routes and Pathways

When used therapeutically, intravenous injection or infusion is the most common route of administration. Epinephrine is available in nebulized racemic dosage form for inhalation.

Intoxication from catecholamine usually results from iatrogenic overdoses, accidental intravenous administration, and the injection of solution intended for nebulization.

Toxicokinetics

Epinephrine is well absorbed after oral administration but is rapidly inactivated in the gut mucosa. When intravenously injected or infused, the onset of drug effect is rapid (within 5 min for dopamine and 3–10 min for epinephrine) and the duration of drug effect is short (10 min for dopamine, 1 or 2 min for norepinephrine, and 15 min to hours for epinephrine depending on route of administration). Exogenous catecholamine in the circulation is rapidly and efficiently taken up by adrenergic neurons. Catecholamine is metabolized by monoamine oxidase, which is localized largely in the outer membrane of neuronal mitochondria, and by catechol-O-methyl transferase, which is found in the cytoplasm of most animal tissues, particularly the kidneys and the liver.

The primary metabolites of dopamine are homovanillic acid and dihydroxyphenylacetic acid (75%) and norepinephrine (25%). The primary metabolites of epinephrine and norepinephrine are vanilylmandelic acid and 3-methoxy-4-hydroxyphenethyleneglycol. Catecholamine metabolites and their conjugates are excreted in urine.

Mechanism of Toxicity

Catecholamines are sympathomimetic drugs. These drugs increase heart rate and cardiac output and may produce cardiac arrhythmias. Administration of norepinephrine also results in increased peripheral vascular resistance. Both effects may cause serious systemic hypertension, which may cause cerebral hemorrhage. Reduced hepatic and renal blood flow may cause tissue ischemia, increased glycolysis, and serum lactic acidosis. In very high doses, a paranoid state may be induced. Production of reactive oxygen species and formation of quinone during the metabolism of dopamine are involved in dopamine toxicity. Recent studies have demonstrated that norepinephrine may enhance or inhibit immune function under certain conditions.

Acute and Short-Term Toxicity (or Exposure)

Animal

Overdose of catecholamines may result in animal death. In test animals, there is evidence that death is the result of respiratory arrest caused by hypertension following overdose of epinephrine.

Human

At high infusion rates of dopamine, ventricular arrhythmias, and hypertension may occur. Nausea, vomiting, and angina pectoris are occasionally seen. Gangrene of the extremities may occur in patients with profound shock given large doses of dopamine for long periods of time. Norepinephrine may cause dose-related hypertension (sometimes indicated by headache), reflex bradycardia, increased peripheral vascular resistance, and decreased cardiac output. High doses of norepinephrine (in excess of 8–12 mg of base per min) cause intense vasoconstriction, which results in 'normal' blood pressure but decreased tissue perfusion. Local necrosis may result from perivascular infiltration and angina, mesenteric ischemia, and peripheral ischemia. Epinephrine may cause dose-related restlessness, anxiety, tremor, cardiac arrhythmias, palpitation, hypertension, weakness, dizziness, and headache. Anginal pain may occur when coronary insufficiency is present. A sharp rise in blood pressure from overdosage may cause cerebral hemorrhage and pulmonary edema.

Chronic Toxicity (or Exposure)

Human

Prolonged use and repeated injection of epinephrine may lead to tolerance and local necrosis. Prolonged use of norepinephrine may cause edema, hemorrhage, focal myocarditis, necrosis of the intestine, or hepatic and renal necrosis. It may also cause plasma volume depletion, which may result in

perpetuation of the shock state or recurrence of hypotension when the drug is discontinued.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Treatment is directed at ameliorating tachycardias, shock, cardiac arrhythmias, systemic hypertension, pulmonary edema, and lactic acidosis. In the case of severe toxicity, administration of a rapidly acting α-adrenergic blocking drug such as phentolamine may be considered.

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CCA-Treated Wood

C Charles Barton and Thomas T Newton

Chromated copper arsenate (CCA) is a chemical mixture registered by the US Environmental Protection Agency (EPA) for use as a wood preservative. It has been demonstrated to protect wood from dry rot, fungi, molds, termites, and other pests that can threaten the integrity of wood products. CCA-treated wood (also known as pressure-treated wood) is most commonly used in outdoor settings. Over 90% of all outdoor wooden structures are made with CCA-treated lumber. Around the home, CCA-treated wood is commonly used for decks, walkways, fences, gazebos, boat docks, and playground equipment. Other common uses of CCA-treated wood include highway noise barriers, signposts, utility poles, and retaining walls.

Untreated wood generally deteriorates within 3–5 years, depending on its exposure to soil and environmental conditions. CCA-treated wood, on the other hand, is relatively strong and long-lasting and maintains its integrity in conditions under which untreated wood would quickly degrade. CCA-treated wood products often retain their structural integrity 10–20 times longer than untreated woods.

In the pressure-treatment process, lumber is loaded into a horizontal cylinder. The cylinder door is sealed, and a liquid solution containing CCA is pumped in. The pressure in the cylinder is then raised, forcing the CCA into the wood. At the end of the process, the excess treatment solution is pumped back to a storage tank for reuse. The CCA solution is toxic. Therefore, it can be applied only by EPA-certified operators. However, wood that has been treated with CCA is not classified as hazardous because the CCA 'fixes' to the wood in a way that makes the chemical insoluble and somewhat leach resistant. Thus, CCA-treated wood is not considered to be a health risk unless burned in fireplaces or wood-stoves.

The arsenic penetrates deeply into the wood and remains there for a long time. However, some of the chemical may migrate from treated wood into surrounding soil over time and may also be dislodged from the wood surface upon contact with skin. The amount and rate at which arsenic leaches, however, varies considerably depending on numerous factors, such as local climate, acidity of rain and soil, age of the wood product, and how much CCA was applied. Interestingly, the leaching occurs more with newer structures and decreases with time.

Since excessive exposure to arsenic can be hazardous to health, precautions should be taken to decrease exposure. Applying a sealant on a regular basis (e.g., one reapplication every other year depending upon wear and weathering) should prevent the migration of arsenic from the wood. One should wash hands thoroughly after contact with treated wood, especially prior to eating and drinking; and ensure that food does not come into direct contact with any treated wood. Furthermore, workers should take certain precautions: wear gloves when handling wood, wear goggles and dust-mask when sawing and sanding, always wash hands before eating, and never burn CCA-treated wood.

During an 8 year investigation, the EPA examined the safety of using and handling CCA-treated wood. None of the EPA's investigations produced any findings showing increased risks of cancer or other toxic effects on humans handling CCA-treated wood.

In 1985, the EPA concluded that the benefits of CCA-treated wood far outweighed any risks. The EPA established modest use precautions, which the treating industry agreed to disseminate in a voluntary consumer-awareness program. The actual exposure levels to arsenic in CCA are considered to be minuscule. In 1990, the Consumer Product Safety Commission (CPSC) measured dislodgeable arsenic in eight samples of CCA-treated wood. In five of the samples, the amount was undetectable. Two other samples yielded small quantities of arsenic. The eighth sample, which yielded the greatest amount of arsenic, was rough-sawn lumber, a material classified by the wood-treatment industry as not acceptable for playground equipment. The CPSC concluded that the amounts of arsenic that people may be exposed to are below the level that may cause a health concern, and deemed it safe. Plants grown in soil touched by CCA-treated wood have the same minuscule exposure levels. For decades, CCA-treated wood has been used commercially near crops in the form of tomato stakes, vineyard supports, banana props, and mushroom trays. No problems have ever been recorded that indicate that the preservative migrates into plants and causes any health effects.

On February 12, 2002, the EPA announced a voluntary decision by industry to move away from using CCA to treat wood used in residential settings. This transition affects virtually all residential uses of wood treated with CCA, including wood used in play-structures, decks, picnic tables, landscaping timbers, residential fencing, patios, and walkways/boardwalks. Effective December 31, 2003, no woodtreater or manufacturer may treat wood with CCA for most residential uses. This decision will facilitate the transition in both the manufacturing and retail sectors to wood preservatives that do not contain arsenic, as well as other alternatives, such as naturally resistant woods and plastic alternatives. EPA does not believe there is any reason to remove or replace CCA-treated structures, including decks and playground equipment. Furthermore, the EPA is not recommending surrounding soils be removed or replaced. Also, CCA-treated wood can be disposed of with regular municipal trash (i.e., municipal solid waste, not yard waste).

See also: Arsenic; Wood Dust.

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Cell Cycle

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The cell cycle is the orderly progression of cells through specific stages during which DNA is replicated and distributed to two daughter cells resulting in cell proliferation. Precise regulation of the passage of cells through this cycle is necessary to assure the maintenance of DNA integrity through multiple generations. Cell cycle regulation also ensures that cell proliferation occurs only under defined conditions in response to growth factors and in the presence of a suitable environment. Loss of cell cycle regulation is a characteristic of cancer.

The cell cycle comprises four stages, which are called G1, S, G2, and M phases (**Figure 1**). S (for DNA synthesis) is the stage in which DNA is duplicated. G1 is the stage immediately prior to S during which the cell prepares for DNA synthesis. M (for mitosis) is the stage in which the cell divides and G2 is the stage preceding M during which the cell prepares for cell division. Two major points of regulation are at the transitions between G1 and S and between G2 and M phases. The progression of cells through late G1/S requires the presence of growth factors. A *restriction point* in late G1 marks the point at which cycle progression becomes growth factor independent. Cells that are actively proliferating progress from M phase back to G1 where preparations for DNA synthesis immediately start anew. Cells that are not actively proliferating are said to be quiescent and are in G0 phase. The entry of cells from G0 into the cell cycle is also a closely regulated step and requires an extracellular stimulus or growth factor. We describe below the critical proteins that have been identified to date that regulate G1/S and G2/M transitions. We emphasize that the cell cycle paradigm is rapidly evolving and expanding and that this description is likely incomplete.

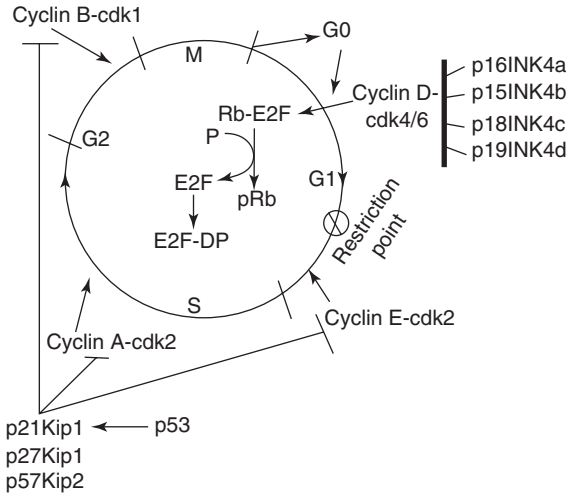

Figure 1 Overview of the different phases of the cell cycle. Quiescent cells are in G0 phase and reenter the cell cycle at G1 during which cells prepare for DNA synthesis. After passing the restriction point in late G1 cells are committed to enter S phase, during which DNA replication occurs. Cells in G2 phase prepare for mitosis (M phase). Cell cycle progression is controlled by various positive and negative cell cycle regulatory proteins including cyclins (A, B, D, E); cyclin dependent kinases (cdk 1, 2, 4, 6); cdk inhibitors (p15, p16, p18, p19, p21, p27, p57), retinoblastoma (Rb) and p53.

Cyclins and Cyclin-Dependent Kinases

Numerous proteins have been identified that stringently regulate the passage of cells at G1/S and G2/M phase transitions. Conserved serine/threonine kinases, called cyclin-dependent kinases (cdks), phosphorylate and activate specific regulatory proteins that drive cell cycle progression. The activity of cdk is controlled at three levels. First, cdks are activated by their interaction with proteins, called cyclins. Cyclins are proteins with very short half-lives of less than 30–60 min. Whereas the cdks are constitutively expressed throughout the cell cycle, the level of the cyclins varies throughout. Cyclin levels are controlled by both regulated synthesis and ubiquitin-mediated proteolysis. Specific cyclin–cdk complexes function at different cell cycle phases. Formation of the heterodimers cyclin D/cdk4, cyclin D/cdk6, and cyclin E/cdk2 are necessary for entry into and progression through G1. The induction of cyclin D family members is provoked by an extracellular signal or growth factor and initiates the entry of quiescent cells from G0 into G1. Cyclin D/cdk heterodimers phosphorylate and inactivate retinoblastoma protein (pRb) causing the release and activation of the E2F family of transcription factors. This family of transcription factors drives transcription of genes necessary for the G1/S transition, including cyclin E. Cyclin E/cdk2 also phosphorylates pRb but unlike cyclin D heterodimers, its activity is mitogen-independent. Both cyclin E/cdk2 and cyclin A/cdk2 drive entry and progression through S phase via the phosphorylation of non-Rb proteins that initiate DNA synthesis. Cyclins A and B form complexes with cdk1 (also called cdc2) and are called the mitotic cylins since these complexes regulate mitosis. Cyclin B/cdk1 controls the G2/M transition. Cyclin B is synthesized as the cell progresses through G2. Upon binding of

cyclin B to cdk1, the activated heterodimer phosphorylates proteins that are involved in mitosis. Activity of the cyclin/cdk complexes is also regulated by phosphorylation/dephosphorylation by cdk activating kinases (CAKs) and phosphatases. A third level of regulation is achieved by control of protein levels of cdk inhibitors. Cdk inhibitors are proteins that accumulate in response to multiple environmental stimuli including DNA damage, hypoxia, cell–cell contact and cytokines, and inhibit the activity of cyclin/cdk heterodimers. The cdk inhibitors include two classes of proteins. The INK 4 proteins, which include $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, and $p19^{INK4d}$, specifically inhibit the activity of cdk4 and cdk6 by competitive inhibition of cyclin D binding to the monomeric kinases. Mutations and deletions of the $p16^{INK4a}$ gene and inactivation by hypermethylation, have been shown to play a role in tumorigenesis in many different types of tumors. The Kip/Cip proteins include three structurally related proteins, p21, p27, and p57. In contrast to the INK4 proteins, the Kip/Cip proteins inhibit most cyclin/cdk heterodimers. Specific Kip/Cip proteins are induced by upstream events. p21 is induced in response to DNA damage and specifically inhibits cyclin E/cdk2. Protein levels of p27 are highest in quiescent cells and induce G1 arrest in response to conditions that typically result in cell quiescence such as growth factor deprivation or contact inhibition. Both the INK4 and the Kip/Cip proteins inhibit the phosphorylation and inactivation of pRb.

Retinoblastoma

The retinoblastoma gene (Rb) was the first tumor suppressor to be identified. Rb mutations were first shown to be causal in familial and sporadic retinoblastoma, a rare tumor of the eye, but have since been associated with many other tumors including osteosarcoma, small cell lung cancer, and prostate and breast cancer. In addition, mutations in the upstream Rb signaling pathway that result in the functional inactivation of the Rb gene product, pRb, are found in virtually all malignancies. Three Rb homologs have been described, including Rb, Rb 107, and Rb 130. All Rb homologs are characterized by a ‘pocket’ domain, which is highly conserved and necessary for pRb’s tumor suppressor function. All the Rb homologs bind viral oncoproteins as well as E2F family members. Binding of viral oncoproteins disrupts the pocket domain of pRb and impairs pRb’s tumor suppressor function. All pRb homologs cause G1 arrest. The primary role of pRb is the inhibition of transcription of genes that mediate passage across the G1/S transition. There are two mechanisms by which pRb inhibits transcription. First, pRb binds to and inhibits the E2F family of transcription factors. The binding characteristics of the homologs vary slightly as, whereas pRb binds preferentially to E2F1-4, p107 and p130 bind preferentially to E2F4 and E2F5. Phosphorylation of pRb regulates its interaction with E2F. The phosphorylation status of pRb fluctuates throughout the cell cycle. Hypophosphorylated pRb is active and binds to E2F family members thus sequestering E2F and inhibiting its transcriptional activity. Hyperphosphorylated pRb is inactive and releases E2F, which results in the transcription of genes that allow the cell to progress to S phase. Upon release from pRb, E2F binds to DP-1 or DP-2 and the resulting heterodimer activates genes necessary for DNA replication. The mechanism by which pRb inhibits E2F transcriptional activity is still debated but it may be via the recruitment of chromatin remodeling enzymes such as histone deacetylases (HDACs) which directly repress transcription by removing acetyl groups from chromatin which causes the chromatin to be less accessible to transcription factors. The role of the pRb-bound HDAC may be to counteract the activity of the E2F-bound acetyltransferase protein, p300/CBP, which transfers acetyl groups to chromatin and enhances transcriptional activity. In addition to its inactivation of E2F resulting in a decrease in transcription of E2F-responsive genes, the complex of pRb and E2F actively represses transcription, which may also be via the recruitment of HDACs to the promoter regions. The regulation of pRb activity is complex. There are 16 possible sites for cdk-mediated phosphorylation and data suggest that phosphorylation at each different site regulates a distinct pRb function. pRb is phosphorylated by multiple cyclin/cdk complexes. Cyclin D/cdk4/6 initiates phosphorylation in early G1 and cyclin E/cdk2 hyperphosphorylates pRb in late G1. Cyclin A/cdk2 maintains phosphorylation of pRb thoughout S phase. pRb may perform other roles in addition to regulation of G1/S including the regulation of apoptosis. A decrease in functional pRb results in the activation of p53-induced apoptosis, which appears to be mediated via the release of E2F1. Free E2F1 activates transcription of ARF (alternate reading frame of the p16INK4a locus), which inhibits a protein called mdm-2 ubiquitin ligase (mdm-2). mdm-2 targets proteins for ubituitin-mediated proteolysis. Since mdm-2 initiates the degradation of p53, its inhibition results in an increase in p53 and a corresponding increase in apoptosis. Thus, a decrease

in functional pRb, which could otherwise result in unchecked cell proliferation, triggers an apoptotic response. A decrease in functional pRb also creates a selection pressure for p53 mutations, since only cells that have mutated dysfunctional p53 survive. Not surprisingly, p53 mutations are often found to coexist with Rb mutations in malignant tumors.

Checkpoints

Checkpoints are surveillance mechanisms comprising numerous genes that detect DNA damage and induce either cell cycle arrest and DNA repair mechanisms, or, in the presence of extensive DNA damage, apoptosis. The data elucidating this surveillance network are very incomplete but have been advanced significantly since the isolation of the mutation that is associated with ataxia telangiectasia (AT). AT is a rare pediatric disease that is associated with immune deficiency and an increased susceptibility to cancer. Prior to the isolation of the AT mutation, it had long been observed that stimuli that induce DNA damage delay progression through the cell cycle. For years this phenomenon was assumed to be the passive response of the cell as a direct result of the DNA damage itself. By contrast, cells that harbor the AT mutation demonstrate a marked decrease in cell cycle arrest after DNA damaging radiation. These data suggested that an active system exists in normal cells that retards cycle progression in the presence of DNA damage. The checkpoint surveillance system comprises sensor proteins (proteins that detect DNA damage and initiate a signaling cascade); transducers (modifying enzymes such as kinases that relay the signal to effector proteins); and effectors (downstream target proteins that, upon activation by modifying enzymes, cause cycle arrest). Of these proteins, the least is known about sensor proteins, although several candidate genes have been suggested. The effector proteins include kinase inhibitors such as p21, or cyclin/cdk heterodimers that are either activated or inhibited to cause cycle arrest. Major transducer proteins include p53, ATM (AT mutated) and ATR (ATM and RAD-3 related).
p53 is a transcription factor that activates the transcription of genes that cause cell cycle arrest at either G1/S or G2. In addition, p53 activates genes that initiate DNA repair and cause apoptosis. Mutations of p53 are commonly described in association with human tumors. The result of p53 activation is cell type-specific and depends on the type and severity of injury. p53-induced G1 cell cycle arrest is mediated via the induction of p21 and p16. p53 has a very short half-life and is generally undetectable in healthy cells. In the presence of DNA damage induced by either ultraviolet or g-irradiation, p53 is activated by posttranscriptional modifications including phosphorylation and acetylation, that either enhance its stability or alter its affinity for binding proteins. ATM and its related protein, ATR, phosphorylate p53 which decreases its binding to mdm-2. A decrease in the interaction between p53 and mdm-2 causes a decrease in ubiquitin-mediated proteolysis of p53 and a resulting increase in p53 protein levels. As described previously, ARF also activates p53 via the inactivation of mdm-2. In addition to phosphorylation, the acetylation status of p53 also determines its stability. p53 is acetylated and stabilized by p300/ CBP which increases apoptosis. The recently described NAD-dependent deacetylase protein, SIRT1, removes acetyl groups from p53 and decreases apoptosis. ATM and ATR are closely related phosphoinositide 3-kinases that are activated by DNA damage. Upon activation, ATM phosphorylates and activates multiple proteins in addition to p53, including mdm-2 and a serine/threonine kinase called Chk-2. Activated Chk-2 phosphorylates p53. ATR phosphorylates many but not all of the same substrates as ATM. ATR phosphorylates and activates Chk-1, which also phosphorylates p53. p53, ATM, and ATR also contribute to G2 arrest. Upon activation, cdk1 initiates mitosis. cdk1 is activated via its interaction with cyclin B and via dephosphorylation by cdc25C phosphatase. Upon phosphorylation by ATM and ATR, Chk-2 and Chk-1 phosphorylate and inhibit cdc25C, which prevents the activation of cdk1. p53 activates transcription of two genes that inhibit cdk1 activity including GADD45 and 14-3-3 s. GADD45 disrupts the cyclin B/cdk1 heterodimer. The protein product of 14-3-3 s sequesters cdc25C, which prevents the dephosphorylation of cdk1. In the face of overwhelming DNA damage, checkpoints, in particular p53, induce cell death by apoptosis rather than cell cycle arrest. Apoptosis, or programmed cell death is an evolutionarily conserved, energy-requiring mechanism by which unwanted or irreparably damaged cells are removed from the organism. Apoptosis is a fundamental component of both normal embryogenesis and adult homeostasis. Apoptosis is also a physiologic response to diverse toxic stimuli including viral infection, DNA damage induced by irradiation or reactive oxygen species, hypoxia, growth factor deficiency or genetic aberration. Apoptosis is carried out by caspases, which are proteases that contain a cysteine nucleophile and cleave proteins whose sequence contains specific motifs that include an aspartic acid residue. Upstream or initiator caspases are activated by the binding of an extracellular ligand to a

death receptor. Death receptors are members of the tumor necrosis superfamily and are characterized by an intracellular death domain. An important example of a death receptor is CD95, or fas, which binds fas ligand. Upon binding of a ligand, the death receptor binds to intracellular adaptor proteins. Adapter proteins bind to initiator caspases 2, 8, 9, or 10, which provokes their autocleavage and activation. Initiator caspases activate downstream effector or executioner caspases, such as caspase 3 or 7, or proapoptotic BCL-2 proteins. The BCL-2 family includes proteins that contain BCL-2 homology domains. These domains allow for heterdimerization by which BCL-2 proteins activate other family members. BCL-2 proteins modulate the intrinsic apoptotic pathway and may have either pro or anti-apoptotic effects. Proapoptotic BCL-2 proteins increase mitochondrial membrane permeability which allows for the release of cytochrome c. Cytochrome c release from mitochondria results in dimerization of an adaptor protein called Apaf-1 (apoptotic protease activating factor) which binds procaspase 9 resulting in its cleavage and activation. Caspase 9 activates the downstream effector, caspase 3. Antiapoptotic proteins in the BCL-2 family inhibit the proapoptotic members and prevent the increase in mitochondrial membrane permeability. The downstream effector caspases target multiple proteins for degradation including enzymes, nuclear structural proteins such as lamins, cytoskeletal proteins such as actin, proteins critical for cell–cell interaction such as b-catenin, and DNA repair enzymes. p53 activates multiple genes that are involved in apoptosis, including genes that encode proteins that function via receptor-mediated signaling and those which encode proteins that modulate downstream effectors. p53-activated IGF-BP3 inhibits binding of IGF-1 to the IGF-1 receptor, which can induce apoptosis. p53 activates transcription of the death receptor ligands fas/Apo1/CD95 and the death receptor KILLER/DR5. p53 also induces the proapoptotic BCL-2 protein bax, as well as other proteins that enhance cytochrome c release from mitochondria, including p53, AIP1, PUMA, and Noxa. Apoptosis may also be induced via an increase in oxidative stress generated by multiple p53-induced genes that are homologous to NADPH-quinone oxidoreductase. Importantly, no singular p53-activated gene product has been conclusively shown to initiate apoptosis. It appears that many p53-induced proapoptotic genes need to be activated concurrently in order for apoptosis to occur. It is not clear which variables determine whether p53 induces cell cycle arrest or apoptosis. Certain cell types, such as T lymphocytes, are especially sensitive to apoptosis whereas fibroblasts are more likely to undergo cell cycle arrest. Whereas p53 induces arrest or senescence in normal cells, p53 activation usually causes apoptosis in transformed cells. The reason for the enhanced sensitivity to p53-induced apoptosis in transformed cells may be related to the deregulation of E2F due to the inactivation of pRb. Cycle arrest induced by p21 may protect the cell from apoptosis. Other factors that may predispose the cell toward p53-induced apoptosis include alterations in the bax/bcl-2 ratio, concurrent absence of growth factors, a greater intensity of stress and higher protein levels of p53. Posttranslational modifications may also determine p53 promoter specificity, which may play a major role in determining whether p53 expression results in cell cycle arrest or apoptosis.

Clinical Application

The normal regulation of the cell cycle plays an important role in tissue repair and inflammation. All tissues may be stratified by proliferative capability into three categories including labile, quiescent or permanently nondividing cells. Labile cells are continuously dividing and include surface epithelial cells such as stratified squamous epithelial cells of the skin and columnar epithelial cells of the gastrointestinal tract. Quiescent cells are nondividing under normal circumstances but can be induced to reenter the cell cycle by exposure to growth factors. Quiescent cells include parenchymal cells of the liver, kidney, and pancreas and mesenchymal cells such as fibroblasts. The cytokine-induced reentry of quiescent cells into G1 phase is an important component of the inflammatory response, which has been well characterized in the kidney. Glomerular mesangial cells proliferate in many models of glomerular disease, including lupus nephritis and diabetes. The proliferation of mesangial cells occurs in response to cytokines such as platelet-derived growth factor and basic fibroblast growth factor. Inhibition of mesangial cell proliferation may abrogate the glomerulosclerosis or the glomerular scarring that occurs as a result of inflammation. Permanently nondividing cells have lost all capacity for proliferation and include nerve cells and cardiac muscle cells. The deregulation of the cell cycle resulting in unchecked cell proliferation is a hallmark of cancer. All human cancers are characterized by defects of restriction point control, checkpoints, DNA repair, or apoptosis. Defects of restriction point control allow for uncontrolled proliferation and result in loss of terminal differentiation. While some cancers are characterized by loss of function mutations of Rb or by disruption of the Rb pocket domain by viral oncoproteins, many more are caused by functional

inactivation of pRb through cyclin D overexpression or INK4a mutations. Mutations of p53 are the most common mutations associated with cancer and occur in almost 50% of all human cancers.

Conclusion

The regulation of the cell cycle plays an important role in normal tissue repair and regeneration. Loss of cell cycle regulation is a chief characteristic of cancer. Cell cycle regulation involves numerous signaling pathways that determine whether cells will proliferate, remain quiescent, arrest or undergo apoptosis. While enormous progress has been made in the elucidation of these signaling pathways, our understanding of cell cycle regulation remains incomplete. Further studies may allow better understanding of diseases that result from deregulation of these pathways.

See also: Tissue Repair.

Further Reading

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Cell Proliferation

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Unicellular organisms, like yeasts, bacteria, or protozoa, have a strong selective pressure to grow and divide as rapidly as possible. The rate of cell division in these cases is limited only by the rate at which nutrients can be taken from the medium and converted into cellular materials. Multicellular organisms, on the other hand, are made up of different kinds of cells performing a variety of functions, and the survival of the organism as a whole is at stake rather than the survival or proliferation of one individual type of cell population. For the multicellular organism to survive, some cells must refrain from dividing even when nutrients are plentiful. Some cells do not divide after a certain stage of development as in the case of central nervous system. However, when need arises for new cells, as in the case of tissue injury, previously nondividing cells must be rapidly triggered to reenter the cell division cycle as part of the overall survival strategy.

Division Cycle of Cells

An adult multicellular animal must divide and supply millions of new cells just to replace the dead or dying cells. Cells go through a division cycle through a highly regulated process known as cell cycle progression (**Figure 1**). Cells divide by going through a cell cycle with the end-product being a duplication of the contents of the mother cell in two daughter cells. In an adult animal most of the cells are in resting or in G_0 (G = gap) phase of cell cycle. When needed to divide they enter the G_1 phase of cell cycle. In most cells the DNA in the nucleus is replicated during only a limited portion of the cell cycle called the S or synthesis phase of the cell cycle. After the S phase, the cells go into a second interval called G_2 phase. In mitosis or M phase, the contents of the nucleus condense to form visible chromosomes, which through an elaborately orchestrated series of movements, are pulled apart into two equal sets and then the cell itself splits into two daughter cells. Upon loss of tissues (e.g., a population of cells and a portion of the tissue) due to injury, the division cycle of cells is stimulated in tissue- or organ-specific fashion so that the lost tissues can be replaced promptly to restore tissue function. Each of the above phases of cell division is highly regulated and orchestrated by an intricate series of signaling mechanisms. When the lost tissue is replaced the entire repertoire is brought down to the normal resting level, thereby reestablishing the cellular, organ, and tissue homeostatic mechanisms.

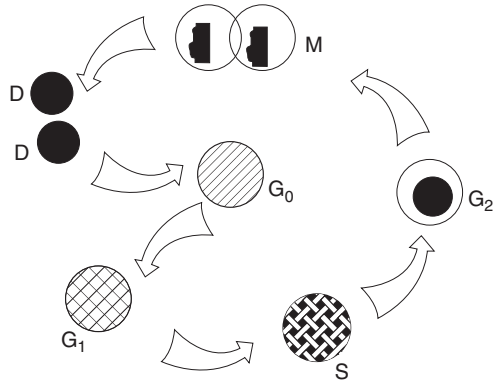

Figure 1 In adult organisms normally cells are in resting phase (G_0) of the cell division cycle. Upon appropriate stimulus the cells enter the division cycle which is characterized by G_1, S, G_2, and M phases. After division, the daughter cells (D) may either reenter the division cycle or enter the resting phase depending on the stimulus.

Genetic Control of Cell Structure and Function during and after Embryonic Development

Almost every multicellular animal is a clone of cells descended from a single original cell, the fertilized egg. Thus, the cells in the body, as a rule, are genetically alike. However, phenotypically they are different; some are specialized as muscles, others as neurons, others as hepatocytes, and so on. The different cell types are arranged in a precisely organized pattern, and the whole structure has a well-defined shape. All these features are ultimately determined by the DNA sequence of the genome, which is reproduced in every cell. Each cell must act according to the same genetic instructions, but it must interpret them with due regard to time and circumstance so as to play a proper part in multicellular organization. The development of vertebrates can be divided into three phases. In the first phase, the fertilized egg cleaves to form many smaller cells and these become organized into an epithelium and perform a complex series of gastrulation and neurulation movements, whose outcome is creation of a rudimentary gut cavity and a neural tube. In the second or organogenesis phase, the various organs, such as limbs, eyes, heart, and so on, are formed. In the third phase, the generated structures go on to grow to their adult size. These phases are not sharply distinct but overlap considerably in time.

Terminal Differentiation and Cell Division

After the embryonic development, cells in the normal adult human body divide at very different rates. Some, such as neurons and skeletal muscle cells, do not divide at all; others, such as liver cells, normally divide once every year or two; and certain epithelial cells in the gut divide more than twice a day so as to provide constant renewal of the gut lining. Most cells in the vertebrates fall somewhere between these extremes; they can divide but normally do so infrequently. Almost all the variation lies in the time cells spend between mitosis and the S phase, with slowly dividing cells remaining arrested after mitosis for weeks or even years. By contrast, the time taken for a cell to progress from the beginning of S phase through mitosis is brief (typically 12–24 h in mammals) and remarkably constant, irrespective of the interval from one division to the next. The time cells spend in a nonproliferative, so-called G_0 state varies not only according to the cell type but also according to the circumstances. Sex hormones stimulate the cells in the wall of the human uterus to divide rapidly for a few days in each menstrual cycle to replace the tissue lost by menstruation; blood loss stimulates proliferation of blood cell precursors; and acute liver damage provokes surviving liver cells to proliferate with a cycle time of only a day or two. Similarly, epithelial cells in the neighborhood of a wound are stimulated to divide so as to repair the injured epithelium. Delicately adjusted and highly specific controls exist to govern the proliferation of each class of cells in the body according to the need.

Role of Growth Factors and Cytokines in Cell Division

When put in an artificial culture medium completely devoid of serum, vertebrate cells will not normally pass the restriction point, even though all the requisite nutrients are present in the medium, and they will halt their growth as well as their progress through the chromosome cycle. Essential components of serum are some highly specific proteins (growth factors and cytokines), usually present in very small concentrations (in the order of 10^{-9}–10^{-11} mol l^{-1}). Different cells require different sets of these proteins. Some of these proteins are involved directly in stimulating cell division and are called complete mitogens. Some can directly inhibit cell cycle progression and thus can control cell division in the body. These are called growth inhibitors. Some of the proteins can cause cell cycle progression in an indirect way and are called growth triggers. **Table 1** provides examples of some of the growth factors and cytokines with their functions.

Cell Senescence and Reluctance to Divide

Most normal cells in the body of a mammal show a striking reluctance to continue proliferating forever. Fibroblasts taken from a normal human fetus, for example, will go through about 50 population doublings when cultured in a standard growth medium; toward the end of this time, proliferation slows down and finally stops, and the cells, after spending some time in quiescent state, die. Similar cells taken from a 40-year-old stop dividing after $\sim$40 doublings, while cells from an 80-year-old stop after $\sim$30 doublings. Fibroblasts from animals with shorter life span stop after a smaller number of division cycles in culture. Because of the correspondence with aging of the body as a whole, this phenomenon is called cell senescence. According to one theory, cell senescence is the result of a catastrophic accumulation of self-propagating errors in a cell's biosynthetic machinery that is unimportant under the conditions of life in the

Table 1 Example of growth factors and cytokines known to regulate cell proliferation

Factor	*Representative functions*
Platelet-derived growth factor (PDGF)	Stimulates proliferation of connective tissue cells and neuroglial cells
Epidermal growth factor (EGF)	Stimulates proliferation of many cell types
Insulinlike growth factors I and II (IGF-I and -II)	Work with PDGF and EGF to stimulate fat cell proliferation
Fibroblast growth factor (FGF)	Stimulates proliferation of many cell types including fibroblasts, endothelial cells, and myoblasts
Interleukin-2 (IL-2)	Stimulates proliferation of T lymphocytes
Transforming growth factor β (TGF-β)	Inhibits cell cycle progression of different cell types
Interleukin-1 (IL-1)	Inhibits proliferation of hepatocytes and other cell types
Hepatocyte proliferation inhibitor	Inhibits hepatocyte proliferation
Nerve growth factor (NGF)	Promotes axon growth and survival of sympathetic and some sensory and CNS neurons
Hematopoietic cell growth factors (IL-3, GM-CSF, M-CSF, G-CSF, and erythropoietin)	Promote division of different blood cells and various other types of cells

wild where most animals die from other causes long before a significant number of cells become senescent. An alternative theory is that the cell senescence is the result of a mechanism that has evolved to protect us from cancer by limiting the growth of tumors.

Cell Proliferation as a Compensatory Response to Toxic Tissue Injury

Human beings are exposed to numerous toxic insults every day. The body has several lines of defense mechanisms to combat the toxicants. Some of the toxicants are filtered out by virtue of their particle size, even before they can enter the body. Toxicants that enter the body can be metabolized and/or conjugated to be excreted out of the body. When these first lines of defense mechanisms are overcome, then the toxic substances cause cell death in the body. The site of cell death depends on the site of action of the toxicant. At this point the tissue can respond by stimulating its healthy cells to divide and to restore tissue structure and function. In response to cell death because of toxic insult, cells in the affected tissues (with the exception of neurons) start dividing in order to replace the dead or dying cells. One surviving cell can go through several cell cycles depending on the severity of the damage. The cell division stops at the precise point when all the dead cells have been replaced with new cells. At high doses of toxicants, the ability of the cells to go through the cell cycle is sometimes inhibited. This leads to two consequences. First, the dead cells are not replaced and failed cell division means loss of the organ and sometimes death. Second, in the absence of compensatory cell division, injury to the tissue can progress in an unrestrained manner. The ability of the cells to go through the cell cycle as needed decreases with age. This is why an 80-year-old can be more susceptible to the same dose of a toxicant as a 40-year-old.

Tissues vary in their compensatory responses to toxic chemicals. Skin, intestine, liver, and kidney are examples of tissues that can respond to toxic injury by stimulating cells to replace the lost tissue. Epithelial cells lining the criptae of the intestines are known to renew themselves every 72 h. Since these cells are subject to many foreign chemicals in the diet, many cells might be expected to be injured or affected in other ways. Therefore, these cells are completely renewed every 3 days. Although not as rapid, skin injury can result in replacement of injured skin rather promptly. The adult liver is normally a quiescent tissue with only an occasional cell dividing to replace dying cells to retain normal tissue homeostasis. Upon injury, however, the liver will respond promptly by stimulating its cells to divide and thereby restoring the lost tissue and function. Surgical removal of portions of the liver leads to restoration of the original liver mass through a very rapid cell proliferation and tissue repair response. Similarly, the kidney is also able to replace its cells upon toxic injury.

Stem Cells and Terminally Differentiated Cells

There are cell populations in the body that are renewed simply by duplication and then there are those that are renewed by means of stem cells. The defining properties of stem cells are (1) they themselves are not terminally differentiated cells – that is, they are not at the end of the pathway of differentiation; (2) they can divide without limit throughout the lifetime of the organism; and (3) when they divide, each daughter cell can either remain as a stem cell or it can take the path leading irreversibly to terminal differentiation.

Stem cells are required wherever there is a recurring need to replace differentiated cells that cannot themselves divide. There may be several reasons why a cell is terminally differentiated. The cell nucleus is digested, as in the outermost layers of the skin, or is extruded, as in the mammalian red blood cells. Alternatively, the cytoplasm may be heavily encumbered with structures, as in myofibrils of the striated muscle cells, which would hinder cell duplication. In other terminally differentiated cells the chemistry of differentiation may be incompatible with cell division. In any case, renewal must depend on stem cells.

The job of the stem cell is not to carry out the differentiated function but rather to produce the cells that will. Those stem cells that give rise to only one type of differentiated cells are called unipotent, those that give rise to a small number of cell types are called oligopotent, and those that give rise to many cell types are called pluripotent.

Cell Proliferation and Cancer

In multicellular organisms there are genes called social control genes, which are involved specifically in the social controls of cell division. A cell that undergoes a mutation or a set of mutations that disrupt the social restraint on cell division will divide without regard to the needs of the organism as a whole, and its progeny will become apparent as a tumor. Cancers, by definition, are malignant tumors; that is, the tumor cells not only divide in an ill-controlled way but also invade and colonize other tissues of the body to create widespread secondary tumors or

metastases. Approximately 10^{16} cell divisions take place in a human body in the course of a lifetime. Even in an environment that is free of mutagens, mutations occur spontaneously at an estimated rate of about 10^{-6} mutations per gene per cell division – a value set by fundamental limitations on the accuracy of DNA replication and repair. Thus, in a lifetime, every single gene is likely to have undergone mutations on about 10^{10} separate occasions in any individual human being. Among the resulting mutant cells, one might expect that there would be many that have disturbances in genes involved in the regulation of cell division and consequently disobey the normal restrictions on cell proliferation. To generate cancer, a cell must undergo a number of mutations occurring together to escape the multiple controls on cell division and then accumulate further changes to become endowed with the capacity for invasion and metastasis. From statistics it has been estimated that somewhere between three and seven independent random events, each of low probability, are typically required to turn a normal cell into a cancer cell; the smaller numbers apply to leukemia and the larger to carcinomas.

A protooncogene is a normal social control gene which can undergo mutation to become an oncogene. Oncogenes and protooncogenes contain DNA sequences that are closely similar but not identical. The mutations in the protooncogenes can result from spontaneous mutations or in response to chemical carcinogens or exposure to radiations. To date, more than 50 protooncogenes have been identified. It is likely, however, that many more social control genes remain to be discovered. Genes that stimulate cell division can be identified readily with current techniques, but there are several genes that have inhibitory effects on cell proliferation and recessive mutations in them are a common cause of cell transformation and cancer. The protooncogenes, code for various growth factors, growth factor receptors, and various intracellular mediators are involved in signaling cells to divide.

Importance of Understanding the Mechanisms in Control of Cell Division

Understanding of the mechanisms in control of cell division offers promising opportunities for developing new avenues for therapeutic intervention, with the aim of restoring and boosting tissue repair mechanisms in cases in which tissues have been damaged or lost as seen in burn wounds, trauma cases, in cases of drug overdoses, chemical poisoning, etc. The current armamentarium of clinical treatments for patients with drug overdoses or chemical poisoning aims to prevent additional injury either by blocking further formation of toxic metabolites or by increasing clearance out of the body. While this is important in preventing further damage to the affected tissue, or damage to unaffected tissue, survival depends heavily on the remaining cells in the tissue to proliferate to replace the dead or dying cells; this, in turn, depends on how soon after the injury the patient commences active treatment. In cases in which either there is a delay in treating the patient or the initial injury itself was massive, death or loss of the organ usually occurs because the damage compromises the regenerating ability of the cells, thereby paving the way for unrestrained progression of damage. If cellular regeneration could be 'actively' stimulated, even after the massive damage, by some therapeutically compatible mechanism, then it might be possible to prevent death or loss of organ. For example, animal experiments have shown that even after massive liver injury, liver failure and animal death can be obviated by stimulating tissue repair in the liver. The importance of cell division in tissue repair and recovery from injury is evident in experiments in which liver failure and animal death are observed in animals receiving an ordinarily nonlethal dose of a toxic chemical, if cell division is blocked by antimitotic agents. Perhaps carefully induced suppression of growth factors, cytokines, and protooncogenes involved in cell death or overexpression of those factors needed for cell division could stop the progression of injury and could restore the organ structure and functions. With the advent of gene therapy, specific genes could, one day, be delivered directly to the organ to induce expression/suppression of any of the factors implicated in fast recovery.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogenesis; Liver; Molecular Toxicology–Recombinant DNA Technology; Tissue Repair.

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Centipedes

Elizabeth J Scharman

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- SYNONYMS: Arthropoda (phylum); Chilopoda (class); Scutigeromorpha, Lithobiomorpha, Geophiulomorpha, and Scolopendromorpha (four orders)

Background Information

Over 2800 species of centipedes of various sizes and colors are found throughout the world in tropical and subtropical locations. The majority of species are 2.5–5.5 cm; tropical species may grow to 25 cm or longer. Centipedes are recognized by their long, multisegmented, flattened body. The body is composed of 15–181 segments, each of which (except the last one) has a pair of legs; the number of segments is always an odd number. Eyes may be simple, complex, or absent. The cranial segment bears multijointed antennae, three pairs of mouth parts, and a modified pair of legs, forcipules, which act as fangs.

Exposure Routes and Pathways

A bite is the usual route of envenomation from the centipede. The hand is a common bite site. There is one case reported in the literature of accidental ingestion.

Mechanism of Toxicity

The venom gland is found at the base of the forcipules. Venom passes through the ducts of the forcipules and is injected into the bite site. The components of centipede venom have not been completely identified. Known components include 5-hydroxytryptamine, histamine, lipids, polysaccharides, and enzymes including proteinases and esterases. Toxin S, which is a cardiotoxic protein, has been isolated from the species *Scolopendra subspinipes*. Cytolysin is found in the North American giant centipede, *Scolopendra heros*.

Acute and Short-Term Toxicity (or Exposure)

Human

Bites may result in localized burning pain, edema, erythema, and paresthesia. Superficial necrosis may occur in 1–2 h. The site may bleed. Swelling may persist for hours to days and may wax and wane during that time. Bullae near the bite site, and rashes at the bite site or other parts of the body, may also appear. Bites from tropical species may result in lymphangitis and lymphadenopathy; these effects are less common with other species. In the one reported case of ingestion, the 6-month-old child developed pallor, hypotonia, vomiting, and lethargy with full recovery.

Envenomation producing death has not been reported in the United States. In the Philippines, a 7-year-old girl is reported to have died following the bite from *Scolopendra subspinipes*, a tropical centipede.

Clinical Management

Treatment is symptomatic and supportive. The wound should be cleaned with soap and water; tetanus prophylaxis should be administered. Application of ice packs or the topical application of a corticosteroid, antihistamine, or local anesthetics may be useful in relieving symptoms. Severe pain has been treated with injection of a local anesthetic. Antibiotics are reserved for documented infections.

See also: Animals, Poisonous and Venomous.

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Cephalosporins

Shayne C Gad

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- Chemical/Pharmaceutical/Other Class: β-Lactam antibiotics (as are penicillins)
- Example Compounds: Cefaclor; Cefadroxil; Cefamandole; Cefazolin; Cefepidime; Cefoperazone; Cefotaxime; Cefoxitin; Ceftriaxone; Cephalexin; Cephalothin; Cephradine; Cephaprin; Cefmetazole; Cefonicid; Ceforanide; Cefotetan; Cefprozil; Loracarbef; Cefperazone; Cefpodoxime; Cefixime; Ceftazidime; Ceftizoxime; Moxalactarn

Uses

Cephalosporins induce their antimicrobial effect by inhibiting the integration of bacterial peptidoglycan. Individual peptidoglycan units are synthesized in the cytoplasm of the bacterial cell and are transported across the cytoplasmic membrane where they are inserted by peptidase enzymes into a crosslinked lattice that forms the structural support of the bacterial cell wall. The peptidase enzymes present in the outer cytoplasmic membrane are referred to as penicillin-binding proteins and represent the target sites for antibacterial action of cephalosporins and other β-lactam antibiotics. Cephalosporins are active *in vitro* against many gram-positive aerobic bacteria and some gram-negative aerobic bacteria. There are substantial differences among the cephalosporins in their spectra of activity as well as levels of activity against susceptible bacteria. Later-generation cephalosporins also are used in the later stages of livestock raising for adding weight.

Background Information

The cephalosporins have sustained their position as a very significant class of antibiotics worldwide for many years, comprising over one-half of the available β-lactam antibiotics. Initially, cephalosporin compounds produced by the fungal organism *Cephalosporium acremonium* were isolated in the early 1940s from fungus in sewage seawater in Calgiari, Sardinia, after it was observed that a natural pattern of periodic clearing of microbes was taking place from a local harbor area. Filtrates from *C. acremonium* cultures were found to have antimicrobial activity against infections in animals and humans, including the injection of filtrates into 'boils' and other cutaneous infections. The initial work later expanded to result in the discovery of cephalosporin C, the structural nucleus for cephalosporin compound development over the next four decades. Ongoing research and development have led to several additional cephalosporin antibiotics that have been released since the 1960s, with 24 unique, yet structurally similar compounds currently available for clinical use in the United States. The first three generations of cephalosporin antibiotics include both parenterally administered (i.e., intravenously or injection) and orally administered agents. The fourth-generation compound, cefepidime, is available for parenteral administration.

Exposure Routes and Pathways

The routes of exposure to cephalosporins are commonly oral, intravenous, or intramuscular. Accidental ingestion of oral dosage forms by children is the most common poisoning exposure.

Toxicokinetics

Cephalosporins are generally well absorbed following administration with bioavailability being greater than 75%. Many of these compounds are not stable in the acid environment of the stomach; therefore, only a limited number are useful for oral administration. The distribution is limited to the extracellular fluid space, with volumes of distribution for most ranging from 0.25 to 0.5 l kg^{-1}. Protein binding is primarily to albumin. Most cephalosporins are widely distributed to tissues and fluids, including pleural fluid, synovial fluid, and bone. Some of the third-generation compounds have good distribution to the cerebrospinal fluid. The metabolites possess antibacterial activity. The cephalosporins and their metabolites are rapidly excreted by the kidneys by glomerular filtration and/or tubular secretion. Serum half-lives of these compounds range from 0.4 to 10.9 h. Patients with immature renal systems or with renal compromise are at risk for toxicity due to decreased elimination. Cefamandole, cefmetazole, cefmenoxime, cefoperazone, and moxalactam have been associated with coagulopathies due to inhibition of platelet aggregation and prolongation of bleeding time.

Mechanism of Toxicity

Hematologic when given in higher doses, cephalosporins bind to cell membrane proteins and

act as haptens. High-dose treatments can induce a hemolytic anemia. They can also prolong bleeding times by reducing platelet adhesion and activation.

Many cephalosporins are substrates for the organic anion transport system in the proximal tubules and can accumulate in the kidney, competing for and inhibiting the transport system leading to renal necrosis. There is great variability in the potential for renal toxicity among different members of the family of compounds. Renal toxic members deplete glutathione levels in the renal cortex. There is evidence that this nephrotoxicity is due to action of the drugs on mitochondria.

Chronic Toxicity (or Exposure)

Animal

The extent of renal accumulation and effect is species dependent (rabbit > guinea pig > rat). There is also great variability in toxicity between different compounds.

Examples of the toxicity profiles of cephalosporins include:

- *Cefotan*: Has adverse effects on the testes of prepubertal rats. Subcutaneous administration of $500\,mg\,kg^{-1}\,day^{-1}$ ($\sim$8–16 times the usual adult human dose) on days 6–35 of life (thought to be developmentally analogous to late-childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence of severity of lesions was dose dependent; at $120\,mg\,kg^{-1}\,day^{-1}$ ($\sim$2–4 times the usual human dose) only one of 10 treated animals was affected, and the degree of degeneration was mild. Similar lesions were observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to $1000\,mg\,kg^{-1}\,day^{-1}$ subcutaneously for 5 weeks, or in infant dogs (3 weeks old) that received up to $300\,mg\,kg^{-1}\,day^{-1}$ intravenously for 5 weeks. *Pregnancy category B*: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan.
- *Cefixine*: Lifetime studies in animals to evaluate carcinogenic potential have not been conducted. SUPRAX did not cause point mutations in bacteria or mammalian cells, DNA damage, or chromosome damage *in vitro* and did not exhibit clastogenic potential *in vivo* in the mouse micronucleus test. In rats, fertility and reproductive performance were not affected by cefixine at doses up to 125 times the adult therapeutic dose. *Pregnancy category B*: Reproduction studies have been performed in mice and rats at doses up to 400 times the human dose and have revealed no evidence of harm to the fetus due to cefixine.
- *Cefoperazone*: Long-term studies in animals have not been performed to evaluate carcinogenic potential. The maximum duration of cefoperazone animal toxicity studies is 6 months. In none of the *in vivo* or *in vitro* genetic toxicology studies did cefoperazone show any mutagenic potential at either the chromosomal or subchromosomal level. Cefoperazone produced no impairment of fertility and had no effects on general reproductive performance or fetal development when administered subcutaneously at daily doses up to 500–$1000\,mg\,kg^{-1}$ prior to and during mating, and to pregnant female rats during gestation. These doses are 10–20 times the estimated usual single clinical dose. Cefoperazone had adverse effects on the testes of prepubertal rats at all doses tested. Subcutaneous administration of $1000\,mg\,kg^{-1}\,day^{-1}$ ($\sim$16 times the average adult human dose) resulted in reduced testicular weight, arrested spermatogenesis, reduced germinal cell population, and vacuolation of Sertoli cell cytoplasm. The severity of lesions was dose dependent in the 100–$1000\,mg\,kg^{-1}\,day^{-1}$ range; the low dose caused a minor decrease in spermatocytes. This effect has not been observed in adult rats. Historically, the lesions were reversible at all but the highest dosage levels. However, these studies did not evaluate subsequent development of reproductive function in the rats. *Pregnancy category B*: Reproduction studies have been performed in mice, rats, and monkeys at doses up to 10 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefoperazone.

Human

Like penicillins, cephalosporins are a relatively nontoxic group of antibiotics. The primary adverse effect reported is hypersensitivity, a rare event. Cross-allergenicity with penicillins may occur. Toxicity is unlikely in children less than 6 years of age who acutely ingest less than $250\,mg\,kg^{-1}$. Nephrotoxicity is a possible, but rare, occurrence with acute ingestion. Coagulopathies have been reported following chronic intravenous use of certain cephalosporins. At higher

concentrations, cephalosporins cause renal tubular injury, characterized by decreased glomerular filtration rate, glucosuria, enzymuria, and proteinuria.

Clinical Management

If a toxic or unknown amount of a cephalosporin has been ingested, gastric decontamination and the administration of activated charcoal is usually all that is needed. In the symptomatic patient, evaluation of renal function and electrolytes may be necessary. Chronic exposure usually requires discontinuation of the drug and supportive care. Anaphylaxis should be treated with epinephrine and/or diphenhydramine.

See also: Hemocompatibility; Kidney.

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Cerium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-45-1
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Ce^{3+}; Ce^{4+}

Uses

Although cerium is a rare earth element, it is relatively abundant in the earth's crust. Among the lanthanides, it is the most abundant. It is one of the 78 common elements in the earth's crust, and ranks 25th in occurrence at an average distribution of 20–60 ppm. Cerium is used in metallurgy as a stabilizer in alloys and in welding electrodes; in glass as a polishing agent, decolorizer, and to render glass opaque to near-ultraviolet radiation. It is also used in ceramics and as catalyst. Cerium is used as a component of some diesel fuel additives, and may be added to residual fuel oils to improve combustion. Cerium is found in portable rechargeable batteries.

Background Information

Cerium is a rare earth metal and the most abundant member of the lanthanide series discovered in 1803. It is the only material known to have a solid-state critical point.

Exposure Routes and Pathways

Inhalation, dermal, and oral are the possible exposure routes.

Toxicokinetics

Cerium is poorly absorbed by the intestine.

Mechanism of Toxicity

Cerium resembles aluminum in its biologic and chemical properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} values reported in rats ranged from 4 to 50 mg kg^{-1} for cerium nitrate with female rats being more sensitive than males. After peritoneal injection, the LD_{50} of cerium nitrate was 470 mg kg^{-1} in female mice and 290 mg kg^{-1} in female rats; the LD_{50} of cerium chloride was 353 mg kg^{-1} in mice and 103 mg kg^{-1} in guinea pigs. The oral toxicity of cerium nitrate was much lower (LD_{50} of 4200 mg kg^{-1} in female rats and 1178 mg kg^{-1} in female mice) than after intravenous or intraperitoneal administration. The LD_{50} of ingested cerium oxide could not be determined in rats when delivered at a dose of 1000 or 5000 mg kg^{-1}. An LD_{50} of 622 mg kg^{-1} has been reported for cerium oxide ingested by mice. The LC_{50} after inhalation of cerium oxide in rats was greater than 50 mg m^{-3}. The primary targets after

inhalation of cerium are the lung and the associated lymph nodes; other organs could be affected via clearance through the blood. Studies of cerium injected systemically have shown that, once in the circulation, cerium can cause liver toxicity with a no-observed-adverse-effect level of $1\,mg\,kg^{-1}$ after a single intravenous injection and a lowest-observed-adverse-effect level (LOAEL) of $2\,mg\,kg^{-1}$ for effects on liver detoxifying enzymes. Effects on other organs where cerium can accumulate (such as spleen, bones, and kidney) have not been studied. A single-dose study on the effects of *in utero* intravenous administration reported reduced weight in newborn mouse pups, with an LOAEL of $80\,mg\,kg^{-1}$. Cerium has been found to depress certain behaviors in mice administered this chemical, and cerium administered to pregnant mice on day 7 or 12 of gestation or 2 days postpartum caused significant decreases in open field activity of offspring. Fetal growth was impaired, as evidenced by weight decreases of 7–19%. The potential carcinogenicity of cerium-containing particles has not been studied in conventional rodent bioassays; *in vivo* mutagenicity studies have been negative.

Human

Cerium can increase blood coagulation rate and produce gastrointestinal effects. Inhalation can lead to polycythemia.

Chronic Toxicity (or Exposure)

Animal

An animal inhalation study involved exposure of rats to cerium oxide particles substantially larger than those in diesel emission. The exposure concentrations ranged between 5 and $500\,mg\,m^{-3}$ for 13 weeks. Effects observed included lung discoloration, enlargement of lymph nodes, and increased lung and spleen weight at all concentrations.

Human

Case reports of workers occupationally exposed to rare earth metals (including cerium) describe a condition termed rare earth pneumoconiosis with pathologic features including interstitial fibrosis, granulomatosis, and bilateral nodular chest X-ray infiltrates. Although the disease sometimes is associated with accumulation of cerium in particles, the role of cerium in this complex disease is unclear relative to other metals or gases to which workers may also have been exposed.

In Vitro Toxicity Data

In vitro mutagenicity studies have been negative.

See also: Aluminum; Metals.

Further Reading

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Relevant Website

http://www.healtheffects.org – Evaluation of Human Health Risk from Cerium Added to Diesel Fuel. Health Effects Institute Communication 9 (August 2001).

Cesium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-46-2
- Synonym: Caesium
- Chemical/Pharmaceutical/Other Class: Alkali metals
- Chemical Formula: Cs^+

Uses

Cesium is used in photovoltaic cells, vacuum tubes, scintillation counters, and atomic clocks.

Background Information

Cesium was discovered in 1860 by Robert Bunsen and Gustaff Kirchoff. It is used in the most accurate atomic clocks. Cesium melts at 28.4°C (just below body temperature) and occurs in Earth's crust at 2.6 ppm.

Exposure Routes and Pathways

Inhalation and ingestion are the routes of exposure.

Toxicokinetics

The metabolism and tissue distribution of cesium-137 were studied in rats injected intraperitoneally and sacrificed 1–300 days postinjection. In a chronic study, rats were administered cesium-137 in their drinking water daily. In the acute study, with the exception of the brain, muscle, and total animal, all tissues showed retention curves resolvable into three exponential components with half-lives of 1.5–2, 5–8, and 15–17 days. Retention in muscles was resolvable into a two-exponential function with half-lives of 8 and 16 days. In the chronic study, the highest equilibrium cesium-137 concentrations, 10% of the average daily intake per gram, occurred in the muscle. The authors concluded that the muscle should be considered the formal critical organ for cesium-137.

Mechanism of Toxicity

Cesium displaces potassium.

Acute and Short-Term Toxicity (or Exposure)

Animal

Primary skin and eye irritation, cutaneous sensitization, and oral acute toxicity studies were conducted on the hydroxide and iodide of cesium. Evaluation of cesium hydroxide has produced somewhat conflicting results. In one study, cesium hydroxide was markedly more toxic than the iodide, but was irritating only to abraded skin. Cesium iodide did not affect the eye or skin. These results indicate that cesium is only slightly toxic acutely and would pose an acute health hazard only when ingested in large quantities. In another study, cesium showed a low or very low acute toxicity orally or intraperitoneally to rats, except for cesium hydroxide, which had an intraperitoneal LD_{50} of $89\,mg\,kg^{-1}$. In a review of cesium hydroxide, it was concluded from the only animal studies available that cesium hydroxide is extremely irritating and corrosive to the eyes and skin after acute exposure.

Human

Cesium has been reported to cause hyperirritability and muscle spasms. Human data for cesium hydroxide are unavailable.

Chronic Toxicity (or Exposure)

Exposure to the radioactive form, cesium-137, can result in an increased risk of cancer. No data on long-term exposure, genotoxicity, mutagenicity, carcinogenicity, and reproduction toxicity have been found for cesium hydroxide.

Clinical Management

Prussian blue is administered by a duodenal tube to act as a chelating agent.

See also: Lithium; Metals; Potassium; Sodium.

Further Reading

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Johnson GT, Lewis TR, and Wagner WD (1975) Acute toxicity of cesium and rubidium compounds. *Toxicology and Applied Pharmacology* 32: 239–245.

Relevant Website

http://www.epa.gov – Cesium. US Environmental Protection Agency.

Channel Blockers *See* Calcium Channel Blockers.

Charcoal

William S Utley

- Chemical Abstracts Service Registry Number: CAS 16291-96-6
- Synonyms: Carbon; Activated charcoal; Wood charcoal

Uses

Charcoal is produced by the incomplete combustion of plant or animal products. The major use of charcoal is for outdoor cooking. The second largest use of charcoal is in industrial applications in the form of activated charcoal. The activation process involves heating the charcoal subjecting it to steam or treating with a chemical to both remove substances that have adhered to it as well as break it down into finer particles and thus increase the surface area. Activated carbon has been used for its adsorptive properties as a 'universal antidote' in cases of poisonings, as a filter aid agent, and in decolorization processes.

Exposure Routes and Pathways

The primary route of exposure is via inhalation of fine dust and ingestion.

Toxicokinetics

Charcoal is not absorbed through the skin or the gastrointestinal tract. When ingested it is excreted in the feces. This is readily apparent from the black color of the feces.

Mechanism of Toxicity

Charcoal is not generally considered to be toxic. It is possible to overwhelm pulmonary defense mechanisms if excessive dust is inhaled over a long period of time. The ability of charcoal to adsorb vitamins and enzymes can lead to nutritional deficiencies if ingested on a chronic basis.

Acute and Short-Term Toxicity (or Exposure)

Human

No reported human toxicity. The absorptive properties of charcoal may interfere with the enterohepatic circulation of certain drugs if it is taken orally.

Clinical Management

If toxicity should manifest, general life support should be maintained. Symptoms should be treated and decontamination undertaken if necessary.

Relevant Website

http://science.howstuffworks.com – Howstuffworks: What is activated charcoal and why does it work in filters?

Chemical Hazard Communication and Material Safety Data Sheets

Michele R Sullivan and Patricia M Nance

Introduction

The production and use of chemicals is fundamental to all economies. Chemicals directly or indirectly affect our lives and are essential to our food (e.g., as fertilizers, pesticides, food additives, and components of packaging), our health (e.g., in pharmaceuticals and in cleaning materials), and our life style (e.g., in appliances, fuels). The omnipresence of chemicals has resulted in the development of sector-specific regulations (e.g., transport, workplace, agriculture, trade, and consumer products). Having information on the hazardous properties and control measures of chemicals available throughout their life cycle allows their production, transport, use, and disposal to be managed safely, thus protecting human health and the environment.

What is Chemical Hazard Communication?

The sound management of chemicals includes systems through which chemical hazards are communicated to workers, consumers, and the public. It is important to know what chemicals are present

and/or used, their hazards to human health and the environment, and the means to control them. A number of classification and labeling systems, each addressing specific use patterns and groups of chemicals, exist at the national, regional, and international levels. The existing hazard classification and labeling systems address potential exposure to chemicals in all types of use settings, including production, storage, transport, workplace use, consumer use, and presence in the environment.

The primary purpose of all existing classification and labeling systems is to provide information to people who are potentially 'exposed' to chemicals, in order to minimize the possibility of adverse effects resulting from that exposure. While the audiences (workers, consumers, etc.) vary with the system, the purpose remains the same.

A goal of hazard communication is to ensure that employers, employees, and the public are provided with information on the hazards of chemicals so that they can take effective preventive and protective measures for their health and safety. This is sometimes referred to as the 'right-to-know' principle.

The first step in the safe handling of chemicals is knowing their identity, their hazards to health and the environment, and the means to control them. This complex information on the hazards and corresponding protective measures needs to be conveyed in a manner that is easily understood. The information can be conveyed in a variety of ways.

Information in the form of labels, placards, posters, or markings provided on or with the container of the hazardous material is common to all the systems currently in existence. This information generally includes some indication of the hazard(s), in text form and/or symbols. In addition to the hazard information, the container information may also include statements regarding safe use or handling, or other types of precautionary measures.

More detailed information may also be provided to those exposed to hazardous chemicals. In the workplace, for example, material safety data sheets (MSDSs) may be available. In the transport sector, a document such as the *North American Emergency Response Guidebook* may supplement the information on placards or markings. The details on these information documents vary from system to system for the same chemical.

In most workplace and transport chemical hazard communication systems, training is also a component. In consumer settings, however, the container label is the only communication mechanism available to provide information on safe handling and use.

Classification of Chemicals: A Starting Point for Hazard Communication

Classification is the identification of the hazard(s) of a chemical or mixture by assigning a category of hazard/danger using defined criteria. Hazard classification generally involves the following steps:

1. Identification of relevant data for a substance or mixture.
2. Comparison of the data to hazard classification criteria to determine whether the product is hazardous and the degree or level of hazard.

Health and physical hazards, and sometimes environmental hazards, are included in all systems. Typical hazards include:

- *Physical hazards*: Flammable liquids, solids, and gases, flammable aerosols, pyrophoric liquids and solids, self-heating substances, substances which in contact with water release flammable or toxic gases, oxidizing liquids, solids, and gases, organic peroxides, self-reactive substances, explosives, corrosive to metals, gases under pressure.
- *Health hazards:* Acute toxicity, skin irritation/corrosion, eye irritation/corrosion, respiratory or skin sensitization, mutagenicity, carcinogenicity, toxic to reproduction, target organ toxicity.
- *Environmental hazards:* Hazardous to the aquatic environment, hazardous to the terrestrial environment, hazardous to the ozone layer.

Chemical Hazard Communication Tools

Once a substance has been classified, the hazard(s) must be communicated to target audiences. The main tools of chemical hazard communication are 'labels' and MSDSs (sometimes called Safety Data Sheets (SDSs) and Material Data Sheets (MDSs)) that contain the hazard information. Their purpose is to identify the hazardous properties of chemicals that may constitute a health, property, or environmental risk during normal handling or use.

Labels

A label on a container of a product is designed to inform persons handling or using the chemical of its hazards. The label is the basic tool to keep the user informed of the hazards and the most important safety precautions. The label can be regarded as a snapshot of the chemical hazard(s) to be used as an alert for the worker who can get more detailed information from an MSDS or SDS, training, etc.

Many countries have developed hazard communication systems with their own standards for how

chemical information is to appear on a label. While the systems vary, the basic components of a label are similar. For example, labels commonly include the following information:

- product identifier/identities of the hazardous components,
- signal word,
- hazard statements,
- hazard symbols/pictograms,
- precautionary information, and
- supplier information.

Signal Words

Some systems require the use of signal words to give some indication of the severity of the hazards involved. Commonly used terms include 'danger', 'warning', and 'caution', depending on the type and severity of known or potential hazard.

Hazard Statements

Most existing systems require a statement of the hazards or effects of the chemicals. In some cases, all effects are required to be provided. In others, there is an established precedence of hazards and a limitation on what is provided.

Hazard Symbols

In addition to the written statements, several of the existing hazard communication systems use symbols to convey hazards. The United States (US) allows the use of symbols in the workplace, but does not require them. The placement and design of the symbols varies among systems. The European Union (EU) system places symbols in a square. The Canadian system requires the symbols to have a circle around them. And the United Nations (UN) transport system requires the symbols be placed in a diamond. These differences result in different labels even when the symbol itself is the same.

Precautionary Information

In addition to the signal words and statement of hazards, many labels are required to include other information that is precautionary in nature and provides more information on safe handling and use. The approach to these warnings and precautions varies among systems. In some systems, manufacturers have the discretion to determine what statements are necessary, and how they will be presented. In other systems, the regulatory authority has developed standard phrases and a decision logic to apply them to the label.

Colors

In addition to text and symbols, colors may be used to denote hazard or classification information. Under the EU system, an orange background is required for symbols. The transport system uses a variety of colors, such as a red background for the flammability symbol. The use of colors is intended to make the symbols stand out, or to more clearly delineate the hazard.

Label Format/Layout

There are a number of requirements in the existing systems that relate to size, appearance, and placement of the labels. For example, the Canadian Workplace Hazardous Materials Information System (WHMIS) system requires a border around the label that distinguishes it from labels of other systems.

The needs of the intended target audience influence what label components are used. In 'transport', for example, the label, placard, and transport documents are all used. In the 'workplace', the label is one element of a multicomponent system of chemical hazard communication, the other elements being the MSDS and training. In communicating the potential hazard of 'consumer products', the label plays the major role in providing the user with information about all the potential health, environmental, and physical hazards of the product and advice on using the product safely.

Material Safety Data Sheets (MSDS, or an SDS or MDS)

The supplier, manufacturer, or importer should be able to provide detailed information about the product on an MSDS (or an SDS or MDS). Chemicals that are used in the workplace are usually accompanied by an MSDS. Safety data sheet information can be found under several names, such as:

- chemical safety card,
- chemical info-sheet,
- SDS,
- MSDS,
- product safety data sheet, and
- health and safety data.

The MSDS provides comprehensive information about a chemical substance or mixture, including potential health, safety, and environmental hazards, and guidance on safe handling, use, and storage. Employers, workers, regulatory professionals, emergency personnel, and others use MSDSs as a source of information about hazards, advice on safety precautions, and regulatory information. The

information in an MSDS acts as a reference for the management of hazardous chemicals. The MSDS is product related and does not provide information for a specific workplace, although where products have specialized end-uses the MSDS information may be more specific.

An MSDS is usually composed of several sections, each containing a different kind of information. Each section is designed to provide useful information to users of the material and to individuals concerned with a variety of health, safety, and environmental issues. For example, the MSDS section on fire-fighting describes measures to be taken to extinguish fires involving the material. The toxicology section contains relevant toxicological information about the material.

In the United States, MSDSs originated in the shipbuilding industry using a format designated as the US Department of Labor, Occupational Safety & Health Administration (OSHA) Form 20. Chemical manufacturers expanded the original 2-page OSHA Form 20 in order to more adequately provide health and safety data on chemical products. Under the OSHA Hazard Communication Standard (29 Code of Federal Regulations (CFR) 1910.1200) issued in 1983, MSDS requirements are performance oriented and do not require a specific format. However, there is a nonmandatory OSHA Form 174.

Originally, MSDSs were intended to be used by health and safety professionals, workers, employers, and customers. In 1986, SARA or the Emergency Planning and Community Right-to-Know Act (EPCRA) expanded the use of MSDSs to fire departments, emergency responders, state and local emergency planning groups, and members of the community. In addition, a number of countries, regions, and international organizations developed guidance or requirements pertaining to MSDSs. Several of these are discussed below.

As result of these activities, MSDSs became diverse in overall format and content, making their use more complicated. In due course, the advantage of harmonizing the MSDS format to make information easier to find was recognized.

A 16-section MSDS format was developed in the 1990s and is common to many standards such as International Organization for Standardization (ISO) 11014-1, the European Union (EU) SDS, the International Labor Organization (ILO) standard under Chemicals Recommendation R177, and the American National Standards Institute (ANSI) Standard Z400.1. The initial 16-section MSDS sequence has been modified. According to the newly developed Globally Harmonized System (GHS) for the Classification and Labelling of Chemicals, information in the MSDS should be presented using the following 16 ordered headings:

1. Identification
2. Hazard(s) identification
3. Composition/information on ingredients
4. First-aid measures
5. Fire-fighting measures
6. Accidental release measures
7. Handling and storage
8. Exposure controls/personal protection
9. Physical and chemical properties
10. Stability and reactivity
11. Toxicological information
12. Ecological information
13. Disposal considerations
14. Transport information
15. Regulatory information
16. Other information

An example of a GHS 16-section MSDS for a fictional product is shown in the Appendix.

Chemical safety data sheets are prepared by various type organizations: chemical safety data sheets prepared by groups of experts and peer-reviewed; and chemical safety data sheets prepared by manufacturers or distributors. There are websites, such as the US National Institute for Occupational Health and Safety (NIOSH) that provide a listing of MSDS for various substances and mixtures. Peer-reviewed data sheets on chemical substances, the International Chemical Safety Cards (ICSCs) are available from the International Program on Chemical Safety (IPCS).

NJ RTK Hazardous Substance Fact Sheets are data sheets for over 1500 individual hazardous chemicals prepared under the New Jersey Right-To-Know Law. Information is available New Jersey State website.

Comprehensibility

The purpose of providing chemical hazard information is to effect a change in behavior causing the user to follow appropriate precautionary measures and avoid the occurrence of an adverse effect from handling or using the chemical. In order to bring about this behavior change, it is important that the information provided to the chemical user or handler is 'comprehensible'. Comprehensibility refers to the ability of the individual reading a label, warning, or safety data sheet to understand the information sufficiently to take the desired action.

Comprehensibility is different from readability because the latter is simply a measure of the educational level of the written material, while the former is a measure of how well the receiver of

the information understands it. A warning about incompatible chemicals may be written at the correct reading level for a specific target audience, for example, but may do such a poor job explaining the hazard that the warning is not understandable by most of the intended audience. Additionally, the same warning may be highly comprehensible to a population of chemical workers, but poorly understood by firefighters with the same educational level but different work experiences.

Training

In addition to labels and MSDS, appropriate 'training' for target audiences who are required to interpret label and/or MSDS information and take corresponding precautionary measures is a component of many hazard communication systems. Training is usually keyed to the nature of the work or exposure; and the target audiences that may include workers, emergency responders, those involved in label and MSDS preparation, and in the transport of hazardous chemicals.

Sectors/Audiences Involved in Chemical Hazard Communication

There are different sectors or target audiences that are users of chemical hazard information. The four primary sectors include industrial production, agriculture, consumers, and transport. Different target audiences receive and use the information conveyed about hazardous chemicals in different ways.

Industrial Production Sector

Workers at factories, storage facilities, construction sites, and at small- and medium-sized enterprises are potentially exposed to industrial chemical hazards. The elements common to workplace hazard communications systems include labels, MSDS/SDS, and training.

Agriculture Sector

Farmers and farm workers are potentially exposed to agricultural chemicals, such as pesticides and fertilizers. Labels are the primary source of information. Visual symbols and orally communicated information are particularly important in the agricultural setting. An MSDS may not be readily available or easily understood.

Consumer Sector

Consumers are exposed to a wide variety of chemicals in their daily lives, from bleaches and dyes, to flammable hair care products and pesticides used in gardens. Since consumers rely solely on label information, comprehensibility is of particular importance.

Transport Sector

The transport sector has long been a focus of international efforts on hazard communication. There is a wide range of target audiences, including transport workers and emergency responders, and also carriers and those who load and unload dangerous goods. Those involved in the transport sector need information concerning safe practices that are appropriate for all transport situations. Labels, placards, transport documents, and MSDSs are key tools.

Other Affected and Interested Sectors

There are others with an interest in chemical hazard communication. Emergency responders involved in responding to chemical emergencies such as spills, leaks, or explosions need hazard and safety information. Firefighters and those first at the scene of a transport accident also need information. Medical personnel responsible for treating victims require specialized information.

Chemical Classification and Hazard Communication Systems

As early as the 1950s, there was international work on the classification and labeling of chemicals. Initial efforts focused on the safe transport of dangerous goods. National and regional systems have more recently been created concerning chemical safety in the workplace. Several of these systems are discussed below. The principles for the various classification, labeling, and hazardous communication systems are related but there are differences among them.

Regional and National Examples

OSHA Hazard Communication Standard (29 CFR 1910.1200) The US OSHA's Hazard Communication Standard (HCS) ensures that information about chemical hazards and associated protective measures is provided to workers and employers. This is accomplished by requiring chemical manufacturers and importers to evaluate the hazards of the chemicals they produce or import, and to provide information through labels on shipped containers and MSDSs. Employers with hazardous chemicals in their workplaces must prepare and implement a written hazard communication program, and must ensure that containers are labeled, employees are provided access to MSDSs, and an effective training program is conducted for all potentially exposed employees. The HCS provides workers the 'right-to-know' the

hazards and identities of the chemicals they are exposed to in the workplace.

ANSI Z400.1: Hazardous Industrial Chemicals – Material Safety Data Sheets – Preparation The ANSI has adopted a voluntary consensus standard that gives guidance regarding preparation of MSDSs. The standard applies to the preparation of MSDS for chemicals and materials used under industrial occupational conditions. It presents basic information on how to develop and write a 16-section MSDS. It identifies information that must be included to comply with the performance oriented OSHA HCS.

Canada's Workplace Hazardous Materials Information System The WHMIS is Canada's hazard communication standard for the workplace. The key elements of the system are labeling of containers of WHMIS 'controlled products', MSDSs, and worker education programs. WHMIS is implemented through coordinated federal, provincial, and territorial legislations. Supplier labeling and MSDS requirements are set out under the Hazardous Products Act (HPA) and associated Controlled Products Regulations. The HPA and its regulations are administered by the Product Safety Bureau of the Government of Canada Department of Health, commonly referred to as Health Canada. Each of the 13 provincial, territorial, and federal agencies responsible for occupational safety and health has established employer WHMIS requirements within their respective jurisdiction. WHMIS includes a mechanism concerning disclosure of confidential business information.

Emergency Response Guidebook The *Emergency Response Guidebook* was developed jointly by Canada, the United States, and Mexico for use by fire fighters, police, and other emergency service personnel who may be the first to arrive at the scene of a transportation incident involving dangerous goods. It is primarily a guide to aid 'first responders' in quickly identifying the specific or generic hazards of the material(s) involved in the incident, and protecting themselves and the general public during the initial response phase.

EU Requirements for Classification and Labeling In keeping with Directive 67/548/EEC and its amendments, dangerous substances which are placed on the EU market have to be labeled according to their classification in the Annex I list, which contains ~2350 existing and 214 new substances. For dangerous substances not in the Annex I list, the manufacturer, distributor, and importer is obliged to apply a provisional classification and labeling following the criteria in Annex VI of the directive. Mixtures or preparations are also regulated by the EU (under 88/379/EEC, 99/45/EC, etc.), as well as SDSs (under 91/155/EC, 2001/58/EC, etc.).

EU Directives contain provisions on:

- Classification of dangerous substances and mixtures into categories characterizing the type and severity of the hazards.
- Packaging of dangerous substances and mixtures.
- Labeling of dangerous substances and mixtures with hazards and safety measures.
- SDSs for dangerous substances and mixtures.

International Agreements and Standards

'Earth Summit' – Agenda 21 At the 'Earth Summit' in 1992, governments agreed to language on the classification and labeling of chemicals in regards to the 'Sound Management of Chemicals':

- Adequate labeling of chemicals and the dissemination of safety data sheets such as ICSCs (International Chemical Safety Cards) and similarly written materials, based on assessed hazards to health and environment, are the simplest and most efficient way of indicating how to handle and use chemicals safely.
- For the safe transport of dangerous goods, including chemicals, a comprehensive scheme elaborated within the United Nations system is in current use. This scheme mainly takes into account the acute hazards of chemicals.

The mandate for the GHS originated at the 'Earth Summit':

- A globally harmonized hazard classification and compatible labeling system, including material safety data sheets and easily understandable symbols, should be available, if feasible, by the year 2000.

ILO Chemical Convention 170 and Recommendation 177 The purpose of the International Labor Organization (ILO) Convention 170 and Recommendation 177 concerning safety in the use of chemicals at work is to protect workers who use chemicals in their workplace. The Convention, which came into force in November 1993, covers hazard classification and communication of hazards, chemical identity, and precautions on labels. It also requires that chemical safety data sheets for hazardous chemicals be provided to employers. Chemical suppliers are responsible for ensuring that chemicals

have been classified, marked, and labeled and have chemical safety data sheets.

UN Recommendations on the Transport of Dangerous Goods The transport of dangerous goods is coordinated globally at the United Nations level by the 'Recommendations on the Transport of Dangerous Goods', also called the 'Orange Book'. The UN Recommendations on the transport of dangerous goods address the following main areas:

- Lists of commonly carried dangerous goods.
- Their classification.
- Labeling, marking, and transport documents.
- Packaging requirements including multimodal tank-containers.

The transport system is based mainly on physical and acute hazards. The elements of this system are widely adopted for the purpose of transporting dangerous goods by sea, air, road, rail, and inland waterways.

ISO 11014-1: International Standard for Safety Data Sheets In 1994, the International Organization for Standardization (ISO) developed a standard format for safety data sheets to create consistency in providing information on safety, health, and environmental matters for chemical products. In order to establish uniformity, certain requirements are provided as to how information on the chemical product shall be given (the titles and sequence of the headings and section content). The ISO SDS standard uses the 16-heading format.

Food and Agriculture Organization (FAO)

FAO International Code of Conduct on the Distribution and Use of Pesticides The 1985 International Code of Conduct, amended in 1989, was developed to address the use of pesticides in developing countries until the countries have established regulatory infrastructures for pesticides. The Code sets forth responsibilities and establishes voluntary standards of conduct for public and private entities engaged in or affecting the distribution and use of pesticides. The Code specifically addresses 'Labeling, packaging, storage and disposal' of pesticides.

FAO Guidelines on Good Labeling Practice for Pesticides The 1995 FAO *Guidelines on Good Labeling Practice for Pesticides* gives guidance on the preparation of labels and specific advice on content and layout. They are intended for use by industry and also by national pesticide regulatory authorities. The Guidelines contain: information that must appear on a label; comprehensibility considerations; pictograms for communicating safety information to users; and toxicity and hazard classifications for a product. The appendices contain examples of labels, hazard statements, agricultural practice statements, and other specific and generic label contents.

WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification

The World Health Organization (WHO) recommended classification of pesticides by hazard, first issued in 1978, has gained wide acceptance. The classification is based primarily on the acute oral and dermal toxicity to rats since these determinations are standard procedures in toxicology.

The Globally Harmonized System for the Classification and Labeling of Chemicals (GHS)

An important new tool to develop/harmonize chemical hazard communication systems is the UN Globally Harmonized System for the Classification and Labeling of Chemicals (GHS). While the existing chemical hazard communication systems are similar in intent, there are differences in their specific provisions. The GHS is an important tool to harmonize systems worldwide. As noted above, the need for a global system was endorsed internationally by the 1992 'Earth Summit'.

The harmonized elements of the GHS may be seen as a collection of 'building blocks' from which to form a regulatory approach for chemical hazard communication within the workplace, for the transportation system, for those involved in chemical work-related activities, and for consumers.

Overview

The GHS provides a comprehensive tool for chemical classification and hazard communication. It applies to all chemicals and mixtures of chemicals. The GHS includes the following elements:

- Harmonized criteria for classifying substances and mixtures according to their health, environmental, and physical hazards.
- Harmonized hazard communication elements, including requirements for labeling, symbols, and safety data sheets.

The GHS addresses the hazard communication elements common to the existing systems:

- Labeling: minimum data elements; hazard pictograms (symbols, colors, frames); comprehensibility;

signal words, hazard statements and precautionary measures.
- Chemical safety data sheets (MSDS/SDS): format; minimum data elements.
- Principles for hazard communication training.

The technical work on developing the GHS was completed in 2001. The technical GHS Document (the 'Purple Book') gives the classification criteria and the hazard communication elements, as well as examples of labels and classification of chemicals to illustrate how to apply the criteria. The UN Sub-Committee on the Globally Harmonized System (SCEGHS) will maintain update, and promote the technical GHS Document as well as manage implementation issues. More information about the GHS and the GHS Document is available at the Relevant Websites section.

Appendix

MSDS Example

1. Identification

Name of the product: Sticky Stuff
Recommended use: General adhesive

Producer:	GHS Ltd., UK London, SE, Southwarkbridge 1 Telephone no. +44-171717-555-555-5 Emergency no. +44-171717-333-333-3

2. Hazard(s) Identification

Classification:	Flammable liquid, Category 2 Eye irritation, Category 2A Hazardous to the aquatic environment; Acute Category 3
Labeling:	
Symbol:	Flame, Exclamation mark
Signal word:	Danger
Hazard statement:	Highly flammable liquid and vapor Causes severe eye irritation Harmful to aquatic life
Precautionary statement:	Keep away from heat, spark, or flame Do not smoke Wear safety glasses Wear impervious gloves Do not breathe fumes Use only with adequate ventilation

3. Composition/Information on Ingredients

Chemical identity: Component A 70–80%
Common name: Solvent A
Number of identity: CAS No.: 111111-11-1
Impurities: None
Chemical identity: Component C 20–25%
Common name: Not applicable
Number of Identity: CAS No.: 44444-44-4
Impurities: none

4. First-Aid Measures

Inhalation: Remove person to fresh air. If respiratory irritation, dizziness, nausea, or unconsciousness occurs, seek immediate medical assistance. If breathing has stopped, give artificial respiration.

Skin contact: Wash the contaminated area with soap and water. Remove contaminated clothing and wash before reuse. If irritation develops get medical attention.

Eye contact: Hold eyelids apart and flush eyes with plenty of water for at least 15 min. Get medical attention.

Ingestion: If swallowed, do NOT induce vomiting. Seek immediate medical attention.

Note to Physicians: Material if ingested may be aspirated into the lungs and can cause chemical pneumonitis. Treat appropriately.

5. Fire-Fighting Measures

Suitable extinguishing media: Foam, extinguishing powder, carbon dioxide, water fog. In case of fire, cool endangered containers with water fog.

Unsuitable extinguishing media: High-pressure water jet.

Specific hazards in case of fire: None are known.

Special protective equipment and precaution for fire fighters: For fires in enclosed areas, wear self-contained breathing apparatus. Do not inhale combustion gases.

6. Accidental Release Measures

Personal precautions: Depending on extent of release consider the need for fire-fighters/emergency responders with adequate personal protective equipment for cleaning-up.

Do not eat, drink, or smoke while cleaning up. Use a self-contained respirator, a mask with filter (type A class 3) or a filtering mask (e.g., EN 405). Wear protective clothing, safety glasses, and impervious gloves (e.g., neoprene gloves). Ensure adequate ventilation. Avoid all sources of ignition, hot surfaces, and open flames (see also Section 7).

Environmental precautions: Prevent spills from entering storm sewers or drains and contact with soil.

Methods and materials for containment and cleaning up: Eliminate all ignition sources. Runoff may create fire or explosion hazard in sewer system. Absorb on fire retardant liquid-absorbing material (treated sawdust, diatomaceous earth, sand). Shovel up and dispose of at an appropriate waste disposal facility in accordance with current applicable laws and regulations, and product characteristics at time of disposal (see also Section 13).

7. Handling and Storage

Precautions for safe handling: Avoid contact with eyes. Avoid prolonged repeated skin contact and breathing mists/vapours.

Use in well ventilated area away from all ignition sources. Switch off all electrical devices such as parabolic heaters, hot plates, storage heaters, etc., in good time for them to have cooled down before commencing work. Do not smoke, do not weld. Do not empty waste into sanitary drains. Take measures to prevent the build up of electrostatic charge.

Conditions for safe storage, including incompatibilities: Storage containers must be grounded and bonded. Store away from all ignition sources in a cool area equipped with an automatic sprinkling system. Ensure adequate ventilation. Store at temperatures between $+5^{\circ}C$ and $+50^{\circ}C$. Store only in the original container.

8. Exposure Controls/Personal Protection

Information on the system design: Draw off vapours directly at the point of generation and exhaust from the work area. In the case of regular work, provide bench-mounted extraction equipment.

Exposure Limits:

Component Name (CAS No.)	*Reference*	*TWA*		*STEL*	
		Ppm	*mg m^{-3}*	*ppm*	*mg m^{-3}*
Component A (111111-11-1)	ACGIH	500	1200	–	–
	UK OEL	500	1200	–	–
Component C (4444-44-4)	German MAK	200	950	–	–

Ventilation: Use in well ventilated area with local exhaust.

Respiratory protection: Approved respiratory equipment must be used when airborne concentrations are unknown or exceed the exposure limits. When processing large amounts use a light duty construction compressed air line breathing apparatus (e.g., in accordance with EN1835), a mask with filter (type A class 3, colour brown), or a filtering half mask (e.g., in accordance with EN 405) when there is inadequate ventilation.

Eye protection: Safety glasses with side shields or chemical goggles must be worn.

Skin protection: If prolonged or repeated skin contact is likely, neoprene gloves should be worn. Good personal hygiene practices should always be followed.

9. Physical and Chemical Properties

Physical state	Liquid
Color	Colourless, transparent
Odor	Solvent, ester-like
Odor threshold	Not available
PH value	Not applicable
Melting point	Not available
Freezing point	Not available
Initial boiling point	56°C
Flash point	− 22°C DIN 51755
Evaporation rate	Not available
Flammability (solid, gas)	Not applicable
Explosion limits	Lower limit = 1.4 vol.%; upper limit = 13.0 vol.% (literature)
Vapour pressure	240 mbar (highest partial vapour pressure) at 20°C
Vapour density	Not available
Relative density	0.89 g cm^{-3} at 20°C
Solubility	Partially soluble in water at 20°C
Partition coefficient	$\log K_{ow} = 3.3$
Autoignition temperature	Not available
Decomposition temperature	Not available
Viscosity	5 cSt at 40°C (ASTM D445)

10. Stability and Reactivity

Chemical stability: No decomposition, if used according to specifications

Possibility of hazardous reactions: None are known

Conditions to avoid: Heat, sparks, flame, and build-up of static electricity

Materials to avoid: Halogens, strong acids, alkalies, and ozidizers

Hazardous decomposition products: None are known

11. Toxicological Information

Acute Toxicity:

Test	*Results*	*Basis*
Oral toxicity (rats)	Not classified	Based on ingredients
Dermal toxicity (rats)	Not classified	Product test data
Inhalation toxicity, vapor (rats)	Not classified	Based on testing of similar materials
Eye irritation (rabbits)	Eye irritant category 2A	Based on testing of similar materials
Dermal irritation (rabbits)	Not classified	Product test data

Summary Comments: May cause severe eye irritation like ocular lesions, which are reversible.

Subchronic/Chronic Toxicity:

Test	*Results*	*Comments*
Dermal sensitization (guinea pig)	Not classified: negative response in Bueller, guinea pig test; 0% animals considered positive	Product test data

Summary Comments: Component A may have a drying effect on the skin, frequent or prolonged contact may cause flaking or cracking of the skin.

12. Ecological Information

Persistence and degradability: The total of the organic components contained in the product is not classified as 'readily biodegradable' (OECD-301 A-F). However, this product is expected to be inherently biodegradable.

Bio-accumulative potential: There is no evidence to suggest bioaccumulation will occur.

Mobility: Accidental spillage may lead to penetration in the soil and groundwater. However, there is no evidence that this would cause adverse ecological effects.

Aquatic Toxicity:

Test	*Results*	*Comments*
Acute toxicity	Acute Category 3: 96 h $LC_{50} = 65\ mg\ l^{-1}$	Product test data

13. Disposal Considerations

Waste Disposal: Product is suitable for burning in an enclosed, controlled burner for fuel value or disposal by supervised incineration. Such burning may be limited by local regulation. The product is suitable for processing at an appropriate government waste disposal facility. Use of these methods is subject to user compliance with applicable laws and regulations and consideration of product characteristics at time of disposal.

Recommended European waste code (EWC): 080406

14. Transport Information

UN number: 1993

UN proper shipping name: Flammable liquid, N.O.S. (Contains Component C)

Transport hazard class: 3

Packing group: II

Marine pollutant: No

15. Regulatory Information

Inventory Status: All components are on TSCA, EINECS/ELINCS, AICS, and DSL.

German: Regulations governing combustible liquids (German-VbF) class: AI

German water endangering class (WGK) = 1, slightly water-endangering product (manufacturer classification).

Australian Regulations:

AS 1940 Class: PGII

Poisons Schedule: S5

US Regulations:

US Superfund Amendments and Reauthorization Act (SARA) Title III:

SARA (311/312) Hazard Categories: Fire, Acute

SARA 313: This product contains the following SARA 313 Toxic Release Chemicals:

Chemical name	*CAS number*	*Concentration*
Component A	111111-11-1	70–80%
Component C	4444-44-4	20–25%

The following product components are cited on the lists below:

Chemical name	*CAS number*	*List citations*
Component A	111111-11-1	NJ RTK, TSCA 12b
Component C	4444-44-4	Prop. 65, NJ RTK

16. Other Information

The information contained herein is accurate to the best of our knowledge. My Company makes no warranty of any kind, express or implied, concerning the safe use of this material in your process or in combination with other substances.

See also: Hazard Identification; Hazard Ranking; Hazardous Waste; Risk Communication.

Further Reading

American National Standard for Hazardous Industrial Chemicals – MSDS Preparation (ANSI Z-400.1-2004).
American National Standard for Hazardous Industrial Chemicals – Precautionary Labeling (ANSI Z-129.1-2000).
Globally Harmonized System of Classification and Labelling of Chemicals (GHS), United Nations, 2003.
ISO 11014-1:2003 Draft Safety Data Sheet for Chemical Products.
OECD Series on Testing and Assessment Number 33, Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures (2001).
OECD Series on Testing and Assessment Number 27, Guidance Document ON THE Use of the Harmonized System for The Classification Of Chemicals that are Hazardous for the Aquatic Environment (2001).
UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria (4th revised edition.)
UN Recommendations on the Transport of Dangerous Goods, Model Regulations (13th revised edition, 2003).

Relevant Websites

http://www.ilo.org – International Chemical Safety Cards (ICSC) in many languages.
http://www.state.nj.us – NJ RTK Factsheets.
http://www.unece.org – GHS Document/'Purple book' in Arabic, Chinese, English, French, Spanish and Russian.
http://www.cdc.gov – A list of MSDS resource sites.
http://hpd.nlm.nih.gov – (US) National Library of Medicine, Household Products Database.
http://www.pp.okstate.edu – Oklahoma State University.
http://www.ilpi.com – Where to find MSDS on the Internet.

Chemical Interactions

Carey N Pope

In reality, people are never exposed to individual chemicals in isolation. For example, multitudes of chemicals comprise the food we eat (both in the food itself and as contaminants on/in food) and there are many contaminating chemicals in air and drinking water, around the home and in the workplace. Thus, there is a continual possibility of interactions among chemicals within our bodies. There are three basic types of interactions between chemicals that can modulate toxicity: antagonism, synergism, and potentiation. Antagonism refers to the ability of one chemical to impair or limit the toxicity of another, or the joint interference with the toxic action of two or more chemicals. To illustrate these examples, let us consider two chemicals referred to as A and B. In measuring functional toxicity, a graded scale has been devised for recording the severity of response from 0 (i.e., no signs of toxicity) to 6 (lethality). If chemical A alone elicits an average response of 4 on the toxicity scale while chemical B elicits no toxicity on its own (i.e., an average response of 0), but both chemicals given together yield an average toxicity score of only 2, then it can be concluded that chemical B antagonized the toxic action of chemical A.

Treatment	*Toxicity score*
Chemical A	4
Chemical B	0
Both A and B	2

If on the other hand, Chemical A and Chemical B both elicited some degree of toxicity using this same scale, for example, chemical A caused an average response of 3 and B also elicited an average response of 3, but when given together a response of only 1 was noted, it is concluded that both chemicals antagonized the toxic actions of each other.

Treatment	*Toxicity score*
Chemical A	3
Chemical B	3
Both A and B	1

A simple mathematical description of antagonism is

$$T_{\text{Both}} < T_{\text{A}} + T_{\text{B}}$$

where T_{Both} is the degree of toxic response when both chemicals are given together while T_{A} and T_{B} represent the degree of toxic response when either chemical A or chemical B is given alone.

Antagonism is a relatively common phenomenon, and antidotal strategies are often based on this type of interaction. Chemical antagonism is a simple interaction between two chemicals in which the formed complex is less toxic. Functional antagonism occurs when two chemicals have opposing actions on physiology, thus their combined effects counteract each other. Kinetic or dispositional antagonism occurs when the absorption, distribution, elimination, or biotransformation of a chemical is altered by

another such that less toxicant reaches its target site. Finally, receptor antagonism occurs when two chemicals bind to a specific receptor in the body, and competition between the two leads to lesser toxicity.

Synergism refers to a greater toxic response with exposure to two chemicals than would be expected based on the toxic response elicited by either of the chemicals alone. Potentiation is similar to synergism in that greater toxicity is seen with exposure to two chemicals than expected based on the responses elicited by those chemicals alone, but in this case one of the chemicals has no capacity to elicit the toxic response on its own. Using the toxicity grading scale above, examples of synergism and potentiation can be considered.

Treatment	*Toxicity score*
Synergism	
Chemical A	1
Chemical B	1
Both A and B	5
Potentiation	
Chemical A	2
Chemical B	0
Both A and B	6

A simple mathematical description of synergism or potentiation is

$$T_{\text{Both}} > T_{\text{A}} + T_{\text{B}}$$

where T_{Both} is the degree of toxic response when both chemicals are given together while T_{A} and T_{B} represent the degree of toxic response when either chemical A or chemical B is given alone.

As noted above, people are exposed to many chemicals at any given time through various environmental media. While interactions between two chemicals are relatively straightforward and easy to understand, with more than two chemicals the analysis becomes exceedingly complicated. However, study of the interactive effects of chemicals can often provide specific information on their mechanisms of action.

See also: Interactive Toxicity.

Further Reading

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Chemical Mixtures, Toxicology and Risk Assessment *See* Mixtures, Toxicology and Risk Assessment.

Chemical Warfare Agents *See* Anthrax; Arsenical Vomiting Agents; Bio Warfare and Terrorism:Toxins and Other Mid-Spectrum Agents; Blister Agents/Vesicants; Botulinum Toxin; BZ; Chlorine.

Chemical Warfare Delivery Systems*

Thomas Cain and George O Bizzigotti

From the initial inception of chemical warfare, various delivery systems have been developed for chemical warfare agents. Chemical weapons were generally designed with two objectives in mind:

- Compatibility with existing weapons systems, that is, chemical artillery shells could have been fired from the same guns as conventional artillery shells, chemical bombs could have been dropped

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from the same airplanes as conventional bombs, etc.
- Efficient dispersal of the chemical agent at the target.

This article surveys the various types of chemical weapons delivery systems that were developed during the twentieth century, concentrating on weapons produced by the United States. In general, chemical weapons delivery systems fit into one of several general types; weapons produced by other nations tend to have similar designs.

Cylinders

Figure 1 shows a diagram of a cylinder configured for a chemical warfare agent release. The German practice was to dig deep narrow trenches below the surface of the main trench. Gas cylinders were carried in at night and placed in deep trenches so that the tops were flush with the bottom of the main trench. The cylinders were covered with boards, on top of which were placed bags filled with peat moss and soaked with potash solution; these were intended to absorb any slow leaks. Three layers of sand bags were added to the top of the absorbent bags. The night before an attack, a protective cap was removed from the valve and a lead pipe attached, with the end directed over the parapet and weighted by a sand bag.

The first toxic chemical attacks of World War I employed gas cylinders. In March 1915, the Germans emplaced 1600 large and 4130 small cylinders of chlorine gas opposite Allied troops defending Ypres, Belgium. This attack illustrated the benefits and weakness of using cylinders: although the attack covered a much larger area than could be dispersed from artillery shells, it was at the mercy of the wind. The Germans waited for 43 days for the wind to shift to a westerly direction, releasing the gas on April 22, 1915. The Germans made more chlorine releases from cylinders, and in September 1915 the Allies launched their own chlorine attacks at Loos from cylinders. In this attack, the wind shifted shortly after the release, and some British troops were overcome when the gas blew back across their lines. There were ~200 chemical attacks during World

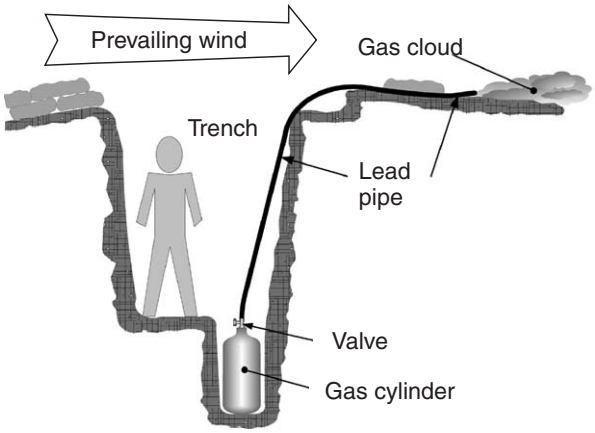

Figure 1 Setup of a gas cylinder for a chemical warfare agent release.

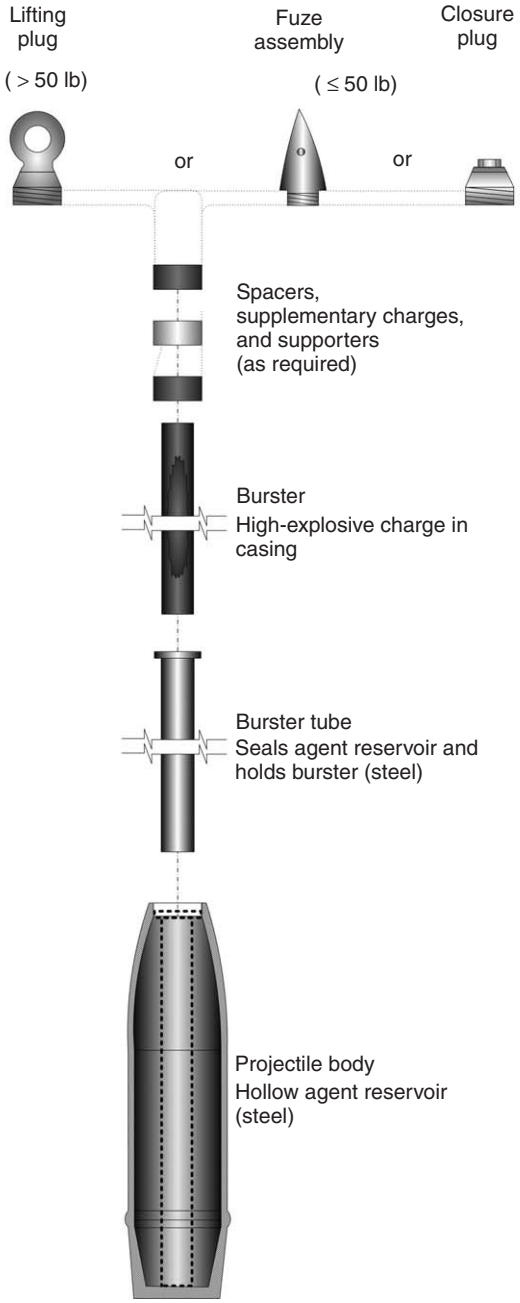

Figure 2 Configuration of a typical chemical artillery shell (projectile).

War I using chlorine and phosgene gas released from cylinders. The largest cylinder attack occurred in October 1915, when the Germans released 550 tons of chlorine from 25 000 cylinders at Rhiems.

Munitions

Chemical munitions consist of a chemical reservoir with a sequence of explosive devices, called the 'explosive train', to rupture the reservoir, disseminating the fill. Typically, an explosive train consists of a fuze with booster, a supplementary charge, and one or more bursters. Munition configurations are described by category in the following subsections.

Projectiles

Chemical projectiles consist of weapons that are fired through the use of an explosive charge and include artillery and mortar shells as well as other projectors. Artillery shells are fired from breech-loaded artillery (i.e., mechanically transported cannons, howitzers, etc., such as those on ships, tanks) while mortars are transported manually and their shells are fired by loading from the muzzle. Projectiles consist of a thick-wall reservoir filled with a chemical agent surrounding an axial explosive charge. When artillery shells are configured with the casing and propulsive charges or when mortar shells have the aft propulsive charge installed, they are referred to as cartridges. Examples of artillery shells are shown in **Figure 2** and a mortar cartridge (i.e., with propulsive charge) is shown in **Figure 3**.

Typical US projectiles range from 75 to 203 mm (3–8 in.) nominal diameters. In storage, small-caliber projectiles that can be lifted by one person (typically <50 lb) have fuzes or closing plugs in place, while larger caliber projectiles, too heavy to lift easily, have lifting lugs (unless recovered as 'duds'). If the projectile is unfuzed, the plug is removed and a fuze assembled to the projectile prior to adjusting the charge and loading the cartridge into the weapon. Fuzes may function on impact, instantaneous, or delay; they can function above ground either at a predetermined height based upon time of flight or function in proximity with the target area. Fuze function detonates the burster charge, resulting in projectile rupture and dispersal of the chemical agent as an aerosol. In the past, the United States has used several munitions that have the same configuration(s) and use identical shell bodies, bursters, and fuzes as chemical weapons, but contain different fills, including incendiary and obscurant chemicals.

Stokes Mortar The 4 in. Stokes mortar developed for chemical agent delivery was first fielded by the British in September 1915 at Loos, and was in wide use by the Somme battles of 1916; this represented the first use of projectiles filled with lethal chemicals in World War I. Chemical artillery shells (or 'projectiles') and mortars remained in chemical arsenals throughout the twentieth century. During World War I, the Germans produced chemical agent-filled projectiles for 77, 105, and 150 mm artillery pieces,

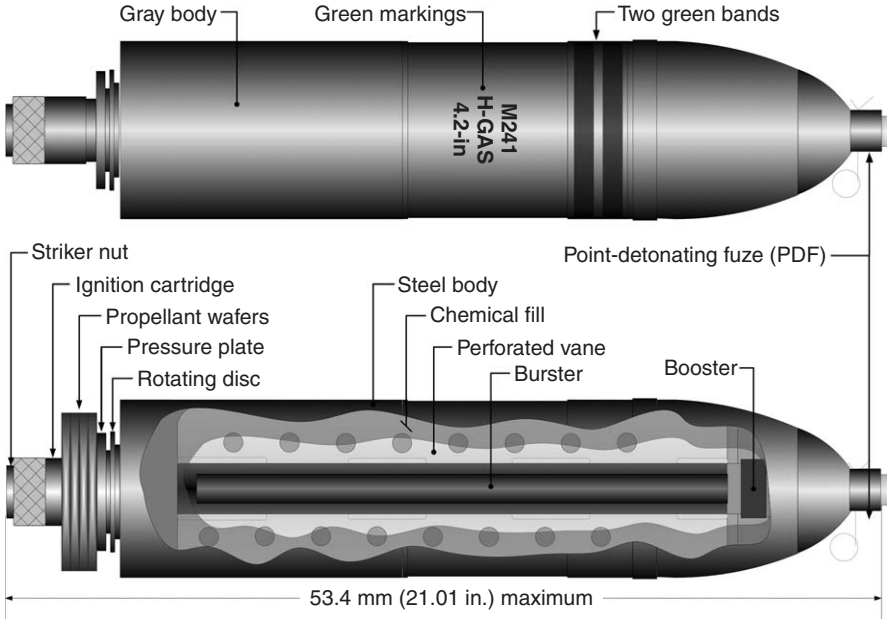

Figure 3 Configuration of a typical chemical mortar cartridge.

and the French produced agent projectiles for their 75 mm rapid firing gun. The United States used Stoke mortars, 75 mm, 4.7 in., 155 mm, 8 in., 9.2 in., and 240 mm projectiles during World War I; projectiles of the same sizes as well as 5 and 6 in., remained in the US arsenal through World War II. The 4.2 in., mortar was standardized in 1928; these remain in the current US stockpile. 105 mm., 5 in., naval, 155 mm, 175 mm and 8 in., naval projectiles were used by the US during the early Cold War years, with 105 mm, 155 mm, and 8 in., naval projectiles in the current stockpile. Virtually all chemical warfare agents have been used to fill artillery projectiles; US mortar shells were filled with mustard, phosgene, Lewisite, Tabun, and other older agents.

Livens Projector The Livens Projector was a large-scale mortar developed for delivering large amounts of chemical warfare agent. The Livens Projector, first used during the Somme offensive in 1916, consisted of a simple tube mortar closed at one end with charge box on which the projectile rested. The Livens Projectors were used in large numbers, often using electrical firing to deliver large numbers of projectiles simultaneously. The Livens Projectors could deliver quantities of agent similar to those in a cylinder attack, but originating a mile from the point of discharge. The Livens Projector was originally developed by the British, and remained in the US arsenal from World War I through the 1930s. **Figure 4** shows the configuration of Livens projectile.

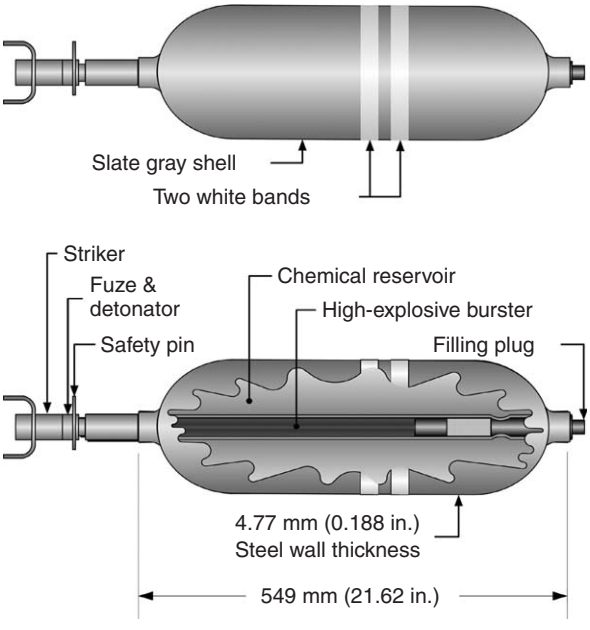

Figure 4 Configuration of a typical Livens projector.

Aerial Bombs, Submunitions, and Bomblets

Aerial chemical bombs were dropped from aircraft and range in weight from 14 to 454 kg (30–1000 lb). Bombs are typically transported and stored without explosive components (bursters, boosters, fuzes, or submunitions) or guidance systems (e.g., tail fins). Explosive components and guidance are generally installed on bombs just before loading onto an aircraft. Chemical bombs contain a reservoir filled with chemical agent surrounding an axial explosive charge. After the bomb is dropped, the fuze is armed. When the bomb arrives at its target, the fuze detonates the axial explosive train, shattering the bomb casing and dispersing the chemical fill as an aerosol. Illustrated examples of some aerial bombs are shown in **Figure 5**. An illustrated example of a bomb with submunitions is shown in **Figure 6**.

Aerial bombs containing chemical warfare agents were first developed around the end of World War I. The first US aerial bombs were 30 lb bombs that held roughly 10 lb of mustard agent. By World War II, 100 lb mustard bombs, 115 and 125 lb bombs filled with mustard, Lewisite, or GA, and 500 and 1000 lb bombs filled with hydrogen cyanide, phosgene, or

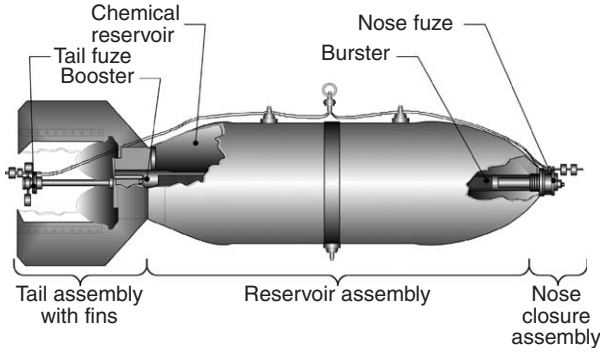

Figure 5 Configuration of typical aerial bombs.

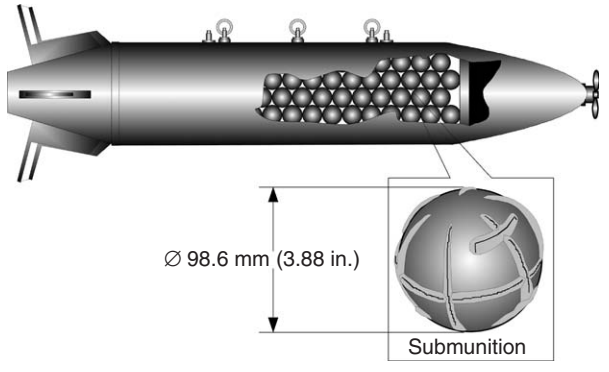

Figure 6 Configuration of bombs with submunitions.

cyanogen chloride were stockpiled. Moreover, 500 and 750 lb GB bombs were developed in the 1950s and remain in the US stockpile.

The payload of some larger munitions contain of a number of submunitions or 'bomblets', small munitions ranging in size from ~25–100 mm (1–4 in.) in diameter. Submunitions and bomblets frequently have explosive components. Bomblets are small bombs used to fill a larger munition. Bomblets are small cylindrical or spherical containers equipped with an axial explosive charge. After the cluster bomb is dropped, the bomblets are released. After release, aerodynamic forces cause the bomblets to spin, which arms the fuze. When the bomblets arrive on target, the fuze detonates the axial explosive charge, or burster. The burster shatters the bomblet casing and causes the chemical agent to disperse as an aerosol. **Figure 6** shows the configuration of a typical chemical cluster munition.

The first chemical cluster munitions were mustard-filled and were developed at the end of World War II. A 1000 lb GB-filled cluster bomb was developed during the Cold War; the weapon held 76 bomblets. Approximately 356 GB-filled bomblets filled the Honest John missile from the 1960s; a smaller warhead holding 52 bomblets was developed for the Little John missile. The Sergeant long-range missile had a warhead containing 330 bomblets; it had a range of 75 miles.

Rockets

Rockets or missiles are fully assembled, self-propelled munition that may have a single chemical reservoir or bomblets (bomblets are not normally present while in storage). Chemical rockets range in size from 60 mm (2.36 in.) diameter bazooka rounds to the Honest John Rocket with a 762 mm (30 in.) diameter and 364 bomblets. **Figure 7** illustrates the exterior configuration of some US chemical rockets.

The M55 chemical agent rocket was first developed in the early 1960s and remains in the US chemical stockpile. The rocket is in a shipping and firing container. The rocket includes (from the rear

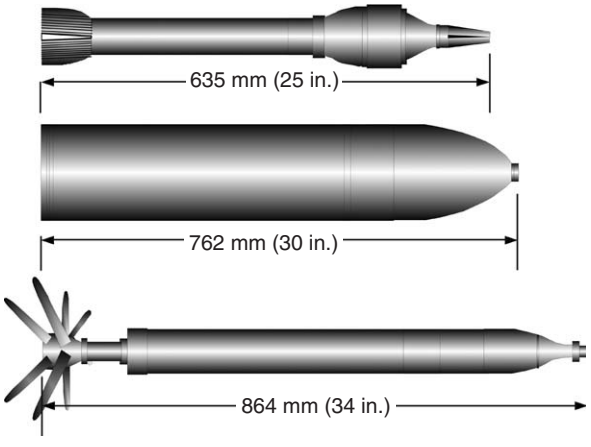

Figure 7 Configuration of typical US chemical rockets.

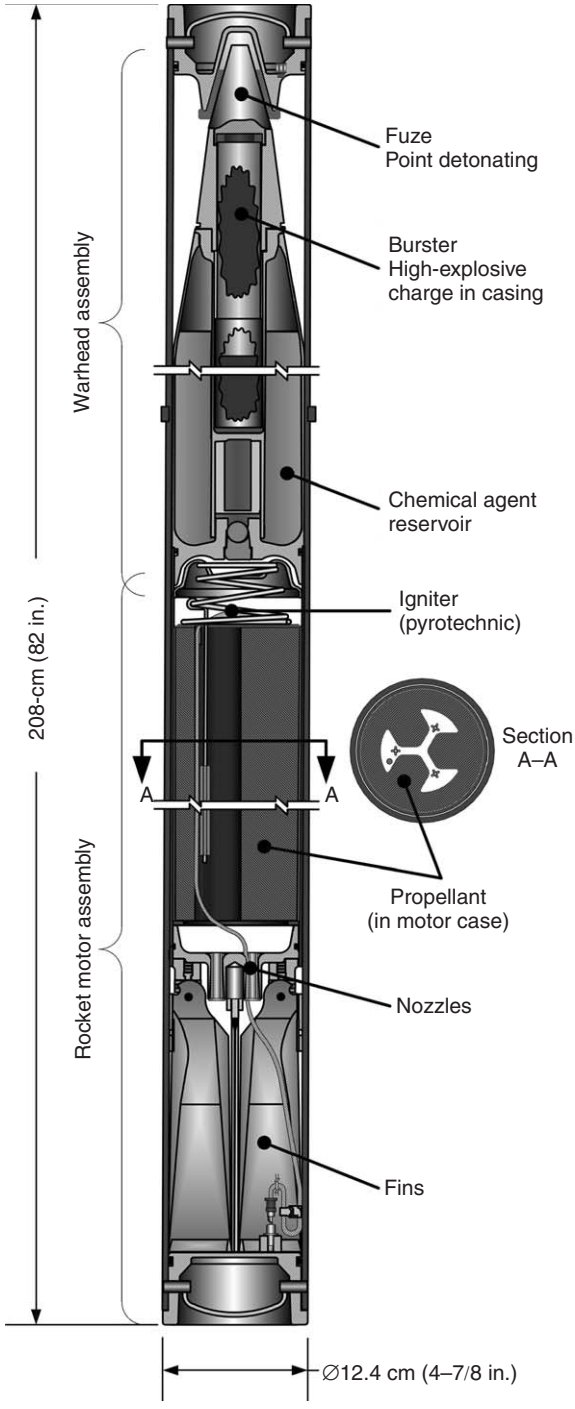

Figure 8 Configuration of the US M55 115 mm Chemical Rocket (82 in. long × 4–7/8 in. diameter in shipping and firing container).

forward) a set of spring-loaded guidance fins, an M28 double-base rocket propellant, a reservoir filled with chemical agent GB or VX surrounding an axial explosive charge, and a fuze. When the rocket propellant is ignited, it thrusts the rocket out of the shipping and firing container. Spring-loaded fins extend and the rocket follows a trajectory based on the angle on firing; the fuze is armed in flight. When the rocket arrives at its target, the fuze detonates the axial explosive charge, or burster. The burster shatters the rocket casing and causes the chemical agent to disperse as an aerosol. **Figure 8** shows an annotated cutaway of the M55 115 mm Chemical Rocket, which exists in the US stockpile.

Chemical rockets were first developed during World War II. The Germans produced 15 cm tractor rockets during the war, and the US standardized a 7.2 in rocket filled with cyanogen chloride or phosgene in 1945; these were stockpiled through the early Cold War. During the 1950s, a GB reservoir was developed for adaptation to a 3.5 in. heat rocket.

Spray Tanks

Spray tanks or spray apparatus range in size from portable units carried by one person up to tanks weighing ~900 kg (1 ton). These tanks were not designed to use explosives to disseminate chemicals but may have explosive components designed to either open valves or eject the spray tank from the aircraft. Spray tanks typically have an agent reservoir that feeds chemical warfare agent to a spray nozzle via a discharge tube. The pressure required to spray the agent can be generated in different ways. Some US spray tanks used a pressure tank filled with compressed gas; others used a scoop to generate pressure using ram air. **Figure 9** shows the configuration of the TMU-28 spray tank.

Spray tanks that allowed warplanes to deliver chemical warfare agents were initially developed during World War II and designed for use with mustard or lewisite. The current stockpile includes the TMU-28 spray tank filled with VX. Chemical spray tanks are containers designed for external use on aircraft for the dissemination of toxic chemical agents, smoke, and incapacitating chemical agents. The Aero 14B Spray Tank is pressure-controlled while the TMU-28/B uses a ram air system for airborne dispersion of chemical warfare agents.

Manually Delivered

Manually delivered munitions are small items that are placed by hand or delivered by an individual soldier.

Placed Placed munitions are delivered to a target by hand and include land mines and smoke pots. These weapons usually contained from 3.8 to 191 (1–5 gallons) of liquid so they could be transported by one person. Explosive components, when used, were either installed or stored in the same container used to store the weapon. As with other munitions, fuzes or activators were not typically installed until ready for delivery. An example of a chemical land mine is shown in **Figure 10**. Smoke canisters typically used a slow-burning material to cause chemical fills to form a smoke.

Chemical landmines were first developed during World War II; these mines were essentially a 1 gallon storage can filled with mustard and having an attached detonator. The M23 chemical agent landmine, first developed in 1960, contains a reservoir filled with chemical agent surrounding an explosive charge. The top of the land mine has a pressure

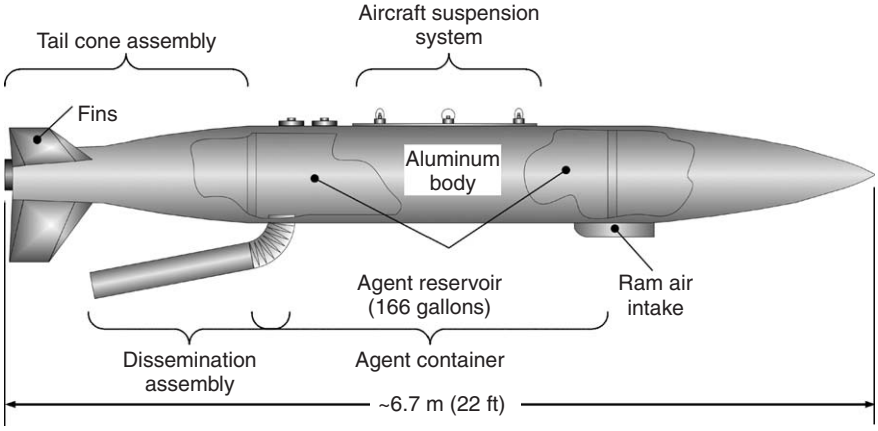

Figure 9 Configuration of the US TMU-28 chemical spray tank.

plate; when the pressure plate is depressed, the fuze detonates the explosive charge, or burster. The burster shatters the mine casing, blows away the soil covering the mine, and causes the chemical agent to disperse as an aerosol. The M23 could also be connected to a remote detonator via a wire. **Figure 10** shows the configuration of the M23 chemical landmine.

Thrown Thrown munitions are manually thrown or ejected by a small explosive charge and include grenades, ejection smoke canisters, and the like. These could be explosive, smoking, or frangible. Frangible, or breakable, munitions were designed to rely on the force of gravity and their impact with the ground to cause the munition case to rupture. The chemical in the munition would then be disseminated by splashing or evaporation.

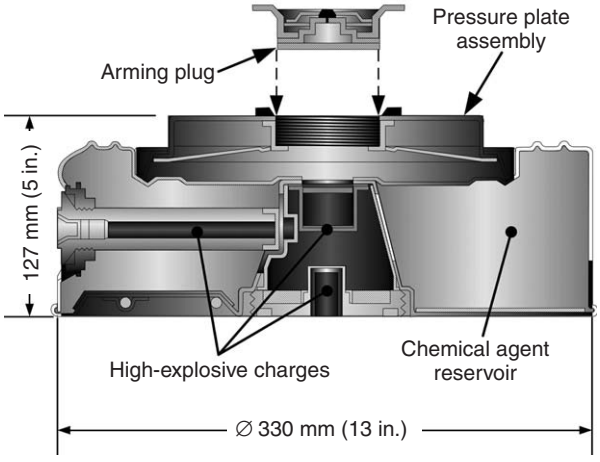

Figure 10 Configuration of the US M23 chemical land mine.

Binary Weapons

The United States began investigating binary weapons in the 1950s. Conventional 'unitary' chemical munitions contain the actual lethal agent, whereas binary weapons contain two components until the weapons are used. At the point that a binary projectile is fired or a binary bomb is dropped, the two components undergo a chemical reaction that forms the lethal agent. The US produced binary weapons during the 1980s; most of these weapons have already been destroyed.

In binary projectiles, the two components were contained in two separate sealed plastic containers. These containers could be stored separately and loaded into the rear of the shell immediately prior to firing. Upon firing, the setback and spin forces caused the containers to rupture, allowing the reactants to combine *en route* to the target. The burster for the binary projectile is container in the front of the projectile. **Figure 11** shows the configuration of a binary chemical projectile.

Storage Containers

Bulk chemical agent is stored in containers. Several different types of containers have been used to store or transport chemical agent. Types of containers that have been used include glass ampoules and bottles, drums, metal cylinders meeting US Department of Transportation specifications of various sizes, and up to a '1 ton container', which is a commercial metal

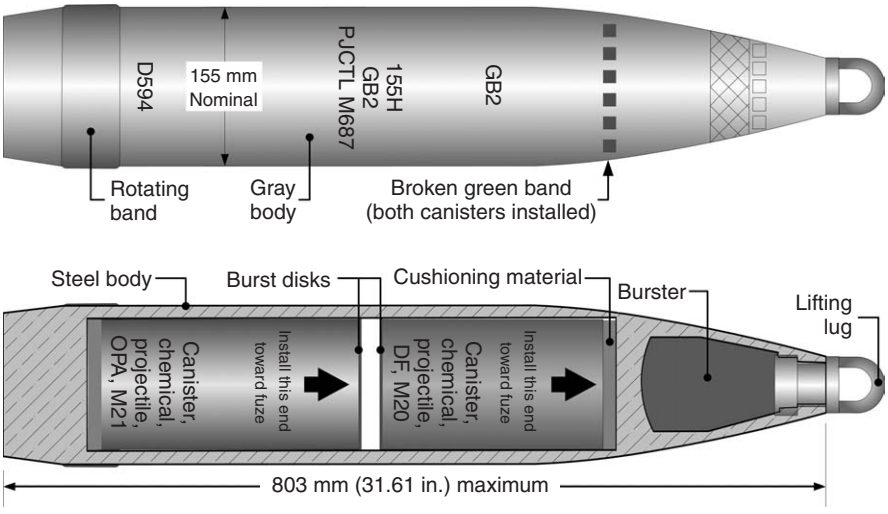

Figure 11 Configuration of a typical binary artillery shell.

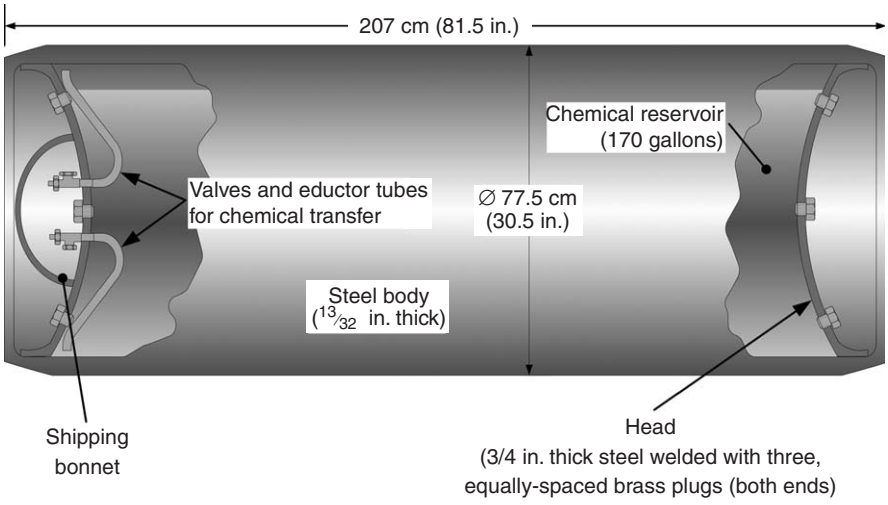

Figure 12 Configuration of a ton container.

cylinder used to transport chlorine gas and other chemical compounds.

The US uses containers similar to containers in general use in the chemical industry. They are probably the least hazardous way to store chemical agent because they do not contain any explosive or energetic components. **Figure 12** shows the configuration of a ton container used for bulk chemical agent storage.

See also: Ancient Warfare and Toxicology; Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents; Chemical Warfare During WW1.

Chemical Warfare During WW1*

George O Bizzigotti

Chlorine Gas

At 5 p.m. on April 22, 1915, German troops at Ypres discharged 180 000 kg of chlorine gas from 5730 cylinders on the line between Steenstraat on the Yser Canal, through Bixschoote and Langemark, to Poelcappelle. The gas cloud blew with the wind, and either killed or caused the French and Algerian troops in the opposing trenches to flee, opening an 8–9 km gap in the Allied line. On April 24, 1915, the Germans conducted a second chlorine gas attack at Ypres, this time against Canadian troops.

On May 31, 1915, chlorine was first employed on the eastern front, by the Germans at Bolimow, near Skierniewice, 50 km southeast of Warsaw. This attack employed 12 000 cylinders, releasing 264 tons of chlorine along a 12 km line. There were ~200 chemical attacks during World War I using gas released from cylinders; the largest of these occurred in October 1915 when the Germans released 550 tons of chlorine from 25 000 cylinders at Rhiems.

Prof. Fritz Haber was chief of the German chemical warfare service during World War I and personally directed the first chlorine gas attack. Haber, a Nobel laureate and known for his discovery of a process for synthesizing ammonia by the combination of nitrogen and hydrogen, is often referred to as the father of chemical warfare.

The Antecedents of Chemical Warfare

Many chronicles of the history of chemical warfare begin with "At 5 PM on 22 April 1915..." What is perhaps less appreciated is that the first chlorine attack represented merely an escalation of an existing use of irritant chemicals. The use of irritating smokes dates to antiquity; the Chinese used arsenical smokes as early as 1000 BC. The use of smoke from burning sulfur against enemy fortifications was a feature of classical Greek warfare described by Thucydides. The first international agreement limiting the use of chemical weapons, concluded in 1675 between

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France and Germany, prohibited the use of poison bullets.

Chemical warfare was debated at several points during the nineteenth century. Sir Lyon Playfair's proposed the use of cacodyl cyanide-filled shells to break the siege of Sebastopol during the Crimean War was rejected by the British Army. During the American Civil War, Mr. John Doughty suggested the use of chlorine-filled artillery shells, but this plan was never implemented. Several other proposals for chemical warfare appeared during the siege of Petersburg in 1864, but were not acted upon. During the late part of the century, there were several efforts to ban chemical warfare. The Brussels Convention of 1874 on the Law and Customs of War banned the use of poison gases. Signatories of Declaration (IV,2) at International Peace Conference at the Hague in 1899 agreed to abstain from the use of projectiles which had the sole objective of the diffusion of asphyxiating or deleterious gases. In addition, the vagaries of wind and weather and the lack of advanced chemical production technology had served as an effective limitation to the employment of chemicals in warfare prior to 1914.

In fact, several belligerents in World War I had been using munitions filled with irritants from almost the beginning of hostilities. The French first used shells filled with ethyl bromoacetate in August 1914, less than 1 month into the war, and chloroacetone was introduced into the French arsenal in November 1914. On October 27, 1914, the Germans at Neuve-Chappelle used the 'Ni-Schrapnell' 105 mm shell, which consisted of lead balls embedded in powdered *o*-dianisidine chlorosulfonate; the inclusion of shrapnel in the shell is generally considered to have been an effort to make the weapon not solely a chemical munition to comply with the Hague declaration. On January 31, 1915 at Boloimow, the Germans introduced 150 mm shells filled with 'T-Stoff', a mixture of brominated aromatics including xylyl bromide, xylylene bromide, and benzyl bromide. All these compounds are extreme irritants capable of severely limiting the effectiveness of unprotected troops. The use of chlorine in April 1915 was thus not entirely a novel type of warfare.

Development of Additional Agents

As the war continued, many toxic compounds in addition to chlorine were tested for utility as chemical warfare agents:

- Bromine was expensive and in limited supply, and was considered more valuable as a feedstock for the manufacture of brominated organic agents.
- Trichloromethylsulfuryl chloride.
- Phosgene (CG) was released from 4000 cylinders (88 tons) on December 19, 1915, at Nieltje in Flanders; phosgene remained a significant chemical warfare agent through World War II.
- Monochloromethyl chloroformate was first used by the Germans at the Somme in the summer of 1916.
- Trichloromethyl chloroformate (DP, diphosgene) was first used in the summer of 1915. It eventually replaced monochloromethyl chloroformate in German gas shells and was used extensively through the end of the war.
- Hydrogen cyanide (AC), an agent, was used extensively by the French. There was considerable debate about whether the French weapons systems were capable of creating lethal cyanide concentrations on the battlefield.
- Hydrogen sulfide.
- Trichloronitromethane (PS, chloropicrin), was mixed with diphosgene in the German 'Green Cross' shell.
- Cyanogen bromide.
- Cyanogen chloride (CK).
- Phenylcarbamine dichloride (phosgene anilide) was used in German gas shells.
- Dichloromethyl ether.
- Dibromomethyl ether.
- Methyl cyanoformate.
- Ethyl cyanoformate.
- Methanesulfonyl chloride.
- Ethanesulfonyl chloride.
- Ethyldichloroarsine, first used in March 1918.
- Methyldichloroarsine.
- Ethyldibromoarsine.
- Bischloroethyl sulfide (HS, mustard gas) was first used in an artillery attack on July 12, 1917 by the Germans. This agent caused the most casualties of any agent used during World War I and remained in chemical stockpiles for nearly a century; it is currently slated for destruction in the United States and Russia.

Of the many chemical warfare agents tried, chlorine, phosgene, diphosgene, chloropicrin, hydrogen cyanide, cyanogen chloride, and mustard were produced and used in significant quantities. Total production of the major agents is given in **Table 1**.

Development of Additional Means of Delivery

At the same time as they experimented with more lethal chemical agents, both sides worked to develop more effective methods of agent delivery. Gas cloud

Table 1 Production of chemical warfare agents during World War I (in tons)[a]

Country	*Chlorine*	*Phosgene*	*Diphosgene*	*Mustard*	*Chloropicrin*	*Cyanides*	*Total*
Germany	58 100	18 100	11 600	7600	4 100		99 500
France	12 500	15 700		2000	500	7700	38 400
Britain	20 800	1 400		500	8 000	400	31 100
United States	2 400	1 400		900	2 500		7 200
Austria	NA	NA	NA	0	NA	NA	5 245
Italy	NA	NA	NA	0	NA	NA	4 100
Russia	NA	NA	NA	0	NA	NA	3 650
Total	93 800	36 600	11 600	11 000	15 100	8100	189 195

[a]NA denotes figures not available. Note that a portion of the chlorine, phosgene, and cyanide were used for nonchemical warfare purposes. A total of 150 000 tons of chemicals were produced for chemical warfare purposes, and 125 000 tons of that were actually used on the battlefield.

attacks relied on the wind; in the absence of wind or if the wind blew from the wrong direction, gas cylinders were useless. If the wind shifted shortly after a release, the gas would blow back onto the attacking forces. Thus, a number of new means of delivering chemical warfare agents to the opposing forces were introduced:

- The 4 in. Stokes mortar developed for chemical agent delivery, first fielded in September 1915 at Loos; this represented the first use of projectiles filled with lethal chemicals in World War I.
- The Germans produced chemical agent-filled projectiles for 77, 105, and 150 mm artillery pieces, and the French produced agent-filled projectiles for their 75 mm rapid firing gun.
- The British Livens Projector was a large-scale mortar developed for delivering large amounts of chemical warfare agent.

The Toll of Chemical Warfare, 1914–18

It is difficult to find a definitive figure for the numbers of men injured and killed by chemical warfare agents during World War I. British casualties alone can be estimated at 185 000 injured and 8700 dead. Prentiss gives a figure of 1 296 853 casualties produced by ~125 000 tons of chemical warfare agents used by the combatants, but it is known that in many cases the official figures underestimate the number of casualties. Furthermore, it is unclear to what degree the official figures include individuals who survived gas attacks with little apparent effect but who developed serious complications only after the war. Given Prentiss' estimate of 10 000 000 battle deaths from the war, it is arguable as to whether chemical warfare was more or less horrific than the other methods of conducting the war.

See also: Chemical Warfare Delivery Systems; Chlorine; Phosgene.

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Chemicals of Environmental Concern

Steve J D'Surney and Mike D Smith

Introduction

Environmental contaminants are any physical, chemical, biological, or radiological substance or matter that has an adverse effect on air, water, soil, or living organisms. In some cases environmental contamination is a clear-cut phenomenon, whereas in others, the perception lies largely in the eyes of the beholder. Toxic organochlorine solvent residues leached into water supplies from a hazardous waste dump are pollutants in anybody's view. Frequently, time and place determine what may be called a pollutant. The phosphate that the sewage treatment plant operator has to remove from wastewater is chemically the same as the phosphate that a nearby farmer has to buy at high prices for fertilizer.

A reasonable definition of a pollutant is a substance present in greater than normal concentration as a result of human activity and having a detrimental effect upon its environment or upon something of value in that environment. Contaminants, which are not classified as pollutants unless they have some detrimental effect, cause deviations from the normal composition of an environment.

Pollutants can enter through direct dumping, piped outflow, and channeled waste streams as localized point sources, or as diffuse nonpoint sources they can enter rivers, lakes, streams, and groundwater through runoff and soil percolation. Nonpoint sources are considered to be major contributors to air, water, and soil pollution which include: runoff from paved streets and parking lots, agricultural lots, soil erosion from logging, atmospheric deposition of acidic or toxic air pollutants (**Table 1**). The source is particularly important, because it is the logical place to eliminate pollution. After a pollutant is released from a source, it may act upon a receptor. The receptor is anything, both biotic and abiotic, that is affected by the pollutant. Humans whose eyes water from atmospheric oxidants are receptors. Juvenile trout that die after exposure to pesticides in water are also receptors. Eventually, if the pollutant is long-lived, it may be deposited in a long-term sink, such as aquatic sediments and soils.

Mineral and Energy Exploration

The largest quantitative source of contamination derives from mining and energy extraction. Mining and mineral processing use a variety of chemicals for extraction, ore processing, water treatment, and many other supporting activities such as overburden removal. Mining and energy extraction generate large volumes of waste and have the potential to cause a number of environmental problems if improperly managed. Water and soil degradation can result from salinization, acidification, and chemical contamination. Streams and rivers can also experience severe siltation.

Coarse tailings and rock blasting produce large amounts of dust and mobilize heavy metal contaminants, such as lead, copper, aluminum, and zinc, which can leach into surface and subsurface waters. Cyanide and mercury are used to extract gold from soil and pulverized rock. Mineral processing generates a great deal of particulate matter released from bauxite and coal processing. Acid leaching into soil, ground water and riparian environments from mine wastes is common. Acid drainage from mine tailings, ore and waste dumps, contains sulfur and sulfides such as iron sulfide, which can be converted to acids through bacterial oxidation in the presence of moisture and oxygen.

Table 1 Common sources of contaminants to the environment

Sources	*Contaminants*
Mining and mineral processing	Heavy metals, chemicals via cyanide and acids, hydrocarbon products resulting from spills and coal mining, and metallic salts
Fossil fuel combustion	Sulfur dioxide, carbon dioxide, nitric oxide, heat, acids, acid rain, ozone, soot, polycyclic aromatic hydrocarbons, and volatile organic compounds
Agriculture and forestry	Pesticides, nitrates, phosphates, greenhouse gases, and mineral salts
Industrial production	Numerous synthetic organic and inorganic compounds, organochlorines, dioxins, heavy metals, hydrocarbons, chlorinated phenols, sulfates, sulfides, surfactants, solvents, acids, bases, salts, pharmaceuticals, plastics, resins, explosives, and natural organics
Consumerism	Residential and commercial chemicals, pesticides, fertilizers, hydrocarbons, solvents, surfactants, paints, sealants, medicines, volatile organic compounds, resins, plastics, metals, salts, acids, and bases

Metal contaminants may become mobilized to cause potential health and environmental problems resulting from leaching into soil, water, and sediment. Acid mine drainage and slag leachate can contain high concentrations of heavy metals and acids. Sulfuric acid can be formed via oxidation of sulfides. As a great deal of attention is paid to the containment and remediation of acid mine drainage, the neutralization of acid pH usually results in the precipitation of many contaminants usually as metallic salts. These salts would then become soluble and may enter surface and ground water.

Oil spills and coal mining command considerable attention from the media because they are often large scale and visually very dramatic. Nothing seems worse than a mass of toxic crude oil and tarry hydrocarbons smeared over the natural habitats of some foreshore or the sight of strip mining operations. As a result, there is a massive public response and a frenzy of activity by agencies, community groups, and politicians.

There are demands for 'the environment to be saved'. Often, as in the case of large spills such as 'Torrey Canyon' or 'Exxon Valdez', very large sums of money change hands in order to mobilize whatever resources can be found to clean up the mess. For many spills, however, the ecological issues are different from those being touted in public discussion. There is, in fact, plenty of evidence that for many marine organisms large, dramatic sudden impacts are not really the most serious threat. Long-term, chronic, low-level contamination of habitats by complex exogenous agents may be more compromising in terms of environmental outcomes. In addition, coupled with the destruction, deterioration, and fragmentation of natural habitats, there exists considerably greater threats to long-term sustainability of coastal biodiversity. Attempts to disperse oil spills with surfactants may be potentially hazardous.

Fossil Fuel Combustion

Humanity's major sources of energy are derived from fossil fuels, principally oil, gas, coal, and wood. The major combustion by-products of fossil fuel burning include sulfur dioxide (SO_2), carbon dioxide (CO_2), and nitric oxide (NO_2), and partially oxidized hydrocarbons. The process of burning fossil fuels in thermal power plants, factories, homes, and motor vehicles emits enormous amounts of the aforementioned pollutants. The most important environmental concerns resulting from fossil fuel use are global climate change, acid rain, surface ozone, and particulate-/aerosol-bound toxins.

Many scientists now believe that global warming is taking place, though the magnitude of the change and the contribution from anthropogenic sources are controversial. A component of the warming observed since the 1880s may be attributed to increases in the concentration of the so-called 'greenhouse gases' such as CO_2 and methane (CH_4) in the atmosphere.

Another side effect of fossil fuel burning is acid rain. In the process of burning organic fuels, some gases, in particular SO_2 and NO_2, combine with atmospheric water vapor to form sulfuric and nitric acids. Acidified rainwater can attain pH values below 3. Acid rain can cause damage to plant life, in some cases seriously affecting the growth of forests and lakes due to acid-stimulated metal leaching from soils and rock.

Besides gaseous fossil fuel emissions that contribute to global warming and acid rain, emissions of particulate matter from incomplete burning also contribute to poor air quality. Coal burning and diesel engines are a major source of particulate organic particles. Additionally, fuel combustion and evaporative emissions from motor vehicles are also major sources of anthropogenic volatile organic compounds (VOCs). Motor vehicles account for a considerable fraction of the total emissions of nitrogen oxides, particulate hydrocarbons, and VOCs in developed countries. Of particular concern is the production of polycyclic aromatic hydrocarbons (PAHs) resulting from incomplete combustion of fossil fuels. These compounds, especially diesel soot emissions, contain some of the most potent mutagens/carcinogens known to mankind.

Compared with solid fossil fuels, natural gas and oil are less polluting. Natural gas is the least polluting fossil fuel. The main environmental problems resulting from the production and transportation of primary energy are related to mining of solid fuels (mainly coal) and oil transportation. Coal mining operations produce large amounts of slag wastes and results in acid water drainage. The continuous acid discharges from mines seriously affects aquatic ecosystems, since acid waters containing heavy concentrations of dissolved heavy metals will support only limited water flora, and will not sustain fish and many invertebrates. The major impacts from oil are associated with accidental spillages during transportation both at sea and on land. The resultant damage to coastal areas and marine life can be dramatic in the short term and may also have long-term consequences. Solid wastes and ash disposal (spoil tips) from coal mines lead to the contamination of water percolating through slag heaps that cause groundwater and soil pollution. The combustion of liquefied petroleum gas (LPG) causes the problems of liquid residual disposal.

Agriculture and Forestry

The global concentration of greenhouse gases has increased measurably over the past 250 years, partly due to land use activities such as agriculture and forestry. Carbon dioxide, methane, and nitrous oxide emissions have increased by ~31%, 131%, and 17%, respectively, since 1750. Agriculture and forestry practices have contributed to trends in emissions of these greenhouse gases through fuel consumption, land use conversions, cultivation and fertilization of soil, production of ruminant livestock, and management of livestock manure. Additionally, the irrigation of formerly arid lands leaches minerals from soils at accelerated rates resulting in toxic concentrations of agricultural pollutants which include nutrients (nitrogen and phosphorus), pesticides, pathogens, selenium, and salts. While farmers do not intend for these materials to move from the field or enterprise, they often do, carried by rainfall, snowmelt, or irrigation water. After passage of the Reclamation Act of 1902, the United States Government began building and subsidizing irrigation projects to foster settlement and development of the arid and semiarid areas of the western USA.

A wide variety of pesticides are applied to agricultural crops to control insect pests (insecticides), weeds (herbicides), fungi (fungicides), and rodents (rodenticide). Annually, 500 million pounds of pesticides are applied to farmland, and certain chemicals can travel far from the point of application. Pesticide residues reaching surface water systems may harm freshwater and marine organisms, damaging recreational and commercial fisheries. Pesticides in drinking water supplies may also pose risks to human health. Long-lived pesticides such as dichloro-diphenyl-trichloro-ethane (DDT), Aldrin, Dieldrin, mercuric and arsenic compounds still persist in the environment. Shorter lived pesticides such as chlorpyrifos, methyl parathion, 2,4 D herbicides, and numerous new compounds are a global concern.

Industrial Production

The global expansion of industrial and consumer-oriented societies is linked to large-scale industrial production and consumerism that utilize a vast array of numerous chemical compounds. The listings of such chemicals are too vast to present in this paper but some examples will be discussed here. Environmental contaminants in nature typically involve complex mixtures, partitioning factors, chemical transformations, and abiotic and biotic interactions. The biological and environmental effects are complex and may be additive, synergistic and even antagonistic in nature.

Pulp and paper mill sludge is a complex and changeable mixture of dozens or even hundreds of compounds. Some are well known, like natural wood extractives, organochlorines, organosulfides, and dioxins. Priority pollutants and chemicals of concern that must be analyzed in pulp mill residues include heavy metals, chlorinated hydrocarbons, chlorobenzenes, PAHs, chlorinated phenols, chlorinated catechols, chlorinated guaiacols, phthalates, resin acids, alkylphenols and alkylphenol ethoxylates, and plant sterols.

In 1775, PAHs were the first group of compounds known to cause cancer in humans. Nowadays, many of these compounds are well-known carcinogens in humans and animals. PAHs are produced in the environment as the result of heating organic matter to high temperatures like tobacco smoke, soot, coal tar, creosote production, wood burning, smoked foods, roasted coffee, charbroiled meat, and fossil fuel combustion exhaust. However, the major environmental source comes from asphalt, tar, used motor oil, diesel exhaust, and coal burning.

Dioxins, a by-product of herbicide and pulp and paper production, are highly toxic members of a class of organochlorine chemicals including chlorinated dibenzo-*p*-dioxins (CDDs), dibinzofurans (CDFs), polychlorinated biphenals (PCBs), brominated dibenzo-*p*-dioxins (BDDs), brominated dibenzofurans (BDFs), and polychlorinated pesticides. Dioxins and its related compounds are cytotoxic and genotoxic, and have hormonal effects that may disrupt the endocrine system and cellular signaling pathways in wildlife and humans. Dioxins have both estrogenic and antiestrogenic effects depending on the organ or tissue affected. Exposure to relatively low levels of these chemicals has had catastrophic effects on populations of Beluga whales, alligators, turtles, mink, otters, bald eagles, osprey, cormorants, terns, herring gulls, migratory birds, chickens, lake trout, chinook and coho salmon, etc., throughout the United States and Canada.

Polychlorinated dibenzofurans (PCDFs) are formed as inadvertent by-products in the production and use of polychlorinated biphenyls (PCBs), formerly used as an insulator in electrical transformers and, in combination with polychlorinated dibenzo-*p*-dioxins (PCDDs), in the production of chlorophenols and have been detected as contaminants in these products. PCDFs and PCDDs also may be produced in thermal processes such as incineration and metal processing and in the bleaching of paper pulp with free chlorine. PCDFs are also found in residual waste from the production of vinyl chloride and the chlor-alkali process for chlorine production. The relative amounts of PCDF and PCDD congeners produced

depend on the production or incineration process and vary widely.

Like PCDDs, PCDFs are ubiquitous in soil, sediments, and air. Excluding occupational or accidental exposures, most background human exposure to PCDFs occurs as a result of eating meat, milk, eggs, fish, and related products, as PCDFs are persistent in the environment and accumulate in animal fat. High exposures have occurred in relation to incidents in Japan (*yusho*) and Taiwan (*yucheng*) involving contamination of rice oil and in accidents involving electrical equipment containing PCBs. Occupational exposures also may occur in metal production and recycling, and in the production and use of chlorophenols and PCBs.

Chemical wood preservatives account for the single largest pesticide use in the United States and one of the greatest pesticide threats to public health and the environment. Wood preservatives protect wood products from fungus and insect decay. The three principle wood preservatives include chromated copper arsenate (CCA), pentachlorophenol (penta), and creosote. The Environmental Protection Agency (EPA) has classified many chemicals and even certain heavy metal contaminants, as known or probable carcinogens, teratogens, cellular toxins, endocrine disrupters, and reproductive toxins. The arsenic in CCA, certain PAHs, and dioxins are known human carcinogens and are linked to disorders and birth defects.

Consumerism

Everything we put down the drain or flush (down the commode) ends up in our watersheds which can affect the health of terrestrial and aquatic wildlife, plants, the atmosphere, and the water quality in our area. Residential and commercial use of chemicals constitutes a very large, nonpoint source of environmental contamination. A typical source of environmental contaminants are products used for household use such as cleaning agents, surfactants, pesticides, fertilizers, lawn and garden treatments, paints, sealants, and even discarded or flushed medicines. It is imperative that those seeking a healthy lifestyle and reduction in pollutant exposure choose with care the products they use to clean and maintain their homes, yards, and pets.

The three primary ways household hazardous products impact our health and the environment are through their manufacture, usage, and disposal. When one purchases a hazardous product for the home, it creates a market for these toxic chemicals. Once we open the container to use the substance, the vapors released and the water contaminated can have an unhealthy effect on humans, marine life, and water and air quality. Long after the need to use that cleansing agent, these products still have lasting effects. Once disposed of, they release chemicals into the ground and wastewater stream which may contaminate our groundwater and present a problem to wastewater treatment facilities. Most often, hazardous products are flushed into the wastewater system or disposed of in landfills which may leach toxins for many years.

The invention of chlorofluorocarbons (CFCs) in the late 1920s and early 1930s stemmed from the call for safer alternatives to the sulfur dioxide and ammonia refrigerants used at the time. Chlorofluorocarbons were chosen for their safety and for their advantageous chemical properties. These compounds are low in toxicity, nonflammable, noncorrosive, and nonreactive with other chemical species, and have desirable thermal conductivity and boiling point characteristics. These features led to increased demand as more applications arose for CFC use. However, CFCs are human-made substances that release chlorine atoms which destroy ozone in the atmosphere, which causes an increase in UV radiation at the ground level. The amount of CFCs produced (and therefore likely released into the atmosphere) steadily increased over several decades until an international agreement called the Montreal Protocol was signed in September 1987. In this agreement the world's nations agreed to phase out CFC production.

In 1962, Rachel Carson's novel, *Silent Spring*, implicated pesticides such as DDT for the decline of some wildlife populations. Continued research in the late 1960s and 1970s discovered elevated use of manufactured organic chemicals in industrial and domestic applications. Chemicals of concern included PCBs, 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD), and related dioxin and furan congeners, PAHs, and various organic solvents. Regulatory efforts by the Environmental Protection Agency resulted in a decreased production of these xenobiotics. In addition to concerns involving genotoxins, cytotoxins and teratogens are reproductive and developmental effects associated with endocrine disruptors. An endocrine disruptor has been broadly defined as an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones responsible for the maintenance of homeostasis and the regulation of developmental processes.

Suspected endocrine-disrupting chemicals are found in insecticides, herbicides, fumigants, and fungicides that are used in agriculture as well as in the home. Other endocrine disruptors are found in industrial chemicals such as detergents, resins, plasticizers, organometals, halogenated aromatic hydrocarbons, and monomers in many plastics. Exposure to these chemicals occurs through direct contact in

the workplace or at home, or through ingestion of contaminated water, food, or air. Studies have found that some of these chemicals do leach out of plastics, such as the PVC plastics used to make IV bags. When these plastics, or other materials, are burned (as well as in their production) many unwanted by-products that are endocrine disruptors or suspected endocrine disruptors are released into the air or water.

Cancer is the uncontrolled proliferation of cells of the body caused by chemical (carcinogens), physical (radiation, X-rays, UV light), and biological agents (viruses). A carcinogen is an agent that causes or induces neoplasia. Most carcinogens react with the growth control genes of the DNA of cells, causing mutations that lead to cancer. Other carcinogens cause cancer through other mechanisms that do not involve DNA. Some of these cause hormonal imbalances that cause rapid cell proliferation. One example is DDT which mimics the actions of the hormone estrogen. Estrogen stimulates cell division in the breasts and uteri of women. DDT is thought to cause certain forms of breast and uterine cancer.

Conclusions

The twentieth century has brought with it tremendous gains in science and technology as well as gains in the quality of human life and longevity. However, these gains have been accompanied by certain hazards, many associated with the 100 000 chemicals which are now commonly in use.

As stated earlier, environmental contaminants are materials that can pollute our surroundings and adversely impact living organisms. Often these pollutants are chemical compounds produced by human endeavors, although environmental contamination can also come from nonhuman sources such as naturally occurring metals, animal waste, oil seeps, and algal blooms. Environmental contaminants may pollute soil, surface water, or aquatic sediments. Many compounds also leach through soils into groundwater, potentially impacting drinking water supplies. Numerous pollutants are discharged directly into the atmosphere by human industry, where winds may transport them to Earth's most remote corners. It is important, however, to note that industry is not the sole source of contaminants; individuals also contribute to this problem through the use of household pesticides and fertilizers, improper disposal of hazardous materials (e.g., used motor oil, paints, cleaning products), and even by driving the family car. Consequently, sites with one predominant contaminant are a rarity; complex mixtures and subsequent exposures define the real world.

See also: Coke Oven Emissions; Dioxins; Organochlorine Insecticides; Polychlorinated Biphenyls (PCBs); Polycyclic Aromatic Hydrocarbons (PAHs); Volatile Organic Compounds (VOC).

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Chemical-Specific Adjustment Factor (CSAF)

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Introduction

In the development of reference or tolerable concentrations or doses, where kinetic and/or dynamic data are adequate, commonly adopted default values for interspecies differences and human variability can be replaced by more certain chemical-specific adjustment factors (CSAFs). These CSAFs represent part of a broader continuum of approaches to incorporate increasing amounts of data to reduce uncertainty, ranging from default (presumed protective) to more 'biologically based predictive' approaches. Guidance

for the adequacy of data to serve as the basis for development of CSAFs is available.

Framework for Development of CSAFs

A framework was proposed to address kinetics and dynamics separately in considering uncertainty related to interspecies differences and interindividual variability in the development of reference or tolerable concentrations/doses. Quantitation of this subdivision is supported by data on kinetic parameters and pharmacokinetic–pharmacodynamic (PKPD) modeling for a range of pharmacological and therapeutic responses to pharmaceutical agents (Renwick, 1993; Renwick and Lazarus, 1998). This framework allows the incorporation of quantitative chemical-specific data, relating to either toxicokinetics or toxicodynamics, to replace part of the usual 100-fold default uncertainty factor for interspecies differences or interindividual variability but collapses back to the usual 100-fold default in the absence of appropriate information (**Figure 1**). Owing to the nature of the data on which the subdivision is based, in the context of the framework, 'toxicokinetics' relates to the movement of the chemical around the body (i.e., the absorption, distribution, metabolism, and excretion). 'Toxicodynamics' relates specifically to the processes occurring in the target tissue(s), including metabolism.

Chemical-Specific Toxicokinetic Adjustment Factors [AK_{AF}, HK_{AF}]

The CSAFs for the toxicokinetic components of interspecies differences and interindividual variability are ratios of measurable metrics for internal exposure to the active compound such as area under the plasma or tissue-concentration time curve (*AUC*), the maximum measured concentration in blood (C_{max}) or clearance (*Cl*). For interspecies differences, this is generally determined on the basis of comparison of the results of *in vivo* kinetic studies with the active compound in animals and a representative sample of the healthy human population. For humans, relevant data on *AUC*, C_{max}, or *Cl* are generally derived from *in vivo* experimentation in volunteers given very low doses of the relevant chemical. Alternatively, relevant information on such parameters may be derived from *in vitro* enzyme studies combined with suitable scaling to determine *in vivo* activity.

For interindividual variability, most often, factors responsible for clearance mechanisms are identified (e.g., renal clearance, CYP-specific metabolism, etc.) and a CSAF derived based on measured or physiologically based pharmacokinetically (PBPK) modeled human variability in the relevant physiological and biochemical parameters. The population distribution for the relevant metric (e.g., *AUC*, C_{max}, *Cl*) for the active entity is analyzed and the CSAF (HK_{AF}) calculated as the difference between the central values for the main group and given percentiles (such as 95th, 97.5th, and 99th) for the whole population (**Figure 2**). These differences are analyzed separately for any potentially susceptible subgroup (**Figure 2**).

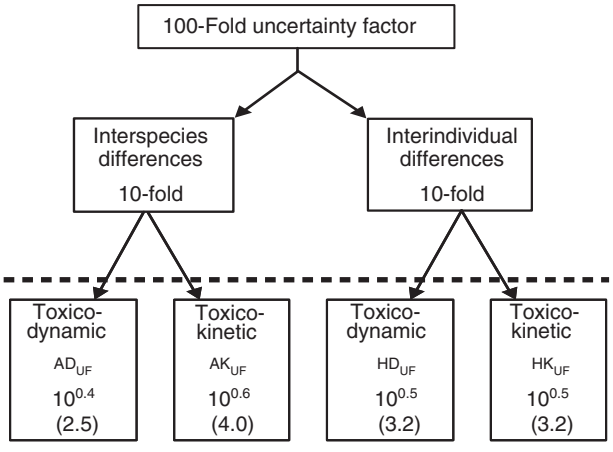

Figure 1 Subdivision of the 100-fold uncertainty factor to allow chemical-specific data to replace part of the default factor. AD_{UF} – Animal to human dynamic uncertainty factor; AK_{UF} – Animal to human kinetic uncertainty factor; HD_{UF} – Human variability dynamic uncertainty factor; and HK_{UF} – Human variability kinetic uncertainty factor. Chemical-specific data can be used to replace a default uncertainty factor (UF) by an adjustment factor (AF).

Chemical-Specific Toxicodynamic Adjustment Factors [AD_{AF}, HD_{AF}]

The CSAFs for toxicodynamic components are most simply, ratios of the doses which induce the critical toxic effect or a measurable related response *in vitro* in relevant tissues of animals and a representative sample of the healthy human population (interspecies differences) or in average versus sensitive humans (interindividual variability). At its simplest, then, replacement of the dynamic component of the default factor for interspecies differences is the ratio of the effective concentrations in critical tissues of animals versus humans (e.g., $EC_{10\ animal}/EC_{10\ human}$) for interspecies differences and in healthy human and susceptible subpopulations for interindividual variability (e.g., the $EC_{10\ average}/EC_{10\ sensitive}$).

Guidance for Development of CSAF

The International Programme on Chemical Safety provides guidance on several aspects of the

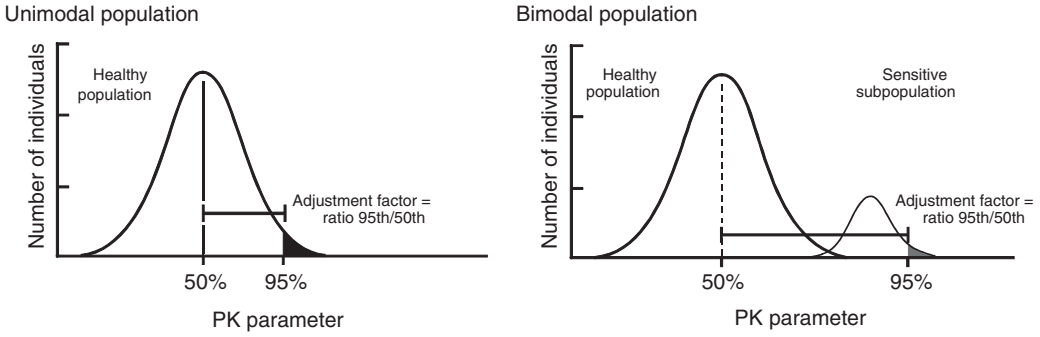

Figure 2 Development of CSAFs for interindividual variability.

development of CSAF, which are only briefly outlined here. For example, data for application in the four components of the framework must relate to the active form of the chemical. For the components of the framework addressing toxicokinetics [AK_{AF}], [HK_{AF}], choice of the appropriate metric is also an essential first step.

Choice of the appropriate end point is critical for the components addressing toxicodynamics [AD_{AF}], [HD_{AF}]. The selected measured end point must either be the critical effect itself or intimately linked thereto (with similar concentration-response and temporal relationships) based on an understanding of mode of action.

In addition, the metric for toxicokinetics or the measure of effects for toxicodynamics as a basis for CSAF needs careful consideration in relation to the delivery of the chemical to the target organ. Measures of various endpoints *in vivo* may represent purely toxicokinetics, or toxicokinetics and part or all of the toxicodynamic processes, as defined based on the subdivision of defaults. This necessitates consideration of the impact of specific data to replace the toxicokinetic and potentially a proportion or all of the toxicodynamic components of the default uncertainty factors.

For data that serve as the basis for all components, relevance of the population, the route of exposure, dose/concentration and adequacy of numbers of subjects/samples must also be considered and the potential impact on the validity of the calculated ratio addressed. For example, for *in vitro* studies which inform primarily dynamic components [AD_{AF}] and [HD_{AF}], the quality of the samples should be considered, and evidence provided that they are representative of the target population, e.g., viability, specific content, or activity of marker enzymes.

Conclusions

Consideration of relevant data in the context of a framework that addresses kinetic and dynamic aspects, explicitly, should result in greater understanding of contributing components and transparency in risk assessment. It is also anticipated that consideration in this context will lead to clearer delineation and better common understanding of the nature of specific data required which would permit development of more informative measures of dose response.

See also: Risk Assessment, Human Health; Uncertainty Factors.

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Chemotherapeutic Agents *See* Cancer Chemotherapeutic Agents.

Chernobyl

Amy Bickham Baird and Ronald K Chesser

Introduction

The meltdown of Reactor IV at the Chernobyl nuclear power plant on April 26, 1986 was the worst nuclear disaster in history. Radioactive fallout from the accident impacted not only Ukraine and its residents, but countries along the path of the radiation plume all the way to Scandinavia. A total of 31 people died as a result of the accident but the complete extent of the health effects are unknown. Despite obvious negative effects, some good came of the accident in terms of helping shape safety measures in nuclear power plants, as well as furthering our understanding of the consequences of exposure to radiation. But what about the long-term effects of exposure to radiation? Now, over 15 years after the accident, we are able to obtain a clearer understanding of these long-term effects. Scientists have been interested in the health effects of those exposed to high amounts of radiation following the meltdown, as well as effects on the nearby environment, where there still remains a high level of contamination.

Dose Estimates

Dose rates in the regions surrounding Reactor IV were highly variable due to distinct plumes of radioactive fallout released subsequent to the explosion. The first plume, designated the Western Trace, yielded doses in excess of $6\,\mathrm{Gy\,h^{-1}}$ in some areas, resulting in the death of over 400 ha of pine (*Pinus sylvestris*) forest. Radiation doses to firemen and reactor personnel exposed shortly after the explosion reached as high as 15 Gy, leading to the deaths of 29 persons (two died as a direct result of the explosion) within 4 months. Exposure of adolescents to ^{131}I in Chernobyl fallout has contributed to elevated cases of thyroid cancers in northern Ukraine and southern Belarus. Thyroid cancers are usually treatable and have not lead to substantial increases in deaths attributable to Chernobyl.

Dose rates rapidly declined subsequent to the Chernobyl accident and ensuing fire. Most of the isotopes released had short half-lives, so their energy rapidly generated absorbed radiation doses. Ninety-eight percent of the isotopes released at Chernobyl has now dissipated. The predominant radionuclides remaining are ^{137}Cs and ^{90}Sr, each having half-lives of $\sim$30 years. These isotopes, however, have high biological affinities and are readily incorporated into living tissues. Because of this affinity, the animals living in the regions near the reactor (Red Forest) are the most radioactive organisms living in otherwise natural environments. Some rodents, for example, are receiving up to $0.1\,\mathrm{Gy\,day^{-1}}$ from cesium and strontium in their muscle and bone. Documenting the doses received at Chernobyl is an important step in performing empirical studies for observing and noting the responses to exposure to the radiation.

Environmental and Genetic Impacts – Empirical Evidence

Living in a radioactive environment such as the areas at Chernobyl could have many potential impacts on the surrounding ecosystem, such as a reduction in lifespan of resident species, increased cancer risks, and a reduction in diversity (both genetic and species diversity) to name a few.

Several recent studies have examined the genetic effects of chronic exposure to radiation using naturally occurring species in the Chernobyl area. One examined population structure of the bank vole, *Clethrionomys glareolus*, from both contaminated and noncontaminated sites. This species was chosen as a model system because it contained the highest levels of internal radiation (^{137}Cs and ^{90}Sr) among rodents in the area. The researchers used DNA sequence data from the mitochondrial control region to define haplotypes and compared haplotype frequencies of chronically exposed individuals to those in control sites. This study, along with a subsequent study, monitored the structure of these populations over several years. They found that genetic diversity was consistently higher in contaminated sites as compared to control sites. They concluded that these observations could be caused by several factors, including increased mutation rate as a result of exposure to radiation, or increased genetic variation due to immigration into the exposed areas. Another study done with the same model system looked at the genetic effects above the DNA level showed that frequencies in micronuclei (chromosome breaks) are not increased in bank voles living in Chernobyl when compared to those outside the exposed area.

Additional experiments were designed to show whether or not an increase in mutation rate was occurring in animals chronically exposed to radiation at Chernobyl. One particular study exposed a transgenic mouse, Big Blue, to radiation in the most contaminated area at Chernobyl (along with individuals at a control site) for 90 days. The mice received a cumulative dose of 3 Gy of gamma-irradiation. Following exposure, mutation rates were estimated from control and experimental groups. They found that mutation rates in exposed individuals were not significantly different from those not exposed to radiation. This study was important not only for understanding the genetic consequences to animals living at Chernobyl, but it showed that chronic exposure to low doses of radiation does not have the same effect as acute doses of the cumulative amount.

Experiments like these performed at Chernobyl have immensely increased the understanding of genetic effects of exposure to radiation in a natural setting. Although there were undoubtedly extreme negative effects on populations immediately following the meltdown, populations have rebounded and are flourishing. The studies examining the effects on rodents can be used to estimate the risks to humans exposed to the same toxicant. Future studies are being planned to examine the possibility of hormesis occurring, such that exposure to small amounts of radiation could increase the production of repair mechanisms in order to decrease the amount of DNA mutations. With all of the advancements in our understanding of the effects radiation on genetics and the environment, there is still much work to be done. The disaster at Chernobyl, though detrimental in many ways, has been a valuable learning experience for many disciplines.

See also: Ecotoxicology; Radiation Toxicology, Ionizing and Nonionizing; Three Mile Island.

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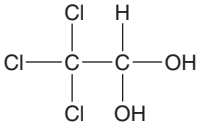
Chloral Hydrate

Michael Wahl

This article is a revision of the previous print edition article by Gregory P Wedin, volume 1, pp. 298–299, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 302-17-0
- Synonyms: Noctec; 'Knockout drops'; 'Mickey finn'; Choral; Hydrated choral; Chloralex; Chloralvan; Novochlorhydrate; Chloraldural; Chloraldurat; Tricholralacetaldehyde, hydrated; Trichloroethylidene glycol; 2,2,2-Trichlorethane-1,1-diol
- Chemical/Pharmaceutical/Other Class: Chloral derivative
- Chemical Formula: $CCl_3CH(OH)_2$
- Chemical Structure:



Uses

Chloral hydrate is used as a sedative–hypnotic agent. It is also a drug of abuse.

Exposure Routes and Pathways

Ingestion of oral dosage forms is the most common route of both accidental and intentional exposures to chloral hydrate. It is available as capsules, tablets, an oral solution, and rectal suppositories.

Toxicokinetics

Chloral hydrate is rapidly absorbed from the gastrointestinal tract following oral or rectal administration. It produces its pharmacologic action within ~30 min. Chloral hydrate is rapidly metabolized by alcohol dehydrogenase to trichloroethanol, which is pharmacologically active. A small amount is metabolized to an inactive metabolite, trichloroacetic acid. Trichloroethanol, in turn, is either conjugated with glucuronic acid to form urochloralic acid or oxidized to trichloroacetic acid. Chloral hydrate has a half-life of only a few minutes, whereas the half-life of trichloroethanol ranges from 4 to 14 h. The half-life of trichloroethanol may be prolonged following overdose.

Both chloral hydrate and trichloroethanol are highly lipid soluble. The apparent volume of distribution of chloral hydrate and trichloroethanol is 0.6–0.75 and 0.6–1.6 l kg^{-1}, respectively. Trichloroethanol is ~40% protein bound. The active and inactive metabolites of chloral hydrate are excreted primarily in the urine. The principal metabolite excreted in the urine is trichloroacetic acid and its glucuronide conjugate.

Mechanism of Toxicity

Chloral hydrate is a central nervous system (CNS) depressant. It is probably responsible for early depressant effects, but prolonged CNS depression is largely due to trichloroethanol. The mechanism by which chloral hydrate and trichloroethanol depress the CNS is not completely known.

Acute and Short-Term Toxicity (or Exposure)

Animal

As in humans, chloral hydrate is used in veterinary medicine as a sedative and hypnotic. Similar therapeutic and toxic effects are seen in humans and in animals.

Human

Chloral hydrate is an irritant to the gastrointestinal tract. Ingestion of chloral hydrate may cause nausea, vomiting, and diarrhea. Gastric perforation and esophageal stricture have been reported in cases of chloral hydrate overdose. Acute ingestion of 2 g is likely to lead to toxic symptoms. The lethal dose in adults is ~5–19 g. Lethargy progressing to deep coma, respiratory depression, hypotension, and hypothermia are characteristic toxic manifestations of chloral hydrate overdose. Unlike most other sedative–hypnotic agents, overdose with chloral hydrate may result in serious atrial and ventricular arrhythmias. Hepatic and renal dysfunction may develop.

Chronic Toxicity (or Exposure)

Animal

Two carcinogenicity studies of chloral hydrate in drinking water in rats showed no increase in tumors at any site. In a separate study of chronic chloral hydrate exposure in female mice, a slight increase in hyperplasia and slight increase in the incidence of adenoma in

the pituitary gland pars distalis were noted at the highest dose. Some studies have shown that chloral hydrate causes hepatocellular tumors in male mice.

Human

Prolonged administration of chloral hydrate may lead to the development of gastritis, skin eruptions, and renal damage. Chronic use of high doses may produce psychologic and physical dependence. Abrupt discontinuation may lead to delirium and seizures.

In Vitro Toxicity Data

Studies of chloral hydrate on porcine brain tubulin assembly assay demonstrated reduced assembly. Chinese hamster embryonic diploid cell studies of carcinogenicity demonstrated chromosomal damage only at the higher concentrations tested.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Gastrointestinal decontamination procedures should be used as appropriate based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used to adsorb chloral hydrate. The patient's level of consciousness and vital signs should be monitored closely. Obtunded patients with reduced gag reflex should be intubated. Respiratory support including oxygen and ventilation should be provided as needed. There is no antidote for chloral hydrate. Hypotension should be treated with standard measures including intravenous fluids, Trendelenburg positioning, and dopamine hydrochloride by intravenous infusion. Cardiac arrhythmias have been succesfully managed with beta blockers such as propenolol or esinolol. Class IA antiarrhythmics should be avoided, as these may worsen conduction disturbances especially with Torsades. Forced diuresis is of no value as a means to enhance the elimination of chloral hydrate. Hemodialysis and hemoperfusion may be useful in severe cases in which standard supportive measures are inadequate. Withdrawal reactions should be managed with barbiturates or other sedative–hypnotic agents.

See also: Charcoal; Gastrointestinal System.

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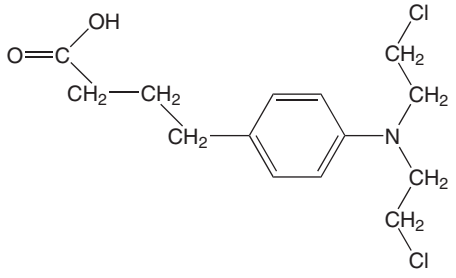
Chlorambucil

Larry J Dziuk

- Chemical Abstracts Service Registry Number: CAS 305-03-3
- Synonyms: CB 1348; 4-[Bis(2-chloroethyl)amino]benzenebutanoic acid; 4-[*p*-Bis(2-chloroethyl)aminophenyl]-butyric acid; Amboclorin; Leukeran; Chloraminophene
- Chemical Formula: $C_{14}H_{19}Cl_2NO_2$
- Chemical Structure:



Uses

Chlorambucil is an alkylating agent that retards or stops growth of cancer cells. It is used to treat chronic lymphocytic leukemia, malignant lymphomas such as lymphosarcoma, giant follicular lymphoma, ovarian cancer, and Hodgkin's disease.

Chlorambucil is also used as an immunosuppressive agent for treatment of lupus erythematosus, Waldenstrom's macroglobulinemia, glomerular nephritis, nephritic syndrome, psoriasis, Wegener's granulomatosis, chronic hepatitis, vasculitis associated with rheumatoid arthritis, and autoimmune hemolytic anemia with cold agglutins. The Food and Drug Administration (FDA) approved chlorambucil for use as a prescription drug in 1969.

The chemical also has been investigated for use as an insect chemosterilant.

Exposure Routes and Pathways

Ingestion is the primary route of exposure. Chlorambucil is administered orally in tablets containing

2 mg active ingredient. Continuous and intermittent oral schedules are used as part of the treatment regimen. Continuous treatment consists of initial daily doses of 0.1–0.2 mg kg^{-1} of body weight for 3–6 weeks. Intermittent treatment consists of 2 week treatments of 10–20 mg daily followed by rest periods of 2–4 weeks.

Occupational exposure may occur during production, formulation, packaging, and administration of the pharmaceutical. Possible exposures consist of inhalation, incidental ingestion, and dermal contact.

Toxicokinetics

Orally administered, chlorambucil is 70–80% bioavailable. It is extensively metabolized in the liver. From 15% to 60% of metabolites of the drug appear in urine after 24 h. Less than 1% of urinary excretion is the intact drug.

Mechanism of Toxicity

Chlorambucil is a cytotoxic agent. As a bifunctional alkylating agent, it causes breaks in DNA thus interfering with DNA replication and transcription.

Acute and Short-Term Toxicity (or Exposure)

Animal

When administered intraperitoneally, the LD_{50} values were 30 and 14 mg kg^{-1} in mice and rats, respectively.

Human

Possible effects of overexposure in the workplace include bone marrow toxicity and symptoms of hypersensitivity (skin rash, hives, itching, and difficulty breathing). Seizures rarely occur after administration of high doses. Children with nephritic syndrome and patients receiving high pulse doses of chlorambucil or with a prior history of seizures are at greatest risk of developing seizures. Myelosuppression, hyperuricemia, and pulmonary toxicity characterized by dry cough, fever, rales, and tachypnea may develop.

Nausea, vomiting and loss of appetite may occur during treatment. Chlorambucil chemotherapy may cause a temporary reduction in the production of blood cells by the bone marrow. Reduction in blood cells can result in anemia, risk of bruising or bleeding, and infection. This effect can begin from 10 to 14 days after the treatment has been given and may last for a few days. The extent to which the blood cell count is reduced depends on the dose of chemotherapy received and which other chemotherapy drugs, if any, were given in combination.

Chronic Toxicity (or Exposure)

A known or probable human mutagen, chlorambucil has been shown to be teratogenic, mutagenic, and carcinogenic in experimental models.

Animal

Chlorambucil is carcinogenic in experimental animals. When administered intraperitoneally to rats, lymphosarcomas, myelogenous leukemia, and reticulum cell sarcomas were noted. Mice receiving the material intraperitoneally developed lymphosarcomas, lung adenomas, and adenocarcinomas. Female mice developed ovarian neoplasms.

Human

Chlorambucil is known to be a human carcinogen based on sufficient evidence of carcinogenicity in humans. Leukemia was reported in a number of epidemiological studies in which chlorambucil was used as a chemotherapeutic agent either alone or in combination with other agents. The risk of developing cancer increases with increasing dose and time of treatment.

In Vitro Toxicity Data

Mutagenic in bacterial mutation assay (Ames), *in vitro* cytogenetics assay, and mouse micronucleus test.

Clinical Management

Medical treatment in cases of overexposure should be treated as an overdose of a cytotoxic agent. No specific antidotes are recommended. Symptoms of adverse reaction include mouth blistering, fatigue, rash, seizures, dizziness, unusual bruising or bleeding, cough, sore throat, congestion, difficulty in breathing, black tarry stools, and red urine. Because chlorambucil is cytotoxic, bone marrow toxicity can occur. Dosages are reduced if the results of medical monitoring indicate that blood cell counts are lowered.

Environmental Fate

Chlorambucil has limited water solubility, will not readily enter air, will not partition into fats, and is not likely to partition into and persist in soil or sediments. It is chemically unstable in water; hydrolysis

may be a significant depletion mechanism. Photolysis is likely a significant depletion mechanism as well.

Ecotoxicology

Chlorambucil is harmful to aquatic organisms such as daphnids. Solutions as low as 0.13 mmol caused mortality in sea urchin embryos.

The material is not toxic to activated sludge microorganisms.

Other Hazards

Chlorambucil is noncombustible, but toxic, corrosive, or flammable thermal decomposition products such as carbon monoxide, hydrogen chloride, and oxides of nitrogen may form if involved in a fire. Because of possible aquatic toxicity concerns, measures should be taken to keep firefighting water from entering surface waters.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration regulates chlorambucil under the Hazard Communication Standard and as a chemical hazard in laboratories, although there is no specific occupational exposure standard for the chemical. The Food and Drug Administration regulates clinical use of the drug and labeling requirements under the Food, Drug, and Cosmetic Act.

Relevant Website

http://ehp.niehs.nih.gov – The National Toxicology Program's Tenth Annual Report on Carcinogens.

Chloramphenicol

Greene Shepherd

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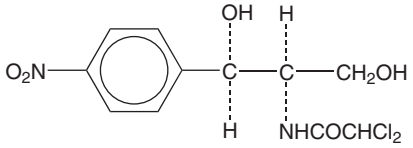
- Chemical Abstracts Service Registry Number: CAS 56-75-7
- Synonyms: AK-Chlor; Alcon opulets chloramphenicol; Amphicol; Aquamycetin; Antibiopto; Arcomicetina; Biomicin; Bioticaps; Cafenolo; Cèbenicol; Chemyzin; Chloramol; Chloromycetin; Chloroptic; Chemicetina; Chlomin; Chloratets; Chloramex; Chlorofair; Chlorsig; Chlorcol; Chloromycetin; Cloromicetin; Cloramffen; Cloramplast; Clorbiotina; Clorfenicol wolner; Clorofenicina; Cloromisol; Cloromoin; Cloroptic; Cloromicetin; Cutispray No. 4; Detreomycin
- Chemical/Pharmaceutical/Other Class: Antibiotic with both bacteriocidal and bacteriostatic properties
- Chemical Formula: $C_{11}H_{12}Cl_2N_2O_5$
- Chemical Structure:



Uses

Chloramphenicol is used as an antibiotic to treat infections of gram negative and anaerobic microorganisms.

Exposure Routes and Pathways

Oral and parenteral are the most common routes of exposure with chloramphenicol.

Toxicokinetics

Chloramphenicol is well absorbed from the gastrointestinal tract; peak serum concentrations are reached 1 or 2 h after an oral dose. Peak serum concentrations after ingestion equal those achieved after intravenous administration. Absorption after intramuscular injection is highly variable with peak concentrations achieved being 5–65% of those reached after intravenous or oral administration. The apparent volume of distribution is 0.6–1.6 l kg^{-1}. Approximately 50% of the drug is bound to plasma proteins (primarily albumin). Chloramphenicol diffuses into breast milk and readily crosses the placenta; fetal blood levels are 30–80% of maternal serum concentrations. Inactivation occurs primarily by hepatic glucuronidation. Hepatic insufficiency is known to decrease metabolism but rarely requires dose modification. Chloramphenicol has an elimination half-life of 1–4 h. Urinary excretion of unchanged

chloramphenicol is ~12% in adults and 20% in children; the remainder is eliminated as drug metabolite. Dosage modifications may be necessary for patients with renal insufficiency.

Mechanism of Toxicity

Chloramphenicol has a narrow therapeutic index with serum concentrations of 10–20 $\mu g\,ml^{-1}$ considered therapeutic and $>25\,\mu g\,ml^{-1}$ is considered toxic. Toxicity occurs through suppression of DNA and RNA synthesis in human as well as bacterial cells.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chloramphenicol is used as a veterinary antibiotic. It has a low level of animal toxicity.

Human

Acute, single overdoses of chloramphenicol in children and adults produce no significant toxicity. However, in neonates, a syndrome ('gray baby syndrome') of vomiting, irregular respiration, abdominal distension, diarrhea, cyanosis, flaccidity, hypothermia, and death may occur. The gray baby syndrome typically begins 2–9 days after the initiation of chloramphenicol. It has been associated with the administration of doses >200 mg daily. Its etiology may be the combination of immature hepatic glucuronidation in conjunction with diminished urinary excretion of the parent drug, secondary to an immature renal system.

Chronic Toxicity (or Exposure)

Animal

Three-day-old Swiss mice given IP injections of 20, 40, or 100 $mg\,kg^{-1}$ body weight for 3 months developed splenomegaly, hepatomegaly, and adenopathy. Mice chronically fed chloramphenicol in drinking water for 2 years had higher rates of lymphomas than controls.

Human

Bone marrow suppression occurs in all individuals who take chloramphenicol regularly. This is primarily manifested by a reversible fall in reticulocyte count. Depressions in platelet count may also occur. Dose-related bone marrow suppression is associated with serum concentrations $\geqslant 25\,\mu g\,ml^{-1}$. Chloramphenicol can also produce severe, idiosyncratic bone marrow toxicity resulting in aplastic anemia. This may occur more commonly in those who undergo prolonged therapy. The reported incidence of severe bone marrow toxicity ranges from 1:30 000 to 1:100 000. Greater myelotoxicity occurs in uremic patients. Other adverse effects include hypersensitivity reactions (including rash and fever), paresthesias, and optic neuritis. Chloramphenicol may also inhibit hepatic microsomal activity, specifically impairing the clearance of drugs including warfarin, phenytoin, and many other drugs.

In Vitro Toxicity Data

Chloramphenicol has been shown to decrease DNA synthesis on rat bone marrow and liver as well as in rabbit bone marrow cells *in vitro*.

Clinical Management

No specific treatment is available for chloramphenicol exposure. Hemodialysis is ineffective. Charcoal hemoperfusion or whole blood exchange transfusion has been recommended in infants with serum concentrations of $>50\,\mu g\,ml^{-1}$. Management is primarily supportive.

See also: Toxicity, Chronic; Warfarin.

Further Reading

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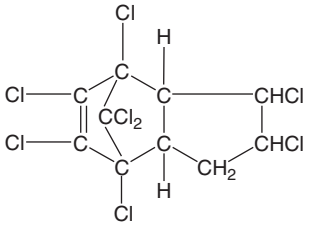
Scott JL, Finegold SM, and Belkins GA (1965) A controlled double-blind study of the hematologic toxicity of chloramphenicol. *New England Journal of Medicine* 272: 1137–1142.

Chlordane

Benny L Blaylock

- Chemical Abstracts Service Registry Number: CAS 57-74-9
- Synonyms: 1,2,4,5,6,7,8,8-Octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan; Chlordane; Chlordane; Belt; Chlor Kil; Chlortox; Corodane; Gold Crest C-100; Kilex Lindane; Kypchlor; Niran; Octachlor; Synklor; Termex; Topiclor 20; Toxichlor; Velsicol 1068
- Chemical/Pharmaceutical/Other Class: Synthetic organochlorine cyclodiene insecticide
- Chemical Formula: $C_{10}H_6Cl_8$
- Chemical Structure:



Uses

Chlordane is used as an insecticide.

Exposure Routes and Pathways

Oral, dermal, and inhalation are all primary exposure pathways. In addition, since chlordane readily crosses the placenta, *in utero* exposure may also occur.

Toxicokinetics

Absorption of chlordane occurs through the skin, the gastrointestinal tract, and the lungs. This is enhanced when the agent is in an organic solvent. Rats retained 77% of the dose absorbed through the respiratory tract. Gastrointestinal absorption is dependent on the amount of lipid-containing material in the gut.

As with other organochlorine cyclodiene compounds, chlordane is metabolized mainly by the liver microsomal cytochrome P-450 system. Several metabolites are produced including chlordene chlorohydrin, monohydroxylated dihydrochlordene, oxychlordane, and relatively smaller but similar amounts of 1,2-dichlorochlordene, 1-hydroxy-2-chlorochlordene, 1-hydroxy-2-chloro-2,3-epoxychlordene, 1,2-hydroxychlordene, trihydroxydihydrochlordene, and β-glucuronide-1-hydroxydihydrochlordene. Oxychlordane is considered the main toxic metabolite and is 20- to 25-fold more toxic than the parent compound.

Due to its high degree of lipophilicity, chlordane is readily sequestered in adipose tissue and in the kidneys, muscles, liver, and brain. The α isomer is primarily stored in adipose tissue while the γ isomer is stored to a greater extent in kidney than in fat.

Due to slow absorption and metabolism, $\sim$80% of an oral chlordane dose is excreted in urine and feces. Chlordane has also been found in human breast milk. Excretion of orally administered chlordane is slow and can take days or weeks. In two accidental poisoning reports, the half-life of chlordane in blood serum of the patients was 88 and 21 days.

Mechanism of Toxicity

Chlordane blocks the neuronal uptake of chloride ions by blocking the activity of γ-amino butyric acid. This results in only a partial depolarization of activated neurons leading to an uncontrolled excited condition. Additionally, chlordane inhibits Ca^{2+},Mg^{2+}-adenosine triphosphate (ATPase) and Na^{+},K^{+}-ATPase functions, leading to increased concentrations of intracellular free calcium in neurons and the release of neurotransmitters. This neurotransmitter release potentiates depolarization of adjacent neurons in a chain reaction manner, propagating stimuli through the central nervous system (CNS).

Acute and Short-Term Toxicity (or Exposure)

Animal

Rats exposed to 413 mg m^{-3} by inhalation showed neurological signs of toxicity including death, abnormal respiratory movements, salivation, and convulsions. Epithelial degeneration and debris in the alveoli were also observed. Hepatotoxicity included centrilobular hepatocytes enlargement; elevated liver enzymes have been reported after acute exposure. Oral exposure also produced convulsions, paralysis, and death. The acute oral LD_{50} for rats is 200–700 mg kg^{-1}, while in mice it is 145–430 mg kg^{-1}. Animals experience toxic effects from chlordane similar to those of other organochlorine insecticides except that tremor is absent. CNS

involvement produces hyperexcitability and convulsions.

Human

Acutely, the first symptoms may be convulsions or they may be nausea, vomiting, and gastrointestinal pain. Convulsions usually occur within 0.5–3 h after ingestion or dermal exposure. They are often accompanied by confusion, incoordination, excitability, or, in some cases, coma. Respiratory arrest may result from exposure to high doses. Chlordane alters liver function that can lead to therapeutic drug interactions. Recovery following convulsions has been observed in infants with dosages of 10–28 mg kg^{-1} and in adults at a dosage of 32 mg kg^{-1}. Death has been observed after dermal exposure to 425 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

Rats exhibited decreased body weights, liver lesions, and increased kidney weights. There are also reports of CNS damage including convulsions. Mice prenatally exposed to chlordane have a suppressed immunity as measured by decreased contact hypersensitivity to oxazolone and delayed macrophage activation. No reproductive or teratogenic effects have been observed in animal testing. In rats undergoing a 2 year chronic feeding study, marked liver and kidney damage was noted at 150 and 300 ppm. The incidence of hepatomas is increased in mice, but not rats, fed chlordane.

Human

Chronically, symptoms include anemia, leukopenia, thrombocytopenia, jaundice, and abnormal blood serum chemistry results. Reproductive or teratogenic effects do not appear to be present. Mutagenicity testing generally indicates that chlordane is not mutagenic. Chlordane is classified as group 2B (possibly carcinogenic to humans) by the IARC.

Clinical Management

Treatment is symptomatic. Anticonvulsive treatment with diazepam or phenobarbital is usually effective for control of convulsions. Cholestyramine treatment has been suggested for increased elimination; this treatment has not been proven beneficial for chlordane, although contaminating heptachlor excretion was increased. Activated charcoal administered as a slurry is recommended. Gastric lavage may be useful if performed quickly after ingestion (within 1 h). Emesis is not recommended due to potential CNS depression or seizures.

Environmental Fate

The half-life of chlordane in soil is approximately 4 years. Residues of this highly persistent chemical have been found in excess of 10% of the initially applied amount 10 years or more after application. Chlordane does not chemically degrade nor does it biodegrade in soils. Chlordane binds rapidly to soil particles and stays adsorbed to clay soil. Combined with its insolubility in water, this produces a low potential for ground water contamination. Sandy soil, on the other hand, allows chlordane to pass into ground water.

Evaporation is the major route by which chlordane is removed from soil. Photochemical breakdown by exposure to sunlight plays a very minor role in eliminating chlordane from soil. In water, the major mechanism by which chlordane exits is by volatilization or by adsorption to sediments. Therefore, surface water almost always has very little chlordane while the higher concentrations are found in suspended solids and sediments.

Ecotoxicology

The toxicity of chlordane for fish and fresh water invertebrates is high. Bioaccumulation is a significant factor for chlordane. It has been estimated that bioaccumulation factors for chlordane are in excess of 3000 times background water concentration. The LC_{50} (96 h) for chlordane in bluegill is 0.057–0.075 mg l^{-1} and 0.042–0.090 mg l^{-1} in rainbow trout.

In birds, chlordane is only moderately to slightly toxic. The LD_{50} in bobwhite quail is 83 mg kg^{-1}. The 8 day dietary LC_{50} for chlordane is 858 ppm in mallard ducks, 331 ppm in bobwhite quail, and 430 ppm in pheasant. In addition, chlordane has been shown to be highly toxic in earthworms and in bees.

Other Hazards

Chlordane itself is noncombustible. However, the materials used as carrier solvents (e.g., kerosene) are often flammable making the mixture in use a flammable or combustible mixture. Toxic gases and vapors such as hydrogen chloride, chlorine, phosgene, and carbon monoxide may be released in a fire involving chlordane.

Chlordane is incompatible with strong oxidizers and alkaline reagents. Chlordane will attack some

forms of plastics, rubber, and coatings. It is corrosive to iron and zinc.

Exposure Standards and Guidelines

- ADI is $0.000\,5\,mg\,kg^{-1}\,day^{-1}$.
- MCL is $0.002\,mg\,l^{-1}$.
- RfD is $0.000\,06\,mg\,kg^{-1}\,day^{-1}$.
- PEL is $0.5\,mg\,m^{-3}$ (8 h).

See also: Carbon Monoxide; Charcoal; Chlorine; Chlorine Dioxide; Cyclodienes; Diazepam; Organochlorine Insecticides; Phosgene; Pollution, Soil; Pollution, Water.

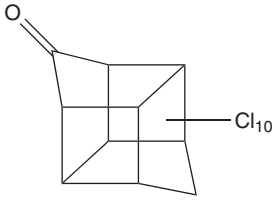
Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicology Profile for Chlordane.
http://extoxnet.orst.edu – Extension Toxicology Network, a collaborative effort of University of California, Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho.
http://toxnet.nlm.nih.gov – Specialized Information Systems, National Library of Medicine. Search for Chlordane.
http://www.osha-slc.gov – US Department of Labor, Occupational Safety and Health Administration.
http://risk.lsd.ornl.gov – Toxicity Summary for Chlordane, Risk Assessment Information System.

Chlordecone

Harihara M Mehendale and Zhengwei Cai

- Chemical Abstracts Service Registry Number: CAS 143-50-0
- Synonyms: 1,3,4-Metheno-2*H*-cyclobuta-(cd)pentaten-2-one; 1,lα,3,3α,4,5,5,5α,5β,6-Decachloroctahydro; Kepone; GC 1189; Ciba 8514; ENT 16,391; NCI-C00191; Decachloroketone; Decachlorotetracyclodecanone; Decachlorotetrahydro-4,7-methanoindeneone
- Chemical/Pharmaceutical/Other Class: Polycyclic chlorinated hydrocarbon
- Chemical Formula: $C_{10}Cl_{10}O$
- Chemical Structure:



Uses

Chlordecone was used as an insecticide and fungicide. Its use was largely terminated following contamination of the James River estuary (Virginia, USA) as a result of improper production practices.

Background Information

Kepone is the registered trade name of Allied Chemical Corporation for chlordecone. Kepone was invented in 1940 by Everett Gilbert, a researcher in the General Chemical division of Allied Chemical Corporation. It was used as an ingredient of several pesticides. Kepone was produced from 1940 to 1965 in small amounts either by Allied itself or by outside firms which sold their product to Allied. In 1966, Allied moved the Kepone project to Hopewell, Virginia. In early 1970s, Allied's international sales of Kepone began to expand and the company started to explore other arrangement for Kepone production. From 1974 to 1975, the Life Science Products, which was associated with Allied, became the sole producer of Kepone in the United States. During the 16 months of operation, over half of the 133 employees in the factory and many residents of the immediate vicinity had evidence of chlordecone intoxication. The illegally discharged chlordecone into the nearby James River by the factory resulted in extensive contamination of the water and marine life throughout the Tide-water Region of Virginia. In July 1975, the Life Science Products plant was officially closed.

Exposure Routes and Pathways

Ingestion is the most common route of exposure to chlordecone. Exposure to this agent may also occur via respiratory and dermal routes in industrial workers.

Toxicokinetics

Chlordecone is readily absorbed (>90%) from the gastrointestinal tract. Absorbed chlordecone rapidly (within 24–48 h) establishes an equilibrium of distribution among most tissues. Absorption of chlordecone may also occur through skin, especially in patients with dermatitis or skin rash. Compared to

other organochlorine pesticides, the ratio of chlordecone concentration in whole blood to that in fat tissue of patients exposed to chlordecone is much higher (1:7) and 75% chlordecone in the blood binds with albumin and high-density lipoprotein. Chlordecone is primarily stored in the liver tissue followed by the adipose tissue in both man and animals. In human, chlordecone is bioreduced to chlordecone alcohol in the liver followed by glucuronide conjugation of the alcohol metabolite. However, this metabolism is quantitatively very minor and not important toxicologically. Chlordecone is not subject to metabolism in animals studied so far, except Mongolian gerbils, which metabolize chlordecone similar to humans.

Fecal excretion is the major route of elimination and only minimal amounts of chlordecone are eliminated through urine. By 84 days, 65% of the dose is excreted in the stool and only 1.6% in the urine. A substantial amount of chlordecone representing as much as 1% of the total body content enters the intestine via biliary excretion. However, the major part of biliary chlordecone (90–95%) is reabsorbed by the intestine and recirculated to the liver (enterohepatic recirculation), while the remaining 5–10% of the biliary chlordecone entering the upper intestine appears in the feces. Elimination of chlordecone from the body is slow. The half-life of chlordecone in the blood and fat tissue is 165 and 125 days, respectively. Lactating women can also excrete substantial amounts of accumulated chlordecone through breast milk.

Mechanism of Toxicity

Chlordecone inhibits brain mitochondrial and synaptisomat membrane-bound Na^+,K^+-ATPase and oligomycin-sensitive Mg^{2+}-ATPase activity and thus may result in blocked cellular uptake and storage of neurotransmitters such as catecholamine and γ-aminobutyric acid, leading to neurotoxicity. Inhibition of Mg^{2+}-ATPase by chlordecone and the consequently deceased hepatic mitochondrial energy production have been postulated for the mechanism of chlordecone-induced hepatic biliary dysfunction. Chlordecone is an inducer of hepatic microsomal drug metabolizing system at high doses. Dietary exposure to nontoxic levels of chlordecone (10 ppm) has been shown to cause a 67-fold increase in lethality of nonlethal dose of carbon tetrachloride in laboratory rats. The mechanism for chlordecone amplification of chloromethane hepatotoxicity and lethality is incapacitated cell division due to decreased energy owing to the disrupted intracellular calcium homeostasis. Because tissue repair cannot occur, limited liver injury progresses unabated, leading to hepatic failure and animal death. Chlordecone impairs the reproductive system by mimicking the effects of excessive estrogen.

Acute and Short-Term Toxicity (or Exposure)

Animal

Exposure to chlordecone in animals may result in increased excitability and liver to body weight ratio, tremor, loss of weight, dermatologic changes, testicular atrophy and increases in hepatocellular carcinoma. Rats, mice, chicks, and quails are sensitive to the toxicity of chlordecone. The LD_{50} for oral administration of chlordecone in corn oil is 71, 126, 250, and 480 mg kg^{-1} for rabbits, rats, dogs, and chicks, respectively. In rabbits, the dermal LD_{50} for chlordecone is 434 mg kg^{-1}. When mice were given daily doses of 50, 25, or 10 mg kg^{-1}, 90% of the animals died within 5, 9, or 24 days, respectively. Chlordecone tremendously potentiates lethal effects and hepatotoxicity of chloromethane in rodents.

Human

There are no reports of death in humans exposed to chlordecone. The major target organs of chlordecone toxicity are the nervous system, the liver, and the testes.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to chlordecone resulted in significant weight loss in rats fed diets containing more than 10 ppm chlordecone for many months. Depressed growth has also been observed in pregnant rats given 2 mg kg^{-1} day^{-1} of chlordecone, in mice fed 40 ppm chlordecone, in laying hen fed 75 ppm and in quail fed 300 ppm chlordecone. Neuromuscular toxicity as evidenced by tremor following chronic exposure to chlordecone was observed in rats, chicks, Japanese quail, and mice. Liver toxicity as evidenced by the increased size of the liver relative to total body weight has been observed in rat, quail mice and dogs chronically treated with chlordecone. Chlordecone exposure through dietary consumption results in blocked and impaired reproductive function in birds and rodents and causes carcinogenesis in rats and mice.

Human

Chlordecone poisoning may cause loss of weight and skin rash. The symptoms of neuromuscular toxicity include an irregular nonpurposive waking tremor (rate, 6–8 Hz) involving the extremities, head, and trunk, and opsoclonus, an unusual oculomotor disorder consisting of chaotic eye movements causing blurred vision. Onset of tremor varies from 5 days to 8 months after initial exposure to chlordecone depending on the duration and intensity of exposure to this compound. Chlordecone poisoning causes liver and spleen enlargement, mitochondrial changes, fatty infiltration of hepatocytes, proliferation of endoplasmic reticulum, and impairment of biliary excretion of selective organic anions. Chlordecone poisoning also causes a decline in sperm number associated with abnormally low percentages of motile sperm. In most cases, these symptoms are reversible upon cessation or exposure. Removal of this chemical from the tissues is accompanied by the disappearance of clinical manifestation of toxicity.

Clinical Management

Removal of chlordecone from the body is the most important measure to take. In cases of ingestion, emesis is indicated as the treatment in other chlorinated hydrocarbon insecticide intoxications, unless the patient is comatose or has lost the gag reflex. Emesis should be followed by administration of activated charcoal and saline cathartics. Oil-based cathartics should be avoided. Administration of cholestyramine, which has been shown to bind chlordecone in the intestinal tract, is an effective way to increase fecal excretion of chlordecone and to accelerate the removal of chlordecone from the blood and other tissues.

Ecotoxicology

Chloredecone is a persistent compound. When it enters water system, marine animals accumulate the residue through the ingestion of plankton which absorb the poison. While these animals may not be killed, they become chlordecone carriers and contribute to the bioaccumulation of the compound in higher animals, including man.

See also: Organochlorine Insecticides. Toxicity, Chronic; Toxicity, Subchronic.

Further Reading

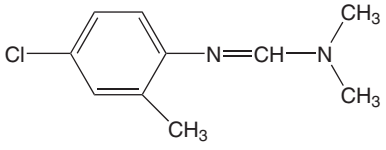
Guzelian PS (1982) Comparative toxicology of chlordecone (kepone) in humans and experimental animals. *Annual Review of Pharmacology and Toxicology* 22: 89–113.

Mehendale HM (1990) Potentiation of halomethane hepatotoxicity by chlordecone: A hypothesis for the mechanism. *Medical Hypotheses* 33: 289–299.

Chlordimeform

Paul R Harp

- Chemical Abstracts Service Registry Numbers: CAS 6164-98-3 (base); CAS 19750-95-9 (salt)
- Synonyms: *N′*-(4-Chloro-*o*-tolyl)-*N*,*N*-dimethylformamidine; Bermat; Chlordimeforme; Chlorodimeform; Chlorophedine; Chlorophenamidine; Fundal; Fundex; Galecron; Spanone; ENT 27335 (base); ENT 27567 (salt); OMS 1209; SHA 059701
- Chemical/Pharmaceutical/Other Class: Organonitrogen acaricide
- Chemical Structure:



Uses

Chlordimeform was used to control mites, ticks, and some members of the Lepidoptera order. Chlordimeform is no longer used in the United States due to its carcinogenic potential and has been withdrawn by the Codex Alimentarius Commission (FAO/WHO).

Exposure Routes and Pathways

The most common accidental exposure pathway was dermal. Inhalation during processing and packaging was also reported as well as suicide attempts through ingestion.

Toxicokinetics

The base formulation readily penetrates the skin but the salt form, which is much more water soluble, does not. Chlordimeform is rapidly demethylated to

demethylchlordimeform and didemethylchlordimeform, both of which are more toxic (based on acute oral LD_{50} values in mice) than the parent compound. Other active but less toxic metabolites include *N*-formyl-4-chloro-*o*-toluidine, 4-chloro-*o*-toluidine, 3-(4-chloro-*o*-tolyl)urea, 1,1-dimethyl-3-(4-chloro-*o*-tolyl)urea, and 1-methyl-3-(4-chloro-*o*-tolyl)urea. Neither chlordimeform nor any of its metabolites have been shown to accumulate in any specific tissue.

Studies using animals treated with radiolabeled chlordimeform indicated the majority of the radioactivity to be excreted in the urine within 24 h of the last treatment. Small amounts of radioactivity were detected in the bile and feces.

Mechanism of Toxicity

Animal studies have detected a variety of pharmacological and biochemical changes in response to chlordimeform exposure. The cause of death following acute exposure appears to be cardiovascular collapse. Chlordimeform interacts directly with and inhibits α2-adrenergic receptors in mammalian systems. Lethal doses of chlordimeform cause decreases in cardiac contractility and peripheral resistance resulting in severe hypotension. Respiratory arrest also occurs but is thought to be secondary to the cardiovascular effects. The effects of chlordimeform on the cardiovascular system share similarities with those seen with local anesthetics such as procaine. Chlordimeform also inhibits monoamine oxidase and acts as an uncoupler of oxidative phosphorylation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chlordimeform elicits cardiovascular toxicity. α2-Adrenergic receptors are modulated in rat brain regions following systemic chlordimeform treatment. One study reported that chlordimeform influenced endocrine regulation within the reproductive system (decreased gonadotropin and testosterone levels) by disrupting hypothalamic α-adrenergic activity.

Human

In addition to the cardiovascular and respiratory effects identified in animal studies, severe hemorrhagic cystitis, gross hematuria, proteinuria, swollen liver, decreased appetite, fatigue, vertigo, and dermatitis have been reported in humans following exposure to chlordimeform.

Chronic Toxicity (or Exposure)

Animal

Chlordimeform was negative in the mouse *in vivo* heritable translocation assay when administered by gavage at the maximum tolerated dosage for 7 weeks.

Human

A carcinogenic potential of some chlordimeform metabolites has been demonstrated. The US EPA's Office of Pesticide Programs has classified chlordimeform as a Group B2 – Probable human carcinogen based on findings of malignant hemangioendothelioma in mice.

In Vitro Toxicity Data

Chlordimeform was negative in a number of *in vitro* tests for DNA damage or mutagenicity.

Clinical Management

Ingestion should be treated with gastric lavage or by administration of activated charcoal, either of which is most effective if performed shortly after ingestion. For inhalation exposure, the victim should be removed from the exposure area and observed for signs of respiratory distress. In cases of dermal exposure, contaminated clothing should be removed and discarded. Any exposed areas of skin should be repeatedly washed with soap and water. For eye contact, flush the eyes with generous amounts of lukewarm water for a minimum of 15 min.

Treatment is basically symptomatic and supportive; no specific antidotes are available. Artificial ventilation with 100% humidified oxygen is necessary in cases of respiratory distress. If patient is cyanotic and cyanosis does not respond to oxygen administration, methemoglobin levels should be determined. Methemoglobinemia can be treated by intravenous administration of methylene blue. Support of cardiovascular function may also be required. Bladder damage can be determined by urinalysis. Hypotension may be treated with isotonic intravenous fluids. Dopamine or norepinephrine may be used if hypotension does not respond to infusion of fluids. Convulsions may be treated with intravenous benzodiazepines (diazepam or lorazepam); phenobarbital may be used if the convulsions are recurrent. Because chlordimeform is a monoamine oxidase inhibitor, foods with large amounts of

tryptophan or tyramine should be avoided and sympathomimetic drugs are contraindicated.

Ecotoxicology

Chlordimeform was only slightly toxic in frog tadpoles (48 h $LC_{50} > 35\,mg\,l^{-1}$), catfish (96 h $LC_{50} > 20\,mg\,l^{-1}$), and snails (48 h $LC_{50} > 60\,mg\,l^{-1}$).

See also: Monoamine Oxidase Inhibitors; Pesticides.

Relevant Website

http://www.inchem.org – Chlordimeform (Environmental Health Criteria 199); International Programme on Chemical Safety.

Chlorination By-Products

S Satheesh Anand and Harihara M Mehendale

Drinking water disinfection is a worldwide practice to eliminate the microbial contaminants and is considered to be one of the greatest public health advances in this century. Ever since the use of disinfectants, there is a huge drop in waterborne infectious diseases such as typhoid fever, cholera, hepatitis, and polio, which for many years posed threat to public health. The most widely used disinfectants are chlorine, ozone, chlorine dioxide, and chloramines. Among these, chlorine is the most efficient in removing microbes. The noteworthy biocidal effects of chlorine have been somewhat offset by the formation of potential toxic and carcinogenic chlorination by-products (CBPs). Hence, in order to balance the microbial and chemical risks, it is essential to understand better the chemistry, toxicology, and epidemiology of CBPs.

Formation of CBPs

Chlorine is applied as chlorine gas, powdered calcium hypochlorite ($Ca(OCl)_2$), or liquid sodium hypochlorite (NaOCl; bleach). Chlorine reacts with the organic (natural organic matter, NOM) or inorganic (bromide ion, Br^-) precursors in the water to form chlorine disinfection by-products (CBPs), including trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs), haloketones, chloral hydrate, and chloropicrin. Humic and fulvic acids are the predominant NOMs. When bromine exists, the chlorine oxidizes it to hypobromous acid/hypobromite ion ($HOBr/OBr^-$) to form bromo THMs (bromodichloromethane, BDCM, and dibromochloromethane, DBCM), HAAs, and HANs.

The formation of CBPs is influenced by pH, temperature, ammonia, carbonate alkalinity, chlorine dose, contact time, removal of natural organic matter before chlorine application, etc. Moreover, the composition of these mixtures may change seasonally resulting in higher CBPs during warm season compared to cold season. Generally, chlorinated THM, HAA, and HAN species dominate over brominated species, although the opposite may be true in high-bromide waters. A significant percentage of the total organic halogens still remain unaccounted for. CBPs are rapidly formed during the first 4–8 h, and nearly 90% of the final concentrations are formed within the first 24 h of chlorine addition to waters containing NOM.

Toxicology of CBPs

Since water chlorination produces carcinogenic, mutagenic, or possibly teratogenic by-products, several countries have laid down standards for various CBP levels.

The dominance of chlorine CBP groups generally decreases in the order of THMs, HAAs, and HANs. Among the THMs, chloroform ($CHCl_3$) and BDCM are the first and second most dominant species. Among HAAs, dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are the first and second most dominant species.

Drinking water ingestion is a predominant pathway of CBP exposure. However, exposure via inhalation, dermal contact, and also during showering, bathing, and swimming can occur. Generally, the metabolism of CBPs is higher in mice relative to rats and human metabolism is found to be similar to rats. The toxicity of the CBPs is highly dependent upon the species and strain of the rodents, some of the CBPs, especially THMs, cause kidney damage only in male rats and mice. A brief review of findings relevant to the toxicity of important CBPs follows.

Trihalomethanes

The THMs are volatile liquids at room temperature and a variety of toxic effects have been associated with short-term and long-term exposure of experimental animals at high doses. Each of the four most common THMs – $CHCl_3$, BDCM, DBCM, and

bromoform – has been shown to be carcinogenic to rodents in high-dose chronic studies. $CHCl_3$ is generally the predominant and the most extensively studied chemical of this class. The maximum contaminated limit for the THM is $100\,\mu g\,l^{-1}$. THMs administered by corn oil gavage cause significantly more toxicity than equivalent doses administered in an aqueous emulsion. However, administration via drinking water did not show any signs of toxicity. Nonetheless, bulk of the studies has been conducted using an oil vehicle.

Chloroform

Toxicokinetics $CHCl_3$ absorption is rapid and extensive after oral, dermal, and inhalation routes. $CHCl_3$ appears to distribute widely throughout the body, with high levels in liver and fat and lower levels in blood, brain, muscle, lung, and kidney. However, high levels were found in the kidney of male mice. $CHCl_3$ is rapidly metabolized and it undergoes both oxidative and reductive biotransformation through cytochrome P-450. While the oxidative metabolism produces phosgene, causing toxic effects, the reductive biotransformation forms dichloromethyl radical, which may react with the phospholipids to form adducts. The balance between the oxidative and reductive pathways depends on oxygen and $CHCl_3$ concentrations, animal species, strain, and the site of metabolism. However, oxidative metabolism is predominant. It is metabolized primarily by CYP2E1, whereas at high levels CYP2B1/2 is also involved in the metabolism. $CHCl_3$ biotransformation occurs mainly in the liver and kidney (only in male mice). In humans, $\sim$80% and 90% of the $CHCl_3$ is absorbed under inhalation and oral exposures, respectively. Absorption of dermal exposure was approximately equivalent to inhalation exposure. Human cytochrome P-450 2E1 catalyzes the oxidation of $CHCl_3$.

Acute and Short-Term Toxicity (or Exposure)
Animal The LD_{50} values ranging from 36 to $3245\,mg\,kg^{-1}$ in rats and mice depending upon strain, vehicle, and route of exposure have been reported. Acute and short-term exposures to $CHCl_3$ cause effects on the liver, kidney, central nervous system, and immune system. In male mice, kidney injury followed by cellular proliferation was evident at a dose as low as $60\,mg\,kg^{-1}$ and these effects were seen in liver at $240\,mg\,kg^{-1}$. In rats, such effects were seen at doses above $180\,mg\,kg^{-1}$. Higher lipid peroxidation and depleted GSH were observed following single $CHCl_3$ administration.

Corn oil administration of $CHCl_3$ to male mice (34, 90, 138, or $277\,mg\,kg^{-1}$) and rats (10, 34, 90, or $180\,mg\,kg^{-1}$) for 4 days or 3 weeks caused dose-dependent liver and kidney damage and cell proliferation at 4 days. While these effects were reversed with low doses, the toxic effects were severe with two high doses at 3 weeks. Mice exposed to 49, 147, 490, or $1470\,mg\,m^{-3}$ for 7 days exhibited severe liver damage at two high doses. Kidneys were affected only in the $1470\,mg\,m^{-3}$ group. In rats, the liver and kidney were affected only at highest dose. The extent of $CHCl_3$-induced damage via drinking water is significantly lower than other modes of administration. Drinking water $CHCl_3$ exposure to female mice for 90 days (34, 66, 92, 132, 263, and $400\,mg\,kg^{-1}$) caused central nervous system depression. Mild adaptive response with $66\,mg\,kg^{-1}$ or more was observed. $CHCl_3$ is not teratogenic, but induces fetotoxic effects (decreased fetal body weight). Signs of maternal toxicity (decreased body weight and changes in organ weight) were reported in rats, rabbits, and/or mice.

Human As with animals, $CHCl_3$ anesthesia may result in death in humans due to respiratory and cardiac arrhythmias and cardiac failure. Because of the relatively high frequency of 'late $CHCl_3$ poisoning' (liver toxicity), its use as anesthetic has been abandoned. There are considerable inter-individual differences in susceptibility. Some persons presented serious illness after an oral dose of 7.5 g of $CHCl_3$, whereas others survived a dose of 270 g $CHCl_3$. The mean lethal dose for an adult is estimated to be about 45 g. Chloroform can cause severe toxic effects in humans exposed to $9960\,mg\,m^{-3}$ (2000 ppm) for 60 min.

Chronic Toxicity (or Exposure)
Animal The predominant effects of long-term exposure occur in the liver and kidney, similar to those observed with short-term exposures. Hepatic effects were reported in mice, rats, and dogs administered $15–180\,mg\,kg^{-1}\,day^{-1}$. Reduced fertility, litter size, gestation index, and viability index were reported in a three-generation study in mice. Majority of the long-term exposure studies have been conducted for $CHCl_3$-induced cancer. $CHCl_3$ causes both benign and malignant tumors in experimental animals. $CHCl_3$ in corn oil given to male and female mice for 78 weeks caused liver tumor at 138 and $477\,mg\,kg^{-1}$and 238 and $477\,mg\,kg^{-1}$. However, tumor incidence was negative when $CHCl_3$ was exposed via drinking water or inhalation except liver and kidney tumors in Wistar rats and male Osborne–Mendel rats, respectively. Inhalation exposure to $CHCl_3$ in male BDF mice at 90 ppm for 2 years caused kidney tumor. $CHCl_3$ did not induce cancer in

other strains of rats and mice, though it causes acute and subchronic toxicity.

Human Higher frequency of hepatitis was observed in workers exposed to occupational $CHCl_3$ concentrations of 10–1000 mg m^{-3} for 1–4 years. In another study, workers exposed to 112–1158 mg m^{-3} for 1 or more years, nausea, lassitude, dry mouth, flatulence, depression, and scalding urination were reported without any liver abnormalities. There have been numerous reports over the last 15 years which have evaluated the relationship between chlorinated water and the incidence of bladder and colorectal cancer. Since $CHCl_3$ is the predominant by-product of water chlorination, it is believed to have caused the bladder and colorectal cancers observed in humans. However, there is no conclusive evidence to support this observation. The weight-of-the-evidence evaluation by International Agency for Research on Cancer (IARC) concluded that there is inadequate evidence for the carcinogenicity of chlorinated drinking water in humans. IARC classified $CHCl_3$ as possible carcinogen (group 2B) and EPA classified it as probable carcinogen (group B2).

Mode of Action A substantial body of data demonstrates a lack of direct *in vivo* or *in vitro* genotoxicity of $CHCl_3$. There is, however, compelling evidence that $CHCl_3$ produces cancer in rodents through a nongenotoxic/cytotoxic mode of action, with carcinogenesis resulting from events secondary to $CHCl_3$-induced cytolethality and regenerative cell proliferation. Thus, sustained toxicity would result in tumor development.

Bromodichloromethane

Bromine substitution generally decreases volatility and enhances lipid solubility (uptake into tissues), which increases biotransformation. Among the four THMs commonly found in drinking water, BDCM appears to be the more potent rodent toxicant and carcinogen. However, studies concerning BDCM toxicities are limited.

Toxicokinetics Absorption of BDCM appeared to be rapid and fairly complete. The highest levels were found in the liver, stomach, and kidney. The half-life of BDCM is estimated to be 1.5 h in rat, 2.5 h in mouse, and 0.45–0.63 min for humans. With an aqueous vehicle, the absorption and elimination were rapid as compared to an oil vehicle.

Like $CHCl_3$, BDCM also undergoes P-450-mediated oxidative and reductive metabolism and produces phosgene and dichloromethyl radical, respectively. Cytochrome P-450, CYP2E1, and CYP2B1/2, as well as a theta-class GST, have been implicated in the metabolism of BDCM. CYP2E1 is responsible for BDCM metabolism in humans.

Acute and Short-Term Toxicity (or Exposure)
Animal The oral LD_{50} values ranging from 450 to 969 mg kg^{-1} were reported in mice and rats. Following acute exposure, pathological changes in liver and hemorrhagic lesions in the kidney, adrenals, lung, and brain and clinical observations including ataxia, sedation, and anesthesia were noted. Males appear to be slightly more susceptible than females.

Five consecutive daily BDCM doses to female rats and mice by aqueous gavage proved to be both hepatotoxic and nephrotoxic to female rats (150–300 mg kg^{-1}), but only hepatotoxic to female mice (75–150 mg kg^{-1}). In subchronic studies of 10–14 days in mice and rats, mild effects on liver have been noted at doses as low as 37 mg kg^{-1} and the effects become more pronounced at 125–300 mg kg^{-1}. In the same study, kidney toxicity was noted at 74–148 mg kg^{-1}. Increased incidence of *sternebral* anomalies in fetus in rats at 50–200 mg kg^{-1} during 6–15 days of gestation was noted and the same doses produced maternal toxicity as evidenced by a 40% reduction in body weight gain.

Human Studies are not available for acute or short-term toxicity of BDCM in humans.

Chronic Toxicity (or Exposure)
Animal BDCM administration in drinking water to male rats and mice for 1 year in average daily doses of 4.4, 21, and 39 mg kg^{-1} for rats and 5.6, 24, and 49 mg kg^{-1} for mice caused proximal tubular damage. BDCM administered via corn oil gavage for 102 weeks, 5 days per week, to rats at 50 or 100 mg kg^{-1} and to mice at 25 or 50 mg kg^{-1} (males) and 75 or 150 mg kg^{-1} (females) resulted in non-neoplastic effects in liver and kidney. BDCM exposure to rats in drinking water at 22 and 39 mg kg^{-1} of body weight per day for 52 weeks resulted in decreased sperm motility.

Animal studies provide convincing evidence that BDCM is carcinogenic. BDCM caused cancer at lower doses and at more target sites than for any of the other THMs. In a 2 year bioassay, a corn oil gavage study, compound-related tumors were found in the liver, kidneys, and large intestine in rats. However, only renal and hepatic tumors were evident in mice.

Human No studies were located regarding long-term or carcinogenic effects in humans following exposure to BDCM. IARC concluded that there is sufficient evidence for its carcinogenicity in experimental animals and inadequate evidence for its carcinogenicity in humans and assigned to 2B class. EPA grouped BDCM in B2 class.

Mode of Action BDCM is a relatively weak mutagen, and its conjugation with GSH may lead to genotoxicity. It is proposed that BDCM induces cancer through cytotoxicity leading to regenerative hyperplasia and direct mutation of metabolites. The extent to which each of these processes contributed to the induction of tumors is at present unclear.

Dibromochloromethane

Compared to $CHCl_3$ and BDCM, the toxic potency of DBCM is lower.

Toxicokinetics The pattern of distribution and elimination of DBCM was very similar to that observed with BDCM, but it is the least studied THM. Presumably, metabolism proceeds via the same routes of biotransformation as described for BDCM. Oxidative metabolism of DBCM would be expected to yield a bromochlorocarbonyl rather than phosgene, and reductive dehalogenation would produce a bromochloromethyl radical. Half-lives of DBCM in rats and mice were estimated to be 1.2 and 2.5 h, respectively.

Acute and Short-Term Toxicity (or Exposure)
Animal Acute oral LD_{50} values ranging from 800 to 1200 mg kg^{-1} in mice and rats were reported. A DBCM dose of 500 mg kg^{-1} produced ataxia, sedation, and anesthesia in mice. Acute or repeated administration of DBCM caused decreased response rates and reduced aggressive behavior in mice and hamsters. The administration of DBCM in an aqueous vehicle for 14 days to male and female mice produced hepatotoxicity in both sexes at 250 mg kg^{-1}. Depressed immune function was also observed in both sexes at 125 and 250 mg kg^{-1}. DBCM-induced cardiotoxicity was reported in male rats after 4-week exposure. Corn oil gavage to mice for 13 weeks at 15, 30, 60, 125, or 250 mg kg^{-1} produced dose-dependent lesions and necrosis in kidney, liver, and salivary glands. Exposure on gestational days 6–15 caused a depression of maternal weight gain, but no fetal malformations. Short-term exposure of female mice in drinking water at 685 mg kg^{-1} day^{-1} caused reduction in fertility, reduced litter size, postnatal survival, and postnatal body weight.

Human Reports on acute or short-term effects of DBCM in humans are not available.

Chronic Toxicity (or Exposure)
Animal The chronic oral administration to rats (40 or 80 mg kg^{-1}) and mice (50 or 100 mg kg^{-1}) by corn oil gavage for 104 weeks caused only mild liver and kidney damage. Decreased serum cholesterol at 540 mg kg^{-1} and decreased triglycerides at 20 mg kg^{-1} for 2 years were reported in mice. In a two-generation reproductive study in mice caused reduction in fertility and gestational index in F_1 generation and only fertility was decreased in F_2 generation at 685 mg kg^{-1} in drinking water.

DBCM was not carcinogenic in rats in a 104 weeks corn oil gavage study at 40 or 80 mg kg^{-1}. However, according to NTP, there is equivocal evidence of DBCM carcinogenicity in male mice and some evidence of carcinogenicity in female mice.

Human Clinical case findings resulting from human exposure to DBCM have not been reported. Due to inadequate evidence for its carcinogenicity in humans and limited evidence for its carcinogenicity in experimental animals this compound was assigned to group 3: not classifiable it as to carcinogenicity to humans by IARC and EPA classified it as a possible carcinogen, group C.

Mode of Action The greater propensity for the metabolism of this compound and bromoform as compared with BDCM is difficult to reconcile with its lower carcinogenicity. A possible explanation is less bioavailability resulting from the greater lipophilicity of this compound and the use of corn oil as the vehicle of administration. However, *in vitro* studies showed that DBCM is more potent mutagenic than other THMs.

Bromoform

Toxicokinetics The distribution and elimination of bromoform resembled those of $CHCl_3$. Bromoform (and organic metabolite) elimination via exhaled breath was greater than that for all other THMs in the rat, but less than that for all other THMs in the mouse. The estimated half-life of bromoform was 0.8 h in rats and 8 h in mice. While both oxidative and reductive pathways were involved in bromoform metabolism, oxidative metabolism seems predominant. Bromoform, like DBCM, has a much greater

potential than BDCM to be conjugated by GSH to form a mutagenic intermediate.

Acute and Short-Term Toxicity (or Exposure)
Animal The acute oral LD_{50} values ranging from 1147 to 1550 mg kg^{-1} in mice and rats were noted. Liver is the major target organ. Hepatocelluar vacuolization was reported in mice administered with 300 mg kg^{-1} in drinking water. In rats, lethargy, shallow breathing, and ataxia were observed at 600 and 1000 mg kg^{-1}.

In a 14 day corn oil gavage study, 600 and 800 mg kg^{-1} were found to be lethal to both sexes of rats. Liver and kidney toxicity as well as decreased antibody-forming cells were evident at 250 mg kg^{-1} in male and female mice when administered for 14 days. The magnitude of the effects was less than that observed with the other THMs. Inhalation exposure to 240 or 24 ppm bromoform for 10 or 60 days, respectively, caused effects in liver and kidney. Fetotoxic response was observed after gavage administration at 50, 100, or 200 mg kg^{-1} on gestation days 6–15.

Human Bromoform was used in the late nineteenth and early twentieth centuries as a sedative to children suffering from whooping cough and several deaths due to overdoses have been reported. Hence, its use was discontinued. The principal causes for death were severe central nervous system depression and respiratory failure. No studies are available for short-term bromoform toxicity in humans.

Chronic Toxicity (or Exposure)
Animal Slight liver and kidney damage occurred after chronic (1 or 2 years) exposure to high dose of bromoform. No developmental and reproductive effects were observed. Dose-dependent fatty changes and minimal liver necrosis were observed at 100 or 200 mg kg^{-1} by corn oil gavage, for 103 weeks, to rats and female mice. Rats exposed for 2 years appeared to have decreased resistance to viral infection due to functional impairment of immune system. Two-year exposure to 200 mg kg^{-1} resulted in dose-related incidences of squamous metaplasia of the prostate gland in male rats.

A significant increase in female rats and nonstatistically significant increase in male rats in the incidence of adenomatous polyps or adenocarcinomas was observed at 200 mg kg^{-1} for 2 years. No neoplastic effects were associated with the exposure of mice to bromoform. Bromoform showed positive for *in vitro* mutagenicity tests.

Human Studies are not available to evaluate the chronic human toxicity of bromoform except the deaths reported following overdose of bromoform containing sedative. IARC classified bromoform as group 3 and EPA classified it as B2.

Mode of Action Although bromoform seems to have a greater propensity for metabolism and is a more potent mutagen than BDCM, it appears to be a less potent toxicant and carcinogen. As with DBCM, a possible explanation is less bioavailability resulting from the greater lipophilicity of this compound and the use of corn oil as the vehicle of administration. This concept may be supported by the occurrence of bromoform-induced tumors in the intestinal tract, but not in the liver or kidneys.

Halo Acids

Halo acids are the second most frequently found CBPs after THMs. To date, the chlorinated acetic acids have been more thoroughly characterized toxicologically than their brominated analogs. HAA, unlike THMs, are nonvolatile and they have low dermal absorption (at low concentrations). The dichloroacetates (DCA) and trichloroacetates (TCA) occur in significantly higher concentrations than the monohaloacetates. TCA and DCA are metabolites and ultimate carcinogenic forms of rodent carcinogens, TCE and PERC. The maximum contaminated limit for the haloacetic acid is 60 μg l^{-1}.

Dichloroacetic Acid

This compound exists in drinking water as the salt; however, most of the experiments have been conducted with free acid. Therefore, the applicability of the results of such studies to estimating human risks will be uncertain because of the large pH artifacts that can be expected when administering a strong acid.

Toxicokinetics Absorption of DCA is rapid from the intestinal tract into the bloodstream. Once in the bloodstream, DCA is distributed to the liver and muscles, and then in smaller quantities to the fat, kidney, and other tissues such as the brain and testes. The systemic clearance of DCA is significantly higher. The metabolism of DCA is mediated by a novel GST, GST-zeta found in cytosolic fraction. This enzyme appears to be subjected to autoinhibition by DCA. Although there are substantial species differences in the metabolism of DCA, autoinhibition seems to be true across the species including humans. The half-life of DCA in dogs and rats are between

17.1–24.6 and 2.1–4.4 h, respectively, and 1.5 h in mice. The DCA half-life in humans is much closer to rats.

Acute and Short-Term Toxicity (or Exposure)
Animal DCA is not very toxic when administered acutely to rodents. The LD_{50} values of 4.5 and 5.5 g kg^{-1} in rats and mice, respectively, have been reported for sodium salt of DCA. Increased lipid peroxidation was reported at 300 mg kg^{-1} in rats and mice.

DCA was administered by gavage at 125, 500, or 2000 mg kg^{-1} to rats and at 50, 75, or 100 mg kg^{-1} to dogs for 3 months, dogs were more sensitive than rats. One of three female dogs died at 75 mg kg^{-1}, and one of four male dogs died at 100 mg kg^{-1}. The most overt toxicity in rats was hind limb paralysis at the highest dose and relative liver weights were significantly increased at all doses. Histopathological changes were observed in the brain and testes of both species. Testicular germinal epithelial degeneration was observed in rats at doses of 500 mg kg^{-1} and above and at all doses in dogs, with severity increasing with dose. Repeated short-term exposure to DCA led to higher glycogen accumulation and decreased plasma glucose and lactic acid in rats and mice. The effects such as reduced weights of accessory organs (epididymis, cauda epididymis, and preputial gland), changes in sperm motion, delayed spermiation and formation, and distorted sperm heads have been observed when administered in drinking water. DCA induces soft tissue abnormalities in fetal rats when administered by gavage in a water vehicle at 140 mg kg^{-1} to their dams during gestation days 6–15.

Human DCA was used as a potential orally effective hypoglycemic agent. Only a slight sedation was noted in some patients. More recently, DCA has been evaluated with success in the treatment of lactic acidosis associated with severe malarial mitochondrial myopathy and liver transplantation. Although DCA is used in a variety of medical conditions, it presents little acute risk probably due to the smaller doses.

Reduced plasma triglycerides, increased β-hydroxybutyrate, and increased plasma uric acid were noted over 6 days of administration of DCA as a hypoglycemic agent. Although there is no conclusive evidence, DCA is proposed to cause neurotoxic effects in humans based on the fact that DCA inhibits its own metabolism. These effects are expected to occur at therapeutic doses, 25–100 mg kg^{-1}.

Chronic Toxicity (or Exposure)
Animal DCA produces a severe hepatomegaly in mice at concentrations in drinking water of 1 g l^{-1} and above in 1 year.

DCA produced multiple tumors per animal at 2 g l^{-1} and above for 1 year and only hepatic tumor was reported with 0.5 g l^{-1} for 2 years. In rats, a statistically significant increase in carcinogenicity was observed at 0.5 or 1.6 g l^{-1} after 2 years.

Human The main reason that DCA was not fully developed as a hypoglycemic agent was that long-term administration to patients induced a reversible polyneuropathy. IARC classified DCA as a group 3 compound for its carcinogenicity. EPA classified it as a group 2B: possibly carcinogenic to humans because there is evidence of carcinogenicity in experimental animals, but there is either no evidence or not sufficient evidence of carcinogenicity in humans.

Mode of Action DCA induces tumor by nongenotoxic mechanisms. Most data now suggest that it is the parent compound that is responsible for the effects related to carcinogenicity by interfering with the cellular signaling mechanisms. This, in addition to autoinhibition of its metabolism, suggests that the actual mechanism is by tumor promotion rather than by cytotoxicity and reparative hyperplasia.

Trichloroacetic Acid

Like DCA, TCA exists almost exclusively in the salt form at pH found in drinking water.

Toxicokinetics TCA is readily absorbed from the gastrointestinal tract in experimental animals and humans and its clearance from blood is relatively slow relative to other HAAs. Approximately half of the administered dose was eliminated unchanged. There are substantial differences in the clearance by different species. Clearance is much faster in mice than in rats and human clearance is very slow. The half-life is 5.8 h in mice, 9.3 h in rats, 50 h in humans and approximately 200 h in dogs. TCA produces same metabolites as DCA with or without being converted to DCA.

Acute and Short-Term Toxicity (or Exposure)
Animal The oral LD_{50} of TCA (neutralized to pH 6–7) is found to be 3.32 g kg^{-1} in rats and 4.97 g kg^{-1} in mice when administered in aqueous solution. Acute administration of TCA reduces the blood glucose and increases lipid peroxidation in

rats. The most obvious target organ for TCA is the liver. Repeated administration of TCA in drinking water at a dose as high as $7.5\,g\,l^{-1}$ only produced minimal evidence of liver toxicity. TCA exposure to male rats in drinking water for 90 days caused a small but statistically significant increase in peroxisome proliferation markers. TCA is clearly without substantive cytotoxic effects at doses of less than $300\,mg\,kg^{-1}$. TCA administration at $800\,mg\,kg^{-1}$ via aqueous vehicle from 6 to 15 days of gestation resulted in body weight reductions, soft tissue malformations, and interventricular septal defect.

Human TCA is a strong acid. It is widely recognized that skin contact of TCA has the potential to produce acid burns, and ingestion of TCA has the potential to damage tissues of the gastrointestinal tract or produce systemic acidosis, even though specific studies of these effects do not appear in the literature. TCA is frequently utilized for chemical peeling by physicians practicing dermatologic surgery. The patient developed marked conjunctivitis of the affected eye and abrasions involving 25% of the cornea.

Chronic Toxicity (or Exposure)
Animal While TCA (neutralized) induces cancer in male mice when administered via drinking water at $1–5\,g\,l^{-1}$, such an effect was not observed in rats.

Human Indirectly, it may be presumed that TCA presents little overt hazard to human health because it is a major metabolite of commonly used solvents such as TCE and Perc. Occupational exposures to these solvents have been quite high in the past, but few, if any, effects of the solvents in humans have been attributed to TCA. Therefore, one would surmise that TCA is relatively nontoxic to humans under circumstances of low exposures such as those encountered in chlorinated drinking water. In addition, the mode of tumor induction – peroxisomal proliferation – in animals does not seem to be operating in humans. Hence, humans appear minimally sensitive to the tumorigenic effects of these compounds. IARC has classified TCA as group 3 compound for its carcinogenicity and EPA classified as a group 2B compound.

Mode of Action In the HAA class, significant differences in mode of action have been demonstrated for DCA and TCA. Despite the close structural resemblance of DCA and TCA and their common target organ (liver cancer induction), it is becoming clear that the mechanisms by which they act are different. TCA is a peroxisome proliferator and DCA induced tumor via epigenetic mechanism.

Other By-Products of Chlorination

Brominated HAAs are formed in waters that contain bromide. There are very limited data available on the toxicity of these chemicals. There are no studies on the health effects of brominated HAAs in humans. No formal reports have been made of carcinogenic or mutagenic effects of brominated HAAs. Metabolism of brominated HAAs has received little attention.

Haloaldehydes and haloketones have received very little attention. Members of this class have been identified as key metabolites of chemicals such as TCE, vinyl chloride, and dibromochloropropane. Trichloroacetaldehyde and chloral hydrate are important compounds of this group. Chloral hydrate is primarily known for its depressant effects on the central nervous system and doses of 500–2000 mg produce central nervous system depression in humans. It is also known to cause liver damage. This compound is classified as group 3 by IARC. TCA and DCA are major metabolites of chloral hydrate.

Toxicological data in experimental animals and humans for the haloketones and haloacetaldehydes are extremely limited. Slight liver and CNS effects were observed. Hepatocellular carcinomas in mice were reported, probably due to mutagenic effect. There is a potential carcinogenic hazard associated with halogenated aldehydes.

Epidemiological Studies

Numerous epidemiological studies have attempted to assess the association between cancer and the long-term consumption of disinfected drinking water. In most studies, disease incidence or mortality was compared between populations supplied with chlorinated surface water and those supplied with unchlorinated groundwater. A wide range of cancer sites such as gall bladder, esophagus, kidney, breast, liver, pancreas, prostate, stomach, bladder, colon, and rectum was found to be statistically associated with the use of chlorinated surface water in humans. Additionally, published epidemiological data suggest the possibility that increased spontaneous abortion rates may be related to DBPs in drinking water. The epidemiological evidence is inconclusive and equivocal for an association between cancer and noncancer effect exposure to CBPs in drinking water. The quality of information about water disinfection exposures and potential confounding characteristics differs dramatically between these studies. The confounding factors such as smoking, drinking, exposure to other

chemicals, etc. make the matter more complicated. In addition, occurrence of cancer incidence at one place and nonoccurrence at other places further complicate the interpretation.

It is noteworthy that there is little support in the animal data for certain target organs that are prominently associated with chlorinated drinking water in epidemiological studies (e.g., bladder cancer). Therefore, the possibility has to be left open that the carcinogenic effect of DBPs may be dependent on genetically determined characteristics of a target organ (or tissue) that make it more susceptible than the same organ in test animals.

Conclusions

Chlorination has been the major disinfection process of drinking water in many countries for many years despite the availability of alternative disinfectants. There is a widespread concern about cancer, noncancer and reproductive effects of CBPs based on animal and epidemiological studies. However, most of these studies were conducted with a single chemical, at high doses and using corn oil as vehicle, a potentially confounding factor in toxicological evaluations of drinking water contaminants. These conditions are irrelevant to human exposure. Importantly, carcinogenic effects of individual CBPs may not represent the risk posed by the mixtures, as disinfected drinking water is a very complex mixture of chemicals. Although some epidemiology studies linked CBPs to the incidence of cancer and adverse reproductive effects in humans, there is no scientific basis for the proposed association and none of the chlorination by-products studied individually to date is a potent carcinogen at concentrations normally found in drinking water. In addition, the toxic effects of many CBPs remain largely unknown and many of them remain unidentified. Hence, it is not possible to make sound scientific judgment. It is important to evaluate all CBPs individually or as mixtures in a systematic manner to provide comparative toxicity and to better understand the exposure concentrations in the drinking water based on daily ingestion (approximately $2\,l\,day^{-1}$), inhalation, swimming, bathing, etc. in the risk assessment paradigm. A complicating factor when assessing risk from CBPs is that they occur in complex mixtures that vary by location, disinfection process, distance from the treatment plant, changing conditions of the source water, and even weather conditions. Moreover, the effects may be altered by factors such as coexposure to other compounds, age, lifestyle, etc. Nonetheless, safe drinking water is a substantive health concern and a balance should be achieved between reducing exposure to CBPs and maintaining control of waterborne diseases.

See also: Bromoform; Chlorine; Chlorine Dioxide; Chlorobenzene; Chloroform; Organochlorine Insecticides; Polychlorinated Biphenyls (PCBs); Trihalomethanes.

Further Reading

IPCS (2000) *Environmental Health Criteria, 216: Disinfectants and Disinfectant By-Products*. Geneva: International Programme on Chemical Safety, WHO.

US EPA (1998) National primary drinking water regulations. Disinfectants and disinfection by-products; notice of data availability. Proposed rule. *Federal Registry* 63.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for CBPs.

Chlorine

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 7782-50-5
- Synonyms: Bertholite; Chloor; Chlor; Chlore; Molecular chlorine; Cloro; RTECS FO2100000; UN1017
- Chemical/Pharmaceutical/Other Class: Disinfectant; Bleaching agent

Uses

Chlorine is used to bleach all types of fabric, to disinfect relatively clean impervious surfaces, to purify water, and to control biofouling in cooling systems. It is used in the processing of meat, fish, vegetables, and fruits. It is also used in the manufacturing of synthetic rubber, plastics, pesticides, antifreeze, refrigerants, antiknock compounds, chlorinated hydrocarbons, polyvinyl chloride, and chlorinated lime. Chlorine is also used in detinning

and dezincing iron and as an ingredient in special batteries.

Background Information

For more than 100 years now, industry has exploited this highly reactive chemical produced from one of nature's most plentiful and inexhaustible minerals – common salt. Today, chlorine is used in a vast range of processes to create thousands of often indispensable products that serve our everyday needs at work, home, and play. More than 2 million jobs in European industry are related to this chemical building block. It underpins the manufacture and use of products with an annual value of more than 380 000 million Euros.

Exposure Routes and Pathways

Dermal or ocular contact and inhalation are the most common exposure pathways.

Toxicokinetics

Chlorine persists as an element only at a very low pH (<2), and at the higher pH found in living tissue it is rapidly converted into hypochlorous acid. In this form, it apparently can penetrate the cell and form *N*-chloro-derivatives that can damage cellular integrity.

Mechanism of Toxicity

Chlorine reacts with body moisture to form acids. The acids form acid proteinates.

Acute and Short-Term Toxicity (or Exposure)

Animal

Exposure of cats to a concentration of 900 mg m^{-1} (300 ppm) for 1 h may cause death after a period during which the conjunctiva is inflamed; coughing and dyspnea are also present. Dogs rarely die following a 30 min exposure to 650 ppm and never die following a 30 min exposure to less than 280 ppm. The pulse rate of dogs is retarded during exposure to concentrations of 200 ppm or greater. In guinea pigs, the inhalation of small quantities of chlorine accelerates the course of experimental tuberculosis.

Human

Liquid chlorine causes burns to skin and eyes and will cause frostbite. It may cause lung injury if inhaled. Chlorine causes smarting of the skin and first-degree burns on short exposure; it may cause secondary burns in long exposures. Inhalation of low concentrations causes mild mucous membrane irritation and irritation of the upper respiratory tract. Inhalation of high concentrations of the gas causes necrosis of the tracheal and bronchial epithelium as well as pulmonary edema, atelectasis, emphysema, and damage to the pulmonary blood vessels. Acute exposure may also cause anxiety and vomiting. Exposure to 500 ppm can be lethal over 30 min, while exposure to 1000 ppm can be lethal within a few minutes.

Chronic Toxicity (or Exposure)

Animal

Chlorine gas was not carcinogenic in mice and rats exposed to varying concentrations. Chlorine administered in drinking water produced lymphomas and/or leukemia in rats, but was not carcinogenic in a third study.

Human

Not classifiable as a human carcinogen. Chronic exposure causes permanent, although moderate, reduction in pulmonary function and corrosion of teeth.

In Vitro Toxicity Data

In a series of *in vitro* experiments on a human lymphocyte culture system, it was reported that chlorine induced chromatid and chromosome breaks, translocations, dicentric chromosomes, and gaps.

Clinical Management

Exposure should be terminated as soon as possible by removal of the patient to fresh air. The skin, eyes, and mouth should be washed with copious amounts of water. A 15–20 min wash may be necessary. Contaminated clothing and jewelry should be removed and isolated. Contact lenses should be removed from the eye to avoid prolonged contact of the chemical with the area. Affected areas should not be rubbed. If breathing has stopped, artificial respiration should be given. If breathing is difficult, oxygen should be given.

Environmental Fate

The stability of free chlorine in natural water is very low because it is a strong oxidizing agent and rapidly oxidizes inorganic compounds. It also oxidizes

organic compounds, but more slowly than inorganic compounds.

Ecotoxicology

Chlorine is highly toxic to all forms of aquatic life; there is no potential for bioaccumulation or bioconcentration.

Exposure Standards and Guidelines

Occupational Safety and Health Administration: 8 h time-weighted average (0.5 ppm). Federal drinking water standards (Environmental Protection Agency): 4000 $\mu g\,l^{-1}$.

See also: Detergent; Pollution, Air; Pollution, Soil; Surfactants, Anionic and Nonionic.

Further Reading

Abdel-Rahman MS, Suh DH, and Bull RJ (1984) Pharmacodynamics and toxicity of chlorine in drinking water in the rat. *Journal of Applied Toxicology* 4: 82–86.

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Vetrano KM (2001) Molecular chlorine: health and environmental effects. *Review of Environmental Contamination and Toxicology* 170: 75–140.

Winder C (2001) The toxicology of chlorine. *Environmental Research* 85: 105–114.

Chlorine Dioxide

Zhengwei Cai

- Chemical Abstracts Service Registry Number: CAS 10049-04-4
- Synonyms: Chlorine-oxide; Alcide; Chlorine oxygen acids; Chlorine peroxide; Chloroperoxyl; Doxcide 50; Hatiox E-100; NA 9191; Chlory radical
- Chemical/Pharmaceutical/Other Class: Chlorine dioxide and its by-products are collectively called oxychlorines
- Chemical Formula: ClO_2
- Chemical Structure: O = Cl = O

Uses

Chlorine dioxide is a strong oxidizing agent, bactericide, and antiseptic. It is used in bleaching cellulose, paper pulp, leather, flour, fats and oils, textiles, and beestpwax, and in deodorizing and purifying water. It is currently considered as an alternative to chlorine, as a disinfectant for public water supplies in the United States. It is also used in the manufacture of many chlorite salts.

Exposure Routes and Pathways

Consumption of drinking water is the most probable route of exposure to chlorine dioxide and its by-products. Patients undergoing hemodialysis may be directly exposed to chlorine dioxide through dialysis water disinfected with chlorine dioxide. Chlorine dioxide is a gas; therefore, inhalation is also an exposure pathway.

Toxicokinetics

Chlorine dioxide can be rapidly absorbed through the gastrointestinal tract. Peak blood concentration levels can be reached within 1 h after a single dose administered orally. It can also be slowly absorbed through shaved skin with a half-absorption time of 22 h. Chlorine dioxide is metabolized to chlorite, chlorate, and mostly chloride. Most administered chlorine dioxide and its metabolites remain in plasma followed by kidneys, lungs, stomach, intestine, liver, and spleen. About 43% of orally administered chlorine dioxide is eliminated in the urine and feces within 72 h. It is not excreted via the lungs.

Mechanism of Toxicity

The toxicity of chlorine dioxide is attributed to the oxidative stress caused by this compound and its by-products or metabolites. Animal studies and *in vitro* experiments with human red blood cells indicate that chlorine dioxide and its by-products, especially chlorite, oxidize hemoglobin to methemoglobin by inhibiting methemoglobin reductase, decreasing erythrocyte glutathione levels, stimulating erythrocyte hydrogen peroxide production, and causing hemolytic anemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Delayed death occurred in animals after exposure to 150–200 ppm for less than 1 h. Rats repeatedly exposed to 10 ppm died after 10–13 days of exposure. Rats are more sensitive than mice to the

developmental effects associated with chlorite-treated drinking water.

Human

Chlorine dioxide gas is highly irritating to the skin and mucous membranes of the respiratory tract. Symptoms of exposure by inhalation include eye and throat irritation, headache, nausea, nasal discharge, coughing, wheezing bronchitis, and delayed onset of pulmonary edema. It is explosive in the form of concentrated vapor or solution (10 vol.% in the air). When involved in a fire, chlorine dioxide is a source of oxygen. Daily ingestion of 1 l of water containing 0.7 mg of chlorine dioxide has been reported to cause nausea. Exposure of a worker to 19 ppm for an unspecified time has been reported fatal.

Chronic Toxicity (or Exposure)

Human

The human experience with chlorine dioxide, both in controlled prospective studies and in actual use situations in community water supplies, has failed to reveal adverse health effects. However, glucose-6-phosphati dehydrogenase-deficient individuals and infants are groups thought to be at higher risk to chlorine dioxide toxicity due to their susceptibility to oxidant-induced methemoglobinemia. The chronic toxicity signs are mainly dyspnea and asthmatic bronchitis, and in certain cases irritation of the gastrointestinal tract.

Exposure Standards and Guidelines

The US EPA has recommended standards of $0.06\,mg\,l^{-1}$ for chlorine dioxide and $0.007\,mg\,l^{-1}$ for chlorite and chlorate in drinking water. The exposure limits have been set at TLV–TWA 0.1 ppm ($0.3\,mg\,m^{-3}$) by ACGIH, MSHA, OSHA, and NIOSH; TLV–STEL 0.3 ppm by ACGIH; and IDLH 10 ppm by NIOSH.

See also: Pollution Prevention Act, US; Pollution, Water.

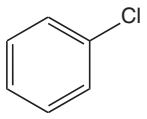
Further Reading

Hose JE, Di Fiore D, Parker HS, and Sciarrotta T (1989) Toxicity of chlorine dioxide to early life stages of marine organisms. *Bulletin of Environmental Contamination and Toxicology* 42(3): 315–319.

Chlorobenzene

Linda A Malley

- Chemical Abstracts Service Registry Number: CAS 108-90-7
- Synonyms: Benzene chloride; Benzene chloro-; Chlorbenzene; Chlorbenzol; Chlorobenzol; Monochlorbenzene; Monochlorobenzene phenyl chloride; NCI-C54886; Caswell No. 183A; EPA Pesticide Chemical Code 056504; MCB, CP 27; I P Carrier T 40; Tetrosin SP
- Chemical/Pharmaceutical/Other Class: Inorganic compounds
- Chemical Formula: C_6H_5Cl
- Chemical Structure:



Uses

Chlorobenzene is used as a solvent for pesticide formulations, in auto parts degreasing, and in the manufacture of adhesives, paints, polishes, waxes, pharmaceuticals, and natural rubber. It is a chemical intermediate in the production of diphenyl oxide, diisocyanates, and nitrochlorobenzene. It has also been used as a fiber-swelling agent and as a dye carrier in textile processing.

Background Information

Chlorobenzene production has been declining since its peak in 1969, and is likely to continue declining due to the substitution of more environmentally acceptable chemicals. Chlorobenzene is produced by chlorination of benzene in the presence of a catalyst, and is produced as an end product in the reductive chlorination of di- and trichlorobenzenes.

Exposure Routes and Pathways

The vapor pressure of chlorobenzene is relatively high (11.8 mmHg); therefore, inhalation is a

potential route of exposure. Since chlorobenzene is soluble in water (448 ppm) and has been detected in wastewater and drinking water, there is potential for oral exposure. In addition, as a result of its solvent and degreasing properties, the potential for accidental skin contact with the material also exists.

Toxicokinetics

Data in rabbits indicate that the toxicity from a single dermal application is minimal with only slight reddening of the skin observed. Continuous skin contact with chlorobenzene for 1 week resulted in moderate erythema and slight superficial necrosis. Absorption in amounts sufficient to cause toxicity can also occur as a result of ingestion or inhalation. Because chlorobenzene is highly lipophilic and hydrophobic, it is thought to be distributed throughout the total body water, with body lipids being a major deposition site.

The kinetics of metabolism and excretion were investigated in rabbits administered a single oral dose of $0.5\,mg\,kg^{-1}$ or doses of 0.5 g twice daily for 4 days. In the single-dose study, 27% of the administered dose was excreted unchanged in the expired air. The majority of the remainder was excreted in the urine as a glucuronide (25%), ethereal sulfate (27%), and mercapturic acid (20%). Similarly, rabbits administered repeated doses of chlorobenzene excreted the majority of the dose in the urine, and only small amounts were detected in the tissues and feces. Rats administered a single, intraperitoneal dose of chlorobenzene also excreted metabolites in the urine which were identified as 4-chlorocatechol, 2-chlorophenol, 4-chlorophenol, and 3-chlorophenol. In addition, chlorobenzene was covalently bound to DNA, RNA, and proteins in the liver, kidney, and lung 22 h following a single intraperitoneal injection. Chlorobenzene is first oxidized to the 3,4-epoxide, which then can follow one of several pathways. One leads to the formation of the I-mercapturic acid conjugate following glutathione conjugation. A second pathway results in the formation of 4-chlorocatechol, and the third pathway ends with the formation of 4-chlorophenol and its conjugates. Data collected from exposed workers and volunteers indicate that for humans, the primary pathways are formation of the *p*-mercapturic acid conjugate and 4-chlorocatechol.

Mechanism of Toxicity

Similar to other volatile organic chemicals, chlorobenzene is a nervous system depressant. In addition, lesions of the liver and kidneys have also been observed following toxic doses.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values for rats, mice, and rabbits were 2290, 2300, and $2830\,mg\,kg^{-1}$, respectively. The approximate inhalation LD_{50} (2 h) is 4300 ppm for mice. Application of chlorobenzene to the skin of rabbits caused slight reddening; prolonged skin contact was irritating. Ocular contact in rabbits caused a transient conjunctival irritation which resolved within 48 h. Tremors, central nervous system depression, and death were observed in cats administered a single inhalation exposure of 3700 ppm and above.

Several repeated-exposure oral studies have been conducted in various species. Although the doses at which effects were observed are variable between species, the primary effects of chlorobenzene were observed in the liver and kidneys. Rats and mice were administered daily doses of 60–$750\,mg\,kg^{-1}$, 5 days per week, for 13 weeks. Survival was lower in rats at $500\,mg\,kg^{-1}$ and above and in mice at $250\,mg\,kg^{-1}$ and above. Pathological changes in the liver and kidneys and changes in the hematopoietic system (spleen, bone marrow, and thymus) were observed in both species at $250\,mg\,kg^{-1}$ and above. In another study, rats were administered doses ranging from 14.4 to $376\,mg\,kg^{-1}\,day^{-1}$, 5 days per week, over a period of 192 days. Doses of $144\,mg\,kg^{-1}\,day^{-1}$ and above caused changes in liver and kidney weights and changes in liver morphology. Doses of $18.8\,mg\,kg^{-1}\,day^{-1}$ and below did not cause any adverse effects. Dogs were administered oral doses ranging from 27.2 to $272.5\,mg\,kg^{-1}\,day^{-1}$, 5 days per week, for 93 days. There were no effects at $54.5\,mg\,kg^{-1}\,day$ and below. At $272.5\,mg\,kg^{-1}\,day^{-1}$, changes in clinical chemistry parameters were observed, four of eight dogs died, and pathological changes were observed in the liver, kidney, gastroenteric mucosa, and hematopoietic tissue.

Repeated-exposure inhalation studies have been conducted in several species. Rats, rabbits, and guinea-pigs were exposed to airborne concentrations ranging from 200 to 1000 ppm for 7 h per day, 5 days per week, for a total of 32 exposures. At 475 ppm and above, organ weight changes and histopathological changes were observed. There were no effects detected at 200 ppm. In another study, changes in hematology parameters and pathological changes in the adrenal cortex, kidney, and liver were observed in rats and rabbits exposed to airborne concentrations of 75 or 250 ppm chlorobenzene vapors for 7 h per day, 5 days per week for 24 weeks.

Exposure of rats to atmospheric concentrations up to 450 ppm did not have any adverse effects on reproductive performance or fertility of male or female rats through two consecutive generations. Chlorobenzene caused minor skeletal alterations in fetuses collected from pregnant rats exposed to atmospheric concentrations up to 590 ppm (a maternally toxic dose) for 6 h per day during the period of organogenesis. Pregnant rabbits exposed to chlorobenzene at concentrations up to 590 ppm did not exhibit evidence of embryotoxicity or teratogenicity.

Human

The human literature primarily consists of case reports. In the industrial environment, symptoms including headache, numbness, skin irritation and redness, eye irritation and redness, irritation and redness of the upper respiratory tract, bronchitis, dizziness, somnolence, loss of consciousness, hematopoietic effects, gastritis, hepatitis, and neuromuscular changes have been reported. Accidental ingestion of 5–10 ml of a cleaning agent containing chlorobenzene caused loss of consciousness, vascular paralysis, and heart failure in a child ($\sim$2 years old).

Chronic Toxicity (or Exposure)

Animal

In a study determining the carcinogenic potential of chlorobenzene, rats were administered daily doses of 0, 60, or 120 mg kg^{-1} day^{-1}, 5 days per week, for 103 weeks, and mice were similarly administered 30 or 60 mg kg^{-1} day^{-1}. No increased tumor incidences were observed in female rats or in male or female mice. Male rats administered 120 mg kg^{-1} day^{-1} had an increased incidence of hepatic neoplastic nodules (8% for untreated control, 4% for vehicle control, 8% for 60 mg kg^{-1}, and 16% for 120 mg kg^{-1}). Based on these results, the US Environmental Protection Agency (EPA) classified chlorobenzene as 'D' (not classifiable as to carcinogenicity in humans).

Human

There were no epidemiology studies in humans regarding long-term exposure to chlorobenzene. However, based on the results of a chronic toxicity study in rats, the US EPA classified chlorobenzene as 'D' (not classificable as to carcinogenicity in humans). In addition, the American Conference of Governmental Industrial Hygienists classified chlorobenzene as 'A3' (confirmed animal carcinogen with unknown relevance to humans).

In Vitro Toxicity Data

Chlorobenzene was not mutagenic in several bacterial strains of *Salmonella typhimurium* or *Escherichia coli* and was negative in rat hepatic DNA repair assays; however, it was weakly positive in a mouse micronucleus assay. Chlorobenzene induced transformation in Fischer 344 adult rat liver cell lines, but was not genotoxic to hepatocytes. In addition, it did not induce DNA repair in the rat hepatocyte primary culture DNA repair assay.

Clinical Management

Treatment is symptomatic and supportive. For ocular contact, the eyes should be irrigated immediately with abundant running water. If the material contacts the skin, the affected areas should be washed with soap and water promptly. If inhalation exposure occurs, the exposed person should be moved to fresh air immediately and provided with respiratory support (oxygen or artificial respiration) if necessary. If the material has been ingested, vomiting should not be induced. For ingestion, gastric lavage (followed by saline catharsis) should be performed or activated charcoal should be administered. The trachea should be protected from aspiration. Renal and hepatic function should be monitored and supported if necessary. Hypotension should be treated symptomatically.

Environmental Fate

In the ambient atmosphere, chlorobenzene will exist as a vapor, and will be degraded by reaction with photochemically produced hydroxyl radicals, with an estimated half-life of 21 days. Photolysis half-lives of 4–18 h were measured in aqueous media. Chlorobenzene is expected to volatilize from soil, and is predicted to have high mobility based on the K_{oc} values. Biodegradation results are variable based on soil type and bacteria type. In river water, the biodegradation half-life was reported to be 150 days, and 75 days in the sediment. Volatilization is expected to occur from water surfaces. Hydrolysis is not predicted to occur.

Ecotoxicology

Chlorobenzene was acutely toxic to rainbow trout, with a 24 h LC_{50} of 1.8 mg kg^{-1}. In addition, the 14 days LC_{50} in guppies was 19 ppm. The 96 h LC_{50} in fathead minnows was 16.9 mg l^{-1} under flow-through conditions.

Table 1 Summary of exposure criteria for chlorobenzene

Agency	*Criteria*	*Averaging time*
ACGIH	TLV – TWA, 10 ppm	8 h/40 h week
NIOSH	IDLH, 1000 ppm	NA
OSHA	PEL (TWA), 75 ppm (350 mg m^{-3})	8 h/40 h week

Conversion: 1 ppm = 3.19 mg m^{-3}. OSHA, Occupational Safety and Health Administration; NIOSH, National Institute of Occupational Safety and Health; ACGIH, American Conference of Governmental Industrial Hygienists; TLV – TWA, threshold limit value – time-weighted average; IDLH, immediately dangerous to life or health; PEL, permissible exposure limit; NA, not applicable.

Other Hazards

Chlorobenzene is highly flammable, and the vapors are heavier than air. They will spread along the ground and collect in low or confined areas. The lower flammable limit is 1.8%, the upper flammable limit is 9.6%, and the flash point is 85°F (29.2°C closed cup). Combustion of chlorobenzene can form phosgene and hydrogen chloride gases. Chlorobenzene reacts with strong oxidizing materials, powdered sodium, and phosphorus trichloride and sodium.

Exposure Standards and Guidelines

Based on the results of a chronic toxicity study in rats, the US EPA classified chlorobenzene as 'D' (not classifiable as to carcinogenicity in humans). In addition, the American Conference of Governmental Industrial Hygienists classified chlorobenzene as 'A3' (confirmed animal carcinogen with unknown relevance to humans).

The current exposure standards and guidelines are summarized in **Table 1**.

See also: Carcinogen Classification Schemes; Chlorophenols; Pesticides.

Further Reading

DHHS/NTP Toxicology and Carcinogenesis Studies of Chlorobenzene in F344/N Rats and B6C3F1 Mice (Gavage Studies) Technical Report Series No. 261 (1985) NIH Publication No. 86-2517.

US EPA Ambient Water Quality Criteria Document; Chlorinated Benzenes (1980) EPA 440/5-80-028.

US EPA Health Assessment Document; Chlorinated Benzenes (1985) EPA 600/8-84-015.

US EPA Health Criteria Document for Chlorobenzene NTIS/PB89-192116 (June 1988).

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chlorobenzene.

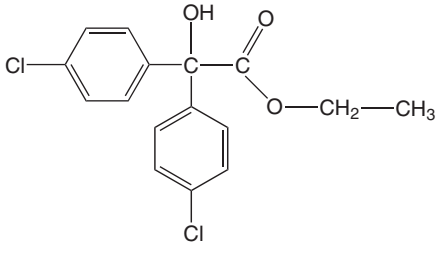
Chloroethyl Sulfide, Bis-2 *See* Mustard Gas.

Chlorobenzilate

David Janz

This article is a revision of the previous print edition article by Tamal Kumar Chakraborti, volume 1, pp. 300–301, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 510-15-6
- Synonyms: Ethyl-4,4-dichlorobenzilate; Ethyl-4, 4-dichlorodiphenylglycollate; Akar; Folbex; Acaraben
- Chemical/Pharmaceutical/Other Class: Chlorinated hydrocarbon
- Chemical Formula: $C_{16}H_{14}Cl_2O_3$
- Chemical Structure:



Uses

The primary use of chlorobenzilate is as an acaricide for mite control on citrus crops and in beehives. Historically, it was used as a synergist for DDT. Although classified as a Restricted Use Pesticide in

the United States and not registered for use in Canada, chlorobenzilate is believed to be used on crops other than citrus in other countries.

Exposure Routes and Pathways

Occupational exposure to chlorobenzilate may occur through inhalation or dermal contact during its production and use as an acaricide. Exposure to the general population may occur via contaminated food and drinking water.

Toxicokinetics

Chlorobenzilate is readily absorbed from the gastrointestinal tract. Dermal absorption occurs following exposure to commercial (oil-based) formulations. No significant storage of chlorobenzilate in adipose tissue of dogs was reported following daily oral administration of 12.8 mg kg^{-1} for 35 weeks. Dichlorobenzilic acid, dichlorobenzyhydrol, chlorobenzoic acid, and dichlorobenzophenone were the major metabolites produced when chlorobenzilate was incubated in the presence of rat liver homogenates. Urinary excretion of these metabolites in addition to significant excretion of unchanged chlorobenzilate in the feces was reported in dogs and rats after oral administration. Although structurally similar to DDT, chlorobenzilate is much more rapidly excreted following absorption.

Mechanism of Toxicity

Similar to other organochlorine pesticides in this structural class, chlorobenzilate causes disruption of normal flow of Na^+ and K^+ across axonal membranes in the central (CNS) and peripheral nervous systems, and may also antagonize GABA-mediated inhibition in CNS. The net result is a hyperexcitable state of neurotransmission.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for chlorobenzilate in rats is 2784–3880 mg kg^{-1}. The dermal LD_{50} is greater than 10 000 mg kg^{-1} in rats and rabbits.

Human

Symptoms of acute poisoning following ingestion, inhalation, or dermal absorption of chlorobenzilate are similar and include nausea, dizziness, vomiting, incoordination, confusion, and muscle weakness or pain. Death may result from respiratory collapse or arrythmias. Chlorobenzilate is a severe eye irritant and causes conjunctivitis following chronic exposure.

Chronic Toxicity (or Exposure)

Animal

A three-generation reproductive study in rats reported testicular atrophy but no effect on reproduction. No mutagenic or teratogenic effects have been reported in animals. Chlorobenzilate has produced hepatocellular carcinoma in mice but the evidence for carcinogenicity in rats is equivocal.

Human

Chronic skin exposure may cause skin inflammation. Chlorobenzilate is considered a possible human carcinogen.

In Vitro Toxicity Data

Chlorobenzilate did not inhibit human placental CYP 19 aromatase activity and did not express estrogen receptor activation *in vitro*.

Clinical Management

Only symptomatic treatment is available. An airway should be established and if necessary assisted ventilation provided. The cardiac rhythm should be monitored and treatment for arrhythmia should be given if required. For eye exposure eyes must be flushed immediately with water or saline and irrigation maintained during transport. For ingestion, oral administration of activated charcoal is indicated. For skin contamination, the exposed area should be washed with soap and water.

Environmental Fate

If released to soil, chlorobenzilate is expected to have low mobility, and therefore unlikely to leach into groundwater. Volatilization from soils is not expected to be a significant fate process. The half-life of chlorobenzilate in fine sandy soils was estimated to be 10–35 days, and degradation was primarily microbial. In silty clay loam and clay soils, the half-life of chlorobenzilate was estimated to be 10.8–15.1 and 29.5–169.1 days, respectively. If released into water, chlorobenzilate is expected to adsorb to particulate matter and sediment. Bioconcentration factors in carp were 224–709, indicating the potential for moderate to high accumulation in aquatic organisms. If released into air, chlorobenzilate will exist in both vapor and particulate phases. The half-life of

vapor-phase chlorobenzilate in ambient air was estimated to be 3.2 days.

Ecotoxicology

Chlorobenzilate is only slightly to practically nontoxic in birds. The 7 day dietary LC_{50} for chlorobenzilate is 3375 ppm bobwhite quail. The 5 day dietary LC_{50} in mallard ducks is greater than 8000 ppm. The LC_{50} (96 h) in rainbow trout is $0.7\,mg\,l^{-1}$ and in bluegill it is $1.8\,mg\,l^{-1}$. Chlorobenzilate is practically nontoxic to bees.

Exposure Standards and Guidelines

The reference dose and the acceptable daily intake for chlorobenzilate is $0.02\,mg\,kg^{-1}\,day^{-1}$.

See also: Carbamate Pesticides; DDT(Dichlorodiphenyltrichloroethane); Organochlorine Insecticides.

Further Reading

Smith AG (2001) DDT and its analogs. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1305–1355. San Diego, CA: Academic Press.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Chloroform

Anna M Fan

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 67-66-3
- Synonyms: Trichloromethane; Formyl trichloride; Methane trichloride; Trichloroform; Freon 20; COBEHN spray-cleaner; Methenyl trichloride; Methyl trichloride; NCI-C02686; R 20; RCRA waste No. U044; UN1888
- Chemical/Pharmaceutical/Other Class: Chlorinated aliphatic hydrocarbon
- Chemical Formula: $CHCl_3$

Uses

Chloroform is a volatile, low-molecular weight, lipophilic compound and a chlorinated trihalomethane. Most of the chloroform produced in the United States is used to make fluorocarbon 22 (HCFC 22) and the rest is produced for export and miscellaneous uses. In the past it was used as an inhalation anesthetic and as an extraction for, fats, oils, greases and other products, as a dry cleaning spot remover, in fire extinguishers, and as a fumigant. It is available as emulsions, spirits, tinctures, and chloroform water. Chloroform is also formed as a by-product of chlorination of water, wastewater, and swimming pool. Other sources include pulp and paper mills, hazardous waste sites, and sanitary landfills.

Exposure Routes and Pathways

Inhalation, ingestion, and dermal contact are the most common routes of exposure. Inhalation exposure can be from indoor or outdoor air, especially in the workplace related to its manufacture and use. Other possible sources of exposure include drinking water, beverages, and food using water containing chloroform and swimming pool water, but chronic poisoning is unlikely by this route. Acute poisoning may be by accidental or deliberate ingestion.

Toxicokinetics

Chloroform is readily absorbed through the lungs when inhaled and through the gastrointestinal tract when ingested. It is readily distributed throughout the body and is highly fat-soluble. The rate of pulmonary uptake in humans is initially rapid, but it decreases as the concentration reaches equilibrium. The total quantity absorbed through the lungs is directly proportional to the following: the concentration in the inspired air; the exposure time; the blood/air Ostwald solubility coefficient; the solubility in the various body tissue; and physical activity. The basic kinetic parameters of chloroform absorption by inhalation and its equilibrium in the body apply equally to both low and high concentrations. Chloroform may be absorbed through intact skin in both humans and animals, but absorption is slow and limited by the moderate lipophilicity of the chemical. It can readily cross the human placenta.

Liver is the principal site of chloroform metabolism which involves two major pathways, both of which are catalyzed by the cytochrome P-450 enzymes in the presence of NADPH. The oxidative pathway produces phosgene and the reductive pathway produces the dichloromethyl free radical. Other metabolites of chloroform include chloromethanol, hydrochloric acid, hydrogen chloride, and digluathionyl dithiocarbonate, with carbon dioxide as the predominant end product of metabolism.

Chloroform is rapidly eliminated from the body. It is primarily excreted by the lungs as carbon dioxide. The half-life for elimination following a single oral dose of 500 mg in two subjects was 1.5 h.

Various physiologically based pharmacokinetic models for chloroform have been described using physiological and metabolic parameter values for rats, dogs, and humans to exercise the models.

Mechanism of Toxicity

Chloroform causes progressive depression of the central nervous system (CNS), ultimately producing deep coma and respiratory center depression. The exact mode of action for chloroform-induced toxicity in the liver and the kidneys is not certain, but metabolism to toxic metabolites by the cytochrome P-450-dependent pathways is likely to play a critical role. Chloroform is converted to chloromethanol, which rapidly dechlorinates to produce hydrochloric acid and phosgene. Phosgene is a poisonous gas that can cause injury to tissues. Phosgene reacts with water to produce carbon dioxide and chloride ion. These by-products can bind to glutathione to produce diglutathione dithiocarbonate. The hepatotoxicity and nephrotoxicity of chloroform is thought to be due largely to phosgene.

For the carcinogenicity of chloroform, there is increasing yet not conclusive evidence that it is due to an epigenetic process, that is, that the mode of carcinogenic action of chloroform is largely due to oxidative metabolism leading to cytotoxicity and cell proliferation in the liver and probably in the kidneys. The hypothesis for this mechanism is based on the theory that chloroform acts as a promoter of previously initiated cells by virtue of regenerative hyperplasia which occurs in response to renal and hepatic toxicity. Some noted that mechanisms of carcinogenicity have not been sufficiently developed to discount carcinogenic effects observed in rodents from predicting cancer hazards to humans, and that other possible mechanisms for chloroform have not been studied. Genotoxicity studies have shown both negative and positive findings. Thus current scientific information suggests that cytotoxicity/cell proliferation appears to be a major factor in chloroform-induced carcinogenesis, but this may not be sufficient to explain the underlying mechanism(s), and multiple mechanisms may be operating concurrently. In recognition that this mode of action may not be exclusive, in the absence of definitive evidence, it would be premature to draw a conclusion on a single, genetic or epigenetic, mechanism.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chloroform in animals is known to cause acute toxicity similar to that in humans. The following lethal dose (LD) values have been reported:

Oral LD_{50} in rats	300, 1194 mg kg^{-1}
Oral LD_{50} in guinea pigs	820 mg kg^{-1}
Oral LD_{Lo} in dogs	100 mg kg^{-1}
Inhalation LC_{Lo} in cats	35 g m^{-3} (4 h)$^{-1}$
Inhalation LC_{50} in cats	47 g m^{-3} (4 h)$^{-1}$
Subcutaneous LD_{50} in mouse	704 mg kg^{-1}
Intravenous LD_{Lo}	75 mg kg^{-1}
Skin LD_{50} in rabbits	$>$20 000 mg kg^{-1}

Acute and subchronic exposures result in toxicity to the liver, kidneys, respiratory system and CNS.

Human

Chloroform causes similar toxicity in humans and animals. Chloroform is an irritant and a CNS and cardiovascular system depressant. Exposure to chloroform can cause liver and kidney toxicity.

Inhalation and ingestion are harmful and may be fatal. The major effect from acute inhalation is CNS depression. It produces dizziness, tiredness, headache at lower concentrations ($<$1500 ppm), anesthesia in the range of 1500–3000 ppm, and may cause death at high levels (e.g., 40 000 ppm). A dose of 10 ml (14.8 g) of chloroform can cause CNS depression and death due to respiratory and cardiac arrest. The oral lethal dose is estimated to be between 0.5 and 5 g kg^{-1} (1 oz to 1 pint) for an average 70 kg man. Short-term inhalation of chloroform at 900 ppm can cause dizziness, fatigue, and headache. Skin contact may result in irritation and redness and high levels can cause sores. Eye contact with liquid chloroform may result in painful irritation of the superficial eye structures, burns, and may cause corneal necrosis and ulcers.

Chronic Toxicity (or Exposure)

Animal

As is with acute and subchronic exposures, chronic exposures result in toxicity to the liver, kidneys, respiratory system and CNS. The majority of the animal data are based on oral exposures, with limited data on inhalation. In rats, liver toxicity consisted of degenerative and foamy vacuolization and necrosis, and increased liver weights in males. Kidney toxicity showed cloudy swelling and nephritis. A chronic reference dose (RfD) of $0.01\,mg\,kg^{-1}\,day^{-1}$ was developed by United States Environmental Protection Agency (US EPA) based on observation of moderate/marked fatty cyst formation in the liver and elevated serum glutamic pyruvic transaminase (SGPT), in a dog chronic oral bioassay, with an LOEL of $15\,mg\,kg^{-1}\,day^{-1}$ (converted to $12.9\,mg\,kg^{-1}\,day^{-1}$), and an uncertainty factor of 1000.

Inhalation exposures to chloroform in animals have shown developmental effects, such as decreased fetal body weight, fetal resorptions and malformations in the offspring. Reproductive effects following inhalation exposures included decreased conception rates, decreased ability to maintain pregnancy, and abnormal sperms. Oral exposures have shown decreased fetal weight, increased fetal absorptions but not birth defects.

Cancer of the liver and kidneys were observed in rats and mice following administration of chloroform by the oral route. These include the following studies: (1) a long-term study by the National Cancer Institute in Osborne–Mendel rats and B6C3F1 mice treated with chloroform by gavage in corn oil; (2) a carcinogenicity study conducted in male Osborne–Mendel rats and female B6C3F1 mice administered chloroform in drinking water; and (3) a series of three studies using ICI, CBA, C57BL, and CF/1 mice administered chloroform in toothpaste. Kidney tumors were found in male rats and liver tumors in male and female mice.

Human

Chloroform may cause dry mouth, headache, hallucinations, dysarthria, ataxia, loss of reflexes, gastrointestinal distress, hepatotoxicity, and psychotic behavior. Inhalation has been associated with liver effects, including hepatitis and jaundice, and CNS effects, such as depression and irritability. Oral exposure can lead to effects on the blood, liver and kidneys. Prolonged or repeated skin contact may cause dermatitis.

There are no epidemiologic data available that attribute human cancer to exposure to chloroform *per se*. Epidemiologic studies suggest an association between cancer of the large intestine, rectum and/or bladder and the constituents of chlorinated water. Chloroform is listed as a group 2B probable human carcinogen by the International Agency for Research in Cancer. It is classified by the US EPA as a Group B2 chemical, a probable human carcinogen, based on 'sufficient evidence' of carcinogenicity in animals. Under the US EPA's Proposed Guidelines for Carcinogen Risk Assessment, chloroform is considered by the Agency as likely to be carcinogenic to humans by all routes of exposure under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues. Chloroform is also considered by the US EPA as not likely to be carcinogenic to humans by any route of exposure under exposure conditions that do not cause cytotoxicity and cell regeneration. Therefore, US EPA has not derived an oral carcinogenic potency slope or an inhalation unit risk for chloroform.

In Vitro Toxicity Data

Chloroform has shown both negative and positive results in genotoxicity studies. The studies included gene mutation in bacteria, yeast, and mammalian cells; DNA damage; sister chromatid exchange; micronuclei induction, recessive lethality, and sperm abnormalities. Positive findings included dose-dependent increases in sister chromatid exchange in mouse (*in vivo*) bone marrow and in chromosomal aberrations in rats (*in vivo*) treated orally or i.p. with chloroform, positive mouse micronuclei assay, induction of intrachromosomal recombination in yeast and reduction in recombination in the presence of a free radical scavenger, DNA binding *in vivo*, and a threefold increase in micronucleated kidney cells in rats exposed orally to a high dose of chloroform. This last finding is of particular interest because of damage at the chromosome level in the animal species (rat) and tissue (kidney cells) most relevant to the carcinogenicity assessment of chloroform.

Clinical Management

Features of chloroform poisoning following ingestion include headache, impaired consciousness, convulsions, respiratory paralysis, dizziness, abdominal pain, nausea, vomiting, and diarrhea. Inhalation may result in dizziness and shortness of breath.

In cases of ingestion, ipecac-induced emesis is not recommended. Activated charcoal slurry with or without saline cathartic or sorbitol can be given in cases of oral exposures. Exposed skin should be decontaminated by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of water at room temperature for at least 15 min.

Management includes early decontamination, supportive and symptomatic treatment with respiratory and cardiac monitoring (respiratory assistance, defibrillation, possible fluid replacement), avoiding catecholamine drugs and treatment of liver and/or kidney failure (renal dialysis) if they occur. No specific antidote is available.

Environmental Fate

Chloroform evaporates easily into the air where it breaks down slowly to form products including phosgene and hydrogen chloride. It dissolves well in water where it may form breakdown products. It does not adsorb well onto soil and can travel through soil to groundwater where it persists for a long time.

Ecotoxicology

Scenedesmus subspicatus	48 h	EC_{10} 225 mg l^{-1} (biomass)
		EC_{50} 560 mg l^{-1} (biomass)
Chlamydomonas angulosa		EC_{50} 382 mg l^{-1} (cell count, biomass, carbon dioxide update)
Selenastrum capricornutum		EC_{50} > 1000 mg l^{-1} (cell count, biomass, carbon dioxide update)
Daphnia magna	48 h	LC_{50} 28.9–353 mg l^{-1}
Rainbow trout		LC_{50} 1.24–2.03 mg l^{-1} (200–50 mg calcium carbonate l^{-1})
Bluegills	96 h	LC_{50} 18.2 mg l^{-1} (flow through)

Amphibians appear to be quite sensitive to the effects of chloroform. Very little information is available on terrestrial microorganisms and vertebrates. No information was identified on the toxicity of chloroform to birds or wild animals.

Other Hazards

Persons with higher risk are those who have concurrent exposure to chemical that induce liver cytochrome P-450, and those with underlying liver, kidney, or neurologic conditions.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration has set a maximum allowable concentration of chloroform of 50 ppm in workroom air during an 8 h work day in a 40 h work week.

The US EPA has set a federal drinking water standard (called maximum contaminant level, or MCL) of 100 μg l^{-1} for total trihalomethanes (a class of drinking water disinfectant by-products).

The US EPA requires that spills or accidental releases of 10 pounds or more of chloroform into the environment be reported.

In 2001, US EPA set an RfD of 0.01 mg kg^{-1} day^{-1} based on an increase in the incidence of moderate to marked hepatic fatty cyst formation in the liver of dogs and SGPT in a chronic oral study. The same RfD value was obtained using the lowest-observed-adverse-effect level (NOAEL)–lowest-observed-adverse-effect level (LOAEL) approach and the Benchmark Dose approach. A point of departure (POD or LED_{10}) of 23 mg kg^{-1} day^{-1} was calculated using quantitative modeling of kidney tumor dose–response data in a drinking water bioassay. When compared to the RfD of 0.01 mg kg^{-1} day^{-1} for noncancer end point based on kidney toxicity, a margin of exposure (MOE) of 2000 is obtained. Therefore, the RfD for non-cancer effect is also considered by US EPA as adequately protective of public health for cancer effects by the oral route, on the basis of the nonlinear dose response for chloroform and the mode of action for both cancer and noncancer effects having a common link through cytotoxicity. No cancer slope factor or unit risk values were developed.

The Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, has developed an inhalation reference exposure level of 50 ppb (300 μg m^{-3}) based on a whole-body inhalation study in rats, 7 h day^{-1}, 5 days $week^{-1}$, for 6 months. The critical effects are pathological changes in the liver and kidney, with an (average experimental exposure) LOAEL of 5.3 ppm, and a total uncertainty factor of 300.

The Agency for Toxic Substances and Disease Registry has established an acute inhalation minimal risk level (MRL) of 0.5 mg m^{-3} (0.1 ppm) based on liver effects in mice, an intermediate inhalation MRL of 0.2 mg m^{-3} (0.05 ppm) based on liver effects in workers, and a chronic inhalation MRL of 0.1 mg m^{-3} (0.02 ppm) based on liver effects in humans.

Miscellaneous

An assessment by the World Health Organization for swimmers exposed to chloroform while using indoor

pools disinfected with chlorine showed that pool users could exceed the tolerable daily intake when concentrations of chloroform in the water and air are relatively high.

See also: Carcinogen Classification Schemes; Carcinogenesis; Cytochrome P-450.

Further Reading

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World Health Organization (WHO) (2004) *Chloroform*. Concise International Chemical Assessment Document 58. Geneva: World Health Organization.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chloroform.

http://193.51.164.11 – International Agency for the Research on Cancer (IARC).

http://www.who.int – World Health Organization (WHO).

Chloromethyl Ether, Bis-

C Vaman Rao

- Chemical Abstracts Service Registry Number: CAS 542-88-1
- Synonyms: BCME; *sym*-Dichloromethyl ether; Dichloromethyl ether
- Chemical/Pharmaceutical/Other Class: Alkyl organic synthetic compound with a strong unpleasant odor
- Chemical Formula: $C_2H_4OCl_2$
- Chemical Structure: $ClCH_2—O—CH_2Cl$

Uses

Bis(choromethyl) ether (BCME) is primarily used in the synthesis of polymers, ion exchange resins, and plastics. It used as a chemical intermediate for the synthesis of other complex organic alkyl compounds as well as chloromethylating (cross-linking) reaction mixture in anion exchange resins. It is used as a dental restorative material.

In textile industry it is used in laminating and as adhesive in the flocking of fabrics and in the finishing product of the fabrics as a mixture with formaldehyde containing reactants and resins. Nonwoven textile industry uses it as binder and thermosetting of acrylic emulsion.

Exposure Routes and Pathways

Primary routes of human exposure to BCME are inhalation and dermal contact, which might occur in chemical plants that make or use BCME. Also, some BCME may exist in chemical waste sites, which may be inhaled by breathing the air containing BCME vapors. The risk of potential occupational exposure to BCME is greatest for chemical plant workers, ion exchange resin makers, laboratory workers, and polymer makers. BCME is highly unstable in water, quickly breaking down into formaldehyde and hydrochloric acid. Therefore, exposure through water pollution is limited.

Toxicokinetics

BCME is rapidly absorbed through skin and lung surface. On contact with body fluids, it is quickly broken down to formaldehyde and hydrochloric acid, and interacts with cells and tissues at various levels. Absorption by the body depends on the proximity to the source of production at the industry and at the waste dump site. BCME is mainly metabolized in the liver but to some extent it is also metabolized in the lung tissue. On metabolism, BCME is converted into an epoxide, which is a reactive species of free radical capable of reacting with any organic substance. Metabolites of BCME cause alkylation of DNA leading to mutagenesis and carcinogenesis.

Glutathione *S*-transferase, sulfotransferase, and glucuronidation help in removal of toxic metabolites.

Mechanism of Toxicity

In humans, acute exposure to BCME may cause skin, mucous membrane, and respiratory tract irritation. Lung irritation, congestion, edema, and hemorrhage have been observed in rats and hamsters following acute inhalation exposure. BCME is irritating to the skin of mice and rabbits. Corneal opacity has been observed in rabbits. Acute animal tests in rats, mice, hamsters, and rabbits have demonstrated BCME to have extreme acute toxicity via inhalation and high acute toxicity via oral and dermal exposure. Chronic bronchitis, chronic cough, and impaired respiratory function have been observed in humans following chronic inhalation exposure. However, exposure to BCME usually occurs concurrently with exposure to chloromethyl methyl ether, which itself is a lung irritant. Chronic inhalation exposure of mice to BCME has been reported to cause respiratory distress.

Chronic Toxicity (or Exposure)

Animal

The International Agency for Research on Cancer (IARC) (1974, 1979, 1982, 1987) reported that there is sufficient evidence of carcinogenicity of BCME. When BCME is administered through subcutaneous route to mice of both sexes, it induced pulmonary tumors, papillomas, and firbrosarcomas; local sarcomas in female mice; and fibromas and fibrosarcomas in female rats. BCME is also an initiator of skin tumors in mice. It produced low incidence of tumors of respiratory tract in rats and hamsters after exposure by inhalation. When administered by inhalation, BCME induced lung tumors in mice and squamous cell carcinoma of the lung and esthesioneuroepitheliomas of the nasal cavity in rats. When applied topically, BCME induced papillomas, most of which developed into squamous cell carcinoma in female mice.

Human

BCME is known to be a human carcinogen based on sufficient evidence of carcinogenicity in humans. Numerous epidemiological studies and case reports from around the world have documented that workers exposed to BCME have an increased of lung cancer. Two studies of workers exposed to BCME showed an increased risk of lung cancer, mainly small cell carcinoma. Two subsequent studies have shown a positive association between atypical cells in bronchial excretion on exposure to BCME. Among heavily exposed workers, the relative risk of cancer is 10-fold or greater. Risks increase with duration and cumulative exposure. Maximal relative risks appear to occur 15–20 years after first exposure, and latency is shortened among workers with heavier exposure. Excess respiratory cancer mortality was most markedly increased in workers less than 55 years of age.

The American Conference of Governmental Industrial Hygienists time-weighted average threshold limit value is 0.001 ppm ($0.0047\,mg\,m^{-3}$) with the notation that material is a confirmed human carcinogen.

Clinical Management

There is no antidote recommended for poisoning by BCME. Administration of free radical scavengers should alleviate the toxicity.

Environmental Fate

No information is available on the transport and partitioning of BCME in the environment. Due to the relatively short half-life in both air and water, it is unlikely that significant partitioning between media or transport occurs. Primary process for BCME degradation in air is believed to be reaction with photochemically generated hydroxyl radicals to yield chloromethyl formate ClCHO, formaldehyde, and HCl. Atmospheric half-life due to reaction with hydroxyl radicals is estimated to be 1.36 h. Hydrolysis in the vapor phase is found to be slower with an estimated half-life of 25 h.

BCME is rapidly hydrolyzed in water to yield formaldehyde and HCl, and the hydrolysis rate constant is estimated to be $0.018\,s^{-1}$ at 20°C, which is equal to a half-life of ~ 35 s.

No information is available on the fate of BCME in soil. It is probable that BCME would rapidly degrade upon contact with moisture in soil. Due to its high volatile nature, it is not expected that BCME would persist in soil for significant periods.

Exposure Standards and Guidelines

The US Environmental Protection Agency (EPA) recommends that levels in lakes and streams should be limited to 0.0000038 parts per billion (ppb) parts of water to prevent possible health effects from drinking water or eating fish contaminated with BCME. Any release to the environment greater than 10 lbs of BCME must be reported to the EPA.

The EPA calculated an inhalation unit risk estimate of $0.062\,\mu g^{-1}\,m^3$. The EPA estimates that, if an

individual were to continuously breathe air containing BCME at an average of $0.000016\,\mu g\,m^{-3}$ ($1.6 \times 10^{-8}\,mg\,m^{-3}$) over his or her entire lifetime, that person would theoretically have no more than a 1 in 10^6 increased chance of developing cancer as a direct result of breathing air containing this chemical. Similarly, the EPA estimates that breathing air containing $0.00016\,\mu g\,m^{-3}$ ($1.6 \times 10^{-7}\,mg\,m^{-3}$) would result in not greater than a 1 in 10^5 increased chance of developing cancer, and air containing $0.0016\,\mu g\,m^{-3}$ ($1.6 \times 10^{-6}\,mg\,m^{-3}$) would result in not greater than a 1 in 10^4 increased chance of developing cancer.

The EPA has calculated an oral cancer slope factor of $220\,mg^{-1}\,kg\,day$. The Occupational Safety and Health Administration (OSHA) has set a limit of 1 ppb as the highest acceptable level in workplace air, and strict controls have been established to minimize exposure to this chemical.

The Agency for Toxic Substances and Disease Registry (ATSDR) has established an intermediate inhalation minimal risk level (MRL) of $0.0014\,mg\,m^{-3}$) (0.0003 ppm) based on respiratory effects in rats. The MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. It is not a direct estimator of risk but rather a reference point to gauge the potential effects. At exposures increasingly greater than the MRL, the potential for adverse health effects increases.

Further Reading

American Conference of Governmental Industrial Hygienists (ACGIH) (1999) *1999 TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents. Biological Exposure Indices.* Cincinnati, OH: ACGIH.

Budavari S (ed.) (1989) *The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals*, 11th edn. Rahway, NJ: Merck and Co. Inc.

International Agency for Research on Cancer (IARC) (1974) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man: Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous Alkylating Agents*, Vol. 4. Lyon: World Health Organization.

International Agency for Research on Cancer (IARC) Monographs (1974, 1979, 1982, 1987).

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Bis(chloromethyl) ether.

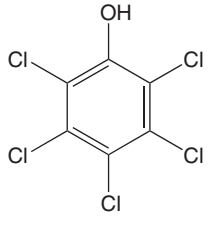
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Bis(chloromethyl) Ether.

Chlorophenols

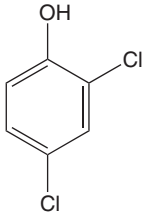
Murali Badanthadka and Harihara M Mehendale

This article is a revision of the previous print edition article by Stephanie E Foster and Paul W Ferguson, volume 1, pp. 312–313, © 1998, Elsevier Inc.

- REPRESENTATIVE CHEMICALS: Pentachlorophenol; 2,4-Dichlorophenol
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Aromatic alcohols
- CHEMICAL STRUCTURES:



Pentachlorophenol



2,4-Dichlorophenol

Uses

Chlorophenols are used in dye synthesis, fungicides, herbicides, wood preservatives, and as ingredients in alcohol denaturants.

Exposure Routes and Pathways

Exposure to chlorophenols may occur through ingestion, inhalation, or dermal contact.

Toxicokinetics

Absorption of pentachlorophenol is rapid through oral, dermal, or inhalation exposure. The major tissue deposits vary somewhat between species. In humans, liver, kidney, brain, spleen, and fat are the major deposition sites. In the mouse, the gall bladder is a principal storage site. In the rat, it is the kidney. The primary route of elimination is by the kidneys in

unchanged form. Labeled pentachlorophenol given to rats by injection or oral route yielded 41–43% unchanged pentachlorophenol in the urine. One metabolite, tetrachlorohydroquinone (5–24%), was identified. Elimination half-life for pentachlorophenol may be up to 20 days in chronically exposed individuals.

In a study, a single dose of 15 mg pentachlorophenol per kg was administered intravenously and orally to B6C3F1 mice. After intravenous administration, the values of clearance and volume of distribution were $0.057 \pm 0.007 \,l\,h^{-1}\,kg^{-1}$ and $0.43 \pm 0.06 \,l\,h^{-1}\,kg^{-1}$, respectively. The elimination half-life was 5.2 ± 0.6 h. After oral administration, peak plasma concentration ($28 \pm 7 \,\mu g\,ml^{-1}$) occurred at 1.5 ± 0.5 h and bioavailability (1.06 ± 0.09) was complete. The elimination half-life was 5.8 ± 0.6 h. Only 8% of the pentachlorophenol dose was excreted unchanged in the urine. Pentachlorophenol was primarily recovered in urine as glucuronide and sulfate conjugate metabolites. A portion of the dose was recovered in urine as tetrachlorohydroquinone (5%) and its conjugates (15%). For both pentachlorophenol and tetrachlorohydroquinone, sulfates accounted for 90% or more of the total conjugates.

There is marked gender difference in biological half-life in non-human primates. Biological half-life for excretion in the Rhesus monkey was 41 and 92 h in males and females, respectively.

Mechanism of Toxicity

Chlorophenols block adenosine triphosphate (ATP) production, without blocking the electron transport chain. They inhibit oxidative phosphorylation, which increases basal metabolic rate and increases body temperature. As body temperature rises, heat-dissipating mechanisms are overcome and metabolism is accelerated. Adenosine diphosphate (ADP) and other substrates accumulate, and stimulate the electron transport chain further. This process demands more oxygen in a futile effort to produce ATP. Oxygen demand quickly surpasses oxygen supply and energy reserves of the body become depleted.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} for pentachlorophenol in laboratory animals ranges from 30 to $100 \,mg\,kg^{-1}$.

Human

Most prevalent signs and symptoms after ingestion of 30–250 ml of chlorophenols are corrosion of tissue, profuse sweating, intense thirst, nausea, vomiting, diarrhea, convulsions, pulmonary edema, cynosis, and coma. If death from respiratory failure is not immediate, jaundice and oliguria or anuria may occur.

Chronic Toxicity (or Exposure)

Human

Repeated exposure may cause symptoms of acute poisoning. Skin sensitivity reactions occur occasionally. Prolonged skin contact with chlorophenols may cause bladder tumors, hemolytic anemia, and lens opacities.

Pathologic findings in deaths by chlorophenols include necrosis of mucous membranes, cerebral edema, and degenerative changes in the liver and kidneys.

Clinical Management

Upon exposure by ingestion, where corrosive injury is absent, the decontamination to prevent further absorption may be achieved by use of activated charcoal. Emesis by syrup of ipecac may be considered, but not preferred. Next, milk should be given to drink. Gastric lavage and emesis are contraindicated in the presence of esophageal injury. In the case of dermal exposure, the poison should be removed by washing the affected skin or mucous membrane with copious amounts of water for at least 15 min.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is $0.5 \,mg\,m^{-3}$ for 8 h time-weighted average (TWA). The threshold limit value is $0.5 \,mg\,m^{-3}$ for 8 h TWA. The National Institute for Occupational Safety and Health recommended exposure limit is $0.5 \,mg\,m^{-3}$ for 10 h TWA.

See also: Chlorophenoxy Herbicides; Drugs of Abuse; Dyes.

Further Reading

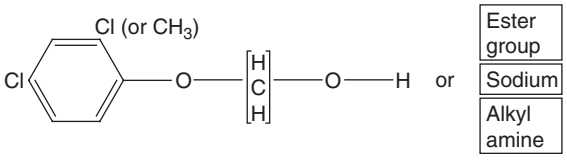
Jensen J (1996) Chlorophenols in the terrestrial environment. *Reviews of Environmental Contamination and Toxicology* 146: 25–51.

Chlorophenoxy Herbicides

Subramanya Karanth

This article is a revision of the previous print edition article by Thuc Pham, volume 1, pp. 313–315, © 1998, Elsevier Inc.

- Representative Chemicals: 2,4-D (2,4-dichlorophenoxy acetic acid); 2,4-DP (2-(2,4-Dichlorophenoxy)propionic acid); 2,4,5-T (2,4,5-Trichlorophenoxy acetic acid); Dicamba (3,6-Dichloro-*o*-anisic acid); MCPA (4-Chloro-2-methyl-phenoxy acetic acid); MCPP (2-(4-Chloro-2-methylphenoxy)propionic acid); Silvex (2-(2,4,5-Trichlorophenoxy)propionic acid)
- Chemical Abstracts Service Registry Numbers: CAS 94-75-7 (2,4-D); CAS 120-36-5 (2,4-DP); CAS 93-76-5 (2,4,5-T); CAS 94-76-4 (MCPA); CAS 93-65-2 (MCPP); CAS 93-72-1 (Silvex); CAS 1918-00-9 (Dicamba)
- Chemical/Pharmaceutical/Other Class: Herbicides
- Chemical Structure (General):



Uses

Chlorophenoxy herbicides are commonly used for controlling broadleaf weeds in agriculture. They are extensively used for the control of vegetation along highways, maintenance of parks, golf courses, home lawns, and gardens.

Background Information

Agent Orange, a mixture of the chlorophenoxy herbicides 2,4-D and 2,4,5-T, was extensively used by US military during Vietnam War in order to destroy forest and other vegetation from the premises of US bases. About 19 million gallons were used on ~3.6 million acres of land in Vietnam and Laos during the period from 1962 to 1971. Some lots of 2,4,5-T were contaminated with dioxins formed during manufacturing. Because dioxins resist degradation and remain in the environment for years, they are considered persistent organic pollutants. The particular dioxin present in Agent Orange, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or TCDD, is notoriously known as the most acutely toxic synthetic chemical. TCDD levels were found to be higher among veterans serving in Vietnam compared to those serving elsewhere at the same time. Concerns that these and other health problems may have been associated with exposure to Agent Orange stimulated a series of scientific studies, health care programs, and compensation programs directed in support of veterans. A large class-action lawsuit filed in 1979 was settled out of court in 1984 resulting in the Agent Orange Settlement Fund, which distributed nearly $200 million to veterans between 1988 and 1996.

A number of studies were conducted to evaluate a possible link between Agent Orange and cancer. The Air Force Health Study compared ~1200 Ranch Hand veterans directly involved in herbicide distribution to 1300 veterans not involved. Periodic physical exams, medical records reviews, and blood dioxin measurements were taken over a 20 year period. About a dozen states, mostly in the Midwest and Northeast, conducted health studies of Vietnam veterans, some of which included cancer information. A series of studies of Australian Vietnam veterans also evaluated cancer incidence. Because of some limitations of the Vietnam veteran studies, other studies were used to draw conclusions on Agent Orange and cancer. No association with soft tissue sarcoma was seen in the Ranch Hand study, in a study of over 10 000 Marines who had served in Vietnam, a large study of sarcoma patients in VA hospitals, the Selected Cancers Study, or studies of veterans in several US states. Most studies have not reported an increase in non-Hodgkin lymphoma (NHL) or Hodgkin disease and respiratory cancers (lung, trachea, bronchus, and larynx). While the VA and Ranch Hand studies did not show an excess of prostate cancer, the Australian studies showed an increased incidence of this type of cancer. Three studies demonstrated an association between paternal Agent Orange exposure and acute myeloid leukemia in children. There were no links established between Vietnam service and cancers of the gastrointestinal tract or brain. Thus, a number of studies were conducted on the possible association of Agent Orange exposure and cancer incidence, with considerable variation in outcome across studies.

The 'Agent Orange Act of 1991' required the US Secretary of Veterans Affairs to request an evaluation of the health effects of Agent Orange in Vietnam Veterans by the National Academy of Sciences. The Institute of Medicine (IOM) formed a 'Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides'. The Committee initiated a series of studies to evaluate cancer and noncancer

health effects in veterans. The IOM concluded that there was sufficient evidence of an association between Agent Orange and soft tissue sarcomas, NHL, Hodgkin disease, and chronic lymphocytic leukemia. Regulatory agencies have relatively similar conclusions regarding carcinogenicity of chlorophenoxy herbicides and TCDD. The US National Toxicology Program (NTP) does not list chlorophenoxy herbicides (including Agent Orange) as carcinogens, but lists 2,3,7,8-TCDD as 'known to be a human carcinogen'. The International Agency for Research on Cancer has not rated Agent Orange itself, but the chlorophenoxy herbicides including 2,4-D and 2,4,5-T are categorized as 'possibly carcinogenic to humans' while 2,3,7,8-TCDD is categorized as 'known to be carcinogenic to humans'. In contrast, the US Environmental Protection Agency has not classified either chlorophenoxy herbicides or TCDD as to carcinogenicity.

Exposure Routes and Pathways

Chlorophenoxy herbicides can be absorbed into the body by inhalation of its aerosol, through the skin, and by ingestion. The most common exposure pathway is accidental or intentional ingestion.

Toxicokinetics

Chlorophenoxy herbicides are readily absorbed through the gastrointestinal tract and distributed throughout the body. They are excreted unchanged mainly in the urine and are generally not stored in the body. Studies in laboratory rats given 1, 5, or $10\,mg\,kg^{-1}$ of ^{14}C 2,4-D have shown that 94–99% is eliminated from the body unchanged within 72 h. Biological half-life ranged from 10 to 33 h. Metabolic conversions may occur more with higher doses.

Mechanism of Toxicity

The mechanism of action of chlorophenoxy herbicides in mammals is not clearly known. They are believed to elicit toxicity by cell membrane damage, uncoupling of oxidative phosphorylation, or disruption of acetylcoenzyme A metabolism. Myotonia (stiffness and incoordination of hind extremities) is commonly observed following overdose of 2,4-D. In addition, high doses can cause significant metabolic acidosis and renal failure in humans. Formulations of chlorophenoxy herbicides were often contaminated by complex chlorinated hydrocarbons, e.g., dibenzodioxins. TCDD, which is highly toxic to mammals, was one of the common dioxin pollutants in Agent Orange.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chlorophenoxy herbicides exhibit low mammalian toxicity. The acute oral LD_{50} for 2,4-D in rats is $>300\,mg\,kg^{-1}$. Dogs are more susceptible to 2,4-D poisoning with an oral LD_{50} of $100\,mg\,kg^{-1}$.

Human

Ingestion of high doses can lead to burning of the mouth, vomiting, abdominal pain, and gastrointestinal hemorrhage. Acute exposure may also cause severe metabolic acidosis, myotonia, and muscle weakness, which can persist for a long period of time.

Chronic Toxicity (or Exposure)

Animal

Long-term feeding studies in rats have revealed that exposure to a daily dosage of $300\,mg\,kg^{-1}\,day^{-1}$ does not cause any adverse effects while exposure to higher doses can result in body weight loss and liver changes.

Human

Chronic exposure to some common chlorophenoxy herbicides such as 2,4-D through drinking water can potentially cause damage to the nervous system, kidneys, and liver. Chronic exposure to 2,4-D has also been linked to immune system suppression and endocrine disruption. Carcinogenic potential of these herbicides is not clear. 2,4-D and MCPA, which are commonly used in wheat production, have been linked to birth defects.

Clinical Management

Chlorophenoxy compounds are moderately irritating to skin. In case of dermal or eye exposure, the contaminated area should be bathed or flushed with copious amounts of water for $\sim$15 min and if irritation persists a physician should be contacted. Ingestion of substantial amounts of these chemicals results in spontaneous emesis. If the patient is fully alert and there are no apparent signs of emesis, emesis is induced with syrup of ipecac (adults, 30 ml; children $<$12 years, 15 ml), followed by one to two glasses of water. In order to limit the absorption of the herbicide in the gut, 30–50 g of activated charcoal is administered in $\sim$6–8 ounces of water. Severe intoxication with chlorophenoxy compounds may result in renal failure. To avoid

toxicant buildup in the kidney and to accelerate excretion, intravenous fluids (saline or dextrose) are administered and serum electrolytes monitored. Early initiation of forced alkaline diuresis with sodium bicarbonate may be useful in the management of acute poisoning.

Environmental Fate

Chlorophenoxy herbicides are generally not persistent in the environment. Common herbicides such as 2,4-D, MCPP, and dicamba are readily biodegraded by soil and aquatic microorganisms. 2,4-D and dicamba are commonly found in public drinking water systems. Typical half-life in water ranges from 10 to 15 days.

Ecotoxicology

Chlorophenoxy herbicides are moderately toxic to nontarget species. Some of the commercial products are highly toxic to aquatic invertebrates and other beneficial nontarget plants.

See also: Carcinogen Classification Schemes; Carcinogenesis.

Relevant Websites

http://www.cancer.org – American Cancer Society.
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://infoventures.com – US Department of Agriculture, Forest Service.

Chloropicrin

Priya Raman

- Chemical Abstracts Service Registry Number: CAS 76-06-2
- Synonym: Trichloronitromethane
- Chemical/Pharmaceutical/Other Class: Fumigant insecticide
- Chemical Formula: CCl_3NO_2

Uses and Background Information

Chloropicrin is a widely used fungicide that is primarily used for preplant soil fumigation. Chloropicrin is used to fumigate stored grain and to treat soil against fungi, insects, and nematodes. It is also a tear gas agent for military use. Chloropicrin was first synthesized in 1848. It was patented as an insecticide in 1908 and has been extensively used since then as a soil fumigant at high application rates of $18\,lb\,acre^{-1}$. Chloropicrin, which has a total US production of ~10–11 million pounds per year was also used as a war gas agent during World War I.

Exposure Routes and Pathways

Chloropicrin has strong lacrimatory properties and is a potent skin irritant. Thus, dermal and eye exposures are the most common routes of chloropicrin toxicity. It is also an inducer of vomiting, bronchitis, and pulmonary edema in humans. As a fumigant, the respiratory tract is the principal target of chloropicrin toxicity. The primary lesion following ingestion of chloropicrin is manifested by corrosive effects on the forestomach tissue. The intraperitoneal LD_{50} in mice is $25\,mg\,kg^{-1}$. Human exposure to chloropicrin also occurs from trace levels in drinking water disinfected by chlorination.

Toxicokinetics

The toxic effects of chloropicrin occur very rapidly. The liver is the primary site of metabolism of this compound. Reductive dechlorination of chloropicrin serves as the basis for its multiple types of toxic action. Following an intraperitoneal or oral administration of chloropicrin, urine is the major route for excretion of its metabolites, mostly (43–47%) within the first 24 h. The urinary metabolites at 24 h are polar and nonvolatile.

Mechanism of Toxicity

Recent studies identify a new metabolic pathway for chloropicrin involving a rapid dechlorination to $CHCl_2NO_2$ and conversion of glutathione (GSH) to GSSG plus possible adduct formation with thiol proteins. In this newly discovered pathway, chloropicrin is metabolized to thiophosgene, characterized as the cyclic cysteine adduct (raphanusamic acid) in mice urine. The initially formed GS-CCl_2NO_2 metabolite is proposed to either react further with GSH or is cleaved by cysteine-β-lyase, ultimately leading to raphanusamic acid, which is excreted. Chloropicrin is an

SN_2 alkylating agent with an activated halogen group and reacts with sulfhydryl groups, 'fixing' enzymes. Oxidation of protein thiols with chloropicrin is accompanied with the formation of internal and cross-linked disulfide bonds leading to the suggestion that inhibition of pyruvate dehydrogenase (PDH) and succinate dehydrogenase (SDH) with critical SH groups in their active sites, are involved in acute mammalian toxicity. Both PDH and SDH complexes are inhibited *in vitro* by chloropicrin with moderate potency ($IC_{50} = 4–13\,\mu mol\,l^{-1}$). Chloropicrin also has the additional toxic effect of interfering with oxygen transport by its reaction with SH– groups in hemoglobin. Thus, chloropicrin toxicity in mice is linked to the accumulation of oxyhemoglobin in tissues, particularly the liver. Chloropicrin may also undergo a photochemical transformation to phosgene.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chloropicrin is 10 times more potent than its dehalogenated metabolites. It has been reported to inhibit porcine heart pyruvate and mouse liver succinate dehydrogenase complexes with IC_{50} values of 4 and $13\,\mu mol\,l^{-1}$ respectively. Mice treated intraperitoneally with choloropicrin at $50\,mg\,kg^{-1}$ showed a dose-dependent increase in liver oxyhemoglobin, hemoprotein, and total hemoglobin levels. Acute toxicity of chloropicrin in mice is due to the parent compound or metabolites other than $CHCl_2NO_2$ or CH_2ClNO_2. In rats, the respiratory tract is the primary target on inhalation exposure. Chloropicrin is intensely irritating with an intraperitoneal LD_{50} of $25\,mg\,kg^{-1}$ in mice. Rabbits exposed to an intravenous injection of chloropicrin at a dosage of $15\,mg\,kg^{-1}$ died within 15–240 min; clinical and autopsy findings were typical of acute pulmonary edema. Chloropicrin induces lesions in the lower respiratory tract with an RD_{50} (concentration which elicits a respiratory rate decrease of 50%) values of 8 ppm. The no-observed-adverse-effect level (NOAEL) for systemic toxicity following chloropicrin exposure is reported to be 1.0 ppm and greater than 1.5 ppm for developmental toxicity and reproductive parameters. The NOAEL for maternal toxicity has been established to be 0.4 ppm and the NOAEL for fetal toxicity is 1.2 ppm suggesting that the developing fetus is not a target tissue for chloropicrin toxicity.

Human

Exposure to chloropicrin causes eye and respiratory tract irritation accompanied by vomiting and diarrhea. The primary signs and symptoms following inhalation exposure to chloropicrin include coughing, nasal and pharyngeal mucosal edema, and erythema, lacrimation, and rhinorrhea. Fatal pulmonary edema has been reported with an onset of 3 h post-exposure. Chloropicrin is a strong eye irritant, producing ocular burning, eye pain, and lacrimation following eye exposure. These effects may last up to 30 min or longer. Redness and edema may be noted 1 or 2 days following exposure. Dermal exposure to chloropicrin produces severe skin irritation.

Chronic Toxicity (or Exposure)

Animal

Following an oral gavage for 90 days, the no-effect-dose is $8\,mg\,kg^{-1}\,day^{-1}$, with severe forestomach tissue lesions characterized by inflammation, necrosis, acantholysis, hyperkeratosis, and epithelial hyperplasia. Mice exposed to 8 ppm chloropicrin vapor for $6\,h\,day^{-1}$ for 5 days developed moderate to severe degeneration of the respiratory and olfactory epithelium as well as fibrosing peribronchitis and peribronchiolitis of the lung.

Human

Following exposure to chloropicrin vapor in an agricultural chemicals facility, persistent chest wall pain as well as an increase in creatine phosphokinase levels has been reported. Severity of symptoms and degree of biochemical abnormalities were reported to occur in a dose-dependent pattern. Inhalation exposure to very high levels of chloropicrin can lead to pulmonary edema, unconsciousness, and even death.

In Vitro Toxicity Data

Chloropicrin is a bacterial mutagen and induces sister chromatid exchanges in cultured human lymphocytes, but is not considered as carcinogenic. Mutagenicity assays establish chloropicrin to be toxic but not mutagenic at 500 nmol per plate.

Clinical Management

Following an eye exposure to chloropicrin, the affected eyes should be irrigated with copious amounts of tepid water for at least 15 min. If irritation persists following decontamination, ophthalmic corticosteroids or local anesthetic ointments may be used. In case of an inhalation exposure, the patient should be monitored for respiratory distress. Emergency airway support and 100% humidified supplemental oxygen with assisted ventilation may be needed.

Following dermal exposure to chloropicrin, the exposed area must be washed thoroughly with soap and water. If dermatitis persists, topical treatment with wet dressings of Burow's solution 1:40, followed by corticosteroid creams or calamine lotion, may be given. Secondary infection may necessitate antibiotic therapy. Oral antihistamines may be useful for pruritis.

Environmental Fate

The half-life of chloropicrin in sandy loam soil was 8–24 h and 4.5 days with carbon dioxide being the terminal breakdown product. Chloropicrin can be produced during chlorination of drinking water in the presence of nitrated organic contaminants. Chloropicrin is efficiently photolyzed in the atmosphere. The half-life of chloropicrin in air exposed to stimulated sunlight is reported to be 20 days, the photoproducts being phosgene, nitric oxide, chlorine, nitrogen dioxide, and dinitrogen tetroxide.

Ecotoxicology

Chloropicrin is highly toxic to fish, the 96 h LC_{50} for trout and bluegill being 0.0165 and 0.105 mg l^{-1} respectively. However, little information is available about the effects of chloropicrin on birds.

Exposure Standards and Guidelines

Chloropicrin is a class I toxicity, restricted use pesticide (RUP), labeled with the signal word 'Danger'. The only exposure guidelines available for chloropicrin are that of permissible exposure level and threshold limit value, both having a value of 0.1 ppm. It has an inhalation reference exposure level of 0.4 μg m^{-3}.

Miscellaneous

Chloropicrin is a clear, colorless oily liquid with a sharp, highly irritating odor. It has a molecular weight of 164.38 and a water solubility of 1.6 g l^{-1} at room temperature. Chloropicrin is miscible with most organic solvents, and has a melting point of −64°C. Some trade names for products containing chloropicrin include Chlor-O-Pic, Metapicrin, Timberfume and Tri-Chlor.

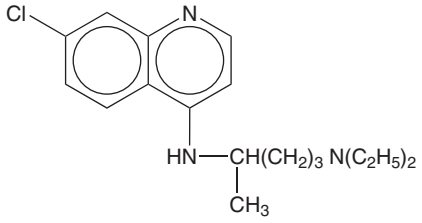
See also: Pesticides.

Chloroquine

F Lee Cantrell

This article is a revision of the previous print edition article by Michael Shannon, volume 1, pp. 316–317, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 54-05-7
- Synonyms: SN 7618; Sanoquin; Tresochin; Silbesan; Artichin; Bipiquin; Avlocluor; Tanakan; Resochin; Resoquine; Aralen
- Chemical/Pharmaceutical/Other Class: Aminoquinoline antimalarial/antirheumatic
- Chemical Formula: $C_{18}H_{26}ClN_3$
- Chemical Structure:



Uses

Chloroquine is used as an anti-inflammatory and antimalarial drug.

Exposure Routes and Pathways

Chloroquine is available in oral and intravenous forms.

Toxicokinetics

Chloroquine is absorbed rapidly and almost completely from the gut; peak serum concentrations are attained within 1 or 2 h. Chloroquine plasma protein binding is ~55%. Its volume of distribution is 116–285 l kg^{-1}. The drug may be found in 500 times greater concentration within the liver, spleen, kidneys, lungs, and leukocytes (compared with plasma). Chloroquine appears to cross the placenta readily. A very small amount is transmitted into breast milk.

The primary route of metabolism is deethylation, producing desethylchloroquine. Elimination is significantly reduced in the presence of hepatic disease.

Nearly 50% of chloroquine is recovered in urine as unchanged drug. The terminal half-life of chloroquine varies from 12 to 60 days.

Mechanism of Toxicity

The mechanism of action of chloroquine is not completely understood but involves inhibition of DNA and RNA polymerase. Chloroquine is also a direct myocardial depressant that impairs cardiac conduction through membrane stabilization.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chloroquine is not used therapeutically in domestic animals. Toxic manifestations of overdose in animals are undefined.

Human

Symptoms of overdose include nausea, vomiting, transient visual or auditory deficits, drowsiness, and seizures followed by severe cardiac arrhythmias, shock, or cardiorespiratory arrest. Hypotension may be severe and intractable, producing metabolic acidosis and end-organ failure. Cardiac conduction disturbances include complete atrioventricular dissociation, QRS and QT prolongation, severe bradycardia, and ventricular fibrillation. Acute ingestions of 30–50 mg kg^{-1} of chloroquine in adults and as little as 300 mg in children are potentially fatal.

Chronic Toxicity (or Exposure)

Animal

Rats chronically administered chloroquine in food for up to 2 years demonstrated dose related inhibition of growth compared with controls. High-dose (from 100 up to 1000 mg kg^{-1} diet over 2 years) studies in rats showed myocardial and other muscle damage centrilobular liver necrosis and testicular damage.

Human

Chronic use of chloroquine may produce cinchonism, a syndrome characterized by headache, visual changes, and gastrointestinal disturbances. Visual disturbances are associated with retinal artery spasm. Ototoxicity may also occur. Dermatologic reactions, particularly a lichenoid skin eruption, may result from chronic chloroquine use.

In Vitro Toxicity Data

Studies in cultured chick brains demonstrated inhibition of retinal pigment epithelium viability at concentrations similar to those seen *in vivo* for patients experiencing chloroquine-induced retinopathy.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. In patients presenting within 1 h of ingestion, activated charcoal should be administered. In the event of depressed consciousness or seizures, airway protection should first be secured. Sodium bicarbonate, epinephrine, and high-dose diazepam should be used to treat cardiotoxicity. Diazepam is recommended for the treatment of seizures. Methods of extracorporeal drug removal, such as hemoperfusion and hemodialysis, are ineffective.

See also: Charcoal; Diazepam.

Further Reading

CDC (1988) Childhood chloroquine poisoning – wisconsin and washington. *Morbidity and Mortality Weekly Report* 37: 437–439.

Piette JC, Guillevin L, and Chapelon C (1987) Chloroquine cardiotoxicity. *New England Journal of Medicine* 317: 710–711.

Relevant Website

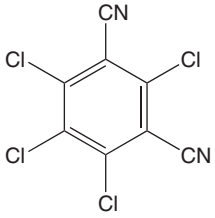
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Chloroquine.

Chlorothalonil

Priya Raman

- Chemical Abstracts Service Registry Number: CAS 1897-45-6
- Synonyms: 2,4,5,6-Tetrachlorobenzenedicarbonitrile; Tetrachloroisophthalonitrile
- Chemical/Pharmaceutical/Other Class: Industrial, agricultural, and horticultural fungicide

- Chemical Structure:



Uses and Background Information

Chlorothalonil is an extensively used pesticide in agriculture, silviculture, and urban settings. This is an important broad-spectrum nonsystemic fungicide that has been widely used for more than 30 years as an effective disease management tool for potatoes, peanuts, turf, and vegetable and fruit crops. Typical chlorothalonil application rates are $1\,kg\,ha^{-1}$ with four to nine applications per growing season. Chlorothalonil can enter surface waters through rainfall runoff, spray drift, or atmospheric deposition, subsequently having an impact on the aquatic biota. It is also used as a fungicide, bactericide, and nematocide. It is used as a wood preservative in some countries. Chlorothalonil is also used as a mildew-preventing agent in paints.

Exposure Routes and Pathways

Dermal and eye exposure are the most common routes of accidental exposure to chlorothalonil. It may also be ingested or inhaled.

Toxicokinetics

Chlorothalonil is rapidly absorbed both orally and via inhalation. However, the amount of material absorbed is limited and dose related. Thus, while ~30% of an administered dose of up to $50\,mg\,kg^{-1}$ is absorbed, absorption at high doses decreases relatively. There is also a marked species-dependent variation in chlorothalonil absorption. Chlorothalonil is only poorly absorbed by the dermal route of exposure. Absorbed chlorothalonil undergoes rapid distribution among body tissues in rats, tissue concentration being in the order of kidney > liver > whole blood. Conjugation with glutathione constitutes the primary route of metabolism of chlorothalonil. Liver represents the major site for chlorothalonil conjugation. In the enzymatic reaction, 4-(glutathione-*S*-yl)-2,5,6-trichloroisophthalonitril is formed initially. This is also a substrate for glutathione-*S*-transferases (GSTs), resulting in the substitution of a second chlorine atom to give 4,6-bis(glutathione-*S*-yl)-2,5-dichloroisophthalonitril. Hydrolysis studies indicated that the metabolism of chlorothalonil is pH dependent. Thus, 4-hydroxy-2,5,6-trichloroisophthalonitrile and 3-cyano-2,4,5,6-tetrachlorobenzamide are formed at pH 9 but not at pH 7. The metabolism of chlorothalonil was recently investigated in liver and gill cytosolic and microsomal fractions from channel catfish using high-performance liquid chromatography. The reports indicate that chlorothalonil is detoxified *in vitro* by GST-catalyzed glutathione (GSH) conjugation. However, no human data are currently available for the biotransformation of chlorothalonil. Chlorothalonil is primarily eliminated via the kidneys. Following administration of $1\,mg\,kg^{-1}$ chlorothalonil endotracheally, orally, or dermally to rats, less than 6% was recovered in blood or urine within 48 h. The major route of elimination following oral administration to rats is in the feces (>80%) with 5.4–11.5% being excreted in the urine as the dose increases from 5 to $200\,mg\,kg^{-1}$. Marked species differences exist in the pharmacokinetic behavior of chlorothalonil. Thus, following oral administration of $50\,mg\,kg^{-1}$ chlorothalonil, dogs and rhesus monkeys excrete up to 98% and 92% of the dose, respectively, in the feces compared with ~82% in rats.

Mechanism of Toxicity

Glutathione conjugation represents a bioactivation reaction for chlorothalonil resulting in the formation of *S*-conjugates toxic to the kidney. Chlorothalonil acts as an alkylating agent and reacts with cellular sulfhydryl compounds. Alkylation of biological molecules results in effects on cellular function and viability. Chronic damage to the proximal tubular epithelium may be involved in the mechanism of chlorothalonil tumorigenicity to the kidneys.

Animal Toxicity

Forestomach and the renal proximal tubule are the primary target tissues of chlorothalonil toxicity in Sprague–Dawley rats. Toxicity is characterized by hypertrophy, hyperplasia, vacuolization, and degeneration of renal tubular epithelium and acanthosis, hyperkeratosis, and hyperplasia of the squamous epithelium of the forestomach. Chlorothalonil is a well-known skin and eye irritant. Sustained contact with the squamous epithelium of the forestomach can lead to an inflammatory response. The earliest observation following chlorothalonil administration at $175\,mg\,kg^{-1}\,day^{-1}$ to rats for varying periods of time for up to 91 days has been characterized by multifocal ulceration and rosion of the mucosa,

subsequently progressing to hyperplasia and hyperkeratinosis. These lesions have been observed in subchronic and chronic studies in rats and mice (no-observed-effect level (NOEL) $\sim 2\,mg\,kg^{-1}\,day^{-1}$) and in chronic studies appear to be closely related with incidence of neoplasia (NOEL ~ 4–$21\,mg\,kg^{-1}\,day^{-1}$). Undiluted chlorothalonil is a strong irritant and produces irreversible corneal, iridal, and conjunctival effects in rabbits. Weakness and sedation precedes death in animals given acute toxic doses intraperitoneally. Chronic oral administration to rats results in ataxia. Hematuria, vaginal bleeding, and epistaxis are seen in rats following chronic oral exposure. In chronic dermal exposures to chlorothalonil dissolved in acetone, the no-effect level for irritation is 0.001%. The 0.01% concentration is a mild irritant and 0.1% a moderate irritant. Prolonged exposure of rodents to chlorothalonil results in nephrotoxicity and renal tubular hyperplasia and these effects, if sustained, can lead to a tumorigenic response. Chlorothalonil produced a dose-related increased incidence of renal tubular adenomas and adenocarcinomas in rats. The oral LD_{50} in rats is greater than $10\,g\,kg^{-1}$. Chlorothalonil is predicted to be a rodent carcinogen via a nongenotoxic mechanism.

Human Toxicity

Facial dermatitis has been reported in occupational exposures and can occur in the absence of direct skin contact, presumably due to the high volatility of chlorothalonil. Chlorothalonil is a strong primary skin irritant and may also cause allergic contact urticaria and anaphylaxis. Patch testing with concentrations greater than 0.01% may produce primary irritant reactions. Hypersensitivity reactions characterized by facial erythema, periorbital erythema and edema, eczema, and pruritis have been observed following chlorothalonil exposure. Photosensitivity reactions were seen in some individuals. High concentrations of chlorothalonil produce delayed irritant reactions. Delayed dermal irritant effects have also been noted 48–72 h after cessation of exposure. Immediate respiratory reactions such as tightness of chest and throat, may occur following inhalation exposure to chlorothalonil. A recent review of the potential cancer risks of chlorothalonil to operators and consumers conducted in the United Kingdom for the Pesticide Advisory Committee (UK, 1994) provided evidence that chlorothalonil is not genotoxic. The primary potential for human exposure to chlorothalonil is to forest service applicators applying the fungicide.

Clinical Management

One of the primary forms of treatment is to support respiratory and cardiovascular function. Dilution and dermal/eye decontamination are primary considerations. Following oral exposure, immediate dilution with 4–8 oz of milk or water is recommended. Vomiting must be induced if victim is conscious. If inhaled, victim must be immediately moved to fresh air. And if not breathing, artificial respiration should be provided. In case of dermal exposure to chlorothalonil, the exposed area should be thoroughly washed with soap and water. Allergic contact dermatitis may be treated with antihistamines, topical steroids, and/or systemic steroids. Following an eye exposure, the affected eyes should be irrigated with copious amounts of tepid water for at least 15 min. Immediate medical attention for the eyes is also recommended. Exposure may cause temporary allergic side effects. Symptoms include redness of the eyes, mild bronchial irritation, and redness or rash on exposed skin. Temporary allergic reactions can be treated with antihistamines or steroid creams and/or systemic steroids upon consultation with the physician.

Environmental Fate

Chlorothalonil is moderately persistent in soil, having a half-life of up to 3 months in moderately moist soil. The principal breakdown product of chlorothalonil in the soil is 4-hydroxy-2,5,6-trichloroisophthalonitrile, which is slightly toxic to aquatic organisms and moderately toxic to birds and mammals. Chlorothalonil is almost insoluble in water and does not evaporate easily.

Ecotoxicology

Chlorothalonil is highly toxic to fish and other aquatic invertebrate animals. However, it is relatively nontoxic to birds, mammals, and bees.

Exposure Standards and Guidelines

Chlorothalonil is classified as a general use pesticide (GUP) by the US Environmental Protection Agency (EPA). Owing to its potential for causing eye irritation, chlorothalonil is also classified as a toxicity class II-moderately toxic chemical. Based on the increased incidence of renal tumor in female rats, EPA currently lists chlorothalonil as a group B2 (probable human) carcinogen; the Q^* value being $0.00766\,mg\,kg^{-1}\,day^{-1}$. Chlorothalonil has an acceptable daily intake and a reference dose value of 0.03 and $0.015\,mg\,kg^{-1}\,day^{-1}$, respectively.

Miscellaneous

Chlorothalonil is an aromatic halogen compound that appears as a grayish to colorless crystalline solid that is odorless or has a slightly pungent odor. It has a molecular weight of 265.92, water solubility of $0.6\,mg\,l^{-1}$ at room temperature, and a melting point of 250–251°C. Chlorothalonil is only slightly soluble in acetone, dimethyl sulfoxide, cyclohexane, and xylene. Some popular trade names for chlorothalonil include Bravo, Daconil 2787, Echo, Exotherm Termil, Nopcocide, Repulse, and Tuffcide. This compound can be found in formulations with many other pesticide compounds.

See also: Nematocides.

Further Reading

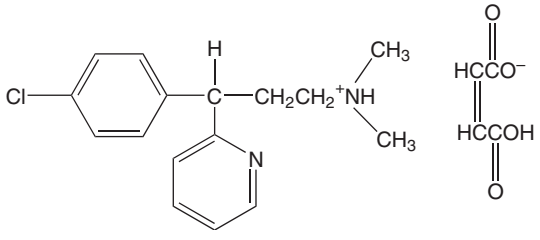
Andre V, Lebailly P, Pottier D, *et al.* (2003) Urine mutagenecity of farmers occupationally during a 1-day use of chlorothalonil and insecticides. *International Archives of Occupational and Environmental Health* 76(1): 55–62 (Epub Oct 2002).

Sherrard RM, Murray-Gulde CL, Rodgers JH Jr., and Shah YT (2003) Comparative toxicity of chlorothalonil: *Ceriodaphnia dubia* and *Pimephales promelas*. *Ecotoxicology and Environmental Safety* 56(3): 327–333.

Chlorpheniramine

Brenda Swanson-Biearman

- Chemical Abstracts Service Registry Number: CAS 132-22-9
- Synonym: Chlortrimeton®
- Chemical/Pharmaceutical/Other Class: A propylamine-derivative (alkylamine) H-1 receptor antagonist
- Chemical Formula: $C_{16}H_{19}ClN_2$
- Chemical Structure:



Uses

Chlorpheniramine, like other antihistamines, is most often used to provide symptomatic relief of allergic symptoms caused by histamine release. It is often included in multisymptom cold preparations.

Exposure Routes and Pathways

Ingestion and injection are the routes of both accidental and intentional exposures to chlorpheniramine.

Toxicokinetics

Chlorpheniramine is well absorbed following oral administration with peak plasma levels occurring within 2–6 h. The drug undergoes substantial metabolism in the gastrointestinal mucosa during absorption and first pass through the liver. The volume of distribution of chlorpheniramine is $3.4–7.5\,l\,kg^{-1}$ and ~69–72% of the drug is bound to plasma proteins. Chlorpheniramine and its metabolites (desmethylchlorpheniramine and didesmethylchlorpheniramine) are excreted almost completely in the urine. Urinary excretion is enhanced with an acidic urine pH, but this is not a viable treatment option. The elimination half-life is more rapid in children than adults, 9.5–13 and 14–24 h, respectively.

Mechanism of Toxicity

The toxicity of antihistamines is related to their anticholinergic (antimuscarinic) activity. The action of acetylcholine at the muscarinic receptors is blocked resulting in signs and symptoms of anticholinergic poisoning.

Acute and Short-Term Toxicity (or Exposure)

Animal

Absorption in dogs is rapid and complete from the gastrointestinal tract, reaching peak plasma concentrations 30–60 min after oral administration. Plasma half-life is ~24 h. Central nervous system (CNS) changes, including sedation or hyperexcitability, salivation, and vomiting, have occurred in dogs and cats following acute low-dose exposures to antihistamines. Seizures and cardiac effects have occurred following acute high-dose exposures. Symptomatic and supportive care, followed by appropriate gastrointestinal decontamination procedures, is the mainstay of treatment.

Human

The alkylamine derivatives are among the most potent antihistamines producing more CNS stimulation and less drowsiness than other antihistamines. In overdoses, CNS stimulation is more common in children. Adult overdose usually causes CNS depression with drowsiness or coma followed by excitement, seizures, and postictal depression. Anticholinergic symptoms including fixed and dilated pupils, flushed skin, dry mouth, fever, urinary retention, hallucinations, and seizures. Cardiovascular effects including tachycardia (prolonged QTc and QRS intervals and nonspecific ST and T-wave changes), hypertension or hypotension, dysrhythmias, and cardiovascular collapse may occur. Severe toxicity may result in cerebral edema, deep coma, cardiorespiratory collapse, or death. Onset of symptoms may occur within 30 min to 2 h after ingestion; death may occur several days after onset of toxic symptoms.

Chronic Toxicity (or Exposure)

Animal

Chronic animal feeding models designed to test for carcinogenicity and mutagenicity have so far proved negative.

Human

Chronic dosing studies in adults and children have demonstrated predictable side effects of drowsiness and sedation in therapeutic doses.

In Vitro Toxicity Data

Ames *Salmonella* and mouse lymphoma tests for mutagenicity have been negative.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Appropriate time-dependent gastrointestinal decontamination should be performed based on the history of the ingestion and the patient's level of consciousness. Electrocardiogram monitoring should be considered in patients who have taken large overdoses. Sinus tachyarrhythmias rarely require treatment. In agitated patients, sedation with benzodiazepines will generally control tachycardia. The use of class Ia antidysrhythmics (quinidine, disopyramide, procainamide, aprindine) and most class III antidysrhythmics (*N*-acteylprocainamide, sotalol) should be avoided since they may further prolong the QT interval and have been associated with torsades de pointes. Intravenous benzodiazepines are recommended for the treatment of seizures. Physostigmine administration may be necessary in a limited number of patients suffering from severe central and peripheral anticholinergic symptoms refractory to conventional therapy.

See also: Benzodiazepines.

Further Reading

Wogoman H, Steinberg M, and Jenkins AJ (1999) Acute intoxication with guaifenesin, diphenhydramine, and chlorpheniramine. *American Journal of Forensic Medicine and Pathology* 20: 199–202.

Wyngaarden JB and Seevers MH (1951) The toxic effects of antihistaminic drugs. *Journal of the American Medical Association* 145: 277–282.

Chlorpromazine

Linda A Malley

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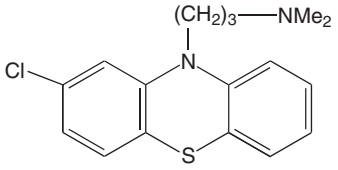
- Chemical Abstracts Service Registry Number: CAS 50-53-3
- Synonyms: 10H-Phenothiazine-10-propanamine; 2-Chloro-*N*,*N*-dimethyl-(9CI CA index name); Phenothiazine; 2-Chloro-10-[3-(dimethylamino)propyl]-(7CI, 8CI); 2-Chloro-10-[3-(dimethylamino)propyl]phenothiazine; 2-Chloropromazine; 4560 R. P.; Aminazin; Aminazine; Ampliactil; Amplictil; BC 135; Chlorpromanyl; Chlordelazin; Chlorderazin; Chlorpromados; Contomin; CPZ; Elmarin; Esmind; Fenactil; Fenaktyl; Fraction AB; HL 5746; Largactil; Largactilothiazine; Largactyl; Megaphen; Novomazina; Phenactyl; Proma; Promactil; Promazil; Propaphenin; Prozil; Sanopron; SKF 2601-A; Thorazin; Thorazine; Torazina; Wintermin
- Chemical/Pharmaceutical/Other Class: Phenothiazine antidepressants
- Chemical Formula: $C_{17}H_{19}ClN_2S$

- Chemical Structure:



Uses

Chlorpromazine is a phenothiazine type of antidepressant and is used as a medication for both humans and animals. In humans, it is employed primarily in the treatment of psychiatric patients as an effective treatment for the management of psychotic disorders, manic depressive illness, apprehension, and anxiety, as well as for the treatment of severe behavioral problems in children. It is also used for short-term treatment of hyperactive children who exhibit excessive motor activity with accompanying conduct disorders. Chlorpromazine is also used to control nausea and vomiting, intractable hiccups, for reduction of choreiform movement in Huntington's disease, and, prior to surgery, acute intermittent porphyria. It is also used as adjunct in the treatment of tetanus, amphetamine toxicity, and migraines. In animals, chlorpromazine is recommended in excitable sows following farrowing, especially in those reluctant to accept their newborn; to capture African lions; as an adjunct to restraint and anesthesia; and as a neuroleptanalgesia (inducing a state of quiescence) in bears, and in reptiles prior to the administration of barbiturate anesthesia.

Background Information

Chlorpromazine is manufactured by heating 2-chloro-phenothiazine and 3-chloropropyl dimethylamine in the presence of sodomide, followed by reaction with hydrogen chloride.

Exposure Routes and Pathways

Chlorpromazine is administered orally, intravenously, intramuscularly, and via suppository. Pharmacists, physicians, and nurses dispensing or administering chlorpromazine could be exposed through dermal or inhalation contact.

Toxicokinetics

Absorption

Absorption of orally administered chlorpromazine is dependent on dosage form, with the elixir giving highest plasma concentration. Absorption of chlorpromazine tablets is erratic. The presence of food in the stomach changes the absorption rate. Antacids decrease the absorption of chlorpromazine.

Distribution and Excretion

Peak plasma levels are reached at 2–4 h, although wide variations (at least 10-fold) in plasma concentrations occur among individuals. Chlorpromazine is 92–97% bound to plasma proteins, principally albumin, at plasma chlorpromazine concentrations of 0.01–1 $\mu g\,ml^{-1}$. Concentrations in the brain are four to five times higher than the plasma. Following a single oral chlorpromazine dose of 120 $mg\,m^{-2}$ to four healthy men, $<1\%$ of the dose was excreted unchanged in the urine within 72 h. Following continuous oral administration of chlorpromazine to a limited number of psychiatric patients in dosages ranging from 0.1 to 1.4 g daily, an average of 37% of the dose was excreted in urine, principally as metabolites. Although intestinal absorption is complete, oral bioavailability is 32% because of variable metabolism in intestinal wall and liver. Intramuscular administration avoids much of the first-pass metabolism in the liver (and possibly also gut) and provides measurable concentrations in plasma within 15–30 min; bioavailability may be increased up to 10-fold.

Chlorpromazine is strongly bound to protein, crosses blood–brain barrier, and concentrates in the brain against plasma gradient. More than 90% of the drug in plasma is bound to proteins, is metabolized in the liver, and is excreted in both urine and feces. There is some evidence that chlorpromazine can cause hepatic microsomal enzyme induction, which indicates that it may accelerate its own metabolism.

Rapid placental transfer was reported in goats and mice. In goats, the fetal plasma levels approached 50% of maternal values within 10 min of the mother receiving an intravenous dose, and the fetal/maternal plasma ratio remained at 0.5 for 1 h, whereas ratios in the liver, kidney, heart, and brain all approached 1 and showed a marked effect on fetal heart rate. In pregnant mice, radiolabeled chlorpromazine rapidly crossed the placenta and accumulated in the eyes of both fetuses and mothers. Marked radioactivity remained in tissues of the eye for 5 months after the drug had been eliminated from other tissues.

Markedly variable half-life values reported for chlorpromazine vary from a relatively short plasma half-life of 2–6 to 18 h. Disappearance of chlorpromazine from plasma includes a rapid distribution phase (half-life $\sim$2 h) and slower early elimination phase (half-life $\sim$30 h). One study reported that after

an oral dose of 120 mg m^{-2} to human volunteers, chlorpromazine displayed a mean elimination half-life of ~18 h (range, 6–119 h). Half-life of elimination from human brain is unknown. The pharmacokinetics of chlorpromazine was investigated in 25 pediatric patients (aged 0.3–17 years) who received an intravenous infusion of 1 mg kg^{-1} chlorpromazine with intravenous metoclopramide administered concomitantly. Compared with previously reported values for adults, the pharmacokinetics of chlorpromazine appeared to be accelerated in children. This was especially evident for half-life and clearance values.

Metabolism

Chlorpromazine undergoes considerable metabolism during absorption (in gastrointestinal mucosa) and first-pass through the liver. As many as 10 or 12 chlorpromazine metabolites have been found to occur in appreciable quantities in humans. Quantitatively, the most important of these are nor2-chlorpromazine (doubly methylated), chlorphenothiazine (removal of entire side chain), methoxy and hydroxy products, and glucuronide conjugates of hydroxylated compounds. In urine, 7-hydroxylated and dealkylated (nor2) metabolites and their conjugates predominate.

In patients, chlorpromazine and various metabolites may be detected in urine 6–18 months after termination of treatment. Little or no chlorpromazine is eliminated in urine of the dog. The primary excretory product in the dog is chlorpromazine sulfoxide, but only 10–15% of the dose is eliminated as such. In horses, metabolites have been detected in urine up to 96 h following intramuscular injection. Following oral administration, metabolites are no longer detected after 80–96 h. The percentage of the dose recovered in equine urine is low, with the average being 10% after intramuscular administration and 27% after oral administration. Unconjugated metabolites excreted by the in horse represented only 1–1.5% of dose after either route of administration; these were excreted entirely as sulfoxide derivatives. Glucuronide-conjugated metabolites are predominantly excreted by the horse in a ratio to unconjugated metabolites of ~7:1 after intramuscular injection and 18:1 after oral administration. Sulfate-conjugated metabolites make up ~5% of the total after oral administration but are detected only in trace amounts after intramuscular injection. Phenothiazine derivatives were not detected in feces with spectroscopic analytical methodology.

Chlorpromazine and its metabolites were found in the maternal plasma and urine, in the fetal plasma and amniotic fluid, and in neonatal urine after doses of 50–100 mg of chlorpromazine were given intramuscularly to pregnant women shortly before delivery.

Mechanism of Toxicity

The action of chlorpromazine upon the brain stem reticular system is to increase reticular activity and stimulate filtering mechanisms in the reticular formation that act to reduce inflow of stimuli in a selective manner. Low doses of chlorpromazine also depress vasomotor reflexes mediated by either the hypothalamus or the brain stem. It inhibits the release of growth hormone, perhaps by action on the hypothalamus; may antagonize the secretion of prolactin release-inhibiting hormone; and appears to cause the reduction in a secretion of corticotropin-regulatory hormone in response to certain stresses. Chlorpromazine has significant adrenergic antagonistic activity and can block the pressor effects of norepinephrine. Extrapyramidal reactions appear to be mediated by blockade of central dopaminergic receptors involved in motor function.

Chlorpromazine also inhibits the reuptake of norepinephrine and 5-hydroxy-tryptamine in rat cerebral cortex but does not affect reuptake of gamma-aminobutyric acid. It is also a potent competitive inhibitor of the stimulatory effects of dopamine on adenylate cyclase.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} value for rats was 225 mg kg^{-1}. Both single and repeated oral exposures to chlorpromazine have been conducted in several species including rats, dogs, cats, and horses. The primary effects of chlorpromazine were the reduction in hematocrit, onset of cardiac arrhythmias, ocular lesions, photosensitization, stimulation of hepatic microsomal enzyme activity, delay in fetal bone ossification, effects on the nervous system, histological changes in lungs and kidneys, ocular lesions, reduction in bile flow, and hormonal changes.

A single dose of 2–4 mg kg^{-1} chlorpromazine caused tachycardia, hypotension, and depression in horses and reduced the number of red blood cells in hemoglobin for up to 2 weeks in repeated dose studies. Clinical signs of instability, lunging forward in an uncoordinated manner, stumbling, and falling were also observed. Parenteral doses of 2.5–5 mg kg^{-1} chlorpromazine caused cardiac arrhythmias in both

unanesthetized and anesthetized dogs, and dose levels of 30 mg kg^{-1} day^{-1} caused ocular lesions in two strains of dogs after 73 days. Subcutaneous doses of 1.5 mg kg^{-1} produced no signs of toxicity, but when administered intravenously caused moderate depression and ataxia which lasted between 6 and 12 h. Marked CNS depression and ataxia were noted for 24–48 h when the intravenous dose was increased to 3 mg kg^{-1}.

When a single dose of chlorpromazine hydrochloride was administered to CF rats on the 14th day of gestation, the ischium and pubis remained unossified until the 20th day of gestation; ossification of skull bones was also delayed. Ossified vertebral bodies and arches were less affected. Ossification was delayed by 1–3 days in long bones of extremities, by 1 day in scapula, and by 2 or 3 days in the ilium. The ribs were also late in maturing.

Fetal rats collected by cesarean section from dams treated with 0, 5, 15, 30, or 45 mg kg^{-1} day^{-1} chlorpromazine during gestation days 6–15 were evaluated on gestation day 21. Fetuses in the 5, 15, 30, and 45 mg kg^{-1} day^{-1} groups had reduced body weight. Body weight and/or weight gain of the dams in the 5, 15, 30, and 45 mg kg^{-1} day^{-1} groups was also reduced, and absolute maternal liver weight was reduced in the 30 and 45 mg kg^{-1} day^{-1} groups. The proportion of litters with one or more resorptions or nonlive fetuses was increased at 15 mg kg^{-1} day^{-1} and above. Pregnant mice were treated with 0, 2.5, 5, 15, or 30 mg kg^{-1} day^{-1} chlorpromazine during gestation days 6–15. Maternal mortality was increased in the 30 mg kg^{-1} day^{-1} group, and maternal weight/weight gain was reduced in dams administered 15 or 30 mg kg^{-1} day^{-1}. The percentage per litter of resorbed and nonlive fetuses was increased in the 30 mg kg^{-1} day^{-1} group, and fetal weight was reduced at 15 mg kg^{-1} day^{-1} and above. Fetal malformations were increased in the 30 mg kg^{-1} day^{-1} group and included open eye, cleft palate, hydronephrosis, missing ribs, and fused ribs. Offspring that were born and matured through weaning from rats treated with chlorpromazine during gestation had impaired basal body temperature control, and impaired performance in behavioral tests.

Human

The minimum lethal or toxic dose is not well established in the literature. The acute fatal dose is thought to be in the range of 15–150 mg kg^{-1} depending on the formulation However, adults have survived ingestion of 9.7 g, and a 350 mg dose was lethal in a 4-year-old child. Four deaths were reported in children following ingestion of 20–74 mg kg^{-1} chlorpromazine.

Adverse Reactions Adverse reactions may accompany the use of products containing chlorpromazine. A representative sample is provided below; however, the *Physician's Desk Reference* should be reviewed for specific product information.

Some adverse effects of chlorpromazine may be more likely to occur, or occur in greater intensity, in patients with preexisting medical conditions. Adverse effects include drowsiness/sedation, jaundice, dermatoses, icterus, hepatotoxicity, hematological disorders, blood dyscrasias, agranulocytosis, hypertension, neurological disorders (dystonias, motor restlessness, pseudo-parkinsonism, tardive dyskinesia), endocrine disorders (inhibition of ovulation and lactation; suppression of menstrual cycle, infertility; growth hormone release, reduction in secretion of corticotropin regulatory hormone, galactorrhea, mastalgia, gynecomastia), autonomic disorders, changes in skin pigmentation, ocular changes (blurred vision, corneal opacities, deposits in the cornea or lens), peripheral anticholinergic effects (decreased sweating, decreased gastric secretion and motility), hypotension, central nervous system (CNS) depression to the point of somnolence and coma (monitoring should be performed especially for neck/upper airway problems), agitation, restlessness, seizures, pyrexia (impairs the body's ability to regulate temperature), ECG changes (including widened QRS complexes) arrhythmias, ventricular tachycardia, possibly ventricular fibrillation, photosensitivity, itching, chromosomal breaks, sudden death, and immune system dysfunction (lupus).

Interactions with Other Agents Chlorpromazine may inhibit or enhance the effects of other agents or medications. Chlorpromazine has potentiated the effects of the following classes of compounds: oral hypoglycemic agents, beta blockers, dopamine, appetite suppressants, antiemetics, barbiturates, thiazide diuretics, lithium, hypotensives, tricyclic antidepressants, anticholinergics, antithyroid medication, monoamine oxidase inhibitors, ophthalmics, anticonvulsants, antidyskinetics, antihistamines, cold remedies, and opioid analgesics. Concurrent use of chlorpromazine with methoxsalen, trixsalen, or tetracycline can potentiate intraocular photochemical damage. Concurrent use of metrizamide can lower the seizure threshold. Concurrent use of chlorpromazine with medications known to alter hepatic microsomal enzyme activity may result in increased hepatotoxicity. In addition, concurrent use of chlorpromazine with the following substances altered the effects of both chlorpromazine and the agent: epinephrine, ethanol, nicotine, dopamine, levodopa, probucol, phenylephrine, mephentermine,

metaraminol, amantadine, maprotiline, physostigmine, dichlorvos, paraquat, and ithium. Chlorpromazine should not be taken or administered in combination with any other prescription or over-the-counter medications or herbal agents without approval from the physician.

Chronic Toxicity (or Exposure)

Animal

Chronic studies in animals were not reported for chlorpromazine.

Human

The human literature consists primarily of case reports. A woman treated for 6 years with chlorpromazine developed multifocal tics and vocalizations following discontinuation of the chlorpromazine therapy. These symptoms suggest that chronic receptor site blockade can result in hypersensitivity of dopamine receptor sites.

Pigment granules have been reported on the anterior lens surface following large doses over long periods of time. The granules became incorporated into the lens and caused loss of transparency.

Lupus was reported to develop in some patients administered chlorpromazine.

In Vitro Toxicity Data

Chlorpromazine was mutagenic in the Ames assay with activation.

Clinical Management

Any suspected overdose patient should be transported to a health care facility as soon as possible. Any patient with clinical signs of phenothiazine overdose should be admitted.

A careful patient history should be taken by physicians to determine the patient's previous and present exposure to neuroleptic agents. Emesis should not be induced. Gastric lavage should be considered; activated charcoal may be administered with or without a cathartic. Positioning, fluids, and dopamine may be useful in the treatment of hypotension. Treatment of seizures may be necessary in patients with CNS excitation. Ventricular tachyarrhythmias should be treated with lidocaine followed by pacing if needed. Neuroleptic malignant syndrome should be treated with dantrolene or bromocriptine along with conservative treatment. Acute renal failure may need to be addressed in patients who develop rhabdomyolysis. Extrapyramidal symptoms may be relieved by barbiturates, methylphenidate hydrochloride, or benztropine. Symptoms resembling withdrawal may occur if administration of chlorpromazine is stopped abruptly.

Environmental Fate

In the ambient atmosphere, chlorpromazine is expected to exist as a vapor and a particulate. The vapor would be degraded by photochemical-produced hydroxyl free radicals, with an estimated half-life of 2 h. Particulates would be expected to have no mobility, and chlorpromazine is not expected to volatilize. In water, chlorpromazine is expected to absorb to suspended particulates. There is a high potential for bioconcentration.

Ecotoxicology

There is no aquatic toxicity information available for chlorpromazine.

Other Hazards

When heated to decomposition, fumes of hydrogen chloride, nitroxides, and sulfoxides are emitted.

Exposure Standards and Guidelines

There are no exposure standards for chlorpromazine.

See also: Tricyclic Antidepressants.

Further Reading

Booth NH and McDonald LE (eds.) (1982) *Veterinary Pharmacology and Therapeutics*, 5th edn. Ames, IA: State University Press.

Department of Health and Human Services/National Institute of Environmental Health Sciences. National Toxicology Program; Chlorpromazine Hydrochloride (CAS No. 69-09-0) Administered to Fischer 344 Rats on Gestation Days 6 through 15, NTP Study No. TER82055 (March 7, 1983).

Hatanaka T, Sato S, Endoh M, Katayama K, Kakemi M, and Koizumi T (1988) Effect of chlorpromazine on the pharmacokinetics and pharmacodynamics of pentobarbital in rats. *Journal of Pharmacobiodynamics* 11(1): 18–30.

Jain AK, Kelwala S, and Gershon S (1988) Antipsychotic drugs in schizophrenia: Current issues. *International Clinical Psychopharmacology* 3(1): 1–30.

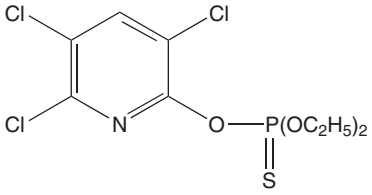
Klawans HL, Falk DK, Nausieda PA, and Weiner WJ (1978) Gilles de la Tourette syndrome after long-term chlorpromazine therapy. *Neurology* 28(10): 1064–1066.

Saillenfait AM and Vannier B (1988) Methodological proposal in behavioural teratogenicity testing: Assessment of propoxyphene, chlorpromazine, and vitamin A as positive controls. *Teratology* 37(3): 185–199.

Chlorpyrifos

Anuradha Nallapaneni and Carey N Pope

- Chemical Abstracts Service Registry Number: CAS 2921-88-2
- Synonyms: Dursban; Lorsban
- Chemical/Pharmaceutical/Other Class: Synthetic organophosphorus insecticide in the phosphorothionate class
- Chemical Structure:



Uses

Chlorpyrifos is a broad-spectrum insecticide useful in controlling a variety of agricultural, urban, and household insects. In the United States, the household use of chlorpyrifos has been recently eliminated but it is still used considerably in agriculture.

Exposure Routes and Pathways

Oral and inhalation routes are primary exposure pathways.

Toxicokinetics

Chlorpyrifos is absorbed rapidly and effectively by the oral route but less effectively by the dermal route. Chlorpyrifos undergoes activation via the P-450–mixed-function oxidase pathway to the oxygen analog, chlorpyrifos oxon. Metabolites include diethylphosphoric acid, 3,5,6-trichloro-2-pyridyl phosphate, and 3,5,6-trichloro-2-pyridinol. Differences in serum A esterase activity appear to contribute significantly to species differences in sensitivity to chlorpyrifos. Major conjugates include glucuronide (80%) and glycoside (4%) metabolites. An $-SCH_3$ addition on the pyridinol ring has also been reported. The water-soluble glucuronide and glycoside conjugates are eliminated primarily via the urine (90%). A trace amount of the parent compound is also eliminated via the urine.

Mechanism of Toxicity

Similar to other organophosphorothionate insecticides, the toxicity of chlorpyrifos is due to inhibition of acetylcholinesterase following its oxidative metabolism to the oxon (i.e., chlorpyrifos oxon). Extensive inhibition of this enzyme results in stimulation of the central nervous system (CNS), the parasympathetic nervous system, and the somatic motor nerves. The IC_{50} values for the insecticide and its oxon are approximately 5×10^{-3} and 5×10^{-9} mol l^{-1}, respectively.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chlorpyrifos is moderately toxic in rodents, with oral LD_{50} values in rats of 95–270 mg kg^{-1} and 60 mg kg^{-1} in mice. Young rodents are markedly more sensitive than adults to chlorpyrifos and a number of other organophosphorus insecticides. Rabbits are relatively resistant to chlorpyrifos, with LD_{50} values $\sim$1 g kg^{-1}. In contrast, many birds are highly sensitive to chlorpyrifos, e.g., the LD_{50} in chickens is 32 mg kg^{-1}. Resistance in rabbits and sensitivity in birds is likely due to differences in A-esterase mediated detoxification. The dermal LD_{50} in rats is $>$2 g kg^{-1}. The 4 h inhalation LC_{50} for chlorpyrifos in rats is $>$0.2 mg l^{-1}.

Human

Exposure to chlorpyrifos can affect the central nervous system, heart, and respiratory system. Symptoms of acute exposure to chlorpyrifos may include paresthesia, incoordination, headache, dizziness, tremor, nausea, cramping, excessive sweating, blurred vision, dyspnea, and bradycardia. Very high exposures may lead to unconsciousness, convulsions, and death. Chlorpyrifos is a skin and eye irritant. Ocular contact may cause pain, moderate eye irritation, and slight temporary corneal injury. Prolonged skin exposure may cause skin irritation. Toxicity is moderate for a single oral dose; swallowing larger amounts may cause serious injury, even death. If aspirated, chlorpyrifos formulations may cause airway damage or even death due to chemical pneumonia.

Chronic Toxicity (or Exposure)

Animal

While young rats are more sensitive than adults to cholinergic toxicity following acute chlorpyrifos exposures, some results suggest that low-level repeated chlorpyrifos exposures elicit lesser degrees of age-related differences in toxic response. A number

of studies suggest, however, that chlorpyrifos may adversely affect neurodevelopmental processes through interaction with a number of macromolecular targets other than acetylcholinesterase.

Human

Cholinesterase inhibition can sometimes persist for weeks; thus, repeated exposures to small amounts of this material may result in accumulation of acetylcholinesterase inhibition with possible sudden-onset acute toxicity. Chlorpyrifos may be capable of causing organophosphate-induced delayed neurotoxicity in humans; a massive overdose resulted in signs characteristic of delayed neurotoxicity. Animal studies generally indicate, however, that doses several times higher than the LD_{50} would be required to initiate delayed neurotoxicity.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered, followed by 2–4 mg (in adults) or 0.015–0.05 mg kg^{-1} (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Environmental Fate

Chlorpyrifos is moderately persistent in soils, with a half-life from 2 weeks to 1 year depending on soil type, climate, pH, and other conditions. Half-life was not affected by organic matter content, however. Chlorpyrifos undergoes degradation by UV light, chemical hydrolysis, and by microbial processes. In moist conditions, 62–89% of the chlorpyrifos remained in soil after 36 h. Chlorpyrifos adsorbs strongly to soil particles and has low water solubility, thus there is little potential for contaminating groundwater. The breakdown product, trichloropyridinol, adsorbs only weakly to soil particles and is more mobile and persistent. Concentrations of chlorpyrifos diminish rapidly in water following application as emulsiable preparations but less so when applied as granules and controlled release formulations. Volatilization is likely the principle mode of chlorpyrifos loss from water. Chlorpyrifos is relatively unstable in water. Hydrolysis is constant in acidic or neutral conditions higher in alkaline water.

Ecotoxicology

Chlorpyrifos is moderately to very highly toxic to birds, with oral LD_{50} values from 8 to 112 mg kg^{-1} in a number of species. Reduction in eggs was noted in mallard ducks at dietary exposure of 125 ppm chlorpyrifos but no effect on egg laying in hens given 50 ppm. Chlorpyrifos is very highly toxic to aquatic vertebrate and invertebrate organisms. Application of chlorpyrifos at concentrations as low as 0.01 pounds per acre can lead to death in aquatic organisms. The 96 h LC_{50} for chlorpyrifos was 0.009 mg l^{-1} in rainbow trout, 0.098 mg l^{-1} in trout, 0.806 mg l^{-1} in goldfish, 0.01 mg l^{-1} in bluegill, and 0.331 mg l^{-1} in fathead minnow. One study reported deformities in fathead minnow at 0.002 mg l^{-1} exposure for 30 days. Chlorpyrifos bioaccumulates in tissues of aquatic organisms with concentration factors of 58–5100. Chlorpyrifos can pose hazard to other wildlife species including bees.

Exposure Standards and Guidelines

The chronic reference dose for chlorpyrifos is 0.005 mg kg^{-1} day^{-1}; the population adjusted dose is 0.0005 mg kg^{-1} day^{-1}. The 8 h dermal permissible exposure limit is 0.02 mg m^{-3}.

See also: A-Esterases; Cholinesterase Inhibition; Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphates.

Further Reading

Cochran RC (2002) Appraisal of risks from nonoccupational exposure to chlorpyrifos. *Regulatory Toxicology and Pharmacology* 35(1): 105–121.

Meyer A, Seidler FJ, Cousins MM, and Slotkin TA (2003) Developmental neurotoxicity elicited by gestational exposure to chlorpyrifos: When is adenylyl cyclase a target? *Environmental Health Perspectives* 111: 1871–1876.

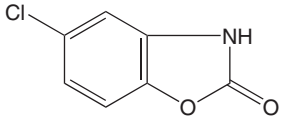
Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Chlorzoxazone

Kashyap N Thakore and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 95-25-0
- Synonyms: Chlorozaxone; 5-Chloro-2(3*H*)-benzoxazolone; 5-Chloro-2-benzoxazolol; 5-Chloro-2-hydroxybenzoxazole; 2-Hydroxy-5-chlorobenzoxazole; Paraflex; Biomioran; Solaxin
- Chemical/Pharmaceutical/Other Class: Strong oxidizing agent
- Chemical Formula: $C_7H_4ClNO_2$
- Chemical Structure:



Uses

Chlorzoxazone is used as a centrally acting skeletal muscle relaxant and as an analgesic. It is also a strong oxidizing agent.

Exposure Routes and Pathways

When heated to decompose, chlorzoazone emits acrid smoke and irritating fumes.

Toxicokinetics

The oral suspension is rapidly absorbed and eliminated. After intravenous administration, the decay of the plasma concentration is rapid. The half-life for an oral dose is ~ 1 h.

Chlorzoxazone is rapidly metabolized in the liver by carbon hydroxylation at position 6 mediated by CYP1A2 as well as by CYP2E1 and the 6-hydroxychlorzoxazone formed is conjugated with glucuronide. The concentration in fat is two times the plasma levels. Chlorzoxazone is eliminated mainly as the glucuronide conjugate by urine.

Mechanism of Toxicity

The toxic metabolite 6-hydroxychlorzoxazone is formed by the action of CYP1A2 and CYP2E1.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the oral LD_{50} is $763\,mg\,kg^{-1}$ body weight (bw) and the intraperitoneal LD_{50} is $150\,mg\,kg^{-1}$ bw. In the mouse, the oral LD_{50} is $440\,mg\,kg^{-1}$ bw, the intraperitoneal LD_{50} is $50\,mg\,kg^{-1}$ bw, and the subcutaneous LD_{50} is $170\,mg\,kg^{-1}$ bw. In the hamster, the oral LD_{50} is $662\,mg\,kg^{-1}$ bw and the intraperitoneal LD_{50} is $166\,mg\,kg^{-1}$ bw.

Human

Chlorzoxazone is harmful if swallowed, inhaled, or absorbed through skin. It causes drowsiness; central nervous system effects such as headache, dizziness, and blurred vision; nausea and vomiting; and eye, skin, and mucous membrane irritation. The target organs are the liver, nerves, and skeletal muscles. Although morbidity and mortality are low in pure compound ingestion, they may be increased in multiple ingestions.

Workers exposed to this compound should wear personal protective equipment and their work should be carried out only in restricted areas. Technical measures should prevent any contact with the skin and mucous membranes. Clothing and equipment after use should be placed in an impervious container for decontamination or disposal.

Chronic Toxicity (or Exposure)

Information on animal and human data is not available.

Clinical Management

In case of contact, the eyes and skin should be flushed immediately with water for at least 15 min. If the victim is not breathing, artificial respiration should

be administered; if breathing with difficulty, oxygen should be given. If patient is in cardiac arrest, cardiopulmonary resuscitation should be provided. If swallowed, the mouth should be washed out with water provided the person is conscious. Life-support measures should be continued until medical assistance has arrived. An unconscious or convulsing person should not be given liquids nor induced to vomit.

See also: Skeletal System.

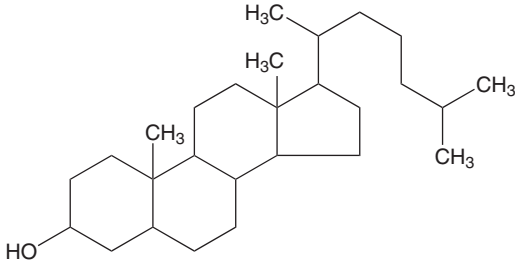
Further Reading

Powers BJ, Cattau EL Jr., and Zimmerman HJ (1986) Chlorzoxazone hepatotoxic reactions: An analysis of 21 identified or presumed cases. *Archives of Internal Medicine* 146(6): 1183–1186.

Cholesterol

Brad Hirakawa

- Chemical Abstracts Service Registry Number: CAS 57-88-5
- Synonyms: (−)-Cholesterol; 5,6-Cholesten-3β-ol; 5-Cholesten-3β-ol; Cholest-5-en-3β-ol; Cholesterin; Cholesterine; Cholesterol base H; Cholesteryl alcohol; Cordulan; D [5](-Cholesten-3β-ol); Dusoline; Dusoran; Dythol; β-Hydroxycholest-5-ene; Hydrocerin; Kathro; Lanol; Nimco; Nimco cholesterol base No. 7l2; Provitamin D; Super hartolan; Tegolan
- Chemical Class: Endogenous sterol
- Chemical Formula: $C_{27}H_{46}O$
- Chemical Structure:



Uses

A natural product, cholesterol is a fat-soluble compound that is present in our daily diet. It can form esters with fatty acids. Most of the cholesterol found in the plasma is in the form of cholesterol esters. It can also be endogenously formed in the cells of the body. The majority of the cholesterol is produced by the liver cells; however, all other cells also form cholesterol. The membrane structure of the cells is partially made up of cholesterol. It is synthesized from multiple molecules of acetyl-CoA. The major use of cholesterol in the body is to form cholic acid in the liver, which conjugates with other substances to form bile salts. Bile salts help in digestion and absorption of fats. Cholesterol is used in small amounts to form adrenocorticoid hormones, progesterone, estrogens, and testosterone. Cholesterol is also used in a multitude of pharmaceutical and cosmetic preparations.

Exposure Routes and Pathways

Cholesterol is an endogenous compound. Oral exposure is the most common exposure pathway.

Mechanism of Toxicity

Cholesterol is transported to and from the cells by special carriers called lipoproteins. There are two types, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). It is believed that excess LDL cholesterol can clog arteries, increasing risk of heart attack and stroke. Conversely, studies suggest that higher levels of HDL cholesterol reduce risk of heart attack. That is, the ratio of LDL to HDL appears to be important. The adverse effects of a prolonged (i.e., chronic) history of excess LDL relative to HDL is of much greater concern than acute exposures to cholesterol.

Chronic Toxicity (or Exposure)

Animal

Evaluations of the carcinogenicity of cholesterol to experimental animals did not result in clear positive results.

Human

LDL cholesterol levels above 100 mg dl^{-1} and HDL cholesterol levels below 40 mg dl^{-1} are risk factors for cardiovascular disease.

In humans, the association between cholesterol and cancer risk contains some apparent contradictions. Depending on whether cholesterol is measured in the diet, blood, or feces, its association with cancer risk may tend to be either positive or negative. Therefore, there is inadequate evidence from scientific studies that cholesterol is carcinogenic to humans.

Clinical Management

Statins are a class of cholesterol-lowering drugs that affect serum cholesterol levels by a variety of mechanisms. Examples include Lipitor, Zocor, Pravachol, Lescol, and Mevacor. Recent evidence suggests regular consumption of soluble fibers (via diet or supplementation) can lower serum cholesterol levels.

See also: Cardiovascular System; Liver.

Relevant Websites

http://www.americanheart.org – American Heart Association.
http://www.inchem.org – International Programme on Chemical Safety INCHEM.
http://www.nhlbi.nih.gov – US National Heart, Lung, and Blood Institute, National Institutes of Health – National Cholesterol Education Program.

Choline

Brad Hirakawa

- Chemical Abstracts Service Registry Numbers: CAS 62-49-7; CAS 123-41-1 (choline hydroxide)
- Synonyms: Bursine; Ethanaminium; Fagine; Gossypine; Luridine; Sincaline; Sinkalin; Sinkaline; Vidine; (2-Hydroxyethyl)trimethylammonium hydroxide
- Chemical/Pharmaceutical/Other Class: Cholinergic agonist
- Chemical Formula: $C_5H_{15}NO_2$
- Chemical Structure: $[HOCH_2CH_2N^+(CH_3)_3]$

Uses

Choline is used as a direct cholinergic agonist in therapeutics and as a research tool.

Exposure Routes and Pathways

Dermal and oral contacts are the most common exposure pathways.

Toxicokinetics

Choline is metabolized to trimethylamine, which is excreted in skin, lungs, and kidney.

Mechanism of Toxicity

Choline is a cholinergic agonist, choline acetyltransferase substrate; therefore, it exerts toxicity by directly hyperstimulating the postganglionic cholinergic receptors. This may lead to stimulation of gastrointestinal, urinary, uterine, bronchial, cardiac, and vascular receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute choline overexposure results in hyperstimulation of the cholinergic nervous system.

Human

The estimated oral lethal dose for humans is 200–400 g. Oral doses of 10 g produce no obvious effect. Vital signs may include bradycardia (decreased heart rate), hypotension, hypothermia, miosis (small pupils), salivation and lacrimation (tearing), ocular pain, blurred vision, bronchospasm, muscle cramps, fasciculation (muscle twitching), weakness, nausea, vomiting, diarrhea, and involuntary urination.

Chronic Toxicity (or Exposure)

Little is known about the chronic toxicity of choline in laboratory animals or humans.

Clinical Management

Atropine sulfate is the drug of choice. Epinephrin may assist in overcoming severe cardiovascular or bronchoconstriction. Diazepam, phenytoin, and phenobarbital may be given in cases of seizures. Induction of emesis is not necessary due to spontaneous vomiting. Activated charcoal slurry with or without

saline cathartic may be used. Sorbitol should not be used because it may contribute to the nausea and diarrhea. Skin decontamination should be accomplished by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min.

Other Hazards

Choline base solutions are corrosive and are extremely destructive to tissue of the mucous membranes and upper respiratory tract, eyes, and skin.

See also: Anticholinergics; Cholinesterase Inhibition; Neurotoxicity.

Relevant Website

http://www.cdc.gov – National Institute for Occupational Safety and Health (NIOSH) website.

Cholinesterase Inhibition

Barry W Wilson

Introduction and History

Cholinesterases (ChEs) are a ubiquitous group of enzymes that hydrolyze esters of choline. A well-known example is acetylcholinesterase (AChE, acetyl choline hydrolase, EC 3.1.1.7), the enzyme responsible for hydrolyzing the important neurotransmitter acetylcholine (ACh). Another ChE is butyrylcholinesterase (BuChE, acylcholine acylhydrolase, EC 3.1.1.8), also known as nonspecific cholinesterase. The preferred substrate for AChEs is ACh; BuChEs prefer to hydrolyze esters like butyrylcholine and propionylcholine. Both AChE and BuChE are inhibited by some organophosphate (OP) and carbamate (CB) esters and also by other chemicals.

Many ChE inhibitors act at the catalytic site of the enzyme, forming enzyme–inhibitor complexes that are slow to hydrolyze. The use of ChE inhibitors as insecticides and as chemical warfare agents, their toxicity to humans, and their impact on wildlife have made them important to toxicology researchers, public health and environmental health officials.

This entry focuses on ChE inhibitions by OPs and CBs. Other chemicals, such as tacrine, cocaine, and succinylcholine, are briefly discussed.

One of the first ChE inhibitors to be studied was a CB, physostigmine (eserine), an alkaloid from the calabar bean (*Physostigma venenosum*) used in a 'trial by ordeal' in West Africa. The accused were forced to eat the poisonous beans; survivors were proclaimed innocent. The drug has been used as a treatment for glaucoma since 1877. In 1931, Englehart and Loewi showed it blocked ChE activity. Soon after, neostigmine, an analog, was shown to be effective in the symptomatic treatment of myasthenia gravis.

OPs with high toxicity were synthesized as chemical warfare agents in the late 1930s and early 1940s. During this period Schrader discovered the insecticidal properties of OPs resulting in the synthesis of tetraethyl pyrophosphate in 1941 and of parathion in 1944. Synthetic CBs developed as pesticides have been in commercial use since the 1950s. Some OPs and CBs exhibit toxicities in addition to their direct inhibitions of ChEs. These include long-term and short-term damage to nerves and muscles, mutagenicity, and effects on reproduction.

Acetylcholinesterase, Butyrylcholinesterase, and Other Esterases

AChEs and BuChEs are specialized carboxylic ester hydrolases that preferentially hydrolyze choline esters. They are classed among the B-esterases, enzymes that are inhibited by OPs. Another B-esterase is neuropathy target esterase (NTE), an enzyme associated with organophosphate-induced delayed neuropathy (OPIDN). Enzymes that actively hydrolyze OPs are known as A-esterases. They provide an important route of detoxification. Examples are paraoxonase and DFPase (**Table 1**). The tertiary structure and amino acid sequences of several AChEs and BuChEs have been elucidated.

ChEs are widely distributed in the body. AChEs regulate excitation at cholinergic synapses by destroying the neurotransmitter ACh. These enzymes are some of the most active known, cycling within a few milliseconds. AChEs are found in excitable tissues at synapses, neuromuscular junctions, myotendinous junctions, central nervous system (CNS) neuron cell bodies, axons, and muscles (**Table 2**). AChEs are also found in the erythrocytes (red blood cells (RBCs)) of mammals, in the serum of some birds and mammals, and in the blood platelets of

Table 1 Esterase classes

A-esterases
Hydrolyze OPs to inactive products
Found in liver and HDL in plasma
High activity in mammals
Lower activity in birds
Examples: Paraoxonase and DFPase
B-esterases
Widely distributed in cells and tissues
Inhibited by OPs and CBs
Slow hydrolysis of OP–enzyme complex
Relatively rapid hydrolysis of CN–enzyme complex
Examples: AChE, BuChE, CaE, and NTE

OP, organophosphate ester; HDL, high-density lipoprotein; CB, carbamate; AChE, acetylcholinesterase; BuChE, butyrylcholinesterase; CaE, carboxylesterase; NTE, neuropathy target estarase.

Table 2 Cholinesterase properties

All
Hydrolyze ACh and other choline esters
AChE
Prefers ACh, is inhibited by excess substrates
Found at neural junctions and in mammal RBCs and plasma and platelets of some vertebrates
BuChE
Prefers butyrylcholine, propionylcholine
Widely distributed in vertebrate tissues and plasma

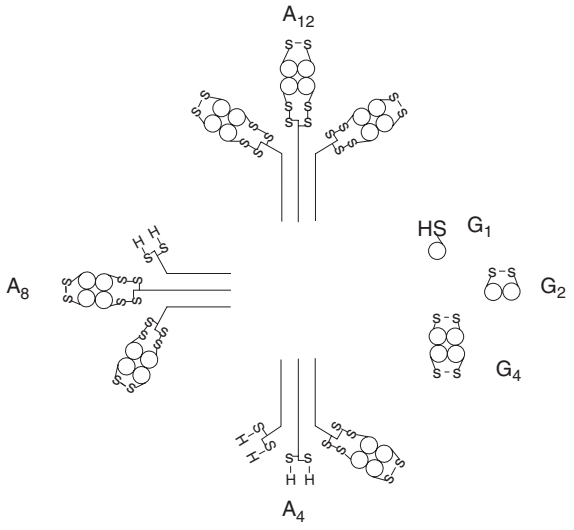


Figure 1 Subunit structure of the multiple molecular forms of ChEs. G, globular forms; A, asymmetric forms with collagen-like tails. Each circle is a catalytic subunit; disulfide bridges indicated by S–S as found in the electric organ of the electric eel. (Modified from Brimijoin WS (1992) US EPA Workshop on Cholinesterase Methodologies.)

rodents (rats and mice) and ruminants (sheep). (For example, the serum ChE activity of the American Kestrel, a small falcon, consists almost entirely of AChE and the serum ChE of the laboratory rat is high in both AChE and BuChE activities.) The AChE activity of human blood is localized to its RBCs. AChE activity occurs in the serum of developing mammals and birds and in precursors of formed blood elements in some species; it decreases to adult levels after birth.

BuChEs are also widely distributed. They are found at synapses, motor endplates, and muscle fibers together with AChE. BuChE activity in blood is restricted to serum.

Substrate preferences of AChE and BuChE enzymes vary with species. For example, although both mammal and bird AChEs rapidly hydrolyze ACh and its thiocholine analog acetylthiocholine (AcTh), avian AChEs also readily hydrolyze acetyl β-methylcholine and acetyl β-methylthiocholine, while mammalian AChEs do not. AChEs and BuChEs respond differently to increasing substrate concentration. AChEs are inhibited by excess substrate (often above 2 mM); BuChEs are less sensitive to substrate inhibition.

AChEs and BuChEs have multiple molecular forms and complicated life histories (**Figures 1** and **2**). Some of the forms move from site to site within cells, others are secreted into body fluids. AChEs consist of asymmetric and globular forms. The asymmetric forms tend to be localized at synapses and motor endplates; they have glycosylated heads joined together by sulfhydryl groups containing the active sites, and collagen tails that attach the enzymes to cell surfaces. The globular forms lack collagen tails; they are made up of the catalytic subunits.

AChE and BuChE subunits are synthesized within cells (e.g., nerve, muscle, liver, and some megakaryocytes), glycosylated within the Golgi apparatus, and secreted. Collagen-tailed forms become attached to the cell surface at specific binding sites. Globular forms are released into body fluids or bind to cell surfaces by ionic bonds. Antibodies have been prepared to several purified AChEs and BuChEs, and protein and nucleic acid sequences have been determined.

The three-dimensional structure of AChE from the electric organ of *Torpedo californica* has been established. One interesting feature is that the active site is embedded in a 'gorge' of $\sim$20 Å that reaches halfway into the protein. The postulated 'anionic site', theoretically invoked to bind the quaternary ammonium ion of ACh, appears to be represented by aromatic amino acids in the gorge itself; these and charges in the active center are believed to stabilize the choline group. In addition, some inhibitions, such as that due

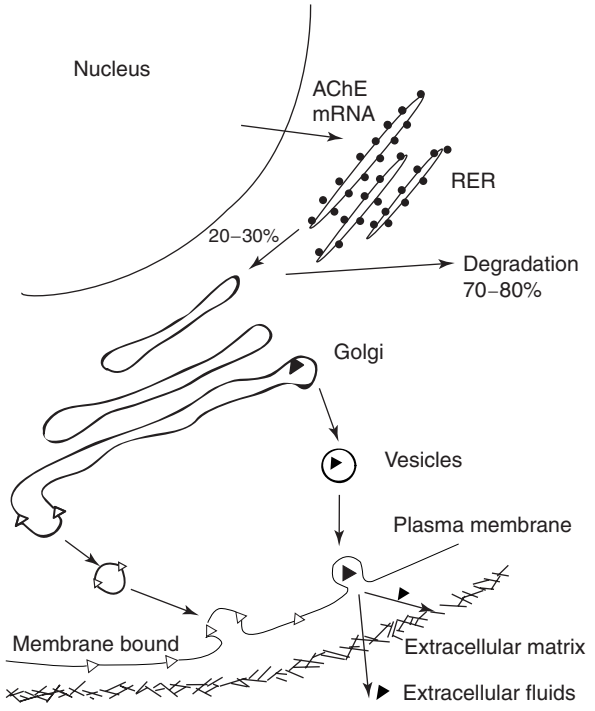


Figure 2 Life cycle of ChEs. AChE is synthesized as a monomer globular form (G_1). Up to 80% is degraded by intracellular processes. Secretory forms are separated from membrane-bound forms, collagen tails are added to asymmetric forms, and the enzyme is glycosylated and becomes enzymatically active. After secretion, globular forms may escape into the body fluids, while asymmetric forms are bound to the synaptic basal lamina. (Modified from Brimijoin WS (1992) US EPA Workshop on Cholinesterase Methodologies.)

to excess substrate, are believed to be due to a 'peripheral site'. Elucidation of the structure of ChE molecules may open the way to a new generation of 'designer' anti-ChE agents with improved specificities of action.

Functions of Cholinesterases

$$E + AX \underset{k_{-1}}{\overset{k_{+1}}{\longleftrightarrow}} EAX \overset{k_2}{\longleftrightarrow} EA \overset{k_3}{\longleftrightarrow} E + A$$

where E is the enzyme, AX is the substrate (ACh) or inhibitor, EAX is the reversible enzyme complex, and *k*s are reaction rate constants.

A 100 years of research has established that a major function of AChE is to hydrolyze the ACh released by cholinergic neurons, regulating the course of neural transmission at synapses, motor endplates, and other effector sites. The reaction is multistep: first is the formation of a reversible enzyme–substrate complex (EAX); second is the acetylation of the catalytic site of the enzyme (EA); and third is the hydrolysis of the enzyme–substrate complex yielding acetic acid, choline, and the regenerated enzyme (E + A). The generally accepted mechanism has been (1) an electrostatic attraction between the positive charge on the quaternary nitrogen atom of Ach and the negative charge on the so-called 'anionic site' on the enzyme forms the enzyme–substrate complex, (2) a basic imidazole moiety (histidine) and an acidic moiety (tyrosine hydroxyl) at the active site catalyze the acetylation of a serine hydroxyl, followed by (3) a rapid deacetylation restoring the enzyme and cleaving acetylcholine into acetate and choline. A similar reaction scheme is believed to apply to BuChEs. The new information on the conformation of these molecules should result in a greater understanding of the biophysical mechanisms underlying their catalytic actions.

In contrast to the functional information available for the roles of ACh and AChE, the function or functions of RBC and serum ChEs are still matters for speculation. One idea is that they protect the body from natural anti-ChE agents (e.g., phyosostigmine) encountered during the evolution of the species; another idea is that they have specific but still unknown roles in tissues. For example, there are reports that inhibition of BuChE activity blocks adhesion of neurites from nerve cells in culture and that AChE promotes outgrowth of neurites as if the enzymes had roles in cell adhesion and differentiation.

Toxicities

The toxicities of OPs and CBs often roughly parallel their effectiveness as inhibitors of brain AChE. For example, **Figure 3** shows the relationship between the toxicity *in vivo* of directly acting OPs and their inhibition of AChE *in vitro*, plotting intraperitoneal LD_{50} versus the PI_{50} in mice. (The LD_{50} is the dose resulting in 50% mortality; the PI_{50} is the negative logarithm of the concentration of toxicant resulting in 50% inhibition of the enzyme.) Only two of the chemicals tested did not 'fit' the curve.

In general, many of the physiological effects of anti-ChEs are those attributable to excess ACh at junctions in the nervous system. The precise symptoms and the time course of ChE inhibition depend on the chemicals and the localization of the receptors affected. The properties of some cholinergic receptors are listed in **Table 3**. Cholinergic junctions are classified into several categories based on their pharmacological sensitivities to nicotine, muscarine, atropine, and curare. Early symptoms of cholinergic poisoning represent stimulation of neuroeffectors of the parasympathetic system. These effects are termed muscarinic – stimulated by muscarine and are blocked by atropine. Such effects include slowing

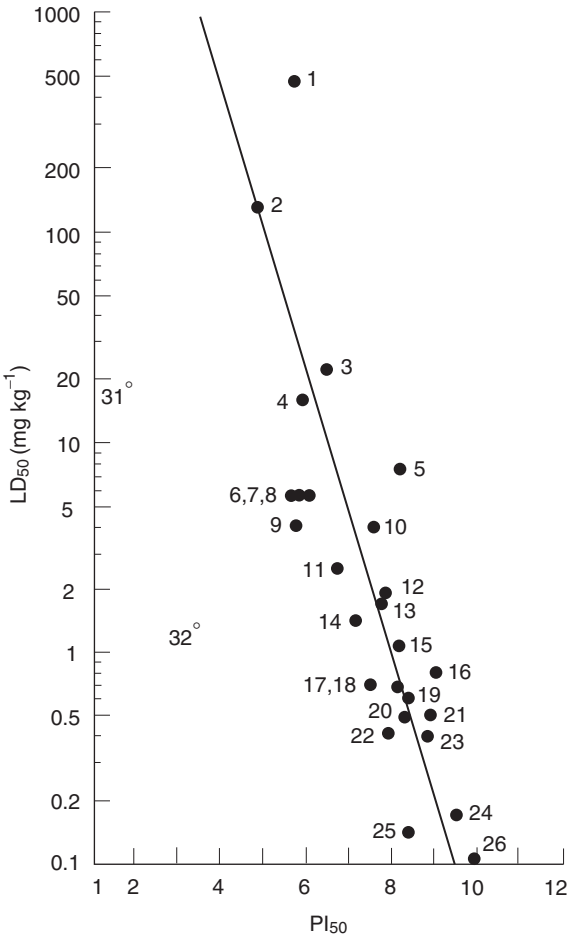


Figure 3 Relationship of the toxicity *in vivo* (LD_{50}) of directly acting OPs to AChE inhibition *in vivo* (PI_{50}). 1, dipterex; 2, *O*,*O*-diethyl-4-chlorophenylphosphate; 3, *O*,*O*-diethyl-bis-dimethylpyrophosphoramide(*sym*); 4, TIPP; 5, *O*,*O*-diethylphosphostigmine; 6, isodemeton sulfoxide; 7, isodemeton; 8, isodemeton sulfone; 9, DFP; 10, diethylamidoethoxy-phosphoryl cyanide; 11, *O*,*O*-dimethyl-*O*,*O*-diisopropyl pyrophosphate (asym); 12, diethylamidomethoxyphosphoryl cyanide; 13, tetramethyl pyrophosphate; 14, *O*,*O*-diethylo phosphorocyanidate; 15, *O*,*O*-dimethyl-*O*,*O*-diethyl pyrophosphate (asym); 16, soman; 17, TEPP; 18, *O*-isopropyl-ethylphosphonofluoridate; 19, tabun; 20, amiton; 21, diethylamido ispropoxyphosphoryl cyanide; 22, *O*,*O*-diethyl-*S*-(2-diethylaminoethyl)phosphorothioate; 23, sarin; 24, *O*,*O*-diethyl-*S*-(2-triethylammoniumethyl)thiophosphate iodide; 25, echothiophate; 26, methylfluorophosphorylcholine iodide; 27, methylfluorophosphoryl-*B*-methylcholine iodide; 28, *O*-ethyl-methylphosphorylthiocholine iodide; 29, methylfluorophosphoryl-homo-choline iodide; 31, schradan; 32, dimefox (27–30, LD_{50} values 0.03–0.07). (Adapted from Gallo L (1991) Organophosphorus insecticides. In: Hayes WJ Jr. and Laws ER Jr. (eds.) *Handbook of Pesticide Toxicology*, vol. 2, p. 932. San Diego, CA: Academic Press.)

of the heart (bradycardia), constriction of the pupil of the eye (miosis), diarrhea, urination, lacrimation, and salivation. Actions at skeletal neuromuscular junctions (motor endplates) are termed nicotinic – stimulated by nicotine, blocked by curare, but not by atropine. Overstimulation results in muscle fasciculation (disorganized twitching) and, at higher doses, muscle paralysis. A third site of action of anti-ChEs is the cholinergic junctions of the sympathetic and parasympathetic autonomic ganglia. These junctions are also nicotinic – stimulated by nicotine but not affected by muscarine, atropine, or curare, except at high concentrations. Their actions affect the eye, bladder, heart, and salivary glands, with one set often antagonizing the actions of another. Finally, there are the junctions of the CNS: some are stimulated by nicotine, and some are affected by atropine. They are not responsive to muscarine or curare. CNS symptoms include hypothermia, tremors, headache, anxiety, convulsions, and coma. Death generally occurs when the agents extensively affect the respiratory centers in the brain. Whether or not there are consistent behavioral effects at low dose levels of OPs and CBs, such as deficits in learning and memory, is a matter of current research, especially on drugs under development to treat Alzheimer's disorder.

Table 3 Properties of cholinergic receptors

Muscarinic peripheral NS
Parasympathetic nervous system
Muscarine stimulates
Atropine blocks
Nicotinic peripheral NS
Skeletal muscle motor endplates
Nicotine stimulates
Curare blocks
Atropine has no effect
Nicotinic CNS
Autonomic NS antagonist
Sympathetic and parasympathetic NS
Nicotine stimulates respiratory center

NS, nervous system; CNS, central nervous system.

The excess ACh produced at the motor endplate brings about a transient myopathy in experimental animals. Experiments *in vivo* and *in vitro* of Dettbarn, Wecker, Salpeter, and others using cholinergic drugs and ACh receptor blockers indicate that excess ACh leads to an influx of Ca^{2+} ions and other cations into the postsynaptic cell, resulting in regions of necrosis in the muscle fiber around the motor endplates. From 10% to 30% of the fibers may be damaged and recovery may take several weeks or more. A disorder known as intermediate syndrome in humans involves prolonged muscle weakness and some muscle damage lasting several weeks or longer after exposure to high levels of some OPs, including methyl parathion, fenthion, and dimethoate.

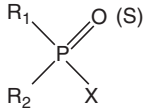
Although most of the effects of OPs and CBs are considered to be caused by AChE inhibition, there is evidence that anti-ChEs directly affect ACh receptors in the CNS and PNS and that some anti-AChE pesticides depress the immune system in experimental animals.

A few OPs such as tri-ortho cresyl phosphate (TOCP), leptophos, mipafox, methamidophos, isofenphos, and chlorpyrifos, cause OPIDN, a neuropathy that results in the death of some motor and sensory neurons in humans and experimental animals. Some, such as chlorpyrifos and isofenphos, require very high dose levels to be neuropathic – higher levels than could occur if the chemicals were used as directed. TOCP, an industrial chemical, has been responsible for the paralysis of thousands of people since the turn of the century. Inhibition of ~70% or more of the carboxylesterase NTE is often associated with the disorder. It is known as a 'delayed' neuropathy because onset of the disorder is usually 10 days to several weeks after exposure. Discussion of this neuropathy is beyond the scope of this article, except to note that neuropathic chemicals that are the most dangerous often are those that are better NTE inhibitors than AChE inhibitors, permitting a higher dose of the chemical to be reached before cholinergic symptoms or death occurs. Agricultural chemicals are routinely screened for OPIDN using hens since chickens are sensitive to the disorder.

The action of many toxicants, including anticholinergic compounds, often involves specific sites on molecules and cells. Such finely tuned molecular events suggest the possibility of discovering 'genocopies', genetic abnormalities that mimic chemically induced disorders. For example, patients have been reported with smaller than normal motor endplates, defective in AChE, and suffering from muscle weakness. There are no reported human AChE-less mutants; it is likely that such a genetic disaster would be lethal. There are humans with inherited differences in their serum BuChEs with decreased activity of the enzyme in their blood. Possessors of these genotypes usually are symptomless, unless they are given succinylcholine (or a similar drug) during surgery to bring about muscle relaxation. Lack of sufficient blood BuChE to speedily destroy the drug intensifies and prolongs the activity of succinylcholine, sometimes with fatal consequences. BuChEs may also play a detoxifying role in cocaine intoxication by hydrolyzing the drug. Several studies on experimental animals indicate that depressing ChEs with anti-ChEs intensifies the toxic effect of cocaine. A 'knock-out' genetically manipulated mouse lacking AChE studied by Oksana Lockridge and colleagues seems to use BuChEs as a substitute to destroy excess ACh.

Organophosphorus Cholinesterase Inhibitors

OP inhibitors are substituted phosphoric acids of the form



where R_1 and R_2 are usually alkyl or aryl groups linked either directly or via O or S groups to the P atom. According to one classification, X, termed the leaving group, may be (1) a quaternary nitrogen; (2) a fluoride; (3) a CN, OCN, SCN, or a halogen other than F; or (4) other groups. (See **Figure 4** for representative organophosphorus cholinesterase inhibitors.)

1. OPs containing quaternary nitrogen (phosphorylcholines) are strong inhibitors of ChEs and directly acting cholinergics. One, ecothiophate iodide, is used in the treatment of glaucoma.
2. Fluorophosphates are also highly toxic and relatively volatile. Sarin and soman are chemical warfare agents. Diisopropyl fluorophosphate (DFP) is often used by biochemists to study serine–active enzymes. Mipafox and DFP cause OPIDN in humans and experimental animals.
3. An example of a CN-containing nerve gas is Tabun.
4. Most OP pesticides are in the fourth and largest category. Many are dimethoxy or diethoxy compounds. OPs used in agriculture tend to be manufactured in the relatively stable P = S form. They are less toxic than OPs with the P = O (oxon) group. (Phosphates lack a sulfur atom, phosphorothioates have a single sulfur atom, and phosphorodithioates have two sulfur atoms.) Many pesticides such as parathion, methyl parathion, diazinon, and chlorpyrifos are phosphorothionates.

Three important chemical reactions that underlie ChE inhibitions are hydrolysis, desulfuration, and alkylation.

- *Hydrolysis*: The rate of hydrolysis is a function of the acid and alcohol groups, pH, and temperature. It usually increases with increasing pH, temperature, and UV light.
- *Desulfuration*: An important oxidation is the conversion of the P = S group of phosphorothionates to P = O, the oxon form, increasing the intensity of ChE inhibition.

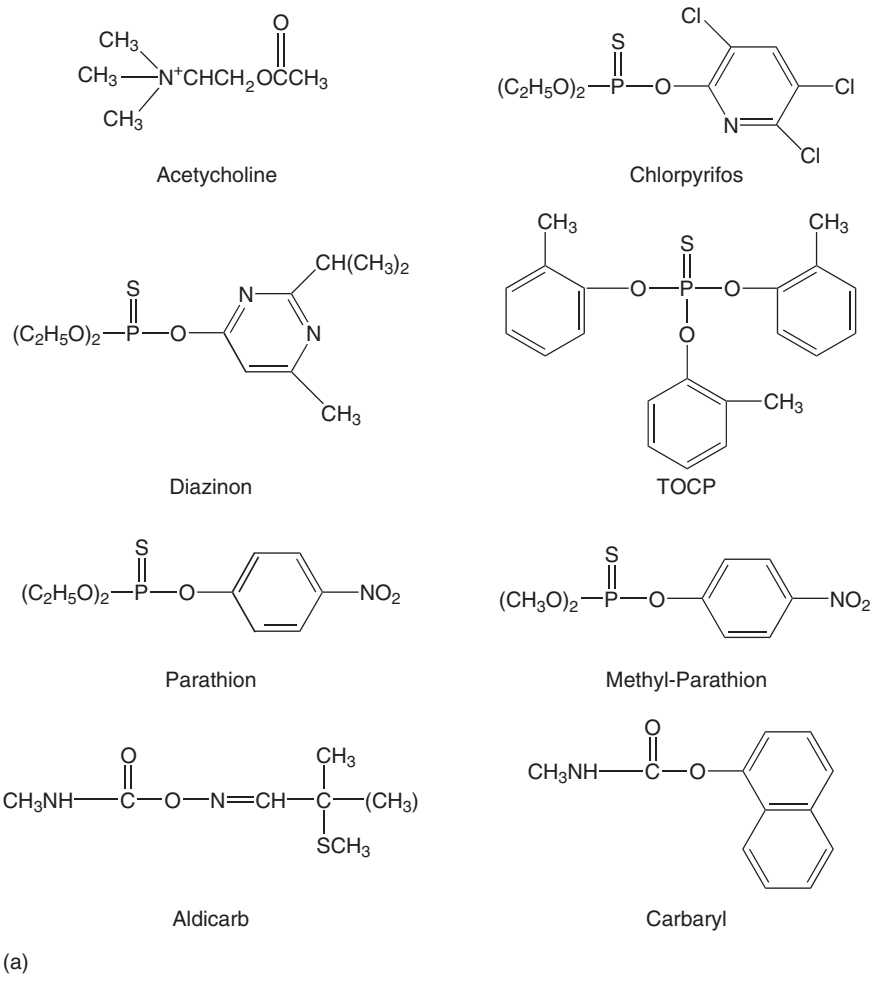


Figure 4 Representative organophosphorus and organocarbamate cholinesterase inhibitors.

- *Alkylation*: Alkyl substituents, especially methoxy groups, may act as alkylating agents. They are capable of altering nucleic acids, leading some to be concerned about OPs as mutagens.

Carbamate Cholinesterase Inhibitors

The CBs used as pesticides are N-substituted esters of carbamic acid. CBs developed in the 1950s as insect repellents were found to have insecticidal activity, leading to the development of the napthyl CBs with high anti-ChE activity and selective toxicity against insects. One example is carbaryl; it is widely used because of its low toxicity to mammals and its degradability. Aldicarb, a plant systemic, is more toxic than carbaryl. A few years ago aldicarb was associated with a July 4th holiday incident when West Coast residents complained of anticholinergic symptoms after eating aldicarb-contaminated watermelon.

Most *N*-methyl and *N*,*N*-dimethyl carbamates are better AChE inhibitors than BuChE inhibitors. However, *N*-carbamylated AChE spontaneously reactivates faster than *N*-carbamylated BuChE. AChE activity may recover as rapidly as 30 min following exposure – much faster than after exposure to OPs.

Although phosphorylation of AChE by OPs is heavily influenced by the electron-withdrawing power of the leaving group, carbamylation by methyl carbamates is also greatly dependent on molecular complementarity with the conformation of the enzyme as well as reactivity of the molecule. In general, phenolic and oxime moieties are more reactive than benzyl alcohol groups.

N-methyl carbamates do not need activation to inhibit ChEs. However, at least in the case of aldicarb, inhibition increases with metabolism. Aldicarb is rapidly oxidized to the relatively stable aldicarb sulfoxide, which in turn is more slowly metabolized to aldicarb sulfone, a stronger AChE inhibitor. These products are then detoxified by conversion to oximes and nitriles, which in turn are degraded to aldehydes, acids, and alcohols. Procarbamate derivatives were

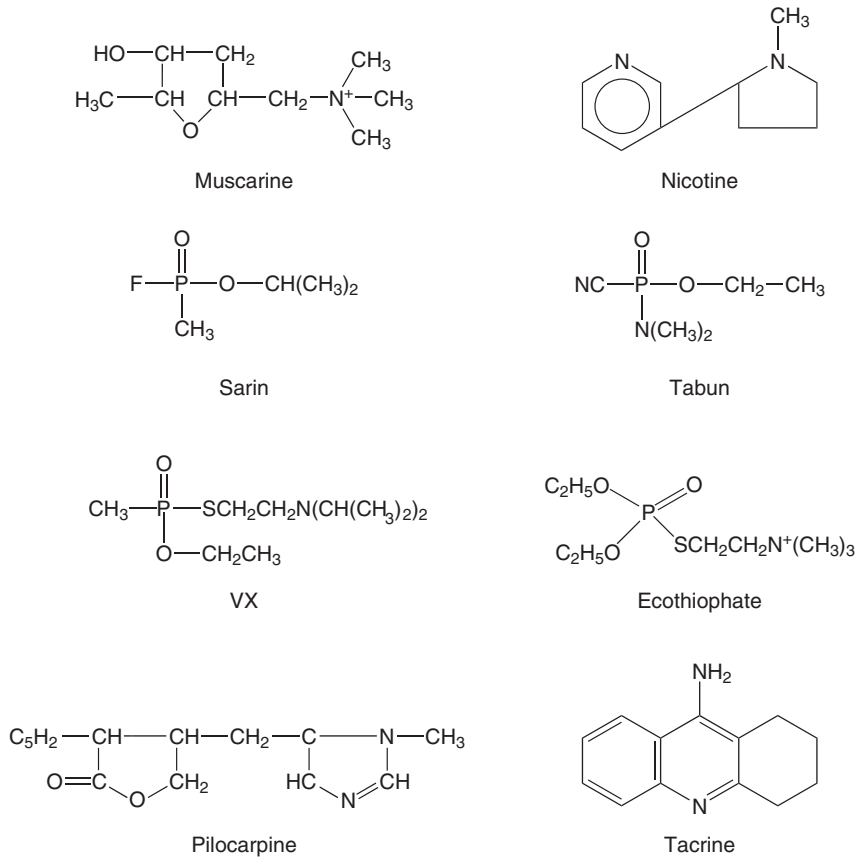


Figure 4 Continued.

developed to reduce the toxicity of CBs to mammals; the hydrogen atom on the carbamate nitrogen is replaced by a wide variety of nucleophiles – many with a sulfur atom – causing reduction in anti-ChE activity. The bond is rapidly broken in insects, restoring the activity and toxicity of the parent compound.

The rapid spontaneous reactivation of carbamates can be a problem in determining ChE activity. For example, some testing routines require that animals be put on a control diet for 24 h before sampling. With CBs, the inhibitions may have disappeared by the time the assays are performed. In addition, the dilutions specified in some assays may reduce the inhibition and high concentrations of substrate may compete with the carbamate to further reactivate the enzyme.

Chemical Warfare Anticholinesterase Agents

Anticholinesterase chemical warfare agents have been stockpiled since their development immediately before and during World War II. Several countries have active research programs into their toxicity and control. P = O groups confer potent anti-ChE inhibition properties and, in addition, the toxicity of agents such as soman and VX may be due in part to their actions on receptors and perhaps other proteins too. The toxicity of the nerve agents is greater than that of agricultural chemicals. For example, the dermal LD_{50} of agent VX is estimated to be 0.04–0.14 mg kg^{-1} for humans, which is at least an order of magnitude more toxic than most pesticides. LD_{50}s for representative agricultural OPs and CBs are shown in **Table 4**.

Assay Techniques

An early assay for ChE activity was a manometric method in which the change in pH due to ACh hydrolysis released CO_2 from a reaction buffer. A common technique (that of Michel) directly determines ACh hydrolysis by changes in pH. Another assay, that of Hestrin, utilizes the reaction of ACh with hydroxyl amine and ferric chloride, producing a reddish-purple complex. A test developed by Okabe and

Table 4 Representative acute LD_{50}s of selected organophosphates and carbamates

Compound	LD_{50} (mg kg^{-1})	
	Oral	*Dermal*
Organophosphates		
Dimethoxy compounds		
Azinphosmethyl (*O*,*O*-dimethyl-*S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl]phosphorodithioate)	13	220
Malathion (*O*,*O*-dimethyl-*S*-(1,2-dicarbethoxyethyl)phosphorodithioate)	1375	>4000
Methyl parathion (*O*,*O*-dimethyl-*O*-(*p*-nitrophenyl)phosphorothioate)	14	67
Diethoxy compounds		
Parathion (*O*,*O*-diethy-*O*-(4-nitorphenyl)phosphorothioate)	13	21
Diazinon (*O*,*O*-diethyl-*O*-(2-isopropyl-6-methyl-4-pyrimidnyl)phosphorothioate)	108	200
Carbamates		
Aldicarb (2-methyl-2-(methylthio)propylideneamino-*N*-methylcarbamate)	0.8	3.0
Carbaryl (1-naphthyl-*N*-methylcarbamate)	850	>4000

Adapted from Gaines TB (1969) Acute toxicity of pesticides, *Toxicology and Applied Pharmacology* 14: 515–534.

co-workers, oxidizes the choline released from ACh hydrolysis and determines the H_2O_2 produced. Several assays use radioactive ACh; one method counts the acetate produced by the reaction by separating it into an organic phase, leaving the unhydrolyzed ACh behind in an aqueous phase. Another common approach utilizes thioanalogs of ACh and other esters. In the assay developed by Ellman and co-workers, hydrolysis of thiocholines such as acetylthiocholine (AcTh) is measured at 410 nm with the color reagent dithionitrobenzoate. Although assays that rely on pH or radioactivity of ACh have the advantage of using a natural substrate, assays utilizing thiocholine esters are inexpensive, readily automated, and do not require expensive disposal of radioactive wastes. Negative features are the possibility of interference of hemoglobin (Hb) in RBC samples and a nonlinear reaction of the reduced glutathione in some RBCs with the color reagent. Some of the methods have been adapted for field use. Whatever the assay, it is important that its conditions be validated for the species, tissues, and chemicals under study. Unfortunately, there are no national or international standards for ChE assays making it difficult to compare results from one clinical laboratory with another. Recently the state of California specified a version of the Ellman assay as its clinical standard and required all laboratories monitoring blood ChEs to comply or harmonize with this standard methodology.

Biochemistry of Cholinesterase Inhibition

The inhibition of the activity of ChEs by OPs and CBs proceeds in a manner similar to the action of the enzymes on ACh. However, instead of forming a rapidly hydrolyzed acetyl–enzyme complex, the OPs and CBs, respectively, phosphorylate and carbamylate the catalytic sites of the enzymes. The major biochemical features of the inhibition of ChEs by OPs and CBs involve (1) activation of the inhibitors; (2) detoxification; (3) reaction of the inhibitor with the serine-active site of the enzyme and loss of a 'leaving group'; (4) hydrolysis of the complex and spontaneous reactivation of the enzyme; (5) loss of a second group, known as aging; and (6) recovery by synthesis of new enzyme.

One way to visualize the biochemical mechanisms underlying the toxicity of OPs and CBs is to trace the fate of an OP such as parathion from its entry into the body. Mixed function oxidases (MFO) in the liver (or in other tissues) convert parathion, a thionophosphate, to its oxygen analog, paraoxon, increasing its anti-ChE potential by orders of magnitude. The paraoxon may exert its toxic action by inhibiting AChE or be inactivated by conjugation with glutathione, reaction with glutathione transferases, further oxidation by MFO, or hydrolysis by A-esterases, in this case paraoxonase. Such reactions may lead to a loss in toxicity of either parathion or paraoxon. Paraoxon may also be inactivated by binding and reacting with B-esterases other than AChE, such as BuChE and carboxylesterases.

The reaction of an OP with AChE, BuChE, or other B-esterases is similar to the reaction of AChE with ACh, except that the hydrolysis step is much slower or, in some cases, may not occur at all. Its basis is a phosphorylation of the enzyme via a nucleophilic attack. The electronegative serine hydroxyl at the catalytic site reacts with the electropositive phosphorus atom of the inhibitor to form an OP–ChE complex and loss of a side group on the phosphorus atom, known as the leaving group (X). The phosphorylated enzyme may, in time, reactivate by

rehydrolysis. A similar set of reactions leads to carbamylation, except that the spontaneous reactivation tends to be more rapid than that for an OP. Spontaneous reactivation of an OP may take hours to days, whereas CBs may reactivate as soon as 30 min. In addition, OPs undergo a further reaction known as 'aging' in which a second group (often an alkyl group) is lost from the phosphate, stabilizing the OP–ChE complex.

Structure/Activity

Some general rules for OPs based on their structures include the following:

1. The P = O group is more toxic than the P = S group because it is more reactive. It is more reactive due to its higher electronegativity, which causes a more electropositive P atom, facilitating its reaction with the serine hydroxyl at the active site.
2. The electron-withdrawing ability of the leaving group X is predicted by the strength of its acid. For example, fluoride is a more powerful leaving group than nitrophenol since HF is a strong acid.
3. Reactivity of the R groups is in the order methoxy ⩾ ethoxy ⩾ propoxy ⩾ isopropoxy ⩾ amino groups. The more difficult a compound is to hydrolyze, the weaker is likely to be its ChE inhibition.
4. Steric effects are also important. The longer and more branched a compound, the more reduced is its rate of inhibition, probably because of the conformation of the proteins around the catalytic site.

The terms 'reversible' and 'irreversible' are often misused in describing ChE inhibitions. For example, statements such as 'OPs are irreversible inhibitors and CBs are reversible inhibitors' are useful insofar as they refer to the stability of the aged OP–enzyme and to the more rapid hydrolysis of the CB–enzyme compared to that of the un-aged OP–enzyme. Technically one could argue that the term 'reversible' should be reserved for cases in which there is an equilibrium between the substrate and the enzyme–substrate complex.

Spontaneous Reactivation of Organophosphates

Table 5 lists the half-lives of recovery for some OP-inhibited AChEs. In general OP–AChE complexes from dimethoxy-substituted OPs (e.g., malathion) spontaneously dephosphorylate faster than diethoxy (e.g., parathion) or diisopropoxy (e.g., DFP) complexes. Eto pointed out in 1974 that the stability of a phosphorylated AChE may be predicted from the stability of the specific OP inhibitor itself. One possibility is that methyl groups have less steric hindrance and greater electronegativity than ethyl or isopropyl groups.

Chemical Reactivation of Organophosphates

It has been almost 40 years since B. Wilson *et al.* observed that nucleophiles, oximes like hydroxamic acid, reactivated OP-inhibited AChE above and beyond that occurring from spontaneous reactivation, opening the way to a treatment for OP poisoning. The oxime registered for use in the United States is 2-PAM Cl (Protopam); its methanesulfonate salt (P2S) is used in Europe. Oxime therapy should be recommended with caution for carbamate poisonings. Although beneficial in the case of aldicarb, there is evidence that 2-PAM treatment increases the toxicity of carbaryl.

The mechanism of action of oxime reactivation involves transfer of the substituted phosphate or phosphonate residue from the catalytic site of the

Table 5 Spontaneous reactivation of aging of selected organophosphates

Compound	*Tissue*	*Spontaneous* (h)	*Aging* (h)
Malathion	Human RBC	0.85	3.9
Methamidophos	Bovine RBC	0.13	0.54
Chlorpyrifos	Bovine RBC/mouse brain	58	36
Diazinon	Human RBC	58	41
Parathion	Rat brain/bovine RBC	103	58
Tabun	Human RBC	ND	13
Sarin	Human RBC	ND	3.0
DFP	Human RBC	ND	4.6
Soman	Human RBC	ND	0.02

Adapted from Wilson *et al.* (1992) In: Chambers JE and Levi PE (eds.) *Organophosphates, Chemistry, Fate, Effects*. New York: Academic Press.

enzyme to the oxime. In addition, 2-PAM may react directly with the free OP molecule itself. Other oximes such as TMB-4, obidoxime, and HI-6 are reported to be superior to 2-PAM as reactivators and antidotes to chemical warfare agents. Oxime therapy should not be used in the absence of ChE inhibition since 2-PAM itself is a weak ChE inhibitor. In addition to the reactions discussed previously, direct effects of these compounds on muscle contraction and nicotinic receptors led Albuquerque and colleagues to propose that oximes also act directly on cholinergic receptors.

Aging

Research on oximes revealed an important phenomenon: the extent of reactivation of an OP–AChE complex decreased with time and depended on the OP used. This 'aging' prevents both spontaneous and chemical reactivation. Evidence indicates aging is due to the loss of a second group from the phosphorus atom. Harris and colleagues in 1966 demonstrated the loss of an alkyl group from a soman–AChE complex and showed that the percentage of enzyme losing an alkyl group correlated with the percentage of enzyme resistant to oxime activation. In general, OP–ChE complexes that spontaneously reactivate slowly tend to age rapidly. Exceptions are dimethoxy-phosphorylated AChEs, which both rapidly age and spontaneously reactivate. In general, agricultural chemicals (e.g., malathion, parathion, and diazinon) have half-lives of aging of hours and longer, while chemical warfare agents age rapidly (e.g., 10 min for soman).

Treatment for Anticholinesterase Poisoning

The information included here is educational; it should not be construed as specific recommendations for treatment of patients.

Inhibition of AChE by OPs or CBs is one of the few types of toxicity for which there are antidotes. The usual treatment for OP poisoning is atropine and 2-PAM (**Table 6**). The presence of atropine reduces the effectiveness of the ACh receptors, counterbalancing the excess ACh present. The recommended doses for humans are 1 g 2-PAM Cl (intramuscular or intravenous) two or three times a day and 2 mg atropine (intravenous) at 15–30-min intervals as needed. Higher doses may be used depending on the extent of the OP intoxication. Environmental Health Criteria No. 63 describes the case of a patient who drank a large amount of dicrotophos while inebriated. Treatments were progressively increased up to 6 mg atropine intravenously every 15 min with continuous infusion of 2-PAM Cl at 0.5 g h^{-1}. All told, 92 g of 2-PAM and 3912 mg of atropine were given to the patient, who was discharged after 33 days.

Table 6 Treatments for anticholinesterase poisoning

Atropine
2 mg intravenously, at 15 to 30 min intervals as needed to suppress symptoms 2 Pralidoxime
1 g either intramuscularly or intravenously two or three times per day or to suppress symptoms
Diazepam
10 mg subcutaneously or intravenously, repeated as required

Adapted from Environmental Health Criteria 63, 1986.

Much of the research on treatments of ChE inhibitions has concerned chemical warfare agents, providing little direct information for the treatment of agricultural chemicals.

Considerable attention has been given to prophylactic treatments to protect military units and civilian populations in the event of either accidental or deliberate release of nerve gas agents. One kit contains a combination of atropine, 2-PAM, and the anxiolytic, diazepam. Another contains pyridostigmine, a carbamate with actions similar to physostigmine. Diazepam is included to lessen CNS symptoms. The use of pyridostigmine is based on the idea that a readily rehydrolyzable carbamate will compete for AChE catalytic sites with the high-affinity binding nerve gas agents, reducing the percentage of AChE that becomes 'irreversibly' inhibited. Using these agents is not without risk since they are themselves toxic. Issuance of atropine kits to the general population of Israel during the Persian Gulf crisis led to the accidental injection of more than 200 children; some had systemic effects but fortunately there were no fatal consequences.

The discovery of methods to isolate relatively large amounts of ChE enzymes in essentially pure form has led to a unique method of treating OP intoxication – that of adding ChEs to the blood. Several experiments indicate enough of the OPs bind to the ChEs to reduce their toxicity in experimental animals. One issue is that of possible immune responses to what might be recognized by the body as a foreign protein, but to date there is no evidence suggesting this to be a problem.

Treatments with Anticholinesterase Agents

Several anticholinesterase agents have been used to treat human disorders.

Alzheimer's Disease and Tacrine

The finding that senile dementia of the Alzheimer's type was accompanied by a loss of AChE activity (as well as other neurochemical markers for cholinergic neurons) in parts of the brain has stimulated study of cholinergic nerve activity, learning and memory, and the use of anti-ChE compounds in the treatment of Alzheimer's disease. The strategy is to increase the effective level of ACh by reducing the activity of the AChE present. Tacrine (tetrahydroaminoacridine) was the first drug to be evaluated for this purpose. Tacrine is a weakly binding anti-ChE agent approved for treatment by the US FDA. The dose of Tacrine recommended ($100\,mg\,day^{-1}$) was chosen on the basis of the side effects the drug has on liver function rather than on unequivocal demonstration of its effectiveness. (In some trials, up to a third of the patients were removed from the studies due to side effects of the drug.) A subsequent cholinesterase inhibitor approved for use was donepezil (E 2020; Aricept). This drug appears less capable of eliciting adverse side effects. Other cholinesterase inhibitors evaluated for use in Alzheimer's disease include physostigmine and trichlorfon.

Glaucoma

Glaucoma is a disorder of vision accompanied by an increase in ocular pressure. Although mostly replaced by other drugs (e.g., beta blockers and pilocarpine), anti-ChE drugs such as ecothiopate are still used in the treatment of these common disorders.

Myasthenia Gravis

Myasthenia gravis is a progressive disorder characterized by muscle weakness; eye muscles are often the first affected. Research has shown it to be an autoimmune disease in which the victim forms antibodies to his or her nicotinic acetylcholine receptors at motor endplates. It is characterized by fatigability and weakness of the skeletal muscles, especially those of the eyes. Approximately 90% of the patients have droopy eyelids and double vision. Treatments include corticosteroids and thymectomy to reduce the actions of the immune system and anti-ChE agents such as pyridostigmine to improve the effectiveness of the receptors that remain.

Wildlife and Domestic Animal Exposures

The recognition that chlorinated hydrocarbons are a persistent danger to wildlife led to a decrease in their use as agricultural chemicals and to an increase in the use of OPs and CBs. In general, OPs and CBs do not bioaccumulate as do chlorinated hydrocarbons and they are relatively biodegradable. However, they are more acutely toxic than chlorinated hydrocarbons to humans and wildlife. A thorough discussion of the comparative toxicology of OPs and CBs is outside the scope of this entry. ChE inhibitions are generally the same, regardless of the animal; differences between species are often in the overall pharmacokinetics and metabolism. For example, although birds have higher brain AChE activities than mammals, they also have less hepatic MFOs to activate OPs and less A-esterases to hydrolyze them. Much research has been done on the toxicology of OPs to wild birds from sparrows to hawks and eagles. For example, Hill *et al*. of the US Fish and Wildlife Service studied the toxicity of 19 OPs and eight CBs to 35 species of birds. In general, such studies showed that over 50% of OPs and 90% of CBs have LD_{50}s of $<40\,mg\,kg^{-1}$ for most birds.

Route of exposure may have much to do with the recovery from OPs. When pigeons were treated orally with an OP, inhibition of blood ChE was rapid, and recovery of activity occurred within a few days. However, when the treatment was conducted dermally, putting the OP on the feet, recovery of enzyme activity took several weeks, implying the presence of a depot for OPs and the possibility that birds can accumulate OPs by flying from site to site. The possibility of bioaccumulation of OPs in a food chain (usually considered to be a characteristic of chlorinated hydrocarbons) was demonstrated by the report of an eagle poisoned by an OP (Warbex) in magpies that, in turn, had obtained the OP by ingesting hair from a steer that had been treated with it for parasites.

Beef cattle, horses (more than sheep), goats, and swine are treated several times each year with OPs to control parasites and some are fed tetrachlorvinphos to prevent fly larvae hatching in their feces. Carbaryl is commonly used for flea and tick control. Oehme states that insecticides are a common cause of poisoning of domestic animals and that "the majority of insecticide problems in domestic animals result from ignorance or mismanagement." Indeed, there is some epidemiological evidence that animal technicians in pet grooming and veterinary hospitals are exposed to the OP and CB chemicals used to control fleas and ticks while washing the animals. Sheep 'dipping' methods have been changed to minimize exposure to the worker.

Exposures in the Workplace

Worldwide, estimates of the number of humans requiring treatment due to anti-ChE chemicals run into many thousands annually. Concern for those who

manufacture and use agricultural chemicals has resulted in studies of pesticide residues, protective clothing, urinary metabolites, and blood ChE levels of farmworkers, greenhouse workers, and spray applicators. In general, the rule has been to consider decreases of blood cholinesterases of 30% or more as meaningful, signifying the worker should be removed from contact with the agent. In the United States, California requires workers to be monitored; however, even there, until recently, there has been no single standard method to determine ChE activities.

Chemical Warfare and Terrorism

The use of chemical weapons, nerve gases, mustard gases, and blistering agents is banned by international treaty. Nerve agents were inadvertently released from storage sites during the Persian Gulf conflict. A decade later the role that nerve agents may have played, whether alone or in company with other chemicals, in a baffling set of symptoms known as the Gulf War syndrome is still under investigation.

Millions of pounds of chemical warfare agents are stored throughout the world. Their destruction by incineration at high temperatures, up to 2500°F (1480°C) is planned or under way in several countries. These include eight sites in the United States, such as the Tooele Army Depot in Utah and Johnston Atoll in the Pacific, which is 750 miles from Hawaii. Some of the ordinance has been stored since World Wars I and II. Complaints have been lodged by citizens groups concerned about possible risks to residents during the destruction of the chemicals.

Sadly, chemical warfare weapons are dangerous instruments of terror. Two recent episodes in which sarin was used by terrorists in Japan cast a cloud over attempts to control the use of these weapons. Sarin was released in a residential area of the city of Matsumoto on June 27, 1994, and in a crowded Tokyo subway less than a year later, on March 20, 1995. In Matsumoto, about 600 residents and rescuers were affected and seven died. More than 5500 people were poisoned and 12 died in the Tokyo incident. Many more might have perished if it were not for the quick action and bravery of firemen, police, and others and the availability of antidotes in Japanese hospitals. (Two subway attendants died removing containers of sarin from subway cars.)

Significance of Blood Cholinesterase Levels

There has been a continuing discussion of the significance of monitoring blood ChEs of humans and wildlife. The setting of no-observed-adverse-effect levels (NOAELs) is an example. (NOAELs are the highest dose levels at which no important effect of a toxicant is observed.) Determining NOAELS is an important step in assigning risks and safe levels for the use of a toxic chemical. Some propose that batteries of behavioral tests performed under controlled laboratory conditions provide the best data for setting safe levels of exposure. Under field conditions others propose that measurements of residues on skin and clothing, urinary metabolites of agricultural workers, and fecal metabolites of wild animals provide evidence of exposure to chemicals without invasive procedures. Proponents of the use of ChE levels point out that they represent standardized, relatively inexpensive measurements that directly demonstrate a biochemical effect of an exposure to a toxic chemical rather than merely providing evidence of the exposure itself. Recent technology permits determinations of enzyme activities on 100 µl or less of blood, obtainable by a finger prick.

Regardless, as long as millions of pounds of OPs and CBs are used annually, ChE measurements will be an important tool in the protection of humans, domestic animals, and wildlife from overexposure to these toxic agents.

See also: A-Esterases; Anticholinergics; Carbamate Pesticides; Carboxylesterases; Neurotoxicity; Nerve Agents; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates; Pesticides; Veterinary Toxicology.

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Cholinesterases *See* Cholinesterase Inhibition.

Chromated Copper Arsenate *See* CCA-Treated Wood.

Chromium

Abbi Heilig

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- Chemical Abstracts Service Registry Number: CAS 7440-47-3
- Synonyms: Chrome; Chromium
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Cr^{3+}; Cr^{5+}

Uses

Chromium is a transitional element with many industrial uses. It is mainly used in imparting a shiny appearance to metal surfaces. In the early 1800s, the mineral, now known as chromite, was widely used in the production of paint as well as in the production of chromium compounds. These compounds can be used in a variety of applications. For example, potassium dichromate is used in the dyeing industry and chromium salts are used in leather tanning and wood preservation. Today, perhaps its most important use is in the production, in combination with iron, of stainless steel.

Background Information

Chromium as a metallic element was first discovered two hundred years ago, in 1797. But the history of chromium really began several decades before this. In 1761, in the Beresof Mines of the Ural Mountains Johann Gottlob Lehmann obtained samples of an orange-red mineral, which he called 'Siberian red lead'. He analyzed this mineral in 1766 and discovered that it contained lead "mineralised with a selenitic spar and iron particles." The mineral he found was crocoite, a lead chromate ($PbCrO_4$).

In 1770, Peter Simon Pallas also visited the Beresof Mines and observed the same type of mineral. He described it as "a very remarkable red lead mineral which has never been found in any other mine. When pulverised, it gives a handsome yellow guhr which could be used in miniature painting...." Chromium from the Beresof Mines and Siberia was used as a paint pigment. Due to its rarity, this later became a collector's item and increased in popularity in the paint industry. A bright yellow made from crocoite fast became the fashionable color for the carriages of the nobility in both France and England.

In 1797, chromium received its name from a professor of chemistry and assaying at the School of Mines in Paris, Nicolas-Louis Vauquelin. He received some samples of crocoite ore and his subsequent analysis revealed a new metallic element, which he called chromium after the Greek word khrôma, meaning color. After further research he detected trace elements of chromium in precious gems – giving the characteristic red color of rubies and the distinctive green of emeralds, serpentine, and chrome mica.

In 1798, Lowitz and Klaproth independently discovered chromium in a sample of a heavy black rock found further north from the Beresof Mines and in 1799 Tassaert identified chromium in the same mineral from a small deposit in the Var region of South-Eastern France. The chromite ore deposits discovered in the Ural Mountains greatly increased the supplies of chromium to the growing paint industry and even resulted in a chromium chemicals factory being set up in Manchester, England around 1808. In 1827, Isaac Tyson identified deposits of chromite ore on the Maryland-Pennsylvania border and the United States became the monopoly supplier for a number of years.

But high-grade chromite deposits were found near Bursa in Turkey in 1848 and with the exhaustion of the Maryland deposits around 1860, it was Turkey that then became the main source of supply. This continued for many years until the mining of chromium ore started in India and Southern Africa around 1906. And although paint pigments remained the main application for many years, chromium was finding other uses: Kochlin introduced the use of

potassium dichromate as a mordant in the dyeing industry in 1820. The use of chromium salts in leather tanning was adopted commercially in 1884. While chromite was first used as a refractory in France in 1879, its real use started in Britain in 1886.

The first patent for the use of chromium in steel was granted in 1865 – but the large-scale use of chromium had to wait until chromium metal could be produced by the alumino-thermic route, developed in the early 1900s and when the electric arc furnace could smelt chromite into the master alloy, ferrochromium.

Among the products chromium is used in, shiny finishes on surfaces and stainless steel are the most popular.

Exposure Routes and Pathways

Most chromium exposure in the general population is through ingestion of the chemical in food containing chromium(III), although exposure is also possible as a result of drinking contaminated well water, or living near uncontrolled hazardous waste sites containing chromium or industries that use chromium. Inhalation of chromium dust and skin contact during use in the workplace are the main routes of occupational exposure.

Studies have shown that inhalation, oral, and dermal exposures can result in chromium deposits in liver, kidney, heart, and lungs. Chromium in the breast milk of mothers can be passed down to infants and fetuses can be exposed to chromium that passes through the placenta.

Toxicokinetics

The toxicokinetics of a given chromium compound depend on the valence state of the chromium atom and the nature of its ligands. In contrast to chromium(III), which is bound to plasma proteins such as transferrins, chromium(VI) entering the blood stream is taken up selectively by erythrocytes, reduced, and bound predominantly to hemoglobin.

The absorption of chromium(VI) into the blood system through the skin has been reported but not investigated extensively, mainly because the reported health effects are rare. Once absorbed into the blood system there are various antioxidants that act as reducing agents, such as glutathione and ascorbate, which rapidly reduce chromium(VI) to chromium(III). Chromium absorbed through the lungs into the blood system is excreted by the kidneys and the liver. The kidney appears to absorb chromium from the blood through the renal cortex and releases it into the urine. Thus, sampling of urine for chromium can be used for biological monitoring of certain types of welding fumes that contain water-soluble chromium(VI).

Mechanism of Toxicity

Chromium may cause adverse health effects following inhalation, ingestion, or dermal exposure. The toxicity of chromium is mainly caused by hexavalent compounds as a result of a higher cellular uptake of chromium(VI) compounds than chromium(III). This is explained by the fact that the chromate anion $(CrO_4)^{2-}$ can enter the cells via facilitated diffusion through nonspecific anion channels (similarly to phosphate and sulfate anions). Absorption of chromium(III) compounds is via passive diffusion and phagocytosis.

Hexavalent chromium is unstable in the body and is reduced intracellularly (by many substances including ascorbate and glutathione) providing very reactive pentavalent chromium and trivalent chromium. Both of these intermediates can alter DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chromium can cause irritation in both the eyes and skin. Hexavalent chromium is corrosive to the skin and eyes.

Human

Ingesting large amounts of chromium(VI) can cause stomach upsets and ulcers, convulsions, kidney and liver damage, and even death. Skin contact with certain chromium(VI) compounds can cause skin ulcers. Some people are extremely sensitive to chromium(VI) or chromium(III). Allergic reactions consisting of severe redness and swelling of the skin have been noted.

Chronic Toxicity (or Exposure)

Animal

The hexavalent form of chromium is a potent teratogen, primarily affecting bone formation. However, trivalent chromium was not found to be teratogenic. Animal studies also show an increase in the risk of cancer after exposure to chromium(VI) compounds.

Human

Chromium(III) is an essential nutrient that helps the body use sugar, protein, and fat. Chronic liver and

kidney damage due to long-term exposure of chromium(VI) has been reported. However, chronic low-level exposure to chromium does not appear to produce measurable renal damage. Dermal exposure to chromium compounds can cause irritant dermatitis and skin ulcerations (chrome holes). Breathing high levels of chromium(VI) can cause irritation to the nose, such as runny nose, nosebleeds, and ulcers and holes in the nasal septum. Inhalation of chromium(VI) compounds is also associated with lung cancer and these compounds are classified as human carcinogens.

Clinical Management

There are no specific antidotes for chromium poisoning. Since most human overexposure is by ingestion, gastric lavage is appropriate in some cases. However, emesis should not be induced. Maintaining the proper fluid balance is critical due to impact on the kidney's ability to reabsorb fluid. It is necessary to establish that there is no impairment with breathing due to fluid accumulation in the lungs. Another important step is to decrease the intake of dietary supplements that contain chromium.

Environmental Fate

Chromium enters the air, water, and soil mostly in the chromium(III) and chromium(VI) forms. In air, chromium compounds are present mostly as fine dust particles, which eventually settle over land and water. Chromium can strongly attach to sediment and soil and only a small amount is expected to dissolve in water and leach though the soil to groundwater. Fish do not accumulate much chromium in their bodies.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit time-weighted averages (TWAs) for chromium compounds are: for, chromium(0) and salts – 1.0 $mg\,m^{-3}$; for chromium(II) and chromium(III) – 0.5 $mg\,m^{-3}$. The American Conference of Governmental Industrial Hygienists threshold limit value – TWA for chromium(VI) is 0.01 $mg\,m^{-3}$. The US Environmental Protection Agency maximum contaminant level in drinking water is 0.1 $mg\,l^{-1}$.

See also: Cardiovascular System; Chromium Hexavalent Compounds; Metals; Respiratory Tract.

Further Reading

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chromium.

Chromium Hexavalent Compounds

Robert Kapp

Chromium occurs in three basic forms: metallic chromium (Cr(0)), trivalent chromium (Cr(III)), and hexavalent chromium (Cr(VI)). Hexavalent chromium can exist as chromium hexavalent ion and as part of a number of compounds including calcium chromate, chromic acid, chromium trioxide, lead chromate, strontium chromate, potassium dichromate, and zinc chromate.

- Chemical Abstracts Service Registry Numbers:

Chemical	*CAS nos.*	*EINECS nos.*
Chromium	7440-47-3	231-157-5
Hexavalent chromium ion	18540-09-9	Not Listed
Ammonium dichromate	7789-09-5	232-143-1
Calcium chromate	13765-19-0	237-366-8
Chromic acid	13530-68-2	236-881-5
Chromium trioxide	1333-82-0	215-6-7-8
Lead chromate	7758-97-6	231-846-0
Strontium chromate	7789-06-2	232-142-6
Potassium dichromate	7778-50-9	231-906-6
Zinc chromate	13530-65-9	236-878-9

- Synonyms:
  - Hexavalent chromium ion – Chromium hexavalent ion; Chromium(6+); Chromium(6+) ion; Chromium(VI); Cr(VI); Chromium ion (Cr^{6+})
  - Ammonium dichromate – Ammonium bichromate; Ammonium dichromate(VI); Ammonium chromate; Chromic acid, diammonium salt; Diammonium dichromate
  - Barium chromate – Barium chromate(VI); Barium chromium oxide; Baryta Yellow; C.I. Pigment Yellow 31; Chromic acid, barium salt; Lemon Yellow; Lemon chrome; Ultramarine yellow
  - Calcium chromate – Calcium chrome yellow; Calcium chromium oxide; Calcium monochromate; Yellow ultramarine
  - Chromium trioxide – Chromic(VI) acid; Chromic oxide; Chromium oxide; Chromium(6+) oxide, Monochromium trioxide
  - Chromic acid – Dichromic acid; Dichromic(VI) acid
  - Lead chromate – Lead chromate(VI); Phoenicochroite; Plumbous chromate
  - Strontium chromate – C.I. pigment yellow; Deep lemon yellow; Strontium yellow; Strontium chromate; Sutokuro T
  - Potassium dichromate – Bichromate of potash; Chromium potassium oxide; Dipotassium dichromate heptaoxide; Iopezite; Potassium dichromate(VI)
  - Zinc chromate – Basic zinc chromate; Buttercup yellow; C.I. 77955; Chromium zinc oxide; Pigment yellow 36; Zinc chromate hydroxide; Zinc hydroxychromate; Zinc tetraoxychromate; Zinc yellow; Zincro ZTO
- Chemical/Pharmaceutical/Other Class: Metals

Uses

Metallic chromium is used in the following applications: to harden steel, the manufacture of stainless steel, the manufacture of other alloys, in electroplating, and as a catalyst. Trivalent chromium is used in the following applications: to make metal alloys, to make high-temperature bricks for industrial furnaces, and in leather tanning. Hexavalent chromium is used in the following applications: the production of pigments, metal finishing, and in wood preservatives.

Ammonium chloride is used in the following applications: as a fluxing agent, as an electrolyte for plating baths and batteries, in personal care products, in pharmaceuticals, and as a general anticaking agent.

Barium chromate is used in the following applications: as an anti-corrosive agent, as a pigment in paint, in ignition control devices, in safety matches, as a constituent in pyrotechnic compositions, and as a coloring agent in ceramics.

Calcium chromate is used in the following applications: as a pigment, as a corrosion inhibitor, as an oxidizing agent, and as a coating for light metal alloys.

Chromium trioxide is used in the following applications: in chromium plating, in copper stripping, in aluminum anodizing, as an anticorrosive, in photography, in hardening microscopic preparations, and in purifying oil and acetylene.

Lead chromate is used in the following applciations: as a pigment, in printing fabric, in chemical analysis of organic material, and as a constituent in pyrotechnic compositions.

Potassium dichromate is used in the following applications: as an anticorrosive agent, in the manufacture of other potassium/chromium compounds, in the manufacture of glass and glazes, and as a constituent in pyrotechnic compositions.

Strontium chromate is used in the following applications: as a pigment, as an anticorrosive agent in aluminum and magnesium alloys, in vinyl sheeting, and in chemical-resistant coatings.

Zinc chromate is used in the following applications: in pigments in paints, varnishes and oil colors, as an anticorrosive in primer coatings, as metal conditioners prior to priming, and as a catalyst.

Background Information

Elemental chromium is an odorless, hard, steel gray, lustrous metal that is available in crystal or powder. The symbol is Cr and the atomic weight is 52. Johann Gottlob Lehmann originally found chromium as a metallic element in 1761 on a visit to the Beresof Mines in the Ural Mountains of Siberia. He obtained samples of an orange-red mineral, which he named Siberian red lead. He later analyzed the sample and discovered it contained lead "mineralized with a selenitic spar and iron particles." The mineral was later found to be crocoite – which is lead chromate ($PbCrO_4$). Peter Simon Pallas also visited the Beresof Mines and observed "a very remarkable red lead mineral which had never been found in any other mine. When pulverized, it gives a handsome yellow guhr which could be used in miniature painting." Subsequent to that finding, the red lead mineral was used as a paint pigment and the crocoite yellow became a fashionable color for the carriages of the French and English nobility. Professor Nicolas-Louis Vauquelin, of the School of Mines in Paris,

discovered trace amounts of chromium in some precious gems – giving the characteristic red color to rubies and the distinctive green color to emeralds, serpentine, and chrome mica. In 1797 Vauquelin subsequently analyzed a sample of crocoite ore and found a new metallic element that he termed 'chromium' after the Greek word khrôma, meaning color because of the many different colors of its compounds. This metal can be in several forms, the most common are the metals chromium(0), chromium(III), and chromium(VI) compounds. Chromium(III) occurs naturally in the environment and types (0) and (VI) are produced in industrial reactions. The divalent state (chromous) is readily oxidized to the more stable trivalent (chromic) state. The hexavalent (chromate) state is more stable than the divalent state, but it is not found in nature. Hexavalent chromium compounds are highly corrosive and are strong oxidizers. They are generally reduced to the trivalent state in nature.

Other hexavalent chromium compound descriptions include the following:

- ammonium chromate occurs as a yellow crystalline substance;
- barium chromate occurs as a yellow powder;
- calcium chromate occurs as yellow monoclinic prisms;
- chromic acid occurs as brownish-red flakes;
- chromium trioxide occurs as odorless purplish to red rhombus crystals;
- lead chromate occurs as yellow to orange monoclinic crystals;
- potassium dichromate occurs as orange-red crystals;
- strontium chromate occurs as monoclinic yellow crystals; and
- zinc chromate occurs as lemon yellow prisms.

Chromium metal does not react with air, oxygen, nitric acid, alkalis, or water at room temperature.

Exposure Routes and Pathways

Human exposure to chromium and hexavalent chromium compounds can occur by inhalation, ingestion and skin contact; however, ingestion is the main route of exposure for the general population. Chromium compounds are widely distributed in the air, water, soil, and food. The trivalent form in trace amounts is considered to be an essential dietary component. Chromium has been detected in vegetables, fruits, grains, cereals, eggs, meat and fish at concentrations between 20 and 520 $\mu g\,kg^{-1}$. The mean daily intake of chromium from the air varies from <0.2 to 0.6 μg; from water is calculated to be <4 μg while the intake from food is approximately 60 μg. Skin exposure can occur through contact with wood treated with chromated copper arsenate. The Agency for Toxic Substances and Disease Registry estimates that citizens that live near industrial facilities or waste sites that can release chromium to the environment would have a higher exposure level than the average citizen.

The highest exposures occur occupationally. The National Occupational Hazard Survey conducted by the National Institute for Occupational Safety and Health from 1972 through 1974 concluded that some 2.5 million workers could be exposed to chromium and its compounds in the workplace. The National Occupational Exposure Survey conducted a decade later from 1981 through 1983 estimated a total of almost 200 000 workers were exposed to hexavalent chromium compounds (barium chromate, calcium chromate, chromium trioxide, lead chromate, strontium chromate, and zinc chromate). Occupational exposure occurs primarily from stainless steel production and welding, chromate production, chrome plating, ferrochrome alloys, chrome pigment and tanning industries.

Toxicokinetics

Both hexavalent and trivalent chromium are poorly absorbed from the lung or gastrointestinal (GI) tract.

Gastric absorption of hexavalent chromium is more efficient than absorption of trivalent chromium; however, absorption of ingested hexavalent chromium is less than 5%. Hexavalent chromium is reduced to the trivalent form upon contact with gastric juices, which appears to significantly reduce its absorption by the oral route of exposure. The size, oxidation state, and solubility of the chromium particles and the activity of the alveolar macrophages can affect absorption by inhalation exposure. In most cases, hexavalent chromium is more readily absorbed from the lungs than the trivalent compounds due, in part, to differences in the capacity of biological membranes. A significant amount of chromium is absorbed into the bone. Chromium is also concentrated in tissues of the liver, kidney, and spleen. Once absorbed, hexavalent chromium readily enters red blood cells through the phosphate and sulfate anion-exchange carrier pathway and some residual may remain in the plasma for an extended time period Dermal absorption is dependent upon the state of the chromium, the vehicle and the integrity of the skin.

Mechanism of Toxicity

Hexavalent chromium is significantly reduced to the trivalent state by glutathione in all tissues. During this reduction process, it has been shown that chromium may interact with cellular macromolecules and DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hexavalent chromium compounds are severely irritating to skin, eyes, and respiratory tissues.

Human

Humans who ingest toxic amounts of hexavalent chromium present clinical features of vomiting, diarrhea, hemorrhagic diathesis, and GI bleeding.

Chronic Toxicity (or Exposure)

Animal

Hexavalent chromium is nephrotoxic and tumorigenic. It has been reported in experimental animals that the hexavalent form of chromium can affect bone formation in fetal development. The mechanisms for this effect have yet to be elucidated; however, it is suggested that the potent peroxidant properties of hexavalent chromium may be involved. Animal studies have revealed a deficiency in lactation and male sterility resulting from hexavalent chromium exposure.

Intrabronchial implantations of zinc chromate and strontium chromate produced bronchial carcinomas.

Human

It is generally accepted that chromium is an essential element for humans. World Health Organization has estimated that the minimum chromium requirement is $33\,\mu g\,day^{-1}$. Some investigators have proposed that chromium deficiency may cause postnatal growth retardation and impaired glucose tolerance. There are no reports documenting chromium deficiency in human reproduction.

Chronic exposure to excess hexavalent chromium results in irritation of the skin and mucous membranes. Exposure to low doses of any form of chromium can induce allergic reactions causing skin rashes and swelling of the skin in sensitive individuals. Ulcerations (or chrome holes) can occur among workers who are exposed to high concentrations of chromic acid, sodium or potassium dichromate or chromate or ammonium dichromate. The ulcers generally occur in nail root areas, the creases over the knuckles, finger webs and forearms. Primary irritation can be attributed to hexavalent chromium. Cross hypersensitization to other metals, such as cobalt and nickel are not uncommon upon exposure to chromium. Systemic toxicity has been noted in humans following dermal exposure to chromium compounds.

Chronic inhalation exposure to hexavalent chromium may give rise to nasal septum bleeding and perforation with an accompanying loss of the sense of smell and taste. Bronchial asthma may result from chronic exposure to chromate dust or chromium trioxide.

Hexavalent chromium compounds are classified as substances known to be carcinogenic to humans. This is based upon sufficient evidence of carcinogenicity in humans exposed in chrome production facilities, chromium-alloy facilities, in the chrome plating industry as well as in chrome pigment industries. This exposure results in an increased incidence of lung cancer among these workers. The incidence of cancers at other sites may be increased in these occupational workers There is not sufficient evidence to show that barium chromate, calcium chromate, chromium trioxide, lead chromate, sodium dichromate and strontium chromate are carcinogenic in humans.

There are no reports documenting excess chromium as a teratogen in the human fetus.

In Vitro Toxicity Data

Hexavalent chromium has been shown to be a strong clastogen in experimental animals producing chromosome aberrations, sister chromosome exchanges, DNA strand breaks, oxidized base damage, DNA – DNA and DNA – protein cross-links. Human genetic studies have shown mixed results and have been limited by insufficient numbers of subjects.

Clinical Management

Upon exposure to any chromium compounds, the initial approach includes an assessment of the clinical status with subsequent support of basic cardiopulmonary functions. Once the airway has been stabilized, further measures can be taken.

After ingestion, emesis should not be induced due to the corrosive effects of chromium compounds. Ascorbic acid should be administered orally or nasogastrically to help reduce the hexavalent compounds to the trivalent forms. Dilution of the GI tract contents is indicated if the dilution can be accomplished within a few minutes after the ingestion.

Dilution may be accomplished with water or with demulcent fluids such as milk. Gastric lavage is indicated in certain circumstances. Hemodialysis and charcoal hemoperfusion may be employed in the event of renal failure. Fluid balance should be monitored and supportive measures taken as indicated.

After dermal exposure, the skin should be irrigated copiously with water. Topical application of freshly made 10% ascorbic acid solution or of a barrier cream containing 2% glycine and 1% tartaric acid has been beneficial in reducing thermal and chemical burns.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value – time-weighted average for chromium(VI) is $0.01\,\mathrm{mg\,m^{-3}}$.

See also: Metals.

Further Reading

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Chromium.

Chromosome Aberrations

Antone L Brooks

DNA is the genetic material responsible for passing genetic information from one cell to its daughter and in whole organisms passing the information from one generation to the next. In each cell, the DNA is packaged with proteins into units called chromosomes. The chromosomes in each cell have a characteristic number, size, and shape depending on its species. These characteristics provide what is called the karyotype for any cell from that species. For example, the human karyotype has 46 chromosomes, each of which can be identified and evaluated to determine if the cell or individual is normal. Such evaluation can predict many genetic diseases.

Chromosomes provide the units or trains that the genes ride on during their travels during cell replication and division. This genetic material (DNA) is organized into genes that are responsible for the production of proteins that regulate cell function. Each of the chromosomes has a unique location in the cell and the chromosomes maintain their respective locations during cell division. In mammals and other higher organisms, each chromosome is divided into two chromatids containing the same information. The chromosome also contains a centromere. This is the site where spindle fibers attach to the chromosome during cell division to ensure that each of the daughter cells has identical numbers and types of chromatids as the parent cell. This provides a way for genetic material to be carefully transmitted with very few errors during cell division, one cell to another, and during reproduction from one generation to the next.

Any physical or chemical agent that causes chromosome damage or aberrations is known as a clastogen or chromosome breaker. Chromosome aberrations can be induced by physiological changes as well as physical and chemical agents. The frequency of aberrations increases with age, cigarette smoking, or exposure to other environmental insults such as exposure to ionizing radiation. The number and type of aberrations can be used to determine if individuals have been exposed to specific environmental insults and to estimate the amount or magnitude of that exposure. The aberrations in this case can serve as a biomarker of radiation or chemical exposure or dose.

With the advances in molecular biology it has become possible to label or 'paint' each of the human chromosomes a different color. This was done by sorting the chromosomes according to their size, isolating the DNA from each chromosome, and making a probe using a different color to match the unique DNA on the sorted chromosome. This chromosome painting makes it possible to accurately determine which chromosomes are involved in each chromosome aberration or rearrangement. With these paints it has been possible to determine that many of the chromosome aberrations produced by ionizing radiation that in the past were thought to be between two chromosomes actually involve many chromosomes.

Such specific identification of aberrations has made it possible, in some cases, to determine which environmental insult is responsible for the chromosome alterations. The chromosome aberrations thus become biomarkers of exposure.

Chromosome damage is involved during the induction of cancer and birth defects. That is why it is important to be able to characterize the type of aberrations and the chromosomes that are involved. For example, if there is an exchange of one piece of a chromosome with a piece of another chromosome it is called a translocation. All the genes are still present after these translocations have occurred, but they are located next to different genes. This can cause a change in the way the genes produce messages and result in alterations in the message and proteins produced. These alterations change the physiology of the cells. For example, in many individuals who have leukemia there is a translocation between human chromosomes 9 and 22. This change results in a unique protein being produced from the altered karyotype. These changes in karyotype and protein provide useful markers of this disease. The understanding of the production of these proteins has been important in developing molecular medicines that can block the action of the new protein. Such medicines have resulted in useful cancer treatments.

The study of chromosome aberrations is important to provide useful biomarkers of exposure, dose, and disease. Understanding chromosome aberrations can result in a better understanding of genetics and the role that genetics plays in the induction of disease in exposed populations.

See also: Biomarkers, Environmental; Biomarkers, Human Health; Carcinogenesis; Radiation Toxicology, Ionizing and Nonionizing.

Further Reading

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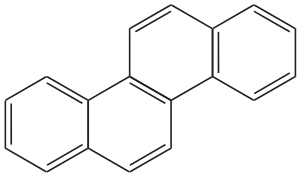
Chronic Toxicity *See* Toxicity, Chronic.

Chrysene

Linda A Malley

This article is a revision of the previous print edition article by Linda A Malley and David M Krentz, volume 1, pp. 344–346, © 1998, Elsevier Inc.

- Representative Chemicals: Polynuclear aromatic hydrocarbons
- Chemical Abstracts Service Registry Number: CAS 218-01-9
- Synonyms: 1,2,5,6-Dibenzonaphthalene; 1,2-Benzophenanthrene; 1,2-Benzphenanthrene; Benz(*a*)-phenanthrene; Benzo(*a*)phenanthrene
- Chemical/Pharmaceutical/Other Class: Polynuclear aromatic hydrocarbons
- Chemical Formula: $C_{18}H_{12}$
- Chemical Structure:



Uses

Chrysene is not produced commercially except for research purposes.

Background Information

Chrysene occurs as a product of combustion of fossil fuels and has been detected in automobile exhaust. Chrysene has also been detected in air samples collected from a variety of regions nationally and internationally. The concentrations were dependent on proximity to nearby sources of pollution such as traffic highways and industries, and was also dependent on seasons (generally higher concentrations were noted in winter months). Chrysene has also been detected in cigarette smoke and in other kinds of soot and smoke samples (carbon black soot, wood smoke, and soot from premixed acetylene oxygen flames). It has been detected as a component in petroleum products including clarified oil, solvents, waxes, tar oil, petrolatum, creosote, coal tar, cracked petroleum residue, extracts of bituminous coal, extracts from shale, petroleum asphalts, and coal tar pitch.

Exposure Routes and Pathways

Occupational exposure to chrysene may occur through inhalation of air contaminated with products of incomplete combustion and dermal contact with soot, motor oil, and coal tar. It has been detected in air samples from the following types of industrial operations and locations: coke ovens, furnaces for silicon carbide process plants, carbon anode plant, graphite plant, metal recycling plant, bitumen paving plant, aluminum refinery, smoking kilns for meat processing, and chimney sweeping. Chrysene has also been detected in surface water and soil samples and in a variety of cooked foods (particularly charcoal broiled/smoked); therefore, exposure to chrysene by ingestion is also possible. Dermal exposure to chrysene can also occur as a result of skin contact with soot or petroleum products.

Toxicokinetics

In general, polynuclear aromatic hydrocarbons (PAHs; the generic class name for chrysene) are highly soluble in lipids and adipose tissue and are expected to be readily absorbed by the dermal, oral, or inhalation routes of exposure.

Following oral administration in rats, peak concentration of chrysene occurred within 1 h in the blood and liver. Chrysene concentrated in the adipose and mammary tissue, and the majority of the dose was eliminated in the feces within 2 days.

In vitro rat liver and mouse skin preparations have been reported to metabolize chrysene to its 1,2-, 3,4-, and 5,6-dihydrodiols and to some monohydroxy derivatives including 1- and 3-phenols. In addition, the dihydrodiols have been reported to undergo further transformation to form 1,2-diol-3,4-epoxide and 3,4-diol-1,2-epoxide.

Mechanism of Toxicity

The primary toxic effect of concern for chrysene is carcinogenicity, which is most likely the result of the mutagenic activity of its metabolites, 1,2-dihydrodiol and 1,2-diol-3,4-epoxide. The 1,2-dihydrodiol and the 1,2-diol-3,4-epoxide have been shown to be mutagenic *in vitro* in bacterial and mammalian cells and have induced pulmonary adenomas when administered to newborn mice. In addition, the 1,2-dihydrodiol was active as a tumor initiating agent on mouse skin. DNA adducts in hamster cells resulting from a reaction of the DNA with 1,2-diol-3,4-expoxide have also been detected.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} of chrysene in mice, administered by intraperitoneal injection, is $320\,mg\,kg^{-1}$. Application of chrysene (0.1% in a petroleum hydrocarbon mixture known to have low embryotoxicity) to the eggshell of Mallard duck embryos resulted in embryotoxic and teratogenic effects in the ducklings. A single oral dose of chrysene administered to pregnant rats on day 19 of gestation induced hepatic P-450 enzymes in the fetal rat liver. In another study, chrysene induced benzo(*a*)pyrene hydroxylase activity in the placenta of pregnant female rats.

Human

As a general class of compounds, PAHs have low acute toxicity.

Chronic Toxicity (or Exposure)

Animal

The primary toxic effect elicited by chrysene is oncogenicity. Several studies have been conducted in mice in which chrysene (diluted in a variety of agents) was applied dermally either as a single dose (followed by a tumor promoting agent) or as multiple doses. Increased incidences of dermal tumors (papillomas and carcinomas) were observed in mice administered chrysene and a tumor promoting agent. Several studies were also conducted in which mice or rats received intramuscular or subcutaneous injections of chrysene

in various dilutants (single or multiple doses). Most of the treatment protocols resulted in an increased incidence of tumors at the injection site. In addition, male mice that were treated on days 1, 8, and 15 after birth with an intraperitoneal injection of chrysene had a higher incidence of pulmonary and liver tumors and lymphosarcomas compared to similarly treated controls.

Human

Chronic exposure to PAHs can produce a variety of effects. Exposure to the eyes can result in irritation and photosensitivity. Dermal exposure can result in erythema, burns, and 'coal tar warts' (precancerous lesions enhanced by ultraviolet light exposure). Inhalation exposure may cause irritation to the respiratory tract accompanied by cough and bronchitis. Oral exposure may produce a thickening and/or whitening of the oral mucous membranes. In addition to the local effects at the site of entry, systemic toxicity may occur, which could result in hepatic or renal effects. Some PAH compounds have been noted to cause hematological effects (anemia, leukopenia, and pancytopenia) in animals and suppression of selective components of the immune system.

Chrysene is classified as A2 (suspected human carcinogen) by the American Conference of Governmental Industrial Hygienists (ACGIH) and B2 (probable human carcinogen) by Environment Protection Agency (EPA) Integrated Risk Management System based on its carcinogenic effects in other species. Specific reports of toxicity or carcinogenicity in humans resulting from exposure to chrysene were not found. However, increased incidences of skin, bladder, and lung tumors and tumors of the gastrointestinal tract have been reported among workers exposed to PAHs.

In Vitro Toxicity Data

The potential for mutagenic effects was determined in bacterial, fungal, and mammalian cell systems *in vitro*. Although chrysene produced negative results in *Escherichia coli* and *Saccharomyces*, it produced positive results in *S. typhimurium* in TA100. In mammalian cells, chrysene produced positive effects in Syrian hamster embryo cells *in vitro*. Administration of chrysene to Chinese hamsters by intraperitoneal injection also produced increased sister chromatid exchanges in bone marrow cells. Increased aberrations were also noted in phase II oocytes collected from NMRI mice treated orally with chrysene. Chrysene induced aryl hydrocarbon hydroxylase in cultured human lymphocytes.

Clinical Management

Toxicity from PAH-containing compounds generally occurs following chronic exposure. Toxicity following acute ingestion is unlikely, and gastric decontamination is generally not necessary. For inhalation exposure, the patient is moved to a place with fresh air. If cough or difficulty breathing develops, oxygen is administrated and assisted ventilation provided as needed. Any broncho-spasm can be treated with inhaled beta-2-agonist and/or oral or parenteral corticosteroids or inhaled sympathomimetic agents. For eye exposure, eyes are irrigated with copious amounts of water for at least 15 min and then a follow-up evaluation should be made. For dermal exposure, the exposed area is washed with soap and water. If dermal hypersensitivity develops, reactions may require topical or systemic corticosteroids or antihistamines.

Since PAH compounds have been noted to cause hepatic, renal, and hematopoietic abnormalities, liver function tests, renal function tests, and a complete blood count are recommended for patients with chronic exposure. If tests indicate organ function abnormalities, supportive treatment should be undertaken. Treatment for cancer should follow standard therapeutic protocols for the type and location of the cancer.

Environmental Fate

Chrysene is expected to be immobile in soil and is not expected to volatilize from either moist or dry soil. Biodegradation rates in soil range from 77 to 387 days depending on soil type. In water, chrysene is expected to absorb to suspended solids and sediment. Bioconcentration in marine organisms is expected to range from low to high. Chrysene is not expected to undergo hydrolysis due to lack of hydrolyzable functional groups. In the atmosphere, chrysene is expected to exist in the particulate phase and may be physically removed by wet and dry depositions.

Ecotoxicology

The 96 h LC_{50} in fish was greater than 1 mg l^{-1}. The 24 h LC_{50} in amphibians was greater than 6.7 mg l^{-1}. The 24 h LC_{50} in insects was 1.7 mg l^{-1}. The 2 h LC_{50} in *Daphnia magna* was 1.9 mg l^{-1}.

Other Hazards

Chrysene emits acrid smoke and fumes when heated to decomposition.

Exposure Standards and Guidelines

Chrysene is classified as A2 (suspected human carcinogen) by the ACGIH and B2 (probable human carcinogen) by EPA Integrated Risk Management System based on its carcinogenic effects in other species.

Table 1 Summary of exposure criteria for chrysene

Agency	*Criteria*		*Averaging time*
ACGIH		A3 Confirmed animal carcinogen	NA
OSHA	PEL (TWA)	$0.2\,mg\,m^{-3}$	8 h/40 h week

OSHA, Occupational Safety and Health Administration; ACGIH, American Conference of Governmental Industrial Hygienists; PEL, permissible exposure limit; NA, not applicable.

Specific reports of toxicity or carcinogenicity in humans resulting from exposure to chrysene were not found. However, increased incidences of skin, bladder, and lung tumors and tumors of the gastrointestinal tract have been reported among workers exposed to PAHs (see **Table 1**).

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Polycyclic Aromatic Hydrocarbons.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Chrysene.

Ciguatoxin

David Elridge and Christopher P Holstege

This article is a revision of the previous print edition article by Gaylord P Lopez, volume 1, p. 346, © 1998, Elsevier Inc.

- Synonyms: Ichthyosarcotoxism; Ciguatera poisoning
- Chemical Formula: $C_{60}H_{86}O_{19}$
- Implicated Sources (Fish): Amberjack; Barracuda; Cinnamon; Coral trout; Dolphin; Eel; Emperor; Spanish mackerel; Surgeon fish; Grouper; Kingfish; Paddletail; Parrot fish; Red snapper; Reef cord; Sea bass; Swordfish; Yankee whiting

Background Information

Ciguatoxin is a lipophilic, heat-stable compound composed of multiple ether rings. Though overall a global problem that can be imported anywhere, this toxin is largely found in coral reef waters between 35° south to 35° north latitude.

Exposure Routes and Pathways

Ciguatoxin is produced by the dinoflagellate, *Gambierdiscus toxicus*, and related dinoflagellates. These microorganisms live attached to microalgae that are subsequently eaten by fish found in the area described above. This toxin is then stored in the viscera and flesh of the fish where it can remain for years. Ciguatoxin becomes progressively concentrated as one follows up the food chain (i.e., typically more toxin is found in larger, carnivorous fish). Human exposure may occur upon ingestion of these fish. Ciguatoxin is tasteless and odorless and does not degrade with cooking or freezing.

Toxicokinetics

After ingestion, the onset of signs and symptoms is highly variable and can range from 30 min to 30 h (average time is 6 h). The toxin may be found in the muscle, mucous, skin, and internal organs of fish. The highest concentration of toxin is typically found in the liver, gonads, and intestines.

Mechanism of Toxicity

Ciguatoxin binds tightly to voltage-sensitive sodium channels. This binding leads to increased opening of sodium channels and subsequent increased cell membrane permeability to sodium. As a result, the electrical potential of involved cells is altered.

Acute and Short-Term Toxicity (or Exposure)

Human

A multitude of signs and symptoms, predominantly neurological and gastrointestinal, can be seen following ingestion of ciguatoxin. In the early stages, nausea, vomiting, diarrhea, and abdominal pain are

common. Neurological abnormalities include tremors, tingling, and numbness of the lips and extremities, disturbance of temperature sensation (classically described as hot and cold reversal), and pruritus. Pain is common and is seen as myalgias, arthralgias, and toothaches. Bradycardia, hypotension, and respiratory failure may occur. Death is rare. Typically, the gastrointestinal symptoms resolve in 24–48 h while the neurological effects may last up to 2 months.

Chronic Toxicity (or Exposure)

Human

Humans exposed repeatedly to ciguatoxins have greater sensitivity compared to those with single exposures. Symptoms may be more pronounced and of greater duration in those with multiple exposures.

In Vitro Toxicity Data

Studies on human embryotic cells demonstrated that ciguatoxins target and bind to site 5 voltage gated sodium channels in both cardiac and muscle cells.

Clinical Management

Treatment is primarily symptomatic and supportive. Basic and advanced life support should be used as necessary. Bradycardia may be treated with IV atropine. Intravenous mannitol infusion has been advocated to alleviate neurological effects in the acute phase. Its utility, however, is controversial and has been questioned in recent studies. Exercise and the ingestion of nuts, grains, alcohol, seafood, opiates, and barbiturates have been reported to aggravate neurological signs and symptoms. Relief of chronic neurological effects has been described with the use of oral amitriptyline. Primary prevention consists of avoiding ingestion of characteristic fish.

See also: Atropine; Fish Consumption Advisory.

Further Reading

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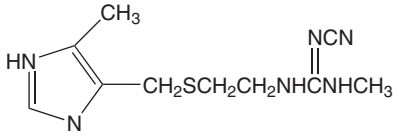
Geller RJ, Olson KR, and Senecal PE (1991) Ciguatera fish poisoning in San Francisco, California, caused by imported barracuda. *The Western Journal of Medicine* 156: 639–642.

Cimetidine

Michael D Reed

- Chemical Abstracts Service Registry Number: CAS 51481-61-9
- Synonyms: Numerous salts and brand names available; Tagamet
- Chemical/Pharmaceutical/Other Class: Histamine-2(H-2) receptor antagonist
- Chemical Formula: $C_{10}H_{16}N_6S$
- Chemical Structure:



Uses

Cimetidine is indicated for the treatment of disorders associated with hypersecretion of gastric acid, for example, gastric and duodenal ulcer disease, gastroesophageal reflux. The drug competitively antagonizes the H-2 receptor of the parietal cells.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to cimetidine. The drug is available as a parenteral formulation for intravenous administration and with improper dosing may result in acute toxicities.

Toxicokinetics

Cimetidine is rapidly and well absorbed (~70% bioavailability) after oral administration. The drug is

extensively distributed throughout the body with a V_d of $\sim 2\,l\,kg^{-1}$ and minimal protein binding, 13–25%. The vast majority of administered cimetidine is excreted unchanged via the kidney (70%) with ~15% undergoing hepatic metabolism and the remainder excreted via the bile. The drug's elimination half-life in patients with normal renal function is ~2 h. Cimetidine inhibits the activity of a number of cytochrome (CYP) P450 drug metabolizing enzymes, including CYP 1A2, CYP 2C9, and CYP 3A4. This interaction with these important human drug-metabolizing enzymes is responsible for a large number of clinically important metabolic-based drug–drug interactions.

Mechanism of Toxicity

Blockade of the cardiac H-2 receptors is the postulated mechanism for the cardiovascular toxicity associated with cimetidine overdosage. Cimetidine penetrates the blood–brain barrier and is associated with central nervous system effects in predisposed individuals, including elderly, and patients with poor renal function.

Acute and Short-Term Toxicity (or Exposure)

Human

Adverse effects associated with cimetidine overdose are rare. Patients are usually asymptomatic or experience minor to moderate gastrointestinal side effects/discomfort. Serious effects are rare with only case report descriptions of possible cimetidine-associated tachycardia. Rapid intravenous cimetidine administration in seriously ill patients has resulted in hypotension, bradycardia, and cardiac arrest. Large therapeutic or an overdose in patients with poor renal function, particularly the elderly, have resulted in central nervous system effects including confusion, delirium, hallucinations, and slurred speech.

Chronic Toxicity (or Exposure)

Animal

Rats given cimetidine at doses up to 4–48 times the recommended human dose over 2 years developed a slight increase in the incidence of benign Leydig cell tumors compared with the control group. There is no evidence of impaired mating or fertility in rabbits, rats, or mice at doses up to 40 times the human dose.

Human

Chronic exposure to cimetidine may be associated with gynecomastia in males and galactorrhea in females, possibly resulting from the drug's affinity for the androgen receptors combined with CYP inhibition of estradiol hydroxylation. Reductions in sperm count and impotence have been described in men chronically receiving the drug.

In Vitro Toxicity Data

Studies of radioprotective effects of cimetidine have demonstrated reduced frequency of radiation-induced micronuclei and chromosomal aberrations at various doses in rat bone marrow cells.

Clinical Management

Though the clinical need for such measures would be expected to be rare, basic and advanced life-support measures as well as aggressive decontamination may be instituted as clinically necessary. Gastric decontamination with a single dose of activated charcoal will effectively adsorb ingested cimetidine.

See also: Charcoal; Cytochrome P-450; Gastrointestinal System.

Further Reading

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MacMahon B, Bakshi M, and Walash MJ (1981) Cardiac arrhythmias after intravenous cimetidine. *New England Journal of Medicine* 305: 832–833.

Price W, Coli L, and Brandstetter RD (1985) Ranitidine-associated hallucinations. *European Journal of Clinical Pharmacology* 29: 375–376.

Ciprofloxacin

Teresa Dodd-Butera and Molly Broderick

- Chemical Name: 1,4-Dihydro-1-cyclopropyl-1,6-fluoro-4-dihydro-oxo-7-(1-piperazinyl)-3-quinolinehydroxycarboxylic acid
- Chemical Abstracts Service Registry Number: CAS 85721-33-1
- Chemical/Pharmaceutical/Other Class: Synthetic broad spectrum antibiotic
- Chemical Structure: $C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$. Ciprofloxacin has a fluorine atom at the 6-position, a piperazine moiety at the 7-position, and a cyclopropyl ring at the 1-position

Uses

Ciprofloxacin hydrochloride is a fluoroquinolone antimicrobial used in the treatment of infections from a wide range of aerobic Gram-positive and aerobic Gram-negative microorganisms. It has been shown to be effective against the following: inhalational anthrax, some types of respiratory infections, urinary tract infections, typhoid fever, gonorrhea, and septicemia. It is used as a secondary agent in the treatment of tuberculosis and has been used, occasionally, for conditions associated with cystic fibrosis.

Exposure Routes and Pathways

Ciprofloxacin is available in oral dosage forms, both pills and suspension. It may also be administered intravenously.

Toxicokinetics

Ciprofloxacin is well absorbed in the gastrointestinal tract. Serum concentration peaks in 1.5–2 h postingestion. The elimination half-life with normal renal function is 4 h. Ciprofloxacin is 20–40% bound to serum proteins and distributed widely throughout the body. Four metabolites in human urine have been identified, which account for ~15% of an oral dose. The metabolites have antimicrobial activity, which are less active than the parent compound of ciprofloxacin. Ciprofloxacin inhibits CYP3A4 enzyme system.

Mechanism of Toxicity

Ciprofloxacin acts by inhibiting the bacterial enzymes DNA gyrase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ciprofloxacin has produced tonic and clonic seizures in high doses in animal studies. In addition, areas of retinitis were noted on investigation of the effects of the drug to the eyes of rabbits; however, this was not a consistent finding in all studies. Hypotensive episodes were found with rapid administration.

Human

Nausea, vomiting, and diarrhea are most common from the oral dosage form. Central nervous system effects have been reported with intravenous administration, including increased intracranial pressure, dizziness, and convulsions. Local skin irritation at the intravenous site may also occur with rapid infusion of the drug. Rarely, cardiovascular symptoms have also been reported. All routes of exposure should be avoided in persons who are sensitive to the hypersensitive effects of ciprofloxacin or any other quinolones, as allergic reactions are possible. Interstitial nephritis has been attributed to hypersensitivity. Ciprofloxacin decreases the clearance of the drug, theophylline. Fatal reactions have been reported when these two drugs are concurrently administered. Theophylline levels must be monitored if the drugs are given together.

Chronic Toxicity (or Exposure)

Animal

Crystalluria and secondary nephropathy were noted, especially under conditions of alkaline urine. No impairment of fertility was noted and no congenital defects could be directly related to the administration of the drug.

Human

Abnormalities of liver, renal, and hematological parameters have been reported. As with other antibiotic regimens, pseudomembranous colitis may occur during or after treatment. Fluoroquinolones have the potential to cause adverse effects on developing cartilage and bone; thus, ciprofloxacin should be used with caution in pregnant women and young children.

Clinical Management

Symptomatic and supportive treatment is recommended, as is monitoring liver, renal, and hematological

parameters and drug levels especially with concurrent administration of other agents.

See also: Gastrointestinal System.

Further Reading

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Zimpfer A, Propst A, Mikuz G, *et al.* (2004) Ciprofloxacin-induced acute liver injury: Case report and review of literature. *Virchows Archiv* 444(1): 87–89.

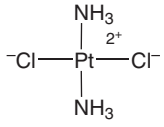
Relevant Website

http://www.fda.gov – US Food and Drug Administration.

Cisplatin

Linda A Malley

- Representative Chemicals: Platinum compounds
- Chemical Abstracts Service Registry Number: CAS 15663-27-1
- Synonyms: Biocisplatinum; CDDP; *cis*-DDP; *cis*-Diaminodichloroplatinum(II); *cis*-Diaminedichloroplatinum; *cis*-Platin; *cis*-Platinous diaminodichloride; *cis*-Platinous diamine dichloride; *cis*-Platinum; *cis*-Platinum diaminodichloride; *cis*-Platinum(II) diaminedichloride; Cisplatyl; Diaminedichloroplatinum; Neoplatin; NSC-119875; Platiblastin; Platinol
- Chemical Formula: $C_{12}H_6N_2Pt$
- Chemical Structure:



Uses

Cisplatin is primarily used for the treatment of a variety of malignancies.

Background Information

Cisplatin is a solid, ranging in color from white to yellow; and is soluble in water.

Exposure Routes and Pathways

Cisplatin is only available for intravenous use. It is generally supplied in vials as a solution or as a lyophilized powder. The possibility exists for dermal, oral, or inhalation exposure during production, and during preparation of dosing formulations.

Toxicokinetics

Following an intravenous injection, several mammalian species demonstrate a similar general organ distribution. Cisplatin is rapidly distributed to all tissues, followed within the first hour by an accumulation in kidneys, liver, skin, bone, ovaries, and uterus. Approximately 60–80% is excreted in the urine within 24 h. However, up to 4 weeks after a single dose, platinum is still detectable in kidneys, liver, skin, and lung. Following a single oral dose of cationic platinum, little absorption occurred and almost the entire dose was excreted in the feces, indicating that it would be unlikely for significant absorption to occur following oral administration of cisplatin.

The chloride atoms of the molecule may be displaced directly by reaction with neucleophiles such as thiol groups. However, hydrolysis of the chloride ion may also occur and may be responsible for formation of an active metabolite, which then reacts with nucleic acids and proteins. Investigation of metabolism of cisplatin in rat liver and kidneys following an intraperitoneal dose was conducted over a 24 h period. Maximum platinum concentrations in kidney cortex and medulla were reached within 1 h after dosing. In addition, the parent compound and five platinum-containing metabolites were present at 1 h postdosing, with cisplatin being the primary species detected. Similarly, there were five platinum-containing metabolites

detected in the liver at 1 h postdosing; however, in contrast to the kidneys, cisplatin was not the primary species detected in the liver.

Mechanism of Toxicity

Cisplatin reacts with nucleosides and nucleic acids and can cross-link cellular DNA. The effects on cross-linking with DNA appear to differ among cell type; however, the effects on cross-linking are most pronounced during the S-phase of the cell cycle. In addition, cisplatin inhibits a number of enzymes that contain a catalytically active sulfydryl group. Ribonucleotide reductase is extremely sensitive to the effects of cisplatin, with greater than 90% inhibition observed *in vitro* in the presence of a two-molar excess of cisplatin. The inhibition was nearly instantaneous and was irreversible.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the oral LD_{50} was $20\,mg\,kg^{-1}$, the intravenous LD_{50} was $8\,mg\,kg^{-1}$, and the intramuscular LD_{50} was $9200\,\mu g\,kg^{-1}$. The toxic effects observed in rats included leukopenia, decreased numbers of circulating platelets, lymphoid depletion, intestinal epithelial injury, bone marrow depression, and sloughing of the renal tubular epithelium. The LD_{50} in mice following a single intraperitoneal injection was $13.0\,mg\,kg^{-1}$, and following a single intravenous injection the LD_{50} was $13.36\,mg\,kg^{-1}$ for males and $12.32\,mg\,kg^{-1}$ for females. The minimum lethal dose for dogs was a single intravenous injection of $2.5\,mg\,kg^{-1}$ or five daily consecutive injections of $0.75\,mg\,kg^{-1}$. Toxic symptoms in dogs included severe hemorrhagic enterocolitis, severe damage to the bone marrow and lymphoid tissue, and marked renal necrosis. Occasionally, pancreatitis was also observed in dogs. In monkeys, the minimum lethal dose was five daily intravenous injections of $2.5\,mg\,kg^{-1}$. Toxic effects observed in monkeys included nephrosis, myocarditis, and some degeneration of spermatogenic cells.

Cisplatin ($13\,mg\,kg^{-1}$) administered to pregnant mice by intraperitoneal injection on gestation day 8 was lethal to all the fetuses. A dose of $8\,mg\,kg^{-1}$ cisplatin was lethal to 98% of the fetuses, and a dose of $3\,mg\,kg^{-1}$ was lethal to 31% of the fetuses. Surviving fetuses exhibited growth retardation and minor skeletal anomalies.

Female rats were administered twice weekly intraperitoneal injections of cisplatin for a cumulative dose of 15 or $34\,mg\,kg^{-1}$. Following the last dose, sensory and motor nerve conduction velocities were determined. Both doses of cisplatin significantly decreased sensory nerve conduction velocity. In addition, the level of cisplatin DNA binding in dorsal root spinal ganglion satellite cells equaled that in liver cells; however, the level of cisplatin DNA binding in spinal cord and brain was very low.

Rats were administered a single intravenous dose of $6\,mg\,kg^{-1}$ cisplatin, which was either preceded (30 min prior) or was followed by (30 or 60 min) $500\,mg\,kg^{-1}$ of reduced glutathione. The reduced glutathione, administered 30 min before or 30 min after the cisplatin, offered significant protection from toxicity and did not interfere with the antitumor effectiveness of cisplatin in a tumor model. However, in mice, the protective effect of reduced glutathione was only partial for some strains.

Human

Cisplatin is corrosive to the skin, and dusts cause eye and respiratory irritation. Renal dysfunction is the major toxic effect of this drug. It reduces the single-nephron glomerular filtration rate and causes a back leak of inulin across the renal tubule. In addition, myelosuppression, peripheral neuropathy (paraesthesias), central extrapyramidal disorders, loss of deep tendon reflexes, metabolic acidosis, headaches, taste disturbance, retrobulbar neuritis, seizures, ototoxicity, nausea, vomiting, diarrhea, thirst, metallic taste, leukopenia, allergic reactions, azotemia, hypokalemia, hypophosphatemia, hypocalcemia, and hypomagnesemia are commonly reported side affects of treatment with cisplatin. Some patients have also experienced anaphylactic reactions to treatment with platinum-containing compounds.

Chronic Toxicity (or Exposure)

Animal

Studies in animals indicate that cisplatin increases the occurrence of tumors. Cisplatin was administered once weekly by intraperitoneal injection to mice for 10 weeks for a total dose of $108\,mol\,kg^{-1}$. Cisplatin-treated mice had a significantly higher incidence (100%) of pulmonary adenomas compared to similarly treated control mice (26%). In another study, mice received weekly intraperitoneal injections of cisplatin 1.62 mg in $5\,ml\,kg^{-1}$ saline for 16 weeks, followed by dermal application of croton oil, dermal application of croton oil alone (control), or saline alone (control). Mice treated with croton oil and cisplatin had a higher incidence of skin papillomas compared to cisplatin alone or the control groups.

Human

Cisplatin caused azoospermia in humans within 2 months after initiation of treatment. Recovery of sperm counts occurred in most patients within 1–2 years after cessation of treatment. Cisplatin administered in combination with etopsoside is carcinogenic in humans.

In Vitro Toxicity Data

Cisplatin was mutagenic in *Salmonella typhimurium* strains G46/pkM101, TA100, and TA98, without metabolic activation. Cisplatin also induced increased mutations in Chinese hamster ovary cells and V79 Chinese hamster cells. Postreplication repair was induced in V79 cells and in HeLa cells; and sister chromatid exchanges were induced in V79 cells. Chromosomal damage and sister chromatid exchanges were also induced by cisplatin in human lymphocyte cultures. Similar to the *in vitro* data, intraperitoneal injection of mice with 13.85 mg kg^{-1} cisplatin induced a significant increase in sister chromatid exchanges and in chromosome aberrations.

Clinical Management

Nephrotoxicity may be prevented or diminished by prehydration with 2 l of normal saline administered over a 6–8 h period, followed by continued hydration during and after the cisplatin infusion. Nausea and vomiting may be managed with antiemetics. Electrolyte concentration should be monitored and supplemented as needed. Treatment for an anaphylactic reaction would include antihistamines, administered with or without epinephrine. If accidental exposure to the eyes or skin occurs, the affected skin area should be washed thoroughly with soap and water, and eyes should be flushed with copious amounts of tepid water for at least 15 min. Seizures should be treated with diazepam, lorazepan, phenobarbital, or phenytoin.

Environmental Fate

During production, *cis*-diaminedichloroplatinum is released, which could leach through soil. Abiotic or biotic processes can convert this to an ionic species, which could enhance its adsorption to soil. *cis*-Diaminedichloroplatinum will slowly convert to *trans*-diaminedichloroplatinum in water. This form will remain dissolved unless it precipitates to the sediment or is adsorbed to suspended particulates.

Ecotoxicology

Toxicity of cisplatin to aquatic organisms was not reported.

Other Hazards

Cisplatin is incompatible in solutions having low chloride content. Cisplatin interacts with aluminum, and only administration equipment that does not contain aluminum should be used for this medication. When heated to decomposition, toxic fumes of hydrogen chloride and nitrogen oxide are emitted.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists recommends an 8 h time-weighted average of 0.002 mg m^{-3}.

See also: Platinum.

Further Reading

Barnes KR and Lippard SJ (2004) Cisplatin and related anticancer drugs: Recent advances and insights. *Metal Ions in Biological Systems* 42: 142–177.

US Department of Health & Human Services/National Toxicology Program; Tenth Report on Carcinogens. *cis*-Diaminedichloroplatinum (15663-27-1).

Clean Air Act (CAA), US

Robert Kapp

- TITLE: CAA
- AGENCY: US Environmental Protection Agency (EPA)
- YEAR ENACTED: 1970; amended 1977, 1990, and 1997

Background

The first US federal legislation dealing with controlling air pollution at its source was Public Law 84-159, the Air Pollution Control Act of 1955. The legislation granted $5 million annually for five years for research

by the Public Health Service. While the act did not prevent any air pollution, it did provide research and technical assistance to air pollution control efforts. In 1960, the Act was amended to extend research for four additional years. In 1962, the Act was further amended to add research by the US Surgeon General to determine the health effects of various motor vehicle exhaust substances.

The Clean Air Act of 1963 (Public Law 88-206) was passed to improve, strengthen, and accelerate programs for the prevention and abatement of air pollution. This legislation granted $95 million over a 3 year period to state and local governments and air pollution control agencies to conduct research and create control programs. This Act encouraged the development of emissions standards for motor vehicles and from stationary sources, and it led to research on the removal of sulfur from high sulfur coal and oil fuels. The Act was amended in 1965 to establish standards for automobile emissions. It was further amended in 1966 and 1967 to expand local air pollutions control programs. The 1967 Amendment established national emissions standards for stationary sources and created Air Quality Control Regions as a means of monitoring ambient air. The states were given fixed timetables in which to implement State Implementation Plans to meet emission standards.

Overview of Clean Air Act

The Clean Air Act (CAA) is the federal law designed to assure that the air is safe to breathe. While public health is the primary goal, the Act also seeks to prevent environmental damage caused by air pollution. The fundamentals of the CAA were set up in the Clean Air Act of 1970 and then were amended several times. The basic framework of the Act and the objective of public health have remained intact.

The Clean Air Act of 1970 (Public Law 81-604) essentially rewrote the original Clean Air Act of 1963, by making it a more effective program to improve the quality of the ambient air. The legislation set ambitious National Ambient Air Standards to protect the public health with six 'criteria' pollutants, which included:

1. carbon monoxide,
2. nitrogen dioxide,
3. ozone,
4. sulfur dioxide,
5. particulate matter with aerodynamic size less than or equal to 10 μm (PM_{10}), and
6. lead.

The CAA also set New Source Performance Standards that strictly regulated emissions of any new sources of air pollution entering an area. It also established two categories of air-quality standards: Primary Standards set limits to protect public health, and Secondary Standards set limits to protect against public welfare effects, such as damage to farm crops and vegetation. The CAA further required leaded gasoline be phased out by the mid-1980s. In addition, the Act allowed citizens the right to take legal action against anyone or any organization, including government itself, who was in violation of the emissions standards.

In 1977, this Act was amended to extend the deadline of meeting the motor vehicle emissions standards. These amendments also made a first attempt to control stratospheric ozone and created the New Source Review, which required older 'grandfathered' facilities to install pollution control technologies as they modernized.

In 1990, the Clean Air Act was again amended and rewritten (Public Law 101-549). These amendments extended the prohibition of leaded gasoline to 1995. However, additional changes drastically strengthened the measures for attaining air quality standards provided in the CAA including:

- Provisions relating to mobile pollution sources
- Expanding the regulation of hazardous air pollutants
- Requiring substantial reductions in power plant emissions for control of acid rain (SO_2 and NO_x abatement). Utilities had the choice of using any of the following ways to meet the standard annual emissions allowance limit:
  - using cleaner fuel or choosing lower sulfur coal or fuel blending;
  - obtaining additional allowances;
  - installing flue gas desulfurization equipment (scrubbers);
  - using previously implemented controls;
  - retiring units;
  - boiler repowering;
  - establish operating permits for all major sources of air pollution;
  - establish provisions for stratospheric ozone protection; and
  - expand enforcement powers and penalties.

Section 112 of the CAA provides a list of 189 hazardous air pollutants for which the Environmental Protection Agency (EPA) must establish emissions standards for sources that emit any listed pollutant. There was considerable debate concerning the costs of emissions control, which came to a head in 1986

when CAA issued a standard for vinyl chloride. This standard was set aside by the courts; however, the court case (Natural Resources Defense Council, Inc. vs. United States Environmental Protection Agency, 1987) set a precedent by recognizing that the EPA could, in fact, consider costs in deciding if any additional margin of safety was necessary.

The 1990 amendments replaced the health-based standard with a two-tiered system of regulation. With this system, EPA must first issue standards that are technology based, designed to require the maximum achievable control technology (MACT) available. If the MACT values are insufficient to protect human health with an 'ample margin of safety', EPA must issue residual risk standards as well. These amendments define a sufficient margin of safety for carcinogens by requiring EPA to establish residual risk standards for any pollutant that poses a lifetime excess cancer risk of greater than 1:1 000 000.

In 1997, the Act was again amended to tighten the permissible ozone levels from 0.12 ppm per 1 h to 0.08 ppm per 8 h. In addition, the amendment revised the 24 h particulate matter up to 10 μmin diameter (PM_{10}) to simplify data handling requirements. Finally, new particulate matter up to 2.5 μm ($PM_{2.5}$) standards with an annual limit of 15 $\mu g\,m^{-3}$ were also added to the Act.

See also: Environmental Protection Agency, US; Environmental Toxicology; Pollution, Air.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency (EPA), Clean Air Act information.

Clean Water Act (CWA), US

Robert Kapp

- Agency: US Environmental Protection Agency (EPA)
- Year of Enactment: 1948, reauthorized 1972; amended 1977–83, 1987, 1988, 1990–92; 1994, 1995, and 1996; reauthorized in 1997

Background

Federal legislation on water began when Congress enacted the River and Harbor Act of 1886 that was recodified in the Rivers and Harbors Act of 1899. The Federal Water Pollution Control Act is a comprehensive statute aimed at restoring and maintaining the chemical, physical, and biological integrity of the US water supply. It was originally enacted in 1948. The Water Pollution Control Act Amendments of 1956 strengthened the enforcement by no longer requiring the federal government to receive consent from the States. The Water Quality Act of 1965 (Public Law 89-234) provided for the setting of enforceable water quality standards and the basis for interstate water quality standards. The Clean Water Restoration Act of 1966 (Public Law 89-753) imposed fines ($100 per day) on polluters who failed to submit a required report. The Water Quality Improvement Act of 1970 (Public Law 91-224) further expanded federal authority to certify water quality. These various amendments created cumbersome legislation that was, at best, difficult to implement. Growing public concern for controlling water pollution in the environment led the enactment of the Federal Water Pollution Control Act Amendments of 1972 (Public Law 92-500). Subsequently, this law was amended in 1977 and became known as the Clean Water Act.

Overview of Clean Water Act (CWA)

In a step toward resolving numerous administrative and implementation problems with the previous water pollution legislation, the 1972 amendments to the Federal Water Pollution Control Act restructured water pollution control under the authority of the Administrator of the US EPA. The objective of the 1972 reauthorization was to restore and maintain the chemical, physical, and biological integrity of the nation's waters. Initially there were two critical goals:

1. The elimination of the discharge of all pollutants into the navigable waters by 1985.
2. The creation of an interim level of water quality to provide for the protection of fish, shellfish, and wildlife and recreation by July 1, 1983.

The 1972 legislation also required federal effluent limitations and state water quality standards,

required permits for the discharge of pollutants into navigable water, provided enforcement mechanisms, authorized funding for wastewater treatment works, and provided construction grants to states and tribes for their water quality programs. The 1972 legislation changed the enforcement from regulating the quality of existing water to regulating the amount of effluents being discharged from particular point sources. (Point source is defined as "any discernible, confined, and discrete conveyance from which pollutants are or may be discharged.")

The EPA Administrator originally published guidelines for 63 chemicals and many other materials – sewage, garbage, dirt, discarded equipment, even heat – that could not be indiscriminately dumped in the water. The Act was amended in 1977 (Public Law 95-217) to include 126 materials, which EPA had identified as toxic under newly-developed health-based 'water quality criteria'. The Act also addressed previously unrecognized but widespread sources of water pollution such as municipal storm water, and new sources such as land application of manure from Confined Animal Feeding Operations. To meet this challenge, the most recent water act, the 1998 National Pollutant Discharge Elimination System focused not just on waterways but on watersheds. The Act authorized numerous research programs to study the prevention, reduction and elimination of water pollution. The act authorized the development of plans for the control of pollution within all or any part of the watersheds of the Great Lakes. In 1992, it authorized EPA to conduct a comprehensive survey of data on aquatic sediment quality and report the findings to Congress. The Act prohibited the discharge of pollutants except those in compliance with the effluent limitations with the best practicable control technology.

The Act provided for construction grants and loans to publicly owned treatment works (POTWs) to implement improved water pollution control measures. Another significant feature of the Act was the creation of a national pollutant discharge elimination system (NPDES). The NPDES required POTWs as well as industrial sources to acquire a permit that mandated certain effluent limitations had to be met before any discharges could occur in navigable waters. Other water pollution issues covered by the Act included the Clean Lakes Program, thermal discharges, non-point source pollution, estuaries, marine sanitation devices, oil and hazardous substance liability, and sewage sludge.

The pollutants regulated under the CWA include biochemical oxygen demand; fecal coliform; total suspended solids, oil and grease; and pH ('conventional pollutants'). Also included in the CWA are 'priority pollutants', that is, toxic pollutants as well as 'nonconventional pollutants' not identified as either conventional or priority.

The critical requirements of the CWA include the following:

1. Direct discharges from 'point source' limitations. Point sources include sewers, pipes, drainage ditches, etc. Any facility that intends to discharge into a lake or stream or river must obtain a permit prior to initiating the discharge. The discharge must meet conditions and effluent limitations set by the state and/or EPA.
2. Pretreatment requirements for indirect discharges into POTWs must meet pretreatment requirements as set forth in 40 CFR 403.6 National Pretreatment Standards: Categorical Standards. Effluent guidelines for direct discharges and pretreatment standards for specific chemical industry manufacturers and users are listed in the Code of Federal Regulations as follows:
   - 40 CFR 414 Organic Chemicals, Plastics, and Synthetic Fibers;
   - 40 CFR 415 Inorganic Chemicals Manufacturing;
   - 40 CFR 417 Soap and Detergent Manufacturing;
   - 40 CFR 418 Fertilizer Manufacturing;
   - 40 CFR 422 Phosphate Manufacturing;
   - 40 CFR 428 Rubber Manufacturing;
   - 40 CFR 446 Paint Formulation;
   - 40 CFR 447 Ink Formulation;
   - 40 CFR 454 Gum and Wood Chemicals Manufacturing;
   - 40 CFR 455 Pesticide Chemicals;
   - 40 CFR 457 Explosives Manufacturing; and
   - 40 CFR 458 Carbon Black Manufacturing.
3. Storm water runoffs were addressed in the 1987 CWA Amendments. These regulations required that manufacturers with any sort of storm sewer connected with any aspect of the chemical process apply for a permit under these conditions: (1) a discharge is associated with industrial activity; (2) a discharge from a large or medium municipal storm sewer system; or (3) a discharge, which has been determined to contribute to a violation of any water standard or is a significant contributor of pollutants to waters of the nation. These specific regulations are located in 40 CFR 122.26.
4. Oil and hazardous substance spill prevention and responses are generally incorporated into the Comprehensive Environmental Response, Comprehension, and Liability Act and EPCRA regulations. However, the Spill, Prevention, Control and

Countermeasure Plan applies to any facility that has oil or hazardous materials that has the potential to reach the nation's waters. These regulations are located in 40 CFR 112.

5. Wetlands modifications and/or the placement of dredge and fill materials into surface waters is covered by a permit program administered by the US Army Corp of Engineers. The CWA defines surface waters to include wetlands, hence, activities that involve any modification of wetlands are covered by the US Army Corps of Engineers. The regulations governing this permit program are located in 40 CFR 404.

See also: Comprehensive Environmental Response, Compensation, and Liability; Effluent Biomonitoring; Environmental Toxicology; Pollution, Water; Safe Drinking Water Act, US; Toxicity Testing, Aquatic.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency, Clean Water Act.

Clinical Chemistry

Shayne C Gad

The function of clinical chemistry in toxicology (as well as in human and veterinary medicine) is to provide, via laboratory analysis, evaluations of the qualitative and quantitative characteristics of specific endogenous chemical components present in samples of blood, urine, feces, spinal fluid, and tissues. The purpose is to help identify abnormal or pathological changes in organ system functions. The most common specimens used in clinical chemistry are blood and urine, and many different tests exist to test for almost any type of chemical component in blood or urine; for example, blood glucose, electrolytes, enzymes, hormones, lipids (fats), other metabolic substances, and proteins. The tests used were all initially applied to human clinical medicine, and may not possess the same utility when performed as part of nonclinical toxicity studies in a wide variety of other species.

Clinical chemistry evaluations are commonly recommended in animal toxicology studies. Regulatory agencies such as the US Food and Drug Administration and the US Environmental Protection Agency have set guidelines for clinical pathology testing in nonclinical toxicity and safety studies. Measurement of chemical components of biological fluids allows the toxicologist to do serial sampling, detect metabolic injury or organ-specific effects, and perhaps gain additional information helpful in establishing the no effect level and determining the mechanism of toxicity. When using serum enzymes as markers of tissue or organ damage, the enzyme of interest must reasonably reflect pathological change in a specific tissue, organ, or group of organs and must be easily measured.

The tests that are routinely performed provide information concerning hepatocellular and biliary integrity and function, renal function, carbohydrate, protein and lipid metabolism, and mineral and electrolyte balance. Modern analytical techniques require only small sample volumes to make accurate determinations, allowing in-life evaluations of effects in rats and larger species at multiple times during the course of a study without compromising animal health.

Table 1 summarizes the commonly measured endpoints and the probable causes behind findings.

See also: Toxicity, Chronic; Toxicity, Subchronic.

Further Reading

Burtis CA and Ashwood ER (1999) *Tietz Textbook of Clinical Chemistry*, 3rd edn. Philadelphia: Saunders.

Relevant Websites

http://www.aacc.org – American Association for Clinical Chemistry (AACC) website. Also considering accessing the AACC Listservs

http://www.clinchem.org – Clinical Chemistry: International and Journal of Molecular Diagonistics and Laboratory Medicine.

http://www.e-c4.org – European Communities Confederation of Clinical Chemistry and Laboratory Medicine.

Table 1 Association of changes in biochemical parameters with actions at particular target organs

Parameter	*Blood*	*Heart*	*Lung*	*Kidney*	*Liver*	*Bone*	*Intestine*	*Pancreas*	*Notes*
Albumin				↓	↓				Produced by the liver. Very significant reductions indicate extensive liver damage
ALP					↑	↑	↑		Elevations usually associated with cholestasis. Bone alkaline phosphatase tends to be higher in young animals
Bilirubin (total)	↑				↑				Usually elevated due to cholestasis, either due to obstruction or hepatopathy
BUN				↑	↓				Estimates blood filtering capacity of the kidneys. Does not become significantly elevated until the kidney function is reduced 60–75%
Calcium				↑					Can be life threatening and result in acute death
Cholinesterase				↑	↓				Found in plasma, brain, and RBC
CPK		↑							Most often elevated due to skeletal muscle damage but can also be produced by cardiac muscle damage. Can be more sensitive than histopathology
Creatinine				↑					Also estimates blood-filtering capacity of kidney as BUN does
Glucose								↑	Alterations other than those associated with stress are uncommon and reflect an effect on the pancreatic islets or anorexia
GGT					↑				Elevated in cholestasis. This is a microsomal enzyme and levels often increase in response to microsomal enzyme induction
HBDH		↑			↑				
LDH		↑	↑	↑	↑				Increase usually due to skeletal muscle, cardiac muscle, or liver damage. Not very specific
Protein (total)				↓	↓				Absolute alterations are usually associated with decreased production (liver) or increased loss (kidney). Can see increase in case of muscle 'wasting' (catabolism)
SGOT		↑		↑	↑			↑	Present in skeletal muscle and heart and most commonly associated with damage to these
SGPT					↑				Elevations usually associated with hepatic damage or disease
SDH					↑↓				Liver enzyme that can be quite sensitive but is fairly unstable. Samples should be processed as soon as possible

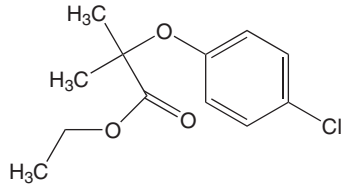
↑: increase in chemistry values; ↓: decrease in chemistry values.

ALP, alkaline phosphatase; BUN, blood urea nitrogen; CPK, creatinine phosphokinase; GGT, gamma glutamyl transferase; HBDH, hydroxybutyric dehydrogenase; LDH, lactic dehydrogenase; RBC, red blood cells; SDH, sorbitol dehydrogenase; SGOT, serum glutamic oxaloacetic transaminase (also called AST (aspertate amino transferase)); SGPT, Serum glutamic pyruvic transaminase (also called ALT (atanine amino transferase)).

Clofibrate

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 637-07-0
- Synonyms: 2-(*p*-Chlorphenoxy)-2-methylpropionic acid ethyl ester; α-*p*-Chlorophenoxyisobutyryl ethyl ester; Amotril; Angiokapsul; Anparton; Antilipid; Ateculon; Ateriosan; Atheropront; Atromid; Atromidin; Hyclorate; Lipavil; Liponorm; Liporil; Lipofaction; Neo-Atromid; Normet; Regelan; Serotinex
- Chemical/Pharmaceutical/Other Class: Antihyperlipoproteinemic agent
- Chemical Structure:



Uses

Clofibrate is a drug used to lower plasma concentration of very low density lipoprotein and also to lower plasma triglyceride concentration.

Background Information

Clofibrate, a well-known hypolipidemic agent, has drawn attention over a past few years with regard to its efficacy in coronary artery disease. It has been shown to exert protective influence against the gross morbid effects in experimental myocardial infarction, development of new infarction, and sudden death in clinical cases. Furthermore, it has recently been reported to reduce incidence and severity of arteriosclerosis, an important risk factor in precipitating myocardial infarction. However, the enthusiasm for prophylactic use of clofibrate has received a major setback following a 5 year US multicenter study report regarding failure of clofibrate in reducing or preventing mortality in cardiovascular disorders.

Exposure Routes and Pathways

Accidental overdose or ingestion is the most common exposure pathway.

Toxicokinetics

Clofibrate is rapidly and completely absorbed after oral administration. It is hydrolyzed to clofibric acid during absorption and in its passage through the liver. The acid binds strongly to plasma proteins. Sixty percent of it is excreted as glucuronide conjugate in the urine. Some is secreted into the bile and reabsorbed. The plasma elimination half-life is 25 h. Clofibrate appears in plasma as *p*-chlorophenoxyisobutyric acid. An acyl-linked metabolite of clofibrate has been identified in human urine.

Mechanism of Toxicity

Clofibrate acyl glucuronide is an electrophilic metabolite that reacts with sulfhydryl groups and causes hepatotoxicity.

Acute and Short-Term Toxicity (or Toxicity)

Animal

Rats given food containing 1% clofibrate or subcutaneous injections of 0.3–0.9 g kg^{-1} daily for 2 days showed spontaneous electromyographic responses in hind limbs. Clofibrate has induced relative enlargement of the liver in proportion to the body of newborn rats as well as an abnormal postnatal fetal thrombosis syndrome. The postnatal thrombosis consisted of an extension of the normal thrombosis in the umbilical arteries, and this caused necrosis of the tail or parts of the hindlimbs. Reproduction studies in both dogs and monkeys using clofibrate dosages approximately four to six times the usual human dosage have demonstrated arrest of spermatogenesis.

Human

Nausea, diarrhea, skin rash, alopecia, weakness, flu-like syndrome, and severe muscle cramps are symptoms of acute toxicity.

Chronic Toxicity (or Exposure)

Animal

Clofibrate causes hepatic tumors in rodents. Fifteen male Fischer 344 rats, weighing 84–100 g, were fed clofibrate at dietary concentration of about 250 mg kg^{-1} body weight per day in ground rat chow for up to 28 months. One or more hepatocellular

carcinomas developed in 10/11 rats, compared with 0/14 controls. Five of the animals showed metastasis. In addition, pancreatic exocrine acinar carcinomas were found in 2/11 rats, a dermatofibrosarcoma in one rat, and a leiomyoma of the intestine in one rat in the clofibrate-fed group. No such tumors were seen in controls.

Human

Chronic administration increases the incidence of cholesterolic gallstones twofold. It also causes a small increase in thromboembolic phenomenon, pulmonary embolism, intermittent claudication, and angina pectoris. The drug may increase the incidence of bowel cancer. Overall, clofibrate cannot be classified as carcinogenic in humans.

In Vitro Toxicity Data

Clofibrate at a concentration of 0.5 mmol in culture medium maintained the cytochrome P-450 content of rat hepatocytes for up to 96 h. This effect was associated with a marked induction of lauric acid hydroxylation whereas little effect was observed on the metabolism of three other cytochrome p450 dependent mixed function oxidase substrates.

Clinical Management

Exposure should be terminated as soon as possible. If toxic amount in overdose is unknown, then gastric decontamination is required. Gastric decontamination is probably usually not necessary and should only be considered if several times the daily therapeutic dose were ingested. Activated charcoal as a slurry (240 ml water/30 g charcoal) should be given. The usual dose is 25–100 g in adults/adolescents, 25–50 g in children (1–12 years), and 1 g kg^{-1} in infants less than 1 year.

Exposure Standards and Guidelines

Manufacturers, packers, and distributors of drug and drug products for human use are responsible for complying with the labeling, certification, and usage requirements as prescribed by the Federal Food, Drug, and Cosmetic Act.

See also: Charcoal; Cytochrome P-450.

Further Reading

Clofibrate (1996) IARC Monographs. *Evaluation of Carcinogenic Risks to Humans* 66: 391–426.

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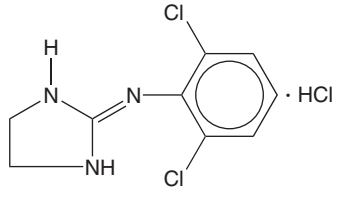
Clonidine

Elizabeth J Scharman

- Chemical Abstracts Service Registry Numbers: CAS 4205-90-7; CAS 4205-91-8 (hydrochloride)
- Synonyms: Clonidine hydrochloride; 2-(2,6-Dichloroanilino)-2-imidazoline hydrochloride; Catapres®
- Chemical/Pharmaceutical/Other Class: Imidazoline-derivative hypotensive agent; A selective α-2 adrenergic receptor agonist
- Chemical Formula: $C_9H_9Cl_2N_3$
- Chemical Structure:



Uses

Clonidine is used in the management of hypertension, attention-deficit hyperactivity disorder, opiate or nicotine withdrawal, vascular headache prophylaxis, and as an aid in the diagnosis of pheochromocytoma. It is also used as an epidural infusion for pain management.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to clonidine. Toxicity may also occur via dermal exposure from transdermal patches.

Toxicokinetics

Clonidine is well absorbed (75–90%) orally with peak plasma concentrations occurring in 2–4 h. Following transdermal application, therapeutic plasma concentrations are reached within 2–3 days and last for 8 h after patch removal. Peak analgesia occurs within 30–60 min when clonidine is given epidurally. Clonidine is metabolized by hydroxylation of the phenol ring and cleavage of the imidazole ring to six inactive metabolites. The volume of distribution is 2.1–4 l kg^{-1}. Clonidine is distributed into the cerebral spinal fluid and into breast milk; it also crosses the placenta. Approximately 65% is excreted by the kidneys; 32% as unchanged drug. The half-life is 6–20 h; 18–41 h in patients with renal insufficiency.

Mechanism of Toxicity

Clonidine acts at postsynaptic α-2 adrenergic receptors in the lower brainstem and medulla oblongata, resulting in inhibition of sympathetic discharge. This results in a decrease in cardiac output and heart rate. Following overdose, peripheral α-2 receptors may be stimulated, resulting in transient hypertension followed by hypotension.

Acute and Short-Term Toxicity (or Exposure)

Animal

Clonidine produces decreases in blood pressure and heart rate in animals as those seen in humans. In a rat model of seizure activity secondary to soman administration, clonidine pretreatment provides some seizure protection compared with controls.

Human

As little as 0.1 mg of clonidine has produced toxicity in children; determination of adult toxicity is based on observation as there is no milligram per kilogram toxic dose established. Clonidine levels are not clinically useful. Toxicity can result from ingestion of used clonidine transdermal patches as residual clonidine remains after full therapeutic use. Symptoms generally begin within 30–90 min and include hypotension, central nervous system depression, bradycardia, and miosis; cardiac dysrhythmias and hypothermia may be noted. Hypotension may be preceded by transient hypertension. Toxic symptoms may persist for up to 24–48 h; longer durations of toxicity have been reported.

Chronic Toxicity (or Exposure)

Animal

Albino rats on therapy with clonidine for 6 months or greater have developed retinal degeneration.

Human

Side effects of clonidine therapy include dry mouth, drowsiness, sedation, and constipation. Abrupt discontinuation of therapy may result in a withdrawal syndrome manifested as restless and headache in addition to significant rebound hypertension. Withdrawal can be avoided by tapering therapy over 2–4 days. The incidence of a local dermatitis or an extended dermal reaction with use of the transdermal patch is $\sim$15–20%.

Clinical Management

Induction of emesis with syrup of ipecac is not recommended due to the potential for rapid onset of lethargy and coma. Clonidine is adsorbed by activated charcoal. Whole bowel irrigation may be useful if a transdermal patch is ingested. Respiratory depression, hypotension, and coma may respond to naloxone (initial dose, 0.4–2 mg intravenously repeated as necessary) although this therapy is not routinely effective. Standard supportive therapies, such as vasopressors, should be utilized as clinically indicated. Paradoxical hypertension following an acute overdose is transient and normally does not require treatment. Therapy for rebound hypertension, which follows abrupt discontinuation of clonidine therapy, includes reinstitution of clonidine or the combined administration of an α- and β-adrenergic blocking agent (e.g., phentolamine or prazosin with a beta blocker such as propranolol).

See also: Charcoal; Nicotine; Opium.

Further Reading

Anderson RJ, Hart GR, and Crumpler CP (1981) Clonidine overdose: Report of six cases and review of the literature. *Annals of Emergency Medicine* 10: 107–112.

Wiley JF, Wiley CC, and Torrey SB (1990) Clonidine poisoning in young children. *Journal of Pediatrics* 116: 654–658.

Clostridium perfringens

Lee R Shugart

Description

Clostridium perfringens is a gram-positive, obligate anaerobic spore-forming rod. It is widely distributed in the environment and frequently occurs in low numbers in the intestines of humans and domestic animals. Spores of the organism persist in soil, sediments, and areas subject to human or animal fecal pollution. Any raw food may contain spores or the bacteria.

Mechanism of Toxicity

In all clostridial diseases, pathogenesis is attributable to potent exotoxins released by the organism. *C. perfringens* strains produce numerous toxins; over 20 have been described scientifically. For toxicological identification, *C. perfringens* strains have been divided into five types (A–E) based on the production of four main toxins (alpha, beta, epsilon, and iota), which combine with other toxic substances created by the bacteria to produce ~25 different diseases.

Nature of Disease

Perfringens food poisoning is the term used to describe the common acute food-borne illness caused by *C. perfringens*. In most instances, poor temperature control is the cause of perfringens food poisoning especially where large quantities of food such as meats, meat products, gravy, and poultry are prepared several hours before serving. Under these conditions, *C. perfringens* (usually type A) proliferates with little or no toxin production. Upon ingestion, the organism starts to sporulate after encountering the acidic conditions found in the stomach and produces toxins, which are released in the gastrointestinal tract. Food poisoning by *C. perfringens* is characterized by intense abdominal cramps and diarrhea, which begin 8–22 h after consumption of tainted food. The illness is usually over within 24 h but less severe symptoms may persist in some individuals (elderly or infirm) for 1–2 weeks. Ingesting food contaminated with the type C strain of the organism may causes a more serious but rare illness in humans that involves invasion of the intestine. In animals, especially domesticated ones, enteritis is almost always fatal. *C. perfringens*-related livestock infections have been reported in every state in the United States and in most parts of the world.

C. perfringens is the most important of the histotoxic clostridia that cause tissue infections in humans, especially of the muscle tissue (clostridial myonecrosis or gas gangrene). The organism is more aerotolerant than most other anerobes. In addition to toxins and enzymes, many of which have lethal, cell-destroying and hemolytic properties, a number of nonlethal enzymes are also produced and apparently contribute to the invasiveness of the organism in the tissue. These include collagenase, deoxyribonuclease, and hyaluronidase.

Control

In most instances, poor temperature control of prepared food is the cause of acute *C. perfringens*-related food poisoning. Therefore, it is important to keep hot foods hot (above 140°F) and cold foods cold (below 40°F) before serving. Perfringens food poisoning is a mild, self-limiting disease. Symptomatic and supportive therapy, including fluid and electrolyte replacement, is normally adequate intervention. The toxicity is produced by a toxin and is not an invasive infectious process; therefore, antibiotics play no role in its management. In some instances where a particular strain of *C. perfringens* has been isolated from domesticated animals, a vaccine can be produce to target the disease.

See also: Gastrointestinal System.

Further Reading

Brynestad S and Granum PE (2002) *Clostridium perfringens* and foodborne infections. *International Journal of Food Microbiology* 74(3): 195–202.

Salyers AA and Whitt DD (1994) *Bacterial Pathogenesis: A Molecular Approach*. Washington, DC: American Society for Microbiology.

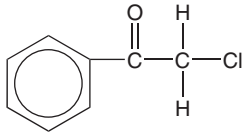
Sarker MR, Singh U, and McClane BA (2000) An update on *Clostridium perfringens* enterotoxin. *Journal of Natural Toxins* 9(3): 251–266.

CN Gas

Harry Salem, Bryan Ballantyne, and Sidney A Katz*

Published by Elsevier Inc.

- Military Designation: CN (Mace)
- Chemical Name: Chloroacetophenone
- Chemical Abstracts Service Registry Number: CAS 532-27-4
- Synonyms: Tear gas; Less-than-lethal; Nonlethal; Lacrimator, Harassing agent; Incapacitant; 2-Chloro-1-phenylethanone; 2-Chloroacetophenone, chloroacetophenone, phenacyl chloride; Chloromethyl phenyl ketone
- Chemical Formula: C_8H_7ClO
- Chemical Structure:



Pharmacological Action

Riot control agents such as CN are those that cause disabling physiological effects when they come into contact with the eyes or skin, or when inhaled. They have the capacity to cause intense sensory irritation of the skin and mucous membranes of the eye and respiratory tract. They are peripheral sensory irritants that pharmacologically interact with sensory nerve receptors in skin and mucosal surfaces at the site of contamination resulting in local pain and discomfort sensations with associated reflexes. The reflex associated with the inhalation exposure of irritants is the Kratschmer reflex. This reflex causes apnea, bradycardia, and a biphasic fall and rise in aortic blood pressure.

Pharmacological Class

CN is a peripheral sensory irritant, lacrimator, and incapacitant.

Uses

CN is used as a nonlethal or less-than-lethal chemical in riot control situations, to distract, deter, incapacitate, disorient, or disable disorderly people, to clear facilities, areas, deny areas, or for hostage rescue. It can also be used in peacekeeping operations. It is also used in military training as a confidence builder for the protective mask.

Exposure Routes and Pathways

CN is a white solid with low vapor pressure that can be dispensed as a fine powder or as a jet or stream of solution from small or large spray tanks, as well as aerosols or smokes by pyrotechnic generation. Its solubility in water is limited, but it is soluble in organic and chlorinated organics. Exposure of eyes, nose, mouth, skin, and respiratory tract produces irritation and pain. If swallowed, CN may produce vomiting.

Toxicokinetics

Evaporation of organic solvent may concentrate CN in the eyes and intensify damage. Hydrolysis of CN is very slow in water and is difficult to decompose. Environmental contamination may be persistent and difficult to remove.

Mechanism of Toxicity

CN is considered less than lethal or nonlethal because it has a large safety ratio. That is, its effective dose or concentration ECt_{50} is low compared to its lethal dose or concentration (LCt_{50}). In the body, CN is converted to an electrophilic metabolite. It is an SN2 alkylating agent that reacts with SH groups and other nucleophilic sites of biomolecules. Alkylation of SH-containing enzymes leads to enzyme inhibition with disruption of cellular processes. CN was found to inhibit human plasma cholinesterase via a non-SH interaction, and some of the toxic effects may be due to alkylation of SH-containing enzymes.

CN as well as CS is an SN2-alkylating agent with activated halogen groups that react readily at nucleophilic sites. The prime targets include sulfhydryl-containing enzymes such as lactic dehydrogenase. Alkylation of SH-containing enzymes leads to enzyme inhibition with disruption of cellular processes. It has been suggested that tissue injury may be related to inactivation of certain of these enzyme systems. The initial response to the inhalation of CN or other sensory irritants is consistent with the Kratschmer reflex and the Sherrington pseudoaffective response. These aerosols stimulate the pulmonary irritant receptors to produce bronchoconstriction and

*The views of authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

increased pulmonary blood volume by augmenting sympathetic tone. The chlorine atoms released from CN on contact with skin and mucous membranes are reduced to hydrochloride acid that can cause local irritation and burns. CN was also found to inhibit human plasma cholinesterase via a non-SH interaction.

Human Toxicology

The incapacitant effects of CN in human volunteers during exposure included lacrimation, some blurring of vision, and conjunctivitis. On the nose and throat, CN causes a tingling sensation, irritation, pain, and some increase in secretions; while on the respiratory track it causes irritation, burning, and pain. CN on the skin causes burning in the periorbital area, and other areas of tender skin, especially where sweating is present. Occasionally, nausea and gagging occur during and soon after exposure. Most of these effects disappear within 20 min after exposure, but conjunctivitis and blepharospasm usually disappear after a few days leaving no aftereffect. Incapacitating dosages (ICt_{50}) of CN have ranged from 20 to 50 mg min m^{-3}. The estimates of human LCt_{50} values, extrapolated from animals exposed to CN dispersed from a solvent, is 7000 mg min m^{-3}, and 14 000 mg min m^{-3} when dispersed from commercially available grenades. Other estimates range from 8500 to 25 000 mg min m^{-3}. The maximum safe inhalation dosage of CN for humans is estimated to be 500 mg min m^{-3}. Acute injuries to the eyes, primarily from effects of blast and missiles, may occur from tear-gas weapons such as pen guns. The immediate effects of these injuries include swelling and edema of the lids with penetration of skin, conjunctivitis, cornea, sclera, or globe by gunpowder and CN. Conjunctival ischemia and chemosis, corneal edema, erosion, inflammation or ulceration, and focal hemorrhage have been reported. A few hand injuries resulting from accidental discharges of tear gas guns at close range have been reported. Surgery was required in all to relieve pain and to remove the foreign material. All of these few victims suffered continuing pain and some loss of sensation, apparently from the toxic action of CN on nerves.

Clinical Management

Effects of exposure in open air are generally self-limiting and require no specific therapy. Most effects disappear in 15–30 min following exposure, although erythema may persist for an hour or longer.

CN can produce intense blepharospasm, pain, lacrimation, conjunctival erythema, periorbital edema, and a rise in intraocular pressure. These generally diminish within 30 min postexposure. CN also produces rhinorrhoea, nasal irritation and congestion, bronchorrhoea, sore throat, cough, sneezing, and unpleasant taste and burning of the mouth immediately after exposure. These effects rapidly resolve within minutes postexposure. Symptomatic treatment of ocular irritation consists of use of a topical solution to relieve the irritation with topical antibiotics. The eyes should be examined for corneal abrasions. Treatment with oral analgesics, topical antibiotics, and mydriatics should be considered. Since CN is a solid, it is possible for a particle or clump to become embedded in the cornea or conjunctiva and cause tissue damage. Medical care for eye pain after exposure should include thorough decontamination of the eyes and a thorough ophthalmological examination. The injured eye should be carefully irrigated with isotonic saline and the remaining powder removed with a cotton swab. Any remaining stromal particles should be removed with a needle tip under slit lamp illumination. Airway problems may occur in individuals with lung disease, especially if exposed to higher than average field use concentrations. If these occur, the immediate priority is the removal from the exposure and to ensure a patent airway.

Severe and prolonged erythema or severe dermatitis may occur several hours after exposure followed by vesiculation. These are generally second-degree burns and should be treated as such.

If the release of irritant incapacitants is in a confined, unventilated space, exposure may be to very high concentrations. Some individuals may be more susceptible to high concentrations, possibly because of an existing medical condition such as asthma, and will require intensive supportive medical treatment post exposure.

Animal Toxicology

Acute and sublethal effects following aerosol exposure from commercially available thermal grenades or from acetone solutions in experimental animals were lacrimation, conjunctivitis, copious nasal secretions salivation, hyperactivity, lethargy, and dyspnea, which occurred in all animals. Effects on the skin of exposed animals were primarily erythema. The estimated LCt_{50} values calculated for CN in the various animal species were 8878 mg min m^{-3} in the rat, 7984 mg min m^{-3} in the guinea pig, and 7033 mg min m^{-3} in the dog. The pathological findings in the animals that died from inhalation of CN consisted of congestion of the alveolar capillaries, alveolar hemorrhage, and excessive secretions in the bronchi and

bronchioles, as well as areas of acute inflammatory cell infiltration of the trachea, bronchi, and bronchioles. The early deaths exhibited lesions of the upper respiratory tract, with marked pseudomembrane formation, excessive salivation, and nasal secretion. The animals that died later exhibited edema, and hemorrhage of the lungs. In repeated exposures for 10 consecutive days in guinea pigs, dogs, and monkeys, the toxicity of CN was found to be considerably less when administered in divided doses. Overall, studies demonstrated a lack of cumulative toxicity. Changes in biochemical endpoints measured following multiple exposures of CN in mice were a decrease in hepatic glutathione and increased lipid peroxidation. Hepatic acid phosphatase increased after the 5 day exposure to CN, and the glutathione levels decreased after 10 day CN exposures. CN-induced elevation in acid phosphatase levels reflected the release of lysosomal enzymes from the liver, indicative of tissue injury. Additionally, hyperglycemia was observed after exposure to CN. Stress-mediated release of epinephrine is known to elevate glucose levels and thus may be responsible for the hyperglycemia. Significant decreases in body weight gain were also noted on exposure to CN. Histopathologic changes following CN exposures included hemorrhage, perivascular edema, congestion of the alveolar capillaries, occluded bronchioles, and alveolitis. Renal histopathology demonstrated congestion and coagulative necrosis in the cortical renal tubules in CN-exposed mice. Hepatic histopathology consisted of cloudy swelling and lobular and centrolobular necrosis of hepatocytes following CN exposures.

At high concentrations, CN may result in chemical injury to the eye with corneal and conjunctival edema, erosion or ulceration, chemosis, and focal hemorrhages. CN-induced ocular effects on the rabbit eye following exposure to various formulations included lacrimation, chemosis, iritis, blepharitis, and keratitis, with severity dependent on the formulation.

CN is also a potent skin irritant that may cause serious injury to the skin that includes severe generalized itching, diffuse and intense erythema, severe edema, and vesication. CN is considered a potent skin irritant, and sensitizer.

In 2 year carcinogenicity inhalation bioassays in rats and mice, there were no indications of carcinogenicity in male rats, while equivocal evidence was found in female rats. These findings were evidenced by increased fibroadenomas of the mammary gland. In these 2 year studies in mice, there was no evidence of carcinogenic activity in males and females.

Decontamination

Contaminated clothing should be removed and sealed in a plastic bag. Disposable rubber gloves should be used when handling contaminated clothes. The eyes should be irrigated copiously with saline for 15–20 min. Contaminated skin should be washed thoroughly with copious amounts of water, alkaline soap and water, a mildly alkaline solution (sodium bicarbonate or sodium carbonate), or mild liquid soap and water. The use of sodium hypochlorite solution will exacerbate the skin lesions and should not be employed. Only a saline irrigation should be used over vesiculated skin.

See also: CS Gas; Non-Lethal Weapons, Chemical; Riot Control Agents.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Coal Tar

Richard D Phillips

- Chemical Abstracts Service Registry Number: CAS 8007-45-2
- Synonyms: Coal tar; Creosote; Coal tar pitch

Uses

Coal tar is a viscous liquid mixture of hydrocarbon compounds, derived along with coke, from the destructive distillation of coal in coking ovens. Coal tar itself may be subjected to distillation, a process that separates groups of the components of coal tar from groups of others. The substances derived from this process are often called ‘coal tar distillates’.

Coal tar products are ingredients in medicines used to treat skin diseases such as psoriasis. Coal tar, along with coal tar pitch and car tar pitch volatiles, are used or produced in several industries including roofing, aluminum, smelting, road paving, rubber production, and coking.

Exposure Routes and Pathways

Most people are exposed to very low levels of coal tar. In the general population, exposure is most likely through products that contain coal tar or similar materials to improve a health problem such as eczema or psoriasis. Occupational exposure to coal tar could occur through contact with the skin or by inhalation exposure to volatile fractions when coal tar is heated.

Toxicokinetics

Coal tar is a complex mixture of hydrocarbons including polyaromatic hydrocarbons (PAHs). Coal tar constituent can enter the body through the lungs, skin, and by ingestion. There is no information that describes how fast or how much of coal tar might enter the body after one or several exposures.

Generally, the PAH components of coal tar are metabolized by oxidative enzymes in the liver and lungs to generate active metabolites that can bind to macromolecules. Principal metabolic products include phenols, dihydrodiols, quinones, anhydrates, and conjugates (e.g., glutathione) of these products.

Mechanism of Toxicity

Defining a general mechanism of toxicity for coal tar is difficult because of the diversity and variability in biological effect and composition. However, PAHs are a major constituent, and their carcinogenic activity is related to their metabolism to reactive intermediates that bind to macromolecules, thus initiating a carcinogenic response. The degree and magnitude will vary based on the composition of the coal tar.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute and prolonged periods of dermal exposure to coal tar in animals are associated with dermal irritation and more severe damage.

Dermal exposure to coal tar products is also toxic to animals but at fairly high doses. For example, the dermal LD_{50} in rabbits is greater than $7950\,mg\,kg^{-1}$.

Inhalation exposure of rats and mice to high doses of coal tar has produced some toxic effects in the form of weight and morphologic changes to the lymphoreticular tissues.

Coal tar products do not appear to be potent reproductive toxins based on experimental findings. Treatment of pregnant rats with coal tar by all routes of exposure produced reduced growth of offspring and an increase with incidence of cleft palate and small lungs.

Human

Coal tar exerts its acute toxic effects in humans primarily via dermal contact, causing structural damage to the tissues that it comes in contact with, such as the skin and eyes. In addition, respiratory effects are likely to occur after inhalation exposure to coal tar products. Over exposure to coal tar is likely to produce a number of systemic effects in the central nervous system, liver, and kidney.

Cutaneous photosensitivity from coal tar pitch has been described.

Chronic Toxicity (or Exposure)

Animal

Coal tar applied to the skin of 259 mice every third or fourth day resulted in skin papillomas in half of the mice that survived 100 days.

Light and heavy coal tar oil were applied dermally for the lifespan of mice or until persistent papillomas developed at the application site. Test solutions were applied three times weekly in male and female mice. Both produced skin tumors: the light coal tar oil contained benzene, toluene, xylene, solvent naphtha, and was the residual oil drained from a naphthalene recovery operation; the heavy coal tar oil was a mixture of creosote, anthracene oils, and the oil drained from the naphthalene recovery operation. The heavy oil was less potent compared to the effects observed in the BaP group of the study.

Five different coal tars were tested for carcinogenic potency in mice. Four samples were crude tars from the coking of bituminous coal, and one sample was produced by the coking of lignite coal. Lengths of exposure were not reported for any of the groups studied. Tumors developed in all five groups. Onset of tumors was delayed when animals were washed with aqueous detergent 5–60 min after application.

In a study with hard coal tar pitch (50% in benzene) and soft coal tar pitch (50% in benzene), 30 mice per group plus controls were skin-painted with one drop of test material twice weekly for 5 months. The hard-pitch solution produced an average of one papilloma/mouse in 21 surviving mice. The soft-pitch solution produced an average of 2.9 papillomas/mouse in 28 surviving mice, plus 14 mice with malignant tumors. Soft pitch was more carcinogenic than hard pitch, and pitches were more carcinogenic than coal tars tested in the same study.

Human

Various case reports and the results of cross-sectional occupational surveys associated chronic occupational exposure to coal tar products with the development of skin cancer. Disease etiology included the development of dermatoses, such as squamous papillomas, that progressed to carcinoma, usually squamous-cell carcinoma. Cancer of the scrotum in chimney sweeps has also been associated with prolonged exposure to coal tar creosote. The latency period for the development of dermatoses, such as squamous papillomas, was usually 20–25 years. Worker exposure in the past was much greater than it now is because of less sophisticated industrial practices used in the past, the lack of knowledge concerning occupational hygiene, and the current recognition of the dangers of excessive exposure to the health of workers.

However, no association between exposure to coal tar products and cancer in humans has been found.

In Vitro Toxicity Data

Coal tar is mutagenic in the *Salmonella typhimurium* assay and the mouse lymphoma assay in the presence of an exogenous mammalian metabolic system.

Clinical Management

Emergency management of direct cutaneous exposure to creosote is prompt and comprehensive decontamination. Treatment commonly includes removal of all contaminated clothing and washing of the skin, hair, and nails with large volumes of soapy water. In order to reduce dermal irritation from creosote-contaminated soil and water, wearing protective clothing, and immediate washing of exposed skin will limit exposure.

The treatment to manage exposure from ingestion also focuses on the acute effects of the phenolic and PAH components. Phenolic compounds cause corrosive esophageal burns, and there may also be a risk of causing pneumonitis in the patient by aspiration of PAHs in coal tar derivatives, so emesis is contraindicated as a means of elimination.

The treatment for contaminated eyes commonly includes irrigation with copious amounts of room temperature water, or saline if available, for at least 15 min.

Should an inhalation exposure occur, treatment commonly includes moving the exposed individual to fresh air and monitoring for respiratory distress.

Environmental Fate

As with other chemical mixtures, the fate and transport processes affecting coal tar can be extremely complex. Coal tar components may partition to the air, water, soil, or biota depending on their physical and chemical properties. Compounds initially released into the atmosphere may undergo atmospheric deposition and reach surface water directly or through runoff carrying soil-bound compounds.

Coal tar constituents released into surface waters will differentially partition to the water column or to sediments depending on their water solubility and sorptive properties. For example, PAHs, the major constituents of coal tar, generally tend to sorb strongly to soil and sediment particulates, and often have low aqueous solubilities and mobility. Many components in the PAH fraction, particularly the higher molecular weight PAHs, will remain in a virtually stationary tar-like mass at the place where they were deposited.

Many of the same bacteria and fungi capable of biodegrading coal tar components in aqueous systems can be found in soils. Especially where the coal tar is close to the surface and under aerobic conditions, the vast majority of the phenolics can be consumed in less than a year. The majority of the lighter fractions of the PAH components (from 53 to 75% by weight) can be biodegraded within 2 months.

Ecotoxicology

For fish and wildlife, the aromatics of coal tar pose the most danger. The aquatic toxicity in the water-soluble fraction is mostly from the aromatics.

See also: Charcoal; Coke Oven Emissions; Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

IARC (1985) *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, vol. 35, pp. 83–159.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Creosote (including Wood Creosote, Coal Tar Cresote, and Coal Tar).

Cobalt

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-48-4
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Co^{2+}

Uses

Cobalt is a relatively rare metal produced primarily as a by-product of the mining of other metals, chiefly copper. It is the essential trace element found in cyanocobaltamine (vitamin B_{12}). This vitamin protects against pernicious anemia and is required in the production of red blood cells. Medicinally, cobalt salts have been used to stimulate the formation of red blood cells in individuals suffering from anemia.

Commercially, cobalt is used primarily in high-temperature alloys, in tungsten carbide tools, and (with iron and nickel) in permanent magnets. Cobalt salts are used in pigments, in paint dryers, and as catalysts in the petroleum industry.

Background Information

Cobalt exists in valence states from 0 to 5, with the most stable ($+2$ and $+3$) being most common. While there is only one stable isotope of cobalt, there are a number of unstable isotopes. Two of these, cobalt-60 and cobalt-57, are in use commercially. Cobalt-60 is used for cancer treatment and for food irradiation. Cobalt-57 has research applications.

Exposure Routes and Pathways

For the general population, ingestion is the primary exposure pathway for cobalt. For persons working in industrial settings, inhalation is a significant pathway (e.g., carbide industry emissions and airborne particulate from grinding processes) as is dermal exposure. There can also be internal exposure from implanted medical devices.

Toxicokinetics

Oral ingestion of cobalt salts results in ready absorption, probably in the jejunum. Although cobalt is readily absorbed, increased levels do not tend to cause significant accumulation. The majority (80%) of cobalt is excreted in the feces of rats and cattle. In contrast, in humans, ~80% of absorbed cobalt is excreted via the urine and 15% is excreted in the feces by an enterohepatic pathway. Breast milk and sweat are secondary routes of excretion. The total body burden for the average person is estimated as 1.1 mg. Muscle contains the greatest mass of cobalt but the highest concentrations are found in fat. Cobalt present in the blood is associated with the red blood cells.

Mechanism of Toxicity

Cobalt most often depresses the activity of enzyme including catalase, amino levulinic acid synthetase, and P-450, enzymes involved in cellular respiration. The Krebs citric acid cycle can be blocked by cobalt resulting in the inhibition of cellular energy production. Cobalt can replace zinc in a number of zinc-required enzymes like alcohol dehydrogenase. Cobalt can also enhance the kinetics of some enzymes such as heme oxidase in the liver. Cobalt interferes with and depresses iodine metabolism resulting in reduced thyroid activity. Reduced thyroid activity can lead to goiter.

Acute and Short-Term Toxicity (or Exposure)

Human

Ingestion of cobalt may result in the production of an unusually high number of red blood cells (similar to a cancer of red blood cells (polycythemiavera)). Ingestion of cobalt salts (once added to beer as a defoaming agent) has resulted in cardiomyopathy. The signs and symptoms of cardiomyopathy due to beer consumption are similar to those of congestive heart failure. Autopsy results indicated a 10-fold increase in cobalt concentrations in heart tissue. The alcohol may have potentiated cobalt absorption or toxic effects.

Chronic Toxicity (or Exposure)

Animal

When implanted intramuscularly in rats, cobalt metal produced fibrosarcomas at the site but no other routes of exposure have elicited a carcinogenic response.

Human

Cobalt is an essential nutrient at low levels ($\sim 40\,\mathrm{mg\,day^{-1}}$). In industrial settings, inhalation of high concentrations of cobalt compounds has led to hard-metal pneumoconiosis, which may result in interstitial fibrosis. Workers with this condition typically develop hypersensitivity to cobalt compounds (symptoms include coughing and wheezing). A few workers have developed skin hypersensitivity after dermal contact with cobalt and its compounds. Cobalt can cause cardiomyopathy and (if inhaled as a dust) interstitial lung disease.

Clinical Management

The oil-soluble BAL (British Antilewisite; 2,3-dimercaptopropanol) appears to be the antidote of choice for cobalt poisoning.

Environmental Fate

The sources of cobalt in the environment are both natural and man-made (anthropogenic). Natural sources include soil, seawater spray, volcanic eruptions, and forest fires. Anthropogenic sources include combustion of fossil fuels, metal smelting, sewage sludge, and processing of cobalt alloys. Cobalt is found in the atmosphere in particulate form and returns to the Earth's surface through dry deposition and with rain or snow. Once in surface water, cobalt generally moves into sediment. Cobalt does not appear to biomagnify significantly in the aquatic food chain. The cobalt that does accumulate in fish is largely found in the nonedible parts of the fish.

Under normal environmental conditions, cobalt is expected to bind strongly to soil and thus migration through soil would be very limited. Cobalt in soil can be taken up by plant roots and root vegetables but is not translocated to the aboveground parts of plants.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value – time-weighted average for cobalt (elemental and inorganic compounds) is $0.02\,\mathrm{mg\,m^{-3}}$. ACGIH classifies cobalt as an animal carcinogen.

See also: Metals.

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Cobalt.

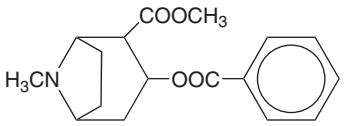
Cocaine

Michael Wahl

This article is a revision of the previous print edition article by William A Watson, volume 1, pp. 355–356, © 1998, Elsevier Inc.

- SYNONYMS: Ecgonine methyl ester benzoate; Benzoylmethylecgonine; [1*R*-(*exo*–*exo*)]-3-(benzoyloxy)-8-methyl-8-azabicyclo[3,21]octane-2-carboxylic acid methyl ester; Crack; Rock; Toot; Blow; Snow; Dama blanca; Coke; Lady
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: A naturally occurring alkaloid with local anesthetic and vasoconstrictor properties and central nervous system stimulant and euphoric effects
- CHEMICAL FORMULA: $C_{17}H_{21}NO_4$

- Chemical Structure:



Uses

Cocaine is used for topical local anesthesia of mucous membranes. It is also a drug of abuse.

Exposure Routes and Pathways

Smoking the base alkaloid (as crack or freebase) and nasal insufflation of the hydrochloride salt are the most common routes of exposure in abuse. Intravenous injection and application of the hydrochloride salt to mucous membranes are also methods of abuse. In therapeutic use, a solution of hydrochloride salt is applied to mucous membranes.

Toxicokinetics

Smoked cocaine is absorbed in seconds from the lungs, which results from volatilization of the alkaloid. Peak plasma concentrations occur within a few minutes. Absorption through mucous membranes is initially rapid, then slowed secondary to the vasoconstrictive effects of cocaine. Peak plasma concentrations occur within 1 h after oral ingestion and nasal application. After oral administration, bioavailability is decreased secondary to presystemic hydrolysis in the gastrointestinal tract.

Cocaine is metabolized by hydrolysis to benzoylecgonine, by cholinesterase to ecgonine methyl ester, and hepatically to norcocaine and ecgonine. In the presence of ethanol, cocaine is also metabolized to cocaethylene. Cocaine metabolism is probably dose dependent at the high doses that are abused, especially with binge use. Cocaine is widely distributed in the body with an apparent volume of distribution of 1.2–1.9 l kg^{-1}. It rapidly appears in the central nervous system (CNS) and crosses the placenta. The elimination half-life of cocaine is ~1 h at doses of less than 2 mg kg^{-1}. The measurement of low concentrations of cocaine in chronic users suggests half-lives as long as 3 days. Low doses of cocaine are excreted in the urine primarily as metabolites, with less than 10% of the dose found as unchanged cocaine.

Mechanism of Toxicity

Cocaine toxicity is primarily secondary to its ability to prevent the reuptake of neurotransmitters including serotonin, dopamine, and norepinephrine. Direct cardic toxicity may be due to inhibition of sodium influx across the nerve cell membrane (type 1C antidysrhythmias). The role of cocaine metabolites in producing clinical toxicity is unclear. Cocaethylene, ecgonine methyl ester, and benzoylecgonine may produce some toxicity. Additional effects of cocaine, which are important in clinical toxicity, include direct vasoconstriction, increased cellular oxygen consumption, increased platelet aggregation, and direct organ toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal models have demonstrated acute toxicity similar to that present in humans. Dogs develop toxicity at lower doses than rats, and death appears to be associated with the development of hyperthermia. The functional status of organ enervation and the presence of anesthetics may alter cocaine toxicity in animal models.

Human

Cocaine toxicity can present after a wide range of doses, with reports of toxicity at doses of less than 1 mg kg^{-1}. Toxicity includes most organ systems, and cocaine use and culture increases the risk of trauma and infections. Acute tolerance to many CNS and cardiovascular effects of cocaine develops. Kindling, the lowering of the seizure threshold with repeated subtoxic doses, can also occur. The CNS toxicity of cocaine includes stimulation, euphoria, agitation, seizures, intracranial hemorrhage, and, with larger doses, coma. Cardiovascular toxicity can include tachycardia, hypertension, coronary artery spasm, myocardial ischemia and infarction, bradycardia, hypotension, cardiovascular collapse, dysrhythmias, and sudden death. Pulmonary toxicity after smoking the alkaloid form of cocaine includes hemorrhage, barotrauma including pneumomediastinum, pulmonary edema, and 'crack lung', a hypersensitivity reaction that includes fever, productive cough, pulmonary infiltrates, and bronchospasm. Other toxicities seen with cocaine use include hyperpyrexia, rhabdomyolysis, metabolic acidosis, and respiratory alkalosis. Cocaine use during pregnancy can result in an increased risk of abruptio placentae, spontaneous abortion, and low-birth-weight infants with congenital malformations and potentially neurobehavioral impairment. Nasal insufflation of single doses of cocaine results in plasma concentrations of 100–500 ng ml^{-1}. Blood cocaine concentrations in fatalities are described as averaging ~6 mg l^{-1}, with a very wide range of reported concentrations of cocaine and metabolites.

Chronic Toxicity (or Exposure)

Animal

Animal models of chronic, self-administered cocaine use show regular patterns of use and abstinence. Administration of cocaine during a period of self-induced abstinence from cocaine restarts this cycle. Animals with free access 24 h a day to cocaine showed weight loss, self-mutilation, and death within 2 weeks.

Human

Toxicity associated with chronic use is not as well described as acute toxicity, but it appears to include cerebral atrophy, cardiomyopathy, and chronic pulmonary disease. Cocaine and its metabolites are most commonly identified in patient urine. An immunoassay directed toward identification of benzoylecgonine will frequently indicate the presence of cocaine and its metabolites for many days after use. The duration of qualitatively detected cocaine and metabolites in urine is probably dose dependent and may be up to 3 weeks in length. Chronic use of cocaine may lead to dependence.

In Vitro Toxicity Data

Cocaine has been demonstrated to covalently modify proteins *in vitro*. This finding has been seen in animals and humans chronically exposed to cocaine. Modified proteins are immunogenic and may explain why some people develop autoimmune effects after chronic cocaine exposure.

Clinical Management

The initial management of acute cocaine intoxication should include assessment and management of the patient's airway, breathing, and circulation. Supplemental oxygen and a benzodiazepine are frequently indicated for agitation and CNS stimulation. Many findings, such as hyperpyrexia, seizures, and rhabdomyolysis, should be managed using the basic treatment approaches for the complication. Concurrent use of alcohol and other drugs is frequent and should be considered during initial assessment. Treatment of cardiac toxicity should also include supportive care, oxygen, and a benzodiazepine. Beta-blockers can theoretically cause unopposed alpha stimulation; this may lead to paradoxical worsening of hypertension and vasoconstriction. If a beta-blocker is to be used, a short-acting beta-blocker (e.g., esmolol) is preferred. Nitrates, opiates, thrombolytics, and/or cardiac catherterization may be employed when appropriate based on the clinical and laboratory findings.

See also: Neurotoxicity.

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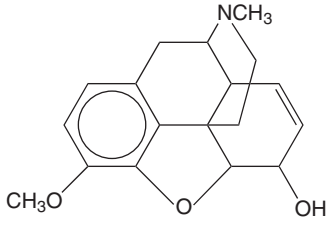
Codeine

F Lee Cantrell

- Chemical Abstracts Service Registry Number: CAS 76-53-3
- Synonyms: Methylmorphine; Morphine monomethyl ether
- Chemical/Pharmaceutical/Other Class: Opioid analgesic/antitussive
- Chemical Formula: $C_{18}H_{21}NO_3$
- Chemical Structure:



Uses

Codeine is used as an analgesic and an antitussive.

Exposure Routes and Pathways

Codeine is customarily ingested in the form of tablets and liquid preparations.

Toxicokinetics

Codeine is well absorbed via oral and intramuscular routes of administration; it is two-thirds as effective orally as parenterally. Peak serum levels are attained in 30–60 min. Codeine is metabolized in the liver by *O*-demethylation and *N*-demethylation and partial conjugation with glucuronic acid. The volume of distribution is $3.5\,l\,kg^{-1}$. Greater than 95% of a single dose is eliminated in 48 h by the kidneys. The elimination half-life is 1.9–4 h. Codeine metabolites are conjugated codeine, norcodeine, conjugated norcodeine, conjugated morphine, and hydrocodone.

Mechanism of Toxicity

Codeine stimulates opiate receptors in the central nervous system (CNS), producing sedation and respiratory depression.

Acute and Short-Term Toxicity (or Exposure)

Animal

Opioids have an excitatory effect on the CNS of cats and horses. Dogs experience similar CNS depressant effects as seen in humans. Death can occur within 12 h. Naloxone has been effective in treating animals.

Human

Depressant effects on the CNS are the most profound. Nausea, vomiting, and miosis may develop within 1 h. Infants and children may demonstrate unusual sensitivity while habituated adults may have extreme tolerance to opioids. In children, greater than $1\,mg\,kg^{-1}$ may produce serious symptoms and $5\,mg\,kg^{-1}$ may cause significant respiratory depression. The estimated lethal adult dose of codeine is $7–14\,mg\,kg^{-1}$.

Chronic Toxicity (or Exposure)

Animal

There is no evidence of carcinogenicity in 2 year feeding studies of mice and rats at doses up to 3000 ppm.

Human

Codeine is often subject to abuse and can cause a withdrawal syndrome after abrupt discontinuation of use.

In Vitro Toxicity Data

In vitro clearance of opiates is altered in hepatic cells obtained from mice with human alpha-globin and sickle beta-globin transgenes than in control mice.

Clinical Management

Basic and advanced life-support measures should be performed as necessary. Gastrointestinal decontamination procedures should be considered for substantial recent ingestions. Activated charcoal will adsorb codeine. Patients with respiratory or CNS depression can be treated with intravenous boluses of naloxone. A continuous naloxone infusion may be necessary if the toxic effects of codeine persist longer than the duration of action of naloxone.

See also: Charcoal; Hydrocodone; Morphine.

Further Reading

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Coke Oven Emissions

Shashi K Ramaiah and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 8007-45-2
- Synonyms: Primary synonym is coal tar pitch volatiles such as benzene soluble organics. However, synonyms vary depending on the specific constituents in the emissions; RTECS No. GH0346000

Uses

The primary use of coke is a fuel reductant and support for other raw materials in iron-making blast furnaces. Coke is also used to synthesize calcium carbide and to manufacture graphite and electrodes, and coke-oven gas is used as a fuel. Coal tar, a by-product of the production of coke from coal, is used in the clinical treatment of skin disorders such as eczema, dermatitis, and psoriasis.

Background Information

Petroleum coke is a chunky powdered carbon product derived from petroleum. If petroleum coke is heated to a high temperature, it may emit volatiles such as polynuclear aromatic hydrocarbons, which could be suspect carcinogens. Such exposures can occur in coke oven workers.

The production of coke by the carbonization of bituminous coal leads to the release of chemically complex emissions from coke ovens that include both gases and particulate matter of varying chemical composition. The chemical and physical properties of coke oven emissions vary depending on the constituents. The emissions include coal tar pitch volatiles (e.g., particulate polycyclic organic matter, polycyclic aromatic hydrocarbons, and polynuclear aromatic hydrocarbons), aromatic compounds (e.g., benzene and β-naphthyl amine), trace metals (e.g., arsenic, beryllium, cadmium, chromium, lead, and nickel), and gases (e.g., nitric oxides and sulfur dioxide).

Exposure Routes and Pathways

The primary routes of potential human exposure to coke oven emissions are inhalation and dermal contact. Occupational exposure to coke oven emissions may occur for those workers in the aluminum, steel, graphite, electrical, and construction industries. Coke oven emissions can have a deleterious effect on human health. Coke oven emissions contain literally several thousand compounds, several of which are known carcinogens and/or cocarcinogens including polycyclic organic matter from coal tar pitch volatiles, β-naphthylamine, benzene, arsenic, beryllium, cadmium, chromate, lead, nickel subsulfide, nitric oxide, and sulfur dioxide. Most regulatory attention has been paid to coal tar pitch volatiles.

Toxicokinetics

Since coke oven emissions are complex mixtures of coal and coke particles, specific information is not available. In general, coke oven emissions are well absorbed from the respiratory tract, skin, and the conjunctiva.

Mechanism of Toxicity

Complex chemical mixtures in coke oven emissions is known to result in DNA adduct formation. Free oxygen radicals and CYP450 are implicated in the pathogenesis. Polycyclic aromatic hydrocarbons, which are primary compounds in coke oven emissions generated by the coking process, cause cancer and mutagenesis by a multitude of mechanisms including DNA adduct formation and metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal studies have reported weakness, depression, dyspnea, general edema, and effects on the liver from acute oral exposure to coke oven emissions.

Human

Acute exposure to coke oven emissions produces irritation of the eyes, respiratory symptoms like cough, dyspnea, and wheezing.

Chronic Toxicity (or Exposure)

Animal

In animals, extracts and condensates of coke oven emissions were found to be carcinogenic in both inhalation studies and skin painting bioassays. The mutagenicity of whole extracts and condensates, as well as their individual components, provides supportive evidence for carcinogenicity. In addition, several inhalation exposure studies in laboratory animals have provided evidence of the carcinogenic effect of aerosols of coal tar and its fractions. The extract was found to produce papillomas and skin carcinomas in the mice and acted as an initiating agent, although the extent to which this extract is representative of coke oven emissions is uncertain since the sample was contaminated with particulate matter from ambient air. Numerous carcinogenicity studies have shown that coal tar samples applied topically to the skin of laboratory animals produce local tumors.

Human

The emissions are investigated as a carcinogen, tumorigen, and mutagen. The cancer sites include

the skin, respiratory system, kidneys, and urinary bladder. Studies of coke oven workers have shown increased risk of mortality from cancer of the lung, trachea, and bronchus; cancer of the kidneys; cancer of the prostrate; and cancer at all sites combined.

Chronic occupation-related exposure is associated with significant excess mortality from cancer of the respiratory system and of the prostate. Depending on the segment of the population considered, the respiratory cancer risk for coke oven workers was as high as 4.5 times the risk for nonoven workers. To evaluate a biologically effective exposure dose in human biomonitoring studies, DNA carcinogen adduct analysis is frequently used.

Occupational Safety and Health Administration (OSHA) has not identified thresholds for carcinogens that will protect 100% of the population. It usually recommends that occupational exposures to carcinogens be limited to the lowest detectable concentration. To ensure maximum protection from carcinogens through the use of respiratory protection, only the most reliable and protective respirators are recommended. The OSHA permissible exposure limit (PEL) for benzene-soluble fraction of coke oven emissions is $0.150\,\mathrm{mg\,m^{-3}}$.

In Vitro Toxicity Data

In vitro toxicity data are not available for coke oven emissions.

Clinical Management

The exposed person should be moved to fresh air at once. If breathing has stopped, mouth-to-mouth resuscitation should be performed. The affected person should be kept warm and at rest. Exposed eyes should be washed immediately with large amounts of water; the lower and upper lids should be lifted occasionally. Medical attention should be obtained immediately.

Ecotoxicology

There is no aquatic toxicity information available for coke oven emissions.

Exposure Standards and Guidelines

The OSHA standard for coke oven emissions is a PEL of $0.15\,\mathrm{mg\,m^{-3}}$ as an 8 h time-weighted average (TWA). Under this standard, specific engineering and work practice control requirements became effective. OSHA has also promulgated a PEL of $<0.2\,\mathrm{mg\,m^{-3}}$ as an 8 h TWA for coal tar pitch volatiles. National Institute for Occupational Safety and Health (NIOSH) and OSHA have recommended work practices to minimize the harmful effects of exposure to coke oven emissions.

Miscellaneous

Coke production in the United States steadily increased between 1880s and 1950s, peaking at 72 million tons in 1951. In 1976, the United States ranked number two in the world with 52.9 million tons of coke or $\sim$14.4% of the world production. Although, the by-product process is designed to collect the volatile materials given off during the coke process, emissions escape because of structural defects around the doors or changing lids, improper use of engineering controls, improper work practices, and insufficient engineering controls.

See also: Combustion Toxicology; Pollution, Air; Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

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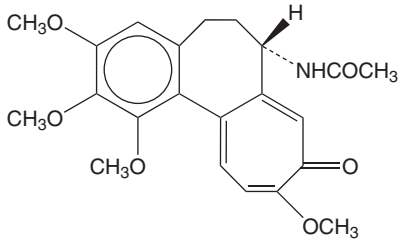
Colchicine

Henry A Spiller

- Chemical Abstracts Service Registry Number: CAS 64-86-8
- Chemical/Pharmaceutical/Other Class: Naturally occurring alkaloid
- Chemical Formula: $C_{22}H_{25}NO_6$
- Chemical Structure:



Uses

Colchicine is used in the treatment of acute gouty arthritis. Unlabeled uses include treatment of familial Mediterranean fever, neoplasms of the skin, and cirrhosis of the liver. It is available as a pesticide for moles and gofers.

Background Information

Colchicine is obtained from the autumn crocus, *Colchicum autumnale*, or the glory lily, *Gloriosa superba*. *C. autumnale* was first introduced for the treatment of gouty arthritis in 1763 by Von Storck.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to colchicine. It is available as an oral tablet and solution for injection.

Toxicokinetics

Colchicine is readily absorbed from the gastrointestinal tract. In therapeutic dosing, peak serum levels occur in 30–120 min. Colchicine undergoes deacetylation and hydrolysis in the liver. It has a rapid initial distribution phase, with a plasma half-life of 19 or 20 mm, suggesting swift uptake by the tissues. The volume of distribution is $2.2\,l\,kg^{-1}$. Up to 40% of colchicine is excreted in the urine, with 20–30% of this as unchanged drug. The majority of the drug undergoes enterohepatic recirculation and is excreted via bile and feces. The average elimination half-life is 20 h.

Mechanism of Toxicity

Colchicine binds to tubulin and prevents its polymerization into microtubules, subsequently disrupting microtubule function. Consequently, it alters nuclear structure, intracellular transport, and cytoplasmic motility, ultimately causing cell death. Colchicine is a potent inhibitor of cellular mitosis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal toxicity is primarily related to ingestion of the plant *C. autumnale*. Colchicine is available as a pesticide for burrowing animals. The estimated toxic dose for cows is $10\,g\,kg^{-1}$ with fresh leaves or 2 or $3\,g\,kg^{-1}$ with dried leaves. Symptoms may include gait disorders, hypersalivation, bloody vomitus, and diarrhea. Death within 72 h has occurred secondary to shock. It is only slightly toxic to cold-blooded and hibernating animals.

Human

Colchicine toxicity has been divided into three stages. The first stage, from 2 to 24 h, is the gastrointestinal phase, notable for abdominal pain, vomiting, diarrhea, and a prominent leukocytosis. The gastrointestinal symptoms may be relieved by atropine, but this does not prevent or alter the onset of the second stage. The second stage is marked by multisystem failure. Most life-threatening symptoms occur 24–72 h postexposure. Confusion, delirium, coma, seizures, and cerebral edema may occur. Progressive respiratory distress and pulmonary edema can occur. After an initial leukocytosis, bone marrow depression is seen with a nadir between the fourth and seventh days. Bone marrow depression, coupled with potential gastrointestinal hemorrhages and a hemolytic anemia, may produce profound anemia. Consumptive coagulopathy may also be seen. Renal function may be affected by direct organ damage as well as by decreased perfusion from profound and persistent hypotension. Cardiovascular instability along with metabolic acidosis may develop due to volume depletion, cardiac failure, and arrhythmias. Most deaths result from shock in the 24–72 h period. Stage three is the recovery phase. If patients survive to this convalescent phase, the main complication is sepsis.

Chronic Toxicity (or Exposure)

Animal

Colchicine has been shown to be teratogenic in mice and hamsters.

Human

Recently, a case report of development of colchicine induced cardiomyopathy was described. The patient also reported respiratory difficulty. Three weeks after discontinuation of colchicines, the patient returned to baseline status.

In Vitro Toxicity Data

Genotoxicity studies (micronucleus tests for chromosome aberrations) in mammalian polychromatic erythrocytes and nonhuman mammalian cell culture were positive. Ames *Salmonella* tests for mutagenicity have been negative.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Treatment of colchicine toxicity is largely supportive. Activated charcoal effectively adsorbs colchicines and should be administered for substantial recent ingestions. Aggressive early gastrointestinal decontamination may be life saving. Severe anemia may require packed red blood cell replacement. Coagulopathies may respond to vitamin K and fresh frozen plasma. Hypotension may be unresponsive to fluid replacement and pressor support. Due to rapid tissue distribution and the large volume of distribution, hemoperfusion and hemodialysis are ineffective. Colchicine Fab fragments have effectively reversed hypotension and increased survival in animals and humans in the research setting but are not commercially available. Colony stimulating factors have also been used for patients with profound bone marrow suppression from colchicine overdose.

Environmental Fate

No information is currently available on breakdown in soil groundwater or surface water. Colchicine alkaloids withstand storage, drying, and boiling.

See also: Atropine; Charcoal; Genomics, Toxicogenomics; Pesticides.

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Combustion Toxicology*

Barbara C Levin and Erica D Kuligowski

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Combustion toxicity research is the study of the adverse health effects caused by exposure to fire atmospheres. A fire atmosphere is defined as all of the effluents generated by the thermal decomposition of materials or products regardless of whether that effluent is produced under smoldering, nonflaming, or flaming conditions. The objectives of combustion toxicity research are to identify potentially harmful products from the thermal degradation of materials, to distinguish those materials that produce unusual or greater quantities of toxic combustion products, to determine the best measurement methods for the identification of the toxic products as well as the degree of toxicity, to determine the effect of different fire exposures on the composition of the toxic combustion products, and to establish the physiological effects of such products on living organisms. The ultimate goals of this field of research are to reduce human fire fatalities due to smoke inhalation, to determine effective treatments for survivors, and to prevent unnecessary suffering of fire casualties caused by smoke inhalation.

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Fire Death Statistics

The latest statistics from the (US) National Fire Protection Association (NFPA) report that the fire death rate in the United States was 1.3 times higher (2.1

times more if the New York City deaths from the September 11, 2001, terrorist attacks are included) than in the United Kingdom, 1.16 times more than in Sweden, 1.3 times less than Japan (or 1.3 times more if the deaths from the September 11, 2001 terrorist attacks are included), and about the same as that in Canada. Although the reasons are still being debated, the number of fire deaths per capita since 1977 have been higher in the United States and Canada than in most of the other industrialized countries outside of the former Soviet Union.

Fire statistics collected by NFPA indicated that 1 687 500 fires were reported in the United States in 2002, the latest year for which complete statistics are available at the time of this writing. Calculated another way, these statistics translate into a reported fire occurring in the United States every 19 s, in an outside property every 38 s, in a structure every 61 s, in a residence every 67 s, and in a motor vehicle every 96 s. These fires caused ~3380 civilian deaths and 18 425 reported injuries in 2002. Excluding New York City's World Trade Center deaths from the September 11, 2001, terrorist attacks in which 2326 civilian deaths occurred, the number of deaths in 2002 decreased by almost 10% from the previous year. However, there still was one civilian fire death every 156 min and one fire injury every 28 min. The number for injuries is believed to be less than the actual number, since many injuries are not reported. The property loss due to fires in 2002 is estimated at 10.3 billion dollars and indicates a decrease of 2.2% from the previous year, if one excludes the World Trade Center deaths from the 2001 numbers.

In 2002, residential fires accounted for only 24% of the total fires, but were responsible for 79% of all fire deaths and 76% of the reported injuries. Although in the years 1977–2002, the number of civilian fire fatalities in homes dropped from 5865 to 2670, fires in homes still cause the greatest concern to the fire community. Statistics show that children under five and adults over 65 years of age are the most frequent casualties of residential fires. This is attributed to their inherent difficulties in trying to escape. Statistics also show that males are more likely to die in fires than females. More fires and higher fire death rates occur in the South than any of the other geographical areas of the United States; the geographical region with the next highest fire death rate and number of fires is the Northeast.

One must distinguish between the causes of fires and the causes of fire deaths. The primary causes of residential fires have been shown to be heating and cooking. Lack of central heat and the incorrect use of portable space heaters are two of the reasons given for the high fire and death rate in the South. Heating fires result in the highest property losses, primarily because cooking fires are usually noticed and extinguished before getting out of control. Fire deaths, however, usually result from fires ignited by cigarettes. The most common fire scenario leading to fire deaths is one in which a person (usually intoxicated) falls asleep in an upholstered chair while smoking. The cigarette falls into a crevice and starts the upholstered chair smoldering. The individual awakes and goes to bed. The chair can smolder for an extended period of time (in laboratory tests, an hour was not unusual) before bursting into flames. It is after the flaming starts that the smoke fills the room and escapes to the other rooms. It is common to find people who have died from smoke inhalation (not burns) in or near their beds, indicating that the little or no effort to escape was probably due to lack of awareness of the danger. Smoke detectors in this type of scenario would save many lives. Statistics have shown that working smoke detectors double one's probability of escaping alive. Recent statistics have also shown that many homes have nonfunctioning smoke detectors due to being disconnected after a false alarm (usually from smoke from cooking or a wood stove) or when the beeping indicating the need for a new battery became annoying.

Since most of the deaths from fires occur in residences, the NFPA proposes the following safety initiatives to improve fire safety: (1) increase fire safety education on fire prevention and what to do if a fire occurs; (2) install smoke detectors in all homes and check them periodically to ensure they are working properly; (3) practice escape plans with the family; (4) install residential home sprinklers to prevent fires from spreading once they start; (5) develop products for the home that are more fire safe and produce less toxic combustion products (the latter is proposed by the authors); and (6) study the needs of the populations most at risk (the young, the elderly, and the poor) and implement preventive measures.

The US Fire Administration has issued the following 'Home Fire Safety Checklist':

- *Smoke detectors*, are they: (1) placed near bedrooms, (2) on every floor, (3) placed away from air vents, and (4) checked regularly for working batteries.
- *Electrical wiring*, is it: (1) replaced if frayed or cracked, (2) not placed under rugs, over nails or in high traffic areas. Are the outlets: (1) not overloaded, (2) cool to the touch, not hot, and (3) not exposed, that is, have cover plates.
- *Electric space heaters*, are they: (1) plugged directly into wall socket with no extension cords and (2) unplugged when not in use.

- *Kerosene heaters*, are they: (1) used only in permitted geographic areas, (2) filled only with K-1 kerosene, never gasoline or camp stove fuel, (3) only refueled outdoors, and (4) only refueled when cool.
- *Woodstoves and fireplaces*, are they: (1) used only with seasoned wood, never with green wood, Wolmanized chromated copper arsenate (CCA)-treated wood, pressure-treated wood, artificial logs or trash, (2) protected by screens, and (3) cleaned regularly along with the flues, interiors, hearths and chimneys. After December 30, 2003, Wolmanized CCA-treated wood (also called Wolmanized pressure-treated wood) will no longer be produced for nonindustrial applications. This wood has been preserved by pressure-treatment with a US EPA-registered pesticide containing CCA to protect it from termite attack and decay. Wood treated with CCA should be used only where such protection is important. Exposure to CCA or its combustion products presents certain hazards. Therefore, the following precautions should be taken both when handling the treated wood and in determining where to use and how to dispose of the treated wood. Treated wood should not be burned in open fires or in stoves, fireplaces or residential boilers because toxic chemicals are produced as part of the smoke and ashes (see section 'Relevant Websites'). The *Journal of the American Medical Association* (in 1984) reported that a Wisconsin family who had burned CCA-containing wood scraps in their home furnace for heating purposes experienced their hair falling out, rashes, severe reoccurring nosebleeds, extreme fatigue, and debilitating headaches. The parents spoke of black out periods that would last for several hours with long periods of extreme disorientation following it. The two children also reported experiencing frequent seizures. Since arsenic affects not just humans, but any pets and wildlife, the family noticed their houseplants and fish had died as well. Later, all the serious health effects were attributed to the family breathing in minute amounts of arsenic dust that had occurred from the ashes.
- *All alternate heaters*, are they: (1) used only in well-ventilated rooms, (2) stable such that they cannot be easily knocked over, (3) never used to dry clothing or other items, and (4) kept at a safe distance from curtains or furniture.
- *Home escape plan*: (1) is it practiced every 6 months, (2) are the emergency numbers, a whistle, and a flashlight kept near the telephone, and (3) is the outside meeting place identified.

Generation of Toxic Gases in Fires: Adverse Effects of Particulates

Eighty percent of the residential fire deaths are attributed to smoke inhalation, not to burns. Smoke is defined by ASTM International (ASTM, formerly the American Society for Testing and Materials) as "the airborne solid and liquid particulates and gases evolved when a material undergoes pyrolysis or combustion." The adverse effects from smoke inhalation are believed to be due mainly to the exposure to toxic gases, although the role of the particulates alone and in combination with fire gases needs further investigation. The importance, therefore, of determining the identities and concentrations of toxic gases produced from materials thermally decomposed under various fire conditions is evident. In addition, the increased variety of plastics in buildings and homes has raised the issue of whether synthetic materials may produce unusually or extremely toxic[1] combustion products. In 1975, the journal *Science* documented a case in which an experimental rigid polyurethane foam containing a fire retardant produced a very unusual toxic combustion product identified as 4-ethyl-1-phospha-2,6,7-trioxabicyclo [2.2.2]octane-1-oxide (commonly referred to as a bicyclic phosphate ester). Bicyclic phosphate compounds have been shown to cause seizures at very low concentrations. Based on these test results, this product never became commercially available. To a large extent, however, it was this case that generated the burgeoning interest in the field of 'combustion toxicology' and the widespread concern about the potential formation of 'supertoxicants'. Although research since the 1970s has shown that this concern is largely unfounded, the bicyclophosphate ester case and at least one other product that generated extremely toxic combustion products have indicated the need to test new formulations or materials containing new combinations of compounds to ensure that extremely or unusually toxic products are not generated.

The gas composition of smoke depends on the chemical composition, the molecular structure and polymer formulation of the burning material, which may include a variety of additives, plasticizers, stabilizers, flame retardants, cross-linking agents, fillers, and blowing agents. In addition, the conditions of

[1] Here, the phrase 'extremely toxic' is a relative term indicating that the effluent from the thermal decomposition of very small quantities of a material has been noted to cause death of experimental animals (usually rats or mice) under controlled laboratory conditions. 'Unusually toxic' indicates that the toxic effect cannot be totally attributable to the combustion gases (either singly or in combination) that are normally considered the main toxicants.

thermal degradation, for example, temperature, oxygen availability and ventilation, will affect the nature of the combustion atmosphere. In a series of reviews of the combustion products and toxicity of seven plastics (acrylonitrile-butadiene-styrenes (ABS), nylons, polyesters, polyethylenes, polystyrenes, poly(vinyl chlorides) (PVC), and rigid polyurethane foams) commonly found in residences, and decomposed under various thermal and atmospheric conditions, over 400 different decomposition products were noted. Many of these products were common to more than one plastic. In addition, there are probably many other combustion products that were not detected. The toxicity of most of these individual compounds is currently unknown and little has been done to tackle the enormous problem of determining the toxicity of combinations of these compounds. It is important to note that lack of detection of a specific combustion product from a material may only mean that the particular analytical techniques used were not suitable to detect that compound or that the investigator did not specifically analyze for that combustion product. Toxicity testing, for example, on animals, becomes important to ensure that an unsuspected and therefore, undetected toxic by-product has not been formed.

Since the number of compounds one can reasonably analyze in any one test is limited, knowledge of the chemical composition, molecular structure, and formulation of the polymer can be used to provide some indication of the main gaseous products which may or may not be generated under specified experimental conditions. However, one needs to be cautious when predicting the combustion products from generic materials of unknown formulations. For example, one would expect nitrogen-containing materials (e.g., ABS, nylons, rigid and flexible polyurethanes) to produce hydrogen cyanide (HCN) and not expect HCN from a material like PVC. However, a PVC containing zinc ferrocyanide[2] (an additive designed to suppress smoke) as well as a vinyl chloride–vinylidene chloride copolymer were found to generate HCN. In a similar fashion, based on the chemical composition, PVC is the only one of the seven plastics mentioned above that would be expected to generate chlorinated combustion products. However, widespread usage of halogenated fire retardants in plastic formulations makes predicting which materials will produce halogenated products extremely difficult.

[2]This material was never made commercially available after toxicity testing indicated that its combustion products produced very rapid deaths of experimental animals (rats).

Temperature also plays an important role in influencing the production of decomposition products. In general, as the temperature and thus the rate of decomposition increases, the quantity of the more complex compounds and heavier hydrocarbons decreases and the concentrations of carbon monoxide (CO), carbon dioxide (CO_2), and nitrogen dioxide (NO_2) increase. The generation of HCN has also been shown to increase as a function of temperature. Another example is hydrogen chloride (HCl), the detection of which begins when stabilized PVC is heated to $\sim 200^{\circ}C$; rapid dehydrochlorination then occurs at $\sim 300^{\circ}C$. On the contrary, more acrolein was generated from polyethylene under lower temperature, nonflaming conditions than under higher temperature flaming conditions.

As mentioned earlier, more work is needed to examine the adverse effects of the particulate matter produced when these materials are thermally decomposed. Examination of the smoke particulate and condensable matter is important for a number of reasons. First, many of the thermal degradation products may condense or be adsorbed by the soot particles and be transported along with the smoke into the body. Hydrogen chloride is one example of a compound that may be transported in such a fashion and can form a corrosive acid mist in moist air, such as that found in the lung. One study of the particulate matter that formed during the smoldering decomposition of rigid polyurethane foam showed that many of the compounds detected in the soot fraction were not found in the volatile fraction. Free radicals, which form in fires and are of toxicological concern due to their high reactivity, are usually considered to have very short life spans; however, if adsorbed onto soot particles, their lifetimes can be considerably longer and, if the soot particles are the correct size, they can be inhaled into the individual's deep lungs. In addition, the particulate matter may interfere with the escape and rescue of individuals by causing the obscuration of vision, eye irritation (the eyes clamp shut and the victim is unable to see), and upper respiratory distress. An extreme case indicating the adverse effect of particulates was noted in experiments conducted at the (US) National Institute of Standards and Technology (NIST). Rats exposed for 30 min to the smoke from polystyrene died during the exposures and the level of CO in the blood, even in combination with CO_2, was too low to be fatally toxic. Pathological examination of these rats showed that their respiratory passages were completely blocked by soot and that suffocation was the likely cause of death.

Toxic Potency versus Fire Hazard versus Fire Risk

Death in a fire may be caused by:

1. Carbon monoxide (CO)
2. Toxic gases in addition to CO
3. Oxygen (O_2) at levels too low to sustain life
4. Incapacitation – either physical (inability to escape) or mental (incorrect decision-making)
5. Bodily burns from flame contact
6. Very high air temperatures
7. Smoke density or irritants in smoke that affect vision and interfere with ability to escape
8. Psychological effects (e.g., fear, shock, and panic)
9. Physical insults (e.g., building or ceiling collapses, broken bones from jumping from upper floors)

Research in the field of combustion toxicology is primarily concerned with items 1–4, all of which are related to the toxic potency of the fire gas effluent. Toxic potency is defined by ASTM as "a quantitative expression relating concentration (of smoke or combustion gases) and exposure time to a particular degree of adverse physiological response, for example, death on exposure of humans or animals." This definition is followed by a discussion, which states, "The toxic potency of smoke from any material or product or assembly is related to the composition of that smoke which, in turn, is dependent upon the conditions under which the smoke is generated." One should add that the LC_{50}[3] is a common end point used in laboratories to assess toxic potency. In the comparison of the toxic potencies of different compounds or materials, the lower the LC_{50} (i.e., the smaller the amount of material necessary to reach the toxic end point), the more toxic the material is.

It is important to note that a toxicity assessment based on lethality due to toxic gases is only part of the total fire hazard that needs to be evaluated especially when one is making choices as to the best material for a specific end use. ASTM defines 'fire hazard' as the potential for harm associated with fire. The discussion that follows this definition states, "a fire may pose one or more types of hazard to people, animals or property. These hazards are associated with the environment and with a number of fire-test-response characteristics of materials, products or assemblies including but not limited to ease of ignition, flame spread, rate of heat release, smoke generation and obscuration, toxicity of combustion products and ease of extinguishment." Other factors that need to be evaluated when considering a material for use in a given situation include the quantity of material needed, its configuration, the proximity of other combustibles, the volume of the compartments to which the combustion products may spread, the ventilation conditions, the ignition and combustion properties of the material and other materials present, the presence of ignition sources, the presence of fire protection systems, the number and type of occupants, and the time necessary to escape.

'Fire risk' is defined by ASTM as "an estimation of expected fire loss that combines the potential for harm in various fire scenarios that can occur with the probabilities of occurrence of those scenarios." The discussion following the definition of fire risk states, "risk may be defined as the probability of having a certain type of fire, where the type of fire may be defined in whole or in part by the degree of potential harm associated with it, or as potential for harm weighted by associated probabilities. Risk scales do not imply a single value of acceptable risk. Different individuals presented with the same risk situation may have different opinions on its acceptability." A simple way to explain the difference between fire hazard and fire risk is to compare the fire to sky diving, a very hazardous sport; however, if one never goes sky diving, no risk is incurred.

Toxicity Assessment: Animal Exposures

In most combustion toxicology experiments, the biological end point has been lethality or incapacitation of experimental animals, usually rats or mice. Incapacitation in a fire can be as perilous as lethality if an individual becomes incapable of correct decision-making or physically unable to move. Under these circumstances, the ability to escape will be lost and death will occur unless the individual is rescued. Therefore, many fire scientists are concerned with the levels of combustion products or amounts of materials which when combusted will cause incapacitation. However, an incapacitation model for use in laboratory testing has been especially difficult to develop. Most of the tests for incapacitation that have been designed are based on the physical-motor capability of an experimental animal to perform some task (e.g., running in a motorized wheel, jumping onto a pole or lifting a paw to escape a shock, running in a maze, or pushing the correct lever to open a door to escape an irritating atmosphere). The concentration of toxic combustion products that cause the loss of these types of

[3]The LC_{50} value is the result of a statistical calculation based on multiple experiments, each with multiple animals, and indicates the concentration at which 50% of the experimental animals exposed for a specific length of time would be expected to die either during the exposure time or the post-exposure observation period.

physical-motor capabilities is usually close to the concentration that is lethal and does not usually add much additional information. More recently, however, there have been attempts at examining neurological end points such as measuring the increased number of errors by humans doing mathematical problems while exposed to low levels of CO or exposing rats and pigeons to a complete neurobehavioral battery of 25 tests following nonlethal toxic exposures.

Whether one needs to examine incapacitation or lethality depends upon the problem one is trying to solve. To determine the best material for a particular end use application, the lethality end point has proved to be more definitive and will flag the materials that produce extremely toxic combustion products better than an incapacitation end point. There are at least two reasons for this: (1) Incapacitation is only measured during the exposure, which is usually 30 min or less, but lethality can also occur during the postexposure observation period, which can be 2 weeks or longer. A material that only causes delayed effects during the postexposure period (e.g., a material that generates HCl) can thus have an LC_{50} value that is lower than the incapacitation EC_{50}[4] value. The amount needed to kill can be less than the amount needed to incapacitate because the amount of thermally decomposed material necessary to cause postexposure deaths can be less than the amount needed to cause incapacitation during the exposure. (2) In many cases in which the combustion products contain high concentrations of irritant gases, the animals would only appear to be incapacitated (i.e., they would stop responding to the test indicator due to the high irritant quality of the smoke), but when removed from the combustion atmosphere, would immediately start responding normally.

Other delayed effects from exposures to combustion atmospheres, such as tissue or organ injury, mutagenicity, carcinogenicity, and teratogenicity also need to be studied since they may ultimately lead to permanent disability or death. The current advances in the field of genetics provide investigators with new opportunities to examine the effects of combustion products at the molecular level. One objective could be to determine whether these toxic products cause DNA damage and/or mutations. Specific problems of interest include: does the damage occur in nuclear DNA and/or mitochondrial DNA, are certain areas of the DNA more prone to these mutations (i.e., are there hot spots?), can we categorize the types of mutations (e.g., transitions, transversions, deletions, insertions), and how efficient are the repair mechanisms? Are these mutagens also known to be carcinogens?

Toxicity Assessment: Predictive Models

In the 1970s, there were essentially two experimental strategies to examine the issues raised by the field of combustion toxicology: (1) an analytical chemical method and (2) an animal exposure approach. In the analytical chemical method, investigators thermally decomposed materials under different experimental conditions and tried to determine every combustion product that was generated. This approach generated long lists of compounds. The toxicity of most of these individual compounds was unknown and the concept of examining the toxicity of all the various combinations of compounds was and still is considered a formidable task. An additional problem with the analytical approach was that, as mentioned earlier, the detection and identification of the toxic combustion products depended on the analytical method used. Therefore, one could not be certain that every toxic product was detected and identified. This approach enabled one to identify many of the multiple products that were generated, without knowing the toxic potency of all the identified compounds, either singly or combined.

In the animal exposure approach, the animals (usually rats or mice) serve as indicators of the degree of toxicity of the combustion atmospheres. The materials of concern are thermally decomposed under different combustion conditions and the animals are exposed to the combined particulate and gaseous effluent. Multiple animal experiments (each with multiple animals) with different concentrations of material are conducted to determine an EC_{50} (incapacitation) or an LC_{50} (lethality) for a specific set of combustion conditions. Each material would then have a particular EC_{50} or an LC_{50} value that can be used to compare the toxicities of different materials decomposed under the same conditions. The lower the EC_{50} or LC_{50} value, the more toxic the combustion products from that material are considered to be. In this approach, one knows the relative toxicity of a material as compared to another material, but does not know which of the toxic gases are responsible for the adverse effects.

In the 1980s, investigators at NIST began examining the possibility of combining the analytical chemical method with the animal exposure approach to develop empirical mathematical models to predict the toxicity. These predictions were based

[4] The definition of the EC_{50} is essentially the same as that of the LC_{50} except incapacitation rather than lethality is the end point and incapacitation is monitored only during the exposure and not during the post-exposure period.

on actual experiments with animals and their response to each of the main toxic combustion gases; CO, CO_2, low O_2, HCN, NO_2, HCl, hydrogen bromide (HBr), and various combinations of these gases. The advantages of these predictive approaches are: (1) the number of necessary test animals can be reduced by first predicting the toxic potency from a limited chemical analysis of the smoke; (2) smoke may be produced under conditions that simulate any fire scenario of concern; (3) fewer tests are needed, thereby reducing the overall cost of the testing; and (4) information is obtained on both the toxic potency of the smoke (based on the mass of material burned) and the responsible gases (based on the primary toxic gases in the mixture). The prediction is checked with one or two animal tests to assure that an unexpected gas or toxic combination has not been generated. The results of using these empirical mathematical models indicated that, in most cases, one could predict the toxic potency of a combustion atmosphere based on the concentrations of the main toxic gases and did not need to worry about the effects of minor or more obscure gases.

Primary Toxic Combustion Gases

Complete combustion of a polymer containing carbon, hydrogen, and oxygen in an atmosphere with sufficient O_2 yields CO_2 and H_2O. It is during incomplete combustion under various atmospheric conditions in either flaming or nonflaming modes that compounds of greater toxicological concern are generated. When O_2 is limited, the primary gases formed during the combustion of most materials are CO, CO_2, and H_2O. If the materials contain nitrogen, HCN and NO_2, two principal thermo-oxidative products of toxicological concern, are also likely to be generated. Halogenated or flame-retarded materials generally produce HCl or HBr. Other commonly found fire gases include nitrogen oxides (NO_x), ammonia (NH_3), hydrogen sulfide (H_2S), sulfur dioxide (SO_2), and fluorine compounds. One also needs to consider that in fire situations, O_2 levels drop and exposure to low O_2 atmospheres will have additional adverse physiological effects. Some of these toxic combustion gases (e.g., CO, HCN, low O_2) produce immediate asphyxiant symptoms, while others (e.g., HCl, HBr, NO_2) fall into an irritant category and produce symptoms following the exposures.

The *N*-Gas Models

The *N*-gas models for predicting smoke toxicity were founded on the hypothesis that a small number (*N*) of gases in the smoke will account for a large percentage of the observed toxic potency. These predictive models were based on an extensive series of experiments conducted at NIST on the toxicological interactions of the primary gases found in fires. Both the individual gases and complex mixtures of these gases were examined. To use these models, materials are thermally decomposed using a bench-scale method that simulates realistic fire conditions, the concentrations of the primary fire gases – CO, CO_2, low O_2, HCN, HCl, HBr, and NO_2 – are measured, and the toxicity of the smoke using the appropriate *N*-gas model is predicted. The predicted toxic potency is checked with a small number of animal (Fischer 344 male rats) tests to assure that an unanticipated toxic gas was not generated or an unexpected toxicological effect (e.g., synergism or antagonism) did not occur. The results indicate whether the smoke from a material or product is extremely toxic (based on mass consumed at the predicted toxic level) or unusually toxic (the toxicity cannot be explained by the combined measured gases). These models have been shown to correctly predict the toxicity in both bench-scale laboratory tests and full-scale room burns of a variety of materials of widely differing characteristics chosen to challenge the system. The six-gas model (without NO_2) is now included in two national toxicity test method standards (ASTM E1678), approved by ASTM, and NFPA 269, approved by the NFPA. It is also included in an international standard (ISO 13344) that was approved by 16 member countries of the International Organization of Standardization (ISO), Technical Committee 92 (TC92). All three of these standards were published in 1996.

The objectives for developing the *N*-gas models were:

- To establish the extent to which the toxicity of a material's combustion products could be explained and predicted by the interaction of the major toxic gases generated from that material in the laboratory or whether minor and more obscure combustion gases needed to be considered.
- To develop a bioanalytical screening test and a mathematical model which would predict whether a material would produce extremely toxic or unusually toxic combustion products.
- To predict the occupant response from the concentrations of primary toxic gases present in the environment and the time of exposure.
- To provide data for use in computer models designed to predict the hazard that people will experience under various fire scenarios.

The Six-Gas *N*-Gas Model The six-gas model (see eqn (1)) was based on studies at NIST on the toxicological interactions of six gases: CO, CO_2, HCN, low O_2 concentrations, HCl, and HBr. First, individual gases in air were tested to determine the concentrations necessary to cause 50% of the laboratory test animals (Fischer 344 male rats) to die either during the exposure (within exposure LC_{50}) or during the exposure plus a 14 day postexposure observation period (within plus postexposure LC_{50}). The studies on HCl and HBr were conducted at Southwest Research Institute (SwRI) under a grant from NIST. Similar measurements for various combinations of these gases indicated whether the toxicity of the mixtures of gases was additive, synergistic, or antagonistic.

Based on these empirical results, the following six-gas model was developed:

$$\frac{m[\mathrm{CO}]}{[\mathrm{CO_2}]-b}+\frac{[\mathrm{HCN}]}{\mathrm{LC_{50}HCN}}+\frac{21-[\mathrm{O_2}]}{21-\mathrm{LC_{50}O_2}}+\frac{[\mathrm{HCl}]}{\mathrm{LC_{50}HCl}}+\frac{[\mathrm{HBr}]}{\mathrm{LC_{50}HBr}}=N\text{-gas value} \quad (1)$$

where the terms in brackets indicate the time-integrated average atmospheric concentrations during a 30 min exposure period ((ppm × min)/min or for O_2 (% × min)/min). The other terms are defined in the following paragraphs.

Under the experimental conditions used at NIST and with Fischer 344 male rats, the 30 min LC_{50} value of CO_2 is 47% (470 000 ppm) with 95% confidence limits of 43–51%. No deaths occurred in rats exposed to 26% CO_2 for 30 min. In a real fire, the highest theoretically possible concentration of CO_2 is 21%, a concentration that could only occur if all the atmospheric O_2 were converted to CO_2, a highly improbable event. Therefore, CO_2 concentrations generated in fires are not lethal. However, CO_2 is a respiratory stimulant causing an increase in both respiratory rate and tidal volume. It also increases the acidosis of the blood. When combined with any of the other tested gases, CO_2 has a synergistic toxicological effect, that is, the toxicity of the other gases is increased in the presence of CO_2 (**Table 1**). Empirically, however, it was found that the effect of CO_2 can only be added into the *N*-gas equation once. Therefore, the CO_2 effect was included with the CO factor since there was more data on the effect of different concentrations of CO_2 on the toxicity of CO, and CO is the toxicant most likely to be present in all fires. The results on the synergistic effect of CO_2 on CO indicated that as the concentration of CO_2 increases (up to 5%), the toxicity of CO increases. Above 5% CO_2, the toxicity of CO starts to revert back toward the toxicity of the CO by itself. The terms m and b in eqn (1) define this synergistic interaction and equal −18 and 122 000, respectively, if the CO_2 concentrations are 5% or less. For studies in which the CO_2 concentrations are above 5%, m and b equal 23 and −38 600, respectively.

Table 1 Synergistic effects of CO_2

Gas[a]	*LC_{50} values*	
	Single gas	*With 5% CO_2*
CO_2	470 000 ppm[b]	
CO	6600 ppm[b]	3900 ppm[c]
NO_2	200 ppm[d]	90 ppm[d]
O_2	5.4%[b]	6.4%[c]

[a] All gases were mixed in air; 30 min exposures of Fischer 344 rats.
[b] Deaths occurred during the exposure.
[c] Deaths occurred during and following the exposures.
[d] Deaths during the postexposure period.

In rats, the 30 min LC_{50} for CO is 6600 ppm and with 5% CO_2, this value drops to 3900 ppm. Exposure to CO in air only produced deaths during the actual exposures and not in the postexposure observation period; however, exposures to CO plus CO_2 also caused deaths in the postexposure period. Carbon monoxide is a colorless, odorless, tasteless and nonirritating poisonous gas. The toxicity of CO comes from its binding to the hemoglobin in red blood cells and the formation of carboxyhemoglobin (COHb). The presence of CO on the hemoglobin molecule prevents the binding of O_2 to hemoglobin (O_2Hb) and results in hypoxia in the exposed individual. Since the binding affinity of hemoglobin for CO is 210 times greater than its affinity for O_2, only 0.1% CO (1000 ppm) is needed to compete equally with O_2 which is normally present at 20.9% in air (20.9%/210 ~ 0.1%). Thus, only 1000 ppm of CO in the atmosphere is enough to generate 50% COHb, a value commonly quoted (but not necessarily proved) as the concentration which is lethal to humans. The time to get to 50% COHb at 1000 ppm CO would be longer than 30 min.

The LC_{50} value of HCN is 200 ppm for 30 min exposures or 150 ppm for 30 min exposures plus the postexposure observation period. HCN caused deaths both during and following the exposures.

The 30 min LC_{50} of O_2 is 5.4%, a value that is included in the model by subtracting the combustion atmospheric O_2 concentration from the normal concentration of O_2 in air, that is, 21%. The LC_{50} values of HCl or HBr for 30 min exposures plus postexposures times are 3700 and 3000 ppm, respectively.

HCl and HBr at levels found in fires only cause postexposure effects.

The pure and mixed gas studies showed that if the value of eqn (1) is 1.1 ± 0.2, then some fraction of the test animals would die. Below 0.9, no deaths would be expected and above 1.3, all the animals would be expected to die. Since the concentration–response curves for animal lethalities from smoke are very steep, it is assumed that if some percentage (not 0% or 100%) of the animals die, the experimental loading is close to the predicted LC_{50} value. Results using this method show good agreement (deaths of some of the animals when the *N*-gas values were above 0.9) and the good predictability of this approach.

This model can be used to predict deaths that will occur only during the fire exposure or deaths during and following the fire. To predict the deaths that would occur both during and following the exposures, eqn (1) is used as presented. To predict deaths only during the exposures, HCl and HBr, which only have postexposure effects, should not be included in eqn (1). In small-scale laboratory tests and full-scale room burns, eqn (1) was used successfully to predict the deaths during and following exposures to the combustion products from numerous materials. In the case of PVC, the model correctly predicted the results as long as the HCl was greater than 1000 ppm; therefore, it is possible that HCl concentrations under 1000 ppm may not have any observable effect on the model even in the postexposure period. More experiments are necessary to show whether a true toxic threshold for HCl does exist.

Although most of the work at NIST concentrated on deaths during or following 30 min exposures, the LC_{50} values of many of these gases, both singly and mixed, were determined at other times ranging from 1 to 60 min and in all the cases examined, the predictive capability of eqn (1) holds if the LC_{50} values for the other times are substituted into the equation.

The Seven-Gas Model; Addition of NO_2 to the *N*-Gas Model Nitrogen dioxide is an irritant gas that will cause lachrymation, coughing, respiratory distress, increases in methemoglobin (MetHb) levels, and lung edema. Single brief exposures to less than lethal concentrations can cause lung damage, emphysema, or interstitial fibrosis. Low levels have been alleged to increase one's susceptibility to respiratory infections, and aggravate one's reactions to allergens. Impairment of dark adaptation has also been noted. Delayed serious effects can be observed as late as 2–3 weeks following exposures. In the lungs, NO_2 forms both nitric (HNO_3) and nitrous (HNO_2) acids, which are most likely responsible for the observed damage to the lung cells and connective tissue.

In fires, NO_2 may arise from atmospheric nitrogen fixation, a reaction that is material independent, or from the oxidation of nitrogen from nitrogen-containing materials. To examine the generation of NO_2 from nitrogen fixation, a small study was undertaken at NIST. In two full-scale fires of rooms in which the main source of fuel was polystyrene-covered walls, only low levels of NO_x (10 and 25 ppm) were found indicating little nitrogen fixation under these conditions. A real example of burning nitrogen-containing materials was the 1929 Cleveland Clinic (Ohio, USA) fire in which 50 000 nitrocellulose X-ray films were consumed. The deaths of 97 people in this fire were attributed mainly to NO_x. An additional 26 people died between 2 h and 1 month following the fire, and 92 people were treated for nonfatal injuries. In laboratory tests of nitrogen-containing materials under controlled conditions, 1–1000 ppm of NO_x were measured. In military tests of armored vehicles penetrated by high temperature ammunition, NO_2 levels above 2000 ppm were found.

Individual and binary mixtures. In small-scale laboratory tests of NO_2 in air, deaths of Fischer 344 male rats occurred only in the postexposure period and the LC_{50} value following a 30 min exposure is 200 ppm. Carbon dioxide plus NO_2 show synergistic toxicological effects. The LC_{50} for NO_2 following a 30 min exposure to NO_2 plus 5% CO_2 is 90 ppm (postexposure deaths) (i.e., the toxicity of NO_2 doubled) (**Table 1**).

As mentioned above, CO produces only within-exposure deaths and its 30 min LC_{50} is 6600 ppm. In the presence of 200 ppm of NO_2, the within-exposure toxicity of CO doubled (i.e., its 30 min LC_{50} became 3300 ppm). An exposure of $\sim$3400 ppm CO plus various concentrations of NO_2 showed that the presence of CO would also increase the postexposure toxicity of NO_2. The 30 min LC_{50} value of NO_2 went from 200 to 150 ppm in the presence of 3400 ppm of CO. A concentration of 3400 ppm of CO was used as that concentration would not be lethal during the exposure and any postexposure effects of CO on NO_2 would become evident; the LC_{50} of CO (6600 ppm) would have caused deaths of the animals during the 30 min exposure.

The 30 min LC_{50} of O_2 is 5.4% and the deaths occurred primarily during the exposures. In the presence of 200 ppm of NO_2, the within-exposure LC_{50} of O_2 and its toxicity increased to 6.7%. In the case of O_2, increased toxicity is indicated by an increase in the value of the LC_{50} since it is more toxic to be adversely affected by a concentration of O_2 ordinarily capable of sustaining life. Exposure of the animals

to 6.7% O_2 plus various concentrations of NO_2 showed that the NO_2 toxicity doubled (i.e., its LC_{50} value decreased from 200 to 90 ppm).

One of the most interesting findings was the antagonistic toxicological effect noted during the experiments on combinations of HCN and NO_2. As mentioned above, the 30 min LC_{50} for NO_2 alone is 200 ppm (postexposure) and the 30 min within-exposure LC_{50} for HCN alone is also 200 ppm. This concentration of either gas alone is sufficient to cause death of the animals (i.e., 200 ppm HCN or 200 ppm NO_2 would cause 50% of the animals to die either during the 30 min exposure or following the 30 min exposure, respectively). However, in the presence of 200 ppm of NO_2, the within-exposure HCN LC_{50} concentration increases to 480 ppm or stated in other words, the toxicity of HCN decreases by 2.4 times.

The mechanism for this antagonistic effect is believed to be as follows. In the presence of H_2O, NO_2 forms nitric acid (HNO_3) and nitrous acid (HNO_2). These two acids are the most likely suspects responsible for the lung damage leading to the massive pulmonary edema and subsequent deaths noted following exposure to high concentrations of NO_2. Nitrite ion (NO_2^-) formation occurs in the blood when the nitrous acid dissociates. The nitrite ion oxidizes the ferrous ion in oxyhemoglobin to ferric ion to produce MetHb (eqn (2)). MetHb is a well-known antidote for CN^- poisoning. MetHb binds cyanide, forming cyanmethemoglobin, which keeps the cyanide in the blood and prevents it from entering the cells. In the absence of MetHb, free cyanide will enter the cells, react with cytochrome oxidase, prevent the utilization of O_2, and cause cytotoxic hypoxia. If, on the other hand, cyanide is bound to MetHb in the blood, it will not be exerting its cytotoxic effect. Therefore, the mechanism of the antagonistic effect of NO_2 on the toxicity of cyanide is believed to be due to the conversion of oxyhemoglobin [$O_2Hb(Fe^{2+})$] to methemoglobin [$MetHb(Fe^{3+})$] in the presence of nitrite (see eqn (2)).

$$2H^+ + 3NO_2^- + 2O_2Hb(Fe^{2+}) = 2MetHb(Fe^{3+}) + 3NO_3^- + H_2O \qquad (2)$$

Tertiary mixtures of NO_2, CO_2, and HCN. Earlier work indicated that the presence of 5% CO_2 with either HCN or NO_2 produced a more toxic environment than would occur with either gas alone. The antagonistic effects of NO_2 on HCN indicate that the presence of one LC_{50} concentration of NO_2 ($\sim$200 ppm) will protect the animals from the toxic effects of HCN during the 30 min exposures, but not from the postexposure effects of the combined HCN and NO_2. Thus, it was of interest to examine combinations of NO_2, CO_2, and HCN. In this series of experiments, the concentrations of HCN were varied from almost 2 to 2.7 times its LC_{50} value (200 ppm). The concentrations of NO_2 were approximately equal to one LC_{50} value (200 ppm) if the animals were exposed to NO_2 alone and approximately half the LC_{50} (90 ppm) if the animals were exposed to NO_2 plus CO_2; the concentrations of CO_2 were maintained at $\sim$5%; and the O_2 levels were kept above 18.9%. The results indicated that CO_2 does not make the situation worse, but rather provided additional protection even during the postexposure period. In each of six experiments, some or all of the animals lived through the test even though they were exposed to greater than lethal levels of HCN plus lethal levels of NO_2. In addition, in four tests, some of the animals lived through the postexposure period even though the animals were exposed to combined levels of HCN, NO_2, and CO_2 that would be equivalent to 4.7–5.5 times the lethal concentrations of these gases. One possible reason that CO_2 seems to provide an additional degree of protection is that NO_2 in the presence of 5% CO_2 produces four times more MetHb than does NO_2 alone.

Mixtures of CO, CO_2, NO_2, O_2, and HCN. The initial design of these experiments was to look for additivity of the CO/CO_2, HCN, and NO_2 factors keeping each at about one-third of its toxic level, while keeping the O_2 concentration above 19%. When these initial experiments produced no deaths, we started to increase the concentrations of CO up to one-third of the LC_{50} of CO alone (6600 ppm), HCN was increased to 1.3 or 1.75 times its LC_{50} depending on whether the within-exposure LC_{50} (200 ppm) or the within- and postexposure LC_{50} (150 ppm) is being considered, and NO_2 was increased up to a full LC_{50} value (200 ppm). The results indicated that just adding an NO_2 factor (e.g., [NO_2]/LC_{50} NO_2) to eqn (1)] would not predict the effect on the animals. A new mathematical model was developed and is shown as eqn (3). In this model, the differences between the within-exposure predictability and the within-exposure and postexposure predictability are: (1) the LC_{50} value used for HCN is 200 ppm for within-exposure or 150 ppm for within-exposure and postexposure, and (2) the HCl and HBr factors are not used to predict the within-exposure lethality, only the within-exposure and postexposure lethality. According to eqn (3), animal deaths will start to occur when the *N*-gas value is above 0.8% and 100% of the animals will die when the value is above 1.3. The results indicated that in those few cases where the values were above 0.8 and no deaths occurred, the animals were severely incapacitated

(close to death) as demonstrated by no righting reflex or eye reflex.

$$N\text{-gas value} = \frac{m[\mathrm{CO}]}{\mathrm{CO_2} - b} + \frac{21 - [\mathrm{O_2}]}{21 - \mathrm{LC_{50}(O_2)}} + \left(\frac{[\mathrm{HCN}]}{\mathrm{LC_{50}(HCN)}} \times \frac{0.4[\mathrm{NO_2}]}{\mathrm{LC_{50}(NO_2)}}\right) + 0.4\left(\frac{[\mathrm{NO_2}]}{\mathrm{LC_{50}(NO_2)}}\right) + \frac{[\mathrm{HCl}]}{\mathrm{LC_{50}(HCl]}} + \frac{[\mathrm{HBr}]}{\mathrm{LC_{50}(HBr)}} \quad (3)$$

The N-gas model including NO_2. For an explanation of these terms, see the paragraphs following eqn (1). Equation (3) should be used to predict the within-exposure plus postexposure lethal toxicity of mixtures of CO, CO_2, HCN, reduced O_2, NO_2, HCl, and HBr. The LC_{50} values will be the same as those given for eqn (1) using 150 ppm for HCN and 200 ppm for NO_2. If one wishes to predict the deaths that will occur only during the exposure, the LC_{50} value used for HCN should be 200 ppm and the HCl and HBr factors should not be included. To predict the lethal toxicity of atmospheres that do not include NO_2, eqn (1) is to be used.

Combustion Toxicity Test Methods

The toxicity of the combustion products from any new material formulation or product containing additives or new combinations of additives needs to be examined. Material and polymer chemists are currently trying to develop new 'fire safe' materials. The terms 'fire safe' or 'fire resistant' are not the same as noncombustible. Unless these new materials are truly noncombustible, some thermal decomposition will occur when the materials are exposed to fire conditions. The toxic gases and the irritants that are present in all smoke need to be considered potential dangers. The toxic products can cause both acute and delayed toxicological effects. It is the acute and extremely short-term effects that prevent escape from burning buildings by causing faulty judgment, incapacitation, and death. The irritants in the smoke can also interfere with the passengers' ability to escape by causing severe coughing and choking and by preventing them from keeping their eyes open long enough to find the exits. In addition, the delayed effects, such as tissue or organ injury, mutagenicity, carcinogenicity, and teratogenicity, need to be studied since they may ultimately lead to permanent disability and postexposure deaths.

Toxicity screening tests for both the acute and delayed effects are, therefore, needed to evaluate the combustion products including any irritants that may be present in newly proposed materials and products. It is imperative that the materials and products be tested under experimental conditions that simulate realistic fire scenarios of concern (e.g., flash-over conditions emanating from first, smoldering and then, flaming of upholstered furniture in homes or smoldering fires in concealed spaces in aircraft). The ideal tests should be simple, rapid, inexpensive, use the least amount of sample possible (since, in many cases, only limited amounts of new experimental materials may be available), use a minimum number of test animals, and have a definitive toxicological end point for comparison of the multiple candidates. While faulty judgment and incapacitation are significant causes of worry since they can prevent escape and cause death, they are extremely difficult and complex end points to define and measure in nonhuman test subjects. Death of experimental animals (e.g., rats), on the other hand, is a more definitive and easily determined end point and can be used to compare the relative toxicities of alternate materials deemed suitable for the same purpose. The assumption made here is that if the combustion products of material X are significantly more lethal than those of material Y, the combustion products of X would probably cause more incapacitation and more impairment of judgment than Y as well. The number of experimental animals can be significantly reduced by utilizing one of the predictive mathematical models developed for combustion toxicology such as the *N*-gas models previously discussed in this chapter. The six-gas *N*-gas model is currently included in two national standards (ASTM E1678 and NFPA 269) and one international standard (ISO 13344).

Many test methods for the determination of the acute toxicity of combustion products from materials and products have been developed over the last two decades and continue to be developed and/or improved. In 1983, 13 of the methods published up to that time were evaluated by Arthur D. Little, Inc. to assess the feasibility of incorporating combustion toxicity requirements for building materials and finishes into the building codes of New York State. On the basis of seven different criteria, only two methods were found acceptable. These two methods were the flow-through smoke toxicity method developed at the University of Pittsburgh and the closed-system cup furnace smoke toxicity method developed at NIST (known at that time as the National Bureau of Standards (NBS)). Standard Reference Materials and protocols (SRM 1048 and SRM 1049) were developed at NIST and are available to the users of these methods to provide assurance that they are performing the methods correctly (see 'Relevant Websites'

section). Based on the results of the Arthur D. Little report, the state of New York under Article 15, Part 1120 of the New York State Fire Prevention and Building Code decided to require that building materials and finishes be examined by the method developed at the University of Pittsburgh and that the results be filed with the state. It is important to note, however, that although the results are filed, the state of New York does not regulate any materials or products based on the results of this or any other toxicity test. Although not regulated, the process of testing by the developer should prevent any unduly toxic products from appearing in the marketplace.

New methods that have been developed since 1983 to examine acute combustion toxicity include the University of Pittsburgh II radiant furnace method, a radiant furnace smoke toxicity protocol developed by NIST and SwRI, and the National Institute of Building Sciences (NIBS) toxic hazard test method. All three use radiant heat to decompose materials.

The NIST radiant test and the NIBS toxic hazard test use the same apparatus, consisting of three components: a radiant furnace, a chemical analysis system, and an animal exposure chamber. The chemical analysis system and animal exposure system are identical to that developed for the NBS cup furnace smoke toxicity method. Although the apparatus of both methods are essentially the same, they have different toxicological end points. In the NIST method, an approximate LC_{50}, based on the *mass* of material needed to cause lethality in 50% of the test animals during a 30 min exposure and/or a 14 days postexposure period, is the determinant of toxicity. The number of animals needed to run the test is substantially reduced by first estimating the LC_{50} by the six-gas *N*-gas model. This estimate is then verified with one or two animal tests to assure that no unforeseen gas was generated. The toxicological end point of the NIBS toxic hazard test is the IT_{50}, the irradiation *time* (the time that the material is exposed to the radiant heat) that is required to kill 50% of the animals during a 30 min exposure or 14 days postexposure time. The actual results of the NIBS test with 20 materials indicated that the test animals died in very short periods of time (personal communication) and the test was unable to discriminate very well between materials. These results substantiate the thesis that mass (the smaller the mass necessary for an LC_{50}, the more toxic the material) is a better indicator of acute toxicity than time.

Both the NIST and NIBS test procedures are designed to simulate a postflashover scenario. The premise for simulating a postflashover fire is that most people who die from inhalation of toxic gases in *residential* fires are affected in areas away from the room of fire origin. Smoke and toxic gases are more likely to reach these distant areas following flashover. This scenario may not be relevant in certain circumstances (e.g., aircraft interior fires, where a smoldering fire in a concealed space may cause significant problems if the plane is over a large body of water and unable to land for a considerable period of time or the Station Nightclub in the early 2000s fire in West Warwick (Rhode Island, USA) that killed 100 and injured over 200 people and resulted from a combination of the use of pyrotechnics inside the nightclub, the use of a highly flammable foam on the walls, no sprinkler system and outdated fire codes).

The NIST radiant test has been accepted by ASTM as a US national standard designated ASTM E1678 and entitled "Test Method for Measuring Smoke Toxicity for Use in Fire Hazard Analysis." In 1995, the International Organization for Standardization, Technical Committee 92, Subcommittee 3 (ISO/TC92/SC3) on Toxic Hazards in Fire published an international standard for combustion toxicity that was approved by 16 countries. This standard – ISO/IS 13344 entitled "Determination of the Lethal Toxic Potency of Fire Effluents" – describes the mathematical models (including the six-gas *N*-Gas Model) available for predicting the toxic potency of fire atmospheres based on the toxicological interactions of the main combustion gases present. In the international standard, investigators have the flexibility of designing or choosing a system that will simulate conditions relevant to their fire scenario, rather than having to accept a designated combustion system.

Toxicant Suppressants

Fire scientists are very familiar with fire-retardant chemicals, which are defined by ASTM as "chemicals, which, when added to a combustible material, delay ignition and combustion of the resulting material when exposed to fire." The discussion adds "a fire-retardant chemical can be a part of the molecular structure, an admixture or an impregnant." The term 'toxicant suppressant', however, is a new term arising from research at NIST which demonstrated that the addition of copper compounds to flexible polyurethane foam (FPU) significantly reduced the generation of hydrogen cyanide (HCN) as well as the toxicity of the combustion products when the foam was thermally decomposed. These experiments were designed to simulate the nonflaming and then flaming stages of a chair ignited by a cigarette (a two-phase heating system which simulates the fire scenario that results in the most fire deaths in the United States). The term 'toxicant suppressant' may be defined as a chemical, which, when added to a

combustible material, significantly reduces or prevents one or more toxic gases from being generated when that material undergoes thermal decomposition. The resultant gas effluent should be less toxic than that from the untreated material, that is, the toxic gas, whose concentration is being reduced, should not be converted to an equally or more toxic product.

The results of these studies at NIST indicated that:

1. Hydrogen cyanide concentrations in the thermal decomposition products from a flexible polyurethane foam were reduced ~85% when the foam was treated with 0.1% or 1.0% Cu_2O by weight and thermally decomposed via a two-phase heating system in the NIST Cup Furnace Smoke Toxicity Apparatus.
2. The copper or copper compounds could be added to the foams during or after the foams were formulated and still reduce the HCN yield and toxicity of the combustion products. (NIST added the copper after formulation; the BASF Corporation added the Cu_2O during formulation.) The addition of the copper or copper compounds during formulation did not affect the foaming process or the physical appearance on the foams except for a slight change of color.
3. Low levels of the copper compounds were effective. In particular, when cupric oxide (CuO) was used, the concentration of copper needed was only 0.08% by weight and when cuprous oxide (Cu_2O) was used, only 0.07% by weight was needed to significantly reduce the generation of HCN.
4. Full-scale room burns indicated that the presence of Cu_2O in the flexible polyurethane foam reduced the HCN generation by ~50–70% when the experimental plan was designed to simulate a realistic scenario (the foams contained 1.0% Cu_2O by weight, were covered with a cotton upholstery fabric and arranged to simulate a chair; smoldering was initiated with cigarettes and flaming occurred spontaneously).
5. Under small-scale conditions, less than 3 ppm of NO_x was generated from the untreated foams, whereas a range of 3–33 ppm of NO_x was measured from 0.1% to 1.0% by weight Cu_2O-treated foams. About 6% of the HCN appeared to be converted to NO_x. In the full-scale room tests, ~23% of the HCN appeared to be converted to NO_x. Since we have shown at NIST that NO_2 acts as an antagonist to HCN, this amount of NO_x may also act to counteract the immediate toxic effects of any residual HCN.
6. Since atmospheric oxygen (O_2) concentrations can reach very low levels in real fires, it was important to know whether the reduction of HCN by copper would occur under low O_2 conditions. Small-scale tests with the ambient O_2 concentrations as low as 6% indicated that the HCN levels were reduced by as much as 82% when the flexible polyurethane foam was treated with 0.1% Cu_2O by weight.
7. The toxicity of the gas effluent was also reduced (an indication that HCN was not being converted into some compound that was even more toxic). Fewer animal (Fischer 344 rats) deaths occurred during the 30 min exposures to the flexible polyurethane foam treated with the copper and copper compounds compared to the untreated flexible polyurethane foam. Toxicity based on LC_{50} values was reduced 40–70% in the small-scale tests with 0.1% Cu_2O-treated foams. The blood cyanide levels in the animals exposed to combustion products from the CuO-treated foams for 30 min were 1/2 to 1/4 of those measured in the animals exposed to the smoke from the same amount of untreated foam.
8. Postexposure deaths were also reduced in the animals exposed to the combustion products from the Cu and Cu_2O-treated FPU foams in the small-scale tests. These delayed postexposure deaths have *not* been observed in animals exposed to combustion products from flexible polyurethane foams decomposed in large-scale room fire tests. The specific cause of these postexposure deaths is not known.
9. No differences in flammability characteristics between the 0.1% Cu_2O-treated and untreated flexible polyurethane foam were observed. These characteristics were examined to assure that the positive effect on toxicity was not contradicted by negative effects on the flammability properties. The flammability characteristics examined were: (1) ignitability in three systems (the NIST Cup Furnace Smoke Toxicity method, the Cone Calorimeter, and Lateral Ignition and Flame Spread Test (LIFT)), (2) heat release rates under small-scale (Cone Calorimeter) and medium-scale (furniture calorimeter) conditions, (3) heats of combustion under small-scale (Cone Calorimeter) and medium-scale (furniture calorimeter) conditions, (4) CO/CO_2 ratios under small-scale (Cone Calorimeter) and medium-scale (furniture calorimeter) conditions, (5) smoke obscuration (Cone Calorimeter), and (6) rate of flame spread (LIFT).
10. Research conducted at the BASF Corporation indicated that the physical properties of the

1.0% Cu_2O-treated flexible polyurethane foam were not significantly different from the comparable untreated flexible polyurethane foam. The physical properties examined were tensile strength, elongation, tear strength, resilience, indentation force deflection, support factor, compression sets, and airflow.

11. One of the additives being used in combustion-modified flexible polyurethane foams is melamine. Small-scale tests conducted at NIST indicated that a melamine-treated flexible polyurethane foam generated six times more HCN than an equal amount of foam that did not contain melamine. The presence of Cu_2O reduced the HCN from the melamine foam by 90%. Melamine-treated flexible polyurethane foam is one of two flexible polyurethane foams currently allowed in Great Britain.

In the late 1970s, research by Jellinek *et al*. at Clarkson College of Technology also showed that the concentrations of HCN generated from the thermal decomposition of a polyurethane at 300°C and 400°C decreased when flowed through copper compounds. In their studies, the polyurethane films were usually 15 μm thick (50 mg). In some experiments, the metal powder was mixed with the polymer and, in others, copper metal films of 400–1000 Å were deposited on top of the polymer films. In most cases, the percentage of copper was 10% or greater. The lowest concentration that they tested was a 2.6% copper film, which inhibited the evolution of HCN by 66%. Their experiments indicated that copper probably acts as an oxidative catalyst that decomposes gaseous HCN into N_2, CO_2, H_2O, and small amounts of nitrogen oxides. Further research is needed to determine whether this is the actual molecular mechanism that allows copper to act as a HCN toxicant suppressant.

Research by Levin *et al*. at NIST differed from that of Jellinek in that much larger samples of flexible polyurethane foam (including full-scale room burns of cushions and simulated chairs), and much smaller concentrations of copper were used. The toxicity of the combustion products from the copper-treated flexible polyurethane foam was also examined.

Unpublished data of Levin *et al*. also indicated that a wool fabric treated with copper would generate 50% less HCN than the untreated fabric. These results demonstrate a more universal effect, namely that treating nitrogen-containing materials with copper compounds will probably reduce the HCN generated when that material is exposed to fire conditions. Taking these results one step further, one could develop other toxicant suppressants, which, when added to materials and products, would now prevent or significantly reduce the toxic effluents that are generated when they are thermally decomposed. Since 80% of fire deaths are the result of smoke inhalation, a less toxic smoke could significantly increase the time available for escape and reduce the number of injuries and deaths from fire.

See also: Carbon Dioxide; Carbon Monoxide; CCA-Treated Wood; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50).

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Relevant Websites

http://www.wolmanizedwood.com – Information on Wolmanized pressure-treated wood.

http://ts.nist.gov – NIST website providing information of standard reference materials and protocols developed.

Common Mechanism of Toxicity

Beth Mileson

Common mechanism of toxicity is a phrase used to characterize the toxicological actions of two or more agents that act by the same cellular and molecular mechanisms leading to a common adverse effect on the structure or function of a living organism. An understanding of all steps that comprise a common mechanism of toxicity for given toxicants is rarely achieved, but identification of the crucial events following chemical interaction with an organism can be sufficient to describe a common mechanism of toxicity. For example, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and its congeners are a group of toxicants that have been characterized as acting by a common mechanism of toxicity, mediated by binding to the aryl hydrocarbon (Ah) receptor in animals.

The concept of common mechanism of toxicity gained prominence in the United States after Congress passed the Food Quality Protection Act of 1996 (FQPA), which requires the US Environmental Protection Agency (EPA) to consider the effects of human exposure to all pesticides and other chemicals that act by a common mechanism of toxicity when they derive tolerances (acceptable levels) for pesticide use on crops. As a result of the FQPA, the term common mechanism of toxicity has a regulatory connotation in addition to a toxicological definition. The US EPA Office of Pesticide Programs (OPP) makes an official determination to identify specific pesticides that act by a common mechanism of toxicity. After the common mechanism group is identified, OPP conducts a cumulative risk assessment of exposure to all the pesticides in the common mechanism group when establishing, modifying, leaving in effect, or revoking a tolerance for a pesticide chemical residue, as specified in the FQPA.

The US EPA OPP has issued guidance to identify pesticides and other chemicals that act by a common mechanism of toxicity. The first step in this guidance is to determine whether a group of pesticides should be considered a 'preliminary grouping' of compounds that may act by a common mechanism of toxicity. Characteristics that OPP suggests may be an indication that substances act by a common mechanism of toxicity include: (1) compounds share a structural similarity; (2) pesticides have a similar mechanism of insecticidal action; (3) compounds act by the same general mechanism of mammalian toxicity, and/or (4) cause a particular toxic effect.

The EPA OPP refines the evaluation of a preliminary common mechanism group by considering the biochemical and toxicological actions of each toxicant to determine if they all act by a common mechanism of toxicity, or if they should be separated into more than one common mechanism group for cumulative risk assessment. To determine if a preliminary group of compounds all act by a common mechanism of toxicity, the actions of these chemicals are evaluated based on whether they cause the same critical effect, act on the same molecular target at the same target tissue, act by the same biochemical mechanism, and/or share a common toxic intermediate. To evaluate mechanisms of toxicity, the US EPA OPP relies on data from studies submitted in support of pesticide registration, data from the public literature, and data from government reports.

The Pest Management Regulatory Agency (PMRA) of Health Canada proposed to harmonize with the US EPA policy on common mechanism of toxicity. PMRA issued guidance for identifying pesticides that have a common mechanism of toxicity that was adapted from the US EPA guidance document.

An example of a group of toxicants that act by a common mechanism of toxicity is the organophosphorus (OP) pesticides. OP pesticides are an otherwise structurally diverse group of chemicals that all contain phosphate atoms that are pentavalent and tetracoordinate. The primary molecular mechanism of action of most of the OP pesticides is initiated by inhibition of acetylcholinesterase (AChE), a serine esterase that occurs throughout the central and peripheral nervous system of vertebrates. The normal physiological action of AChE is to hydrolyze the neurotransmitter acetylcholine (ACh) so that activation of cholinergic receptors is transient. Inhibition of AChE results in accumulation of ACh at the synapses, overstimulation of cholinergic receptors on muscle fibers, neurons, and autonomic end organs, and resultant signs of cholinergic toxicity. Clinical signs of cholinergic toxicity include: increased lacrimation and salivation, bronchoconstriction, bronchosecretion, miosis, gastrointestinal cramps, diarrhea, urination, bradycardia, tachycardia, hypertension, muscle fasciculations, tremors, and muscle weakness, among other signs. OP pesticides have been determined to act by a common mechanism of toxicity if they inhibit AChE by phosphorylation and elicit any spectrum of cholinergic effects in exposed animals or humans.

Many classes of pesticides are composed of structurally similar compounds that act by a common

mechanism of toxicity. Examples of pesticide groups that contain multiple compounds that act by a common mechanism of toxicity in addition to the OP pesticides include: methyl carbamates, triazines, and pyrethroids. The fact that groups of pesticides act by a common mechanism of toxicity is predictable since these chemicals were designed to resemble one another structurally and elicit similar pesticidal effects.

See also: Food Quality Protection Act, US; Pesticides; TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin).

Further Reading

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Relevant Website

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Comprehensive Environmental Response, Compensation, and Liability Act, US

Robert Kapp

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- TITLE: CERCLA; revised as the Superfund Amendments Reauthorization Act (SARA): also known as the Superfund
- AGENCY: US Environmental Protection Agency (EPA)
- YEARS ENACTED AND REVISED: CERCLA: December 11, 1980; SARA: October 17, 1986

Background

Until the 1970s, US citizens were mostly unaware of how the indiscriminate dumping of chemical wastes might affect public health and the environment. The common practice was to simply abandon waste chemicals on properties and in landfills with no consideration for their ultimate fate. This resulted in thousands of uncontrolled and abandoned hazardous waste sites throughout the nation. Although the Environmental Protection Agency (EPA) had been created in 1970, there were no specific provisions to deal with this ever-growing environmental problem. The Clean Water Act – CWA (1977), the Resource Conservation and Recovery Act – RCRA (1976), the Water Pollution Control Act (1972), and the Rivers and Harbors Act (1899) set the stage for more comprehensive legislation. However, in 1979, the events at Love Canal, New York brought to a head the fact that abandoned hazardous waste could be a serious threat to any community. Citizen concern was high over the magnitude of the ever-growing problem and that led Congress to establish The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) to locate, investigate and clean up the worst sites nationwide.

Overview of CERCLA (Superfund)

On December 11, 1980, Congress passed the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, also known as the Superfund). This law strengthened the EPA and State authorities to investigate and respond to the release of waste and hazardous materials into the environment. The Office of Emergency and Remedial Response at EPA administers the program in cooperation with states and tribal governments. This legislation essentially created a tax on the chemical and petroleum industries, and provided broad Federal authority to respond directly to chemical releases or potential releases of hazardous substances that are deemed threatening to public health or the environment. The taxes collected from inception through 1986 totaled US$1.6 billion.

The legislation was amended on October 17, 1986 with the enactment of the Superfund Amendments and Reauthorization Act (SARA). One of the changes in SARA was to increase the fund from $1.6 to $8.5 billion, and SARA required the EPA to make changes to the Hazard Ranking System to more accurately note the level of danger of sites to be placed on the National Priorities List (NPL). In addition, the NPL must be revised and republished every 2 years, and informally reviewed annually. The term ‘Superfund’ is derived from the fund of money that is collected by EPA to investigate sites and to

pay for cleanups where no responsible parties can be determined.

There are four basic components to the CERCLA/Superfund Legislation:

1. The legislation sets up an information-gathering and analysis system that enables federal, state and tribal governments to designate chemical dumpsites and develop priorities for cleaning them up. The EPA Administrator issues regulations that, in the Administrator's opinion, identify 'hazardous' or 'toxic' substances. The owners and operators of sites containing any listed chemical must notify the EPA of the amount and types of identified wastes their sites contain. This information assists the EPA in developing the NPL to plan responses.
2. The legislation also gives the EPA the authority to respond to toxic substance emergencies where immediate short-term response is deemed necessary for the public welfare.
3. The legislation further established a Hazardous Substance Trust Fund to pay for removing wastes and for remedial actions associated with the cleanup where no responsible parties can be determined. SARA revised and expanded the 1980 CERCLA legislation from its original $1.6 billion budget to $8.5 billion.
4. The legislation holds responsible persons and companies liable for toxic wastes cleanup and restitution costs. Unfortunately, the legislation was not clear on this point and not all federal courts have applied the same standards to determine parent corporation liability for CERCLA infractions.

Site Cleanups – Remediations and Removals

- Remediations are conducted according to the National Contingency Plan and refer to permanent cleanups.
- Removals are cleanups other than permanent, that is, emergency or temporary cleanups.
- EPA generally takes remedial actions only at sites listed on the NPL (which currently lists ~1300 sites).
- EPA assigns responsibility by looking at all potentially responsible parties (PRPs), including current owners/operators, previous owners/operators, facilities that generated the waste and transporters that delivered the waste.
- 'Strict' liability is defined as parties being responsible regardless of how careful they were in their practice of disposing of the waste.
- 'Joint and several' liability is defined as any one PRP is potentially liable for all costs of the cleanup no matter how much of the total contamination is directly due to their disposal activities.
- General remediation process is specified under the Superfund's National Contingency Plan as follows:
  - emergency removal to address immediate environmental problems;
  - a remedial investigation/feasibility study (RI/FS) to determine cleanup approaches;
  - a Record of Decision to document the approach EPA has selected for cleanup; and
  - the design, construction, operation and maintenance of the final cleanup.
- In addition, the cleanup process is required to meet all other environmental requirements during its operation. These are referred to as applicable or relevant and appropriate requirements.

New Releases

The CERCLA hazardous substance release reporting regulations in the US Code of Federal Regulations (40 CFR Part 302) are intended to minimize environmental releases of current manufacturing processes. A designated person at the facility must report the release of any hazardous material when it exceeds the reportable quantity to the National Response Center. The hazardous substances and reportable quantities are defined and listed in 40 CFR §302.4.

The National Response Center telephone number is: 1-800-424-8802

US EPA's RCRA/Superfund/UST Hotline to answer questions regarding guidance for the Superfund Program: 1-800-424-9346.

See also: Love Canal; National Environmental Policy Act, US; Pollution Prevention Act, US; Resource Conservation and Recovery Act, US; Valley of the Drums.

Relevant Websites

http://www4.law.cornell.edu – Cornell University Law School, US Code covering CERCLA.
http://www.epa.gov – US EPA CERCLA information.

Computational Toxicology

S Satheesh Anand and Harihara M Mehendale

Definition

Computational toxicology is the application of mathematical and computer models for prediction of effect of toxic agents and understanding the mechanism.

Background

Protecting human health from the possible hazardous effects of toxic chemicals is a challenging task. A number of scientific uncertainties exist along a 'source-to-adverse outcome' continuum, beginning with the presence of the chemical in the environment, frequency and duration of exposure, disposition of the chemical, the presence of the active chemical at a systemic target site, and the series of biological events that lead to the manifestation of an adverse outcome. Unexpected toxicity due to interaction and altered toxicity by lifestyle such as smoking, drinking, etc. are additional issues. Present risk assessment methods rely on laboratory testing of chemical-to-chemical basis to obtain toxicity data and the quantitative relationship between dose level and likelihood of toxic response to estimate human risk. The large number of chemicals in commerce coupled with time and expense limit the testing to a few chemicals. Moreover, the question pertaining to high to low dose and animal to human extrapolation still remains. In view of some 87 000 chemicals under consideration, it would be beneficial if rapid testing methods were developed to assist prioritization of chemicals for further testing and reduce the existing uncertainties in risk assessment.

Over the last several years, there has been increasing pressure to reduce the animal use in toxicology and to utilize novel technologies such as *in vitro* methods, and computational chemistry for rapid identification of chemical risks. The *in vitro* data are not sufficiently validated to address the uncertainties in risk assessment. Hence, the interest has shifted toward using computers, which are capable of performing a series of complex arithmetic or logical operations and have the ability to process, store, and retrieve data without human intervention. The use of computers in toxicology has increased significantly in recent years. Computational toxicology involves the application of various mathematical and computer models to predict effects and understand the cascade of events that result in an adverse response, or its mode of action. It would improve linkages across the source-to-outcome continuum, including the areas of chemical transformation and metabolism, better diagnostic/prognostic molecular markers, improved dose metrics, characterization of toxicity pathways, metabonomics, systems biology approaches, modeling frameworks, and uncertainty analysis.

Computational toxicology includes several computational disciplines including:

- Computational chemistry, which refers to physical–chemical mathematical modeling at the molecular level and includes such topics as quantum chemistry, force fields, molecular mechanics, molecular simulations, molecular modeling, molecular design, and cheminformatics.
- Computational biology or bioinformatics, which refers to development of molecular biology databases and the analysis of the data.
- Systems biology, which refers to the application of mathematical modeling and reasoning to the understanding of biological systems and the explanation of biological phenomena.

Three strategic objectives of the computational toxicology initiative are to:

1. improve understanding of the linkages in the continuum between the source of a chemical in the environment and adverse outcomes;
2. provide predictive models for screening and testing; and
3. improve quantitative risk assessment.

Computer in Contemporary Toxicology

Computational toxicology is a rapidly emerging and developing area, combining theoretical models with computers to investigate a variety of toxicological problems. In order to achieve the precise predictive methodology, it is necessary to link adverse outcomes to initiating events and the target organ's response to injury. Computational toxicology techniques have excellent promise to focus research on reducing uncertainties in both ecological and human health risk assessments. The use of computer in toxicology has steadily increased from literature survey, data mining, and statistical analysis to predicting toxic outcome and reducing uncertainties in risk assessment.

Several computer software programs are available for compartmental modeling of pharmacokinetic

data (such as WinNonlin, PK analyst, Summit, SAS). In general, concentration and time data are entered into a spreadsheet format. The operator then chooses a user-defined model or a specified model from a built-in library to fit curves to concentration versus time data. Program outputs include pharmacokinetic parameter estimations and descriptive statistical estimations. A wide variety of options and costs are available that can fit users' needs.

The following are the emerging approaches in toxicology in which use of computer is pivotal:

'Omics'

The unprecedented advances in molecular biology during the last two decades have resulted in a dramatic increase in knowledge about gene structure and function, an impressive set of efficient new technologies for monitoring genetic sequences, genetic variation, and global functional gene expression. These advances have led to a new subdiscipline of toxicology, 'toxicogenomics', which includes studies of the cellular products controlled by the genome (messenger RNAs, proteins, metabolites, etc.). The new 'global' methods of measuring families of cellular molecules, such as RNA, proteins, and intermediary metabolites, have been termed 'omic' technologies. The computer is an integral part in the 'omics' field and it is very difficult to handle the data generated by these technologies without computers. The development of 'omic' technologies has evolved into three scientific disciplines. Systems biology allows one to integrate the complex information developed by three areas of 'omics' at the organismic level.

Genomics

Genomics is the study of genes and their biological function. It has been done using the microarray technique (also called DNA chips), which contains many hundreds or thousands of short DNA strands, each in its own compartment. By washing a solution of a substance over the whole chip at once, the section of DNA affected can be made to fluoresce, thus indicating which genes are turned on or off by the substance and suggesting its likely effect on the body. Presently, each chip contains a particular number of genes and it may soon be possible to include the whole human genome on such a chip and test all of it at once for possible adverse effects. The advantage of using the genomic technology is that it will capture the changes in the genes, which are not originally under investigation. It is expected that genomics will greatly help in defining and characterizing sensitive subpopulations and producing signature profile for each toxin. There are number of software such as arraySCOUT, GeneSpring, Spotfire DecisionSite for Functional Genomics available to read and interpret the genomic data.

Proteomics

Proteins are involved in all biological processes and can therefore be considered the functionally most important biological molecules and are crucial for the description of biological systems. The systematic identification and characterization of proteins is called proteomics. A predominant technology platform in proteomics, two-dimensional gel electrophoresis, is used to separate complex protein mixtures allowing individual protein spots on the gel to be identified by computer-operated mass spectrometry. Mass spectrometric data are then processed through a series of computer algorithms such as Mass Lynx and ProteinLynx software to determine the sequence identity of the proteins.

Metabonomics

Metabonomics is a systems approach for studying *in vivo* metabolic profiles and is still a relatively new technology in comparison to the other 'omics'. Genomics and proteomics allow for the measurement of response to chemicals on the genetic and cellular protein levels, respectively; however, neither provides a complete description of metabolism and chemical toxicity. To fully understand the xenobiotics metabolism, it is crucial to understand the metabolic status of the whole organism. Metabonomics complements genome and proteome responses and provides connection between these and tissue function. The application of metabonomics to toxicity testing involves the elucidation of changes in metabolic pattern associated with chemical toxicity based on the measurement of component profiles in biofluids, cells, or tissues. Metabolites are assayed in biofluids using nuclear magnetic resonance spectroscopy. Like proteomics and genomics, metabonomics provides a fingerprint of the small molecules contained in a given biofluid. Software such as Eclipse and MATLAB are used for bioprofiling of metabolites.

Systems Biology

Systems biology is a new field, which integrates genomic, proteomic, and metabonomic information into a coherent picture. Systems biology is brought by joining computer science, biology, and medical programs and could lead to the development of virtual biological systems. Systems biology uses computational methods to reconstruct an integrated physiologic and biochemical model of an organism's

or cell's biology that allows validation and simulation experiments that build confidence in predictive ability of adverse effects. In this regard, it is targeted at studying how normal biological processes are governed, and how alterations can lead to diseases or other unwanted outcomes.

The 'omics' technology is a high throughput separation of genes, proteins, and metabolites and has the potential to be a powerful tool in risk assessment. However, it is still in its infancy. The advantage of using these approaches is to capture the effects of compounds at low doses, which are not possible in animal testing. These techniques generate huge amount of data and it is not easy to analyze. Bioinformatics aids in making meaningful conclusions from a deluge of data points. Advanced computer software are now able to interpret the data with an increasing degree of accuracy. In addition to reading and interpreting the data, computers are making it possible to apply complex analytical techniques used in 'omics'.

It is anticipated that these new technologies will: (1) lead to new families of biomarkers that permit characterization and efficient monitoring of cellular perturbations; (2) provide an increased understanding of the influence of genetic variation on toxicological outcomes; and (3) allow definition of environmental causes of genetic alterations and their relationship to human disease. With the development of 'omics', the discipline of toxicology is acquiring exciting new tools in safety screening, problem solving, and mechanistic investigation. Assuming a group of compounds induces similar changes in the gene, protein, or metabolic profile, it may be possible to classify compounds based on their profiles; i.e., it would provide a 'fingerprint'.

Quantitative Structure–Activity Relationships

Quantitative structure–activity relationship (QSAR) dates back to the nineteenth century and is a computer-based tool that attempts to correlate variations in structural or molecular properties of compounds with their biological activities. These physicochemical descriptors, which include parameters to account for hydrophobicity, topology, electronic properties, and steric effects, are determined empirically or, more recently, by computational methods. The premise is that the structure of a chemical determines the physiochemical properties and reactivities that underlie its biological and toxicological properties. Being able to predict potential adverse effects not only aids in the designed development of new chemicals but also reduces the need for animal testing. It may ultimately or potentially lead to better health and environmental protection through the strategic application of limited testing resources and existing information assets to help sort out or identify the most hazardous chemicals. These principles have already been successfully applied to the prediction of skin permeability coefficients, the skin corrosivity of organic acids, bases, phenols, and electrophilic organic chemicals, and the eye irritation potential of neutral organic chemicals. QSARs are currently being applied in many disciplines pertaining to drug design and development. Lately, interest has grown in utilizing this tool in environmental risk assessment to group the compounds of similar biological activity. Software such as TOPKIT and CaseTox are used in QSAR modeling.

Biological variability can be demonstrated quite readily by building a QSAR model, which discriminates between chemicals with different toxicological hazard classifications. Application of computer-based QSARs has resulted in developing novel predictive capabilities for representing chemical structures as a distribution of conformations and properties rather than discrete structures.

Physiologically Based Pharmaco (Toxico) Kinetics

Knowledge of the concentration–time relationships of toxicants in the biological systems is paramount in predicting toxicological effects. The commonly used pharmacokinetic models cannot be used for extrapolation due to the lack of actual anatomical, physiological, and biochemical realism. The extrapolation is possible when the mathematical descriptions of the uptake and disposition are combined with the knowledge of anatomy, physiology, and biochemistry and is termed physiologically based pharmaco (toxico) kinetics (PBPK) models. These models are used to gain insights into the dosimetry and mode of action of chemicals. A PBPK model is founded on known physiological processes (blood flow rates, tissues volumes, breathing rates, etc.), on chemical-specific processes (partition coefficients, chemical density, metabolic constants, molecular weight, etc.), and on species-dependent processes. Thus, a PBPK model can be developed and validated against the more readily available experimental animal data, and then extrapolated to predict concentrations of toxic compounds in human tissues following exposure. PBPK models can be used to extrapolate from a single acute to a chronic exposure, from a continuous to an intermittent exposure, from a high to a low exposure, and from a single chemical to a complex mixture exposure. Perhaps the greatest impact in using a PBPK model to convert exposure into a target

tissue dose is the flexibility to perform these calculations on an individual basis. Models can be developed to individually track each person's unique physiological and/or metabolic characteristics. The metabolic rates in a model can be individually adjusted to account for enhanced (or decreased) metabolism of a particular compound when the person is on a specific pharmaceutical agent. PBPK modeling is a powerful toxicological tool designed to convert an exposure (regardless of route) into a target tissue dose.

In PBPK models, the body is divided into compartments with blood flow to each compartment. Chemicals can be introduced into the body by several routes, metabolized, and excreted in breath, feces, and urine. The compartments and flows are described by a system of differential mathematical equations. In order to write and solve the mathematical equation and interpret the data, the help of a computer is essential. The PBPK model equations, along with the integration algorithms, can be written and solved using programming language (FORTRAN, BASIC, etc.), simulation software (ACSL, acslXtreme, Matlab, Simusolv, STELLA, etc.), or spreadsheets on many type of computers – mainframes, workstations, microcomputer, or minicomputers. The processing speed, hard disk space, and run time memory of the currently marketed computers are quite helpful in PBPK modeling.

Computational Toxicology in Quantitative Risk Assessment

Following are the areas in which computational toxicology is expected to reduce or eliminate the uncertainties:

Dose–Response Assessment

Computer-based technologies would help in determining the shape of the dose–response curve in the low dose range based upon *in vivo* and *in vitro* data, and to correlate with low dose adverse effects. These technologies may also result in the identification of useful biomarkers, adverse effect, and mode of action of the low dose range.

Cross-Species Extrapolation

One of the major challenges in regulatory toxicology is the prediction of toxicity of a chemical across the species; presently, the effects in humans are predicted from animal studies. The concept of extrapolation is based on the knowledge that all the species arise from common evolutionary ancestors. However, it is not trivial because chemicals vary as a function of an animal's physiology and its environment and pathways of metabolism can differ significantly across species, even closely related animals. These can be addressed by biologically based dose–response models. Although there is remarkable similarity in basic biology among animals, there are also significant species-specific differences in genes, proteins, biochemistry, and physiology. These differences lead to uncertainties in extrapolation. To address this, characterizing the toxicity pathways is critical.

Chemical Mixtures

Multichemical exposures are ubiquitous. A number of uncertainties exist from the mode of action, type of interaction to toxic outcome in the mixture risk assessment. Computer-based technologies have huge potential to elucidate the mechanisms and predict the outcome for real-world mixtures rather than the defined mixtures at high-dose concentration.

Sensitive Population

There is an increasing concern that some people are more susceptible to toxic effects of one chemical compared to others. This group includes children, pregnant women, elderly, genetic background, lifestyle such as smoking, alcohol consumption, diet, and existing disease conditions. With application of computational toxicology it is expected that we will be able to fine-tune our ability to predict the mechanisms behind the susceptibility and reduce the uncertainty.

Conclusions

Protecting human health and the environment carries with it the challenge of assessing the risk that is posed by tens of thousands of chemicals. The large numbers of chemicals, various uncertainties and difficulties associated with the regulation have made it impossible to evaluate the data on all the chemicals. Today, however, toxicology is getting a facelift with the help of recent computer-based approaches (e.g., genomics, proteomics, metabonomics, QSAR, and PBPK), and scientists may have the ability to develop a more detailed understanding of the risks posed by a much larger number of chemicals. When the biology is complex and the database is large (modern technologies generate prodigious amounts of data), then these models play an essential role in organizing data, identifying key data gaps, and generating predictions of dose–response behaviors. Computational

toxicology is expected to increase the accuracy or precision of risk assessment based on science. It is designed to increase the capacity to prioritize, screen, and evaluate chemicals by enhancing the predictive understanding of toxicities. Success will be measured by the ability to improve risk assessments by understanding the potential of chemicals to affect molecular and biochemical pathways of concern. Computational methods will not assure 100% accuracy in the prediction of toxicity, and they will not eliminate the need for testing, but these methods will dramatically slash the time, choice, and expense of testing chemicals and make regulatory decision-making quicker and science-based.

See also: Bioinformatics; Risk Assessment, Human Health; Safety Testing, Clinical Studies; Toxicity Testing, Validation.

Further Reading

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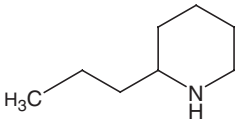
Relevant Websites

http://www.niehs.nih.gov – National Center for Toxicogenomics (NCT).
http://www.epa.gov – US EPA. Computational Toxicology.

Coniine

Murali Badanthadka and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 458-88-8
- Synonyms: S-2-Propylpiperidine; Cicutine; Conicine; *N*-Methylconine; Conhydrine; Pseuedoconhydrine
- Chemical/Pharmaceutical/Other Class: Piperidine alkaloid
- Chemical Formula: $C_8H_{17}N$
- Chemical Structure:



Background Information

Poison hemlock (*Conium maculatum*) and dog parsley (*Aethusa cynapium*) are poisonous plants of the parsley family, which contain coniine.

Exposure Routes and Pathways

The most common route of coniine exposure is by ingestion, although there are reports of dermal and eye irritations upon direct contact.

Toxicokinetics

Coniine is rapidly absorbed from the gastrointestinal tract.

Mechanism of Toxicity

Coniine acts on the autonomic ganglia to produce initial stimulation of skeletal muscle followed by neuromuscular blockade. The actions of coniine are similar to those of nicotine but produce paralysis of greater numbers of central nervous system (CNS) and skeletal muscle nerve endings.

Acute and Short-Term Toxicity (or Exposure)

Animal

Certain small birds (skylarks, chaffinches, and robins) are not susceptible to coniine poisoning. Coniine toxicity has been reported in cows, goats, horses, pigs, sheep, ewes, rabbits, and chickens. The oral LD_{50} of coniine in the mouse is $\sim 100\,mg\,kg^{-1}$. There are limited data in cattle, goats, and sheep suggesting developmental abnormalities to the musculoskeletal system of offspring when pregnant mothers are exposed orally to coniine ($70\,mg\,kg^{-1}$ for cattle and $484\,mg\,kg^{-1}$ for goats and sheep).

Human

Toxic doses of the plant extract are difficult to determine due to differing concentrations of eight piperidinic alkaloids in the plant. The concentrations of alkaloids vary with the age of the plant. Plants up to ~1-year-old have very low alkaloid content in roots, ~0.15% in stems and 0.3–0.6% in the leaves. Plants in their second year have an alkaloid content of ~1% in all parts of the plant. Geographic latitude and drying will also affect the coniine content of the plant. A toxic dose of coniine is estimated to be

60 mg and a lethal dose is estimated to be 100–300 mg for an adult person.

The principal manifestations of coniine poisoning are nausea and vomiting, salivation, fever, and gradually increasing muscular weakness followed by paralysis with respiratory failure.

Clinical Management

No antidote exists for coniine. Treatment is directed at removing ingested toxin and providing supportive care. Gastric lavage may be used to remove the ingested plant or plant extract. However, this method may not effectively remove large pieces of plant material. Intragastric administration of activated charcoal is recommended to reduce absorption in the gastrointestinal tract. Due to the rapid onset of CNS depression and seizures, emesis is generally not recommended. Difficulty to breath is treated by artificial respiration with oxygen. Convulsions are controlled with diazepam.

See also: Hemlock, Poison; Plants, Poisonous.

Further Reading

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Consumer Products

Nancy Linde

Introduction

There are products that we use everyday and products that we use only occasionally. Among both categories, there are products that are very tempting for children by the nature of the way the look, smell, sound, feel, or simply because they can be reached. According to the Federal Hazardous Substances Act of 1960, labeling of hazardous products must contain instructions for special handling and storage, as well as warning statements for accidental exposure prevention and treatment. Several common household products are briefly discussed here for their most common toxicological concerns.

Specific Categories and Concerns

Air Fresheners/Deodorizers

These products commonly contain formaldehyde. Accidental exposures, depending on the amount, may lead to eye, nose, throat and skin irritation, as well as nausea, nosebleeds, headaches, dizziness, memory loss, and shortness of breath. While these symptoms are typically reversible, formaldehyde is a regulated as a carcinogen.

Ammonia

Ammonia is a respiratory irritant when inhaled, and large doses may affect the nervous and circulatory system with rapid heart beat, weak pulse, bluish lips and fingers, restlessness, and temporary blindness. When ingested it can cause severe pain in the mouth, chest, throat, and stomach. Dermal contact can cause severe irritation, burning, and even permanent blindness.

Antifreeze

Consumption of small amounts of antifreeze can be deadly. Poisonous constituents are typically ethylene glycol and methanol. There is no home treatment aside from standard first-aid and cardiopulmonary resuscitation (CPR) for signs of shock or cardiac arrest. Gastric treatment and dialysis may be immediately necessary for survival depending on the dose, and long-term kidney and brain damage are possible.

Bleach

Powdered and liquid household bleach contain sodium hypochlorite. This is a respiratory irritant and can cause burning from inhalation, ingestion, or dermal contact. Contact with other chemicals can

lead to chlorine fumes, and when mixed with ammonia can result in formation of a nonodorous deadly methane gas. The extent of damage will depend on the amount consumed and how rapidly it is diluted and neutralized. Accidental ingestion should be followed by immediate consumption of milk or water to dilute, and vomiting should not be induced due to the burning potential. Long-term damage to the mouth, throat, eyes, lungs, esophagus, nose, and stomach are possible, some of which may continue to occur for weeks after exposure.

Detergents

Household detergents generally contain simple soaps and corrosive alkalis. Ingestion may lead to respiratory irritation, severe abdominal pain, vomiting, hypotension, and dangerous change in blood pH. Ingestion should be followed by immediate consumption of milk or water if possible, then immediate medical attention. The prognosis will depend on how rapidly the alkali was diluted and neutralized. Extensive damage to the mouth, throat, eyes, lungs, esophagus, nose, and stomach are possible, and depending on the severity of the damage, death could occur up to 1 month after ingestion.

Disinfectants

Common household disinfectants contain sodium hypochlorite, phenols, or ammonia.

Drain Cleaners

Drain cleaners may contain sodium or potassium hydroxide (lye), hydrochloric acid, or trichloroethane. Sodium or potassium hydroxide is a caustic irritant that can affect the central nervous system (CNS) inhibiting reflexes, cause burns to skin and eyes, and is poisonous if swallowed due to severe tissue damage. Hydrochloric acid is a corrosive irritant, causes damage to the kidneys, liver, and digestive system. Trichloroethane is a skin and eye irritant, causes central nervous system depression, and liver and kidney damage when ingested.

Dyes

Household dyes for cloth are typically nontoxic mixtures of pigments, salts, and mild soaps. Some contain small amounts of corrosive alkali detergent that can only be hazardous if consumed in large quantity.

Fertilizers and Household Plant Foods

Plant fertilizers are mildly toxic in small doses, and may lead to gastrointestinal upset and or skin irritation. Larger doses can be more harmful, especially to children, and may cause severe burns, therefore, vomiting should not be induced, but milk or water should be given to dilute poisonous ingestions. Poisonous ingredients are typically nitrates and nitrites.

Glue

Household glues such as Elmer's and Krazy Glue are generally nontoxic and would require large consumption to cause abdominal pain and obstruction of the gastrointestinal tract. Elmer's glue can be easily washed off the skin with soap and water, whereas Krazy Glue and other super glues (containing cyanoacrylates) generally require rinsing with nail polish remover or acetone. For eye contact, rinsing should be done only with water and/or an eye doctor should be seen if symptoms persist or the eyelids cannot be opened. Symptoms are generally treatable with full recovery. For effects related to inhalation of glue solvents, see section on Inhalants.

Hydrogen Peroxide

Household hydrogen peroxide is typically sold in a 3% solution. Short-term dermal exposures may whiten the skin temporarily. Ingestion can lead to burns in the oral cavity and throat, and abdominal pain. Dermal and ocular exposures should be immediately rinsed, and vomiting should not be induced.

Inhalants

Inhalant abuse is a drug abuse problem. Inhalants can be any substances that have volatile hydrocarbons as their base. Examples of hydrocarbons are acetone, benzene, toluene, turpentine, and gasoline. Volatile hydrocarbons can be classified into several groups. They range from high volatility, minimal viscosity substances such as methane, butane, benzene and petroleum ether to minimal volatility, high viscosity products such as lubricating oil, mineral oils and asphalt. The inhalation of these substances, especially those with high and intermediate volatility, can rapidly displace alveolar gas, causing difficulty in breathing. In addition, they can easily cross the capillary membrane of the lungs and affect the CNS. Most hydrocarbons are CNS depressants and the early effects of inhalation resemble alcohol intoxication. Continued inhalation however may lead to increased symptoms of intoxication, confusion, hallucination, and aggressive behavior.

As most of the harmful effects of inhalant abuse are not felt immediately, chronic abuse of inhalants is associated with a variety of medical problems with a real risk of death. There are a number of solvents that have become the target for abuse. A great number of them are known to be toxic (see **Table 1**).

Table 1 Common targets for consumer product inhalation abuse

Products	*Major volatile compounds*
Adhesive/glues	Acetone, ethyl acetate, butanone, toluene, cyclohexane, trichloroethylene, *n*-hexane, xylene
Aerosol propellants	Liquid petroleum gas, dimethyl ether, fluorocarbon
Anesthetics and analgesics	Nitrous oxide, cyclopropane, diethyl ether, halothane, enflurane, isoflurane
Commercial dry cleaning and degreasing agents	Dichloromethane, methanol, trichloroethane, toluene
Domestic spot removers and dry cleaners	Dichloromethane, trichloromethane, tetrachloroethylene
Fire extinguishers	Bromochlorodifluoromethane
Cleaning solutions	Trichloroethylene, petroleum products, carbon tetrachloride
Fuel gases	Liquid petroleum gas, propane, butane
Nail varnish and nail varnish remover	Acetone and esters
Paint and paint thinners	Acetone, butanone, esters, hexane, toluene, trichloroethylene, xylenes
Typewriter correction fluid	Trichlorethane
Industrial solvents	*n*-Hexane
Lighter fluids	Naphtha, aliphatic hydrocarbons

Source: Razak Lajis, pharmacist at the National Poison Centre, Universiti Sains Malaysia, Penang, Malaysia.

Table 2 The health hazards of some solvents used in substances that are inhaled

Solvent	*Health hazard summaries*
Acetone	Vapors mildly irritating to eyes and respiratory tract. A CNS depressant at high levels. Ataxia and seizures have been reported
Chloroform	Vapors slightly irritating to eyes and respiratory tract. A CNS depressant. Mild to moderate systemic toxicity include headache, nausea, vomiting, confusion, and drunkenness. More severe exposures may cause respiratory arrest and coma. A carcinogen in animals
n-Hexane	Vapors mildly irritating to eyes and respiratory tract. Light-headedness, giddiness, nausea, and headache. Greater exposure may cause unconsciousness and death
Toluene	Acute exposure results in euphoria, excitement, dizziness, headache, nervousness, ataxia, convulsion, and coma. Deaths have been recorded from acute exposure to toluene in 'sniffers'
Trichloroethane	A respiratory and CNS depressant. The symptoms of acute inhalation may include nausea, euphoria, ataxia, dizziness, agitation, and lethargy. Severe exposure will lead to respiratory arrest, seizures, and coma
Xylene	Dizziness, excitement, flushing of the face, drowsiness, incoordination, tremor, confusion, respiratory depression, and coma

Taken in smaller doses, they can cause euphoria, delusions, and hallucinations. Higher doses may lead to convulsions and coma. Chronic abuse of certain substances such as toluene-containing products can produce severe organ damage involving the liver, kidneys, and brain. Inhalation of glues remains hazardous and can be fatal. Glues containing *n*-hexane and toluene (see **Table 2** for a list of health hazards posed by solvents used in substances that are inhaled) have been associated with the development of muscle weakness and atrophy. Three major clinical presentations are common with people who sniff toluene-containing glues. They will experience muscle weakness, gastrointestinal complaints, and neuropsychiatric disorders. Glue sniffers may also develop signs of renal toxicity. The euphoria of mild intoxication may be accompanied by nausea and vomiting. Some of the signs and symptoms of acute intoxication are breathing difficulties, chest pain and discomfort, eye irritation, double vision, ringing ears, diarrhea, and muscle and joint pain. After prolonged inhalation or rapid inhalation of highly concentrated vapor, the sniffer may experience a phase of excitement followed by loss of consciousness and coma.

Inks and Dyes

Artistic inks and dyes, as well as those used in textiles are generally nontoxic, though occasionally irritating or allergenic to some. They are known to have high contents of heavy metals, such as cobalt, lead, mercury, and others, many of which are common skin irritants, and some of which are carcinogens and reproductive toxicants therefore large and frequent

ingestions should be avoided. Dermal and ocular contact should be followed by rinsing with water to prevent irritation. Eye irritation and staining of skin and other mucous membranes is generally reversible.

Pesticides

Typical household pesticides can be moderately toxic with inhalation, but are generally nontoxic for human dermal contact or low-level ingestion (carbon, sulfur, potassium nitrate). Revenge Rodent Smoke Bombs, for example, contain a variety of volatile gases and solids that will become airborne and respirable upon ignition. After igniting, cartridge produces foul-smelling smoky mixture of gases (including carbon monoxide, carbon dioxide, and nitrogen) and solids (including potassium carbonate, potassium sulfate, sulfides, and uncombined carbon). Fumes may be harmful if inhaled. If inhaled, and person has poison symptoms (headache, nausea, dizziness, and weakness), the victim should be transferred to fresh air. The victim should be made to lie down and kept warm. If response is adequate recovery will be rapid. If breathing has stopped, artificial respiration should be provided. A physician should be contacted immediately.

Pesticide residues are common to many types of food as they protect against molds, fungi, and insects. The actual types of pesticides, and residuals found on foods are tightly regulated by the US Department of Agriculture. While allowed pesticides residues already have low in toxicity, rinsing/washing fresh produce in cold water is recommended.

Herbicides

Common household herbicides such as Ortho Weed B Gon Crabgrass Killer generally contain pesticides that are relatively nontoxic to humans, such as calcium acid methanearsonate at 8–10%. Eye and skin irritation may be mild with quick recovery, and small ingestions and inhalation are nonirritating.

Fungicides

Common household fungicides, such as Scotts Lawn Fungus Control and Ortho Multi Purpose Fungicide Daconil 2787 Plant Disease Control generally contain carbamates that are moderately toxic, cause skin and eye irritation upon exposure, may be harmful if swallowed, and with prolonged or excessive inhalation may cause respiratory tract irritation.

Insecticides

Typical household bug sprays are generally mixtures of pyrethrins. These are generally not highly toxic to humans; however, inhalation may cause asphyxiation. Industrial insecticides occasionally found in households, garages, and greenhouses may contain more toxic organophosphates, carbamates, and paradichlorobenzenes. Exposure to these compounds may result in breathing difficulty, gastrointestinal upset, multiple nervous system effects such as weakness, sweating, convulsions, and other treatable symptoms. Dermal exposures should be followed by immediate rinsing, CPR should be administered if breathing stops, and all exposures should be immediately treated by a health professional. Some multipurpose landscaping/yard insecticides, such as Ortho Bug B Gon Multi Purpose Insect Killer Ready may contain permethrin at 2.5%, or other pyrethroids. Canine flea products contain higher amounts, such as 45–65% permethrin. Agricultural insecticide applications of pyrethrins are in the range of 0.14–0.25 lb per acre on various nuts and vegetables. In most mammals, there is generally a rapid metabolic breakdown of pyrethroids by the liver; therefore, no signs of toxicity are likely to occur. Permethrin is detoxicified by ester hydrolysis or oxidation, followed by hepatic hydroxylation and conjugation to either glucuronides or sulfates. Because cats are generally deficient in metabolizing substances through hepatic glucuronidation, they are limited in their ability to metabolize permethrin quickly. Cats will exhibit sensitivity to high concentration of this insecticide. The clinical signs of permethrin toxicosis in cats may include muscle tremors, hyperexcitability, depression, ataxia, vomiting, seizures, anorexia, and death. Signs may develop within a few hours to 3 days following exposure. Treatment of permethrin toxicosis in a cat consists of tremor and/or seizure control, dermal decontamination, and supportive care.

Other household garden insecticides, such as Ortho Bug B Gon Ready Spray contain dizainon, a common pesticide that is relatively non-toxic when inhaled or ingested in small accidental amounts, though are moderately irritating to the eyes and skin. Larger ingestions can cause cholinesterase inhibition with symptoms occurring within 12 h. Effects may include headache, dizziness, weakness, nausea, vomiting, diarrhea, pupil constriction, blurred vision, excessive salivation or nasal discharge, profuse sweating, and abdominal cramps. Incontinence, unconsciousness, convulsions and breathing difficulties are indicative of severe poisoning.

Human dermally applied insecticides such as Cutter, DEET, Off Skintastic and others may contain *N*,*N*-diethyl-*m*-toluamide up to 35%. They are generally safe at the recommended doses. Several case examples have reported symptoms in children receiving higher and frequent doses, especially of DEET, of disorientation, slurred speech, flexing of

fingers and dorsiflexing of toes, shaking, and convulsions. As these products are sold at diluted concentrations, the active compound is generally mildly irritating to mucous membranes and if it gets into the eyes, can be immediately relieved by rinsing with water. Few have reported skin irritation with frequent use, especially those with other skin ailments such as psoriasis or severe acne.

Flea Powder

Flea powders may contain carbaryl, dichlorophene, chlordane, and other chlorinated hydrocarbons. Carbaryl is very toxic, interfering with the CNS, respiratory system, and cardiovascular system, and may also cause skin damage. Dichlorophene is a skin irritant and may cause damage to the liver, kidneys, spleen, and central nervous system. Chlordane and other chlorinated hydrocarbons are biocumulative, and may damage the eyes, lungs, liver, kidneys, and skin.

Mineral Spirits

Mineral spirits are solvents. Ingestion, skin contact, or inhalation of fumes may lead to breathing difficulty, severe pain in the throat, burning in nose, eyes, ears, lips, or tongue, loss of vision, abdominal pain, vomiting, blood in vomit and/or stool, hypotension, collapse, skin irritation including burns and necrosis (holes) in skin and underlying tissues. Accidental skin exposures should be rinsed immediately, accidental ingestions should be followed by immediate consumption of milk or water, and all exposures should follow emergency treatment of the symptoms by a health professional.

Oven Cleaners

Oven cleaners contain corrosive alkalis that can cause burning of the skin and respiratory tract if inhaled. Large doses may lead to breathing difficulty, pain in throat, nose, eyes, ears, lips, and tongue, abdominal pain, vomiting, blood in vomit and stool, hypotension, collapse, skin irritation, burns, and necrosis (holes) in skin and underlying tissue, and severe changes in blood pH. Dermal contact should be treated with immediate washing, ingestion with consumption of milk or water, inhalation with fresh air, and in all cases treatment of the symptoms by a health professional.

See also: Arts and Crafts Materials and Processes; Consumer Product Safety Commission; Corrosives; Deodorants and Antiperspirants; Detergent; Food Additives; Food, Drug, and Cosmetic Act, US; Glyphosate; Mouthwash; Pesticides.

Relevant Websites

http://www.nlm.nih.gov – National Institutes of Health and the National Library of Medicine. Household Products Database. Provides information on health and safety of everyday products.

http://www.cpsc.gov – United States Consumer Product Safety Commission (CPSC). Find recalled products by product type.

http://www.kidsource.com – KidSource – Education and Healthcare Information.

Copper

Shayne C Gad

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- Chemical Abstracts Service Registry Number: CAS 7440-50-8
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Cu^{2+}

Uses

Copper is an essential trace element. Adequate daily requirements are 2–3 mg day^{-1}. It is widely distributed in nature and extensively used in industry. It is used as an electrical conductor, as a component in a variety of alloys (including gold and silver alloys), and as a constituent in paints and ceramic glazes. Because it corrodes at a very slow rate, it is used extensively for water pipes. In addition, copper sulfate mixed with lime is used as a fungicide.

Medicinally, copper sulfate is used as an emetic. It has also been used as an antihelminthic (antiparasitic agent) based on its astringent and caustic actions.

Background Information

Copper and its compounds are naturally present in the earth's crust. Natural discharges to air and water

may be significant. Therefore, it is important to consider the background levels that are commonly found and distinguish these from high levels that may be found as a result of anthropogenic activity.

Copper is emitted into the air naturally from windblown dust, volcanoes, and anthropogenic sources, the largest of which are being primary copper smelters and ore processing facilities. It is associated with particulate matter. The mean concentration of copper in the atmosphere is 5–200 $ng\,m^{-3}$.

Exposure Routes and Pathways

The primary exposure pathway for copper is ingestion (e.g., food and water). Many foods contain copper, especially legumes, organ meats, and oysters. Water carried through copper pipes is also a source of this element. Inhalation is only a significant exposure pathway in industrial settings (e.g., near copper refineries).

Many workers are exposed to copper in agriculture, industries connected with copper production, metal plating, and other industries. Little information is available concerning the forms of copper to which workers are exposed. Copper has been identified at many National Priorities List hazardous waste sites in the United States.

Toxicokinetics

Approximately 50% of ingested copper is absorbed from the stomach. Although copper can be absorbed from the gastrointestinal tract, a modifying biological mechanism regulates total copper absorbed. Copper is transformed in the blood by first binding to albumin and then to a copper-specific protein (ceruloplasmin). Copper also binds to metallothionein more firmly than zinc or even cadmium. Copper is stored in the liver and bone marrow as the metallotheionein.

Copper-dependent enzymes include tyrosinase (which is involved in melanin pigment formation) and the various oxidases (i.e., cytochrome oxidase, superoxide dismutase, amine oxidase, and uricase). Copper plays a major role in the incorporation of iron into the heme of hemoglobin. Copper deficiency is characterized by hypochromic, microcytic anemia resulting from defective hemoglobin synthesis.

Copper levels in the human body vary with age. Copper levels in the brain increase with age, whereas in some tissues (e.g., liver, lungs, and spleen), copper levels are higher in newborns than in adults. Tissue levels gradually decline up to age 10 and remain relatively constant thereafter. Copper is normally excreted in bile, which plays a primary role in copper homeostasis.

Mechanism of Toxicity

Copper reduces glutathione, which is necessary for normal cell viability. The amino acid transferases are inhibited in the presence of excess copper; lipid peroxidation also occurs. Copper combines with thiol groups, which reduces the oxidation state II to I in copper and oxidizes the thiol groups to disulfides, especially in the cell membrane.

Acute and Short-Term Toxicity (or Exposure)

Animal

Copper produces lung damage by inhalation. Intratracheal administration of copper has produced lung damage in rodents; macrophages increased with degenerative membrane structure and hemoglobin values decreased. In larger animals, excess copper intake resulted in iron-deficient anemia and gastric ulcers.

Human

Although copper is an essential element, it is much more toxic to cells than such nonessential elements as nickel and cadmium. Acute poisoning from ingestion of excessive amounts of copper salts, most frequently copper sulfate, results in nonspecific toxic-symptoms, a metallic taste, nausea, and vomiting (with vomitus possibly a blue-green color). The gastrointestinal tract can be damaged by ulceration.

Chronic Toxicity (or Exposure)

Animal

No statistically significant increases in tumor formation were noted in mice fed copper for $\sim$1 year. Subcutaneous and intramuscular injection of copper compounds showed a low incidence of sarcomas. The current data are adequate to assess the carcinogenicity of copper.

Human

Severe symptoms include hypotension, coma, jaundice, and death. Liver necrosis has also been observed. In some cases, copper toxicity can result in an inability to urinate. Treatment with copper compounds can induce hemolytic anemia.

It is believed that the increased susceptibility to copper toxicity seen in infants and children is due to

the normally high hepatic copper levels in early life and the fact that homeostatic mechanisms are not fully developed at birth.

Copper is associated with two genetic inborn errors of metabolism. The first, Menke's disease or Menke's kinky-hair syndrome is associated with severe copper deficiency, due to a defect in an ATPase gene resulting in the inability of the gastrointestinal tract to absorb copper. It is a sex-linked trait characterized by peculiar hair, failure to thrive, severe neurological degradation in the brain, and death before 3 years of age. The cerebral cortex and white matter degenerates; mental retardation ensues before death. The second disease, Wilson's disease or heptolenticular degeneration, is associated with severe copper excess, due to a defect in another ATPase gene resulting in the inability of the liver to excrete copper in the bile. It is characterized by an unusual concentration of copper in the brain, kidneys, cornea, and especially in the liver (which may become abnormally large). Mental retardation is not associated with this disease. This disease is usually treated with a chelating agent such as penicillamine or triethylene tetramine.

In Vitro Toxicity Data

Mutagenesis results are dependent on the bacterial strain and copper compound evaluated. Mammalian cell tests indicate a positive mutagenic response.

Clinical Management

For acute toxicity, emesis is recommended. Treatment is symptomatic. A combination of BAL (British AntiLewisite; 2,3-dimercaptopropanol) and calcium-ethylene diamine tetraacetic acid has been used successfully in a poisoned infant. Penicillamine has also been used. Recently, oral administration of 2,3-dimercapto1-propane sulfonate was found to be effective in experimental rodents. Electrolyte balance must be maintained when gastric lavage is indicated. Potassium ferrocyanide should be added to precipitate the copper.

Environmental Fate

The largest release of copper by far will be to land, and the major sources of release are mining and milling operations, agriculture, solid waste, and sludge from publicly owned treatment works. Sediment is an important sink and reservoir for copper. In relatively clean sediment, the copper concentration is <50 ppm; polluted sediment may contain several thousand ppm of copper.

Copper is released to water as a result of natural weathering of soil and discharges from industries and sewage treatment plants. Copper compounds may also be intentionally applied to water to kill algae. Of special concern is copper that gets into drinking water from the water distribution system.

The bioconcentration factor (BCF) of copper in fish obtained in field studies is 10–100, indicating a low potential for bioconcentration. The BCF is higher in molluscs (i.e., oysters), where it may reach 30 000, possibly because they are filter feeders. There is a good deal of evidence that there is no biomagnification of copper in the food chain.

The major species of soluble copper found in freshwater, seawater, and a combination of the two over a range of pHs is Cu^{2+}, $Cu(HCO_3)^+$, and $Cu(OH)_2$. At the pH values and carbonate concentrations characteristic of natural waters, most dissolved Cu(II) exists as carbonate complexes rather than as free (hydrated) cupric ions.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value time-weighted average is $0.2\,mg\,m^{-3}$ for copper fume and $1\,mg\,m^{-3}$ for copper dusts and mists.

The Environmental Protection Agency drinking water limit is 1.3 ppm. The median concentration of copper in natural water is 4–10 ppb.

Daily intakes of copper and other essential minerals are estimated and can be found as part of the Food and Drug Administration's Total Diet Study.

See also: Metallothionein; Metals; Pollution, Water.

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Copper.

Coprine *See* Mushrooms, Coprine.

Corrosives

Greene Shepherd

- Representative Chemicals: Glacial acetic acid; Oxalic acid; Acetic acid; Nitric acid; Hydrofluoric acid
- Synonyms: Irritants; Acids/bases

Acute and Short-Term Toxicity (or Exposure)

Human

After ingestion, corrosive injury to the esophagus and stomach are commonly found. With skin contact, the symptoms are severe pain and brownish or yellow stains. Burns usually penetrate the full thickness of the skin, have sharply defined edges, and heal slowly with scar formation. With eye contact, conjunctival edema and corneal destruction is prevalent. Symptoms include pain, tearing, and photophobia.

The Occupational Safety and Health Administration permissible exposure limits for various corrosives are as follows: glacial acetic acid, 10 ppm; acetic anhydride, 5 ppm; hydrofluoric acid, 3 ppm; sulfuric acid, 1 mg m^{-3}; oxalic acid, 1 mg m^{-3}; nitric acid, 2 ppm; bromine, 0.1 ppm; chlorine, 1 ppm; fluorine, 1 ppm; hydrochloric acid, 5 ppm.

Chronic Toxicity (or Exposure)

Human

Long-term exposure to acid fumes (inhalation exposure) may cause erosion of the teeth followed by jaw necrosis. Bronchial irritation with chronic cough and frequent attacks of bronchial pneumonia are common.

Clinical Management

In case of ingestion, neither gastric lavage nor emesis should be used. Activated charcoal is unlikely to bind significant amounts of corrosive agents and can make endoscopic evaluation difficult. Ingested corrosives may be diluted by drinking 4–6 oz (113.4–170.1 ml) of water or milk. If vomiting is persistent, do no attempt to administer additional fluids. Avoid neutralization therapies as the resultant exothermic reaction may cause additional tissue injury.

In case of eye contact, the corrosive should be diluted by irrigating the area with tap water or saline for 30–60 min. With alkali exposures prolonged irrigation may be necessary.

In case of skin contact, the corrosive should be removed by flooding the affected area with water for at least 15 min. With alkali exposures prolonged irrigation may be necessary. If the exposure is in the form of a powder rather than a liquid the excess powder should be brushed off prior to irrigation.

See also: Acetic Acid; Bromine; Chlorine; Fluorine; Hydrochloric Acid; Hydrofluoric Acid; Sulfuric Acid.

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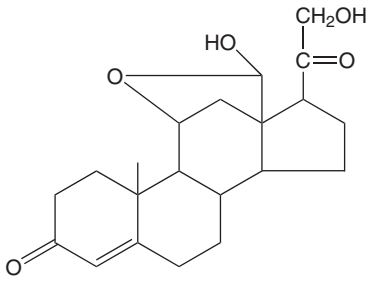
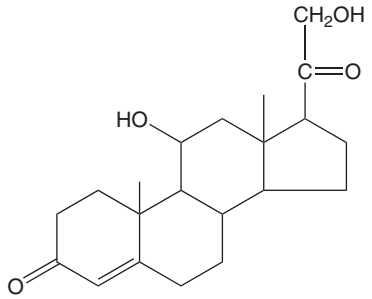
Corticosteroids

Prathibha S Rao

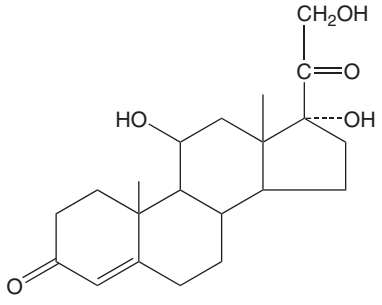
- Representative Chemicals: Mineralocorticosteroids (e.g., aldosterone); Glucocorticoids (e.g., corticosterone)
- Chemical/Pharmaceutical/Other Class: Natural or synthetic hormones
- Chemical Structures:



Aldosterone



Corticosterone



Cortisol

Uses

Corticosteroids cause a wide range of physiological effects, including impacts on protein and lipid metabolism; electrolyte and water balance; and functions of the cardiovascular system, kidneys, skeletal muscle, the nervous system, and other organs and tissues. Corticosteroids are used to treat a wide variety of clinical conditions including adrenal insufficiency, asthma, allergic disorders, and collagen and autoimmune diseases.

Exposure Routes and Pathways

Corticosteroids are administered orally, parenterally, and topically. A certain degree of absorption into the systemic circulation occurs with all forms of topical administration. With respiratory aerosols, the total absorption is similar to that from parental or oral administration.

Toxicokinetics

Generally, the biological half-lives of corticosteriods can be classified as short (8–12 h), intermediate (12–36 h), or long (36–72 h). Cortisone and cortisol are examples of short-lived corticosteroids. Prednisone, prednisolone, and triamcinolone are of the intermediate class. Dexamethasone and β-methasone are associated with the longer-lived class.

The adrenocortical steroids and their synthetic congeners require a double bond in the 4,5 position and a ketone group at C3 for biological activity. The reduction of the 4,5 double bond, resulting in an inactive compound, occurs by both hepatic and extrahepatic metabolisms. Most of the ring A-reduced metabolites can be conjugated at the 3-hydroxyl position with sulfate or glucuronic acid forming water-soluble metabolites enhancing excretion.

Mechanism of Toxicity

The corticosteroids, like other steroid hormones, act by altering the nature of protein synthesis in target tissues. Corticosteroids interact with specific receptor proteins found in the cytoplasm of cells in many tissues to form a steroid–receptor complex. This complex then translocates into the nucleus, where it combines with DNA sequences within the regulatory region of affected genes (termed glucocorticoid response elements). Subsequently, target genes are expressed and appropriate proteins are synthesized. Although there are many similarities between the mechanisms of action of the glucocorticoids and mineralocorticoids, several processes have been identified, such as tissue-restricted receptors for

mineralocorticoids, to explain differences in effects of these two major corticosteroid classes.

Acute and Short-Term Toxicity (or Exposure)

Animal

No relevant information is available on acute toxicity of corticosteroids in animals.

Human

Two categories of toxic effects are observed in the therapeutic use of corticosteroids: those resulting from withdrawal and those resulting from continuous use of large doses. Too rapid withdrawal causes (1) acute adrenal insufficiency and (2) fever, myalgia, arthralgia, and malaise.

The use of corticosteroids for days or few weeks does not lead to adrenal insufficiency but prolonged therapy may result in suppression of pituitary–adrenal function.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to high serum levels of corticosterone induced a significant impairment of inhibitory avoidance learning in rats. In another study, corticosterone elevated over a period of 21 days impaired the formation of a longer-term form of memory, most likely reference memory. Impairments in spatial working memory were seen only after longer durations of corticosterone administration.

Human

In addition to pituitary–adrenal suppression, prolonged therapy with corticosteroids can cause fluid and electrolyte disturbances, hypertension, hyperglycemia, and glycosuria. It also increases the susceptibility to infections including tuberculosis; causes peptic ulcers, osteoporosis, behavioral disturbances, posterior subcapsular cataracts, growth arrest, Cushing's habitus, 'buffalo hump', enlargement of supraclavicular fat pads, 'central obesity', striae, ecchymoses, acne, and hirsutism.

Clinical Management

Acute overdose probably would not result in toxicity. Should oral overdosage occur, standard emergency and supportive care procedures should be followed. If anaphylaxis should occur, epinephrine may be given as 0.3–0.5 ml of a 1:1000 solution for adults (children should receive 0.01 ml kg^{-1}). Mild anaphylaxis may be treated with antihistamines alone. If chronic toxicity should occur, it is important to reduce the dosage of corticosteroid to a minimal maintenance dose at the first sign of toxicity.

Environmental Fate

Not much information is available.

Ecotoxicology

Not much information is available.

See also: Anabolic Steroids; Lipid Peroxidation.

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Corticosterone *See* Corticosteroids.

Cortisone *See* Corticosteroids.

Cosmetics and Personal Care Products

Paul Sterchele

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Introduction

Cosmetics are natural or synthetic toiletry products that are used to maintain hygiene and include externally applied products used to enhance appearance. This class includes dental products, bath supplies (e.g., bubble baths, body washes, and bath beads), powders, lotions, lipsticks, perfumes, colognes, shampoos, depilatories, and hair coloring/waving products. Most of these products contain alcohols, aromatic hydrocarbons, perborates, and anionic and nonionic surfactants. Use of cosmetics is as old as civilization itself. Centuries ago wealthy women would apply the white lead pigment known as ceruse to their faces to appear fashionably pale – sometimes with lethal consequences. Women also used belladonna alkaloids like atropine to dilate pupils to enhance the attractiveness of the eyes in the late nineteenth century.

Product Formulations and Human Toxicity

Most cosmetics are nontoxic, although composition and the magnitude and route of exposure are important determinants. In general, accidental ingestion of small quantities might be expected to elicit some minor, transient gastrointestinal distress, but frank toxicity is rare. However, caution should be exercised to prevent children from being overexposed to these products.

Hair-Coloring Products

Permanent hair colors contain an oxidizer (usually 6% hydrogen peroxide) and a dye intermediate (*p*-phenylenediamine, resorcinol, aminophenols along with water, ammonia, glycerin, isopropanol, and propylene glycol). Semipermanent hair colors contain propylene glycol, isopropanol, fatty acids, fragrance, alkanolamines, and dyes. Some Grecian hair formulations contain lead in the form of lead acetate.

Large ingestions of hydrogen peroxide may produce mild gastritis due to decomposition resulting in release of oxygen.

Hair-Waving Products

Waving lotions contain thioglycolic acids and ammonia sulfides, and neutralizer solutions contain hydrogen peroxide, sodium bromate, or perborate in mildly acidic solutions. Some permanent wave fixatives contain 2–8% (weight/volume) mercuric chloride.

Sodium borate decomposes into borate and peroxide and is less toxic than potassium bromate. From 3 to 6 g and from 15 to 30 g boric acid is potentially fatal to children and adults, respectively. Cutaneous manifestations include desquamating, erythematous rash commonly over palms, soles, buttocks, and scrotum. The lesion may progress to exfoliation. Central nervous system (CNS) effects range from irritability, restlessness, and headache to coma and convulsions in severe cases. Gastrointestinal symptoms include anorexia, nausea, vomiting, and diarrhea. Acute renal tubular necrosis may lead to renal failure in moderate to severe cases.

Bromate salts are extremely toxic; they are capable of causing deafness and renal failure at doses between 240 and 500 mg kg^{-1}. Potassium bromate, also used as neutralizer in cold waves, is an extremely toxic compound that produces nausea, vomiting, diarrhea, deafness, acute renal failure, hypotension, CNS depression, and hemolysis. Both otic symptoms and renal impairment may be permanent. Primary tubular damage can progress to interstitial fibrosis and glomerular sclerosis.

Hair-Straightening Products

Hair straighteners contain 1–3% sodium hydroxide solution. The solution is highly caustic.

Depilatories

Similar to other hair preparations that are formulated to modify the molecular configuration of hair, hair-removal products typically contain thioglycolate salts to dissolve the keratin protein. Because of the caustic nature of these products, care should be taken to minimize irritation to the surrounding skin.

Hair Sprays and Conditioners

Hair sprays contain ethanol as a solvent with resin polymers composed of vinyl acetate, acrylamide, and methyl vinyl ether. Hair conditioners contain cationic surfactants, perfumes, and alcohols.

Bath Preparations

Bubble baths usually contain anionic and nonionic surfactants along with alcohols and preservatives. Bath salts may contain borax, while bath oils contain vegetable and mineral oils.

Nail Polish and Removers

Nail polish contains hydrocarbon solvents (xylene, toluene, and acetone), alcohol solvents, plasticizers, and resins. Nail polish removers are solvents containing acetone or ethanol.

Colognes, Perfumes, Toilet Waters

Colognes, perfumes, and toilet waters usually contain ethanol (at concentrations ranging from 50% to 95%) and volatile or essential oils.

Volatile or Essential Oils

Sage, eucalyptus, turpentine, pine, pennyroyal, and cinnamon contain hydrocarbons, ethers, alcohols, esters, and ketones. These components can cause allergic contact dermatitis, which begins 12 h within sensitization and peaks at 48–72 h. Essential oils are mucosal irritants leading to gastrointestinal distress and salivation. Concentrated formulations of essential oils can cause convulsions and CNS depression at 10 ml doses. Aspiration can cause chemical pneumonitis. Alcohol produces intoxication, which may be complicated by hypoglycemia, especially in children.

Dental Products

Toothpastes, powders, and tooth liquids contain calcium phosphates, alumina, abradants, and anionic surfactants. Mouthwashes usually contain alcohol, flavoring (essential oils), and sweeteners. (For mouthwash toxicity information, see section on Colognes, Perfumes, Toilet Waters.) Denture cleaners contain bicarbonates, borates, phosphates, and carbonates. (For toxicity information on borates, see section on Hair-Waving Products.) Acrylic denture material contains methacrylate.

Deodorants

Deodorants contain aluminum and zinc salts, and fragrance to mask the smell of perspiration.

Clinical Management

Since most ingested cosmetics are nontoxic, only supportive care and dilution are required.

1. Induction of emesis depends on product toxicity, quantity, time since exposure, patient's weight, and the presence of symptoms. Cationic surfactants, perborates, and substantial ingestion of essential oils may benefit by administration of syrup of ipecac. Syrup of ipecac can be used in hydrocarbon ingestion only if the total dose of hydrocarbons exceeds 1 or 2 ml kg^{-1}.
2. Potassium borate: Lavage with 2% sodium bicarbonate solution and administer 10–50 ml of 10% sodium thiosulfate solution intravenously at the rate of 3 ml min^{-1} to reduce the bromate to the less toxic bromide ion. An alternative therapy is the administration of 100–500 ml of 1% sodium thiosulfate. Patients should be observed for development of renal toxicity and ototoxicity.

See also: Acetone; Acrylamide; Alkalies; Ammonia; Atropine; Belladonna Alkaloids; Boric Acid; Deodorants and Antiperspirants; Ethanol; Food, Drug, and Cosmetic Act, US; Fragrances and Perfumes; Hydrogen Peroxide; Isopropanol; Lead; Surfactants, Anionic and Nonionic; Surfactants, Perfluorinated; Toluene; Xylene.

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Relevant Websites

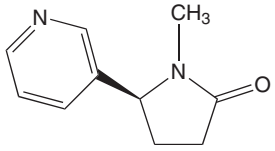
http://www.ctfa.org – US Cosmetic, Toiletry, and Fragrance Association (CTFA).

http://hpd.nlm.nih.gov – US National Library of Medicine, 'Household Products Database' and 'Tox Town'. The Household Products Database links several thousand US Consumer brands to Health effects from Material Safety Data Sheets (MSDSs) provided by manufacturers, and allows scientists and consumers to research products based on chemical ingredients.

Cotinine

Sanjay Chanda and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 486-56-6
- Synonyms: 1-Methyl-5-(3-pyridinyl)-2-pyrrolidinone; *N*-Methyl-2-(3-pyridyl)-5-pyrrolidone
- Chemical/Pharmaceutical/Other Class: Research chemical; Antidepressant
- Chemical Structure:



Uses

Cotinine is primarily used in research.

Background Information

Cotinine is the major metabolite of nicotine. Nicotine is rapidly metabolized and has a short half-life, but cotinine is metabolized and eliminated at a much lower rate. This results in higher cotinine-to-nicotine ratio in various tissues, including the brain. In studies on conscious, freely moving rats, intravenous administration of either nicotine or cocaine induced the release of dopamine in the nucleus accumbens, as assayed by microdialysis. Prior intravenous administration of a high dose of cotinine ($500\,\mu g\,kg^{-1}$) inhibited this nicotine- or cocaine-induced dopamine release. The effect of cotinine is not due to altered metabolism of nicotine or its binding at the receptor site, because cotinine, unlike nicotine, does not affect the binding of the nicotinic ligand cystine. The findings suggest that cotinine affects a putative component of the reward mechanism, and as such could have therapeutic value.

Exposure Routes and Pathways

Cotinine is a viscous liquid. Dermal or ocular contact is the most common exposure pathway. Because it is a predominant metabolite of nicotine, systemic exposure occurs after consumption of tobacco products.

Toxicokinetics

Cotinine is formed as a major metabolite of nicotine after tobacco smoking. The average half-life of cotinine is 19 h. It can be detected in plasma, urine, and saliva. Cotinine is also formed in the body after intake of some vegetables (e.g., eggplant, tomato, and green pepper) primarily of the family solanaceae. These vegetables contain nicotine as their natural defense mechanism against fungi, bacteria, insects, and animals. It had been thought that the presence of cotinine in human urine could be used as evidence of smoking or tobacco use. However, because cotinine can also arise from consumption of some vegetables, use of cotinine as a biomarker of exposure to tobacco smoke or other forms of tobacco products may not be reliable.

Mechanism of Toxicity

Cotinine stimulates the nicotinic receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity in animals is similar to that observed in humans. The intraperitoneal LD_{50} in mice is $930\,mg\,kg^{-1}$; the oral gavage LD_{50} in mice is $1604\,mg\,kg^{-1}$.

Human

Symptoms of acute toxicity include nausea, salivation, abdominal pain, vomiting, diarrhea, cold sweat, headache, dizziness, and disturbed hearing and vision.

Chronic Toxicity (or Exposure)

Animal

Reported to be a carcinogen.

Human

Reported to be a carcinogen.

Clinical Management

Vomiting should be induced with syrup of ipecac or gastric lavage should be performed. Respiratory assistance and treatment of shock may be necessary.

See also: Nicotine; Tobacco; Tobacco Smoke.

Further Reading

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Stevens KR and Munoz LR (2004) Cigarette smoking: Evidence to guide measurement. *Research in Nursing & Health* 27: 281–292.

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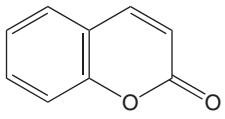
Coumarins

Betsy D Carlton

- Chemical Abstracts Service Registry Number: CAS 91-64-5
- Synonyms: Coumarin; 2*H*-1-Benzopyran-2-one; 1,2-Benzopyrone; *cis*-*o*-Coumaric acid lactone; Coumarinic anhydride; 2-Oxo-1,2-benzopyran; Tonka bean camphor (*note*: coumadin and warfarin are not synonyms for coumarin)
- Chemical/Pharmaceutical/Other Class: Benzo-α-pyrone
- Chemical Formula: $C_9H_6O_2$
- Chemical Structure:



Uses

Coumarin is a naturally occurring benzo-α-pyrone compound that is most often used as a fragrance ingredient, where it functions as a fragrance, as a fragrance enhancer, and as a stabilizer. Coumarin is widely used in perfumes, hand soaps, detergents, and lotions at concentrations from 0.01% to 2.4%. It is used to give pleasant aromas to household products or to mask unpleasant odors. The conservative estimate for systemic exposure of humans by using cosmetic products is $0.13\,mg\,kg^{-1}\,day^{-1}$, disregarding any corrections that should be made for absorption that is $<100\%$. Coumarin is used as a pharmaceutical for the treatment of high protein lymphedema, for improved venous circulation, and has been in clinical trials as an antineoplastic. Unlike coumadin (or warfarin), coumarin has no anticoagulant activity and is not used clinically as an anticoagulant or as a rodenticide.

Coumarin is found in a large number of plants belonging to many different families including tonka beans, woodruff, lavender oil, cassia, melilot (sweet clover), and other plants. It is found in edible plants such as strawberries, cinnamon, peppermint, green tea, carrots, and celery, as well as in partially fermented tea, red wine, beer, and other foodstuffs. Although coumarin's use in foods is allowed via naturals such as cinnamon, at the present time coumarin is not permitted for use as a direct food additive, although it is used as a tobacco flavor.

Coumarin is also used in the electroplating industry.

Exposure Routes and Pathways

Due to its common use in fragrances and fragrance-containing products, dermal exposure to coumarin is common. Coumarin is readily absorbed dermally, a fact that makes dermal dosing for lymphedema treatment a consideration. Human exposure to coumarin also occurs orally via natural foodstuffs, from pharmaceutical use, and from tobacco products.

Toxicokinetics

The absorption, metabolism, and excretion of coumarin have been widely studied for many years. Advances in synthetic and analytical chemistry techniques in recent years have allowed a significant revision to our understanding of how coumarin is handled in the body, particularly in rodents and humans. While coumarin is readily available by both the oral and dermal routes in animals and humans, blood levels and toxicity profiles are influenced by the specific exposure mode. Plasma levels more than 20×higher than those observed following exposure via the diet have been reported following a bolus oral dose at similar milligram coumarin per kilogram body weight levels.

Dermal exposure by-passes the 'first-pass' effect of initial metabolism by the liver. Coumarin in the blood first passes through the lung, where significant amounts can be exhaled, prior to being metabolically processed by the liver. Metabolic pathways are highly species- and, sometimes, strain-specific. DBA/2J mice have been reported to have a high level of coumarin hydroxylase activity, resulting in metabolism mainly to 7-hydroxycoumarin. CH3/HeJ mice, on the other hand, have been reported to have very little hydroxylase activity.

In rats and many strains of mice other than the DBA/2J, oral coumarin exposure results in hepatic metabolism of coumarin, with the formation of the coumarin 3,4-epoxide (CE), which spontaneously rearranges extremely rapidly to the *o*-hydroxyacetaldehyde (*o*-HPA), the toxic metabolite. The *o*-HPA is then further metabolized to the nontoxic *o*-hydroxyacetic acid (*o*-HPAA) and *o*-hydroxyethanol (*o*-HPE). Rodents also metabolize coumarin by several lesser pathways, to the nontoxic 3-hydroxycoumarin and several other more minor metabolites. It is the balance between the formation of the toxic *o*-HPA and the nontoxic *o*-HPAA and *o*-HPE that is critical to the determination of hepatotoxicity at high exposure levels of coumarin. Mice form more *o*-HPA than do rats, but detoxify it much more rapidly and efficiently than do rats. The result is that hepatotoxicity at doses $\geqslant 150\,\text{mg coumarin kg}^{-1}$ body weight is observed in rats, but not mice. Similarly, when high doses of coumarin result in high plasma levels, mice demonstrate pulmonary toxicity whereas rats do not. This is the result of the formation of higher levels of CE and *o*-HPA in the Clara cells in the lungs of mice, which is not observed in rats.

In contrast to rodents, humans primarily metabolize coumarin not to the epoxide and *o*-HPA, but rather to the nontoxic metabolite, 7-hydroxycoumarin. Very high levels of coumarin are required to generate any *o*-HPA in human liver, and what is formed is rapidly detoxified. Humans have very few Clara cells in the lungs and do not generate CE and *o*-HPA in the lung, even at high coumarin doses. In a study using human hepatic microsomes with various CYP2A6 7-hydroxylation capacities, those samples that demonstrated a low capacity to utilize the 7-hydroxylatation pathway also showed a decreased capacity to form CE.

Mechanism of Toxicity

Coumarin toxicity is a function of blood and target tissue levels of coumarin relative to the metabolic capacity of the target organ. Cellular toxicity results when the formation of the toxic moieties exceeds the capacity of the cell to detoxify. This can have significant impact when comparing dosing by gavage to dietary exposure (see section 'Toxicokinetics').

Acute and Short-Term Toxicity (or Exposure)

Animal

LD_{50} values ranging from 160 to $780\,\text{mg kg}^{-1}$ body weight have been reported. The differences may relate to the species/strain of animal used and whether the animals were fasted at the time of dosing. Coumarin can be slightly irritating to the eye and skin.

In rat studies, dietary exposure at levels $\geqslant 2500$ ppm for 4 weeks or more may result in decreased food consumption, with resulting decreased body weight, and microscopic changes in the liver. Doses as high as 1–2% in the rodent's diet (10 000–20 000 ppm) have been given, usually resulting in a refusal of food and mortality.

In contrast to coumadin, coumarin is not teratogenic. In rats exposed to doses that are sufficiently high to significantly effect food consumption, coumarin can decrease reproductive success. At lower dose levels, no adverse effects on reproduction or development have been reported.

Human

Coumarin exposure is common via cinnamon, green tea, sweet clover honey, and other foodstuffs and adverse effects have not been reported. When used as a pharmaceutical, doses have ranged from 70 to $7000\,\text{mg day}^{-1}$. The most common pharmaceutical dosage appears to be 200 mg once or twice per day. Infrequently, hepatotoxicity has been reported following pharmaceutical use. Hepatic enzyme changes have been reported to be reversible following cessation of administration, and occasionally are reversible despite continued use. The incidence rates reported have ranged from $<0.1\%$ to 6%, depending on the study population and, to a lesser extent, on the dose administered. Some deaths have been reported, but confounding factors such as preexisting medical conditions have precluded interpretation in most cases. Studies of CYP2A6 polymorphism in humans have not shown an association with coumarin-associated liver dysfunction.

Coumarin has been tested for its ability to cause sensitization in several test systems including dermal application in guinea pigs, the mouse ear swelling test, and the local lymph node assay (LLNA). In all cases where pure coumarin was tested in animals, results were negative, including when tested at up to 50% in four recent LLNA studies. Also in LLNA studies, a chlorinated impurity (6-chlorocoumarin) and less-pure coumarin derived from *o*-cresol have been shown to be sensitizers, confirming reports of sensitization from various substituted derivatives of coumarin. In humans already sensitized to certain other substances such as Balsam of Peru, coumarin has been reported to cross-react. While laboratory species are not likely to have been exposed to coumarin or cross-reacting substances before being tested, the human population is likely to have been

previously exposed. This can make testing in humans more difficult to interpret.

Coumarin has no anticoagulant activity, is not used to prevent blood clots, and does not interact with the vitamin K-dependent clotting factors. It is not teratogenic in humans (does not cause hemorrhagic syndrome) and has no reported adverse effects on human reproduction. In contrast to overexposure to coumadin, coumarin overexposure does not result in bruising and hemorrhage.

Chronic Toxicity (or Exposure)

Animal

Numerous long-term toxicity and/or carcinogenicity studies have been conducted. In general, the primary effects reported are decreased food consumption with resulting decreased body weight and liver toxicity (in rats). At doses that produce a significantly decreased body weight gain ($\geqslant 150\,mg\,kg^{-1}$ body weight), liver toxicity and liver tumors are reported in rats. These tumors are nonmetastatic and nonlethal. Lung tumors have been reported in mice exposed by bolus (via stomach tube) administration at doses $\geqslant 150\,mg\,kg^{-1}$ body weight, but not in mice exposed to comparable doses in the diet.

Human

In workers, dusts may be irritating to the respiratory system. Coumarin may be a weak dermal sensitizer in sensitive individuals. Purity of the material can play a significant role in this regard. No other long-term effects of coumarin have been reported in humans. The International Agency for Research on Cancer (IARC) reviewed coumarin in 2000 and classified it in group 3, not classifiable as a carcinogen in humans.

In Vitro Toxicity Data

Coumarin is not mutagenic and does not bind to DNA. It is not clastogenic (i.e., it has no significant effect on chromosomes).

Environmental Fate

Coumarin is readily biodegradable. Coumarin is unlikely to bind to soil. Coumarin does not bioaccumulate; the bioconcentration factor has been determined to be <10–40. Various environmental fate studies have shown that coumarin in the environment would biodegrade and be lost to volatilization. Losses resulting from photolysis may also occur.

Ecotoxicology

Coumarin is not very environmentally toxic, with 96 h LC_{50} values in fish of $56\,mg\,l^{-1}$ and a 24 h EC_{50} in *Daphnia magna* of $55\,mg\,l^{-1}$. Algal respiration was depressed at laboratory test concentrations of $50\,\mu mol\,l^{-1}$. Coumarin in the environment will readily degrade.

Miscellaneous

Coumarin is a white crystalline solid. Its odor has been described both as vanilla-like and as having a note of 'newly mown hay'. Odor thresholds of 0.33–2 ppb have been reported. Coumarin occurs naturally in many plants such as tonka beans, lavender, and cassia and in many natural food stuffs such as cinnamon, green tea, peppermint, and sweet clover honey. Concentrations range from 87 000 ppm in cassia and 40 000 ppm in cinnamon to 20 ppm in peppermint and 5 ppb in tangerines.

See also: Fragrances and Perfumes.

Further Reading

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Born SL, Hu JK, and Lehman-McKeeman LD (2000) *o*-hydroxyphenylacetaldehude is a toxic metabolite of coumarin. *Drug Metabolism and Disposition* 28(2): 218–223.

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Ford RA, Hawkins DR, Mayo BC, and Api AM (2001) The *in vivo* dermal absorption and metabolism of [4-^{14}C] coumarin by rats and by human volunteers under simulated conditions of use in fragrances. *Food and Chemical Toxicology* 39: 153–162.

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Lake B (1999) Coumarin metabolism, toxicity and carcinogenicity: Relevance for human risk assessment. *Food and Chemical Toxicology* 37: 423–453.

Crafts Materials *See* Arts and Crafts Materials and Processes.

Creosote

William S Utley

- Chemical Abstracts Service Registry Number: CAS 8001-58-9 (Coal tar creosote); CAS 8021-39-4 (Wood creosote)
- Synonyms: Coal tar oil; Brick oil; Heavy oil; Naphthalene oil; Liquid pitch; Wood creosote
- Chemical/Pharmaceutical/Other Class: Phenolic Compound
- Chemical Formula: A distillate of coal that contains an estimated 162 different compounds. Estimated make-up is as follows: aliphatic hydrocarbons (7%), polycyclic aromatic hydrocarbons (69%), and nitrogen containing polycyclic aromatic hydrocarbons (11%). Some of the polynuclear aromatic hydrocarbons identified in creosote are: anthracene, benz(*a*)anthracene, benzo(*a*)pyrene, and pyrene

Uses

Creosote is primarily used as a wood preservative in the United States. It has been used as a disinfectant, antiseptic, and a germicide, as a hop defoliant antifungal preparation, and as an animal or bird repellent. The leaves of the creosote bush may be used in herbal remedies or dietary supplements.

Exposure Routes and Pathways

The primary route of exposure is dermal through handling treated wood or inhalation, particularly when treated wood is burned in a poorly ventilated area.

Toxicokinetics

Absorption

Creosote is readily absorbed through the skin and the gastrointestinal tract. As creosote is diluted the absorption rate may actually increase.

Distribution

The K_{ow} (log of the octonal to water partition coefficient) is 1 and therefore is not expected to bioconcentrate.

Elimination

Creosote appears to be primarily excreted in the urine. Conjugation with sulfuric and hexuronic acids as well as oxidation leads to a 'smoky' appearance of the urine.

Clinical Management

Acute episodes are treated similar to phenolic poisonings with initial stabilization of breathing and cardiac monitoring. Dermal decontamination is accomplished by swabbing the affected area with olive oil. For ingested material the preferred method is administration of activated charcoal followed by a cathartic. Phenol and phenolic substances tend to exhibit an increased absorption rate at dilute concentrations and have a rapid onset of acute symptoms; therefore, there is a potential for seizures.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cattle have been poisoned by licking treated lumber, the estimated dose being 4–6 g kg^{-1}. A mixture of fuel oil and creosote was once widely distributed as a cure for ringworm. Excessive application of this material has caused poisoning of animals. Skin painting studies with mice have produced skin tumors. Creosote was mutagenic in the Ames test when metabolically activated with S9. The acute LD_{50} to rats is 725 mg kg^{-1}.

Human

Toxicity is expressed either via general depression with cardiac collapse or via the irritating/corrosive nature by irritation and burns of the skin and eyes. Brief exposures via inhalation may cause respiratory irritation. Oral exposure to larger quantities of creosote may result in stomach pains and burning of the mouth. Large doses (7 g for adults and 1–2 g for children) have been associated with death 14–17 h after ingestion. Cardiovascular collapse appears to be the primary cause of death. Nonlethal symptoms include salivation, vomiting, thready pulse,

headache, and loss of pupillary reflexes. Reports of long-term self-medication have indicated symptoms of intoxication and visual disturbances.

Chronic Toxicity (or Exposure)

Animal

Animal studies have demonstrated that creosote oils derived from coal tar are capable of producing skin carcinomas and papillomas when applied directly to the skin.

Human

Creosote is carcinogenic to humans x-udd occupational studies that show an increased incidence in scrotal cancer in workers exposed to creosote from wood and coal burning fire places.

Environmental Fate

Given the low water content and persistence of creosote, the material will tend to end up in the soil and sludge columns of the environment.

Ecotoxicology

Creosote is highly toxic to aquatic organisms with typical acute values of 76 ppb for *Daphnia magna* (48 h LC_{50}) and 600 ppb for rainbow trout (96 h LC_{50}).

Regulatory Levels

Creosote is regulated as a combustible/flammable liquid for transport. The US Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) classify creosote as a probable human carcinogen, class B 1 and 2A, respectively. The 8 h time-weighted average for creosote established by the EPA is 0.2 mg m^{-3}.

Regulations

- Creosote is included in the EPA high production volume program.
- Creosote is listed as an RCRA hazardous waste U051.
- IARC 2A carcinogen based on limited human and sufficient animal data.
- EPCRA SARA 313 reportable substance.
- CERCLA reportable quantity (RQ) of 1 pound.
- Regulated as a California Proposition 65 carcinogen.
- Classified in the European Union as toxic, may cause cancer (T: R-45).

See also: Coal Tar; Petroleum Distillates.

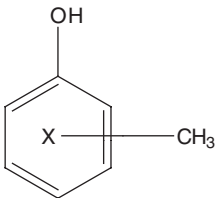
Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Creosote.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Creosote.

Cresols

Murali Badanthadka and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 1319-77-3
- Synonyms: Cresol; Tricresol
- Chemical Formula: C_7H_8O
- Chemical Structure:



Uses

It is used as a disinfectant, fungicide, bactericide, wood preservative, local antiseptic, parasiticide, and insecticide.

Background Information

Pure cresol is colorless, yellowish, brownish-yellow, or pinkish liquid. *o*-Cresol, *m*-cresol, and *p*-cresol are the three structural isomers of cresol. The names of the three compounds indicate which of the hydrogens on the benzene ring portion of the molecule have been replaced. They are obtained from coal tar or petroleum. Because the boiling points of these three compounds are nearly the same, a separation of a mixture of the three into its pure components is impractical.

The mixture of cresols obtained from coal tar is called cresylic acid, an important technical product used as a disinfectant and in the manufacture of resins and tricresyl phosphate. Cresols are useful as raw materials for various chemical products, disinfectants, and synthetic resins. The isomer *o*-cresol is a starting material for the herbicides 4,6-dinitro-*o*-cresol and 2-methyl-4-chlorophenoxyacetic acid. The isomers *m*-cresol and *p*-cresol are used in phenol–formaldehyde resins and are converted to tricresyl phosphate (a plasticizer and gasoline additive) and to di-*t*-butyl cresols (antioxidants called BHT).

Exposure Routes and Pathways

Cresols are released to the atmosphere in auto and diesel exhaust, during coal tar and petroleum refining, wood pulping, and during its use in manufacturing, metal refining, etc. Wastewater from these industries as well as municipal wastewater treatment plants contains cresols. Exposure may occur through inhalation, drinking water, dermal contact, food, and beverage ingestion.

Toxicokinetics

Cresols are absorbed across the respiratory and gastrointestinal tracts, and through the skin. Gastrointestinal and dermal absorption are rapid and extensive. Cresols are distributed to all the major organs. The primary metabolic pathway for cresols is conjugation with glucuronic acid and inorganic sulfate. Minor metabolic pathways include hydroxylation of the benzene ring and side-chain oxidation. The major route for elimination is renal excretion in the form of conjugate metabolites.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cresols are highly irritating to the skin and eyes of rabbits, rats, and mice. Short-term exposure to inhaled mixtures of cresol aerosol and vapor results in irritation of the respiratory tract, small hemorrhages in the lung, body weight reduction, degeneration of heart muscle, liver, kidney, and nerve cells.

Short-term oral exposure resulted in decreased body weight, organ weight, histopathological alterations in the respiratory and gastrointestinal tracts of rats. More severe effects were reported in mice. At the highest concentrations death resulted from exposure to *o*-, *m*-, and *p*-cresols but not from exposure to cresol itself.

Human

Cresols are highly irritating upon dermal contact, eye contact, and contact with any mucous membranes. Ingestion of cresols results in burning of the mouth and throat, abdominal pain, and vomiting. The target tissues/organs affected are the blood, kidneys, lungs, liver, heart, and central nervous system (CNS). In acute exposures, severe burns, anuria, coma, and death may result. Dermal exposure has been reported to cause severe skin burns, scarring, systemic toxicity, and death. Very few data are available regarding reproductive effects and there are no data on carcinogenicity in humans. At concentrations normally found in the environment, cresols do not pose any significant risk for the general population. However, under conditions of high exposure, people with renal insufficiency or enzyme deficiency will develop potential adverse health effects.

Chronic Toxicity (or Exposure)

Animal

Exposure to vapors of *o*-, *m*-, and *p*-cresol resulted in weight loss, reduced locomotor activity, inflammation of nasal membranes and skin, and changes in the liver. Thirteen-week oral exposures to mice, rats, and hamsters resulted in mortality, tremor, reduced body weights, hematological effects, increase in organ weight, hyperplasia of nasal and forestomach epithelium. Oral and inhalation exposures to cresol isomers result in lengthened estrus cycle, histopathological changes in the uterus and ovaries of rats as well as mice. No adverse effects on spermatogenesis are observed. Mild fetotoxic effects have been reported upon exposure of pregnant mice. Some evidence of genotoxicity has been reported from *in vitro* experiment using sister-chromatid exchange assay. However, cresol is not genotoxic after *in vivo* exposure.

Human

Prolonged or repeated absorption of low concentrations of cresol through the skin, mucous membranes, or respiratory tract may cause chronic systemic poisoning. Symptoms and signs of chronic poisoning include vomiting, difficulty in swallowing, salivation, diarrhea, loss of appetite, headache, fainting, dizziness, mental disturbances, and skin rash. Death may result if there has been severe damage to the liver and kidneys.

Clinical Management

Oral exposure: Liquid intake should be avoided because dilution may enhance absorption. Immediate

administration of activated charcoal is recommended to limit systemic toxicity. Ipecac-induced emesis is not recommended because of the potential for CNS depression, seizures, and aspiration. Gastric lavage is effective only within 1 h after ingestion. Patients should be treated symptomatically. Convulsions are controlled with diazepam.

In case of inhalation exposure victims should be removed to fresh air. Respiratory distress should be monitored and healthcare personnel consulted.

Dermal exposure: Decontamination with water is necessary. Copious dilution with room temperature water is appropriate after dermal and eye exposures.

Environmental Fate

Atmospheric fate: Cresols are not expected to persist in the atmosphere because: (1) cresols have low estimated half-lives (less than 1 day); (2) they are sensitive to photolysis; and (3) the water solubility of cresols may cause transport of cresols from the atmosphere to the soil or aqueous environment. The photodegradation half-life of cresol isomers during the daytime is 8–10 h while at night it is ~2–4 min. Daytime half-lives would be reduced under smog conditions. Cresols are highly soluble compounds, and gas scavenging will be an efficient removal process as is reflected by high concentrations in rain.

Terrestrial fate: While there is substantial release of cresols to the soil, this route of environmental exposure is not expected to be a problem. Cresols are readily biodegraded by soil microflora and move to lower layer of soil. Therefore, cresols will not persist in soils and will probably be leached, due to their water solubility, into the aquatic environment where they will be degraded by microorganisms. The degradation rates of cresols in soil may decrease at lower temperatures (-2°C to 5°C).

Aquatic fate: Cresols do not contain any functional groups that are hydrolyzable. Therefore, hydrolysis of these compounds in aquatic media is unlikely. However, it will degrade primarily due to biodegradation in eutrophic waters although photolysis may make a contribution in oligotrophic lakes based on modeling studies. Biodegradation generally occurs within 8 h after several days of acclimation, except in oligotrophic lakes, estuarine, and marine waters where degradation takes several days. Degradation is much slower under anaerobic conditions especially for the *o*-isomer.

Exposure Standards and Guidelines

Occupational Safety and Health Administration permissible exposure limit is 5 ppm (22 mg m^{-3}) for 8 h time-weighted average (TWA). The threshold limit value for cresol and its isomers is 5 ppm for 8 h TWA. National Institute for Occupational Safety and Health recommended exposure limit is 2.3 ppm (10 mg m^{-3}) for 10 h TWA for all isomers.

See also: Coal Tar; Pesticides.

Further Reading

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Lesaffer G, De Smet R, D'Heuvaert T, *et al.* (2003) Comparative kinetics of the uremic toxin *p*-cresol versus creatinine in rats with and without renal failure. *Kidney International* 64: 1365–1373.

Morinaga Y, Fuke C, Arao T, and Miyazaki T (2004) Quantitative analysis of cresol and its metabolites in biological materials and distribution in rats after oral administration. *Legal Medicine (Tokyo)* 6: 32–40.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Cresols.

Criminal Enforcement of Environmental Laws

Grant R Trigger

Scope of Potential Environmental Tort Criminal Liability

Initial environmental laws in the United States were focused on reducing the amount of contamination released to the environment. Expanding wastewater treatment systems and requiring improved pollution control equipment with attendant regulatory permit requirements were the primary vehicles for improving the environment. Over time those who failed to comply with these requirements became the focus of enforcement officials and the desire to encourage more widespread compliance resulted in pressuring individual corporate officials with the threat of

personal liability to ensure greater compliance. As discussed below, these trends have eroded traditional principles of criminal law such that there may actually be greater jeopardy of being convicted of an environmental related crime than of a drug or robbery related offense. The 'shrinking' *mens rea* requirement of knowingly causing a violation of law is a potential issue for any environmental criminal litigation matter according to Marshall, Sims, and Castella (see Further Reading section). In other words, traditional criminal law punished the defendant for knowingly causing harm; however, the trend in environmental crimes is to convict due to the consequences rather than intent. For example, if a plant operator allows a discharge, he may be potentially criminally liable even if he did not know the content of the discharge was hazardous. For a contrary view see the note authored by Escobar listed in the 'Further Reading' section.

From an international perspective, growing attention to chemical use and waste disposal practices has resulted in a proposal by the European Union to enact new legislation, which would require the registration and control of certain chemicals. Under the proposed Registration, Evaluation, and Authorization of Chemicals Act (REACH) chemical producers would be required to provide authorities, the public, and customers with basic toxicity and exposure information. If that information is not supplied the chemical will not be allowed on the market. The more dangerous chemicals cannot be used without permission and the user must demonstrate that there is no alternative to the use of that chemical. Interestingly, REACH as proposed does not specify civil or criminal penalties but instead defers to the member states for selection and enforcement of specific penalties. In the meantime, potential criminal investigations and prosecutions of environmental matters are underway in international forums such as Paris (1999 Erika oil spill) and Malaysia (industrial sludge from Taiwan imported under falsified import documents). More information on these matters can be found in the 'Further Reading' section.

Evolution of Environmental Criminal Liability

Tampering with monitoring equipment and falsifying consumer certifications has been the basis of criminal convictions. In May, 1998, Louisiana-Pacific Corp. pleaded guilty to Clean Air Act (CAA) and consumer fraud violations at its strand board manufacturing plant near Montrose, Colorado, agreed to pay a $5.5 million criminal fine, $31 million for consumer fraud violations, and make a $500 000 donation to seven groups working to improve air quality. The criminal fine was the largest ever received in the CAA's 28 year history. The company pleaded guilty to tampering with emissions monitoring equipment, lying to the Colorado Department of Public Health about the number of times the mill violated its permits, creating nonrepresentative samples for the American Plywood Association that were used in quality assurance testing, and misrepresenting to customers, through use of the Association's quality assurance certification mark, that the product met the requirements of the Association. The investigation began when a former employee filed suit against the company when he was discharged after refusing to tamper with the equipment. *US v. Louisiana-Pacific Corp.*, No. 95-CR-215 (D. Colo. 1998).

In addition, inadequate resources devoted to environmental compliance can lead to criminal liability. In May 1991, United Technologies Corp. pleaded guilty to six felony violations of Resource Conservation and Recovery Act (RCRA) and agreed to pay a $3 million fine for hazardous waste violations. The violations were the result of improper disposal of cleaning solvents at a Stratford, Connecticut site, discovered during an Environmental Protection Agency (EPA) inspection of the facility. The company's in-house environmental compliance officer became aware of the illegal disposal, but it was not discontinued until the following year. The company had only one full-time person responsible for environmental compliance for all of its facilities in the United States. The US Attorney issued a statement that "companies creating hazardous wastes have a clear duty to aggressively devote adequate manpower and financial resources to protecting our environment." The EPA Regional Administrator also issued a statement that "it should now be abundantly clear that criminal sanctions are not reserved only for the flagrant and deliberate violations of the environmental laws, but also for violations that result from a company's plain or institutional indifference to meet its legal responsibility." *US v. United Technologies Corp.*, No. 2:91CR00028 (D. Conn. 1991).

Liability exposure to individual officers or employees has expanded the scope of criminal liability and responsibility for not only the actions of individuals but also their respective corporate employers. In the first conviction for the newly created Multi-Agency Environmental Task Force in the US District Court for the Eastern District of Michigan, the owner of an environmental laboratory pled guilty to mailing falsified environmental test results and bills for tests his company never performed. Jerry Martin, owner of Martin Environmental

Laboratories, was sentenced to 1 year in prison and payment of $16 781 to former customers. The company was fined $5000. *US v. Martin Envt'l Labs.*, No. 01-90040 (E.D. Mich. May 2, 2002). Because no environmental statutes prohibited laboratory fraud, Mr Martin was charged with mail fraud, a federal felony with sentencing guidelines that include prison. The case started with a tip from a former employee to the Task Force. The Task Force consists of the US Attorney for the Eastern District of Michigan, the Michigan Attorney General, the Federal Bureau of Investigation, the US Coast Guard, and the US Customs Service.

Summaries of Selected Environmental Criminal Actions Involving Individuals

Expanding the 'reach' of the federal racketeering statute to environmental matters has broadened the basis for criminal environmental liability. On August 10, 2001, the US District Court for the Eastern District of Michigan, in the first successful case for convictions under the federal racketeering law for environmental crimes, sentenced the president/owner and operations manager of Hi-Po, Inc. to multiple-year prison terms. They were charged with dumping materials such as diesel fuel into streams and sewers so that their company would win contracts to clean up the polluted waters. Aaron Smith, president and owner, was sentenced to 33 months in prison, for violation of the Racketeer Influenced and Corrupt Organizations Act (RICO), followed by 3 years of supervised release. He was also ordered to pay $505 000 in restitution and to forfeit $500 000. Stephen Carbeck, the company's operations manager, was sentenced to 27 months in prison, for violation of RICO, followed by 3 years of supervised release, and was ordered to pay $430 000 in restitution. The company itself was fined $50 000 for pleading guilty to two counts of violating the Clean Water Act (CWA). *US v. Smith*, No. 00-80528 (Aug. 10, 2001).

In a case that further widened the scope of environmental criminal liability the court concluded that a defendant did not have to know that his conduct violated the law to be held criminally liable. On September 16, 1998, the US Court of Appeals for the Sixth Circuit upheld a trial court's conviction of a Louisville, Kentucky paint manufacturer and its vice president for violations of RCRA for the illegal storage and disposal of hazardous waste materials. The court found that government prosecutors were not required to prove that the paint manufacturers and its vice president knew that materials stored or dumped at its facility were hazardous substances or that such storage or disposal required a permit and distinguished this case from the *Ahmad*, *infra*, case because the *Ahmad* case did not discuss the requirement for knowledge of the law and dealt with the issue of mistakes in facts. The government prosecutors were only required to prove that the defendant had knowledge of the storage or disposal, that the material was waste, and that it was harmful to others or the environment. The court held that the vice president did not have to know the materials were RCRA hazardous wastes to support a 'knowing' violation under RCRA. The trial court's 21 month prison term and $5000 fine for the vice president was upheld. The company had not appealed its $225 000 fine. The violations were discovered in 1992 during a Kentucky Department of Environmental Protection inspection. The vice president was in charge of manufacturing operations and had responsibility for environmental compliance at the company's facilities. *US v. Kelley Technical Coatings, Inc.*, No. 96-6282, 157 F.3d 432 (6th Cir. 1998).

On May 29, 1998, four executives of the former LCP Chemicals plant in Brunswick, Georgia, were indicted on 42 counts of violating federal environmental laws. The plant had been placed on the National Priorities List (NPL) and was closed in 1994 as state officials were in the process of revoking the facility's permits. Each defendant was charged with one count of conspiring to violate the CWA, RCRA, the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), and the Endangered Species Act. Christian Hansen, chairman and CEO of the corporate owner of the company and *de facto* plant manager, and Randall Hansen, COO of the corporate owner, and Alfred Taylor, plant and operations manger of the plant, were charged with 20 counts of violating the CWA. Brent Hanson, technical and environmental manager of the corporate owner, was charged with 17 counts of violating the CWA. The CWA violations were for alleged discharges of mercury and chlorine into a creek in violation of the facility's NPDES permit. The RCRA charges were for illegal storage of mercury-contaminated hazardous waste in buildings and tanks at the plant and for filing false and misleading reports. Christian Hansen was sentenced to 9 years in prison; Randall Hansen was sentenced to 46 months. *US v. Hansen*, No. CR-298-23 (S.D. Ga. Jan. 1999). The Hansens appealed and on August 24, 2001 the US Court of Appeals for the Eleventh Circuit upheld the convictions. *US v. Hansen*, No. 99-11638, 262 F.3d 1217 (11th Cir. Aug. 24, 2001). On June 3, 2002, the US Supreme Court declined to overturn the convictions or to review the limits of the responsible corporate officer doctrine.

On July 11, 1997, a US appeals court upheld the 1996 conviction of Timothy Sinskey, a former vice president and plant manager, and Wayne Kumm, the plant engineer, of a John Morell & Co. meat packing facility in Sioux Falls, South Dakota. *US v. Sinskey*, No. 96-3962, 119 F.3d 712 (8th Cir. 1997); *US v. Kumm*, No. 96-3965 (8th Cir. 1997). The court distinguished this case from *Ahmad, infra*, and held that while *Ahmad* was based on a 'mistake of fact' defense, this case was based on a 'mistake of law' and stated, "We have repeatedly held that, in other statutes with similar language, the word 'knowingly' refers only to knowledge of the relevant activities" and that knowledge of the illegal nature of the acts need not be proved. 119 F.3d at 715. The two employees were charged with manipulating the flow of discharge material and selectively sampling the effluent to minimize the number of exceedances at the plant. In resolution of criminal charges against the company, it had previously agreed to pay a $2 million fine and $1 million to establish an environmental cleanup fund. *US v. John Morrell & Co.*, No. 96-CR-40004 (D.S.D. May 28, 1996). Mr Sinskey was convicted on felony counts that included conspiracy, rendering a discharge monitoring method inaccurate, illegally discharging hazardous waste, and falsifying discharge monitoring reports and was sentenced to 24 months in prison, placed on probation for 2 years, and ordered to pay a $5000 fine and $600 to a federal victim witness fund. *US v. Sinskey*, No. 96-400/001 (D.S.D. Nov. 4, 1996). Mr Kumm was convicted on one felony count of rendering a monitoring device inaccurate and was sentenced to 6 months in jail, 6 months of home confinement, a $2000 fine, and $8000 to an environmental group. *US v. Kumm*, No. 96-400/002 (D.S.D. Nov. 4, 1996).

In *US v. Ahmad*, 101 F.3d 386 (5th Cir. 1996), a federal appeals court held that the government must meet 'traditional' intent requirements of criminal law to obtain a conviction under the CWA. The court reversed and remanded the lower court's conviction and held that the law required the government to prove defendant's knowledge of each actual element of an offense and that the defendant had to know all the facts that made his actions illegal. The court also narrowed the 'public welfare doctrine' that allows the government to dispense with traditional intent requirements in prosecuting some environmental regulatory crimes and held that serious felonies should not fall within the public welfare exception absent a clear statement from Congress. Mr Ahmad was convicted of discharging gasoline into a city sewer when, in fact, he thought it was water – a mistake in fact which, he claimed, meant that he lacked the 'knowledge' required for a criminal conviction. Later decisions have distinguished themselves from *Ahmad* as being convictions based on 'mistakes in law' and not 'mistakes in fact'.

On April 3, 1991, the US Court of Appeals for the First Circuit upheld the conviction and 2-day prison sentence of David Boldt, a chemical engineering manager employed for 6 months by Astro Circuit Corporation, for knowingly discharging wastewater containing excessive amounts of copper into a city sewer. Mr Boldt was one of four defendants charged in a 52-count indictment for numerous violations of the CWA and he was found guilty of preparing false written statements to authorities for two incidents. *US v. Boldt*, No. 90-1454, 929 F.2d 35 (1st Cir. 1991).

On August 16, 1999, the US District Court for the Middle District of Florida sentenced the owner of a company to 13 years in prison and fined him $14 000 for intentionally and continuously dumping toxic waste into the Tampa, Florida sewer system over a period of 9 years in violation of the CWA and RCRA. Gary Benkovitz, also known as Gary Blake, owned Bay Drum and Steel, Inc., and admitted that he directed employees to empty drums into a storm sewer that empties into McKay Bay. The prison sentence is the maximum allowed under the federal sentencing guidelines for environmental crimes. One of the reasons Mr Benkovitz received the maximum sentence is because he allegedly continued the violations while he was awaiting sentencing for an earlier felony charge for illegal dumping. The company is estimated to have discharged 3 million gallons of contaminated wastewater and more than 450 000 lb of hazardous solid waste into the sewer system. *US v. Benkovitz*, Nos. 97-331, 98-349 (M.D. Fla. Aug. 16, 1999).

Criminal Convictions Related to Toxic Tort Exposures

In the context of personal injury toxic tort liability a defendant's apparent intentional disregard for the safety of his employees led to a criminal conviction. On June 26, 1998, a five-count indictment was lodged against the owner of an Idaho fertilizer company who allegedly exposed workers to cyanide in 1996 without protective equipment, causing permanent brain damage to one employee. Allan Elias, the owner, was charged with endangering the safety and health of employees by ordering employees to clean out a 25 000 gal storage tank that contained cyanide. Count one was for knowing endangerment; and counts two through five were for illegal disposal of hazardous waste on three occasions and making a

false statement by fabricating and backdating a safety plan on worker entry of the tank. Mr. Elias ordered the workers to continue after they complained about sore throats and stopped work inside tank. Mr. Elias informed emergency workers that the tank contained water and that he did not know what caused the injury to the 20 year old employee who ultimately sustained permanent brain damage. Mr Elias was sentenced to 17 years of imprisonment and ordered to pay $6 million in restitution to the family of one of his employees who suffered brain damage as a result of cleaning the tank without wearing appropriate protective gear. *US v. Allan Elias*, No. CR-98-070-BLW (D. Idaho June 2, 1998). On October, 23, 2001, the US Court of Appeals for the Ninth Circuit upheld the conviction, but remanded the case to the district court to amend the sentence by deleting the restitution provision because the particular provision under which Mr. Elias was ordered to pay restitution (18 U.S.C. § 3663) did not allow the imposition of restitution. *US v. Elias*, No. 00-30145, 27 Fed. Appx. 750 (9th Cir. Oct. 23, 2001).

In 1995, a Florida jury awarded $500 million to the parents of one of two children who died in 1992 from acute toluene exposure while playing in an unlocked dumpster where the chemical was illegally discarded. *Perez v. William Recht Co.*, No. 92-8983-B (Hillsborough County Cir. Ct., Sept. 1995). As a result of the incident, a plant manager and a shop foreman received federal prison sentences, and the company pleaded no contest to criminal charges of knowing endangerment and received a $1.5 million fine. *US v. Whitman*, No. 94-70-CRT-1B (M.D. Fla. 1994). On July 28, 1998, a federal grand jury indicted the owner of William Recht Co. for eight counts of criminal hazardous waste storage without a permit. The indictment alleges that Mr Recht refused at least four times to provide funds for proper disposal of drums of hazardous and nonhazardous waste after the death of the children. *US v. Recht*, No. 98-280-CR-T-24A (M.D. Fla. 1998).

The potential liability for corporate officials has expanded even to those who are not in direct management control of environmental compliance activities. In *Doe Run Resources Corp. v. Neill* No. SC85451, 123 S.W. 3d 502 (Mo. S. Ct Feb. 10, 2004), the Missouri Supreme Court concluded that a lead smelter's chief financial officer likely would have had enough knowledge of the company's alleged polluting activities that he could have stopped or influenced those activities by his control over the company's finances and budget. As a result the court held he could be sued personally under applicable Missouri law for harm caused by the company's breach of state and federal environmental laws. Although this case did not address criminal liability it suggests that if a CFO can be held liable for environmental non-compliance of his company, that a criminal case based on little more may under case specific circumstances lead to potential criminal liability.

US Environmental Protection Agency Criminal Enforcement Activities

As of the end of 2003, the US EPA had charged an average of about 330 criminal defendants per year (1998–2003) with no trends suggesting a decline in prosecutions. An average of over 183 years of sentences were issued and an average of over $84 million dollars in fines were collected each year during this same time period. While these numbers do not reflect any assessment of tort claim recoveries they do demonstrate the fact that criminal matters are not insignificant on a national scale and must be considered in reviewing any potential toxic tort matter.

See also: Chemicals of Environmental Concern; Clean Air Act (CAA), US; Clean Water Act (CWA), US; Resource Conservation and Recovery Act.

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Cromolyn

F Lee Cantrell

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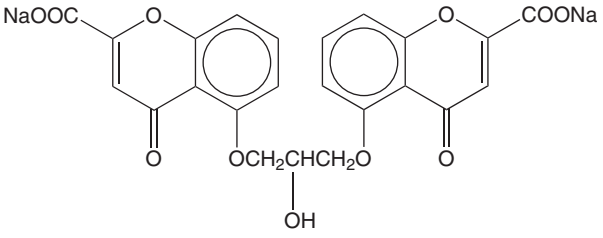
- Chemical Abstracts Service Registry Number: CAS 16110-51-3
- Synonyms: Cromolyn sodium; Disodium cromoglycate; Disodium salt of cromolyn; Nasalcrom; Intal; Crolom; Gastrocrom
- Chemical/Pharmaceutical/Other Class: Mast cell stabilizing antiallergic agent
- Chemical Formula: $C_{23}H_{14}Na_2O_{11}$
- Chemical Structure:



Cromolyn sodium

Uses

Cromolyn is used primarily for the prophylaxis of various types of asthma and in the treatment of mastocytosis and vernal conjunctivitis.

Exposure Routes and Pathways

For use in asthma, cromolyn is administered by inhalation using solutions delivered by aerosol spray or nebulizer as well as a powdered drug mixed with lactose and delivered by a turbo inhaler. For use in mastocytosis, cromolyn is ingested in a liquid form. Cromolyn is available in ocular drop form for the treatment of vernal conjunctivitis.

Toxicokinetics

Oral absorption of cromolyn is less than 1%, although up to 10% of an inhaled dose of cromolyn can be absorbed systemically. After complete absorption, cromolyn is excreted unchanged in urine and bile in about equal proportions. Peak plasma concentrations occur 15 min after inhalation. The distribution of cromolyn in the lung and the extent of systemic absorption are enhanced by bronchodilation during drug delivery. The biological half-life following inhalation ranges from 45 to 100 min.

Mechanism of Toxicity

The major prophylactic effect of cromolyn is centered on inhibition of the degranulation of pulmonary mast cells causing a reduction in histamine release, reduced leukotriene production, and inhibition of release of inflammatory mediators from several cell types.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of cromolyn, measured as the LD, has been determined in the rat ($>2150\,mg\,kg^{-1}$ orally and $6000\,mg\,kg^{-1}$ subcutaneously) and the mouse ($3300\,mg\,kg^{-1}$, intravenous; $1000\,mg\,kg^{-1}$, intraperitoneal; and $4400\,mg\,kg^{-1}$, subcutaneous).

Human

None reported.

Chronic Toxicity (or Exposure)

Animal

Studies of reproduction in mice, rats, and rabbits have not demonstrated fetal toxicity at doses up to 338 times the usual human dose.

Human

Because of its low toxicity, cromolyn is generally well tolerated. Adverse side effects, such as bronchospasm, cough, wheezing, laryngeal edema, joint swelling, joint pain, angioedema, headache, rash, and nausea, are rare (less than 1 in 10 000 patients). Documented instances of anaphylaxis are also been rare.

In Vitro Toxicity Data

Peripheral blood mononuclear cells obtained from allergy-dependent asthmatics demonstrated antigen-specific anti-allergic inflammatory effects.

Clinical Management

Toxicity is unlikely, but should adverse effects occur, general emergency management and supportive care procedures are indicated.

See also: Aerosols.

Further Reading

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Crude Oil *See* Oil, Crude.

CS Gas

Harry Salem, Bryan Ballantyne, and Sidney A Katz*

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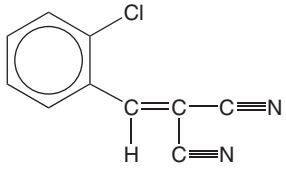
- Military Designation: CS
- Chemical Name: Chlorobenzylidene malononitrile
- Chemical Abstracts Service Registry Number: CAS 2698-41-1
- Synonyms: Tear gas; Less-than-lethal; Nonlethal; Lacrimator, Harassing agent; Incapacitant; (2-Chlorophenyl) methylene; Propanedinitrile, (*o*-Chlorobenzylidine) malononitrile; 2-Chlorobenzalmalononitrile
- Chemical Formula: $C_{10}H_5ClN_2$
- Chemical Structure:



Pharmacological Action

Riot control agents such as CS are those that cause disabling physiological effects when they come into contact with the eyes or skin, or when inhaled. They have the capacity to cause intense sensory irritation of the skin and mucus membranes of the eye and respiratory tract. They are peripheral sensory irritants that pharmacologically interact with sensory nerve receptors in skin and mucosal surfaces at the site of contamination resulting in local pain and discomfort sensations with associated reflexes. The reflex associated with the inhalation exposure of irritants is the Kratschmer reflex. This reflex causes apnea, bradycardia, and a biphasic fall and rise in aortic blood pressure.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Pharmacological Class

CS is a peripheral sensory irritant, lacrimator, sternutator, and an incapacitant.

Uses

CS is used as a nonlethal or less-than-lethal chemical in riot control situations, to distract, deter, incapacitate, disorient, or disable disorderly people, to clear facilities, areas, deny areas, or for hostage rescue. It can also be used in peacekeeping operations. It is also used in military training as a confidence builder for the protective mask. In addition to the nonpersistent form of CS, two hydrophobic variations were created, CS1 and CS2. CS1 is a micronized powder formulation containing 5% hydrophobic silica aerogel, which can persist for up to 2 weeks in normal weather conditions, and CS2 is a siliconized microencapsulated form of CS1 with a long shelf life, persistence, resistant to degradation, and ability to float on water, which could restrict or deny the use of water for military operations. CS is commonly used as a riot control agent and a simulant for training. Members of military organizations and law enforcement agencies are routinely exposed to heated CS during training. The heat vaporizes the CS for dispersion, which then condenses to form an aerosol.

Exposure Routes and Pathways

CS at room temperature is a white solid, stable when heated and with a low vapor pressure. The vapor is several times heavier than air. It can be dispersed as a fine powder, or as a jet or stream of solution from small or large spray tanks, as well as aerosols or smokes by pyrotechnic generation. Its solubility in water is limited, but it is soluble in organic and chlorinated organics. High-temperature dispersion may produce a number of organic thermal degradation

products through rearrangements and loss of cyano and chlorine substituents on CS, possibly HCN and HCl. CS is rapidly hydrolyzed in water with a half-life of ~15 min at room temperature at pH 7. In alkaline solution (pH 9), the half-life is ~1 min. Therefore, CS can be easily inactivated by a water/alkaline solution, or by washing with soap and water.

Toxicokinetics

CS reacts covalently with plasma proteins to form compounds that may be antigenic. On contact with water, it hydrolyses into *o*-chlorobenzaldehyde and malononitrile. The kidney excretes *o*-chlorobenzaldehyde as the metabolites *o*-chlorohippuric acid (major) and *o*-chlorobenzoic acid (minor). The malononitrile is metabolized to thiocyanate. The cyano groups of 2-chlorobenzylidene malononitrile are unlikely to cause systemic cyanide toxicity since no significant amounts of free cyanide appear in the plasma.

Mechanism of Toxicity

These agents are considered less than lethal and nonlethal because they have a very large safety ratio. That is, their effective dose or concentration ECt_{50} is very low compared to their lethal dose or concentration (LCt_{50}).

CS as well as CN is an SN2-alkylating agent with activated halogen groups that react readily at nucleophilic sites. The prime targets include sulfhydryl-containing enzymes such as lactic dehydrogenase. In particular, CS reacts rapidly with the disulfhydryl form of lipoic acid, a coenzyme in the pyruvate decarboxylase system. It has been suggested that tissue injury may be related to inactivation of certain of these enzyme systems. CS causes the release of bradykinin, which can cause pain without tissue injury. The initial response to the inhalation of CS or other sensory irritants is consistent with the Kratschmer reflex and the Sherrington pseudoaffective response. These aerosols stimulate the pulmonary irritant receptors to produce bronchoconstriction and increased pulmonary blood volume by augmenting sympathetic tone. The chlorine atoms released from CS on contact with skin and mucus membranes are reduced to hydrochloride acid that can cause local irritation and burns.

Human Toxicity

When CS was disseminated using spray nozzles from a 10% solution in acetone or in methylene dichloride, or from a miniature M8 thermal grenade, the mass median diameter of CS produced was 3.0 μm for the CS in acetone, 1.0 μm for the CS in methylene dichloride, and 0.5 μm for the miniature M18 CS thermal grenade. When properly fitted, protective masks fully protected against exposure to CS. In those who were unable to mask rapidly, panic was evident. Concentrations of 9–10 $mg\,m^{-3}$ forced 50% of the subjects to leave the chamber within 30 s, 99% left when exposed to ~17 $mg\,m^{-3}$, and 100% left and were considered incapacitated at 40 $mg\,m^{-3}$ or greater.

Persons who had been exposed previously to a high concentration developed a fear of the agent, and even though subsequently exposed to lower concentration, the time to incapacitation for trained men was shorter than expected. There were no significant differences noted in the time to incapacitation in subjects exposed to CS at 0–95°F, although it was apparent that the subjects appeared unable to tolerate the agent as well as those exposed at ambient temperature. At 95°F and a relative humidity of 35% or 97%, the skin-burning effects were much more prominent, possibly because of the excessive diaphoresis. Hypertensive subjects reacted similarly to and tolerated CS as well as normotensive individuals. However, their blood pressure elevation was greater and lasted longer than in normotensives, possibly because of the stress of exposure. The hypertensive subjects recovered as rapidly as normotensives.

Subjects with a history of peptic ulcer, jaundice, or hepatitis, and those between the ages of 50 and 60 years reacted similarly to normal subjects. Persons with a history of drug allergy, hay fever, asthma, or drug sensitivity were able to tolerate CS exposure as well as the normal subjects; however, a higher percentage of this group had more severe chest symptoms than the normals. Although many of these lay prostrate on the ground for several minutes, no wheezing or ronchi were heard on auscultation, and recovery time was slightly prolonged, but only by 1 to 2 min. Although not significantly different, subjects exposed to CS disseminated from methylene dichloride appeared to tolerate the agent for a slightly longer period than those subjected to CS in acetone solution, nor was there many differences from CS disseminated from the miniature M18 CS smoke grenade. CS was effective within seconds. Although high concentrations for prolonged exposure in closed spaces can produce severe effects, no validated deaths in humans have been reported for CS. These effects were acute laryngo-tracheobronchitis in an infant, reactive airway dysfunction syndrome, hemoptysis and hypoxia, and erythroderma in adults, which were all treated successfully. Ingestion

of CS may lead to abdominal cramping, pain, and diarrhea.

Clinical Management

Ocular exposure to CS produces intense blepharospasm, pain, lacrimation, conjunctival erythema, periorbital edema, and a rise in intraocular pressure. These effects generally diminish within 30 min postexposure. CS also produces rhinorrhea, nasal irritation and congestion, bronchorroea, sore throat, cough, sneezing, unpleasant taste, and burning of the mouth immediately after exposure. These effects rapidly resolve within minutes postexposure. Symptomatic treatment of ocular irritation consists of use of a topical solution to relieve the irritation with topical antibiotics. The eyes should be examined for corneal abrasions. Treatment with oral analgesics, topical antibiotics, and mydriatics should be considered. Since CS is a solid, it is possible for a particle or clump to become embedded in the cornea or conjunctiva and cause tissue damage. Medical care for eye pain after exposure should include thorough decontamination of the eyes and a thorough ophthalmologic examination. The eye with ocular injuries should be carefully irrigated with isotonic saline and the remaining powder removed with a cotton swab. Any remaining stromal particles should be removed with a needle tip under slit lamp illumination. Airway problems may occur in individuals with lung disease, especially if exposed to higher than average field use concentrations. If these occur, the immediate priority is the removal from the exposure and to ensure a potent airway.

Severe and prolonged erythema or serve dermatitis may occur several hours after exposure that is then followed by vesiculation. These are generally second-degree burns and should be treated like second-degree chemical burns.

If the release of irritant incapacitants is in a confined, unventilated space, exposure may be to very high concentrations. Some individuals may be more susceptible to high concentrations, possibly because of an existing medical condition such as asthma, and will require intensive supportive medical treatment postexposure.

Animal Toxicity

Various experimental animal species were exposed to aerosols of CS generated by various methods from exposure from 5 to 90 min. The toxic signs observed in mice, rats, guinea pigs, rabbits, dogs, and monkeys were immediate, and included hyperactivity, followed by copious lacrimation, and salivation within 30 s of exposure in all species except the rabbit. The initial level of heightened activity subsided, and within 5–15 min following initiation of the exposure, exhibited lethargy and pulmonary stress, which continued for about an hour following cessation of the exposure. All other signs had disappeared within 5 min following removal from the exposure. When toxic signs were observed, they occurred following exposure by all of the dispersion methods.

Lethality estimates were expressed by calculation of LCt_{50} values. From acute exposures to CS dispersed from a 10% CS in methylene dichloride the LCt_{50} values (in mg min m^{-3}) were as follows: mice, 627 000; rats, 1 004 000; and guinea pigs, 46 000. No deaths occurred in rabbits exposed to up to 47 000 mg min m^{-3}. CS at dosages up to 30 000 mg min m^{-3} did not cause any deaths in any of the monkeys with pulmonary tularemia. The combined LCt_{50} for mice, rats, guinea pigs, and rabbits was calculated to be 1 230 000 mg min m^{-3} for CS dispersed from methylene dichloride. Goats, pigs, and sheep did not exhibit hyperactivity on exposure to CS, and they were also resistant to its lethal effect. Therefore, no LCt_{50} values could be calculated for goats, pigs, or sheep. However, a combined LCt_{50} was calculated for all of the species tested, including mice, rats, guinea pigs, rabbits, dogs, monkeys, goats, pigs, and sheep, and was estimated to be 300 000 mg min m^{-3}. LCt_{50} values were also calculated for CS dispersed from M18 and M7A3 thermal grenades. These were (in mg min m^{-3}): 164 000 for rats and 36 000 for guinea pigs exposed to the M18 thermal grenade dissemination; for the M7A3 thermal grenade they were (in mg min m^{-3}) as follows: rats, 94 000; guinea pigs, 66 000; rabbits, 38 000; goats, 48 000; pigs, 17 000; dogs, 30 000; monkeys, 120 000.

All of the acute exposure results were combined and LCt_{50} values were calculated for all rodents to be 79 000 mg min m^{-3}, and for all nonrodent species tested to be 36 000 mg min m^{-3}, and for all the species it was 61 000 mg min m^{-3}. The LCt_{50} values for CS2 were also calculated. CS2 is 95% CS, 5% Cal-o-Sil R, and 1% hexamethyldisilazane, and the LCt_{50} values are: rats, 68 000 mg min m^{-3}; guinea pigs, 49 000 mg min m^{-3}; dogs, 70 000 mg min m^{-3}; and monkeys, 74 000 mg min m^{-3}. The lethal effects in animals following inhalation exposures are caused by lung damage leading to asphyxia and circulatory failure, or bronchopneumonia secondary to respiratory tract injury. Pathology involving the liver and kidneys following inhalation of high dosages of CS is also secondary to respiratory and circulatory failure.

The acute inhalation toxicity of CS, generated in smoke and as an aerosol, was studied in several

Table 1 Acute inhalation toxicity LCt_{50} (mg min m^{-3})

Animal	*CS smoke*	*CS aerosol*
Guinea pig	35 800	67 000
Rabbit	63 600	54 090
Rat	69 800	88 480
Mouse	70 000	50 110

animal species, and the LCt_{50} data are presented in **Table 1**.

Repeat exposures of thermally dispersed CS were conducted in rats and dogs. They were exposed from 4 to 5 min per day, 5 days a week for 5 weeks. The 25 day cumulative dosage (Ct) to which the rats were exposed was 91 000 mg min m^{-3} (3640 mg min m^{-3} per day), while the dogs were exposed to a cumulative dosage of 17 000 mg min m^{-3} (680 mg min m^{-3} per day). No lethality occurred in the dogs, while the rats became hyperactive and aggressive, biting noses and tails of other rats, and scratching their own noses. No changes were found in blood values for sodium, potassium, protein, albumin, or creatinine throughout the tests. Five of the 30 rats exposed died, two following the cumulative dosage of 25 000 mg min m^{-3}, and three died after 68 000 mg min m^{-3}. Gross pathological examinations of the rats that died were negative, as were those of six other rats that were sacrificed after 5 weeks' exposure. The exposed rats lost ~1% of body weight, while unexposed rats gained ~20% during the 5 weeks. There were no significant differences in organ to body weight ratios for heart, kidneys, lungs, liver, or spleen following the 5 week exposures. It was concluded that repeated exposures did not make the animals more sensitive to the lethal effects of CS. The animals that died after exposure to CS showed increased numbers of goblet cells in the respiratory and gastrointestinal tracts and conjunctiva, as well as necrosis in the respiratory and gastrointestinal tracts, pulmonary edema, and occasionally hemorrhage in the adrenals. Death appeared to result from poor transfer of oxygen from the lungs to the blood stream, probably because of edema, and hemorrhage in the lungs, and obstruction of the airways.

The effects of repeated exposures to CS in mice, rats, and guinea pigs to neat CS aerosols for 1 h per day, 5 days per week for 120 days demonstrated that high concentrations of CS were fatal to the animals after only a few exposures, while mortality in the low and medium concentrations did not differ significantly from the controls. It was concluded that CS concentrations below 30 mg m^{-3} were without deleterious effects.

The effects of CS inhalation were studied on embryonic development in rats and rabbits at concentrations consistent with those expected in riot control situations (~10 mg m^{-3}). Although the concentrations were low and the duration of exposure (5 min) may not have been adequate to assess the fetotoxic and teratogenic potential of CS, no significant increase in the numbers of abnormal fetuses or resorptions were noted.

CS2 was evaluated for carcinogenicity in the US National Toxicity Program (NTP) 2 year rodent bioassay. Compound related non-neoplastic lesions of the respiratory tract were observed. The pathologic changes observed in the exposed rats included squamous metaplasia of the olfactory epithelium, hyperplasia, and metaplasia of the respiratory epithelium. In mice, hyperplasia and squamous metaplasia of the respiratory epithelium was observed. Neoplastic effects were not observed in either rats or mice. It was concluded that the findings suggests that CS2 is not carcinogenic to rats and mice. CS in methylene chloride was also tested in mice and rats for carcinogenicity in a 2 year study, and no tumorigenic effects were observed in the CS-exposed animals.

In Vitro Toxicity Data

The mutagenic potential of CS and CS2 were studied in microbial and mammalian bioassays. CS was positive in the Ames Assay, while others reported questionable genotoxicity for *S. typhimurium*, and negative when tested in *S. typhimurium* strains TA 98, TA 1535, and TA 1537 with and without metabolic activation. The mutagenic potential for CS and CS2 in mammalian assays such as the Chinese hamster ovary test for the induction of sister chromatid exchange (SCE) and chromosomal aberration (CA), and the mouse lymphoma L5178Y assay for induction of trifluorothymidine (Tfi) resistance indicated that CS2 induced SCE, CAs, and Tfi resistance. The Committee on Toxicology of the (US) National Research Council (1984) reported that, taken in their totality, the test of CS for gene mutation and chromosomal damage provide no clear evidence of mutagenicity. Although most of the evidence is consistent with nonmutagenicity, in the committee's judgment, it is unlikely that CS poses a mutagenic hazard to humans.

Decontamination

Contaminated clothing should be removed and sealed in a plastic bag. Disposable rubber gloves should be used when handling contaminated clothes.

The eyes should be irrigated copiously with saline for 15–20 min. Contaminated skin should be washed thoroughly with copious amounts of water, alkaline soap and water, a mildly alkaline solution (sodium bicarbonate or sodium carbonate) or mild liquid soap and water. The use of sodium hypochlorite solution will exacerbate the skin lesions and should not be employed. Only a saline irrigation should be used over vesiculated skin.

Decontamination of material/clothing after contamination with CS can be done with sodium bicarbonate or carbonate 5–10% solution. If this means of decontamination cannot be accomplished (e.g., contaminated rooms and furniture), then the only other means is by intensive air exchange – preferably with hot air.

If clothing is to be washed, cold water should be used because hot water will cause any residual CS to volatilize leading to symptoms in attending staff.

See also: CN Gas; Non-Lethal Weapons, Chemical; Riot Control Agents.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

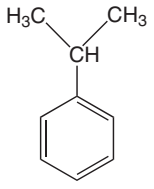
http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Culture and Toxicology *See* Toxicology in the Arts, Culture, and Imagination.

Cumene

Ralph Gingell

- Chemical Abstracts Service Registry Number: CAS 98-82-8
- Synonyms: Isopropyl benzene; (1-Methylethyl)-benzene; 2-Phenylpropane
- Chemical/Pharmaceutical/Other Class: Alkyl aromatic family of hydrocarbons
- Chemical Formula: C_9H_{12}
- Chemical Structure:



Uses

Cumene is an industrial intermediate in the manufacture of phenol and acetone via cumene hydroperoxide. It also has minor applications as a solvent.

Exposure Routes and Pathways

Cumene is a naturally occurring constituent of crude oil and may be released to the environment from a number of anthropogenic sources, including processed hydrocarbon fuels; for example, diesel fuel contains 0.86 wt.% of cumene; furnace oil (no. 2) contains 0.60 wt.%. Humans can be exposed to cumene via industrial emissions, petrol station or motor vehicle emissions, and accidental releases. The general population would be exposed to cumene primarily by inhalation, although occupational populations may be exposed by the dermal route. Minor exposure may result from contact with refined petroleum products and ingestion of contaminated foods and possibly drinking water.

Toxicokinetics

Cumene is absorbed readily via the inhalation route in man and animals, and is metabolized efficiently, within the body, to water-soluble metabolites that are excreted into the urine. The secondary alcohol 2-phenyl-2-propanol and its conjugates are major metabolites. Neither cumene nor its metabolites are likely to accumulate within the body. Based on controlled studies in humans, the average retention of inhaled cumene in the respiratory tract was 50%.

Mechanism of Toxicity

The signs of toxicity after exposure to cumene vapors are consistent with central nervous system (CNS) depression, and eye and respiratory irritation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cumene has low toxicity to laboratory animals by inhalation, oral, or dermal routes of exposure. A 4 h inhalation LC_{50} of 39 200 mg m^{-3} (8000 ppm) in rats was reported by several investigators. Acute oral LD_{50} values for rats range from 1400 to 2900 mg kg^{-1} body weight. Acute dermal LD_{50} values for cumene applied undiluted to rabbit skin range from 3160 mg kg^{-1} body weight to 12.3 g kg^{-1}. In acute exposures, animals exhibit damage to the spleen and fatty changes in the liver, but no renal or pulmonary effects. The concentration of cumene causing a 50% reduction in the respiratory rate in mice (a measure of respiratory irritation) was determined to be 2058 ppm (10 117 mg m^{-3}).

Cumene is a CNS depressant characterized by slow induction and long duration of effects. Acute behavioral effects following a single 20 min inhalation exposure to cumene at 2000–8000 ppm were short-lived and completely reversible. CNS depressant effects were reported only at quite high concentrations (>500 ppm).

In Fischer 344 rats, exposure to cumene vapor for 13 weeks resulted in mild toxicity at 1200 ppm, minimal effects at 500 ppm, and no-observed effects at 50 and 100 ppm; the main effects were reversible decreased activity, reversible organ weight changes, and male rat renal hyaline droplet formation, which is not believed to be relevant to humans. Neurotoxicological effects were not observed in this study, which included complete batteries of functional and motor activity tests and neurohistopathology.

Cumene is not a primary developmental toxicant. Exposure of rats to cumene vapor during organogenesis resulted in clinical signs of maternal irritation and toxicity at 500 and 1200 ppm with a no-observed-effect level of 100 ppm. No developmental toxicity was observed at any dose level tested. Exposure of New Zealand rabbits to cumene vapor during organogenesis also resulted in clinical signs of irritation and maternal toxicity at 2300 ppm with less severe effects at 1200 and 500 ppm; the no-observed-effect level was <500 ppm. No developmental toxicity was observed at any dose level tested.

Human

The main hazard with low volatility, low viscosity hydrocarbons such as cumene is aspiration pneumonitis, which may occur after vomiting accidentally ingested material. Cumene is irritating to the eyes and skin. Prolonged skin contact may result in skin drying, defatting, and rashes. Exposure to vapor concentrations may cause CNS depression indicated by dizziness, slight incoordination, and unconsciousness.

Chronic Toxicity (or Exposure)

Animal

Cumene is being tested for carcinogenicity by inhalation in rats and mice by the US National Toxicology Program; reports are not available. Cumene is not expected to be a genotoxic carcinogen.

Human

No information is available regarding the toxicity of cumene in humans following acute, subchronic, or chronic exposure. No epidemiology, case reports, or clinical studies of humans were located.

In Vitro Toxicity Data

Cumene was negative with or without activation in the Ames *Salmonella*/mammalian-microsome preincubation mutagenicity assay. Cumene was negative, with or without metabolic activation, in the HGPRT mutation assay with Chinese hamster ovary (CHO) cells, and the CHO chromosome aberration assay. Cumene was negative in the unscheduled DNA synthesis test using rat primary hepatocytes. Cumene was negative in the BALB/3T3 mouse embryo cell morphological transformation assay.

Clinical Management

As with other petroleum hydrocarbon products, management in most cases is symptomatic. Attention should be paid to possible aspiration pneumonitis after ingestion exposure; vomiting should not be induced. Oral or high concentration vapor exposure may cause CNS depression; the patient should be removed to fresh air. Liquid may cause skin or eye irritation; contaminated clothing should be removed, and skin and eyes should be flushed with water.

Environmental Fate

Cumene is a volatile liquid and exists mainly in the vapor phase in the atmosphere. It degrades in the atmosphere via reaction with hydroxyl radicals. Although small amounts of cumene may be removed from the atmosphere by precipitation, cumene is not expected to react with ozone or directly with light. In water, cumene can be volatilized, undergo biodegradation, or adsorb to sediments. It is

expected to biodegrade rapidly in soil under aerobic conditions; in water, it can readily adsorb to soil or volatilize.

Ecotoxicology

Although cumene is considered moderately toxic to aquatic organisms under rigorous laboratory conditions, its volatility and biodegradability greatly reduce its hazard to the aquatic environment. The 96 h LC_{50} values for rainbow trout, sheepshead minnow, and mysid shrimp, based on mean measured concentrations, were 4.8, 4.7, and 1.3 mg l^{-1}, respectively. The 48 h daphnid EC_{50} was 4.0 mg l^{-1}. Because of cumene's high volatility (vapor pressure, 3.2 mmHg at 20°C), all tests were conducted under flow-through conditions using a proportional diluter system.

Other Hazards

Cumene is flammable and vapors may result in explosive mixtures.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (time-weighted average) is 50 ppm (246 mg m^{-3}) based on irritation and CNS depression. The (US) Occupational Safety and Health Administration permissible exposure limit, (US) National Institute for Occupational Safety and Health recommended exposure limit, and Deutsche Forschungsgemeinschaft (DFG) MAK are all also 50 ppm, with skin notations. The odor threshold is 0.039 ppm.

Miscellaneous

Vapors are heavier than air and may travel across the ground and reach remote ignition sources causing a flashback fire danger. Electrostatic charges may be generated during pumping and may cause fire. Exposure prevention includes proper eye, skin, and face protection and a cartridge-type of self-contained breathing apparatus.

See also: Fuel Oils; Oil, Crude; Petroleum Distillates.

Further Reading

Seymour FK and Henry JA (2001) Assessment and management of acute poisoing by petroleum products. *Human and Experimental Toxicology* 20: 551–562.

Relevant Websites

http://ntp-server.niehs.nih.gov – NTP, National Toxicology Program (2003).

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Cumene.

Cumulative Risk Assessment

Jeffrey H Driver

Human health risk assessments with chemicals and other agents (biological, physical) typically follow a paradigm that involves four steps – hazard identification, dose–response assessment, exposure assessment, and risk characterization. The process was recommended by the US National Research Council in the 1980s, and is usually applied to a single agent and exposures associated with one or more routes (oral, dermal, inhalation). This has been more recently referred to as aggregate exposure and risk assessment.

It has been recognized that humans may be exposed, on a daily basis, to a plethora of synthetic and natural agents, by different routes of exposure. Concerns have been raised regarding the possibility that exposures to multiple agents, for example, chemical mixtures, could cause unanticipated adverse effects on human health through a variety of toxicological interactions. Various researchers and regulatory agencies have evaluated chemical mixtures previously, with respect to toxicity testing, exposure assessment, or risk estimation. However, only recently, in part because of the US Food Quality Protection Act (FQPA) of 1996, have cumulative risk assessments been developed for integrated food, water, and residential safety evaluations of agricultural chemicals. The FQPA requires that cumulative risk assessment should be considered in situations where there is exposure to two or more chemicals acting through a common mechanism of toxicity. This also implies that a determination must be made regarding the likelihood of concurrent exposure, for given subpopulation, to the chemical mixture of interest during a toxicologically relevant

time period (e.g., daily, 30 day moving average period).

General principles for defining the existence of a common mechanism of toxicity have been addressed by an expert working group convened by the International Life Sciences Institute (ILSI). The working group proposed that a common mechanism might exist if two or more chemicals cause the same critical effect, act on the same molecular target at the same target tissue, act by the same pharmacological mechanism of action, and may share a common toxic intermediate. With the exception of a few groups of chemicals, such as the organophosphate and carbamate insecticides, precise mechanistic information on the animal and/or human effects of chemical agents is limited. Common mechanism determinations will therefore be difficult to establish with the degree of rigor implied by the ILSI working group.

Concurrent exposure, a critical component of cumulative risk assessment, refers to coexposure to two or more chemicals, presumed to act via a common toxicological mechanism. It is important to distinguish between simultaneous concurrent 'external' exposure (timing of oral, dermal and inhalation exposures) and 'internal' exposure or the actual absorbed dose attained in a given biological compartment (e.g., plasma) or at a specific target tissue, as a function of time. It is the temporal dose profile at target tissues that provides the most accurate exposure assessment accounting for purposes of health risk estimation. In the case of chemical mixtures, variations in the timing and frequency of exposure and subsequent absorption, distribution, metabolism, and excretion of different chemical agents will result in differences in dose–response interactions. Therefore, assessing potential temporal patterns of concurrent exposure (via multiple pathways and routes) become a critical underpinning of a credible cumulative risk assessment.

The major steps required for cumulative risk assessment include:

1. Development and application of methodology (and associated data) for determination of the probability that an individual person in a reference population will actually be concurrently exposed to two or more chemicals with a presumed common mechanism of toxicity.
2. Development of an appropriate absorbed dose metric (including time scale) to cumulative toxicologically equivalent doses across the chemicals of interest.
3. Development of an appropriate cumulative risk metric to characterize the potential health risks of the mixture of interest.

It is important to emphasize that a significant degree of scientific uncertainty exists regarding the methodology, underlying data sources, and interpretation of cumulative exposure and risk assessments. Consequently, efforts are being taken to evaluate assessments that have been conducted to date, conduct uncertainty analyses, and then target the areas of data development (e.g., product use, time–activity, and biological monitoring surveys that include demographic, geographic, and temporal specificity for a representative reference population). These efforts will improve the quality of cumulative risk assessments and provide a basis for more scientifically sound risk management decision-making.

See also: Exposure Assessment; Risk Assessment, Ecological; Risk Assessment, Human Health.

Further Reading

CDC (US Centers for Disease Control) (2003) Second National Report on Human Exposure to Environmental Chemicals. Department of Health and Human Services, Centers for Disease Control and Prevention. NCEH Publication Number 02-0716.

ILSI (International Life Sciences Institute) (1999) *A Framework for Cumulative Assessment: An ILSI Risk Science Institute Workshop Report*. Washington, DC: ILSI Press. ISBN 1-57881-055-8.

Mileson BE, Chambers JE, Chen WL, *et al.* (1998) Common mechanism of toxicity: A case study of organophosphorus pesticides. *Toxicological Sciences* 41: 8–20.

NRC (National Research Council) (1983) *Risk Assessment in the Federal Government: Managing the Process*. Washington, DC: National Academy Press.

NRC (National Research Council) (1988) *Complex Mixtures: Methods for In Vivo Toxicity Testing*. Washington, DC: National Academy Press.

Safe SH (1998) Hazard and risk assessment of chemical mixtures using the toxic equivalency factor approach. *Environmental Health Perspectives* 106(suppl. 4): 1051–1058.

Seed J, Brown RP, Olin SS, and Foran JA (1995) Chemical mixtures: Current risk assessment methodologies and future directions. *Regulatory Toxicology and Pharmacology* 22: 76–94.

US EPA (US Environmental Protection Agency) (1999) *Guidance for Conducting Health Risk Assessment of Chemical Mixtures*. Washington, DC: Risk Assessment Forum.

US EPA (US Environmental Protection Agency) (1999) *Guidance for Performing Aggregate Exposure and Risk Assessments*, October 29. Washington, DC: US EPA, Office of Pesticide Programs.

US EPA (US Environmental Protection Agency) (2002) *Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity*, January 14. Washington, DC: US EPA, Office of Pesticide Programs.

US EPA (US Environmental Protection Agency) (2002) *Organophosphate Pesticides: Revised OP Cumulative Risk Assessment*, June 10. Washington, DC: US EPA, Office of Pesticide Programs.

Wilkinson CF, Christoph GR, Julien E, *et al.* (2000) Assessing the risks of exposures to multiple chemicals with a common mechanism of toxicity: how to cumulate? *Regulatory Toxicology and Pharmacology* 31: 30–43.

Curare

Susan M Stejskal

- Chemical Abstracts Service Registry Number: CAS 8063-06-7
- Synonyms: Intocostrine; Ourari; Urari; Woorali; Woorari; Wourara
- Chemical/Pharmaceutical/Other Class: Toxic alkaloid (D-tubocurare) found in South American woody vines including *Strychnos toxifera*, *S. castelnaei*, *S. crevauxii*, and *Chondodendron tomentosum*

Uses

Curare is often used as a general term to describe a wide variety of highly toxic plant extracts. Curare was originally used by South American Indians as an arrow poison that caused paralysis of skeletal muscle of prey being hunted. Curare was first used medically as a muscle relaxant in 1912. An extract from *Chondrodendron tomentosum* has been used clinically to reduce spasms in patients with tetanus and those treated with shock therapy, and to treat muscular rigidity and spastic paralysis. Curare is also used as an adjunct to general anesthesia.

Exposure Routes and Pathways

Only effective when enters the bloodstream.

Toxicokinetics

Curare is effective only when it enters bloodstream, but does not cross the blood–brain barrier. It is easily broken down following ingestion.

Mechanism of Toxicity

Curare mimics acetylcholine by binding to receptor at muscle synapses, preventing nerves from stimulating muscular contraction and causing death by respiratory paralysis. It is a neuromuscular nondepolarizing agent and a nicotinic antagonist.

Acute and Short-Term Toxicity (or Exposure)

Animal

As a potent muscle relaxant, curare can cause death quickly by inducing asphyxia due to rapid relaxation of diaphragmatic muscles. According to one source, death from respiratory arrest can take place within a few minutes in birds and small prey, and up to 20 min in larger mammals. Curare is considered to be highly toxic. The LD_{50} values are as follows:

- intravenous LD_{50}, dog: $1200\,\mu g\,kg^{-1}$;
- intravenous LD_{50}, mouse: $140\,\mu g\,kg^{-1}$;
- intravenous LD_{50}, rabbit: $1300\,\mu g\,kg^{-1}$;
- intraperitoneal LD_{50}, mouse: $3200\,\mu g\,kg^{-1}$; caused flaccid paralysis;
- subcutaneous LD_{50}, mouse: $500\,\mu g\,kg^{-1}$;
- subcutaneous LD_{50}, rabbit: $2700\,\mu g\,kg^{-1}$; and
- oral LD_{50}, rabbit: $270\,mg\,kg^{-1}$.

Human

Curare is acutely toxic. The lowest published lethal dose, route unreported: $375\,\mu g\,kg^{-1}$.

Clinical Management

Respiratory failure should be treated supportively until the effect subsides.

See also: Neurotoxicity.

Further Reading

Baden M (1992) *Unnatural Death: Confessions of a Medical Examiner.* Ivy Books.

Sumner J (2000) *The Natural History of Medicinal Plants*. Portland, OR: Timber Press.

Relevant Website

http://rain-tree.com – Curare (*Chondrodendron tomentosum*) (from the Raintree Rainforest Database).

Cuyahoga River

Lee R Shugart

The Cuyahoga River, flowing through the city of Cleveland in northwest Ohio, was so polluted that it caught fire on June 22, 1969. The cause of the fire was undetermined but investigations pointed to the discharge of highly volatile petroleum derivatives as the possible cause. The fire was not entirely unexpected as a fireboat patrolled the Cuyahoga River daily checking for oil slicks and clearing them away.

The ignited floating oil slick was extinguished in less than half an hour but not before the fire scorched two key railroad trestles as it passed under them. Flames from the burning oil slick reached heights of roughly five stories and were battled by a fireboat from the river and three fire battalions along the shore. Damage to the trestles was estimated to be ~$50 000. There had been previous fires in 1936 and one in 1952 that caused ~30 times the amount of damage as the 1969 fire.

Cleveland mayor Carl Stokes and others felt that the polluted state of the Cuyahoga was "a long-standing condition that must be brought to an end" and used the river fire as a reminder of the importance of continued support for cleanup of The Cuyahoga River and Lake Erie. *Time Magazine* published an article in August of the same year that dramatized the state of the Cuyahoga River and focused national attention on the deteriorating plight of our nation's waterways. The 1969 Fire was an event that mobilized America's commitment to cleanup its rivers and became the rallying point for the passage of the Clean Water Act of 1972.

At the time of the 1969 Fire, the river was characterized as being 'Chocolate-brown, oily, bubbling with subsurface gases, it oozes rather than flows. The river has no visible life, not even low forms such as leeches and sludge worms that usually thrive on wastes'. The Clean Water Act of 1972 was responsible for repairing many of these problems. Today, the areas around the Cuyahoga River are being revitalized. Pleasure boats crowd the river and restaurants pack the riverbank in downtown Cleveland. The greatest testament to the improvement of the Cuyahoga River is the return of fish and other organisms.

See also: Clean Water Act (CWA), US; Environmental Protection Agency, US.

Relevant Website

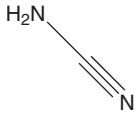
http://www.epa.gov – Cuyahoga River Area of Concern (from the US Environmental Protection Agency).

Cyanamide

Leonard I Sweet

This article is a revision of the previous print edition article by Heriberto Robles, volume 1, pp. 386–387, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 420-04-2
- Synonyms: Amidocyanogen; Carbamonitrile; Carbimide; Cyanoamine; Carbodiamide; Carbodiimide; Cyanogenamide; Hydrogen cyanamide; Cyanogen nitride
- Chemical/Pharmaceutical/Other Class: Cyanamides
- Chemical Formula: CH_2N_2
- Chemical Structure:



Uses

Cyanamide is used in the production of synthetic rubber, cyanide, fumigants, and metal cleaners. It has also found use as an intermediate for pesticides, herbicides, detergents, medicines (e.g., antihistamines, hypertension, sedatives), in the photography industry, as an additive for fuels, lubricants, and cements, and as a paper preservative. The dimer (dicyandiamide) is a raw material for melamine and guanidine.

Exposure Routes and Pathways

Exposure to cyanamide can occur via inhalation, ingestion, skin or eye contact, and there is potential for skin absorption. Inhalation and dermal contact is expected to be the primary route of occupational exposure.

Toxicokinetics

The major urinary metabolite of cyanamide is n-acetylcyanamide. *In vitro* studies suggest that cyanamide is metabolized to cyanide; however, results *in vivo* suggest this biotransformation pathway is irrelevant in humans. Rapid absorption is anticipated, though bioavailability is incomplete with estimates ranging from 53% to 70%. The estimated half-life in humans after oral administration is expected to be less than 2 h.

Mechanism of Toxicity

Cyanamide is not expected to release cyanide as part of its mechanism of toxicity. The principal toxicological mechanism of cyanamide is inhibition of aldehyde dehydrogenase. Cyanamide can produce acetaldehyde syndrome with concurrent exposure to alcohol, resulting in symptoms that include vomiting, parasympathetic hyperactivity, difficulty in breathing, and confusion.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cyanamide causes irritation of the eyes and mucous membranes, as well as inhibition of the liver enzyme aldehyde dehydrogenase. Cyanamide is very toxic by the oral exposure route and moderately toxic by the dermal route. The oral LD_{50} for rats is $125\,mg\,kg^{-1}$, and the dermal LD_{50} in the same species is $84\,mg\,kg^{-1}$. The intravenous LD_{50} for rats is $56\,mgkg^{-1}$. For rabbits, the dermal LD_{50} value is $590\,mg\,kg^{-1}$. Instillation of 100 mg of cyanamide into the eyes of rabbits resulted in severe irritation. Cyanamide induced ulceration on contact with the moist skins of experimental animals.

In rats, cyanide poisoning produces overactivity of the parasympathetic nervous system causing miosis, salivation, lacrimation, and twitching. Symptoms of severe poisoning in rats include constricted pupils followed by markedly dilated pupils, congested vessels of the iris and retina, and possibly papilledema.

Human

Cyanamide is irritating and caustic to the eyes, skin, mucous membranes, and respiratory and gastrointestinal tracts of humans. Effects after a single mild overexposure are expected to be transient, and diminish after a few hours. The typical signs and symptoms of acute overexposure to cyanamide include the following: flushing of the face and upper body, nausea, fatigue, difficulty in breathing, swelling, lacrimation, skin and eye burns, constricted pupils, excessive salivation, twitching, shivering, vasodilatation, tachycardia, bradycardia, and hypotension. Cyanamide poisoning produces overactivity of the parasympathetic nervous system. The most serious effects associated with acute, high-dose exposure are coma and cardiovascular collapse. The estimated fatal dose in humans ranges from 40 to 50 g cyanamide. Contact with cyanamide in dust or liquid form can cause severe irritation of the eyes and ulceration of moist skin.

Chronic Toxicity (or Exposure)

Animal

The reproductive toxicity of cyanamide has been studied in a two-generation study of reproduction-fertility in rats, involving oral daily doses of 2, 7, or $25\,mg\,kg^{-1}$ cyanamide. The highest dose level resulted in decreases in weight, number of corpora lutea, number of implantations, and numbers of neonates.

Cyanamide has been studies in mice for carcinogenicity, at dose levels administered in drinking water of 0, 70, 200, and 600 ppm for up to 104 weeks. Findings suggest elevated incidence of benign granulosa theca tumors of the ovary at the 600 ppm dose level, as compared to controls. Pathological findings indicated presence of ovarian hyperplastic lesions.

Hydrogen cyanamide has been investigated for genotoxicity, and has generally been shown to lack significant activity.

Human

Cyanamide also acts as a potent inhibitor of the enzyme aldehyde dehydrogenase, which results in a disulfiram-like reaction in individuals concomitantly exposed to alcohol. Potentiated by the ingestion of alcohol, the accumulation of acetaldehyde in the body presents as a syndrome of vasodilation characterized by facial flushing, headache, nausea, vomiting, difficulty in breathing, sweating, chest pain, hypotension, weakness, blurred vision, and confusion. Calcium cyanamide has been used in aversion therapy for alcoholism.

Chronic overexposure may produce the following: pneumonitis and pulmonary edema upon repeated inhalation; throat ulceration and esophageal irritation upon oral ingestion; dermal ulceration, allergic dermatitis, and sensitization upon skin exposure; and keratitis, conjunctivitis, or corneal ulceration upon

repeated contact with the eyes. Chronic overexposure may also affect the liver and nervous system.

Clinical Management

Management of individuals overexposed to cyanamide begins with removing those individuals from the source of exposure, flushing eyes and skin with copious amounts of water, and removing contaminated clothing. Skin contamination should be removed by washing with soap and water. Treat dermal irritation or burns with standard topical therapy. Patients developing dermal hypersensitivity reactions may require treatment with topical or systemic corticosteroids or antihistamines.

If ingested, vomiting should not be induced. If large doses have been ingested within an hour of exposure, gastrointestinal decontamination should be considered. If dosage was small or treatment is delayed, oral administration of activated charcoal and sorbitol may prove beneficial. Gastric lavage treatment may be given with caution and avoided if tracheal or esophageal ulceration is suspected.

Hypotension or 'antabuse'-type reactions should be treated by placing the patient in the Trendelenburg position, providing intravenous fluids, including plasma or blood if necessary, and vasopressor drugs.

In cases of respiratory overexposure, the victim should be moved to fresh air immediately and treated according to severity of irritation. The presence and severity of respiratory irritation, bronchitis, and pneumonitis should be evaluated. If respiratory tract irritation or respiratory depression is evident, arterial blood gases, chest X-ray, and pulmonary function tests should be monitored. For acute lung injury, ventilation and oxygenation should be maintained and evaluation should be done with frequent arterial blood gas or pulse oximetry monitoring.

Monitoring complete blood count, urinalysis, and liver and kidney functions test is suggested for patients with significant exposure. Assisted ventilation (100% humidified supplemental oxygen) should be provided as required, arterial blood gases should be monitored, and institution of basic life-support systems as necessary.

Environmental Fate

If released to the environment, cyanamide is expected to preferentially partition to the soil and water. Bioconcentration and bioaccumulation potential is expected to be low, based on the estimated bioconcentration factor and experimental octanol–water partition coefficient. Aerobic biodegradation is expected to occur. Volatilization is not expected to be an important fate and transport process based on the Henry's law constant and vapor pressure. When released into the air, vapor phase cyanamide is expected to have a half-life of less than 1 day.

Ecotoxicology

If released to the environment, cyanamide is expected to have a low potential for aquatic toxicity to invertebrates and fish (with estimated effective/lethal concentrations of $>1000\,\mathrm{mg\,l^{-1}}$).

Other Hazards

Cyanamide is a highly reactive chemical and is a dangerous explosion hazard. It can release toxic fumes of cyanides and nitrogen oxides when heated to decomposition, or contacted with acids, acid fumes, moisture, or 1,2-phenylenediamine salts. It is combustible when exposed to heat or flame. Cyanamide reacts with acids, strong oxidants, strong reducing agents, and water, causing explosion hazard.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for cyanamide include the following:

- American Conference of Governmental Industrial Hygienists ($2\,\mathrm{mg\,m^{-3}}$ ppm time-weighted average (TWA));
- Australia ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- Belgium ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- Canada ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- China ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- Denmark ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- France ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- Germany ($2\,\mathrm{mg\,m^{-3}}$ TWA inhalable fraction);
- Mexico ($2\,\mathrm{mg\,m^{-3}}$ TWA);
- Sweden ($4\,\mathrm{mg\,m^{-3}}$ short-term exposure limit);
- United Kingdom ($2\,\mathrm{mg\,m^{-3}}$ TWA); and
- US Occupational Safety and Health Administration vacated permissible exposure limit ($2\,\mathrm{mg\,m^{-3}}$ TWA).

Miscellaneous

Cyanamide is a colorless, orthorhombic, hydrophilic, crystalline solid with a mild odor. It is commonly used in liquid solution, and is expected to be soluble in water, ether, benzene, acetone, phenols, amines, ketones, and alcohols.

See also: Acetamide; Cyanide; Formamide.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Cyanamide.

http://www.inchem.org – International Chemical Safety Card from the International Programme on Chemical Safety. Cyanamide.

http://www.state.nj.us – Hazardous Substance Fact Sheet from the New Jersey Department of Health and Senior Services. Cyanamide.

Cyanide

Zhengwei Cai

- Chemical Abstracts Service Registry Numbers: CAS 57-12-5 (CN); CAS 74-90-8 (Hydrogen cyanide); CAS 143-33-9 (Sodium cyanide); CAS 151-50-8 (Potassium cyanide)
- Synonyms: Carbon nitride ion; Cyanide anion; Cyanide ion; Cyanure (French); Hydrocyanic acid; Isocyanide; Hydrocyanic acid sodium salt; Hydrocyanic acid potassium salt
- Chemical/Pharmaceutical/Other Class: Cyanide is any one of a group of compounds containing the monovalent combining group CN. Inorganic cyanides are regarded as salts of hydrocyanic acid (hydrogen cyanide). Organic cyanides are usually called nitrites
- Chemical Formulas: HCN (hydrogen cyanide); NaCN (sodium cyanide); KCN (potassium cyanide); CH_3CN (acetonitrile)
- Chemical Structure: $-C\equiv N$

Uses

Cyanide compounds are widely used in industry. Sodium cyanide and potassium cyanide are used extensively in the extraction of gold and silver from low-grade ores. The cyanide ion can form a wide range of complex ions with metals. These complex metal cyanide ions are extensively used in electroplating. Cyanide compounds are also used in case-hardening of iron and steel, metal polishing, photography, and the fumigation of ships and warehouses. Organic cyanide compounds are used in ynthetic rubber, plastics, and synthetic fibers; they are also used in chemical synthesis. Cyanides are used in rodenticide and fertilizer production.

In addition, cyanides can be found in the seeds of the apple, peach, plum, apricot, cherry, and almond in the form of amygdatin, a cyanogenic glycoside. Amygdatin (Laetrile) has been used as an antineoplastic drug, but such beneficial effects have not been scientifically proven.

Background Information

Cyanide poisoning causes a high incidence of severe symptomatology and fatality. Between 1926 and 1947, death rates from cyanide poisoning in America ranged between 79 and 416 per 10 million population and gradually declined thereafter. The availability of the antidote kit may have contributed to this decreasing death rate. There are numerous sources of potential cyanide exposure. With the increased use of plastic building materials, the potential hazards of cyanide poisoning as a component of smoke inhalation in closed space fires still exist.

Exposure Routes and Pathways

Humans may be exposed to cyanide in a number of different forms. These include solids, liquids, and gases. Sources include industrial chemicals, natural products, medications, and combustion products. Inhalation of toxic fumes and ingestion of cyanide salts, cyanide-containing fruit seeds, and cyanide waste-contaminated drinking water are the most common exposure pathways. The respiratory route represents a potentially rapidly fatal type of exposure. Exposure to cyanides may also occur via the dermal route in industrial workers.

Toxicokinetics

Cyanide is rapidly absorbed from the skin and all mucosal surfaces; it is most dangerous when inhaled because toxic amounts are absorbed with great rapidity through the bronchial mucosa and alveoli. Once absorbed, distribution of cyanide through the body is rapid. Within a few minutes, cyanide is distributed through the body and its conversion to thiocyanate starts. The majority of cyanide in the body is protein-bound (60%). In sublethal doses, cyanide reacts with sulfane sulfur to form nontoxic

thiocyanate through an enzymatic reaction involving rhodanase and mercaptopyruvate sulfur transferase. Within 3 h, 90% of the dose of cyanide is converted to thiocyanate appearing in blood. Cyanide is also trapped as cyano of vitamin B_{12}, oxidized to formate and carbon dioxide, and incorporated into cysteine. In nonfatal cases, metabolized cyanide (thiocyanate) is excreted in the urine. Although cyanide is volatile, excretion through the lungs is not a significant route of elimination of cyanide.

Mechanism of Toxicity

Cyanide is described as a cellular toxin because it inhibits aerobic metabolism. It reversibly binds with ferric (Fe^{3+}) iron-containing cytochrome oxidase and inhibits the last step of mitochondrial oxidative phosphorylation. This inhibition halts carbohydrate metabolism from citric acid cycle, and intracellular concentrations of adenosine triphosphate are rapidly depleted. When absorbed in high enough doses, respiratory arrest quickly ensues, which is probably caused by respiratory muscle failure. Cardiac arrest and death inevitably follow.

For this reason, cyanide action has been described as 'internal asphyxia'. Although some cyanide combines with hemoglobin to form a stable nonoxygen-bearing compound, cyanhemoglobin, this substance is formed only slowly and in a small amount. Therefore, death is not due to cyanhemoglobin but to inhibition of tissue cell respiration.

Recent studies have shown that cyanide also inhibits the antioxidant defense enzymes (such as catalase, superoxide dismutase, and glutathione peroxidase) and stimulates neurotransmitter release. These effects of cyanide may also contribute to its acute toxicity. The prolonged energy deficit and the consequent loss of ionic homeostasis, which may result in activation of calcium signaling cascade and eventually cell injury, contribute to cyanide toxicity resulting from subacute exposure or in the postintoxication sequela.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cyanide toxicity varies with the animal species, type of cyanide compound, route of uptake, metabolic state, and other factors. The LD_{50} for cyanide has been ported in various species. Potassium cyanide, if injected, has a 24 h LD_{50} of 6.7–7.9 mg kg^{-1} in mice. The lethal dose of potassium cyanide infused at a rate 0.1 mg kg^{-1} min^{-1} is 2.4 mg kg^{-1} in dogs breathing room air. When hydrogen cyanide is inhaled by mice, the LD_{50} is 177 ppm with a lethal time of 29 min. The time to death is greater than 17 min for exposure to less than 266 ppm, but falls to 40 s at 873 ppm. The LD_{50} for sodium cyanide is 4.6–15 mg kg^{-1} in rats. Male gerbils are 50-fold more sensitive to methacrylonitrile, which is metabolized to cyanide in rodents, than Sprague–Dawley rats, and about fivefold more sensitive than albino Swiss mice. Single and repeated low-dose cyanide intoxication can result in demyelinating lesions of the cerebral white matter in monkeys, but high doses of cyanide are required to produce similar brain lesions in rat.

Human

Cyanide is a chemical asphyxiant, which renders the body incapable of utilizing an adequate supply of oxygen. Exposure to high dose of cyanide is often lethal. The lethal dose of cyanide in humans is 0.5–1.0 mg kg^{-1}. The lethal dose of hydrocyanic acid is $\sim$50 mg for an adult and the lethal dose of the potassium or sodium salt is 200–500 mg. The threshold limit value (TLV) of HCN for inhalation is 4.7 ppm. This is defined as the maximum safe average exposure limit for a 15 min period by the Occupational Safety and Health Administration. Exposure to 20 ppm of HCN in air causes slight warning symptoms after several hours; 50 ppm causes disturbances within an hour; 100 ppm is dangerous for exposures of 30–60 min; and 300 ppm can be rapidly fatal unless prompt, effective first aid is administered. The median lethal dose for skin contamination is $\sim$100 mg kg^{-1}.

Following the inhalation of toxic amounts of cyanide, symptoms usually appear within a few seconds, whereas it may take a few minutes for symptoms to appear following oral ingestion or skin contamination by the salts. The symptoms include a flushed skin, tachypnea, and tachycardia. Stupor, coma, and seizure immediately precede respiratory arrest and cardiovascular collapse. Death shortly occurs. If large amounts have been absorbed, collapse is usually instantaneous-the patient falling unconscious and dying almost immediately. With smaller doses, weakness, giddiness, headache, nausea, vomiting, and palpitation usually occur. With the rise of the blood cyanide level, ataxia develops and is followed by lactic acidosis, convulsive seizures, coma, and death. At higher cyanide doses, cardiac irregularities are often noted, but heart activity always outlasts the respiration.

Chronic Toxicity (or Exposure)

Animal

Ingestion of cyanogenic plants, such as cassava and sorghum, has been associated with development of

goiter and tropical pancreatic diabetes in both human and animals. However, results from animal studies indicate this association in animals is controversial. Chronic cyanide exposure has been reported to reduce memory along with reduction in the levels of dopamine and 5-hydroxytryptamine in the rat brain.

Human

Chronic low-level exposure to cyanide produces various signs and symptoms. Exposure to small amounts of cyanide compounds over long-term periods of time is reported to cause loss of appetite, headache, weakness, nausea, dizziness, and symptoms of irritation of the upper respiratory tract and eyes. The most widespread pathologic condition attributed to cyanide is tropical ataxic neuropathy associated with chronic cassava consumption. This is a diffuse degenerative neurological disease with peripheral and central signs. Cassava is the major staple food in various tropical areas; the plant has a high content of cyanogenic glycoside (linamarin). With continued ingestion over a period of time, tropical neuropathy gradually develops. The syndrome is characterized by optic atrophy, nerve deafness, and ataxia due to sensory spinal nerve involvement. Other signs include scrotal dermatitis, stomatitis, and glossitis. Chronic low-level exposure to cyanide may also lead to ultrastructural changes of heart muscle. In addition, with chronic cyanide ingestion, the thyroid may be affected due to enhanced formation of thiocyanate. Thiocyanate can block uptake of iodide by the thyroid gland, and myxedema, thyroid goiter, and cretinism may occur. This chronic effect of cyanide may pass to the fetus through maternal exposure.

Clinical Management

To be of any value, treatment of cyanide poisoning must be rapid and efficient. The rapid and early recognition of cyanide poisoning is usually difficult because most of the clinical manifestations are nonspecific. Potentially valuable cyanide blood levels are usually available for confirmation of diagnosis. Arteriolization of venous blood has been used as a significant symptom of cyanide poisoning. If cyanide was ingested, removing the unabsorbed poison by ravaging the stomach with copious amounts of water through a gastric tube is necessary. This should be continued until all odor of cyanide is gone from the lavage fluid. Artificial respiration with 100% oxygen is often used in the treatment of cyanide poisoning, although oxygen is not a specific antidote. It is theorized that oxygen therapy increases the rate of displacement of cyanide from cytochrome oxidase, and the increased intracellular oxygen tension nonenzymatically converts the reduced cytochrome to the oxidized species, enabling the electron transport system to function again. The nitrite-thiosulfate antidotal combination is still one of the most effective treatments of cyanide poisoning. If the victim is conscious and speaking, no treatment is necessary. If the victim is unconscious but breathing, an open ampoule of amyl nitrite can be placed under the victim's nose for 15 s and it can be repeated 5 to 6 times. A fresh ampoule should be used every 3 min until the victim regains consciousness. Amyl nitrite is a powerful cardiac stimulant and should not be used more than necessary. If the patient is not breathing, 0.3 g (10 ml of a 3% solution, adults) of sodium nitrite should be administered intravenously at the rate of 2.5 ml min^{-1} followed by 12.5 g (50 ml of a 25% solution) of sodium thiosulfate at the same rate. Inhalation of amyl nitrite should also be performed. Nitrite will convert hemoglobin to methemoglobin, which has higher affinity for cyanide than hemoglobin. A methemoglobin level of $\sim$25% is desired for maintaining normal hemoglobin function and detoxification. Thiosulfate is a sulfur donor for converting cyanide to nontoxic thiocyanate. For children weighing less than 25 kg, sodium nitrite should be dosed on the basis of their hemoglobin level and weight. The patient should be observed for the next 24–48 h and if the signs of intoxication persist or reappear, injection of nitrite thiosulfate at one-half of the recommended dose should be repeated. Hydroxocobalamine has been effectively used in France as an antidote for acute cyanide poisoning. Hydroxocobalamine (vitamin B_{12a}) is currently approved by Food and Drug Administration, but is not popularly used in the United States as an antidote for cyanide poisoning. Because of its extremely low adverse effect, hydroxocobalamine is ideal for out-of-hospital use in suspected cyanide intoxication. It is actively proposed to be used in the United States.

Ecotoxicology

The toxicity of cyanide in the aquatic environment or natural waters is a result of free cyanide, that is, as HCN and CN^-. Fish are extremely sensitive to cyanide. Most fish can tolerate a free cyanide stream concentration of 0.05 mg l^-, but some species are even more sensitive.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration permissible exposure limit: time-weighted average (TWA) 5 mg (CN) m^{-3}.
- American Conference of Governmental Industrial Hygienists TLV: CL 5 mg m^{-3} (skin)

- DFG MAK: 5 mg m^{-3}.
- National Institute of Occupational Safety and Health recommended exposure limit (cyanide) TWA CL 5 mg m^{-3} per 10 min.

See also: Cyanamide; Cyanogen Chloride.

Further Reading

Eckstein M (2004) Cyanide as a chemical terrorism weapon. *JEMS* 29(8): Suppl 22–31.

Gracia R and Shepherd G (2004) Cyanide poisoning and its treatment. *Pharmacotherapy* 24(10): 1358–1365.

Hall AH and Rumack BH (1986) Clinical toxicology of cyanide. *Annals of Emergency Medicne* 15: 1067–1072.

Patnaik P (1999) *A Comprehensive Guide to the Hazardous Properties of Chemical Substances*, 2nd edn. New York: Wiley.

Sauer SW and Keim ME (2001) Hydroxocobalamin: Improved public health readiness for cyanide disasters. *Annals of Emergency Medicine* 37: 635–641.

Cyanogen Chloride

Leonard I Sweet

- Chemical Abstracts Service Registry Number: CAS 506-77-4
- Synonyms: Chlorcyan; Chlorine cyanide; Chlorocyan; Chlorocyanide; Chlorocyanogen; Mauguinite
- Chemical/Pharmaceutical/Other Class: Cyanogens
- Chemical Formula: CNCl
- Chemical Structure: N≡C – Cl

Uses

Cyanogen chloride is used as a military poison gas, a warning agent in fumigant gases, tear gas, as an insecticide, metal cleaner, in ore refining, in production of synthetic rubber, and in a variety of chemical syntheses.

Exposure Routes and Pathways

Exposure to cyanogen chloride can occur via inhalation, skin absorption, ingestion, and with skin or eye contact. Cyanogen chloride is particularly hazardous when inhaled due to the strong potential for absorption in toxic amounts through bronchial mucosa and alveoli.

Toxicokinetics

Cyanogen chloride is converted to cyanide in the body by a reaction with hemoglobin and glutathione. Rapid absorption of the cyanide ion is anticipated from all tissues, and it is expected to distribute to organs and tissues via the blood; where it has the potential to concentrate in red blood cells, possibly due to binding to methemoglobin. Absorbed cyanide is excreted unchanged in the lungs, sweat, and urine, whereas greater amounts are converted by sulfurtransferase/rhodanase enzymes to thiocyanate. The estimated half-life in humans for the conversion of cyanide to thiocyanate from a nonlethal dose ranges from 20 to 60 min. The toxicity of cyanogen chloride rests largely on its ability to yield hydrocyanic acid *in vivo*.

Mechanism of Toxicity

Cyanogen chloride is similar in toxicity and mode of action to hydrogen cyanide, but is a much more potent irritant partly due to its greater volatility and chlorine moiety. Target organs include the eyes, skin, respiratory system, central nervous system, and cardiovascular system. Cyanogen chloride can cause marked irritation of the respiratory tract with hemorrhagic exudate of the bronchi and trachea, and pulmonary edema. In addition to severe potential for local irritation, systemic toxicity occurs by liberating cyanide molecules that target and bind to cells, interrupting electron transport and metabolism. Cyanides interfere with cellular oxygen uptake and transport by inhibition of cytochrome oxidase enzymes. The most oxygen-dependent organ systems are typically most affected, including the heart and brain.

Acute and Short-Term Toxicity (or Exposure)

Animal

Cyanogen chloride causes severe irritation to the eyes, skin, mucous membranes, and respiratory system, as well as dizziness, congestion of the lungs, interference with cellular metabolism, and loss of appetite. The adverse effects of overexposure to cyanogen chloride, such as pulmonary toxicity, may be delayed. The oral LD_{50} for cats is 6 mg kg^{-1}.

The short-term inhalation LC_{50} values for laboratory animals range from 3800 to 6000 mg m^{-3}. Acute poisoning with cyanogen chloride results in signs of cyanide poisoning and pulmonary edema including difficulty in breathing, bloody nasal exudates, cyanosis, and possibly death.

Human

Cyanogen chloride is a rapidly acting and severe eye, skin, mucous membrane, and respiratory tract irritant. Effects of overexposure are similar to those for cyanide and other cyanogenic compounds. Overexposure may cause tearing, cellular hypoxia, burning of the eyes, lacrimation, rapid respiration, flushing, irregular heartbeat, vomiting, hemorrhagic changes, drowsiness, pulmonary edema, convulsions, and possibly death by asphyxia. The adverse effects of overexposure may be delayed for several hours. Irritant concentrations can occur as low as 1 ppm can cause severe eye and nasal irritation. Skin contact with liquid may cause frostbite injury. The lowest published toxic concentrations for humans via inhalation are 10 mg m^{-3} for eye effects, and 2 g m^{-3} for skin. Inhalation overexposure to cyanogen chloride concentrations of 48 ppm (for 30 min) or 159 ppm (for 10 min) has caused death in humans. The estimated lethal dose by ingestion of cyanogen chloride is approximately 13 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

The chronic effects of exposure to cyanogen chloride include hoarseness, conjunctivitis, and edema of the eyelid. Short-term fatal concentrations in animal models range from 48 to 500 ppm. The carcinogenic or mutagenic potential of cyanogen chloride is not well characterized.

Human

Repeated inhalation exposure of low concentrations may cause dizziness, weakness, congestion of the lungs, conjunctivitis, hoarseness, loss of appetite, mental deterioration, weight loss, and possibly enlarged thyroid glands.

Clinical Management

Management of individuals overexposed to cyanogen chloride begins with rapid action to remove those individuals from the source of exposure, flushing eyes and skin with copious amounts of water, and removing contaminated clothing. Skin contamination should be removed by washing with soap and water. If frostbite injury has occurred, the area should not be rubbed or flushed with water, and attempt to remove frozen clothing from frostbitten areas should not be made. A cyanide antidote kit should be kept in immediate work areas.

Triage should be conducted, and asymptomatic victims should be monitored and histories taken. Where exposure has resulted in acute signs and symptoms, oxygen and antidotes should be administered immediately. Antidotes include amyl nitrite, hydroxocobalamin, hyperbaric oxygen, sodium nitrite, and sodium thiosulfate. For irritation of the eyes, washing with a weak solution of boric acid may prove beneficial. Dermal irritation should be treated with soothing lotions, such as calamine. If inhaled and if breathing is difficult, respiration should be supported and 100% oxygen administered. If typical nitrile effect is observed, amyl nitrite should be administered. Lung function and electrocardiogram should be monitored. Additional diagnostic procedures include blood anion gap, arterial blood gases, blood cyanide levels, blood electrolytes, blood methemoglobin, and blood or urine thiocyanate. Medical observation is recommended for days after breathing overexposure, as pulmonary edema may be delayed. As first aid for pulmonary edema, administration of corticosteroid spray may prove therapeutic. If ingested, water or milk should be consumed. Gastrointestinal decontamination should be considered, with lavage or activated charcoal with cathartic. Emesis is contraindicated due to rapid course of the neurologic symptoms. For acidosis, sodium bicarbonate should be given. Hemodialysis, charcoal hemoperfusion, and chelation may prove useful in enhancing elimination.

Environmental Fate

If released to the environment, cyanogen chloride is expected to preferentially partition to the air, soil, and water. It is expected to slowly convert to cyanides, and will react slowly with water or water vapor to form hydrogen chloride. Photolysis may also be an important abiotic removal process. Bioconcentration and bioaccumulation potential is expected to be low. Volatilization is expected to be an important fate and transport process based on the vapor pressure. Cyanogen chloride is expected to persist in air if released.

Ecotoxicology

If released to the environment, cyanogen chloride and its decomposition products are expected to have high potential for aquatic toxicity to invertebrates

and fish (with effective/lethal concentrations for 50% of the organisms tested $< 1\,mg\,l^{-1}$).

Other Hazards

Flammable when exposed to heat or flame. When heated to decomposition or on contact with water or steam, it will react to produce highly toxic and corrosive fumes of hydrogen cyanide, hydrochloric acid, and nitrogen oxides. Contact with alcohols, acids, acid salts, amines, strong alkalis, olefins, and strong oxidizers may cause fire and explosion. Cyanogen chloride may polymerize violently if contaminated with chlorine.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for cyanogen chloride include the following:

- American Conference of Governmental Industrial Hygienists (0.3 ppm ceiling);
- Australia (0.3 ppm peak);
- Belgium (0.3 ppm short-term exposure level, STEL);
- Canada (0.3 ppm ceiling);
- China ($0.75\,mg\,m^{-3}$ ceiling);
- Denmark (0.1 ppm time-weighted average, TWA);
- France (0.3 ppm STEL);
- Germany ($0.75\,mg\,m^{-3}$ TWA);
- Sweden (0.1 ppm threshold limit value);
- United Kingdom (0.3 ppm STEL); and
- US Occupational Safety and Health Administration vacated permissible exposure limit (0.3 ppm ceiling).

Miscellaneous

Cyanogen chloride is a colorless liquid or gas, with a pungent acrid, bitter almond like, or choking odor that is generally detected at concentrations ~ 1 ppm. It is expected to be soluble in water, alcohol, ether, and most organic solvents.

See also: Cyanide.

Further Reading

World Health Organization; WHOTAC, Technical Report Series, 1211 Geneva, 27, Switzerland.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Cyanogen Chloride.

http://www.inchem.org – Cyanogen Chloride (International Chemical Safety Card from the International Programme on Chemical Safety).

http://www.state.nj.us – Cyanogen Chloride (Hazardous Substance Fact Sheet from the New Jersey Department of Health and Senior Services).

Cyclodienes

Benny L Blaylock

Cyclodienes are chlorinated hydrocarbon insecticides with a polycyclic structure and, as the name implies, two unsaturated bonds. Not all of the insecticides in this class meet these criteria. Chlordane, for example, contains only one double bond in its polycyclic structure. Endrin and dieldrin are epoxides of the cyclodienes isodrin and aldrin, respectively.

Cyclodienes appear to act more in the central nervous system than in the peripheral nervous system. One major mode of action is the inhibition of γ-aminobutyric acid-regulated Cl^- ion flux in neurons. Cyclodienes also exert effects on membrane-bound adenosine triphosphatases (ATPases), altering Na^+, K^+, and Ca^{2+} ion transport. The result is a partial depolarization of neurons rather than repolarization after activation. The accumulation of Ca^{2+} ions intracellularly in the terminal ends of neurons promotes the release of neurotransmitters from storage vesicles and the depolarization of adjacent neurons.

Symptomatology is essentially the same as that described for organochlorine insecticides. In many cases, convulsions are the first sign of toxicity without the progression of nerve hyperactivity seen in other classes of organochlorine insecticides. More recently, evidence of endocrine disruption in both mammalian and other species has been accumulating for several cyclodiene pesticides including chlordane, aldrin, dieldrin, lindane, and endosulfan.

Clinical management is symptomatic, as described for organochlorine insecticides.

See also: Aldrin; Chlordane; Dieldrin; Endosulfan; Endrin; Lindane.

Further Reading

Colborn T, vom Saal FS, and Soto AM (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspectives* 101: 378–384.

Narahashi T (2001) Neurophysiological effects of insecticides. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 335–351. San Diego, CA: Academic Press.

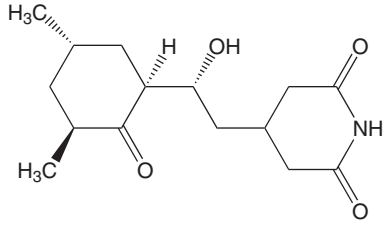
Relevant Website

http://npic.orst.edu – National Pesticide Information Center, Oregon State University and the US Environmental Protection Agency.

Cyclohexamide

Midhun C Korrapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 66-81-9
- Synonyms: 1*S*-(1α(*S*),3α,5*β*)-4-(2-(3,5-Dimethyl-2-oxo-cyclohexyl))-2-hydroxyethyl-2,6-piperidinedione; Naramycin
- Chemical/Pharmaceutical/Other Class: Bactericidal, antifungal, antibiotic, and antipsoriatic
- Chemical Formula: $C_{15}H_{20}O_4N$
- Chemical Structure:



Uses

Cyclohexamide is used as a fungicide, plant growth regulator, and as protein synthesis inhibitor. This is also used in laboratory media as a selective agent to permit isolation of pathogenic and nonpathogenic fungi.

Exposure Routes and Pathways

Cyclohexamide is produced as a by-product during the chemical synthesis of streptomycin, an antibiotic. Therefore, waste releases to the environment from streptomycin production may contain cyclohexamide. Occupational exposure to cyclohexamide may occur through dermal contact with this compound found in the waste stream at workplaces where streptomycin is produced.

Mechanism of Toxicity

Cyclohexamide is a potent inhibitor of protein synthesis in animals. It causes an increase in adrenal RNA and increased production of glucocorticoids.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals given toxic doses exhibit salivation, bloody diarrhea, tremors, and excitement, leading to coma and death due to cardiovascular collapse. A single dose of cyclohexamide ($2\,mg\,kg^{-1}$, ip) produced progressive decrease in bile flow in rats. The oral LD_{50} in monkeys and dogs is $\sim 500\,mg\,kg^{-1}$ and that for rats is only $2\,mg\,kg^{-1}$. In all three species, toxic symptoms include excessive salivation and diarrhea. Bloodstained feces may arise from vascular lesions of colon (monkey) or stomach and small intestines (dogs). Rats and dogs show transient central nervous system (CNS) excitement with tremors and in the dog perhaps meningeal irritation. Death is due to cardiovascular collapse and is preceded by coma in all species. Autopsies on rat revealed enlarged adrenals, stomach hemorrhage, liver congestion, and kidney damage.

Human

Cyclohexamide is a potent irritant. When ingested gastrointestinal symptoms of nausea, vomiting, diarrhea, and excessive salivation have been reported. Other signs of poisoning are transient CNS excitement and tremors.

Chronic Toxicity (or Exposure)

Cyclohexamide has been shown to be mutagenic in both animals and humans.

Clinical Management

Intragastric administration of charcoal as a slurry (240 ml water/30 g charcoal) should be undertaken

to minimize absorption of life-threatening levels of cyclohexamide by ingestion.

Environmental Fate

Cyclohexamide is expected to have very high mobility in soil. Volatilization from moist soil surfaces is not expected to be an important disbursement process. It is not expected to adsorb to suspended solids and sediment. Volatilization from water surfaces is not expected. It is expected to exist solely in the particulate phase in the ambient atmosphere.

Ecotoxicology

The cyclohexamide LD_{50} in male mallard duck and female pheasant by oral administration is 82.5 and 9.38 mg kg^{-1}, respectively.

Exposure Standards and Guidelines

Extremely hazardous substances that are solids are subject to either of two threshold planning quantities. The lower quantity applies only if the solid exists in powdered form and has a particle size less than 100 μm, or is handled in solution or in molten form. If the solid does not meet any of these criteria, it is subject to the upper threshold planning quantity. Cyclohexamide is an extremely hazardous substance that is subject to reporting when stored in amounts in excess of its threshold planning quantity of 100 or 10 000 lbs.

See also: Charcoal; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Occupational Exposure Limits.

Further Reading

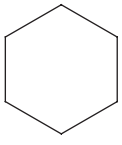
Parry EW (1981) Cycloheximide treatment modifies the pattern of 'metastasis' following intravenous injection of ehrlich ascites tumour cells. *Gann* 72: 464–467.

Satav JG, Katyare SS, Fatterparker P, and Sreenivasan A (1997) Study of protein synthesis in rat liver mitochondria use of cycloheximide. *European Journal of Biochemistry* 73: 287–296.

Cyclohexane

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 110-82-7
- Synonyms: Cicloesano; Cyclohexaan; Cyclohexan; Exahydrobenzene; Hexahydro-benzene; Hexamethylene; Hexanaphthene
- Chemical/Pharmaceutical/Other Class: Saturated alicyclic hydrocarbon
- Chemical Formula: C_6H_{12}
- Chemical Structure:



Uses

Cyclohexane is used as a solvent for lacquers, resins, fats, oils, and waxes, in paint and varnish remover, in the manufacture of nylon, in the extraction of essential oils, and in analytical chemistry for molecular weight determination. In addition, it is used in the manufacture of adipic acid, benzene, cyclohexanone, cyclohexanol, cyclohexyl chloride, nitrocyclohexane, and solid fuel for camp stoves. Further, it is used in industrial recrystallization of steroids and in fungicidal formulations (it has a slight fungicidal action).

Background Information

Cyclohexane is obtained in the distillation of petroleum or by hydrogenation of benzene. It constitutes 0.5–1.0% of petroleum.

Exposure Routes and Pathways

Cyclohexane was a predominant pollutant in shoe and leather factories in Italy, associated with the use of glue. Occupational exposure to cyclohexane may occur through inhalation and dermal contact with this compound where cyclohexane is produced or used. The general population may be exposed to cyclohexane via inhalation of ambient air, ingestion of drinking water, and dermal contact with products containing cyclohexane. The general population may also be exposed to cyclohexane due to its presence in gasoline. It has been found in mother's milk and has been detected in studies of the air in various cities.

Toxicokinetics

Cyclohexane is readily absorbed via inhalation and the oral routes of exposure. Animal studies indicate dermal absorption to be high, probably due to the defatting action of the compound. Cyclohexane absorption into the lungs is rapid, with the concentration in the lungs reaching 42–62% of the air concentration. No information is available on the rate of absorption through the gastrointestinal tract. Cyclohexane is metabolized by cytochrome P-450 enzymes in the liver and other tissues. Several metabolites have been identified including cyclohexanol and *trans*-cyclohexane-1,2-diol. These compounds have been identified in the urine of human subjects and experimental animals within 48 h of exposure.

Mechanism of Toxicity

The precise mechanism of toxicity of cyclohexane has not been identified, but is likely similar to other central nervous system (CNS) depressants and general anesthetics. These compounds are believed to exert their effects through a general interaction with the CNS, and interference with neuronal membrane functions has been postulated as a mechanism of action. Disruption of membrane enzymes and the corresponding alterations in cell functions may account for the behavioral and anesthetic effects observed following exposure to various solvents.

Acute and Short-Term Toxicity (or Exposure)

Animal

The reported oral LD_{50} in rabbits is 5.5–6.0 mg kg^{-1} indicating the relatively low oral acute toxicity of cyclohexane. Vapor concentrations of 92 000 mg m^{-3} produced rapid narcosis and death in rabbits. In mice, concentrations of 51 000 mg m^{-3} caused narcosis and death occurred at 61 200–71 400 mg m^{-3}. The oral LD_{50} was reported as 12 705 mg kg^{-1} for rats and 813 mg kg^{-1} for mice.

Human

Cyclohexane is a CNS depressant and may produce mild anesthetic effects. Inhalation exposure can cause headache, nausea, dizziness, drowsiness, and confusion. Very high concentrations may cause unconsciousness, convulsions, and death. Vapors may be irritating to the nose and throat. Severe lung irritation, damage to lung tissues, or death may result from aspiration into the lungs. Direct dermal contact with liquid may cause mild irritation, which may become more severe if exposure is prolonged. Eyes may become irritated upon exposure to vapors or liquid; however, the effect is generally mild and temporary unless exposure is prolonged. Ingestion of cyclohexane may cause sore throat, nausea, diarrhea, or vomiting.

Chronic Toxicity (or Exposure)

Animal

Lower doses (1.0–5.5 mg kg^{-1}) produced mild to extensive hepatocellular degeneration and glomerulonephritis. Microscopic changes in the liver and kidneys were observed in rabbits exposed to 2700 mg m^{-3} for 50 exposures. No changes were noted at 1490 mg m^{-3}. In subchronic inhalation studies in rats and mice, the no-observed-effect level (NOEL) in rats for acute, transient effects was 500 ppm based on a diminished/absent response to an auditory alerting stimulus at 2000 ppm and above. The NOEL for subchronic toxicity in rats was 7000 ppm based on the lack of adverse effects on body weight, clinical chemistry, tissue morphology, and neurobehavioral parameters. In mice, the NOEL for acute, transient effects was 500 ppm based on behavioral changes during exposure at 2000 ppm and above. The NOEL for subchronic toxicity in mice was 2000 ppm based on hematological changes at 7000 ppm.

Human

Prolonged exposure may produce liver and kidney damage. Cyclohexane is not a carcinogen or a developmental toxicant.

In Vitro Toxicity Data

Negative results were obtained in Ames and sister chromatid exchange, mouse lymphoma, and unscheduled DNA synthesis assays.

Clinical Management

If inhalation exposure occurs, the source of contamination should be removed or the victim should be moved to fresh air. Artificial respiration should be administered or, if the heart has stopped, cardiopulmonary resuscitation provided. If dermal contact has occurred, contaminated clothing should be removed and the affected area should be washed with water and soap for at least 5 min or until the chemical is removed. Contaminated eyes should be flushed with lukewarm, gently flowing water for 5 min or until the chemical is removed. If ingestion

occurs, vomiting should not be induced. Water should be given to dilute the compound. If vomiting occurs naturally, the victim should lean forward to reduce risk of aspiration. Aspiration of the compound into the lungs may produce chemical pneumonitis requiring antibiotic treatment and administration of oxygen and expiratory pressure.

Environmental Fate

If released to air, cyclohexane will exist solely as a vapor in the ambient atmosphere, and will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals (the half-life for this reaction in air is estimated to be 45 h). Little mineralization was detected in a soil biodegradation test and aqueous screening biodegradation tests. Cyclohexane is highly resistant to biodegradation and is catabolized chiefly by cooxidation (use of other organic matter as a carbon and energy source). A bacterium that grows aerobically on cyclohexane was isolated from the wastewater plant of a petroleum refinery. An estimated bioconcentration factor of 89 suggests the potential for bioconcentration in aquatic organisms is moderate. Hydrolysis is not expected to occur due to the lack of hydrolyzable functional groups.

Ecotoxicology

LC_{50} for fathead minnow is 95 mg l^{-1} (static).

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average, is 100 ppm.

See also: Cyclohexene; Hexane.

Further Reading

Baxter CS (2001) Alicyclic hydrocarbons. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 4, pp. 161–165. New York: Wiley.

Kreckmann KH, Baldwin JK, Roberts LG, *et al.* (2000) Inhalation developmental toxicity and reproduction studies with cyclohexane. *Drug and Chemical Toxicology* 23: 555–573.

Lewis RJ, Sr. (ed.) (2000) Cyclohexane. In: *Sax's Dangerous Properties of Industrial Materials*, vol. 2, pp. 1037–1038. New York: Wiley.

Malley LA, Bamberger JR, Stadler JC, *et al.* (2000) Subchronic toxicity of cyclohexane in rats and mice by inhalation exposure. *Drug and Chemical Toxicology* 23: 513–537.

Rouvière PE and Chen MW (2003) Isolation of *Brachymonas petroleovorans* CHX, a novel cyclohexane-degrading proteobacterium. *FEMS Microbiology Letters* 227: 101–106.

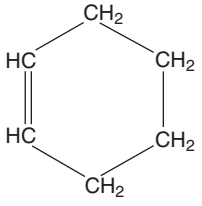
Relevant Website

http://ecb.jrc.it – European Commission (2004) Cyclohexane. European Risk Assessment Report Vol. 41.

Cyclohexene

Patricia J Beattie

- Chemical Abstracts Service Registry Number: CAS 110-83-8
- Synonyms: 1,2,3,4-Tetrahydrobenzene; Tetrahydrobenzene
- Chemical/Pharmaceutical/Other Class: Cycloalkene
- Chemical Formula: C_6H_{10}
- Chemical Structure:



Uses

Cyclohexene is used in oil extraction and in the manufacture of adipic, maleic, and hexahydrobenzoic acids and aldehydes. It is also used as a stabilizer for high-octane gasoline and as a catalyst solvent.

Exposure Routes and Pathways

Exposure occurs most commonly through either inhalation or skin contact.

Toxicokinetics

Cyclohexene is readily hydroxylated by microsomal oxidases to the corresponding dihydroxy derivatives. These are then further conjugated and eliminated in urine.

Mechanism of Toxicity

Cyclohexene is an irritant and defats skin on direct contact. It is also an anesthetic and central nervous system (CNS) depressant on inhalation exposure. The mechanism for this toxicity is unknown.

Acute and Short-Term Toxicity (or Exposure)

Animal

In general, cyclohexene appears to be only mildly toxic. Mice exposed acutely by inhalation to 8830 ppm exhibited a loss of righting reflexes; at 13 400–14 900 ppm death occurred. Dogs inhaling cyclohexene at unknown concentrations exhibited symptoms characterized by muscular quivering and incoordination.

Human

No acute effects have been reported in humans. By analogy to effects reported with structurally similar compounds and in animals, cyclohexene is regarded as a mild respiratory irritant and CNS depressant. When ingested, it represents a low to moderate pulmonary aspiration hazard.

Chronic Toxicity (or Exposure)

Animal

Rats, guinea pigs, and rabbits were exposed to cyclohexene vapors at 75, 150, 300, and 600 ppm for 6 h day^{-1}, 5 days $week^{-1}$ for 6 months. At low doses, an increase in alkaline phosphatase was reported. At 600 ppm, in rats, the same increase in alkaline phosphatase was observed along with a decrease in weight gain. Other blood and biochemical measures were within normal limits.

Human

No chronic health effects have been reported in humans.

Clinical Management

Overexposure to vapors of cyclohexene should be treated by removing the patient to fresh air. If skin or eye contact occurs, the affected areas should be flushed with water for at least 15 min to remove residual solvent. If ingestion of cyclohexene occurs, vomiting should not be induced. This could result in aspiration of solvent into the lungs leading to chemical pneumonitis and pulmonary edema, which can be fatal.

Environmental Fate

Cyclohexene biodegrades in aerobic soils, has a low to moderate mobility, and rapidly volitalizes to the atmosphere. In the atmosphere, it then undergoes a rapid, aerosol-forming reaction with ozone with an estimated half-life of 8.3 h. In water, cyclohexene degrades under aerobic conditions. Based on its water solubility and log octanol/water partition coefficient, cyclohexene does not bioconcentrate in fish or aquatic organisms.

Exposure Standards and Guidelines

- OSHA permissible exposure limit is such that the 8 h time-weighted average is 300 ppm (1015 mg m^{-3}).
- The American Conference of Governmental Industrial Hygienists guideline is such that the 8 h time-weighted average is 300 ppm.
- The NIOSH recommended exposure limit is such that the 10 h time-weighted average is 300 ppm.
- The NIOSH immediately dangerous to life or health at 2000 ppm.

See also: Gasoline.

Further Reading

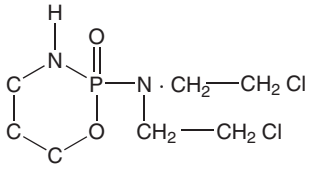
Health Council of the Netherlands: Committee on Updating of Occupational Exposure Limits (2004) *Cyclohexene: Health-Based Reassessment of Administrative Occupational Exposure Limits*. The Hague: Health Council of the Netherlands.

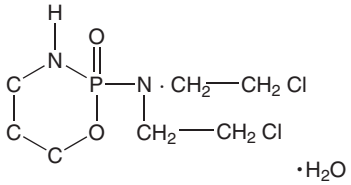
Cyclopeptide *See* Mushrooms, Cyclopeptide.

Cyclophosphamide

Greene Shepherd

- Representative Chemicals: Cyclophosphamide; Cyclophosphamide monohydrate
- Chemical Abstracts Service Registry Numbers: CAS 50-18-0; CAS 6055-19-2 (monohydrate)
- Synonyms: 2*H*-1,3,2-Oxazaphosphorin-2-amine, *N*,*N*-bis(2-chloroethyl) tetrahydro-, 2-oxide; 2*H*-1,3,2-Oxazaphosphorine; 2-chloroethyl)amino]tetrahydro-, 2-oxide; Asta B 518; B 518; Bis(2-chloroethyl)phosphoramide cyclic propanolamide ester; Clafen; Claphene; Cyclophosphamid; Cyclophosphan; Cyclophosphane; Cytophosphan; Cytoxan; Endoxan; Genoxal; *N*,*N*-bis-*O*-Trimethylenephosphoric acid ester diamide; *N*,*N*-Bis(2-chloroethyl)-*N*′,*O*-propylenephosphoric acid ester diamide; NSC 26271; Procytox; Sendoxan; Cyclophosphamide monohydrate; Cyclophosphamide hydrate; Endoxan monohydrate
- Chemical/Pharmaceutical/Other Class: Nitrogen mustard
- Chemical Formula: $C_7H_{15}Cl_2N_2O_2P$
- Chemical Structures:





Anhydrous form

Uses

Cyclophosphamide is used in human medicine as an antineoplastic (anticancer) agent in a variety of applications. Cyclophosphamide is a potent immunosuppressive agent and is used to prevent rejection episodes following renal, hepatic, and cardiac transplantation; and in non-neoplastic disorders in which there is altered immune activity, such as Wegener's granuloma, rheumatoid arthritis, the nephrotic syndrome in children, or autoimmune ocular diseases. Cyclophosphamide has also been used in veterinary practice for defleecing sheep, and it has been tested as an insect chemosterilant.

Exposure Routes and Pathways

Exposure to this odorless, white, crystalline powder may occur during its manufacture, formulation, or distribution as an antineoplastic drug. During manufacture and experimental use, exposure may be by inhalation or skin absorption. Therapeutically, patient exposure is by the oral, intramuscular, intraperitoneal, intravenous, or intrapleural route.

Toxicokinetics

In most species, cyclophosphamide is rapidly absorbed, metabolized, and excreted. In patients, cyclophosphamide was distributed rapidly to all tissues and exhibited a half-life of 6.5–7 h. The majority of an administered dose (50–68%) was excreted in the urine and no parent compound or metabolite was detected in expired air or feces. Carboxyphosphamide and phosphoramide mustard were detected in the urine. Cyclophosphamide is a racemer, and stereoselective metabolism by cytochrome P-450 of the enantiomers has been demonstrated in mice, rats, and rabbits. The primary metabolite is the 4-hydroxy derivative, and it exists in equilibrium with aldophosphamide, its ring-opened tautomer. Either metabolite can be converted by mammalian enzymes to 4-ketocyclophosphamide or to the propionic derivative. Both metabolites are relatively nontoxic and represent major urinary metabolites.

Mechanism of Toxicity

Cyclophosphamide works by interrupting the cell cycle in a nonphase specific manner. It prevents cell division by forming cross-linkages in DNA. An extensive study of cyclophosphamide analogs, some of which release acrolein but no phosphoramide mustard and others that cannot undergo complete metabolism, has provided strong evidence that (1) the phosphoramide mustard metabolite is responsible for the drug's antitumor activity, (2) the toxic side effects are probably due to the phosphoramide mustard and acrolein, and (3) nor-nitrogen mustard is responsible for the renal damage that occurs in some cases.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD in mice ranges from 370 mg kg^{-1} (subcutaneous) to 310 mg kg^{-1} (intravenous). The LD in rats was 160 mg kg^{-1} (intravenous), 180 mg kg^{-1} (oral), and 400 mg kg^{-1} (intraperitoneal in rats bearing tumors). The intravenous LD was 400 mg kg^{-1} in guinea pigs and 40 mg kg^{-1} in dogs. In mice, rats, and dogs, the predominant hematological effect of cyclophosphamide is leucopenia; some depression of thrombocytes was also noted. Prolonged treatment of rodents with cyclophosphamide has produced pathological structural changes in a variety of organs including lung, gut, pancreas, and liver. In rats, cyclophosphamide given orally decreases mitosis in crypts, decreases the height of villi, and causes degeneration of the intestinal mucosa. A single intraperitoneal dose of cyclophosphamide caused marked necrosis of the bladder and necrosis of the renal tubular and renal pelvic epithelium in mice, rats, and dogs. Cyclophosphamide is teratogenic in the rhesus monkey when given intramuscularly for various periods between 25 and 43 days of pregnancy at doses ranging from 2.5 to 20 mg kg^{-1} body weight. Placental transfer of cyclophosphamide has been demonstrated in mice, and a positive correlation between alkylation of embryonic DNA and the production of congenital abnormalities has been reported in mice.

Human

Patients treated with cyclophosphamide have been reported to exhibit various side effects such as flushing of the face, swollen lips, cardiotoxicity, pneumonitis or interstitial fibrosis, agitation, dizziness, tiredness, weakness, headache, nausea, vomiting, diarrhea, stomatitis, hemorrhagic colitis, hepatitis, hemorrhagic cystitis, fever, chills, sore throat, sweating, pancytopenia, leukopenia, alopecia, changes in the nucleoli of lymphocytes, water and sodium retention, pulmonary fibrosis, and visual blurring. Birth defects, such as limb reductions or pigmentation of the fingernails and skin, were also noted. Cystitis, hemorrhagic cystitis, and fibrosis of the bladder wall have been reported in patients treated for cystitis, rheumatoid arthritis, lupus erythematosus, and neoplasia, respectively. Fatal cardiomyopathy may result when very large doses of cyclophosphamide are given as conditioning for bone marrow transplantation. Cyclophosphamide has teratogenic and mutagenic potential and can cause sterility of either sex. It can damage germ cells in prepubertal, pubertal, and adult males and cause premature ovarian failure in females. It is most toxic to the human fetus during the first 3 months and congenital abnormalities have been detected after intravenous injections of large doses to pregnant women during this period of pregnancy. Mothers taking cyclophosphamide should avoid breast-feeding.

Chronic Toxicity (or Exposure)

Animal

Mice dosed at 7% of the LD_{50} cyclophosphamide per week subcutaneously for 1 year had higher rates of leukemias, mammary carcinomas, ovarian carcinomas, and lung tumors compared to controls.

Human

Chronic overmedication with cyclophosphamide would be expected to produce bone marrow suppression. Decreased ability to fight infection, inability for blood to clot with subsequent bleeding, hair loss, and other toxic effects may develop in patients.

In Vitro Toxicity Data

Studies of the teratorgenic potential of cyclophosphamide have demonstrated that cyclophosphamide must be metabolized in order to become teratogenic.

Clinical Management

Treatment is largely supportive. Cyclophosphamide is adsorbed to activated charcoal and charcoal should be used for substantial, recent ingestions. Patients may require aggressive fluid support. Standard supportive therapies, such as vasopressors, should be utilized as clinically indicated. Patients may require prolonged observation due to the delay in the development of adverse effects. Antibiotics may be needed due to development of immunosuppression. MESNA has been used for management of cyclophosphamide-induced hemorrhagic cystitis.

Environmental Fate

Cyclophosphamide may be released into the atmosphere secondary to production or through waste streams. If released into the air, it is expected to be present in both vapor and particulate phases at ambient temperature and pressure. Vapor-phase degradation by hydroxyl radicals in general produces a half-life of 5.5 h. Particulate cyclophosphamide will

be broken down by both wet and dry decompositions as well as through photodegradation.

See also: Acrolein.

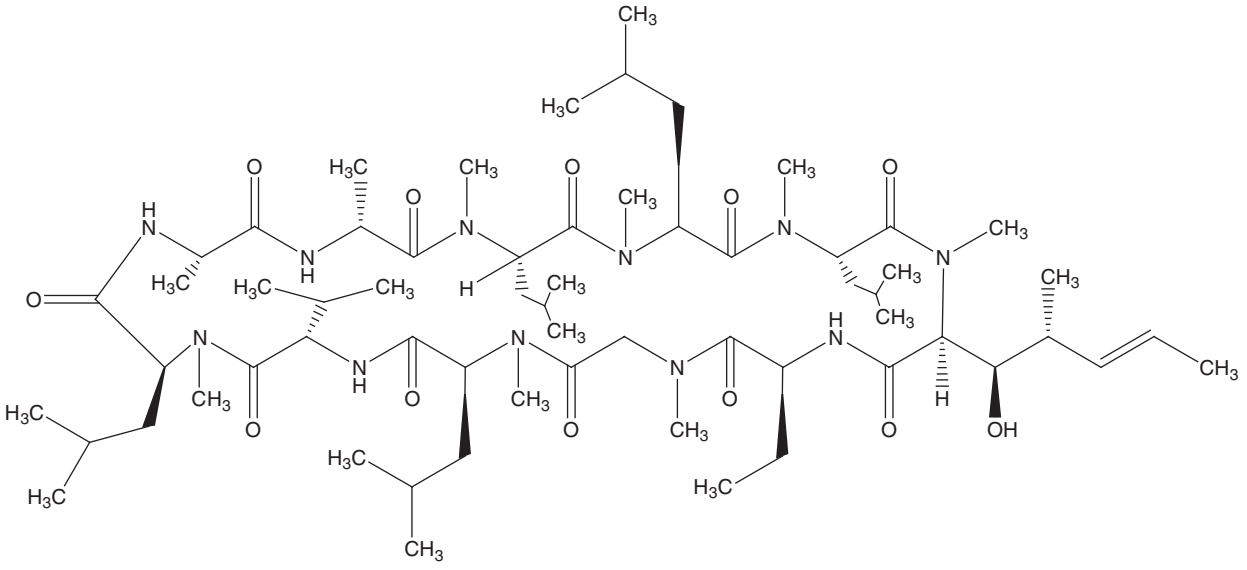
Further Reading

Braverman AC, Antin JH, and Plappert MT (1991) Cyclophosphamide cardiotoxicity in bone marrow transplantation. *Journal of Clinical Oncology* 9: 1215–1223.

Cyclosporine

Teresa Dodd-Butera and Molly Broderick

- Chemical Abstracts Service Registry Number: CAS 59865-13-3
- Synonyms: Cyclosporin A; Neoral; Sandimmune (brand names)
- Chemical/Pharmaceutical/Other Class: Immunosuppressive agent
- Chemical Structure:



Uses

Cyclosporine was approved for transplant immunosuppression in 1983. It is administered to prevent organ rejection after transplant of kidney, liver, lung, heart, or bone marrow. In addition, it has been given to patients with nephrotic syndrome, rheumatoid arthritis, psoriasis, severe Crohn's disease, and for other medical conditions.

Background Information

Cyclosporine is a macrolide antibiotic cyclopeptide with 11 amino acids. It inhibits cytokines (primarily interleukin-2) produced by T-cells in response to antigen exposure. Cyclosporin A can be biosynthesized from soil fungus *Tolypocladium inflatum* or synthetically manufactured. Hazardous combustion and decomposition products of this process include carbon monoxide, carbon dioxide, nitrogen oxide, hydrogen chloride gas, and phosgene.

Exposure Routes and Pathways

Cyclosporine is available in liquid, pill, capsule, opthalmic drops, and injectable forms. Oral and intravenous routes are the most common pathways of exposure.

Mechanism of Toxicity

The mechanism of cholestasis from cyclosporine may result due to interference with uptake and transport of bile salts. Cyclosporine also causes prerenal vasoconstriction and decreased glomerular perfusion, which is sometimes dose-dependent. Cyclosporine induces enzymes in porphyrin production, which can exacerbate the symptoms of porphyria.

Toxicokinetics

Cyclosporine is biotransformed primarily in the liver by CYP3A4; however, alternate metabolic pathways may yield toxic metabolites. Most of the cyclosporin dose is excreted in bile as an active metabolite. Less than 1% is excreted as unchanged drug. Inducers of CYP3A increase cyclosporine metabolism, whereas competitors may increase cyclosporine to potentially toxic levels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal studies show immunosuppression, renal and cardiovascular, and neurotoxicity. Studies in rats and rabbits have shown that large doses may cause death to the fetus or birth defects, depending on timing of exposure in gestation.

Human

Symptoms may include nausea, high blood pressure, bleeding, headache, disorientation, and diminished renal and hepatic functions. Some of these may occur as side effects of therapeutic doses. Allergic reactions may occur with parenteral administration of the drug and in persons allergic to polyoxyl 40 hydrogenated castor oil (present in capsules and solution).

Chronic Toxicity (or Exposure)

Animal

There was an increased incidence of lymphoma in grafted macaques and male mice also receiving cyclosporine. Rats exposed to cyclosporine A developed renal and hepatocellular tumors.

Human

There is an increased risk of getting infections; however, use of this drug demonstrated lower rates of infection than some other regimens given for immunosuppression and autoimmune diseases. Additionally, cyclosporine A is considered a human carcinogen, due to the increased risk of lymphoma and skin cancer in patients taking the drug. In some cases, tumor regression occurred after discontinuing cyclosporine. Hyperkalemia, loss of renal function, gum sensitivity, noncardiogenic pulmonary edema, and, rarely, myopathies may occur in patients on cyclosporine therapy.

Women who take cyclosporine during pregnancy may be at increased risk for delivering prematurely, though controlled scientific studies are necessary. In addition, cyclosporine passes into breast milk. Due to the potential risk to the infant for immunosuppression, impact on growth, and carcinogenicity, breastfeeding is not recommended with cyclosporine therapy.

In Vitro Toxicity Data

Most *in vitro* tests were negative for genetic damage; however, there was a slight increase in sister chromatid exchange in human lymphocytes exposed *in vitro*.

Clinical Management

Multiple-dose activated charcoal has been used to increase elimination of cyclosporine. Dose reduction when needed and close monitoring of the patient for symptoms of toxicity are required. However, many of the issues of clinical management are related to adverse effects and interactions with other substances. The following precautions are recommended for patients taking cyclosporine. Medication should not be taken with grapefruit or grapefruit juice, as this interferes with biotransformation and elimination of the drug. Patients should be advised to avoid alcohol, cough and cold remedies, diet pills, stimulants, ibuprofen, and certain herbs and supplements. Discuss any medications with a healthcare professional before taking. Similar brands are not necessarily interchangeable. No double dosing for missed doses. Patients using cyclosporine should avoid others with diseases and infections, and live vaccinations. Vaccinations received by patients on cyclosporine may not be as effective.

See also: Charcoal; Sister Chromatid Exchanges.

Further Reading

Goldfrank L, Flomenbaum N, Lewin N, *et al.* (eds.) (2002) *Goldfrank's Toxicologic Emergencies*, 7th edn. New York: McGraw-Hill Medical Publishing.

Klaassen C (ed.) (2001) *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.
Rezzani R (2004) Cyclosporine A and adverse effects on organs: histochemical studies. *ProgHistochemCytochem* 39(2) 85–128.

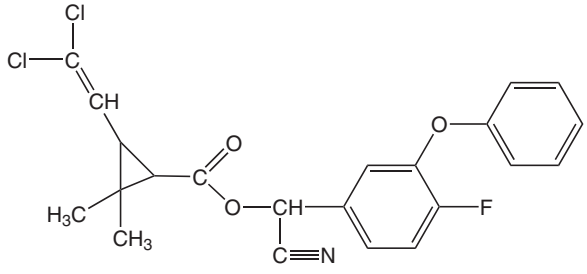
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Cyclosporine.

Cyfluthrin

Subramanya Karanth

- Chemical Abstracts Service Registry Number: CAS 68359-37-5
- Synonyms: Attatox; Baythroid; Contur; Solfac
- Chemical/Pharmaceutical/Other Class: Pyrethroid insecticide. Technical-grade cyfluthrin consists of a mixture of eight stereoisomers, consisting of two *cis* and two *trans* isomeric pairs
- Chemical Formula: $C_{22}H_{18}Cl_2FNO_3$
- Chemical Structure:



Uses

Cyfluthrin is a broad-spectrum nonsystemic insecticide used to control cockroaches, ants, termites, mosquitoes, flies, tobacco budworms, and common chewing and sucking insects of cotton, cereal, potatoes, and peanuts. It is also used effectively in the control of public health pests.

Exposure Routes and Pathways

Common routes of cyfluthrin exposure include dermal, ingestion, and inhalation.

Toxicokinetics

Cyfluthrin is excreted mainly as urinary metabolites but a portion of it is also excreted unchanged in feces. Toxicokinetic studies with ^{14}C-cyfluthrin in rats have shown that the initial step of biotransformation includes ester hydrolysis resulting in 3-phenoxy-4-fluorobenzyl alcohol intermediate and permethric acid. 3-Phenoxy-4-fluorobenzyl alcohol is further oxidized to 3-phenoxy-4-fluorobenzoic acid, which is either hydroxylated to 4′-hydroxy-3-phenoxy-4-fluorobenzoic or conjugated with glycine to 4′-hydroxy-3-phenoxy-4-fluorobenzoic acid.

Mechanism of Toxicity

Cyfluthrin elicits toxicity by modifying the voltage-sensitive sodium channels in neuronal membranes. It binds to a receptor site on the alpha subunit of the sodium channel, which results in prolonged opening of sodium channels. This delay in the closure of sodium channels leads to a protracted sodium influx causing repetitive firing of sensory nerve endings and hyperexcitation. High doses may result in complete depolarization of the nerve membrane and blockade of nerve conduction.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity studies in laboratory animals have shown that cyfluthrin is moderately toxic to mammals with an oral LD_{50} of 850–1200 mg kg^{-1} in rats.

Human

Cyfluthrin is slightly irritating to skin and eyes in humans. One of the common symptoms of cyfluthrin poisoning is paresthesia (a stinging, burning, and itching skin particularly on the face), progressing to numbness. Dermal irritation may worsen if exposed to sun or heat. Large doses of cyfluthrin may cause excessive salivation, irritability, tremors, convulsions, and death. Inhalation exposure may result in labored breathing and nasal discharge.

Chronic Toxicity (or Exposure)

Animal

Long-term exposure to cyfluthrin has been reported to cause diarrhea, reduced body temperature, and weight loss in laboratory animals. A 24 month chronic

feeding study in rats demonstrated a no-observed-adverse-effect level (NOAEL) of 2.6 mg kg^{-1} day^{-1}. No organ-specific toxicities were observed in laboratory animals following long-term dietary exposure. Mutagenicity and carcinogenicity studies have shown no evidence of potential effects in rats and mice.

Human

Little is known regarding chronic effects of cyfluthrin in humans. As cyfluthrin is commonly used on food crops, risks of dietary exposure and through water and air are relatively high. Exposure may also occur through inhalation and contact from indoor and outdoor uses.

Clinical Management

In the case of dermal exposure, the contaminated area must be washed with plenty of water and soap. Topical application of vitamin E preparations may help to reduce the severity of skin reactions. The affected eye must be irrigated with lukewarm water for at least 10 min. The contaminated clothing is removed and the airway cleared. In the case of ingestion, gastric lavage is avoided as solvents present in cyfluthrin formulations may increase the risk of aspiration pneumonia. Atropine (adults and children >12 years: 0.6–1.2 mg kg^{-1}; children <12 years: 0.02 mg kg^{-1} by IV infusion) may be useful to control excessive salivation but care should be taken to avoid excess administration. If prolonged and frequent seizures appear, diazepam should be used for treatment (5–10 mg kg^{-1}, IV).

Environmental Fate

Cyfluthrin is a photosensitive compound and following exposure to sunlight, it readily breaks down. It is highly immobile in soil and unstable in water. On soil surfaces its half-life is 2–3 days. Under anaerobic conditions, its half-life in soils is $\sim$2 months. Cyfluthrin does not move through soils and is not a groundwater contaminant. It rapidly breaks down in surface waters as it floats on the surface where it is subject to photodegradation.

Ecotoxicology

Cyfluthrin is least toxic to birds while acute toxicity studies have shown that it is highly toxic to marine and freshwater organisms. It is extremely toxic to honey bees and other beneficial insects.

Exposure Standards and Guidelines

The reference dose for cyfluthrin is 0.008 mg kg^{-1} day^{-1}, while the acceptable daily intake is 0.02 mg kg^{-1} day^{-1}.

See also: Neurotoxicity; Pyrethrins/Pyrethroids.

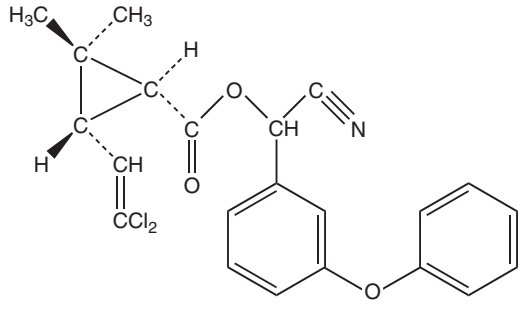
Relevant Websites

http://pmep.cce.cornell.edu – Cornell University.
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Cypermethrin

Paul R Harp

- Chemical Abstracts Service Registry Numbers: CAS 52315-07-8; CAS 69865-47-0; CAS 86752-99-0; CAS 86753-92-6; CAS 88161-75-5; CAS 97955-44-7
- Synonyms: (*R*,*S*)-α-cyano-3-phenoxybenzyl-2,2-dimethyl (1*R*,1*S*)-*cis*,*trans*-3-(2,2-dichlorovinyl) cyclopropane carboxylate; Agrothrin; Ammo; Arrivo; Cymbush; Cymperator; Cynoff; Demon; Folcord; Prevail; Polytrin; Ripcord; Stockade; CCN 52; FMC 30980; OMS 2002; NRDC 149; PP 383; SHA 109704
- Chemical/Pharmaceutical/Other Class: Type II pyrethroid insecticide
- Chemical Structure:



Uses

Cypermethrin is a broad-spectrum insecticide used in a variety of agricultural, commercial, and residential

applications. It is available as a wettable powder, an emulsifiable concentrate, or a concentrate for ULV application. Technical cypermethrin is a mixture of eight isomers.

Exposure Routes and Pathways

Dermal contact is probably the most common route of exposure but cases of ingestion and inhalation have also been reported.

Toxicokinetics

Pyrethroids are poorly absorbed through the skin and are only moderately absorbed in the gastrointestinal tract. In one case of dermal exposure, absorption was estimated to be ~3%. Metabolism of cypermethrin occurs rapidly through ester cleavage and hydroxylation. Adipose tissue acts as a storage depot and has varying affinities for the different isomeric forms; elimination half-lives of 3.4 and 18 days have been reported in rats for *trans* and *cis* isomers, respectively. Urinary excretion is a primary route of elimination but fecal excretion may also be significant depending on species of animal and isomeric configuration.

Mechanism of Toxicity

Several mechanisms of action have been identified for the pyrethroids with the primary mechanism related to a selective high affinity for membrane sodium channels. Closing of the channel, which ends the action potential, is slowed resulting in a prolonged 'tail' current and repetitive firing of presynaptic and accompanying postsynaptic cells following a single action potential. High enough doses can cause complete depolarization and blockade of nerve conduction. Cypermethrin also inhibits Ca^{2+},Mg^{2+}-ATPase and type II pyrethroids such as cypermethrin have been shown to act on γ-aminobutyric acid-mediated chloride ionophores and voltage-sensitive calcium-independent chloride channels.

Acute and Short-Term Toxicity (or Exposure)

Animal

In mammals, cypermethrin produces type II motor symptoms characterized by hyperactivity, incoordination, choreoathetosis, and convulsions.

Human

In humans, extensive dermal exposure to cypermethrin may cause temporary effects of paresthesia (stinging, burning, tingling) and numbness. Symptoms following ingestion include nausea, vomiting, tenesmus, diarrhea, unconsciousness, and death due to respiratory failure.

Chronic Toxicity (or Exposure)

Animal and Human

Chronic effects in humans following cypermethrin exposure have not been reported. Studies have demonstrated possible genotoxicity in mouse spleen and bone marrow. US Environment Protection Agency has classified cypermethrin as a possible human carcinogen based on findings of benign lung adenomas in mice.

Clinical Management

Exposed skin should be washed promptly with soap and water. Dermal application of vitamin E oil preparations may be used for both prophylaxis and treatment of paresthesia. For contact with eyes, flush immediately and for an extended period with generous amounts of clean water or saline. Gastric lavage is indicated if patient has ingested a large amount of pyrethroid and can be treated soon after exposure. For ingestion of smaller amounts or if treatment has been delayed, activated charcoal and catharsis are indicated. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenytoin or phenobarbital may be helpful for recurrent seizures. No specific antidotes for pyrethroid-induced neurotoxic effects have been approved for use in humans. Spontaneous recovery usually occurs with mild or moderate intoxication.

Environmental Fate

Cypermethrin is moderately persistent in soils. Cypermethrin degrades more rapidly in sandy compared to clay soils, and in soils with low organic content. Under aerobic conditions, the half-life is 0.5–8 weeks. Cypermethrin is more persistent under anaerobic conditions. Cypermethrin is subject to photodegradation and microbial degradation under aerobic conditions. Cypermethrin binds strongly to soil particles and poses minimal leaching concerns.

Cypermethrin hydrolyzes slowly under acidic or neutral conditions but more rapidly under alkaline conditions. Concentrations decrease rapidly due by adsorption to sediment, particles, and plants.

Ecotoxicology

Cypermethrin is practically nontoxic to birds but is highly toxic to bees. Fish and crustaceans are extremely sensitive to cypermethrin and pyrethroid compounds in laboratory settings. However, various factors (e.g., sediment binding) may reduce pyrethroid toxicity to these nontarget organisms in a natural environment.

Exposure Standards and Guidelines

The reference dose for cypermethrin is 0.01 mg kg^{-1} day^{-1}. The acceptable daily intake is 0.05 mg kg^{-1} day^{-1}.

See also: Neurotoxicity; Pyrethrins/Pyrethroids.

Further Reading

Vijverberg HPM (2000) Pyrethroids. In: Spencer PS and Schaumburg HH (eds.) *Experimental and Clinical Neurotoxicology*, 2nd edn. New York: Oxford University Press.

Relevant Websites

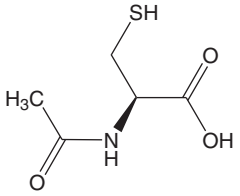
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Cysteine, *N*-Acetyl-L

Stephen R Clough

This article is a revision of the previous print edition article by David M Krentz and Linda A Malley, volume 1, pp. 392–393, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 616-91-1
- Synonyms: L-α-Acetamido-β-mercaptopropionic acid; Acetylcysteine; Airbron; Broncholysin; Brunac; Fabrol; Fluatox; Fluimucetin; Fluimucil; Fluprowit; Inspir; L -α-Acetamido-β-mercaptopropionic acid; Mercapturic acid; Mucocedyl; Mucolator; Mucolyticum; Mucomyst; Muco Sanigen; Mucosil; Mucosol; Mucosolvin; Mucret; *N*-Acetyl-L-cysteine (NAC); *N*-Acetyl-L-(+)-cysteine; *N*-Acetyl-3-mercaptoalanine (IUPAC); Neo-Fluimucil; Parvolex; Respaire; Tixair
- Chemical Formula: $C_5H_9NO_3S$
- Chemical Structure:



Uses

N-Acetyl-L-cysteine (NAC) is a natural sulfur-containing compound that is produced in living organisms from the amino acid cysteine. It is involved in the intracellular synthesis of a chemical called glutathione (or GSH). Cells (particularly liver cells) use glutathione to detoxify chemicals by making them more water soluble and thus easier to excrete from the body. NAC is also a powerful antioxidant.

NAC is primarily marketed and used as a mucolytic agent to break up mucus (by reducing disulfide bonds in mucoproteins) in persons having bronchopulmonary diseases including chronic bronchitis, cystic fibrosis, asthma, sinusitis, and pneumonia. It is also used extensively as an antidote for acetaminophen (paracetamol) overdose or toxicity. Because it is a precursor of glutathione, it has been proven useful in replenishing depleted glutathione levels in the liver. Other studies have shown it can be used as a chelating agent for the treatment of heavy metal (mercury, lead, cadmium) poisoning. Other reports (primarily animal studies) have suggested that NAC can find use as a detoxifying agent for a number of toxicants, such as paraquat, urethane, aflatoxin, *Escherichia coli*, carbon tetrachloride, chloroform, and carbon monoxide.

Exposure Routes and Pathways

The most common route of exposure to NAC is (voluntary) inhalation through the respiratory tract. Although not approved by the US Food and Drug Administration, it may be given intravenously in emergency situations. According to a National Institute for Occupational Safety and Health survey conducted between 1981 and 1983, over 30 000 workers in the United States are exposed to NAC on a daily basis. Over two-thirds of those people are inhalation therapists and clinical laboratory technicians, with the remaining majority in some type of medical profession.

Toxicokinetics

NAC is rapidly absorbed after oral administration, with peak plasma levels occurring in 2 or 3 h. With intravenous administration, peak plasma levels occur immediately. Orally administered NAC appears to distribute primarily to the kidneys, liver, and lungs. It is detectable in pulmonary secretions for at least 5 h after the dose. NAC is rapidly absorbed and exists as the free species in plasma with a concomitant increase both in plasma L-acetylcysteine levels and in protein and nonprotein –SH concentrations. Protein binding is more than 50%. The volume of distribution in humans is 0.337–0.471 kg^{-1}.

Thirty percent of intravenously administered NAC is renally cleared. Acetylcysteine elimination is not impaired in patients with severe liver damage. The terminal half-life of NAC is 2–6 h. This may be increased to 13 h after an intravenous injection.

Mechanism of Toxicity

Fatalities from normal doses and overdoses of intravenous NAC have not been reported. This is most probably due to the fact that the body produces this compound naturally and can rapidly metabolize it in the liver. Toxicity is usually limited to anaphylactoid reactions and nausea/vomiting. The average time for the onset of adverse effects following commencement of the infusion of NAC was 30 min (range, 5–70 min). *In vivo* and *in vitro* tests indicate that NAC is an inhibitor of allergen tachyphylaxis by inhibition of prostaglandin E synthesis. Adverse reactions are anaphylactoid in type and have been attributed to cause histamine release.

Acute and Short-Term Toxicity (or Exposure)

N-Acetylcysteine is used primarily in the treatment of acetaminophen overdose and/or toxicity. It is also nebulized for mucolytic effects and less often used to treat corneal ulcers. It has a very low potential to cause acute toxicity in either animals or humans.

Animal

Oral formulations of *N*-acetylcysteine are used intravenously in the clinical treatment of animals, although it has not been approved for this use. The Registry of Toxic Effects of Chemical Substances (RTECS) lists an acute oral, intravenous, and intraperitoneal LD_{50} in dogs of 1.0, 0.7, and 0.7 g kg^{-1} body weight, respectively. For mice, RTECS lists an oral, intravenous, and intraperitoneal LD_{50} of 4.4, 3.8, and 0.4 g kg^{-1} body weight, respectively. For rats, RTECS lists an oral and intravenous LD_{50} of 5.05 and 1.14 g kg^{-1} body weight, respectively. Acute effects cited for mice include central nervous system depression and somnolence; rats showed gastrointestinal changes.

Human

The primary toxicity of NAC consists of nausea/vomiting, particularly after oral therapy, and an anaphylactoid reaction that may be life-threatening. Many cases of anaphylactic reactions have been reported with symptoms primarily consisting of rash, nausea, hypotension, bronchospasm, angioedema, tachycardia, and respiratory distress. NAC may also have some neurological toxicity that includes dizziness, intracranial hypertension, hypoactivity, ataxia, and seizures. There have been reports of mucosal damage with full strength (20%) NAC, which causes hyperemia and hemorrhages of bowel mucosa. During inhalation therapy, irritation or soreness of the mouth may occur. The RTECS cites a 'lowest published toxic dose' reported for a child of 8.48 g kg^{-1} over a 3 day period. This is a very large dose and places this substance in the acute category of 'practically nontoxic'.

Chronic Toxicity (or Exposure)

Animal

N-Acetylcysteine has not been shown to be teratogenic in rats or rabbits. When administered to rabbits during the critical phase of embryogenesis, no malformation resulted.

Human

Experience in 59 pregnant patients suggested that use of *N*-acetylcysteine in pregnancy did not result in toxic effects on the fetus. In practice, the risk to the mother and baby of paracetamol-induced liver damage probably far outweighs any potential risk of *N*-acetylcysteine, and pregnancy should not be considered a contraindication to the use of this agent.

In Vitro Toxicity Data

N-Acetylcysteine is negative in the Ames mutagenicity test and also reduces the mutagenic affect of chemical carcinogens in the same assay.

Clinical Management

Since 1974, it has been known, and generally accepted, that NAC is hepatoprotective, especially for treating overdoses of acetamenophen. Basic and advanced life-support measures should be utilized as

necessary. For acetaminophen overdose, a $140\,mg\,kg^{-1}$ dose followed by $70\,mg\,kg^{-1}$ every 4 h for an additional 17 doses should be administered. Since NAC has not been approved for intravenous administration, assistance is available through the Rocky Mountain Poison Center.

Environmental Fate

Because NAC is a natural compound that contains no halogen atoms or substitutions, it would be expected to be easily metabolized by microorganisms in the environment and thus not present a risk from the standpoint of persistence or bioaccumulation.

Ecotoxicology

NAC is produced naturally in the body and is therefore not anticipated to be a hazard to ecological receptors.

Exposure Standards and Guidelines

There are no regulatory exposure standards or guidelines for NAC. Acute doses of $140\,mg\,kg^{-1}$ are recommended for the initial 'loading' dose in humans (i.e., for paracetamol poisoning) and $1330\,mg\,kg^{-1}$ can be tolerated by humans over a 72 h period.

Further Reading

Meredith TJ, Jacobsen T, Haines JA, and Berger J-C (eds.) (1995) *IPCS/CES Evaluation of Antidotes Series, vol. 3: Antidotes for Poisoning by Paracetamol*. Published by Cambridge University Press on behalf of the World Health Organization and of the Commission of the European Communities.

Relevant Website

http://www.intox.org – IPCS INTOX Data Bank.

Cytochrome P-450

Kartik Shankar and Harihara M Mehendale

Evolution of P-450s

Cytochrome P-450 proteins are one of the largest superfamily of enzyme proteins. Genes encoding cytochrome P-450 (called CYP) are found in virtually all genomes. Sequence comparisons indicate that the diverse superfamily originated from a common ancestral gene some three billion years ago. The origin of the P-450 superfamily lies in prokaryotes, before the advent of eukaryotes and before the accumulation of molecular oxygen in the atmosphere. As a comparison, *Mycobacterium tuberculosis* has 20 P-450 genes, baker's yeast has three, and the fruit fly *Drosophila melanogaster* has 83 genes. Humans have 57 different active P-450 genes and a similar number (58) of pseudogenes. There is particularly the large number of P-450 genes in the plants with 323 genes in the rice and 249 genes in the thale cress genomes. In the human genome, the P-450 genes are arranged into 18 families and 42 subfamilies.

Biochemistry of P-450 Enzymes

In vertebrates, the liver is the richest source of P-450 and is also the most active organ in the oxidation of xenobiotics. P-450 enzymes are expressed in the microsomal fraction (smooth endoplasmic reticulum) of the cell where they are anchored in the lipid bilayer. In addition to the liver, P-450s are also ubiquitously expressed in the lung, kidney, skin, nasal mucosal, gastrointestinal tract, placenta, bladder, nervous system, and blood platelets among other tissues. Although they are expressed in a variety of tissues, the function of P-450s seems to differ in each case. The liver, lung, and small intestine carry out mainly xenobiotic biotransformation. Placental P-450s are devoid of the ability to metabolize any appreciable xenobiotics but function mainly as a steroid hormone metabolizing system. Kidney P-450s are involved in some metabolism of xenobiotics, but are involved in cholecalciferol and salt balance regulation. Cytochrome P-450s are hemoproteins (iron-containing) of the *b* cytochrome type and derive the name P-450 from the wavelength (450 nm) at which the carbon monoxide derivative of the reduced cytochrome has an absorption maximum. Cytochrome P-450s, like other monooxygenases, carry out oxidation reactions in which one atom of molecular oxygen is reduced to water while another is incorporated into the substrate. Reducing equivalents are transferred from NADPH to P-450 by a flavoprotein enzyme called the NADPH–cytochrome P-450 reductase (P-450 reductase).

Reactions Catalyzed by P-450s

P-450s catalyze a large number of substrates that may be exogenous or endogenous compounds.

P-450s carry out aliphatic and aromatic hydroxylations, aromatic epoxidations (leading to stable epoxides like dieldrin from aldrin, or arene oxides), O-, N-, and S-dealkylations and oxidations, oxidative deaminations, and desulfurations among other reactions. Although the primary evolutionary role of the P-450 enzymes is to convert hydrophobic xenobiotics into more hydrophilic compounds and enhance their removal from the body, P-450s also catalyze reactions that lead to more reactive (and hence toxic) compounds. Several xenobiotics are converted into potential carcinogens via the cytochrome P-450 system.

Major CYP Families

Human cytochrome P-450s that metabolize xenobiotic compounds are almost exclusively in the *CYP1*, *CYP2*, *CYP3* and, to a small degree, *CYP4* families. The CYP1 family consists of three genes and two subfamilies. Genes in this family are controlled by the aryl hydrocarbon (Ah receptor), which is activated most notably by components of incineration products and cigarette smoke. CYP1A1 and 1B1 are expressed in varying amounts in different tissues and are most efficient in metabolizing polycyclic aromatic hydrocarbons (PAHs), while CYP1A2 preferentially metabolizes arylamines and *N*-heterocyclics. In addition, CYP1A2 metabolizes ~10–20 drugs, whereas CYP1B1 and 1A1 do not seem to act mainly as drugs. The *CYP2* family is the largest P-450 family in humans containing 16 individual isozymes. Human CYP2C8, CYP2C9, CYP2C18, and CYP2C19 together metabolize to varying amounts greater than half of all frequently prescribed drugs. Results from *in vitro* assays show that CYP2D6 metabolizes more than 75 drugs. CYP2E1, a toxicologically important isozyme, bioactivates several compounds, including acetaminophen, benzene, chloroform, carbon tetrachloride, butadiene, and vinyl chloride. The *CYP3* family has four members and has the most abundantly expressed P-450s in the liver. CYP3A4 and 3A5 are known to metabolize more than 120 frequently prescribed drugs. The CYP3A family is regulated via the pregnane-X-receptor, a nuclear receptor that can be induced by pregnenolone-related compounds.

Endogenous Functions of P-450s

Following the sequencing of the human genome, all the human P-450s have been identified. However, the endogenous physiological role of the majority of P-450s remains unknown. The critical roles of several individual isozymes are now becoming clear with the use of gene knockout and transgenic animals. Three important biological systems that are intrinsically regulated by several P-450 enzymes require special mention:

1. *Cholesterol metabolism and bile acid biosynthesis*. At least seven cytochrome P-450 enzymes play critical roles in the conversion of acetate into sterols and bile acids. Key among these are CYP51A1, CYP7A1, CYP7B1, and CYP39A1. The roles of each of these enzymes are beyond the scope of this article, but some excellent reviews and texts are available on the topic.
2. *Steroid synthesis and metabolism*. Six P-450s participate in steroid synthesis. CYP11A1 catalyzes the synthesis of pregnenolone. CYP17A1 is required for the biosynthesis of cortisol, testosterone, and estrogen. CYP19A1 converts androgenic steroids to estrogens. CYP11B2, 21A1, and 21A2 are also involved in the intermediary steps in the formation of corticosterone and aldosterone.
3. *Vitamin D_3 biosynthesis and metabolism*. The vitamin D_3 system, which acutely controls calcium status in addition to a host of other physiological functions, is a classical example of P-450s in multiple tissues being involved in the biosynthesis of a biologically active metabolite. Cholecalciferol is hydroxylated at the 25-position by either CYP27A1 or CYP2D25, both of which are expressed in the liver. The 25-hydroxycholecalciferol undergoes another P-450-mediated hydroxylation at the 1-α position by the renal CYP27B1 to the active 1,25-dihydroxycholecalciferol (vitamin D_3). Most of the biological function is attributed to this metabolite, although recent studies suggest that even the 25-hydroxy metabolite may be exerting certain biological effects. The degradation of the active 1,25-vitamin D_3 is catalyzed by the renal CYP24A1 enzyme, which catalyzes a third 24-hydroxylation leading to 1,24,25-vitamin D_3 metabolite.

See also: Biotransformation; Genomics, Toxicogenomics; Mechanisms of Toxicity; Vitamin D; Xenobiotics.

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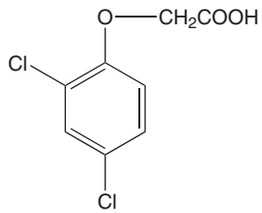
D

2,4-D (2,4-Dichlorophenoxy Acetic Acid)

Raja S Mangipudy and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 94-75-7
- Synonyms: 2,4-Dichlorophenoxyacetic acid; Dichlorophenoxyacetic acid
- Chemical/Pharmaceutical/Other Class: Chlorinated phenoxyacetic acids. A closely related compound is 2,4,5-T
- Chemical Structure:



Uses

2,4-D free acids, esters, and salts are formulated in water suspensions or solutions, or in various organic solvents, for application as systemic herbicides. Some esters are fairly volatile, whereas salts are not. The acid is corrosive. There are many commercial formulations available for weed and brush control, for certain agricultural uses, and for lawn and garden weed control.

Exposure Routes and Pathways

2,4-D may be encountered as a vapor, liquid, or as a component of mixtures. It may cause damage at the point of contact (skin, eyes, lungs, and gastrointestinal tract). Occupational exposure may occur through inhalation and dermal contact when 2,4-D is produced or used.

Toxicokinetics

Rapid and complete absorption of chlorphenoxy compounds from the gastrointestinal tract has been reported. Nearly complete absorption of 2,4-D occurs within 24 h in humans. 2,4-D is primarily metabolized by acid hydrolysis, and a minor amount is conjugated. It is highly protein bound and widely distributed. The chief organs of deposition are kidneys, liver, and the central and peripheral nervous systems.

2,4-D is primarily excreted unchanged (90%) in the kidneys via the renal organic anion secretory system. It may be conjugated to glycine or taurine. A minor fraction of 2,4-D is filtered by the glomerulus. The estimated half-life of 2,4-D is $\sim$18 h.

Mechanism of Toxicity

2,4-D is a plant-growth regulator that stimulates nucleic acid and protein synthesis and affects enzyme activity, respiration, and cell division. It is absorbed by plant leaves, stems, and roots and moves throughout the plant. It accumulates in growing tips.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in mice is 368 mg kg^{-1}. The oral LD_{50} in rats is 375 mg kg^{-1}.

2,4-D and all of its derivatives have been either embryotoxic, fetotoxic, or teratogenic in animals. Rats treated orally with 100 mg kg^{-1} day^{-1} 2,4-D from birth to 25 days of age had lower levels of acetylcholinesterase in the olfactory bulb and hippocampus regions of the brain. Monoamine neurotransmitters were also reduced. These results may account for slower learning in rats that were exposed to 2,4-D during postnatal development of the brain.

Human

Acute ingestion can cause miosis, coma, fever, hypotension, emesis, tachycardia, muscle rigidity, possible respiratory failure, pulmonary edema, and rhabdomyolysis. Alteration in liver functions such as elevated lactate dehydrogenase and aspartate aminotransferase has also been reported. In humans, the

causal relationship between these effects and chlorphenoxy herbicides such as 2,4-D remains controversial and not yet proven.

An extensive review of the published literature concluded that there is 'reasonable evidence' that occupational exposure to phenoxy herbicides is associated with increased risk for non-Hodgkin's lymphoma. Evidence is weaker or conflicting for soft-tissue sarcomas. In another review, however, case–control epidemiological studies were called inconclusive; occupational exposure studies have generally not shown associations between exposure to 2,4-D and cancer.

There appears to be sufficient evidence to regard 2,4-D as a suspect human carcinogen for non-Hodgkin's lymphoma and, possibly, for soft-tissue sarcomas and Hodgkin's disease as well. The possibility that 2,4-D may be a human carcinogen needs further study.

Chronic Toxicity (or Exposure)

Animal

In 2-year dietary tests in mice and rats, 2,4-D was not oncogenic (tumor causing). Toxic effects in the animals' kidneys were seen at low dosages in these tests.

Human

Long-term exposure to 2,4-D has been reported to cause liver, kidney, digestive, muscular, or nervous system damage. Symptoms may include weakness, fatigue, headache, dizziness, loss of appetite, nausea, eye and nasal irritation, skin irritation, hypertension, and slowed heart rate.

In Vitro Toxicity Data

2,4-D has been active in many different short-term genetic assays, including DNA damage and repair, mutations in yeast and human cells, sex-linked recessive mutations in fruit flies, and chromosome aberrations *in vitro*.

Clinical Management

No specific antidote is available. The patient must be monitored for seizures; gastrointestinal irritation; possible liver, kidney, or muscle damage; arrhythmias; acidosis; dyspnea; headache; coma; hyperthermia; and hypotension. Gastric lavage and activated charcoal/cathartic are probably more useful decontamination methods.

Environmental Fate

2,4-D has a relatively short half-life and is rather immobile in the soil. In 35 recent studies across the United States, the average lowest depth detected ranged from 6–12 in. in soils of the southern United States to 16–24 in. in low organic soils. Soils were sampled to a depth of 48 in. Its average half-life in soils ranged from 6.4 days in southern soils to 8.3 days in high organic matter soils. The average half-life was 6.1 days in grass and 6.9 days in thatch. The half-life in natural water was 2–4 weeks, although in areas such as a treated rice paddy, the half-life was as short as 1 day. The acid form of 2,4-D, as well as the amine and ester chemical groups, metabolized to compounds of nontoxicological significance and ultimately to forms of carbon. Thus, 2,4-D is considered a biodegradable compound. Under normal conditions, 2,4-D residues are not persistent in soil, water, or vegetation.

Other Hazards

To keep residues of 2,4-D out of meat or milk, dairy cattle should not be grazed on treated areas for 7 days after application. Also, hay should not be cut for 30 days and meat animals should not be slaughtered for 3 days. Contact with dried residues on vegetation is not expected to be hazardous. Inert ingredients found in 2,4-D products may include ethylene glycol, methanol, sequestering agents, petroleum hydrocarbons, and surfactants. Ethylene glycol is moderately toxic to humans; it may cause tearing, anesthesia, headache, cough, respiratory stimulation, nausea or vomiting, pulmonary, kidney, and liver changes. Methanol is moderately toxic to humans; it may cause damage to the optic nerve, tearing, headache, cough, difficult breathing, other respiratory effects, nausea, or vomiting. Some commercially formulated 2,4-D products have LD_{50} values that are much higher than the 2,4-D acid. This indicates that these formulations may have considerably less acute toxicity than the acid form. However, exposure to these formulated products may have other health effects similar to those reported for 2,4-D alone or for inert ingredients in commercial formulations. Some 2,4-D formulations may be contaminated with halogenated dibenzo-*p*-dioxins (but not TCDD), dibenzofurans, or *N*-nitrosamines. Dibenzodioxins and dibenzofurans may cause disorders of the skin, blood, and gastrointestinal tract; they may also cause headaches, numbness, birth defects, or fetal toxicity. Nitrosamines are carcinogenic.

The Material Safety Data Sheet (MSDS) should always be referred to for detailed information on handling and disposal.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.3\,mg\,kg^{-1}\,day^{-1}$.
- Maximum contaminant level is $0.07\,mg\,l^{-1}$.
- Reference dose is $0.01\,mg\,kg^{-1}\,day^{-1}$.
- Permissible exposure limit is $10\,mg\,m^{-3}$ (8 h).

See also: Pesticides; 2,4,5-T.

Further Reading

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Charles JM, Cunny HC, Wilson RD, and Bus JS (1996) Comparative subchronic studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in rats. *Fundamental and Applied Toxicology* 33: 161–165.

Charles JM, Dalgard DW, Cunny HC, Wilson RD, and Bus JS (1996) Comparative subchronic and chronic dietary toxicity studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in the dog. *Fundamental and Applied Toxicology* 29(1): 78–85.

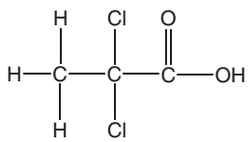
EPA, Health Effects Division (1997) Carcinogenicity Peer Review Committee Report on 2,4-D, January 29, 1997.

Rowland J (1996) 2,4-Dichlorophenoxyacetic Acid: Review of a Chronic Toxicity/Carcinogenicity Study in Rats: A Carcinogenicity Study in Mice, and a Re-review of a Developmental Toxicity Study in Rats, EPA Memorandum.

Dalapon

Priya Raman

- Chemical Abstracts Service Registry Number: CAS 75-99-0
- Preferred Name: Dalapon sodium salt
- Synonyms: Basfapon; Basfapon B; Basfapon/Basfapon N; BH Dalapon; Basinex; Crisapon; Dalapon 85; Ded-Weed; Devipon; 2,2-Dicloropropionic acid; α-Dichloropropionic acid; α,α-Dichloropropionic acid; Dowpon; Dowpon M; Gramevin; Kenapon; Kyselina; Liropon; Proprop; Radapon; Revenge; Unipon
- Chemical/Pharmaceutical/Other Class: Chlorinated aliphatic herbicide
- Chemical Structure:



Uses

Dalapon is used as a herbicide primarily to control annual and perennial grasses including Bermuda grass and Johnson grass. Use of dalapon on food crops is primarily with sugarcane and sugar beets. Dalapon is also used on fruits, potatoes, carrots, asparagus, alfalfa, and flax, and in forestry, home gardening, and to control reed and sedge growth in aquatic environments.

Exposure Routes and Pathways

Dermal inhalation and ocular exposures of liquid or vapor are the most common routes of exposure. If the chemical leaches into groundwater there is the possibility of ingestion.

Toxicokinetics

Dalapon is a polar compound that is not readily absorbed by tissues. It causes irritation to the tissue with which it comes into contact. If absorbed in the gastrointestinal tract, it is principally eliminated as the parent compound in the urine.

Mechanism of Toxicity

The mechanism of action of dalapon is the same as for most acids. The acid denatures tissue proteins upon contact.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values were $7.5–9.3\,g\,kg^{-1}$ in male and female rats. Relatively similar high oral LD_{50} values were noted in rabbits and guinea pigs ($3.9–4.6\,g\,kg^{-1}$). Dalapon is a moderate skin and eye irritant. The sodium salt of dalapon caused irritation, severe conjunctivitis, and corneal injury in rabbits, with recovery over several days.

Human

Dalapon can cause corrosive injury to tissues. Burning and irritation are the predominant acute toxicities

seen with exposure to dalapon. Skin lesions are more likely with moistened skin. Eye exposure may cause corneal destruction and conjunctival edema accompanied by pain and tearing. Permanent eye damage can result from ocular exposure. Ingestion can lead to oral, throat, and gastrointestinal irritation. Inhalation of vapors causes irritation of the eyes, nose, and throat with destruction of mucus membranes. Severe inhalation exposure may cause respiratory distress accompanied by pulmonary edema. Some other symptoms of high acute exposure to dalapon include loss of appetite, slowed heartbeat, and gastrointestinal disturbances such as vomiting, diarrhea, tiredness, pain, and irritation of respiratory tract.

Chronic Toxicity (or Exposure)

Animal

Long-term feeding studies in dogs and rats indicated increased kidney weights at high dosages. The no-observed-adverse-effect level (NOAEL) in a 2 year feeding study in rats was $15\,mg\,kg^{-1}\,day^{-1}$. The NOAEL in a 1 year feeding study in dogs was $50\,mg\,kg^{-1}\,day^{-1}$.

Human

Long-term exposure may cause increased kidney and liver weights. Repeated or prolonged exposure to dalapon may cause irritation to the mucuous membrane linings of the mouth, nose, throat, lungs, and to the eyes. Chronic skin contact can lead to moderate irritation or even mild burns.

In Vitro Toxicity Data

Dalapon was negative in a variety of mutagenic test assays.

Clinical Management

In the event of dermal exposure to dalapon, contaminated clothing should be removed quickly and the exposed area should be flushed with copious amounts of water for 15 min. With eye exposure, the affected eye should be flushed with water for 30 min, occasionally lifting the upper and lower lids. With inhalation exposure, the victim should be moved to fresh air. If the victim is not breathing, artificial respiration should be administered. If swallowed, vomiting should not be induced. If the victim is conscious, he or she should drink plenty of water or milk.

Environmental Fate

Dalapon is somewhat persistent in soil but does not readily adsorb to soil particles. It remains active in soil for several months when applied at high rates. Dalapon has a relatively high mobility in soil, with leaching possible. Soil microorganisms are efficient at degrading dalapon, however, such that dalapon is not typically found in groundwater. High temperatures and increased moisture accelerate dalapon degradation in soil. Dalapon can also be degraded by ultraviolet light. In aquatic environments, dalapon is degraded by microorganisms (most important), hydrolysis, and photolysis. Dalapon is absorbed by both plant roots and leaves and translocates. With high applications, dalapon precipitates and leads to local corrosive effects on plants.

Ecotoxicology

The LC_{50} of dietary (5 day exposure) dalapon was more than 5000 ppm in mallards, ring-necked pheasants, and Japanese quail. The acute oral LD_{50} of dalapon in chickens was $>5\,g\,kg^{-1}$. Reproduction may be affected in birds at nonlethal exposures. LC_{50} values for dalapon in a variety of fish species was $\sim 100\,mg\,l^{-1}$. Dalapon is only slightly toxic to mollusks. Crustaceans and insects are most sensitive of aquatic invertebrates. Dalapon is relatively nontoxic to honeybees and terrestrial insects.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists Threshold Limit Value is 1 ppm; the Maximum Contaminant Level is $0.2\,mg\,l^{-1}$; and the reference dose is $0.03\,mg\,kg^{-1}\,day^{-1}$.

See also: Pesticides.

Relevant Websites

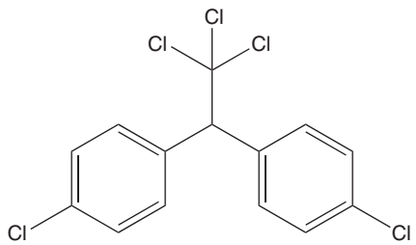
http://www.envirotools.org – Envirotools.org, Michigan State University.

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

DDT (Dichlorodiphenyltrichloroethane)

Benny L Blaylock

- Chemical Abstracts Service Registry Number: CAS 50-29-3
- Synonyms: Dichlorodiphenyltrichloroethane; *p*,*p*′-DDT; 1,1′-(2,2,2-trichloroethylidene)-bis-(4-chlorobenzene); Anofex; Cesarex; Chlorophenothane; Dedelo; Dinocide; Didimac; Digmar; ENT 1506; Genitox; Guesapon; Guesarol; Gexarex; Gyron; Hildit; Ixodex; Kopsol; Neocid; OMS 16; Micro DDT 75; Pentachlorin; Rukseam; R50; and Zerdane
- Chemical/Pharmaceutical/Other Class: Synthetic organochlorine insecticide
- Chemical Formula: $C_{14}H_9Cl_5$
- Chemical Structure:



Uses

DDT is an insecticide whose use has been banned in the United States. It is still used in some parts of the world.

Exposure Routes and Pathways

The most common route of exposure is ingestion. Data indicate that, even with relatively high doses, there is minimal absorption of DDT through skin. Therefore, exposure via dermal absorption was considered to be negligible. DDT and its metabolites are ubiquitous in the atmosphere but are present in such low concentrations that exposure via inhalation is negligible. Potential inhalation of relatively high levels of DDT should be possible only in areas of production or formulation.

Toxicokinetics

Gastrointestinal absorption of DDT is slow with symptoms delayed by several hours. DDT dissolved in solvents containing vegetable or animal fat is absorbed several times faster than the undissolved compound. Due to DDT's large particle size, absorption through the respiratory tract is less important to toxicity. Skin absorption is considered almost negligible.

Metabolism of DDT proceeds at a very slow rate. Liver microsomal P450 and other microsomal enzymes initially dechlorinate DDT to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) and reduce to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD). The conversion of DDD to bis(*p*-chlorophenyl)acetic acid (DDA) involves the formation of an acyl chloride intermediate by hydroxylation followed by hydrolysis to yield the final product.

DDT, like most other organochlorine insecticides, is highly lipophilic. It is stored in all tissues with higher levels generally found in adipose tissue. Most species store DDE more tightly than DDT.

DDA is the main form in which DDT is excreted. Most excretion takes place through bile with ~2% in urine and less than 1% in feces. Cows excrete ~10% of DDT doses in their milk; rodent females also excrete DDT in mother's milk.

Mechanism of Toxicity

The nervous system is the main site of toxicity for DDT. Effects are observed on both the central nervous system (CNS) and peripherally. There is significant alteration of neuronal membrane enzymatic and electrophysiological properties. In particular, sodium channels are altered such that once activated they close slowly, prolonging the depolarization of the nerve by interfering with the active transport of Na^+ ions out of the axon. Potassium channels are also affected. DDT specifically affects Na^+,K^+-adenosine triphosphatases (ATPases) and Ca^{2+}-ATPases, which inhibit repolarization of neurons. The membrane remains partially depolarized and is extremely sensitive to complete depolarization by very small stimuli. DDT also inhibits calmodulin that is necessary for Ca^{2+} transport essential for the subsequent release of neurotransmitters.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values in rats range from 133 to 800 mg kg^{-1} and from 150 to 300 mg kg^{-1} in mice. In animal models, the main toxic effect of DDT is neuronal hyperactivity. Alterations in the previously

mentioned ion transport channels in axon membranes produce paresthesia, hyperexcitability, irritability, fine tremors, and convulsions. Administration of 50 mg kg^{-1} as a one-time dose in rats resulted in decreased thyroid function. Monkeys dosed with 50–160 mg kg^{-1} showed increased liver enzymes.

In a recent retrospective study (US Collaborative Perinatal Project), it was suggested that DDT may be responsible for premature births and low birth weights in mothers with high DDE blood levels.

Human

Most human exposures are from ingesting very large amounts of DDT. The nervous system appears to be one of the primary target systems for DDT toxicity in humans after acute, high exposures. Symptoms range from nausea, fatigue, and vomiting to tremor and convulsions in severe poisoning. The vomiting is not due to irritation of the gastrointestinal tract and is probably central in origin. Symptoms following high oral doses include paresthesia of the tongue, lips, and face, apprehension and hypersensitivity to external stimuli, tremor with a gradual onset and usually mild effects, and, in extremely high doses, convulsions.

Liver involvement has not been a prominent symptom in DDT poisonings. Jaundice has been reported after 4–5 days following ingestion of 5000–6000 mg of DDT.

Chronic Toxicity (or Exposure)

Animal

In the liver, hepatocyte hypertrophy and centrilobular necrosis are seen. There is also an increase in liver cancers. Immune modulation has been described in both chickens and mice showing decreased antibody titers. Nervous system effects include tremors, loss of equilibrium, and changes in cellular chemistry in monkeys.

Sterility in rats and decreased fetal weights in rabbits have been reported after chronic or subchronic exposure. Gestational and lactational exposures in mice resulted in impaired learning performance. Abnormal tail development was reported in rats in a two-generational study with dosages of 10 mg kg^{-1} day^{-1}.

Carcinogenicity evidence in animals is equivocal with the production of liver and lung tumors in rats, mice, and hamsters in some studies but not in others.

Human

Almost continuous daily exposure to aerosols, sufficient to leave a white deposit of DDT on nasal vibrissae of volunteers, produced moderate irritation of nose, throat, and eyes. Except for this irritation during exposure, there were no symptoms. Anemia and thrombocytopenia have been reported after exposure in a house sprayed with the pesticide over a period of 4 months. Peripheral neuropathy has been rarely reported after chronic occupational exposure.

Neither reproductive nor teratogenic effects in humans have been reported. International Agency for Research on Cancer classifies DDT as 2B (possibly carcinogenic to humans).

Clinical Management

Diazepam may be beneficial to control convulsions. Activated charcoal is administered as a slurry. Phenobarbital is used if seizures recur after the use of diazepam. Gastric lavage may be of benefit if the patient has ingested a large amount of DDT and the procedure can be done within 1 h after ingestion. Emetics are contraindicated.

Environmental Fate

DDT is highly environmentally stable with a reported half-life of between 2 and 15 years. The insecticide is immobile in most soils and loss is the result of runoff, volatilization, photolysis, and biodegradation. Due to its extremely low solubility in water, DDT will be retained to a greater degree by soils and soil fractions. However, due to its persistence, DDT may be able to eventually leach into groundwater, especially in soils with little soil organic matter.

DDT may reach surface waters primarily by runoff, atmospheric transport, drift, or by direct application (e.g., to control mosquito-borne malaria). The reported half-life for DDT in the water environment is 56 days in lake water and $\sim$28 days in river water. The main pathways for loss are volatilization, photodegradation, adsorption to waterborne particulates, and sedimentation. Aquatic organisms, as noted above, also readily take up and store DDT and its metabolites.

Ecotoxicology

Birds are generally only slightly affected or not affected by direct exposure to DDT. Exposure is through the food chain by consuming organisms such as fish and earthworms with significant body burdens of DDT. The major concern has been eggshell thinning and altered reproductive success. It is thought that DDE is responsible for thinning of the egg shell. This seems more pronounced in predator birds. Alterations in mating behavior have also been linked to DDT exposure.

DDT is highly toxic to aquatic invertebrates. Early developmental stages are more susceptible than are the

adults. The same level of toxicity is also seen in a large number of fish including coho salmon, rainbow trout, northern pike, black bullhead, bluegill sunfish, largemouth bass, and walleye. In addition, DDT may bioaccumulate in fish, leading to long-term exposure as well as to exposure of other species that prey on fish.

Nontarget species such as bees and earthworms are not affected.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.02\,mg\,kg^{-1}$.
- Reference dose is $0.0005\,mg\,kg^{-1}\,day^{-1}$.
- Permissible exposure limit is $1.0\,mg\,m^{-3}$ (8 h).

Further Reading

Jaga K and Dharmani C (2003) Global surveillance of DDT and DDE levels in human tissues. *International Journal of Occupational Medicine and Environmental Health* 16(1): 7–20.

Turusov V, Rakitsky V, and Tomatis L (2002) Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence, and risk. *Environmental Health Perspectives* 110(2): 125–128.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for DDT, DDE, DDD.

http://extoxnet.orst.edu – Extension Toxicology Network, a collaborative effort of University of California, Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho; search for DDT.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for DDT.

Decane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 124-18-5
- Synonyms: Decane; UN2247 (DOT) (also called Alkane C(10); Decyl hydride; *n*-Decane)
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon (C10)
- Chemical Formula: $C_{10}H_{22}$

Uses

Decane is a constituent in the paraffin fraction of petroleum and is also present in low concentrations as a component of gasoline. It is used as a solvent, in organic synthesis reactions, as a hydrocarbon standard, in the manufacture of petroleum products, in the rubber industry, in the paper processing industry, and as a constituent in polyolefin manufacturing wastes. Decane is a flammable liquid (at room temperature) that is lighter than water.

Exposure Routes and Pathways

Because decane can exist as a liquid and a vapor at normal temperature and pressure, exposure could occur by either dermal contact or inhalation; oral exposure would most likely be either incidental or accidental. Decane can be detected in urban air (up to 3 ppb) as a result of automobile emissions.

Mechanism of Toxicity

Decane is generally considered to be fairly nontoxic, relative to other aliphatic hydrocarbons. This is probably due to the fact that it is less volatile than octane or heptane and may not be as readily transferred across either the pulmonary alveoli or the blood–brain barrier. If it is aspirated into the lungs, however, decane will cause adverse effects similar to those seen with heptane or octane.

Using *in vitro* and/or microbial systems, decane has been shown to be metabolized to decanol and is thus thought to be readily biodegradable in the natural environment.

Human Toxicity

Adverse effects to humans would be expected to be similar to those seen in laboratory animals (see below). There is currently no industrial air standard for occupational exposure to decane.

Animal Toxicity

Decane has been shown to have narcotic effects in both mice and rats, primarily in experiments documenting acute exposure at high concentrations. One study estimated a 2 h LC_{50} of $72\,300\,mg\,m^{-3}$ in

rats. In mice, an intravenous dose of 912 mg kg^{-1} is expected to cause death in 50% of the experimental animals. Another rat study showed that a concentration of 540 ppm in air (18 h day^{-1}, 7 days week^{-1}, 8 weeks) had a significant positive effect on weight gain. This exposure also caused some slight adverse effects (e.g., decreased white blood cell count) but no significant toxic effects overall.

Clinical Management

Persons who are exposed to high concentrations of decane in air should vacate or be removed from the source and seek fresh air. Upon oral ingestion, vomiting should not be induced as pulmonary aspiration may occur, resulting in severe narcosis and/or death. In areas of expected increased concentration, extreme care must be taken to use explosion-proof apparatus and keep the areas free from ignition sources, such as sparks from static electricity.

Aquatic Toxicity

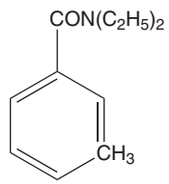
The US Environmental Protection Agency ECOTOX database has very few records for decane. An acute (48–96 h) no-observed-effect concentration of 500 mg l^{-1} was recorded for the sheepshead minnow (saltwater fish). Acute effects (48 h exposure) on the water flea (*Daphnia magna* LC_{50}) ranged from 1.3 to 29 mg l^{-1} (freshwater cladocercan).

See also: Heptane; Octane.

DEET (Diethyltoluamide)

Mark L Winter

- Chemical Abstracts Service Registry Number: CAS 134-62-3
- Synonyms: Detamide; Autan; I-Delphene; Metadelphene; Black Flag; Tabarad; Delphene; Dieltamide; Flypel; Muskol; Naugatuck Det; Off; 612 Plus; Jungle plus; Pellit; DETA; DET; *N*,*N*-Diethyl-3-methylbenzamide; *N*,*N*-Diethyl-*m*-toluamide
- Chemical/Pharmaceutical/Other Class: Methyl benzamide repellent
- Chemical Structure:



Uses

DEET is used as an insect repellent.

Exposure Routes and Pathways

DEET is available in solutions, lotions, gels, aerosol sprays, sticks, impregnated towlettes, and wristbands. Dermal and ocular exposures are the most common exposure pathways.

Toxicokinetics

Approximately 50% of each topically applied dose of DEET is absorbed within 6 h. Peak plasma levels are attained within 1 h. Ingestion of DEET may result in symptoms within 30 min, implying very rapid absorption. Oxidative enzymes in the liver metabolize DEET. Metabolites of DEET have not yet been characterized. Following movement through the skin, DEET is absorbed and distributed rather rapidly. Some studies indicate, however, that DEET and metabolites can remain in the skin and fatty tissues for 1 or 2 months after topical application. Ingestion of 50 ml of 100% DEET by adolescents or adults has resulted in severe toxicity and death. Ingestion of 25 ml of 50% DEET by a 1-year-old child resulted in severe toxicity.

Mechanism of Toxicity

DEET is primarily toxic to the central nervous system (CNS). The mechanism of toxicity is still unknown.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral LD_{50} values were reported as 1800–2700 mg kg^{-1} in male rats and 1750–1800 mg kg^{-1} in females. Rats given an LD_{50} dose showed signs of

toxicity that included lacrimation, chromodachryorrhea, depression, prostration, tremors, asphyxial convulsions, and respiratory failure. Ocular administration of DEET led to mild to moderate edema of the nictitating membrane, lacrimation, conjunctivitis, and corneal injury. These signs dissipated within 5 days. DEET is not a sensitizer in guinea pigs.

Human

A toxic syndrome consisting of ataxia, hypertonicity, tremor, and clonic jerking, and progressing to coma and seizures, may occur after dermal or oral exposure. Symptoms may occur within 30 min after an acute ingestion. Dermal exposure may cause irritation, sensitization, and erythema. A study with volunteers using 75% DEET showed that 48% had severe dermal reactions at the crease of the elbow but not at other dermal application sites.

Chronic Toxicity (or Exposure)

Animal

Dietary exposure to DEET for 200 days at 10 000 ppm led to growth retardation. Relative increases in testes and liver weights were noted in male rats, liver, and spleen in female rats, and kidneys of both sexes. No significant changes were noted at 500 ppm. Dermal application of DEET ($1\,g\,kg^{-1}\,day^{-1}$) led to reproductive toxicity in rats. No teratogenic response was noted in rabbits treated with dermal dosages as high as $5\,g\,kg^{-1}\,day^{-1}$. DEET may cause testicular and renal hypetrophy with repeated dosing.

Human

Chronic application of 70% DEET solution caused paranoid psychosis, pressurized speech, flight of ideas, and delusions after 2 weeks of daily application for the inappropriate treatment of a skin rash. Repeated application causes erythema. Extensive daily dermal application of 10–15% DEET for 2 days to 3 months has resulted in encephalopathy in children. Toxic encephalopathy has been associated with DEET in children. Signs of toxicity included agitation, weakness, disorientation, ataxia, seizures, coma, and, in three cases, death. As part of the Reregistration Eligibility Decision on DEET released in 1998, however, the US Environmental Protection Agency reviewed all available data on the toxicity of DEET and concluded that "normal use of DEET does not present a health concern to the general US population."

Clinical Management

Basic life-support measures for respiratory and cardiovascular function should be utilized. Dermal decontamination should be accomplished by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min. If eye irritation persists after irrigation, medical assistance should be sought. Ipecac-induced emesis is not recommended in cases of accidental oral exposure as coma and seizures can occur rapidly within 30 min to 1 h of ingestion. Gastric lavage should be performed cautiously with a small-bore soft nasogastric tube with small aliquots of water or saline. Activated charcoal can also be used. Initial control of seizure activity may be attempted with a benzodiazepine. Respiratory depression, hypotension, dysrhythmias, and the need for endotrachael intubation should be monitored.

Environmental Fate

Little information is available on the environmental fate of DEET. DEET is stable to hydrolysis at environmental pH levels.

Ecotoxicology

DEET is slightly toxic to birds: the acute LD_{50} in quail was $1375\,mg\,kg^{-1}$. DEET appears only slightly toxic to fish. The 24 h and 96 h LC_{50} values in trout were 125 and 172 ppm. DEET is slightly toxic to Daphnia, with a 48 h EC_{50} of 75 ppm.

See also: Benzodiazepines; Charcoal; Toxicity Testing, Dermal.

Further Reading

Sudakin DL and Trevathan WR (2003) DEET: A review and update of safety and risk in the general population. *Journal of Toxicology. Clinical Toxicology* 41: 831–839.

Relevant Websites

http://pmep.cce.cornell.edu – Cornell University.
http://www.epa.gov – US Environmental Protection Agency.

DEF (Butyl Phosphorotrithioate)

Priya Raman

- SYNONYM: Tributyl *S,S,S*-phosphorotrithioate
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Organophosphorus herbicide
- CHEMICAL STRUCTURE: $(C_4H_9\text{–}S)_3P{=}O$

Uses

DEF is used as a cotton defoliant to facilitate mechanical harvesting.

Exposure Routes and Pathways

The major route of exposure to DEF is skin contact.

Toxicokinetics

DEF is readily absorbed through the skin. It is metabolized to *n*-butyl mercaptan in the gastrointestinal tract by hydrolysis. The metabolite is excreted in the urine. A urinary metabolic profiling following oral administration of DEF to a lactating goat revealed that DEF is efficiently metabolized to many metabolites. The amount of DEF in liver, kidney, and muscle represented $<1\%$ of the total residue. A major metabolite, 3-hydroxybutylmethyl sulfone was found in the tissue, milk, and urine. The hydrolytic products of DEF, *S,S*-dibutyl phosphorodithioate and *S*-butyl phosphorothiate were identified as minor components in urine, comprising 5% and 4% of the total residue, respectively.

Mechanism of Toxicity

DEF is a relatively weak inhibitor of acetylcholinesterase. The compound is hydrolyzed to a large extent in the intestine to *n*-butyl mercaptan, which is responsible for the late acute effects of DEF. The putative molecular target in neural tissue for initiation of delayed neuropathy is neurotoxic esterase or neuropathy target esterase (NTE).

Acute and Short-Term Toxicity (or Exposure)

Animal

DEF produces profound hypothermia in rats, mice, and guinea pigs by inhibition of thermogenesis. Its actions on heat conservation and motor control are, however, minimal. It is effective against both shivering and nonshivering thermogenesis and completely blocks the increase in body temperature evoked by anterior hypothalamic stimulation. The toxicologic effect of DEF, the extent and permanence of injury, and the progression or improvement of clinical signs of toxicity depended on the dose, duration, and route of exposure.

Human

Late acute poisoning from DEF is related to the release of its breakdown product, *n*-butyl mercaptan. Signs of toxicity appear within 1 hr after exposure and include general weakness, malaise, sweating, nausea, vomiting, anxiety, and drowsiness. DEF affects the lymphocyte NTE in exposed workers.

Chronic Toxicity (or Exposure)

Animal

A subchronic administration of DEF caused three toxicologic effects in hens, depending on the route of exposure: (1) an acute cholinergic effect resulting from inhibition of acetylcholinesterase, relieved by atropine, not associated with neuropathological lesions; (2) a late acute effect in chickens resulting from *n*-butyl mercaptan toxicity 4 days after oral administration of daily large doses of DEF resulting in darkening and drooping of the comb, loss of appetite and weight, weakness, emaciation, paralysis, and death, not relieved by atropine nor associated with histopathological changes in nerve tissues; and (3) delayed neurotoxicity after a delay period following topical application causing axonal and myelin degeneration resulting in ataxia, paralysis, and death.

Human

Both intensity and length of exposure play important roles in determining the extent of inhibition of NTE in lymphocytes; 50% of preexposed values of NTE activity were obtained when measured 3 or 4 weeks after the beginning of DEF exposure. However, there is no direct evidence of a correlation between a high level of lymphocyte NTE inhibition and development of neuropathy in humans. Blood acetylcholinesterase and plasma butyrylcholinesterase levels remained unchanged during the study period. There is no available weight-of-the-evidence summary assessment for DEF as a developmental or reproductive toxin.

In Vitro Toxicity Data

DEF has been reported to cause extensive alterations in morphological features of erythrocyte and nuclear membranes and affected the permeability properties of rat liver mitochondrial membrane. A reduction in the activity of cytochrome-*c*-oxidase and NAD. H-oxidase has also been observed. Content of both DNA and RNA decreased in tissues studied within 1 month of DEF intoxication and was usually restored within 3 months. Histological study showed development of necrodystrophy in liver tissue and of fibroplastic glomerulonephritis in kidney. The deteriorating effect of DEF on cellular genome functions relates not only to its cytotoxicity but also to the cancerogenic and mutagenic properties of the pesticide.

Clinical Management

Supportive and symptomatic treatment should be provided to the patient following accidental or intentional exposure to DEF.

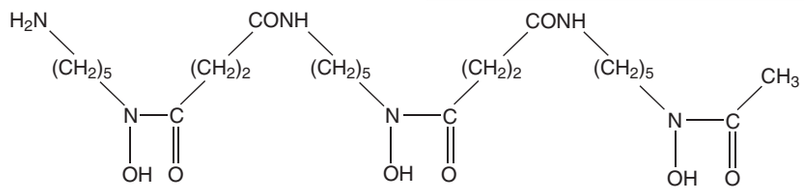
See also: Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphates; Pesticides.

Deferoxamine

Greene Shepherd

This article is a revision of the previous print edition article by Zhengwei Cai, volume 1, pp. 403–404 © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 70-51-9
- Synonyms: *N*-Benzoylferrioxamine B; Deferoxaminum; Deferrioxamine; Desferal; Desferral; Desferrin; DFO; DFOA; DFOM; Desferrioxamine B; Propionohydroxamic acid
- Chemical/Pharmaceutical/Other Class: Deferoxamine is an iron-chelating agent. It is an iron-free derivative of ferrioxamine B, which belongs to a group of siderophores, growth factors for certain microorganisms
- Chemical Formula: $C_{25}H_{48}N_6O_8$
- Chemical Structure:



Uses

Deferoxamine is widely used for treatment of both acute iron intoxication and iron-overload anemia. Recently, it has been used in trials of malaria treatment.

Exposure Routes and Pathways

In acute cases, intravenous injection and oral ingestion are the most common routes of administration. Nightly subcutaneous infusions combined with monthly intravenous infusions are used in chronic illness.

Toxicokinetics

Deferoxamine is poorly absorbed from the gastrointestinal tract. When given orally in the setting of an iron overdose, deferoxamine has been shown to bind with iron-forming ferrioxamine, which promotes absorption. The absorbed complex is cleaved during first-pass metabolism resulting in free iron capable of damaging hepatic tissue.

Deferoxamine metabolism takes place in primarily the plasma and occurs rapidly. Of the three metabolites produced, the major one is known as metabolite C. Other organs may also have some metabolizing capacity. Deferoxamine has a very high affinity and specificity for the ferric iron and chelates it in a 1:1 molar ratio. Its elimination half-life in human plasma is between 10 and 30 min. Deferoxamine mainly distributes in blood, but bile excretion is a possible elimination route. Renal clearance is the major elimination route of deferoxamine in humans. It accounts for about one-third of the total body clearance; 0.296 and $0.234\,l\,h^{-1}$ in healthy and hemochromatotic adults, respectively. Once formed in the blood ferrioxamine is rapidly excreted

unchanged in the urine. Its elimination half-life is 5.9 and 4.6 h in normal and hemochromatosis adults, respectively.

Mechanism of Toxicity

Deferoxamine has some serious side effects including infusion rate related hypotension, renal insufficiency, neurotoxicity, growth retardation, and bacterial infections. Deferoxamine may induce venous dilation leading to poor venous return, depressed cardiac output, and eventually hypotension. An acute decrease in glomerular filtration rate and renal plasma flow is the possible mechanism underlying the nephrotoxicity induced by deferoxamine. Depletion of iron, translocation of copper, and chelation of other trace elements including zinc, due to excessive deferoxamine, may interfere with critical iron-dependent enzymes and cause oxidative damage within neural tissue. These are possible mechanisms responsible for deferoxamine-induced neurotoxicity and growth retardation.

The iron–deferoxamine complex, ferrioxamine, is a growth factor for many bacteria and by providing easily utilized iron, deferoxamine has been associated with *Yersenia enterocolitica* overgrowth with prolonged therapy. *In vitro* studies have shown that deferoxamine inhibits the synthesis of prostaglandin, hemoglobin, ferritin, collagen, and DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} of deferoxamine in mice and rats is $>325\,mg\,kg^{-1}$ with intravenous administration and $>1000\,mg\,kg^{-1}$ with oral administration. Deferoxamine-induced hypotension, tachycardia, and renal insufficiency have been also reported in rats and dogs.

Human

Rapid infusion of deferoxamine over 15 min results in hypotension and tachycardia. An infusion rates of $15\,mg\,kg^{-1}\,h^{-1}$ or longer is recommended. Intravenous deferoxamine administration has been reported to cause renal insufficiency indicated by a progressive increase in serum creatinine and decrease in creatinine clearance.

Patients treated with deferoxamine chronically may develop neurotoxicity manifested as visual and hearing losses, growth retardation, and bacterial infections.

Chronic Toxicity (or Exposure)

Animal

Dogs given subcutaneous injections of high-dose deferoxamine developed lens opacities.

Human

Patients with inherited or acquired anemias that require regular blood transfusions frequently have symptoms or laboratory evidence of iron overload. Deferoxamine given subcutaneously at $40\,mg\,kg^{-1}$ over 8–12 h has been the standard of therapy for these patients. Patients receiving higher doses (e.g., $125\,mg\,kg^{-1}$) demonstrated ocular toxicity of blurriness, loss of night vision, and optic neuropathy. Auditory toxicity has been noted as well. Up to 25% of patients on chronic deferoxamine have some impairment of high-frequency hearing.

In Vitro Toxicity Data

Studies of a rat model of intracerebral hemorrhage have noted that deferoxamine treatment reduced oxidative stress from iron release. Iron chelators may have a role in preventing damage associate with strokes.

Clinical Management

For patients who develop hypotension secondary to deferoxamine, the infusion should be discontinued and restarted at a slower rate after recovery of the blood pressure. Fluids and pressors should be used to support the patient. Patients experiencing anaphylactic reactions should discontinue deferoxamine and start treatment with antihistamines and steroids. Patients who develop infections with *Yersinia* species secondary to deferoxamine use should be started on antibiotics (e.g., sulfamethoxasole–trimethoprim) while cultures and sensitivities are pending. Patients experiencing neurologic complications should have the deferoxamine discontinued and a neurologist should be consulted.

See also: Iron; Neurotoxicity.

Further Reading

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Howland MA (1996) Risks of parenteral deferoxamine for acute iron poisoning. *Journal of Toxicology: Clinical Toxicology* 34(5): 491–497.

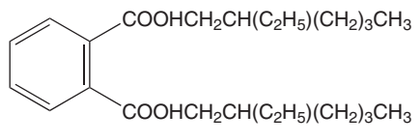
Olivieri NF, Buncic JR, and Chew E (1986) Visual and auditory neurotoxicity in patients receiving subcutaneous deferoxamine infusions. *New England Journal of Medicine* 314: 869–873.

Tenenbein M, Kowalski S, and Sienko A (1992) Pulmonary toxic effects of continuous deferoxamine administration in acute iron poisoning. *Lancet* 339: 699–701.

DEHP (Di-Ethyl Hexyl Phthalate)

Raja S Mangipudy and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 117-81-7
- Synonyms: Di(2-ethylhexyl) phthalate; Dioctyl phthalate; DOP; BEHP; Compound 889; DAF 68; Octyl phthalate; Ethylhexyl phthalate; OCTOIL
- Chemical Structure:



Uses

Di-ethyl hexyl phthalate (DEHP) is a softening agent commonly used in plastics such as polyvinyl chloride (PVC). It is found in products such as telephone cords, kidney dialysis machine tubing, medical plastic bags, shower curtains, vinyl wall coverings, and children's toys.

Exposure Routes and Pathways

DEHP is readily absorbed via ingestion; it is not absorbed significantly via dermal contact. Inhalation exposure is not likely because of the compound's low vapor pressure.

Toxicokinetics

DEHP is absorbed by the oral and parenteral routes. It is not absorbed in any significant amount through intact skin. Intravenously or orally administered DEHP is rapidly metabolized to derivatives of mono-(2-ethylhexyl) phthalate (MEHP). The MEHP metabolites are mainly excreted in the urine and the bile. The estimated half-life of DEHP in humans following intravenous administration is 28 min.

Mechanism of Toxicity

DEHP is a peroxisome proliferator, so named because it causes extensive proliferation of hepatic peroxisomes in susceptible species. As peroxisome proliferators are uniformly carcinogenic in rats and mice, there is concern as to whether this effect is rodent specific or whether it can manifest in other species, including humans via drugs used in long-term therapy or through blood transfusion bags.

Acute and Short-Term Toxicity (or Exposure)

Animal

The maternal–fetal transfer of DEHP and its major metabolite, MEHP, has been demonstrated in rats, and associated with the inhibition of brain and liver steroidogenesis in exposed offspring. In female rats, DEHP administration can suppress estradiol levels and ovulation. A possible mechanism for this effect is the inhibition of the enzymatic formation of estradiol by MEHP.

The oral administration of DEHP to pregnant mice on days 0–17 of gestation can cause exophthalmia and exencephaly, as well as malformations of the tail, major vessels, ribs, and vertebrae. Similar teratogenic effects were observed when larger doses of DEHP were fed to pregnant mice on gestational days 6, 7, 8, 9, or 10. Although in sufficient doses DEHP is fetotoxic in rats, several studies did not find DEHP to produce an increase in malformations in rats at any dose tested and a 1984 summary source found available data insufficient to conclude that DEHP increases congenital malformations in this species. A detailed analysis of the developmental toxicity of DEHP in rats, completed in the fall of 2000, reviewed many of the more recent studies and found minimal indications of congenital malformations in the absence of maternal toxicity, except for effects on genital development in males. In rats, a maternal dose of 750 mg DEHP per kilogram per day during late gestation reduced testosterone levels in male offspring and reduced anogenital distance by an average of 36%. The authors of this report did not find evidence that DEHP interferes with the binding of testosterone to the androgen receptor, but suggested that these effects were a result of the testicular toxicity of DEHP (see below).

An evaluation of the developmental effects of DEHP by the National Toxicology Program estimated that the no-observed-adverse-effect level (NOAEL) for DEHP in sensitive species to be 44 mg kg^{-1} day^{-1}, more than 500 times the estimated daily human intake. A more recent estimate found a similar wide disparity between typical oral exposures to DEHP in humans and an NOAEL in rodents of 4–14 mg kg^{-1} day^{-1}.

The testicular toxicity of DEHP and other phthalic acid esters is well documented in various animal species. The monoester (MEHP) appears to be the active toxicant and young animals appear more susceptible than older animals. Human testicular toxicity from these compounds has not been demonstrated but concern has been registered about the potential for testicular toxicity in male infants who are exposed to procedures involving PVC tubing, such as extracorporeal membrane oxygenation. Estimates for DEHP exposure in these children are imprecise, but range up to 30 mg kg^{-1} day^{-1}. The concern about this level of exposure in neonates was addressed in part by a study of neonatal male rats treated for 3 weeks with either intravenous or oral DEHP. The intravenous doses were 60, 300, and 600 mg kg^{-1} day^{-1}, and the oral doses were 300 and 600 mg kg^{-1} day^{-1}. There were no adverse effects of 60 mg kg^{-1} day^{-1} intravenously. The 300 and 600 mg kg^{-1} day^{-1} doses were associated with decreased testicular weight and associated histologic changes consistent with impaired spermatogenesis. By 90 days of age, after being permitted to recover, the animals that had been treated intravenously had no residual histologic changes, although testis weight had not completely recovered. The orally treated animals had residual decreases in testis weight and abnormal histopathology findings. There were no changes in sperm motility, epididymal and testicular sperm count, or morphology at any dose at any time.

Human

The effects of DEHP on humans have not been established.

Human data on the possible pregnancy effects of intravenous DEHP are limited to a 1981 study of 19 gravid women exposed to hemodialysis for renal failure or drug overdose. These pregnancies resulted in two stillbirths and more than half of the infants were premature and of low birth weight; however, no malformations were observed and neonatal growth was normal. There is no evidence that the adverse outcomes reported were due to DEHP rather than to the medical complications or other drug exposures that occurred during the pregnancies. This report did not specifically monitor or address the likely exposure to DEHP from dialysis equipment in the population studied. Despite an absence of defined embryotoxic effects, the restriction of exposure to DEHP during pregnancy has often been recommended. A specific focus has been placed on the use of freshly drawn blood products for transfusion during pregnancy to avoid the intravenous administration of DEHP, which may reach a concentration of 5 mg dl^{-1} in whole blood when it has been stored in PVC blood bags for 21 days.

More recently, the use of other plasticizers in blood and intravenous fluid delivery systems has obviated concerns associated with DEHP.

Chronic Toxicity (or Exposure)

Animal

DEHP has been shown to produce liver cancer in mice and rats after lifetime exposure.

Clinical Management

The potential for esophageal or gastrointestinal tract irritation following ingestion suggests that emesis should not be induced. Other measures to prevent absorption may be beneficial. Exposed skin and eyes should be copiously flushed. Liver function and blood glucose must be monitored.

See also: Peroxisome Proliferators; Polymers.

Further Reading

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Delaney Clause

Robin C Guy

This article is a revision of the previous print edition article by Shayne C Gad, volume 1, p. 405, © 1998, Elsevier Inc.

Background Information

In 1958, the US Food and Drug Administration's (FDA) Food, Drug, and Cosmetic Act (FD&C Act) was amended to include a clause that essentially banned the use of food additives and pesticides which were shown to cause cancer in humans or animals. The Delaney Clause was contained in Section 409 (348(c) (3) (A)) of the FD&C Act. Section 409 lays out requirements for the use of food additives, including pesticide residues. The Delaney Clause states "no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal."

This clause regulates pesticide residues in processed foods to mean that carcinogenicity potential is the only factor, and that any benefits of the pesticide or food additive may not be considered. In addition, it set up a 'zero-cancer-risk' standard for food additives. If residues of carcinogenic pesticides are found to concentrate in processed foods, the US Environmental Protection Agency (EPA) cannot set a tolerance or maximum legal limit for that pesticide/food combination. Later, Congress added the same zero-cancer-risk clause for amendments governing new animal drugs and color additives (1960 Color Additives Amendment).

The birth of the Delaney Clause can be traced back to a 1950 resolution in the US House of Representatives that charged a House select subcommittee to investigate the use of chemicals in foods. Among the subcommittee's responsibilities was an examination of the 'nature, extent, and effect' of 'chemicals, compounds, and synthetics' on all facets of food production. The subcommittee was chaired by James J. Delaney, a New York Democrat.

While the Delaney Clause prevented the use of possibly dangerous chemicals such as diethyl-stilbestrol (DES), some prospectively useful substances were banned because improved analytical testing procedures were able to detect very small quantities of possible carcinogens. When the Delaney Clause was introduced, analytical testing procedures detected substances in concentrations of parts per million. It later became possible to detect substances in concentrations of one part per billion or trillion, making it far more probable that traces of a carcinogen be detected. Worsening this problem was the fact that tested substances are administered to animals at the maximum tolerated dose, far more than would be normally ingested. The Delaney Clause was criticized by many scientists who believed that its zero-tolerance standard was impossibly high.

The Delaney Clause was eventually replaced with a new law, the Food Quality Protection Act (FQPA) of 1996, which advanced a new standard of 'reasonable certainty of no harm'. Prior to the passage of the FQPA, the FDA had been employing Delaney in the case of food additives and animal drugs in a similar manner, that is, 'reasonable certainty of no harm'. FDA incorporated the idea of safety into its color additive regulations. Currently, under 21 CFR 70.3(i), a color additive is 'safe' if 'there is convincing evidence that establishes with reasonable certainty that no harm will result from the intended use of the color additive'. The EPA was following the zero-tolerance standard. The FQPA ended the application of the Delaney Clause to pesticide tolerance levels. FQPA would allow EPA to determine what level of risk will be adequate to protect the public health as long as the dietary risk posed to food consumers is negligible.

See also: Carcinogenesis; Food Quality Protection Act, US; Pesticides.

Further Reading

Merrill RA (1997) Food safety regulation: reforming the delaney clause. *Annual Review of Public Health* 18: 313–340.

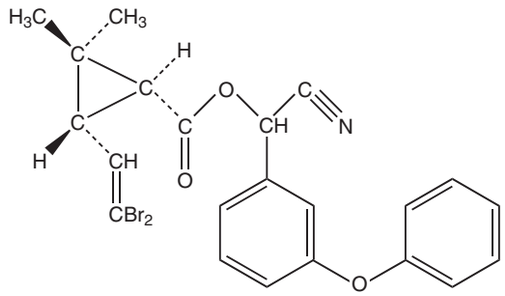
Relevant Websites

http://www.epa.gov – US Environmental Protection Agency (EPA). Environmental laws that establish EPA's authority.
http://vm.cfsan.fda.gov – US Food and Drug Administration (FDA). What are FDA requirements for Food Additives?

Deltamethrin

Paul R Harp

- Chemical Abstracts Service Registry Numbers: CAS 52918-63-5; CAS 62229-77-0; CAS 55700-96-4
- Synonyms: *S*-α-Cyano-3-phenoxybenzyl-(1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate; Butox; Decis; K-Othrine; Kordon; Sadethrin; AEF 032640; NRDC 161; OMS 1998; RU 22974; SHA 209400
- Chemical/Pharmaceutical/Other Class: Type II pyrethroid insecticide
- Chemical Structure:



Uses

Deltamethrin is a broad-spectrum insecticide used in a variety of agricultural applications. It is available as a wettable powder, granule, emulsifiable concentrate, concentrate for ULV application, and as a concentrate for thermal fogging.

Exposure Routes and Pathways

Exposure to deltamethrin has occurred through ingestion, inhalation, and dermal contact.

Toxicokinetics

Pyrethroids are poorly absorbed through the skin and are only moderately absorbed in the gastrointestinal tract. Metabolism of deltamethrin occurs rapidly through ester cleavage and hydroxylation. Deltamethrin is eliminated more slowly from adipose tissues than from other sites such as brain or blood. In one case of dermal exposure, absorption was estimated to be ~3%. Urinary excretion is the primary route of elimination.

Mechanism of Toxicity

Several mechanisms of action have been identified for the pyrethroids with the primary mechanism related to a selective high affinity for membrane sodium channels. Closing of the channel, which ends the action potential, is slowed resulting in a prolonged 'tail' current and repetitive firing of presynaptic and accompanying postsynaptic cells following a single action potential. High enough doses can cause complete depolarization and blockade of nerve conduction. Deltamethrin also inhibits Ca^{2+},Mg^{2+}-ATPase and type II pyrethroids such as deltamethrin have been shown to act on γ-aminobutyric acid-mediated chloride ionophores and voltage-sensitive calcium-independent chloride channels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Symptoms include hyperactivity, incoordination, choreoathetosis, and convulsions.

Human

Extensive dermal exposure causes temporary effects of paresthesia (stinging, burning, tingling) and numbness. Symptoms following ingestion include nausea, vomiting, tenesmus, diarrhea, unconsciousness, and death due to respiratory failure.

Chronic Toxicity (or Exposure)

Animal

Chronic effects following deltamethrin exposure have not been reported.

Human

Chronic effects following deltamethrin exposure have not been reported.

Clinical Management

Exposed skin should be washed promptly with soap and water. Dermal application of vitamin E oil preparations may be used for both prophylaxis and treatment of paresthesia. For contact with eyes, flush immediately and for an extended period with generous amounts of clean water or saline. Gastric lavage is indicated if patient has ingested a large amount of pyrethroids and can be treated soon after exposure.

For ingestion of smaller amounts or if treatment has been delayed, activated charcoal and catharsis are indicated. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenytoin or phenobarbital may be helpful for recurrent seizures. No specific antidotes for pyrethroid-induced neurotoxic effects have been approved for use in humans. Spontaneous recovery usually occurs with mild or moderate intoxication.

Environmental Fate

Deltamethrin degrades in soil within 1–2 weeks. Deltamethrin is rapidly adsorbed primarily by sediment, is rapidly taken up by plants, and evaporates from surface water. No detectable residues exist on plants after $\sim$10 days.

Ecotoxicology

Deltamethrin is toxic to bees. Fish and crustaceans are extremely sensitive to pyrethroid compounds in laboratory settings. However, various factors (e.g., sediment binding) may reduce pyrethroid toxicity to these nontarget organisms in a natural environment.

Exposure Standards and Guidelines

The acceptable daily intake for deltamethrin is $0.01\,\mathrm{mg\,kg^{-1}\,day^{-1}}$. The reference dose is $0.0001\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.

See also: Neurotoxicity; Pesticides; Pyrethrins/Pyrethroids.

Further Reading

Vijverberg HPM (2000) Pyrethroids. In: Spencer PS and Schaumburg HH (eds.) *Experimental and Clinical Neurotoxicology*, 2nd edn., pp. 1028–1044. New York: Oxford University Press.

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http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Deodorants and Antiperspirants

Zhengwei Cai and Pertti J Hakkinen

The main function of sweating is to control body heat. Sweat glands are most numerous in the armpits, groin, and feet, and the numbers have a genetic basis varying from person to person. Sweat is not a significant route for eliminating toxins from the body. In addition to producing sweat, sweat glands have been implicated in being able to secrete sex pheromones and other substances. Some substances in sweat can react with the bacteria normally found in the armpit, and the reaction can produce an unpleasant odor; however, the genetic variation in the ability to smell various substances means that people may perceive the same body odor in different ways.

Reducing perspiration in the underarm does not affect the body's ability to regulate temperature, or the body's ability to excrete substances of any kind. There are many sweat ducts all over the body, and only a small percentage of them are in the underarms. Thus, reducing the flow of perspiration in the underarms does not affect the body's ability to regulate temperature since the sweat glands in other parts of the body can adequately control the overall body temperature.

There are basically two types of deodorants: simple deodorants and antiperspirants. Deodorants help control odor primarily by masking the odor caused by the bacteria interacting with perspiration and by reducing odor-causing bacteria. Deodorants have no effect on decreasing sweat. Antiperspirants help control wetness, and thereby odor, by slowing the flow of perspiration to the surface of the skin.

A simple deodorant consists of an antibacterial agent in a cream base. Antiperspirant ingredients ('aluminum salts') such as aluminum chlorohydrate, activated aluminum chlorohydrates, and aluminum–zirconium–glycine (AZG) complexes work by forming superficial plugs in the sweat ducts, reducing the flow of perspiration. Antiperspirants are available in four product types: cream, liquid, powder, or stick. They usually include aluminum salts, titanium dioxide, oxyquinoline sulfate, zirconium salt, alcohol, and antibacterial agents. Some liquid forms are propellant dispensed (aerosols). Waxes, soap, and humectants may be present in minor proportion in stick forms. Roll-on types may be added with

emulsifiers and thickeners. The amounts of ingredients present in these products are usually small, and unless a large quantity is ingested, no ill effect should ensue.

Deodorant efficacy is evaluated by sensory assessments performed by an expert panel. The sensory assessment by an 'expert panel' can be somewhat misleading since it can involve professional armpit sniffers, a profession classified by some websites as one of the 'jobs your mother may never have wanted you to have' and among the worst possible jobs! Various measurement methods are used to demonstrate the efficacy of antiperspirants, including a gravimetric method, water evaporation quantification, electrodermal measurements, staining procedures, dye injections, and cyanoacrylate skin surface strippings and casting replicas. Other useful methods include visualization of apocrine gland excretion, and the collection of sweat and volatile compounds. Microbiological assessments and chromatographic analysis are also performed.

In case of oral ingestion, the mouth can be rinsed out, and milk can be given for soothing and diluting effect. These products are nonirritating to most people, but sensitization may occur in some individuals. For these people, the preparation should be washed off thoroughly and a substitute brand may be chosen. Discontinuing use of deodorant may be necessary. Shaving the underarms can impact the outer layers of the skin that protect the body, causing susceptibility to cuts, rashes, and various forms of skin irritation. Because of this, some formulations now contain chemicals that help to renew and protect underarm skin. Aerosol products may cause eye irritation. The eyes should be washed carefully with lukewarm water for a few minutes, and soothing eye drops may be helpful.

The (US) Food and Drug Administration has issued a final rule in 2003, in the form of a final monograph establishing conditions under which over-the-counter antiperspirant drug products (including deodorants) are generally recognized as safe and effective. This rule has been in effect from December 2004.

Antiperspirants have been implicated in breast cancer and in the 'aluminum hypothesis' of Alzheimer's disease. However, various experts and health organizations, e.g., the American Cancer Society and (US) National Cancer Institute, have reported that there is no apparent link with breast cancer. Further, expert reviews have found no consistent relationship between Alzheimer's disease and exposures to aluminum-containing medications, antiperspirants, drinking water, or other materials or products.

A sudden change in sweat production can signal other problems ranging from damage to the autonomic nerves controlling the sweat pores, to various hormonal disorders and obesity. A high level of sweating is called hyperhidrosis, and botulism toxin injections have been used to control hyperhidrosis for several months at a time. Another approach used to control hyperhidrosis is endoscopic thoracic sympathectomy, which involves severing the nerves controlling the hyperactive sweat glands.

See also: Aluminum; Cosmetics and Personal Care Products.

Further Reading

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http://www.ctfa.org – US Cosmetic, Toiletry, and Fragrance Association (CTFA)

http://cis.nci.nih.gov – US National Cancer Institute, Cancer Facts on Antiperspirants/Deodorants and Breast Cancer.

http://hpd.nlm.nih.gov – US National Library of Medicine, "Household Products Database"; and "ToxTown." The Household Products Database links several thousand US consumer brands to health effects from Material Safety Data Sheets (MSDSs) provided by the manufacturers, and allows scientists and consumers to research products based on chemical ingredients.

http://www.heraproject.com – The Human and Environmental Risk Assessment (HERA) project.

Deoxyribonucleic Acid *See* Aneuploidy; Carcinogen–DNA Adduct Formation and DNA Repair; Chromosome Aberrations; DNA Phosphoramidites; Genetic Toxicology; Genomics, Toxicogenomics.

Dermal Toxicity Testing *See* Toxicity Testing, Dermal.

Desferrioxamine *See* Deferoxamine.

Desipramine *See* Tricyclic Antidepressants.

Detergent

Zhengwei Cai and Pertti J Hakkinen

Detergents are various surface-active agents (surfactants) particularly effective in dislodging foreign matter from soiled surfaces and retaining it in suspension. Soap, which is made from fats or fatty acids, is a detergent. However, in common usage the term 'detergent' applies to the synthetic nonsoap *substances*, not to soap, and also to *products* made from synthetic surfactants.

Surfactants and builders are the major components of cleaning products, with the builders serving to enhance or maintain the cleaning efficiency of the surfactants, primarily by reducing the water hardness. Other ingredients are added to formulations to provide functions such as increasing cleaning performance for specific soils/surfaces, ensuring product stability, and supplying a unique identity to a product. Examples include foam stabilizers, optical brighteners or whiteners, anti-redeposition agents, bleaching agents (chlorine-releasing agents) or bactericidal agents (mild concentrations of quaternary ammonium compounds), enzymes, fragrances, and dyes. Water is likely to be the major component of a liquid version of a detergent product.

Soaps and detergents are important for personal and public health. The (US) Soap and Detergent Association has noted that, through their ability to loosen and remove soil from a surface, soaps and detergents can (1) contribute to good personal hygiene, (2) reduce the presence of germs that cause infectious diseases, and (3) extend the useful life of clothes, tableware, linens, surfaces, and furnishings.

Soaps and detergents found in the home can be grouped into four general categories: personal cleansing, laundry, dishwashing, and household cleaning. The surfactants used in detergents have been developed to perform well under a variety of conditions, and are less sensitive than soap to the hardness minerals in water. Most surfactants will not form a film. Detergent surfactants were developed in response to a shortage of animal and vegetable fats and oils during World War I and World War II. In addition, a substance that was able to perform in hard water was desired to make cleaning more effective. Petroleum was used for the manufacture of these initial surfactants since it was widely available; however, detergent surfactants are now made from a variety of petrochemicals (derived from petroleum) and/or oleochemicals (derived from fats and oils).

Surfactants are usually classified by their ionic properties in water. Anionic surfactants are used in laundry and hand dishwashing detergents, household cleaners, and personal cleansing products. Linear alkylbenzene sulfonate, alcohol ethoxysulfates, alkyl sulfates, and soap are common anionic surfactants. Nonionic surfactants are low sudsing, and are typically used in laundry and automatic dishwasher detergents and rinse aids. The most widely used nonionic surfactants are alcohol ethoxylates. Cationic surfactants are used in fabric softeners and in fabric-softening laundry detergents. Other cationics are the disinfecting/sanitizing ingredients used in some household cleaners. Quaternary ammonium compounds are the major cationic surfactants. Amphoteric surfactants are used in personal cleansing and household cleaning products for their mildness, sudsing, and stability. Imidazolines and betaines are major amphoteric surfactants.

Even the different product types within a category of detergents are formulated in different product forms and with different ingredients selected to meet consumer desires for a selection of product types, to perform a broad cleaning function, and to deliver properties specific to that product. For example, laundry detergents and laundry aids are available as liquids, powders, gels, sticks, sprays, pumps, sheets and bars. They have been formulated to meet a variety of soil and stain removal, bleaching, fabric softening and conditioning, and disinfectant needs

under varying water, temperature, and use conditions. Further, the laundry detergents are either general purpose or light duty, with general-purpose detergents being suitable for all washable fabrics, and liquids working best on oily soils, and for pretreating soils and stains. Light-duty detergents are used for hand or machine-washing lightly soiled items and delicate fabrics.

Water alone will not remove oily, greasy soil on clothing since the oil and grease repel the water molecules; however, a surfactant's hydrphobic end is attracted to the oil and the hydrophilic end is attracted to the water molecules. These opposing forces loosen the soil and suspend it in the water. Warm or hot water helps dissolve grease and oil in soil. The agitation of the water and clothing in a washing machine, or rubbing clothing with the hands or an implement helps to pull the soil free from the clothing.

Exposure and risk assessors, and the developers of consumer products need to understand the reasonably foreseeable ways that consumers will use the products. Even a task as simple as dispensing a laundry detergent powder from its box into the washing machine could be done in several different ways by a consumer, resulting in different types and magnitudes of potential exposures. For example, the laundry powder could be poured from different heights above the washing machine, directly into the washing machine from the box, from the box into a measuring cup, etc. These and other possible differences in just how the product is dispensed could lead to meaningful differences in the types and magnitudes of inhalation and skin exposures to the powder.

Further, exposure and risk assessors, and the developers of consumer products need to understand the reasonably foreseeable ways that consumers will use a product in combination with other products. For example, laundry detergents are often used in combination with other products that might be useful for particular needs. For example, laundry aids contribute to the effectiveness of laundry detergents and provide special functions. Boosters enhance the soil and stain removal, brightening, buffering, and water softening performance of detergents, and are used in the washing machine in addition to the detergent. Enzyme presoaks are used for soaking items before washing to remove difficult stains and soils. Fabric softeners are added to the final rinse as a liquid, or to the clothes dryer on a nonwoven sheet. Pre-wash soil and stain removers are used to pretreat heavily soiled and stained garments. Starches, fabric finishes, and sizings are used in the final rinse or after drying. Water softeners are added to the wash or rinse to inactivate hard water minerals and increase cleaning power since detergents are more effective in soft water. Bleaches (chlorine and oxygen) are used to whiten and brighten fabrics and help remove stubborn stains, and liquid chlorine bleach (e.g., a sodium hypochlorite solution) can disinfect and deodorize fabrics.

In addition to laundry products, detergents are used in dishwashing products for hand and machine dishwashing. They are available as liquids, gels, powders, and solids. Further, many types of household cleaning products are available for consumers because no single cleaning product can provide optimum performance on all surfaces and soils. Thus, a broad range of products has been formulated to clean efficiently and easily, including liquids, gels, powders, solids, sheets and pads for use on painted, plastic, metal, porcelain, glass and other surfaces, and on washable floor coverings.

Human Safety

Human safety evaluations begin with the specific ingredients, and then move on to the whole product. The effects for all ingredients are considered as the product is formulated. Human safety-related data for a chemical used in a detergent or soap product (or in another type of consumer product), and for an entire formulation, can come from *in silico* data (from computer programs that estimate toxic properties based on data for similar chemicals, and/or from the physical chemical properties of the chemical of interest), *in vitro* data (from the results of 'alternatives to animal' tests, e.g., from cell cultures used to assess eye or skin irritation potential), animal (toxicological) studies (e.g., to assess eye or skin irritation potential), and human data (examples are discussed below).

The human data include premarketing (i.e., before a product has begun to be sold to consumers) clinical and 'controlled use' studies of the entire formulation. Further, the human data could include post-marketing (i.e., after a product has begun to be sold to consumers) studies conducted by physicians or dermatologists, and epidemiological studies developed by Poison Control Centers, companies, academia, etc.

Examples of human testing that may be very useful in the safety evaluation of detergents and other consumer products include human clinical studies, e.g., patch tests to confirm the absence of meaningful human skin irritation potential predicted from *in vitro* and any animal studies. Possible human studies also include 'controlled use' studies, e.g., from studies designed to assess the skin effects from wearing a type of fabric laundered with a new detergent formula. Further, examinations of the 'real-world' experiences consumers have had using a product are very helpful in confirming the absence of meaningful safety issues, or

could lead to changes in product composition, labeling, package design, etc., if the risk assessor judges that the data indicate a need to refine the product to lower risks. As noted above, these real-world data can come from human epidemiological studies and other studies developed by Poison Control Centers, companies, academia, and others to look at the health effects associated with the use of a consumer product under reasonably foreseeable conditions.

Even though manufacturers formulate and package their cleaning products to ensure that they are safe or have very low risk, human health effects can still result from normal uses and unintended exposures. To warn consumers about a specific hazard, household cleaning products carry cautionary labeling whenever necessary, e.g., CAUTION or WARNING or DANGER, along with first aid instructions. A laundry product label might look like:

Caution. Eye irritant. Harmful if swallowed. KEEP OUT OF REACH OF CHILDREN. If swallowed, give a glassful of water. Call a physician. In case of eye contact, flush with water.

The manufacturer's safety data and material safety data sheet (MSDS) supporting this labeling might indicate the following:

> Acute Health Effects:
>
> Inhalation: Transient irritation with prolonged exposure to concentrated material.
>
> Ingestion: May result in nausea, vomiting, and/or diarrhea.
>
> Eye Contact: May cause stinging, tearing, itching, swelling, and/or redness.
>
> Skin: Prolonged contact with concentrated material may be drying or transiently irritating to skin.

In addition, companies marketing detergents, soaps, and other products tend to work closely with poison control centers to assure that, should an accidental exposure occur, treatment information is available to health care providers and concerned parents.

While most laundry detergents are not strong enough to do significant harm, some laundry products, automatic dishwashing detergents, wall cleaners, drain or oven cleaners, disinfectants, and ammonia can cause extensive injury. In addition, extensive eye and skin exposure to some detergents may also cause toxic effects. Further, product interactions might occur. For example, the mixing of a toilet bowl cleaner or any acid with a chlorine-type bleach may produce chlorine gas, causing respiratory irritation with coughing, labored breathing, and inflammation of the eyes and mucous membranes, and the addition of ammonia to bleach produces a toxic gas, chloroamine. As the title of one risk communication book states, 'Read the label', especially if the product is a new one for a consumer.

Occupational Safety

Occupational allergy and occupational asthma were safety issues many years ago with the manufacture of detergents. However, comprehensive pre-clinical, clinical, and industrial hygiene programs have been developed to successfully control allergy and asthma to enzymes used in the detergent industry. The detergent industry has developed guidelines for the safety assessment of enzymes, control of exposure to enzymes, and medical surveillance of enzyme-exposed workers, and occupational allergy and asthma to enzymes in the detergent industry have become uncommon. The cases that have been documented in some manufacturing sites have had poor adherence to the guidelines. Those manufacturing sites that have adhered to the guidelines have had few cases of allergy and asthma to enzymes among exposed workers. Further, reviews of medical data from these sites have shown that workers who have developed IgE antibody to enzymes can continue to work with enzymes and remain symptom-free. The basic principles of these programs can be applied to other industries where occupational allergy and asthma to proteins are safety issues.

Environmental Safety

Most household cleaning products are formulated to be used with water and 'go down the drain' into wastewater treatment systems (municipal sewage treatment plants or septic tank systems). To assure that these types of products are safe for the environment, manufacturers evaluate the impacts of product ingredients in wastewater treatment systems, streams, rivers, lakes, and estuaries. Environmental risk assessment considers the exposure concentrations and effects of individual ingredients.

Two sets of information are used in these assessments. One set enables industry scientists to predict the concentration of the ingredient from all sources, including cleaning products, at various locations in the environment (the predicted exposure concentration). The other set is used to find the highest concentration of the ingredient at which no harm will occur to animals, plants or microorganisms living in the environment, i.e., the no-effect concentration. Comparing the predicted exposure concentration and the no-effect concentration enables scientists to determine whether the use of an ingredient is safe for the environment.

An example of an environment issue for detergents is the finding that high levels of phosphates in detergents discharged into water systems can lead to a build-up of nutrients that results in a large amount of algae and water plant growth (a complex process called eutrophication). Public, academic, and government concerns have led to adverse publicity, to legislation banning the use of phosphate detergents, and to the development of nonphosphate versions of products.

Environmental Quality

The (US) Soap and Detergent Association has noted that manufacturers of cleaning products have been leaders in reducing packaging waste and encouraging sound waste disposal practices. For example, "Advances in technology have resulted in products that are more concentrated, products that combine two functions in one, products with refill packages and packages that use recycled materials. Concentrated products need less energy to manufacture and transport, and require less packaging. Multifunctional products eliminate the need for separate packages. Refill packages allow consumers to reuse primary packages many times, decreasing the amount of packaging used and the volume of trash generated. Plastic and paperboard that would otherwise be thrown away become usable materials through recycling."

Finally, life cycle assessment (LCA) is being used to improve the environmental quality of detergents. LCA provides a 'cradle-to-grave' or 'cradle-to-cradle' evaluation of the environmental impacts of a product and its package, usually all the way from acquiring the raw materials, manufacture and distribution, and consumer usage and disposal. The use of LCA can help assess whether reducing an environmental impact in one area, e.g., manufacturing, moves the impact to disposal or another area. LCA also helps highlight where environmental improvement efforts should be focused.

See also: Alkalies; Consumer Products; Exposure Assessment; Life Cycle Assessment; Poisoning Emergencies in Humans; Surfactants, Anionic and Nonionic.

Further Reading

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Development Toxicity Testing *See* Toxicity Testing, Developmental.

Developmental Toxicology

Calvin C Willhite and Philip E Mirkes

The Problem of Birth Defects

Two-third of all infant deaths occur in the first 27 days of life. Congenital malformations and chromosome abnormalities account for 20% of all infant deaths, and infants born too small (<2.5 kg) or too soon (<37 weeks gestation) have much higher risk of death than those born at term. For the year 2002, the five leading causes of the 27 970 infant deaths in the United States were:

1. congenital malformations and chromosome abnormalities (5630 deaths);

2. premature birth and low birthweight (4686 deaths);
3. sudden infant death syndrome (2295 deaths);
4. maternal complications (1704 deaths); and
5. placental and membrane complications (1013 deaths).

In 2002, the three leading causes (congenital malformations/genetic defects, low birthweight, and sudden infant death syndrome) were responsible for 45% of all infant deaths. In 2001, United States infant morality was 6.8 per 1000 live births and this death rate increased to 7.0 per 1000 live births in 2002.

Non-Hispanic Black and American Indians experience the highest infant mortality rates. In 2002, infant death ranged from a low of 3.0 per 1000 live births to Chinese mothers to a high of 13.8 for Black mothers. For the years 1995–2002, infant mortality rates for Black mothers ranged from 13.3 to 14.6 per 1000 and those for American Indians ranged from 8.3 to 10.0 per 1000. In contrast, rates for Whites over those same years were 5.7–6.3 per 1000.

Infant mortality varies by location as well as race. Southern states have higher rates. Rates are lowest in Western and Northeastern states. Infant mortality is higher in Mississippi (10.5 per 1000 live births) than in Massachusetts (4.8 per 1000 live births). Between 2001 and 2002, the highest total rate was that in Washington, DC (11.4 per 1000 live births). Infants born to non-Hispanic Black mothers in Wisconsin experienced a rate of 17.9.

Other well-known factors also contribute to elevated risk of infant death. United States infant mortality rates increased significantly between 2001 and 2002 for teenage mothers (10.7–11.5). Infant mortality rates also increased for mothers who smoke tobacco (10.5–11.1). In 2002, infant morality was 68% higher for smoking mothers (11.1) than for mothers who did not smoke during their pregnancy (6.6).

Nearly 50% of the annual hospital charges ($29.3 billion) in the United States for delivery and neonatal care are associated with prematurity. Hospitalization costs for a normal delivery average $1300 where costs where for premature infants average $75 000. These initial charges do not include the public and private health care costs for the 25% of these infants who survive with blindness, cerebral palsy, and other chronic conditions.

The science of teratology (a word coined in 1832 by Geoffrey Saint-Hilares as literally 'the study of monsters') has a history predating that of medicine as we know it today. The contemporary definition of teratology is 'the science dealing with the causes, mechanisms, and manifestations of a structural or functional nature of abnormal prenatal development'. Teratology can be considered a subdivision of developmental biology. Developmental toxicology encompasses embryonic and fetal death, reduced fetal growth, and other manifestations of abnormal development brought on by exposure to xenobiotics (literally 'foreign chemicals'). Virtually all chemical compounds (including common sugars like glucose or normal amino acids like phenylalanine) can induce embryotoxicity and fetotoxicity if the dose given is sufficiently large and the time and duration of exposure in pregnancy is appropriate. Maternal disease like diabetes and phenylketonuria can predispose a patient to an abnormal pregnancy outcome. Nevertheless, a compound is not usually considered a teratogen (a chemical that causes birth defects) if the dose required causes maternal poisoning in animal studies. Human teratogens can be seen where there is frank intoxication; there is no better example of this than ethanol in alcoholic mothers and their offspring who exhibit features of the fetal alcohol syndrome (FAS). Thus begins the dilemma for the clinician, the teratologist, the government regulator, and the family of an affected child – teratogens may seem to be everywhere, but on close inspection they seem to be nowhere. The birth of a malformed child has always been a matter of intense concern and sorrow; the same question always follows: 'What caused it?' Ancient peoples formulated their own explanations on the cause(s) of these diseases and some remnants of their hypotheses are still with us today. As we will see in the following discussion, we have advanced from stoning or cremation of mothers of infants with birth defects. However, we have replaced the hypotheses of antiquity (termed superstition today) with more subtle (but in some instances equally preposterous) and damaging ideas in our search for responsible agents and parties.

It is often said that the cause of a particular birth defect is 'multifactorial', generally taken to mean that it is the interaction of one or more environmental agents (a drug, a hazardous waste site, or a drinking water contaminant) with the genetic makeup (genotype) of the mother and her embryo. This notion of multifactorial causation stems from the writings of the French surgeon Ambroise Pare (1510–90) in *Chyrurgery* (1579) recounting the influence of maternal impressions (see below), demonic intervention, and environmental or mechanical factors. Actually, until the thalidomide tragedy of the twentieth century, it was generally accepted that the embryo was well protected and that the placenta functioned as a 'barrier', which insulated the conceptus from noxious agents. Today, the multifactorial explanation is either applied correctly, when describing the interplay between a susceptible genotype (e.g., inborn errors of metabolism) and exposures of interest or out of

frustration on the part of teratologists, obstetricians, or pediatricians, who cannot otherwise account for the empirical observations at hand. This tendency taken together with the oft held public view that certain birth defects are inevitable, the result of random chance, or 'God's will', lead to dismay that these conditions can ever be 'cured' or prevented. In contrast, the frequency of these conditions and the natural desire to ascribe one or another particular factor or agent (e.g., a drug or a workplace chemical or practice) as the cause leads today to significant legal and financial consequences that, depending on the particular circumstance, may or may not be warranted. The science of teratology has at its core the emotional distress that accompanies the birth of a malformed child. This factor is neither lost in tort adjudication nor lost in the nation's abortion (right to life) debate. Professional scientific societies, such as the Teratology Society and the American College of Obstetricians and Gynecologists, are dedicated to the study and prevention of birth defects. Over the past 20 years, it has become clear that a great many of these common, costly, and deadly conditions can indeed be prevented. It is surprising that prevention can sometimes be accomplished by simple and inexpensive steps once the etiologic agent(s) has been identified.

Generally speaking, developmental toxicology focuses on abnormal morphogenesis induced by xenobiotics; however, a discussion of the extent or nature of birth defects must consider other possible causes – infectious microbes, abnormal chromosomes, radiation, hormones, maternal disease, and nutritional status. These factors must be added to the 'normal' or 'background' rate of embryonic demise known from the classic studies of Hertig AT and Rock J carried out during the middle of the last century in couples of proven fertility under optimal conditions for pregnancy. In these studies, 15% of the oocytes failed to fertilize, 15% of the fertilized oocytes started cleavage but failed to implant, and of the 70% that implanted 58% survived. Of those surviving, 16% were abnormal.

Embryos die for any number of reasons (e.g., degeneration of the corpus luteum or a defective trophoblast) and they are aborted spontaneously with the next menstrual period – usually without producing any of the maternal signs associated with pregnancy. Thus, by the end of the first expected menstrual period more than one-half of all human eggs exposed to sperm under the best of conditions die for one reason or another.

The biological context of the word 'development' covers the changes from conception through birth, neonatal life to adulthood, and to old age. The word is restricted here, however, to embryonic and fetal life ranging from subtle changes detectable only in studies of children or young laboratory animals to embryonic or fetal death. A brief discussion of functional delay or deficit (commonly referred to as behavioral teratology) is presented. It is these functional or behavioral deficits that represent insidious to overt manifestations of developmental toxicology.

To understand the causes and pathogenesis of congenital malformations, one must possess at least a working knowledge of embryology as can best be gained from completion of an undergraduate course in the subject or as is commonly taught in medical school anatomy. For purposes of the current discussion, a rudimentary understanding of biology and mammalian embryology is assumed.

Historical Lessons

While it may seem obvious, birth defects are not new. In the vast majority of cases, birth defects and their causes cannot be linked to modern consumer products, occupational exposures, therapeutic or recreational drugs, or environmental pollutants. Congenital defects are perhaps the greatest source to have influenced the myths of antiquity (second only to belief in divinity or the study of the heavens), fairy tales of Rumpelstiltskin and other dwarfs, elves and hunchbacks or otherwise twisted (arthrogryposis, torticollis, and scoliosis) trolls, or contemporary book and film scripts of the macabre.

Cyclops are first recorded as subterranean beings who serve Hephaestus (Sanskrit Yavishta and the Vedic god of fire), the Greek divine blacksmith. The sons of Uranus and Gaea (Arges, Steropes, and Brontes) are all cyclops. These cyclops forged the trident for Poseidon and the bronze helmet for Hades and were then killed in furious revenge by Apollo. The cyclops of Homer's *Odyssey* (Polyphemus, who Ulysses blinded with a sharpened, burning stake driven into his eye) inhabited the southwest coast of Sicily and lived in caves, killing and devouring any stranger who chanced upon them. According to Callimachus, the cyclops Brontes, Steropes, Acamas, and Pyracmon, who lived on Mount Etna (the active volcano near the Sicilian city of Taormina on the Ionian Sea), were

> *Enormous giants, big as mountains and their single eye, under a bushy eyebrow, glittered menacingly. Some made the vast bellows roar, others, raising one by one their heavy hammers struck great blows at the molten bronze and iron they drew from the furnace.*

One of the colonial American explanations regarding the etiology of cyclopia was hybridization

between species. The birth of a cyclopic infant or farm animal (whose mother lived near a person with features thought to resemble that of the malformed newborn) was suspect. In the *Records of the Colony and Plantation of New Haven* (1638–48), the story of one such unfortunate neighbor is recounted, Mr. George Spencer, who happened to live near a sow who gave birth to a cyclopic pig that had 'but one eye in the middle of the face'. The jury concluded that Mr. Spencer who 'had but one eye…the other hath (as it is called) a pearle in it' was guilty of bestiality. The 'pearle' apparently bore some superficial resemblance to the cyclopic pig's eye. The sow was 'slaine in his sight, being run through with a sworde' and poor Mr. Spencer was put to death for his crime on April 8, 1642.

Cyclopia is, of course, the most severe manifestation of holoprosencephaly – a condition in which the embryonic forebrain fails to separate into right and left hemispheres. The etiologic agent(s) of human cyclopia is not known. In ruminants (sheep, cattle, and goats), however, ingestion of the plant *Veratrum californicum* on even a single day (e.g., day 14 in ewes) reliably produces the condition. Subsequent investigations confirmed the presence of a teratogenic alkaloid, 11-deoxojervine, in the plant. That compound is termed most appropriately cyclopamine. Cyclopamine illustrates one of the many factors that must be taken into account for interspecies extrapolation of teratology data. Cyclopamine and its congeners cannot be held accountable for human cyclopia. Cyclopamine is not teratogenic in monogastric animals (e.g., rabbits) because it is degraded by stomach acid to an inactive compound (called veratramine). Nevertheless, when cyclopamine was fed to pregnant rabbits along with sufficient alkali so as to reduce stomach acid, cyclopamine was definitely teratogenic and, in fact, induced cyclopia.

Another example of historical explanations for birth defects is the theory of maternal impression. For better or worse, such theories find their way into laws, regulations, and even (in a modified fashion) into litigation. The eighteenth-century Philadelphia surgeon, Dr. John Morgan, described the birth of a 'piebald Negro girl with splotches all over her body' – a condition attributed to her mother's habit of evening star watching. Sirenomelia and ptergium colli were attributed to the mother seeing a snake or cobra during pregnancy and anencephalus to the mother looking at monkeys. Pregnant Spartan women were legally required to concentrate on statues and pictures of beautiful gods and warriors so as to ensure strong and healthy babies. One historical account involves an Italian nobleman whose wife happened to employ a black male servant. One day during her pregnancy, she attended a cultural exhibit at the local museum. After gazing at a portrait of a Moor, she subsequently gave birth some time later to a mulatto. The nobleman protested and the legislature responded, of course, by passing a law to the effect that pregnant women were to be banned from visiting art museums.

Although it may seem odd to consider it so, even structurally normal identical (monozygotic) twins can be considered a developmental aberration. Thirty percent of the 1.08–1.36% of the normal incidence of twin births in the United States are monozygotic. The factors responsible for splitting of the single fertilized egg (zygote) into two blastocysts (each with its own inner cell mass or embryo proper) are – like the vast majority of other developmental anomalies – unknown, but maternal genotype is a predominant contributor. The stage at which splitting occurs determines whether the embryos develop each with their own placenta and amniotic cavity, whether the embryos develop having a common placenta and separate amniotic cavities, or whether the embryos share a common placenta and a common amniotic cavity. Failure of complete separation of the inner cell mass results in any of a variety of conjoined (Siamese) twins (diploterata or 'double monster').

The term 'Siamese twins' was first coined by the nineteenth-century circus king, Barnum PT, in reference to Eng and Chang Bunker, who were joined at the sternoxiphoid. (After retiring from the circus, Eng and Chang farmed in North Carolina and married at age 44, fathered a total of 22 normal children by two sisters, and died of arteriosclerosis in 1874 at age 63 – within 2 h of one another.)

Conjoined twins can be joined at the chest (thoracopagus), lower spine (pygopagus), or skull (craniopagus), with the latter being variable in fusion at the dorsal (occipital craniopagus), parietal, or ventral (syncephalus frantalis) aspect. Symmetrical conjoined twins occur about once in every 50 000 births and craniopagus twins are born about once in 3 million births (one in every 58 cases of conjoined twins).

The most extreme occipital craniopagus (janiceps) is Janus, the Roman god of gates and doorways represented artistically with his double faces (*Janus bifrons*) each in opposite directions so as to observe the interior and exterior, the entrance and exit, of public buildings. Janus arose from the god Chaos when earth, air, fire, and water took form during the creation of the world. His two faces represent his confusion in his initial state; thus, not only was Janus the god of departure and return but also he was the god of daybreak and new beginnings. Janus was revered even more than Jupiter and was honored on the first

day of every month. The first month of the year (Januarius) still bears his name.

Dicephalic (double-headed) monsters populate not only 1950s matinee movies but also appear in sculpture, drawings, and carvings throughout history – Catal Huyuk (6500 BC) in southern Turkey and the South Pacific (dicephalus dibrachius), clay figurines in Mexico and South America (500 BC–800 AD), and figures on Babylonian clay tablets found near the Tigris (Cuneiform Texts from Babylonian Tablets, British Museum, London).

Neural Tube Defects

The belief that Satan, witches, sorcerers, and other diabolic and demonic forces were responsible for congenital malformations was prevalent during the fifteenth and sixteenth centuries, and this belief found its way to the new world. Not that these concepts were new – far from it. A mummified anencephalus – a condition considered the most severe malformation compatible with intrauterine life (**Figure 1**) – was discovered in 1825 at the Egyptian catacombs of the Hermopolis sarcophagus. This individual's area cerebrovasculosa (rudimentary brain) had been ceremonially removed through the nose, as was the custom. Based on the mummy's location and condition, and the inscription on the sarcophagus, the malformed individual was considered the product of fornication between the mother and an ape. This anencephalus was brought to the Berlin Museum by the King of Prussia and was unfortunately destroyed in the Second World War. By the 1600s, mothers of anencephalics were condemned in ways not dissimilar to the execution of witches in Salem, Massachusetts. One theory as to the origin of anencephalics was consanguinity with a troll – particularly dangerous being those who lived near roadways or under bridges – whose sons and daughters resembled their fathers.

Anencephalus is one of a constellation of malformations known collectively as neural tube defects (NTDs). Anencephalus is the end result of failure of neural fold elevation and fusion, a deficiency that can occur only in the most anterior region (**Figure 1**), along the entire axis (craniorachischisis totalis; **Figure 2**), or localized in areas along the spine (spina bifida; **Figure 3**). Spina bifida is a common

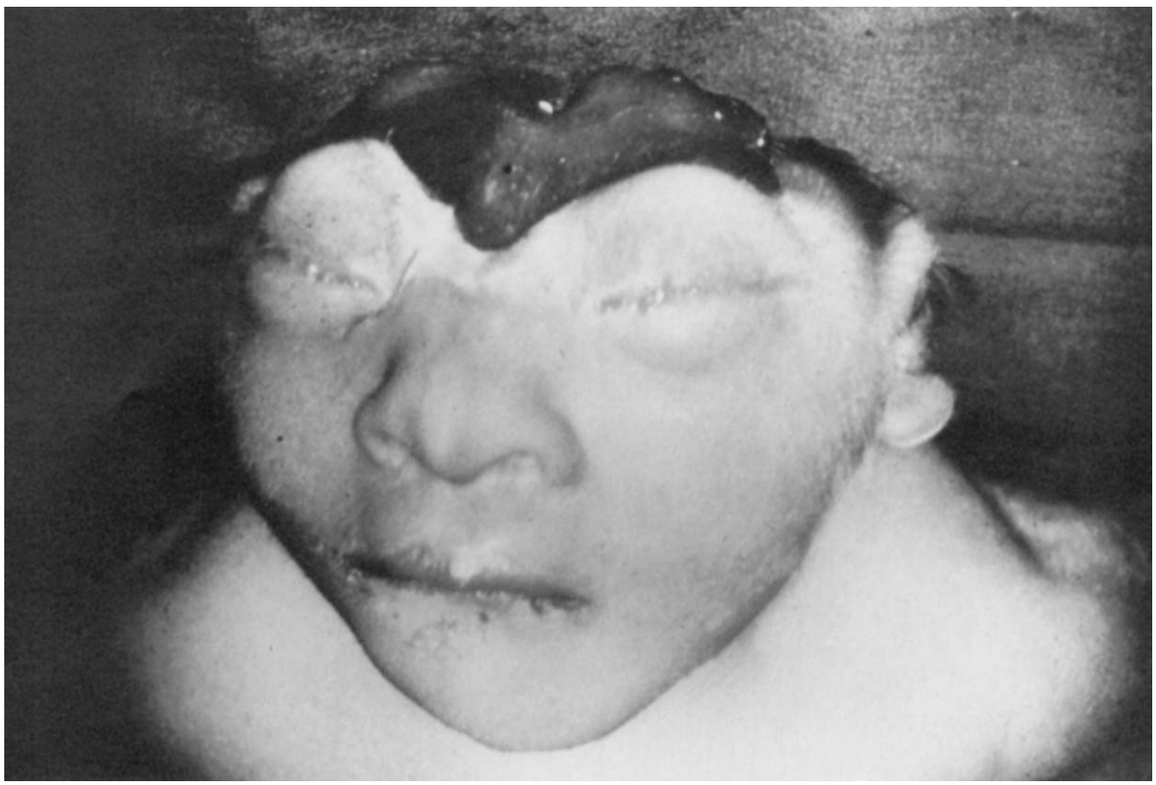


Figure 1 Anterior view of an anencephalic human fetus. Notice the low-set ears, elevated nose and maxilla, the short neck (due to anomalies of the cervical vertebrae), and the prominent, protruding rudimentary brain. (Reproduced from Marin-Padilla M (1991) Cephalic axial skeletal-neural dysraphic disorders: Embryology and pathology. *Canadian Journal of Neurological Sciences* 18: 153–169, with permission.)

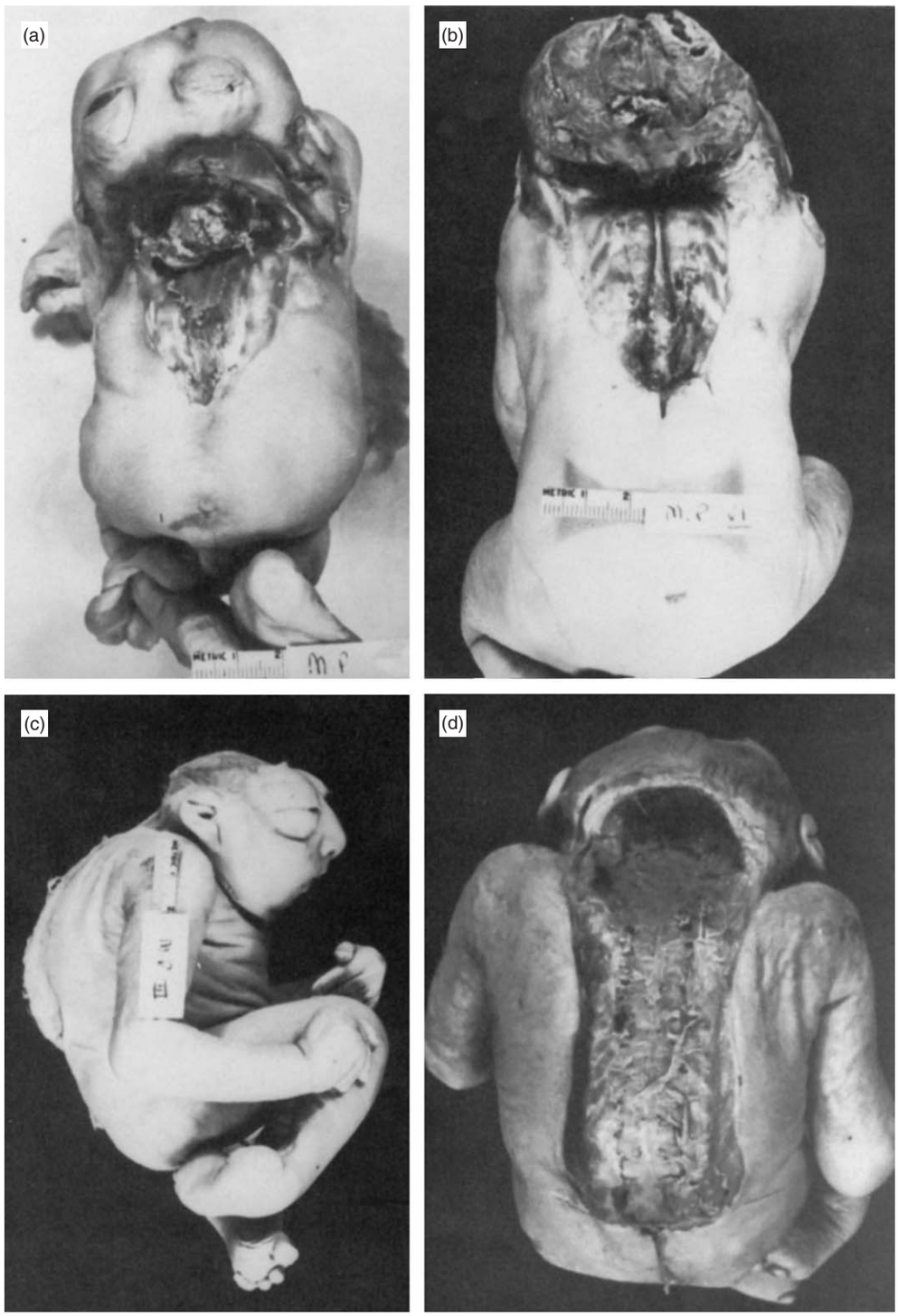


Figure 2 External appearance of various types of human craniorachischisis totalis (total myeloschisis) illustrating the severity of the dysraphic disorders. The first fetus (a) illustrates the severity of the lordosis and the shortness of the axial skeleton which can occur in these disorders. The exposed areas of the central nervous system are totally destroyed. In (b), note the exencephalic brain (termed area cerebrovasculosa). (c, d) Lateral and posterior view. Compare (c) with **Figure 1**. In (d), the destroyed areas of brain and spinal cord tissues have been removed to show the severity of the malformations of the vertebrae. (Reproduced from Marin-Padilla M (1978) Clinical and experimental rachischisis. In: *Congenital Malformations of the Spine and Spinal Cord. vol. 32. Handbook of Clinical Neurology*. Amsterdam: North-Holland, with permission from Elsevier.)

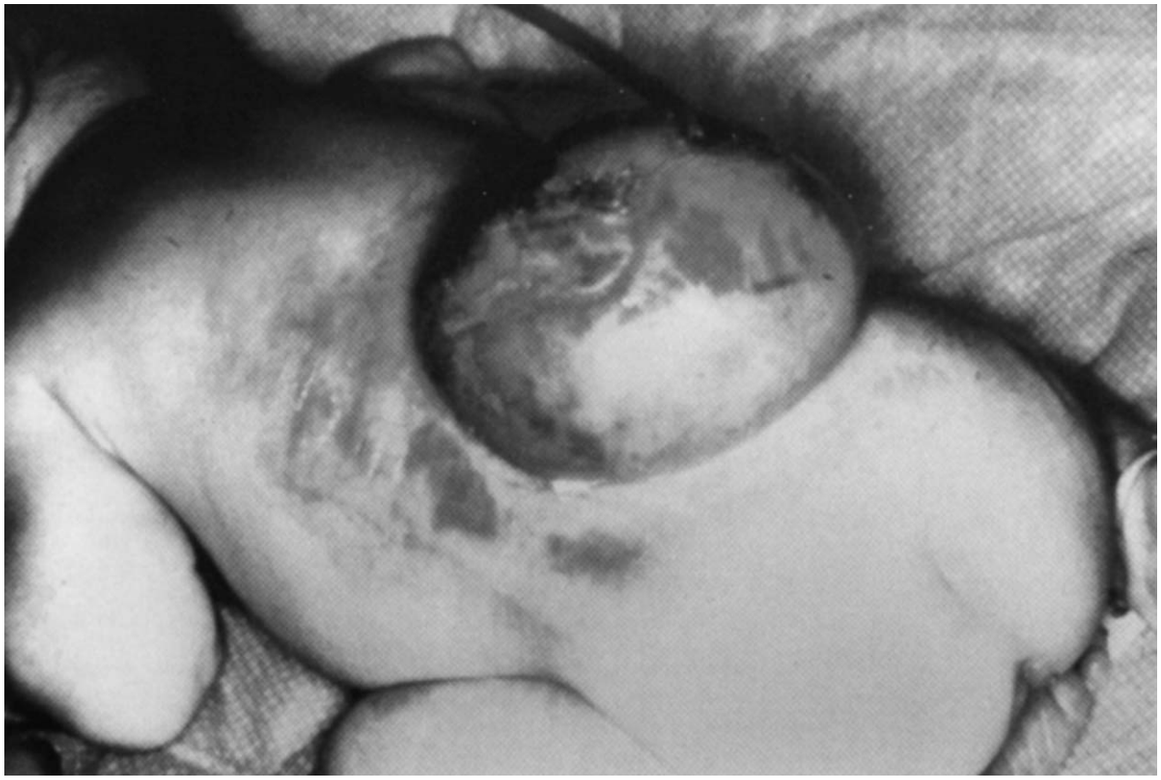


Figure 3 Newborn infant with spina bifida. Note the large meningocele on this child's back.

term used to describe a range of defects of the axial skeleton, involving the vertebrae and to various degrees the cord itself. If only the vertebrae show incomplete spinous process fusion and the subarachnoid space remains within normal limits, this is a subclinical condition known as occult spina bifida. If the vertebral arch is only rudimentary, the overlying tissues are weak, and cerebrospinal fluid pressure contributes to expansion of the subarachnoid space and the meninges herniate dorsally, this condition is diagnosed as spina bifida meningocele (cystica). If the vertebrae are so rudimentary that only the body of the bones is thickened, the spinal cord itself is displaced into the subarachnoid space (now a gross, protruding meningeal sac), the condition is classified as spina bifida with myelomeningocele (**Figure 3**). If there is complete failure of neural fold elevation in cranial, cervical, thoracic, and/or lumbar regions and the neuroectoderm is left exposed on its dorsal aspect, the spinal cord then develops with its ependymal layer in open contact with amniotic fluid and its lumen cannot be recognized. This latter condition (**Figure 4**) is termed spina bifida aperta (rachischisis or myeloschisis). Thus, spina bifida can range from a partial failure of neural tube closure, manifest as a benign subclinical condition of no practical consequence, to damage that is permanently disabling, affecting the patient's ability to walk and control normal bodily functions.

Just as partial failure of neural fold apposition and fusion can occur along the spine, it can also occur in the skull. Cephalic malformation can be complete in anencephalus (with little or no involvement of the lower spine) or incomplete as in encephalocele and Arnold–Chiari malformation (**Figure 5**). Encephalocele (**Figure 5**) is one such condition in which the brain extends through dura and membranous bone and comes into contact with the scalp.

From the early 1600s through the latter part of the nineteenth century, there was a great deal of interest on the part of physicians and surgeons in describing and classifying NTDs as well as in speculating on the cause(s) of NTDs and their pathogenesis. Dissections of fetuses with spina bifida and anencephaly were described in detail. These early studies provided a basis for understanding the morphogenesis of these malformations but gave little indication as to the actual etiologic agent(s) responsible. The 1801–96 manuscripts by the German authors Arnold J and Chiari H describe hindbrain anomalies in spina bifida and provide for four different categories of herniation of the cerebellum into the foramen magnum. In that case a cervical sac or encephalocele forms; in others there is a simple cerebellar hypoplasia

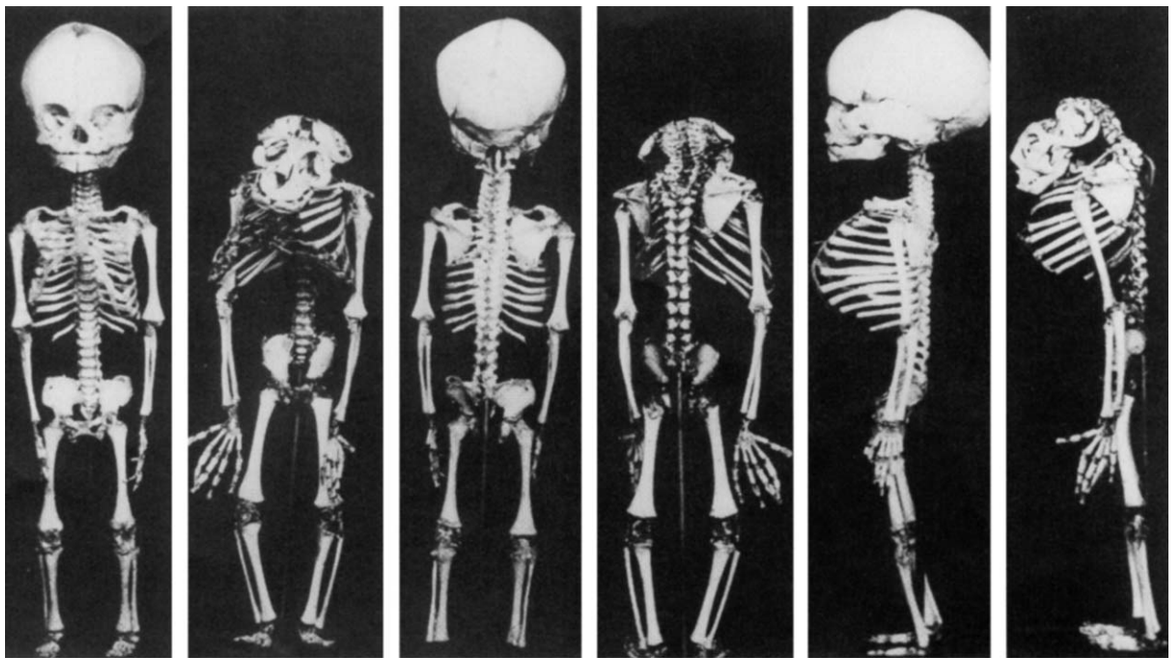


Figure 4 Posterior, anterior, and left lateral views of the entire skeletons of a 7 month premature infant and of a human anencephalic with cervical myeloschisis. Note the severity of the skeletal defects in the anencephalus, the anomalous facial bones, the short cervical column, and the absence of a proper skull. (Reproduced with permission from Marin-Padilla M (1991) Cephalic axial skeletal-neural dysraphic disorders: Embryology and pathology. *Canadian Journal of Neurological Sciences* 18: 153–169.)

(termed collectively Arnold–Chiari malformations). This condition often results in hydrocephalus (accumulation of cerebrospinal fluid followed by marked expansion and thinning of the skull with subsequent compression and atrophy of the brain). Blockage of the roof of the fourth ventricle and continued production of cerebrospinal fluid by the choroid plexus leads to increased intracranial pressure. As a result, the medulla oblongata is forced into the cervical canal, the herniated cerebellum is compressed, and interrupted cerebrospinal fluid flows into the subarachnoid space producing an extensive internal hydrocephalus (**Figure 5**). In those cases of Arnold–Chiari in which the lumen of the spinal cord is open at some point (e.g., myeloschisis), hydrocephalus does not occur since cerebrospinal fluid either accumulates at another point (**Figure 3**) or, in cases in which the cord is exposed on the surface of the skin, cerebrospinal fluid drains to amniotic fluid and relieves increased intracranial pressure. It is by this route that α-fetoprotein (a normal serum component synthesized in the embryonic yolk sac to 12 weeks and then by the fetal liver) escapes into the amniotic fluid. Radioimmunoassay of this protein is used in routine management of high-risk pregnancy where abnormally high concentrations are indicative of NTDs. Acute hydrocephalus is also a consequence of Dandy–Walker malformation, a condition characterized by defects of the ventricular system and stenosis (constriction) of the foramina of the fourth ventricle and it may present as a severe occipital encephalocele.

Anencephalus is a relatively common condition, affecting on average one in every 1000 births (or five or six embryos per 1000 pregnancies, given published studies of fetuses examined at 8 weeks gestation). Anencephalus occurs four times more often in males than in females and four times more often in Caucasians than in blacks. Even lower rates occur among North American Indians (0.5 per 1000), Japanese (0.4 per 1000), and Central and South Americans (0.1–0.3 per 1000). Spontaneous early pregnancy abortion of anencephalic embryos ranges from 54% (London) to 87% (Japan).

Although the term anencephalic suggests a lack of all but the bones of the face, in fact, all of the bones of the skull are present (**Figure 6**). The anomalies of the facial bones and those of the remainder of the skull are the consequence of early disruption of the notochord, the mesoderm, and the neuroepithelium. Early deficiency in neural tube closure exposes the developing brain (neuroepithelium) to mechanical abrasion from the fourth week of gestation until birth. The hindbrain (often enclosed and therefore protected by the rudimentary neurocranium) can remain intact – containing those structures responsible for control of respiration. Thus, the newborn

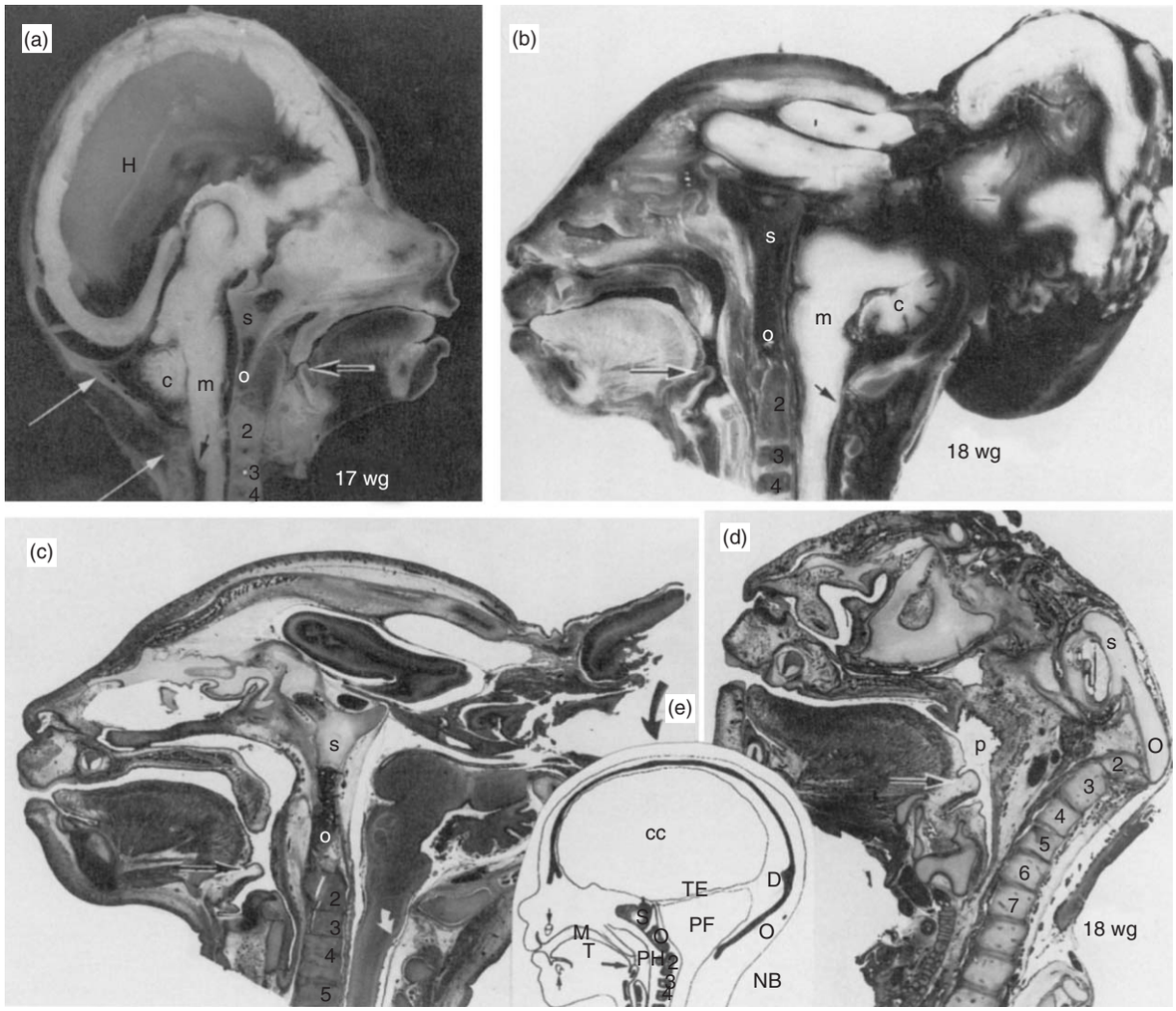


Figure 5 (a) Midsagittal section of the head of a premature infant with Arnold–Chiari malformation and secondary hydrocephalus. H, hydrocephalus; s, sphenoid; o, squama occipitalis; m, medulla; c, cerebellum; wg, weeks gestation. The cervical vertebrae are numbered. (b) Premature infant with partial failure of the anterior neural tube closure and a large occipital encephalocele. (c) Glass slide section of the infant shown in (b). (d) Premature infant with anencephalus, occipital schisis, and cervical rachischisis. The gross appearance of this infant was similar to that shown in **Figures 1** and **3**. (e) Arrow indicates schematic ink drawing of the midsagittal section of a normal newborn's head. For comparison to (a)–(d), locate the size and shape of the cerebral cavity (CC), the location of the tentorium (T), its angle in relation to that of the spine (D), the size and shape of the posterior fossa (PF), and the mouth (M), tongue (T), teeth (small arrows), and the nasal passage (P), pharyngeal (PH) and laryngeal (large arrow) cavities. NB, newborn. The black and white arrows in (a)–(d) point to the location of the epiglottis in relation to the base of the skull. The black arrows in (a) and (b) point to the bend in the medulla caused by the downward displacement of the subtentorial central nervous system. Note in (a)–(c) the short base of the skull, its angle to the spine, and the small posterior fossa. In (d), note the angle of the spine and relatively large facial skeleton compared to that shown in (e). (Reproduced from Marin-Padilla M (1991) Cephalic axial skeletal-neural dysraphic disorders: Embryology and pathology. *Canadian Journal of Neurological Sciences* 18: 153–169, with permission.)

anencephalic can survive hours to (at most) a few days in the absence of mechanical ventilation and aggressive life support. Anencephalic humans with larger skull bones (thus having a more representative brain) can survive for as long as a few weeks. The characteristic facial features of the anencephalus (low-set ears, protruding tongue, short maxilla, and elevated pointed nose; **Figure 1**) are direct consequences of the malformations of the bones of the base of the skull. The base of the skull (which in reality is composed of modified vertebrae) is small and, rather than forming a normal 90° angle with the spine, can be tipped to ~45°. The early collapse of the cephalic neural folds leads to gross malformation of the base of the skull. The normal sphenoid (with which all of these bones articulate directly or indirectly) resembles a bird in flight (**Figure 7a**), whereas the anencephalic sphenoid resembles a bat with folded

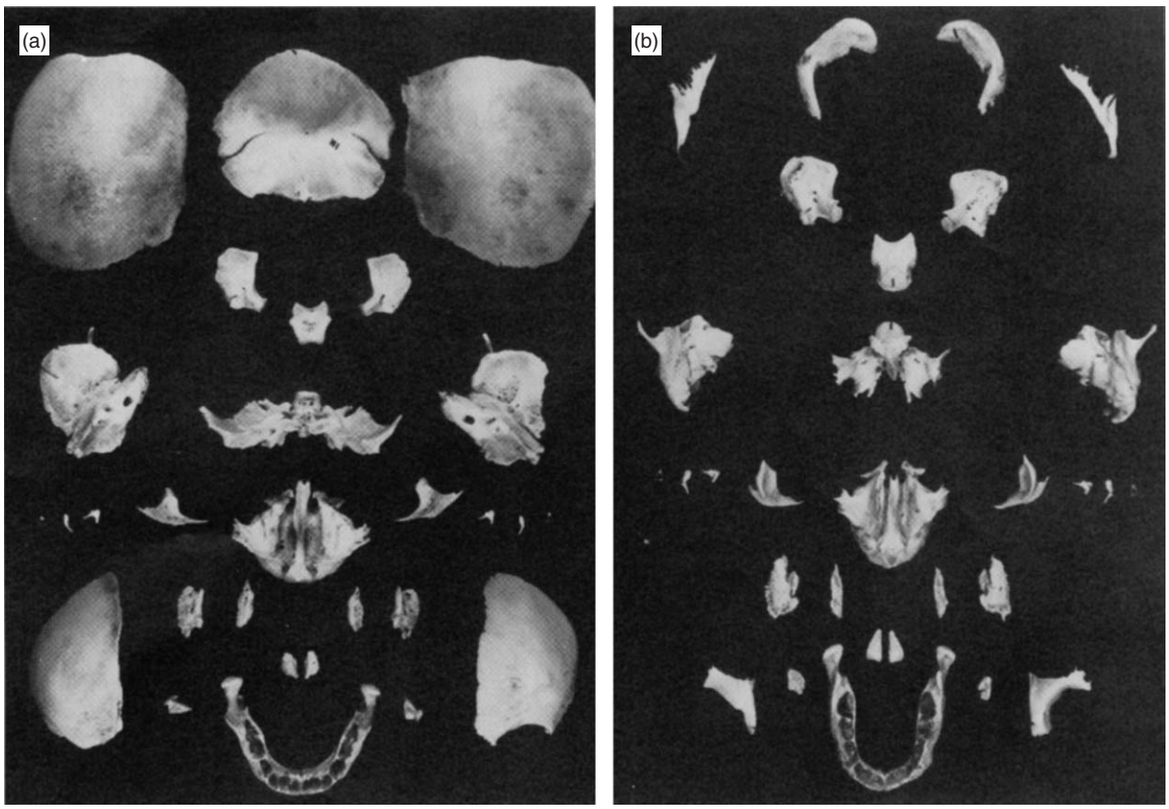


Figure 6 Disassembled skull of a (a) normal and (b) anencephalic infant. The following bones, starting from the upper corner, are the parietals and the squama of the occipital (which in the anencephalus are represented by two small fragments); the basilar portion of the occipital with its two lateral portions; the temporals (note the rudimentary squamas in the anencephalus); the sphenoid (located in the center of each figure); the ossicles, the zygomatics, and the maxilla with the vomer and palantines; the lateral masses of the ethmoid and the turbinate bones; and the frontals, the lacrimals, the mandible, and the two nasals. The rudimentary bones of the cranium and the normal (but narrowed) bones of the face are obvious. (Reproduced with permission from Marin-Padilla M, (1976) Morphogenesis of anencephaly and related malformations. In: *Current Topics in Pathology*, vol. 51, pp. 145–174. New York: Springer.)

wings (**Figure 7b–f**). Studies of disassembled normal and anencephalic skulls (**Figure 6**) show that it is the sphenoid malformations that precipitate the other gross malformations of the skull. The sphenoid is a bone that arises from mesodermal consolidation around the notochord prior to closure of the anterior neuropore. Since there is intimate communication between the embryonic brain (neuroectoderm), face (originating principally from neural crest), and the developing bones of the skull proper (neurocranium), teratogens that act on neuroectoderm, mesoderm, or both can initiate a cascade of abnormal events which ultimately give rise to anencephalus.

The geographic distribution of NTDs is most remarkable. The incidence among people living in Ireland is four times that of people living in the United States (including those of Irish descent living in New England). Such striking differences have precipitated speculations and generated hypotheses on the cause of NTDs. One such theory, advanced in the early 1970s, was that the high incidence of anencephalus and spina bifida in Ireland was caused by ingestion of potatoes containing unidentified teratogens, potatoes infected with fungi (blighted) after storage, or compounds produced by such potatoes in response to blight. Subsequent epidemiologic studies failed to support any of these theories; nonetheless, numerous studies have confirmed an excess number of NTD births in winter and a slow (but steady) decline in NTD births over the past five decades in the United States.

It is well known that the risk of NTDs is greatest in mothers 35 years or older (being particularly high in those who have borne several children) and that there is a marked increase in risk for mothers whose previous pregnancies ended in fetal or neonatal death (relative risk = 3.5–3.8). When one compares population prevalence of anencephalus and spina bifida (0.13–0.75% including New York, New England, British Columbia, Hungary, London, and South Wales), it is clear that recurrent risk is increased (from 0.1% in the general population to 1.8–7.1%)

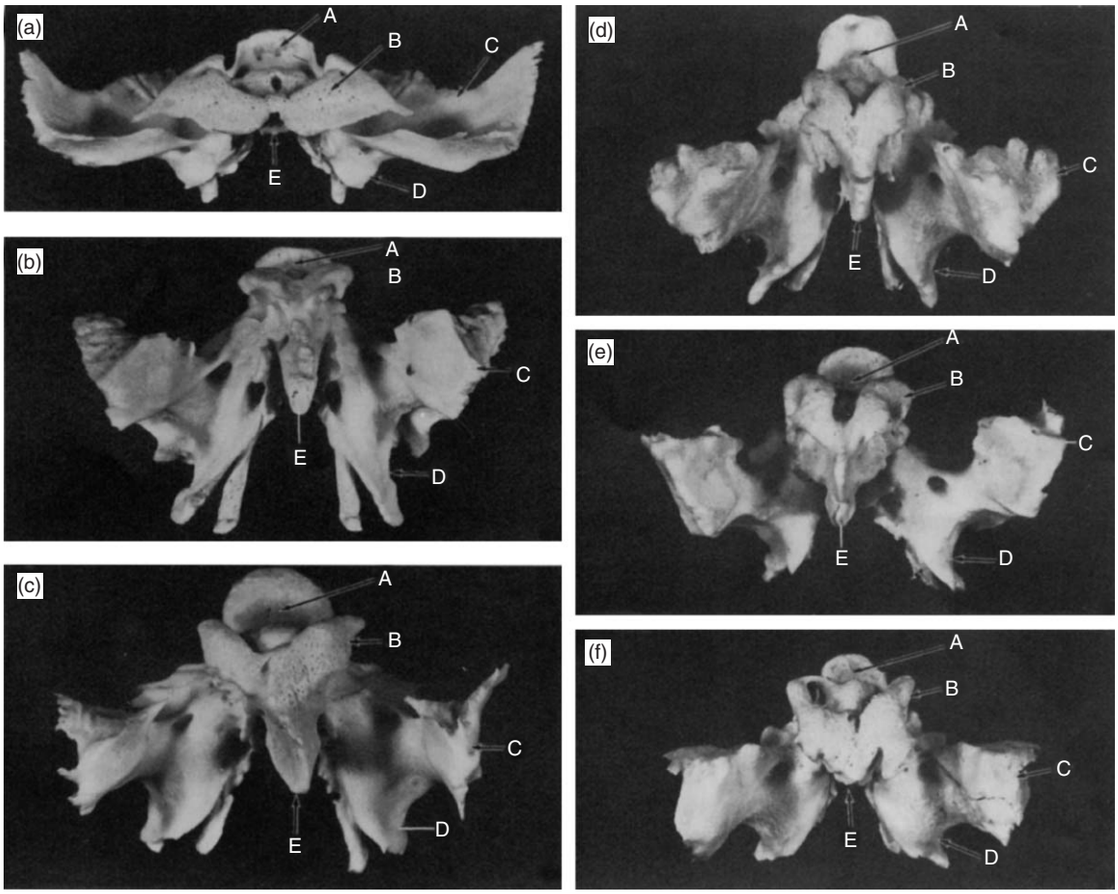


Figure 7 (a) Anterosuperior aspect of the sphenoid bone of a normal newborn infant as shown in **Figure 6** (a) (× 1.8). A Body of the sphenoid bone; B the lesser wings; C the greater wings; D the pterygoid process; E the rostrum. (b) Anterosuperior aspect of the sphenoid bone of a newborn infant with partial anterior cranioschisis (× 2). (c) Anterosuperior aspect of the sphenoid bone of a newborn premature infant with complete (simple) cranioschisis (**Figure 6**) (× 2.5). (d) Anterosuperior aspect of the sphenoid bone of a newborn premature infant with anencephalus and cervical spina bifida (× 2.5). (e) Anterosuperior aspect of the sphenoid bone of a newborn premature infant with complete open spina bifida and anencephalus. The gross appearance of this infant is shown in **Figure 3** c, d (× 3). (f) Anterosuperior aspect of the sphenoid bone of a newborn infant with complete open spina bifida, anencephalus, a diaphragmatic hernia, and a large omphalocele (× 3). (Reproduced from Marin-Padilla M (1965) Study of the sphenoid bone in human cranioschisis and craniorachischisis. *Virchows Archives in Pathological Anatomy* 339: 245–253, with permission.)

for siblings of fetuses with NTDs. In some families, as many as four anencephalics have been born to one mother. Although rare, anencephalics have been born to sisters who were daughters of women who had NTD pregnancies. Although the risk is greater among monozygotic compared to dizygotic twins, a simple genetic mechanism cannot be held responsible for NTDs. (The reader is reminded of those who advanced genetic causes for tuberculosis and Creutzfeldt–Jakob disease – a disease first found in the Fore cannibals of New Guinea.) More to the point, there is increased risk of NTDs in families with two or more affected pregnancies, and this increased risk for subsequent pregnancies is consistent "with a causal role of an environmental agent to which certain families are more exposed than others" (Yen S and Macmahon B as quoted in Elwood and Elwood, 1980). Indeed, one clue to the etiology of NTDs came from the observations of increased incidence in urban compared to rural areas – an observation consistent with a lifestyle or dietary hypothesis.

In 1992, the results of a landmark study by Czeizel A and Dudas I were published in the *New England Journal of Medicine*. Using a carefully controlled double-blind protocol (considered the 'gold standard' by which clinical trials of new pharmaceuticals are routinely conducted), these investigators found that 0.8 mg day^{-1} of folic acid (a water-soluble B vitamin) in a multivitamin preparation prevented spina bifida and anencephalus in the Hungarian women in their study. A subsequent study by Werler M and associates of Boston University (published in

1993) confirmed that folic acid supplementation of the diet could have prevented a large proportion of spina bifida and anencephalus that occurred in the United States from 1988 through 1991. These results also confirmed those published by the United Kingdom Medical Research Council, which had conducted a randomized controlled clinical trial among women who had previously experienced an NTD pregnancy in 1991. The Hungarian trial was so successful that ethical considerations dictated its prompt discontinuation and those women who had been assigned to the placebo group were given the vitamin. These studies were extensions of previous trials on multivitamin supplements conducted in Europe and the United States in the mid-1960s and 1970s. Although it had been known since the 1950s that two cancer chemotherapeutic drugs known to induce folate deficiency (methotrexate and aminopterin) also induced terata in animals and in humans, interpretation of those data was confounded by the fact that folate antagonists have a number of pharmacologic actions, NTDs are not produced uniformly after exposure to either of these drugs, and NTDs are not the only malformations produced. In previous prospective studies of serum and erythrocyte folate, two important epidemiologic and clinical observa tions emerged as indicators of maternal folate status: (1) Folate deficiency can be documented in 66% of mothers of NTD pregnancies and (2) low folate mirrors the woman's socioeconomic status. When matched with mothers of normal children for age, parity, time of conception, and pregnancy, 69% of mothers with NTD pregnancies were folate deficient compared with 17% of the referent controls.

In 1992 and 1993, Australian, Scottish, and Welsh departments of health and social services recommended widespread folic acid supplementation of breakfast cereals and breads. In order to avoid possible excess folate in one's diet, these groups recommended that some unfortified breads and breakfast cereals continue to be available. These groups recommended that women with spina bifida, or those who had a previous child with an NTD, consume 5 mg of folate each day if pregnancy was possible and that supplementation continue through the 12th week of gestation. It was recommended that all other women consume 0.4 mg daily, increase their consumption of folate-rich foods (fruit and vegetables), and avoid overcooking these foods. The United States Public Health Service recommended that all women capable of becoming pregnant should consume 0.4 mg of folic acid per day for the purpose of reducing their risk of having a pregnancy affected by spina bifida or other NTDs. Nevertheless, the debate on folate supplementation of women's diets continues. Money is not an issue (400 μg of folate is currently sold at 4/100ths of a penny and food fortification for 50 servings at 200 μg per serving amounts to one US cent). Rather, the benefits of folate supplementation are weighed against the possibility of masking vitamin B_{12} deficiency. This debate takes place in light of the following: only 30% of low-income women consume the US recommended daily amount of folate and fewer than 10% of women of childbearing age consume 400 μg of folate per day. Only half of the women with incomes 130% of the poverty line or less ate one serving of any vegetable over any 4 days; 18% did not eat any vegetables. With regard to fruit, fully one-third did not consume any fruit or juice and only 5% of White women 19–29 years of age and 4% of Black women of that age ate two or more fruits or three or more vegetables each day. To compound the problem, cigarette smoking reduces blood folate levels. Cigarette smoking is increasingly popular among young women and is found more often among lower socioeconomic groups. Thus, numerous social considerations confront the practical implementation of the prevention of NTDs by folate. It is not known how folate prevents NTDs or what precise role folate plays in early embryonic neural fold elevation and fusion.

Although we are far from understanding why folic acid plays such a significant role in preventing NTDs, some clues are available. To understand the discussion that follows and why clues are so hard to come by, it is necessary to appreciate the complex pathways involving folate metabolism (see **Figure 8**). For example, ~150 genes and their respective proteins are involved in folic acid metabolism and transport. Because gene mutations are known to result in alterations in proteins that are related to various diseases, scientists have focused on key genes involved in folic acid metabolism and transport in an effort to determine whether specific mutations (termed single nucleotide polymorphisms) in 'folic acid genes' are causally related to NTDs. The most extensively studied 'folic acid genes' include folate receptor alpha (FRα), reduced folate carrier (RFC), 5,10-methylene-tetrahydrofolate reductase (MTHFR), methonine synthase (MTR), methionine synthase reductase (MTRR), methylenetetrahydrofolate dehydrogenase (MTHFD), and serine hydroxymethyltransferase (SHMT).

Folates are transported from the extracellular milieu to the inside of cells by either receptor-mediated (FRα) or carrier-mediated uptake (RFC). Because folates are essential for proper cellular function and necessary for neural tube closure, it is easy to envision how mutations in these receptor genes could lead to reduced levels of intracellular folates and thereby NTDs. Thus, several groups have searched for

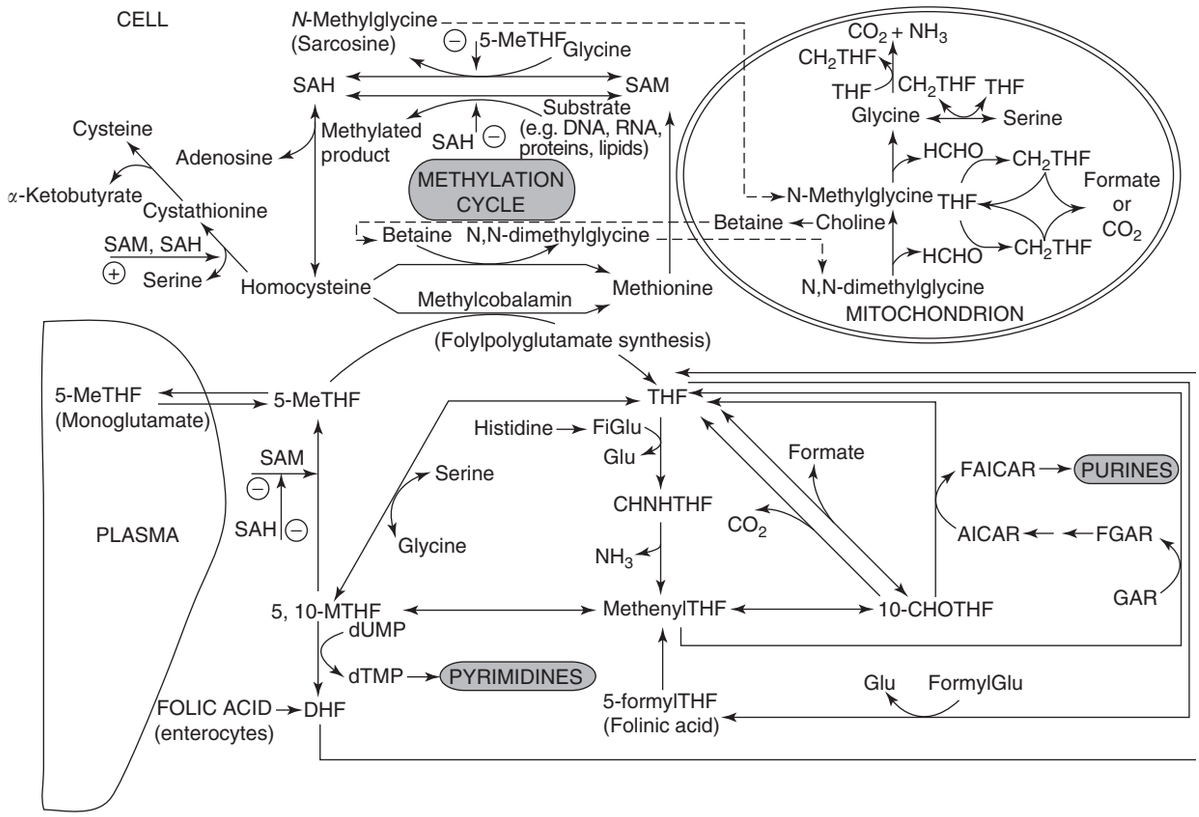


Figure 8 Extended folate metabolism, including compartmentation. *MTHFR*, methylenetetrahydrofolate reductase; *SHMT*, serine hydroxymethyltransferase; *BHMT*, betaine homocysteine methyltransferase, *MAT*, methionine adenosyltransferase; *SAH-hydrolase, S*-adenosylhomocysteine hydrolase; *MT*, methyltransferase; *CBS*, cystathionine β-synthase; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; THF, tetrahydrofolate; and 5-MeTHF, 5-methyltetrahydrofolate. (Reproduced from Van der Put *et al.* (2001) Folate, homocysteine and neural tube defects: An overview. *Experimental Biology and Medicine* 226: 243–270.)

nucleotide polymorphisms in FRα and RFC to determine whether specific mutations are associated with an increased risk for NTDs. Several polymorphisms were found in FRα (631T > C, 610A > G, and 762G > A); however, none of these alterations was associated with increased NTD risk. In addition, other studies failed to find any polymorphisms in RFC. Although studies completed to date have not identified any polymorphisms in FRα or RFC associated with NTD risk, it is important to remember that complete loss of the folate binding protein 1 gene (the mouse homolog of human FRα) resulted in mice with NTDs. This leaves open the possibility that specific mutations in FRα and/or RFC may play a role in the genesis of NTDs.

Once inside the cell, folates participate in a number of interconnected metabolic pathways involving (1) thymidine and purine biosynthesis necessary for DNA synthesis, (2) methionine synthesis via homocysteine remethylation, (3) methylation reactions involving *S*-adenosylmethionine (AdoMet), (4) serine and glycine interconversion, and (5) metabolism of histidine and formate (see **Figure 8**). Via these pathways, folates play an indispensable role in such critical processes as DNA synthesis and gene expression, to name a few. Because DNA synthesis and gene expression are critical processes during embryogenesis, one can see how alterations in these processes due to limited folates could negatively affect important developmental events like neural tube closure. Because methionine is such a critical amino acid, researchers have focused considerable effort on characterizing nucleotide polymorphisms in MTHFR, the rate-limiting enzyme in the synthesis of methionine from homocysteine. Mutations in MTHFR can result in elevated levels of homocysteine, which in turn have been associated with an increased risk for NTDs. The first and most well-studied MTHFR polymorphism is the 677C > T, with TT homozygotes exhibiting reduced MTHFR activity and increased homocysteine. Although several research groups have reported a three- to sevenfold increased risk for NTDs associated with the 677C > T mutation, other studies have reported either a smaller or no associated risk for NTDs. More research is required to determine the

linkage between this mutation and increased risk for NTDs; however, currently available information confirms that this mutation is associated with an increased risk. In addition to this mutation, complete sequencing of the MTHFR coding sequence identified a second common polymorphism, 1298A > C. The homozygous CC allele is, like the TT allele, associated with decreased MTHFR activity, but this CC allele it is not associated with increased homocysteine or an increased NTD risk. Polymorphisms in two other genes, MTR and MTTR, have also been studied. A polymorphism in the MTR gene 2756A > G was found; however, this polymorphism is not associated with increased homocysteine levels or increased risk for NTDs. In contrast, a common polymorphism in MTTR, 66A > G, is associated with increased risk for NTDs when the cobalamin (B_{12}) levels are low or the MTHFR 677 TT allele is present in the infant. Finally, SHMT catalyzes the reaction of serine and tetrahydrofolate to form glycine and 5,10-methylene-tetrahydrofolate and is, therefore, a major entry point for one-carbon units from serine into folate-dependent metabolism. Because of this key role in folate metabolism, SHMT could be a candidate gene involved in NTDs. Two polymorphisms have been identified in the cytosolic form of SHMT, 1181G > A and 1420C > T and two in the mitochondrial form of SHMT, 850C > T and a 4 base pair deletion (delTCTT); however, none of these alterations were associated with an increased risk for NTDs.

While there is little doubt that genetic factors are involved in the etiology of NTDs, proving a causal link between a specific gene (mutation) and an increased NTD risk remains a challenge. Nonetheless, some putative genetic risk factors have been identified. Hopefully, future studies in humans taking advantage of information gained from animal studies will identify not only genetic risk factors but also environmental factors that together culminate in an increased risk for NTDs. Identification of genetic and/or environmental factors contributing to an increased risk for NTDs is a critical step in reducing the numbers of babies born with NTDs.

The Cause of Birth Defects

In the vast majority of cases, the cause of a birth defect is unknown (**Figure 9**).

Maternal infections account for no more than 3% or 4% of the total load of congenital malformations. The most well known of these infections is rubella (German measles). Infection during various stages of gestation corresponds to the particular malformations produced. Malformation of the eye, including cataract and microphthalmia (literally small to nearly absent eye), can be induced by infection during the sixth week. Congenital deafness is induced by infection during the ninth week. Malformations of the heart (patent ductus arteriosus and ventricular septal defect), teeth, and brain (resulting in profound mental retardation) follow infection during weeks 5–10 and weeks 13–28, respectively. As with all known teratogens, not all children exposed to rubella *in utero* develop congenital defects, but the risk of malformation is greatest (to at least 47%) when rubella exposure occurs shortly after implantation.

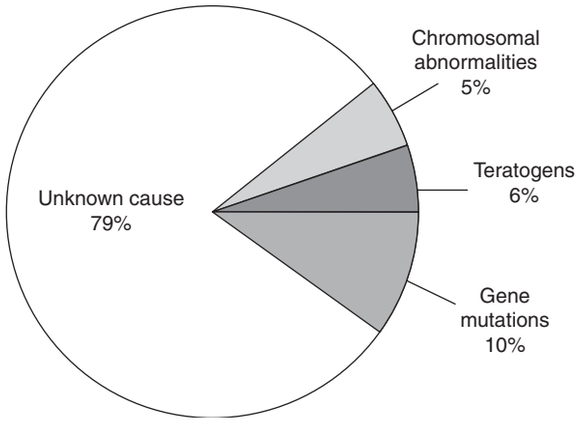


Figure 9 Pie chart of the relative percentages of the causes of human birth defects. It has been estimated by various authorities that cytogenetics contributes to no more than 5% of all malformed live births, Mendelian inheritance to no more than 15–20%, maternal infections 3% or 4%, maternal disease 3% or 4%, problems of constraint *in utero* (amniotic bands) 2%, and all drugs, chemicals, and radiation no more than 1% of the total load of structural birth defects in human beings.

Other infections clearly associated with increased risk of terata include toxoplasmosis, cytomegalovirus, and herpes simplex. Microphthalma, blindness, hydrocephalus, and cerebral calcification can occur after infection with these organisms.

The contribution of genetic disorders is estimated to account for no more than ~15% of the total load of congenital malformations (**Figure 9**). Inherited conditions account for (at most) 20% of the total, and abnormal cytogenetics accounts for no more than 5% of the total. An entire branch of the science of genetics is concerned with abnormally high or low numbers of chromosomes. Variable degrees of mental retardation, frank structural malformation, and sterility are common consequences of abnormal numbers of somatic chromosomes (autosomes) or of sex chromosomes. Two well-known syndromes arising from an abnormally high number of sex chromosomes are Klinefelter's (male only) and Triple X (female only), which originate as failures in normal chromosome number reduction (meiosis) during in spermatogenesis and oogenesis. A syndrome resulting

from too few sex chromosomes is Turner's syndrome, a condition characterized phenotypically by a webbed neck and congenital absence of the ovaries.

An excess number of autosomes or the absence of one or more autosomes can either be lethal to the embryo or result in well-known conditions. For example, the risk of Down's syndrome (trisomy 21 or mongolism) increases with maternal age – being one in 2000 for mothers aged 40 or more years. Extra chromosomes 17 and 18 result in micro- or anophthalmia (congenital absence of the eyeball), mental retardation, cleft lip, cleft palate, and deafness; this condition occurs on average once in every 5000 births.

Phenylketonuria is a genetic disease that results in abnormally high concentrations of the amino acid phenylalanine in the blood. Children of mothers with high plasma phenylalanine ($>3\,\mathrm{mg\,dl^{-1}}$) are at increased risk for microcephaly, mental retardation, heart malformations, esophageal atresia, tracheoesophageal fistula, and low birth weight. There is a clear concentration–response relationship between plasma phenylalanine and abnormal pregnancy outcome; head circumference and low birth weight are inversely (and linearly) related to maternal blood phenylalanine concentrations. Offspring of mothers with plasma phenylalanine $>20\,\mathrm{mg\,dl^{-1}}$ experience a 92% incidence of congenital heart disease; those exposed *in utero* to maternal plasma phenylalanine levels higher than 3 but lower than $11\,\mathrm{mg\,dl^{-1}}$ experience a 21% incidence of congenital heart malformation. These defects can be prevented with adherence to a strict diet to control phenylalanine intake, total energy, protein, and weight gain during pregnancy; however, normal pregnancy outcome can occur only when dietary control occurs at or before conception. Women who consume a 'relaxed diet' experience a 0.6% malformation rate in their children (compared to 0.0% for those on a strict diet). Women placed on a phenylalanine-restricted diet after conception (but before the second trimester) typically experience pregnancies with malformation rates on the order of 19–20%.

Maternal disease – not necessarily of either microbial or genetic origin – can cause or contribute to adverse pregnancy outcome. One common example is diabetes mellitus. If uncontrolled, diabetes mellitus can result in mental retardation, congenital malformation, and embryonic death. Glucose appears to be the teratogenic agent (perhaps potentiated by acetone and β-hydroxybutrate ketone bodies) in uncontrolled diabetes. The extent of hyperglycemia is related directly to the risk of holoprosencephaly (also usually accompanied by microcephaly, cleft lip and palate, mental retardation, and epileptiform seizures), situs inversus (complete transposition of the viscera), and ureteral duplex (complete or partial double ureter). These risks are 400, 84, and 23 times that in uncomplicated pregnancy, respectively. Sacral, vertebral, and pelvic malformation are also associated with elevated maternal glucose levels. To illustrate that stage of development determines susceptibility to teratogenic insult, women who develop diabetes relatively late in gestation (second or third trimester) do not have an increased risk of adverse pregnancy outcome. Only those women with uncontrolled diabetes prior to conception and through the first 8 weeks of pregnancy are at risk. Insulin control of maternal diabetes produces marked reduction in neonatal mortality (from 33% in the decade 1920–30 to 6.5% from 1975 to 1979). By careful management of these mothers, the number of diabetes-induced late fetal deaths (stillbirths) and depressed or abnormally high birth weights (macrosomia) have been reduced to the point that birth defects are now the leading cause of death among offspring of diabetic mothers.

Another example of a maternal condition that contributes to birth defects is low circulating iodine. Cretinism is one of the most profound, but completely preventable, syndromes of malformation known. Characteristic consequences of prenatal iodine deficiency include pervasive mental and physical retardation, deaf-mutism (due to primary malformation of the inner ear), lack of muscle tone with a spastic or rigid walk, and failure to attain a height at maturity of more than 1 m. Today, this condition (known as endemic cretinism) is most prevalent in impoverished areas of African and East Asian countries. Prior to implementation of a national program of iodized salt in the early part of the twentieth century, endemic cretinism was commonplace in Switzerland. After institution of iodized salt, deaf-mutism declined 50% within 8 years and no cretins have been born in that country since 1930.

Maternal disease in addiction contributes to infant morbidity and mortality. Two of the most common agents, ethanol and tobacco smoke, are illustrative. A host of clinical and epidemiologic studies have confirmed a distinctive pattern of congenital malformations in babies born to alcoholic mothers. These malformations include microcephaly, short palpebral fissures, epicanthal folds, maxillary hypoplasia, cleft palate, micrognathia, joint disease, cardiac anomalies, capillary hemangiomata, anomalous genitalia, and retarded fetal and neonatal growth and development (a pattern referred to as fatal alcohol syndrome (FAS)). FAS children have abnormal motor and psychological development and abnormal dermatoglyphic characters. Miscellaneous terata also found among these children are arthrogryposis, limb

reduction defects, and gastroschisis. With ethanol, there is a definite dose–response relationship: a 9% malformation rate in light drinkers, a 14% rate for moderate drinkers, and a 32% rate for heavy drinkers. In addition, as with almost all teratogens, the severity of the malformations is greatest in those infants born to mothers consuming the highest dose. Some authors have argued that consumption of one type of alcoholic beverage (e.g., wine, beer, and schnapps) was more or less dangerous than another; however, the relationship holds true for all drinking. Pregnancy outcome is directly related to the quantity of absolute ethanol consumed per day. Ethanol doses consumed by these mothers range from 'social' (1 oz of absolute ethanol day^{-1} or $350\,mg\,kg^{-1}\,day^{-1}$) to 'heavy' (2.3–15 oz of absolute ethanol day^{-1} or $800–5600\,mg\,kg^{-1}\,day^{-1}$). Statistically significant reductions in birth weight (91 g for ethanol exposure before pregnancy and 160 g for exposure in late pregnancy), decreased infant length, increased stillbirth and second-trimester spontaneous abortion (0.5–1 oz of absolute ethanol day^{-1}), and increased risk of early spontaneous abortion (1 oz of absolute ethanol, twice per week) are well documented. Notwithstanding these data, results from the National Institute of Child Health and Human Development (NICHHD) study of drinking habits and pregnancy outcome in 32 870 women who had two drinks or less each day showed that those women had the same overall risk of birth defects as pregnant women who did not drink ethanol at all. NICHHD defined moderate drinking for purposes of the study on a daily basis (e.g., wine with a meal) but excluded binge drinking (foregoing drinking during the week but consuming several drinks on the weekend).

The most common addiction during pregnancy is tobacco smoking; 30.9% of all US women smoke before pregnancy and 25.5% continue to smoke during pregnancy. This practice continues despite the fact that the first reports of adverse effects on the human fetus were published more than 80 years ago and despite the legally required warnings on tobacco advertisements and product packaging. At least 19 major epidemiologic studies of more than 300 000 pregnancies have been published. The results of those studies lead to the following dose–response conclusions. Smoking 10–20 cigarettes per day throughout pregnancy increases the risk of early spontaneous abortion and reduces birth weight by as much as 92 to 316 g. Women who cease smoking during the early part of their pregnancy deliver babies with birth weights near those of babies born to mothers who have never smoked. The frequency of spontaneous abortion is directly related to the number of cigarettes smoked; the frequency for pack-a-day mothers being double that of nonsmoking mothers. The data hold true after correcting for maternal age, race, height, weight gain during pregnancy, socioeconomic status, gestational age, and parity. It appears that it is the nicotine and carboxyhemoglobin content of maternal blood that is responsible for decreased placental blood flow and anoxia leading to or contributing to the low birth weight. Perinatal mortality increases exponentially with decreasing birth weight; perinatal mortality is increased 20% in offspring of mothers who smoke less than one pack per day, and it is increased 35% in mothers who smoke more than one pack a day. Tobacco use is the single most important preventable determinant of low birth weight and its associated perinatal mortality in the United States. Of the 39 000 excess low-birth-weight babies in the United States, 5900 could be prevented each year by smoking cessation. The hospitalization and medical cost on a national basis for these babies is enormous – so much so in fact that for every $1 spent on smoking cessation during prenatal care, at least $6 in hospitalization and related medical care costs can be saved. This one intervention alone could double the cost savings gained by prenatal care.

Fear of Birth Defects

Two of the most questionable practices by those who are not familiar with the principles of teratology are (1) making lists of chemicals or other agents known or suspected to be teratogenic or otherwise toxic to the embryo and fetus and (2) assuming that the results of animal studies mimic those in human beings.

Perhaps the most obvious example of the first practice is California's Proposition 65, a set of laws enacted by public vote in 1986 (known as the Safe Drinking Water and Toxic Enforcement Act). Proposition 65's list, like other similar lists, includes chemical compounds ranging from known human teratogens to compounds having adverse effects demonstrated to occur only in laboratory animals. This list reinforces the notion that compounds are either 'positive' (teratogenic) or 'negative' (nonteratogenic) and that providing printed or other warnings will prevent birth defects. This is simply not the case. All compounds can cause the various manifestations of developmental toxicity provided the exposure (dose) is large enough, the route of exposure is appropriate, and the timing of exposure occurs during a susceptible period of development.

One clear example of a known teratogen for which warning can be effective is ethanol, but warnings alone are not the whole story. Alcohol consumption

during pregnancy is a definite problem that has increased over time (**Figure 10**). Studies of children in the general population indicate that from 0.1% to 0.2% show signs of FAS, but this figure does not represent the true measure of this problem. Among Native American tribes of the Great Plains or in northern British Columbia, the Yukon and the Southwest, studies have found 5% of the children affected. On the Pine Ridge reservation in South Dakota, more than 25% of the children show signs of FAS. Many of these mothers are so disabled by their disease that they are unable to care for their children. These children are difficult to place in foster homes because of their mental deficiency and inability to develop appropriate emotional and logical responses to everyday situations.

In the case of compounds that are merely suspected teratogens, however, there can be dark consequences. For example, in August 1973, the United States Consumer Product Safety Commission (CPSC) banned the sale of spray adhesives and published national warnings that these products caused birth defects and chromosome abnormalities. The CPSC warned all pregnant women who may have had contact with these sprays to see their physician and inquire about the chromosomes of their fetus. The minimum consequences of this regulatory action were 1273 working days logged by 130 US diagnostic and genetic counseling centers on spray adhesives, at least 380 chromosome studies, 11 amniocenteses, and at least nine elective abortions out of concern for exposure to spray adhesive. Eight of these abortions were performed without first performing diagnostic amniocentesis, and one was performed in a woman who had chromosome breaks in her amniotic fluid. The genetic counselor in the latter case had informed the mother that he was unable to determine the health of her fetus with the information he had on hand; she elected abortion because of fear of possible birth defects and without telling the counselor of her decision. The aborted fetus was fixed in formalin and a detailed autopsy was performed: not only was there no evidence for any congenital abnormality, but the chromosome change first observed was also found to be due to viral contamination of her amniotic fluid sample. In those areas of the country where local newspapers had given the CPSC warnings the greatest publicity, the greatest numbers of inquiries to local genetic counseling services were made. Six months later, the CPSC withdrew the ban on these sprays because no toxicity of the substances in the spray could be demonstrated and the original observations on chromosome damage could not be confirmed.

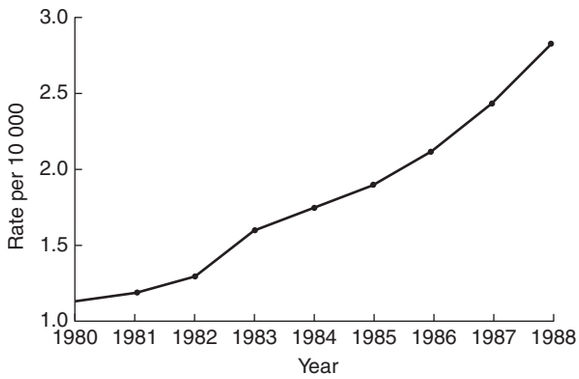


Figure 10 Fetal alcohol syndrome rates in the United States, 1980–88. These rates have shown a steady increase due to increased recognition and reporting of this condition by physicians and not necessarily due to increased alcoholism among women of childbearing age. (Reproduced from *Birth Defects and Infant Mortality*, Infant Mortality Report Series, vol. 1, no. 2, March of Dimes Birth Defects Foundation, with permission from March of Dimes.)

The 1973 US CPSC action and its consequence is not an isolated or rare example. Pregnant women are often very worried about birth defects. Women not exposed to any teratogenic agent appearing on any lists believe they have a one in four chance of having a child with major structural malformations – a risk equivalent to that after prenatal thalidomide exposure. Single women have a significantly higher (probability of less than 0.05) tendency to terminate their pregnancy than do married women; published studies demonstrate a greater willingness on the part of single mothers to abort their fetus when exposed to nonteratogens compared to married women in similar circumstances. The data show that the economic and social factors cited by single mothers in decisions to continue their pregnancy are compounded by distorted perceptions of teratogenic risk.

That the young, the minority, and the educationally and economically disadvantaged are placed in a particularly vulnerable position with respect to this misinformation is highlighted by the following example. United States hospitalization and census data show that 61% of all pregnancies end in live birth, 26% in induced abortion, and 13% in early embryonic and late fetal death. Definite ethnic- and age-related differences underlie these overall rates. United States pregnancy rates for nonwhite women (80% of whom are Black) average 68% higher than for White women. Although current census data show reduced numbers of births to teenage mothers (a 10% decline in the number of teenage pregnancies since the 1970s), those figures mirror the decline in the total number of teenagers (9%) over the same period. Pregnancy rates among teenagers as a group increased because of the decline in use of oral

contraceptives and increased sexual activity. For US teenagers 15 years of age, more than 50% of all pregnancies terminate in elective abortion; for all US teenage pregnancies, 40% end in abortion and 10% in fetal loss. For women aged 15–19 years, there has been a 24% increase in elective abortion since 1976. Rates of induced abortion for US nonwhite women are significantly greater than those for White women with the differential increasing to age 34 (after which this differential declines). These patterns underlie the fact that, in North America and Europe, there is one induced abortion for every live birth.

Among the most problematic issues raising the specter of death and disability is radiation. After the Hiroshima and Nagasaki atomic bombs, spontaneous abortion in survivors who were pregnant increased to the point that one-third of the embryos died and of those that lived, at least 25% were afflicted with a structural malformation of one type or another (microcephaly, spina bifida, ocular defects, or oral cleft). There is no question that exposure to radiation from atomic bombs, or from X-rays or other medical procedures, has been responsible for instances of human congenital malformation. Other experience illustrates an ironic association between radiation and abortion. Following the Chernobyl meltdown and disaster in the former Soviet Union, fear and rumor were responsible for the abortion of at least 2500 otherwise wanted pregnancies in Greece. This occurred despite the fact that the radiation drifted north, to Scandinavia, and that effective exposure in Greece was 100 mrem – much less than the amount that could cause terata. In all of Western Europe, the total number of panic-induced abortions resulting from that episode has been estimated from hospital records at 100 000–200 000.

The second questionable practice by those unfamiliar with the principles of teratology concerns an overemphasis of animal data. Several thousand compounds have been identified as developmentally toxic in animal bioassays, but only relatively few are known human teratogens. There is a tendency among those who have not actually conducted laboratory studies, those who substitute a strength-of-evidence approach for the weight-of-evidence approach, or those who are otherwise unfamiliar with the principles of teratology to assume that the effects seen in animal (including bird) studies do or could occur in people. This is evident in: (1) epidemiology studies in which investigators focus on a specific malformation in human populations after those defects have been observed in animal studies and (2) laboratory studies in which investigators have attempted to confirm or reproduce the human syndrome in animals. Concordance between animal and human data is the exception rather than the rule. Five teratogens are offered here to illustrate this point: acetazolamide, aspirin, caffeine, lead, and trypan blue. Finally, the retinoids (a large and diverse number of compounds of which vitamin A is a member) are presented to demonstrate the fact that although species concordance is relatively rare, it does occur.

Acetazolamide (Diamox or Hydrazol), a prescription diuretic, is a classic example of a teratogen that produces malformations in a highly species-dependent fashion. Administration of acetazolamide to pregnant mice, hamsters, or rats causes right forelimb postaxial ectrodactyly (absent digit) and only on very rare occasions is any other malformation induced. Acetazolamide is not teratogenic in monkeys and, despite its widespread use in the 1950s in early and late human pregnancy, there has been no evidence that acetazolamide caused developmental toxicity in humans.

Acetazolamide exerts its pharmacologic action through inhibition of the enzyme carbonic anhydrase. It is believed that inhibition of this enzyme in rodent placenta results in disruption of the normal potassium ion balance, producing the malformation of the right forepaw. Replacement of the potassium lost to acetazolamide inhibition of carbonic anhydrase prevents the terata that would otherwise be induced by acetazolamide. Because the rodent fetus remains in constant orientation *in utero* with its right side against the placenta, it is thought that local disruption of potassium in that area dictates the constant malformation of only the right digit. In those rodent strains having genetic situs inversus, only the corresponding left digit is affected. In primate embryos, there is no detectable carbonic anhydrase at the stage of development where acetazolamide would be expected to induce these malformations.

Aspirin is the most widely used of any medication in the United States. Aspirin is a reliable, reproducible teratogen in rodents, cats, dogs, ferrets, and monkeys when 50–500 mg kg^{-1} oral doses are administered on a susceptible day of gestation. Malformations of all major organ systems, growth retardation, embryonic/fetal death, and behavioral deficits in the survivors are all consequences of prenatal aspirin exposure in common laboratory animals. Epidemiologic studies have failed to demonstrate any syndrome of terata; increased embryonic, fetal, or neonatal mortality; or reduced birth weight that could be attributed to *in utero* aspirin exposure. Some epidemiologic data indicate that mothers of children with congenital malformations actually consumed less aspirin during the first trimester than did mothers of normal children. To be sure, there are case reports of limb reduction defects, cardiac malformations, and even

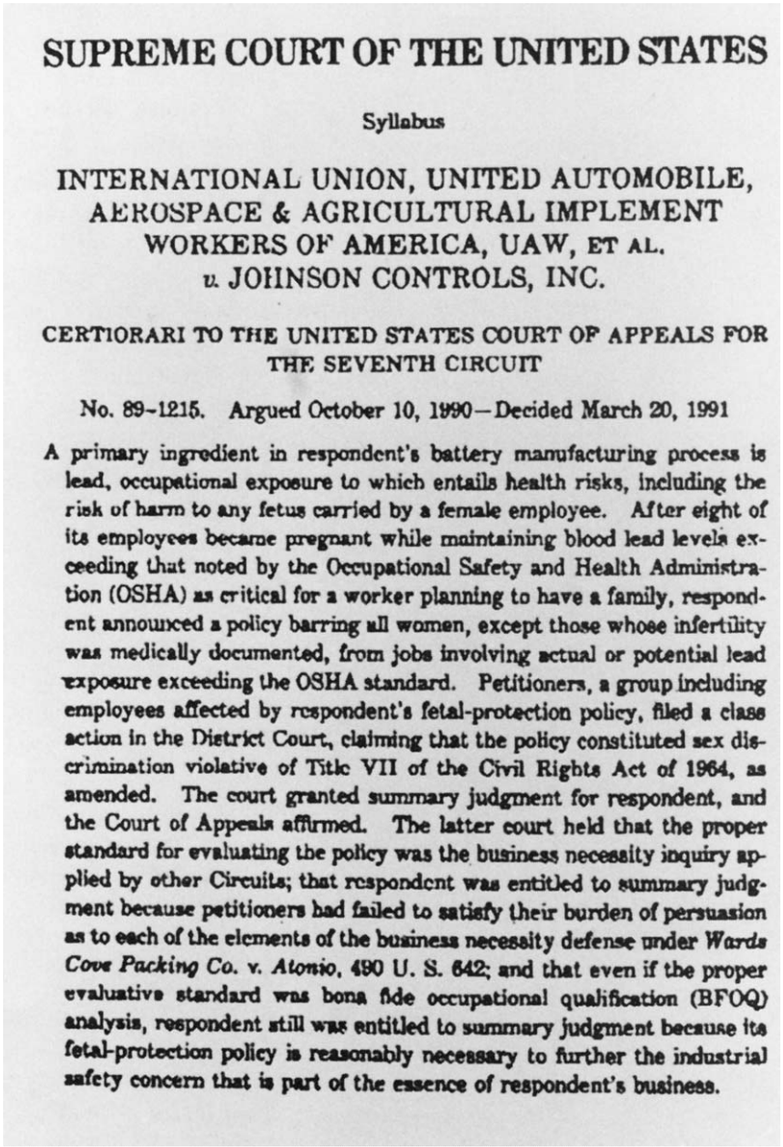
cyclopia associated with prenatal aspirin exposure. When circulating aspirin concentrations in the blood of pregnant rodents or monkeys given teratogenic doses of aspirin are compared with concentrations found in human blood, the values in the animals are of the same order of magnitude as (and in some instances even less than) those found in human blood. Aspirin can be present in human umbilical cord blood and in normal newborn blood at concentrations of up to 10 times those associated with embryotoxicity in animals.

It is acknowledged that, given aspirin's widespread, unrestricted use among the 6 million pregnant women per year in the United States and given the 7.5% total malformation and minimum 13% fetal loss (spontaneous abortion and stillbirth) rates, a great many of these adverse pregnancy outcomes will have experienced aspirin exposures. It is also acknowledged that, if aspirin were a newly developed drug submitted today for regulatory evaluation, it is highly unlikely that it would be approved for marketing. The data published to date demonstrate that, at the aspirin doses usually consumed and at doses less than those causing overt intoxication (salicylism), the risk of adverse pregnancy outcome is no greater than the norm. Like acetazolamide, it appears that there is a physiologic insensitivity on the part of the human embryo compared to other species (which explains the differential teratogenic potency).

Caffeine is, without question, teratogenic in common laboratory animals. It is in the methylxanthine of chocolate and cocoa (2–20 mg/5 oz serving), coffee (74 mg/8 oz serving), soft drinks (30–58 mg/12 oz serving), prescription drugs (32–100 mg), and over-the-counter drugs (30–200 mg). In rodents and rabbits, oral doses of 80–150 mg kg^{-1} day^{-1} induce a consistent pattern of limb reduction defects and delayed development (usually measured as reduced skeletal ossification). A single, large oral bolus is more effective in producing limb malformations than is the same total dose given over several days. More than a dozen published epidemiologic and clinical studies on thousands of pregnant women and their caffeine consumption have failed to confirm any relationship between caffeine and human congenital malformation. Some studies have shown a possibility of increased risk (1.7 times normal) of early spontaneous abortion in selected subgroups consuming total doses in excess of 3 mg kg^{-1}; however, these studies failed to take into account covariates like ethanol and cigarette smoke or measured caffeine consumption before (but not during) pregnancy. A study of offspring of 1529 women who consumed three, four, or six cups of coffee per day (corresponding to 222, 296, and 444 mg caffeine per day, respectively) during early and mid-gestation at birth through age 7 and adjusted for use of ethanol, tobacco, aspirin, acetaminophen, prescription drugs, and also dietary composition found no evidence of functional deficit. There is no question that caffeine crosses the rodent and human placenta and that caffeine has been detected in human umbilical cord and newborn blood. Here dose is the parameter that underlies the differential response in humans and laboratory animals; it is theoretically possible that daily ingestion of caffeine at concentrations equivalent to that of 75 cups of coffee, 125 cups of tea, or 200 cans of a caffeine-containing soft drink could induce terata in humans. Exposures to high doses such as these occurred in the early 1980s from ingestion of mail-order caffeine pills (each pill containing $\sim$500 mg caffeine). These doses produced stroke, convulsions, tachycardia, coma, and at least 12 deaths in acute cardiopulmonary arrest. None of the individuals involved appear to have been pregnant at the time.

Lead is another example of an agent that induces terata in laboratory animals that have no direct concordance in human beings. When pregnant hamsters are given a sufficiently large dose of a water-soluble lead salt early in gestation, the otherwise normal young are born without tails. When first reported, some advocated the position that since humans do not normally grow tails, the observations in hamsters were only a laboratory curiosity. Today, however, the effects of lead on the developing human are well documented. There are at least six major prospective epidemiologic studies on prenatal lead exposure and postnatal cognitive development. Deficits in Bayley Mental Development Index scores and problems with language skills in young children are linked with maternal or umbilical cord lead concentrations in the 20–30 $\mu g\,dl^{-1}$ range. In some children, slowed intellectual development has been noted after exposure to umbilical cord lead concentrations of as little as 10 $\mu g\,dl^{-1}$ during gestation. (By comparison, background blood lead in healthy US adults without occupational lead exposure ranges from 2 to 6 $\mu g\,dl^{-1}$.) It is important to note here that these studies also show that the early postnatal delay in intelligence is not permanent and generally cannot be detected at more than 5 years of age. These data have had a profound influence on social policy worker protection and reproductive health in the United States (**Figure 11**).

Trypan blue, a teratogenic azo dye, is yet another example of a compound which produces malformations in a highly species-specific fashion. Exposure of pregnant rodents to trypan blue during early gestation produces a uniform lumbosacral spina bifida in their offspring (**Figures 12–14**). These malformations are anatomically indistinguishable from those in



SUPREME COURT OF THE UNITED STATES

Syllabus

INTERNATIONAL UNION, UNITED AUTOMOBILE, AEROSPACE & AGRICULTURAL IMPLEMENT WORKERS OF AMERICA, UAW, ET AL. v. JOHNSON CONTROLS, INC.

CERTIORARI TO THE UNITED STATES COURT OF APPEALS FOR THE SEVENTH CIRCUIT

No. 89-1215. Argued October 10, 1990—Decided March 20, 1991

A primary ingredient in respondent's battery manufacturing process is lead, occupational exposure to which entails health risks, including the risk of harm to any fetus carried by a female employee. After eight of its employees became pregnant while maintaining blood lead levels exceeding that noted by the Occupational Safety and Health Administration (OSHA) as critical for a worker planning to have a family, respondent announced a policy barring all women, except those whose infertility was medically documented, from jobs involving actual or potential lead exposure exceeding the OSHA standard. Petitioners, a group including employees affected by respondent's fetal-protection policy, filed a class action in the District Court, claiming that the policy constituted sex discrimination violative of Title VII of the Civil Rights Act of 1964, as amended. The court granted summary judgment for respondent, and the Court of Appeals affirmed. The latter court held that the proper standard for evaluating the policy was the business necessity inquiry applied by other Circuits; that respondent was entitled to summary judgment because petitioners had failed to satisfy their burden of persuasion as to each of the elements of the business necessity defense under *Wards Cove Packing Co.* v. *Atonio*, 490 U. S. 642; and that even if the proper evaluative standard was bona fide occupational qualification (BFOQ) analysis, respondent still was entitled to summary judgment because its fetal-protection policy is reasonably necessary to further the industrial safety concern that is part of the essence of respondent's business.

Figure 11 Summary statement of the famous Johnson Controls decision by the United States Supreme Court in the matter of lead and developmental toxicity.

humans with spina bifida (**Figure 2**d). After trypan blue treatment, the dye distributes to all maternal organs except the brain because it is unable to cross the blood–brain barrier. The embryo does not contain any of the dye, but the cells of the trophoblast, the parietal and the visceral yolk sac, and derivatives of the gut show visible accumulation of the compound. The site of action of trypan blue is the visceral yolk sac and the associated endoderm where it inhibits pinocytosis, causes increased mineral ion absorption, disrupts local osmotic balance, uncouples oxidative phosphorylation, and ultimately interferes with histiotrophic nutrition. Trypan blue does not reach or act on the embryo itself and there are no cellular or subcellular pathologies that can be attributed to the temporal presence of the dye. The interference with embryonic nutrition results in accumulation of fluid (intercellular edema) in the paraxial mesenchyme that is located above the affected endoderm. This is followed by extravasation from the blood islands of the yolk sac placenta into these expanded intercellular spaces, giving rise to failure of neural fold apposition and fusion in that area leading to myeloschisis (**Figure 11**). The cell cycle in the neuroepithelium of the affected area maintains normal generation times and the neuroepithelium continues to grow, only in a mechanically distorted fashion.

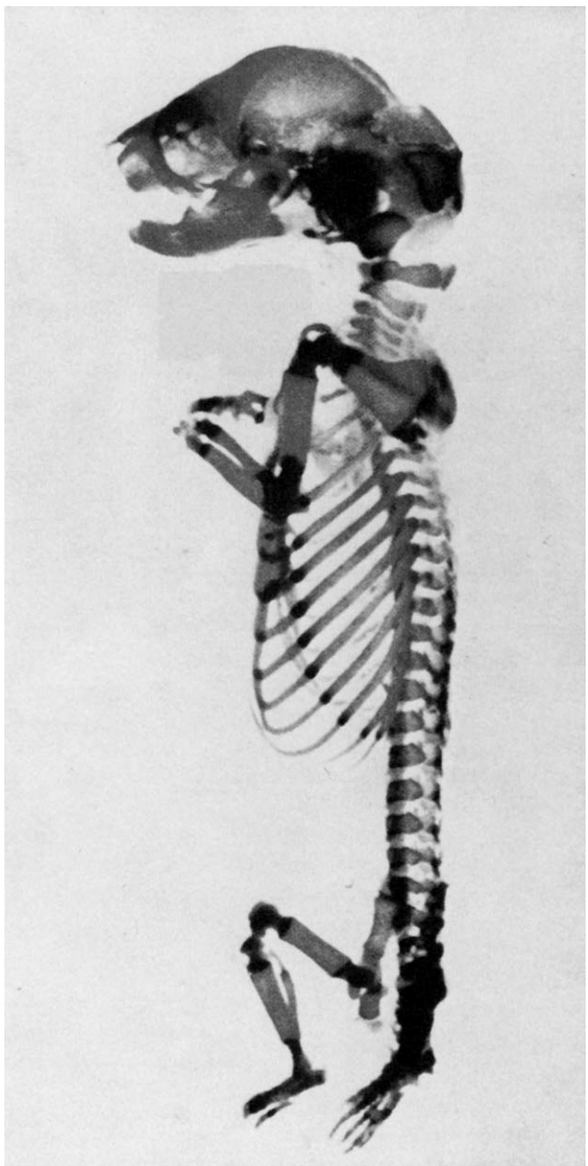


Figure 12 Skeleton of a day 20 control rat fetus. The ossification in the skull, vertebrae, ribs, and sternebrae is nearly complete. Endochondral ossification of the pectoral and pelvic girdle and limbs is progressing ($\times$ 3.2).

In rodents and rabbits, two placentas function during organogenesis: the yolk sac (choriovitelline) and the chorioallantoic placenta. In humans, the chorioallantoic placenta is the organ in which exchange between maternal and embryonic blood takes place. The choriovitelline placenta of rodents and lagomorphs is a selective adaptation in those species which have a very high reproductive potential of short gestation (hamster = 17 days) and large (six to 17 littermates) litters. Trypan blue does not induce its teratogenic response in those species that do not depend on the (comparatively primitive) yolk sac placenta.

Retinoids (vitamin A and its congeners), on the other hand, are agents capable of inducing terata across diverse species. Excess retinoic acid (RA) in human, hamster, and macaque embryos induces a nearly identical syndrome of craniofacial, cardiac, and central nervous system (CNS) terata. The craniofacial defects are due to disruption of the neural crest. The gestational stage during which embryos must be exposed to elicit craniofacial terata corresponds to a time when neural crest cells are still associated with the neuroepithelium. Retinoid teratogenesis displays a classic U-shaped dose–response relationship and has a definite structure–activity relationship. Experimental (proprietary) retinoids have been synthesized which are among the most powerful teratogens known, some being as potent as the most active dioxin. Retinoid deficiency, however, is also teratogenic. Retinoids related to RA (including the 13-*cis* isomer and the oxidized and glucuronide metabolites) are normal constituents of human and animal tissues; thus, one can debate what constitutes a 'safe' dose.

The effects of retinoids on embryos occur through changes in gene expression. Two families of nuclear receptors, termed retinoic acid receptor (RAR) and the related RXRs, have two endogenous retinoids, RA and its 9-*cis* isomer, as normal ligands. These receptors mediate retinoid changes in gene expression. When these receptors bind their particular ligand, they induce transcription by complex with the promoter region of the target genes (termed response element or RARE). The nucleic acid sequence GGTCA is present in at least two parts of the response element and this DNA sequence is the minimum (termed half-site) for binding of one receptor molecule. Subdivisions (RAR-α, RAR-β, and RAR-γ) of each family are recognized, each with a number of isoforms. Retinoid nuclear receptors are subsets of the steroid/thyroid hormone superfamily of nuclear receptors, all of which recognize an AGGTCA sequence, and it is the spacing of this sequence which determines response element specificity for the cognate receptor. The RXR receptor recognizes the AGGTCA half-site separated by one base pair and the RXRs form heterodimers with those nuclear receptors like RARs that bind to direct repeats of the half-site. RARs require heterodimerization with RXRs for efficient DNA binding; after RXR–RAR interaction, binding to the specific DNA sequence is stabilized. The RAR–RXR heterodimers are gene activators in the presence of ligand and are repressors in the absence of ligand.

The expression of the retinoid nuclear receptors and related proteins for cytosol retinoid transport,

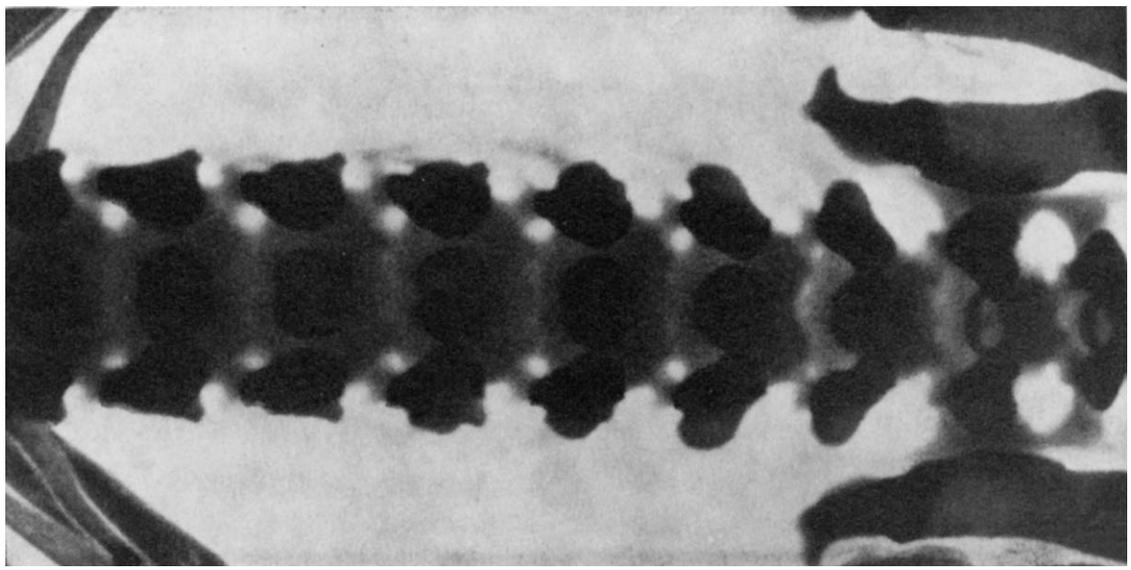


Figure 13 Higher magnification of the vertebrae, ribs, pelvic girdle, and limbs of the fetus shown in **Figure 12**. Note how the ossification centers of the bodies and arches of the vertebrae are regularly oriented ($\times$ 9).

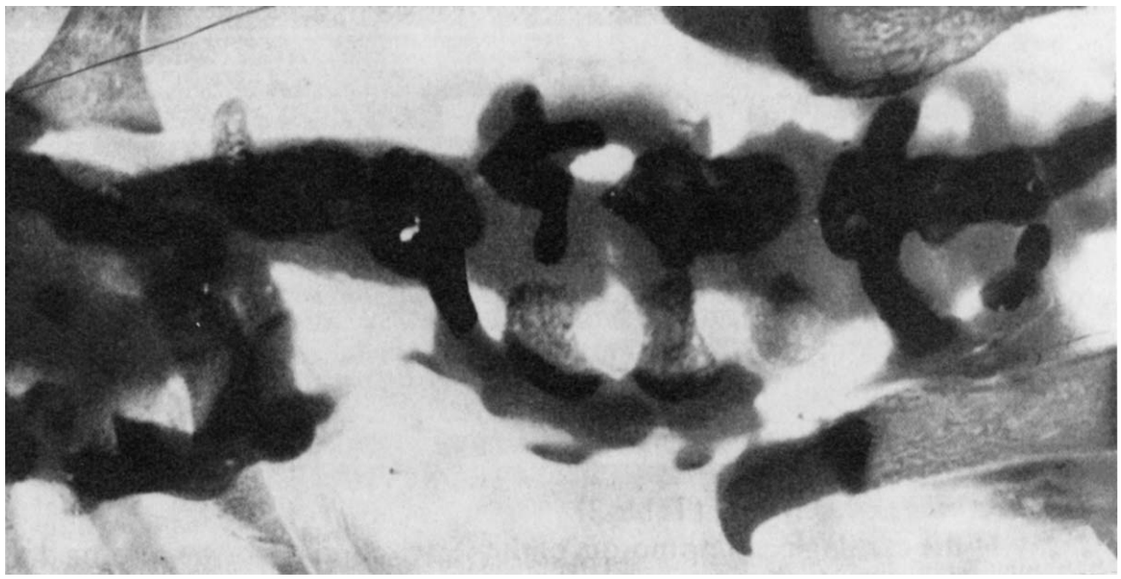


Figure 14 Magnification of the thoracic, lumber, and sacral regions of the spine of a rat fetus with trypan blue-induced spina bifida aperta on day 21 of gestation. Note the severe malformations of the vertebral column when compared to the control (**Figure 13**). The vertebral arches and bodies are so malformed that it is difficult to identify those structures. (Reproduced from Peters PWJ, Verhoef A, De Liefde A, and Berkvens JM (1981) Development of the skeleton in normal rats and in rats with trypan blue-induced spina bifida. *Acta Morphological Neerlando-Scandinavica* 19: 21–34, with permission.)

metabolism, and control of local concentrations of free ligand (termed the CRABPs and CRBPs) in developing embryos are carefully controlled in space and time. Embryonic CRABP is differentially expressed in those structures sensitive to retinoid teratogenesis (e.g., branchial arch mesenchyme and portions of the embryonic CNS), but this cellular binding protein is not directly involved in retinoid mechanism of action. The RAR-α serves a common 'housekeeping' function, and it is present in nearly all embryonic tissues. RAR-β is abundant in the lateral nasal processes and hindbrain neuroectoderm. RAR-γ predominates in somitic mesoderm, frontonasal and precartilaginous mesenchyme, and skin. RAR-β

does not colocalize in embryos with RAR-γ and neither does it occur in the limb, except in the interdigital mesenchyme (which is doomed to die in the formation of normal digits). RAR-β is expressed in inner ear mesenchyme, which gives rise to structures damaged by retinoids and producing deafness in those children who survive (**Figure 15**).

It is the binding and transactivation by the retinoid nuclear receptors on target genes in embryos which is held responsible for retinoid teratogenicity. It is the disruption of normal embryonic segmentation and alterations of normal gene expression by exogenous retinoid which leads to the defects (**Figure 15**). Homeobox (Hox) gene expression is one of the targets. The gene *Hox 2.9* (found normally in rhombomere 4 during stages sensitive to RA insult) is inappropriately expressed in rostral areas, and *Krox-20* (a gene found normally in rhombomere 3) is suppressed after exposure to RA. *Hox 2.9* expression is also disrupted in neural crest and mesoderm – tissues that are known targets in retinoid teratogenesis. Normal segmented patterns of temporal gene expression are either disrupted or lost altogether in RA-treated embryos and it is these genes which have roles in normal cardiac and craniofacial and CNS morphogenesis.

The paradigm emerging is that of a cascade (a series of biochemical steps using secondary, tertiary, or more messenger molecules, each successive step amplifying those of the preceding step). After retinoid absorption from the gut, biotransformation in the liver, transport through the blood and across the

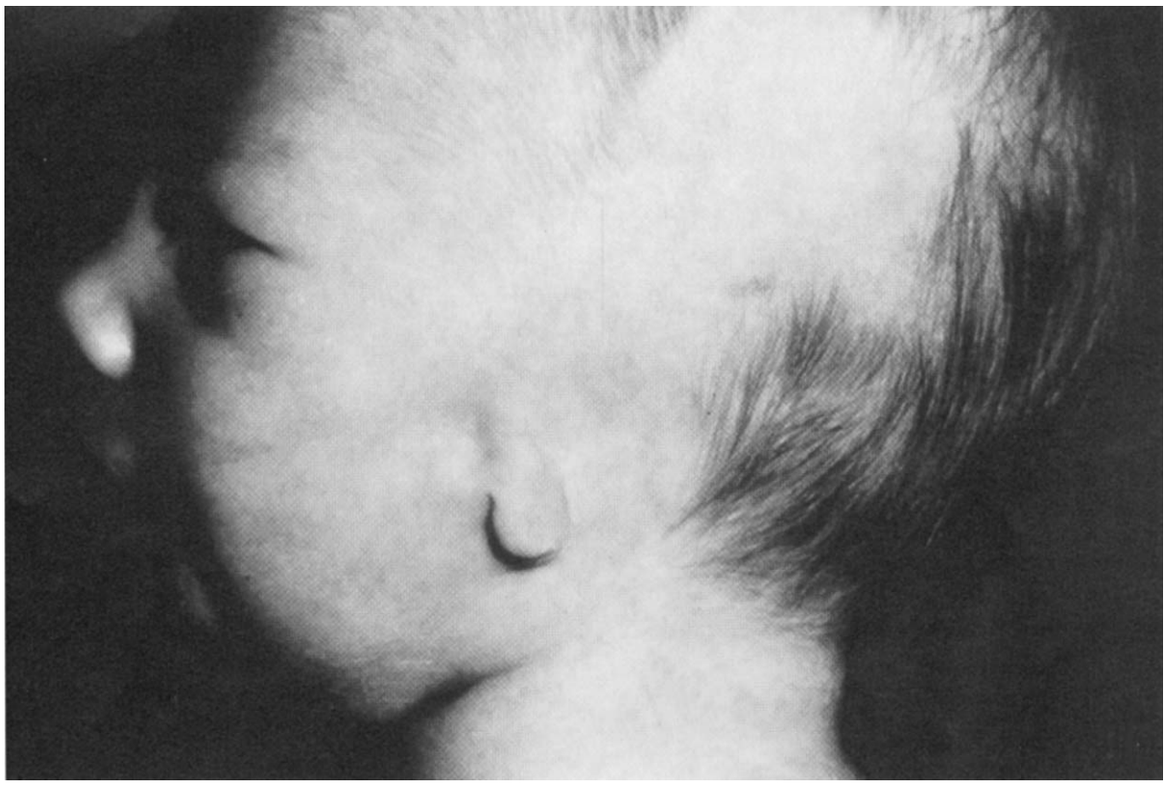


Figure 15 Lateral view of a 2.8 kg newborn at 38 weeks gestation exposed to 80 mg day^{-1} isotretinoin (13-*cis*-retinoic acid) from days 0 through 42 of pregnancy. Note the rudimentary pinna, imperforate external auditory canal, micrognathia, depressed nasal bridge, prominent occiput, and narrow sloping forehead. This child is also afflicted with paralysis of the left side of the face, abnormal visual-evoked potential, hyperterlorism, latent auditory brain stem response, reduced muscle mass and tone in his legs, and eyes that cannot follow. This child also has a cleft palate, ventricular septal defect, aortic stenosis, pulmonary stenosis, dysplastic pulmonary aortic arch, and at 2 months of age he required a tracheostomy and gastrostomy. This collection of malformations is known as retinoid embryopathy. The syndrome is common in infants born to mothers ingesting from 20 to 50 mg day^{-1} isotretinoin in treatment of acne from conception (day 0) or shortly thereafter (days 14–16) through the first trimester (days 35–84). Identical malformations can be induced in fetal hamsters after isotretinoin exposure during equivalent stages of embryogenesis (early primitive streak stage in hamster occurs on day 8); in humans, retinoid embryopathy occurs after exposure during implantation (day 7), the primitive streak stage (day 14), closure of the anterior neuropore (at 10 somites or about day 25), and through appearance of limb buds (days 27 and 28). Thus, the most sensitive time for exposure occurs before the mother knows she is pregnant (i.e., from the first missed menstrual period). (Reproduced from Willhite CC, Hill RM, and Irving DW (1986) Isotretinoin-induced craniofacial malformations in humans and hamsters. *Journal of Craniofacial Genetics and Developmental Biology* 2(Suppl.):193–209.)

placenta, and sequestration in target cells in the embryo the first biochemical actions are triggered by retinoid binding to their cognate nuclear receptor(s). The retinoid–receptor complex binds as a heterodimer to specific upstream regions of the clusters of Hox genes. Hox genes contain the information for patterning of the vertebrae, brain, and branchial arches (Hox codes). The proteins produced by transcription of Hox genes are themselves transcription factors (called homeoproteins). The homeoproteins have DNA-binding domains that are remarkably consistent from amphibians to birds, rodents, and primates – just as are the highly conserved regions of the retinoid nuclear receptors. (It is the highly conserved nature of the morphogenetic mechanism of action of this cascade which forms the basis for the similar teratogenic profiles of retinoids across diverse species – a reflection of the most ancient of vertebrate pattern formation maintained through prehistoric evolution.) Abnormal expression of Hox codes in target cells precipitates magnified local disruption of the ordinary careful control and coordination in vertebrate body pattern formation. Once the small window in space and time has closed and the affected progenitor cells have not arrived at their appointed station in sufficient numbers, all is lost. There is a cascade of abnormal events at the next higher level of organization – when the neural crest cells remain clustered together just under the neuroepithelium instead of migrating down to the branchial arches, insufficient numbers of these cells are available for proliferation and differentiation into bone, muscle, cartilage, thymic, and cardiac tissue and the syndrome known as retinoid embryopathy ensues.

For decades, it has been known that high levels of vitamin A during pregnancy (on the order of 40 000–250 000 international units (IU); 10 000 IU = 3.3 mg retinol) cause structural and behavioral disorders (termed functional deficits or behavioral teratology) in rodents. Prenatal hypervitaminosis A delays and disrupts motor coordination, activity, and learning-related development. In fact, vitamin A has been used for years as a reference teratogen or 'positive control' in the study of potential behavioral teratology of drugs, workplace chemicals, or other agents. After vitamin A doses that do not cause overt structural malformation, neonatal survival and weight may not be affected, but performance of the offspring on a variety of tests designed to assess mental and physical ability (e.g., rotorod balance, righting reflex and other reflex development, running wheel, water T maze, active avoidance, open field, and shock avoidance) is reduced. Thus, postnatal growth and development can be affected by exposures (dose) to retinoids less than those causing obvious structural terata. Behavioral terata induced by retinoids in rodents mirror the human experience. The risk for major craniofacial, cardiac, thymic, and CNS malformations after prenatal isotretinoin (13-*cis*-retinoic acid) exposure (**Figure 15**) is at least 25%, a value excluding those spontaneously or otherwise aborted embryos and fetuses exposed to the drug. Longitudinal follow-up of children who were exposed to isotretinoin *in utero* and survived to 5 years of age show that at least 20% are mentally retarded and that more than 50% are of substandard intelligence (**Figure 16**). The retinoids are an example of the principle that postnatal growth and development can be the most sensitive endpoint in teratogenesis. Retinoids are perhaps the best example of species concordance, dose–response in behavioral disorders at lower exposures, structural terata and growth retardation at somewhat increased exposures, and increased embryonic/fetal death at higher exposures. The abnormal transactivation of the retinoid nuclear receptors and subsequent molecular events precipitate abnormal events at the level of the cell, leading to abnormal anatomic or mechanical disorganization in tissues (e.g., the cranial neural crest) precipitating abnormal morphogenesis of whole organs and systems relating to or surrounding those abnormal organ structures (e.g., malformation of the inner and external ear, the jaw, and CNS). As in retinoid-induced or other CNS malformation (**Figures 4–7**), malformation of the skeleton cannot be separated in the final analysis from malformation of the brain.

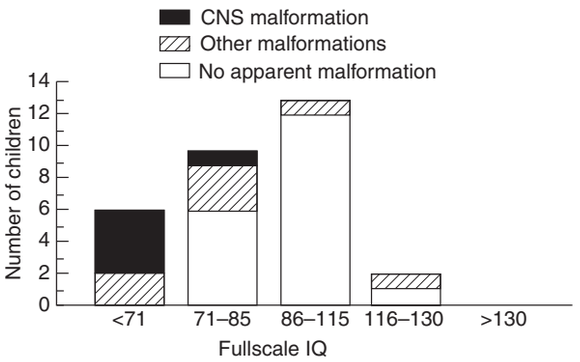


Figure 16 Relationship between congenital malformation and functional deficit in isotretinoin-exposed children at 5 years of age. All of the children classified as mentally retarded (IQ of less than 71) have major malformations. Of those with marginal intellectual ability (full-scale IQ of 71–85), 40% have major structural malformations. Among all children with significant intellectual deficits, 38% have no major malformations. (Reproduced from Adams J and Lammer EJ (1991) Relationship between dysmorphology and neuropsychological function in children exposed to isotretinoin *in utero*. In: *Functional Neuroteratology of Short-Term Exposure to Drugs*, pp. 159–170. Tokyo: Teikyo University Press.)

Principles of Teratology

Evaluation of new pharmaceuticals, pesticides, and the like for developmental toxicity is required by law. Such testing is actually a special type of toxicity testing and the rules for these studies are based on a few generally accepted principles and a number of assumptions. The principles are listed in this section and the assumptions implicit in these studies are discussed. The overall predictive ability of the animal studies to give reliable indication of potential adverse effects in humans is then presented.

There are four general principles of developmental toxicity testing:

1. Developmental stage determines susceptibility to insult.
2. There is a dose–response continuum. The magnitude and duration of the exposure determines the response, which can range from subtle change to frank malformation to death.
3. Genotype influences response.
4. Maternal systems may or may not be influenced by exposure to the insult. Although the ultimate target in the conceptus, the primary site of toxic action may be elsewhere.

Developmental toxicity testing is carried out using animal bioassays. Guidelines published by domestic and international regulatory agencies specify whole animal, mammalian systems. A number of *in vitro* screening methods using invertebrate, amphibian, chick, and cultured normal or neoplastic mammalian cells have been developed, but none of these are currently accepted by regulatory agencies as adequate measures of potential developmental toxicity. These policies stem from experience; as one moves away from whole animal mammalian systems, prediction of teratogenic potential in humans becomes tenuous. *In vitro* and other systems (nematodes (*Caenorhabditis elegans*), fruitfly (*Drosophila*), amphibians (*Xenopus*), and Hydra) are valuable in studies of teratogenic mechanisms of action. Nearly all the basic work in understanding homeotic gene complexes, their arrangements in tandem clusters, their segmented expression in anteroposterior domains which determine position, and the basic tenant that transcription of these genes in space and time is related to their order on the chromosome comes from *Drosophila*. Using homeobox and zinc finger probes developed in *Drosophila*, research is underway to characterize homologous and related genes in mammals, including those homeotic gene complexes which control anteroposterior orientation and organization of the neural tube. The examples discussed in the previous section (aspirin, acetazolamide, caffeine, lead, trypan blue, and retinoids) illustrate that as knowledge of a mechanism is increased, the accuracy of interspecies extrapolation of laboratory data to human beings is increased and one can extend with confidence those conclusions to related compounds or exposure situations. Since the genes involved in embryogenesis have been conserved during evolution, alteration in homeobox sequence and expression as measured in nematode worms, sea urchins, mollusks, annelid worms, fish, chickens, and mice can have similar consequences in human embryos.

There is no perfect animal model for accurate prediction of human teratogenic sensitivity – not all primates respond to all known or suspected human teratogens. Monkeys yield the most predictive dose–response in no more than half of all cases. New methods offer much promise in improving this situation. Using homologous recombination to 'knock out' particular genes in embryos can yield chimeric offspring that provide excellent models of phocomelia, osteogenesis imperfecta, and congenital degeneration of Purkinje cells; inappropriate gene expression is most often lethal but, in some cases, the offspring are phenotypically normal. After insertion of bacterial genes like that for β-galactosidase (lacZ) under control of a weak constitutive promoter, those genes which fall near a strong promoter region are transcribed and the mRNA is translated into a functional enzyme detectable by histochemical methods – producing an insoluble, blue salt in those groups of cells (like neurons) expressing those DNA sequences that can be isolated. Injecting lacZ constructs into fertilized eggs and screening the transgenic embryos yields models like 'Blue mice', giving straightforward indications of disrupted genes. The tools of molecular biology and the application of rigorous interspecies dose scaling based on the principles of physiologically based pharmacokinetics (discussed in a following section) are keys to accurate identification of those agents and levels of exposure with which the public needs to be concerned. The high degree of uncertainty currently associated with interpretation of developmental toxicity data leads to marked discord between the regulated community and those agencies and groups charged with protecting the public health.

Recent developments in biomedicine promise to reduce this uncertainty. One of these new developments involves the completed human genome sequencing project. As a result of this project, we now know, or will shortly know, all of the genes that combine to orchestrate human development. This knowledge, combined with similar information from a variety of animals, has led to the development of a new field

called genomics, literally the study of the genome. Because of recent developments, researchers have tools, for example, DNA microarrays (so-called DNA chips), to study the thousands of genes that must be turned on and off according to a precise development schedule to ensure normal development. Currently, these DNA chips, actually microscope slides, are engineered to contain sequences representing 20 000 or more genes. In fact, it will not be long before all the genes for humans and a variety of animal species will be contained on chips smaller than the size of a microscope slide.

Why is this such a significant development? For an answer, we must go back to the central dogma of biology, that is, DNA makes RNA makes protein makes phenotype. During development from the fertilized egg to the newborn, the genes in the human genome are temporally and spatially read out and translated into their respective proteins. Subsequently, a complex interaction of proteins culminates, in ways only dimly understood, in the phenotype. Prior to the advent of genomics, investigators typically studied genes one at a time, attempting to relate the expression of a particular gene and its encoded protein with a particular developmental phenotype, for example, the eye. Scientists used techniques such as Northern blot analysis, *in situ* hybridization, and immunohistochemistry to study one particular gene or at most a small group of genes. This approach was taken despite the knowledge that literally thousands of genes and proteins were operative at any particular stage of development. With the advent of DNA microarray technology, scientists can now study all genes in the genome simultaneously. How is this capability of monitoring gene expression on a global scale going to reduce the uncertainty in extrapolating developmental toxicity data from animals to humans? One possibility is to use microarrays to identify patterns of gene expression that are associated with congenital defects in animal models (e.g., mouse, rat, rabbit) induced by different individual or classes of developmental toxicants. Such patterns of gene expression could then be used to design appropriate experiments in nonhuman primates to determine whether specific genes or groups of genes are related to abnormal phenotypes. In the end, the hope is that gene expression profiling using DNA microarrays will identify biomarkers of exposure/effect/susceptibility so that data from animal studies can be used to more accurately predict whether a particular developmental toxicant poses a risk of causing birth defects in humans. Gene expression profiling using DNA microarrays has enormous potential; however, much research needs to be completed before we know whether this hope is realized.

Although genomics in general and gene expression profiling in particular offer great promise, scientists are already looking beyond genes and their messenger RNAs to the cellular molecules that actually produce the phenotype, that is, proteins. Although it is currently estimated that there are $\sim$30–40 000 genes in the human genome, it is also estimated that there may be an order of magnitude more proteins because of alternative splicing of RNA and various post-translational modifications of proteins, for example, phosphorylation. Thus, the task of monitoring changes in all cellular proteins, termed the proteome, is daunting. Nonetheless, new developments in protein analysis, particularly developments in mass spectrometry (MS), have spawned a new field of research called proteomics. The goal of proteomics is to develop high-throughput techniques for the simultaneous analysis of all proteins (and their modifications) in the proteome. While currently far from this goal, exciting new developments are coming online frequently. One example is ICAT analysis. ICAT stands for isotope-coded affinity tag. ICATs are small molecules that consist of three elements: (1) an affinity tag (biotin), which is used to isolate ICAT-labeled proteins (peptides), (2) a linker region that can incorporate stable isotopes (e.g., light = hydrogen or heavy = deuterium), and (3) a reactive group that can form a covalent linkage with cysteine residues in proteins. ICATs exist in two forms, heavy (containing eight deuteriums) and light (containing no deuteriums). Using these ICATs, two protein mixtures representing two different states (e.g., embryos exposed to a developmental toxicant compared to unexposed embryos or exposed embryos of a sensitive strain of mice compared to exposed embryos of a resistant strain) are treated with isotopically light and heavy ICAT reagents (e.g., lysates from treated embryos labeled with light ICAT compared to lysates from untreated embryos labeled with heavy ICAT). In each lysate, any one protein is covalently tagged at cysteine residues with either a heavy or light ICAT. The two 'labeled' protein mixtures are then combined and proteolyzed to peptides. ICAT-labeled peptides are isolated utilizing the biotin tag and then separated by microcapillary high-performance liquid chromatography (LC). Each pair of ICAT-labeled peptides is chemically identical, but can be distinguished by LC–MS on the basis of the 8 Da mass difference between the heavy and light ICATs. Because the ratios of the original amounts of proteins from the two lysates are strictly maintained in the peptide fragments, the relative levels of any protein in the two lysates can be determined from the ratio of the peptide pairs. Every other scan is devoted to recording sequence information about an eluting

peptide (tandem mass spectrum), which is subsequently used to identify the parent protein by computer searching the recorded sequence information against large protein databases. Although ICAT analysis has not yet been used to identify specific proteins that play important roles in developmental toxicity, this approach has tremendous potential. Moreover, the combination of gene expression profiling using DNA microarrays and protein expression profiling using ICAT analysis offers the real possibility of identifying genes and their respective proteins that orchestrate the developmental processes that culminate in structural and/or functional birth defects. It is the hope that such information will lead to new strategies for preventing birth defects.

Developmental toxicity testing in laboratory animals is based on the assumption that those species are, in one way or another, similar to humans in their reaction to the compound or agent of concern. The apparent concordance between human and animal data is quite poor – even when normal maternal–placenta–embryo relationships remain intact. In some cases, rodents are more sensitive to the particular chemical insult than rabbits, monkeys, or humans; in other cases, the converse is true. Drug registration, pesticide registration, and other regulatory testing protocols make use of either articulated or implicit assumptions. Two of the assumptions made in the absence of rigorous data are that the metabolic fate and pharmacokinetic parameters of the chemical in animals are similar to those in humans, and that the embryonic and fetal structural and metabolic development in animals is similar to humans. Since no one species is always suitable, regulatory agencies require testing in more than one species in the hope that at least one species will show some relevant adverse effect at a particular dose on which a regulatory decision can be based. In practice, this is usually the no-observed-adverse-effect level (NOAEL), the lowest-observed-adverse-effect level (LOAEL), or a benchmark dose (BMD) in the most sensitive species tested.

Two practical compromises occurred at the outset of these testing protocols. First, the laboratory species used most commonly (rodents and rabbits) are relatively inexpensive, can be bred year-round, and are small, which allows for efficient husbandry, dosing, and ease of handling and permits use of the rather large numbers needed for statistical evaluation. Second, dosing is carried out for a prolonged period of gestation (usually days 6–15 of pregnancy in rats and 6–18 days in rabbits) to cover organogenesis and early fetal life, even though it is recognized that a single exposure during a critical period can be sufficient to induce terata. The latter practice stems from the fact that, at the outset, the investigator cannot be sure which particular gestational stage will be most sensitive; however, this approach is compromised by the possibility that some compounds (like retinoic acid and cadmium) induce or otherwise alter their own metabolism after repeated exposures so that teratogenic potency can be altered.

Methods

Of the 50 or so mixtures, compounds, or agents known to elicit human developmental toxicity under a particular regimen or exposure, the majority were first identified by astute physicians. Some human teratogens have been identified by epidemiologic studies and were only later confirmed in animals. Only a few were identified first in animal studies. Nonetheless, for every known human teratogen, there is at least one animal model. The only way the predictive ability of animal studies can be evaluated is by epidemiologic studies of sufficient statistical power. Some believe that the results of animal developmental toxicity studies have little value in predicting human response; others maintain the opposite point of view. This divergence is due to the physiologic and metabolic differences between humans and common laboratory animals and the differences in how the animal studies have been designed. For example, scientists have generally been unable to reproduce folic acid deficiency-induced NTD in wild-type rodents, probably not because these animals are insensitive to the reproductive consequences of inadequate dietary folate, but because rodents practice coprophagy (feeding on dung) from which they obtain microflora-produced nutrients. Recent studies with 'knockout' (genetically modified) mice lacking normal folate binding and transport capabilities found craniofacial defects in these animals.

The general application of epidemiologic methods to developmental toxicity is described below and followed by a discussion of laboratory studies in rodents and rabbits. The bulk of data available in developmental toxicology are based on these protocols. Since the difference in human and animal response appears to rest in large part on differences in behavior, physiologic parameters, and xenobiotic absorption, distribution, metabolic fate, and elimination, a brief description of transplacental pharmacokinetics is also provided.

Human Studies

The major advantage of studies of humans is that we have the data in the right species. The major

disadvantage is that usually we have no reliable quantification of exposure (dose). It is, therefore, difficult if not impossible to construct a dose–response relationship.

Clinical Data

Malformations like cyclopia occur on average 60 times more often in early, aborted embryos than in infants at term. Cleft lip and palate occur 10 times more often in early abortions than at term. Study of early human embryos could be a very useful tool in identification of developmental toxins, but tabulation of anomalies in aborted embryos and fetuses is not routine. Since the total malformation rate in these spontaneous abortions ranges from 3% to 5%, it is difficult to sort out cause and effect for the comparatively small contribution of exposure to one compound or situation of relatively low teratogenic potential. It is, by comparison, relatively straightforward to identify potent, unique, or unusual teratogens in contrast to a general or common effect (e.g., ventricular septal defect). In some instances, the more rare the malformation (e.g., cyclopia), the more difficult it is to correlate etiology because of the very small number of affected infants at term; conversely, a cluster of very unique findings (e.g., thalidomide-induced phocomelia) has been the best clue historically to identify human teratogens.

Epidemiology

Birth certificates are notoriously poor sources of information because of unintentional and intentional deletion of important data. Three descriptive approaches are useful for generating hypotheses: case reports, correlation studies, and birth defect registries. Four methods are useful in testing these hypotheses: cohort studies, case–control studies, cross-sectional studies, and intervention studies. The greatest limitation to all these approaches is quantifying exposure and correcting for potentially confounding factors (e.g., inherited disorders, maternal parity, disease, age, prepregnancy weight, weight gain during pregnancy, socioeconomic status, ethnicity, and diet).

Case reports can be very useful and have been the mainstay in identification of human teratogens. Usually there is no information on confounding variables, and the actual cause and effect relationship cannot be established by the case report alone. Many known human teratogens were established by a consensus of case reports, later to be confirmed by epidemiologic evaluation. Correlation studies seek relationships between geographic location, time of exposure, personal characteristics, and pregnancy outcome, but it is extremely difficult to correct for those correlations that have no actual influence on the malformation of interest. Birth defect monitoring programs (surveillance registers) can be conducted on an area-wide basis (e.g., California Birth Defects Monitoring Program) or on an industry-wide basis (e.g., Finnish Occupational Registry); however, dose estimation and comparison to control populations are difficult. These programs can provide promising leads only through laborious, expensive follow-up of the mothers with efforts to correct for recall bias. All these methods rely on identification of subgroups placed at increased risk because of their lifestyle, habits, occupations, or medical needs.

Cross-sectional studies can identify prevalence rates based on the distribution of a particular syndrome or malformation, but the study design makes it difficult to identify cause and effect relationships. Case–control protocols match the affected pregnancy to an unaffected pregnancy but, again, it is difficult to account for maternal recall bias and perhaps equally difficult to control for bias (even unconscious) on the part of the investigator. Cohort studies, while suffering from problems of dose determination, are usually prospective, the largest, the most expensive, the slowest, and usually the most statistically powerful to detect reliable associations. Double-blind intervention studies, like those conducted with folic acid and prevention of NTD, usually yield the most conclusive data.

All these epidemiologic approaches are judged by the following criteria:

1. consistency with other epidemiologic findings;
2. specificity of risk with those having highest exposure;
3. strength of statistical association;
4. dose–response relationship;
5. biological plausibility;
6. temporal relationship between exposure and outcome; and
7. statistical significance.

Animal Bioassays

The first step in the laboratory study of developmental toxicity is determination of the substance to be tested. This might be straightforward in drug or pesticide registration studies because only a single pure compound is of concern. In the case of complex mixtures, however, selection of the test substance(s) for the particular condition(s) and route(s) of exposure can be very difficult. For example, gasoline, diesel fuel, or aviation fuel each contain more than 250 diverse hydrocarbons, which change with source of the crude oil, the products are formulated differently

by different refineries and the composition can change with the season of the year. While bioassays can be carried out with complex mixtures or technical-grade materials, interpretation or extension of those data to related or slightly altered materials can be problematic.

The second step in the laboratory study of developmental toxicity is selection of the test species and strain. International (and even state) regulatory requirements differ; most look favorably on data from outbred rats and rabbits from laboratories with cumulative and comprehensive histories of malformation incidence in untreated or control animals. All regulatory agencies recommend selection of a test species that absorbs, distributes, and metabolizes the compound(s) of concern in a manner similar to that of humans; however, human pharmacokinetic data are rare and even those parameters in animals may be lacking. The animals should be housed and treated in accordance with the *Guide for Care and Use of Laboratory Animals* (US National Institutes of Health (NIH) Publication No. 85-23; DHEW Publication No. 74-23). The only difference between the treated and control groups should be exposure to the agent of concern; both groups should be provided with controlled humidity, temperature, light cycle, and the same basic, nutritionally adequate diet.

The third step in the laboratory study of developmental toxicity is the determination of dose, exposure route, and number of animals to be studied. Most regulatory agencies require at least three dose levels in addition to a sham or vehicle control group. The highest dose is expected to produce toxicity in either the offspring or the dams; a reduction in maternal body weight gain or increased mortality (usually not more than 10% maternal death) are accepted as the highest required dose. Some agencies permit substitution of a 'limit test'; that is, if a dose of 1000 mg kg^{-1} fails to induce embryotoxicity or teratogenicity, then a full developmental toxicity bioassay may not be necessary. The lowest dose should elicit no adverse effect. Initial dose selection can be based on the anticipated therapeutic dose (or multiple thereof), on results of a range-finding toxicity study, or on an LD_{50} value in the same or a closely related species. The route of administration should be the same as the route by which humans are exposed. If the compound is to be given orally, it is preferable that intubation be used since incorporation into the feed can yield palatability problems and it provides only an approximation of dose. Regulatory agencies stipulate that 20 litters for rodents and eight to 12 litters for nonrodents per dose group are necessary. Taking note that rodents can have as many as 15–18 offspring per litter, large numbers of animals at each dose can be required for statistical evaluation. It is the litter – not the individual implantation site – that is considered the experimental unit. However, when one encounters a potent teratogen, as few as five litters per dose group may be sufficient to provide meaningful results.

The fourth step in the laboratory study of developmental toxicity is selection of duration of exposure. The reader should be aware that different laboratories count the days of gestation differently; some count the day after breeding as day 0 and others count it as day 1. Regulatory agencies stipulate that treatment begin on day 6 (first day counted as day 0) and continue each day through day 15 in rodents and from days 6 through 18 in rabbits to cover organogenesis and early fetal life. The animals should be weighed prior to dosing, regularly during treatment, and at term. Careful daily inspection for clinical signs of intoxication is needed. Just before term (e.g., day 18 in mice and day 15 in hamsters), the fetuses are collected by cesarean section. Conventional teratology study in rodents requires that the dams and their fetuses be killed just prior to term since malformed or otherwise defective offspring are preferentially cannibalized by the mother. Regulatory requirements for sacrifice of the dam date from 1970 US Food and Drug Administration (FDA) safety evaluation guidelines. Cannibalism of malformed offspring occurs during the immediate postpartum period perhaps precipitated by the pup's inability to move about and/or nurse normally. If the dams are disturbed during this period, this will also precipitate cannibalism.

In contrast to primates, which abort dead embryos, dead rodent embryos are resorbed and the implantation site is recorded as a resorption site. The numbers of living and dead fetuses, and the number of resorption sites, are counted, and fetal weight, sex, and external malformations are recorded. Fetuses can either be inspected fresh in evaluation of internal soft tissues (known as the Staples technique) or can be placed in a fixative (usually Bouin's solution) and sectioned at a later time (Wilson technique). Other fetuses are fixed in ethanol, cleared in potassium hydroxide, and the cartilage and bone are stained with Alcian Blue and Alizarin Red, respectively. The US FDA recommends that one-third of the rodent fetuses be subjected to visceral examination and that two-thirds be studied for abnormalities of cartilage and bone. The US Environmental Protection Agency (EPA) recommends that one-third to one-half of each litter be examined for skeletal anomalies. For rabbits, all fetuses are to be examined for both visceral and skeletal malformations.

There is no rigid, universal definition of what constitutes a malformation, a deviation, a retardation,

an aberration, or a functional disorder. Only death is unequivocal. All species show anatomic variations, particularly in patterns of skeletal ossification. In some cases, delays in ossification can be a reflection of retarded growth, often found in concert with reduced fetal body weight; however, in other cases these changes may be a reflection of terata found after exposures to higher doses.

Just as with an LD_{50} in adult animals where, by definition, one-half of the animals die and one-half live after acute chemical insult, and the reason(s) why one particular animal that is apparently identical to the other members of the group lives and another dies is not known. Some fetuses in a litter can be grossly malformed and the neighboring fetus can be normal. The 'litter effect' is a term applied to the finding that, at some dose, different females treated in the same way vary in the degree and even type of response.

Mention of *in vitro* methods is appropriate here. Study of teratogens with chick embryos can be extremely valuable, as illustrated by the elegant mechanistic studies of retinoids in cultured wing bud; however, the chick embryo suffers from ill-defined metabolic capabilities and the numbers of false positives due to the placement of the test substance directly on the embryo and toxic surface actions are high. Two of the more useful *in vitro* methods are cultured whole rat or mouse embryos and cultured rat and mouse limb buds. For whole embryo culture, the embryos can develop normally during 48 h of head-fold stage and precise control of exposure and experimental conditions can be achieved. The technique is inexpensive and it has been said that, with a little practice, one skilled person can explant and culture 50 embyros in 1 day. The embryos are placed in bottles containing culture medium and the bottles are attached to a rotating drum; during incubation, oxygen and 5% CO_2 are bubbled continuously into the bottle to maintain adequate oxygen and a steady pH. The embryos are very sensitive to changes in temperature and artifacts can be induced. Although novel approaches, like including human serum from patients being treated with various drugs can help in relating the rodent culture results for human health risk assessment, the patterns of malformation induced in cultured rodent embryos can be quite different from those seen in embryos exposed to the same compound *in vivo*. A great many *in vitro* methods have been developed: cultured ova, cultured embryonic pancreas, palate, teeth, lens, kidneys, gonads, bone, thyroid, fish, sea urchin, dissociated adult invertebrate cells, micromass (dispersed embryonic limb and lung), and whole invertebrate or amphibian tail regeneration. None of these, however, have been accepted by regulatory agencies in safety assessment. *In vitro* studies of developmental toxicants are generally regarded as methods for elucidation of mechanism of action and those data can be used to explain and support conclusions reached from conventional protocols.

It has become increasingly important to collect pharmacokinetic and transplacental transfer data in studies of developmental toxicants. These approaches require that quantification of parent compound and metabolites be carried out in a rigorous manner; measurements of total radioactivity from labeled test compounds in blood and tissues are of little utility here. The exception is use of autoradiography of early embryos, where one can obtain an approximation of compound localization in tissues of developing embryos that cannot be otherwise achieved. It may be very important to determine species-specific plasma or erythrocyte binding of the active compound since small differences here can make for large differences in the concentration of free drug and can explain what otherwise looks like a major difference in species sensitivity to the teratogen.

Classical methods for analysis of pharmacokinetic data ('box models') fit sums of exponential functions for two or three mathematical compartments, and these methods usually have no identifiable compartment other than that from which the data arise (e.g., plasma). These methods are useful first steps, yielding concentration:time curves with rates of uptake, the maximum concentration, the rates of elimination, and total dose (measured as the area under the concentration:time curve, AUC). Box models are not particularly useful in scaling dose between species as there are at least four different kinds of dose: administered dose, absorbed dose, metabolized dose, and delivered (or target tissue) dose. Physiologically based pharmacokinetic (PBPK) models have been developed to overcome these limitations (**Figure 17**). These methods require anatomical information (organ-specific blood flow and volume) and physiologic information (rate of metabolism), whereas traditional compartment models assume all input to a central compartment (blood) and free distribution into other compartments. The results from PBPK analyses show the influence of dose-dependent changes in drug behavior and it may be that one species appears most sensitive when compared using an administered dose basis ($mg\,kg^{-1}$ body weight), but these species may be of equivalent sensitivity when one compares the two on a target tissue (delivered) dose basis (e.g., embryonic AUC). Advantages of PBPK analyses include considerations of blood flow and metabolic limitations, anatomic volumes, and other characteristics (such as plasma

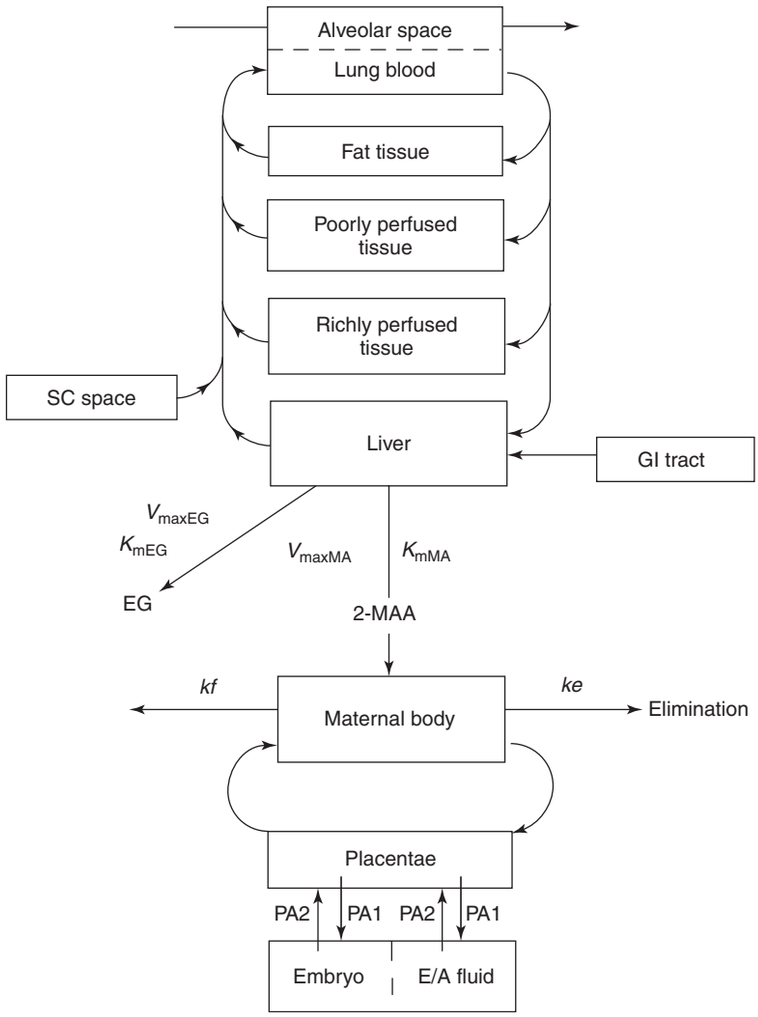


Figure 17 Schematic diagram of a physiologically based pharmacokinetic model used to describe the uptake, distribution, transplacental transfer, and elimination of a 2-methoxyethanol (2-ME) and its teratogenic metabolite, 2-methoxyacetic acid (2-MAA) in the pregnant mouse. Extraembryonic (E/A) fluid is the combined execoelomic and amniotic fluids that surround the embryo. The results of pharmacokinetic studies show that it is the total exposure (concentration $\times$ the exposure time) of the embryo to 2-MAA rather than the peak concentration of this toxic metabolite which is most closely related to teratogenic outcome. (Reproduced from Clarke DO, Elswick BA, Welsch F, and Conolly RB (1993) Pharmacokinetics of 2-methoxyethanol and 2-methoxyacetic acid in the pregnant mouse: a physiologically based pharmacokinetic model. *Toxicology and Applied Pharmacology* 121: 239–252, with permission from Elsevier.)

protein binding). These methods provide increased accuracy for interspecies extrapolation of dose.

Lessons

Three examples of mistakes known to every teratologist and the major lessons learned from each are described below. These lessons provide a warning and a caution to the reader. These lessons demonstrate how data can be misinterpreted or missed altogether and illustrate the responsibilities borne by those who make mistakes in developmental toxicology.

Diethylstilbestrol

For a great many years, the only thought given to transplacental carcinogenesis was the teratoma. Teratomas are – in a word – bizarre. Ordinarily, the primitive streak mesenchyme degenerates at its caudal terminus, but in rare cases (and mostly in female embryos and under unknown circumstances) some of these cells can persist and can become malignant either *in utero* or in infancy. In other cases, the primordial germ cells (which migrate by ameboid movements from the yolk sac along the dorsal mesentery to the gonadal ridge at week 3 and normally differentiate into male and female germ cells) remain

and give rise to teratomas in or on testes or ovaries. These uncommon tumors can arise from all three germ layers, giving rise to a disorganized mass or ball of pancreas mixed with teeth or an eyeball, cartilage, and neural tube. Teratomas can also present as an independent growth, as a partial 'parasitic twin' or 'fetus in fetu'.

Diethylstilbestrol (DES) changed forever the view of transplacental carcinogenesis. DES is a drug that was prescribed to 1 or 2 million pregnant women at doses ranging from 1.5 to 150 mg day^{-1} from 1945 to 1970 to reduce the risk of spontaneous abortion and premature delivery. Female offspring of these women have risk for vaginal and cervical clear-cell adenocarcinoma of 1.4 per 1000. Latency is between 7 and 30 years and peak age of diagnosis is 19 years. These women may also be afflicted with structural terata of the uterus, fallopian tubes, cervix, and vagina, and may present with adenosis. Male offspring experience an increased incidence of urogenital, hypotropic, and capsular induration to testicular tissue and have associated terata of the reproductive system. The total DES dose in mothers of women with vaginal adenocarcinoma ranges from 135 to 18 200 mg.

Conventional developmental toxicity protocols can be conducted as independent studies or as part of a multigeneration or continuous breeding reproduction toxicity test. The animal bioassay for developmental toxicity has traditionally focused on gross congenital malformations, usually induced by exposure during early embryogenesis. By contrast, DES exposure late in gestation by oral dosing or subcutaneous injection in pregnant mice and rats (days 17–21), hamsters (days 14 or 15), and rabbits (days 12–14) with 0.1–40 mg kg^{-1} can increase embryonic mortality, induce cryptorchidism, and cause feminization of male genitalia. Histologic examinations of female and male mice or hamsters born to DES-treated dams reveal cystic metaplasia and neoplasia in segments of the reproductive tract. In animals raised to maturity, reduced fertility, ovarian tumors, endometrial hyperplasia, and uterine adenocarcinoma develop. In female rhesus monkeys born to mothers given oral DES at 1 mg day^{-1} from day 130 to term, vaginal ridging, cervical hooding, and vaginal adenosis develop.

Three lessons are demonstrated by the DES experience:

1. Transplacental carcinogenesis can and does occur in humans and animals.
2. To focus attention on gross terata is to miss important manifestations of developmental toxicity.
3. Exposure during late gestation can have at least as great a consequence as exposure during early embryogenesis.

As a result of lessons learned from DES, an *in utero* exposure phase was added to the carcinogenicity testing requirements for food additives, drugs, and pesticides. The results of multigeneration reproduction studies must be considered together with those from carcinogenicity bioassays and conventional developmental toxicity studies in hazard evaluation.

Thalidomide

No discussion of developmental toxicity can be complete without mention of the thalidomide epidemic of the 1950s and early 1960s, which affected 10 000 children. Thalidomide ranks with methylmercury as the most infamous of all teratogens. Thalidomide was prescribed in Western Europe and Australia as a sedative antiemetic in early pregnancy; it is still used today under careful supervision in the treatment of Hansen's disease (leprosy) and other special situations. Thalidomide phocomelia and associated limb reduction defects (oligodactyly and bone fusions) usually of the radius, humerus, and ulna occurred in at least 96% of those embryos exposed. These defects were usually bilateral and symmetrical and occurred after consumption of 0.5–1.0 mg kg^{-1} day^{-1} (corresponding to maternal circulating concentrations of 1 μg ml^{-1}) from days 34 to 50 of pregnancy. Of the children whose mothers received thalidomide only after day 50, 103 of 104 were normal. Of equal (but perhaps less dramatic) importance were the drug-induced malformations of the ear (anotia and microtia), the congenital deafness, epilepsy, anophthalmia, and the eye muscle and facial paralysis. Nearly one-half of these infants died in their first year, due principally to patent ductus, aortic and ventricular septal defects, and other cardiac malformations that occurred in at least 30% of these infants.

Common laboratory rodents are refractory to thalidomide embryopathy; doses up to 4 g kg^{-1} increased the numbers of abnormal rat and mouse fetuses in only a few of the more than 60 studies. Rabbits do show limb reduction defects (classified as terminal incomplete longitudinal paraxial radial hemimelia) after thalidomide administration, and it is this observation that forms the basis for inclusion of rabbits in current animal testing requirements. Many species have been studied, including the armadillo, baboon (*Papio cynocephalus*), bonnet monkey (*Macaca radiata*), bushbaby, cat, cynomolgus monkey (*Macaca fascicularis*), dog, ferret, green monkey (*Cercopithecus aethiops*), guinea pig, hamster, Japanese monkey (*Macaca fuscata*), marmoset (*Callithrix jacchus*), mouse, pig, rabbit, rat, rhesus monkey (*Macaca mulatta*), and stump-tailed monkey (*Macaca arctoides*). However, only certain strains of rabbit

(given 150 mg kg^{-1} day^{-1}) and nonhuman primates (given 4–45 mg kg^{-1} day^{-1}) respond in a manner similar to that of humans. Not all primates respond to thalidomide (e.g., *Galago crassicaudatus*).

The mechanism of the species-specific action of thalidomide is not known. The compound crosses the placenta and has similar pharmacokinetic parameters in susceptible species (rabbit) and resistant species (rodent). The molecular structure of thalidomide and requirements for malformation are very specific. Thalidomide decomposes rapidly in water to at least 12 different products – all of which are inactive as teratogens and all of which undergo biotransformation *in vivo* to other products. These data prompted the suggestion that it is the parent thalidomide molecule that is the ultimate teratogen. Studies with supidimide analogs and other members of the phthalimide family (**Figure 18**), including side chain or phthalimide ring-modified analogs, show precise structural requirements. Of the numerous phthalimides with their *in vivo* metabolites and related compounds examined, only thalidomide and its EM12 congener are teratogenic. Using topological parameters, geometric parameters, electronic parameters, and physiochemical parameters, several groups have attempted to derive generalized predictors for structure–activity relationships of teratogens; the structure–activity relationships with phthalimides

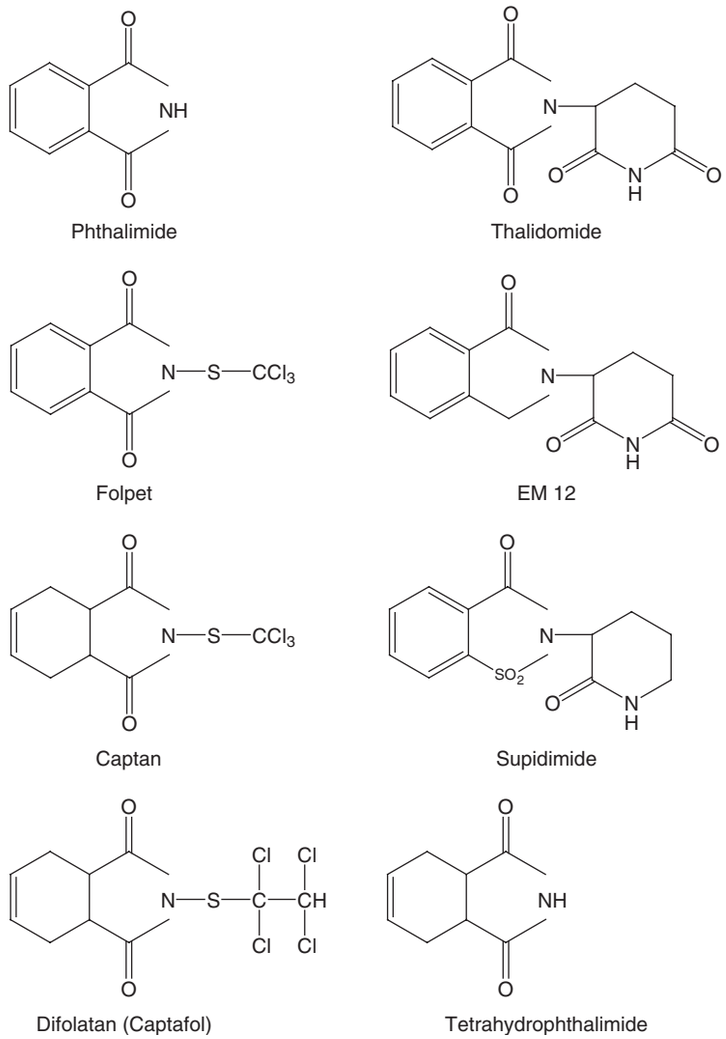


Figure 18 The phthalimide family. In the presence of heat and ammonia, cyclic anhydrides lose a molecule of water and the two acyl groups (R–C = O) become attached in a ring to the ammonia nitrogen, forming an imide. In this way, phthalimide is produced from phthalic anhydride. Phthalimide is acidic and when heating with potassium hydroxide in alcohol along with an alkyl halide, the corresponding *N*-substituted imide is produced. Neither phthalimide nor tetrahydrophthalimide are teratogenic; no derivative of tetrahydrophthalimide (captan and difolatan) is teratogenic. The *N*-substituted imides, folpet and supidimide, are not teratogenic. Of the phthalimides, only thalidomide and its close relative *N*-(2′,6′-dioxopiperiden-3′-yl)-phthalimidine (EM 12) are teratogenic.

point out the severe limitations of this approach. Regulatory agencies use structure–activity relationships to guide recommendations for collection of certain kinds of toxicity data; structure–activity relationships (even for compounds like retinoids in which these are well described) are no substitute for actual developmental toxicity data.

Four lessons are demonstrated by the thalidomide experience:

1. Terata can occur in the offspring of apparently healthy mothers.
2. Human teratogens may have little or no such activity in common laboratory animals.
3. Human terata can occur after exposures less than those used in animal studies.
4. Just because a compound has a chemical structure that appears closely related to an established teratogen does not imply that the congener is teratogenic.

Bendectin

Bendectin (Debendox) was the name given to the 1:1:1 mixture of dicyclomine hydrochloride, doxylamine succinate, and pyridoxine hydrochloride, a prescription drug first marketed in 1956 used to control nausea and vomiting in pregnancy. In 1976, dicyclomine hydrochloride was dropped from the formulation after large clinical trials showed that it did not contribute to the efficacy of the drug. Bendectin use was very common; from 20% to 25% of all expectant mothers used the drug. Approximately 30 million pregnancies were exposed over the 27 years that the drug was available. The customary daily dose was one to four tablets day^{-1}, each containing 20 mg of active ingredient (1 or 2 $mg\,kg^{-1}\,day^{-1}$).

Bendectin is not teratogenic in laboratory animals (including nonhuman primates), but developmental toxicity (reduced fetal body weight and delayed skeletal ossification) can be induced in animals after exposures (500–800 $mg\,kg^{-1}\,day^{-1}$) that also cause frank maternal intoxication and/or increased maternal death. There are six published *in vitro* studies of Bendectin using cultured rodent embryos or embryonic cells; of those, only one, of mesenchymal cells, showed any indication of toxicity and that occurred after exposures to concentrations far greater than can be achieved in humans after ingestion of therapeutic doses (25 mg or more). There are at least 14 cohort and 18 case–control epidemiologic investigations on Bendectin and pregnancy outcome in addition to one, by the NIH, in which the occurrence of congenital malformations was prospectively studied in 31 564 newborns. The results of the NIH study, like those of others, found that the odds ratio for any of 58 major categories of malformation and Bendectin exposure was 1.0 – exactly that which is expected by chance alone. Of those categories with trends or suggestive positive associations, the magnitude of those associations was as great as that from vomiting during pregnancy without Bendectin use as with Bendectin use. There was no increase in malformation rate after exposure to Bendectin *in utero* than would otherwise be expected by chance; there are no objective data to conclude that exposure to Bendectin in animals or humans has any adverse effects on embryonic or fetal development at the doses used by these 30 million women.

As of 1987, at least 300 lawsuits had been filed alleging that Bendectin caused congenital malformations. The drug was dropped from commerce not because of lack of efficacy, or because it caused toxicity or because there was no market for the drug, but because of the excessive cost incurred by the manufacturer in defending the drug in litigation. The major focus here was the allegation that prenatal Bendectin exposure caused phocomelia and assorted limb reduction defects. Given the 'background' or 'spontaneous' rate of major congenital malformation in the United States (3%), it would be expected that 900 000 malformed children would be born to those 30 million mothers even in the absence of any drug use. Given the US background rate for limb reduction defects (one per 3000 births), 10 000 such defects would be expected to occur even in the absence of any drug use.

How then can it be that a compound which has no detectable teratogenic activity in either animal or human studies be held responsible for human congenital malformations? The history of Bendectin can be traced to ignorance of the principles of teratology, compounded by precedential case law following the first erroneous decision and to two articles appearing in the popular press. In the September 1979 issue of the *National Enquirer*, the following was published:

> *Experts Reveal…Common Drug Causing Deformed Babies. In a monstrous scandal that could be far larger than the thalidomide horror, untold thousands of babies are being born with hideous defects after their mothers took an anti-nausea drug (Bendectin) during early pregnancy.*

At least seven women are known to have elected abortions as a consequence. In the magazine, *Mother Jones* ('The Bendectin Coverup', November, 1980), the authors counseled women to use – instead of Bendectin – 'natural alternatives' including 100 mg of pyridoxine (a dose 10 times that of the same compound in the Bendectin formulation).

The Bendectin lawsuits stem from the age-old question that always follows the birth of a malformed child, 'What caused it?', together with today's substantial monetary rewards that can befall successful litigation or out-of-court settlement, particularly with a large, impersonal, and wealthy corporation. Compounded by parental shock, denial, anger, and sadness, the potential astronomical medical costs and social costs for the child, the financial rewards offered by expert witness testimony, the inadequate research and reporting necessary for sensationalization in the popular press, and successful settlement or judgment in preceding cases all combine to perpetuate the myth. Juries – being people – are by nature very sympathetic to the plight of the child and his or her family who are through no fault of their own in need of financial and other assistance to address their situation. The desire to help can be overwhelming. The original Bendectin lawsuits arose from failure to understand proper interpretation of the animal data, the desire to identify a responsible agent, and from substituting a strength-of-evidence approach for the weight-of-evidence evaluation of the data. Fundamental here was the failure to apply properly the concept of dose response. Only after the accumulated weight of many consistent epidemiologic findings of sufficient statistical power, accounting for confounding factors and where exposures are of sufficient magnitude and during appropriate periods in gestation, can conclusions on excess risk be drawn. In the strictest sense then, the best the science of teratology can offer on the concept of safety is the statement that the exposure of concern represents no measurable excess risk.

Three lessons demonstrated by the Bendectin experience are the following:

1. Human teratogens can be confirmed only by consistent findings in epidemiologic studies, recognizing that at the 95% confidence limit, the results in one of 20 studies of equal statistical power can differ by chance alone.
2. Maternal intoxication in animal studies in and of itself can contribute to abnormalities in the offspring.
3. Overall weight of evidence should be used in identification of compounds, agents, and exposures of concern.

Data Interpretation and Regulatory Policy

Some have criticized those groups charged with protection of the public health for failure to designate a code or notation which can be assigned to a chemical showing it to be a reproductive or developmental toxicant (see Further Reading, US EPA, 1991). Developmental toxicity is usually not the only kind of toxicity associated with exposures of concern. A 1985 US EPA reevaluation of 18 pesticides (avermectin, cacodylic acid, captafol, captan, cyanazine, dinocap, EDBC, endrin, fenarimol, folpet, fusilad, nitrofen, pentachlorophenol, 2,4,5-T, silvex, TPTH, triadimefon, and warfarin) and six industrial chemicals (arsenic, two glycol ethers, lead, chloromethane, and mercury) found that in no case was embryonic or fetal toxicity the sole documented effect. Teratogenic activity almost always occurs in tandem with other adverse effects on health, including mutagenicity, male or female reproductive toxicity or carcinogenicity. Gender-specific requirements like the protective work clothing promulgated for women in the case of endrin and silvex cannot be justified, not only on the basis of US Supreme Court decisions (**Figure 11**) or because gender-specific regulations concerning 'women of child-bearing age' restrict the activities of those women who are not fertile but also because such regulations carry with them high social costs. Regulations concerning only pregnant women would most likely fail to protect the embryo since it is exposures which occur before the patient recognizes that she is pregnant which are most likely to damage her embryo. Correct determination of safe levels of exposure rests with identification of the end point of toxicity associated with the LOAEL, regardless of whether it is developmental toxicity or another expression of toxicity.

The conventional method for interspecies extrapolation of developmental toxicity data involves empiric determination of an NOAEL in the test species followed by application of an uncertainty (or 'safety') factor to calculate the safe dose for the species or individual of interest. These calculations are often based on the administered dose and are usually expressed on a body weight basis. The default safety factor used can range from 100 to 1000 or more. Dose scaling on a body weight or surface area basis is fraught with problems. For example, scaling of a child's sulfonamide dose to the adult by body weight, surface area, age, or caloric expenditure (as a surrogate for metabolic rate) yields an adult daily and grossly excessive dose of 7 g. In the case of teratogens, the problem is even more acute. The absorbed dose can be so small (e.g., cyclopamine) or the site of drug action so restricted (e.g., trypan blue) that the quantitative differences between the species are so great that the result is judged an inherent qualitative difference. In the case of vitamin A and its metabolites, the conventional safety factor approach results in calculation of vitamin A doses so small that the risk of vitamin A deficiency-induced terata is increased.

Thirty years ago, Karnofsky D listed the two basic tenets used today in interpretation of animal developmental toxicity data. All compounds can produce embryotoxicity if applied in sufficient dosage at an appropriate stage of development, and the purpose of evaluating chemicals for teratogenic potential is not to eliminate from use, but rather to estimate the hazard its use presents to the human embryo. Teratologists believe that developmental toxicity can be viewed most accurately from the threshold concept; that is, there exists an exposure below which no adverse effect will occur. This belief arises from experience with human teratogens, from pharmacokinetic considerations, and because embryos – to a point – have the remarkable ability to compensate for lost or damaged cells. For instance, early rodent and rabbit embryos can develop quite normally after surgical obliteration of at least 50% of the entire inner cell mass. Implicit here is that one must distinguish between theoretical risk and practical risk. Using statistics, arbitrary safety factors, or subjective means, theoretical risk can always be calculated and upper-bound confidence levels assigned. Multiple and consistent epidemiologic studies of high quality showing no association between developmental morbidity or mortality and the exposure of concern can never prove that no hazard exists; these data can be used only to demonstrate that the risk, if any, is so small that for all practical purposes it can be disregarded.

The weight-of-evidence evaluation takes into account the human experience and the animal data. Interpretation of human data depends on the design of the study, definition of the cohort, quantification of exposure, validity of ascertainment, control for confounding factors, size of the study population, and appropriate statistical methods. In practice, identification of human teratogens has relied on answers to two questions:

1. Is there a distinctive pattern of malformation which can be associated with the exposure of concern?
2. Have there been sufficient numbers of cases to substantiate the conclusion?

The principal disadvantage to using human data in this way is, of course, that recognition occurs after the fact. Interpretation of the animal data is carried out with the following in mind. Without exception – even with very odd, rare, or unique malformation – there is no case in which the defect has not occurred sporadically; there are no known examples of agents which cause malformation that cannot also be caused by some other agent; some species are prone to particular types of congenital malformation (e.g., cleft palate in mice); and chemicals that are not teratogenic in animals (e.g., coumarin anticoagulants) can be teratogenic in humans. Animal data can only be used to provide an approximation of risk in humans. Interpretation of the animal data rests on answers to these seven questions:

1. Were the studies carried out in a species that handles the compound in a manner similar to that in humans?
2. Were the studies carried out using a route of administration applicable to anticipated human exposure?
3. Does the effect occur in more than one species?
4. Does the effect occur after doses less than those which elicit other types of toxicity?
5. Was a significant increase in the numbers of litters containing abnormal outcome observed?
6. Was a dose–response relationship identified?
7. What populations are at risk of exposure and what is the magnitude of their exposure?

Of great concern are those teratogens, like thalidomide, which are able to induce terata in the absence of any apparent disturbance in maternal well-being. A large difference in the dose required to cause embryotoxicity and a much higher dose required to cause maternal toxicity can be key in identification of those agents. Where maternal toxicity occurs after exposures equivalent to those causing increased embryonic morbidity and mortality, then estimates of safe exposure can be derived using safety factors applied to the NOAEL for adults. Even studies with low statistical power can contribute to the total weight of evidence; a strength-of-evidence (picking and choosing data to support one or another conclusion) approach cannot be justified.

In summarizing the results of a risk assessment, it is important that the reader look for the following three key points: a discussion of the quality of the studies supporting the concern for risk of developmental toxicity, the confidence that one can place in the NOAEL derived, and the list of uncertainties in the assessment. Animal studies (like those for aspirin and caffeine) usually employ doses much greater than those to which humans are or could be exposed. When extremely high doses are used, drug actions in the mother can elicit systemic poisoning which by itself may or may not be responsible for the effects seen. Even very reliable animal teratogens may have no counterpart in humans since the human exposures are relatively small.

Conclusions

What is the solution to the problem of birth defects? If not lists of developmental toxins, printed and electronic warnings, or new laws or judicial decree, then what? Although answers to the question can be simple, true solutions to the problem are complex. Only by reductions in the numbers of infants with birth defects can significant inroads to reducing infant mortality be made. The solutions are complex and expensive, but not as expensive as the cumulative charges for labor, postpartum care, surgery, hospitalization, prostheses, lost wages, and the extra social and educational services required by these patients. For every $1 spent on research into the cause and prevention of birth defects, published data show that $11 can be saved in public expenditure, insurance claims, and legal and medical payments made by individuals to address or attempt to rectify the problem.

Only by knowing the cause of a problem can an intelligent solution be devised. Basic research into the 80% of all birth defects that are of unknown cause (**Figure 9**) sponsored by groups like the March of Dimes, the Deutsche Forschungsgemeinschaft, and the US Public Health Service is key. This effort has two fronts: (1) the systematic collection and evaluation of human data and (2) basic laboratory investigation. These efforts include central birth defect registries in Australia, California, Canada, Finland, Japan (Ishikawa, Kanagawa, Osaka, Tottori, and the National Association for Maternal Welfare), New York, Sweden, the Commission on Professional and Hospital Activities (Michigan), the US Centers for Disease Control (Georgia), and the March of Dimes Nationwide Information Center (Massachusetts). For example, the first indication of valproic acid teratogenicity was detected by the Rhone-Alpes birth defect registry.

Understanding how embryos grow is fundamental to understanding and preventing birth defects. How does the embryo know its back from front, top from bottom? How does it control shape change from an initial ball of rapidly dividing cells to form the segmented cylinder of the torso? It is remarkable that the molecular biology of *Drosophila* has a direct bearing on understanding human embryonic development. Of equal import is understanding what the animal data are trying to tell us; as has been said by toxicologists on more than one occasion, 'The animals never lie' – it is only our flimsy interpretation of the results of the animal studies upon which doubt can be cast. To make use of those data and to develop credible public health standards based on those data, fundamental knowledge of control mechanisms in normal embryonic development and rigorous methods for interspecies extrapolation of animal and *in vitro* data are urgently needed. It makes little sense to generate animal developmental toxicity data if in the end we do not know how, if at all, those data relate to human beings. Fortunately, progress in two areas of research provide optimism that we may, in the not too distant future, gain the information needed to make accurate predictions of human risk. One advancement comes from developmental biology, a field of study that focuses on how the fertilized egg develops into an adult capable of producing gametes of the next generation. Over the past 20 years, research using roundworms (*C. elegans*), fruitflies (*Drosophila*), fish (zebrafish), frogs (Xenopus), birds (chicken), and mice has revealed several revolutionary findings. First, research has shown that development in these widely divergent organisms proceeds using similar mechanisms of communication both within and between cells of the embryo. Second, communication within and between cells in embryos from the roundworm to the mouse (and undoubtedly the human as well) involves a relatively small number (estimated to be 17) of signal transduction pathways. These pathways are used at different times and in different contexts in different organisms; however, in all animals these pathways orchestrate the complex processes that facilitate development from the fertilized egg to the newborn. Third, these signaling pathways are highly conserved from roundworms to chordates. What is different between roundworms and chordates is the way in which these pathways are used. Different organisms use different combinations of pathways at different times and in different places in the embryo to achieve species-specific gene expression patterns and thereby normal development. Nonetheless, the study of how specific signaling pathways are regulated during normal and abnormal fruitfly development, for example, has direct relevance to the study of human development and how developmental toxicants cause birth defects. Thus, the addition of new information from developmental biology continues to generate new insights relevant to understanding human development and preventing birth defects. Another avenue of great promise is the development of accurate PBPK models (**Figure 17**) scaling dose from rodent to rabbit to nonhuman primate to human. Only on very rare occasions is there an opportunity to measure the compound(s) of concern in human tissue after quantifiable maternal exposure, but it has been done and every effort must be made to use the results to confirm and validate the confidence that can be placed in the PBPK results. While PBPK interspecies scaling of dose can be handled, these methods cannot scale response.

Once one knows the problem and has devised a solution, then the real job begins. National Center for Health Statistics data show a decline in total US infant mortality from 1982 to 1992, but marked geographic and racial differences remain. The 1992 overall US rate of infant death was 8.5 per 1000 live births (California, 6.9; Texas, 7.7; New York, 8.5; New Jersey, 8.5; Pennsylvania, 8.6; Ohio, 8.7; Florida, 9.1; Illinois, 10.0; Georgia, 10.4; Michigan, 10.5) – a decline attributed not to reductions in the numbers of birth defects or premature births but to improved neonatal intensive care units and the introduction of synthetic pulmonary surfactants and consequent reductions in death from acute neonatal respiratory distress syndrome. Still, the years of potential life lost due to birth defects ranks fifth, just behind that of homicide and suicide (1, unintentional injury; 2, cancer; 3, cardiovascular disease); prematurity/low birth weight ranks sixth and sudden infant death syndrome seventh. Ethnic discrepancy remains pronounced; rates of White (5.8 per 1000 live births) and Cuban Hispanic (3.7 per 1000 live births) infant death are similar, but the 2002 rate for Blacks (13.9 per 1000 live births) increased compared to the previous year.

Aggressive public health efforts to combat cigarette smoking during pregnancy can pay for themselves at least six times over. Targeting low-income and other women to increase their consumption of fruit and vegetables and to improve their folate status can prevent at least 50% to perhaps 80% of all cases of anencephalus and spina bifida. Ethanol has without doubt been responsible for congenital mental retardation for thousands of years; compassionate and performance-driven intervention is critical for Indian reservations where whole towns and villages are affected. Not that these measures are popular or easy; they are not attractive high technology and they suffer from the fact that one cannot identify a villain or other responsible party upon which to place blame, extract compensation, and with whom one can take free journalistic license. That these steps are not easy can best be illustrated by iodine. Today's residents of wealthy industrialized countries give not a second thought to iodized salt; supplementary dietary iodine in prevention of goiter and cretinism is so common that these problems have disappeared. Why, then, does this completely preventable problem persist in the Third World? In impoverished countries, it is routine that a salt merchant equipped only with sack, shovel, and camel or mule should go to the salt mine and return to the market to sell his wares. The consumer, whose total annual income may be only a few hundred or less US dollars and who is likely illiterate, faced with the choice between the more expensive iodized variety (which looks, tastes, and for all intents and purposes works just as well as the 'natural product') and the less expensive native rock salt, will select the less expensive item. The cultural resistance and practical problems faced by the World Health Organization and United Nations in widespread dissemination of iodine or vitamin A supplements are legend, but their successful introductions can make and have made tremendous improvements in the quality of life and have reduced the incidence of birth defects.

While it remains important to identify anthropogenic and iatrogenic contributions to the etiology of birth defects, it must be recognized that despite all the resources devoted to laboratory testing, regulation, banning, restricting or listing of drugs, pesticides, industrial chemicals, and hazardous waste, birth defects occurred long before these chemical products and their wastes were introduced to our environment. Despite our best efforts, only modest progress has been made in preventing congenital malformations. Embryogenesis is exceedingly complex – after one observes the heart form from cardiac jelly (literally by growing back on itself and tying itself into a knot), the partitioning of aortic sac and conotruncus, migration of neural crest to aorticopulmonary septum, and development of associated circulation, it is a wonder things go as well as they do as often as they do. In the final analysis, it is becoming apparent that factors like inadequate or improper nutrition, ethanol, and other lifestyle factors contribute far more to the load of human congenital malformations and other manifestations of reproductive failure than has been appreciated to date.

See also: Carcinogenesis; Chromosome Aberrations; Dominant Lethal Tests; Dose–Response Relationship; Environmental Hormone Disruptors; Epidemiology; Levels of Effect in Toxicological Assessment; Molecular Toxicology–Recombinant DNA Technology; Reproductive System, Female; Reproductive System, Male; Risk Assessment, Human Health; Toxicity Testing, Developmental; Toxicity Testing, Reproductive; Toxicology, History of.

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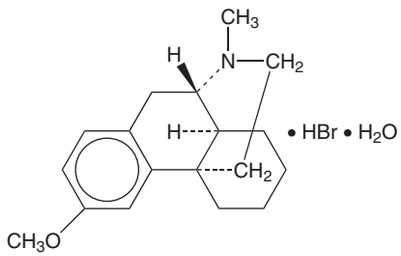
Dextromethorphan

Michael Wahl

This article is a revision of the previous print edition article by Carole Wezorek, volume 1, pp. 453–454, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 125-71-3
- Synonyms: DM; Dextromethorphan hydrobromide; Demorphan; 3-Methoxy-*n*-methylmorphinan; D-Methorphan; Drug store wine; Robowing
- Chemical/Pharmaceutical/Other Class: The methyl ether of the dextrorotatory form of levorphanol, an opiate analgesic
- Chemical Formula: $C_{18}H_{25}NO$
- Chemical Structure:



Uses

Dextromethorphan is used as an antitussive and cough suppressant. It is also used as a drug of abuse.

Exposure Routes and Pathways

Dextromethorphan preparations are administered orally in tablet or liquid form. Ingestion is the most common route of accidental and intentional exposure to dextromethorphan.

Toxicokinetics

Dextromethorphan is rapidly and well absorbed from the gastrointestinal tract. Erratic and slower absorption may occur with high-dose and sustained-release products. In therapeutic doses, peak plasma levels occur at 2.5 h with conventional dosage forms and at 6 h with sustained-release preparations. Dextromethorphan is quickly converted in the liver to an *o*-demethylated product, dextrorphan (3-hydroxy-*N*-methylmorphinan). Dextromethorphan undergoes polymorphic metabolism, depending on variations in cytochrome P450 enzyme phenotype. A total of 5–10% of the Caucasian population are poor metabolizers. The serum half-life of the parent drug is greatly increased in these individuals. Only minor amounts of active metabolites are formed in poor metabolizers. The volume of distribution of dextromethorphan is 5–6.4 l kg^{-1} in dogs. Dextromethorphan and the glucuronide and sulfate ester conjugate, together with (+)-3-hydroxy-*N*-morphinan and traces of unmetabolized dextromethorphan, are excreted in the urine. The plasma half-life is about 2–4 h with conventional dosage forms.

Mechanism of Toxicity

Dextromethorphan is the methylated dextro-isomer of levorphanol. Unlike the L-isomer, it has no analgesic properties. Dextromethorphan acts on the central nervous system (CNS) to elevate the cough threshold. It retains only the antitussive activity of other morphine derivatives. Administration of dextromethorphan may be associated with histamine release. Dextromethorphan is often present in multisymptom products with a combination of ingredients. Toxic effects of concurrent agents such as antihistamines, decongestants, analgesics, and/or alcohol may be exhibited.

Acute and Short-Term Toxicity (or Exposure)

Human

Life-threatening dosage of dextromethorphan is low. CNS depression may result, with amounts of $10\,mg\,kg^{-1}$ or more, in children. Adults may tolerate up to $14\,mg\,kg^{-1}$ with only minor effects. Ingestion of large amounts of dextromethorphan may result in lethargy, respiratory depression, nystagmus, psychosis (euphoria, hallucinations, paranoia, disorientation), and coma. Following a large ingestion of a long-acting preparation, symptoms may persist for 7 or 8 h. Hemodynamic compromise and other severe symptomatology may result from concurrent ingredients in multisymptom cold products.

Chronic Toxicity (or Exposure)

Human

Chronic abuse of dextromethorphan has resulted in psychotic behavior and hallucinations.

In Vitro Toxicity Data

Studies of rat mesencephalic neuron–glia cultures demonstrated decreased glia-induced inflammatory response in the presence of dextromethorphan.

Clinical Management

Basic and advanced life-support measures should be instituted as necessary. Gastric decontamination may be performed as indicated by the patient's symptomatology, the specific product involved, and the history of the ingestion. Activated charcoal may be useful to adsorb dextromethorphan and concurrent ingestants. Naloxone may be helpful in reversing the CNS and respiratory depressant effects of dextromethorphan. The level of consciousness and respiratory status should be carefully monitored. Management of the toxicological consequences of coingestants should be appropriate to the agent involved. Plasma dextromethorphan levels are not clinically beneficial to management of the overdose but may be useful in determining the metabolizer phenotype.

See also: Gastrointestinal System.

Further Reading

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Diabetes, Effect of Toxicity

Kartik Shankar and Harihara M Mehendale

Etiology of Diabetes

Diabetes 'mellitus' is a metabolic disorder characterized by hyperglycemia. Two principal forms of diabetes exist: type 1 diabetes (insulin-dependent diabetes mellitus) and type 2 diabetes (noninsulin-dependent diabetes mellitus). Type 1 diabetes also called as early-onset diabetes occurs due to insufficient insulin secretion by the pancreas, leading to hyperglycemia. This type of diabetes requires treatment with extraneous insulin. The second and more prevalent form of diabetes (90–95% cases of diabetics suffer from type 2 diabetes) is of a more complex etiology. Type 2 diabetes occurs primarily due to peripheral insulin resistance and is (at least in its early stages) accompanied with hyperinsulinemia. Both type 1 and type 2 forms of diabetes have been shown to have some genetic basis. Diet and obesity

significantly affect predisposition of an individual to type 2 diabetes.

Impact of Diabetes

Currently, diabetes afflicts an estimated 194 million individuals worldwide, and this number is expected to rise to at least 300 million by 2025. Sixteen million Americans suffer from diabetes with nearly a million new cases diagnosed every year. Not only is diabetes the sixth leading cause of death in the United States but also the numbers of diabetes-related deaths have gone up a striking 30% in the last decade alone. It is estimated that the total cost of diabetes including medical care and expenses as a result of lost productivity is a staggering US$ 132 billion. The quality of life of diabetics is seriously compromised due to single or in many cases multiorgan complications. Adults with diabetes have two to four times higher chances of heart disease, stroke, and high blood pressure. Diabetes is also the leading cause of blindness and end-stage renal disease among adults. In addition, diabetics are susceptible to nerve disease contributing to lower-extremity amputations.

Diabetes and Idiosyncratic Liver Toxicity

Since the above complications are serious and often life-threatening they have received attention leading to much basic research being done in these areas. However, important and potentially life-threatening complications of the liver in the diabetic condition have escaped attention. In a recent study, the prevalence of drug-induced liver disease among 40 190 type 2 diabetic patients was estimated. Surveys indicated that liver toxicity purely attributable to drug-induced causes were at the rate of 5.0/10 000 person-years, and overall liver disease incidence rate is 53.2/10 000 person-years among type 2 diabetic patients, suggesting a high background rate of liver disease in diabetics. Consistent with these data, a recent epidemiological study of 150 000 diabetic patients indicates that diabetics are twice as likely to suffer from hepatic failure as nondiabetic individuals. In one study, the incidence of acute liver injury in 34 328 patients with type 2 diabetes was evaluated from 1994 to 1998, and it was found that the overall annual incidence was 14.2 and 8.8/100 000 patient-years in the diabetes and general populations, respectively. Neither the mechanisms nor the predisposing factors underlying these liver injuries are clearly understood.

The field of hepatotoxicity in diabetes suddenly came to life when the promising new oral antidiabetic drug, troglitazone, led to severe and, in some cases, fatal hepatotoxicity in diabetics. Fatal episodes of hepatotoxicity resulted in withdrawal of the drug from the US markets. This incident prompted a renewed interest in the area and a need to understand the mechanisms that caused hepatotoxicity. The other members of class of thiazolidienediones, pioglitazone, and rosiglitazone, were also put under scrutiny for overt hepatotoxicity. Till date no major class effect of the thiazolidinediones has been found. So the question, does diabetes increase the sensitivity to potential hepatotoxicants, still lingers. Other incidents of idiosyncratic hepatotoxicity have been observed in diabetic patients on drug therapy with methotrexate, acarbose, and metformin.

Liver Toxicity in Animal Models of Diabetes

Studies using experimental diabetic animal models have indicated that xenobiotic-induced hepatotoxicity is modulated in diabetes. Hepatotoxicity of several structurally and mechanistically diverse chemicals, such as chloroform, thioacetamide, menadione, nitrosoamines, bromobenzene, and CCl_4, is significantly increased in type 1 diabetic rats. It was reported that thioacetamide-induced hepatotoxicity was potentiated in alloxan- or streptozotocin-diabetic rats. Recent studies have confirmed the potentiation of thioacetamide hepatotoxicity in streptozotocin-diabetic rats. Several studies have shown that hepatotoxicity of CCl_4 is potentiated in alloxan- or streptozotocin-induced type 1 diabetic rats.

Almost all of the work examining modulation of xenobiotic-induced hepatotoxicity in diabetes has been done using type 1 diabetic models. Recently, a study reported a novel high-fat diet-induced type 2 diabetic rat model and tested the susceptibility of these diabetic rats to several classical hepatotoxicants. On treatment with nonlethal or sublethal doses of allyl alcohol, CCl_4, or thioacetamide, it was found that hepatotoxicity of all these toxicants is significantly increased in the type 2 diabetic rats.

Chemical-induced liver injury is differentially modified by diabetes in murine type 1 diabetic models. Contrary to the enhanced hepatotoxicity in diabetic rats, diabetes in mice tends to protect animals from severe hepatotoxicity. It has been reported that the induction of diabetes in Swiss mice did not increase the susceptibility of mice to CCl_4 hepatotoxicity as occurs in rats. Development of diabetes also protected mice from acetaminophen toxicity. Further studies also showed that streptozotocin-induced diabetic mice were substantially resistant to lethal doses

of bromobenzene, CCl_4, and thioacetamide. Further, protection against acetaminophen was evident in three strains of diabetic mice. Studies examining the changes in compensatory liver cell division following acetaminophen showed that diabetic mice showed greater cell division compared to control mice. Treatment of diabetic mice with colchicine (an antimitotic agent, known to block cell division) after acetaminophen administration resulted in increased mortality in the diabetic suggesting a critical role of cell division in mediating the diabetic-induced resistance.

Mechanisms Involved in Hepatotoxic Predisposition in Diabetes

Mechanistic studies to examine the altered hepatotoxicity have traditionally focused on bioactivation/detoxification of xenobiotics in diabetic state. Type 1 diabetes, at least in the streptozotocin-induced diabetic rat, increases several phase I drug metabolizing enzymes. However, changes in drug metabolizing enzymes differ depending on the species and type of diabetes. A second and perhaps more determining factor in the ultimate outcome of hepatotoxic challenge is compensatory tissue repair. The liver has an extraordinary ability to regenerate in response to cell loss by physical damage, toxic injury, or infections. In diabetic rats (both type 1 and type 2 diabetes) where hepatotoxicity is potentiated, liver tissue repair is significantly inhibited. On the other hand, diabetic mice, which are resistant to hepatotoxicity, show greater compensatory tissue repair. More mechanistic investigations to decipher the underlying mechanisms that predispose diabetics to hepatotoxicity are underway.

See also: Common Mechanism of Toxicity; Liver; Tissue Repair.

Further Reading

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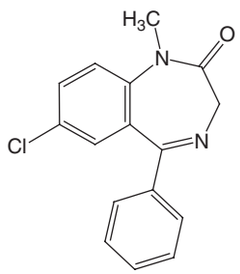
Diazepam

Teresa Dodd-Butera and Molly Broderick

- Chemical Name: 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one
- Chemical Abstracts Service Registry Number: CAS 439-14-5
- Synonyms: Methyl diazepinone; Valium (brand name)
- Chemical/Pharmaceutical/Other Class: Benzodiazepines; Psychoactive; Sedative hypnotics
- Chemical Structure:



Uses

Diazepam is a member of a class of drugs, known as benzodiazepines, introduced in the 1960s. They are relatively safe drugs, in comparison with other types of drugs used to treat anxiety. Diazepam is widely available and has a high therapeutic index; however, the drug also has the potential for abuse.

Diazepam is used primarily in the treatment of mental anxiety. In addition, it acts as a muscle relaxant for a variety of medical conditions. It may also be used as a sedative–hypnotic and anticonvulsant (e.g., for status epilepticus and drug-induced seizures). Diazepam may also be used to alleviate some of the symptoms associated with the following: cholinesterase poisoning, substance abuse withdrawal, antihistamine overdose, Black Widow spider envenomation, and chloroquine overdose. As an anesthetic, diazepam may be used alone or in combination with other drugs for conscious sedation.

Exposure Routes and Pathways

The oral route is a frequent pathway of exposure for therapeutic, accidental, and intentional overdoses.

Intravenous administration may be used to treat seizures. However, rectal suppositories have been used on occasion, if there is no parenteral access. The dermal and inhalational routes have been used in animal models, but are not conventionally used in clinical situations.

Toxicokinetics

Diazepam is rapidly absorbed following oral and parenteral administration, which may contribute to the potential for abuse. It is minimally enterohepatically recirculated, and peaks and declines are seen in serum levels after distribution into the tissue. The volume of distribution has ranged in reports from approximately 1 to 3 l kg^{-1}. Plasma protein binding is greater than 95%, which limits the effectiveness of dialysis after acute overdoses. Elimination half-life is a consideration for both therapeutic and toxic conditions. The range may be 24 h to more than 2 days. Small amounts of the unchanged drug are eliminated in the urine, and the active metabolite is desmethyldiazepam. The *N*-demethylation of diazepam involves CYP2C19 and CYP3A4.

Mechanism of Toxicity

The toxic and therapeutic effects of this drug have been attributed, in large part, to the potentiation of γ-aminobutyric acid (GABA) in the central nervous system (CNS). GABA is a neurotransmitter which mediates pre- and postsynaptic inhibition. Diazepam influences GABA activity by binding to the benzodiazepine receptor complex, thus resulting in increased CNS inhibition.

Acute and Short-Term Toxicity (or Exposure)

Animal

Reports of the lethal dose (50%) in animal studies are as follows: LD_{50} (oral) rat 1200 mg kg^{-1}; LD_{50} (oral) dog 1000 mg kg^{-1}; LD_{50} (oral) mice 700 mg kg^{-1}.

Human

Overdose of diazepam with oral and parenteral administration has the potential to cause impairment of consciousness and judgment, hypotension, bradycardia, coma, and respiratory failure. In addition, deaths have been reported when diazepam is used in combination with other CNS depressants. Sedation and somnolence are common adverse effects from the drug.

The very old and the very young are most susceptible to toxicity. Intravenous exposure results in a more rapid manifestation of symptoms, and toxicity is often iatrogenic. Even in therapeutic doses, parenteral administration may cause apnea and hypotension, especially with rapid administration of the drug.

Chronic Toxicity (or Exposure)

Animal

Carcinogenicity has been suggested, but not confirmed in animal models.

Human

Toxic effects may occur with chronic administration, and patients taking diazepam need medical monitoring. In addition, dependence may develop with regular use of benzodiazepines, and withdrawal symptoms may occur with cessation.

Pregnancy and Breastfeeding Due to its high lipid solubility, diazepam crosses the blood–brain barrier and is present in breast milk. In addition, it crosses the placental barrier. Therefore, it is not recommended during pregnancy and breastfeeding.

In Vitro Toxicity Data

Mutagenic activity of diazepam has been reported in the Ames test (TA 100). In addition, a micronucleus test using mouse bone marrow was found to be genotoxic with diazepam.

Clinical Management

Frequently, diazepam overdoses are adequately managed with clinical observation and supportive care. However, coingestion of ethanol and other CNS depressants which may exacerbate toxicity are common and warrant investigation in the patient history. Flumazenil, a benzodiazepine antagonist, effectively reverses symptoms of CNS toxicity, but is hazardous with the coingestion of other substances such as antidepressants. Therefore, it should not be used routinely.

Withdrawal symptoms may be delayed due to the long half-life and active metabolites of diazepam.

See also: Benzodiazepines.

Further Reading

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Relevant Website

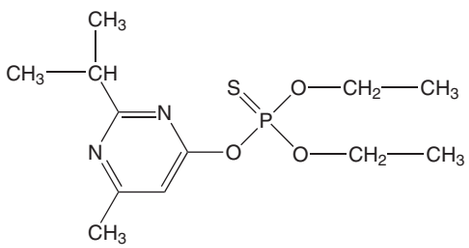
http://www.nlm.nih.gov – NIH Medline plus: Diazepam.

Diazinon

Subramanya Karanth

This article is a revision of the previous print edition article by Thuc Pham, volume 1, pp. 454–455, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 333-41-5
- Synonyms: Basudin; Dazzel; Diazitol; Gardentox; Dipofene; Knox Out; Nucidol; Spectracide
- Chemical/Pharmaceutical/Other Class: Organophosphorus (phosphorothioate) insecticide
- Chemical Structure:



Uses

Diazinon has been commonly used to control household insects such as cockroaches and ants. Household uses were eliminated in the United States in 2002, and lawn and garden uses were cancelled in 2003. Certifications in various agricultural practices were also canceled.

Exposure Routes and Pathways

Dermal and inhalation routes are the primary exposure routes for diazinon.

Toxicokinetics

Diazinon is readily absorbed through the skin, lungs, and gastrointestinal tract. Like other phosphorothioate organophosphorus insecticides, diazinon is bioactivated to diazoxon by microsomal enzymes. Other major metabolites in rats and cows include diethyl thiophosphate and diethyl phosphate. Excretion of diazinon is rapid in laboratory rats, with a half-life of $\sim$12 h. It is mainly excreted in urine (80%) and $\sim$20% is excreted in feces.

Mechanism of Toxicity

Like other organophosphorus insecticides, the active metabolite, diazoxon, elicits toxicity by inhibiting the enzyme acetylcholinesterase in the cholinergic synapse. Acetylcholinesterase inhibition leads to accumulation of the neurotransmitter acetylcholine resulting in neurotoxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} for diazinon was 300–400 mg kg^{-1} in rats. The inhalation LC_{50} (4 h) in rats was 3.5 mg l^{-1}. The dermal LD_{50} in rabbits was 3.6 g kg^{-1}. Very low doses (1 mg kg^{-1}) can produce toxicity in calves.

Human

Acute exposure to diazinon may result in acetylcholinesterase inhibition in the central and peripheral nervous system. Severity of poisoning varies with different formulations. Typical signs of poisoning include weakness, headaches, tightness in the chest, blurred vision, salivation, sweating, nausea, vomiting, diarrhea, and abdominal cramps. Diazinon has been associated with the intermediate syndrome.

Chronic Toxicity (or Exposure)

Animal

Studies in laboratory animals have shown that body weight gain is affected following chronic exposure to

diazinon. The no-observed-adverse-effect level (NOAEL) for chronic dietary exposure in rats is $0.02\,mg\,kg^{-1}$. Diazinon is not carcinogenic to laboratory animals and its mutagenic potential is not clearly understood.

Human

Repeated exposure to diazinon can cause accumulation of acetylcholinesterase inhibition and lower the threshold for subsequent exposures. Chronic exposure has been reported to lower neurobehavioral scores in farm workers. NOAEL for humans is $0.025\,mg\,kg^{-1}\,day^{-1}$.

Clinical Management

General decontamination procedures should be initiated in case of diazinon exposure. For skin decontamination, the exposed area is washed with plenty of water using soap and shampoo. In case of eye exposure, the eyes are flushed with water repeatedly for several minutes. Contaminated clothing is removed and the airway cleared. In case of ingestion, gastric decontamination should be performed immediately (within 30 min). Atropine treatment should be initiated immediately to counteract muscarinic effects. Atropine (adults and children >12 years: 2–4 mg; children <12 years: 0.05–0.1 mg) treatment should be repeated every 15 min until oral and bronchial secretions are controlled and atropinization is achieved. The duration and dosage of atropine treatment is slowly reduced as the condition of the patient improves. Pralidoxime should be administered slowly at the recommended dosage (adults and children >12 years: 1–2 g; children <12 years: 20–50 mg by intravenous infusion in 100 ml saline at $\sim 0.2\,g\,min^{-1}$). This dosage can be repeated at 1–2 h intervals initially and at 10–12 h intervals later depending on the condition of the patient. Repeated atropine treatment over several injections may be necessary for effective control of cholinergic signs.

Environmental Fate

Mobility of diazinon in soil is generally poor and under certain soil conditions it can effectively contaminate groundwater. It has a half-life of 2–4 weeks in soil. It is degraded easily in acidic water and persists for a long time at neutral pH.

Ecotoxicology

Bird kills with diazinon have been reported at all times of the year. Birds are markedly more susceptible to diazinon toxicity than other terrestrial vertebrates. LD_{50} values for birds range from 2.75 to $40.8\,mg\,kg^{-1}$. Diazinon is also very highly toxic to fish and bees.

Exposure Standards and Guidelines

The reference dose for diazinon is $0.0007\,mg\,kg^{-1}\,day^{-1}$, the acceptable daily intake is $0.002\,mg\,kg^{-1}\,day^{-1}$, and the threshold limit value (8 h) is $0.1\,mg\,m^{-3}$.

See also: Acetylcholine; Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates; Pesticides.

Further Reading

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Ibrahim NA and El-Gamal BA (2003) Effect of diazinon, an organophosphate insecticide, on plasma lipid constituents in experimental animals. *Journal of Biochemistry, Molecular Biology and Biophysics* 36(5): 499–504.

Relevant Websites

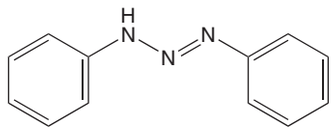
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

http://www.epa.gov – US Environmental Protection Agency.

Diazoaminobenzene

Ruth Custance and Cathy Villaroman

- Chemical Abstracts Service Registry Number: CAS 136-35-6
- Synonyms: Anilinoazobenzene; Benzeneazoanilide; Benzeneazoaniline; DAAB; Alpha-Diazoamidobenzol; *p*-Diazoaminobenzene; 1,3-Diphenyltriazene; 1,3-Diphenyl-1-triazene; DPT; *N*-(Phenylazo) aniline; Diazobenzeneanilide; *p*-Diazoaminobenzene
- Chemical Formula: $C_{12}H_{11}N_3$
- Chemical Structure:



Uses

Diazoaminobenzene (DAAB) is used as a chemical intermediate, a complexing agent, and as a polymer additive. DAAB has been used to promote adhesion of natural rubber to steel tire cords. It has also been used as a blowing agent in the production of a foamed polymeric material. In addition, DAAB is used in the manufacture of dyes and insecticides. DAAB is present in cosmetics, pharmaceuticals, and food products, as a dye contaminant in D&C Red No. 33, FD&C Yellow No. 5, and FD&C Yellow No. 6.

Exposure Routes and Pathways

The presence of DAAB as a dye contaminant in cosmetics and food products could result in low level exposures via the oral and dermal routes. Occupational exposure may occur through inhalation and dermal contact where these chemicals are produced or used as a chemical intermediate and polymer additive. DAAB is harmful to the respiratory tract, skin, and eyes. Most exposures to the general population are typically through consumption of food and use of cosmetics containing DAAB impurities.

Toxicokinetics

DAAB is well absorbed from the gastrointestinal tract but is only minimally absorbed via the dermal route. Regardless of the route of administration, the absorbed portion of the dose is rapidly metabolized and primarily excreted in urine. Within 24 h, ~60% of an oral dose of DAAB was accounted for in the urine of rats and mice as metabolites of benzene, a known human and animal carcinogen, or aniline, a rat carcinogen.

Mechanism of Toxicity

DAAB is a respiratory tract, skin, and eye irritant. DAAB yields benzene and aniline as metabolites. The proposed metabolic pathway for DAAB is that it is cleaved reductively by liver enzymes or gut flora to form aniline, benzene, and nitrogen. DAAB metabolism also results in the formation of a reactive phenyl radical, which could account for an additional risk of toxicity or carcinogenicity. The erythrocyte and lymphoid systems are major targets of DAAB toxicity. Induction of lymphoid atrophy of the thymus and other lymphoid tissues were observed, as well as methemoglobin formation, accompanying anemia, increased spleen weights, and regenerative hematopoiesis.

Acute and Short-Term Toxicity (or Exposure)

DAAB is considered explosive and is harmful to the respiratory tract, skin, and eyes via dermal contact and inhalation. Results from short-term animal toxicity studies suggest that DAAB has toxic effects similar to that from exposures to benzene and aniline. However, DAAB was observed to be more toxic at the application site than benzene or aniline. The mechanism that accounts for the greater acute toxicity of DAAB has not been determined. However, it may be attributable to properties of the parent molecule or to free radicals formed in its metabolism.

Animal

Following dermal application of DAAB in a 16 day study, animals exhibited symptoms that were similar to those from exposures to benzene and aniline. These symptoms included dose-related decreases in thymus weights in rats and mice and increases in the heart weights of rats and mice, liver and spleen in rats, and kidney in male rats and female mice. DAAB also induced hematologic effects in rats and mice, including Heinz-body formation and chemical-related methemoglobinemia. Induction of lymphoid atrophy of the thymus and other lymphoid tissues characteristic of benzene toxicity were also observed. Nonneoplastic lesions were observed in both rats and

mice, which included hyperplasia and inflammation of the skin, and hematopoietic cell proliferation in the spleen. Nonneoplastic lesions in the heart, kidney, and liver were also observed in mice. DAAB also induced toxicity not observed for aniline or benzene, including skin lesions at the application site.

Chronic Toxicity (or Exposure)

DAAB induced a greater number of micronuclei than did a combination of equimolar doses of benzene and aniline. The carcinogenicity of DAAB, specifically, has not been determined. However, the carcinogenicity of its two metabolites, benzene and aniline, has been evaluated. DAAB is considered carcinogenic since benzene is classified as a known human carcinogen.

Animal

In the late 1940s, carcinogenicity studies revealed that dermal exposure to DAAB resulted in skin and lung tumors in some mice. DAAB is metabolized to the known carcinogens aniline and benzene, both of which are carcinogenic in laboratory animals. Oral exposure to benzene induced multiple tumors at multiple sites in rats and mice of both sexes. Rats exposed to aniline in the diet developed sarcomas of the spleen and other body organs. In addition, transgenic mice developed skin tumors and leukemia following dermal exposure to benzene. Therefore, DAAB is considered to be carcinogenic in animals, based on its metabolism to benzene and aniline.

Human

No human studies specifically on DAAB exposure were identified. DAAB is predicted to be a carcinogen because one of its main metabolites, benzene, is classified as a known human carcinogen. A causal relationship between benzene exposure and leukemia has been reported in numerous epidemiological studies. The other main DAAB metabolite, aniline, is not classified as a human carcinogen based on its limited evidence of carcinogenicity in animals and inadequate evidence in humans.

In Vitro Toxicity Data

DAAB was shown to be mutagenic in *Salmonella typhimurium* strains when testing occurred in the presence of induced rat or hamster liver S9 enzymes. No additional genetic toxicity data have been reported for DAAB, but literature exists for benzene and aniline, the two main metabolites. Although benzene and aniline are not mutagenic in the *Salmonella* assay, they are active in other assays, such as in those that detect chromosomal damage.

Environmental Fate

DAAB melts at 98°C, decomposes at 130°C, and explodes at its boiling point of 150°C. The decomposition products of DAAB include benzene, *o*- and *p*-aminodiphenyl, diphenylamine, and azobenzene. DAAB is insoluble in water but soluble in ethyl alcohol, ethyl ether, benzene, pyridine, and hexane.

Exposure Standards and Guidelines

No DAAB-specific regulations have been found. The FDA regulates FD&C Yellow No. 5 and FD&C Yellow No. 6 for use as color additives in foods, drugs, and cosmetics and D&C Red No. 33 for use as a color additive in drugs and cosmetics. The American Conference of Governmental Industrial Hygienists, the National Institute for Occupational Safety and Health, and the Occupational Safety and Health Administration have not established occupational exposure limits for DAAB to date.

See also: Blood; Carcinogenesis; Dyes; Food and Drug Administration, US; Skin.

Further Reading

NTP (2002) *NTP Report on the Metabolism, Toxicity, and Predicted Carcinogenicity of Diazoaminobenzene (CAS 136-35-6),* TR-073. Research Triangle Park: National Toxicology Program.

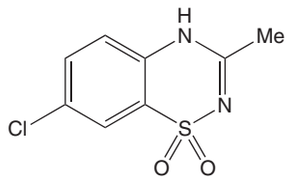
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Diazoaminobenzene.

Diazoxide

William S Utley

- Chemical Abstracts Service Registry Number: CAS 364-98-7
- Synonyms: 2*H*-1,2,4-Benzothiadiazine, 7-chloro-3-methyl-, 1,1-dioxide; Hyperstat; Mutabase proglicem
- Chemical/Pharmaceutical/Other Class: Therapeutic hypoglycemic agent
- Chemical Formula: $C_8H_7ClN_2O_2S$
- Chemical Structure:



Uses

Diazoxide is administered intravenously for the treatment of hypertension and administered orally for the treatment of hypoglycemia.

Exposure Routes and Pathways

Diazoxide is therapeutically administered either intravenously or orally.

Toxicokinetics

Diazoxide is well absorbed orally. It is distributed to the plasma where it is highly bound (>90%) to the plasma proteins. It readily crosses both the placental and blood brain barriers. The volume of distribution is 180 ml kg^{-1}. The half-life of elimination for diazoxide is 15–30 h. Approximately 20–50% of diazoxide is eliminated unchanged by the kidney, with the remainder being metabolized by the liver to 3-carboxy and 3-hydroxymethyl derivatives.

Mechanism of Toxicity

Diazoxide acts as an antihypertensive by relaxing the arteriole smooth muscle. The cardiac output and renin secretion is increased. The result is retention of salt and water and elevated levels of angiotensin II that eventually counteract the hypotensive action of the drug.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute exposures include alterations of neonatal glucose levels, fetal bradycardia, and interference with labor in women treated intravenously with diazoxide. The two most frequently cited side effects are salt and water retention and hyperglycemia.

Chronic Toxicity (or Exposure)

Human

Chronic exposure can lead to hypertrichosis. Diazoxide exhibits the ability to relax smooth muscle and therefore may be contraindicated in late pregnancy. Commonly observed side effects include decreased urination, swelling of feet or lower legs, and rapid weight gain. Occasionally, increased tachycardia may be observed and on rare occasions fever, skin rash, stiffness of arms or legs, trembling and shaking of hands and fingers, unusual bleeding or bruising, may be observed.

Exposure Standards and Guidelines

Diazoxide is regulated in the state of California as a Proposition 65 reproductive toxin.

See also: Hypoglycemics, Oral.

Further Reading

Silvani P, Camporesi A, Mandelli A, Wolfler A, and Salvo I (2004) A case of severe diazoxide toxicity. *Paediatric Anaesthesia* 14(7): 607–609.

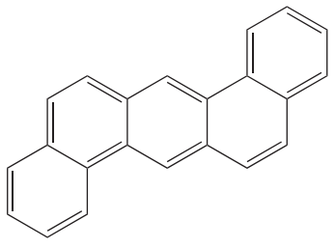
Relevant Website

http://www.nlm.nih.gov – Medline Plus: Diazoxide (Oral).

Dibenz[*a*,*h*]anthracene

William S Utley

- Chemical Abstracts Service Registry Number: CAS 53-70-3
- Synonyms: 1,2,5,6-Dubenzanthracene; DB[*a*,*h*]A; DBA
- Chemical Formula: $C_{22}H_{14}$
- Chemical Structure:



Uses

There are no reported industrial uses for dibenz[*a*,*h*]anthracene. It is used as a research tool. Dibenz[*a*,*h*]anthracene is a by-product of incomplete combustion and therefore is a fairly ubiquitous compound, generally strongly bound to the sediment.

Exposure Routes and Pathways

The primary route of exposure to dibenz[*a*,*h*]anthracene is via the skin, from petroleum-based products. An additional significant route of exposure is through inhalation of cigarette smoke. While ingestion is a route of exposure, the significance of such exposure is debatable. Dibenz[*a*,*h*]anthracene is found in many food items (cereals, fruits, and vegetables) in the low parts per billion (ppb) level. It is found in cigarette smoke at 100–150 ppb and in used motor oil at around 14 000 ppb. It is found in petroleum products such as coal tar, mineral oil, and petroleum waxes.

Toxicokinetics

Dibenz[*a*,*h*]anthracene has an octanol to water partition coefficient ($\log K_{ow}$) of 6.5 and will bioconcentrate in lower organism with less efficient mixed function oxidase systems. Dibenzo[*a*,*h*]anthracene is poorly absorbed via the gastrointestinal track, being excreted primarily unchanged with the feces. The majority of absorbed portion will distribute to the kidney and liver where it is oxidized to the dihydrodiol by mixed function oxidases. Epoxidation of the 3,4-dihydrodiol may lead to the formation of a diol-epoxide, the purported metabolite responsible for its carcinogenicity. In man, dibenz[*a*,*h*]anthracene can be oxidized to the active metabolite where it is quickly eliminated, or can react with target compounds such as the DNA. Dibenz[*a*,*h*]anthracene has not been isolated in the fat tissues, reflecting the effectiveness of the metabolizing enzyme system.

Mechanism of Toxicity

Dibenz[*a*,*h*]anthracene is metabolically activated by the mixed function oxidase (MFO) system of the liver (P448) to form an epoxide that subsequently covalently binds to the DNA. This interaction with the DNA is believed to result in the carcinogenicity of the material. The particular area of the compound oxidized by the MFO system will result in epoxides of varying carcinogenic potency.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, dibenz[*a*,*h*]anthracene has been shown to cause fetal deaths when given at 5 mg kg^{-1} daily for the first days of pregnancy. Tumors in the forestomach were produced in mice given 9–19 mg over a 5–7 month period.

Human

Like many organic compounds, excessive acute exposure to dibenz[*a*,*h*]anthracene can lead to dizziness, nausea, and general central nervous system disturbances that resemble intoxication. Previous exposure to polyaromatic hydrocarbons or genetic predisposition can increase the MFO system that activates dibenz[*a*,*h*]anthracene to the reactive epoxide. In addition, personal habits such as smoking can significantly increase a person's exposure to these compounds.

Chronic Toxicity (or Exposure)

Animal

Dibenz[*a*,*h*]anthracene is a confirmed animal carcinogen. It produced carcinomas in mice both via dermal and oral routes of administration. Skin painting studies have produced mammary tumors. Lung

tumors have been induced in rats receiving intratracheal administration of dibenz[*a*,*h*]anthracene.

Human

Dibenz[*a*,*h*]anthracene is classified as a probable human carcinogen and therefore chronically may produce cancer. In addition, carcinogenic polyaromatic hydrocarbons have been implicated in immunosuppressive activity.

In Vitro Toxicity Data

Dibenz[*a*,*h*]anthracene has been reported to induce DNA damage and gene mutations in bacteria.

Environmental Fate

High K_{oc} values (ranging from 5.7×10^5 to 3.0×10^6) indicate that dibenz[*a*,*h*]anthracene will tend to remain bound to the soil column and not migrate. Neither vitalization nor biodegradation are expected to be significant factors. Reported biodegradation values in nonacclimated soil are in excess of 240–750 days. Acclimated sludge has been reported to metabolize dibenz[*a*,*h*]anthracene in 36 days and may account for a large percentage of its degradation in the environment.

Ecotoxicology

Reported values for *Daphnia magna* range from 0.4 to $0.8\,\mathrm{mg\,l^{-1}}$. Based on the substance's high K_{ow} and modeling, it is predicted to be highly toxic to aquatic life ($LC_{50} < 1\,\mathrm{mg\,l^{-1}}$).

Exposure Standards and Guidelines

The acceptable level in drinking water is $13.3\,\mathrm{ng\,l^{-1}}$. Dibenz[*a*,*h*]anthracene is an resource conservation and recovery act (RCRA) hazardous waste (U063). Dibenz[*a*,*h*]anthracene is classified as a B2 probable human carcinogen, based on sufficient animal data and no human data.

An oral slope factor of $7.3\,\mathrm{mg^{-1}\,kg^{-1}\,day^{-1}}$ has been calculated for benzo[*a*]pyrene based on the incidence of stomach tumors in mice treated with benzo[*a*]pyrene.

The US Environmental Protection Agency (EPA) has calculated the drinking water unit risk as $2.1 \times 10^{-4}\,\mathrm{g^{-1}\,l^{-1}}$. The EPA has calculated an inhalation unit risk of $1.7 \times 10^{-3}\,\mathrm{g^{-1}\,m^{-3}}$.

Dibenz[*a*,*h*]anthracene is listed as an International Agency for Research on Cancer 2A confirmed animal carcinogen and is listed as a California Proposition 65 carcinogen.

Dibenz[*a*,*h*]anthracene is listed in section 112 of the Clean Air Act; listed under sections 304 and 307 of the Clean Water Act; listed as an RCRA hazardous waste U063. Dibenz[*a*,*h*]anthracene is regulated under comprehensive environmental response, compensation, and liability act (CERCLA) with a reportable quantity (RQ) of 1 pound. It is listed as an emergency planning and community right-to-know act (EPCRA) superfund amendments reauthorization act (SARA) 313 reportable substance.

In the European Union, dibenz[*a*,*h*]anthracene is classified as T, N: R-45, R-50/53 (toxic, may cause cancer, and dangerous to the environment).

See also: Benz[*a*]anthracene; Benzo(*a*)pyrene; Clean Water Act (CWA), US; Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

Petry T, Schmid P, and Schlatter C (1996) The use of toxic equivalency factors in assessing occupational and environmental health science associated with exposure to air borne mixtures of polycyclic aromatic hydrocarbons. *Chemosphere* 32: 639–648.

Relevant Website

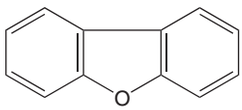
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dibenz[*a*,*h*]anthracene.

Dibenzofuran

Kashyap N Thakore and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 132-64-9
- Synonyms: 2,2′-Biphenylene oxide; 2,2′-biphenylylene oxide; dibenzo(*B*,*D*)furan; diphenylene oxide
- Chemical/Pharmaceutical/Other Class: Strong oxidizing agent; Antiestrogen
- Chemical Formula: $C_{12}H_8O$

- Chemical Structure:



Uses

Dibenzofuran is an industrial chemical or by-product.

Exposure Routes and Pathways

Inhalation is the most common route of exposure. Dibenzofuran is present in cigarette ash and is a by-product of processes in the pharmaceutical industry. When heated to decompose, it emits acrid smoke and irritating fumes. Exposure can also occur by ingestion of contaminated food.

Toxicokinetics

Dibenzofuran may be rapidly absorbed by various routes including oral, nasal mucosal, inhalation, and dermal routes. 2,2′,3-Trihydroxy biphenyl dioxygenase is a key enzyme responsible for meta-cleavage of the first aromatic ring in the degradation pathway. After intravenous or oral administration to rats, most of the compound is quickly distributed to the liver, muscle, skin, and adipose tissue and metabolized. Its metabolites may remain in the adipose tissue for a relatively long period of time. Polychlorinated dibenzofuran is highly lipophilic and is accumulated in adipose and liver tissues at a higher level and in muscle, kidneys, spleen, lungs, brain, and blood at a lower level. The metabolites are rapidly excreted mainly in bile, urine, and feces. They can also be excreted through milk.

Mechanism of Toxicity

Dibenzofuran induces hepatic, skin, and lung cytochrome P450 1A1, 1A2, and aryl hydrocarbon hydroxylase in rats. Thus, toxicity results from aryl hydrocarbon receptor signal transduction pathway. Bioactivation of many polycyclic hydrocarbon carcinogens is mediated by these enzymes.

Acute and Short-Term Toxicity (or Exposure)

Animal

2,3,7,8-Tetrachlorodibenzofuran (TCDF) causes wasting syndrome, thymic atrophy, and immune suppression in rodents, hair and fingernail loss in monkeys, chloracne formation in the rabbit ear, and hyperpigmentation in the rhesus monkey. It is hepatotoxic and has profound effects on both steroid and growth factor receptor systems. Significant species variability is seen in the toxicity of this compound. The LD_{50} for TCDF is 5–10 $\mu g\,kg^{-1}$ body weight in guinea pigs, $\geq 6000\,\mu g\,kg^{-1}$ body weight in mice, and $1000\,\mu g\,kg^{-1}$ body weight in monkeys.

Human

Dibenzofuran may be harmful by inhalation, ingestion, or skin absorption and may cause irritation. It is globally distributed, is persistent in the environment, and tends to accumulate in human tissues. The major primary sources of exposure for the general population are combustion (municipal waste incineration and automobile exhaust), carbon electrode processes (smelters), chemical manufacturing wastes (chlorophenols), open-use agricultural and industrial chemicals (chlorophenols and chlorophenoxy herbicides), polychlorinted biphenyls, and aqueous chlorination (sewage sludge and kraft pulp mills). Exposure can occur through contaminated food (fish, meat, and dairy products; breast milk in the case of infants). All the chlorinated compounds have the potential to cause dermal, hepatic, and gastrointestinal toxicities. The half-life in humans is relatively long.

Chronic Toxicity (or Exposure)

Animal

It is a liver tumor promoter, teratogenic, and immunotoxic affecting natural killer cells.

Human

It is not classifiable as to human carcinogenicity.

Clinical Management

Due to long biological half-life and lipid solubility of dibenzofurans, blood analysis may serve as an index of past cumulative occupational exposure and a means of assessing a person's exposure situation. In case of contact, the eyes and skin should be flushed immediately with water for at least 15 min. If inhaled, the victim should be removed to fresh air. If the person is not breathing, artificial respiration should be given; if breathing with difficulty, oxygen should be given. If the patient is in cardiac arrest, cardiopulmonary resuscitation should be given. In case of ingestion, the mouth should be washed out with water provided the person is conscious. These life-supporting measures should be continued until medical assistance has arrived. Liquids should not be

administered to and vomiting should not be induced in an unconscious or convulsing person.

In the workplace, technical measures should prevent any contact with the skin and mucous membranes. Workers potentially exposed to this compound should wear personal protective equipment and their work should be carried out only in restricted and ventilated areas. After use, clothing and equipment should be placed in an impervious container for decontamination or disposal. Preemployment and periodic medical examination should focus on liver function.

Environmental Fate

It is expected to have very low to no mobility in soil and significantly degrade in soil. It dissolves in water and also volatilizes. It exists primarily in the gas-phase in the atmosphere and reacts with photochemically-produced hydroxy radicals. It is biodegraded at contaminated sites where populations of adapted microorganisms are present; otherwise biodegradation may be slow. Biodegradation is also slow when oxygen is limited. It adsorbs very strongly to sediment and particulate matter in the water column. It has a potential for bioconcentration in aquatic organisms.

Other Hazards

It is not flammable.

Exposure Standards and Guidelines

The US Environmental Protection Agency is required to establish and phase in specific performance based standards for all air emission sources that emit dibenzofuran as one of the listed pollutants.

See also: Chlorophenols; Combustion Toxicology; Polychlorinated Biphenyls (PCBs); Polycyclic Aromatic Hydrocarbons (PAHs); TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin).

Relevant Websites

http://ntp.niehs.nih.gov – National Toxicology Program (NTP) (2004) Summary of Data of Chemical Selection: Dibenzofuran.

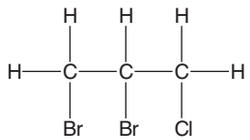
http://www.epa.gov – Environmental Protection Agency (US EPA) (2003) Substance Registry System, Washington DC.

Dibromochloropropane

Mark L Winter

This article is a revision of the previous print edition article by Thuc Pham, volume 1, pp. 458–459, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 96-12-8
- Synonyms: 1,2-Dibromo-3-chloropropane; DBCP; Nemafume; Nemanax; Nemaset; Nemagon; Femafume; Fumazone
- Chemical/Pharmaceutical/Other Class: Halogenated alkane
- Chemical Structure:



Uses

Dibromochloropropane (DBCP) was used in the United States as a soil fumigant and nematocide. The use of dibromochloropropane has been banned in the United States but it is still used in some other countries.

Exposure Routes and Pathways

Respiratory and dermal routes of exposure were most common.

Toxicokinetics

DBCP is well absorbed by any route of exposure. Absorption is almost complete following oral exposure. Microsomal transformation leads to the formation of reactive metabolites. Metabolites undergo conjugation with glutathione. DBCP induces microsomal enzymes in the testes, liver, and kidneys. Covalently bound metabolites accumulate in the liver and kidneys. Urinary excretion is the major route of elimination.

Mechanism of Toxicity

At high exposures, DBCP stabilizes neuronal membranes and leads to nervous system depression.

Reactive metabolites of DBCP (e.g., epoxides) bind to cellular macromolecules. With testicular toxicity, DBCP may act by preventing differentiation of spermatogonia into mature sperm.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral LD_{50} of DBCP in male rats and guinea pigs is $\sim$150–300 mg kg^{-1}. The dermal LD_{50} is >1 g kg^{-1}. DBCP acts as a central nervous system depressant at high vapor concentrations.

Chronic Toxicity (or Exposure)

Animal

Early animal studies demonstrated reduced testicular weights, with testicular atrophy at higher exposure levels. Several studies reported decreased sperm count and infertility with long-term exposure to DBCP. It is an experimental carcinogen, capable of increasing tumor incidence in a variety of tissues.

Human

The most marked chronic toxicity in humans from DBCP exposure is male infertility. Occupational exposure to DBCP has been associated with reduced sperm count and elevated levels of follicle-stimulating hormone and luteinizing hormone in humans. From a group of studies on occupational exposure to DBCP, it was estimated that $\sim$15% of exposed workers were azoospermic. The effects on sperm production last years after exposure has ended, in particular with men exposed for a period of more than 4 years. Upon testicular biopsy, the seminiferous tubules were devoid of spermatogenic cells, with only Sertoli cells remaining. Some authors have concluded that paternal exposure to DBCP, severe enough to cause azoospermia or oligospermia, did not increase the rate of congenital malformations or of impaired health status of offspring conceived during or after exposure.

In Vitro Toxicity Data

DBCP was mutagenic in several bacterial assays.

Clinical Management

Acute exposure to DBCP vapors requires removal from the source and symptomatic treatment. There is no treatment for testicular toxicity.

Exposure Standards and Guidelines

The reference concentration for inhalation is 2×10^{-4} mg m^{-3}. The maximum contaminant level (MCL) is 0.0002 mg l^{-1}.

See also: Pollution, Water; Reproductive System, Male.

Further Reading

Gehring PJ, Nolan RJ, Watanabe PG, and Schumann AM (1991) *Solvents, fumigants, and related compounds Handbook in Pesticide Toxicology*, Vol. 2, pp. 637–730. San Diego: Academic Press.

Hoyer PB (2001) Reproductive toxicology: Current and future directions. *Biochemical Pharmacology* 62: 1557–1564.

Meistrich ML, Wilson G, Shuttlesworth GA, and Porter KL (2003) Dibromochloropropane inhibits spermatogonial development in rats. *Reproductive Toxicology* 17: 263–271.

Potashnik G and Phillip M (1988) Lack of birth defects among offspring conceived during or after paternal exposure to dibromochloropropane (DBCP). *Andrologia* 20(1): 90–94.

Thomas MJ and Thomas JA (2001) Toxic responses of the reproductive system. In: *Casarett and Doull's Toxicology*, 6th edn., pp. 673–709. New York: McGraw-Hill.

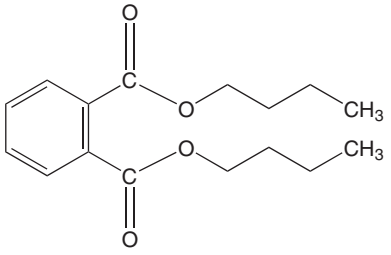
Dibutyl Ether *See* Diethyl Ether.

Dibutyl Phthalate

David R Wallace

This article is a revision of the previous print edition article by Sushmita M Chanda, volume 1, pp. 459–460, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 84-74-2
- Synonyms: Dibutyl-1,2-benzenedicarboxylate; *o*-Benzenedicarboxylic acid; Dibutyl ester; DBP
- Chemical/Pharmaceutical/Other Class: Plasticizer and softener; Aromatic dicarboxylic acid ester
- Chemical Formula: $C_{16}H_{22}O_4$
- Chemical Structure:



Uses

Dibutyl phthalate has multiple uses in a variety of materials. Primary uses for dibutyl phthalate are to soften and increase plastic flexibility, for example, in shower curtains, raincoats, food wraps, and car interiors to name a few. It has been used in insect repellents and as a solvent for perfume oil and resins. Dibutyl phthalate can be used as a plasticizer in nitrocellulose lacquers, elastomers, explosives, nail polish, and solid rocket propellants. Other uses include perfume fixative, textile lubricating agent, safety glass additive, printing inks, and adhesives.

Exposure Routes and Pathways

Exposure to dibutyl phthalate is usually by inhalation. It is known to leech out from finished plastics into blood, milk, and other food materials and, therefore, can be ingested orally. Dermal and ocular exposures are also possible.

Toxicokinetics

In rats, greater than 90% of dibutyl phthalate was excreted in the urine within 48 h following i.v. or oral dosing, but elimination in the feces was low. No accumulation was observed in tissues 24 h after exposure. Dibutyl phthalate was hydrolyzed rapidly by the rat liver esterases. Monobutyl phthalate (MBP) was a common metabolite in different species. The glucuronide conjugate was also detected in rat, hamster, and guinea pig together as well as a small amount of phthalic acid and unchanged compound. Omega or omega-1 oxidation products of MBP were also detected in the urine.

Mechanism of Toxicity

Dibutyl phthalate acts as an uncoupler of oxidative phosphorylation in rats.

Acute and Short-Term Toxicity (or Exposure)

The majority of the studies focusing on dibutyl phthalate exposure has centered on acute exposure via inhalation, ingestion, and dermatological contact.

Animal

Exposures to dibutyl phthalate have caused photophobia, conjunctivitis, edema, and keratitis. Increase in mean liver weight and testicular atrophy have been observed in rats exposed to dibutyl phthalate. The LD_{50} in rats is 8–10 g kg^{-1} (oral) and 4 g kg^{-1} (intraperitoneal). Aerosol (2 h) exposure of dibutyl phthalate at a concentration of 250 mg m^{-3} in mice produced symptoms of irritation to the upper respiratory tract and eyes. Increased concentrations resulted in bronchospasms causing difficulty in breathing, ataxia, weakness, convulsions, and eventually death. The LC_{50} in mice was determined to be 25 g m^{-3}. Dibutyl phthalate has weak estrogenic activity in a number of assays and may act as an anti-androgen.

Human

Dibutyl phthalate has low acute toxicity based on animal studies. It can cause an immediate stinging and burning sensation upon contact by splashing. Following ingestion, it can also cause dizziness and nausea. Contact with the skin has been reported to result in contact dermatitis.

Chronic Toxicity (or Exposure)

Information regarding the long-term, chronic, exposure to dibutyl phthalate is lacking. There is no information on human carcinogenicity and only limited effects in animals. No information is available on human teratogenicity, but animal studies have shown a reduced body weight. No information on dibutyl

phthalate effects on the human reproductive system is available.

Animal

Rats exposed to 0.5 mg m^{-3} for 6 h day^{-1} for 6 days exhibited significantly higher brain and lung weights, smaller overall body weights compared to control groups. The Environmental Protection Agency (EPA) has classified dibutyl phthalate as 'group D' carcinogen, in that no definitive carcinogenic characteristics have been reported.

Human

Generalized symptoms of chronic exposure dibutyl phthalate include pain, numbness, spasms, weakness, and finally, polyneuritis. In an American Conference of Governmental Industrial Hygienists (ACGIH) study of 147 Russian workers exposed to several dibutyl esters for a period of 0.5–19 years and an air concentration of 1.7–66 mg m^{-3} reported significant adverse effects. By the seventh year of work, reports of pain, numbness, and muscle spasms were reported. These symptoms were followed by weakness in the extremities and a 32% rate of polyneuritis.

In Vitro Toxicity Data

Short-term studies with human cells have yielded negative results. Long-term mutagenicity tests have provided inconclusive results.

Clinical Management

Induced emesis is not recommended if the victim has any signs of esophageal or gastrointestinal tract irritation or burns, or decreased sensory response, depressed gag reflex, or impending shock. Activated charcoal slurry with or without saline cathartic or sorbitol can be given in cases of oral exposures. Skin decontamination should be done with repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min. Treatment is supportive and symptomatic and no specific antidote is available.

Environmental Fate

Dibutyl phthalate is considered nonhazardous for air, sea, and road freight. No other reports on dibutyl phthalate are available.

Exposure Standards and Guidelines

Occupational Safety and Health Administration permissible exposure level, the National Institute for Occupational Safety and Health recommended exposure limit, and the ACGIH threshold limit value has been set at 5.0 mg m^{-3} for an 8–10 h day per 40 h week. Although dibutyl phthalate is not subject to EPA emergency planning requirements, if dibutyl phthalate is released in a quantity exceeding 10 pounds over 24 h, the appropriate local, state, and federal authorities must be notified.

See also: Fragrances and Perfumes; Nitrocellulose; Toxicity Testing, Dermal.

Further Reading

ACGIH (1991) *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th edn. Cincinnati, OH: American Conference of Government Industrial Hygienists.

Sittig M (1991) *Handbook of Toxic and Hazardous Chemicals*, 3rd edn. Park Ridge NJ: Noyes Publications.

Relevant Websites

http://www.intox.org – Canadian Centre for Occupational Health and Safety. Cheminfo: Dibutyl phthalate.

http://www.osha.gov – Occupational Safety and Health Administration, US Department of Labor Health Guidelines.

http://www.epa.gov – US Environmental Protection Agency Technology Transfer Network Air Toxics Website: Dibutyl phthalate.

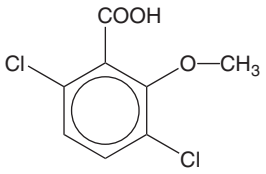
Dicamba

Xun Song

This article is a revision of the previous print edition by Sushmita M Chanda, volume 1, p. 460, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 1918-00-9
- Synonyms: Banlen; Banvel 480; Brush buster; Compound B dicamba; Velsicol compound 'r'; Velsicol 58-CS-11; Banvel herbicide; Banvel 4WS; Banfel®; Banvel®; Banvel CST®; Banvel D®; Banvel XG®; Mediben®
- Chemical/Pharmaceutical/Other Class: Benzoic acid, 3,6-dichloro-2-methoxy-

- Chemical Structure:



Uses

Dicamba is mainly used as herbicide to control weeds, dock, bracken, and brush. Dicamba is frequently applied with other herbicides including atrazine, glyphosate, imazethapyr, ioxynil, and mecoprop.

Exposure Routes and Pathways

Dicamba is available as an odorless, white or brown, crystalline solid. Exposure to dicamba may occur through oral, dermal, or inhalation route.

Toxicokinetics

Dicamba is known to be well absorbed orally. Minimal absorption occurs through the skin. Following ingestion in animals, dicamba is readily distributed in all organs and systems. When given as a food supplement to rats, most dicamba was excreted unchanged in the urine while a small proportion was metabolized into glucuronic acid conjugates. The half-life of elimination in rats was estimated to be 0.83 h.

Mechanism of Toxicity

There is little evidence of dicamba toxicity in mammals. In plants its primary action is to act as a growth regulator.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for dicamba is 757–1707 mg kg^{-1} in rats, 1190 mg kg^{-1} in mice, 2000 mg kg^{-1} in rabbits, and 566–3000 mg kg^{-1} in guinea pigs. In rabbits, the dermal LD_{50} is greater than 2 g kg^{-1} while the inhalation LC_{50} in rats is greater than 200 mg l^{-1}.

Human

Symptoms of poisoning with dicamba include loss of anorexia, vomiting, muscle weakness and spasms, bradycardia, shortness of breath, central nervous system effects (excitation or depression), incontinence, and cyanosis. Inhalation can cause irritation of the nasal passages and lungs and loss of voice. Recovery from severe overdose is generally complete within 2–3 days. Dicamba can cause severe, permanent corrosive damage to the eyes. Dicamba may cause skin burns.

Chronic Toxicity (or Exposure)

Animal

Prolonged dietary dicamba exposure at high dosages in rats led to changes in liver and decreased body weights. Dicamba was negative in reproductive, teratogenic, and carcinogenic tests.

Human

Little is known regarding chronic effects of dicamba in humans but animal studies suggest little potential for chronic toxicity.

In Vitro Toxicity Data

Dicamba is not mutagenic.

Clinical Management

There is no specific antidote; therefore, the treatment is symptomatic and supportive. Skin decontamination should be done with repeated washing with soap. Exposed eyes should be irrigated with copious amounts of water (at room temperature) for at least 15 min. Emesis can be induced if initiated within 30 min of ingestion. Ipecac can be used to induce emesis. Emesis is not encouraged if the patient is comatose or convulsing. Activated charcoal slurry with or without saline cathartic and sorbitol may be used.

Environmental Fate

Dicamba is moderately persistent in soil (half-life is 1–4 weeks). Microbial degradation is predominant. Degradation increases with temperature, increasing moisture, and low pH. When soil moisture increases above 50%, however, dicamba degradation is reduced. Photodegradation occurs to a limited extent. Some dicamba residues volatilize from plant surfaces. Dicamba does not bind to soil particles and is highly water soluble and therefore mobile. Groundwater contamination is possible. In surface waters, microbial degradation is predominant.

Photodegradation can also occur. Dicamba does not significantly bioaccumulate.

Ecotoxicology

Dicamba is practically nontoxic to birds (LD_{50} in mallard ducks was $>2\,g\,kg^{-1}$). The 8 day dietary LC_{50} in mallards and quail was $>10\,000$ ppm. Dicamba is also of low toxicity to fish. The LC_{50} (96 h) for dicamba was 100–135 $mg\,l^{-1}$ in rainbow trout, bluegill, grass shrimp, fiddler crab, and sheepsheadminnow. The LC_{50} (48 h) was 110 $mg\,l^{-1}$ in *Daphnia magna*. Dicamba is not toxic to bees.

Exposure Standards and Guidelines

The reference dose for dicamba is 0.045 $mg\,kg^{-1}\,day^{-1}$.

See also: Pesticides; Pollution, Water.

Relevant Websites

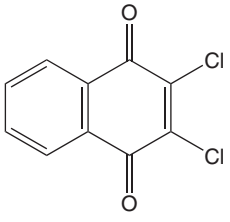
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency and National Pesticide Information Center.

Dichlone

Xun Song

This article is a revision of the previous print edition article by Todd A Bartow, volume 1, p. 461, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 117-80-6
- Synonyms: Dichloronaphthoquinone; 2,3-Dichloro-1,4-naphthoquinone; Phygon; Algistat; Quintar; Sanquinon; Miraclear
- Chemical/Pharmaceutical/Other Class: Naphthoquinone
- Chemical Structure:



Uses

Dichlone is primarily used as a fungicide. It is especially effective for brown rot of stone fruit and scab on apples and pears. Dichlone is also used to control blue algae. There are no registered uses for dichlone in the United States.

Exposure Routes and Pathways

Exposure to dichlone may occur through oral and dermal routes.

Toxicokinetics

Toxicokinetic data for dichlone are limited. It has been demonstrated that dichlone is poorly absorbed from the gastrointestinal tract.

Mechanism of Toxicity

The exact mechanism of toxicity is not clear. *In vitro* studies suggested that incubation of dichlone with normal human erythrocytes induced rapid loss of intracellular potassium, increased the osmotic fragility, and inhibited the Na^+,K^+-ATPase. Dietary dichlone exposure caused inhibition of glycolysis in rat liver. Dichlone can inhibit pyruvate and succinate dehydrogenases. Dichlone was also reported to cause oxidative stress and swelling of mitochondria.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute dermal LD_{50} in rabbits was 5 $g\,kg^{-1}$. The oral LD_{50} in rats was 1.3 $g\,kg^{-1}$. Dichlone is a skin irritant.

Human

Dichlone has relatively low toxicity in humans. It is irritating to the skin and mucous membranes. Irritation of the cornea may occur. Ingestion of large doses usually results in prompt emesis. Large doses may cause central nervous system depression, coma, and death.

Chronic Toxicity (or Exposure)

Animal

Little toxicity was noted in rats given a diet of 1500 ppm dichlone for 2 years. Dietary exposure of dogs to dichlone (500 ppm) for 1 year elicited slight liver changes.

Human

Based on animal studies, dichlone exposure is not expected to lead to chronic toxicity. Little is known, however, regarding long-term exposures in humans.

Clinical Management

If a poisoning is suspected, one should not wait for symptoms to develop. Immediate medical attention should be sought. Emesis may be indicated in recent substantial ingestion unless the patient is or could rapidly become obtunded, comatose, or convulsing. Emesis is most effective if initiated within 30 min. An activated charcoal cathartic may also be employed. Use of any fat should be avoided since these agents may increase the irritant effects If contaminated with dichlone the affected area should be washed vigorously with soap. Contaminated clothing should be removed and discarded.

Ecotoxicology

Dichlone is toxic to fish. Dichlone is relatively nontoxic to bees. The LC_{50} in *Daphnia magna* is 0.014 ppm.

Exposure Standards and Guidelines

The reference dose for dichlone is $0.08\,mg\,kg^{-1}\,day^{-1}$.

See also: Pesticides.

Further Reading

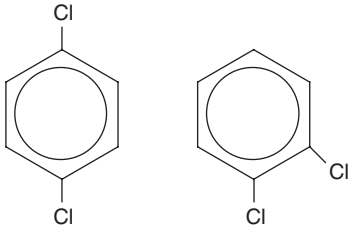
Babich H, Palace MR, Borenfreund E, *et al.* (1994) Naphthoquinone cytotoxicity to bluegill sunfish BF-2 cells. *Archives of Environmental Contamination and Toxicology* 27: 8–13.

Pritsos CA, Pisani DE, and Pardini RS (1985) Inhibition of liver glycolysis in rats by dietary dichlone (2,3-dichloro-1,4-naphthoquinone). *Bulletin of Environmental Contamination and Toxicology* 35: 23–28.

Dichlorobenzene

Elmar Udarbe Zamora

- Chemical Abstracts Service Registry Numbers: 1,2-DCB (CAS 9-5501); 1,3-DCB (CAS 541-73-1); 1,4-DCB (CAS 106-46-7)
- Synonyms: 1,2-DCB: Benzene-1,2-chloro-, *o*-Dichlorobenzene, 1,2-Dichlorobenzene; 1,3-DCB: Benzene-1,3-chloro-, *m*-Dichlorobenzene, 1,3-Dichlorobenzene; 1,4-DCB: Benzene-1,4-chloro-, *p*-Dichlorobenzene, 1,4-Dichlorobenzene, AI13-0050, *p*-Dichloricide, Evola, Globol, Paracide, Para crystals, Parazene, Paradow, Paramoth, Paranuggets, Paradi, PDC, PDCB, *p*-Dichlorobenzol, Persia-parazol, Santochlor, *p*-Chlorophenyl chloride
- Chemical/Pharmaceutical/Other Class: Chlorinated aromatic hydrocarbon
- Chemical Formula: $C_6H_4Cl_2$
- Chemical Structure:



Para- *Ortho-*

Uses

The dichlorobenzenes are used as moth repellants, insecticides, miticides, fumigants, disinfectants, space and air deodorizers, to prevent breakup of stoneware and molding, for molding resins and surface coatings. They are also used as chemical intermediates, for example, in the manufacture of polyphenyl sulfide. These are available as mothballs, flakes, cake, crystals,

and as a 100% concentrate. In some 1,4-dichlorobenzene commercial preparations, 1,2- and 1,3-dichlorobenzenes usually occur in minute amounts.

Exposure Routes and Pathways

The most common way to be exposed to 1,4-dichlorobenzene is by inhaling the vapors from mothballs and toilet deodorizers. However, this route ($35\,\mu g\,day^{-1}$) is not expected to lead to substantial effects. Ingestion and dermal or ocular contact are the other most common routes of exposure.

Toxicokinetics

1,4-Dichlorobenzene is well absorbed orally and by inhalation. The highest concentrations are found in the adipose tissue. It is rapidly oxidized to phenolic compounds and metabolized to sulfate and glucuroate conjugates. The major metabolite is 2,5-dichlorophenol. From 91% to 97% is excreted in the urine within 5 days.

Mechanism of Toxicity

In some species, 1,4-dichlorobenzene can cause nephrotoxicity associated with hyaline droplets. This syndrome requires the presence of $\alpha 2\mu$-globulin protein.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dichlorobenzene is an eye, skin, and upper respiratory tract irritant in animals. It also affects the liver and kidneys and is considered a potential carcinogen. The oral LD_{50} is $500\,mg\,kg^{-1}$ in rats and more than $2\,g\,kg^{-1}$ in rabbits. The lowest-observed-adverse-effect level (LOAEL) is $155\,mg\,kg^{-1}$ per 2 years of intermittent exposure in rats and mice. 1,4-Dichlorobenzene and a primary metabolite were negative in the mouse *in vivo* micronucleus test.

Human

Dichlorobenzene has low acute toxicity. However, people exposed to high levels for relatively short periods of time potentially can develop malaise, nausea, vomiting, headaches, and irritation of eyes, respiratory tract, and the skin (burning sensation upon contact). Central nervous system (CNS) depression may occur at high concentrations that are extremely irritating to the eyes and nose. The TDLo in humans is $300\,mg\,kg^{-1}$.

Chronic Toxicity (or Exposure)

Animal

Rats, rabbits, and guinea pigs exposed through inhalation over a long period of time have reduced food consumption and body weights. CNS signs include tremors and respiratory/dermal effects seen were nasal and ocular discharges. The liver and kidneys increased in weight and showed degenerative and necrotic cellular changes. Livers of rats exposed orally ($300\,mg\,kg^{-1}\,day^{-1}$) for prolonged periods were found to exhibit cellular degeneration, cloudy swelling, and focal necrosis. 1,2-DCB was found to be toxic to Fisher rats. As noted before, the nephrotoxicity is dependent on presence of species-specific expression of $\alpha 2\mu$-globulin protein.

Human

People exposed to levels above the maximum contaminant level goal (MCGL) for prolonged periods of time can potentially have abnormalities in the blood (e.g., anemia), in the CNS, develop skin lesions, experience appetite loss, and have liver damage. Protein, bilirubin, and blood is present in the urine. A woman exposed to *p*-dichlorobenzene for 6 years developed nervous system effects, including severe muscle incoordination, weakness in all limbs, and decreased reflex responses. While 1,4-dichlorobenzene is a carcinogen in rats, mechanistic information suggests that it has little carcinogenic potential in humans.

In Vitro Toxicity Data

Dichlorobenzenes can covalently bind to nucleic acids. In a concentration dependent manner, 1,2-dichlorobenzene and *p*-dichlorobenzene were found to be estrogenic in the yeast estrogen screen with relative potencies in relation to B-estradiol of 2.2×10^{-7} for 1,4-dichlorobenzene and 1.04×10^{-8} for 1,3-dichlorobenzene. No mutagenic potential was observed in the gene mutation assay using mouse lymphoma cells.

Clinical Management

The affected skin or eyes should be immediately flushed with plenty of water for 15 min. The affected skin should be washed with soap and water. Activated charcoal slurry with or without saline cathartic can be given if dichlorobenzene is ingested. Vomiting should not be induced. The victim should be moved to fresh air when exposed through inhalation. Humidified oxygen (100%) can be supplemented with assisted ventilation. If the victim is

unconscious, nothing should be given by mouth, airway, breathing, and circulation should be stabilized, and the victim should be brought to the hospital immediately. Since many of the reported toxicities are due to chronic exposure, treatment is mostly symptomatic and supportive.

Environmental Fate

Most 1,4-dichlorobenzene found in the environment is due to its use as a toilet deodorizer and moth repellant. Since the solid easily vaporizes, much of 1,4-dichlorobenzene is released into the air. Dinitrobenzenes in water readily evaporate. DCB adsorbs to soil and is relatively resistant to degradation.

Ecotoxicology

This chemical was found to be present in fish (400 ppm). It may also accumulate in plants.

Other Hazards

It is incompatible with aluminum and its alloys and could react with plastics, rubber, or coatings. It reacts violently with oxidizing agents like chlorine and permanganate. In cases of spills or leakage, all flammable material or all sources of ignition are removed to prevent fire. The spill is dampened with 60–70% alcohol. The spill is removed by using a paper dampened with 60–70% alcohol, which is disposed in a suitable container. All contaminated clothing are washed with 60–70% alcohol and then washed in soap and water.

Exposure Standards and Guidelines

The MCLG is the level of a contaminant in drinking water below which there is no known or expected risk to health. The Environmental Protection Agency sets the MCLG for *o*-dichlorobenzene at 0.6 ppm.

The National Institute for Occupational Safety and Health sets the recommended exposure limit at 1.7 ppm in an 8 h threshold limit value – time-weighted average (TLV – TWA).

The Occupational Safety and Health Administration (OSHA) sets the permissible exposure limit for *p*-dichlorobenzene as 75 ppm for an 8 h TWA.

The American Conference for Governmental Industrial Hygienists sets the TLV – TWA for *p*-dichlorobenzene at 10 ppm.

The National Fire Protection Assocation rates 1,4-dichlorobenzene as moderately hazardous to health and moderately flammable.

See also: Pesticides.

Further Reading

Barter JA and Sherman JH (1999) An evaluation of the carcinogenic hazard of 1,4-dichlorobenzene based on internationally recognized criteria. *Regulatory Toxicology and Pharmacology* 29: 64–79.

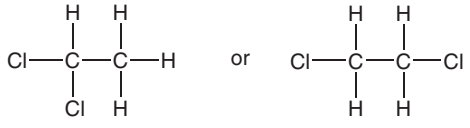
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dichlorobenzenes.

Dichloroethanes

Madhusudan G Soni and Harihara M Mehendale

- Synonyms: Chlorinated hydrochloric ether; Ethylidene chloride; Ethylidine dichloride
- Chemical/Pharmaceutical/Other Class: Chlorinated hydrocarbon
- Chemical Structure:



Uses

1,2-Dichloroethane is utilized as a solvent, pesticide, fumigant, gasoline additive, and in the synthesis of vinyl chloride. 1,1-Dichloroethane is utilized in relatively small quantities, primarily in the chemical, agricultural, and petroleum industries. In the past, 1,1-dichloroethane was used as an anesthetic.

Exposure Routes and Pathways

Inhalation through contaminated air and ingestion of contaminated water are common routes of exposure in humans.

Toxicokinetics

Dichloroethanes are readily absorbed through the lungs following inhalation exposure in both humans and experimental animals. Absorption after oral ingestion in experimental animals is rapid, complete, and essentially linear. Studies in animals have shown that dichloroethanes are well absorbed through the skin following dermal exposure. Dichloroethanes are metabolized to a variety of chlorinated metabolites, some of which (e.g., chloroacetaldehyde and acetyl chloride) are reactive species and are more toxic than the parent compounds. The relatively greater rate of metabolism of 1,1-dichloroethane relative to 1,2-dichloroethane is not consistent with the relatively higher toxicity, mutagenicity, and carcinogenicity of 1,2-dichloroethane. Therefore, it is possible that there is alternative route of metabolism. Dichloroethanes appear to be rapidly distributed in humans. In rats, dichloroethanes are readily distributed throughout body tissue after inhalation or oral ingestion. The highest concentrations were found in fat. Following inhalation exposure in rats, elimination occurred primarily via the excretion of soluble metabolites and unchanged parent compound in urine and carbon dioxide in the expired breath. Urinary metabolites accounted for 84% of the absorbed dose, fecal accounted for 2%, and carbon dioxide accounted for $\sim$7%. Following oral exposure, urinary metabolites accounted for 60%, unchanged in the breath accounted for 29%, and carbon dioxide in breath accounted for 5% of the administered 150 mg kg^{-1} dose.

Mechanism of Toxicity

The mechanism of action of dichloroethane-induced toxicity is not fully elucidated. By most criteria, 1,1-dichloroethane is less toxic than 1,2-dichloroethane. Studies of the possible mutagenicity and carcinogenicity of 1,1-dichloroethane have been negative or inconclusive. In contrast, 1,2-dichloroethane is carcinogenic in rats and mice and mutagenic in several test systems, particularly in the presence of activating enzymes, such as hepatic glutathione transferases and to a lesser extent by hepatic microsomal cytochrome P450 enzymes.

Acute and Short-Term Toxicity (or Exposure)

Animal

An acute oral LD_{50} of 680 mg kg^{-1} has been reported for rats. In mice the reported LD_{50} values for male and female were 489 and 413 mg kg^{-1}, respectively. No adverse clinical effects were noted in rats, rabbits, or guinea pigs exposed to 1000 ppm 1,1-dichloroethane for 13 weeks, which followed a prior 13-week exposure to 500 ppm. However, under the same conditions, renal injury was apparent in cats. Short-term animal studies indicate that the liver and kidneys are the principal target organs of 1,2-dichloromethane. Lowest reported effect levels for ingestion and inhalation were 49–82 mg kg^{-1} body weight per day (increases in liver weight in rats exposed for 13 weeks) and 202 mg m^{-3} (effects on liver and kidney function in rats exposed for 12 months), respectively. According to available evidence, 1,2-dichloromethane does not adversely affect the reproductive or development process in animals except at maternally toxic levels. Results of *in vivo* studies in rats, mice, and insects were consistently positive for genotoxic activity.

Human

Very limited human toxicity data are available for dichloroethanes. Symptoms observed were central nervous system depression, corneal opacity, bronchitis, respiratory distress, myocardial lesions, hemorrhagic gastritis and colitis, increased blood clotting time, hepatocellular damage, renal necrosis, and histopathological changes in brain tissue. Death was most often attributed to cardiac arrhythmia. In the past, 1,1-dichloroethane was used as an anesthetic at levels of $\sim$25 000 ppm. This use was discontinued when it was discovered that cardiac arrhythmias might be induced. In persons with impaired pulmonary function, especially those with obstructive airway diseases, the breathing of dichloroethane might cause exacerbation of symptoms to its irritant properties.

Chronic Toxicity (or Exposure)

Animal

In chronic toxicity studies, liver and kidneys were the principal target organs. Exposure to 1,2-dichloroethane by gavage for 78 weeks induced a significant increase in the incidence of tumors at several sites in both rats and mice. Inhalation exposure of rats or mice did not show significant increases in tumor incidence. However, repeated dermal or intraperitoneal application of 1,2-dichloroethane resulted in an increase in lung tumors in mice.

Human

No information is available on the chronic (long-term) effects of 1,1-dichloroethane in humans. Epidemiological studies regarding the carcinogenic effects of 1,2-dichloroethane are not conclusive, due to concomitant exposure to other chemicals.

In Vitro Toxicity Data

In several *in vitro* assays in prokaryotes, fungi, and mammalian (including human) cells, 1,2-dichloroethane has been consistently genotoxic.

Clinical Management

Respiratory and cardiovascular function should be supported, and the victim should be moved to fresh air and given artificial respiration; if breathing is difficult, oxygen should be given. In case of contact with material, the eyes should be flushed immediately with running water for at least 15 min; the skin should be washed with soap and water. Contaminated clothing and shoes should be removed, and isolated at the site. In case of oral exposure, emesis should not be induced. A charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, should be administered.

Environmental Fate

1,2-Dichloroethane released to the environment partitions to the atmosphere. In the atmosphere, reaction with photochemically produced hydroxyl radicals is the primary degradation mechanism of 1,2-dichloroethane. 1,2-Dichloroethane released to soil or water surfaces is expected to volatilize quickly. Biodegradation occurs slowly in water and soil surfaces. 1,2-Dichloroethane is not expected to undergo hydrolysis and photolysis.

Exposure Standards and Guidelines

The US Occupational Safety and Health Administration regulatory level in workplace air is 1 ppm for an 8 h day, 40 h week. US Environmental Protection Agency has set a limit in water of 0.005 mg l^{-1}. The US National Institute for Occupational Safety and Health recommends that it would be prudent to handle 1,2-dichloroethane in the workplace as if it was a human carcinogen.

See also: Gasoline; Pesticides; Vinyl Chloride.

Further Reading

Agency for Toxic Substances and Disease Registry (ATSDR) (1993) *Toxicological Profile for 1,2-Dichloroethane* (Draft). Atlanta, GA: ATSDR, Public Health Service, US Department of Health and Human Services.

Anonymous (1998) 1,2-Dichloroethane. Concise International Chemical Assessment Document (CICAD) vol. 1, pp. 1–21.

Anonymous (1999) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Human, vol. 71(2), pp. 501–529.

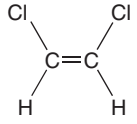
US EPA (Environmental Protection Agency) (1986) *Health Effects Assessment for 1,2-Dichloroethane*. EPA/540/1-86/002. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, Cincinnati, OH.

Dichloroethylene, 1,2-

Sachin S Devi and Harihara M Mehendale

This article is a revision of the previous print edition article by David M Krentz and Linda Angevine Malley, volume 1, pp. 465–467, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 540-59-0 (*sym*); CAS 156-59-2 (*cis*); CAS 156-60-5 (*trans*)
- Synonyms: Ethene, 1,2-dichloro-; Ethylene, 1,2-dichloro-; 1,2-Dichloroethene; Acetylene dichloride; Dioform; *sym*-Dichloroethylene; 1,2-DCE
- Chemical/Pharmaceutical/Other Class: Organic compounds
- Chemical Formula: $C_2H_2Cl_2$
- Chemical Structure:



Uses

1,2-Dichloroethylene is used as a direct solvent for perfumes, dyes, gums and waxes, oils, fats, lacquers, thermoplastics, phenols, and camphor; as a chemical intermediate for chlorinated compounds; and as an agent in retarding fermentation. 1,2-Dichloroethylene is also used as a low-temperature solvent for heat-sensitive substances in the extraction of caffeine, fats, and natural rubber, as well as in organic synthesis for polymers and telomers and as a coolant in refrigeration plants. Miscellaneous applications include use as a dry cleaning solvent, cleaning solution for printed circuit boards, and use in food packaging, adhesives, and germicidal fumigants.

Background Information

1,2-Dichloroethylene is a colorless, volatile liquid with an ether-like, slightly acrid odor. The commercial product is available as either the *cis* or *trans*

isomer or a mixture of the two isomers. The *trans* isomer has an odor threshold concentration of 17 ppm of air.

Exposure Routes and Pathways

- Breathing 1,2-dichloroethylene that has leaked from hazardous waste sites and landfills.
- Drinking contaminated tap water or breathing vapors from contaminated water while cooking, bathing, or washing dishes.
- Breathing 1,2-dichloroethylene, touching it, or touching contaminated materials in the workplace.

Toxicokinetics

1,2-Dichloroethylene is largely excreted through the lungs. In isolated perfused rat liver systems, *cis* and *trans* isomers are metabolized to the same metabolites, dichloroacetic acid and dichloroethanol. In this system, the *cis* isomer is metabolized to a greater extent than the *trans* isomer. The metabolites are formed by an epoxide intermediate. Studies with rat liver microsomes exposed to 1,2-dichloroethylene cause a fall in microsomal cytochrome P450 content without affecting other microsomal enzymes. The decrease in cytochrome P450 only occurred in the presence of reduced nicotinamide adenine dinucleotide, suggesting that the chloroethylene must be converted to a metabolite that exerts its destructive action. The loss of P450 was attributed to the destruction of heme since the fall in cytochrome P450 was always accompanied by parallel decrease in microsomal heme content.

Mechanism of Toxicity

The acute narcotic effects are due to the physical interaction of the material itself on the cells of the central nervous system (CNS). The long-term effects are most likely due to the production of an unstable reactive intermediate during biotransformation.

Acute and Short-Term Toxicity (or Exposure)

Animal

1,2-Dichloroethylene vapor is a CNS depressant and a mild irritant of the mucous membranes. The acute oral LD_{50} for a 60:40 *cis–trans* mixture in rats is reported as greater than 2000 mg kg^{-1}. Inhalation exposure to 16 000 ppm for 4 h was lethal to rats, but 8-min exposures to the same concentration produced anesthesia.

Human

The major effect of 1,2-dichloroethylene is narcosis; it has been used in a combination with ether (Dichloren) as an anesthetic in at least 2000 cases. No evidence of eye toxicity was seen in these cases. In high concentrations, exposure to 1,2-dichloroethylene causes CNS depression; in milder exposures, it can produce nausea, vomiting, weakness, tremor, epigastric cramps, burning of the eyes and vertigo. One fatality has been reported that was due to inhalation of a very high vapor concentration in a small enclosure. Exposure to the vapor of dichloroethylene may cause burning of the eyes. Other symptoms of acute exposure are nausea, vomiting, and epigastric distress. Symptoms of exposure-related narcosis including drowsiness, tremor, incoordination, dizziness, and weakness; these symptoms clear quickly after exposure is terminated.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure studies have shown that repeated inhalation of up to 1000 ppm 1,2-dichloroethylene resulted in no identified ill effects in rats, rabbits, guinea pigs, and dogs. Dogs narcotized by inhaling 1,2-dichloroethylene vapor developed superficial corneal turbidity that cleared within 48 h and did not disturb vision.

Human

1,2-Dichloroethylene is a defatting agent, and repeated skin exposure may cause irritation and dermatitis. No cancer bioassays or epidemiological studies were available to assess the carcinogenicity of 1,2-dichloroethylene. US Environmental Protection Agency has placed *cis*-1,2-dichloroethene in weight-of-evidence group D, not classifiable as to human carcinogenicity, based on the lack of or negative human or animal cancer data. *trans*-1,2-Dichloroethylene has not undergone a complete evaluation and determination under US EPA's IRIS program for evidence of human carcinogenic potential.

Clinical Management

Persons exposed to 1,2-dichloroethylene should have their vital signs closely monitored; the heart should be monitored by EKG. Epinephrine and other catecholamines should be avoided, especially beta agonists since they may increase the risk of arrhythmias.

Pulmonary edema, renal failure, and liver injury should be managed symptomatically. However, based on toxicity similarities to trichloroethylene, data on plasma levels of following 1,2-dichloroethylene overdose/exposure are not clinically very useful. Renal and liver function tests should be monitored in the presence of suspected kidney or liver injury.

Environmental Fate

cis-1,2-Dichloroethylene may be released to the environment in emissions and wastewater during its production and use. Under anaerobic conditions that may exist in landfills or sediment, one is likely to find 1,2-dichloroethylenes that are formed as breakdown products from the reductive dehalogenation of trichloroethylene and tetrachloroethylene. The *cis*-1,2-dichloroethylene is apparently the more common isomer found although it is mistakenly listed as the *trans* isomer. The *trans* isomer, being a priority pollutant, is more commonly analyzed for and the analytical procedures generally used do not distinguish the isomers. If *cis*-1,2-dichloroethylene is released on soil, it should evaporate and/or leach into the groundwater where very slow biodegradation should occur. If released into water, *cis*-1,2-dichloroethylene will be lost mainly through volatilization (half-life 3 h in a model river). Biodegradation, adsorption to sediment, and bioconcentration in aquatic organisms should not be significant. In the atmosphere *cis*-1,2-dichloroethylene will be lost by reaction with photochemically produced hydroxyl radicals (half-life 8 days) and scavenged by rain. Because it is relatively long lived in the atmosphere, considerable dispersal from source areas should occur. The general population is exposed to *cis*-1,2-dichloroethylene in urban air as well as in contaminated drinking water from ground water sources. Occupational exposure will be via dermal contact with the vapor and liquid or via inhalation. 1,2-Dichloroethene evaporates rapidly into air.

Exposure Standards and Guidelines

Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL): The current OSHAPEL for 1,2-dichloroethylene is 200 ppm (790 mg m^{-3} as an 8 h time-weighted average (TWA) concentration.

National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL): The NIOSH has established a REL for 1,2-dichloroethylene of 200 ppm (790 mg m^{-3}) as a TWA for up to a 10 h workday and a 40 h workweek.

American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV): The ACGIH has assigned 1,2-dichloroethylene a TLV of 200 ppm (793 mg m^{-3}) as a TWA for a normal 8 h workday and a 40 h workweek.

Rationale for Limits: The NIOSH limit is based on the risk of narcotic effects and mucous membrane irritation. The ACGIH limit is based on the no-effect level of 1000 ppm in animals.

See also: Catecholamines; Cytochrome P-450.

Further Reading

Barnes DW, Sanders VM, White KL Jr., Shopp GM Jr., and Munson AE (1985) Toxicology of *trans*-1,2-dichloroethylene in the mouse. *Drug and Chemical Toxicology* 8: 373–392.

Clayton G and Clayton F (1982) *Patty's Industrial Hygiene and Toxicology*, 3rd edn. New York: Wiley.

Costa AK and Ivanetich KM (1982) The 1,2-dichloroethylenes: Their metabolism by hepatic cytochrome P-450 *in vitro*. *Biochemical Pharmacology* 31: 2093–2102.

Grant WM (1986) *Toxicology of the Eye*, 3rd edn. Springfield, IL: Charles C Thomas.

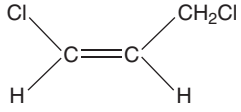
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dichloroethene, 1,2-.

Dichloropropene, 1,3-

Jing Liu

- Chemical Abstracts Service Registry Number: CAS 542-75-6
- Synonyms: 1,3-Dichloro-1-propene; 1,3-Dichloropropylene; Dorlone; Nemex; Telone; Vidden D
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbon nematocide
- Chemical Formula: $C_3H_4Cl_2$
- Chemical Structure:



Uses

1,3-Dichloropropene is used as a preplant soil fumigant for the control of nematodes.

Exposure Routes and Pathways

Most exposures occur through inhalation due to application techniques and to its volatility. Dermal, ocular, and oral exposures are also possible.

Toxicokinetics

1,3-Dichloropropene can be rapidly absorbed through the skin and via the respiratory and gastrointestinal tracts. Blood levels of the glutathione-conjugate of 1,3-dichlororpropene reached steady state within 15 min after oral exposure in rats, indicating rapid absorption. The presence of *N*-acetyl-cysteine conjugates in the recovered urine of field applicators suggested the chemical is readily absorbed via inhalation. 1,3-Dichloropropene has an elimination half-life of less than 10 min from the bloodstream. Urinary elimination half-lives ranged from 5 to 6 h in rats, from 7 to 10 h in mice, and an average of 9.5 h in humans. Dichloropropene primarily undergoes conjugation with glutathione to form a mercapturic acid and oxidation to carbon dioxide. An additional route of metabolism reported in mice involves stereospecific epoxidation to the corresponding 1,3-dichloropropene oxide. The predominant route of excretion is via the urine ($\sim$50–80% in rat and mouse, respectively) as mercapturic acid conjugates. Lesser amounts are eliminated through feces.

Mechanism of Toxicity

Although there is substantial documentation regarding health effects of 1,3-dichloropropene, little data concerning mechanisms of toxicity are available. 1,3-Dichloropropene acts as an irritant and sensitizer. Macromolecular binding of this compound may also contribute to its toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of 1,3-dichloropropene is about 470 mg kg^{-1} in rats and 640 mg kg^{-1} in mice. The dermal LD_{50} in rabbits is 504 mg kg^{-1}. Inhalation LC_{50} in mice is 4650 mg m^{-3} every 2 h.

Lung/tracheal congestion, fluid in the pleural cavity, atelectasis, emphysema, and/or pulmonary edema and lung hemorrhaging were observed in rats exposed to 1,3-dichloropropene through inhalation. Rabbits exhibited stomach and intestinal hemorrhage after dermal exposure. Ocular or dermal exposure may cause conjunctival and corneal irritation, erythema, edema, necrosis, and subcutaneous or skeletal muscle hemorrhage in rats, rabbits, and guinea pigs. 1,3-Dichloropropene was not embryotoxic or teratogenic in inbred rats or rabbits at doses that produced maternal toxicity.

Human

1,3-Dichloropropene has moderate acute toxicity. The health effects of 1,3-dichloropropene may involve many organ systems including liver, kidney, lung, gastrointestinal tract, and mucous membranes. Vapors of 1,3-dichloropropene are irritating to the eyes and respiratory tract, possibly causing delayed pulmonary edema. Individuals may experience eye, nose, and throat irritation, nausea, vomiting, headache, and chest discomfort. Contact dermatitis has been reported in farmers exposed to the compound.

Chronic Toxicity (or Exposure)

Animal

Both male and female dogs ingesting 1,3-dichloropropene (15 mg kg^{-1} day^{-1}) for either 13 weeks or 1 year exhibited primarily regenerative hypochromic, microcytic anemia. Chronic exposure via the oral route has also caused neoplastic and preneoplastic lesions of the stomach in rats. Hyperplasia and hyperkeratosis of the forestomach and urinary bladder hyperplasia were reported in mice exposed to one formulation of 1,3-dichloropropene (Telone IIb) for 2 years through inhalation.

Human

Chronic dermal exposure may cause skin sensitization. 1,3-Dichloropropene has been classified in Group B2 as a possible human carcinogen through both inhalation and oral exposures.

In Vitro Toxicity Data

Using isolated rat hepatocytes, 1,3-dichloropropene was shown to be cytotoxic as measured by increases in phospholipid hydroperoxides and lactate dehydrogenase. 1,3-Dichloropropene also exhibited nephrotoxicity *in vitro* using rat renal cortical slices, where *p*-aminohippurate uptake was decreased. Genotoxicity of 1,3-dichloropropene was observed as increases in sister-chromatid exchange in human lymphocytes *in vitro*.

Clinical Management

Treatment is symptomatic and supportive.

Environmental Fate

1,3-Dichloropropene is mobile and persistent, particularly in colder climates. When injected into the soil, its mobility is controlled by temperature, soil type, and moisture. Even though volatilization occurs from the soil surface, most 1,3-dichloropropene is degraded through hydrolysis to 3-dichloroallyl alcohol. The overall half-life of the compound in soil ranges from a few days to more than 9 weeks depending on conditions. Adsorption to sediment and bioconcentration in fish are not important processes.

Ecotoxicology

1,3-Dichloropropene is highly toxic to invertebrates and moderately toxic to birds, mammals, and fish.

Exposure Standards and Guidelines

The National Institute for Occupational Safety and Health and American Conference of Governmental Industrial Hygienists threshold limit value – time-weighted average for 1,3-dichloropropene is 1 ppm ($5\,mg\,m^{-3}$).

The reference concentration (RfC) established by the Environmental Protection Agency (EPA) is $0.02\,mg\,m^{-3}$ based on hypertrophy/hyperplasia of nasal respiratory epithelium in mice.

The chronic reference dose set by the EPA is $0.0003\,mg\,kg^{-1}\,day^{-1}$ (oral).

See also: Pollution, Water.

Further Reading

Bartels MJ, Brzak KA, Mendrala AL, and Stott WT (2000) Mechanistic aspects of the metabolism of 1,3-dichloropropene in rats and mice. *Chemical Research in Toxicology* 13(11): 1096–1102.

Bartels MJ, Hansen SC, Thornton CM, *et al.* (2004) Pharmacokinetics and metabolism of 14C-1,3-dichloropropene in the fischer rat and the B6C3F1 mouse. *Xenobiotica* 34(2): 193–213.

Kevekordes S, Gebel T, Pav K, Edenharder R, and Dunkelberg H (1996) Genotoxicity of selected pesticides in the mouse bone-marrow micronucleus test and in the sister-chromatid exchange test with human lymphocytes *in vitro*. *Toxicological Letters* 89(1): 35–42.

Schneider M, Quistad GB, and Casida JE (1998) 1,3-Dichloropropene epoxides: intermediates in bioactivation of the promutagen 1,3-dichloropropene. *Chemical Research in Toxicology* 11(10): 1137–1144.

Secondin L, Maso S, and Trevisan A (1999) Different effects of (*cis* + *trans*) 1,3-dichloropropene in renal cortical slices derived from male and female rats. *Human & Experimental Toxicology* 18(2): 106–110.

Stebbins KE, Quast JF, Haut KT, and Stott WT (1999) Subchronic and chronic toxicity of ingested 1,3-dichloropropene in dogs. *Regulatory Toxicology & Pharmacology* 30(3): 233–243.

Relevant Websites

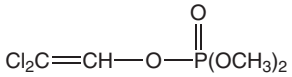
http://ehp.niehs.nih.gov – National Institute of Environmental Health Sciences.

http://www.epa.gov – US Environmental Protection Agency.

Dichlorvos

Nikita Mirajkar and Carey N Pope

- Synonyms: DDVF; DDVP; Canogard; Dedevap; Estrosol; Herkol; Vapona; Apavap; Benfos; Fly-Bate; Fly-Die; Fly-Fighter
- Chemical/Pharmaceutical/Other Class: Synthetic dimethoxy organophosphorus insecticide
- Chemical Structure:



Uses

Because of its high vapor pressure, dichlorvos is useful in the control of insects in closed spaces (e.g., warehouses, greenhouses, animal shelters, homes, and restaurants). It is available in oil solutions, emulsifiable concentrates, aerosols, and baits. Therapeutically, dichlorvos is used as a broad-spectrum anthelmintic (for destroying or expelling intestinal worms). It is also used as a feed through larvicide to control botfly larvae in the manure. It is primarily used for insect control. Dichlorvos is also a breakdown product of the organophosphorus pesticide trichlorfon (metrifonate).

Exposure Routes and Pathways

Exposure to dichlorvos vapor can result in exposure through not only the respiratory route but also the dermal and oral routes (e.g., through contamination of feed).

Toxicokinetics

Animals exposed to dichlorvos vapor were found to absorb at least 50% of the total material by the respiratory route. Dichlorvos can also be absorbed through the oral and dermal routes. Following oral exposure, dichlorvos is rapidly detoxified in the liver. Metabolites include *O*,*O*′-dimethyl phosphate, monomethyl phosphate, *O*-methyl-*O*-2,2-dichlorovinyl phosphate (desmethyl dichlorvos), and inorganic phosphate. Detoxification processes for dichlorvos are also found in plasma.

Under most conditions, dichlorvos is not detectable in any tissues. Dichlorvos is not stored in tissues, it does not accumulate in secretions (e.g., milk), and it is below detection levels in the blood of various species at exposure levels in excess of 10 times those effective for insect control. At exceptionally high concentrations ($90\,mg\,m^{-3}$ or about 2000 times normal exposure levels), dichlorvos was detectable in various tissues of the rat.

Dichlorvos is rapidly metabolized and excreted by mammals following any route of exposure.

Desmethyldichlorvos and dimethylphosphate are rapidly excreted or further metabolized. The glucuronide conjugate of dichloroethanol is excreted in the feces. Species differences in elimination are common. For example, following oral administration, the cow eliminates $\sim$50% through the feces, whereas the rat excretes only about 3% via the feces.

Mechanism of Toxicity

The toxicity of dichlorvos is due to inhibition of acetylcholinesterase and the signs of toxicity are generally similar to those caused by other organophosphorus insecticides. Dichlorvos is a direct inhibitor of cholinesterases; thus, toxicity rapidly follows exposure and recovery is also rapid. With inhalation exposures, airway acetylcholinesterase inhibition is possible in the absence of significant blood enzyme inhibition. The fly head acetylcholinesterase appears more sensitive to inhibition by dichlorvos relative to mammalian brain acetylcholinesterase. At high doses, dichlorvos may cause hyperglycemia and abnormal glucose tolerance.

In Vitro Toxicity Data

Dichlorvos has been shown to be positive in the Ames assay and in other bacterial and mammalian cell mutagenesis assays.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dichlorvos is of moderate acute toxicity, with an oral LD_{50} value in rodents from 50 to $150\,mg\,kg^{-1}$. While the LC_{50} for inhibiting mammalian brain acetylcholinesterase is similar between dichlorvos and paraoxon (i.e., the active metabolite of parathion), the acute LD_{50} values for these agents are considerably different, due in part to the more rapid metabolism and elimination of dichlorvos.

In rabbits, at acute doses, dichlorvos was found to be a mild skin and eye irritant.

Human

Inhalation, dermal, or oral exposure to dichlorvos can result in systemic toxicity through inhibition of acetylcholinesterase. Symptoms of acute exposure to dichlorvos may include blurred vision, nausea, headache, and shortness of breath.

Increased risk from exposure to dichlorvos will occur in persons who have reduced lung function, convulsive disorders, liver disorders, or recent exposure to cholinesterase inhibitors.

Chronic Toxicity (or Exposure)

Human

Symptoms of chronic exposure to dichlorvos are similar to those of acute exposure, in addition to which there could be tension, insomnia, loss of appetite, apathy, trembling, and confusion.

Experimental studies suggest that relatively high exposures may produce delayed neurotoxicity, whereas the dibutyl analog is capable of inducing delayed neurotoxicity at relatively low doses.

In humans, the plasma cholinesterase appears more sensitive than erythrocyte cholinesterase to inhibition by dichlorvos; thus, discrimination between these two activities may be warranted during assessment of exposures.

Dichlorvos is classified as a mutagen and a possible human carcinogen, based on the results of animal studies. It is, however, not classified as a teratogen. It has been shown that dichlorvos may affect the immune system.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered followed by 2 and 4 mg (in adults) or 0.015 and 0.05 mg kg^{-1} (in children) every 10 and 15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Environmental Fate

In soil, dichlorvos is generally not active, and has low persistence, with a half-life of 7 days.

In general, it is not absorbed from the soil by plants. Dichlorvos undergoes hydrolysis and biodegradation. Soil pH influences dichlorvos degradation, with more rapid breakdown under alkaline conditions.

Dichlorvos adsorbs very poorly to soil particles and is soluble in water, in which it degrades primarily by hydrolysis, with a half-life of ~4 days in lakes and rivers.

Ecotoxicology

Dichlorvos is only slightly toxic to plants. Dichlorvos is moderately toxic to fish and highly toxic to aquatic invertebrates. However, in the presence of UV light, toxicity of dichlorvos to aquatic organisms can be increased 5–150-fold. It does not bioaccumulate in fish. The toxicity of dichlorvos to birds and mammals ranges from low to moderate. Dichlorvos is highly toxic to bees. Dichlorvos can be hazardous to endangered species.

Exposure Standards and Guidelines

- Reference dose is 0.0005 mg kg^{-1} day^{-1}.
- Accepted daily intake is 0.001 mg kg^{-1} day^{-1}.
- Threshold limit value is 0.9 mg m^{-3}.

See also: Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates; Pesticides.

Further Reading

Mennear JH (1998) Dichlorvos: A regulatory conundrum. *Regulatory Toxicology and Pharmacology* 27: 265–272.

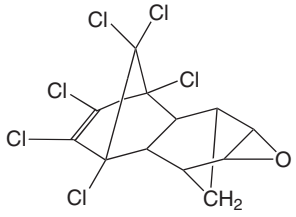
Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dichlorvos.
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Dieldrin

Benny L Blaylock

- Chemical Abstracts Service Registry Number: CAS 60-57-1
- Synonyms: 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene; Alvit; Dieldrix; Octalox; Quintox; Red Shield
- Chemical/Pharmaceutical/Other Class: Organochlorine insecticide
- Chemical Formula: $C_{12}H_8Cl_6O$
- Chemical Structure:



Uses

Dieldrin is used as an insecticide and its use has been significantly restricted in the United States and several other countries. There are currently no Environmental Protection Agency registrations for dieldrin.

Exposure Routes and Pathways

The most important exposure routes for dieldrin are oral and dermal.

Toxicokinetics

Dieldrin is readily adsorbed for the gastrointestinal tract, the respiratory tract, and through the skin. In mammals, two major metabolism routes of dieldrin seem to be predominant: (1) direct oxidation by cytochrome oxidases, resulting in 9-hydroxydieldrin; and (2) the opening of the epoxide ring by epoxide hydrases, resulting in 6,7-*trans*-dihydroxydihydroaldrin. Dieldrin is hydroxylated to 9-hydroxydieldrin by liver microsomal monooxygenases in rats. Metabolism of dieldrin is three to four times more rapid in male than in female rats. Excretion in humans is primarily in the feces via the bile. Dieldrin is also excreted via lactation in nursing mothers.

Mechanism of Toxicity

Dieldrin characteristically stimulates the central nervous system (CNS) causing hyperexcitation and generalized seizures. Both *in vitro* experiments using rat brain membranes and intravenous or intraperitoneal administration of aldrin and dieldrin to rats have shown that these agents are capable of blocking the activity of GABA by blocking the influx of chloride through the $GABA_A$ receptor–ionophore complex.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for rats is $46\,mg\,kg^{-1}$. Convulsions are the principle CNS effect. In birds, acute symptoms include tail feathers spread and pointed either upward or downward, hyperexcitability, jerkiness in gait, ataxia, dyspnea, myasthenia, fluffed feathers, immobility, terminal wing-beat convulsions, or opisthotonos. Mortalities usually occurred 1–9 days following treatment.

Human

CNS excitation culminating in convulsions was the principal toxic effect noted in occupational studies of workers employed in either the manufacture or application of aldrin or dieldrin. Patients exposed to dieldrin may also experience other symptoms including headaches, dizziness, hyperirritability, general malaise, nausea and vomiting, anorexia, muscle twitching, and myoclonic jerking. Dieldrin is not classifiable as to its carcinogenicity in humans by International Agency for Research on Cancer.

Chronic Toxicity (or Exposure)

Animal

In addition to its CNS effects, dieldrin increases hepatocarcinogenesis with chronic exposure. Dieldrin has been shown to suppress macrophage function and T-dependent humoral immune functions. Reproductive effects in rats were observed when pregnant females were dosed with $1.0\,mg\,kg^{-1}$ aldrin subcutaneously. A significant but slight decrease in fertility was observed in female mice exposed to 1.3 or $1.95\,mg\,kg^{-1}\,day^{-1}$ of dieldrin from 4 weeks prior to mating through weaning.

Human

Dieldrin has caused numerous cases of chronic poisoning to workers who have sprayed the compound for several months. Characteristically there is headache, dizziness, and involuntary muscular movements. In severe cases there are epileptic convulsions with loss of consciousness. The only ocular disturbance so far noted in human beings has been blurred vision of undetermined cause, and nystagmus accompanying incoordination and tremor. In a study of five male farm workers exposed to a mixture of herbicides and pesticides including dieldrin, four were found to have suffered impotence after chronic exposure; sexual function recovered after termination of exposure.

Clinical Management

Treatment is symptomatic. Activated charcoal as a slurry has been reported to absorb aldrin and increase its rate of excretion after oral exposure. Emesis is not recommended due to potential CNS depression or seizures. Diazepam or phenobarbital is used when anticonvulsant therapy is necessary.

Environmental Fate

In temperate soil, the half-life of dieldrin is ~ 5 years. Most dieldrin and aldrin found in surface water are the result of runoff from contaminated soil. With this level of persistence, combined with high lipid solubility, the necessary conditions for dieldrin to bioconcentrate and

biomagnify in organisms are provided. Bioconcentration factors of 12 500 and 13 300 have been reported for guppies and sculpins, respectively. It is likely that dieldrin is bioconcentrated by aquatic organisms rather than bioaccumulated. Dieldrin exhibits low water solubility, high stability, and semivolatility. These characteristics favor its long-range transport. In the air, dieldrin is degraded by ultraviolet light to the more persistent photodieldrin within a few days.

Ecological Effects

Dieldrin has low phytotoxicity. Plants are affected only by application rates much higher than suggested use rates. The acute toxicity of dieldrin is quite variable for aquatic invertebrates, with insects being the most sensitive group (values range from 0.2 to 40 $\mu g l^{-1}$). It is highly toxic to most species of fish tested in the laboratory (values range from 1.1 to 41 $\mu g l^{-1}$). In frogs, the 96 h LC_{50} of dieldrin ranged from 8.7 $\mu g l^{-1}$ for *Rana catesbeiana* tadpoles to 71.3 $\mu g l^{-1}$ for the tadpoles of *Rana pipiens*. Spinal deformities in embryo–larval tests were observed at concentrations as low as 1.3 $\mu g l^{-1}$ for *Xenopus laevis* after a 10 day exposure.

There is significant variation in the acute toxicity of dieldrin to avian species. Pigeons have an acute oral LD_{50} values in the range of 26.6 $mg kg^{-1}$ while, in mallard ducks, the acute oral LD_{50} is 381 $mg kg^{-1}$. Mallard ducklings were exposed to dieldrin in the diet for 24 days. A 24 day no-observed-adverse-effect level of 0.3 µg dieldrin per gram diet, based on growth impairment, was determined. Reproduction success in birds has not been consistently affected in the absence of maternal toxicity.

Other Hazards

Dieldrin is a noncombustible substance and does not burn but may decompose upon heating to produce corrosive and/or toxic fumes. It is not compatible with strong oxidizers, active metals such as sodium, strong acids, or phenols.

Exposure Standards and Guidelines

- Acceptable daily intake is 0.0001 $mg kg^{-1} day^{-1}$.
- Reference dose is 0.005 $mg kg^{-1} day^{-1}$.
- Permissible exposure limit is 0.25 $mg m^{-3}$ (8 h).

See also: Aldrin; Diazepam; Organochlorine Insecticides.

Further Reading

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Swaen GM, de Jong G, Slangen JJ, and van Amelsvoort LG (2002) Cancer mortality in works exposed to dieldrin and aldrin: An update. *Toxicology and Industrial Health* 18(2): 63–70.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dieldrin.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Dieldrin.

http://www.osha-slc.gov – US Department of Labor, Occupational Safety and Health Administration.

Diesel Exhaust

Kathryn A Wurzel

- SYNONYMS: Diesel engine emissions; Diesel exhaust particulate; Diesel particulate

Description

Diesel exhaust is a complex mixture of hundreds of constituents in either a gas or particle form. Gaseous components of diesel exhaust include carbon dioxide, oxygen, nitrogen, water vapor, carbon mono xide, nitrogen compounds, sulfur compounds, and numerous low-molecular-weight hydrocarbons. Among the gaseous hydrocarbon components of diesel exhaust that are individually known to be of toxicologic relevance are the aldehydes (e.g., formaldehyde, acetaldehyde, acrolein), benzene, 1,3-butadiene, and polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs. Diesel engines are used to power heavy machinery, locomotives, ships, buses, heavy-duty trucks, and some light-duty trucks and passenger cars.

Exposure Routes and Pathways

Inhalation is the primary route of exposure to diesel exhaust. Incidental ingestion following deposition on soil or vegetation is also a possible route of exposure.

Toxicokinetics

Clearance of diesel particles from the alveolar region of the lung (area of gas exchange) varies from ~2 months in rats to almost 1 year in humans. The slower particle clearance rates in humans may result in greater extraction of organics. High-exposure concentrations reduce the lung clearance in animals, further increasing the lung burden. Biological fluids are relatively ineffective in extracting organics adsorbed to diesel particle surfaces. Phagocytosis by macrophages is much more effective in extracting organics. A fraction of organics was eluted in this manner within hours with the more tightly bound fraction removed with a half-life of ~1 month. Elution rates are generally more rapid than particle clearance rates so most of the organic fraction is assumed to be bioavailable even with no clearance inhibition.

Mechanism of Toxicity

Comparison of toxic responses in animals exposed to whole diesel exhaust or filtered diesel exhaust indicates that the principal etiologic agent of noncancerous health effects in animals is diesel particulate. Animal experiments provide strong support for the premise that diesel exhaust toxicity results from a mechanism that is analogous to that of other relatively inert particles in the lung. Tumor induction at high doses may be primarily the result of lung particle overload with associated inflammatory responses. Although tumorigenic responses could not be detected under nonparticle-overload conditions, the animal experiments lack sensitivity to determine if a threshold exists. Some studies do support the existence of a threshold if inflammation is assumed to be a prerequisite for lung tumor induction. Most of the carcinogenicity appears to be associated with the portion containing PAHs with four to seven rings. Carcinogenic effects may be a result of the formation of covalent adducts with DNA and subsequent alteration of cellular genetic information. Another proposed mechanism is based on the carcinogenic potential of the particle itself. The particle may induce increases in DNA adducts in the lungs or induce release of mediators from macrophages, many of which are considered to act via promotion.

Acute and Short-Term Toxicity (or Exposure)

Animal

Studies of responses associated with acute exposure to diesel exhaust have mainly been associated with high concentrations of carbon monoxide, nitrogen dioxide, and aliphatic aldehydes. Short-term and chronic exposure studies indicate that toxic effects are related to high concentrations of particulate matter. Minimal effects on pulmonary function have been observed in short-term testing even though histological and cytological changes were noted in the lung.

Human

Human studies are comprised of both occupational and human experimental exposures consisting of exposure to diesel exhaust in the occupational environment and exposure to diluted diesel exhaust or diesel particulate matter under controlled conditions.

Symptoms of acute exposure of humans to concentrations above ambient environmental concentrations are irritation of mucous membranes, eyes, and respiratory tract evidenced by early induction of an inflammatory response in healthy humans. Chest tightness and wheezing may occur. Neurophysiological effects include headache, nausea, heartburn, vomiting, weakness, and tingling of extremities and light-headedness. Diesel exhaust odor may cause nausea, headaches, and loss of appetite. Exposure may also increase allergic response to known allergens in some individuals and induce or exacerbate asthma. Studies conducted over the work shifts of individuals exposed to diesel exhaust indicate that reversible changes in pulmonary function in humans can occur due to diesel exhaust exposure, although it is not possible to relate these changes to specific exposure levels.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure studies have been performed on rats, mice, guinea pigs, hamsters, cats, and monkeys. Changes were similar to those noted in short-term exposure studies (accumulation of particles in the lung, increase in lung weight, increase in macrophages and leukocytes, hyperplasia of alveolar epithelium, and thickening of alveolar septa). Decreased resistance to respiratory tract infections has been noted in mice exposed to diesel exhaust. Limited animal data are available indicating alteration in liver structure and function. The lowest exposure levels resulting in impaired pulmonary function varied by species.

Certain extracts of diesel exhaust have been demonstrated to be both mutagenic and carcinogenic in animals and in humans. Lung tumors were induced in female mice and Fischer 344 rats; however, the dose–response relationship is unclear. Dermal, skin painting, and subcutaneous injection in mice also elicited tumorigenic responses.

Human

Epidemiologic studies of exposure to diesel exhaust and occurrence of lung cancer provide evidence that is consistent with a causal association. Overall, the human evidence for potential carcinogenicity for diesel exhaust is considered to be strong, but less than sufficient for diesel exhaust to be considered as a human carcinogen because of exposure uncertainties (lack of historical exposure data for exposed workers) and an inability to address all potential confounding factors.

See also: Pollution Prevention Act, US; Polycyclic Aromatic Hydrocarbons (PAHs); Respiratory Tract.

Further Reading

Kagawa J (2002) Health effects of diesel exhaust emission – a mixture of air pollutants of worldwide concern. *Toxicology* 27(181–182): 349–353.

United States Environmental Protection Agency (2000), Health Assessment Document for Diesel Exhaust (EPA/600/8-90/057E), July 2000.

Diesel Fuel

Shayne C Gad

- Chemical Abstracts Service Registry Numbers: CAS 68334-30-5 (Diesel oil); CAS 68476-34-6 (Diesel fuel no. 2)
- Synonyms
  - Diesel fuel (general) = auto diesel, automotive diesel oil (ADO), diesel engine road vehicle (DERV), diesel, diesel fuel oil, gas oil
  - Diesel fuel no. 1 = no. 1 diesel, kerosene, arctic diesel, diesel fuel oil no. 1, diesel oil no. 1, dipolar
  - Diesel fuel no. 2 = diesel fuel, diesel fuel oil no. 2, diesel oil no. 2
  - Diesel fuel no. 4 = marine diesel fuel, distillate marine diesel fuel
- Chemical/Phamaceutical/Other Class: Petroleum hydrocarbon mixture of branched-chain alkanes, cycloalkanes, aromatic compounds, and sulfurized esters

Uses

Diesel fuel no. 1 is primarily used in city buses. Diesel fuel no. 2 is used in railcars, trucks, and boats. Diesel fuel no. 4 is used in marine vessels.

Background Information

Diesel oil is a complex mixture produced by the distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C9–C20 and boiling points in the range of ~163–357°C (325–675°F).

Diesel fuel no. 1 is a straight-run middle distillate with a boiling range consistent with that of kerosene. It contains branched-chain alkanes (paraffins), cycloalkanes (naphthenes), aromatics, and mixed aromatic cycloalkanes. The boiling point range of diesel no. 1 largely eliminates the presence of benzene and polycyclic aromatic hydrocarbons (PAHs). Kerosene contains less than 0.02% benzene and low levels of PAHs.

Diesel fuel no. 2 is a blend of straight-run and catalytically cracked streams, including straight-run kerosene, straight-run middle distillate, hydrodesulfurized middle distillate, and light catalytically and thermally cracked distillates. The boiling range is generally ~160–360°C (320–680°F). Diesel fuel no. 2 is similar in composition to fuel oil no. 2. Some of the PAHs contained in fuel oil no. 2, and therefore probably present in diesel fuel no. 2, include phenanthrene, fluoranthene, pyrene, benz(*a*)anthracene, chrysene, and benzo(*a*)pyrene.

Diesel fuel no. 4 is also called marine diesel fuel. It is the most viscous of the diesel fuels and contains higher levels of ash and sulfur. Diesel fuel no. 4 may contain more than 10% PAHs.

Exposure Routes and Pathways

The most common exposure pathway is dermal exposure from handling during transfer, fueling, and repair of diesel-powered vehicles. Although the constituents of diesel are not sufficiently volatile for inhalation of vapors to be an exposure route of concern, inhalation of diesel aerosols can occur. Ingestion of diesel, often associated with aspiration into the lungs, can occur as a result of accidental poisoning or suicide attempts.

Toxicokinetics

Since diesel fuel is a mixture of numerous individual substances, absorption, metabolism, and excretion are very complicated and have not been completely characterized. Systemic effects following dermal and oral exposure and inhalation of diesel aerosols have

been demonstrated, indicating that absorption can occur via all routes of exposure.

The alkanes, cycloalkanes, and aromatic compounds present in diesel are lipophilic and tend to distribute to tissues with higher adipose tissue content. The reversibility and short-term nature of many effects observed during acute exposure indicate that retention of the principal diesel fuel components in body tissues is limited.

The alkanes and cycloalkanes in diesel fuel are generally not readily metabolized, and are mostly excreted unchanged through the lungs, with a very small fraction excreted in the urine. The aromatic constituents of diesel are subject to oxidative metabolism and are typically excreted in the urine as water-soluble metabolites.

Mechanism of Toxicity

The mechanism of action for diesel fuels is not well characterized due to the complexity of its petroleum hydrocarbon mixture. The presence of additives that improve fuel combustion or prevent microbial growth may contribute to toxicity. Based on research conducted with individual components of diesel fuels, the primary mechanism of action for central nervous system (CNS) depression from diesel fuel is the reversible, physical interaction of the aromatic and aliphatic hydrocarbons with cell membranes. Renal toxicity is possibly attributed to oxidative metabolites of some of the aromatic constituents. Eye and skin injury are attributable to direct irritant action and the high lipid solubility that may dissolve protective skin oils and allow penetration into the skin tissue. The dermal carcinogenesis observed in rodents subjected to chronic dermal exposure to diesel may be attributed to the genotoxic activity of PAHs and the promoting activity of repeated dermal injury.

Acute and Short-Term Toxicity (or Exposure)

Animal

The principal toxicities observed in animals acutely or subacutely exposed to diesel are dermal irritation by the dermal route and renal toxicity, liver toxicity, and CNS depression from all routes of exposure. Application of marine diesel fuel to the skin of mice resulted in ulceration and in diesel fuel-induced chromosomal aberrations on bone marrow cells of rats.

Diesel fuel has been demonstrated to have a low toxicity in animals following oral exposure. The LD_{50} in rats ranges from 7.5 to $\sim 9\,g\,kg^{-1}$.

Human

Inhalation or ingestion of diesel fuel resulted in acute and persistent lung damage in humans. Kidney toxicity has been observed in dermally exposed individuals using diesel fuel as a skin degreaser or a shampoo. In a suicide attempt, a woman ingested 1.5 l of diesel fuel and developed toxic lung disease and fever, which was resolved over the next 4 months.

Chronic Toxicity (or Exposure)

Animal

In acute irritation tests in rabbits, diesel fuel was only mildly irritating to the eyes but severely irritating to the skin. Male and female mice dosed dermally with 2000–40 000 $mg\,kg^{-1}$ of marine diesel fuel for 14 consecutive days demonstrated skin lesions and acanthosis, parakeratosis, hyperkeratosis, and inflammatory infiltrates of the dermis. Mice receiving $>20\,000\,mg\,kg^{-1}$ displayed 100% mortality.

No treatment-related mortality was observed in mice administered 250–4000 $mg\,kg^{-1}$ marine diesel fuel by dermal application 5 day $week^{-1}$ for 13 weeks; however, the 4000 $mg\,kg^{-1}$ dose group exhibited chronic dermatitis at the site of application. Rabbits exposed to diesel fuel no. 2 for 24 h day^{-1}, 5 day $week^{-1}$, for 2 weeks at doses of 1 and 4 $ml\,kg^{-1}$ exhibited no mortality and 67% mortality, respectively. All dying animals exhibited signs of chronic dermal irritation, severe anorexia, and depression as the test progressed. Primary causes of death were depression and anorexia attributed to dermal irritation with infection rather than any systemic toxicity, although some liver necrosis was noted.

Rats dosed dermally with 1000 $ml\,kg^{-1}$ diesel fuel per day, 5 day $week^{-1}$, for 2 weeks had demonstrated weight loss, reduced liver weights, serum glucose, serum protein, and serum cholesterol, as well as a reduction in hemoglobin, hematocrit, red cell count, and blood lymphocyte counts. Marine diesel fuel produced lesions in the kidneys of mice treated dermally with 50 µl undiluted fuel three times a week for 60 weeks. Kidney lesions were not observed in a second dermal study in which B6C3F1 mice were treated with up to 500 $ml\,kg^{-1}$ of marine diesel fuel diluted in acetone five times per week for 103 weeks.

Rats exposed to an aerosol of diesel fuel no. 2 at 100 $mg\,ml^{-1}$ demonstrated very mild histological changes in the liver and thyroid. No other biochemical effects, hematological effects, or tissue changes were observed in the exposed animals. Continuous 90 day inhalation exposure to 50 or 300 $mg\,m^{-3}$ of marine diesel fuels produced hyaline droplet nephropathy and reduced body weight gain in male rats.

Pregnant rats exposed to 100 and 400 ppm diesel fuel no. 2 via inhalation on days 6–15 of gestation did not produce offspring with developmental or fetotoxic effects.

Carcinogenicity responses are primarily dependent on the type of diesel fuel applied. Diesel fuel no. 2 did not induce a significant increase in carcinogenesis in Swiss mice when applied at 0.05 ml three times a week for 62 weeks, even in the presence of extreme skin irritation. In another study, diesel fuel no. 2 did not produce tumors by itself; however, it did promote the development of skin tumors initiated by other chemicals. In contrast, marine diesel fuel induced a significant increase in the incidence of squamous cell papillomas and carcinomas when applied to the skin of 49 or 50 male and B6C3F1 mice at doses of 250 and 500 ml kg^{-1}, 5 day $week^{-1}$. The 500 ml kg^{-1} group study was terminated at 84 weeks due to development of severe skin ulcerations, and the 250 ml kg^{-1} group study was carried out for 103 weeks. The chemical composition of the marine diesel fuel tested was not completely chemically characterized but consisted of a greater percentage of aromatics and a lesser percentage of alkanes compared to diesel fuel no. 2. It has been suggested that skin carcinogenesis of diesel fuels is probably promoted by chronic irritation and hyperplasia; however, the lack of carcinogenesis in the more refined diesel fuels even in the presence of marked skin irritation indicates that high concentrations of genotoxic PAHs in marine diesel fuel may be involved in the carcinogenic mechanism.

The International Agency for Research on Cancer (IARC) has judged that there is limited evidence for the carcinogenicity in experimental animals of marine diesel fuel. There is limited evidence for the carcinogenicity in experimental animals of straight-run kerosene and sufficient evidence for the carcinogenicity in experimental animals of light vacuum distillates, and of light catalytically cracked distillates and of cracked residues derived from the refining of crude oil.

Human

The predominant effect reported is skin changes from chronic dermal exposure, including cutaneous hyperkeratosis. In a case–control study of cancer at many sites, there was evidence of an increased risk of squamous cell carcinoma of the lung in men estimated to have had substantial exposure to diesel fuel. There was also an indication of an increased risk of cancer of the prostate. No attempt was made to separate the effects of combustion products from those of exposure to diesel fuel itself. Overall, the IARC Working Group has judged that there is inadequate evidence for the carcinogenicity of diesel fuels in humans. Marine diesel fuel is possibly carcinogenic to humans (group 2B). Distillate (light) diesel fuels are not classifiable as to their carcinogenicity to humans (group 3).

In Vitro Toxicity Data

Fuel oil no. 2 gave borderline positive results for mutagenicity in the Ames *Salmonella typhimurium* assay. It was mutagenic to mouse lymphoma cells in forward mutation assays. Another sample did not induce mutation in bacteria or algae; a sample of marine diesel fuel and aliphatic and aromatic fractions of an unspecified diesel fuel were also nonmutagenic to bacteria.

Clinical Management

Skin exposed to diesel fuel should be washed thoroughly with soap and water to minimize local irritation and prevent further absorption. Exposed eyes should be rinsed with large quantities of water for at least 15 min.

Diesel fuel may be aspirated into the lungs following vomiting, causing aspiration pneumonia. Therefore, inducing vomiting following ingestion is not indicated unless the threat of severe renal, liver, or CNS toxicity outweighs potential development of aspiration pneumonia. Inducing vomiting may be indicated if large quantities were ingested or the fuel is suspected to contain highly toxic additives. Vomiting may be induced by administering syrup of ipecac (30 ml for adults and 5 ml for children 1–12 years of age). Activated charcoal may be considered for patients who have ingested another toxic substance.

Environmental Fate

Fuel oil no. 2 is released to the environment during its production, formulation, and use. Direct release to aquatic environments occurs during its use in mosquito control as a coating on breeding waters. If released into soil, fuel oil no. 2 will strongly adsorb. It may biodegrade in water and soil or volatilize from water (half-life of 4.4–4.8 h from a model river) and moist soil surfaces, but adsorption may attenuate the rate of these processes. In water, adsorption to sediment is important. Bioconcentration in aquatic organisms may be limited for the chief components due to metabolism. If released to the atmosphere, degradation of vapor phase components of fuel oil no. 2 by reaction with photochemically produced

hydroxyl radicals (estimated half-life on the order of 1 day or less) will be significant.

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Further Reading

International Agency for Research on Cancer (IARC) (1989) Monographs on the evaluation of the carcinogenic risk of chemicals to humans. *Diesel Fuels* **45**: 219–237.

Scheepers PT and Bos RP (1992) Combustion of diesel fuel from a toxicological perspective. II. Toxicity. *International Archives of Occupational and Environmental Health* 64: 163–177.

Relevant Website

http://www.oehha.ca.gov – (US) State of California. Health Effects of Diesel Exhaust.

Dietary Restriction

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Introduction

Diet restriction (DR), also referred to as caloric restriction, is defined as limitation in the amount of calories consumed by an individual. DR refers to reduction in caloric intake without any reduction in the daily requirement of vitamins and minerals. Diet restriction has been traditionally defined in terms of percent reduction in calories as compared to *ad libitum* caloric intake. Extensive research in the past century has shown that reduction in food consumption (and in turn, reduction in calories) is associated with a number of beneficial effects such as increase in life span, delay in aging processes, decrease in diseases such as cancer, inflammatory disorders and type-2 diabetes, and protection from toxic effects of various toxicants. In recent years, DR has been recognized as the only lifestyle modification that can be used as a potential cure against 'Syndrome X', a combination of various obesity-related disorders such as type-2 diabetes, and cardiovascular problems. Although the most common experimental DR paradigm in rodents and primates is to restrict calories by 30–40%, in humans, reduction even as low as 10% of normal caloric intake has been found to be beneficial. An important finding of human and animal studies is that DR has to be adopted as a lifestyle rather than a 'one time' treatment in order to derive full benefit. Most of the positive changes brought about by DR are lost once the individual starts consuming *ad libitum* calories.

Effect of DR has been studied in a range of models including yeast (*S. cereveacae*), rotifers, worms (*C. elegans*), flies (*Drosophila)*, spiders, fish, rodents (many strains of rats and mice), guinea pigs, primates (rhesus monkeys and chimpanzees), and humans. The beneficial effects of DR have been observed in all the species that have been studied. While most of the mechanistic information regarding the decrease in cancer incidences, delay in aging, and protection against chemical-induced toxicities comes from rodent models, human studies have demonstrated that DR can bring about improvement in general health of an individual and decrease the risk of 'syndrome X' and related disorders such as heart disease.

The beneficial effects of DR can be broadly divided into three categories: antiaging effects, prevention of disease incidence including cancer, and protection from toxicant-induced injury.

Antiaging Effects of DR

Effect on Animals

Most striking effect of DR is increase in lifespan of the diet-restricted individuals. Longevity has been observed in all the species studied from yeast to rodents and long-term studies on primates are currently underway.

The antiaging effects of DR are best understood in rodent species (**Table 1**). It is known that rats and especially the mice subjected to DR either starting from early age or from middle age accrue the benefits of increased life span. In one such study the C3B10F1 mice subjected to 40% DR had a 20% increase in life span. The experiments with rats and mice indicate that the increase in life span is pronounced in mice as compared to rats. The mechanisms proposed for the greater increase in life expectancy in mice include greater decrease in the energy expenditure by mice on DR. On the other hand, the genetic predisposition of rats to obesity makes them less sensitive to physiological changes induced by DR.

There is substantial evidence suggesting that moderate diet restriction, lifelong or started in the middle

Table 1 Antiaging effects of diet restriction

Effect	*Model*
Prolongation of life span	Male/female white rats C3B10RF1 mice *S. cereveacae* (yeast) *C. elegans* F344 × Brown Norway F1 rats
Attenuation of inflammatory disorders	Sprague–Dawley, F344 rats, and Balb/c mice
Prevention of dysregulation of cytokines	C57BL/6 mice
Decreased accumulation of protein carbonyls (markers of oxidative stress) in brain	C57BL/6 mice
Decreased accumulation of protein carbonyls in skeletal muscles	C57BL/6 mice
DR maintains 'young' gene expression profile in aged mice livers	C3B10RF1 mice

age, prevents various age-associated diseases. DR maintains the overall physiology in 'young' state and prevents age-associated diseases in cardiovascular system, brain, liver, muscles, and kidney's. The most prominent effect of DR is the attenuation of inflammation that leads to a variety of disorders. Moderate and long-term DR prevents dysregulation of cytokine levels and cytokine related signaling, especially that of TNF-α, IL-6 and NF-κB. Decrease in oxidative stress has been postulated as a mechanism behind many of the antiaging effects of DR. Decrease in oxidative stress results in lower age-associated dysregulation in inflammation-related signaling and thus delays aging process. This notion is supported by the observation that DR decreases the 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative stress, in brain and other tissues such as liver, heart and skeletal muscles.

It has been recognized that neurodegenerative lesions of brain are one of the major complications in aging. A plethora of studies have shown that DR modulates neurophysiology at the molecular, cellular, and behavioral level thereby preventing loss of nervous function associated with aging. DR prevents aging-induced protein oxidative damage. This is evident from the decreased accumulation of protein carbonyl and sulfhydryl groups, which increasingly accumulate in aging animals. Decrease in accumulation of these protein carbonyls has been correlated to retention of sensorimotor coordination and improvement in learning process in the aged DR mice. DR rats and mice have decreased deposition of lipofuschin granules, another marker of aging in the brain. Similar results have been obtained in skeletal muscles where DR mice did not exhibit the fourfold increase in the levels of mitochondrial carbonyls and lipid peroxides observed in aged *ad libitum* fed mice. DR leads to induction of heat shock proteins (HSPSs) and stress proteins in the brain, which have been viewed as a potential mechanism behind the decreased oxidative stress in aged DR brains. Apart from oxidative stress other relatively less investigated mechanisms have been put forth to explain longevity due to DR. These include changes in the neuropeptide Y levels, decrease in glycation and glycoxidation, decrease in body temperature, and altered gene expression and protein degradation.

With the advent of cDNA based microarray technology during the last decade, understanding gene expression profile in aging and calorie restricted (antiaging) rodents has gained the center stage. The transcriptome of the DR rodents offers evidence and explanations for many of the anti-aging physiological modifications observed in these animals. The gene expression profile of the aged mice indicated marked inflammatory response, oxidative stress, and reduced neuronal plasticity and neurotrophic support. DR selectively attenuated the age-associated induction of the genes encoding inflammatory and stress responses. These data also indicate a metabolic shift towards lowered accrual of macromolecular damage due to increased protein turnover. Aging was accompanied by changes in gene expression of genes associated with increased inflammation, cellular stress, fibrosis, reduced apoptosis, xenobiotic metabolism, normal cell cycle, and DNA replication. DR reversed majority of these gene changes associated with aging and shifted the 'normoaging' genomic profile towards the 'slow-aging' profiles associated in DR mice.

It can be seen that decreased oxidative stress in DR may be a combination of two events. First, a decreased production of reactive oxygen species (ROS), especially at complex I in the mitochondria. The second mechanism seems to be induction of antioxidant mechanisms such as in increase in glutathione and glutathione-s-transferase activity. Similarly, increased expression of HSPs has been observed in DR and postulated to play a role in decrease oxidative stress. Thus the two opposing forces, decreased production of ROS and increased scavenging the ROS, both are induced in DR leading to decreased oxidative stress. The antiaging effects of DR are strongly linked to this decreased in oxidative stress. The prevention of inflammatory disorders by DR may also be directly linked to decreased oxidative stress since it is known that ROS mediate the production of proinflammatory cytokines such as TNF-α and produce inflammation. Thus, the literature evidence supports the antioxidation theory in the light of anti-aging effects of DR.

Effects on Humans

The limited but striking human evidence, from the biosphere studies, indicates that diet restriction improves human physiology that may eventually lead to increase in longevity. In these biosphere studies, eight human volunteers (four male and four female) stayed in a materially closed but energetically open (sunlight, electric power, and heat) environment and sustained on food material grown inside the biosphere. These subjects underwent an ~30% calorie restriction during the 6 months of stay in the biosphere. The clinical data obtained on the body mass index, serum glucose, triglycerides, serum cholesterol, blood pressure, and leukocyte counts indicated striking improvement after the 6 month period. Although these data from short-term experiments cannot predict the antiaging effects of DR in humans, they indicate that DR of humans is certainly possible and the beneficial effects that were observed in animals under experimental conditions can be achieved in humans.

Anticarcinogenic Effects of DR

Decrease in tumor incidence was one of the first observed favorable effects of reduced food intake observed in experiments conducted in early twentieth century. Since then substantial evidence has shown that DR results in inhibition of tumor promotion and decreases in both spontaneous and chemical-induced cancer incidence.

Effect on Animals

DR has been shown to delay onset of a number of other spontaneous tumors in rodents. This includes hepatoms, breast tumors, pancreatic islet cell tumors, renal tumors, mammary gland cancers, pituitary tumors, and pheochromocytomas. Decrease in spontaneous appearance of preneoplastic foci in the liver was observed in the SPF Wistar rats. Similarly, marked reduction in spontaneous hepatoma was observed in B6C3F1 mice diet restricted for 12 months. In rodents, DR has been shown to decrease colon cancer incidence. Recently it has been shown that DR prevents spontaneous sarcomas and lymphomas in $p53^{-/-}$ mice, which are genetically susceptible to a number of neoplasms. The decrease in tumor incidence is linked to the increase in life expectancy of DR animals, especially rodents, where the incidence of spontaneous tumors is the leading cause of death in rodents.

The hallmark of the anticarcinogenic effect of DR is the ability of diet restriction to prevent chemical-induced tumors. The first reports of inhibition of chemical carcinogenesis came in the 1940s. Benzo[*a*]pyrene-induced skin tumors were decreased in diet restricted ABC, C57, and Swiss strains of mice. Interestingly, it was demonstrated that the decrease in tumor incidence is independent of any particular source of calories. Contrary to the popular belief of 'low-fat' or 'low-carb' diets, it was demonstrated that decrease in cancers following DR depends mainly upon reduction in the total number of calories rather than decrease in calories coming from either only fat or carbohydrates. Since the first reports in the 1940s to 1950s, the anticarcinogenic effect of DR on chemical-induced tumors has been extensively studied in various tissues and after exposure to a variety of chemicals.

There are a plethora of reports indicating that DR can prevent chemical carcinogen in a number of tissues. It has been shown that 40% DR completely inhibits growth of mammary tumors in female rats treated with 7,12-dimethylbenz(*a*)anthracene. Colon tumors induced by 1,2-dimethylhadrazine are also inhibited by 40% DR in rats. Short-term fasting inhibits appearance of altered hepatic foci induced by the initiation–promotion model of diethylnitrosamine and phenobarbital. In a classic initiation–promotion model, 40% DR during and following the promotion phase (promoted by 12-*O*-tetradecanoylphorbol-13-acetate) of skin tumors initiated by 7,12-dimethylbenz(*a*)anthracene lead to significant reduction in skin papillomas. DR inhibited mammary tumors induced by 1-methyl-1-nitrosourea in a dose-dependent manner. Other cancers prevented by DR include 6-nitrochrysene-induced liver tumors in B6C3F1 mice, preneoplastic foci and pancreatic tumors initiated by azaserine in Lewis rats, decrease in azoxymethane-induced colonic cell proliferation, a prerequisite to colon cancer, in F344 rats, decreased tumorigenicity of 4-aminobiphenyl and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in CD1 mouse bioassay by DR, and inhibition of whole-body radiation-induced myeloid leukemia. Recently, it has been demonstrated that 30–40% calorie restriction inhibits growth of syngenic CT-2A malignant mouse astrocytoma by almost 80%.

The mechanism(s) by which DR inhibits cancer incidence is not completely understood and is currently under investigation in several laboratories. Three hypotheses have gathered substantial experimental evidence (**Figure 1**): (1) changes in drug metabolizing enzymes leads to decreased bioactivation of carcinogens thereby decreasing DNA adduct formation and tumor formation; (2) decreased DNA damage due to lower oxyradical stress owing to DR leads to lower cancer incidence; and (3) selective removal of initiated and transformed cells by DR animals. Decreased bioactivation of carcinogens

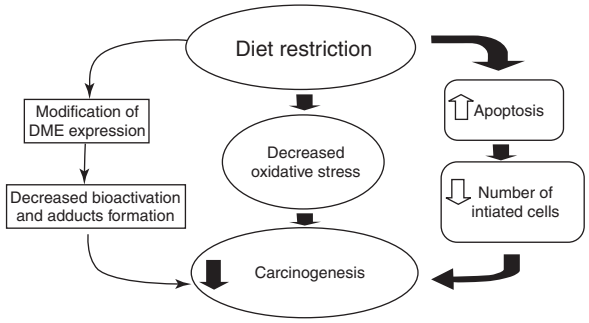

Figure 1 Proposed mechanisms of anticancer effects of DR. Three main mechanisms proposed to explain the anticarcinogenic effects of DR include decreased oxidative stress, increased selective apoptosis of initiated cells, and decreased metabolism of carcinogens.

pertains only to inhibition of chemical-induced carcinogenesis. According to this theory the DR animals have decreased bioactivation of carcinogens. DR decreases expression of sex-specific drug metabolizing enzymes (DMEs) CYP 2C11 (in males) and corticosterone sulfotransferase, which are closely associated with bioactivation and tumor production by carcinogens such as 2-acetylaminofluorene or aflatoxin-B_1. Similarly, decrease in CYP 2A1 in the testicular Leydig cells by DR correlates with the decreased Leydig cell hyperplasia and tumors. Despite the limited experimental evidence, the decreased tumor incidence correlates well with decrease in DMEs by DR.

It has been postulated that increase in DNA damage due to ROS contributes heavily to carcinogenesis. The dividing cells such as hepatocytes have a greater chance of fixing and multiplying the number of the mutations caused by DNA damage leading to transformation of cells. DR is known to decrease the oxidative stress leading to less number of mutations. Decreased oxidative stress in the DR animals is known to occur via decrease in mitochondrial leakage of free radicals. This decrease is not due to lower oxygen consumption but is due to decreased leakage of the reactive species at Complex I in the mitochondrial electron transport chain.

The third hypothesis put forth to explain the anticarcinogenic effects of DR holds that selective removal of initiated cells takes place in DR. It has been shown in the liver tumor model that increased apoptosis and decrease in the rate of cell proliferation in DR animals play an important role in decreasing spontaneous as well as chemical-induced tumors. Similar enhancement in apoptosis has been shown in brain tumor model. The increase in apoptosis is a mechanism devised by the DR animals to cope up with the decreased availability of food. The damaged and weak cells are efficiently removed in the DR animal, which also helps in efficient management of a tight energy budget. Moreover, upregulated apoptosis also removes the cells containing mutated DNA and transformed cells, which are the precursors of tumorigenesis. The successful inhibition of tumors by DR in the $p53^{-/-}$ mice, which are predisposed to tumorigenesis due to deletion of pro-apoptotic gene p53, suggests that the increased rates of apoptosis in DR animals are independent of p53 activation.

The hunt for the mechanisms behind inhibition of chemically induced or spontaneous tumors by DR is still on. Certain other mechanisms such as decreased signaling via the IGF pathway have been postulated specifically in the colon cancer inhibition by DR. It can be seen from the collective evidence that decrease in cancer incidence in DR may be a result of multiple mechanisms. DR induces decrease in oxidative stress mediated by decreased production of ROS, which, in turn, leads to decreased DNA damage. Decrease in DNA damage and lowered mutagenesis is a key for decreased tumor production. On the other hand, increased apoptosis removes those small numbers of cells that inherit the mutated DNA thereby providing a second line of defense. In the mitotic tissues such as liver, kidney, and gastrointestinal tract, DR has been shown to decrease the rate of cell proliferation through more efficient management of energy budget. Decrease in proliferation leads to decreased propagation of mutations as higher cell division provides an opportunity to fix and propagate mutations. A combination of decreased oxidative stress and rate of cell division and increased apoptosis play significant role in inhibition of cancer incidences by DR.

Effect on Humans

The data on anticancer effects of DR on humans are limited. It has been shown in humans that obese persons subjected to DR have decreased rectal cell proliferation, a biomarker related to colon cancer. In a recent retrospective study in Swedish women, it was noted that women with less caloric intake had substantially lower incidence of breast cancer. These reports indicate that the anticancer effects of DR observed in the experimental models are reproducible in humans.

Protection against Acute Toxicity by Diet Restriction

In spite of extensive research on the beneficial effects of DR, the protection offered by DR against acute chemical toxicity has been hardly studied. Nevertheless, there are a few substantial reports indicating that DR can offer protection against acute exposure

Table 2 Protection from acute toxicity by diet restriction

Protection from	*Species*	*Proposed mechanism*
Ozone-induced lung damage	Male F344 rats	Decreased inflammatory response combined with increased antioxidant status
Cardiotoxicity of isoproterenol (IPR)	Male Sprague–Dawley rats	Decreased core body temperature of DR rats after IPR treatment
Aspirin and acidified ethanol-induced gastric toxicity	Male F344	DR prevents decline in GSH and ATP in the gastric tissue
Gancyclovir-induced gastrointestinal tract damage	B6C3F1 mice	Higher tissue repair and regeneration in DR rats
LPS-induced inflammation and liver toxicity	Balb/c mice	Increased levels of corticosterone leading to decrease in inflammation
Thioacetamide-induced lethal liver injury	Male Sprague–Dawley rats	Enhanced compensatory liver tissue repair due to prompt upregulation of promitogenic signaling

to lethal doses of chemicals. These include protection against ozone-induced lung toxicity, lipopolysaccharide (LPS)-induced inflammatory liver damage, cardiotoxicity of isoproterenol, acute toxicity of gancyclovir, aspirin (nonsteroidal anti-inflammatory drug)-induced gastric damage and thioacetamide-induced liver injury (**Table 2**). In all these studies short-term DR (up to 3 months) had been employed. The mechanisms involved in protection from acute toxicities are as diverse as the chemicals inducing the toxicity and the organs in which they produce damage. These studies indicate that long-term DR not only prevents age-associated diseases but short-term DR also protects from acute doses of chemicals.

Effects on Animals

It has been observed that three weeks of DR in F344 rats protects the DR rats from ozone-induced (2 ppm) lung injury. Markers of lung injury such as polymorphonuclear neutrophils (PMNs), IL-6, ascorbate, total glutathione (GSH), α-tocopherol, and fibronectin content, estimated 24 h of ozone exposure in the bronchoalveolar lavage (BAL) fluid indicated lower tissue damage in DR rats. This was accompanied by lower secretion of IL-6, fibronectin, and lower increase in infiltration of PMN. It was concluded that the protection offered by DR is mediated by lower inflammatory response and higher activation of anti-inflammatory mechanisms. This notion is supported by the improved anti-oxidant status of DR lungs illustrated by higher α-tocopherol, urate, and ascorbate in the BAL. These results were confirmed by an independent study in male Sprague–Dawley rats where 20% DR protected the rats from acute exposure to ozone.

Moderate DR also protects from toxicant-induced liver injury. Male Sprague–Dawley rats subjected to 35% DR for three weeks survived a lethal dose of a model hepatotoxicant, thioacetamide. This protection was in spite of a 2.5-fold higher thioacetamide-induced bioactivation-mediated liver injury experienced by the DR rats. This increase in the initial liver injury was due to induction of hepatic CYP2E1, an enzyme involved in the bioactivation of thioacetamide. Further studies indicated that the mechanism behind the protection offered by DR against thioacetamide toxicity is timely stimulation of compensatory liver tissue repair in the DR rats. It has been demonstrated that when toxicants produce injury in liver (and other organs such as kidney) a concomitant stimulation of tissue repair occurs initiated by intricate signaling via cytokines, growth factors, and nuclear receptors. The compensatory liver cell division starts much earlier in DR rats after thioacetamide treatment due to timely expression of signaling molecules such as IL-6, TGF-α, HGF, and PPAR-α. This prompt increase in cell division results in steady decline in liver injury in the DR rats. In the *ad libitum*-fed controls, a delay in initiating cell division was observed. These observations have shed light on a new dimension of DR-mediated control of cell division, where the signaling involved in compensatory cell division is stimulated but mitogenic signaling, which can lead to cancer, is inhibited. During carcinogenesis, a decrease in cell cycle genes is observed in DR animals while a prompt upregulation of these cell division genes is observed during toxicant-induced acute injury when a timely compensatory tissue repair is necessary for survival.

In a recent study, DR mice exhibited lower inflammatory damage induced by LPS. Balb/c mice were subjected to 40% DR and challenged with 25 μg of LPS. The DR mice had attenuated increases in the proinflammatory cytokines consistent with the lower liver damage characterized by lower plasma alanine aminotranferase (ALT), aspartate aminotransferase (AST) and histopathological analysis. The DR mice also had higher circulating corticosterone, a mediator of antiinflammatory action in DR,

after LPS treatment. The authors concluded that the protection from LPS challenge in DR mice is mediated by increased corticosterone, which leads to decrease in inflammatory response. Although data from other DR experiments support the author's notion of increased anti-inflammatory action, a sharp increase in liver cell division and repair that may play role in long-term survival of the LPS-treated mice was not evaluated in this study.

There are other examples of DR-mediated protection from toxicant-induced injury. Forty percent DR for 12 weeks in male Sprague–Dawley rats protected from that cardiotoxicity of a widely used β-adrenergic agonist, isoproterenol (IPR). It took a large dose ($300\,mg\,kg^{-1}$ killed $<10\%$ of DR rats versus $15\,mg\,kg^{-1}$ killed 50% of *ad libitum* fed rats) of IPR to kill DR rats. The underlying mechanisms are not clear. However, the authors observed a rapid decrease in core body temperature in the DR rats after IPR treatment. The decreased body temperature may have led to decreased uptake of the parent compound and its metabolites by heart which may explain the protection offered by DR. DR also protects from gancyclovir-induced gastrointestinal tract (GIT) necrosis. Similar to protection from thioacetamide-induced liver injury, the mechanism behind protection against gancyclovir-induced GIT injury models is the enhanced tissue regeneration and repair in the DR animals. Female B6C3F1 mice diet-restricted for 4 weeks (40% restriction) are resistant to gancyclovir.

Effects on Humans

No data are available on the effects of DR on acute toxicity in humans.

DR and Pharmaceutical Drug Safety Testing

Preclinical toxicology testing of pharmaceutical agents is a very important component of the drug development process. Rodent bioassays (using species such as Sprague–Dawley rats) recommended by the Food and Drug Administration have been traditionally used by the drug industry to monitor toxicological effects and carcinogenic potential of the candidate drug molecules. During such long-term cancer bioassay studies, it was observed that control animals suffer from spontaneous tumors towards the end of the assays. This caused serious problems in the final analysis of the data due to animal variation resulting in decreased statistical significance. To overcome this problem, the rodents used in the assay were moderately diet-restricted (25–30% DR) for the duration of the assays. Moderate DR of rodents has a number of advantages such as reduction in animal-to-animal variation, decrease in occurrence of spontaneous tumors, and reduction in diet-related endocrine, renal, and cardiac diseases. Interestingly, moderate DR did not affect the activities of phase I and phase II drug metabolizing enzymes and the toxicokinetics of the candidate drugs. However, larger doses of the candidate drugs have to be used to produce significant toxicological effects. These observations have led to believe that moderate DR of rodents used in bioassays will significantly improve the quality and accuracy of long-term bioassays.

DR and Human Health

Although extensive evidence has been generated supporting its beneficial effects, DR is far from being adopted as a lifestyle choice by people. Obesity remains a major health risk in the United States at this time. Unlike experimental animals, DR in humans is completely voluntary and it has to be adopted as a lifestyle and done consistently throughout the life to accrue its beneficial effects. Both these conditions pose formidable roadblocks in adoption of DR as a lifestyle choice. Recently a concept called 'caloric restriction mimetics' has been put forth by some investigators. It is argued that since people are less likely to adopt DR as a lifestyle by their own choice, pharmacological agents that induce similar type of effects can be developed, which can help people to accrue the same benefits that long-term DR can provide. Some initial experiments have indicated that 2-deoxy-glucose (2-DG), a glucose analog, can be used as a caloric restriction mimetic. Data obtained from subchronic treatment of rats indicate that 2-DG may be a promising candidate since it reproduced some of the beneficial effects of DR namely, decrease in body weight gain, increased insulin sensitivity, and decrease in mean body temperature. However, other beneficial effects of DR such as increased life span, decreased disease incidence (including cancer), and protection from toxicity of chemicals still remain to be studied following treatment with the caloric restriction mimetics.

Summary

Overall, evidence gathered over last 90 years indicates that DR is the most effective modulation that may result in substantial improvement in quality of health via a number of mechanisms including overall decrease in oxidative stress, prompt tissue repair following injury, decreased disease incidence including cancer and increased life span. The importance of

DR as a lifestyle modification is more than ever today, since obesity and obesity-related disorders such as 'syndrome X' are number one public health concerns in the United States.

See also: Carcinogenesis; Toxicity, Acute.

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Dietary Supplements

Abbi Heilig

Dietary supplements have traditionally been products made of one or more of the essential nutrients, such as vitamins, minerals, and protein. Now, a dietary supplement in the United States is a product taken by mouth that contains a 'dietary ingredient' intended to supplement the diet, as defined by Congress in the Dietary Supplement Health and Education Act (DSHEA) of 1994. As defined in the DSHEA, the 'dietary ingredients' in these products may include: vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandulars, and metabolites. The dietary supplements may make certain claims of function or purpose such as: building strong bones (calcium), maintaining cell integrity (antioxidant), or maintaining bowel regularity (fiber). Some are used to supplement the diet by increasing the total dietary intake.

The DSHEA set up a new framework for the US Food and Drug Administration (FDA) regulation of dietary supplements. It also created an office in the National Institutes of Health to coordinate research on dietary supplements, and it called on then President Clinton to set up an independent dietary supplement commission to report on the use of claims in dietary supplement labeling. It is now easy to spot a supplement because DSHEA requires manufacturers to include the words 'dietary supplement' on product labels. Also, starting in March 1999, a 'Supplement Facts' panel will be required on the labels of most dietary supplements. The DSHEA mandated information that will be required on the labels of dietary supplements includes:

- statement of identity (e.g., 'ginseng');
- net quantity of contents (e.g., '60 capsules');
- structure–function claim and the statement 'This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease';
- directions for use (e.g., 'Take one capsule daily');
- supplement Facts panel (lists serving size, amount, and active ingredient);
- other ingredients in descending order of predominance and by common name or proprietary blend; and
- name and place of business of manufacturer, packer, or distributor. This is the address to write for more product information.

Besides FDA, individual states can take steps to restrict or stop the sale of potentially harmful dietary supplements within their jurisdictions.

Approximately 29 000 dietary supplements are currently available to American consumers, and annual sales of dietary supplements in the United States are approaching $16 billion, and an average of 1000 new products are developed each year. Although manufacturers are restricted from claiming that using their products leads to therapeutic benefits, surveys show that many people take supplements for purposes such as treating colds or alleviating depression. According to other survey data, the majority of consumers believe these products to be either reasonably or completely safe.

Some dietary supplements are extracts or concentrates; however, they may also be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders. Further, they can be in other forms, such as a bar; however, if they are, information on their label must not represent the product as a conventional food or a sole item of a meal or diet. They must be intended for ingestion through the alimentary canal and cannot have been previously subject of an Investigational New Drug Application (IND), New Drug Application (NDA), or Biological License Application (BLA) filing. Regardless of how the dietary supplements are produced, DSHEA places them all in a class of 'foods', not drugs, and requires that they all be labeled as a dietary supplement (**Table 1**).

Issues

Providing the public with truthful and nonmisleading information about products for one's health is the responsibility of the FDA. Due to the thousands of individual dietary supplements produced, FDA cannot monitor or regulate the products enough to ensure protection of unsafe products. Unless the supplement is an NDA, the FDA does not require the manufacturers to submit any safety information on dietary ingredients in the dietary supplement before marketing. Therefore, the agency must depend upon adverse event reports, product sampling, consumer complaints, inspections, market-place surveys, information in scientific literature, and other sources to identify danger. The FDA can restrict certain substances that pose a 'significant and unreasonable risk', however, they cannot act upon this until substantial harm occurs to a consumer. And even though harm may occur, banning a product may take years. For example, products containing ephedra have caused harm to thousands of consumers for several years, but FDA has not been able to restrict its use.

As the dietary supplement industry drastically continues to grow, so does the risk. Possible fraudulent products as well as consumer injury are both widely reported. It is very simple for a consumer to obtain such products of fraud due to advertisements on the internet, TV, and magazines. These articles promote a new product claiming to be a 'miracle cure', 'magical', or 'new discovery', however, if the product was a 'cure' it would already be reported and used by health care professionals. Some promotions claim that the product can cure a wide range of unrelated diseases, but no product can do this. There are even some that claim to be backed by scientific studies, but the references may be inadequate or nonexistent. For instance, if a list of references is provided, the citations cannot be traced, or if they are traceable, the studies are out of date, irrelevant, or poorly designed.

Advertisement and easily available products have caused an increase in the problem of fraudulent products and consumer injury. Certain products such as GHB (gamma hydroxybutyrate) and GLB (gamma butyrolactone), were both sold as 'sleep aids' or 'relaxers', better known as date rape drugs. Both of these products have caused death in some cases, but are still on the market as a dietary supplement.

Another issue with safety of dietary supplements is the purity of the ingredients. The supplements are not required to go through any control measures before they are put on the market unlike drugs. Cases such as the L-tryptophan one in 1989 are therefore subject to be repeated. The impurities of this product led to an epidemic of eosinophilia–myalgia syndrome, with 1500 reported cases and 37 deaths.

Some other examples of problem products are given in **Table 2** and may vary according to the individual consumer.

Great stress, to ensure efficacy, is put on those products containing stimulants, such as caffeine or ephedra, to those on a diet, workout often, or under the age of 18. These products can cause long-term stress to the body if used for extensive amounts of time. Ephedra arises in the concern of the FDA because of the mechanism of ephedra in the human body. The adrenaline-like stimulant can cause dangerous effects to the nervous system and heart. Some of these effects include heart attack, seizure, stroke, and even death. There must be caution because the risk can increase with the dose, and with strenuous exercise. It specifies certain groups (such as women who are pregnant or breast feeding) who should never use these products and lists other conditions, such as diseases and the use of certain medications that rule out the use of ephedrine alkaloids.

Other reported dangers are the kava-containing supplements. Supplements containing the herbal ingredient kava are promoted for relaxation. For instance, kava is known to relieve stress, anxiety, and tension for sleeplessness, and menopausal symptoms. There has not yet been a determination from FDA that kava-containing products have the ability to perform such benefits. Kava-containing products have been associated with liver-related injuries, including hepatitis, cirrhosis, and liver failure.

PC-Specs is a dietary supplement most commonly used to lower blood pressure as well as in trials to treat prostate cancer. PC-Specs is a mixture of eight herbs – seven of them Chinese. However, the danger is that the supplement contains warfarin and alprazolam. It is commonly prescribed as a blood thinner for

Table 1 Commonly used dietary supplements

Herb	*Suggested use*	*Potential toxicity*	*Potential drug interactions*	*Comments*
Black cohosh (*Cimicifuga racemosa)*	Menopausal symptoms	Gastrointestinal discomfort	None known	No long-term studies showing efficacy or safety
Chast tree berries (*Vitex agnus-castus*)	Premenstrual syndrome, mastodynia	Pruritus	May have dopaminergic activity; therefore, avoid with use of dopamine-receptor antagonist (e.g., neuroleptics)	Small, short-term studies suggest efficacy
Cranberry (*Vaccinium macrocarpon)*	Urinary tract infections	Nephrolithiasis (with cranberry concentrate tablets)	None known	Treatment efficacy not proven; small studies show possible efficacy for prevention
Dong quai (*Angelica sinensis*)	Menopausal symptoms	Rash	Increased international normalized ratio in patients taking warfarin	No clinical evidence of efficacy
Echinacea (*E. purpurea, E. pallida, E. angustifolia*)	Upper respiratory infections	Hypersensitivity reactions	Theoretically, may antagonize the effect of immunosuppressive medications	Variations in plant species studied, part of plant used, and extraction methods make conclusion regarding efficacy difficult
Ephedra (*E. sinica,* mahuang)	Asthma, congestion, weight loss	Hypertension, arrhythmia, myocardial infarction, stroke	Avoid use with monoamine oxidase inhibitors and cardiac glycosides; potential for serious toxicity when combined with out stimulants	Probably effective for short-term weight loss when combined with caffeine; long-term data lacking
Evening primrose (*Oenothera biennis*)	Eczema, irritable bowel syndrome, mastalgia, premenstrual syndrome, rheumatoid arthritis	Nausea, vomiting, diarrhea, flatulence	Possible lowering of seizure threshold in patients taking antiepileptic medications	Conflicting efficacy data for number of conditions
Feverfew (*Tanacetum parthenium*)	Migraine prophylaxis	Hypersensitivity reactions	Theoretical risk of increased bleeding when combined with anticoagulants	Few studies support efficacy
Garlic (*Allium sativum*)	Cardiovascular protection	Gastrointestinal upset, bleeding	Theoretical risk of increased bleeding when combined with anticoagulants	Beneficial effects unproven
Ginger (*Zingiberis rhizoma*)	Motion sickness, dyspepsia	None known	Theoretical risk of increased bleeding when combined with anticoagulants	Has also been used for nausea and vomiting of pregnancy and osteoarthritis

Ginkgo biloba	Dementia, claudication, tinnitus	Gastrointestinal upset, headache, dizziness, bleeding, seizure	Theoretical risk of increased bleeding when combined with anticoagulants	May have modest effects on cognitive performance and functioning in patients with Alzheimer disease or multi-infarct dementia; no evidence to support prevention of memory loss or dementia
Ginseng (*Panax* species; Asian ginseng, Korean ginseng, American ginseng)	Fatigue, diabetes	Generally considered safe; rare reports of hypertension, insomnia, headache, and mastalgia	May interact with monoamine oxidase inhibitors and warfarin (decreased prothrombin time)	Currently, little data to support its use
Kava kava (*Piper methysticum*)	Anxiety	Rash, sedation, liver toxicity	May potentiate effects of benzodiazepines; best to avoid with other antioxidants or alcohol because of risk of excess sedation	Studies suggest efficacy; no data on addition potential
Kola nut (*Cola nitida*)	Fatigue	Irritability, insomnia	Caution when used with other stimulants	Contains caffeine
Saw palmetto (*Serenoa repens*)	Prostatic hyperplasia	Mild gastrointestinal effects	None known	Short-term studies show improvement in symptoms; no evidence for prevention of BPH or prostate cancer
St. John's wort (*Hypericum perforatum*)	Depression, anxiety	Headache, insomnia, dizziness, gastrointestinal irritation	Can decrease levels of cyclosporine, digoxin, oral contraceptives, theophylline, and indinavir; serotonin syndrome can occur when combined with prescription SSRIs	May be effective for mild to moderate depression
Valerian (*Valeriana officinalis*)	Insomnia	Headaches	Avoid use with benzodiazepines because of sedation	Theoretical risk of addiction with prolonged use

Table 2 Example of problem products

Agent	*Use*	*Issues*
Ephedra	Weight loss	Through 2001, there were more than 13 000 health complaints and 100 deaths
Kava	Stress relief	Liver damage – 11 cases of liver failure as of March, 2002
PC-Specs	Lowers blood pressure	Product contaminated with warfarin, leading to bleeding. Also use represents a drug claim
St. John's wort	Antidepressant	Interacts with drugs such as indinavir

patients who are prone to, or recovering from, strokes and other blood clotting disorders. Warfarin can interact with other drugs and cause uncontrollable bleeding and susceptibility to bleeding.

St. John's wort is a herb that has been used for centuries for medicinal purposes, including treating depression. St. John's wort interacts with certain drugs, and these interactions can be dangerous. Some patients who take antidepressant drugs such as St. John's wort, do not experience relief from their depression. Other patients have reported unpleasant side effects from their prescription medication, such as a dry mouth, nausea, headache, or effects on sexual function or sleep. St. John's wort has the potential to interact with other drugs that an individual might be taking and cause adverse side effects including lowering effectiveness of the other drugs. Since St. John's wort is not a proven therapy for depression, it is also not regulated by the FDA. There have been no reported deaths due to the intake of St. John's wort.

Many companies and programs are trying to advance the idea of more regulation of ingredients in the dietary supplements to help reduce risks and injury. However, no action has taken place to appease the issues at hand.

The purpose of a dietary supplement is to better one's health when in actuality it rarely does and the person remains in the average state of poor health. They are meant to enhance a healthy diet, not substitute it. With the use of several dietary supplements one could experience several side effects without gaining the possible benefits.

To bolster the FDA's ability to evaluate the safety of dietary supplements, a 2004 report ('Framework for Evaluating the Safety of the Dietary Supplements') from the Institute of Medicine and the National Research Council of the (US) National Academies outlines a science-based process for assessing supplement ingredients, even when data about a substance's safety in humans is scarce. This approach to safety evaluation works within the regulatory parameters set by the Dietary Supplement Health and Education Act (DSHEA), which does not require manufacturers to provide safety data on their products. The report stated that supplement makers, the public, and others need to increase their reporting of health problems related to supplement use in order to further improve the agency's ability to protect consumers.

Further, a 2003 guidance document ('Guidance for the safety assessment of botanicals and botanical preparations for use with food and food supplements') was developed and published by an expert group of the Natural Toxin Task Force of the European Branch of the International Life Sciences Institute (ILSI Europe) and discussed with a wider audience of scientists at a workshop held in May 2002 in Marseille, France.

Finally, supplement users who suffer a serious harmful effect or illness that they think is related to supplement use should call a doctor or other healthcare provider. He or she in turn can report it to FDA MedWatch by calling 1-800-FDA-1088 or going to www.fda.gov/medwatch/report/hcp.htm on the MedWatch Website. Patients' names are kept confidential. To file a report, consumers will be asked to provide:

- name, address, and telephone number of the person who became ill;
- name and address of the doctor or hospital providing medical treatment;
- description of the problem; and
- name of the product and store where it was bought.

Consumers should also report the problem to the manufacturer or distributor listed on the product's label and to the store where the product was bought.

See also: Saint John's Wort.

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Relevant Websites

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http://www.nap.edu – (US) National Academy of Sciences (2004), the Institute of Medicine and the National Research Council, Committee on the Framework for Evaluating the Safety of the Dietary Supplements, National Research Council, Dietary Supplements: A Framework for Evaluating Safety.

Diethyl Ether

Angelica Becaria

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- Chemical Abstracts Service Registry Number: CAS 60-29-7
- Synonyms: Ether; Ethyl ether; Diethyl oxide; Ethyl oxide; Ethoxyethane; Sulfuric ether; Anesthetic ether
- Chemical/Pharmaceutical/Other Class: Ether
- Chemical Formula: $C_4H_{10}O$
- Chemical Structure: $CH_3–CH_2–O–CH_2–CH_3$

Uses

Diethyl ether is used in the production of rubber, plastics, paints, coatings, perfumes, and cosmetics. It is used as a solvent or extractant for fats, waxes, oils, resins, dyes, and alkaloids. It is also used as a fuel additive, alcohol denaturant, and as a medical anesthetic.

Exposure Routes and Pathways

Inhalation is the main route of exposure to diethyl ether. Occupational exposure to diethyl ether may occur through inhalation and dermal contact with this compound at workplaces where diethyl ether is used. Exposure to this chemical may also occur via inhalation of ambient air and ingestion of contaminated drinking water.

Toxicokinetics

Diethyl ether is immediately absorbed from inhaled air into the bloodstream and passes rapidly into the brain. More than 80% will be eliminated through the lungs and another 1–2% excreted in the urine. The remainder may deposit in fatty tissue. Radiotracer studies in rats have shown that diethyl ether can be degraded to carbon dioxide. 90% of diethyl ether applied on skin is absorbed after 20 min.

Mechanism of Toxicity

The mechanism and site of action of diethyl ether are unknown. In the past, most solvents were thought to interfere with the bulk properties of membranes such as membrane fluidity and permeability, thus causing a generalized perturbation to neuronal membranes. In recent years, it has emerged that specific sites such as ion channels and other receptors are the more likely targets.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation of high concentrations of ether produces central nervous system (CNS) changes, such as behavioral effects, excitation, depression, and unconsciousness. Male mice exposed by inhalation to 13 300–30 000 ppm of diethyl ether for 20 min had decreased excitability, reduced muscle tone, and reduced sensorimotor activity. Diethyl ether is a mild eye irritant. The reported toxic doses for mice include the following: LC_{50} (inhalation), 31 000 ppm per 30 min; LD_{50} (intraperitoneal), 2.4 g kg^{-1}; and LD_{50} (intravenous) 996 mg kg^{-1}.

Human

The target organ of ether is the CNS. Inhalation of high concentrations may cause CNS effects including headache, dizziness, unconsciousness, and coma. It is, however, rare to find death due to an inhalation exposure. Ingestion poisonings are of rapid onset, short duration and clinically similar to ethanol overdose. Diethyl ether is an irritant to the eye, skin, and mucous membranes.

Chronic Toxicity (or Exposure)

Animal

Rats exposed orally to $3500\,mg\,kg^{-1}\,day^{-1}$ for 13 weeks to diethyl ether presented signs of toxicity characterized by decrease in appetite, weight loss, and death.

Human

Repeated dermal exposure may cause the skin to become dry and cracked due to oil extraction. Several reports have suggested that long-term exposure to diethyl ether may have health effects, but there is not enough information available to draw firm conclusions.

In Vitro Toxicity Data

Mutagenicity studies in cultured mammalian cells are ambiguous. Positive and negative results have been reported. Bacterial mutagenicity tests have been primarily negative. Aged ether (containing peroxides) has been shown to be mutagenic.

Clinical Management

Contact with the skin should be minimized by thoroughly washing affected areas for at least 15 min. Symptoms of dermatitis should be treated if necessary. If ingested, vomiting should not be induced since ether poses an aspiration hazard and chemical pneumonitis may occur. CNS depression may result from ingestion. Treatment should be symptomatic. There are no known antidotes to diethyl ether.

Environmental Fate

The industrial use of diethyl ether may result in its release to the environment through various waste streams. In air, diethyl ether will exist as a vapor and will be degraded in the atmosphere after reacting with hydroxyl and nitrate radicals. Half-lives of these reactions in air are estimated to be 1.2 and 5.8 days, respectively. In soil and water, diethyl ether is expected to volatilize and biodegradation is likely to be a slow process. Bioconcentration of diethyl ether in aquatic organisms is low.

Ecotoxicology

The LC_{50} for *Poecilia reticulate* (guppy) is shown to be 2138 ppm for 14 days. The LC_{50} for *Pimephales promelas* (fathead minnow) is $2560\,mg\,l^{-1}$ for 96 h.

Other Hazards

Diethyl ether is extremely flammable. Its volatility and low ignition temperature make it one of the most dangerous fire hazards in the laboratory. Ether vapor forms explosive mixtures with air due to the formation of unstable peroxides. Diethyl ether may react violently with halogens or strong oxidizing agents.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is set at 8 h time-weighted average of 400 ppm, which is equivalent to $1200\,mg\,m^{-3}$. Fifteen minutes short term exposure limit is 500 ppm. The 'immediately dangerous to life or health' concentration is 1900 ppm and is based on 10% of the lower explosive limit for safety considerations.

See also: Anesthetic Agents; Volatile Organic Compounds (VOC).

Further Reading

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Diethylamine

Janice McKee

- Chemical Abstracts Service Registry Number: CAS 109-89-7
- Synonyms: *n*-Ethylethanamine; *n,n*-Diethylamine; Diethamine
- Chemical/Pharmaceutical/Other Class: Amine
- Chemical Formula: $C_4H_{11}N$
- Chemical Structure: $(C_2H_5)_2NH$

Uses

Diethylamine is used in the manufacture of rubber-processing chemicals, adhesives, pharmaceuticals,

insect repellents, resins, flotation agents, and dyes. It is used as a corrosion inhibitor in the metal industries and is used in electroplating. It is also used as a solvent for removing impurities from oils, fats, and waxes, and is used as a polymerization inhibitor.

Exposure Routes and Pathways

Occupational exposure may occur through inhalation and dermal contact. The general population may be exposed through the ingestion of food and the use of tobacco products.

Toxicokinetics

Diethylamine is rapidly absorbed, with the rate of skin absorption dependent on the size of the area involved and the duration of contact. Diethylamine is primarily excreted unchanged in the urine.

Mechanism of Toxicity

The toxic effects of diethylamine are due primarily to its corrosive action on tissues.

Acute and Short-Term Toxicity (or Exposure)

Diethylamine is a corrosive skin and eye irritant and severe respiratory tract irritant.

Animal

Diethylamine is a primary skin irritant and is an irritant to the eyes and mucous membranes. The dermal LD_{50} in rabbits was $580\,mg\,kg^{-1}$. An inhalation LC_{50} of 4000 ppm in rats was reported for a 4 h exposure. The oral LD_{50} in rats has been reported to be $540\,mg\,kg^{-1}$. Intraperitoneal injection in rats resulted in a moderate inhibitory effect with respect to liver function and monoamine oxidase activity.

Human

Diethylamine is a severe skin and eye irritant. Eye exposure to diethylamine can cause edema of the corneal epithelium, generally without pain and causing colored halos around lights. This effect generally clears within 24 h. Intense eye exposures cause blurring, photophobia, and discomfort from the roughness of the corneal epithelium. Direct contact with skin has a corrosive effect, causing erythema and blistering. Respiratory tract irritation is expected from inhalation exposures. Ingestion of diethylamine causes severe burns to the oral tissues, with emesis, abdominal pain, and diarrhea.

Chronic Toxicity (or Exposure)

Animal

Rabbits exposed by inhalation to 100 ppm for 6 weeks experienced irritation of the lung tissue and cornea, moderate peribronchitis, nephritis, a slight thickening of the vascular walls, and multiple punctate erosions and edema of the cornea. Changes in the liver were also noted, including parenchymatous degeneration. Parenchymatous degeneration of the heart muscle has been observed in rabbits at these concentrations but has not been confirmed in other species.

Human

Chronic diethylamine exposure could aggravate existing respiratory diseases.

In Vitro Toxicity Data

Diethylamine was evaluated for mutagenicity in the *Salmonella*/microsome preincubation assay. It was negative in these tests up to 3333 μg per plate in the presence and absence of Aroclor-induced rat or hamster liver S9.

Clinical Management

Exposed skin and eyes should be irrigated with copious amounts of water. After inhalation exposure, the victim should be moved to fresh air and monitored for respiratory distress. Humidified supplemental oxygen (100%) should be administered with assisted ventilation as required. If coughing or breathing difficulties are noted, the patient should be evaluated for irritation, bronchitis, or pneumonitis, including chest X-rays and determination of blood gasses. If pulmonary edema is present, positive end expiratory pressure ventilation and steroids should be considered. For ingestion exposures, the use of diluents is controversial. Emesis or lavage should be avoided. A fall in blood pressure may indicate a delayed gastric or esophageal perforation.

Environmental Fate

Diethylamine will exist solely as a vapor in the ambient atmosphere, and will be degraded in the atmosphere by reaction with hydroxyl radicals; the half-life for this reaction in air is estimated to be 5 h. In soil, diethylamine is expected to have high mobility. Volatilization from moist soil surfaces is not expected; however, it may volatilize from dry soil surfaces. Biodegradation may be rapid in water. The

potential for bioconcentration in aquatic organisms is low.

Ecotoxicology

An LC_{50} for the fathead minnow has been reported to be 855 mg l^{-1} for 96 h.

Other Hazards

Diethylamine is highly flammable. It is incompatible with strong oxidizing agents.

Exposure Standards and Guidelines

Diethylamine occupational exposure standards and guidelines include the following:

- *USA*: Occupational Safety and Health Administration (OSHA) permissible exposure limit (Table Z-1) is 25 ppm (75 mg m^{-3}).
- *USA*: The vacated 1989 OSHA permissible exposure limit of 10 ppm (30 mg m^{-3}) and short-term exposure limit (STEL) of 25 ppm (75 mg m^{-3}) is still enforced in some states.
- *USA*: National Institute for Occupational Safety and Health values include a recommended exposure limit of 10 ppm (30 mg m^{-3}), a 15 min STEL of 25 ppm (75 mg m^{-3}), and an 'immediately dangerous to life or health' value of 200 ppm.
- *USA*: American Conference of Governmental Industrial Hygienists recommended exposure limit is 5 ppm, with a 15 min STEL of 15 ppm, with a skin notation.
- *Australia*: 10 ppm, STEL 25 ppm.
- *Germany*: 10 ppm, short-term level 20 ppm for 10 min, four times per shift.
- *Sweden*: 10 ppm, short-term value 15 ppm for 15 min, with a skin notation.
- *United Kingdom*: 10 ppm, and 10 min STEL of 25 ppm.

See also: Corrosives; Respiratory Tract.

Further Reading

American Conference of Governmental Industrial Hygienists, Inc. (1991) *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th edn., vols. I–III. Cincinnati, OH: ACGIH.

Clayton GD and Clayton FE (eds.) (1993–1994) *Patty's Industrial Hygiene and Toxicology*, 4th edn., vols. 2A, 2B, 2C, 2D, 2E, 2F: Toxicology. New York: Wiley.

Relevant Websites

http://www.bibra.co.uk – Diethylamine and its Hydrochloride (BIBRA Toxicity Profile).

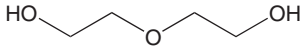
http://www.osha-slc.gov – Diethylamine (Occupational Safety and Health Guideline).

Diethylene Glycol

Lu Yu

This article is a revision of the previous print edition article by Linda Angevine Malley and David M Krentz, volume 1, pp. 475–477, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 111-46-6
- Synonyms: Ethanol, 2,2′-oxybis-; β,β′-Dihydroxydiethyl ether; Bis(β-hydroxyethyl) ether; Bis(2-hydroxyethyl) ether; Brecolane NDG; Deactivator E; Deactivator H; Dicol; Digenos; Diglycol; Digol; Dissolvent APV; DEG; Ethylene diglycol; TL4N; 2,2′-Oxybis[ethanol]; 2,2′-Oxydiethanol; 2,2′-Oxyethanol; 3-Oxapentane-1,5-diol; Glycol hydroxyethyl ether; 3-Oxa-1,5-pentanediol; 2-Hydroxyethyl ether; Ethanol, 2,2′-oxydi-; Glycol ether; 2,2-Di(hydroxyethyl) ether; Carbitol; Diethylenglykol; Dihydroxydiethyl ether; 2,2′-Dihydroxyethyl ether; 2-(2-Hydroxyethoxy)ethanol; Iethyleneglycol; HADB 69; NSC 36391
- Chemical/Pharmaceutical/Other Class: Glycol
- Chemical Formula: $C_4H_{10}O_3$
- Chemical Structure:



Uses

Diethylene glycol is used as dehydrating agent for natural gas processing; as a humectant for tobacco, casein, synthetic sponges; as a lubricating and finishing agent for textiles; as a constituent of brake fluids, lubricants, mold release agents, antifreeze formulations, and inks; as a plasticizer for cork, adhesives, paper, packaging materials, and coatings; as a solvent for printing inks and textile dyes; as an intermediate in the production of diethylene glycol

dinitrate; dioxane; and as an intermediate in the production of some resins, morpholine, polyurethane, triethylene glycol, surfactants, and diethylene glycol esters and ethers. It is also used in lacquer industry and in cosmetics. Diethylene glycol is produced commercially as a by-product of ethylene glycol production.

Exposure Routes and Pathways

Occupational exposure occurs through inhalation, dermal contact, and digestion. Accidental ingestion has been reported, such as the 1985 scandal of Austrian wine diluted with diethylene glycol. If diethylene glycol is released to the atmosphere, it may expose to general population through inhalation of vapor.

Toxicokinetics

After oral administration, diethylene glycol will be absorbed rapidly and almost completely through the gastrointestinal tract. It was reported that 10% of dermal applied diethylene glycol is absorbed by rats. Diethylene glycol was rapidly distributed throughout all organs and tissues following administration with maximum levels in kidney and minimum levels in the adipose tissue, and excreted mainly unchanged through urine. A portion of diethylene glycol is metabolized to oxylate. The half-life for diethylene glycol following a single oral dose of 1, 5, or 10 ml was reported to be between 6 and 10 h in rats in a study.

Mechanism of Toxicity

In large doses, diethylene glycol is a central nervous system (CNS) depressant; and lethality, which occurs within 24 h of a large single dose, is considered to be the result of this effect. Smaller doses, which produce acute toxicity with injury or delayed lethality, primarily affect the kidneys and the liver and are associated with renal insufficiency due to swelling of the convoluted tubules and plugging of the tubules with debris.

Acute and Short-Term Toxicity (or Exposure)

Animal

Diethylene glycol is not irritating to the skin or eyes of the experimental animals. LD_{50} ranged from $7.7\,g\,kg^{-1}$ in rat i.p. study to $26.9\,g\,kg^{-1}$ in rabbit oral study. Symptoms of acute toxicity were similar for rabbits, dogs, mice, and guinea pigs and consisted of thirst, diuresis, and refusal of food, followed several days later by low urine volume, proteinuria, prostration, dyspnea, bloating, coma, low body temperature, and death.

Human

Diethylene glycol is not highly irritating to mucous membranes or by application to skin. Relatively low hazard has been associated with industrial use of diethylene glycol due to its low vapor pressure and low dermal penetration. The average fatal dose in people who drank sulfanileamide elixir with diethylene glycol was $\sim 1\,ml\,kg^{-1}$. Probable lethal dose to human is $0.5–5\,g\,kg^{-1}$. Median diethylene glycol dose that was fatal in 85 of 87 children was estimated to be $1.3\,ml\,kg^{-1}$ (range $0.22–4.42\,ml\,kg^{-1}$). Effects from accidental ingestion or ingestion of contaminated medications include CNS depression, nausea, vomiting, headache, diarrhea, abdominal pain, polyruia, oliguria, anuria, leukocytosis, ascites, hydrothorax, hydropericardium, hypotension, hemorrhages, congestion in the stomach and intestines, distention of the leptomeningeal veins, pulmonary edema, pericardial hemorrhage, centrilobular necrosis with slight jaundice and liver enlargement, enlarged kidneys, cortical necrosis of the kidneys, and acute renal failure. The results of a case–control, cohort study and toxicological evaluation of acute kidney failure among 109 Haitian children (18 years or younger) attributed to diethylene glycol contamination of glycerin used in the local manufacture of acetaminophen oral syrup are reported. Another case control study concluded that paracetamol elixirs with diethylene glycol were responsible for a large outbreak of fatal renal failure in Bangladesh.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to diethylene glycol produces renal and liver lesions. In repeated dose studies, rats given a diet containing 0.25% for 30 days were not affected. Degenerative kidney lesions occurred at dietary concentrations of 1000 ppm (1%) for 30 days. Rats administered diethylene glycol via drinking water exhibited narcosis, weight depression, and mortality at concentrations of 5% and 20%. Diethylene glycol was administered in the diet at concentrations of 2000 ppm (2%) and 4000 ppm (4%) for ~ 2 years. Males administered the 4000 ppm diet had a higher incidence of bladder stones, and one male had a bladder tumor. These data suggest that diethylene glycol is not a primary carcinogen; however, high dietary concentrations can result in formation

of bladder stones, which can lead to development of bladder tumors due to irritation. A dose level without effect was assumed to be between 10 000 and 40 000 ppm in drinking water for rats in a 4 week study. A no-adverse-effect level in rat of 1.25% in the drinking water has been derived from a 2 year study. Diethylene glycol at 3.5% was reproductive toxic in Swiss mice. There was no maternal or developmental toxicity at 1250 mg kg^{-1} day^{-1} of diethylene glycol in Swiss mice; 5000 mg kg^{-1} day^{-1} produced significant maternal toxicity, but no evidence of developmental toxicity; 10,000 mg kg^{-1} day^{-1} caused maternal toxicity and developmental toxicity.

In Vitro Toxicity Data

Diethylene glycol was not shown to be mutagenic in *Salmonella* microsome in tests reviewed. Negative results were also obtained in tests for SCE and chromosome aberrations in Chinese hamster ovary cells, reverse mutation tests in *Saccharomyces cerevisiae* D7, SOS chromotest in *Escherichia coli* PQ37. Diethylene glycol is not genotoxic.

Clinical Management

Usual measures for decontamination (ipecac/lavage, activated charcoal, cathartics) are recommended within 2 h of ingestion. Renal and hepatic function should be monitored and supported. If acidosis occurs, treatment should begin with 1 or 2 mEq kg^{-1} (for children 1 mEq kg^{-1}) of sodium bicarbonate intravenously, repeated every 1 or 2 h as needed. Hemodialysis may be necessary for severe acid/base disturbances or renal failure. Treatment with ethanol has been effective in animals, but efficacy data for humans are not available.

Environmental Fate

Diethylene glycol has been detected in drinking water, ground water, surface water, and indoor air resulting from its release to the environment from its production and use. When released to soil or water, diethylene glycol is highly mobile; adsorption to soil and sediment is very low; volatilization is not expected to be important. It is not susceptible to photolysis on soil surface, but is expected to biodegrade quickly in both soil and water. Bioaccumulation of diethylene glycol in aquatic organism is expected to be low due to an estimated bioconcentration factor of 0.05.

When released to the atmosphere, vapor-phase diethylene glycol is degraded by reaction with photochemically produced hydroxyl radicals with an estimated half-life of 13 h. Diethylene glycol in air in particulate-phase may be removed by wet deposition.

Other Hazards

Flammable, must be preheated before ignition will occur.

Exposure Standards and Guidelines

Workplace environmental exposure level 8 h time-weighted average is 10 mg m^{-3}.

See also: Ethylene Glycol.

Further Reading

Diethylenglykol (1995) *Toxikologische Bewertung*, vol. 11, 114pp. Heidelberg: Berufsgenossenschaft der chemischen Industrie.

Woolf AD (1998) The Haitian diethylene glycol poisoning tragedy. *Journal of the American Medical Association* 279: 1215–1216.

Diethylstilbestrol

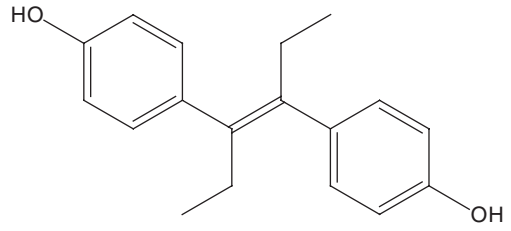
Xuannga Mahini

This article is a revision of the previous print edition article by Bonnie S Dean, volume 1, pp. 477–478, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 56-53-1
- Synonyms: 4,4′-(1,2-Diethyl-1,2-ethene-diyl)bisphenol; 4,4′-Dihydroxy-α,β-diethylstilbene; 3,4-bis(*p*-Hydroxyphenyl)-3-hexene; α,α′-Diethylstilbenediol; α,α′-Diethyl-4,4′-stilbenediol; Antigestil; Bio-des; Bufon; Comestrol; Cyren A; Dawe's Destrol; DEB; DES; Dibestrol '2' premix; Di-estryl; Distilbene; Domestrol; Estilbin 'MCO'; Estrobene; Estrosyn; Fonatol; Grafestrol; Hi-bestrol; Iscovesco; Makarol; Micrest; Microest; Mislestrol; Neo-oestranol I; Oestrogenine; Oestromenin; Oestromensyl; Oestromon; Palestrol; Phenol-4,4′(1,2-diethyl-1,2-ethenediyl)bis-(E); Serral; Sexocretin; Sibol; Stil; Stilbestrol; Stilboestrol;

Stilbetin; Stilboffral; Stilboestroform; Stilkap; Stilrol; Synestrin; Synthoestrin; Synthofolin; *trans*-α,α′-Diethyl-4,4′-stilbenediol; *trans*-Diethylstilbesterol; *trans*-Diethylstilbestrol

- Chemical/Pharmaceutical/Other Class: A nonsteroidal, synthetic stilbene derivative with estrogenic activity. It is an odorless, white crystalline powder, with a molecular weight of 268.36. Its *cis*-isomer tends to revert to the *trans*-form
- Chemical Formula: $C_{18}H_{20}O_2$
- Chemical Structure:



Uses

Diethylstilbestrol is an effective estrogenic agent that was formerly used in estrogenic hormone therapy (for menstrual disorders, postpartum breast engorgement, postcoital contraceptive, prevention of spontaneous abortion), and in chemotherapy of various cancers including postmenopausal breast cancer and prostate cancer. It was also used in biomedical research and veterinary medicine (growth promoter for cattle and sheep; veterinary drug to treat estrogen deficiency disorders). As diethylstilbestrol is a drug once prescribed during pregnancy to prevent miscarriage or premature deliveries, an estimated 5–10 million persons in the United States were exposed to diethylstilbestrol from 1938 to 1971, including pregnant women prescribed diethylstilbestrol and their children. In 1953, published research showed that diethylstilbestrol did not prevent miscarriages or premature births. However, diethylstilbestrol continued to be prescribed until 1971. In 1971, the US Food and Drug Administration (FDA) issued a Drug Bulletin advising physicians to stop prescribing diethylstilbestrol to pregnant women. The FDA warning was based on a study published in 1971 that identified diethylstilbestrol as a cause of a rare vaginal cancer in girls and young women who had been exposed to diethylstilbestrol before birth (in the womb). The US Department of Health and Human Services (HHS) and FDA banned the use of diethylstilbestrol in food animal production in the United States in 1979. Also, FDA implementing regulations (21 CFR Part 530.41) of the Animal Medicinal Drug Use Clarification Act of 1996 prohibit the extra-label use of diethylstilbestrol in food-producing animals.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to diethylstilbestrol. It is available in an oral dosage form. All oral and parenteral drug products contain 25 mg or more of diethylstilbestrol per unit dose. Occupational exposure to diethylstilbestrol may occur through inhalation and dermal contact with this compound at workplaces where diethylstilbestrol is produced or used.

Toxicokinetics

Diethylstilbestrol is readily absorbed through the gastrointestinal tract. It is metabolized in the liver by oxidation and conjugation with sulfuric and glucuronic acids. A certain proportion undergoes enterohepatic circulation. The major metabolites of diethylstilbestrol are the oxides, sulfuric conjugates, and the glucuronic conjugates. Diethylstilbestrol is widely distributed throughout most body tissues with major concentrations in fat tissue. Protein binding is 50–80%. The glucuronides and sulfates of diethylstilbestrol are excreted in the urine. A portion is excreted in the bile but is mostly reabsorbed via the enterohepatic circulation.

Mechanism of Toxicity

Diethylstilbestrol stimulates estrogen receptor-containing tissue. In the 1950s and early 1960s, diethylstilbestrol was an accepted treatment for threatened miscarriages. In 1970, it was first reported that young women whose mothers had been given diethylstilbestrol during the first trimester of pregnancy had an increased incidence of vaginal dysplasia or vaginal adenocarcinoma. Approximately 25% of males exposed to diethylstilbestrol *in utero* exhibit genital lesions and low sperm counts.

Acute and Short-Term Toxicity (or Exposure)

Animal

In cases of diethylstilbestrol intoxication, treated pigs had thickened bladder, with gross distention and acute inflammation, enlarged pelvic urethra, hemorrhages, thickening of mucosa, enlarged prostate gland, and seminal vesicles. Large doses of diethylstilbestrol administered to mice obliterated medullary cavities on long bones, and extramedullary

hematopoiesis occurred in the liver, spleen, and adrenal glands.

Human

Toxicity other than gastrointestinal effects is most common following acute ingestion: nausea, vomiting, abdominal cramps, bloating, and diarrhea. Fullness and tenderness of the breast and edema were also observed. Severe migraine was reported in some. Endometriosis and its attendant pain were also seen.

Chronic Toxicity (or Exposure)

Animal

As in humans, exposure to diethylstilbestrol was shown to cause cancer in animals. Breast tumors were observed in animal administered diethylstilbestrol in the diet. Male and female rats fed with diethylstilbestrol in the diet were also shown to have liver and pituitary cancer. Cancer of the cervix and vagina occurred in female mice injected subcutaneously with diethylstilbestrol. Although male mice did not show unusual tumors in any organ, they developed single or multiple epididymal cysts. Male golden hamsters injected with diethylstilbestrol developed kidney tumors. Ovarian lesions and tumors were found in female dogs that received diethylstilbestrol subcutaneously. Diethylstilbestrol was shown to be teratogenic to Rhesus monkeys. Cattle that had been implanted with diethylstilbestrol failed to conceive or had dead fetuses. Following an oral administration of diethylstilbestrol, abortion was observed in pregnant cattle; following subcutaneous implantation, changes in pelvic morphology was observed.

Ovarian tumors were found significantly in the female offspring of treated mice. A decrease in reproductivity was observed in the female offspring of treated mice, where alteration of reproductive tracts was observed in the male offspring. Results from rodent studies also indicate that potent estrogenic chemicals such as diethylstilbestrol can adversely affect sperm counts and quality.

In laboratory animal studies of elderly third-generation diethylstilbestrol-exposed, female mice, an increased risk of uterine cancers, benign ovarian tumors, and lymphomas was found. Elderly third-generation diethylstilbestrol-exposed male mice were at an increased risk of certain reproductive tract tumors. Both the female and male mice studied were the offspring of female mice exposed to diethylstilbestrol before birth (in the womb).

Human

According to the US Environmental Protection Agency's Office of Health and Environmental Assessment diethylstilbestrol is considered to be a group A human carcinogen, which is based on sufficient evidence in humans and sufficient evidence in animals.

More than 30 years of research has confirmed that health risks are associated with diethylstilbestrol exposure. However, not all exposed persons will experience the following diethylstilbestrol-related health problems.

The known health effect for women who are prescribed diethylstilbestrol while pregnant is a modestly increased risk (30%) for

- Breast cancer (Which means when considering breast cancer risks across a lifetime, one in six women prescribed diethylstilbestrol during pregnancy will get breast cancer. In comparison, only one in eight unexposed women will get breast cancer across their lifetime.).

The known health effects for women exposed to diethylstilbestrol before birth (in the womb), known as diethylstilbestrol daughters, are an increased risk for

- Clear cell adenocarcinoma (40 times more likely than unexposed women), a rare kind of vaginal and cervical cancer.
- Increased risk for clear cell cancer appears to be highest for diethylstilbestrol daughters in their teens and early 20s. However, cases have been reported for diethylstilbestrol daughters in their 30s and 40s.
- Reproductive tract structural differences in the uterus and fallopian tube (T-shaped uterus, small uterine cavity, and/or uterine constrictions).
- Pregnancy complications, including ectopic (tubal) pregnancy and preterm (early) delivery.
- Infertility.

The known health effect for men exposed to diethylstilbestrol before birth (in the womb), known as diethylstilbestrol sons, is an increased risk for

- Noncancerous epididymal cysts (growths on the testicles), hypotropic testes, capsular induration of the testes, and cryptorchidism.

Although laboratory animal studies of mice exposed to diethylstilbestrol before birth (in the womb) suggested an increased risk of autoimmune disease in female mice, studies among humans have reported mixed results. One study indicated

that autoimmune diseases occurred more often in women exposed to diethylstilbestrol before birth (in the womb), known as diethylstilbestrol daughters, than in the general population. However, no one autoimmune disease (such as rheumatoid arthritis or lupus) occurred more often than others. Researchers will continue to explore this issue.

Third-generation children (the offspring of diethylstilbestrol daughters and sons) are just beginning to reach the age when relevant health problems (such as reproductive tract problems), have been studied.

A study of the health risks for the grand-daughters of women prescribed diethylstilbestrol while pregnant or third-generation daughters was published in 2002. The researchers compared findings of pelvic examinations of 28 diethylstilbestrol grand-daughters with findings noted in their mothers (diethylstilbestrol daughters). Even though abnormalities were present in more than 60% of diethylstilbestrol daughters, no abnormalities were found in the diethylstilbestrol grand-daughters. Diethylstilbestrol grand-sons are being studied at the Netherlands Cancer Institute. Early research reported that hypospadias, misplaced opening of the penis, occurred 20 times more frequently among sons of diethylstilbestrol daughters.

Purpura, edema, leg cramps, gynecomastia, porphyria cutanea tarda, and chloasma may be associated with the chronic use of diethylstilbestrol. Various cancers in premenopausal women have been shown to occur. Other chronic toxic effects include thrombocytopenia, gynecomastia, and fluid retention.

In Vitro Toxicity Data

Clastogenic activity was evaluated in three cultures of rat liver cells. Neither chromatid aberrations nor chromosome aberrations were increased significantly.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be used as deemed appropriate to the patient's level of consciousness and the history of the ingestion. Activated charcoal may be used to adsorb diethylstilbestrol or concomitant ingestants.

Environmental Fate

Diethylstilbestrol's production and use in biochemical research, medicine, and also in veterinary medicine may result in its release to the environment through various waste streams. It may also be released to the environment during transport, storage, or disposal. If released to soil, diethylstilbestrol is predicted to strongly adsorb to the soil. Volatilization from the dry or wet soil surface would probably be unlikely. The extent of biodegradation in soil is not known, although diethylstilbestrol has been shown to be resistant to degradation in activated sludge. If released to water, diethylstilbestrol may bioconcentrate in aquatic organisms and strongly adsorb to suspended solids and sediments. Diethylstilbestrol is expected to be essentially nonvolatile on water surfaces. Diethylstilbestrol would not be susceptible to hydrolysis. The extent of biodegradation in natural waters is not certain, although diethylstilbestrol has been shown to be resistant to degradation in activated sludge. If released to the atmosphere, diethylstilbestrol vapors should rapidly oxidize, primarily by reaction with ozone. It is expected to exist solely in the particulate phase in an ambient atmosphere. Particulate-phase diethylstilbestrol may be removed from the air by wet and dry deposition.

Other Hazards

Side effects noted after clinical administration were headache, nausea, vomiting, and, sometimes, vaginal bleeding. Prominent gynecomastia and other feminizing effects were produced in males occupationally exposed to estrogens. Changes in secondary sexual characteristics are fully reversible on cessation of exposure.

Exposure Standards and Guidelines

The Comprehensive Environmental Response, Compensation, and Liability Act reportable quantity for diethylstilbestrol is 1 lb or 0.454 kg when there is a release of this designated hazardous substance. As stipulated in 40 CFR 261.33, when diethylstilbestrol, as a commercial chemical product or manufacturing chemical intermediate or an off-specification commercial chemical product or a manufacturing chemical intermediate, becomes a waste, it must be managed according to Federal and/or State hazardous waste regulations (Resource Conservation and Recovery Act). The state of Florida drinking guideline for diethylstilbestrol is $100\,\mu g\,l^{-1}$.

See also: Developmental Toxicology; Environmental Hormone Disruptors; Reproductive System, Female.

Further Reading

IARC (1972 to present) *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*. Geneva: World Health Organization, International Agency for Research on Cancer (multivolume work), vol. 21, p. 205.
Kaiser J (2000) Endocrine disrupters. Panel cautiously confirms low-dose effects. *Science* 290(5492): 695–697.

Relevant Websites

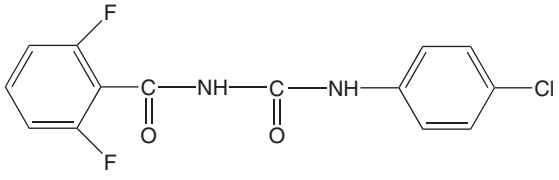
http://www.cdc.gov – DES Update Home.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Diethylstilbestrol.
http://www.epa.gov – NIH/NIEHS. EDRI Federal Project Inventory: 06995-02 Toxicity of Environmental Estrogens.

Diflubenzuron

Nili Jin

This article is a revision of the previous print edition article by Sushmita M Chanda, volume 1, p. 478, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: CAS 35367-38-5; CAS 51026-04-1; CAS 104790-81-0
- Synonyms: Dimilin; Difluron; 1-(4-Chlorophenyl)-3-(2,6-difluorobenzoyl)urea; Micromite; Vigilante; DFB; *N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide
- Chemical/Pharmaceutical/Other Class: Benzoylphenylurea insect growth regulator
- Chemical Formula: $C_{14}H_9ClF_2N_2O_2$
- Chemical Structure:



Uses

Diflubenzuron is used as an insecticide, larvicide, ovicide, and insect growth regulator.

Exposure Routes and Pathways

Ingestion is the primary route for intentional or accidental exposure. Dermal and inhalation exposure are also possible.

Toxicokinetics

Diflubenzuron is well absorbed from the digestive tract and has widespread distribution in the tissues. The major metabolic route in mammals is via hydroxylation. The major route of elimination is via feces; however, urinary elimination is equally important in some species. After a single intravenous dose of diflubenzuron, tissue levels peaked within 15 min in most tissues. Elimination rate after oral administration differs from species to species but is generally completed within 3 days. No literature is available on toxicokinetics in human.

Mechanism of Toxicity

Diflubenzuron inhibits the enzyme chitin synthase, which is required in the final step of chitin synthesis. Chitin is a polysaccharide and a major constituent of the exoskeleton of insects. In insects, the trachea is held open by rings of chitin. The exoskeleton and waxy covering also prevent water loss. Inhibiting chitin synthesis therefore can provide an effective means of pest control. Moreover, vertebrates and most plants do not utilize chitin, thus making diflubenzuron a target-selective pesticide.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of diflubenzuron is low by any route of exposure. The main effects are methemoglobinemia and liver and spleen lesions. The acute oral LD_{50} in rat is $>4.64\,g\,kg^{-1}$ body weight; acute dermal LD_{50} in rat is $>10\,g\,kg^{-1}$ body weight; acute inhalation LD_{50} is $>2.49\,mg\,l^{-1}$. The no-observed-adverse-effect level based on methemoglobinemia is $2\,mg\,kg^{-1}$ body weight per day in rats and dogs and $2.4\,mg\,kg^{-1}$ body weight per day in mice.

Human

No study has reported on the acute toxicity of diflubenzuron in humans.

Chronic Toxicity (or Exposure)

Animal

Long-time exposure of lab animals to diflubenzuron resulted in higher methemoglobin levels and spleen and liver damage. There was no evidence of treatment-related carcinogenicity.

In Vitro Toxicity Data

Diflubenzuron is not mutagenic.

Clinical Management

Diflubenzuron has very low systemic side effects, if absorbed through the skin. The exposed area should be thoroughly washed with soap and water. Eyes should be washed with copious amounts of room-temperature water for 15 min in cases of eye contamination. If small amounts are ingested, no treatment is needed. Low toxicity is seen in nontargeted species. Symptomatic treatment is recommended.

Environmental Fate

Diflubenzuron adsorbs to and remains relatively immobile in soil. Degradation of diflubenzuron is largely through microbial hydrolysis and photolysis with a half-life range of 0.5–1 week. Uptake of diflubenzuron through leaves does not occur. Residual diflubenzuron in soil may be absorbed by plants.

Ecotoxicology

There is no significant acute or chronic toxicity of diflubenzuron on small mammals, birds, fish, or mollusks. All chitin-synthesizing organisms, such as shrimp, lobster, and crabs, are susceptible to diflubenzuron toxicity.

Other Hazards

Diflubenzuron is not flammable. If diflubenzuron is involved in a small fire, the fire should be extinguished with carbon dioxide, dry powder, or alcohol-resistant foam.

Exposure Standards and Guidelines

On the basis of the no-observed-adverse-effect level (NOAEL) of 2 mg kg^{-1} body weight per day derived in long-term toxicity studies on mice, rats, and dogs and applying a 100-fold uncertainty factor, 0.02 mg kg^{-1} day^{-1} exposures are expected to be safe.

See also: Pesticides.

Further Reading

Fischer SA and Hall LW Jr. (1992) Environmental concentrations and aquatic toxicity data on diflubenzuron (dimilin). *Critical Reviews in Toxicology* 22(1): 45–79.

Ishaaya I (1993) Insect detoxifying enzymes: Their importance in pesticide synergism and resistance. *Archives of Insect Biochemistry and Physiology* 22(1–2): 263–276.

US Department of Agriculture (1977) *Common Name for the Pest Control Chemical N-[[(4-Chlorophenyl)amino]carbonyl]-2,6-Difluorobenzamide 'diflubenzuron'*. New York: American National Standards Institute.

WHO (1995) *Diflubenzuron: Health and Safety Guide*. Geneva: World Health Organization.

WHO (1996) *Diflubenzuron: Environmental Health Criteria, 184*. Geneva: World Health Organization.

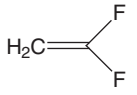
Relevant Website

http://www.inchem.org – Chemical Safety Information from Intergovernmental Organizations.

Difluoroethylene, 1,1-

Kashyap N Thakore and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 75-38-7
- Synonyms: 1,1-Difluoroethene; NCI-C60208; Vinylidene fluoride
- Chemical/Pharmaceutical/Other Class: Flammable gas; Strong oxidizing agent
- Chemical Formula: $C_2H_2F_2$
- Chemical Structure:



Uses

Difluoroethylene is used in the manufacture of polyvinylidene fluoride, which is used as a thermal,

chemical, and ultraviolet light-resistant agent, and as an anticorrosive agent. The monofilament form is used as filter cloth in the pulp and paper industry. It is used as an insulator due to its high melting temperature. Elastomeric copolymers are used for their heat- and moisture-resistant properties, primarily in industrial, aerospace, and automotive applications.

Exposure Routes and Pathways

The primary exposure route is inhalation.

Toxicokinetics

Absorption is very rapid after inhalation and reaches a steady state within minutes of exposure and blood levels decline rapidly at the end of exposure. Biotransformation is very slow; difluoroethylene may produce alkylating intermediate and some acetone. The tissue/air partition coefficients were determined to be 0.07, 0.18, 0.8, 1.0, and 0.29 for water, blood, liver, fat, and muscle, respectively. Difluoroethylene is eliminated as fluoride ions in urine.

Mechanism of Toxicity

Difluoroethylene may interact with the hepatic microsomal monooxygenase to form epoxide. It inhibits microsomal mixed function oxidase *in vitro*.

Acute and Short-Term Toxicity (or Exposure)

Animal

Difluoroethylene is not acutely hepatotoxic at dose levels up to 82 000 ppm by inhalation for 3.5 h in normal rats, whether fed or fasted.

Human

No data are available for occupational exposure. Acute exposure causes nausea, dizziness, and headache. Difluoroethylene is harmful if swallowed, inhaled, or absorbed through skin.

In occupational settings, technical measures should prevent any contact with the skin and mucous membranes. Workers potentially exposed to this compound should wear personal protective equipment and their work should be carried out only in restricted and ventilated areas. Clothing and equipment after use should be placed in an impervious container for decontamination or disposal. Difluoroethylene is listed as a hazardous air pollutant.

Chronic Toxicity (or Exposure)

Animal

It is carcinogenic in long-term bioassays in Sprague–Dawley rats by oral administration.

Human

It may cause heritable genetic damage and may cause tumors of sense organs, skin, and appendages. No data are available for the evaluation of carcinogenicity.

Clinical Management

In case of dermal or ocular contact, the eyes and skin should be flushed with water for 15–20 min. For inhalation exposure, the patient should be moved to fresh air. If necessary, oxygen and artificial respiration should be administered. If the patient is in cardiac arrest, cardiopulmonary resuscitation should be administered. Life-support measures should be continued until medical assistance has arrived. Do not administer liquids to or induce vomiting in an unconscious or convulsing person.

Environmental Fate

It has medium to high mobility in soil and may degrade in soil. Volatilization is a major fate process for 1,1-difluoroethylene in water. Aquatic bioconcentration and adsorption to sediment are not expected to be important fate processes. It exists as a gas in the ambient atmosphere and degrades by reaction with photochemically produced hydroxyl radicals.

Other Hazards

1,1-Difluoroethylene is extremely flammable and explosive. It may polymerize violently under high-temperature conditions or upon contamination with other products.

See also: Pollution, Air.

Relevant Websites

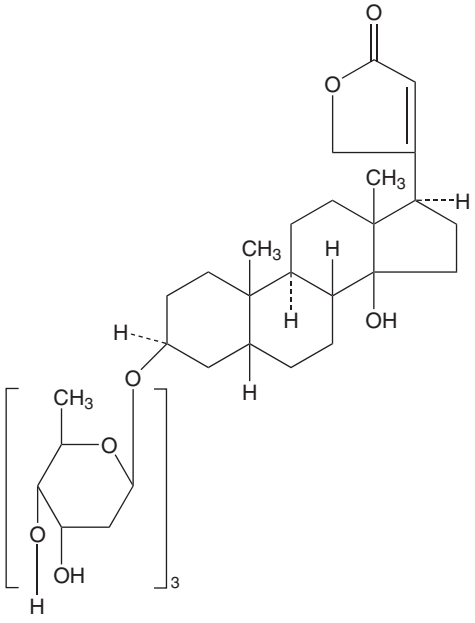
http://www.cdc.gov/niosh – NIOSH (2003) *Pocket Guide to Chemical Hazards*. Cincinnati, OH: National Institute for Occupational Safety and Health.

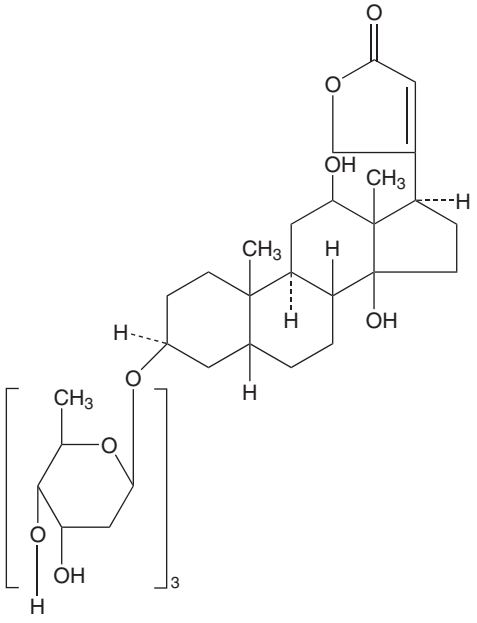
http://www.iarc.fr – IARC (1999): Monographs on the Evaluations of Carcinogenic Risks to Humans: Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide, vol. 71, p.1551.

Digitalis Glycosides

Michael Wahl

- Representative Chemicals: Digoxin; Digitoxin
- Chemical Abstracts Service Registry Numbers: CAS 20830-75-5 (Lanoxicaps); CAS 71-63-6 (Crystodigin)
- Synonyms: Lanoxin; Foxglove
- Chemical/Pharmaceutical/Other Class: Digoxin is a cardiac glycoside congener of digitalis with a hydroxyl group at the C12 position. Digitoxin is a cardiac glycoside congener of digitalis that does not contain a C12 hydroxyl group
- Chemical Structures:





Uses

Digitalis glycosides are positive inotropic agents used in the management of patients with congestive heart failure. They control ventricular rate in supraventricular arrhythmias including atrial fibrillation and atrial flutter.

Exposure Routes and Pathways

Ingestion is the most common exposure pathway following accidental and intentional ingestions. Digoxin is also available for parenteral administration and parenteral toxic exposures can occur.

Toxicokinetics

Digoxin

Oral administration of digoxin tablets and liquid results in 60–85% absorption from the small intestine. Liquid-filled digoxin capsules are 90–100% absorbed. The presence of food or other medications may delay oral absorption. Approximately 80% of digoxin is absorbed following intramuscular administration. Minimal metabolism occurs. Cleavage of the sugar moieties occurs in the liver and via bacteria in the large intestine. Protein binding is 20–30%. Volume of distribution approximates $4\,l\,kg^{-1}$ in adults. Digoxin is excreted in the urine primarily as unchanged drug. In healthy patients, the half-life ranges from 34 to 44 h. The half-life can be prolonged in renal failure.

Digitoxin

Oral absorption of digitoxin is rapid and complete. It is extensively metabolized in the liver to several active metabolites including digoxin. Protein binding is 97%. The volume of distribution approximates

0.47–0.76 l kg^{-1}. Renal, biliary, and fecal elimination occur. The half-life can range from 4 to 14 days.

Mechanism of Toxicity

The digitalis glycosides interfere with the Na^+,K^+-ATPase pump with a resultant intracellular loss of potassium and intracellular increases in sodium and calcium. The net effects of this are increased myocardial contractility and decreased cardiac conduction.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals manifest toxic effects similar to those seen in humans. Cases of cows ingesting large quantities of plants that contain cardiac glycosides demonstrated anorexia, weakness, diarrhea, and arrhythmias.

Human

Nausea and vomiting are frequently seen. Changes in level of consciousness may be observed. Rhythm disturbances are common signs of toxicity. The most common arrhythmias include bradycardia, heart block, and paroxysmal atrial tachycardia. Premature ventricular contractions and ventricular tachycardia are less common. Severe hyperkalemia can also occur following acute ingestion of a digitalis glycoside. Serum potassium levels as high as 13.5 mEq l^{-1} have been reported after acute digitalis ingestion. Digoxin serum concentrations can be extremely high immediately following an acute ingestion. Normal digoxin serum concentration is 0.5–2 ng ml^{-1}. Normal digitoxin serum concentrations are 18–22 ng ml^{-1}. These may decrease over 8–12 h as distribution of the drug occurs.

Chronic Toxicity (or Exposure)

Animal

Digoxin has been used in animals to treat congestive heart failure as in humans. Some concern has been raised about potential effects of drugs like digoxin causing toxicity in aquatic animals from sewer effluent or landfill leachate. In a study using a *Hydra vulgaris* model, digoxin at concentrations of 1 mg l^{-1} for 17 days and did not demonstrate adverse effects in feeding or bud formation.

Human

Anorexia, nausea, vomiting, and diarrhea occur after chronic exposure. Decreases in level of consciousness and delirium may be observed. Visual changes including color changes and snowy vision have been described frequently. Common arrhythmias that occur during chronic toxicity include premature ventricular contractions, ventricular tachycardia, and ventricular fibrillation. Hypokalemia is often present in chronic toxicity and actually precipitates toxicity. Serum digoxin/digitoxin concentrations will be elevated but not as high as those seen in acute toxic exposures.

In Vitro Toxicity Data

Studies have demonstrated that Tween 80, a common nonionic surfactant, increases permeability of digoxin in Caco-2 cells.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. A baseline 12-lead electrocardiogram should be obtained and continuous cardiac monitoring should be utilized. A digoxin/digitoxin serum concentration should be obtained as well as a serum potassium level. Gastrointestinal decontamination procedures should be used as necessary based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used for substantial recent ingestions. In patients with severe dysrhythmias, serum potassium concentrations >5 mEq l^{-1}, and elevated digoxin/digitoxin serum concentrations, digoxin immune Fab should be given. This sheep antibody, which binds digitalis glycosides, is effective in reversing both acute and chronic toxicities. Each 40 mg vial binds 0.6 mg of digoxin/digitoxin. Dosage should be based on serum concentration of digoxin/digitoxin or amount ingested. If these are unavailable, 10 vials can be administered.

Digoxin immune Fab can be administered over 30 min or it can be administered intravenous push to patients in cardiac arrest. Since it will pull digoxin/digitoxin out of tissue sites, serum concentrations of digoxin/digitoxin will rise. These will represent bound digitalis and should not be reacted to clinically. Adverse reactions to digoxin immune Fab include exacerbation of heart failure and atrial arrhythmias as well as hypokalemia. Allergic reactions have not been commonly reported. The antigen/antibody complex should be eliminated within 5 days of administration. This may be delayed beyond 7 days in patients with renal failure. In chronic toxicity, hypokalemia should be treated cautiously with potassium replacement since rapid increases in serum potassium can exacerbate conduction disturbances. Ventricular arrhythmias can he treated with

phenytoin or lidocaine. Overdrive pacing should also be considered. Class IA antiarrhythmics such as quinidine should be avoided since they can cause conduction disturbances. Conduction disturbances should be managed with a transvenous pacemaker.

See also: Cardiovascular System; Foxglove.

Further Reading

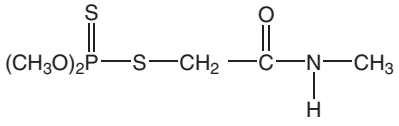
Shumaik GM, Wu AW, and Ping AC (1988) Oleander poisoning: treatment with digoxin-specific Fab antibody fragments. *Annals of Emergency Medicine* 17: 732–735.

Smolarz A, Roesch E, Lenz E, *et al.* (1985) Digoxin specific antibody (Fab) fragments in 34 cases of severe digitalis intoxication. *Journal of Toxicology. Clinical Toxicology* 23: 327–340.

Dimethoate

Nikita Mirajkar and Carey N Pope

- Chemical Name: *O*,*O*-Dimethyl-*S*-2-(methylamino)-2-oxoethyl phosphorodithioate
- Chemical Abstracts Service Registry Number: CAS 60-51-5
- Synonyms: Phosphamide; Cygon; De-fend; Rogor; Rogodial; Roxion; Dimetate; Devigon; Dicap; Dimet; Rogodan
- Chemical/Pharmaceutical/Other Class: Synthetic organophosphorus pesticide of the phosphorothionate class
- Chemical Structure:



Uses

Dimethoate is a systemic and contact insecticide–acaricide used on a range of insects including mites, flies, aphids, and planthoppers. Dimethoate is used very commonly in livestock for the control of botflies and mites. Formulations include aerosols, dusts, granules, and emulsifiable concentrates.

Exposure Routes and Pathways

Dimethoate can be absorbed by the oral, dermal, or inhalation route.

Toxicokinetics

Dimethoate is rapidly absorbed after any route of administration in mammals. It is rapidly metabolized in the liver. Like other phosphorothionate pesticides, the parent compound is activated by cyp450 to the active metabolite, omethoate. A major route of detoxification of the parent compound is hydrolysis of the C–N bond. Pretreatment with phenobarbital increases sensitivity to dimethoate in mice. In male rats, ~60–80% of an orally administered dose of dimethoate is eliminated via the kidneys within 24 h of exposure. Elimination was almost complete within 48 h of exposure. Female rats appear to eliminate dimethoate at a slower rate. The rate of metabolism and elimination of dimethoate varies in different species. Dimethoate produces less toxicity in animals with high rates of dimethoate metabolism and animals with high liver-to-body weight ratios.

Mechanism of Toxicity

Dimethoate exerts toxicity through inhibition of acetylcholinesterase. The oxidative metabolite (i.e., omethoate) is two to three orders of magnitude more potent in inhibiting acetylcholinesterase than the parent compound. The *N*-demethylated omethoate may be the most potent inhibitor of cholinesterases. The enzyme in red blood cells may be more sensitive to inhibition than plasma enzyme following dimethoate exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral LD_{50} for pure dimethoate in rodents is around 500 mg kg^{-1}, but reported values using technical products range from 28 to 400 mg kg^{-1}. Early formulations contained the solvent methyl Cellosolve, which appears to have participated in chemical changes upon storage that increased mammalian toxicity. In studies comparing dermal and oral exposures, the dermal LD_{50} values were generally reported to be about twice as high. In a reproductive toxicity test, dimethoate exposure in the drinking water (~10 mg kg^{-1} day^{-1}) was associated with

60% plasma cholinesterase inhibition in adult mice and altered pup survival and growth but was without teratogenic effects. Teratogenic effects (e.g., fused sternebrae) were reported in rats receiving 12 mg $kg^{-1} day^{-1}$ dimethoate but were absent in animals treated with lower doses (i.e., 3 and 6 mg $kg^{-1} day^{-1}$). Dimethoate was mutagenic in mice. Mutagenic effects were reported to be more prominent in mice given a single high dose of dimethoate than in mice given 1/12 of the same dose daily for 30 days.

Human

Dimethoate shows moderate toxicity after absorption through oral, dermal and inhalation routes.

Characteristic signs of acetylcholinesterase inhibition (e.g., diarrhea, nausea, and abdominal cramps, sweating, blurred vision, difficulty in breathing or respiratory depression, and slow heartbeat) have been reported following dimethoate exposure. High exposures to dimethoate may be associated with a relapse, where the patient stabilizes and then suddenly gets much worse. Dimethoate does not cause delayed neurotoxicity but has been associated with the intermediate syndrome of organophosphate poisoning.

Respiratory ailments, recent exposure to cholinesterase inhibitors, impaired cholinesterase production, or liver malfunction may potentiate the toxicity of dimethoate. Also, high environmental temperatures or exposure of dimethoate to light (visible or UV) may enhance its toxicity.

Chronic Toxicity (or Exposure)

Animal

Rats given oral doses of dimethoate at 5, 15, or 30 mg $kg^{-1} day^{-1}$ for over a year showed an increase in malignant tumor formation. Increased tumor incidence was not dose dependent, however. Hence, there is inconclusive evidence of carcinogenicity.

Human

Chronic exposure to dimethoate in man can produce disorientation, irritability, confusion, impaired memory and concentration, severe depression, speech difficulties, delayed reaction times, headache, nightmares, sleepwalking, and drowsiness or insomnia. It may also produce nausea, weakness, malaise, and loss of appetite.

Under normal conditions, there is little likelihood of impaired reproductive function, teratogenic, mutagenic, or carcinogenic effects in humans.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered, followed by 2–4 mg (in adults) or 0.015–0.05 mg kg^{-1} (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

After effective intervention, patients should be closely monitored for the possibility of sudden relapse.

Environmental Fate

Dimethoate has low persistence in soil with a half-life of $\sim$20 days. It evaporates from dry soil surfaces and is biodegradable. Since dimethoate is broken down rapidly by soil micro organisms, its breakdown is much faster in moist soils. In alkaline soils, it is degraded by hydrolysis. Since it is highly soluble in water and adsorbs very poorly to soil particles, it may leach into groundwater.

The half-life of dimethoate in river water is $\sim$8 days. It does not bioaccumulate in aquatic organisms, nor does it adsorb to suspended particles in water. Dimethoate undergoes significant hydrolysis, especially under alkaline conditions. However, losses by photolysis and evaporation from open waters are not expected to be significant. Dimethoate is not toxic to plants.

Ecotoxicology

In birds, dimethoate is moderately to very highly toxic. Acute oral LD_{50} values of 41.7 mg kg^{-1} in mallards and 20 mg kg^{-1} in pheasants have been reported. Since birds are unable to metabolize dimethoate as rapidly as mammals, it shows greater toxicity in these species.

Dimethoate produces moderate toxicity in fish, with reported LC_{50} values of 6.2 mg l^{-1} in rainbow trout and 6.0 mg l^{-1} in bluegill sunfish. However, it is much more toxic to aquatic invertebrate species like stoneflies and scuds. Dimethoate is highly toxic to honeybees. The 24 h topical LD_{50} for dimethoate in bees is 0.12 μg per bee.

Exposure Standards and Guidelines

- Reference dose is 0.0005 mg kg^{-1} day^{-1}.
- Acceptable daily intake is 0.002 mg kg^{-1} day^{-1}.
- Threshold limit value is 0.7 mg m^{-3}.

See also: Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Dimethyl Ether

Gerald L Kennedy

- Chemical Abstracts Service Registry Number: CAS 115-10-6
- Synonyms: Methyl ether; Dymel
- Chemical/Pharmaceutical/Other Class: Ether
- Chemical Formula: C_2OH_6
- Chemical Structure: CH_3–O–CH_3

Uses

Dimethyl ether is used as a refrigerant, as a propellant in aerosol products, as a starter for gasoline engines in cold weather, as a rocket propellant, and as a specialty solvent in chemical synthesis.

Exposure Routes and Pathways

Inhalation is the main exposure route of dimethyl ether; dermal is possible and ingestion is less likely.

Toxicokinetics

Dimethyl ether is rapidly absorbed by the respiratory tracts with steady-state levels attained within 30 min. The material is cleared rapidly with the mean biological half-life being ~90 min. Absorption was proportional to the dimethyl ether concentration breathed. No tissue storage, particularly in adipose tissue, was seen.

Mechanism of Toxicity

Higher concentrations of dimethyl ether act on the central nervous system to produce narcosis. The effects are rapidly reversible which is consistent with the very rapid bioelimination of the molecule. Dimethyl ether has in the past been considered for use as a human anesthetic. It should be noted that this chemical can produce cardiac sensitization similar to the effects of epinephrine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxic effects on animals from inhalation exposure include anesthetic effects and decreased blood pressure. The 4 h inhalation lethal concentration 50% in rats is 164 000 ppm (16.4%). Cardiac sensitization occurred in dogs exposed to concentrations of 20% or greater.

Human

The main effects of dimethyl ether in humans have been related to its depressant activity on the central nervous system (CNS). Depression of the CNS has been reported at concentrations ranging from 6% to 10% in air. Again, the rapid uptake and clearance of the chemical limits the possibility of other systemic damage in man. However, inhalation of high concentrations of vapor may cause heart irregularities, unconsciousness, and death. Vapor reduces oxygen available for breathing as it is heavier than air. Direct contact with liquid dimethyl ether may produce frostbite.

Vapors of dimethyl ether may produce eye irritation with discomfort, tearing, or blurring of vision. Inhalation exposure may be associated with nonspecific discomfort such as nausea, headache, or weakness. Signs of CNS depression may include dizziness, headache, confusion, incoordination, and loss of consciousness. Abusers of this product could show increased susceptibility to the cardiac arrhythmia effects of epinephrine (cardiac sensitization).

Chronic Toxicity (or Exposure)

Animal

Repeated inhalation exposure to rats and mice caused changes in white blood cell counts, anesthesia, and reduced weight gain. Chronic exposure of rats to 20 000 ppm (2%) caused liver weight reduction and alteration in enzymes associated with liver damage. In another study, observations included decreased red blood cell counts, spleen changes, and decreased survival of males at 10 000 and 25 000 ppm. Hemolysis and red blood cell destruction occurred at 25 000 ppm.

Tests in rats have shown no carcinogenic response at concentrations up to 25 000 ppm. Developmental toxicity including careful evaluations of fetal structural integrity was unaffected in two rat studies, one employing inhalation exposures up to 40 000 ppm, the other to 28 000 ppm. A host-mediated assay in which bacterial indicator organisms incubated in the peritoneal cavity of mice being treated with dimethyl ether showed no increase in mutations.

Human

No information regarding the long-term effects of dimethyl ether in humans has been reported. Again, the first sign of response to the chemical would be expected from the CNS and repeated exposures would most likely behave like a series of acute exposures.

In Vitro Toxicity Data

Dimethyl ether is inactive in genetic tests including the *Salmonella* assay (with and without metabolic activation in at least four strains), HPRT reversion in CHO cells, DNA repair/synthesis in rat liver cells, and the sex-linked recessive lethal test in the fruit fly.

Clinical Management

If high concentrations are inhaled, immediate removal to fresh air should be done. The person should be kept calm. Artificial respiration should be employed on a nonbreathing individual. If breathing is difficult, support oxygen should be given and a physician called. For skin contact, the exposed area should be flushed with water for at least 15 min. The skin should be treated for frostbite if necessary by gently warming the affected area. If irritation is present, it is recommended that a physician be called. For eye contact, flushing should be done with plenty of water for 15 min and a physician called. Ingestion is not considered a potential route of exposure.

It is important to note that because of possible disturbances of cardiac rhythm, catecholamine drugs such as epinephrine should be used with special caution only in situations of emergency life support.

Environmental Fate

Dimethyl ether released to the atmosphere would be expected to exist almost entirely in the vapor phase since the vapor pressure is 4450 mmHg at 25°C. It is susceptible to photooxidation via vapor phase reaction with photochemically produced hydroxyl radicals. An atmospheric half-life of 5.4 days has been calculated. It will also exhibit very high mobility in soil and, therefore, it may leach to groundwater. If dimethyl ether is released to water, it will not be expected either to significantly absorb to sediment or suspended particulate matter, bioconcentrate in aquatic organisms, or directly photolyze. No data concerning the biodegradation of dimethyl ether in environmental media were located but on many ethers are known to be resistant to biodegradation. Dimethyl ether would not be expected to bioconcentrate in aquatic organisms.

Ecotoxicology

Acute toxicity to aquatic organisms is not particularly high with 48 h no-observed-effect concentrations greater than 4000 ppm in both daphnids and guppies.

Exposure Standards and Guidelines

There are no exposure standards but one of the producers for dimethyl ether has an internal guideline that suggests airborne exposure of 1000 ppm both 8 and 12 h time-weighted averages would not be xpected to result in adverse health effects.

See also: Anesthetic Agents.

Further Reading

American Industrial Hygiene Association (1996) *Workplace Environmental Exposure: Dimethyl Ether.*

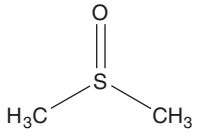
Bohnenn LJM (1979) Dimethyl ether: Alternative aerosol propellant. *Drug and Cosmetic Industry* 125: 58–74.
Caprinol TG (1975) Toxicological aspects of dimethyl ether. *European Journal of Toxicology And Environmental Hygiene* 8: 287–290.
Kirwin CJ and Galvin JB (1993) Ethers. In: Clayton GO, Claytin FE, and Allan RE (eds.) *Patty's Industrial Hygiene and Toxicology*, 4th edn., vol. II, PartA, pp. 445–525. New York: Wiley Interscience.

Dimethyl Sulfoxide

Samantha E Gad

This article is a revision of the previous print edition article by Daniel Steinmetz, volume 1, pp. 483–484, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 67-68-5
- Synonyms: Methyl sulfoxide, DMSO
- Chemical/Pharmaceutical/Other Class: Sulfoxides
- Chemical Formula: C_2H_6OS
- Chemical Structure:



Uses

Dimethyl sulfoxide (DMSO) has excellent solvent properties and acts as a skin penetration enhancer for drugs and other substances by increasing the permeability of the barrier layer of the skin. It is used in the topical administration of drugs, the production of synthetic fibers, the application of pesticides, as an antifreeze, hydraulic fluid, and in the manufacturing of industrial cleaners and paint strippers. Its anti-inflammatory and analgesic effects, and the ability to quench free radicals have been by physicians and others for various therapeutic uses.

Exposure Routes and Pathways

Exposure via dermal absorption and inhalation for hospital and veterinary personnel is common due to the use of DMSO in drugs. Industrial applications may lead to dermal and eye contact, and inhalation, with oral exposure a less likely route. The public may also be exposed via the use of DMSO in drugs, and to low levels via the environment (e.g., air and drinking water) via human uses and from natural productions (see below).

Toxicokinetics

DMSO is readily absorbed by animals and humans by dermal and oral routes and enhances absorption of many other chemicals by those routes. Higher concentrations of DMSO are more readily absorbed than more dilute solutions of DMSO in water. After dermal application, radiolabeled DMSO has been detected in blood within five minutes along with halitosis resulting from a metabolite, dimethyl sulfide. Distribution to other organs has been reported to occur within 20 min. Radiolabeled DMSO was detected in bones and teeth of animals within 1 h. DMSO can affect ionic balance due to its facilitation of the absorption of many other substances through biological membranes. The major metabolites of DMSO in humans are dimethyl sulfone and dimethyl sulfide. Following oral administration, about two-thirds of the dose is excreted in urine as unchanged DMSO, $\sim$20% as dimethyl sulfone, and $<$5% is exhaled as dimethyl sulfide. These metabolites have also been identified in monkeys and rats. In the eyes, the highest levels appear to accumulate in the cornea, and the lowest in the lens. DMSO has a calculated half-life of 16 h in blood; the corresponding value for dimethyl sulfone is 38 h. About 3% of oral doses is exhaled as dimethyl sulfide in humans; the percentage appears to be slightly higher in animals. DMSO can persist in serum for more than 2 weeks after a single exposure.

Mechanism of Toxicity

Most physiological properties of DMSO appear to be related to its penetration properties, its potential to inhibit or stimulate enzymes and to act as a free radical scavenger, and its ability to cause histamine release from mast cells. These properties are largely based on DMSO's chemical characteristics, including its hydrogen bonding behavior, water affinity, ability to interchange with water in membranes, and ability to react with organic molecules.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of DMSO is generally quite low in animals. DMSO causes mild skin and eye irritation in rabbits at as low as 100 mg. Rat oral LD_{50} values range from 14.5 to 28 g kg^{-1} and dermal LD_{50} values range up to 40 g kg^{-1}. The intraperitoneal and intravenous LD_{50} in mice, rats and dogs exceeds 15 g kg^{-1}. Acute lethal doses in experimental animals have been shown to produce rapid breathing, restlessness, coma, hyperthermia, and rapid death, or death after several days caused by renal failure. DMSO is an experimental teratogen and also causes other reproductive effects in experimental animals.

Human

DMSO is an irritant of the eyes, skin, and respiratory system. Absorption rapidly results in a garlic-like taste and odor. The odor has also been described as an oyster – or onion – like breath and body odor. Dermal application can produce erythema, scaling, contact urticaria, and stinging and burning sensations. Nausea, vomiting, abdominal cramps, chills, chest pains, and drowsiness have been reported, along with erythema, and itching, and transient hemolysis with hemoglobinuria. Anaphylaxis has been said to be a rare occurrence, and transient photophobia and color vision disturbances have been reported.

Chronic Toxicity (or Exposure)

Animal

Repeated exposures lead to renal and hepatic lesions. Prolonged eye contact causes corneal injury (opacities), and repeated dermal application results in irritation and urticaria. DMSO has been reported to cause adverse reproductive effects in animals. It is a questionable carcinogen with some experimental tumorigenic data.

Human

Overexposure may result in urticaria, headache, lethargy, nausea, and dizziness. In a few cases, eosinophilia and sulfhemoglobinemia have been reported following intravenous administration of DMSO. The human TD_{Lo} is 1800 mg kg^{-1} for skin, and 606 mg kg^{-1} for intravenous administration. The lenticular changes causing myopia seen in animals following chronic use have not yet been reported in humans.

Clinical Management

Eye exposure should be followed by irrigation with water for at least 15 min; exposed skin should be washed thoroughly with soap and water. Resulting burns or skin irritation should be treated with standard therapy. Cases of dermal sensitization reactions may require topical antiinflammatory agents. If DMSO is swallowed, vomiting should not be induced. Charcoal in water, or with a cathartic should be administered to prevent absorption. Liver and kidney function and blood parameters should be monitored.

Environmental Fate

DMSO is naturally released in the environment, primarily by the oxidation of dimethyl sulfide that is biologically produced in soil, water, and vegetation. It is produced by phytoplankton, and may be released during its production, transport, disposal, and use as a solvent, medicinal analgesic, and other uses. It is fairly resistant to biodegradation based upon screening tests. DMSO may be reduced by some reducing agents that may occur in soil. If released in water, it should disproportionate to dimethyl sulfide and dimethyl sulfone, and may be reduced by reducing agents that may occur in natural waters. In the atmosphere, DMSO will exist primarily in the vapor phase, and will react with photochemically produced hydroxyl radicals with a half-life of $\sim$7 h. It also may be released during its production, transport, disposal, and use as a solvent and medical analgesic.

See also: Sensory Organs.

Further Reading

Aguilar JS, Roy D, Ghazal P, and Wagner EK (2002) Dimethyl sulfoxide blocks herpes simplex virus-1 productive infection *in vitro* acting at different stages with positive cooperativity. Application of micro-array analysis. *BMC Infectious Diseases* [electronic resource] 24: 9.

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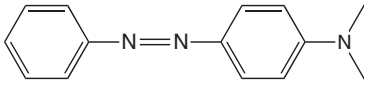
Dart RC (ed.) (2004) *Medical Toxicology*, 3rd edn. Philadelphia: Williams and Williams.

Hostynek JJ (2003) Factors determining percutaneous metal absorption. *Food and Chemical Toxicology* 41: 327–345.

Dimethylaminoazobenzene

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 60-11-7
- Synonyms: 4-Dimethylaminoazobenzene (preferred name); DAB; DMAB; 4-Dimethylaminoazobenzol; 4-Dimethylaminophenylazobenzene; Dimethyl yellow; Brilliant fast oil yellow; Fast yellow; Fat yellow
- Chemical Formula: $C_{14}H_{15}N_3$
- Chemical Structure:



Uses

Dimethylaminoazobenzene was once used as a coloring agent for butter and margarine. Dimethylaminoazobenzene was also used as an intermediate in the production of dyes, photosensitive polymers, and reusable films. Dimethylaminoazobenzene is no longer used as a dye and coloring agent.

Toxicokinetics

In laboratory animals, dimethylaminoazobenzene is metabolized in the liver to 4-aminoazobenzene and *N*-hydroxy-4-aminoazobenzene and excreted in bile.

Mechanism of Toxicity

Metabolites of dimethylaminoazobenzene can bind to liver cell macromolecules. Binding to liver cell macromolecules can then lead to liver carcinomas. Diets high in riboflavin have been shown to reduce the binding of dimethylaminoazobenzene to liver macromolecules. The reduced binding was shown to result in a reduced incidence of liver carcinomas.

Acute and Short-Term Toxicity (or Toxicity)

Animal

The oral LD_{50} has been reported as $200\,mg\,kg^{-1}$ in rats and $300\,mg\,kg^{-1}$ in mice.

Human

Overexposure to dimethylaminoazobenzene has been reported to produce skin, lung, and blood damage. Signs and symptoms of overexposure include contact dermatitis, difficulty in breathing, coughing, bloody sputum, bronchial secretions, methemoglobinemia, bloody urine, and frequent and painful urination.

Chronic Toxicity (or Exposure)

Animal

Oral administration of dimethylaminoazobenzene has been shown to produce liver cancer in rats and bladder tumors in dogs. Carcinogenic effects have also been produced following dermal and subcutaneous applications in rats and mice. Dimethylaminoazobenzene is also a teratogen.

Human

The only long-term effect reported for workers exposed to dimethylaminoazobenzene was contact dermatitis.

In Vitro Toxicity Data

Dimethylaminoazobenzene is mutagenic in genotoxicity studies.

Clinical Management

There is no special clinical treatment for overexposures to dimethylaminoazobenzene. Basic life support measures should be implemented and further chemical exposure and absorption should be prevented by removing contaminated clothing and washing the affected area. If ingested, the esophagus and digestive tract may be irritated and may be burned. Therefore, a careful examination should be performed and gastric lavage instituted only if the esophagus is not damaged. It is believed that lavage may be effective at removing the ingested material. Medical examination should look for signs of irritation, abnormalities, and hypersensitivity.

Environmental Fate

Dimethylaminoazobenzene may be released to the environment as a waste industrial product or from unintentional accidental releases. If released to soil it is expected to adsorb strongly to soil particles and not percolate down to groundwater. However, the chemical exists mostly in its ionized form in soils with neural and basic pHs. Therefore, the degree of water solubility and percolation will be influenced by soil and water pHs. The chemical is soluble in

organic solvents (alcohol, ether, oil) and essentially insoluble in water (its solubility is in the parts per million range). Therefore, if released to water, it may bioconcentrate in aquatic organisms and/or adsorb to organic matter in sediment. No information is available regarding biodegradation rates for the chemical in environmental media.

Exposure Standards and Guidelines

Dimethylaminoazobenzene is listed as a hazardous air pollutant under the Clean Air Act and, when released to the environment, is a hazardous waste. The US Environmental Protection Agency has classified dimethylaminoazobenzene as a probable human carcinogen. This classification is based on the fact that, although there is no epidemiological evidence that links dimethylaminoazobenzene exposure to the development of human cancer, there is sufficient evidence from laboratory animal studies.

National Institute for Occupational Safety and Health considers 4-dimethylaminoazobenzene to be a potential occupational carcinogen. Special precautions must be taken when working with dimethylaminoazobenzene. Personnel handling dimethylaminoazobenzene must follow industrial hygiene and health protection requirements for handing potentially carcinogenic substances. At a minimum dimethylaminoazobenzene exposure should be minimized through the use of engineering controls, work practices, and personal protective equipment, including impervious and disposable gowns and gloves as well as eye and respiratory protection. In addition, working areas and working instruments must be especially designed for handling potentially harmful substances.

See also: Dyes; Food, Drug, and Cosmetic Act, US; Polymers.

Further Reading

Klaassen CD (ed.) (2001) *Casarett & Doull's Toxicology, The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.

Rossoff IS (2002) *Encyclopedia of Clinical Toxicology*. Boca Raton: The Parthenon Publishing Group.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Dimethylaminoazobenzene.

Dimethylmercury

Diem HaMai and Stephen C Bondy

- Chemical Abstracts Service Registry Number: CAS 593-74-8
- Synonyms: Mercury dimethyl; Methylmercury; Methyl mercury
- Chemical/Pharmaceutical/Other Class: Alkyl mercuries
- Chemical Formula: $(CH_3)_2Hg$
- Chemical Structure: $CH_3–Hg–CH_3$

Uses

The primary use of dimethylmercury (DMM) is to calibrate research equipment, as in its application as a standard reference material for ^{199}Hg NMR measurements.

Background Information

History

In 1997, Karen Wetterhahn, an internationally renowned researcher of the carcinogenic effects of heavy metals on DNA repair proteins, was killed within a few months after a single exposure of less than a milliliter of DMM on her latex-covered hand.

Interest

Monomethylmercury (MMM) is the most toxicologically prominent of organic mercury compounds due to its environmental ubiquity and high potential for bioconcentration. Although DMM is less frequently encountered, it exhibits a far more toxic profile. Based on the lethality of only a few drops of the substance, DMM has been classified as a 'supertoxic' chemical. Absorption of $\sim$100 µl of the colorless liquid is equivalent to a severely toxic dose of 100–200 mg of mercury per 100 ml of whole blood. Its synthesis, transportation, and use should be minimized and exercised with only extreme care.

DMM is much more lethal to humans and other mammals than is MMM, yet studies in isolated cell systems reveal that MMM is more toxic than DMM. This paradox arises from the interaction of several factors:

1. DMM is highly lipophilic, and is thus sequestered longer in tissue depots than is MMM. This

also allows more rapid access to the nervous system.
2. DMM exists at room temperature as a liquid with high volatility. This is in contrast to the solid state of pure MMM. Together with its lipophilicity, these physical qualities enable high concentrations of DMM to be rapidly absorbed by the skin and lungs. Effectively, these routes circumvent first-pass elimination, thereby prolonging the systemic circulation of DMM and extending its residence time in the body.
3. The catabolism of DMM to MMM is required in order to enable its neurotoxicity.

Exposure Routes and Pathways

The primary routes of exposure to DMM are dermal contact and inhalation.

This substance has extensive lipid solubility and is absorbed immediately by the skin. Additionally, DMM is able to penetrate many materials including plastic and rubber compounds such as latex, polyvinyl chloride, and neoprene in a matter of seconds. In permeability tests, a Silver Shield glove of a flexible, plastic-laminate, offered skin protection from DMM for ~4 h. This chemically resistant glove, when worn under an outer glove that is resistant to abrasion and tears, may provide limited protection for direct handling of DMM.

The inhalation route of entry is also toxicologically significant. Because of its high vapor pressure (50–82 mmHg at 20°C), a toxic fume with a slightly sweet odor forms at room temperature. To avoid inhalation of mercury vapors, all work with DMM must be conducted under a hood with the handler wearing a face shield.

Exposure to DMM may also occur via ingestion with absorption occurring at an order of 90–95%.

Toxicokinetics

DMM differs significantly from its inorganic counterparts in its toxicokinetics and health effects. The absorption, distribution, metabolism, and excretion of DMM resemble that of other organic mercuries, particularly the alkylated ones.

DMM is rapidly absorbed and distributed, yet its metabolism and excretion is relatively slow. The difference between its rate of uptake and elimination could in part account for the severity of the toxic effects of DMM, its long latency period of several months, and its pronounced effects on the brain.

Absorption and Deposition

The extensive lipophilicity of DMM allows the substance to pass readily through biological membranes. This chemical property facilitates its near instantaneous absorption by the skin, lungs, and gastrointestinal tract, and results in its accumulation in depots of adipose tissue, plasma proteins, and brain. Approximately 10% of the body burden of organic mercury is localized in the brain.

Metabolism and Transport

During an initial lag period, DMM undergoes conversion to the monomethylated form, and distribution from blood to tissues (half-life of uptake into hair, 6 days). This biotransformation may occur at sites rich in metabolic enzymes such as the skin, intestinal flora, the liver, and macrophages. Free-radical mechanisms are proposed as another possible means of dealkylation.

In tissues, its monomethylated metabolite may undergo further biotransformation. Ultimately, conversion into inorganic mercury enables the metal to bind to glutathione for biliary excretion. However, much of this complex can also be reabsorbed by the gastrointestinal tract. Such bile-hepatic recycling permits redistribution of mercury.

In contrast to inorganic mercury, alkyl mercuries do not induce metallothionein synthesis in renal or liver cells.

Elimination

In humans and mice, the excretion of organic mercury occurs largely by the fecal route, and follows first-order kinetics. The whole body clearance times and blood clearance periods are longer than those for inorganic mercury with the half-life of DMM being ~78 days in humans. Other excretory routes are urine, sweat, and hair.

Mechanism of Toxicity

In contrast to the white crystalline solids of the pure forms of MMM and phenylmercury, DMM exists as a colorless liquid at room temperature with high volatility. These physical qualities enable high concentrations of the substance to be absorbed by exposure pathways of the skin and lungs that circumvent first-pass elimination. Effectively, this prolongs the systemic circulation of DMM, and extends its residence time in the body.

The additional alkyl group flanking the mercury imparts DMM with lipophilicity that exceeds its monoaklyated counterpart, and allows DMM to be sequestered in lipid-rich depots. The metabolic delay

allows the neurotoxicity of DMM to remain latent for months.

The gradual conversion into MMM results in the release of DMM from depots such as lipid-rich tissues and plasma proteins, and permits its movement through barriers such as the blood-brain and placenta. A cysteine complex of the monomethylated metabolite penetrates the endotheilial cells of the blood–brain barrier by mimicking methionine and using the large neutral amino acid transporter.

Thus, the toxicity of DMM is mediated by its dealkylation. Cleavage of the carbon–mercury bond generates MMM metabolites, which can form covalent bonds with cellular ligands with amphiphilic properties. The mercury center reacts with sulfur and sulfur-containing thiol groups of enzymes and thereby inhibits them. The metal center of DMM acts as a soft acid, and binds tightly to polarizable donor atoms in soft bases. Within cells, mercury may interact with a variety of proteins, particularly microsomal and mitochondrial enzymes. This can severely impair cell function.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicokinetics and health effects of DMM closely resemble that of MMM. Reports of toxicity due to organic mercury compounds are largely based on the administration of the monomethylated form.

Human

DMM is primarily a neurotoxin, and lethality can result from a single exposure to a few drops. Immediate adverse effects may include irritation to the eyes, respiratory tract, and skin. However, symptoms of intoxication may remain latent for months after the exposure.

The earliest clinical deficits include numbness and tingling sensation of the lips, hands and feet, joint pain, narrowing of vision, hearing difficulties, a widely based gait, and emotional disturbances. These symptoms arise from whole-blood concentrations of mercury that exceed 200 μg $Hg\,l^{-1}$ of whole blood (normal concentration, 1–8 μg $Hg\,l^{-1}$). The progression of symptoms includes incoordination, difficulty in pronouncing words, deafness, emotional disturbances, and ultimately, death.

The small granule cells of the cerebellum are selectively vulnerable to DMM. The cortex is also profoundly affected. Extensive neuronal loss and gliosis occur bilaterally within the primary visual cortex (especially at the calcarine fissure) and auditory cortex, with milder loss in the motor and sensory cortices.

Chronic Toxicity (or Exposure)

Animal

Behavioral, developmental, and systemic effects have been reported for a various rodent species in studies of MMM. In rodents, neuronal degeneration with wide-spread calcium deposition has been found in the brain following chronic daily exposure to DMM.

Human

No reports could be found on the effects of chronic toxicity of DMM in humans.

In Vitro Toxicity Data

Few studies have compared the toxicity of DMM with MMM. In cell culture, MMM is the more toxic of the two compounds. MMM has been shown to inhibit nucleic acid synthesis and decrease cell viability in cultured cells whereas exposure to DMM has resulted in no significant effects on these parameters. DMM induced chromosomal aberrations and mitotic spindle disturbances to a far lesser extent than the monomethylated form. This underscores the significance of its biotransformation to DMM toxicity.

Clinical Management

Chelating agents for mercury, such as cysteine and penicillamine, have been used as intervention measures to reduce the concentration of inorganic mercury. However, chelation therapy has yielded variable success in cases of alkyl mercury poisoning. Studies of MMM suggest that chelators may reduce brain and blood mercury levels if started within a few days after exposure. Surgical gallbladder drains and oral administration of a nonabsorbable thiol resin have been applied in order to interrupt biliary excretion and reabsorption of mercury by the intestine.

In the recent Wetterhahn case, the use of oral succimer (2,3-dimercaptosuccinic acid) and exchange transfusion at the initial onset of symptoms was successful in increasing urinary excretion of mercury and decreasing whole-blood mercury concentration. However, despite repeatedly normal CT and MRI scans of the brain, the patient quickly became unresponsive to all visual, verbal, and light-touch stimuli, 22 days after the first neurological symptoms developed (and 176 days after exposure). This suggests that chelators have little clinical benefit if begun after the onset of neurological symptoms.

Ecotoxicology

Most data is confined to MMM which can be generated from inorganic mercurial wastes by plankton and then progressively bioaccumulated. The final concentration in animals higher up the food chain can be several orders of magnitude higher than those present in the original water. Bacterial synthesis of DMM also takes place in marine and estuarine environments. It is present there at very low levels since it is rapidly hydrolyzed to MMM or vented into the atmosphere.

Other Hazards

DMM is highly flammable in the presence of strong oxidizing agents. Elevated temperatures cause decomposition into explosive hydrocarbon gases.

See also: Methylmercury; Minamata.

Further Reading

Nierenberg DW, Nordgren RE, Chang MB, *et al.* (1998) Delayed cerebellar disease and death after accidental exposure to dimethylmercury. *New England Journal of Medicine* 338: 1772–1776.

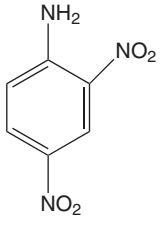
Ostlund K (1969) Studies on the metabolism of methyl mercury and dimethyl mercury in mice. *Acta Pharmacologicaet et Toxicologica* 27(Suppl 1): 1–136.

Weiss B, Clarkson TW, and Simon W (2002) Silent latency periods in methylmercury poisoning and in neurodegenerative disease. *Environmental Health Perspectives* 110(Suppl 5): 851–854.

Dinitroanilines

Robert A Young

- Chemical Abstracts Services Registry Numbers: There are four isomeric forms of dinitroaniline – 2,3-Dinitroaniline (CAS 602-03-9); 2,4-Dinitroaniline (CAS 97-02-9); 2,6-Dinitroaniline (CAS 606-22-4); 3,5-Dinitroaniline (CAS 618-87-1)
- Synonyms:
  - 2,3-Dinitroaniline; 2,3-Dinitrobenzenamine; 2,3-Dinitrophenylamine
  - 2,4-Dinitroaniline; 2,4-Dinitrobenzenamine; 2,4-Dinitrophenylamine
  - 2,6-Dinitroaniline; 2,6-Dinitrobenzenamine; 2,6-Dinitrophenylamine
  - 3,5-Dinitroaniline; 3,5-dinitrobenzenamine; 3,5-dinitrophenylamine
- Chemical/Pharmaceutical/Other Class: Aromatic amine
- Chemical Formula: $C_6H_5N_3O_4/C_6H_3(NH_2)(NO_2)_2$
- Chemical Structure: The structure of 2,4-dinitroaniline, the most common dinitroaniline, is shown below



Uses

2,4-Dinitroaniline is used in the production of azo dyes.

Exposure Routes and Pathways

Exposure to dinitroanilines is most likely to occur in occupational settings. Inhalation and dermal exposure are the primary routes of exposure.

Toxicokinetics

Most of the information on the toxicokinetics of dinitroanilines pertains to 2,4-dinitroaniline. Dinitroanilines are highly toxic to humans and are well absorbed from all routes of exposure. Nine metabolites were detected in rats administered [^{14}C]2,4-dinitroaniline orally or intravenously. 2,4-Dinitrophenylhydroxylamine was the main metabolite and was excreted in the urine as the sulfate conjugate and in bile as the glucuronide. Amine hydroxylation and sulfation of 2,4-dinitroaniline are probable detoxification processes that occur rapidly and facilitate clearance.

In rats administered [^{14}C]2,4-dinitroaniline per kilogram orally or intravenously, there was rapid distribution of the compound to all major tissues. Muscle, skin, and adipose tissue contained 65–70% of the ^{14}C activity in the body during the 45 min after dosing. Approximately 70–85% of the aforementioned doses were cleared from most tissues within 6 h after administration. Three days after administration, only residual levels were detected in the major tissues.

Urinary excretion of ^{14}C activity at 6 and 24 h after dosing accounted for 30% and 63%, respectively, of the administered dose. Fecal excretion over 3 days accounted for 23% of the dose. Elimination of 2,4-dinitroaniline-derived ^{14}C activity in the bile amounted to 12.5% of the dose after 5 h.

Mechanism of Toxicity

Much of the toxicity associated with dinitroaniline exposure is the result of methemoglobin formation in which the iron of the hemoglobin molecule is oxidized causing a deficiency in the oxygen carrying capacity of the blood. This produces the cyanosis and other signs of dinitroaniline-induced toxicity.

Acute and Short-Term Toxicity (or Exposure)

Human

Little definitive information is available regarding the toxic effects of dinitroanilines. Dermal and ocular exposure may result in irritation and pain. Inhalation exposure to dinitroanilines may cause irritation, coughing, and throat soreness. Aniline, a structurally similar compound, is a skin and eye irritant and a mild dermal sensitizer. It is rapidly absorbed by all routes of exposure and induces methemoglobinemia. Signs and symptoms of methemoglobinemia include blue skin, headache, dizziness, weakness, lethargy, loss of coordination, coma, and death. Headache and confusion occur early following poisoning, and restlessness, seizures, and coma may occur following severe poisoning. Acute exposure to 3–5 mg kg^{-1} is associated with signs and symptoms of toxicity that develop within a few hours following exposure. Liver and kidney damage may ensue within 12–72 h post-exposure and are probably secondary, hemolysis-mediated effects. As little as 1 g of aniline has caused human fatalities. The mean lethal dose for humans of the structurally related aniline has been estimated to be in the range of 15–30 g.

Chronic Toxicity (or Exposure)

Animal

The oral LD_{50} values for laboratory species range from 418 mg kg^{-1} (rat) to 1050 mg kg^{-1} (guinea pig). A 4 h inhalation LC_{Lo} of 17 mg m^{-3} is reported for the laboratory rat. No signs of toxicity were observed in male Fischer 344 rats administered up to 90 μmol [^{14}C]2,4-dinitroaniline per kilogram orally or 10 μmol kg^{-1} intravenously. Animal studies have also shown varying effects on thyroid function.

Human

Human data on the effects of chronic exposure to dinitroanilines are lacking. An increase in the severity of damage to the organs affected by acute exposure would be expected. Additionally, the adverse health effects resulting from prolonged methemoglobinemia are likely to be significant.

Clinical Management

For inhalation exposures, the victim should be removed from the exposure environment and 100% humidified supplemental oxygen should be administered with assisted ventilation as required. Exposed skin and eyes should be copiously flushed with water and thoroughly decontaminated to prevent further absorption. For oral exposure, clinical management should focus on decreasing absorption. Emesis may be indicated in recent substantial ingestion unless the patient is or could rapidly become comatose or convulsive. Emesis is most effective if initiated within 30 min. Gastric lavage may be indicated if performed soon after ingestion or in patients who are comatose or at risk of convulsing. If the patient is cyanotic and symptomatic, or the methemoglobin level is greater than 30% in an asymptomatic patient, measures should be taken to correct the methemoglobinemia.

Exposure Standards and Guidelines

There are currently no regulatory or health-based guidance values for dinitroanilines.

See also: Aniline.

Further Reading

Grayson M and Eckroth D (eds.) (1978) *Kirk–Othmer Encyclopedia of Chemical Technology*. New York: Wiley.

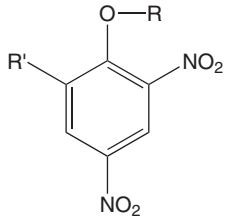
Sax NI and Lewis RJ Sr. (eds.) (1989) *Dangerous Properties of Industrial Materials*, 7th edn. New York: Van Nostrand Rienhold.

Dinitrophenols

David Janz

This article is a revision of the previous print edition article by Tamal Kumar Chakraborti, volume 1, pp. 485–487, © 1998, Elsevier Inc.

- Representative Chemicals: Dinitrophenol (DNP) occurs in six different isomers – 2,3-DNP, 2,4-DNP, 2,5-DNP, 2,6-DNP, 3,4-DNP, and 3,5-DNP
- Synonyms: A number of substituted 2,4-DNPs are sold under different trade names; analogs include DNOC, 2,4-Dinitro-6-methylphenol; Binapacryl, 2-*s*-Butyl-4,6-dinitrophenol-3-methylcrotonate; Dinocap, 2,4-Dinitro-6-(1-methy-*n*-heptyl)-phenylcrotonate; Dinoseb, 2,4-Dinitro-6-*s*-butylphenol
- Chemical Structure:



Uses

Dinitrophenols are used as fungicides, herbicides, or insecticides. The fungicidal, herbicidal, or insecticidal properties depend on minor differences in the chemical structures of the different dinitrophenol compounds. Several dinitrophenol compounds have more than one pesticidal use. The pesticidal use of one dinitrophenol, dinoseb, was eliminated in the United States in 1986. There has recently been a voluntary cancellation of all US product registrations for the fungicide/miticide Dinocap.

Exposure Routes and Pathways

Dinitrophenol compounds can enter the body through inhalation, oral, or dermal routes of exposure.

Toxicokinetics

Dinitrophenols are rapidly absorbed from the gastrointestinal tract, respiratory tract, and intact skin. They can bind to plasma proteins. After absorption, they are transported through the blood to different organs and distributed in the liver, the kidneys, and the eyes.

Dinitrophenols undergo reduction in the presence of NADPH and nitroreductase and conjugation takes place at the phenolic site. Humans can slowly detoxify 2,4-DNP to 2-amino-4-nitrophenol, 2-nitro-4-aminophenol, and 2,4-diaminophenol and their glucuronic acid conjugates. The metabolism of DNP is temperature dependent (i.e., DNP metabolism is greatly diminished at low temperatures). In mice, a reduced LD_{50} and increased toxicity for dinitrophenols were observed with an increase in ambient temperature.

In humans, dogs, and rats, 2-amino-4-nitrophenol was found to be the major excretory product. Humans can slowly eliminate both the unchanged compound and the previously mentioned metabolites. Urinary excretion is considered to be the main route of elimination of dinitrophenols. The half-life in the serum of a severely poisoned farmer was calculated to be 13.5 days. The residence half-life in humans is estimated to be 5–14 days. The elimination half-life for dinitrophenols in mice was ~ 6 h.

Mechanism of Toxicity

Dinitrophenols act as uncouplers of oxidative phosphorylation. Oxygen consumption, body temperature, breathing rate, and heart rate are increased following exposure to toxic levels of dinitrophenols. The permeability of mitochondrial membranes to hydrogen ions was found to be increased with the failure of conversion of ADP to ATP. Dinitrophenols reduce the electrochemical (proton) gradient necessary for oxidative phosphorylation by releasing phenolic protons in the mitochondrial matrix. The energy produced due to oxidation is not utilized for the synthesis of ATP but elevates body temperature, which can lead to fatal hyperpyrexia. Inefficient circulation and respiration cannot meet the body's increased metabolic demand, resulting in anoxia and acidosis. Fat serves as an alternative fuel for metabolism. Weight loss occurs as a result of inhibition of lipogenesis from pyruvate and lactate following exposure to dinitrophenols.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dinitrophenols exhibit high acute toxicity in animals. The oral LD_{50}s in rats, mice, guinea pigs, and dogs were reported to be 30, 20–40, 65, and 30 mg kg^{-1}, respectively.

Human

Dinitrophenols are extremely toxic to humans and are well absorbed from all routes of exposure. Fatal

cases of poisonings have been reported as a result of dermal exposure to dinitrophenols. Fever is a very early sign of dinitrophenol toxicity. Hepatic and renal damage were reported within 12–72 h following acute exposure to dinitrophenols. Typical signs of dinitrophenol toxicity were reported to occur within a few hours following acute exposure to 3–5 $mg\,kg^{-1}$ of dinitrophenol. Acute signs of toxicity include elevation of blood pressure, heart rate, and body temperature; headache; and mental confusion. Severe poisoning may cause restlessness, seizures, and coma. Cerebral edema was reported in two cases of fatal poisoning. Typical gastrointestinal symptoms may include nausea, vomiting, and abdominal cramps.

Chronic Toxicity (or Exposure)

Animal

Signs seen with acute exposures can also be exhibited following repeated oral exposures to as little as $1\,mg\,kg^{-1}\,day^{-1}$ of dinitrophenol. Repeated low-level exposures ($2\,mg\,kg^{-1}\,day^{-1}$) can cause peripheral nerve damage. Dinitrophenols have been reported to cause cataracts (after repeated exposure).

Human

The concurrent use of sulfonamide drugs, which are able to bind preferentially to serum albumin, may greatly enhance the acute toxicity of dinitrophenols.

In Vitro Toxicity Data

Dinitrophenols can suppress germ cell viability *in vitro*.

Clinical Management

Only symptomatic treatment is available. Adequate measures should be taken to maintain fluid and electrolyte balance and keep the body temperature within tolerable limits. Measures should be taken to remove the poison from the body through gastric lavage and saline cathartic. Gastrointestinal absorption may be prevented by administering activated charcoal. Salicylates, which contain a phenolic group, must not be used as antipyretic agents during treatment of DNP poisoning. Therefore, control of temperature should be restricted to physical measures.

Environmental Fate

Dinitrophenols are expected to have high mobility in soil, particularly in moist soils since they will exist primarily as anions. If released into water, dinitrophenols will largely remain in solution and not adsorb significantly to particulate matter or sediment. Volatilization of dinitrophenols from soil or water is not expected to be a significant fate process. Dinitrophenols are not known to undergo hydrolysis in the environment, although photolysis may be an important abiotic degradation process. A bioconcentration factor of <1 in carp suggests that accumulation in aquatic organisms is very low.

Ecotoxicology

Dinitrophenols are very highly toxic to birds and highly toxic to fish. Toxicity is enhanced under acidic conditions. Dinitrophenols do not pose problems with bioaccumulation. Bees are sensitive to these toxicants.

See also: Dinoseb; Pesticides.

Further Reading

Hollingworth RM (2001) Inhibitors and uncouplers of mitochondrial oxidative phosphorylation. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn. San Diego, CA: Academic Press.

Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

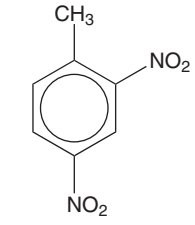
Dinitrotoluene

Robert A Young

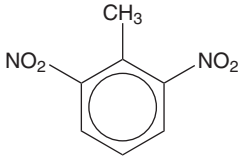
- Chemical Abstracts Service Registry Numbers: Dinitrotoluene (DNT) occurs in six isomeric forms: 2,3-DNT (CAS 602-01-7); 2,4-DNT (CAS 121-14-2); 2,5-DNT (CAS 619-15-8); 2,6-DNT (CAS 606-20-2); 3,4-DNT (CAS 610-39-9); 3,5-DNT (CAS 618-85-9)
- Synonyms:
  - 2,3-DNT: 1-Methyl-2,3-dinitrotoluol; 2,3-Dinitrotoluol

- 2,4-DNT: 1-Methyl-2,4-dinitrotoluol; 2,4-dinitcrotoluol
- 2,5-DNT: 1-Methyl-2,5-dinitrotoluol; 2,5-Dinitrotoluol
- 2,6-DNT: 2-Methyl-1,3-dinitrotoluol; 2,6-Dinitrotoluol
- 3,4-DNT: 1-Methyl-3,4-dinitrotoluol; 3,4-Dinitrotoluol
- 3,5-DNT: 1-Methyl-3,5-dinitrotoluol; 3,5-Dinitrotoluol; Binitrotoloene

- Chemical/Pharmaceutical/Other Class: Aromatic hydrocarbon
- Chemical Formula: $C_7H_6N_2O_4$
- Chemical Structure: The chemical structures of the most prevalent and toxicologically important dinitrotoluenes, 2,4-DNT and 2,6-DNT, are shown below



2,4-Dinitrotoluene



2,6-Dinitrotoluene

Uses

Dinitrotoluenes are intermediates in the production of toluene diisocyanate but are also used as gelatinizing and waterproofing agents in commercial and military explosives and in the production of polyurethane foams.

Exposure Routes and Pathways

Dinitrotoluenes (DNTs) may occur as a contaminant of soil, surface water, and groundwater. Because DNTs are of low volatility, exposure via the air is inconsequential. The primary route of exposure to DNTs is through contaminated groundwater and, due to their mobility, in surface water as well.

Toxicokinetics

Most of the toxicokinetic data for DNTs are for the 2,4- and 2,6-isomers. Data regarding the absorption of DNT following inhalation exposure are not available, but absorption may be inferred from data on urinary metabolites in workers exposed via inhalation. Efficient absorption of various DNT isomers following oral exposure has been verified in several animal species. In animals, ingested DNT appears to be readily absorbed (55–90%) within 24 h. Limited human data suggest that dermal exposure may result in significant absorption.

Urine from workers exposed to dinitrotoluene contained 2,4- and 2,6-DNT, 2,4- and 2,6-dinitrobenzoic acid, 2,4- and 2,6-dinitrobenzyl glucuronide, 2-amino-4-nitrobenzoic acid, and *N*-(acetyl)amino-4-nitrobenzoic acid. The most prevalent metabolites were 2,4-dinitrobenzoic acid and 2-amino-4-nitrobenzoic acid, collectively accounting for 74–86% of the dinitrotoluene metabolites detected. Bioactivation of dinitrotoluene in the rat is thought to occur by oxidation of the methyl group to an alcohol by a cytochrome P450-dependent pathway. The benzyl alcohol is then conjugated with glucuronic acid and excreted in the bile. Intestinal microflora hydrolyze the glucuronide and reduce one nitro group, forming an aminonitrobenzyl alcohol, which can be reabsorbed from the intestine. The amino group is oxidized to a hydroxylamine by hepatic enzymes and conjugated with sulfate. Decomposition of the sulfate ester yields a highly electrophilic nitrenium (or carbonium) ion that can react with DNA and other biological nucleophiles. Urinary excretion of these metabolites peaked near the end of the work shift but declined to low or undetectable concentrations by the start of work the following day. The calculated elimination half-lives of total dinitrotoluene-related material detected in urine ranged from 1.0 to 2.7 h and those of individual metabolites from 0.8 to 4.5 h.

Data regarding the distribution of DNT are limited to 2,4-DNT studies in animals. Following oral administration of 2,4-DNT to various laboratory species, the greatest concentrations of the chemical occurred in the liver, kidneys, and blood. Only small amounts were found in the brain, heart, and spleen. A biphasic increase in hepatic levels of 2,4-DNT in rats suggested that the chemical undergoes enterohepatic circulation.

Urinary excretion of these metabolites peaked near the end of the work shift but declined to low or undetectable concentrations by the start of work the following day. The calculated elimination half-lives of total dinitrotoluene-related material detected in urine ranged from 1.0 to 2.7 h and those of individual metabolites from 0.8 to 4.5 h. Urinary excretion in Fischer 344 rats given 2,6-DNT accounted for half of the dose ($10\,mg\,kg^{-1}$) 72 h after administration of [^{14}C]-2,6-DNT. 2,6-Dinitrobenzoic acid, 2,6-dinitrobenzyl alcohol glucuronide, and 2-amino-6-nitrobenzoic acid accounted for 95% of the urinary ^{14}C. Fecal excretion accounted for one-fifth of the dose in 72 h.

Mechanism of Toxicity

The most prominent toxicologic effect of DNT is the formation of methemoglobin and the subsequent effects of reduced oxygen carrying capacity of the blood, which produce the cyanosis and fatigue characteristic of DNT poisoning. DNT and/or its metabolites produce this effect by oxidizing the iron in the hemoglobin molecule. This process also leads to the formation of Heinz bodies, granule-like aggregates of precipitated hemoglobin, which serve as sensitive indicators of toxic insult to the blood. Hepatotoxic effects are due, in part, to cellular damage resulting in altered hepatocytes and deficiencies in biliary excretion. DNT has also been shown to disrupt Sertoli cell function, which may explain, in part, DNT's effect on the male reproductive system.

Acute and Short-Term Toxicity (or Exposure)

Animal

Most of the toxicity data are for the 2,4- and 2,6-DNT isomers. Oral LD_{50} values for 2,4-DNT are extremely variable ranging from 177 to 609 mg $kg^{-1}\,day^{-1}$ for rats and from 390 to 1647 mg kg^{-1} for mice. Rat and mouse oral LD_{50} values of 216 and 607 mg kg^{-1}, respectively, have been reported for 3,5-DNT. In addition to lethality, acute oral exposures of laboratory animals to 2,4-DNT have resulted in hematologic disorders (methemoglobinemia) and toxic effects in the male reproductive system. Longer-term oral exposures also induce hematologic and reproductive effects in addition to renal and neurologic disorders. For 2,6-DNT, oral LD_{50} values of 665 and 714 mg$kg^{-1}\,day^{-1}$ have been reported for rats and mice, respectively. The toxicologic effects of 2,6-DNT in animals are similar to those of 2,4-DNT.

Human

Most reports of human toxicity involve exposure to technical-grade DNT. Commercial-grade DNT is usually a combination of 2,4-DNT ($\sim$76%) and 2,6-DNT ($\sim$19%), with the remaining composition containing various other isomers.

The primary signs of toxicity regardless of the route of exposure are headache, fatigue, nausea, vomiting, and cyanosis resulting from methemoglobin formation. General signs and symptoms may be similar to those of alcohol intoxication. When methemoglobin levels approach 15%, cyanosis appears; and when the methemoglobin levels exceed 40%, weakness and dizziness occur. Methemoglobin levels above 70% may produce muscle tremors, cardiovascular effects, and death.

Chronic Toxicity (or Exposure)

Animal

2,4-DNT at an oral dose of 40 mg $kg^{-1}\,day^{-1}$ for 2 years produced liver tumors in rats and at a dose of 97 mg $kg^{-1}\,day^{-1}$ for 2 years produced renal tumors in male mice. 2,6-DNT at doses as low as 7 mg $kg^{-1}\,day^{-1}$ produced hepatocellular carcinomas in male rats following a 1 year oral exposure. The available data also indicate strain differences in the carcinogenic response for several of the DNT isomers.

Human

Long-term exposure to low levels of DNT will result in methemoglobinemia, the severity of which depends on the magnitude of the exposure. Although carcinogenic effects of DNT have been demonstrated in animals, there is currently no evidence of DNT carcinogenicity in humans.

The chronic oral reference doses for 2,4- and 2,6-DNT are 0.002 and 0.001 mg $kg^{-1}\,day^{-1}$, respectively. The oral slope factor for both 2,4- and 2,6-DNT is 6.8×10^{-1} $(mg\,kg^{-1}\,day^{-1})^{-1}$. The US Environmental Protection Agency (EPA) classifies the isomer mixture as a B2 carcinogen (probable human carcinogen; sufficient evidence in animals but inadequate or no evidence from epidemiologic studies). The 3,4-DNT isomer is not classifiable as to its carcinogenicity to humans. The American Conference of Governmental Industrial Hygienists threshold limit value for 2,4-DNT is 0.2 mg m^{-3}, the National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit for 2,4-DNT is 1.5 mg m^{-3}, and the NIOSH immediately dangerous to life or health (IDLH) is 50 mg m^{-3}. Both organizations categorize the chemical is a suspected human carcinogen.

In Vitro Toxicity Data

In mammalian cells *in vitro*, it induced DNA strand breaks, gene mutations in mouse lymphoma cells (without activation) but not in Chinese hamster ovary cells, and a low frequency of sister chromatid exchange but not of chromosomal aberrations in Chinese hamster ovary cells. It inhibited intercellular communication but did not induce cell transformation.

Clinical Management

For most cases of DNT poisoning, clinical management involves correction of the methemoglobinemia and associated support therapy.

Environmental Fate

Dinitrotoluenes may enter the environment in wastewater from the processes in which it is made and used. In soil, 2,4-DNT will be slightly mobile (estimated $K_{oc} = 282$). Based on aqueous biodegradation tests, 2,4-DNT may biodegrade in both aerobic and anaerobic zones of soil. 2,4-DNT in water will not bioconcentrate significantly (experimental BCF = 204) and will have a slight tendency to partition to suspended and sediment organic matter ($\log K_{ow} = 1.98$). Volatilization of 2,4-DNT from water will not be significant. Photolysis will probably be the most important removal process for 2,4-DNT in water. Photolytic half-lives for 2,4-DNT in river, bay, and pond waters were 2.7, 9.6, and 3.7 h, respectively, and the reaction was found to be accelerated in the presence of humic material. The importance of biodegradation in natural waters is unknown. In the atmosphere, 2,4-DNT is estimated to have a half-life of 71 days. 2,4-DNT has been detected in drinking water, seawater, river water, and in wastewater from 2,4,6-trinitrotoluene production.

Exposure Standards and Guidelines

The US EPA has calculated a reference dose (RfD) of $0.002\ mg\ kg^{-1}\ day^{-1}$ based on neurotoxicity in dogs and estimates that consumption of this dose or less, over a lifetime, would not likely result in the occurrence of chronic, noncancer effects.

See also: Pollution, Soil.

Further Reading

Grayson M and Eckroth D (eds.) (1978) *Kirk–Othmer Encyclopedia of Chemical Technology*. New York: Wiley.

Tchounwou PB, Newsome C, Glass K, *et al.* (2003) Environmental toxicology and health effects associated with dinitrotoluene exposure. *Reviews in Environmental Health* 18(3): 203–229.

US Air Force (1989) *The Installation Restoration Program Toxicology Guide*. OH: Wright-Patterson AFB Air Force Systems Command, Aerospace Medical Division.

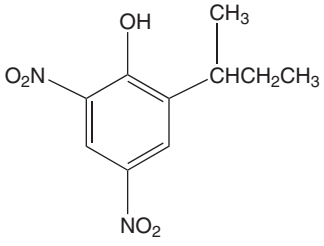
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dinitrotoluene.

Dinoseb

Priya Raman

- Chemical Abstracts Service Registry Number: CAS 88-85-7
- Synonyms: Dinitrobutylphenol (DNBP); 2-*sec*-Butyl-4,6-dinitrophenol; Basanite; Caldon; Chemox; Dynamyte; Elgetol; Gebutox; Hel-Fire; Kiloseb; Nitropone; Premerge; Sinox General; Subitex; Vertac Weed Killer
- Chemical/Pharmaceutical/Other Class: Dinitrophenol
- Chemical Structure:



Uses

Dinoseb is used as an herbicide, corn yield enhancer, insecticide, and miticide. It is used as a herbicide in soybeans, a variety of vegetables, fruits, nuts, on citrus trees, and with other field crops for control of grasses and broadleaf weeds. Dinoseb is used as an insecticide in grapes. It is produced in emulsifiable concentrates or as water-soluble ammonium or amine salts.

Exposure Routes and Pathways

Oral and dermal routes are the most common routes of exposure to dinoseb. Inhalation of dinoseb can also lead to serious complications.

Toxicokinetics

Dinoseb is rapidly absorbed from the gastrointestinal tract, respiratory tract, and intact skin. It undergoes oxidation of either of the two methyl groups on the *sec*-butyl chain, conjugation of the phenolic products,

and formation of many uncharacterized metabolites. Microsomal enzymes of rat liver reduce the *o*-nitro group of dinoseb. The compound is highly bound to plasma proteins. Hepatic and urinary excretions are the primary routes of elimination. Breakdown products are found in liver, kidney, spleen, blood, and urine. Dinoseb can pass through the placenta and into the fetus of experimental animals.

Mechanism of Toxicity

Dinoseb uncouples oxidative phosphorylation from electron transport by carrying protons across the inner mitochondrial membrane, thereby dissipating the pH gradient and membrane electrochemical potential and preventing the formation of adenosine triphosphate. Following exposure to this chemical, metabolism in all body cells is stimulated, resulting in an increase in oxygen consumption, body temperature, breathing rate, and heart rate. Dinoseb-induced weight loss may occur due to inhibition of lipogenesis from pyruvate and lactate. The body fat serves as the major fuel for the extra metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values in rats and guinea pigs were 25–58 $mg\,kg^{-1}$. The dermal LD_{50} in rabbits was 80–200 $mg\,kg^{-1}$ and 200–300 $mg\,kg^{-1}$ in guinea pigs. Dinoseb is not a skin irritant. Neurological and skeletal malformations have been observed in laboratory animals exposed to dinoseb.

Human

Acute exposure to dinoseb is associated with signs and symptoms of toxicity that develop rapidly within a few hours following exposure. Hyperthermia and profuse sweating are the early manifestations of toxicity. Liver and kidney damage may ensue within 12–72 h postexposure. Early symptoms of dinoseb toxicity include headache and confusion followed by restlessness, hyperactivity, seizures, and coma following severe poisoning. The respiratory rate is usually markedly increased. Sinus tachycardia, ventricular tachycardia, and ventricular fibrillation may occur. Following ocular exposure to dinoseb, cataracts, secondary glaucoma, paresis of accommodation, and nystagmus have been reported. Other signs and symptoms following exposure to dinoseb include fatigue, thirst, insomnia, weight loss, flushing of the face, nausea, vomiting, abdominal pain, occasional diarrhea, methemoglobinemia, and hemolytic anemia. Dinoseb has the potential to cause damage to the immune system, liver, kidneys, and spleen. Direct skin contact with dinoseb results in irritation, yellow stains, burns, and dermatitis.

Chronic Toxicity (or Exposure)

Animal

Pregnant rats given 200 ppm dinoseb in their feed showed reductions in fetal survival. Surviving fetuses exhibited lower than normal birth weights. Morphologic abnormalities of the kidney have been noted in the offspring of female rats given dinoseb; however, renal function and morphology subsequently returned to normal. Dinoseb administered intraperitoneally to pregnant rats on gestation days 10–12 at a dose of 10.5 $mg\,kg^{-1}\,day^{-1}$ caused a reduction in body weight in offspring. The no-observed-adverse-effect level of dinoseb for developmental toxicity was 3 $mg\,kg^{-1}\,day^{-1}$. Maternal toxicity and malformations of the eye have been observed among the offspring of pregnant rats fed 200 ppm of dinoseb. Studies in laboratory animals indicate that dinoseb has the potential to cause damage to the immune system. Dinoseb has been reported to cause decreased sperm count and abnormal sperm shape in male rats and mice following 3 weeks of exposure at low levels of $\sim$10 $mg\,kg^{-1}\,day^{-1}$ for 30 days. Dinoseb is a potential teratogen, low levels of which fed to rats and rabbits have been found to cause birth defects in the fetuses of exposed females.

Human

Little is known regarding chronic effects of dinoseb in humans.

In Vitro Toxicity Data

Dinoseb was not genotoxic or mutagenic as assessed by a number of *in vitro* assays.

Clinical Management

Exposure to dinoseb requires symptomatic treatment. Blood glucose, liver function, and renal function tests should be monitored in symptomatic patients. Adequate ventilation and oxygenation should be provided with close monitoring of arterial blood gases. The fluid and electrolyte balances should be maintained. The body temperature should be kept within tolerable limits. Antipyretic drugs are, however, not effective because dinoseb poisoning involves peripheral metabolism, not central nervous system control of temperature. Diazepam is administered to

overcome the accompanying seizure and convulsions following dinoseb exposure. In case of an oral exposure to dinoseb, gastrointestinal absorption may be prevented by gastric lavage and/or activated charcoal administration. Exposed eyes and skin should be irrigated with copious amounts of water following an ocular or dermal exposure to dinoseb.

Ecotoxicology

Dinoseb is highly toxic to birds, with oral LD_{50} values less than $10\,mg\,kg^{-1}$ and 5–8 day dietary LC_{50} values of around 500 ppm. Dinoseb is highly toxic to fish, with 96 h LC_{50} values of $44–118\,\mu g\,l^{-1}$. Dinoseb use can lead to fish kills from runoff following rain. Dinoseb is not bioaccumulated. Dinoseb is also toxic to bees.

Exposure Standards and Guidelines

The reference dose is $1\,\mu g\,kg^{-1}\,day^{-1}$.

See also: Developmental Toxicology; Dinitrophenols; Pesticides.

Further Reading

Hollingworth RM (2001) Inhibitors and uncouplers of mitochondrial oxidative phosphorylation. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1169–1261. San Diego, CA: Academic Press.

Relevant Website

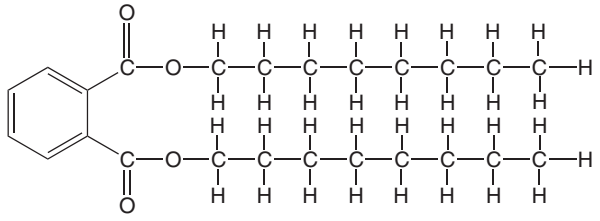
http://www.envirotools.org – Envirotools.org, Michigan State University.

Dioctylphthalate

Robert A Young

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 117-84-0
- Synonyms: Di-*N*-octylphthalate (preferred name); 1,2-Benzenedicarboxylic acid dioctyl ester; Di-octyl-*O*-benzenedicarboxylate; DNOP; *N*-Octyl phthalate; *O*-Benzenedicarboxylic acid dioctyl ester; Octyl phthalate; Phthal\ic acid dioctyl ester; Benzenedicarboxylic acid di-*n*-octyl ester
- Chemical/Pharmaceutical/Other Class: Alkyl phthalate
- Chemical Formula: $C_{24}H_{38}O_4$
- Chemical Structure:



Uses

Dioctylphthalate is used as a plasticizer in cellulose ester resins, polystyrene, and vinyl plastics. It is also a component of some pesticides.

Exposure Routes and Pathways

Humans may be exposed to dioctylphthalate in food (as an indirect food additive) and in drinking water. Although ingestion is the primary route of exposure for the general public, inhalation and dermal exposures may be more significant in occupational settings in which the chemical is used in industrial processes. Exposure via parenteral administration resulting from leaching of dioctylphthalate from plastic tubing and containers used in medical practice has also been documented.

Toxicokinetics

Definitive information regarding the absorption of dioctylphthalate is not available. Absorption may be inferred, however, due to systemic toxic effects following oral administration of the chemical and by analogy to absorption characteristics of similar phthalate esters. The limited information regarding the biotransformation of dioctylphthalate indicates that the chemical undergoes hydrolysis to a monoester within the intestines prior to absorption. However, it is also likely that hydrolysis may occur in the intestinal mucosal cells and in other tissues. Phthalate esters are generally widely distributed in the body. The effects observed in various organs and tissues following exposure to dioctylphthalate affirm its distribution throughout the body.

Chemical-specific elimination data for di-*N*-octylphthalate are not available. However, data from

animal studies using the diisoctylphthalate isomer have shown that it is excreted in the urine and bile as a monoester. Species-dependent quantitative and qualitative differences have been observed for excretion of this isomer as well as di-*N*-butylphthalate and bis(2-ethylhexyl)phthalate. Excretion half-lives of 1.2 and 5.4 h have been reported for these compounds.

Mechanism of Toxicity

Specific data regarding the mechanism by which dioctylphthalate causes toxic responses are not available. There is some evidence that the toxic effects observed for this chemical may be due to its mono-*l*-octyl ester metabolite.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats fed diets containing 20 000 ppm di-*N*-octylphthalate, an accumulation of large droplets of fat around central veins was observed that progressed to mild centrilobular necrosis and increased liver weight within 10 days.

Human

Definitive information regarding the acute toxicity of di-*N*-octylphthalate is not available. An estimated lethal oral dose in humans is between 0.5 and $15\,g\,kg^{-1}$, or between 1 oz equivalent to 29.6 mls and 1 qt equivalent to 0.96 liters in a 70 kg adult. Compounds that are structurally similar to di-*N*-octylphthalate are known to irritate mucous membranes resulting in irritation of the eyes, throat, and upper respiratory tract passages and in gastrointestinal disturbances. There is evidence that some phthalates, such as di-*s*-octylphthalate, may be reproductive and developmental toxicants. Generally, the acute oral toxicity of alkylphthalates is low and the acute oral toxicity decreases as molecular weight increases.

Chronic Toxicity (or Exposure)

Animal

Renal toxicity has been observed in rats and mice given di-*N*-octylphthalate in the diet (1000 ppm) for 48 weeks, and evidence of liver toxicity was noted for rats given a diet containing 3500 ppm for 7–12 months. Although many of the phthalate esters exert toxic effects on the male reproductive system, di-*N*-octylphthalate appears to be among the least potent. Evidence for developmental toxicity is equivocal. There is currently no evidence showing that di-*N*-octylphthalate is genotoxic.

Human

A case report noted the development of an asthmatic reaction in a worker continuously exposed to dioctylphthalate during a manufacturing process. Based on the known toxic effects of di-*N*-octylphthalate in animals, chronic exposure of humans may result in liver and kidney damage.

The chronic reference dose for di-*N*-octylphthalate is $0.02\,mg\,kg^{-1}\,day^{-1}$. No other regulatory or health-based guideline values are currently available for di-*N*-octylphthalate. Neither the US Environmental Protection Agency nor International Agency for Research on Cancer have evaluated the carcinogenicity of di-*N*-octylphthalate.

Clinical Management

The potential for esophageal or gastrointestinal tract irritation following ingestion suggests that emesis should not be induced. Other measures to prevent absorption may be beneficial. Gastric lavage may be indicated if performed soon after ingestion or if the patient is comatose or at risk of convulsing. Exposed skin and eyes should be copiously flushed with water.

Environmental Fate

Based on its vapor pressure, dioctylphthalate will likely exist in both the vapor and particulate phases in the atmosphere. The vapor will be degraded by reaction with photochemically produced hydroxyl radicals; the particulate phase will be removed from the atmosphere by wet and dry deposition. It is likely to adsorb to soil and sediment. As a result, volatilization and hydrolysis are not expected to be important fate processes in water.

See also: Phthalate Ester Plasticizers.

Further Reading

Kavlock R, Bockelheide K, Chapin R, *et al.* (2002) NTP center for the evaluation of risks to human reproduction: Phthalates expert panel report on the reproductive and developmental toxicity of di-*n*-octyl phthalate. *Reproductive Toxicology* 16(5): 721–734.

Kirk RE and Othmer F (eds.) (1998) *Kirk–Othmer Encyclopedia of Chemical Technology.* New York: Wiley.

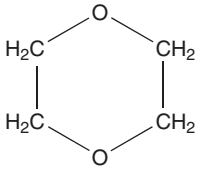
McKee RH, Butala JH, David RM, and Gans G (2004) NTP center for the evaluation of risks to human reproduction reports on phthalates: Addressing the data gaps. *Reproductive Toxicology* 18(1): 1–22.

Dioxane, 1,4-

Julie A Stickney and Eric M Silberhorn

This article is a revision of the previous print edition article by Leyna Mulholland, volume 1, pp. 492–494, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 123-91-1
- Synonyms: 1,4-Dioxane; Dioxane; Diethylene oxide; *p*-Dioxane; Glycolethyleneether; 1,4-Diethylenedioxide; *p*-Dioxan; Tetrahydro-*p*-dioxin; 1,4-Diethylene dioxide; Dioxyethylene ether
- Description: 1,4-Dioxane is a colorless liquid with a mild, ether-like odor. It is miscible in water and most organic solvents. It is relatively stable under normal temperature and pressure
- Chemical Structure:



Uses

1,4-Dioxane is a solvent widely used for a range of organic products, including cellulose acetate, nitrocellulose, other cellulose esters or ethers, fats, oils, waxes, mineral oil, natural and synthetic resins, and polyvinyl polymers. It has been used for wetting and dispersing in textile processing, dye baths, stain, and printing compositions. It is also found in cleaning and detergent preparations, adhesives, cosmetics, deodorants, fumigants, emulsions, and polishing compositions. It has been used as an ingredient in lacquers, paints, varnishes, and paint and varnish removers. 1,4-Dioxane is also used in purifying drugs and in cosmetic products such as shampoos and bath preparations. It has been used in the embedding process for the preparation of tissue sections for histology. Additionally, 1,4-dioxane has been used as a stabilizer for chlorinated solvents, particularly, 1,1,1-trichloroethane, in solvent applications.

Exposure Routes and Pathways

Exposures to 1,4-dioxane may occur through inhalation, ingestion, and dermal contact. In the occupational setting, workers would be exposed primarily through inhalation of vapors and contact with the skin. For the general population, 1,4-dioxane may be ingested as a contaminant of drinking water, soil, or food. Inhalation of vapors and skin contact are not considered to be major exposure routes for the general population.

Toxicokinetics

1,4-Dioxane is absorbed rapidly and completely following oral and inhalation exposure with much less absorption occurring from the dermal route. In both rats and humans, 1,4-dioxane is primarily metabolized to β-hydroxyethoxyacetic acid (HEAA), which is excreted in the urine. Data indicate that the metabolism of 1,4-dioxane is linear at exposure levels up to 50 ppm ($180\,mg\,m^{-3}$). The half-life for elimination of 1,4-dioxane in humans is $\sim 1\,h$ at concentrations of 50 ppm ($180\,mg\,m^{-3}$) or less. A similar half-life was observed in rats given low oral or intravenous doses of 1,4-dioxane ($<10\,mg\,kg^{-1}$). At oral or intravenous doses of $>10\,mg\,kg^{-1}$ in the rat, however, plasma clearance and HEAA excretion are reduced and unchanged 1,4-dioxane concentrations are increased in both urine and breath. Studies demonstrate that clearance of 1,4-dioxane from the blood is markedly nonlinear and dose dependent. The biotransformation of 1,4-dioxane to HEAA is a saturable process that is significantly altered by the magnitude of the administered dose. Multiple daily oral doses were excreted more rapidly than the equivalent single dose suggesting induction of metabolism at high doses and 1,4-dioxane has been shown to induce the mixed function oxidase enzyme system in the mouse liver. Physiologically based pharmacokinetic (PBPK) modeling approaches have been used to evaluate the risk of liver cancer in humans exposed to 1,4-dioxane. These models take into account the nonlinear pharmacokinetics observed in experimental studies as well as physiological differences between the rodent models and humans. Various toxicological data indicate that 1,4-dioxane toxicity occurs only after doses large enough to saturate processes for detoxification and elimination.

Mechanism of Toxicity

Pharmacokinetic and toxicological data indicate that liver and kidney toxicity induced by 1,4-dioxane occurs only after doses large enough to saturate processes for detoxification and elimination. 1,4-Dioxane is one of many carcinogens that have not been demonstrated to react covalently with DNA. Its

mode of action is not sufficiently well understood to permit assignment to a specific class of epigenetic agents. However, the data suggest a tumor promotion mechanism associated with tissue injury and subsequent regeneration. Eye and respiratory irritation occur from direct contact of 1,4-dioxane with mucous membranes.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute effects of exposure to high concentrations of 1,4-dioxane in animals include eye and respiratory irritation, nervous system effects, and liver and kidney damage. Guinea-pigs can tolerate inhalation of 2000 ppm 1,4-dioxane for several hours without serious symptoms. Higher concentrations produced eye, nose, and lung irritation. Dogs given 1,4-dioxane orally over a period of 9 days died after a total consumption of about $\sim 3\,g\,kg^{-1}$ with severe liver and kidney damage. Single doses of 5.66, 5.17, and $3.90\,g\,kg^{-1}$ to mice, rats, and guinea-pigs produced symptoms progressing from weakness, depression, incoordination, and coma to death. Autopsy revealed hemorrhage areas in the pyloric region of the stomach, bladders distended with urine, enlarged kidneys, and slight proteinurea without hematuria.

Human

Exposure to high concentrations of 1,4-dioxane may cause eye, skin, and respiratory irritation, nervous system effects, and liver and kidney toxicity. There are five cases of fatal poisoning in men who inhaled excessive amounts of 1,4-dioxane while working in a textile factory. Symptoms were irritation of the upper respiratory passage, coughing, irritation of eyes, drowsiness, vertigo, headache, anorexia, stomach pains, nausea, vomiting, uremia, coma, and death. Autopsies revealed congestion and edema of the lungs and brain and marked injury of the liver and kidneys. Blood analysis of these victims showed no abnormalities other than considerable leukocytosis. Twelve humans were exposed to 200 ppm 1,4-dioxane for 15 min, considered to be the highest acceptable dose; at 300 ppm it caused irritation of the eyes, nose, and throat. Death was reported in one worker after 1 week on a job where the average concentration of 1,4-dioxane vapor was 470 ppm. Possible skin absorption and damage to the kidneys, liver, and brain were indicated.

Chronic Toxicity (or Exposure)

Animal

1,4-Dioxane causes liver and kidney toxicity in laboratory animals. Limited data regarding developmental toxicity show maternal and embryo effects at similar doses. Several carcinogenicity bioassays have been conducted for 1,4-dioxane in mice, rats, and guinea-pigs. With the exception of one vapor inhalation study, drinking water was the exposure medium used in all cancer bioassays. No treatment-related tumors were reported in the inhalation study. The primary target organs for cancer in the oral studies were the liver and the nasal cavity. Taken together, the bioassay data show that production of liver tumors is more consistent across strains and species of experimental animals than the nasal tumor data. Liver tumors were observed in both sexes and multiple strains of both mice and rats, while nasal tumors were present mostly in rats. With the exception of one study liver tumors also occurred with a much higher incidence rate than nasal cavity tumors. US EPA's B2 (probable human carcinogen) classification for 1,4-dioxane was based on induction of nasal cavity and liver carcinoma in multiple strains of rats, liver carcinomas in mice, and gall bladder carcinomas in guinea-pigs.

Human

Several epidemiology studies on occupational exposure to 1,4-dioxane are available in the literature. Very limited conclusions can be drawn from the negative findings of these studies. All of the studies lack sufficient cohort size and number of cases to enable identification of low-level excess cancer risk. Of note, the reported cancers have various sites of origin and are not similar to those seen in animal models. A mortality study was conducted on employees exposed to 1,4-dioxane. Observed deaths from overall cancer were not significantly different from expected number of deaths. 1,4-Dioxane can be inhaled in amounts sufficient to cause serious systemic intoxication. Injury may become apparent hours after termination of an exposure that had been erroneously considered to be negligible. Prolonged and repeated contact can cause eczema and repeated inhalation exposures to low concentrations have been fatal.

1,4-Dioxane is currently classified as B2, a probable human carcinogen, by US EPA based on adequate animal studies and inadequate human studies. Three epidemiological studies on workers exposed to 1,4-dioxane are available. Two of the deaths were due to cancer: one epithelial carcinoma in a 66-year-old man and one melofibrotic leukemia in a 71 year-old man.

No statistically significant increase was noted based on these few cases of cancer. Among 165 production and processing workers exposed to 1,4-dioxane, 12 deaths were reported. Three of these deaths were due to cancer: one stomach cancer, one alveolar carcinoma, and one mediastinal malignancy. Three deaths were not different from the expected numbers.

In Vitro Toxicity Data

1,4-Dioxane has been described as either a very weak genotoxin or not genotoxic. It produced negative results in most test systems including the *Salmonella* assays (Ames test), DNA alkylation and repair, hepatocyte unscheduled DNA synthesis, and the Chinese hamster ovary chromosome aberration assay. Hepatic DNA damage was seen in the alkaline elution test and positive findings were sometimes reported for the liver micronucleus assay, although data for this parameter are inconsistent.

Incubating 2.5% 1,4-dioxane with human lymphocyte cultures caused an increase in phytohemaglutanin stimulation of DNA synthesis. No significant effects were seen at lower concentrations. 1,4-dioxane was not demonstrated to bind covalently to DNA in the presence of microsomal preparations.

Clinical Management

First-aid procedures for eye or skin contact involve irrigation and water washing. Respiratory support may be required for people exposed to high vapor concentrations of 1,4-dioxane. Immediate dilution with milk or water may be of benefit following ingestion of irritant chemicals like 1,4-dioxane. Activated charcoal binds most toxic agents and can decrease their systemic absorption if administered soon after ingestion. Persons exposed to 1,4-dioxane should be thoroughly medically examined before ipecac alkaloid is administered to induce emesis. If signs of oral, pharyngeal, or esophageal irritation, a depressed gag reflex, or central nervous system (CNS) excitation or depression are present, emesis should not be induced. Gastric lavage may be indicated if performed soon after ingestion or in patients who are comatose or at risk of convulsing. The airway should be protected by placing the patient in the Trendelenburg and left lateral decubitus position or by cuffed endotracheal intubation. After control of any seizures present, gastric lavage may be performed.

In cases of ingestion exposure, the patient should be carefully monitored for the development of any systemic signs or symptoms and symptomatic treatment should be provided as necessary. In cases of inhalation exposure, the victim should be moved to fresh air and monitored for respiratory distress. If cough or difficulty in breathing develops, the patient should be evaluated for respiratory tract irritation, bronchitis, or pneumonitis. Supplemental oxygen (100%, humidified) should be administered with assisted ventilation as required. When eyes are exposed, they should be irrigated with copious amounts of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persist, the patient should be seen in a health care facility. In case pulmonary edema develops due to exposure, ventilation and oxygenation should be maintained with close arterial blood gas monitoring. If P_{O_2} remains less than 50 mmHg, positive end-expiratory pressure or CPAP may be necessary.

Environmental Fate

1,4-Dioxane is a cyclic ether compound that is miscible with water in all proportions and is also moderately volatile. It is resistant to hydrolysis and microbial degradation, but may undergo photolysis at water and soil surfaces. An estimate of the half-life for abiotic degradation in water with addition of ozone was 60 h. The half-life for photo-oxidation in air was 3.4 h. 1,4-Dioxane has a low adsorption potential and a high mobility/leaching potential in soil/water systems. No bioaccumulation of this chemical is expected.

Ecotoxicology

Ecological toxicity data for 1,4-dioxane are available for fish, aquatic, and terrestrial invertebrates, microorganisms, algae, and terrestrial plants. Acute and chronic toxicity levels generally range between 1000 and 10 000 $mg\,l^{-1}$, with the exception of a long-term study in fish that reported a no-observed effect level of approximately 100 $mg\,l^{-1}$.

Other Hazards

1,4-Dioxane is a flammable liquid and a fire hazard. Toxic gases and vapors (which may include carbon monoxide) may be released in a fire involving dioxane.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit for 1,4-dioxane in air is 100 ppm (360 $mg\,m^{-3}$) for an 8 h work shift. The National Institute for Occupational Safety and Health recommended exposure limit (REL) is 1 ppm (3.6 $mg\,m^{-3}$), which should not be exceeded in a 30 min period. The American Conference of Governmental Industrial Hygienists REL is 20 ppm averaged

over an 8 h work shift. The International Agency for Research on Cancer classifies 1,4-dioxane as possibly carcinogenic to humans (group 2B) based on inadequate evidence in humans and sufficient evidence in experimental animals. Similarly, the United States Environmental Protection Agency (USEPA) has classified 1,4-dioxane as a probable human carcinogen (group B2) based on inadequate data from human epidemiological studies and sufficient data from laboratory animal studies including, nasal cavity and liver carcinomas in multiple strains of rats, liver carcinomas in mice and gall bladder carcinomas in guinea-pigs. An oral cancer slope factor (CSF) value of 0.011per(mg per kg per day)$^{-1}$ was published on USEPA's Integrated Risk Information System (IRIS) database.

See also: Carcinogen Classification Schemes; Kidney; Liver.

Further Reading

Stickney JA, Sager SL, Clarkson JR, *et al.* (2003) An updated evaluation of the carcinogenic potential of 1,4-dioxane. *Regulatory Toxicology and Pharmacology* 38: 183–195.

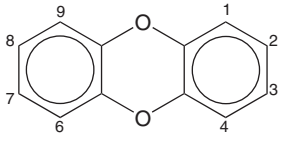
The Netherlands Organization for Applied Scientific Research (TNO) and the National Institute of Public Health and the Environment (RIVM) (1999) *Risk Assessment: 1,4-Dioxane*. The Netherlands: Chemical Substances Bureau, Ministry of Housing, Spatial Planning and the Environment (VROM), Final Version, 5 November, EINECS-No.: 204-661-8.

Dioxins

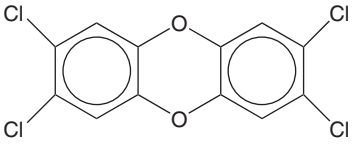
Robert A Young

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 1746-01-6 (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin)
- Chemical/Pharmaceutical/Other Class: Halogenated aromatic hydrocarbon
- Chemical Structure: There are 74 chlorinated dibenzo-*p*-dioxin congeners. The basic structure for unsubstituted dibenzo-*p*-dioxin (showing the carbon numbering scheme that is used to name specific congeners) and the structure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (one of 22 tetrachlorinated dibenzo-*p*-dioxins) are shown below



Dibenzo-*p*-dioxin



2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

Uses

Dioxins are by-products of various chemical syntheses and are usually present as contaminants of end products. There is no known use.

Background Information

There are a wide range of chlorinated dibenzo-*p*-dioxins varying in the extent of their chlorination. Dioxin nomenclature is based on the number and positions of carbon atoms that are chlorinated and include mono-, di-, tetra-, penta-, hexa-, hepta-, and octachlorinated congeners. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD, more commonly referred to as TCDD or dioxin) is usually of greatest concern because of high toxicity in laboratory animal models, its widespread distribution and persistence in the environment, bioaccumulation potential, and because the greatest amount of data exists for this form.

Exposure Routes and Pathways

2,3,7,8-TCDD and other chlorinated dibenzo-*p*-dioxins are released during the combustion of many polychlorophenols and also occur as contaminants in various chemicals such as the herbicide 2,4,5-trichlorophenoxyacetic acid. Most high-level exposure to 2,3,7,8-TCDD and other dioxins results from accidental releases or explosions in chemical plants or storage facilities for dioxin-containing chemicals. Because of the persistence of dioxin congeners in the environment and their potential for bioaccumulation, exposure may occur via the soil, air (especially when dioxins occur as combustion products), or water. When bound to components of the soil, the health hazard from 2,3,7,8-TCDD is reduced compared to ingestion of the pure compound. However, its bioavailability varies with the specific media in which it occurs.

Toxicokinetics

Dioxins are highly lipid soluble and are efficiently absorbed by most routes of exposure although absorption will vary quantitatively depending on the route. Because dioxins are poor substrates for the enzymes typically involved with biotransformation of xenobiotics, they are very poorly metabolized. Dioxins tend to exhibit high concentrations in the liver and tend to accumulate in fatty tissue. Because of their high lipid solubility and poor metabolism, excretion of dioxins is extremely slow. The elimination half-life in humans is ~ 10 years.

Mechanism of Toxicity

Some, but not all, of the toxic effects of 2,3,7,8-TCDD are mediated by the interaction with an intracellular protein called the Ah receptor. This interaction ultimately alters genetic expression leading to deleterious effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal data have shown that 2,3,7,8-TCDD is a highly toxic chemical and capable of exerting a wide range of toxic effects. Oral LD_{50} values in animals vary considerably (e.g., 0.6, 1, 20, 114, and 1157 $\mu g\,kg^{-1}$ for guinea pigs, dogs, rats, mice, and hamsters, respectively). Toxic effects observed in animals following acute exposure include damage of the liver, heart, thymus gland, adrenals, and immunosuppressive effects. The effects, however, vary with species. Toxic effects, including death, have also been observed in animals following short-term dermal exposure to 2,3,7,8-TCDD.

Human

A TD_{Lo} of 107 $\mu g\,kg^{-1}$ has been reported for humans although a more generally accepted minimum toxic dose for humans is 0.1 $\mu g\,kg^{-1}$. Nonlethal effects following short-term exposure to 2,3,7,8-TCDD include headache, fatigue, irritation of the gastrointestinal and respiratory tracts, dehydration, and skin irritation. The acneform skin irritation resulting from exposure to 2,3,7,8-TCDD or chemicals that contain TCDD is referred to as chloracne.

Dioxin was the poisoning agent in a high profile political incident in 2004. It was ultimately identified as the cause of the disfiguring acne-like skin condition suffered by Ukrainian opposition leader Viktor Yushchenko a few months before the first presidential election. The suspicion is that the dioxin was placed into soup ingested by Mr. Yushenko. The acne-like skin condition is the most recognizable hallmark of dioxin poisoning in humans. It is expected that at least most of his skin condition is reversible; however, his situation is unique as a known case of high exposure dioxin poisoning, with severe effects, in a human. further, it is not known what other effects to his body related to the poisoning might surface in future months and years. The actual intake of dioxin in this poisoning is unknown.

Chronic Toxicity (or Exposure)

Animal

In addition to the aforementioned acute effects, chronic exposure in animals has also resulted in carcinogenic responses in the liver and lungs. 2,3,7,8-TCDD has been shown to be carcinogenic and teratogenic in several laboratory species.

Human

The toxic effects associated with chronic exposure to 2,3,7,8-TCDD include chloracne, impaired liver function, peripheral neuropathies, and altered blood chemistry parameters. Other long-term effects may include chromosome damage, heart attacks, reproductive disorders, and cancer, although epidemiologic data regarding these effects are equivocal. Some effects of long-term low-level exposure to dioxins appear to be reversible following cessation of the exposure.

The US Environmental Protection Agency classifies hexachlorodibenzo-*p*-dioxin mixture (HxDD) in cancer group B2 (probable human carcinogen with sufficient evidence in animals but inadequate evidence in humans), and International Agency for Research on Cancer classifies the chemical as 2B (probably carcinogenic to humans; sufficient evidence in animals). The oral slope factor and unit risk for HxDD is $6.2 \times 10^3\,(mg/kg/day)^{-1}$ and $1.8 \times 10^{-1}\,\mu g\,l^{-1}$, respectively. The inhalation unit risk is $1.30\,(g/m^3)^{-1}$. Inhalation unit risk values for other polyhalogenated dioxin congeners have been developed based on a toxicity equivalent factor (TEF) approach where the TEF for 2,3,7,8-TCDD is equal to 1. No reference doses or reference concentrations have been derived for 2,3,7,8-TCDD or any other dioxin.

Clinical Management

There are no clinical procedures specific for dioxin intoxication, but clinical management for acute intoxication by dioxin-containing chemicals such as 2,4-D and 2,4,5-T may be applied. Basically, these procedures include decontamination of the gut and/or skin and possibly alkaline diuresis for severe overdose situations.

Ecotoxicology

It has been difficult to assess the ecotoxicology of dioxin in the field because of the presence of other chemicals that cause similar effects and are present at higher concentrations. However, it appears that the early life stages of fish are most susceptible to the effects of dioxin and that birds may exhibit decreased egg production, embryotoxicity, and fetal malformations as a result of dioxin exposure.

See also: Distribution; Immune System; Pollution, Soil; Polybrominated Diphenyl Ethers (PBDEs); 2,4,5,-T; Toxic Torts.

Further Reading

Barnes DG, Kutz FW, and Bottimore DP (1989) *Update to the Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-Dioxins and -Dibenzofurans (CDDs and CDFs)*. Risk Assessment Forum. Washington, DC: US EPA.

Kirk RE and Othmer F (eds.) (1998) *Kirk–Othmer Encyclopedia of Chemical Technology*. New York: Wiley.

US Air Force (1989) *The Installation Restoration Program Toxicology Guide*. Air Force Systems Command, Aerospace Medical Division. Wright-Patterson AFB, 04.

Relevant Website

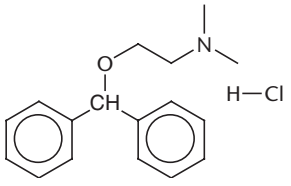
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Dioxins.

Diphenhydramine

Michael Wahl

This article is a revision of the previous print edition article by Carla M Goetz, volume 1, pp. 496–497, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 58-73-1
- Synonyms: Benadryl; Diphenhist; Genahist; Sominex; Nytol; Sleepinal; Caladryl; Dermarest
- Chemical/Pharmaceutical/Other Class: An ethanolamine derivative H-1 receptor antagonist
- Chemical Formula: $C_{17}H_{21}NO$
- Chemical Structure:



Uses

Diphenhydramine, like other antihistamines, is most often used to provide symptomatic relief of allergic symptoms caused by histamine release. The drug is also used as an antitussive, a nighttime sleep aid for the short-term treatment of insomnia, and as a preventive and treatment for motion sickness. Diphenhydramine may be useful in the treatment of parkinsonian syndrome in geriatric patients including drug-induced extrapyramidal reactions. Diphenhydramine has been used topically for the temporary relief of pruritus and pain associated with various skin conditions including minor burns, insect bites, and minor skin irritation.

Exposure Routes and Pathways

Ingestion, injection, and dermal application are the routes of both accidental and intentional exposures to diphenhydramine.

Toxicokinetics

Diphenhydramine is absorbed rapidly after an oral dose with peak plasma levels achieved within 2 h. The drug is also absorbed through abraded skin resulting in systemic toxicity. Diphenhydramine undergoes extensive first-pass metabolism with 40–60% of an oral dose reaching systemic circulation as unchanged drug. Diphenhydramine is 98% protein bound and has an apparent volume of distribution of 3–7 $l kg^{-1}$. Approximately 64% of the dose of diphenhydramine is excreted as metabolites in the urine. The serum half-life is 4–10 h.

Mechanism of Toxicity

The toxicity of antihistamines is related to their anticholinergic (antimuscarinic) activity. The action of acetylcholine at the muscarinic receptors is blocked, resulting in signs and symptoms of anticholinergic poisoning. Diphenhydramine may produce direct toxicity unrelated to its anticholinergic properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

Central Nervous System (CNS) changes including sedation or hyperexcitability, salivation, and vomiting

have occurred following relatively small exposures to antihistamines. Seizures and cardiac effects have occurred following acute high exposures. Symptomatic and supportive care followed by appropriate gastrointestinal decontamination procedures should be administered.

Human

Diphenhydramine overdose results in signs and symptoms of anticholinergic poisoning including dry mouth, fixed dilated pupils, flushed skin, fever, and hallucinations. CNS depression is more common in adults, whereas stimulation including tonic–clonic seizures is more common in children. Cardiovascular effects including tachycardia, hypertension or hypotension, arrhythmias, and cardiovascular collapse may occur. Symptoms of an overdose generally occur within 30 min to 2 h after ingestion. Fatalities have occurred in children at doses under 500 mg and seizures have been described after doses as low as 150 mg. A fatal adult dose is estimated to be between 20 and 40 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies of various concentrations of diphenhydramine in mouse and rat models have shown equivocal evidence of carcinogenesis. Slightly increased rates of unusual cancers were demonstrated (e.g., astrocytomas and alveolar/bronchiolar neoplasms).

Human

Although first-generation anthistamines such as diphenhydramine have been used for decades, they may produce sedation, psychomotor impairment, and may negatively impact sleep patterns.

In Vitro Toxicity Data

Histamine induces CD86 expression and chemokine production in immature human derived monocyte-derived dendritic cells that can be blocked by diphenhydramine and other H1 receptor antagonists.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Appropriate gastrointestinal decontamination procedures should be administered based on the history of the ingestion and the patient's level of consciousness. Treat seizures with benzodiazepines or barbiturates. Consider treatment of prolonged QRS or wide complex tachydysrythmias with sodium bicarbonate. Consider use of benzodiazepines for CNS excitation, hallucinations and movement disorders. Aggressively treat hyperpyrexia with external cooling such as cool mist and fans. In a limited number of cases physostigmine administration may be necessary to treat severe central and peripheral anticholinergic symptoms refractory to conventional therapy. If physostigmine is given intravenously, the rate of administration should not exceed 2 or 3 min. Continual electrocardiogram monitoring is essential.

See also: Cholinesterase Inhibition; Hypersensitivity, Delayed Type.

Further Reading

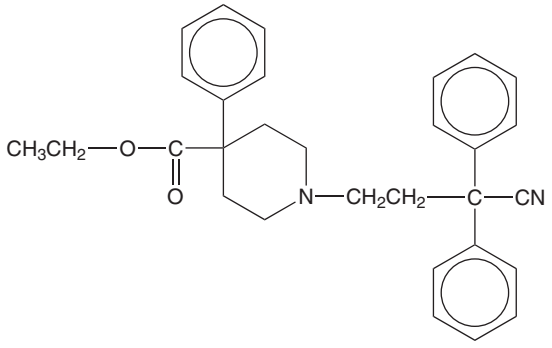
Koppel C, Ibe K, and Tenczer J (1987) Clinical symptomatology of diphenhydramine overdose: An evaluation of 136 cases in 1982 to 1985. *Clinical Toxicology* 25: 53–70.

Pentel P and Peterson CD (1980) Asystole complicating physostigmine treatment of tricyclic antidepressant overdose. *Annals of Emergency Medicine* 9: 588–590.

Diphenoxylate

Alexander B Baer and Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 3810-80-8
- Synonym: Lomotil
- Chemical/Pharmaceutical/Other Class: Antidiarrheal
- Chemical Formula: $C_{30}H_{32}N_2O_2$
- Chemical Structure:



Uses

The only recognized use for diphenoxylate is in combination with atropine for the treatment of acute and chronic diarrhea.

Exposure Routes and Pathways

Exposure is by the oral route; diphenoxylate is available in liquid and tablet forms.

Toxicokinetics

Diphenoxylate is readily absorbed from the gastrointestinal tract. Peak levels occur 3 h after a single oral dose. Diphenoxylate is metabolized rapidly in the liver to both active and inactive metabolites. Enterohepatic circulation may occur. The volume of distribution is $4.6\,l\,kg^{-1}$. The half-life of diphenoxylate is 2.5 h. The major metabolite difenoxin (diphenoxylic acid) has a half-life of 4.4 h after therapeutic dosage and is five times more potent than the parent chemical. After overdose, difenoxin's half-life has been reported to extend greater than 12 h. Diphenoxylate is excreted in the feces (49%) and urine (13%) primarily as metabolites.

Mechanism of Toxicity

Diphenoxylate is a narcotic-like substance that slows gastrointestinal motility and depresses the central nervous system producing coma and respiratory depression. Anticholinergic effects (secondary to the presence of atropine as an abuse deterrent) can be seen early after exposure with opioid effects occurring later. There is no correlation between the dose ingested and the severity of effects in children. Severe poisonings with coma and respiratory depression have been reported in children with small ingestions.

Acute and Short-Term Toxicity (or Exposure)

Animal

Diphenoxylate–atropine has been used in the past to treat cats with diarrhea, but toxicity has limited its usefulness. Lomotil should generally be avoided in cats.

Human

Single therapeutic doses produce little or no opiate-like effects in adults. Effects from larger doses (40–60 mg) are typical of opioid drugs and include miosis, ataxia, lethargy, respiratory depression, seizures, and coma. Onset of symptoms may be delayed 6–8 h. Anticholinergic effects of atropine (tachycardia, urinary retention, irritability, mydriasis, and cutaneous flushing) may be evident before opioid symptoms. Children are more susceptible to the effects and may manifest slower onset. The therapeutic index is low in children; symptoms have resulted with only one tablet.

Chronic Toxicity (or Exposure)

Human

A morphine-like physical dependence can occur with chronic administration.

In Vitro Toxicity Data

In a Caco-2 model of P-glycoprotein influenced cell transport and uptake, diphenoxylate was not dependent on P-glycoprotein.

Clinical Management

In addition to general supportive measures directed to the airway, breathing and circulation, the clinician may consider measures to decrease absorption in the alert patient they suspect has been exposed to a life-threatening dose of diphenoxylate. Charcoal may decrease absorption. Charcoal administration should be cautiously used in a patient with altered mental status to avoid charcoal aspiration pneumonitis. It is important to recognize the potential for delayed toxicity and monitor diphenoxylate poisoned patients for a minimum of 24 h. Transient recovery may be observed before this time. Repeat boluses or continuous infusion of naloxone may be necessary to reverse opioid sedating effects.

See also: Atropine.

Further Reading

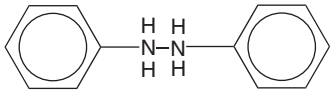
Cutler EA, Barrett GA, and Craven PW (1980) Delayed cardiopulmonary arrest after lomotil ingestion. *Pediatrics* 65: 157–158.

McCarron MA, Challoner KR, and Thompson GA (1991) Diphenoxylate–atropine (lomotil) overdose in children: An update (report of eight cases and review of the literature). *Pediatrics* 87: 694–700.

Diphenylhydrazine

Robert A Young

- Chemical Abstracts Service Registry Numbers: Diphenylhydrazine occurs as the isomers 1,1-Diphenylhydrazine (CAS 530-50-7) and 1,2-Diphenylhydrazine (CAS 122-66-7)
- Synonyms: 1,1-Diphenylhydrazine; *N*,*N*′-Bianiline; 1,2-Diphenylhydrazine; Hydrazobenzene; *N*,*N*′-Diphenylhydrazine; *sym*-Diphenylhydrazine
- Chemical/Pharmaceutical/Other Class: Polycyclic amine
- Chemical Formula: $C_{12}H_{12}N_2/(C_6H_5)_2NNH_2$
- Chemical Structure:



Uses

1,2-Diphenylhydrazine has been used for the production of benzidine, which, in turn, is used in the production of benzidine-based dyes. These dyes, however, are no longer produced in the United States. 1,2-Diphenylhydrazine is also used in the production of the anti-inflammatory pharmaceutical agent phenylbutazone and in the production of sulfinpyrazone, a uricosuric agent.

Exposure Routes and Pathways

The primary route of exposure is likely to be via ingestion or dermal contact with dust of contaminated soil. Because of the low volatility and solubility of diphenylhydrazine, inhalation exposure or exposure via water is not likely to be significant.

Toxicokinetics

For humans, there are no data regarding the absorption of diphenylhydrazines by any exposure route. Gastrointestinal absorption of 1,2-diphenylhydrazine in rats can be inferred by the presence of the parent compound and its metabolites in the urine and by systemic toxic effects following oral administration. No data are available regarding inhalation or dermal absorption in animals. Data are unavailable regarding the metabolism of 1,1-diphenylhydrazine. Limited data regarding the metabolism of 1,2-diphenylhydrazine by rats suggest benzidine and aniline to be major metabolites with minor metabolites including unspecified hydroxy derivatives. Conversion of 1,2-diphenylhydrazine to aniline may occur through intestinal microflora and by acid conversion in the stomach.

Data are not available regarding the distribution of either form of diphenylhydrazine. Limited data in rats indicate that urinary excretion of metabolites and unchanged parent compound occurs following oral administration of 1,2-diphenylhydrazine. No data are available regarding the excretion of 1,1-diphenylhydrazine.

Mechanism of Toxicity

The mechanism of diphenylhydrazine toxicity is not currently known. It is possible that some of the toxic effects observed for diphenylhydrazine may be the result of its major metabolites, aniline and benzidine, which are known animal carcinogens.

Acute and Short-Term Toxicity (or Exposure)

No data are available regarding the acute or chronic toxicity of 1,1- or 1,2-diphenylhydrazine in humans.

Chronic Toxicity (or Exposure)

Animal

Only limited data are available regarding the toxicity of diphenylhydrazine in animals. Oral LD_{50} values of 959 and 301 mg kg^{-1} have been reported for rats, and gastrointestinal hemorrhage and death have been reported for rodents following 4 week dietary exposure to a dose of 390 mg 1,2-diphenylhydrazine per kilogram body weight per day. Chronic exposure (78 weeks) of rats and mice to 1,2-diphenylhydrazine in the diet (equivalent to doses of 4 and 52 mg kg^{-1} day^{-1} for rats and mice, respectively) resulted in hepatocellular carcinomas. Exposure of rats to 1,2-diphenylhydrazine at doses of 5–15 mg kg^{-1} day^{-1} for 78 weeks resulted in effects ranging from death to histopathological changes in the liver and gastrointestinal tract. Other effects of chronic oral exposure of animals to 1,2-diphenylhydrazine include decreased weight gain, interstitial inflammation of the lung, and fatty degeneration and necrosis of the liver. The acute toxicity of diphenylhydrazines in animals following inhalation exposure has not been determined, and no information is available regarding the carcinogenic or noncarcinogenic effects in animals

following chronic inhalation exposure to diphenylhydrazines.

Environmental Fate

If spilled on land, diphenylhydrazine would absorb moderately to soil and be oxidized to azobenzene by air or cations in the soil. If released in water, it will adsorb moderately to sediment and particulate matter where it should undergo rapid reversible oxidation by dissolved oxygen and environmentally common cations (e.g., copper (II)) to azobenzene. If released in the atmosphere, it will degrade by a combination of air oxidation and photolysis.

Other Hazards

1,2-Diphenylhydrazine has been found to be present in drinking water at levels of $1\,\mu g\,l^{-1} = 1$ ppb. Hydrazobenzene was detected in groundwater samples at Love Canal, Niagara Falls, NY, and in 1.2% of 1205 effluents sampled in a national survey at a median concentration of less than $10\,\mu g\,l^{-1}$.

Exposure Standards and Guidelines

Health-based guidance values for 1,2-diphenylhydrazine include an inhalation unit risk of 2.2×10^{-4} $(\mu g/m^3)^{-1}$ and a drinking water unit risk of 2.2×10^{-5} $(\mu g/l)^{-1}$. The US Environmental Protection Agency (EPA) classifies 1,2-diphenylhydrazine as a probable human carcinogen (B2). The cancer slope factor for 1,2-diphenylhydrazine is 8.0×10^{-1} (mg/kg/day)$^{-1}$. No regulatory values or guidance values are available for 1,1-diphenylhydrazine.

The US EPA Reportable Quantity for 1,2-diphenylhydrazine is 1 lb (statutory) with a proposed Reportable Quantity of 10 lbs.

See also: Aniline; Benzidine.

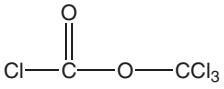
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Diphenylhydrazine, 1,2-

Diphosgene

Fu-Min Menn

- Chemical Name: Carbonochloridic acid trichloromethyl ester
- Representative Chemical: Phosgene ($COCl_2$, CAS 75-44-5)
- Chemical Abstracts Service Registry Number: CAS 503-38-8
- Synonyms: Trichloromethyl chloroformate; Trichloromethyl carbonochloridate; Chloroformic acid trichloromethyl ester; UN1076; Perstoff; Superpalite, DP
- Chemical/Pharmaceutical/Other Class: Choking agent
- Chemical Formula: $C_2Cl_4O_2$
- Chemical Structure:



Uses

Diphosgene has been widely used as a substitute compound for phosgene in different chemical synthesis reactions, including formation of chloroformates, carbonates, urea, and isocyanates, and for chlorination, carboxylation, and dehydration, in various industry settings, such as pharmaceuticals, dyes, perfume, adhesive, and pesticide. It was also used as a chemical weapon agent in World War I.

Background Information

Diphosgene was produced by Germany (~11 600 tons) as a chemical weapon in World War I, and caused mass casualties when first used in 1916. It is classified as a choking agent or lung-damaging agent (pulmonary toxicant). It is denser than air and accumulates in low-lying areas. Diphosgene is a combination of phosgene and chloroform, which destroys gas filters in gas masks. Diphosgene is chemically similar to phosgene and has a slower decomposition rate than phosgene.

Exposure Routes and Pathways

Diphosgene is listed as highly toxic in the United States and as very toxic in the European Union. The primary routes of entry for diphosgene are through

skin, eyes, inhalation, and ingestion. It causes damage primarily to the respiratory tract through inhalation, which irritates the nose, throat, and especially lung tissue. Direct skin exposure to liquid diphosgene can cause surface burns.

Toxicokinetics

Diphosgene is not detoxified in the human body.

Mechanism of Toxicity

The mechanism of action has not been well established. However, its toxicity is very similar to phosgene because diphosgene decomposes to phosgene and nontoxic levels of chloroform on heating and reaction with a nucleophile. Even a trace of moisture leads to the formation of phosgene. Both phosgene and diphosgene react with water to generate hydrochloric acid, which can cause damage to the tissue in the upper respiratory tract. Diphosgene and phosgene are insoluble in water and react directly on alveolar and capillary membranes allowing plasma to flood the alveoli resulting in pulmonary edema.

Acute and Short-Term Toxicity (or Exposure)

Inhalation is the major route of exposure. Diphosgene is extremely damaging to mucous membranes, eyes, skin, and the respiratory tract, and may cause minor irritation to severe tissue damage and death. Toxicity effects vary with the concentration of vapor and the length of exposure. Signs and symptoms of toxicity may be immediate or delayed. The delayed (up to 6 h) acute respiratory distress syndrome is characteristic of chocking agent inhalation.

Animal

Mild pulmonary edema was observed in experimental rats at 6 h after exposure to diphosgene (20 min at 44.9 mg m^{-3}), and became extensive at 24 h. However, lung damage caused by low-dose diphosgene inhalation in most experimental rats was reversed and could not be distinguished from lungs of control animals after 10 days of exposure.

A 10–20 min exposure to diphosgene at a concentration of 0.9 mg l^{-1} caused fatality to tested rabbits.

Human

Diphosgene usually causes irritation to eyes and the upper respiratory tract at low concentration. Inhalation can cause fatality if the concentration is >25 ppm. Surface burns are the common symptoms on humans when exposed to high concentrations of diphosgene. Direct eye exposure to liquid diphosgene can cause corneal abrasions, ulcers, or perforations.

For the respiratory tract, inhalation can cause spasms, inflammation and edema of the larynx and bronchi, dyspnea, cyanosis, pneumonia, and pulmonary edema. Serious symptoms, such as pulmonary edema and asphyxiation, may not be observed for hours after overexposure. Occasionally, cardiac failure occurs as a complication of severe pulmonary edema. With regards to the cardiovascular system, diphosgene can cause rapid heartbeat and hypotension. Gastrointestinal exposure may cause nausea and vomiting in patients and may be fatal.

It has been reported that the LCt_{50} (inhalation dose) of phosgene in humans is ~ 3200 mg min m^{-3}.

Chronic Toxicity (or Exposure)

Human

Pulmonary fibrosis and emphysema can develop after persistent exposure.

Clinical Management

There is no known laboratory test available that can confirm diphosgene exposure. However, evaluation of oxygen saturation and arterial blood gas is recommended for initial treatment for all patients. When inhaled, the patient should be removed to fresh air area immediately. Oxygen supplement can improve tissue oxygenation and reduce the damage due to hypoxemia in patients. Artificial respiration devices with or without positive pressure should be used if necessary.

If diphosgene is swallowed, vomiting should not be induced. For skin contact, contaminated clothing should be removed and the exposed area flushed with water and soap for at least 15 min. Patient should be under medical observation for at least 48 h.

Antibiotics should be used only with the development of bacterial bronchitis or pneumonia. β-adrenergic agonist has been used to relieve bronchospasm by relaxing bronchial smooth muscle and reducing hyperactivity in diphosgene inhalation. An adult dose of albuterol 0.5% (2.5 mg in 2.5 ml saline solution) can be used and repeated as needed.

Environmental Fate

Diphosgene decomposes into phosgene gas, hydrogen chloride gas, carbon monoxide, and carbon dioxide.

Exposure Standards and Guidelines

Phosgene occupational exposure criteria include the following:

- The US Occupational Safety and Health Administration permissible exposure limit is 0.1 ppm time-weighted average (TWA).
- The American Conference of Governmental Industrial Hygienists threshold limit value is 0.1 ppm TWA.

Miscellaneous

Diphosgene is safer than phosgene during production, transportation (in glass container only), and storage because it is stable in liquid form at room temperature. The odor of diphosgene smells like green corn or newly mown hay. It is a colorless liquid at 20°C, and has a specific gravity of 1.66 at 15°C; the boiling point is 128°C. It is heat sensitive and should be stored at 2–8°C. It is insoluble in water and soluble in ether and ethanol.

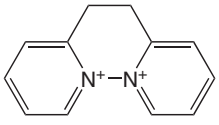
In 1992, a monitoring device that detected phosgene and diphosgene in the air was patented in Germany.

See also: Phosgene.

Diquat

Carey N Pope

- Chemical Name: 1,1′-Ethylene-2,2′-bipyridinium ion
- Chemical Abstracts Service Registry Number: CAS 231-36-7
- Synonyms: Deiquat; Reglone; Aquakill; Dextrone; Reglox; Reward; Tag; Torpedo; Vegetrole; Weedtrine-D
- Chemical/Pharmaceutical/Other Class: Bipyridinium herbicide and desiccant
- Chemical Formulas: $C_{12}H_{12}N_2$; $C_{12}H_{12}N_1Br_2$ (dibromide salt)
- Chemical Structure:



Uses

Diquat is used to control aquatic weeds, for preharvest desiccation of various crops, and for postemergence weed control in cotton production. It is sometimes used in conjunction with paraquat.

Exposure Routes and Pathways

Dermal and respiratory are common accidental routes of exposure.

Toxicokinetics

Absorption from the gastrointestinal (GI) tract is poor (6% absorption in rats) with the remainder primarily eliminated in the feces. Oral diquat is transformed to a minimal degree within the intestines, with fecal elimination of metabolites. Complete elimination occurred within 4 days after oral dosing. Diquat was rapidly eliminated via the urine with dermal, inhalation, or intravenous dosing. With subcutaneous dosing, ~90% was eliminated within 24 h via urine. Following intravenous administration in mice, diquat did not accumulate in lung or muscle.

Mechanism of Toxicity

Diquat free radical is formed by glutathione reductase. Aerobic autooxidation of diquat free radicals leads to superoxide production and oxidative damage (lipid peroxidation).

Acute and Short-Term Toxicity (or Exposure)

Animal

Diquat is moderately toxic via oral dosing, with oral LD_{50} values of 120–233 mg kg^{-1} in rats, mice, rabbits, guinea pigs, and dogs. Cows are more sensitive, with an oral LD_{50} of 30–56 mg kg^{-1}. The acute dermal LD_{50} in rabbits is 400–500 mg kg^{-1}. A single dose of diquat was not irritating to the skin of rabbits, but repeated dosing led to erythema and eschar formation. Moderate to severe eye irritation was

noted in rabbits. Diquat can lead to severe irritation of the mouth, throat, esophagus, and stomach, and nausea, vomiting, diarrhea, severe dehydration, and disruption of fluid balance, GI discomfort, chest pain, kidney failure, and hepatotoxicity. Very large doses can lead to tremors and convulsions. Diquat elicits delayed toxicity, with an onset at ~24 h following dosing with death occurring from 2 to 14 days after dosing.

Human

Acute overdose in humans has been associated with ulceration of the GI tract, acute renal failure, hepatotoxicity, and breathing difficulties. Nausea, emesis, and diarrhea are initial signs of overdose. Inhalation of diquat can lead to nosebleed. Exposure to dusts can cause skin irritation, cough, and chest pain.

Chronic Toxicity (or Exposure)

Animal

Chronic diquat exposures (2.5 or 5 $mg\,kg^{-1}\,day^{-1}$) led to cataracts in dogs and rats. Higher dosages may lead to retinal detachment and hemorrhage. In another study, oral diquat (4 $mg\,kg^{-1}\,day^{-1}$ for 2 years) in rats produced no behavioral changes or alterations in kidneys, liver, or mycocardium but elicited some histopathology in lung. Repeated dermal contact can lead to skin inflammation, and, at sufficient dosages, systemic toxicity. Diquat has little reproductive toxicity, teratogenic, or carcinogenic potential.

Human

There is relatively little information on the chronic effects of diquat in humans. In contrast to animal studies, cataracts have not been noted in workers occupationally exposed to diquat for long periods.

In Vitro Toxicity Data

Diquat was negative in mutagenesis assays using *Escherichia coli* mutants but positive in the Ames assay.

Clinical Management

There are no antidotes for diquat. The airway should be maintained and ventilation assisted. Oxygen therapy should not be used. For oral exposure, gastric lavage should be performed with activated charcoal. Bentonite (7%, 200 ml) every 2 hours for 24 h may be used instead of charcoal. Supportive measures including fluid and electrolyte replacement should then be employed. Hemodialysis is of proven value when renal failure is present.

Environmental Fate

Diquat is highly adsorbed by soil. While being water soluble, its adsorption to soil minimizes leaching. Diquat typically remains in the top inch of soil for long periods after application. Diquat stays bound to soil particles, remaining biologically immobile in surface waters. When applied to open water, it disappears rapidly by binding to suspended particles. Microbial degradation and photodegradation are important pathways of elimination. Diquat is rapidly absorbed into plant leaves but does not translocate due to its rapid toxicity to plant tissues.

Ecotoxicology

Acute oral LD_{50} values in birds range from 200 to 564 $mg\,kg^{-1}$. Diquat is moderately to practically nontoxic to fish and aquatic invertebrates. The 8 h LC_{50} for diquat was 12–29 $mg\,l^{-1}$ in two fish species. The 96 h LC_{50} was 16–245 $mg\,l^{-1}$ in six species of fish. Yellow perch appear sensitive to concentrations of diquat found during control of aquatic vegetation. There is little or no bioconcentration of diquat in aquatic species. Diquat is practically nontoxic to honey bees.

Exposure Standards and Guidelines

The oral reference dose for diquat is 2.2μg $kg^{-1}\,day^{-1}$, the acceptable daily intake for diquat is 2 $\mu g\,kg^{-1}\,day^{-1}$, and the 8 h threshold limit value is 0.1 $mg\,m^{-3}$.

See also: Paraquat.

Further Reading

Jones GM and Vale JA (2000) Mechanisms of toxicity, clinical features, and management of diquat poisoning: A review. *Journal of Toxicology: Clinical Toxicology* 38: 123–128.

Tanen DA, Curry SC, and Laney RF (1999) Renal failure and corrosive airway and gastrointestinal injury after ingestion of diluted diquat solution. *Annals of Emergency Medicine* 34: 542–545.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Diquat.

Disc Batteries

Toby Litovitz

- SYNONYM: Button batteries

Uses

Disc batteries have become ubiquitous. They are used as a power source for hearing aids, games and toys, watches, cameras, calculators, recording devices, remote controls, digital thermometers, lighted jewelry, musical or 'talking' books and greeting cards, phones, and many other devices. While their diameters range from 6 to 23 mm, cells over 15 mm in diameter are more often implicated in severe clinical outcomes.

Background Information

Disc batteries are composed of two wafer-like plates separated by an electrolyte-soaked fabric. The electrolyte is generally an alkaline solution, typically up to 45% sodium or potassium hydroxide. These contents are placed in a steel can with a plastic grommet separating the cathode and anode. Batteries may also contain metals including zinc and cadmium. Significant amounts of mercury have not been present in button batteries sold in the United States since the enactment of the Mercury-Containing and Rechargeable Battery Management Act of 1996.

Exposure Routes and Pathways

Typical exposure to disc batteries is via unintentional ingestion; these ingestions are not limited to small children. Ingestion often occurs unintentionally in those who place them in their mouths while changing the batteries of their instruments or toys, or when batteries are mistaken for pills. Disc battery ingestions are unique among childhood poisonings in that the mean age of victims is higher than that of typical pediatric poison exposures. Batteries may also be placed in aural or nasal cavities.

Toxicokinetics

Seventy-eight percent of ingested disc batteries are eliminated in the feces within 72 h; 86% pass within 4 days. Transit times in excess of a year have been reported without adverse effects.

Mechanism of Toxicity

Toxicity from disc battery exposure occurs through four potential mechanisms: (1) alkaline injury to adjacent tissue following leakage of alkaline constituents; (2) generation of an external current that flows through electrolyte-rich tissue fluids and forms hydroxides locally, also leading to alkaline damage of tissue; (3) aspiration, producing respiratory tract obstruction; and to a lesser extent (4) pressure necrosis as occurs following the ingestion of coins. Heavy metal poisoning is not expected, and symptomatic cases of heavy metal poisoning following battery ingestion have not been reported despite tens of thousands of battery ingestions that have occurred. Battery lodgment, whether in the esophagus, a diverticulum, the nose, or the ear canal, is required for injury to occur. Lodgment, especially in the esophagus, is usually associated with larger diameter cells (20–23 mm diameter). Most of these larger cells are lithium batteries, with 3 V rather than the standard 1.5 V button cell. The greater voltage, in addition to the greater diameter, contributes to the increased likelihood of significant injury.

Acute and Short-Term Toxicity (or Exposure)

Human

Most button battery ingestions are benign. However, if a disc battery becomes lodged in the esophagus, auditory canal, or nasal cavity, severe corrosive injury may occur. Esophageal burns may occur within 4–6 h of the ingestion. Esophageal perforation, esophageal stenosis requiring repeated dilatation or surgical repair, tracheoesophageal fistula, tension pneumo- or hemothorax, perforation through the aortic arch, massive exsanguinations, and cardiac arrest have been reported following button battery lodgment in the esophagus. Two fatal battery ingestion cases have occurred in toddlers. Batteries in the ear or nose may also be associated with severe injury including perforation or destruction of the tympanic membrane, destruction of the ossicles, hearing impairment, nasal septal perforation, saddle deformity of the nose, destruction of the nasal turbinates, facial nerve paralysis, chondritis, or atrophic rhinitis.

Chronic Toxicity (or Exposure)

Human

ged impaction of the battery in the esophagus, ear, or nose markedly increases the severity of the injury.

Clinical Management

Because of the high rate of prompt, uncomplicated passage of disc batteries, clinical management of battery ingestions is focused on ensuring that esophageal lodgment has not occurred. Patients with disc battery ingestion should have prompt radiographic localization, including a chest X-ray to ensure that the disc battery has not lodged in the esophagus. The absence of symptoms is not adequate confirmation that the battery is not in the esophagus as more than one-third of patients with batteries in the esophagus are asymptomatic at the time of the initial diagnosis. Furthermore, although battery diameter is predictive of lodgment, severe esophageal injury has occurred with button cells as small as 11.6 mm diameter. If located in the esophagus, the battery must be promptly removed endoscopically, ideally within 2–4 h of the ingestion.

If the battery is located in the stomach or more distal gastrointestinal tract, removal is not indicated except in the unusual event that the patient develops signs or symptoms suggestive of significant injury such as hematochezia or abdominal pain with tenderness.

Once batteries have passed beyond the esophagus, more careful outpatient follow-up for lithium cells and larger diameter cells (especially in children <6 years of age) is indicated, even in the absence of symptoms. The battery diameter and chemical system can be determined from the imprint code. Assistance with identification of cells and clinical guidance is available through the National Button Battery Ingestion Hotline at 202-625-3333. Batteries in the ear or nose must be removed immediately. Nasal and otic drops should be avoided as these increase injury by enhancing corrosion, leakage, and the generation of an external current.

Conservative management includes a repeat abdominal radiograph in 1–2 weeks if the battery has not been observed to pass. There is no role for gastric emptying efforts (induced emesis or gastric lavage) or use of cathartics. Blood or urine mercury concentrations are not needed.

See also: Alkalies.

Further Reading

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Distribution

Jules Brodeur and Robert Tardif

Distribution is the process by which absorbed chemicals are delivered to the various organs of the body and may produce an effect, be stored, or be eliminated.

One of the major factors regulating the distribution of chemicals throughout the body is the amount of blood perfusing the various organs. Per unit of organ weight, brain, and viscera, especially kidneys, are very well perfused and are therefore presented with large amounts of chemicals. Irrigation of resting skeletal muscle, skin, and bone is less important, while that of fat tissue is poor. If one also considers total tissue mass, however, the amount of blood reaching an organ can be of importance in terms of distribution. For instance, in certain individuals, fatty tissue can represent 30% of the body mass and, despite a low perfusion rate, can become an important repository for lipid-soluble chemicals.

Increasing or lowering blood perfusion will result in more or less rapid distribution of chemicals to their sites of action, storage, or removal. Muscular effort increases the amount of blood ejected by the heart per unit time. The influence of muscular activity on the overall perfusion of body organs is rather complex. For instance, the fraction of blood perfusing skeletal muscles is disproportionately increased at the expense of most of the other organs, including those responsible for elimination; skin perfusion is also markedly increased during muscular effort. On the other hand, the fraction of blood perfusing the brain remains the same. Distribution of chemicals will vary accordingly; elimination processes will be less effective allowing higher concentrations of chemicals to reach target organs, including the brain.

When the ambient temperature is elevated, the rate of skin perfusion increases; the skin then appears red and feels warm. Although skin is not an important route for transfer of chemicals, increasing the local circulation is likely to facilitate percutaneous exchange of chemicals between blood and the environment.

Another major factor determining the distribution of chemicals is their affinity for a given tissue. Affinity depends on the physico-chemical properties of a chemical, the biochemical composition of the various cells in organs, and the ability of the cellular membrane acting more or less as a barrier. Chemicals may penetrate into cells by passive diffusion through the lipid-rich membrane, by special carrier-mediated transport systems, or by filtration through small water channels in the cell membrane. Lipid-soluble neutral molecules easily diffuse across cell membranes and tend to accumulate in lipid-rich tissues. Active transport systems (energy-requiring systems) in the liver help remove certain molecules for elimination into the bile. Distribution is therefore a dynamic process enabling chemicals to reach their sites of action, storage, or removal.

Sites of storage have considerable importance in modulating the action of a chemical or its removal. These sites are usually different from those of major action, but they could be located in organs responsible for removal (e.g., liver and kidneys). While they are stored, most chemicals are temporarily inactivated; they remain available to be released and redistributed as the concentration of free, circulating chemicals in blood decreases. Storage prolongs the residence time of chemicals in the body and helps smooth out rapid fluctuations of the concentration of circulating chemicals. On the other hand, sites of storage can represent a threat in the sense that deposited chemicals may be rapidly released for further redistribution to potential sites of action.

Adipose tissues (fat) can store relatively large amounts of highly lipid-soluble chemicals like chlorinated pesticides, polychlorinated biphenyls, dioxins, furans, a number of organic solvents (benzene, trichloroethylene, and styrene), and certain drugs like anesthetics. In the past, biopsy of subcutaneous abdominal fat tissue has been used to monitor exposure to chlorinated pesticides in rural populations around the world; today, the tendency is to monitor such chemicals using blood lipids since the concentration of chemicals in the latter is in equilibrium with that in fatty tissues. Volatile chemicals, like benzene, temporarily stored in fatty tissues, are slowly released into blood and may be more easily monitored in the air exhaled by the lung. Chlorinated pesticides are also largely stored in adipose tissues of birds during summer. When birds migrate, however, adipose tissues are extensively used as a source of energy; pesticides are then mobilized and redistributed to body tissues including the central nervous system (CNS), where concentrations may reach toxic levels.

Bone is also an important site of storage for certain metals that possess physicochemical properties similar to calcium. More than 90% of absorbed lead is incorporated into bone. Lead will stay there for years, slowly exchanging with lead in the blood and other tissues. When the demand for calcium in bone is high, lead may be rapidly released from its deposit and may reach toxic concentrations in target organs; this is especially true for lead workers who have accumulated large burdens of lead in bone throughout the years. Radioactive strontium is another metal with high affinity for bone. Unfortunately, bone is not only a site of deposit for strontium but also a site of action since radiation emitted by strontium induces bone cancers. Fluorides, which are also deposited in bone, may eventually cause skeletal fluorosis, a disease characterized by an increase in the density and the calcification of bone.

The affinity of liver and kidney for a number of chemicals is also considerable. A protein in the liver, ligandin, has a remarkable degree of affinity for organic acids; it plays a role in the transfer of these chemicals from blood into liver. Both liver and kidney may become storage depots for metals, like cadmium and zinc, due to the presence of small binding proteins called metallothioneins. When the binding capacity of these proteins is exceeded, local toxicity may appear, as is the case for cadmium in the kidney.

Finally, what may be considered as one of the most important storage depots in the body is plasma protein. Albumin, the most abundant protein in plasma, and other plasma proteins may bind reversibly a very large number of chemicals, many of which are therapeutic agents. The protein-bound fraction of a chemical exists in a state of equilibrium with the unbound (also called 'free') fraction; only the free form of a chemical is available for biological effect and disposition. By sequestering chemicals for several hours, in certain cases, protein binding in plasma regulates the pharmacological and toxicological effects of chemicals; distribution to sites of action is delayed and access to elimination processes is slowed down. It is a fact of considerable therapeutic importance that certain drugs may be displaced from their sites of protein binding by other chemicals with higher affinity for the same protein. Displacement of drugs that require precise dosing schedules to produce their therapeutic effect without also inducing toxicity, like anticoagulants and oral hypoglycemic agents, can lead to severe toxic manifestations.

The brain, as a site where chemicals are distributed, is a very sensitive organ. A more or less permeable membrane barrier located at the junction between the bloodstream and the brain acts as a shield to certain noxious chemicals; it is called the 'blood–brain barrier'.

The barrier effect is mainly due to the fact that the cells lining the walls of the capillaries present in the brain tissue are tightly joined, contrary to what prevails with capillaries in other tissues; this leaves very little space between the cells for filtration of small-size, water-soluble molecules. Moreover, the cells of brain capillaries possess very few endocytotic vesicles, which in capillaries of other tissues engulf large molecules and serve as a transfer mechanism; as a result, many neurotoxins, such as diphteria and tetanus toxins, are excluded. Furthermore, the capillaries of the brain are surrounded by prolongations of certain brain cells, thus forcing lipid-soluble chemicals to cross an additional lipid membrane. Finally, the intercellular fluid bathing the brain cells contains lower concentrations of proteins; this results in a reduction of the movement of certain water-insoluble chemicals that are more easily transported when bound to proteins.

The existence of the blood–brain barrier does not preclude the passage of chemicals into the brain. As is the case with all other cellular membranes in the body, lipid-soluble nonionized chemicals enter the brain by passive diffusion. Anesthetics, ethanol, and CNS depressants, for instance, rapidly diffuse into the brain in a matter of a few seconds or minutes. They also exit the brain rapidly when the concentration gradient between blood and brain is reversed. Elemental mercury, methylmercury, and tetraethyl lead are examples of lipid-soluble forms of metals that easily enter the brain, while the ionized, much less lipid-soluble inorganic salts of mercury and lead penetrate only poorly.

In newborn infants, the blood–brain barrier is not fully developed; certain chemicals, like lead, and some endogenous substances, like bilirubin, may therefore enter the brain more easily. Like the brain, but for different reasons, the embryo is also very sensitive to exogenous chemicals circulating in the maternal blood. The placenta is the route by which the developing embryo and fetus exchanges with maternal blood. Its main physiological function is to provide nutrients to the fetus and remove its waste products. In humans, only three layers of cells separate maternal and fetal blood and form what has been termed the placental barrier.

The placental barrier is far from being an absolute shield to the passage of foreign chemicals into the fetal circulation. The free form of lipid-soluble, nonionized molecules crosses the placenta by passive diffusion and reaches equilibrium between maternal and fetal circulations. Large molecules and microorganisms may traverse the placenta by endocytosis. Certain differences between maternal and fetal tissue concentrations of chemicals can be explained by, among other factors, lower plasma concentrations of binding proteins, lower amounts of body fat, and the absence of a fully developed blood–brain barrier in the fetus. Once delivered, most chemicals will diffuse back into the maternal circulation, leaving the fetus unharmed. A few chemicals, however, may have a devastating effect, killing the fertilized egg, inducing birth defects, or retarding the growth of the developing fetus.

Thus, for a number of chemicals and a few molecules of microbiological origin, the placenta does not serve as an efficient barrier. For many years, it has been known that the rubella virus (German measles) may cause human congenital anomalies. Similarly, some chemicals known as teratogens may also produce abnormalities in the development of the human fetus. Among them are vitamins A and D taken at high dosages, certain anticancer drugs, some steroid hormones, and thalidomide, certainly the best known teratogen.

Although less spectacular than birth defects like missing limbs or cleft palate, retardation in the functional development of the fetus may be just as damaging. In this regard, the fetotoxicity of excessive alcohol consumption and tobacco smoking is well known. Governments now issue severe warnings to pregnant women concerning the danger of these actions.

See also: Absorption; Blood; Developmental Toxicology; Excretion; Gastrointestinal System; Kidney; Liver; Metallothionein; Neurotoxicity; Pharmacokinetics/Toxicokinetics; Skeletal System.

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Disulfiram

F Lee Cantrell

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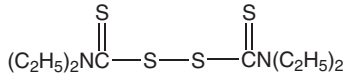
- Chemical Abstracts Service Registry Number: CAS 97-77-8
- Synonyms: Tetraethylthiuram disulfide; Teturamin; Abstensil; Alcophobin; Abstenyl; Antabuse; Antadix
- Chemical/Pharmaceutical/Other Class: Thiuram derivative; Ethanol abuse deterrent
- Chemical Formula: $C_{10}H_{20}N_2S_4$
- Chemical Structure:



Uses

Disulfiram is used as a deterrent to ethanol abuse.

Exposure Routes and Pathways

Disulfiram is available only in an oral form.

Toxicokinetics

Disulfiram is rapidly absorbed, although up to 20% is excreted unchanged in the feces. Pharmacological effects usually occur within 12 h after ingestion and can persist for up to 14 days. Disulfiram has a large volume of distribution secondary to its high lipid solubility. Its protein binding is ~50%. Disulfiram is metabolized in the liver to produce diethyldithiocarbamate, diethylamine, and carbon disulfide. Metabolites are excreted in the urine although a small amount is excreted from the lungs as carbon disulfide.

Mechanism of Toxicity

Disulfiram has multiple mechanisms of toxicity. Its most well-defined action is inhibition of aldehyde dehydrogenase, which thereby diminishes the breakdown of acetaldehyde. Accumulation of carbon disulfide, a disulfiram metabolite, as well as inhibition of dopamine-β-hydroxylase has also been associated with its toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Disulfiram is not used therapeutically in domestic animals. Its toxicity when ingested in overdose is undefined.

Human

Acute overdose of disulfiram, in the absence of concomitant ethanol ingestion, may produce hypotension. When taken with ethanol, a constellation of severe reactions including flushing, vasodilation, pulsating headache, vomiting, and chest pain may occur. Less commonly, severe reactions including hypotension with shock, coma, seizures, and myocardial infarction may occur. An ethanol level as low as 5–10 mg dl^{-1} may produce this reaction with fully developed symptoms appearing when ethanol concentrations exceed 50 mg dl^{-1}. These toxic manifestations correlate with increased serum concentrations of acetaldehyde and may persist for 1–2 weeks after cessation of disulfiram use.

Chronic Toxicity (or Exposure)

Animal

Large doses in laboratory animals produce advanced degenerative changes in liver and kidneys.

Human

In the absence of ethanol ingestion, chronic disulfiram use may produce adverse effects including fatigue, impotence, headache, dermatitis, and a metallic or garlic aftertaste. Neurologic complaints including vertigo, irritability, insomnia, slurred speech, and personality changes may occur. Less commonly, peripheral neuropathy, optic neuritis, delirium, and bizarre behavior may occur. Hematologic and gastrointestinal toxicity include blood dyscrasias and cholestatic hepatitis, respectively. Clinically important drug interactions include impaired metabolism of barbiturates, warfarin, and phenytoin; this may result in toxicity from these agents.

In Vitro Toxicity Data

Disulfiram has been investigated as an agent to prevent the development of multiple drug resistance in cancer cells. Recent studies have demonstrated

inhibition of ATP hydrolysis and modulated P-glycoprotein transport.

Clinical Management

Basic and advanced life-support measures should be utilized as needed. In patients presenting within 1 h of ingestion, activated charcoal should be administered. Supportive care should be provided as needed. Extracorporeal elimination is not indicated for acute disulfiram poisoning but has been effective in treating the acute disulfiram–ethanol interaction.

See also: Alcoholic Beverages and Alcoholism; Carbon Disulfide; Dithiocarbamates.

Further Reading

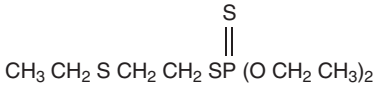
Heath MJ, Pachar JV, and Martinez ALP (1992) An exceptional case of lethal disulfiram–alcohol reaction. *Forensic Science International* 56: 45–50.

Woolley B and Devenyi P (1980) Acute disulfiram overdose. *Journal of Studies in Alcohol* 41: 740–743.

Disulfoton

Jamaluddin Shaikh

- Chemical Abstracts Service Registry Number: CAS 298-04-4
- Synonyms: Dimaz; Disyston; Disystox; Dithiodemeton; Dithiosystox; Solvirex
- Chemical/Pharmaceutical/Other Class: Organothiophosphate insecticide and acaricide
- Chemical Formula: $C_{10}H_{18}O_2S_3P$
- Chemical Structure:



Uses

Disulfoton is a selective, systemic organophosphorus insecticide and acaricide that is particularly effective against aphids, leafhoppers, beet flies, spider mites and coffee leaf miners affecting cotton, tobacco, beets, corn, peanuts, wheat, ornamentals, cereal grains, and potatoes.

Exposure Routes and Pathways

Disulfoton is formulated as a granular product. Dermal and oral routes are the most common exposure pathways. Vapors can also be absorbed by inhalation.

Toxicokinetics

Following absorption, disulfoton distributes throughout the body. It generally does not accumulate in tissues, but is initially bioactivated to the more toxic oxygen analogue, sulfoxide and sulfone, which further degrade to less toxic products. Animal studies showed that within 10 days after dosing, disulfoton and metabolites were eliminated through the urine (81.6%), feces (7%), and exhaled air (9.2%).

Mechanism of Toxicity

Disulfoton mainly causes harmful effects to the nervous system. Sulfoxide and sulfone metabolites inhibit acetylcholinesterase activity in the nervous system, and this action causes neurological effects. Cholinesterase activity in blood is also inhibited by disulfoton and can serve as indicator of exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Disulfoton exhibits gender-dependent differences in toxicity. The oral LD_{50} ranges from 6.2 to 12.5 mg kg^{-1} in male rats and from 1.9 to 2.5 mg kg^{-1} in female rats. The dermal LD_{50} is 15.9 mg kg^{-1} for male rats and 3.6 mg kg^{-1} for female rats. The inhalation LC_{50} for 1 h is 180 ppb for male rats, and 90 μg l^{-1} for female rats.

Human

Disulfoton is considered a highly toxic insecticide and acaricide by all routes of exposure. Early signs and symptoms in humans, whether absorbed through skin, ingested, or inhaled, may include headache, fatigue, blurred vision, dizziness, salivation, tearing, sweating, defecation, urination, and fluid accumulation in the airways. Convulsion and coma can occur at high doses. Ingestion of disulfoton can lead

to a rapid onset of signs. Signs and symptoms following dermal exposure may be delayed up to 12 h. At least 1 week is needed for complete recovery from acute poisoning but complete restoration of blood cholinesterase activity to normal levels may take longer.

Chronic Toxicity (or Exposure)

Animal

Rats have survived for 90 days at a dose of $0.5\,mg\,kg^{-1}\,day^{-1}$. Repeated exposures to disulfoton lead to tolerance, at least partially through downregulation of cholinergic receptors in the central and/or the peripheral nervous systems.

Human

Disulfoton is readily absorbed through the skin. Daily repeated absorption may cause loss of appetite, weakness, flu-like symptoms, and malaise. Some studies report that workers continuously exposed to disulfoton can develop anxiety, irritability, delayed reaction times and cognitive defects. Chronically exposed workers may also suffer from cataract.

Clinical Management

Dermal decontamination should be accomplished by repeated washing with soap. The possibility of disulfoton sequestered under the fingernails should not be overlooked. In case of eye contamination with disufoton, eyes should by flushed with copious amounts of clean water for 15 min. If eye irritation is persistent after decontamination, ophthalmologic consultation is required. Ipecac can be used to induce emesis in case of recent ingestion. Emesis is not encouraged if the patient is comatose or convulsing. Activated charcoal is an effective absorbent and hence used with or without saline cathartic and sorbitol.

Environmental Fate

Disulfoton is relatively stable in water at neutral and acidic pH. It is resistant to hydrolysis with a half-life of 323 days at pH 7. Alkalinity enhances hydrolysis. Disulfoton has been shown to persist for 1 week in sandy loam soil.

Ecotoxicology

Disulfoton is highly toxic to most species of warm water fish. Cold-water species are less sensitive to disulfoton. It has been detected in ground water in several places in the United States. Massive fish kills have been noted in the past with disulfoton use. The metabolites of disulfoton are very toxic to honey bees. It is very toxic to birds. The LD_{50} for Northern bobwhite, Red-winged blackbird, and Mallard are 28, 3.2, and $6.54\,mg\,kg^{-1}$, respectively.

Exposure Standards and Guidelines

The reference dose for disulfoton is $0.043\,\mu g\,kg^{-1}\,day^{-1}$.

See also: Cholinesterase Inhibition; Organophosphates; Pesticides.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Disulfoton.

Dithiocarbamates

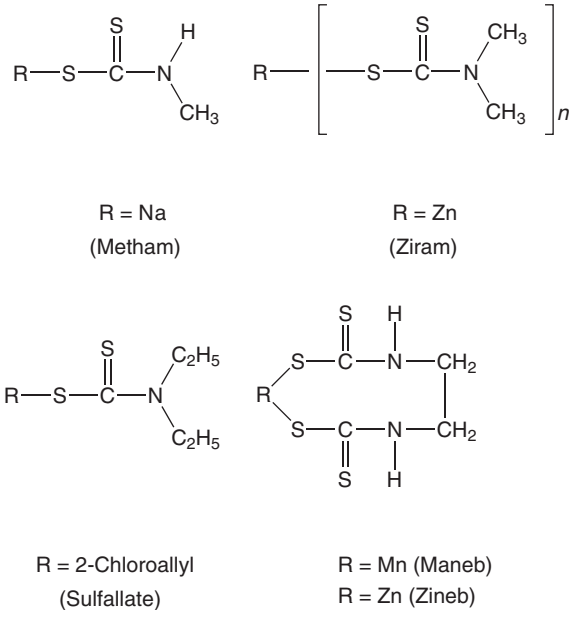
David Janz

This article is a revision of the previous print edition article by Tamal Kumar Chakraborti, volume 1, pp. 504–505, © 1998, Elsevier Inc.

- Preferred Names: Thiram (CAS 137-26-8); Ziram (CAS 137-30-4); Maneb (CAS 12427-38-2); Zineb (CAS 12122-67-7)
- Representative Chemicals: Methyldithiocarbamates (metham); Dimethyldithiocarbamates (DDC, ferbam, thiram, and ziram); Diethyldithiocarbamates (sulfallate); Ethylenebisdithiocarbamates (anobam, maneb, nabam, and zineb)
- Synonyms:
  Thiram–Arasan; Fernasan; Nomersan; Puralin; Tersan; Thiosan
  Ziram–Corozate; Fuclasin; Karbam White; Mathasan; Milbam; Nibam; Zimate
  Maneb–Dithane M-22; Manzate

Zineb–Dithane Z-78; Lodacol; Parzate

- Chemical Structure:



Uses

Most dithiocarbamates are used as fungicides. Dithiocarbamates are also used as herbicides and, at least one compound, metham, is used as nematocide. Ziram is also used as an accelerant in the rubber industry, as a biocide in water treatment, and as an ingredient in adhesives.

Exposure Routes and Pathways

Occupational exposure to dithiocarbamates may occur through inhalation and dermal routes, whereas the general population may be exposed via ingestion of food and water, and through dermal contact with commercial fungicide products. Human poisonings have been reported following oral, inhalation, and dermal exposures to these compounds.

Toxicokinetics

Dithiocarbamates are absorbed from the gastrointestinal tract, lungs, and skin. The absorption of ethylenebisdithiocarbamates from the gastrointestinal tract may be altered by the presence of cations occurring naturally in food.

Little specific information is available regarding the biotransformation of these compounds. Indirect evidence suggests, however, that these chemicals undergo rapid biotransformation and excretion in humans, usually within hours or days following absorption. The metal moiety of ethylenebisdithiocarbamates is eliminated in the metabolic process. The initial metabolites of these ethylenebisdithiocarbamates are not identical to those of dimethyldithiocarbamates. A common metabolic product of all dithiocarbamate fungicides is carbon disulfide. Carbon disulfide may undergo further metabolism to form thiourea (an antithyroid substance), which may partially explain the tendency of different dithiocarbamates to affect thyroid function. Mammalian biotransformation and environmental degradation of ethylenebisdithiocarbamates to ethylene thiourea (ETU) is of concern due to the known mutagenic, carcinogenic, teratogenic, and antithyroidal properties of this chemical. ETU is reported to be metabolized *in vivo* and *in vitro* to ethylene urea with release of atomic sulfur. This sulfur atom can bind to macromolecules in the liver and may alter the activity of some enzymes in the endoplasmic reticulum. It is suggested that binding of this reactive sulfur atom in the thyroid gland may cause a decrease in iodination of tyrosine leading to thyroid dysfunction.

Generally, dithiocarbamates are rapidly excreted through the kidneys. One study reported that the elimination half-life for ethylenethiourea was ~100 h. However, the effect of thiram on acetaldehyde dehydrogenase tends to persist for 10–14 days.

Mechanism of Toxicity

As mentioned above, *in vivo* biotransformation or environmental degradation of ethylenebisdithiocarbamates to form ETU is of toxicological significance due to the known mutagenic, carcinogenic, teratogenic, and antithyroidal properties of this chemical. Neurotoxicity following chronic exposure to maneb has been reported to involve dopaminergic neurotransmission. However, a link to Parkinsonism remains unclear and may be related to manganese content of maneb, to certain metabolites such as carbon disulfide, and/or to the capacity of dithiocarbamates to bind divalent metals and form more lipophilic complexes able to enter the central nervous system. Thiram can precipitate an antabuse (disulfiram) reaction in persons who have consumed a substantial amount of alcohol by inhibiting the enzyme, acetaldehyde oxidase. The ethylenebisdithiocarbamates have the same potential. Several dithiocarbamates have been reported to inhibit cytochrome P450-dependent monooxygenases in animal models.

Acute and Short-Term Toxicity (or Exposure)

Animal

All dithiocarbamates have moderate to low acute toxicity. As opposed to carbamate pesticides, exposure to

dithiocarbamates does not precipitate symptoms of cholinergic crisis. The oral LD_{50} values of these agents vary from 285 to 7500 mg kg^{-1} (average, >2500 mg kg^{-1}). Large doses of thiram caused ataxia, and hyperactivity followed by clonic convulsion, loss of muscle tone, and dyspnea in rats and mice.

Human

Historically, systemic poisoning by dithiocarbamates has been rare. However, an antabuse-like reaction (flushing, sweating, headache, weakness, hypotension, and tachycardia) may occur when ethanol is consumed following exposure to thiram and metallobisdithiocarbamates. Interestingly, this is not typically observed following carbamate, monothiocarbamate, or ethylenedithiocarbamate exposure.

Ataxia, weakness, hypothermia, and ascending paralysis are possible neurologic symptoms after exposure to thiram and ethylenedithiocarbamates. Gastrointestinal signs following dithiocarbamate exposure include nausea, vomiting, and diarrhea. Renal failure has been reported following maneb exposure. Exposure to sprays, solutions, suspensions, and powders of these agents may cause allergic contact dermatitis and mucous membrane irritation. In one case, coma, seizures, and right hemiparesis were reported following exposures to maneb and zineb.

Chronic Toxicity (or Exposure)

Animal

Decreased fertility and impaired thyroid function were reported in cattle after repeated exposure to 200 mg kg^{-1} of zineb for 80 days. Dogs given ziram for 5–9 months (25 mg kg^{-1} day^{-1}) exhibited convulsions and some lethality.

Human

Thiuram, the ethyl analog of thiram, was reported to cause peripheral neuropathy in humans (characterized by pain, numbness, and weakness in the extremities). Occupational exposure to maneb-containing fungicides caused extrapyramidal symptoms in two agricultural workers. In addition to the previously mentioned symptoms, mental confusion, drowsiness, lethargy, and flaccid paralysis were reported to occur with thiram poisoning.

Clinical Management

Symptomatic treatment is recommended as there is no specific antidote available for poisoning by these compounds. In the case of accidental oral poisoning, gastric lavage should be performed soon after ingestion. Absorption of these compounds may be prevented by administering activated charcoal slurry. Conventional anticonvulsant drugs may be used to treat seizures.

Environmental Fate

In general, dithiocarbamates have low to moderate mobility in soils. If released into water, dithiocarbamates are expected to adsorb to particulates and sediments. Volatilization from water or soils is not expected to occur for most dithiocarbamates. If released into air, the low vapor pressures for most dithiocarbamates indicate they will be bound predominantly to particulate matter in the ambient atmosphere. Environmental degradation in air, water, and soil is relatively rapid due to photolysis and/or hydrolysis. Bioconcentration factors ranging from 2 to 90 suggest low accumulation of dithiocarbamates in aquatic organisms.

Ecotoxicology

Dithiocarbamates are potent toxicants in *Daphnia*, with an LC_{50} of <1 mg l^{-1}. Dithiocarbamates are also potent toxicants in fish, with LC_{50} values generally <1 mg l^{-1}. Immature trout are more sensitive than older individuals. Some dithiocarbamates have embryotoxic and teratogenic effects.

See also: Carbamate Pesticides; Pesticides.

Further Reading

Ecobichon DJ (2001) Toxic effects of pesticides. In: Klaassen (ed.) *Casarett and Doull's Toxicology*, 6th edn. New York: McGraw-Hill.

Meco G, Bonifati V, Vanacore N, and Fabrizio E (1994) Parkinsonism after chronic exposure to the fungicide maneb (manganese ethylene-bis-dithiocarbamate). *Scandinavian Journal of Work Environment and Health* 20: 301–305.

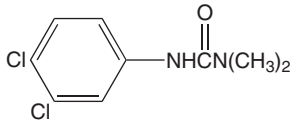
Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Diuron

Jing Liu

- Chemical Abstracts Service Registry Number: CAS 330-54-1
- Synonyms: *N*′-(3,4-Dichlorophenyl)-*N*,*N*-dimethylurea; 3-(3,4-Dichlorophenyl)-1,1-dimethylurea; DCMU; DMU; Cekiuron; Crisuron; Dailon; Diater; Diurex; Duirol; Karmex
- Chemical/Pharmaceutical/Other Class: Substituted urea herbicide
- Chemical Formula: $C_9H_{10}Cl_2N_2O$
- Chemical Structure:



Uses

Diuron is used as a general pre-emergence herbicide for weed control on noncrop lands and among some agricultural crops such as asparagus, pineapple, cotton, and sugarcane. It is also used as a soil sterilant.

Exposure Routes and Pathways

Dermal and inhalation routes are the primary exposure pathways in occupational settings. Ingestion is also a possible route of accidental exposure.

Toxicokinetics

Diuron is absorbed from the gastrointestinal and respiratory tracts. There was no apparent tissue storage of diuron being noted in either rats or dogs after up to 2 months of feeding. It undergoes hydroxylation and dealkylation with the urea moiety generally unchanged. In rats and dogs, the predominant metabolite was *N*-(3,4-dichlorophenyl)-urea, accompanied by small amounts of *N*-(3,4-dichlorophenyl)-*N*′-methylurea, 3,4-dichloroaniline, 3,4-dichlorophenol, and unmetabolized diuron. In mouse liver microsomes, eight metabolites were identified with the *N*-demethylated derivative being the major one, followed in importance by three *N*-hydroxymethyl compounds. Metabolites found in mammals are similar to those found in soil and plants wherein dealkylation and hydroxylation are also the major metabolic pathways. The metabolites are mainly excreted in urine and feces.

Mechanism of Toxicity

Diuron is a selective inhibitor of the Hill reaction in plant photosynthesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Diuron exhibits low acute toxicity. The oral LD_{50} in male rats is $>3\,g\,kg^{-1}$. Dietary protein levels have been reported to influence the acute toxicity of diuron in albino juvenile rats. High acute dosage of diuron (at LD_{50}s) in these young rats may cause drowsiness and ataxia; animals that survived were irritable and hyperexcitable. Diarrhea and clinical signs of renal dysfunction were reported. Subacute exposure of diuron may cause growth retardation and increase erythropoiesis. No skin irritation and sensitization was found in guinea pigs. Diuron was able to induce multiple hepatic microsomal enzymes in rats after as early as 3 days of oral exposure.

Human

Diuron produces little acute toxicity in humans except irritation of the skin, eyes, and nose.

Chronic Toxicity (or Exposure)

Animal

A 14 months feeding study in female rats (0.5–$1\,g\,kg^{-1}$) caused hemolytic anemia and methemoglobinemia. At extremely high subacute dosages, male rats exhibited spleen and bone marrow changes. With repeated high dosages, diuron led to blood chemistry changes, increased mortality, and growth retardation. At a very high dosage ($250\,mg\,kg^{-1}\,day^{-1}$ on gestation days 6–15), diuron caused wavy ribs, extra ribs, and delayed bone formation in rats.

Human

Little is known regarding effects of long-term exposure to diuron in humans.

In Vitro Toxicity Data

Diuron was not mutagenic in a number of bacterial and mammalian cell assays.

Clinical Management

Treatment is symptomatic.

Environmental Fate

Diuron is stable to hydrolysis (at pHs 5, 7, and 9) and photolysis and therefore persistent in the environment. Mobility of diuron in the soil is related to organic matter. It has the potential to leach into ground and to contaminate ground and surface waters. Diuron, however, has low water solubility (42 ppm).

Ecotoxicology

Diuron has an oral LC_{50} of 1730 ppm in bobwhite quail and 5000 ppm in mallard ducks. The compound is relatively nontoxic to honey bees. Diuron is moderately toxic to aquatic animals such as rainbow trout, bluegill sunfish, sheepshead minnow, Eastern oyster, and brown shrimp. The LC_{50}s of diuron in fish range from 4.3 to 42 ppm.

Exposure Standards and Guidelines

The oral reference dose derived from a 2 year dog feeding study using abnormal pigments in blood as the critical endpoint was $0.002\,mg\,kg^{-1}\,day^{-1}$.

The threshold limit value – time-weighted average is $10\,mg\,m^{-3}$.

Oncogenic systemic no-observed-adverse-effect level (NOAEL) in rats is $1.25\,mg\,kg^{-1}\,day^{-1}$.

Reproductive NOAEL in rats is $6.25\,mg\,kg^{-1}\,day^{-1}$.

See also: Pesticides.

Further Reading

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Kinoshita FK and DuBois KP (1970) Induction of hepatic microsomal enzymes by Herban, Diuron, and other substituted urea herbicides. *Toxicology and Applied Pharmacology* 17: 406–417.

Schoket B and Vincze I (1990) Dose-related induction of rat hepatic drug-metabolizing enzymes by diuron and chlorotoluron, two substituted phenylurea herbicides. *Toxicology Letters* 50: 1–7.

Suzuki T and Casida JE (1981) Metabolites of diuron, linuron, and methazole formed by liver microsomal enzymes and spinach plants. *Journal of Agricultural and Food Chemistry* 29: 1027–1033.

Wang SW, Chu CY, Hsu JD, and Wang CJ (1993) Haemotoxic effect of phenylurea herbicides in rats: Role of haemoglobin-adduct formation in splenic toxicity. *Food and Chemicals Toxicology* 31: 285–295.

Liu J (2001) Phenylurea herbicides. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1521–1528. San Diego, CA: Academic Press.

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http://pmep.cce.cornell.edu – EXTOXNET.
http://www.epa.gov – US Environmental Protection Agency.

DNA *See* Aneuploidy; Carcinogen–DNA Adduct Formation and DNA Repair; Chromosome Aberrations; DNA Phosphoramidites; Genetic Toxicology; Genomics, Toxicogenomics; Molecular Toxicology–Recombinant DNA Technology; Toxicity Testing, Mutagenicity.

DNA Adduct *See* Carcinogen–DNA Adduct Formation and DNA Repair.

DNA Phosphoramidites

Sang-Tae Kim

- Representative Chemicals: Deoxyadenosine benzoyl cyanoethyl phosphoramidite; Deoxycytidine benzoyl cyanoethyl phosphoramidite; Deoxyguanosine isobutyryl cyanoethyl phosphoramidite; Thymidine cyanoethyl phosphoramidite
- Chemical Abstracts Service Registry Numbers: Deoxyadenosine benzoyl cyanoethyl phosphoramidite (CAS 98796-53-3); Deoxycytidine benzoyl cyanoethyl phosphoramidite (CAS 102212-98-6); Deoxyguanosine isobutyryl cyanoethyl

phosphoramidite (CAS 93183-15-4); Thymidine cyanoethyl phosphoramidite (CAS 98796-51-1)

- Synonyms:
  - A phosphoramidite; Deoxyadenosine phosphoramidite; Adenosine, *N*-benzoyl-5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-,3′-[2-cyanoethyl bis(1-methylethyl)phosphoramidite]
  - C phosphoramidite; Deoxycytidine phosphoramidite; Cytidine, *N*-benzoyl-5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-,3′-[2-cyanoethyl bis(1-methylethyl)phosphoramidite]; *N*4-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine-3′-*O*-[*O*-(2-cyanoethyl)-*N*,*N*′-diisopropylphosphoramidite]
  - G Phosphoramidite; Deoxyguanosine phosphoramidite; Guanosine, 5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-2′-deoxy-*N*-(2-methyl-1-oxopropyl)-,3′-[2-cyanoethyl bis(1-methylethyl)phosphoramidite]
  - T Phosphoramidite; Deoxythymidine phosphoramidite; Thymidine, 5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-, 3′-[2-cyanoethyl bis(1-methylethyl)phosphoramidite]
- Chemical Formulas:
  - A phosphoramidite: $C_{47}H_{52}N_7O_7P$
  - C phosphoramidite: $C_{46}H_{52}N_5O_8P$
  - G Phosphoramidite: $C_{44}H_{54}N_7O_8P$
  - T Phosphoramidite: $C_{40}H_{49}N_4O_8P$

Uses

DNA phosphoramidites are used in the chemical synthesis of DNA oligonucleotides.

Exposure Routes and Pathways

Exposure to DNA phosphoramidites is most likely to occur in occupational settings. Inhalation and dermal exposure are the primary routes of exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

DNA phosphoramidites were determined to be nontoxic to rats when dosed at 2000 mg kg^{-1} via the oral route. DNA phosphoramidites had no skin irritation potential based upon studies in the rabbit. DNA phosphoramidites had slight eye irritation potential based upon studies in the rabbit. DNA phosphoramidites were tested for their capacity to induce hypersensitivity responses in mice as measured by the proliferation of lymphocytes in the draining lymph nodes. DNA phosphoramidites did not induce hypersensitivity responses and therefore are not considered to be potential sensitizers. In a 28 day oral toxicity study in rats, the major target organ of toxicity was the liver; however, the trends or findings might not necessarily be considered adverse in the 28 day dosing regimen. No other significant tonic effect were noted.

Human

Little information is available regarding the toxic effects of DNA phosphoramidites in humans. Prolonged eye contact to the solid form of DNA phosphoramidites may cause eye irritation.

In Vitro Toxicity Data

DNA phosphoramidites were not mutagenic to *Salmonella typhimurium* and *Escherichia coli* strains with and without metabolic activation.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Molecular Toxicology–Recombinant DNA Technology.

Further Reading

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Poopeikoa NE, Juhlb M, Vesterc B, Sørensen MD, and Wengel J (2003) Xylo-configured oligonucleotides (XNA, xylo nucleic acid): Synthesis of conformationally restricted derivatives and hybridization towards DNA and RNA complements. *Bioorganic & Medicinal Chemistry Letters* 13: 2285–2290.

DNA Repair *See* Carcinogen–DNA Adduct Formation and DNA Repair.

Dominant Lethal Tests

Samantha E Gad

This article is a revision of the previous print edition article by Ann D Mitchell, volume 1, pp. 506–508, © 1998, Elsevier Inc.

Dominant lethal mutations are generally considered to be the result of mutations in germinal tissue which do not cause dysfunction of the gametes but which result in prenatal death of embryos heterozygous for the mutations. Thus, dominant lethal tests, usually conducted in rodents, assess the inheritability of genomic mutations (i.e., mutations that can be passed to the next generation). Obviously, since embryos heterozygous for dominant lethal mutations do not survive, the mutations that result in dominant lethality cannot be passed to succeeding generations. However, the assumption is made that if dominant lethal mutations are present, other dominant and recessive nonlethal mutations could also be present and inherited by future generations.

Pioneering studies that provided the scientific foundation for the dominant lethal test were conducted in the 1930s, but the only known mutagen before the end of World War II was radiation. It was not until the late 1950s and the 1960s that, as a result of genetic research, scientists expressed concerns that chemicals might be hazardous to the germline of humans and suggested that routine toxicity testing of chemicals should include assays for mutagenicity. The dominant lethal test was one of the first genetic toxicology tests to be developed to assess the potential hazards of chemicals. In the early 1970s, the dominant lethal test (together with the host-mediated assay and the *in vivo* cytogenetic assay) was one of the original three screening tests recommended for evaluating the mutagenic effects of chemicals and, in the ensuing time, numerous approaches for examining dominant lethal mutations in rodents have been developed and evaluated.

Dominant lethal tests are usually conducted in mice or rats. The mouse dominant lethal test is more economical, but the rat dominant lethal test is more informative, as corpora lutea may be accurately enumerated in pregnant female rats, but not in mice, to assess preimplantation loss. Although dominant lethal tests may be performed with treated females, the tests are commonly performed with treated males in order to identify stages sensitive to mutation induction during germ cell development because the knowledge of stage sensitivity is important for risk evaluation. In a typical dominant lethal test, males are dosed with three levels of the test chemical with the objective of the highest dose being one that will exhibit some signs of toxicity but that will be low enough for a sufficient number of males to survive through the duration of the test. Following dosing, each treated male is mated to one or more virgin females over each of a series of mating cycles. The females are euthanized mid-gestational term, and the uterine contents are examined to enumerate the number of live implantations (fetuses), early and late dead implantations (dominant lethals), and total implantations. If the test is conducted in rats, the corpora lutea are also enumerated to determine the number of ovulated eggs that fail to develop into fetuses (preimplantation loss). Data, including fertility indices, are then analyzed statistically based on groups of treated males, and each parameter and each mating group is evaluated independently.

Dominant lethal protocols can vary in the route and duration of exposure of the treated males, the number of males per dose level, the number of females per male per mating interval, and the number of mating intervals. However, it is necessary that dominant lethal tests be conducted with sufficient numbers of animals and mating intervals to maximize the test's sensitivity for detecting genomic mutational events and to provide information on germ cell-stage sensitivity of any mutagenic effects that may be observed. Thus, it is necessary to define protocols for each test material that will address these concerns as well as current regulatory requirements. Economies of testing may be achieved by using a laboratory's recently obtained (e.g., within $\sim$12 months) historical positive control values for the same species and strain, with the same number of mating cycles, rather than using a concurrent group of positive control animals. However, even then, one dominant lethal test may involve more than 2000 animals.

The dominant lethal test is no longer used as an initial, or first-tier, test largely because of the time and expense that are involved as well as the numbers of animals that are required to assess the results for statistical significance. However, the dominant lethal test has gained acceptance as a second-tier test for national and international regulatory submissions since the current consensus of experts is that the dominant lethal test can best be used to confirm positive results from lower tier chromosomal aberration-detecting systems (confirming in the sense of indicating the ability of the chemical to penetrate gonadal tissue and to produce cytogenetic damage). Further, the expert consensus is that the dominant lethal assay should not

be used as a risk assessment method. Supporting these views is the evidence indicating that the dominant lethal test is less sensitive than first-tier tests for assessing gene and chromosomal mutations *in vitro*. In addition although the correlation between positive dominant lethal results and carcinogenicity is apparently low, which would be expected, it has been found that dominant lethal results are highly predictive of the outcome of the even more expensive and time-consuming third-tier specific locus and heritable translocation tests for genetically transmissible mutations. Thus, the apparent lack of sensitivity of the dominant lethal test in comparison to *in vitro* tests and the lack of concordance of dominant lethal test results with carcinogenicity may reflect differences between *in vitro* and *in vivo* exposures of target cells, differences in the cellular and genetic mechanisms of carcinogenesis in somatic tissues and the induction of heritable mutations in germinal tissues, and/or the failure of some mutagenic chemical metabolites to reach germinal tissues because the dominant lethal test has high concordance with other mammalian germ cell mutagen tests.

See also: Analytical Toxicology; Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogenesis; Chromosome Aberrations; Developmental Toxicology; Host-Mediated Assay; Molecular Toxicology–Recombinant DNA Technology; Mouse Lymphoma Assay; Genetic Toxicology; Sister Chromatid Exchanges; Toxicity Testing, Mutagenicity.

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Donora: Air Pollution Episode

Michael A Kamrin

Introduction

Donora, Pennsylvania is located in a narrow valley along the Mohongahela River ~35 miles south of Pittsburgh. In the first half of the twentieth century, it was the site of two United Steel Corporation facilities: the Donora Zinc Works and the American Steel and Wire Company. As was the case in many other towns in which industrial facilities were sited during that era, the poor air quality that was associated with these installations was considered a fact of life. Although most people thought of it as a nuisance, some health impacts of the pollution were recognized as early as 1918 and in the 1920s farmers sued the company for loss of crops and livestock. As a result of these health concerns, air quality measurements were made regularly from 1926 to 1935 but were then discontinued. The end of monitoring was not due to great improvements in air quality. Indeed, this remained poor especially when weather conditions led to the accumulation of pollutants in the area.

Donora Air Pollution Incident

The attitude towards local air pollution changed in 1948, when weather conditions led to a prolonged period of very poor air quality which resulted in significant morbidity and mortality among the residents. The incident, which lasted from October 26 to October 31, started as the result of cold anticyclone that approached the area from the west. The cold ground, coupled with the anticyclone, led to an elevated inversion layer that trapped the pollutants in the air above Donora and the surrounding area. This inversion layer lasted for 5 days due to a high-pressure ridge that remained in the area, moving less than a few hundred miles during this time.

As the days went by, visibility gradually decreased as the chemical smog increased in intensity. By the third day of the incident, the percentage of people becoming ill started to rise precipitously despite the best efforts of the local emergency response volunteers and medical personnel. An attempt was made to evacuate citizens with heart or respiratory ailments but the conditions made travel impossible. Volunteers brought oxygen to those suffering respiratory distress but the smog delayed the delivery of oxygen and supplies were limited.

The first death occurred on the third day and on the fourth day, the number of deaths increased so that a temporary morgue had to be set up. The town's eight physicians were too few in number to attend to all who were affected. All during this period, the plants continued to spew pollution, containing particulates, sulfur dioxide/sulfuric acid, zinc, lead and cadmium, into the air. It was not until the morning of the 30th that the Donora Zinc Works shut down and later that afternoon, rains finally arrived and washed the smog from the air.

The incidence of adverse health impacts decreased rapidly as the smog disappeared. It appears from the time course of the toxicity that it was a threshold phenomenon; that is, effects were related to the maximum pollution dose and abated quickly when the dose decreased. Other air pollution episodes; for instance, the 1952 London fog, followed a different response pattern in that the effects were influenced mainly by the total pollution dose (concentration averaged over time).

The toll from the Donora incident was very high; 20 people died, ~50 were hospitalized, and ~6000 (out of the population of 14 000) experienced some adverse effects from the chemical smog. These adverse effects included nasal discharge, constriction of the throat, sore throat and symptoms related to compromised lung function. As might be expected, the older people were most affected; over two-thirds of those hospitalized were over 55. These data reflect only the acute effects of the episode and it is quite possible that some of those who survived suffered chronic or delayed effects that decreased their life expectancy. Indeed, a follow-up study ten years after the incident suggested that there was higher mortality in the individuals who showed adverse effects during the episode.

This incident gained national attention when it was mentioned in a national news broadcast by the well-known Walter Winchell. The seriousness of the episode led to a collaborative investigation by local, state, and national agencies, including the US Public Health Service. This represented the first organized attempt to document the health effects of air pollution in the US. The Public Health Service recommended warnings tied to meteorological conditions and better air sampling to prevent future problems of this sort.

However, this episode did not remain in the public or scientific consciousness long. By 1949, public attention turned to a smog incident in Southern California where irritant effects were of most concern. The lack of scientific interest in the effects of smog was reflected in the absence of discussion of this incident in technical conferences, such as the meetings of the American Chemical Society.

However, the great London fog of 1952 that resulted in ~4000 deaths refocused attention on the public health consequences of air pollution and the lessons from Donora. The tremendous death toll in London made it clear that air pollution was more than just a nuisance and led to the enactment of legislation on both the state and federal level to control this type of environmental insult. The Commonwealth of Pennsylvania passed a state Clean Air Act in 1955, the first such law to control air pollution. This was followed in 1970 by the passage of the Federal Clean Air Act; hearings on this bill were marked by references to the events that happened in Donora in 1948.

Summary

The Donora air pollution incident was an early warning of the potential health impacts of industrial air pollutants and served as an important contributor to the development of regulations to control air pollution and also to increased public concern about environmental contamination of all types. This concern intensified in the 1960s and culminated in the formation of the Environmental Protection Agency in 1970. This was followed soon after by the passage of a number of environmental protection laws and regulations, including the Clean Air Act, during the decade of the 1970s.

See also: Clean Air Act (CAA), US; Pollution, Air; Great Smog of London.

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Dose–Response Relationship

Samantha E Gad

The dose–response relationship measures the correlation that occurs as one modifies the amount (dose) of a chemical substance to which a living material is exposed and the severity of the effect (response). This is commonly used with pharmaceuticals to determine the most effective amount of medication to be administered to have the desired beneficial effect. If the amount of medicine administered is too small (below the therapeutic level), the intended beneficial effect does not occur; if the dose is increased and the amount administered is too large (above therapeutic range) toxicity may become evident. Toxicologists hold that the dose–response relationship applies not only to therapeutic agents but also to all chemical substances, that is, 'the dose makes the poison'. The underlying principal is that the biological effects (beneficial or deleterious) of chemicals are due to the amounts of active material at the site, or sites, of action and that the concentration or the amount of the substance at the site (internal dose) is related to the amount of chemical administered (external dose).

The dose–response relationship can be viewed most simply as what happens in a single individual, where the severity of the effect increases as the dose increases; this is referred to as a 'graded' relationship. This relationship between dose and response can also be described in terms of the numbers of individuals (usually measured and reported as a percentage) of a defined population affected at a given dose level, where the frequency increases as the exposure increases; this is referred to as an 'all-or-none' or 'quantal' relationship. The demonstration of dose–response relationship suggests causality between the degree of exposure and the adverse effect. An adverse effect can be defined as a change in the morphology, physiology, growth, development, or life span of an organism that results in an impairment of its functional capacity or ability to compensate for additional stress, or in an increased susceptibility to the harmful effects of other environmental influences.

In 1983, the National Research Council of the (US) National Academy of Sciences published a report titled *Risk Assessment in the Federal Government: Managing the Process*; this work has had a marked influence on the risk assessment process used by regulatory agencies worldwide. The risk assessment process, in this report, consists of four components: hazard identification, dose–response assessment, exposure assessment, and risk characterization. 'Hazard identification' in the context of the report is concerned with evaluating the potential adverse health effects of a chemical, mixture of chemicals, or process; thus, it is very similar to the traditional term 'toxicity' used by toxicologists.

Dose–Response Curves

The typical dose–response curve is usually sigmoidal in shape, when the response, expressed as a percentage of the frequency, is plotted against the dose, expressed as a logarithmic scale (**Figure 1a**). The sigmoidal curve represents the cumulative curve of a normal (Gaussian) distribution of the response, where the response for each individual dose level is expressed as a percentage of the total and is plotted against the log dose (**Figure 1b**). All of the responses occurring in the dose levels below and above any dose level of particular interest should be included to do the curve, and the background response level in the control group must be kept in mind. The resulting bell-shaped, normal distribution curve that is typically developed from toxicity data reflects the variation in susceptibility among individual subjects in a given population to the effects of the chemical. The subjects responding at the lower doses (left side of the curve) represent hypersusceptible subjects, whereas those responding at the higher doses (right side of the curve) are considered resistant subjects; those situated at the middle doses represent the average responders.

Mathematically, if one plots the cumulative response along a probability unit ('probit') scale, the cumulative sigmoidal dose–response curve is transformed into a straight line. Such a transformation is illustrated in **Figure 1c**. Converting the data to straight lines is essential if one wishes to compare the dose–response characteristics of several chemicals because mathematical procedures exist for determining various parameters of such a linear relationship. One can assess the degree of change in response to the degree of change in dose; this is called the 'slope' of the dose–response curve. With some agents, the change in response may be extremely abrupt (a relatively small change in dose results in a large change in response); this type of chemical is said to possess a 'steep' dose–response curve. With other agents, the change may be quite small (a relatively large change in dose is required to elicit a small change in response); such a substance is said to exhibit a 'flat' dose–response relationship. Linear curves can also be compared mathematically to determine if they are parallel to each other.

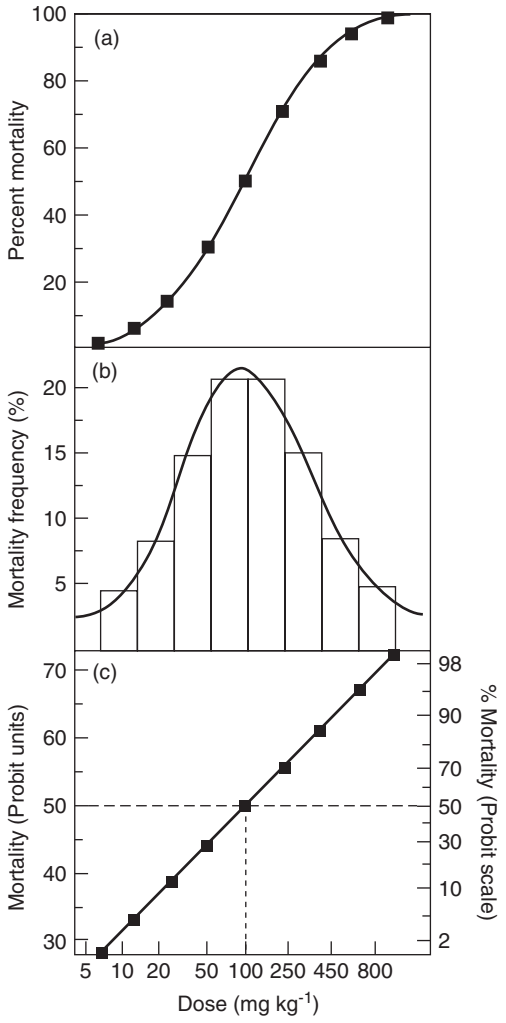

Figure 1 Graphical representation of a typical dose–response relationship for assessing mortality in laboratory animals receiving varying dosages of a toxicant. In all panels, the dosage ($mg\,kg^{-1}$) is plotted as a logarithmic scale. (a) The response (%mortality) is plotted as a cumulative percentage of the total number of dead animals (the number of animals killed at a specific dosage level and all dosages below it are added together and the percentage of the total number is calculated). (b) Each bar (mortality frequency %) represents the percentage of the total number of animals that died at each dosage minus the percentage that died at the immediately lower dosage. The curve that joins the bars is the bell-shaped relationship known as the normal frequency distribution curve. (c) The cumulative percentage of the total number of dead animals seen in the top panel is expressed in probit units on the left ordinate (mortality (probit units)) and as a probit scale on the right ordinate (% mortality (probit scale)). (Reproduced from Klaassen CD and Eaton DL (1991) Principles of toxicology. In: Amdur MO, Doull J, and Klaassen CD (eds.) *Casarett and Doull's Toxicology: The Basic Science of Poisons,* 4th edn, 13. New York: Pergamon.)

While the typical dose–response curve can usually be described by a sigmoidal or linear curve, it may be convex, concave, or even bimodal. These other configurations are usually the exception rather than the rule and depend on the mechanism of action of the material in question and even the presence of multiple toxicity sites. In some instances, the curve may be 'J-shaped' or inverted 'U-shaped', depending on the endpoint being measured. This type of dose–response relationship is observed in a phenomenon known as hormesis, with one explanation being that exposure to small amounts of a material can actually confer resistance to the agent before frank toxicity begins to appear following exposures to larger amounts. However, analysis of the available mechanistic studies indicates that there is no single hormetic mechanism. In fact, there are numerous ways for biological systems to show hormetic-like biphasic dose–response relationship. Hormetic dose–response has emerged in recent years as a dose–response phenomenon of great interest in toxicology and risk assessment.

Threshold

A toxicant must be present at its cellular site of action in sufficient amounts to exert its deleterious effects. When the concentration is too small it is said that the 'threshold' has not been reached; therefore, the material does not exert any adverse action. The distribution of active substances in the body is not uniform, and certain cells can exhibit preferentially high affinities for particular agents. Pharmacokinetic thresholds determine the effective dose of a chemical at its biological target site based on the absorption, distribution, biotransformation, and excretion of the particular chemical.

Specific Dose-Dependent Values

Specific dose-dependent values on the linear dose–response curves can be estimated and statistically compared as well as the degree of variability (confidence limits) representative of the data being analyzed. Perhaps the most common specific toxicity value so determined in laboratory animals is the 'median lethal dosage' or the LD_{50}. This value is the estima ted dosage that would be expected to kill 50% of a given population of animals under the conditions of a particular laboratory test. With medicinal agents, another useful value related to the LD_{50} is the 'therapeutic index'. Here one is interested in the 'median effective dosage' (ED_{50}) for a beneficial pharmacological therapeutic effect and how it compares to the toxic potency of the agent. One way of assessing this situation is to calculate an LD_{50}/ED_{50} ratio; the larger the ratio, the greater the relative safety of the chemical. Another way of assessing the relative safety of medicinal agents is to compare the ED_{99} (the dose that is effective in 99% of a given population) to the

LD_1 (the dose that is lethal to 1% of the same population). The ratio LD_1/ED_{99} is called the 'margin of safety' – the larger the ratio, the greater the relative safety of the medicinal agent.

Chronicity Index

The result of repetitive exposures to a given chemical may be different from when exposure to the material only occurs once or twice. The cumulative effects of repetitive exposures over time may render the agent more hazardous. This property can be estimated in animals by comparing the lethal potency (LD_{50}) of an agent, given only once, to its lethal potency when administered repetitively. The 'chronicity index' is a term that has been applied to a ratio of the 'one-dose LD_{50}' (animals receive the material only once) and the '90-dose LD_{50}' (animals receive the material repetitively each day for 90 days). If the one-dose/90-dose ration is close to 1, this is an indication that repetitive administration does not result in cumulative effects or cumulative retention, whereas if the ratio increases and is larger than one, it is quite likely that the agent exerts cumulative effects or is retained over the repeated exposures.

Dose–Response Limits in Regulatory Toxicology

Although mathematical ratios are not usually derived from subchronic toxicity studies conducted with laboratory animals for regulatory purposes, the dose–response relationship is a very important part of such studies. Different dose levels are utilized in such experiments, and it is desirable to have at least one dose level where no adverse biological effects occur following exposure. One finds various terms used to describe the severity of biological effects observed or extrapolated from such studies. The 'no-observed-effect level' (NOEL) is described as the highest dosage that creates no significant difference in the observed and measured effects between the exposed animals and the unexposed control group. The 'lowest-observed-effect level' (LOEL) is the lowest dose used in a study that results in the appearance of some statistically or sometimes nonstatistically significant biological effect (beneficial or deleterious). The 'lowest-observed-adverse-effect level' (LOAEL) is the lowest dose used that results in the appearance of an adverse effect.

The 'no-observed-adverse-effect level' (NOAEL) is the highest dosage where an adverse effect is not observed. Depending on the doses used in a study, even the lowest effective dose could cause a moderate or severe response although this would not be the ideal situation toxicologically. The same is true with the LOAEL, that is, this dose group could have effects that are more than mild. Finally, the 'frank-effect level' (FEL) is a treatment level that results in the appearance of overt toxic effects. **Figure 2** is a graphical representation of where these various dosage levels might appear in a typical dose–response curve. Given study designs and their fixed number of dose levels, not all of them are observed in each subchronic or chronic study. Nevertheless, one usually sees an FEL, an LOAEL, and either an LOEL or an NOAEL. One can also extrapolate an NOAEL or an NOEL from the data derived in a subchronic or chronic study because these dosage levels can be important for establishing safety guidelines for humans. Extrapolation of an NOAEL, NOEL, or other level of effect or no effect is usually a matter of data analysis. In risk assessment, this is usually achieved by applying uncertainty factor or using a mathematical model. There are many decision-making processes in order to estimate an NOAEL of NOEL by data extrapolation, and readers are referred to some of the 'related topics' of this encyclopedia (see See also section). For example, the benchmark dose (BMD) methodology can be used to identify point-of-departure (POD) estimates for use in derivation of reference doses (RfDs) or for evaluation of margins of exposure.

The dose–response relationship is the basis by which regulatory bodies define under what limits humans can be exposed to potentially toxic chemicals and yet not suffer adverse effects. A number of different government bodies establish regulations to define safe exposure conditions. The 'acceptable daily intake' (ADI) is

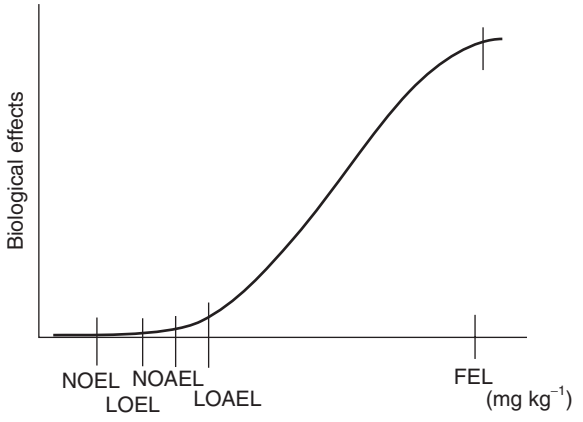

Figure 2 Schematic representation of various dosage limits used in regulatory toxicology and where they usually appear in a typical dose–response curve. (Reproduced from Ecobichon DJ (1992) *The Basis of Toxicity Testing*, Boca Raton, FL: CRC Press.)

defined as the daily intake of a chemical that, during an entire lifetime, appears to be without appreciable risk; the ADI is used by the US Food and Drug Administration (FDA) for calculating permissible levels of nonfood ingredient residues in food (for instance, food additives or pesticides). The US Occupational Safety and Health Administration (OSHA) estimates 'permissible exposure limits' for chemical contaminants in occupational environments, to which workers are exposed for given periods during normal working conditions. Further, the US Environmental Protection Agency (EPA) makes use of the reference concentration (RfC) and RfD to estimate levels of noncarcinogenic environmental chemicals to which humans can be exposed during a lifetime without deleterious effects. All of these various safety indicators use NOAEL or NOEL values as part of the calculation.

Dose–response has received an increasing level of attention in recent years, including the founding of the Dose–Response Specialty Group (DRSG) of the Society for Risk Analysis (SRA) in 1994. This group is open to all members of the SRA interested in biological and mathematical relationships between exposure and effect, with a focus on issues related to the shapes of dose–response curves and their underlying biological meaning, population distributions that describe ranges of response, and probabilistic approaches to extrapolating responses in unknown populations based on observations in experimental populations or epidemiology studies. The group is interested in all exposed populations including humans and environmental species and is closely aligned with the toxicology and environmental sciences fields.

In addition to SRA's DRSG, a group of scientists representing several US federal agencies, the International Society of Regulatory Toxicology and Pharmacology, the private sector, and academia met in 1990 to develop a strategy to encourage the assessment of the biological effects of low-level exposures (BELLE). The meeting was convened because of the recognition at the time that most human exposures to chemical and physical agents are at relatively low levels, yet most toxicological studies assessing potential human health effects involve exposures to levels of orders of magnitude greater than actual human exposures. Consequently, the BELLE founders noted that risks at low levels are estimated by various means, frequently utilizing assumptions about which there may be considerable uncertainty. BELLE is committed to the enhanced understanding of low-dose responses of all types, whether of an expected nature (e.g., linear, sublinear) or of a so-called paradoxical nature. Paradoxical dose–response relationships might include U-shaped dose–response curves and biphasic dose–response curves. The focus of BELLE now encompasses dose–response relationships to toxic agents, pharmaceuticals, and natural products over wide dosage ranges in *in vitro* and *in vivo* systems, including human populations.

See also: Benchmark Dose; Exposure Assessment; Exposure Criteria; Hazard Identification; Hormesis, LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Levels of Effect in Toxicological Assessment; Maximum Allowable Concentration (MAC); Maximum Tolerated Dose (MTD); Pharmacokinetics/Toxicokinetics; Reference Concentration (RfC); Reference Dose (RfD); Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Toxicity, Acute.

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Relevant Websites

http://www.belleonline.com – Biological Effects of Low Level Exposures (BELLE) Advisory Committee.

http://www.sra.org – Society for Risk Analysis (SRA), Dose Response Specialty Group.

Drinking Water Criteria

Betty J Locey

Drinking Water Standards and Criteria

Drinking water is necessary for people, livestock, wildlife, crop irrigation, and for recreation. Although ~70% of the Earth's surface is covered with water, most is salt water (salinity of ~3.5%) and not fit to drink. Only 3% of the water on the Earth is freshwater (water that contains only minimal quantities of dissolved salts) and much of this water is in snow and ice (e.g., glaciers) as well as lakes, streams and groundwater. In some places in the world, freshwater is plentiful (e.g., the Great Lakes); however, in many places water is scarce. Wars have been fought over freshwater resources.

Freshwater generally includes constituents and materials other than water (H_2O). These may be naturally occurring or introduced by human activities. These may include chemicals, microbial agents, radioactive species, and other agents (e.g., asbestos, suspended materials such as sediments). Where drinking water is supplied through public water supply systems, contaminants may also be introduced during treatment (residual disinfection chemicals) and during delivery from water treatment facilities to consumers (e.g., lead leaching from solder, pipes and faucets). Contaminants in drinking water can pose a health threat, may cause cosmetic effects (e.g., consuming water with high levels of fluoride may result in mottled teeth) or may make water less palatable (e.g., disagreeable odor or taste).

Drinking water standards and criteria define acceptable levels of contaminants. That is, they specify levels of contaminants that are at concentrations that pose acceptable risks of adverse health and cosmetic effects during typical consumer exposures. Governments and regulatory agencies around the world develop drinking water standards and criteria. What is acceptable is generally defined under the applicable laws, rules and guidance. Generally, criteria are established to protect human health. However some criteria, particularly those that are legally enforceable standards, also account for feasibility and weigh costs against benefits. A set of criteria may be useful for one contaminant. For example, health-based criteria may be developed for short-term exposure and for long-term exposure, criteria based on aesthetic effects, and for consideration of feasibility.

Generally, health-based criteria incorporate consideration of all potential effects known to be associated with the contaminant and the criteria established to protect for the most sensitive effect (effect that occurs at the lowest concentration). If a constituent is regulated as a carcinogen, the criteria may be set to protect for an acceptable excess lifetime cancer risk based on an assumed rate of water consumption. Approaches used to develop criteria and standards may not be the same from one governing body to another although most agencies do strive to use the best science and risk assessment approaches consistent with science policy and regulations. The following are examples of agencies that develop drinking water criteria and standards:

- The World Health Organization develops drinking water quality guidelines that can be used in developing and developed countries worldwide.
- Health Canada publishes Guidelines for Canadian Drinking Water Quality.
- The United States Environmental Protection Agency (US EPA) develops drinking water criteria consistent with requirements defined under the Federal Safe Drinking Water Act (SDWA).
- States in the United States may develop or adopt drinking water standards and guidance that is equal to or more restrictive than criteria prepared under the Federal SDWA. For example, California develops Public Health Goals (PHGs).

United States Perspective

Drinking water quality in the United States is primarily regulated under the Federal SDWA, passed in 1974 and amended in 1986 and 1996. The SDWA, among other things, provides USEPA the authority to set drinking water standards. Drinking water standards are part of a system, referred to as a 'multiple barrier' approach, which protects drinking water quality. The SDWA provides for evaluation and regulation of drinking water sources, water collection systems, treatment, and distribution systems. Some states have primary enforcement authority for the SDWA and regulate drinking water quality under state laws, regulations, and administrative rules. If a state administers the SDWA, criteria and standards may not be less restrictive than the federal program.

Drinking water supplied by most public water systems in the United States is regulated under the SDWA. Standards apply to public water systems that provide drinking water to at least 15 service connections or regularly serve at least 25 individuals at least 60 days out of the year. Public water systems may

include systems that service schools, businesses, camps, and shopping malls as well as municipal water treatment systems.

Not all drinking water is regulated under SDWA. Private wells are not regulated, but standards and criteria established under the SDWA may be used as guidelines to judge water quality. In addition, drinking water criteria have been developed under a number of remedial programs (e.g., states and US EPA Regional Offices). For example, health-based drinking water criteria are provided for numerous constituents in US EPA Region 9's Preliminary Remediation Goals and US EPA Region 3's Risk-Based Concentrations. Standards and criteria developed under the SDWA fall into the following general categories:

- National Primary Drinking Water Regulations (Primary Standards) are legally enforceable water quality standards that are applied to water regulated under SDWA (public water systems). Primary standards limit the levels of specific constituents in drinking water. Standards are developed for constituents anticipated to be present in drinking water and that can adversely affect the public health. Standards include maximum contaminant levels (MCLs) and treatment technologies. MCLs are generally health/risk based. However, they also take into consideration whether levels can be achieved (feasibility) and include a cost benefit analysis. Some standards are based on the best available treatment technology. Some are expressed as action levels (e.g., lead).
- National Secondary Drinking Water Regulations (secondary standard) are nonenforceable criteria/guidelines. They are set at levels that protect against aesthetic effects (e.g., taste, odor, or color) and/or cosmetic effects (e.g., teeth and skin discoloration). Secondary standards are not enforceable under the federal SDWA, however, states can adopt them as enforceable standards.

Generally, when criteria are developed, the first step is to collect the available data and information and evaluate the potential for exposure through drinking water to cause harm. Then, criteria and standards are developed as appropriate.

Maximum contaminant level goals (MCLGs) are nonenforceable public health goals. MCLGs are set at levels where there is no known or expected risk of adverse effects. They do not consider detection limits or available treatment technology to reduce levels of constituents and may be set at levels that cannot be achieved in certain public water systems. Once the MCLG has been developed an enforceable standard, usually an MCL, is established.

An MCLG may be based on a reference dose (RfD) to protect for effects that are believed to occur only if exposure levels exceed a particular threshold. The RfD is expressed as a daily dose ($mg\,kg^{-1}\,day^{-1}$) that is believed to be protective over a lifetime exposure. Generally this includes most noncancer effects. The standard approach is to multiply the RfD by the assumed adult body weight (70 kg) and then divided by the assumed daily water consumption (2 l). This is called the drinking water equivalent level (DWEL). The DWEL reduced by a percentage (usually 20%) to account for exposure from sources other than drinking water is used to arrive at the MCLG.

For chemicals regulated as carcinogens, the MCLG is generally set as zero. This is based on the assumption that in the absence of data indicating otherwise, any exposure to a carcinogen, no matter how small, is associated with some risk of cancer. If there is sufficient information on the mode of action for a carcinogen that allows for the use of a different approach for developing health protective criteria, the MCLG may not be set at zero. The MCLG is also set at zero for microbial contaminants.

Health Advisories (HAs) are drinking water criteria that provide guidance on levels of constituents that may cause adverse effects in drinking water. HAs are developed to be protective of 1-day exposure, 10-day exposure, and lifetime, which is the DWEL.

USEPA has set $\sim$90 standards

These include the following:

- Inorganic contaminants such as antimony, asbestos, barium, beryllium, cadmium, chromium, copper, cyanide, mercury, nitrate, nitrite, selenium, thallium, arsenic, fluoride, and lead.
- Volatile organic contaminants such as benzene, carbon tetrachloride, chlorobenzene, *o*-dichlorobenzene, *p*-dichlorobenzene, 1,1-dichloroethylene, *cis*-1,2-dichloroethylene, *trans*-1,2-dicholoroethylene, dichloromethane, 1,2-dichloroethane 1,2-dichloropropane, ethylbenzene, styrene, tetrachloroethylene, 1,2,4-trichlorobenzene, 1,1,1,-trichloroethane, 1,1,2-trichloroethane, trichloroethylene, toluene, vinyl chloride, and xylenes.
- Synthetic organic contaminants including pesticides and herbicides such as the following: 2,4-D, 2,4,5-TP (Silvex), acrylamide, Alachlor, atrazine, benzoapyrene, carbofuran, Chlordane, dalapon, di-2-ethylhexyl adipate, di-2-ethylhexyl phthalate, dibromochloropropane, Dinoseb, dioxin (2,3,7,8-TCDD), Diquat, Endothall, Endrin, epichlorohydrin, ethylene dibromide, glyphosate, Heptachlor, Heptachlor epoxide, hexachlorobenzene,

hexachlorocyclopentadiene, Lindane, Methoxychlor, Oxamyl, (Vydate) polychlorinated biphenyls (PCBs), pentachlorophenol, Picloram, Simazine, and Toxaphene.

- Microbiological agents are commonly found in drinking water sources. Levels of certain bacteria (such as coliform bacteria, Fecal Coliform and *Escherichia coli*) and parasites (such as *Cryptosporidium and Giardia lamblia*) are regulated. In addition, turbidity is regulated as it may provide a good medium for microbial growth, may be an indicator of microbial presence, and may interfere with disinfection agents.
- It is common for water suppliers to use disinfectants such as chlorine, chloramines and chlorine dioxide to kill microorganisms such as giardia and *E coli*. Levels of disinfectants used may be higher after rainstorms in summer months. By-products include: trihalomethanes, haloacetic acids, bromate, and chlorite. Levels of disinfection products and by-products are regulated.
- The levels of radionuclides are regulated. These include certain alpha emitters, beta/photon emitters, combined radium 226/228 and radon gas.

In addition to laws, criteria and systems discussed above, compliance with several standards play an important role in maintaining drinking water quality. These standards provide for development of criteria when none are available from the regulating body. For example, compliance with National Sanitation Foundation International/American National Standards Institute (NSF/ANSI) Standard 61, which addresses the potential for constituents to leach from components of drinking water systems into water moving toward the tap, is required under many state laws and regulations.

NSF International is a not-for-profit, nongovernmental organization that is known world wide for standards development and product certification. NSF is accredited by ANSI, US Occupational Safety and Health Administration (OSHA) and the Standard Council of Canada (SCC). Water program standards important to drinking water quality in the United States include the following:

- NSF/ANSI Standard 60: Drinking Water Treatment Chemicals – Health Effects: Standard 60 addresses the potential for adverse health effects to occur because of the use of drinking water treatment chemicals and related impurities. The standard includes a procedure for developing criteria when none are available under the SDWA. Chemicals of interest include those used to control scale and corrosion, used to adjust pH, to soften water, precipitation and sequestering agents, coagulation and flocculation chemicals, disinfection chemicals oxidation chemicals and drilling products.
- NSF/ANSI Standard 61: Drinking Water System Components – Health Effects: Standard 61 addressing constituents that may be indirectly added to drinking water from the well or intake to the tap/faucet. These include pipes and related products, mechanical devices, protective materials, joining and sealing materials, process media, and plumbing devices, including faucets. In addition, certain materials that can support microbial growth (e.g., include solvent-based coatings, gaskets, etc.) must be evaluated to demonstrate compliance with this Standard. The Standard includes a procedure for developing criteria when none are available under the SDWA. Standard 61 is incorporated by reference into many state drinking water laws.

Freshwater resources are precious and necessary for life. Work on cost-effective systems that can desalinate salt water for use as drinking water is ongoing and likely to be important to the future development of certain areas of the world.

See also: Exposure Criteria; Gastrointestinal System; Risk Assessment, Ecological; Risk Assessment, Human Health; Safe Drinking Water Act, US.

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Drugs of Abuse

Molly Broderick and Teresa Dodd-Butera

Certain pharmaceutical drugs and other substances are classified as 'drugs of abuse' because of the tendency for people to use (or overuse) these substances for other than their intended purpose and in some cases become addicted. Because of the adverse health, sociological, and other consequences of using these substances, availability and quantity of many – but not all – of these substances are controlled by regulatory agencies. This article surveys major classes and provides specific examples of drugs of abuse, the main adverse effects, treatments available in overdose situations, and withdrawal symptoms, if applicable.

Stimulants

Controlled stimulants that are frequently abused include amphetamines, methylphenidate, methamphetamine, and cocaine. Amphetamine, methamphetamine, methylphenidate, and cocaine can be smoked, inhaled, ingested, and injected. Methamphetamine's effects can last up to 6 h. Methylphenidate (Ritalin) is a sustained release product and can last up to 12 h. Cocaine's effects last only about 1 h. These drugs have significant potential for abuse and addiction.

Adverse effects include increased risk of seizures, myocardial infarction, rhabdomyolysis, renal failure, and stroke. Other life-threatening adverse effects include hyperthermia, hypertension, vasoconstriction, tachycardia, cardiac ischemia, and paranoia. Prolonged cocaine abuse has been shown to cause cardiomyopathy.

Treatment is aimed at controlling sympathetic stimulation, specifically controlling hypertension, tachycardia, seizures, hyperthermia, and agitation. Medical and psychological withdrawal syndrome starts within 24–48 h after drug discontinuation. Withdrawal symptoms include sleepiness or insomnia, apathy, depression, irritability, and nausea but are not life threatening. A number of medications are available to decrease the craving during withdrawal.

Depressants

This class includes drugs with sedating or amnesic effects. GHB (gamma hydroxybutyric acid), the GHB precursor, 1,4-butanediol (1,4-BD), and Rohypnol or flunitrazepam are sometimes used for sexual assault or for the central nervous system (CNS) effects. Both GHB and Rohypnol have been referred to as date rape drugs. GHB usage has decreased because the Food and Drug Administration (FDA) has banned the drug's usage and has stopped Internet sales of the drug's precursor sold as a dietary supplement. Rohypnol is not legal in the United States but is smuggled into this country and distributed illegally. These medications can be added to a drink or can be taken in pill form. Lethargy, amnesia, ataxia, and confusion can last up to 8 h. In large doses coma and respiratory depression can occur. Effects are compounded when taken with alcohol.

Rohypnol is a benzodiazepine and has a risk of sedation, ataxia, slurred speech, amnesia, respiratory depression, bradycardia, and hypotension. These effects are compounded if other medications are co-ingested. GHB's risk includes coma, respiratory depression, bradycardia, and seizures. Rohypnol ('roofies') and GHB (liquid ecstasy) are not manufactured or sold legally in the United States. Rohypnol is legally sold in Europe, Latin America, and Mexico and is smuggled into this country and transported illegally throughout the country. GHB is easy to produce in clandestine laboratories. Until 1997 it was readily available on the Internet or in health food stores. The FDA made it illegal to sell or produce GHB in 1997.

Respiratory support with oxygen may be required for respiratory depression associated with Rohypnol ingestion. A benzodiazepine antagonist can reverse respiratory depression and coma caused by overdose but is not routinely recommended because it can precipitate withdrawal symptoms and seizures. There is no antidote to GHB overdose. Ventilator respiratory support, seizure control, and supportive care may be required. Symptoms often resolve within 3–4 h. Abuse of both rohypnol and GHB can cause withdrawal symptoms. Long-term use of Rohypnol can cause seizures, tremors, and anxiety. Long-term abuse of GHB withdrawal can last from days to weeks. GHB withdrawal includes anxiety, tremors, disorientation, hallucinations, and insomnia.

Opioids/Narcotic Drugs

Opioids cause a release of endorphins producing a feeling of pleasure. Examples of abuse include heroin, a highly addictive opioid that metabolizes to morphine and readily passes into the brain producing an immediate euphoria. Pharmaceutical or medicinal abused opioids include oxycontin, hydrocodone, codeine, methadone, and propoxyphene.

Opioid toxidrome effects include miosis, respiratory depression, and CNS depression. More serious

effects include hypoxia, hypotension, and coma. Morphine and oxycontin come in extended release forms and can cause prolonged effects. Opioids are available both legally and illicitly. Illegal street drugs can contain adulterants or a highly concentrated preparation. Naloxone is an opioid antagonist and will reverse coma and respiratory depression. Oxygen is also required for respiratory depression.

Opioid Analogs

Dextromethorphan (DM, DTM, skittles) is an opioid analog that is available in hundreds of over-the-counter cough suppressants and cold medications. Abusers describe a feeling of euphoria, disassociation, and visual distortion, CNS depression, and ataxia. It is a popular over-the-counter drug of abuse. This is normally a safe medication when taken as prescribed, but in overdose or intentional misuse adverse effects include distortions in sight and sound and a feeling of being separated from the body. A series of plateaus are described ranging from mild stimulating effects to more serious effects of confusion, lack of coordination, ataxia, and increased heart rate. Respiratory depression, inability to move extremities, and seizures has been reported.

Naloxone, an opioid antagonist, may reverse sedation and the respiratory depressant effects. Oxygen may be required for respiratory depression alone. No specific antidote is available. The treatment mainstay is supportive care.

Inhalants

Young teenagers abuse inhalants because they are easily available in the home and readily available in over 1000 products. They are relatively inexpensive or free and anyone can purchase them regardless of age. Some are also available in most offices and schools as well as in the home. Commonly abused inhalants include gasoline, butane, propane, benzene, toluene, degreasers, cleaning fluids, nail polish removers, whipped cream propellants, glues, and paint thinner. When inhaled they cause a feeling of lightheadedness, tingling, and disorientation. Unfortunately, these solvents can be life threatening and associated with 'sudden sniffing death' resulting in hypoxia, ventricular arrhythmias, and/or cardiac arrest. Inhaling solvents from plastic bags can result in suffocation. Chronic abuse causes brain atrophy, neurological impairment, hepatotoxicity, and nephrotoxicity.

Inhalants cause fast acting intoxicating symptoms because they are inhaled directly into the lungs. The initial symptom is stimulation but with repeated inhalations the symptoms include disinhibition, euphoria, giddiness, dizziness, tingling, stupor, apathy, muscle weakness, and slurred speech. Inhalants can produce a rapid irregular heart rate that can cause heart failure and death within minutes. Death can also occur from suffocation. Abusers inhale fumes from rags or from plastic or paper bags or balloons, or directly from the can. Long-term abuse of inhalants can cause permanent damage to the brain, heart, kidneys, and liver.

There is no specific antidote to inhalant abuse. Treatment may require oxygen and electrocardiogram and blood tests. Detox and drug abuse treatment programs are sometimes not effective and the relapse rate is high. Inhalant abusers build up a tolerance and require increased amounts to achieve the same effects in addition to having cravings for solvents. Detoxification and withdrawal can cause tremors, agitation, irritability, and difficulty in sleeping.

Hallucinogens

Methylenedioxymethamphetamine (MDMA), ecstasy, is a hallucinogenic amphetamine that increases levels of neurotransmitters in the brain. It is most often ingested in pill form but can be inhaled. It is often ingested at 'rave' parties. It is referred to as Adam and XTC. Lysergic acid diethylamide (LSD) (acid) is a mood-altering hallucinogenic agent. It is sold in tablet form, on blotter paper, and sometimes as a liquid. Ecstasy and LSD are illegal but manufactured and sold illicitly.

MDMA causes a feeling of clarity or sharpness, tingling, pleasure, and a feeling of disassociation. It can result in dehydration, seizures, hyperthermia, tachycardia, and renal impairment. LSD is also a hallucinogen but does not have an amphetamine component. The drug causes visual hallucinations. Emotions can be labile and paranoia can occur. Increased body temperature, heart rate, and blood pressure can occur. Some people experience flash-backs.

There are no specific antidotes for ecstasy or LSD. Treatment is supportive and includes fluid replacement, seizure, and temperature control. Keeping patients in a dark quiet room with decreased stimulation may help lessen anxiety. The risk of long-term dependence, addiction, or withdrawal of MDMA is unclear. Growing evidence is that MDMA can affect memory. LSD is not known to be addicting and is not known to cause withdrawal.

Dissociative Drugs

Ketamine (special K, super K, vitamin K) is a short-acting general anesthetic producing what is described

as an out of body experience or a feeling of delusion or dissociation. It is odorless and tasteless and can be added to drinks prior to date rape. It is available in liquid, powder, and pill forms. Phencyclidine (PCP, angel dust, rocket fuel, supergrass) can be smoked, snorted, or ingested. The effects are immediate and can last hours. It is not used medically due to severe risks. PCP is abused for the euphoric, hallucinogenic, out-of-body sensations and dissociative anesthetic effects but often causes violence, agitation, paranoia, and severe hypertension. Treatment often requires sedating and restraining the patient before evaluation of toxicity can be assessed. Ketamine is mainly used in veterinary medicine. It causes a dream-like state and/or hallucinations. In excess it causes delirium, amnesia, lack of coordination, impaired consciousness, and respiratory depression. PCP can cause prolonged hypertension in addition to hyperthermia, seizures, paranoia, combativeness, muscle rigidity, and psychosis.

Both Ketamine and PCP can be life threatening. There is no antidote. Treatment is dependent on symptoms and may require hospitalization. PCP is addictive and withdrawal can cause drug-seeking cravings, fatigue, irritability, and depression.

Marijuana

Marijuana contains a psychoactive resin called D9 tetrahydrocannabinol (THC) and cannabinol. Most resin is found in the flowering female plant. When smoked or ingested it produces a feeling of relaxation, euphoria, and happiness. Other possible effects include poor concentration and decreased reaction time. Used medically under the names dronabinol and marinol, it has been prescribed as an antiemetic and as an appetite stimulant and has also been used in the treatment of glaucoma. Effects last a few hours, are not considered highly toxic, and most often do not require medical treatment.

See also: Amphetamine; Benzodiazepines; Cocaine; Codeine; Dextromethorphan; Heroin; Hydrocodone; LSD (Lysergic Acid Diethylamide); Marijuana; Methadone; Methylenedioxymethamphetamine; Morphine; Phencyclidine; Propoxyphene.

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Dyes

Christophe J Le Coz

Introduction

Dyes are used primarily to impart color in textile, leather, paints, cosmetic, and food industries. Many natural dyes (animal, mineral, or vegetal extracts) have been largely replaced by synthetic dyes that were developed at the end of the nineteenth century. Dyes should be safe, with no toxicity, carcinogenicity, or allergenicity. However, the most frequently reported causes of unexpected side effects of garments are textile dyes, and some dyes formerly used for food like Butter Yellow are known to be carcinogenic. It is actually arduous to routinely detect the exact composition of dyes, because the chemicals used are generally not declared in textiles, contrary to the case with cosmetics or foods. Moreover, the manufacturing modifications in developed countries and the banishment of strongly allergenic, mutagenic, or carcinogenic dyes in Europe and in the United States may be counterbalanced by the high numbers of imported clothing generally treated with historically

older and cheaper dyes from the Far East or underdeveloped countries.

Classification and Characteristics of Dyes

There are thousands of dyes, marketed under different names (more than 100 for some of them), that it is sometimes difficult to rapidly and accurately recognize a specific dye.

Color Index System

Dyes are indicated in the color index (CI), with two systems. A numeric one, with five numbers, corresponds to the CI number, for example, CI 11110. The second system is a CI name, indicating the chemical category, the color, and an identification number, for example, CI Disperse Red 1 for the previous molecule. However, the CI does not contain all information about dyes and some textile dyes have no CI number.

According to their chemical structures and the CI system, dyes can be classified into 17 groups: nitro dyes, triphenylmethane derivatives, xanthenes, acridine derivatives, quinoline derivatives, azines, anthraquinones, indigoid dyes, phthalocyanines dyes, oxydation bases, insoluble azo dye precursors, and azo dyes (classes XII–XVII). In practice, dyes are classified into different application classes: disperse, acid, basic, direct, vat, fiber-reactive, sulfur, premetallic, solvent dyes, and naphthols.

Purity of Dyes

A final color often results from a subtle mixture of several dyes. Because of this, *a priori* unexpected dyes can be employed as yellow, red, orange, or red dyes for black or blue garments. For example, Serisol Black L 1944, used to dye black 'velvet' clothes, contains five disperse dyes, namely Blue 124, Blue 106, Red 1, Yellow 3, and Blue 1. Moreover, a commercial 'pure' dye often comprises one or two major components, and frequently other chemicals and/or impurities. Disperse Yellow 3 is generally pure, Disperse Red 153 or Disperse Blue 35 contain two major fractions, and Disperse Red 1 comprises one major compound and at least two other minor substances. These impurities can also be responsible for sensitization. Moreover, there can be some mistake and confusion between dyes with similar names.

Transformation of Dyes

If they are ingested, dyes and particularly those that have an azo group can be metabolized by the intestinal microflora or by the liver enzymes. So, their effects can occur in organs responsible for metabolism or elimination, like the liver and urinary tract. Skin metabolism may also be responsible for the transformation of dyes, for example, those from colored textiles that can leach from the fabric and migrate to the skin. For example Disperse Orange 3 is degraded to *p*-phenylenediamine (PPD) and nitroaniline in the skin (**Figure 1**). Direct Blue 14 (CI 23850), after azo reduction, converts to the aromatic amine *o*-toluidine and other amines when incubated with cultures of *Staphylococcus aureus*.

The manufacturing processes for textile fabrication are complex and additional procedures such as bleaching can also lead to allergenic products.

Dye Application Classes

Disperse Dyes

Disperse (or plastosoluble) dyes are partially soluble in water and are used to color synthetic fibers like polyester, acrylic and acetate, and sometimes nylon, particularly in stockings. They are not employed for natural fibers. These molecules are the main sensitizers among the dyes. Women seem to be more prone than men to become sensitized but these data are not consistent.

Anthraquinone Dyes These dyes consist of substituted anthraquinones (see **Figure 2** for the structure of anthraquinone). They are plastosoluble and used to stain synthetic fibers such as polyester, acetate, or nylon. Among these substances, Disperse Red 11, Disperse Blue 3, and Disperse Blue 35 have been reported as causes of contact dermatitis from dresses, trousers or nylon stockings. Disperse Blue 35 is also a phototoxic compound. Disperse Blue 3 has a structure close to that of Disperse Blue 7, and was positive in several patient tested with a dye series (chemical structures of these dyes are shown in **Figures 3–6**). With Disperse Orange 76 (an azo dye), Disperse Red 11 was thought to be one of the most common causes of dye allergy in men.

Azo Dyes Azo dyes are characterized by an R1–N=N–R2 chemical structure. They represent the

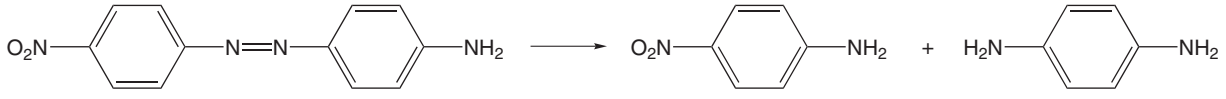


Figure 1 Degradation of Disperse Orange 3 into nitroaniline and *p*-phenylenediamine.

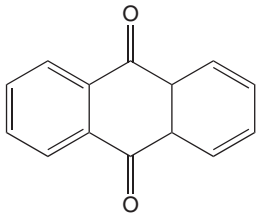


Figure 2 Anthraquinone.

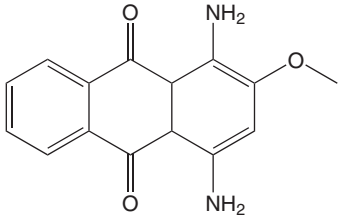


Figure 3 Disperse Red 11.

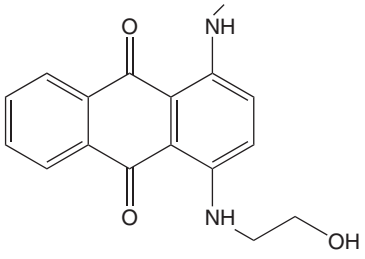


Figure 4 Disperse Blue 3.

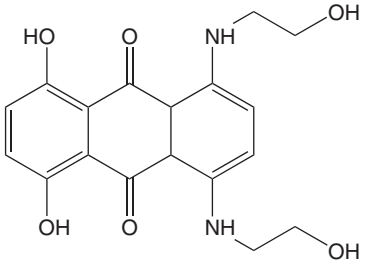


Figure 5 Disperse Blue 7.

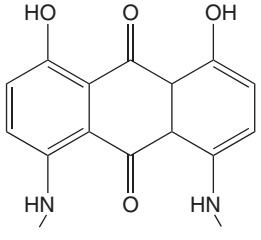


Figure 6 Disperse Blue 35 (major compound).

majority of commercial colorants, enabling a broad spectrum of shades and fastness properties. They are suitable for coloring various substrates, including both synthetic and natural fibers. These molecules are trapped within the fibers in which they are formed during the dyeing process. Azo dyes, disperse type, are used in synthetic fibers. They are the molecules most often implicated in textile dye dermatitis, mainly in nylon stocking, socks, trousers, dresses, and underwear. Disperse Yellow 3, Disperse Orange 3, and Disperse Red 1 were the principal sensitizers in a retrospective 1940–1984 study. Today, Disperse Blue 124 and/or 106, Disperse Orange 3, Red 1, or Yellow 3 are frequently encountered. A recent classification divided them into four chemical subgroups.

The monoazoic compound Disperse Blue 124 (**Figure 7**) is the most frequently positive dye on patch testing with the textile series, particularly in women. It is probably the main cause of textile contact dermatitis today. It is closely related to another azo dye, Disperse Blue 106 (**Figure 8**), marketed since 1985, and both are frequently used together. This latter dye seems to have the stronger sensitizing potential and can provoke infiltrated lesions. Concomitant positive reactions to both Disperse Blue 106 and 124 are expected because of their structural similarity, and are very consistent.

Disperse Orange 3 (**Figure 9**) was cited in reports of stocking dermatitis, and remains a frequent allergen. An average of two-thirds of the patients

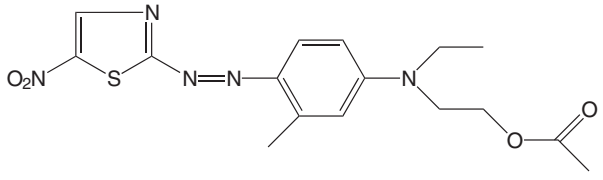


Figure 7 Disperse Blue 124.

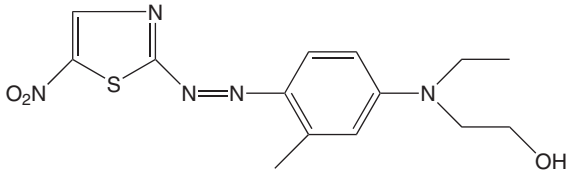


Figure 8 Disperse Blue 106.

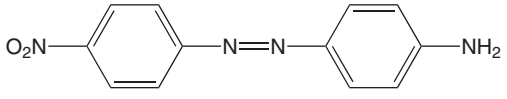


Figure 9 Disperse Orange 3.

sensitized to Disperse Orange 3 are sensitized to PPD, and primary sensitization to Disperse Orange 3 seems to be acquired from PPD contained in hair dyes. *p*-Aminoazobenzene (PAAB, Solvent Yellow 1) and *p*-dimethylaminoazobenzene (PDMAAB or Butter Yellow) are positive in about two-thirds of the patients sensitized to Disperse Orange 3.

Disperse Red 1 (**Figure 10**) was implicated in dermatitis from stocking, and is frequently observed on patch testing, especially in subjects under 12 years of age.

Disperse Red 17 (**Figure 11**) gave positive patch test reactions in patients sensitized to other azo dyes, and was cited as a stocking dye.

Disperse Brown 1 (**Figure 12**) is less frequently positive, as is Disperse Brown 2.

Disperse Orange 76 (**Figure 13**) is often positive and was thought to be one of the main causes of dye allergy in men, together with Disperse Blue 3 (an anthraquinone dye).

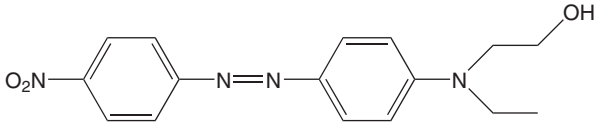


Figure 10 Disperse Red 1.

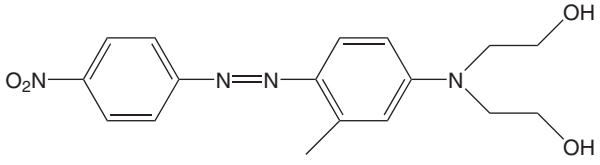


Figure 11 Disperse Red 17.

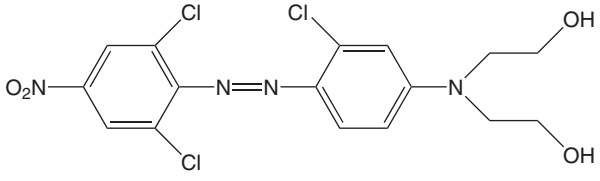


Figure 12 Disperse Brown 1.

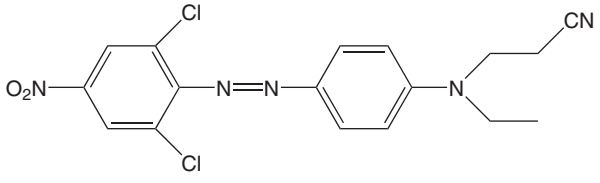


Figure 13 Disperse Orange 76.

Reactions to Disperse Yellow 3 (**Figure 14**) are frequent. The first cases reported concerned nylon stocking dermatitis, and this azo dye is still currently used to dye such garments.

Disperse Red 153 (**Figure 15**) is based on two structurally close compounds.

Disperse Black 1 and 2 are rarely positive.

Methine, Nitro, and Quinoline Dyes

Disperse Yellow 39, a no longer available methine dye, was implicated in trouser dermatitis. The nitro dye Disperse Yellow 9 was cited in some reports.

Acid Dyes

These are used to color silk, wool, and other animal fibers, or nylon (polyamide) when high wet-fastness is needed. Such dyes include monoazoic, diazoic, triphenylmethane, and anthraquinone compounds. Acid Yellow 23, Acid Black 48, Acid Black 63, and Acid Violet 17 (triphenylmethane derived; **Figure 16**) were reported in the literature, mainly before 1985. Acid Yellow 61 (Supramine Yellow GW), Acid Red 359 (Neutrichrome Red SGN), and Acid Red 118 (Supramine Red GW), each tested in 5% petrolatum and removed after 3 days, were positive for skin

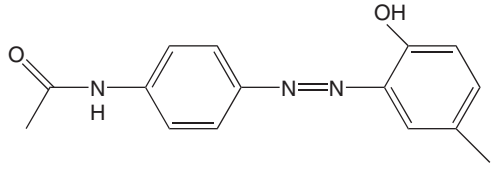


Figure 14 Disperse Yellow 3.

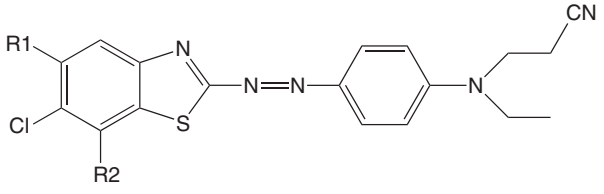


Figure 15 Disperse Red 153. R1 = Cl or H and R2 = H or Cl, respectively.

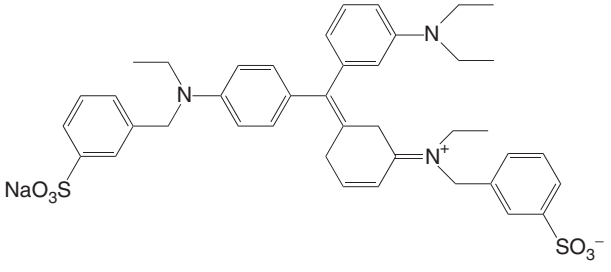


Figure 16 Acid Violet 17.

reactions in 5, 2, and 1 out of 1814 consecutive patients, respectively. The relevance of the patch test results to observed dermatitis was considered possible in four patients.

Basic Dyes

These are mainly used to dye wool and silk, modacrylic, nylon, and polyester. They can be applied to cotton with a mordant (a substance used to set dyes). Basic dyes include monoazoic, diazoic, and azine compounds. Basic Red 46, a monoazoic dye, was implicated in a sweater-induced dermatitis. Basic Brown 1 (**Figure 17**), Basic Black 1, Brilliant Green, Turquoise Reactive and Neutrichrome Red have also been reported as allergens.

Direct Dyes

These dyes are directly applied on fibers, most often cotton, wool, flax, or leather in a neutral or alkaline bath. They have low wet-fastness, and frequently need after-treatments. Direct Black 38 (**Figure 18**), a triazoic compound dye used for cotton, wool, and silk, has been implicated in patients wearing black clothes, with concomitant immediate-type reactions in some cases.

Direct Orange 34 (Arancio Diazol Luce 7 JL), an azo dye, was positive during systematic testing in 8 out of 1814 patients.

Vat Dyes

Such water-insoluble dyes are applied in a reduced soluble form and then re-oxidized to the original insoluble form once absorbed into the fiber. They have high wet-fastness and are used to dye cotton, flax, wool, and rayon fibers. They mostly commonly include Vat Blue 6, responsible for cosmetic dermatitis, and Vat Green 1. Vat Blue 1 (**Figure 19**) is used to dye Levi Strauss 501 'shrink to fit' blue jeans. Vat Green 1, an anthraquinone derivative (**Figure 20**), has been reported as a cause of clothing contact dermatitis, from navy-blue uniforms in nurses.

Reactive Dyes (Fiber)

Reactive dyes were introduced at the end of the 1950s. These synthetic dyes consist of a two-part, direct coloring agent. The first moiety is a chromophore with an azo, anthraquinone, or phthalocyanine derivative structure. This moiety is connected to a second reactive group, which is able to form covalent bonds with the amine or sulfhydryl groups of proteins in the textile fibers (**Figure 21**). The main

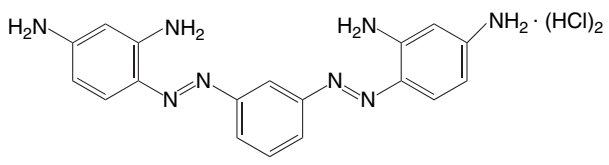


Figure 17 Basic Brown 1 (Bismarck Brown Y).

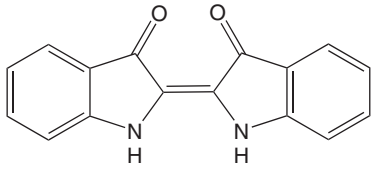


Figure 19 Vat Blue 1 (synthetic indigo).

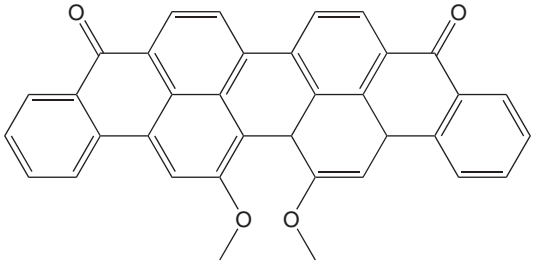


Figure 20 Vat Green 1.

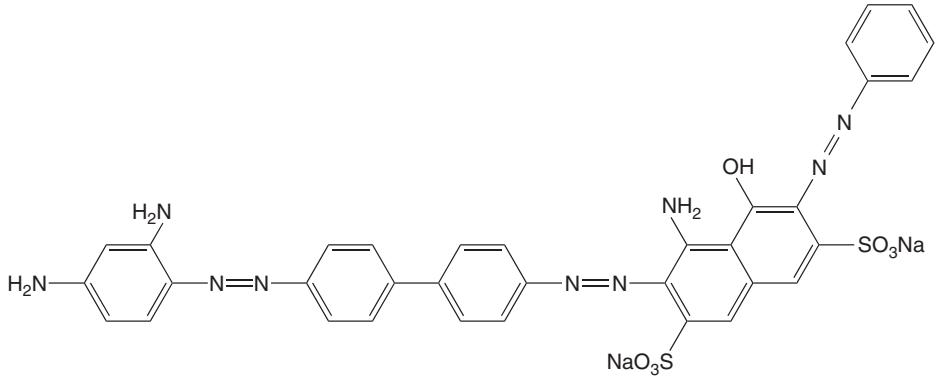


Figure 18 Direct Black 38.

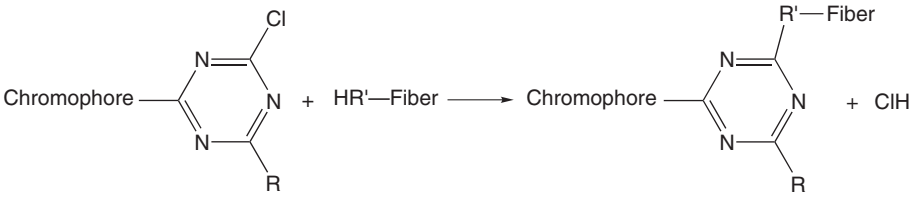


Figure 21 Reaction between textile fibers and the reactive dye.

reactive structures are vinylsulfone, monochlorotriazine, dichlorotriazine, difluoropyrimidine, and dichloroquinoxaline. Such dyes are used for coloring cellulosic fibers (cotton and silk), wool, or polyamides, and are widely used for the production of clothes. They account for more than 10% of the world's production of dyes. Reactive dyes are used in industry in a powdered or granulated form, or as liquids or pastes. They are irritant and sometimes allergenic, and most sources of sensitization are from occupational exposures. Respiratory symptoms in the textile industry have been reported in up to 10% of employees: rhinitis, dyspnoea, and asthma. These dyes can be irritants, inducing nonspecific symptoms. Allergic cases due to a specific IgE production are ascertained by positive prick tests and blood serum IgE levels. Contact dermatitis may occur and may be of irritant or allergic type. In allergic dermatitis, patch tests realized with the dye are positive, proving a delayed type allergic reaction.

In a study of allergic contact dermatitis in consumers, 1813 consecutive patients were tested with an additional textile series of 12 reactive dyes, and 18 patients (0.99%) were found to be sensitized to reactive dyes. However, only five patients had a history of intolerance to garments, and two of the four patch tests performed with pieces of garment were positive. In practice, reactive dyes in clothing should not be sensitizers. If they can be extracted from fibers, they are in a hydrolyzed, nonsensitizing form.

Sulfur, Solvent, and Nondisperse Azoic Dyes

Sulfur dyes are used for cotton in work clothes. Solvent dyes are mono- or diazoic compounds used to dye oils, greases, varnishes, solvents, and cosmetics. Solvent Yellow 1 (PAAB), a monoazoic compound, was positive in patients sensitized to stockings.

Dye-Fixing and Dye-Coupling Agents

β-Naphthol (2-naphthol, azoic coupling component 1) (**Figure 22**) is no longer used.

Naphthol AS (3-hydroxy-2-naphthoic acid anilide, azoic coupling component 2) (**Figure 23**), a coupling agent used for cotton dyeing, has replaced β-naphthol

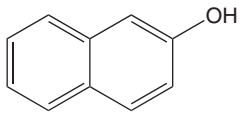


Figure 22 β-Naphthol.

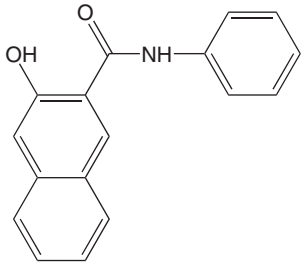


Figure 23 Naphthol AS.

because of a stronger affinity for cellulose. Naphthol AS first caused pigmented contact dermatitis in workers at a textile factory in Mexico in the 1970s, where it was widely used. It has been reported as an agent of pigmented contact dermatitis in several patients, generally due to non-European and non-North American textiles. Its presence in textile can be easily ascertained by thin-layer chromatography.

Side Effects of Dyes

Allergic Contact Dermatitis

Allergic contact dermatitis is a result of a delayed-type immune reaction, due to hapten-specific lymphocytic T clonal expansion. Sensitization to textile dyes in clothing necessitates a transfer of the dye from the garment to the skin, inducing sensitization and/or elicitation of the immune response. However, 'bleeding' of textile dyes, which induces skin discoloration, is a nonallergic phenomenon. Sensitization occurs from the dye itself, from intermediate products during the dying process or after-treatments, or from metabolites arising in the skin. Attributing an allergy to a textile dye is a difficult process and, even if a textile dye is found to be positive on patch testing, the precise identification of the sensitizer in the garment is

extremely difficult. Reports of clothing dermatitis are frequently individual, excepting rare epidemics occurring from furs dyed by PPD and derivatives in the 1920s, from dyed nylon stockings in the 1940s, or from black 'velvet' clothing and blouses in the 1980s. Epidemiological studies regarding this topic are most often not controlled, and habitually report a frequency of positive patch tests to textile additives, mainly dyes or finishes. Thus, the prevalence of sensitization to substances potentially implicated in textile dermatitis is ~1–5% of patch-tested patients, but the clinical relevance of such tests is sometimes questionable. For example, a study in 1012 patients indicated that 31 patients (3%) reacted to at least one clothing dye, but that only 10 reactions were relevant. It is difficult to determine its exact incidence for these reasons, but some data suggest that clothing dermatitis is not rare.

Contact dermatitis from clothing has the clinical feature of a typical eczema, though dry rather than vesicular. The lesions can progress and be severe, generalized or even erythrodermic, as long as contact with the allergen is not avoided. Pigmented contact dermatitis arises mainly in patients with a high phototype (IV or V), and has been described from Naphthol AS as well. In some instances, the lesions can be monomorphic and infiltrated, and even simulating cutaneous lymphoma. They may imitate an atopic dermatitis in popliteal areas, demonstrate a persistent erythematous or urticarial-type dermatitis, or even present solely as diffuse itching. Purpuric clothing dermatitis, described during World War II, was due to textile finishes in British soldiers' uniforms. This rare instance occurred with rubber compounds such as isopropyl-phenyl *p*-phenylenediamine (IPPD), and with the azo dye Disperse Blue 85, or another azo Disperse Yellow 27 available as Serisol Fast Yellow GDW. Cockade lesions are rarely described.

The dermatitis generally occurs on the sites of intimate contact with the garment, and the lesions are sometimes symmetrical. Friction or perspiration sites are preferentially involved, and a clinical pattern of textile dermatitis is generally described: neck, major skin folds, and inner thighs. The areas protected by underclothing or the lining of the skirt of the clothing are often free of symptoms. The face can be involved from the handling of the dyes. Some peculiar localizations in accordance with the form of the garment, are reported in **Table 1**. The delay necessary for the diagnosis may be long, and some patients may have difficulties in understanding the role of an invisible although colored substance in their allergy.

Examination of the garment, since the labeling indicates the fiber composition, can guide the practitioner to specific dyes or textile finishes. The practitioner can examine the different parts of the fabric and take some of them, of different colors or textures, for patch testing or for further chemical analysis. Main reported allergens are indicated in **Table 2**. It has to be noted that all allergens have not been identified and no published data are available on them.

Table 1 Localization of dermatitis according to garment type

Type of garment	*Localization of the lesions*
Socks	Feet, legs
Stockings	Lower legs, feet, toes, popliteal fossea
Blouses	Back, chest, axillary borders
Dresses	Back, neck, elbows, axillary borders, forearms, wrists
Jackets	Dorsum of hands, wrists, forearms
Trousers	Thighs, lower legs, dorsum of hands

Mutagenesis and Carcinogenesis

Monoarylamines Monoarylamines, less or more substituted, can have a weak carcinogenic potential. The prototype of these single ring aromatic amines is aniline (**Figure 24**), which can induce splenic carcinoma by feeding at high doses for a long term. Other monoarylamines (substituted anilines) like *o*-toluidine, *o*-anisidine, and *p*-cresidine are genotoxic upon metabolic activation and induce carcinomas of spleen or urinary balder.

Polycyclic Amines Several polycyclic arylamines have carcinogenic potential. It is, however, important to note that (even slight) molecular modifications can influence solubility, bioavailability, and metabolism, and the mutagenic and carcinogenic potential of molecules.

Naphthylamines The dicyclic arylamine 2-naphthylamine (**Figure 25**) has demonstrated carcinogenicity in several species, including humans. On the other hand, 1-naphthylamine has not revealed carcinogenic potential in experimentation, but the process for its production can generate 2-naphthylamine and other possibly carcinogenic aromatic amines.

Diphenylamines The biphenyl series comprises molecules with two phenyl rings joined by a carbon-to-carbon bond and an exocyclic amino group. The prototype is 4-aminobiphenyl (xenylamine) and one of the most known is 4,4′-diamino-diphenyl (benzidine) (**Figure 26**). Case reports and follow-up studies of workers provide evidence that occupational exposure to benzidine is strongly associated with an increased risk of bladder cancer. In animals, when administered in the diet or by intraperitoneal injections, benzidine

Table 2 Main textile dyes reported as allergens

Name of the dye	*Color index no.*	*Application class*	*Chemical class*
Acid Red 118 (Supramine Red GW)		Acid	Azo dye
Acid Red 359 (Neutrichrome Red SGN)		Premetallic	Azo dye (chrome)
Acid Black 48	65005	Acid	Anthraquinone
Acid Yellow 36	13065	Acid	Azo dye
Acid Yellow 61	18968	Acid	Azo dye
Acid Violet 17	42650	Acid	Triphenylmethane
Basic Black 1	50431	Basic	Azine
Basic Brown 1 (Bismarck Brown R)	21000	Basic	Diazoic
Basic Red 46		Basic	Mono azoic
Direct Black 38	30235	Direct	Triazoic
Direct Orange 34	40215	Direct	Azo (stilbene)
Disperse Black 1	11365	Disperse	Azo
Disperse Black 2	11255	Disperse	Azo
Disperse Blue 1	64500	Disperse	Anthraquinone
Disperse Blue 3	61505	Disperse	Anthraquinone
Disperse Blue 7	62500	Disperse	Anthraquinone
Disperse Blue 35		Disperse	Anthraquinone
Disperse Blue 85	11370	Disperse	Azo
Disperse Blue 106		Disperse	Azo related to DB 124
Disperse Blue 124		Disperse	Azo related to DB 106
Disperse Blue 153		Disperse	Azo
Disperse Brown 1	11153	Disperse	Azo
Disperse Orange 1	11080	Disperse	Azo
Disperse Orange 3	11005	Disperse	Azo
Disperse Orange 13		Disperse	Azo
Disperse Orange 76		Disperse	Azo
Disperse Red 1	11110	Disperse	Azo
Disperse Red 11	62015	Disperse	Anthraquinone
Disperse Red 17	11210	Disperse	Azo
Disperse Red 153		Disperse	Azo
Disperse Yellow 1	10345	Disperse	Nitro
Disperse Yellow 3	11855	Disperse	Azo
Disperse Yellow 9	10375	Disperse	Nitro
Disperse Yellow 27		Disperse	Azo
Disperse Yellow 39		Disperse	Methine
Disperse Yellow 49		Disperse	Methine
Disperse Yellow 54		Disperse	Quinoline
Disperse Yellow 64	47023	Disperse	Quinoline
Naphthol AS	37505	Coupling agent	
p-Aminophenol	76550		Related to some azo dyes
p-Aminoazobenzene (Solvent Yellow 1)	11000		Related to some azo dyes
p-Phenylenediamine	76060		Related to some azo dyes
Vat Green 1	59825	Vat dye	Anthraquinone

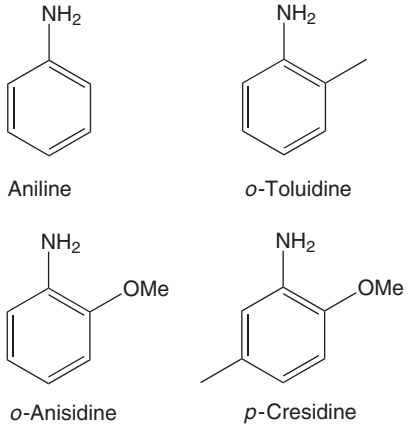


Figure 24 Molecular structures of aniline derivatives.

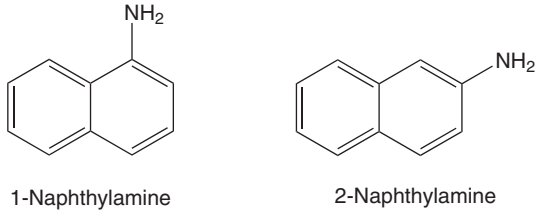
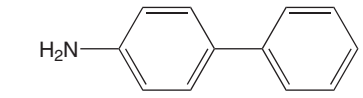


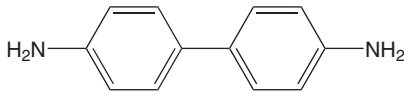
Figure 25 Molecular structures of naphthylamines.

induces urinary bladder carcinomas, mammary carcinoma, and hepatocellular carcinomas.

Benzidine derivatives like 3,3′-dimethylbenzidine and 3,3′-dimethoxybenzidine (*o*-dianisidine) (**Figure 27**), are used as dyes or intermediates for dyestuffs or pigments (e.g., Trypan Blue, Acid Red 14, and Direct

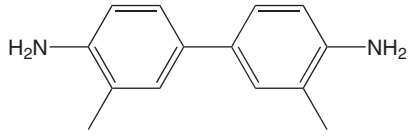


Xenylamine

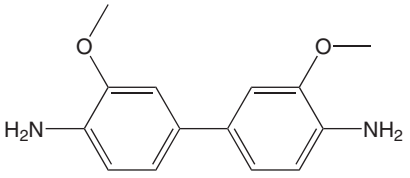


Benzidine

Figure 26 Structures of xenylamine and benzidine.



3,3'-Dimethylbenzidine



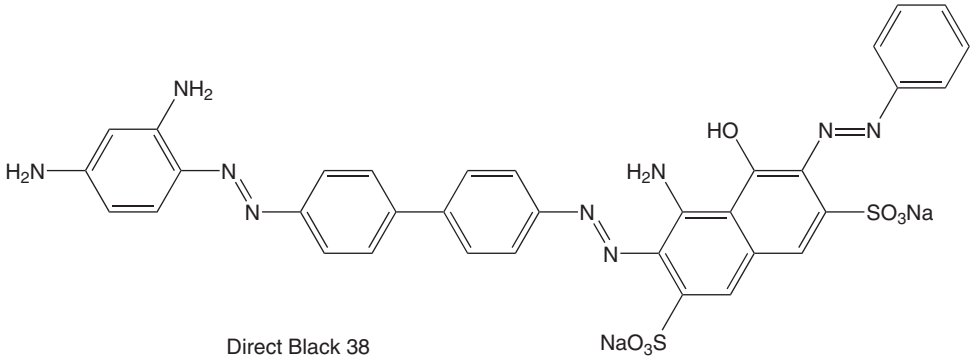
3,3'-Dimethoxybenzidine

Figure 27 Structures of 3,3′-dimethylbenzidine and 3,3′-dimethoxybenzidine.

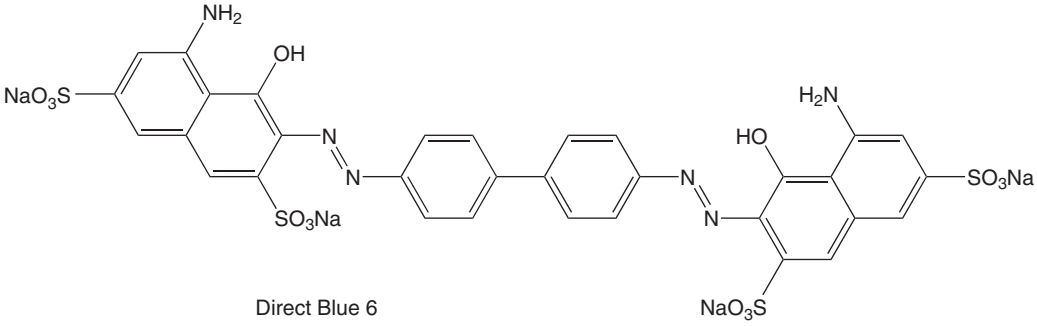
Blue 1, 8, 15, 76, 98, 218, and Pigment Orange 16), coatings, plastics, or in chemical studies. They are currently suspect carcinogens. Carcinogenicity studies in animals have showed induction of several neoplasms and carcinomas (intestine, lung, mammary, liver, and skin). No adequate studies have been reported in humans. Dyes metabolized into such amines are also reasonably anticipated to be human carcinogens.

Azo Compounds Azo dyes are widely used in the food, pharmaceutical, cosmetic, textile, and leather industry. They are synthetic compounds characterized by one (monoazo) or several intramolecular N═N bonds. Azo dyes, if they are systemically absorbed, can be metabolized by the way of azoreductases of intestinal microflora by liver cells and skin surface bacteria. This metabolism leads to aromatic amines that can be hazardous. In the 1930s, some azo derivatives like 4-dimethyl aminoazobenzene (Butter Yellow, CI Solvent Yellow 2, CI 11020) and *o*-aminoazotoluene were experimentally found to be directly carcinogenic to liver and bladder after feeding. Other complex azo dyes like Direct Black 38 or Direct Blue 6 (**Figure 28**) release the aromatic amine benzidine. Some examples of azo dyes metabolized in benzidine and benzidine-congeners are listed in **Table 3**.

Anthraquinone Derivatives 2-Aminoanthraquinone (CAS 117-79-3) (**Figure 29**) is used as an intermediate



Direct Black 38



Direct Blue 6

Figure 28 Structure of Direct Black 38 and Direct Blue 6. See the benzidine precursor at the center of the molecules.

Table 3 Benzidine and benzidine-congener-based dyes

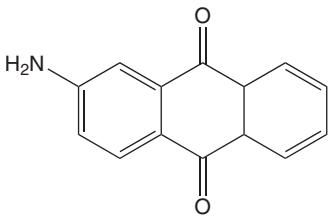
	Color index name	*CI number*	*CAS number*
Benzidine-based	Direct Red 28 (Congo)	22120	573-58-0
	Direct Blue 6	22610	2602-46-2
	Direct Brown 95	30145	16071-86-6
	Direct Black 38	30235	1937-37-7
o-Toluidine-based	Direct Red 2	23500	992-59-6
	Direct Blue 14 (Trypan)	23850	72-57-1
o-Dianisidine-based	Direct Blue 8	24140	2429-71-2
	Direct Blue 15	24400	2429-74-5

in the industrial synthesis of anthraquinone dyes: Vat Blue 4, 6, 12, and 24, and Pigment Blue 22. It is a carcinogen in animals, inducing hepatocellular carcinomas and lymphomas. 1-Amino-2-methylanthraquinone (CAS 82-28-0) is used as a dye and a dye intermediate, for example, for Solvent Blue 13 and Acid Blue 47. It is a liver and kidney carcinogen in animals. Disperse Blue 1, used for semipermanent hair colorations and for coloring fabrics and plastics, induced urinary bladder carcinomas and sarcomas in rats. They are reasonably anticipated to be human carcinogens.

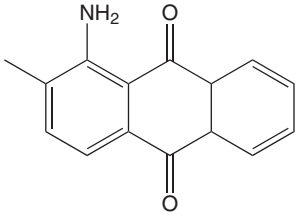
Other Compounds Magenta and Basic Red 9 (CAS 569-61-9), a common constituent of Magenta, have been used to dye textile fibers, to prepare printing inks, and in biological stains. In workers engaged in the manufacture of Magenta, there was a marked excess of cancer of the urinary bladder. It is possible that the workers were also exposed to *o*-toluidine. CI Basic Red 9 (**Figure 30**) was, however, an inducer of hepatocellular carcinoma in mice and rats after oral administration, and induced local sarcomas after subcutaneous administration.

Legislation

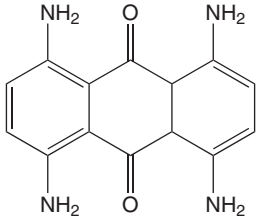
Following the introduction in 1994 of the German Consumer Goods Ordinance that restricted the use of certain azo dyes in consumer goods, several other European Union (EU) member states introduced similar but different regulations. In the interests of transparency and the maintenance of the single market, the European Parliament accepted the nineteenth amendment of the Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations, namely azo dyes. Azo dyes, which can release any of a group of defined aromatic amines, are prohibited from being used in those consumer goods that are considered to have direct and prolonged skin or mouth contact. Such dyes may not be detectable in textiles and leather that can be in contact with skin or mouth, that is, at a level lower than 30 ppm. The EU Directive was published in September 2002 and, since September 2003, all EU countries are required to prohibit the manufacture and sale of those defined



2-Aminoanthraquinone



1-Amino-2-methylanthraquinone



Disperse Blue 1

Figure 29 Structures of 2-aminoanthraquinone, 1-amino-2-methylanthraquinone, and Disperse Blue 1.

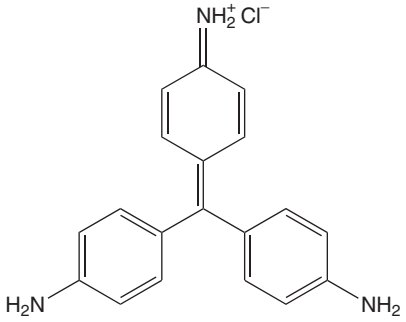


Figure 30 Structure of Basic Red 9 monohydrochloride.

Table 4 List of aromatic amines forbidden in the European Union

CAS number	*Index number*	*EINECS*[a] number	*Substances*
92-67-1	612-072-00-6	202-177-1	Biphenyl-4-ylamine (4-aminobiphenyl xenylamine)
92-87-5	612-042-00-2	202-199-1	Benzidine
95-69-2		202-441-6	4-Chloro-*o*-toluidine
91-59-8	612-022-00-3	202-080-4	2-Naphthylamine
97-56-3	611-006-00-3	202-591-2	*o*-Aminoazotoluene (4-amino-2′,3-dimethylazobenzene (4-o-tolylazo-o-toluidine)
99-55-8		202-765-8	5-Nitro-*o*-toluidine
106-47-8	612-137-00-9	203-401-0	4-Chloroaniline
615-05-4		210-406-1	4-Methoxy-*m*-phenylenediamine
101-77-9	612-051-00-1	202-974-4	4,4′-Methylenedianiline (4,4′-diaminodiphenylmethane)
91-94-1	612-068-00-4	202-109-0	3,3′-Dichlorobenzidine (3,3′-dichlorobiphenyl-4,4′-ylenediamine)
119-90-4	612-036-00-X	204-355-4	3,3′-Dimethoxybenzidine (*o*-dianisidine)
119-93-7	612-041-00-7	204-358-0	3,3′-Dimethylbenzidine (4,4′-bi-*o*-toluidine)
838-88-0	612-085-00-7	212-658-8	4,4′-Methylenedi-*o*-toluidine
120-71-8		204-419-1	6-Methoxy-*m*-toluidine (*p*-cresidine)
101-14-4	612-078-00-9	202-918-9	4,4′-Methylene-bis-(2-chloroaniline) (2,2′-dichloro-4,4′-methylene-dianiline)
101-80-4		202-977-0	4,4′-Oxydianiline
139-65-1		205-370-9	4,4′-Thiodianiline
95-53-4	612-091-00-X	202-429-0	*o*-Toluidine (2-aminotoluene)
95-80-7	612-099-00-3	202-453-1	4-Methyl-*m*-phenylenediamine
137-17-7		205-282-0	2,4,5-Trimethylaniline
90-04-0	612-035-00-4	201-963-1	*o*-Anisidine (2-methoxyaniline)
60-09-3	611-008-00-4	200-453-6	4-Amino azobenzene

[a] European Inventory of Existing Commercial Substances.

consumer goods, which on chemical analysis are found to contain the listed aromatic amines (**Table 4**). Since most colored textile and leather articles are treated with azo dyes and pigments, it is important to underline that only few azo dyes are affected ($\sim$4% of known azo dye structures, a total of $\sim$300 dyes, and these are mainly direct dyes). Articles colored with other azo dyes can be manufactured and sold without restriction. Additionally, over the last years, all European dye manufacturers have stopped manufacturing such azo dyes and test institutes report that the vast majority of samples tested today comply with the EU Directive.

See also: Aniline; Cosmetics and Personal Care Products; Safety Testing, Clinical Studies; Skin; Toluidine.

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Relevant Website

http://www.etad.com – Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD) Website.

E

E. coli (Escherichia coli)

Lee R Shugart

This article is a revision of the previous print edition article by Vittoria Werth, volume 1, p. 515, © 1998, Elsevier Inc.

Description

Escherichia coli is a member of the bacterial family, Enterobacteriaceae, the enteric bacteria. Members of the Enterobacteriaceae are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g., *Salmonella*, *Shigella*, *Yersinia*). Several others are normal colonists of the human gastrointestinal tract (e.g., *Escherichia*, *Enterobacter*, *Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of humans. *E. coli* is a facultative anaerobic, motile, gram-negative rod.

Mechanism of Toxicity

In general, most strains of *E. coli* are avirulent; however, there are strains that cause an impressive variety of different types of diseases, including diarrhea, dysentery, hemolytic uremic syndrome, bladder and kidney infections, septicemia, pneumonia, and meningitis. A strain of *E. coli* associated with a particular disease is due to the fact that the organism has acquired a set of virulence genes.

Before the advent of molecular biology and the identification of virulence factors, surface antigens were a convenient method for 'fingerprinting' *E. coli*. Three surface components formed the basis for the serological classification scheme: O antigens for lipid polysaccharides, H antigens for flagella, and K antigens for capsulated strains. At least 700 serogroups have been identified and are still in use for tracing outbreaks of intestinal disease. There is some correlation between serogroup and virulence.

Nature of Disease

E. coli is responsible for three types of infections in humans: intestinal diseases (gastroenteritis); urinary tract infections (UTI); and neonatal meningitis.

As a pathogen, *E. coli* is best known for its ability to cause intestinal diseases. A classification scheme based on virulence factors (virotyping) is more directly associated with the intestinal disease process than serotyping. The characteristics that form the basis for the virotyping system include patterns of bacterial attachment to host cells, effects of attachment on host cells, production of toxins, and invasiveness. Currently, there are five virotypes: enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EaggEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* (EIEC).

Uropathogenic *E. coli* cause 90% of the urinary tract infections. The bacteria colonize from the feces or perineal region and ascend the urinary tract to the bladder. With the aid of specific adhesins (pyelonephritis-associated pili) they are able to colonize the bladder. Another factor involved in the pathogenicity of the uropathogenic strains of *E. coli* is their resistance to complement-dependent bactericidal effect of serum. This phenomenon is associated with the presence of a capsule, which decrease the ability of antibodies and/or complement to bind to the bacterial surface, which in turn prevents the phagocytes from recognizing and engulfing the bacterial cells.

Epidemiological studies have shown that pregnancy is associated with increased rates of colonization by K-1 strains of *E. coli* and that these strains become involved in the subsequent cases of meningitis in the newborn. The organism invades the blood stream of infants from the nasopharynx or gastrointestinal tract. Neonatal meningitis requires antibiotic therapy and catastrophic sequelae are rare. The K-1 antigen is considered the major determinant of virulence among strains of *E. coli* that cause neonatal meningitis. The K-1 antigen is a capsular homopolymer of sialic acid that inhibits phagocytosis, complement, and other host's immunological mechanisms.

Control

E. coli colonizes the gastrointestinal track of most warm-blooded animals within hours or a few days after birth. A symbiotic relationship exists under normal conditions, as our enteric flora provide for our

source of Vitamin K and B-complex vitamins. Once established, a strain may persist for years, but shifts in resident populations tend to occur over long periods of time, and more rapidly after enteric infection or antimicrobial chemotherapy that perturbs the normal flora. Acquisition of genetic information from other sources (bacterial viruses, plasmids, etc.) may result in the organism becoming a virulent strain. Because of the potential for fecal contamination of water and food, common-sense actions should be taken to minimize risk. *E. coli* food poisoning usually requires hospitalization and most diseases associated with *E. coli* infections respond to antibiotic therapy.

See also: Salmonella; Shigella.

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Relevant Website

http://www.ifst.org – Verocytotoxin-Producing *E. coli*; Food Poisoning and its Prevention (from the Institute of Food Science and Technology, UK).

Echinacea

Lee R Shugart

- SYNONYMS: Black Sampson; Coneflower; Niggerhead; Rudbeckia

Description

Family: *Asteraceae*

Genus and species: *Echinacea angustifolia*, *E. pallida*, *E. purpurea*

Echinacea, better know as the purple coneflower, is a plant native to the United State and can be found growing as a wildflower in the prairies of the Great Plains states and as far south as Texas. The flowers are a rich purple and the florets are seated round a high cone. It has a faint aromatic smell, with a sweetish taste that leaves a tingling sensation, an indication of isobutylamides.

Chemistry and Pharmacology

Echinacea contain a complex mixture of chemicals, the composition of which varies depending upon the part of the plant that is examined and may include caffeic acid derivatives, flavonoids in the free and glycoside forms, alkamides, polysaccharides, inulin and fructans, glycoproteins, sugars, and essential oils.

Usage

Echinacea is a medicinal herb that was widely used by the North American Plains Indians and later by colonial settlers before the nineteenth century. Its popularity decreased markedly with the widespread use of antibiotics during 1940–1950, but the renewed interest currently demonstrated by society for herbal medicines has reversed this trend.

Nearly all parts of the plant are used, including the roots, leaves, flowers, and seeds, to prepare liquid extracts (tinctures), tablets, and teas.

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Ecotoxicology

Chris Theodorakis

Ecotoxicology generally refers to the effects of environmental pollutants at the population, community, or ecosystem levels, although individual-level effects (toxicity, sublethal effects, toxicokinetics) of pollution may also be included if they are part of a field study (rather than in the laboratory).

Field studies can be classified as either manipulative or observational. In manipulative studies, previously unexposed organisms are used, and the experimenter determines the level of contamination to which they are exposed. In contrast, in observational studies the level of contamination to which the organisms are exposed is not under the control of the experimenter. These studies may be part of an independent research project, or may be mandated by a regulatory authority for monitoring organismal health and environmental quality. Manipulative field studies may employ microcosms and mesocosms, enclosures, and field applications of chemicals, while observational studies involve field surveys and collections.

Microcosms are composed of large chambers, terreria, aquaria, or artificial pools; aquatic mesocosms include artificially constructed ponds or streams, while terrestrial mesocosms are large containers filled with soil, plants, and (sometimes) leaf litter. Microcosms and mesocosms typically contain more than one species of test organism, are located outdoors (but may also be located indoors), and often contain sediment and/or vegetation. The rationale is to produce a test system with similarities to the natural environment, but is more controllable. End points examined may include acute toxicity, sublethal effects, or community/population level effects.

The uses and end points measured for enclosure studies are similar to mesocosms, but here a portion of the natural environment is enclosed and manipulated, rather than constructing an artificial system. Manipulations include adding previously unexposed organisms to an enclosure in a contaminated environment, or applying test chemicals to an enclosed portion of a noncontaminated environment. Terrestrial enclosures are usually corrals fenced in by wire or plastic mesh or impermeable barriers such as metal or plastic sheets. They may range from $< 1\,m^2$ to more than a hectare. Aquatic enclosures may include a section of the shoreline fenced off by plastic curtains (littoral enclosures) or boxes made of flexible plastic sheets suspended in open water (limnocorrals). Small enclosures are used to monitor acute toxicity or sublethal effects, while larger enclosures may study population, community, or ecosystem level end points.

Field applications of chemicals may be used to study effects on populations, communities, or ecosystems, or to study movement of chemicals through the environment. In terrestrial systems, the chemical is applied to various plots of land and samples are taken at various times. In aquatic systems, such applications may include adding chemicals to natural ponds or streams. In some studies, lakes were separated by plastic curtains, and the chemical was applied to one side of the lake only. This allowed determination of effects by comparison of impacted and nonimpacted sides. Because of potential of environmental contamination or lasting impacts, field applications are rarely used, and then only for chemicals that are not persistent and easily degradable.

Finally, biotic surveys and collections involve going to contaminated field sites and collecting organisms or samples of environmental media (air, water, soil, sediment) for analysis. They may be used for determining the level of contamination in organisms or media, and/or for determining the toxic effects on organisms living there. Field surveys may also be used for determining the effects of pollution on populations, communities, and ecosystems.

A population is a group of interacting or potentially interacting individuals of the same species. Population effects may be determined by collection of empirical data or simulated with the use of population models. In the former case, the effects of environmental contamination on population density (number of individuals per unit area), size (total number of individuals), age structure, sex ratios, biomass (total mass of all individuals), population growth (change in size or density over time), sustainability, and probability of extinction are determined. Movement between populations, such as immigration, emigration, and colonization, may also be affected by pollution. Population-level effects of pollution may be determined using laboratory exposures (for small organisms) or manipulative or observational field studies. Alternatively, the effects of pollutants on populations can be predicted or simulated using mathematical models, or by using computers to study the effects of pollution on populations. These models use empirical data such as mortality (toxicity), abundance, age distribution, and age-specific mortality and fecundity in order to predict the effects of pollutant exposure on abundance of individuals and rate of population change (e.g., growth or decline).

These types of alterations could also result in modifications of the genetic structure of aquatic and terrestrial populations. Such modifications are manifested as reductions in genetic diversity within the population or changes in gene or allele frequencies. The mechanisms whereby these changes occur include genetic bottlenecks as a result of reductions in population size or recruitment and selection for pollutant-resistant genotypes. A reduction in genetic diversity may affect population growth, sustainability, and ability to adapt to environmental variables. Furthermore, because changes in the genetic makeup of the population involve alterations in

survival and recruitment, such changes may be indicators of adverse chronic effects on population structure and dynamics. They may also be indicators of community-level effects, because it has been found that patterns of genetic diversity and community-level pollution effects are correlated in contaminated streams.

Populations exposed to pollutants may undergo genetic adaptation (short-term evolution) and become resistant to the effects of pollution. Adaptation to anthropogenic toxicants was first documented for pesticide resistance in insect and rodent populations, and plants exposed to heavy metals. Subsequently, populations of other organisms such as fish, frogs, invertebrates, and rodents have been found which have genetic adaptations to cope with polluted environments. Such adaptation may affect the patterns of population responses, and needs to be taken into consideration during biomonitoring and ecological risk assessment programs. In addition, there may be costs associated with developing such resistance, and individuals that have the pollutant-resistant genotypes may be more susceptible to natural stressors, or be at a disadvantage in nonpolluted environments.

Any effects on populations may ultimately be manifested as effects on communities because, by definition, communities are collections of interacting populations of several species (e.g., an aquatic community may consist of populations of fish, worms, plants, insects). Individual populations within a community may interact by competing for resources (food, habitat, etc.) or by predator/prey relationships. Environmental contaminants can affect the structure of communities as well as the interactions of species within them. For example, it is well known that exposure to chemicals may cause a reduction in community diversity (e.g., relative number of species), and changes in community composition. In addition, the trophic structure of fish and invertebrate communities may also be affected by exposure to anthropogenic chemicals. Changes in community structure and diversity may be determined by field sampling or manipulative studies. Alternatively, computer simulations using food web or linked population models may be used to assess community-level effects.

The trophic structure of communities is related to the relative abundance of species that feed on various food items (piscivores, omnivores, detritivores, insectivores, etc.), or have various foraging methods (shredders, scrapers, etc.). These changes in species/trophic composition may come about by direct or indirect mechanisms. The direct effects involve loss of some species due to an increase in pollution-induced mortality or reduced reproductive output. In this case the communities will be dominated by species that are less affected by pollutant exposure. This is the basis of a phenomenon termed 'pollution-induced community tolerance' (PICT), in which algal communities become more pollution tolerant over time due to the replacement of pollution-sensitive species with more tolerant ones.

On the other hand, community structure may change through indirect mechanisms. Indirect effects are those that are not due to toxic effects *per se*. For example, an insecticide may not be toxic to birds, but the birds may disappear because the insecticide kills off the insects on which it feeds. Conversely, the body size and population density of a species of minnow may increase in contaminated sites due to toxic effects on competing species (more food available for the minnow) or on predators such as bass. It has also been suggested that such changes in community structure come about because some species are more genetically plastic than others, and so are better able to adapt to novel stressors such as pollution. Thus, the more sensitive species would not be able to adapt to this stressor and become extinct locally. These types of perturbations in community structure and dynamics may ultimately compromise the stability, sustainability, and productivity of affected ecosystems.

An ecosystem is a collection of interacting populations and communities, plus the abiotic environment (e.g., climate, environmental chemistry, soil type, geology, hydrology). Ecosytem studies focus on end points such as biomass (the total mass of all organisms in an ecosystem), trophic structure, energy flow and carbon cycling (e.g., from plants to herbivores to carnivores), productivity (the amount of biomass in each trophic level produced over time), and cycling of oxygen, nitrogen, or nutrients (e.g., phosphorus) through the ecosystem. Manipulative studies use microcosms or mesocosms to study net ecosystem photosynthesis, respiration, or productivity. Field studies attempt to quantify these effects in natural ecosystems, which may be difficult. The most studied effects of pollutants on ecosystems include (1) effects of acid rain on nutrient cycling and productivity, (2) effects of elevated greenhouse gases or decreased atmospheric ozone on ecosystem structure and function, and (3) hypoxia and anoxia (little or no oxygen in the water) as a result of algal blooms or microbial decomposition due to inputs of fertilizers, sewage or animal wastes, and biodegradable chemicals into aquatic ecosystems. Complex ecosystem computer models also exist, which link biotic populations with climate and other abiotic components of the ecosystem.

Results from individual-level effects in field studies and surveys of population, community, and

ecosystem-level effects of pollution are often complicated by confounding effects. Confounding factors are, for example, temperature, season, soil chemistry that may affect or obscure responses to toxic agents. Moreover, any two sites differ naturally in terms of chemistry, habitat, etc. Thus, when comparing contaminated and noncontaminated sites, it may be difficult to separate natural variation from anthropogenic effects.

At present, one of the 'hot topics' that illustrates the problem of separating anthropogenic effects from natural variation is the status of amphibian populations. Worldwide, amphibian populations are declining, and in many locations, deformed amphibians are being found. Such deformities include missing or extra limbs or digits and facial malformations. Possible causes of these phenomena include air and water pollution, agricultural chemicals, habitat destruction, introduction of nonnative predators, diseases caused by viruses and parasites, and increased exposure or susceptibility to solar ultraviolet light. Many industrial and agricultural chemicals have also been found that are lethal, inhibit metamorphosis, interfere with embryonic development, or alter mating behavior of amphibians at very low levels. Although similar patterns have been found in many 'hot spots' around the world, there is probably no single cause, and multiple factors may be at work in each location. Unraveling this mystery and linking cause and effect remains a major challenge.

The ultimate application of ecotoxicology is in the use of ecological risk assessments. Ecological risk assessments can be defined as determining the probability that an adverse effect will occur, and the magnitude of such an effect, on natural ecosystems as a result of pollution or other anthropogenic (man-made) activities. As set out by guidelines by regulatory agencies such as the US Environmental Protection Agency, ecological risk assessments consist of the following components: (1) hazard definition – in which it is decided what the adverse effects might be and what chemicals might cause them; (2) exposure assessment – which uses chemical characteristics of each contaminant, fate and transport computer simulation models, and/or measured levels of contaminants in environmental media to estimate the magnitude and contamination; (3) effects assessment – which determines predicted or actual effects on living organisms in contaminated environments; and (4) risk characterization – which calculates the probability of an effect of a particular severity and duration, given the estimated magnitude of environmental contamination.

Ecological risk assessments can either be predictive or retrospective. Predictive risk assessments are used to predict ecological consequences for the release of a chemical before it enters the environment. This is done through the integration of toxicity tests, computer simulation models of environmental fate and transport, and computer simulation models of population, community, and ecosystem level contaminant effects. Retrospective risk assessments assess the magnitude of effects, and future consequences of contamination or cleanup, for sites that are already contaminated. Because of the difficulty in separating anthropogenic effects from natural variation between sites due to indirect effects and confounding factors, a 'weight of evidence' approach, using multiple lines of evidence, needs to be used in retrospective assessments. Because this is derived from methods used in epidemiology to demonstrate pathogens as the cause of disease, this approach is termed 'ecoepidemiology'. Central to this theme is demonstrating that bioindicators of exposure coincide with bioindicators of effect. Bioindicators of exposure include chemical contaminants in biological tissues and biomarkers of exposure. Bioindicators of effect include biomarkers of effects, gross injury (tumors, lesions, deformities, and disease), overt mortality (e.g., fish or bird kills), population declines or extinction, and changes in community structure or ecosystem function.

A similar application of ecotoxicological data is hazard assessment. Unlike risk assessment, hazard assessment is nonprobabilistic and relies upon indices rather than probabilities. One such index is the 'hazard quotient', which is the ratio of the expected environmental concentration (based upon field surveys or simulation models) divided by a 'benchmark' concentration. The benchmark concentration is derived from some measure of toxicity such as the LC_{50} or no-observed-effect level. Hazard assessments are often conducted at different levels or 'tiers' of increasing complexity and specificity: if a chemical is identified as potentially hazardous by tier (the least complex and specific test), a decision is made to take action or, if more information is needed, to proceed to tier 2 tests. After tier 2 tests, a decision is made whether to take action or proceed to tier 3 tests, and so on. This process is repeated until it is decided that there is enough information to determine whether or not there is significant ecological hazard. If there is, then regulatory action is taken.

See also: Biomarkers, Environmental; Biomonitoring; Chemicals of Environmental Concern; Ecotoxicology, Aquatic; Ecotoxicology, Avian; Ecotoxicology, Genetic; Ecotoxicology, Invertebrate; Ecotoxicology, Terrestrial; Ecotoxicology, Wildlife; Environmental Processes; Environmental Toxicology.

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Ecotoxicology, Aquatic

Yuan Zhao and Michael C Newman

Ecotoxicology, the science of contaminant fate and effects in the biosphere, emerged as a distinct discipline in the 1960s. Initial ecotoxicological concepts and methods were adopted from classic toxicology, ecology, and geochemistry. Ecotoxicology is now an interdisciplinary science encompassing effects from biomolecules to the entire biosphere, and contaminant fates from chemical speciation to global cycling.

Aquatic ecotoxicology has always been a central component of ecotoxicology because many of the first pollution issues involved the hydrosphere. Early applications of aquatic ecotoxicology included identifying and quantifying point source toxicity in support of water quality regulation. Standard toxicity testing protocols were generated based primarily on effects to individuals but also included some descriptive metrics of ecological community structure. Research in this important field contributed insights needed to formulate key US laws such as the Clean Water Act (CWA) and the Toxic Substances Control Act (TSCA).

In the early 1990s, the activities of ecotoxicologists expanded to support the ecological risk assessment paradigm. Aquatic ecotoxicologists established the conceptual underpinnings for hazard identification and risk characterization, and the data for characterizing exposure and ecological effects. Such knowledge now supports regulatory activities related to federal acts such as the Marine Protection Research and Sanctuaries Act (MPRSA), the Comprehensive Environmental Response, Compensation, Liability Act (CERCLA), and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

Currently, much aquatic ecotoxicological prediction is based on laboratory testing in which organisms are exposed to a contaminant for a specified time and effects are estimated for either lethal or sublethal impacts to individual organisms. Regression models are commonly applied to acute exposure data to predict effect metrics such as the LC_{50} (concentration killing 50% of exposed individuals by the end of the exposure) or EC_{50} (concentration having an effect on 50% of exposed individuals by the end of the exposure). Metrics derived from analysis of variance (ANOVA) and post-ANOVA tests are applied for more chronic exposures or subtle effects that are more difficult to model. The lowest exposure concentration with a statistically significant effect (lowest observed effect concentration or LOEC) and the highest exposure concentration with no statistically significant effect (no observed effect concentration or NOEC) are the most common such effect metrics. Notionally, the LOEC and NOEC bound an effect threshold concentration for the tested contaminant.

Laboratory experiments quantifying contaminant effects commonly involve either exposure via water or sediments; exposure via food is addressed less frequently. The most common laboratory tests expose individual organisms directly to contaminant present

at a range of concentrations in water. In experiments assessing effects of sediment-associated contaminants, benthic organisms are exposed directly to sediments or nonbenthic species are exposed to sediment elutriate. Elutriate tests are designed to provide data on exposure that occurs during sediment disturbances such as that resulting from storm or dredging activities. Other types of experiments include bioaccumulation or bioavailability tests that determine the potential for contaminant accumulation in organisms. In the absence of knowledge of the effects or bioaccumulation potential for a specific chemical, the magnitude of effect or bioaccumulation is sometimes predicted with quantitative structure–activity relationship (QSAR) models that relate contaminant molecular qualities for a class of contaminants such as polychlorinated biphenyls to their bioactivity (i.e., effect or potential for bioaccumulation).

Augmenting results of laboratory experiments, mesocosms, and field studies are commonly used to study the structure and function of impacted aquatic communities. Mesocosms are experimental ponds or streams designed to simulate, in a simplified manner, aquatic ecosystems. Relative to laboratory systems, mesocosms are normally located outdoors and less controlled, but can achieve more ecological realism. Field studies include surveys and natural system manipulations. The former provide relatively inexpensive observation of the consequences of contamination to communities and, although affording the least controlled observation, are the most often used type of study. Used less frequently because of increased costs, the latter involve manipulation of an entire ecosystem such as a lake or a portion of it. Intelligent combining of conclusions from laboratory tests, mesocosms, and field studies allows aquatic ecotoxicologists to assess hazard or risk due to contamination.

Current trends in the aquatic ecotoxicology literature suggest several possible changes in the near future. More insights about population consequences will be gained during assessments through the application of emerging molecular techniques, and of population-based metrics and methods. Conventional, laboratory-generated effect metrics such as the LC_{50}, NOEC, and LOEC will be used with more balance with metrics of community and population effects. This will be required to meet the demands of modern ecological risk assessment. To more directly address the needs of ecological risk assessment, more emphasis will also be placed on methods of quantifying the uncertainty in effect estimates.

See also: Ecotoxicology; Environmental Toxicology; Pollution, Water; Risk Assessment, Ecological.

Further Reading

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Ecotoxicology, Avian

Pierre Mineau

Ecotoxicology is the study of the harmful effects of chemicals on ecosystems. Avian ecotoxicology is the subset that concerns itself with the bird component of these ecosystems. Birds have long been considered an important and valued component of the natural landscape because of their visibility, broad geographical distribution, and the wide variety of ecological niches they occupy. Birds feature prominently in terrestrial ecotoxicology. The harmful effects of chemicals on birds are varied in nature. They include effects on general health, survival, and reproductive potential, generally acting directly on individuals through cellular or physiological mechanisms, or indirectly through a related component of the ecosystem, for example, affecting nesting cover or food sources. Either way, the concern is that these effects can result in repeated losses of individuals creating a population sink. If severe or widespread enough, these sinks can lead to species declines or extirpation, which, in turn, will affect other parts of the ecosystem or will reduce an ecosystem's intrinsic aesthetic, economic, or spiritual value to human beings. As with all other branches of ecotoxicology, avian ecotoxicology is an interdisciplinary endeavor that draws on such fields as analytical chemistry,

toxicology, behavior, physiology, population, as well as landscape ecology.

Early Beginnings – Concerns about a 'Silent Spring'

Some of the earliest efforts at large-scale pest control with arsenicals resulted in observed bird mortality. The plight of birds entered public consciousness following the 1962 publication of *Silent Spring*. The author, Rachel Carson, foretold of a world where "…On the mornings that had once throbbed with the dawn chorus of robins, catbirds, doves, jays, wrens, and scores of other bird voices there was now no sound; only silence lay over the fields and woods and marsh…" unless the situation was redressed and the then profligate and indiscriminate use of pesticides was placed under some form of control. The spraying of ornamental trees, woodlots and forests, especially, exposed large numbers of birds to these new chemical poisons.

The single most important event that put birds in the forefront of the new science of ecotoxicology is undoubtedly the discovery that the insecticide DDT (or more precisely DDE, a breakdown product of the pesticide) caused eggshell thinning in birds. Thin eggshells are more prone to cracking, resulting in higher levels of embryo mortality, in part as a result of pathogen entry and also from excessive water loss and desiccation. Following frequent observations of broken eggs in the nests of British peregrine falcons, time series of eggshell thickness indices suggested that the problem had begun shortly after the introduction of DDT. At higher levels of DDT contamination, some eggs simply crushed under the weight of incubating birds. Correlations between eggshell measurements and residue levels, although not always perfect, added to the evidence and captive reproduction studies removed any doubts that the effect was very real.

Because of the gradual accumulation of residues as one moved to higher levels of trophic food webs, the more serious impacts of DDT were not on songbirds, as feared by Rachel Carson, but on species at the apex of either terrestrial or aquatic food webs such as birds of prey, and fish-eating species such as ospreys, pelicans, and cormorants.

Of course, reduced eggshell thickness can be the result of lower food (and hence calcium) intake in birds and this fact has undoubtedly led to some confusion in establishing clear correlations between eggshell thickness in individual eggs and their DDE content. A vast number of pesticides and environmental contaminants can cause a rapid but transient (i.e., generally less than 5 days) decline in eggshell thickness in the laboratory. These declines are typically associated with a decrease in food intake and, indeed, a similar pattern of thinning can be elicited with a fast. Eggshell thinning has also been seen in response to a decrease in available calcium as can occur in areas impacted by acid precipitations. However, the type of thinning that is elicited by DDE in sensitive species shows a very different pattern altogether, being much more difficult to reverse in exposed birds. DDE-induced thinning is a result of a specific action of the pesticide on calcium transport and deposition in the avian shell gland.

Researchers were also able to establish the link between declines of other predatory species such as the European sparrowhawk and the use of organochlorine pesticides other than DDT. For instance, the cyclodiene insecticides aldrin, dieldrin, and heptachlor used as seed treatments caused massive mortality of both seed-eating species and their predators. All of the insecticides had the following points in common: they were highly soluble in fats and refractory to metabolism. The impacts on the predatory species typically take place in periods of food stress when fat soluble residues are released from fat stores and returned into general circulation. In a food-stressed individual, the brain remains as the most lipid rich tissue and this is where contaminants move to. Toxicity results when threshold values in brain tissue are exceeded. At sublethal levels, documented effects of cyclodiene insecticides in birds have included changes in their reproductive, social, and avoidance behaviors.

Silent Spring also raised concerns about the very high toxicity of another group of insecticides to birds. These were the organophosphorus insecticides, neurotoxic compounds that were offshoots of nerve gas research during the Second World War. Both organophosphorus compounds and another insecticide group, the methyl carbamate insecticides, work by inhibiting acetylcholinesterase, an enzyme that is vital to nerve transmission and the proper functioning of neuromuscular junctions. Unfortunately, this is a nonspecific mechanism of toxicity because acetylcholinesterase is important to both invertebrate and vertebrate life forms; also, birds are typically much more sensitive than mammals to these two chemical groups. The introduction of some organophosphorus insecticides to replace DDT and the use of phosphamidon in forestry operations, for example, resulted in massive songbird mortality in North American forests.

Pesticide Use Since *Silent Spring*

DDT and other organochlorine insecticides were eventually banned in the United States and in many

other countries, in part because of the evidence accumulated on birds, notably declining populations of some species. Despite these bans, birds continue to be exposed to residues of these pesticides in areas of heavy past use such as orchards and even residential lawns. Exposure results from the consumption of earthworms primarily. Deep-burrowing worm species are bringing residues back to the surface and reintroducing them into the food chain.

By the late 1960s, crude screening of pesticides for their impact on birds had begun in a number of countries. Systematic review of pesticide applications for their risk to birds began in 1972 in the United States although uniform test guidelines took another 10 years to develop. These guidelines are virtually unchanged today. They consist of a test of acute toxicity in adult birds, one of dietary toxicity in chicks, and a truncated reproductive test where eggs are collected from dosed birds and artificially incubated. Throughout the 1980s and early 1990s, a number of field trials conducted on the most toxic pesticides registered in the United States were carried out but this practice has been greatly scaled back now in favor of a new approach that emphasizes early attempts at risk mitigation, more sophisticated modeling of the laboratory tests, as well as the consideration of incident data. It is fair to say that, despite a few examples to the contrary, the consideration of birds in the official risk assessment process has not had a huge impact on registration decisions, at least in the United States. Insecticides well known for their ability to kill birds have remained on the market for decades. Several are still in general use today. Indeed, the best available scientific evidence suggests that bird mortality is frequent and largely unavoidable in our farm fields and this would be true as long as insecticides of high acute toxicity continue to be used. The Federal Insecticide Fungicide and Rodenticide Act (FIFRA), which provides the regulatory framework for pesticide use in the United States, uses a risk versus benefit approach to evaluate chemical effects, thus leaving the door wide open for bird mortality to be judged acceptable in light of the perceived economic benefits.

Also, the rapid modernization and intensification of agricultural production in all parts of the world has meant that concerns about the impact of specific pesticides on birds extend beyond national borders. The same toxic pesticide can affect a bird species on its North American breeding grounds as well as on its Latin American wintering and migratory staging grounds.

The cholinergic system targeted by both organophosphorus and carbamate insecticides is ubiquitous in animals. It is therefore not surprising that exposure to these pesticide classes has been linked to a broad range of physiological and behavioral responses in birds. The most relevant adverse effects are undoubtedly those that can result in lowered reproduction and survival. This includes an increased susceptibility to predation, a decreased tolerance to cold, reduced feeding abilities, and disrupted reproductive behaviors such as parental care.

Concerns are also on the increase with another class of pesticides: the coumarin anticoagulants. An increasing number of birds of prey are being found to have been secondarily poisoned by the newer 'single feed' anticoagulant pesticides. Also, a very high proportion of asymptomatic birds appear to be carrying liver residues of these compounds, which is cause for concern.

Finally, many concerns with pesticides currently have to do with the indirect effects of pesticides on birds. The most notable indirect effect is the loss of invertebrate food sources as a result of insecticide or herbicide use. Herbicides can affect the density of phytophagus insects by reducing the food and shelter provided by plants. The link between insect populations and avian breeding success has been documented in the United Kingdom primarily following long-term studies on the grey partridge and a few other farmland species. Very little of this type of work has been carried out in North America.

Birds as Sentinels of Environmental Quality

Although concerns about bird mortality and/or local population declines associated with a specific chemical arise from time to time, most of the past work in avian ecotoxicology has been generated, not with a concern for avian populations but rather with the view that birds could serve as sentinels of environmental quality. The rehabilitation of some species – for example, the bald eagle in the Great Lakes ecosystem – has even been enshrined in policy. Birds are also used in contaminated site remediation programs to indicate biological damage or remediation success. A few well-studied examples of anthropogenic contaminants and their impact on birds are reviewed below.

Persistent Organic Pollutants (POPs)

DDT and the cyclodiene pesticides are only a few components of a large 'soup' of persistent environmental contaminants consisting of halogenated organics – that is, molecules with a carbon skeleton (usually aromatic) deriving some or all of their toxicological activity through the insertion of chlorine,

bromine, or fluorine atoms. Examples are polychlorinated biphenyls (PCBs), dioxins, furans, chlorinated benzenes and terpenes, polybrominated diphenyl ethers (PBDEs), perfluorooctane sulfonates (PFOS), etc. They originate from a combination of agricultural, manufacturing, industrial, and combustion sources. They vary in the extent to which they are easily metabolized and cleared and the extent to which they are fat soluble and accumulate in biota and birds in particular. Because birds often accumulate residues to very high levels making chemical detection easier, sentinel bird species have been used to monitor levels of POPs and assess the efficacy of controls placed on some of the contaminants. Eggs are most often used for establishing contaminant trends. The main issue confronting avian ecotoxicologists working in areas with high levels of POPs (e.g., the North American Great Lakes) has been the attribution of specific biological effects such as elevated rates of malformations or higher levels of chick mortality to specific components of the contaminant 'soup'. Because many of the POPs result in the induction of the same detoxifying enzyme, cytochrome P4501A; the potential of contaminant mixtures is often expressed as 'toxic equivalents'. Enzyme titers in the liver are commonly measured indirectly, by measuring catalytic activity either with the aryl hydrocarbon hydroxylase assay or the 7-ethoxyresorufin *O*-deethylase or similar assay. These are more convenient and less costly than the chemical analysis of the hundreds of congeners of PCBs, dioxins, and furans. However, this technique is not a panacea and actually has been shown not to work very well in at least one key sentinel species, the Herring Gull. There are data, from both field and laboratory, on the possible pervasive effects of some POPs, principally dioxins and some of the conformationally-similar PCBs (coplanar PCBs), on stress and immune responses, hormone levels, vitamin A storage levels, as well as porphyrin synthesis but the significance of these findings to individual well-being and survival is debated.

Hydrocarbons

Birds are exposed to hydrocarbons through a variety of routes. Oil spills, not only the large spills of crude that make the front pages of the newspapers, but the countless small spills that result from leaks, bilge cleaning, and other spills of various types of fuel oils affect birds through fouling of plumage, external coating of eggs (which results in elevated embryonic mortality), ingestion, usually during preening, or habitat degradation. Hydrocarbon ingestion is often lethal but can also have a wide number of sublethal manifestations on behavior, reproductive success, immune function, and other measures of individual health. For example, crude oil ingestion in breeding ducks suppresses follicular development, alters gonadal hormone levels, and reduces eggshell thickness as well as hatchability. The small amount of oil needed to produce these effects suggests that the impact on hatching is not from oil passing into the egg although it is unclear whether eggshell thinning alone can explain the reduced hatching success. Polycyclic aromatic hydrocarbons tend to represent the most toxic fraction of oils and fuels. Because this fraction is more volatile, hydrocarbons tend to be most toxic immediately following release. Large spills of hydrocarbons can have transient effects on local seabird populations, especially those species that try to escape by diving rather than flying away from the slick.

Lead

By far the most important source of lead contamination in birds is lead shot. This problem is especially acute in waterfowl that ingests aquatic sediments in areas of heavy shooting and in their consumers (e.g., eagles). Imbedded shot and bullet fragments also represent a source of exposure for predators and scavengers. Lead poisoning from shot affects waterbird species worldwide. Fortunately, a solution is readily available in the form of a nontoxic shot even though many jurisdictions have been very slow in implementing this change. Lead sinkers also represent a problem for loon (diver) species as well as for swans in areas where recreational fishing is practiced. Again, changing fishing weights from lead to another metal such as steel is not difficult or costly when compared to other environmental remediation measures. Lead bullets will prove more difficult to replace and there still are no readily available replacements. Lead bullet fragments in carrion are proving a major problem for the introduction of the critically endangered California condor into its former range in the western US. Other sources of lead in birds have included paints, automobile exhaust, and industrial emissions or waste. Songbirds nesting downwind from metal smelters have experienced eggshell quality problems and poor hatching success, in large part from the lead contamination. Lead is neurotoxic at very low doses and is particularly effective at disrupting neural development in developing birds.

Mercury

Mercury in its organic form also biomagnifies in food webs. The use of mercury for gold extraction has been responsible for widespread contamination of aquatic ecosystems and the practice continues unabated in many parts of the world. Other sources,

historic and present, include industrial processes, pulp bleaching, as well as combustion and release of natural mercury from flooded sediments, especially under acidic water conditions. There is good evidence that the acidification of poorly buffered lakes receiving acidic precipitation from combustion sources results in higher levels of mercury being biologically available to aquatic biota and thence to fish-eating bird species. Historically, mercury used in pesticide seed treatments caused severe mortality in seed-eating bird species and their predators. Long-term monitoring programs in seabirds suggest that mercury contamination is increasing globally. At sublethal doses, mercury has been found to affect reproduction although there appears to be wide interspecific variation in the levels that prove embryotoxic and teratogenic. Mercury is also a neurotoxicant and, even in small doses, can modify normal behaviours in bird chicks. This has been established in the laboratory through the usual tests such as visual cliffs and open fields but, at least one intriguing example has been documented in the wild: young loons on high mercury lakes spend less time on the backs of their parents.

Selenium

Selenium is an essential element but it is very embryotoxic at high doses. The classic selenium study is the work performed at the Kesterson Wildlife Refuge, an area which received drainwater that had percolated through selenium-rich soils in surrounding agricultural areas. Eggs and embryos are particularly susceptible to maternally acquired selenium. Effects include poor hatching success and poor survival of hatched young. The most dramatic effect, however, consists of very visible malformations in embryos and chicks including small or missing eyes, absence of legs or toes, incomplete beak development, development of the brain outside of the skull case, and fissures into the abdominal cavity. These were seen in wild wader species and replicated in the laboratory. The case of selenium is particularly interesting because, although it passes into the egg, it is not bioaccumulative. Selenium also offers an interesting observation on interspecies sensitivity differences. There was an $\sim$10-fold difference in the threshold for embryotoxicity in two closely related species: the black-winged stilt and American avocet. Thresholds for major malformations were not as divergent: calculated EC_{10} values differed by approximately fourfold.

Endocrine Disruptors

Since the publication of the book *Our Stolen Future* by Theo Colborn and co-authors in 1996 and the publicity that surrounded its publication, there has been renewed interest in understanding the endocrine basis of pesticide- and pollutant-induced effects on reproduction although such interactions have long been established in bird populations exposed to contaminants and pesticides. A number of pesticides and contaminants, several of which have been mentioned in earlier sections, can affect the avian endocrine system at some concentration. The field evidence to date for endocrine effects in birds is exclusively from persistent and bioaccumulative contaminants, typically in the predatory top of the food chain species. The key question that remains to be answered is whether long-term population stability is compromised by such effects. Also, we need to find out whether exposure to modern agricultural chemicals – that is, typically not persistent and not bioaccumulative – is such that some of their demonstrated endocrine modulation potential is likely to be expressed in the wild.

Tools of the Trade

Avian ecotoxicology brings to bear a number of tools to investigate effects from the molecular to the population levels. At the molecular level, current advances are similar to those being made in mammalian toxicology, namely toxicogenomics and the use of such tools as gene amplification and differential display of coding regions of the genome in response to a toxicant as well as microarrays – often those that have been developed for probing the mammalian genome. A wide array of biochemical markers and bioassays have been developed in birds to measure immune function, stress response, and general organismal health and function as well as more targeted disruptions such as cholinesterase inhibition. At the other end of the spectrum are the standard time-honored methods used by avian ecologists worldwide: behavioral assessments, censusing, mark–recapture and telemetry, monitoring of reproductive success, and the placement of these measurements in a population and landscape context. More than 40 years after the publication of *Silent Spring* the field of avian ecotoxicology is vibrant and growing in several directions. We can undoubtedly rejoice at the fact that impacts of chemicals on birds are being studied more intensively now than when Rachel Carson wrote her seminal work, and that the field of avian ecotoxicology is attracting innovative researchers from a multitude of disciplines. We might, however, lament the fact that many of our experimental subjects are in trouble with many more bird species declining than either increasing or holding their own. We might also ask why the regulatory, political, and

social responses to avian toxicology research findings often lag so far behind our understanding of the problems.

See also: Carbamate Pesticides; DDT (Dichlorodiphenyltrichloroethane); Endocrine System; Federal Insecticide, Fungicide, and Rodenticide Act, US; Lead; Pesticides; Polybrominated Diphenyl Ethers (PBDEs); Polychlorinated Biphenyls (PCBs); Polycyclic Aromatic Hydrocarbons (PAHs); Selenium.

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Relevant Website

http://www.ourstolenfuture.org – The book Our Stolen Future deals with research on endocrine disruption. It discusses how common contaminants can affect the development of the fetus. This website is a good resource to check recent developments.

Ecotoxicology, Genetic

Wendy L Rose and Susan L Anderson

Introduction

Genetic ecotoxicology is a discipline that emerged from early studies in radiobiology. In the 1950s, radiation exposure was shown to alter the development, growth, and reproduction of mammals, fishes, and invertebrates. Growth retardation, suppression of cell division, and modified cell differentiation were detected in radiation-exposed organisms. Radiation exposure was also linked to gonad sterility as well as reduced fecundity, hatching success, and fertilization success in vertebrate and invertebrate species. As the field of radiobiology expanded in the late 1960s and early 1970s, scientists began to associate congenital and developmental abnormalities with chromosome damage and mutations resulting from radiation exposure. With increasing awareness of environmental pollution, research originated on the effects of toxicants, in addition to radiation, on the genetic material of aquatic and terrestrial organisms. Moreover, studies began to address the relationship between heritable genetic damage and consequences to populations and communities.

Currently, we define genetic ecotoxicology as the study of the effects of toxicants and radiation on the genetic material of aquatic and terrestrial organisms, and related population- and community-level responses. Genetic ecotoxicology can be distinguished from genetic toxicology, which primarily focuses on the individual-level effects of genotoxicants in humans and model mammalian species. Genetic ecotoxicologists are interested in a range of effects including: DNA and chromosome damage, reduced developmental or reproductive success, diminished population size and distribution, age class alterations, and shifts in community structure. Genetic ecotoxicology is a complex discipline that integrates genetic toxicology, ecology, and population genetics. In the following sections, we hope to clarify the goals of this expanding field by: (1) examining the ways in which toxicant exposure may cause deleterious consequences to aquatic organisms and their populations, and (2) describing widely used methods in genetic ecotoxicology and their application to studies of toxicant effects on populations. The discussion below is limited to studies on aquatic organisms, which have been used as model species in numerous investigations of radiation- and toxicant-induced genetic damage at both the individual and population levels. At the individual level, this overview mainly concentrates on the effects of genotoxicant exposure on aquatic organisms, rather than radiation-induced responses, which are covered elsewhere in this encyclopedia.

How Toxicant Exposure Could Lead to Population-Level Consequences

To best understand the questions driving genetic ecotoxicological research, we have synthesized a model relating toxicant exposure to population-level consequences (**Figure 1**). Below, we discuss some of the factors and processes that may contribute to the decline of a population based on recent reviews in this field.

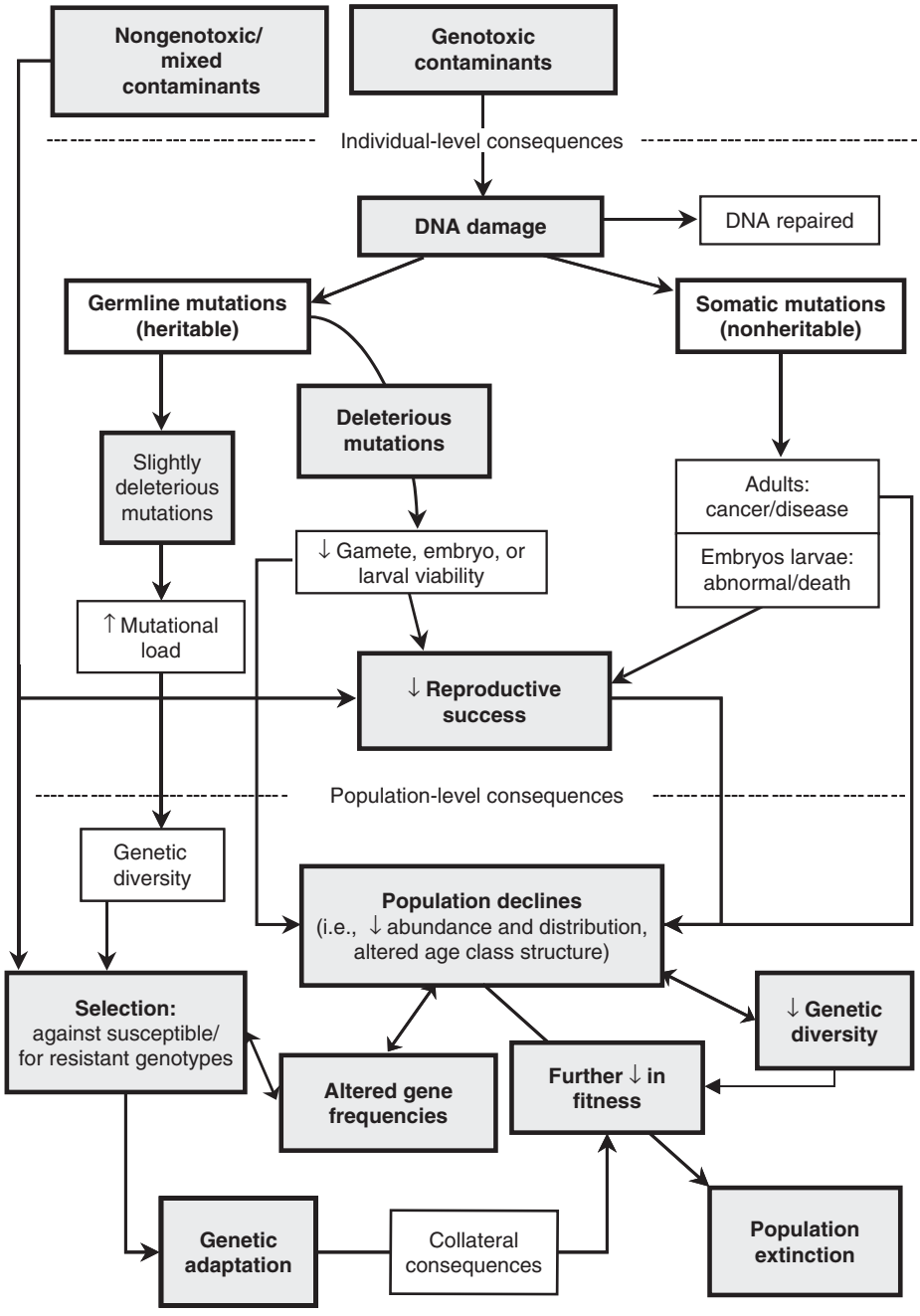

Figure 1 Model of how genotoxic and nongenotoxic contaminant exposure may lead to population-level consequences such as declines and extinction. The gray boxes indicate key steps in the pathways leading to population-level responses of contaminant exposure. Within the boxes, the up arrows suggest increases and the down arrows suggest decreases in the endpoints of toxicant exposure.

Genotoxic contaminants are chemicals that interact with and cause DNA damage such as strand breaks, adducts, pyrimidine dimers, or chromosomal damage in exposed organisms (**Figure 2**). Genotoxicants may cause damage by chemical interaction with the DNA molecule, interference with the structural integrity of DNA, or through indirect mechanisms including induction of oxidative stress, and inhibition of DNA repair. Most DNA damage will be repaired or may occur in introns, sections of DNA that do not code for protein structure or function. However, if DNA damage is not repaired or is misrepaired, gene expression may be compromised or mutations, alterations in DNA sequence, may arise.

The severity of DNA damage depends upon whether mutations arise in germline (heritable) or somatic (nonheritable) cells. Heritable genetic material is found within the germline cells, or sperm and eggs, while nonheritable genetic material is found within somatic cells, or all other cells in an organism. In adults,

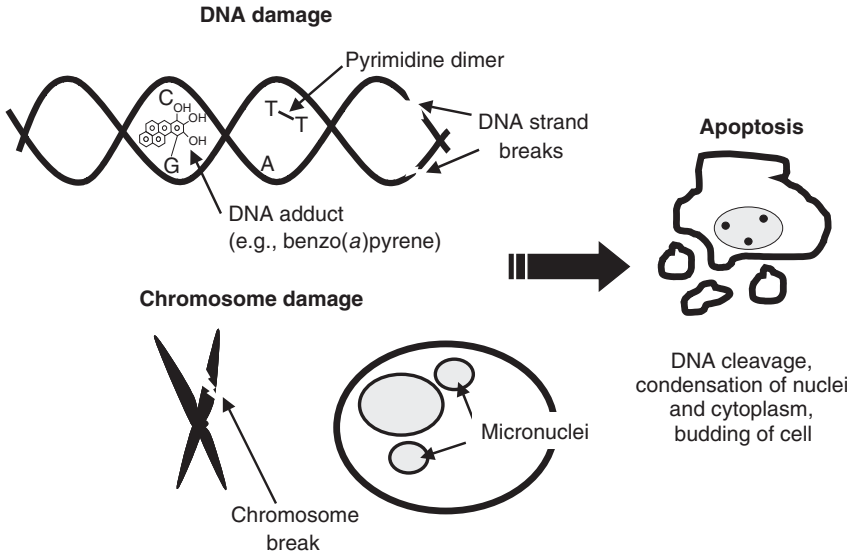

Figure 2 Genetic and cytogenetic endpoints used in genetic ecotoxicology to assess individual-level effects in toxicant-exposed organisms. The large arrow indicates that severe or misrepaired damage to DNA or chromosomes may cause a cell to undergo apoptosis or programmed cell death.

somatic mutations may cause metabolic or physiological impairment, cancer, and decreased survival in exposed individuals. If large numbers of individuals demonstrate chronic toxicities or disease such as cancer, then individual-level consequences of somatic mutations may eventually lead to population declines. However, it is more likely that individual-level effects of toxicants simply contribute to the decline of populations. More acute population-level effects may occur as a result of somatic mutations in embryos and larvae, which represent the youngest segment of a population. Specifically, the age class structure of toxicant-exposed populations may be altered, leading to shifts in reproductive cycles and reduced developmental or reproductive success. Regardless of age, somatic mutations are less severe than germline mutations, which directly affect the integrity of the germline cells, and the next generation of organisms.

Germline cells may incur deleterious or slightly deleterious mutations as a result of toxicant exposure. Germline cells that acquire deleterious mutations may undergo cell death or dysfunction resulting in impaired fertility, fertilization, embryonic development, hatching success, or larval and adult viability, all of which are indicative of diminished reproductive success. As a result, there may be a decrease in population size and ultimately, a reduction in genetic diversity, or the degree of gene variation within a population that allows it to resist harsh environmental changes or epidemics. Slightly deleterious mutations, mutations that do not directly lead to a life-threatening disease, but may contribute to an increased mutational load, also may arise in germline cells. However, few studies have demonstrated with certainty that an increased mutational load, and results thereof, may result from toxicant or radiation exposure.

Dramatic life history alterations that result from toxicant exposure could lead to population extinction through a variety of mechanisms. For example, if one life stage of an organism is more sensitive to toxicant exposure than others, reduced survival and fecundity may bring about population bottlenecks, severe reductions in population size, or alterations in age class structure. Bottlenecks may be accompanied by a decrease in genetic diversity, inbreeding depression, further diminution of fitness, and eventually population extinction. Alternative hypotheses such as decreases in population density also may explain how toxicant exposure could lead to severe population declines or extinction.

Nongenotoxic contaminants are toxic substances that do not directly alter the genetic material but may impair reproductive success or lead to selection and indirect changes in population genetic structure. The well-known dichlorodiphenyltrichloroethane (DDT), once sprayed to eradicate malaria-spreading insects, caused eggshell thinning in brown pelicans and other species and led to impaired reproductive success and widespread population size reductions. Thus, selection against susceptible or for resistant genotypes may occur in populations that have been exposed to nongenotoxic contaminants. Selection is the process that results in the survival and reproductive success of organisms or populations that are best adjusted to their environment. As a result of selection, the following may occur: (1) gene frequency alterations;

(2) modifications in life history characteristics and population viability; (3) population size reductions; and (4) genetic variability decreases. In addition, genetic adaptation may have collateral consequences such as slow growth, late development, reduced longevity, and reduced fecundity.

Methods for Detecting Individual-Level Effects of Genotoxic Contaminants in Organisms

There are many well-known methods for detecting structural damage to DNA as depicted in **Figure 2**. Techniques vary in their selectivity for specific groups of toxicants, sensitivity for detecting low-level toxicant exposure, time requirements, cost, technical difficulty, use of radioactivity, and appropriateness for measuring DNA or chromosome damage in field-exposed organisms. Here, we discuss some of the most widely accepted methods, according to recent reviews in genetic ecotoxicology for evaluating DNA damage in toxicant- or radiation-exposed organisms.

^{32}P-postlabeling analysis is an effective method for the detection of DNA adducts, chemicals that are bound covalently to DNA, and implicated in chemically induced carcinogenesis. The ^{32}P-postlabeling technique involves the isolation and hydrolysis of DNA, labeling of normal and adducted 3′-monophosphates with ^{32}P, resolution of adducts from normal nucleotides by TLC, and detection of adducts by autoradiography or screen phosphor imaging. In a series of studies in the 1990s, Stein *et al.* used ^{32}P-postlabeling analysis to demonstrate that DNA adduct levels in fishes were higher in industrial and urban areas as compared to fishes from reference sites, or relatively noncontaminated aquatic environments. For example, polycyclic aromatic hydrocarbon (PAH)-DNA adducts in oyster toadfish (*Opsanus tau*) liver were significantly higher from a creosote-contaminated site in the Elizabeth River, Virginia, when compared with reference sites, and levels of PAH-DNA adducts decreased with increasing distance from the creosote contamination.

The alkaline unwinding and comet assays are methods used to detect DNA strand breaks that arise as a result of exposure to genotoxicants or normal metabolic processes. DNA strand breaks may be incurred following the loss of a base in the DNA strand, leaving a transitory gap in the DNA subsequent to DNA repair. In the alkaline unwinding method, DNA is isolated and denatured at a specific temperature and pH, and the DNA strand break levels are calculated as the inverse proportion of the Hoecsht-stained double-stranded DNA. Additional modifications of this method are available for the resolution of single- versus double-stranded DNA strand breaks.

In the comet assay, cells are embedded in agarose on slides, lysed, and electrophoresed under alkaline conditions. Upon fluorescence staining and microscopy, cells with DNA damage appear as comets and have DNA strands that have migrated out of the nuclei. The distance or amount of DNA migration indicates the extent of DNA strand breakage. The comet assay has been used to evaluate DNA damage in plants, invertebrates, fishes, and amphibians from sites worldwide. For instance, the comet assay was used to demonstrate that DNA damage in mussels exposed to contaminants in San Diego Bay was significantly higher than DNA damage in reference mussels.

Severe DNA cleavage indicative of apoptosis, or programmed cell death, has been suggested as a possible biomarker of genotoxicant exposure in aquatic and terrestrial organisms. The TdT-mediated dUTP Nick-End Labeling (TUNEL) assay may be used to assess apoptosis or DNA cleavage in field-exposed organisms. Here, tissues or whole organisms are fixed, sectioned and embedded, and proteolytically digested, and fluorescein- or biotinylated-dUTPs are transferred to 3′-OH groups on cleaved DNA strands using a terminal deoxynucleotidyl transferase (TdT) enzyme. Biotinylated DNA fragments are detected with a streptavidin-HRP conjugate and substrate (DAB and hydrogen peroxide) and viewed by light microscopy. Fluorescein-bound DNA fragments are measured using fluorescent or confocal microscopy or flow cytometry.

Techniques are also available for detecting the effects of genotoxicants on chromosome integrity. Chromosomal aberrations are analyzed during metaphase and anaphase of mitosis and represent a method in which the effects of genotoxicants can be measured in tissues with a high mitotic index. For metaphase preparations, organisms are treated with colchicine, graded hypotonic solutions, and fixative. Fixed cell suspensions are spread onto slides, and stained with Giemsa and aceto-orcein. For anaphase preparations, cells are typically fixed in formalin solutions, stained with Giemsa and squashed on slides. Both metaphase and anaphase cell preparations are scored by the percentage and type of chromosomal aberrations. An integrated study of the health of Atlantic mackerel (*Scomber scombrus*) from the New York Bight in 1980 demonstrated that mitotic and chromosomal abnormalities were useful measures of toxicant exposure because they were associated with elevated levels of planktonic hydrocarbon and zinc, embryonic differentiation, and gross embryonic malformations.

In addition to chromosomal aberrations, the micronucleus assay can be used to measure chromosome integrity. Micronuclei, small secondary nuclei or DNA aggregates, arise during cell division when chromosomes break or misalign. Resulting DNA fragments become incorporated into one of the daughter cells during cell division. In the simplified micronuclei method, dividing cells are spread and fixed on slides, stained using Wright–Giemsa or fluorescent stains, and the number of cells with micronuclei are scored.

Chromosomal damage also may be assessed in a large number of cells using flow cytometric analysis. In this method, an increase in DNA content variation in the cells of interest reflects an increase in the amount of chromosomal damage such as chromosome deletions or aneuploidy. The method involves isolation of nuclei from a cell suspension, staining of cells with a DNA stain such as propidium iodide, and measuring DNA content for cells in the G_0/G_1 phase of the cell cycle by flow cytometry. This method was used to measure DNA content variation in redbreast sunfish (*Lepomis auritis*) along a gradient from a toxic effluent release (East Fork Popular Creek, TN), in relationship to other biological and community level responses. DNA content variation and percentage pollutant-tolerant species was highest at the site of a toxic effluent release and decreased with increasing distance, and additional biomarker responses demonstrated similar trends.

In genetic ecotoxicology, experiments should be designed to not only measure exposure or effects, but also to discern differences among treatments due to nontoxicant-related environmental stressors, and to draw linkages between genotoxicity and fitness. To best examine mechanisms through which genotoxicity is related to effects on fitness, multiple endpoints of genotoxicity and fitness should be measured. Careful consideration should be taken to assess whether factors unrelated to toxicant exposure such as handling stress are causing modifications in the measured biological responses. Prior to field sampling, laboratory studies should be performed to evaluate tissue- and time-specific differences, species differences, and seasonal differences in the biological endpoints of interest. Finally, integration of field and laboratory experiments is also recommended; measurements evaluated in field-exposed organisms should be similarly assessed in organisms exposed to toxicants within the same media in the laboratory.

Methods for Detecting Population-Level Effects of Toxicants in Field-Exposed Organisms

There are a variety of methods that can be used to examine population genetic structure. In **Figure 3**, methods for evaluating population-level effects of toxicants in aquatic organisms have been categorized

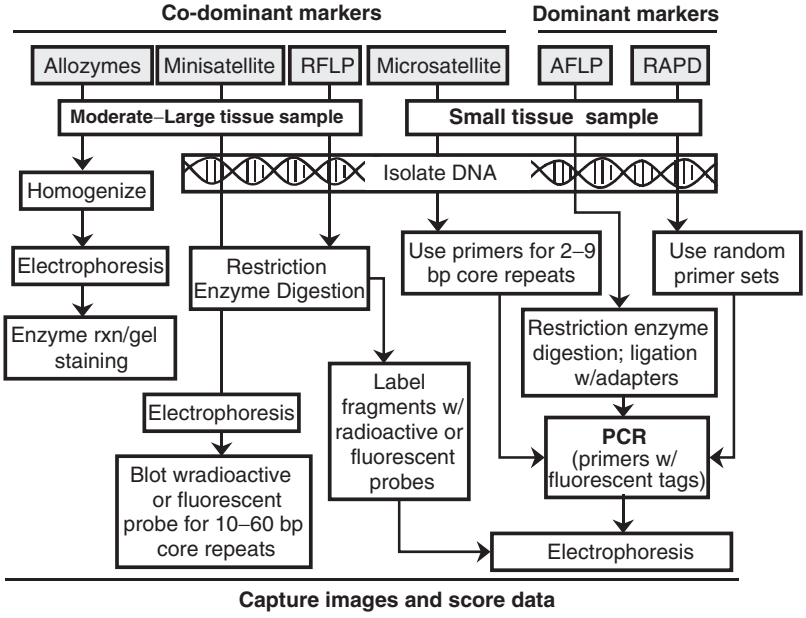

Figure 3 Population genetic methods used in genetic ecotoxicology to examine toxicant effects in populations of aquatic and terrestrial organisms. Methods are categorized as either co-dominant (allozymes, minisatellites, RFLP, microsatellites) or dominant (AFLP, RAPD) markers. As shown here, techniques used to generate these markers are based on similar procedures such as PCR and electrophoresis, and similar methods of analysis. bp, base pairs.

as co-dominant or dominant markers, those in which heterozygous and homozygous dominant genotypes can or cannot be distinguished, respectively. Heterozygous genotypes have different alleles or forms of a gene at corresponding loci (e.g., positions of a gene along a chromosome) on homologous chromosomes. Homozygous genotypes have the same alleles at corresponding loci. Co-dominant markers include allozymes, microsatellites, and restriction fragment length polymorphisms (RFLPs), while dominant markers include amplified fragment length polymorphisms (AFLPs) and randomly amplified polymorphic DNAs (RAPDs). Techniques used to generate this diverse group of markers are based on similar procedures such as PCR and electrophoresis, and similar principles and methods of analysis (**Figure 3**).

Allozymes are co-dominant markers that have been used for several decades to assess the population genetic structure of aquatic and terrestrial populations. Allozymes are enzymes that vary in their electrophoretic mobility and are indicative of different alleles of single genetic loci. The allozyme technique uses starch or acrylamide gel electrophoresis to separate allozymes within tissue homogenates. Allozymes are visualized upon exposure to a particular enzyme catalyst or by staining gels with dyes, and scored by the number of specific allozyme types per individual.

Restriction fragment length polymorphism analysis is another technique that generates co-dominant allele data. In this method, DNA is isolated and digested with restriction enzymes, enzymes that cut DNA at specific positions within target sequences. The DNA fragments are labeled with fluorescent or radioactive tags, and electrophoresed. Images are captured using autoradiography or gel scanners and individuals scored according to the DNA fragment sizes. In the 1990s, Wirgin *et al*. used both allozyme assays and RFLP analysis to determine that there were genetic polymorphisms in the cytochrome P_{450}1A (CYP1A) gene of some Atlantic tomcod (*Microgadus tomcod*) from the Hudson River that were not present in tomcod from the less contaminated Saco River. The alleles were sequenced and evidence was found for selection against the Hudson River CYP1A allele, which was only detected in the heterozygous state.

In addition to selection, RFLP has also been used to demonstrate that decreases in genetic variation due to toxicant exposure may be suggestive of bottlenecks. RFLP analysis was used in the 1990s to demonstrate that copepods exposed to PAHs for several generations in the laboratory and near oil platforms experienced a reduced mtDNA composite genotype diversity compared to controls or those at reference sites.

Microsatellites are hypervariable co-dominant loci composed of arrays of 2–9 bp repeating motifs. Differences in the number of repeat motifs in an array define microsatellite polymorphisms. Method development requires the identification of microsatellite loci, and for each locus, the design of PCR primers to anneal to conserved regions flanking the microsatellite. Analysis involves PCR amplification with fluorescently labeled primers followed by electrophoresis to distinguish microsatellite alleles of different array size.

In contrast to allozyme, RFLP, and microsatellite analyses, the RAPD technique uses the random PCR amplification of DNA sequences to generate dominant allele data. Specifically, the method involves the isolation of DNA, PCR amplification of DNA sequences using a random set of short primers with fluorescent tags, and electrophoresis. The presence or absence of bands is scored upon visualization of gels. In a series of studies in the 1990s, Theodorakis *et al*. provided evidence of selection in radionuclide-exposed mosquitofish (*Gambusia affinis*) from Tennessee ponds using RAPD and allozyme assays. The authors determined that genetic diversity was higher in radionuclide-exposed mosquitofish populations than in reference populations. A lower incidence of DNA strand breaks and higher fecundity were found to correlate with the RAPD banding patterns in radionuclide-exposed mosquitofish. In a later study, similar RAPD banding patterns were detected in another mosquitofish species (*Gambusia holbrooki*) from a separate radionuclide drainage, providing further evidence of natural selection in radionuclide-exposed mosquitofish populations.

The AFLP technique is another method in which dominant allele data are collected. Here, DNA is isolated and digested with restriction enzymes, and DNA fragments are ligated to adaptors, short double-stranded DNA sequences. Ligated fragments are then amplified with fluorescently labeled primers that recognize and bind to adaptors during PCR, and fragments are separated by electrophoresis. Visualization and scoring of gels is similar to that of RAPD analysis. In a recent study, AFLP and microsatellite markers were used to test the null hypothesis that genetic variation among Sacramento sucker (*Catostomus occidentalis*) populations in the California's Central Valley was due to biogeographical influences. The alternate hypothesis was that genetic variation among populations was explained by differences in long-term pesticide exposure history. Using both AFLPs and microsatellites, differences in watershed geography best explained the genetic variation

among sucker populations and demonstrated the importance of testing contaminant exposure hypotheses against the null hypothesis of biogeographical and historical influences.

Regardless of the markers used, population genetic analyses are based on similar principles. First, allele scores are generated from either the presence or absence of bands (dominant markers) or sizes of bands (co-dominant markers) on gels, and a genotype is generated from the composite of alleles. Allele and genotype frequencies at single loci are calculated from the data and multi-locus genotypes are used to further characterize population structure based on population genetic models. For instance, genetic structure may be determined from allele frequencies, whereas genetic variability may be determined from mean heterozygosity and percentage polymorphism.

Meticulous experimental design and clear hypotheses are necessary to distinguish among factors that affect genetic patterns and determine potential mechanisms through which differences arose among populations. Background levels of natural genetic variation among many populations should be determined prior to testing contaminant exposure hypotheses. Chemical analyses and biological responses to toxicants should be measured at field sites to establish toxicant exposure. More than one method is recommended for the examination of population genetic structure to verify that results are accurate. A sufficient sample size should be used to detect differences among treatments, and several sites should be included for each treatment type. Finally, we stress that it is exceedingly important to rigorously test contaminant exposure hypotheses; experiments must be designed to reject the null hypothesis that genetic patterns among populations are accounted for by life history and biogeographical factors.

Conclusion

In summary, many factors contribute to the ongoing success or failure of aquatic and terrestrial populations in the face of environmental pollution. The growing field of genetic ecotoxicology offers many ways for scientists to examine the effects of toxicants and radiation on aquatic and terrestrial organisms, both at the individual and population levels. The methods described here can be used to address a diversity of environmental pollution problems such as assessing impacts of oil spills on aquatic organisms and populations, developing standards for cleanup of hazardous waste sites, evaluating the relative roles of habitat destruction and contaminant exposure in the management of endangered species, and screening industrial and nonpoint source inputs to ecosystems. Because it is difficult to identify all factors contributing to the demise of a species, we propose that careful field sampling designs and rigorous hypothesis testing, coupled with strategic selection of molecular markers and subsequent statistical analyses, will help scientists to best examine these issues.

See also: Apoptosis; Ecotoxicology, Aquatic; Benzo(*a*)-pyrene; Biomarkers, Environmental; Cytochrome P-450; DDT (Dichlorodiphenyltrichloroethane); Genetic Toxicology; Polycyclic Aromatic Hydrocarbons (PAHs); Radiation Toxicology, Ionizing and Nonionizing.

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Ecotoxicology, Invertebrate

Pawel J Migula

The Background and Basic Concepts

Ecotoxicology concentrates on studies of toxic effects on organization levels higher than organisms. Ecotoxicology differs from toxicology and relies on the fundamentals of ecology, which means that ecotoxicologists study pollutants and their effects on different ecosystems. The objectives of ecotoxicology cover both fundamental and applied aspects.

Invertebrate ecotoxicology is a specialized area of ecotoxicology that deals with all aspects of ecological and toxicological effects of toxic substances on invertebrates and their consequences at the population level and above. The main objectives of invertebrate ecotoxicology are similar to those of ecotoxicology itself, related to the areas where invertebrates can be used:

- Obtaining data for risk assessment and environmental management.
- Meeting the legal requirements for the development and release of new chemicals into the environment.
- Developing empirical or theoretical principles to improve our knowledge of the behavior and effects of either natural or anthropogenic chemicals and to predict and evaluate changes caused by them in ecosystems.

There are many important factors which determine the study of invertebrates in ecotoxicology at both population and ecosystem levels. They represent more than 90% of all animal species and are much more abundant than vertebrates. They are present in nearly all types of ecosystems, from the deep oceanic bottom, through the surface water to soil and other terrestrial areas, including those with the most severe conditions for biological life. They often play a key role in different chains of the food web, determining interrelationships within the nets and participating in upward biomagnification of chemicals in the net. They are present in all heterotrophic layers, utilizing variety of food, take part in the decomposition of organic matter, transfer of biogenic substances and xenobiotics. Despite their small dimensions they exist in large quantities and represent animals that are short- or long-lived; they can also be maintained in the laboratory more easily and cheaply than vertebrates. Many species are abundant throughout the year, especially in the soil or in sediments. Invertebrates raise less ethical concern than vertebrates, especially mammals. In cases where mechanisms of toxic action of chemicals are similar to those observed in the case of vertebrates they may replace them in routine toxicity tests of novel compounds or pesticides. Invertebrates are more useful as the key animals in standardized ecotoxicological testing of soils or sediments. They should also provide a useful material for better understanding the interactions of chemicals with the biotic part of the environment and their consequences to an assessed ecosystem. Moreover, short generation time and abundance make some species useful models to study microevolution during long-term *in situ* investigations of populations living in stressed environments for many generations. They can develop resistance to toxic substances such as pesticides or heavy metals.

There are also several disadvantages of the use of invertebrate species in ecotoxicological investigations. Many of them are not suitable for continuous assessments of seasonal effects. Their generation time is limited to certain periods when they are active. Depending on physical conditions and food availability, they may disappear or be present as inactive forms (different diapausing stages, insect pupae, dormant eggs). In many species only adults can be used, because in case of their immature forms there are difficulties in determining their taxonomic position which cannot be overcome without sectioning, and, in turn, cannot be done prior to laboratory assays. In such a case they can serve only for studies at the community or ecosystem level where pooling of samples is acceptable, and their selection is based on similarities of their body size/biomass or feeding habits. More individuals are necessary in a sample than in the case of vertebrates; preparatory techniques are more complicated, more practice and sometimes pooling of individuals in a sample are needed. At the molecular level invertebrates may respond to the same chemical in a way similar to vertebrates. External factors cannot interfere with such responses causing sometimes insensitivity of invertebrates. On the contrary, due to lower activity of microsomal detoxification enzymes, insecticides are often more toxic to invertebrates than to vertebrates.

Population ecotoxicology of invertebrates covers broad ecological and evolutionary aspects of the chemical effects on individuals, changes in population demography and interrelations between various

populations and species. Fitness can be a good measure of management of organisms with toxicants. Changes in relative fitness may lead to the evolution of resistance. The best-known example is the evolution of insect resistance to insecticides, and more recently resistance of various soil-dwelling invertebrates to heavy metals.

Invertebrates in Ecotoxicity Testing

Many ecotoxicological tests are performed by studying invertebrate biochemical fingerprint techniques for risk assessment. Invertebrates are recommended by OECD Test Guidelines to be used for assessing the effects of chemicals on the reproductive output in soil acute toxicity tests at organism, population and community levels for legislative purposes of new or existing chemicals. The OECD-recommended methods for testing physical and chemical properties, effects on human health and wildlife, and accumulation and degradation in the environment are also partially based on the use of invertebrates. Such tests are of little value for the whole ecosystem. They are performed in controlled laboratory conditions, while in nature organisms are exposed to a mixture of pollutants of different concentrations, which differ in bioavailability, routes of reaching the targets in the organism, and interact with a series of abiotic and biotic factors.

Invertebrate Ecotoxicology Gives Tools for Searching and Evaluating Microevolution

Generally, toxic substances are potent selection factors. Invertebrate ecotoxicology is therefore a very attractive science which helps study ecological and evolutionary phenomena directly confirming the existence of microevolutionary processes which could not be investigated in other ways. In a relatively short time many generations of short-living invertebrate species can be used to measure a selective pressure of sublethal concentrations of chemicals on genomic changes. Where tolerance limits are exceeded such changes may lead to the extinction of some populations while other populations may develop resistance through inherited adaptive biochemical/physiological/structural or behavioral features against various toxic substances. A spatial and temporal selection of genotypes with newly inherited features is good evidence of evolved tolerance to metals in populations from heavily contaminated sites. Moreover, the disappearance of newly inherited mechanisms of resistance/tolerance can be studied in populations in conditions of ceased pressure from the chemicals. Studies of metal tolerance were recognized as the best examples of microevolution.

Metal Tolerance and Metal Adaptation in Soil-Dwelling and Aquatic Invertebrates

Exposed and unexposed populations of some terrestrial invertebrate species demonstrate divergences of physiology or tolerance to heavy metals in their food. Adaptation was proved in natural populations of such taxonomic groups as Oligochaeta, Mollusca, Crustacea (Isopoda), Myriapoda, Arachnida, Apterygota, and Insecta. Adaptations are species-dependent and their various strategies have been identified. For example, the terrestrial isopod, *Porcellio scaber*, differs in life-history traits, storage, and excretion patterns. The females from the contaminated site had smaller body size and started to reproduce earlier than the isopods from the unpolluted site. They had been selected for early reproduction and allocated more energy to increase reproduction. A similar life history with an increased reproductive effort under metal stress characterizes populations of a collembolan *Orchesella cincta*. Both species differ in the strategy of metal bioconcentration and bioelimination. *Porcellio scaber* is a slow bioeliminator of metals which effectively accumulates metals mainly in metal-binding intracellular granules stored in hepatopancreas, and partly in metal-binding glycoproteins. *Orchesella cincta*, in contrast to the isopod, is a very quick eliminator of metals. Net assimilation rates of metals are lower in collembolans than in isopods. Genetic adaptation of *O. cincta* may occur within years or decades. The consequence of metal adaptation is reduced genetic variation for tolerance, which indicates additional costs of tolerance. Metal-adapted *O. cincta* shows increased mortality when transferred to uncontaminated conditions.

Invertebrates in Ecotoxicological Studies of Endocrine-Disrupting Substances

Endocrine disruptors are 'chemical substances that interfere with, or have adverse effects on, the production, distribution, or function of these same hormones'. These substances do not affect growth or cause increased mortality. They affect the development of organisms which is manifested in alterations of reproductive capacity or metabolic disorders of many steroid hormones. Some aquatic invertebrate species (cladocerans, mollusks) with a short reproduction time have been intensively studied as the

targets and useful models of hazard assessment for endocrine disrupting pollutants. Impaired reproduction in *Daphnia magna* caused by changes in endocrine metabolism as a result of alterations in biotransformation enzymes has been recently used as an early warning system of reproductive toxicity. The best-known example of an endocrine-disrupting chemical is tributyltin (TBT) – the antifouling agent used for painting boats responsible for abnormalities in the development of female reproductive organs in about 100 marine gastropods. The dogwhelk, *Nucella lapillus*, common on the rocky shores of northern Europe and the North Atlantic, is the best studied neogastropod species which is very sensitive to TBT (lowest-observed-effect concentration, LOEC = $1\,\mathrm{ng\,l^{-1}}$ as Sn). In TBT-affected females a phenomenon known as imposex (or pseudohermaphroditism) has been described. Inhibition of the enzyme converting testosterone to 17E estradiol – P450-dependent aromatase in TBT-exposed females increases the testosterone content which induces development of nonfunctional male characteristics – vas deferens (a channel between prostate and penis) and a penis. The vas deferens occludes the genital papillae and such females become sterile by blocking the release of egg capsules. Any further development of aborted capsules might eventually kill the exposed females. Affected populations may decline or become locally extinct. The measurement of two parameters at the cellular and organism level can be used as potent early warning indices of endocrine-disrupting chemicals in marine ecosystems: changes in the vas deferens sequence (VDS) and the relative size of the female and male penises (RPS). The regulatory restrictions for the use of antifouling paints containing TBT gave positive results and since then the recovery of dogwhelk populations has been documented in many areas along European shores; nevertheless, the toxic effects of TBT appear to persist to some extent in open waters.

Biomarker Concept in Invertebrate Ecotoxicology

Invertebrates are an advantageous group for the use of biomarkers for *in situ* measurements. In a general sense, biomarkers are understood as various biochemical or physiological parameters of an organism (here an invertebrate) which can be used to demonstrate the exposure to environmental chemicals or to detect toxic effects. Biomarkers offer a quick and sensitive detection of chemical stress within the organisms and might indicate health status at the organism level of the key invertebrate species from both terrestrial and aquatic ecosystems.

When effects of pollutants are seen at the community or ecosystem levels, it may be too late to start reclamation activities, which become inefficient and extremely costly. That is why invertebrates have been effectively used as sources of biomarkers. They can be extrapolated to actual or potential changes at the population level, and considered as predictive tools to assess changes and consequences on community and ecosystem levels. Only some biomarkers identified in invertebrates are highly specific and sensitive, the majority are less specific to chemicals and then indicate the exposure or toxic effects of their mixture. In invertebrates biomarkers can be used as the diagnostic tools of their health or would give the basis for predicting the fate of stressed environment and to start remediation activities.

The use of invertebrate biomarkers as tools in assessment procedures is subject to many problems and restrictions. One of them is the lack of knowledge about normal ranges of measured parameters in a given species. Dose–response curves are known for only selected chemicals and the effects of their interactions are usually treated mechanistically. Our knowledge of how various factors – abiotic (season, temperature, pH, salinity, insulation, humidity, type of soil, or sediment), biological (developmental stage, age, sex, physiological state, reproductive state, molting, adaptation) or ecological (competition, abundance, food availability, habitat) – may influence the response of the target species is insufficient. Generally with increased hierarchical level of our study the magnitude of possible interactions increases with increased complexity and concomitantly increased unpredictability. That is why this approach is also inadequate and needs further study. Tolerance of invertebrates to various pollutants can be higher than that of vertebrates due to generally lower energy requirements, the possibility of diapausing or a good survival without food (in utmost cases encysting). In the majority of invertebrate species, a short generation time gives higher probability of genetically based adaptations.

Antioxidant systems as biomarkers of disturbances were studied by using many key species of freshwater, marine, and terrestrial ecosystems. The quality of benthic zone is validated with mussels, mostly bivalves, which are abundant, sedentary, reproduce in the same area, and give a possibility by using various life stages in long-term monitoring studies. Biomarkers demonstrating the antioxidative capacity in *Unio tumidus* were used for the survey of different aquatic ecosystems of the Mediterranean Sea along the coasts of Italy, France, and Spain. End points were activity of glutathione-related antioxidative enzymes and lipid peroxidation. Disturbances should

be measured by using a set of biomarkers, not a single one. These measures can be used in an integrated approach with population and community studies of a given ecosystem. All collected data are necessary to make the diagnosis of ecosystem quality and predict scenarios for the future.

In invertebrates biomarker responses can also be linked with responses at higher levels of organization. Impairments caused by pollutants in aquatic ecosystems could be predicted studying freshwater crustaceans (*Gammarus pulex*, *Asellus aquaticus*, *Cambarus robustus*) as biomonitors. Casual links of the chemical stress responses can be established between various levels of biological organization. For example, reduced energy allocation to growth (scope for growth) in chemically stressed populations correlates well with the responses at the population level (a decrease in brood viability, reduction in offspring size).

Invertebrates in Ecotoxicity Assessment at the Population and Community Level

Population parameters cannot be generally more sensitive to chemical stress than individual parameters. Demographic approaches are practically absent in present risk assessment methodologies which are based generally on the end points for toxicity testing. Demographic methods can be studied using short-lived invertebrates existing in stressing conditions for their entire life. There is a series of potential endpoints to be used for measurements in organisms experimentally exposed to a series of concentrations of a given chemical or a mixture of chemicals. They should be egg-laying, hatching, growth, diapause, molting, rates of development, reproduction, or survival. For all these end points sensitivity to chemicals can be different. It would be perfect to use an integrative approach in which all life-history traits are integrated. Demographic techniques are applied in ecotoxicology to a variety of invertebrates species, mostly short-living freshwater species. A common base which allows comparisons between many species with different life-history traits is r – intrinsic rate of increase. Two important demographic processes determine population dynamics: natality and lethality.

Another convenient parameter in ecotoxicology is net reproduction index (R_0) which indicates how many individuals (generally females) in the next generation (N_{T+1}) can replace the parental generation (N_T): $R_0 = N_{T+1}/N_T$. The development of a population in a real time (at time intervals when important changes could happen – change in the age group or life stage, molting, maturity, production of offspring, death and so on) can be calculated on the basis of appropriate matrix equations where a columnar vector represents the age distribution at time t I multiplied by a matrix L in order to get age distribution at time $T + 1$. Matrix population model allows calculations of the eigenvalue of the projection matrix λ, a factor often called 'finite rate of increase' which shows whether month by month or year by year a given population decreases ($\lambda < 1$), increases ($\lambda > 1$) or remains stable ($\lambda = 1$). Such approaches in ecotoxicology can be found in relation to some soil-dwelling species, gastropods, chilopods, or insects exposed to metals or organophosphates.

Trade-offs are an essential part of the life-history theory. The effects of toxicants at the population level can be theoretically well predicted on the basis of the trade-off in the physiological allocation of energy resources which are not unlimited. In ecotoxicology this concept is used to recognize if there is any correlation between different life-history traits. There can be a compensatory reaction, as in example of metal-adapted isopods *P. scaber* where less energy is allocated to growth (individuals are smaller), more to reproduction. Other examples of trade-offs in invertebrates are negative correlations between the survival of juvenile forms and reproduction at the adult stage. Theoretically it should be clear that detoxification needs higher energy requirements. At the moment this type of trade-off is not well supported empirically and depends on the mode of action of a toxic substance.

Life-history traits are also applied to the analysis of sensitivity distribution and comparisons between populations exposed to similar chemicals as stressing factors. A convenient factor, called sublethal sensitivity index (SSI), can be used to identify populations on the basis of their reproductive sensitivity in a wide range from which they can reproduce at concentrations of a given chemical even exceeding the LC_{50} value (low SSI) to this at which reproduction ceases at concentrations much lower than an appropriate LC_{50} value (high SSI). Sensitivity analysis changed the opinion that effects of chemicals at the population level are determined by the effects on the most sensitive life-history traits, which are generally early stages of development. In these type of studies an important contribution came both from the studies of small invertebrates like population of soil nematodes and avian populations.

The main focus of the ecological risk assessment is to minimize undesired events caused by chemicals. Species sensitivity distribution (SSD) is an example of an ecotoxicological method which is based on such events at above the no-effect level/concentration. We can assume that within a community species differ in

sensitivity to various substances, and a distribution of sensitivity can be expressed by a parametric equation. If we know parameters of no-effect concentration distribution for the community, testing all species for no-effect concentration is not necessary. In practice SSDs have been positively accepted as indicators of scenario analysis of risk to concentration of toxic substances and, vice versa, leading to the protection of sensitive species.

Invertebrates as Tools in Genetic Ecotoxicity Studies

Genetic ecotoxicology focuses on studying alterations in genetic material in the biotic compartments of ecosystems. Ecotoxicology is interested in several consequences of exposure to genotoxic substances such as gamete losses, developmental abnormalities, neoplasia, lethal mutations, or changes in genetic diversity which affect Darwinian fitness (alterations in the growth rates, reproductive output, and viability of offspring). Genotoxic effects which occur in germ cells are transferred to consecutive generations resulting in alterations of gene expression in natural invertebrate populations in a way similar to that observed in vertebrates. Many different highly advanced techniques have been applied to study exposure, and sometimes effects, caused by genotoxic chemicals in invertebrates. The most common assays for screening chemicals for their DNA-damaging properties are DNA fingerprints, differential display mRNA, DNA alkaline unwinding, DNA strand breaks, DNA adducts, comet assay, micronuclei tests, c-K-ras oncogenes, and many others described in textbooks on genetics.

The evidence that chemicals induce neoplasia in invertebrates is scarce and has been demonstrated in mollusks (clams, snails, mussels) and planarians exposed to polychlorinated biphenyls and polycyclic aromatic hydrocarbons. Higher mortality of individuals with tumors is common as they could be easily hunted and are more sensitive to various pathogens.

Ecotoxicological genetics of invertebrates at the population level allows the demonstration of general relations between genetic variability and environmental pollution. The effects of mutagenic agents on DNA are various somatic disorders which might indirectly cause reproductive disorders resulting in increased mortality. Genetic diversity is gradually or rapidly lost (effects of so-called genetic bottleneck) and thus direct effects of toxic chemicals can result in extinction of the population.

In conclusion, studies of ecotoxicology of invertebrates which play a key role in different ecosystems allow better understanding of chemical–biological interactions in various stress conditions. In many cases they can substitute vertebrates in ecotoxicity testing of chemicals. They are more appropriate for integrated *in situ* testing, using the end points which cannot be used with vertebrates, thus their role in the assessment procedures for risk assessment should increase. In ecotoxicology studies we should accept that a high degree of internal complexity is an inherent property. Studies on invertebrates increase the possibility of parallel analysis of many variables, thus increasing the accuracy of our evaluations whether a community operates within normal range or stress can be recognized and defined.

See also: Biomarkers, Environmental; Ecotoxicology; Ecotoxicology, Aquatic; Ecotoxicology, Genetic; Environmental Hormone Disruptors.

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Ecotoxicology, Terrestrial

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Terrestrial ecotoxicology is the study of how environmental pollutants affect land-dependent organisms and their environment. It includes the subdisciplines of: wildlife toxicology (how poisons affect birds, mammals, reptiles, and amphibians), plant toxicology, invertebrate toxicology, soil invertebrate toxicology, soil microbiology (how poisons affect soil microbial functions), biogeochemistry (how pollutants move through air and soil, are biodegraded by soil organisms, or are taken up by plants), and ecology (how plants and animals interact with each other as well as with abiotic (nonliving) parts of the ecosystem).

An ecotoxicological process requires three primary elements: (1) a source (what is the pollutant and where is it coming from?), (2) a receptor (which organism is likely to be affected by the pollutant?), and (3) an exposure pathway (how does the pollutant get from the source to the receptor?). The exposure pathway element also involves quantifying how much pollutant (if any) reaches a receptor. If one of these elements is missing, it is unlikely that a pollutant will be able to affect an organism. If all three are present, an ecotoxicologist can assess the potential for adverse responses in individuals, populations, or communities.

Pollutants and Sources

All manner of pollutants are considered under the discipline of terrestrial ecotoxicology including pesticides, persistent organic pollutants, other organic substances, and metals and metalloids (e.g., selenium and arsenic). Naturally occurring toxins such as those produced by poisonous plants, snakes, or invertebrates generally are not included unless people intentionally apply them for pest control. For example, the pyrethroid pesticides are derived from the naturally occurring pyrethrin toxin that is found in chrysanthemum.

Pollutants may enter the terrestrial environment through direct application, as is the case with pesticides, fertilizers, or biosolids (sewage sludge). Improperly managed landfills and waste sites can contribute mixtures of pollutants to soil systems, through surface runoff of leachates or blowing dusts. Local areas may be contaminated by wet or dry deposition of air pollutants emitted from industrial processes or through land composting or disposal of industrial waste products. Long-range transport of volatile substances can result in contamination of soils or foliage many miles distant from the source, while automobile exhaust and other particulate emissions are generally deposited locally. Floodwaters and other sporadic or episodic surface runoff may leave behind pollutants that settle onto or bind with soils.

Receptors

Typical terrestrial receptors include soil microbes, invertebrates (e.g., beetles) including soil-dwelling invertebrates (e.g., insect larvae, worms, nematodes), plants, amphibians, reptiles, birds, and mammals. Although not shown in **Figure 1**, terrestrial organisms that depend upon aquatic ecosystems for some or all of their food and habitat, such as fish-eating (piscivorous) birds (e.g., cormorants, osprey, eagles) and mammals (e.g., mink, otter) are also potential receptors. Domestic animals such as livestock and pets, or agricultural crops, generally are not considered as receptors, although information about the toxic effects of chemicals in these species may be used in the absence of data about wild organisms.

Exposure Routes and Pathways

Terrestrial organisms can be exposed to pollutants in soil through one or more of six primary pathways (**Figure 1**): (1) direct (dermal) contact with soil, (2) ingestion of the soil itself incidental to grooming or consumption of soil-dwelling organisms, (3) inhalation of pollutants released from soil as vapors, (4) ingestion of plants, (5) ingestion of soil-dwelling organisms, or (6) ingestion of other organisms that have taken up pollutants from the soil. If pollutants in soil are leached or eroded into water sources, consumption of polluted drinking water can become an exposure pathway for terrestrial organisms. Understanding the relative importance of these exposure pathways is necessary both from a risk mitigation standpoint (to identify the exposure pathway(s) that need to be severed) and for an understanding of potential toxic effects. For example, inhalation exposure is more likely to result in pulmonary (lung) damage, while dietary ingestion will produce effects to the liver and general systemic toxicity.

Direct (dermal) exposure can be a significant exposure pathway for humans and for other terrestrial organisms. While feathers, fur, or scales protect the skin of nonhuman receptors, many substances are

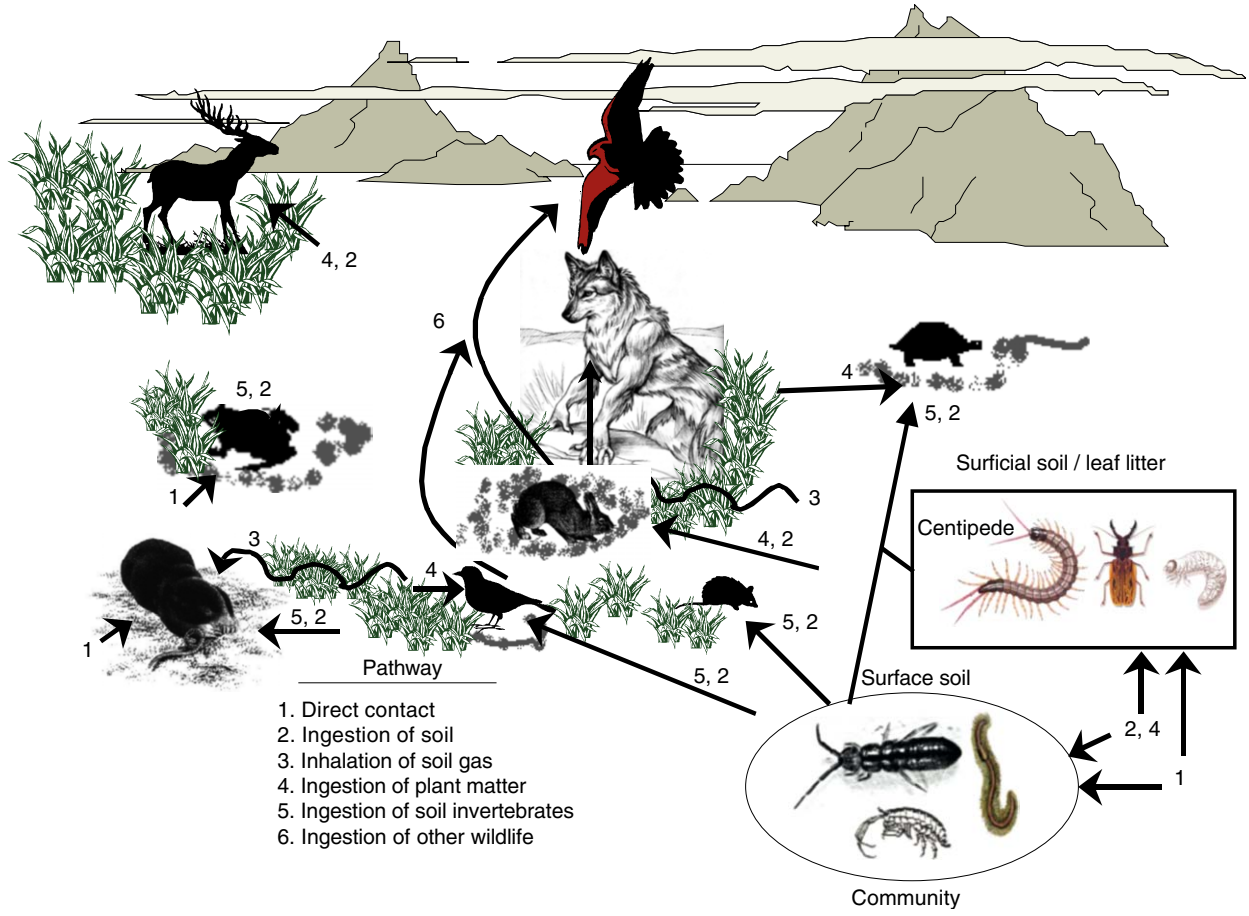

Figure 1 Exposure pathways and receptors in a typical terrestrial food web. (Reprinted with permission from Lanno R (ed.) (2003) Contaminated soils. In: *Soil–Chemical Interactions to Ecosystem Management*, 400pp. Pensacola, FL: Society of Environmental Toxicology and Chemistry; © SETAC, Pensacola, FL, USA.)

able to penetrate to the skin and be absorbed. Additionally, penetration of eggshells of birds or reptiles by pollutants, particularly petroleum oils and certain polycyclic aromatic hydrocarbons, can be a significant exposure pathway.

Plants, soft-bodied soil-dwelling organisms (e.g., earthworms), and soil microbes can be exposed through passive diffusion, and, occasionally active uptake, to pollutants that have moved into the water that fills the spaces between the soil particles (known as 'soil porewater'). Polluted soil particles can be 'splashed' on plants by rain and those that become airborne can be deposited on plants as dust. Such deposition or splashing of soil particles generally is not a significant exposure pathway for plants, as their leaves are relatively impervious to particulates. However, receptors consuming plants may be exposed to polluted particles adhering to the outside of the plant. Highly volatile substances that are released from soil as gas or are brought into an area through the air can be 'inhaled' (absorbed) by plants, resulting in significant toxic effects (ozone is an example of such a pollutant).

Hard-bodied terrestrial invertebrates (e.g., beetles, centipedes) can be exposed through incidental ingestion of pollutant-containing soil particles or through ingestion of food items (e.g., leaf litter or other soil organisms) that have taken up pollutants from soil.

Small mammals (e.g., moles, mice, and voles) and certain bird species that live or nest in subsurface burrows may inhale volatile pollutants that are evaporated out of the soil as a vapor. This pathway is likely to be significant only in those cases where poor ventilation allows vapors to collect and concentrate and where the receptor spends a lot of time (e.g., when nesting) in such a poorly ventilated space. There is, however, little information available about how to quantify this exposure pathway. Small mammals are more likely to be significantly exposed to pollutants that have been taken up in their food items (e.g., plants, soil invertebrates) or through incidental ingestion of polluted soil particles.

Larger avian and mammalian receptors may be directly exposed to soil-related pollutants via incidental ingestion of soil and indirectly exposed through ingestion of contaminated food items (e.g., plants, soil invertebrates, or other animals). The dietary pathway is 'indirect', in that the larger receptor is exposed to soil-related pollutant brought to it in its food through the food web and does not need to be in the vicinity of the pollutant source. In fact, if the contaminated food item is capable of traveling, a larger receptor could be exposed quite some distance from the source.

A terrestrial food web (e.g., **Figure 1**) is a simplified representation of the complex interactions of below-ground processes and above-ground plants and animals. It is used to illustrate the trophic level and predator–prey relationships among selected terrestrial receptors and their potential food items. Terrestrial ecotoxicology uses information about the structure of a local food web to make predictions about how pollutants may move from soil and into plants or animals at various trophic levels, including those possibly some distance from the source of the pollutant.

Pollutants adhering to soil particles can enter a food web in three primary ways: (1) along with nutrients (nitrogen, phosphorus) and essential elements (e.g., zinc, copper, manganese), they can move from soil particles into the soil porewater; they can then be taken up from the porewater by plant roots through active or passive mechanisms and distributed throughout the plant, (2) from porewater into soil-dwelling receptors (e.g., earthworms) through transdermal osmosis, and (3) by being stripped from soil or organic particles as these are digested by soil-dwelling organisms. However, not all of a pollutant that can be measured in soil using chemical analysis methods is necessarily biologically available (bioavailable) to a receptor. For example, only about 3% of the mercury in mine tailings can be extracted with a biologically relevant mild acid (i.e., synthetic stomach acid), whereas over 90% can be recovered if concentrated nitric acid is used ('total recoverable' method). Bioavailability is reduced when the pollutant tightly adheres to soil particles, and is a function of soil pH, amount of organic carbon in the soil, relative amount of clay versus sand, and several other similar factors. The length of time the material is present in the soil can also influence bioavailability, as many chemicals can become more tightly bound with time.

Above- and below-ground plant parts, along with soil invertebrates, are the base (lowest trophic levels) of the terrestrial food web. Receptors at an intermediate trophic level can be exposed to pollutants that have moved from the soil to their food items. Examples would include foliage-feeding invertebrates, invertebrate-eating invertebrates, and birds, mammals, and other animals that feed on plants and invertebrates (e.g., amphibians and reptiles). Pollutants can reach predators at the top of the food web, such as wolves and hawks, when they feed on lower and intermediate trophic level receptors that have taken up pollutants.

Two important processes, which are different but related, govern the movement of a pollutant into and through a terrestrial food web: bioaccumulation and biomagnification. The movement of pollutants from the soil into a plant or animal at a given trophic level of a terrestrial food web is called 'bioaccumulation'. The concentration of a pollutant capable of bioaccumulation will be higher in a plant or animal than in the soil. The concentration of some pollutants increases as it is passed from one trophic level up to a higher level through the food chain. Such pollutants are said to 'biomagnify' and the process is known as 'biomagnification'. As a general rule, chemicals that biomagnify are persistent in the environment for long time periods (months to years) and have a high affinity for lipids (fats). Examples are the organochlorine pesticides such as lindane or DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane); chlorinated hydrocarbons used as fire retardants such as polychlorinated biphenyls (PCBs), or industrial pollutants such as polychlorinated dioxins. An exception is the biomagnifying organometallic compound methylmercury, which has a high affinity for tissue macromolecules and not lipids. Chemicals that degrade very quickly (e.g., many pesticides) or are soluble in water (e.g., metals) will be removed from the soil and not stored in organisms for long periods. Chemicals that biomagnify can pose a high risk to top predators (those at the highest trophic levels) in terrestrial food webs, as a very small amount in soil can magnify into a substantial, and potentially toxic, concentration by the time it reaches the top of the food chain. An excellent example of the consequences of biomagnification is the use of the insecticide DDT prior to its being banned for use in the United States in 1972. DDT is very effective in controlling mosquitoes, as well as agricultural insect pests, and was applied very widely for these and other purposes. It has relatively low mammalian toxicity and is not acutely toxic to birds. However, because DDT is very persistent (it has a half-life of $\sim$30 years; 'half-life' being the time it takes for one-half of the chemical to turn into something else) and is readily stored in lipids within plants and animals, the small amounts applied to the environment biomagnified in the terrestrial food chain to levels that caused eggshell

thinning in birds such as robins, peregrine falcons, and pelicans. Many species of hawks, eagles, and owls were affected to the point where their populations showed significant declines. Since its use was banned, the amount of DDT in the environment has gradually declined, eggshell thickness has returned to normal, and many of the affected bird populations have recovered.

Responses in Individuals (Toxicity)

Toxic responses of plants, soil microbes and invertebrates, and wildlife can be assessed following standard laboratory toxicity test protocols developed by the US Environmental Protection Agency (USEPA), the American Society of Testing and Materials (ASTM), and several international organizations including the Organization for Economic Cooperation and Development (OECD), and the International Organization for Standardization (ISO). There are no standard methods available for toxicity tests with reptiles. For amphibians, tests have been standardized for exposure to the aquatic life phase (eggs and tadpoles), but not for the terrestrial adults. Typically, these tests look at acute mortality following a single high-concentration application to soil or food, or at effects on growth or reproduction when exposed to lower amounts over long time periods. Soil microbial tests look at the functions that are provided by soil microorganisms, rather than effects on the microbes themselves. These include the ability to fix atmospheric nitrogen in the soil (nitrification) and organic decomposition. Because soil microorganisms release carbon dioxide (CO_2) as an end product of their metabolism in a manner similar to higher animals, 'soil respiration' or the amount of CO_2 that comes up out of the soil is another commonly measured function. However, all of these functions are highly variable and depend a great deal on soil conditions (e.g., temperature, moisture) and so must be interpreted with caution.

When conducting laboratory toxicity tests, it can be difficult to incorporate some chemicals into the soil or animal feed, particularly if the chemicals are very insoluble. Furthermore, raising test organisms in the laboratory and conducting studies for sufficient time periods to encompass the reproductive cycle of a plant or animal can be prohibitive (consider, e.g., a life-cycle test on a long-lived tree species). For this reason, as well as for other ethical concerns, most species are not tested directly, but rather a standard set of test organisms is used from which extrapolations are made to other species. It must be recognized that such interspecific extrapolations are highly uncertain, and large safety factors frequently are applied as a result. The emerging science of 'toxicogenomics' may provide a better understanding of the response of genes to pollutant exposures and allow for more accurate interspecific extrapolations. Currently, information derived from laboratory rodents for use in human health toxicity evaluations can also be applied to mammalian wildlife, as well as studies conducted with domestic livestock. Mallard ducks and quail generally represent birds, although chicken or turkey studies conducted for agricultural purposes can also be used. The earthworm is a well-studied representative of soft-bodied soil invertebrates, and the springtail or potworm are used as hard-bodied invertebrates. The African clawed frog and the leopard frog (native to North America) are used in amphibian studies. Plants generally have been represented by domestic species, including lettuce, rapeseed, and cucumber although the use of wild plants is becoming more common.

While laboratory studies are useful for studying toxicological responses of organisms to individual chemicals under controlled conditions, they frequently are not predictive of what occurs in natural environments. Organisms may be exposed to multiple chemicals simultaneously, and are always under stress from environmental conditions (e.g., too much or too little water, heat and cold stress, predator avoidance, etc.). Therefore, bioassays may be conducted in the field to look at potential effects under natural conditions. For plants or soil invertebrates, this might be as simple as collecting soil from a contaminated location, putting it into pots, and adding the standard test organism or unexposed specimens of the same species found at the site and then following the standard protocol. Alternatively, organisms can be observed directly in the field to look for toxic responses. These can be as simple as observing dead plants or animals in a location with high contamination, changes in the way plants look such as leaf color (chlorosis) or spotting, or through health evaluations of animals. 'Biomarkers' also may be studied, which are measurable changes in animal physiology that result from exposure to pollutants. Examples of useful field biomarkers are changes in enzymes associated by hemoglobin synthesis as a result of exposure to lead or reduced activity of the cholinesterase enzyme following exposure to certain classes of pesticides. These indicate both an effect (enzyme change) and that exposure occurred. Other biomarkers such as induction of enzymes in the liver that degrade toxins (e.g., the family of cytochrome P450 enzymes) are only indicators of exposure and not of effects. New methods are emerging from the science of toxicogenomics that may make it possible to measure biological responses to very low levels of

pollutants in the environment, although with a few exceptions the relationship between changes in gene expression and whole animal toxicological response is not yet clear.

For some chemicals, the amount of pollutant present in plant or animal tissues can be used to predict whether or not they will be affected. An organism can tolerate a certain amount of a chemical in its body above which level it is likely that adverse effects will occur. This is known as the 'critical body residue', although it sometimes is specific to certain tissues (e.g., liver or kidney). For example, it is known that a level of $6\,\mu g\,g^{-1}$ wet weight of lead in the liver of a duck will result in toxicity, and at $15\,\mu g\,g^{-1}$ wet weight it is highly likely that the duck will die. For other chemicals, the critical body residue is not known, and the presence of chemicals in the tissues can only be used as an indication that exposure has occurred.

Responses in Populations and Communities

Environmental pollutants primarily cause effects in individual organisms. Assessing effects at the level of the individual is appropriate (and may be legally required) for: species whose numbers are so low that they are protected by special laws (e.g., US Endangered Species Act), birds protected under international treaty (e.g., the Migratory Bird Treaty Act of North America), or other animals afforded special protection (e.g., the US Bald Eagle Act). These special instances aside, terrestrial ecotoxicologists generally are more interested in the sustainability of whole populations or in changes in community biodiversity. Population models can be used to determine whether alterations in reproductive rates, mortality rates, or length of time needed to mature resulting from exposure to environmental pollutants will significantly alter the ability of a population to sustain itself over time. Depending upon the life history of a species, effects on reproduction may outweigh direct toxic (lethal) effects, or conversely may have a negligible impact on population dynamics.

Sometimes a plant or animal species of particular interest is not affected directly by a pollutant, but indirectly as, for example, the pollutant reduces either its prey resource or its predators. Either may result in a change in the community composition of the species. In addition, changes in plant communities may result in different wildlife species using a particular area, resulting in the loss of some species and increases in others. Community diversity surveys can be performed for plants and, to a lesser extent, for soil invertebrates to assess such changes. Because wildlife obtain food and shelter from plant communities, changes in how these communities are distributed across the landscape can also significantly affect the spatial distribution of wildlife. Alterations in the distribution patterns of a wildlife species can change the probability of both its exposure to pollutants and the emergence of any subsequent population-level effects. Some areas are highly conducive to production of wildlife species, whereas others do not have the right combination of habitat requirements and cannot support significant reproductive output of wildlife species. Contamination of a high-production area can result in serious consequences for a population as a whole, whereas contamination of an area that supports little or no reproduction may only expedite the demise of an otherwise nonsustainable local population.

For some environmental pollutants there can be a range of tolerances among individuals within a species such that some respond significantly less than others. Over time, those that are not significantly affected by exposures will have the greatest ecological fitness and so produce more offspring that also have the ability to cope with such exposure. This is termed 'adaptation' of the species to the pollutant, or development of tolerance or resistance. The best examples are insects that develop resistance to pesticides as a result of repeated exposures, necessitating the development of new chemicals to which they have no experience. Additionally, pollution-induced community tolerance (known as 'PICT') has been demonstrated in soil microbial communities, where the types of species may shift to accommodate the soil pollutant but overall functioning of the microbial community remains unaffected (e.g., soil respiration or nitrification continue). Plants and animals can also develop tolerance over time and adapt to some environmental pollutants. In some instances, plants have been observed to recognize pockets of pollutants in soils and avoid exposure by growing their roots around such areas. Some plants can take up large amounts of pollutants without being affected ('hyperaccumulators'; e.g., selenium uptake by locoweed), and have been used as a bioremediation method at contaminated waste sites and mining (tailings) sites.

Risk Assessment

Terrestrial ecological risk assessments are conducted following the standard framework and guidance published by the US EPA and various states. All but the most basic ecological risk assessments require a team of specialists to gather, analyze, and interpret information about fate and transport of pollutants

through the soil system, uptake by plants and soil organisms, and both direct and indirect effects to the organisms of concern. The ecotoxicological information discussed above can be assembled into a risk assessment by conducting toxicity tests or field assessments of effects and estimating expected exposure levels. Exposure and effects data are then combined to estimate the likelihood of adverse effects to individual organisms. Such an estimate can be used to directly assess toxicological risks, but assessing ecological consequences at the population or community level generally requires the use of predictive models.

See also: Ecotoxicology; Ecotoxicology, Avian; Ecotoxicology, Invertebrate; Ecotoxicology, Wildlife; Risk Assessment, Ecological.

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Ecotoxicology, Wildlife

Richard S Halbrook

Wildlife toxicology is a subdiscipline of environmental toxicology and evolved during the mid-twentieth century in response to concerns regarding wildlife exposure to pollutants. The term 'wildlife' generally refers to any nondomesticated species, but often refers to animals rather than plants, and sometimes is further defined as terrestrial vertebrates (mammals, birds, amphibians, and reptiles), separating them from fish and invertebrate species. However, these categories are only a matter of semantics because all of these groups are interconnected within complex trophic groups in the environment. Due to this interconnectedness, Wildlife toxicologists must often integrate accumulation and effects of contaminants among all of these groups.

In studying the impacts of pollutants on wildlife, the wildlife toxicologist evaluates exposure and effects, and determines the mechanisms involved in the assimilation, distribution, and influence chemicals have on specific cells or biological processes that effect the individual, population, or community of organisms living in the area where a pollutant occurs. In addition to evaluating the effects of pollutants on wild species, wildlife toxicologist also have used wild species as sentinels to warn of potential adverse accumulation and distribution of chemicals in the environment. Miners taking canaries into the mines to warn of poisonous gases is a classic example of the use of wildlife as toxicological sentinels. Wildlife also serve in determining the bioavailability of environmental contaminants (i.e., is the chemical able to be absorbed into the body of an organism or is it in a particular form or bound so that it is not absorbed into the body). Because wild species are closely associated with natural environments, they integrate chemicals in their environments over time and space, thus providing a biological system for evaluating bioavailability, accumulation, and effects of single or multiple chemicals.

Effects on Wildlife

The effects of pollutants on wildlife may be either direct or indirect, and these effects are influenced by the mode of action of the specific chemical. Direct effects are those that result in death or biological damage to an exposed individual. For instance, exposure of an organism to a certain chemical may not result in direct mortality, but may affect an animals motor responses and therefore its ability to capture prey or avoid predators; which ultimately

may result in the death of the individual. Direct effects also may include impaired growth or reproduction that results in declining populations. For example, symptoms in wildlife exposed to chlorinated hydrocarbons (instance e.g., dichlorodiphenyltrichloroethane (DDT)) tend to be nonspecific and include nervousness, hyperexcitability, weakness, lack of appetite, and tremors. Several laboratory studies conducted during the 1940s indicated that acute (short term) exposure to some chlorinated hydrocarbon pesticides would not cause direct mortality of wildlife; however, they may cause weakness and loss of appetite that may reduce the general health of the effected species. This in turn leads to increased susceptibility to disease or predation. It was later discovered that exposure of birds of prey to DDT, or its metabolites, resulted in thinning of egg shells, which in turn resulted in population reductions due to breaking of the eggs and death of the developing embryos.

Indirect effects would be those that have an adverse influence even though the individual may not have been directly affected by exposure to a chemical. Elimination of insects, a major food source of many mammalian and avian species, or loss of habitat (cover) due to weed control, may influence the health and welfare of wild species. As insect populations are reduced due to insecticide spraying, wildlife are unable to obtain their nutrient requirements and become malnourished. The outcome may be similar to that observed from direct effects. As the animals general health diminishes because of lack of food, it loses its ability to avoid predation, or to capture prey, or it becomes more susceptible to disease and infection. Similarly, the loss of habitat can reduce an animal's ability to escape predation, or it may reduce breeding and rearing areas, which also may result in population declines in affected species.

History

During the late 1800s and early 1900s, there was a tremendous expansion in the development and use of chemicals in industry and agriculture. At that time, it was a common practice to discharge industrial waste into rivers and streams, or dispose of them in landfills, and it was common for repeated widespread application of pesticides to agricultural land. There were few laws or regulations governing industrial or agricultural chemicals, and potential effects of these chemicals on wildlife were unknown. From 1900 through the early 1930s lead arsenic, lime-sulfur, and nicotine sulfate compounds were a few of the insecticides used in agriculture. Some of the agricultural publications during this time period reported potential adverse effects of insecticides on beneficial insects, such as honey bees, and expressed some concern about effects on other wildlife. However, there were very few studies conducted during this time period that evaluated impacts of pesticides on wildlife.

During the 1930s and the 1940s, several important chemical discoveries sparked a major shift in the types and quantities of chemicals used as agricultural pesticides. The discovery of the insecticidal properties of DDT in 1938 was paramount in these discoveries. Because of its low acute toxicity to mammalian species, perceived environmental safety, and effectiveness, DDT was considered to be an ideal chemical for widespread use in the environment and was widely used as an agricultural and public health insecticide. However, DDT, as well as other chlorinated hydrocarbon insecticides, do not easily degrade and therefore accumulate in the environment over time. In addition, repeated application of DDT resulted in the evolution of more resistant insects, which in turn resulted in the application of more DDT at higher concentrations. Results of laboratory and field studies of potential adverse effects of DDT on wildlife began to appear in the mid-1940s, and some of the first reports of mass mortality among wild species occurred during the mid-1950s. These included deaths of numerous waterfowl species utilizing lakes sprayed with DDT, or one of its daughter compounds (DDD or DDE), to control gnats.

Similar to the expansion in use of agricultural chemicals during the first half of the twentieth century, there was a tremendous industrial expansion occurring in North America and many new chemicals were being developed for industrial use. One of these chemicals was polychlorinated biphenyls (PCBs), another chlorinated hydrocarbon. PCBs were first synthesized in the late 1800s, and because of their chemical properties, were used in various industries, including the electrical industry. As with DDT, adverse environmental effects of PCBs were unknown in the early 1900s, and environmental spilling and landfill disposal of used PCBs were common. During the 1920s and the 1930s, several reports of adverse effects of PCBs to humans were published; however, no field studies of adverse effects of PCBs or other industrial chemicals to wildlife are known to have been conducted. It was not until the 1960s when mink farmers in the Great Lakes Region reported reproductive failure in mink, that the widespread environmental distribution and adverse effects of PCBs on wildlife became known. It was soon discovered that fish from the Great Lakes contained elevated PCB concentrations that caused the reproductive failure observed in mink.

Reports of adverse effects in wildlife from exposure to agricultural pesticides, like DDT, and from exposure to industrial chemicals, like PCBs, that emerged during the 1950s and the 1960s, along with publications like *Silent Spring* by Rachel Carlson in 1963, lead to the increased awareness of potential impacts of chemicals on wild species. This awareness, and the studies that it generated, resulted in the offshoot of a group of toxicologists specializing in the effects of pollutants on wildlife and the birth of wildlife toxicology.

Major Contaminants of Concern

Although any chemical can be toxic to wildlife if exposure occurs at a high enough concentration, those that are used or released in the environment are of the greatest concern. Those that have been associated with adverse effects in wildlife include: the persistent organic pollutants (POPs), cholinesterase inhibiting insecticides, and some metals. Another group of chemicals, the pharmaceuticals, also are becoming a major concern to wildlife.

Persistent Organic Pollutants

POPs are carbon-containing chemical compounds that, to a varying degree, resist photochemical, biological, and chemical degradation. Because of their persistence and high lipid solubility, POPs tend to bioaccumulate in fatty tissues. They also are semivolatile and therefore can vaporize or absorb onto atmospheric particles. This permits the global transport of these chemicals in air and water. The insecticide DDT is a classic example of a POP, and the effectiveness of DDT as an insecticide lead to the synthesis of other chlorinated hydrocarbon insecticides (other POPs) shortly after WWII. These included insecticides such as aldrin, dieldrin, chlordane, and toxaphene. In addition to insecticides, other POP pesticides were developed during the 1940s and were distributed widely in the environment; for example, hexachlorobenzene, a fungicide used on seed grains. Even though use of many of these chlorinated hydrocarbon insecticides have been banned or are strictly regulated, they are often detected in tissues of wild species.

PCBs are another well known POP that has accumulated in the environment with reported adverse effects in wildlife. Previous discharge of PCBs into streams, rivers, and lakes, disposal in landfills, and atmospheric discharge has resulted in widespread environmental distribution of PCBs. The manufacturing of PCBs also may result in the production of by-products, such as dioxins and furans, which also can be highly toxic to wildlife. Even with modern day restrictions on use, PCBs persist in the environment and are still frequently detected in tissues of wild species.

Persistent organic pollutants may persist in the environment for many years, and because they are lipid soluble, can accumulate in greater concentrations (biomagnify) in species feeding higher up the food chain. Wildlife studies conducted since the 1950s have indicated that POPs can disrupt an organism's endocrine system and are often referred to as endocrine disruptors. These studies have reported population declines, reproductive impairment, eggshell thinning, metabolic and behavioral changes, influences on sex determination, and embryonic deformities in a variety of wild species including eagles, cormorants, alligators, and mink, exposed to POPs.

Cholinesterase Inhibiting Compounds

During the 1950s, two other major groups of chemicals were developed for use as insecticides, the organophosphates (OPs) and carbamates. Although some OPs and carbamates may be more acutely toxic than the organochlorine chemicals that preceded them, they are considered a more ecologically acceptable group of chemicals because, in general, they are not as long lived in the environment, and therefore are less likely to accumulate in the environment.

Unlike the nonspecific effects and uncommon occurrence of direct mortality observed in wildlife exposed to chlorinated hydrocarbon pesticides, several studies have documented direct mortality from exposure to OP and carbamate insecticides. The method by which the OPs and carbamate insecticides affect wildlife is quite different from the method by which the chlorinated hydrocarbon insecticides effect wildlife. The OPs and carbamates inhibit cholinesterase, primarily acetylcholinesterase (AChE), which is an enzyme that functions in the breakdown of the neurotransmitter acetylcholine. Acetylcholine functions in the transmission of nerve impulses. Therefore, when AChE is inhibited by an OP or carbamate insecticide, it can no longer breakdown acetylcholine and there is continued transmission of nerve impulses that eventually leads to nerve and muscle exhaustion. The respiratory muscles are a critical muscle group that is affected, often leading to respiratory paralysis as the immediate cause of death. A major difference in the mode of action between OPs and carbamates is that the inhibition of AChE by OPs is, from a biological standpoint, irreversible, while the inhibition from exposure to carbamates is reversible in a biologically relevant time frame. There

are numerous reports in the literature documenting direct adverse effects of cholinesterase inhibiting insecticides, especially regarding effects in wild avian species. In addition to direct effects, indirect effects on wildlife resulting from decreased food availability (insects) are well known. As with all insecticides used in the environment, the timing and method of application greatly influences the occurrence of potential adverse effects.

Metals

Unlike the POPs, OPs, and carbamates, which are not naturally occurring, metals are elements that do occur at low concentrations in the environment. Mining and various industrial activities may have resulted in an increase in concentration of many metals to levels that are potentially toxic. Ignorance, mismanagement, and/or accidental release have resulted in the accumulation of metals in some environments, and metals such as mercury, lead, cadmium, and selenium, have been associated with adverse effects in wildlife. Pollution by metals is usually localized in the vicinity of the polluting source; however, some metals, such as mercury, have been transported in the atmosphere or in water resulting in pollution of areas distant from the source. Wildlife are often exposed through ingestion of metals in food or from incidental ingestion of metals in contaminated soil. Major concerns with regard to wildlife include: ingestion of mercury contaminated fish; ingestion of lead from ammunition associated with waterfowl hunting and skeet shooting, or from of lead sinkers used in fishing; exposure to selenium in agricultural drainage and in the vicinity of phosphate mining; and exposure to cadmium in the vicinity of battery and electroplating plants.

Pharmaceuticals

An emerging concern in wildlife toxicology is the potential adverse effects of pharmaceutical compounds. Every day, hundreds of different pharmaceuticals, including birth control chemicals, antidepressants, and antibiotics, are ingested by humans, and growth hormones and medications are used in the animal production industry. A portion of these compounds, or their by-products, are eliminated in feces and urine, and pass either by direct discharge or via sewage systems into streams and rivers. Small quantities of these pharmaceuticals from many sources result in the potential exposure of fish, amphibians, and other aquatic wildlife to a 'cocktail' of pharmaceuticals. The effects of this exposure is just beginning to be understood, but may include suppression of motor function, reduced or delayed growth, immunological suppression, or impaired reproduction.

Because of adverse effects observed in wildlife due to historic use of chemicals in the environment, and because of accidental spills and releases of various chemicals, laws and regulations have been developed in most countries for the regulation of chemical use. Today, the development of new chemicals intended for environmental use incorporate extensive testing procedures to evaluate potential impacts to wildlife. The intent is to avoid adverse effects to wildlife similar to those that have occurred in the past. Because of existing regulations, wildlife toxicologists today are not usually faced with high concentrations of a specific chemical in the environment, except possibly near a pollution source or as a result of a spill, but rather with exposure to low levels of multiple chemicals. Because wild species integrate contaminants over time and space, they provide a biological mechanism for evaluating the accumulation and effects of these contaminants. A major difficulty for the wildlife toxicologist is determining that an adverse effect has occurred and determining the severity of that effect. In evaluating effects in wildlife, the toxicologist must rely on environmental concentrations (exposure concentrations) or tissue concentrations that can be related to laboratory and field data associated with adverse effects, or upon morphological and physiological changes (biomarkers) that indicate that exposure has occurred and resulted in an adverse effect in the organism. This assessment of risk to wildlife will continue to be a challenge given the potential exposure of wild species to the large number of existing chemicals and to new chemicals that are continuing to be developed.

See also: Chemicals of Environmental Concern; Ecotoxicology; Environmental Toxicology; Polychlorinated Biphenyls (PCBs).

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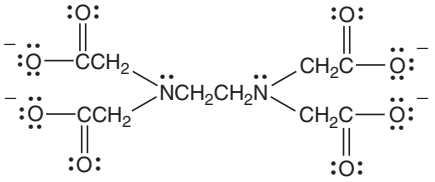
EDTA (Ethylenediaminetetraacetic Acid)

C Charles Barton and Harihara M Mehendale

- Chemical Name: Ethylenediaminetetraacetic acid
- Chemical Abstracts Service Registry Number: CAS 60-00-4
- Synonyms: Ethylenedinitrilotetraacetic acid; Celon A; Cheelox; Edetic acid; Nullapon B Acid; Trilon BW; Versene
- Chemical/Pharmaceutical/Other Class: Organic chelating agent
- Chemical Formula: $C_{10}H_{16}N_2O_8$
- Chemical Structure:



Uses

EDTA is used as a food additive, in herbicides, in pharmaceuticals, and in a variety of consumer products. EDTA is used as a blood preservative by complexing free calcium ion (which promotes blood clotting). EDTA's ability to bind to lead ions makes it useful as an antidote for lead poisoning. Furthermore, EDTA is often used to treat various cardiovascular diseases.

Background Information

EDTA is a white, odorless, crystalline (sugar or sandlike) material. It has a molecular weight of 292.28 and its melting point is 240°C. It is water insoluble.

Exposure Routes and Pathways

The most probable routes of human exposure to EDTA would be ingestion and dermal contact. Workers involved in the manufacture or use of EDTA may be exposed by inhalation and dermal contact. In chelation therapy, EDTA is administered via intravenous infusion.

Toxicokinetics

EDTA is essentially not metabolized by the human body and it is rapidly excreted in the urine. About 50% of EDTA administered intravenously is excreted within 1 h and 90% within 7 h. EDTA and its metal chelates do not permeate the cellular membrane to a significant extent; thus, most of the EDTA remains in the extracellular fluid until excreted in the urine.

Mechanism of Toxicity

The principle toxicity of EDTA relates to the metal chelate, especially in lead poisoning. Lead may be released from the chelate in the kidneys, and then the lead may affect the tubules and glomeruli of the kidneys.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, more live fetuses resulted when calcium disodium EDTA was used to treat lead poisoning. However, in rats that were not lead poisoned, increases in submucous clefts, cleft palate, syndactyly, adactyly, abnormal ribs, and abnormal vertebrae occurred. Furthermore, the doses of EDTA in the study were comparable to those used in man and without noticeable changes in the mother. Since zinc calcium EDTA did not cause teratogenicity at low levels in rats, zinc calcium EDTA should be available for use in pregnancy.

Human

Cases of anuria have been reported when EDTA was administered to treat lead poisoning. Such kidney injury is reversible and is probably not due to the chelate directly, but to the reabsorption of the metal in the tubules. Of 130 children that received dimercaprol and EDTA, 3% developed acute renal failure and 13% had biochemical evidence of nephrotoxicity. However, lead poisoning can cause kidney injury without EDTA therapy. In another study, 122 patients were given EDTA and none showed posttreatment increases in plasma creatinine.

Reversible mild increases in plasma hepatic aminotransferase activities are frequently reported after use of EDTA. Furthermore, extravasation may result in development of painful calcinosis at the injection site.

In a workplace setting, the following acute health effects may occur immediately or shortly after exposure to EDTA: contact may irritate the skin causing a rash or burning feeling; contact with high concentrations may irritate the eyes; and inhalation of EDTA dust may irritate the nose and throat.

Chronic Toxicity (or Exposure)

Animal

Laboratory studies on various animal species as well as reports from veterinary practices have revealed that long-term therapy with EDTA may cause deficiencies in zinc and vitamin B_6.

Human

Prolonged systemic therapy with EDTA has resulted in zinc and vitamin B_6 deficiencies. Furthermore, febrile reactions with headache, myalgia, nausea, vomiting, lachrymation, nasal lesions, glycosuria, hypotension, and electrocardiographic (ECG or EKG) changes have been reported.

In Vitro Toxicity Data

All known pharmacological effects of EDTA result from formation of chelates with divalent and trivalent metal ions in the body. Also, the effects on rat liver glucocorticoid receptor *in vitro* have been studied. At 4°C, 10 mmol EDTA had a stabilizing effect on unbound hepatic glucocorticoid receptors. Apparently, endogenous metal ions are involved in the processes of glucocorticoid–receptor complex stabilization and transformation. Furthermore, EDTA increases the absorption of a number of agents. This effect is nonspecific because EDTA increases the absorption of bases, acids, and neutral compounds. It appears that by chelating calcium, EDTA causes a general increase in membrane permeability.

Clinical Management

In case of contact with EDTA, the eyes should be flushed immediately with running water for at least 15 min. Affected skin should be washed with soap and water. Contaminated clothing and shoes should be removed and isolated at the site.

Exposure Standards and Guidelines

The FAO/WHO acceptable daily intake for calcium disodium edetate as a food additive is 2.5 mg kg^{-1} body weight (1.9 mg kg^{-1} of body weight as the free acid).

See also: Food and Agriculture Organization of the United Nations; Lead.

Further Reading

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Education and Careers in Toxicology *See* Toxicology, Education and Careers.

Effluent Biomonitoring

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Introduction

The Clean Water Act mandates the reduction of water pollution sources, largely through control of point source discharges. Under the National Pollutant Discharge Elimination System (NPDES), municipal and industrial entities that discharge wastewater (e.g., sewage, pulp, and paper) into national waterways are required to obtain a permit and meet imposed effluent limitations. These limitations, designed to be protective of water uses, human health, and aquatic life, are generally expressed in terms of a numerical limitation for a specific chemical (e.g., 10 μg l^{-1} of copper). However, one shortcoming to chemical-specific requirements is that bioavailability and toxicity of multiple chemical species in complex effluents are not directly evaluated,

thereby providing little information on an organism's ability to integrate the effects of prolonged exposure to multiple contaminants.

In addition to chemical-specific requirements, toxicity requirements can also be imposed to ensure that the discharge is meeting the goal of protecting aquatic biota. NPDES permit requirements for aquatic toxicity may be numerical limitations or merely biomonitoring requirements. Whole-effluent toxicity (WET) testing monitors whether the discharge is likely to be toxic to aquatic life by exposing test organisms to various concentrations of the effluent and observing their response. The principal advantage to WET testing over chemical-specific requirements is the direct and integrated biological assessment of antagonistic, synergistic, or additive effects from multiple chemical interactions. During both acute (short-term) and chronic (long-term) WET exposures, generally invertebrate and fish species are challenged with various concentrations of effluent under controlled conditions, and lethal or sublethal end points are measured. When conducting compliance biomonitoring, if toxicity is observed above a certain magnitude, defined in terms of acute or chronic 'toxic units' (TUa and TUc), duration (e.g., time-averaged in-stream concentration), and frequency (e.g., not to be exceeded more than once every 3 years), additional studies may be required to evaluate, treat, and reduce effluent toxicity. (The TUa is defined as 100/median lethal concentration (LC_{50}), and the TUc is defined as 100/no-observed-effect concentration (NOEC), or 100/chronic value which is defined as the geometric mean of the lowest-observed-effect concentration (LOEC) and (NOEC). In addition, under the US EPA Total Maximum Daily Load (TMDL) program, limits have also been established for toxicity, expressed as WET-derived toxic units, considering both point and nonpoint sources.

This entry is intended to provide an introduction and practical analysis of the current standardized aspects of aquatic toxicity biomonitoring of industrial and municipal effluents. These tests and monitoring programs are needed to determine whether management requirements or regulatory criteria are being met as well as to assess the temporal and spatial trends in water quality.

Acute and Chronic Tests

A battery of aquatic biological tests has been developed to evaluate the toxic effects elicited from effluents. For all the types of toxicity tests, selection of exposure concentration and duration, test species and strains, and monitored water quality parameters are critical. The short- and long-term methods for evaluating the potential of toxic discharges have been well characterized.

In general, weaknesses in the results of aquatic biological tests include the following: (1) lack of ecological relevance due to laboratory culture conditions (e.g., test organisms more or less sensitive, more or less crowded or stressed, than indigenous species); (2) restriction to monitoring a single life stage of uniform size under a set of controlled conditions (vs. varying temperatures, seasons, food supply, predation, etc.); and (3) lack of direct relevance to the susceptibility and bioavailability of other life stages.

In addition, laboratory-prepared water used for diluting the effluent is relatively free of suspended solids, humic matter, and other varying components (e.g., hardness level) that may serve as nutrients, provide sorption sites, and have direct effects on the functional expression of toxicity. Thus, toxicity as expressed by the LC_{50} should not be viewed as a biological constant but as a value that varies with age and physical, chemical, and biological factors.

Acute Toxicity Tests

Acute bioassays are designed to assess the effects of toxic substances that occur within a relatively short period after exposure (48–96 h). The effluent toxicity elicited by test organisms is often fatal and rarely reversible. The relevant information to be gained from this type of test is the distribution of the exposure–response relationship and the nature and potency of the toxic effects, such as immobility, percentage mortality, and time interval to mortality.

Ideally, the exposure duration should be long enough to allow for steady-state conditions (body burden and elimination of toxics) to occur, although this is not always achieved.

The responses monitored are generally binary variables – meaning 'all or none' (e.g., mobile or immobile) – with known sample size and unknown probability, versus more graded or continuous observations.

Although crude as an end point, mortality is highly visible, clearly defined, and measurable and has been utilized as a first tier in hazard prediction. The acute procedure is often applied in situations in which the effluent is diluted at least 80 times; if there is less dilution of the effluent by the receiving water, then the 7 day chronic test is performed often.

Acute tests can be further divided with respect to flow regime: typically either static or static-renewal. During the static procedure, test organisms are exposed to the environmental sample in relatively nontoxic and nonreactive cups, glass beakers, or aquaria

and the test solution is not changed. Advantages to this design include ease of operation, minimal space needed, and minimal waste generated. This system is relatively simple and cost-effective for evaluating large numbers of samples or where only limited volumes of effluent are available. However, the concentrations of the effluent components – chemical compounds and their degradation products, dissolved oxygen, metabolic by-products, and hydrogen ions – may change throughout the test due to complex biochemical events: uptake by the test organism, adsorption on the organism or on the walls of the test container, biodegradation, vaporization, and precipitation. The potential accumulation of metabolic or other wastes may lead to undue stress on the test organisms and variable results. The application of the static-renewal test may minimize these difficulties.

During the static-renewal acute test, the exposure solutions are periodically renewed (24 or 48 h) either by carefully transferring the test organisms to a freshly prepared exposure medium or by gently decanting and refilling test containers. In contrast to the static procedure, the static-renewal system is designed to mimic more natural intermittent exposure scenarios (e.g., acid rain and agrochemical applications) and mitigate the changes induced by unstable, volatile, or high oxygen-demanding effluents.

Chronic Toxicity Tests

Chronic whole-effluent toxicity tests involve exposing organisms (usually emphasizing presumably sensitive early life stages, such as neonates or newly hatched larvae) to various effluent concentrations for a period of typically 7 days, during which the exposure solutions are renewed daily. The 7 day cladoceran survival and reproduction test is one of several procedures employed for estimating the chronic toxicity of effluents. Generally, these longer term tests provide more information on effluent effects such as fecundity, growth, reproduction, life span, behavior, and mortality.

Specific exposure concentrations for effluent dilution are often evenly spaced in a linear or geometric series (e.g., 100%, 50%, 25%, 12.5%, and 6.25% effluent). Replicate exposures are required, with test vessels arranged in the incubator in a randomized block design. At the end of the test, statistical calculations of NOECs for survival and reproduction or growth are typically performed as a multiple comparison procedure of effluent-treated groups. The induced effects of effluent pollutants on growth or reproduction and on survival may or may not be equal, suggesting information on the relative sensitivities of the test organisms. The test end points and duration are assumed to demonstrate that the aggregated substances in the effluent have or have not been protective of aquatic life.

Advantages to the chronic test include its attempts to detect longer term, and perhaps more subtle, sublethal, toxic effects by studying relatively susceptible neonates or larvae and their reproduction or growth. The 7 day chronic procedure may mimic intermittent and fluctuating pollutant exposures in nature through daily renewals, thereby allowing for organismal recovery, adaptation, acclimation, or simple stressor avoidance.

In general, a greater proportion of aquatic organisms are exposed to sublethal concentrations of toxics compared to acutely lethal concentrations. There is general acceptance that chronic exposure of fish to sublethal levels of toxics makes them more prone to disease states. Although scientifically controversial, intuitively one can grasp how individual responses such as growth, reproduction rates, and survival probability, as a function of age, could relate to population level responses.

One limitation to chronic WET tests is that they do not indicate which stressor(s) is causing the observed effects. Furthermore, they are generally subject to false positives (type I error) and false negatives (type II error) because of weak correlation between WET tests results and in-stream effects. For instance, during the 7 day fathead minnow survival and growth test, growth rate end points (dry weights compared to controls) may not be a reliable indicator of latent toxic effects since there is potential for reproductive failure in successive generations even in the absence of statistically poor growth.

A related general weakness in WET testing schemes involves the natural 'variability' of effluents, and whole-effluent tests, which may be unrelated to the actual effluent toxicity but related to short-term spatial, temporal, and seasonal variation at a site. Interpretation of WET data is therefore complicated, as one may not be able to easily compare to the reference values like one can with chemical analyses. More frequent effluent testing may identify these atypical toxicity responses.

Another major disadvantage of the chronic WET tests is referred to as 'simulated toxicity', or 'pathogen interference', whereby the observed toxicity is attributable to adverse interactions from biological growth of freshwater pathogenic (e.g., *Aeromonas hydrophilia*) or sheathed (*Sphaerotilus natans*) bacteria or fungi on the test organisms and is not necessarily a manifestation of chemical constituents present in the effluent. The microbial growth, and resulting anomalous WET results, may be a

result of contaminated culture conditions (e.g., masses of bacterial growth surrounding brood pouch eggs), contaminated sampling equipment, or microbial proliferation due to nutrients associated with the effluent or laboratory culture environment.

Statistical disadvantages in chronic testing data analyses have been presented, suggesting that NOECs are misleading and should be phased out of regulatory use. This research suggests that NOEC concepts do not consider basic variability and are artifacts of the test design in terms of effluent concentrations and intervals chosen. Another concern is that WET data are bounded, and it is not possible to measure effects beyond 100% effluent; thus WET data as expressed in toxic units cannot be measured as values <1, leading to difficulties in statistical analysis and data interpretation. As an alternative, regression analysis has been proposed as a more robust procedure than traditional hypothesis testing to mitigate the problems of violation of assumptions, experimental error, and variability in test protocols.

Physical, Chemical, and Biological Test Factors

Although variability is inherent in all environmental measurements, a variety of physical, chemical, and biological factors should be noted in compliance biomonitoring to enhance the consistency and defensibility of toxicity test results. These various parameters may affect the effluent toxicity to aquatic biota, and it is important that the investigator take them into account. Test conditions should mimic receiving water conditions, whenever feasible, to allow accurate assessment of the in-stream effect of an effluent. However, the use of upstream water for dilution should be avoided due to the potential variability in quality, or toxicity, over the testing period.

Physical considerations of particular importance in aquatic toxicity testing include exposure temperature, periodicity and intensity of light, test organism loading, test duration, laboratory equipment, test methods, and data recording. For instance, control and test solution volume should be sufficient to allow organisms free mobility, to provide adequate supply of dissolved oxygen (e.g., $>4\,\mathrm{mg\,l^{-1}}$), and to prevent buildup of metabolic waste products such as ammonia. Chemical considerations of interest that contribute to the observation of WET toxicity include pH, alkalinity, hardness (as well as the ratios of calcium and magnesium), salinity, dissolved gases, organics, inorganics, sediments, and humic substances. For instance, the bioavailability and toxicity of metals to aquatic biota are known to differ with pH, hardness, and oxidation state. Fluctuations of pH levels of a receiving water can result in over- or under-predicted effluent toxicity. Biological factors inherent to biomonitoring include test organism age, class, size, genetic state, acclimation to culture conditions, nutritional health, parental care, and food quality and quantity. For instance, dietary factors such as algal digestibility and availability affect test organism body size, sensitivity, and performance, as evidenced in *Ceriodaphnia* reproduction tests.

Laboratory Dilution Water

Water selected for diluting the effluent should be of uniform quality, free of contaminants (e.g., benzene, pesticides), and available in large enough volumes for culture maintenance, acclimation, and testing. Based on these minimal requirements, synthetic laboratory water prepared with reagent-grade chemicals and deionized water is particularly useful for most small laboratory operations and where precise water quality parameters such as alkalinity, hardness, pH, and specific conductance should be measured to check for consistency between batches, as part of good laboratory practice. However, if a variety and numerous types of toxicity tests are performed routinely, then a high-quality surface water such as a large lake may be desirable. Generally, lake water is less prone to changes in quality (suspended solids, organic matter, and runoff contaminants) that may affect results compared to river water. A municipal water supply is least desirable for laboratory diluent due to potential interferences from the constant oxidation of residuals needed for safe and potable water.

Test Organism Selection and Culture

Invertebrates and fish are typically used in toxicity testing of effluents and are the primary focus of most historical and present methods (ASTM, US EPA, and OECD). For acute and chronic effluent testing, the most typical invertebrate species is the water flea (*Daphnia magna*, *D. pulex*, or *Ceriodaphnia dubia*), and the routine vertebrate test species is the fathead minnow (*Pimephales promelas*). These test species have held regulatory acceptance and meet regulatory guidelines. Another reason these test organisms have been favored is their relatively small size, lending themselves to practical considerations such as availability of the effluent and sensitivity to toxics. Aquatic criteria often require acute and chronic toxicity data on a range of organisms representing multiple trophic levels. Standardized culturing techniques are required, and should be refined, to assure the body size, performance, health, and sensitivity of test

organisms. The quality and quantity of food for cultures – microalgae (*Selenastrum capricornutum*), cereal leaves, trout chow, yeast, rotifers, or brine shrimp – are critical for individual energy assimilation and balance and for precise and quantifiable data.

Good Laboratory Practice

Good laboratory practice regulations govern the planning, experimental design, and conducting of whole effluent toxicity studies and are described in federal publications and toxicity testing manuals (e.g., Code of Federal Regulations, USA). In laboratories, much cost and effort are associated with the handling of samples and information as well as the test itself. Good laboratory quality assurance begins before a sample is accepted, carries through during sample log-in and testing, and continues after completion of the analysis.

This system of quality assurance was introduced to ensure that toxicity tests are competently performed and that data are not fabricated. A sound, written quality assurance program is an essential basis for laboratory operation and is often found in the form of standard operating procedures. The quality assurance program functions not only to monitor the reliability of data recorded and reported but also to control data quality in order to meet regulatory requirements. Clearly written protocols for each procedure employed eliminate or reduce errors in laboratory operation caused by factors such as personnel (qualifications and technical competence), supplies (e.g., purity of reagents), equipment calibration and maintenance, sample storage and handling, and analytical methods. Quality control describes the set of quantifiable measures, including established protocols and standard equipment, used to define daily laboratory activity.

The good laboratory practice criteria for whole effluent toxicity tests include species acceptability, exposure system conditions, physical and chemical conditions, and statistical data analysis methods. For instance, the test acceptability criteria for the larval fathead minnow 7 day chronic tests involves having 80% or greater survival of controls and an average dry weight of surviving control fish equal to or greater than 0.25 mg.

Sample Collection and Handling

Care should be taken that the sample collected is representative and undergoes minimal changes prior to toxicity evaluation. Hence, a 24 h composite sample obtained with a refrigerated, proportional flow sampling device is a good choice. This type of sample could then be readily shipped to the contracting laboratory in a cooler packed with ice to maintain sample integrity. A 'chain-of-custody' form must accompany the shipment indicating the source and type of sample, time of collection, whether pre- or post-chlorination, and the name of the individual who collected the sample.

Upon arrival, the sample should be logged in the laboratory record book indicating time of arrival, sample temperature, and pH. The sample should in all cases be analyzed for residual chlorine and, if present, oxidized with sodium thiosulfate before it is employed for toxicity evaluation. A portion of the sample should also be removed for alkalinity and hardness analyses. Often it is necessary to coarse filter the sample to remove floc or suspended debris before testing; however, this practice may reduce the sample's toxicity. The remainder of the sample should be kept at 4°C for a period not exceeding 72 h after initial sample collection. It is desirable to employ two separate 24 h composite samples for performing a 96 h acute larval fathead minnow test. This would allow renewal after 48 h exposure. In the 7 day tests with *Ceriodaphnia* and *Pimephales*, three separate 24 h composite samples should be employed for daily renewal of the various exposure solutions. Toxicity data summary sheets should include daily routine physico-chemical measurements and sample information. It is essential that good laboratory practices be used in all aspects of sample collection, treatment, and analysis to obtain quality and defensible results.

Data Analysis

Statistical methods in effluent toxicity evaluations enable the investigator to quantify the observed exposure–response relation, with reference to the desired end point. The resulting statistical confidence interval in the data may then be used to ensure test reproducibility, to compare multiple test results, and for regulatory decision-making.

Statistical methods frequently employed in effluent toxicity evaluations include point estimation technique such as probit analysis, and hypothesis testing like Dunnett's analysis of variance (ANOVA). Point estimation technique enables the investigator to derive a quantitative dose–response relationship. This method has been generally applied to statistical analyses of acute effluent monitoring data.

Hypothesis testing is generally used as a qualitative measure to evaluate whether the differences between treatments and control are statistically different, especially in chronic bioassays. However, this approach

has a major limitation in determining biological end points; inasmuch as potential end points are controlled by the selected dose range, results from multiple samples cannot be compared in a quantitative manner.

Commonly used statistical software includes ToxStat (West, Inc., USA), SAS (SAS Institute Inc., Cary, NC, USA), and various US EPA provided programs.

Acute Methods

Many models have been used to calculate EC_{50} or LC_{50} values from acute effluent monitoring data, such as graphic interpolation, moving average, probability unit (probit), logistic unit (logit), Litchfield–Wilcoxon, and Spearman–Karber (often trimmed) methods. The challenge then arises as to which model to choose, given the toxicity data. The most obvious choice is the model that holds the most biological support. However, the answer is that there is not much biological basis for these models, and the investigator is left to choose the most appropriate statistical procedure based on the test results and standard methods.

A parametric probit method can be applied to, for example, determination of LC_{50} only when the test data contain two or more partial mortalities, and are proved to be appropriate for such method by significant chi-square tests. The probit and logit models assume that the organism tolerances have a lognormal distribution, with a typical exposure–response curve given by a sigmoidal-shaped curve. Both methods utilize transformations and curve fitting of the data and require varying observations of partial mortalities. Otherwise, distribution-free Spearman–Karber (often trimmed) method is recommended. Graphical method can be used to obtain LC_{50} values when no partial mortality was observed. The criterion for acceptable acute tests in terms of mortality in the controls is often specified to be less than 10%.

Chronic Methods

In terms of the statistical methods of the partial life cycle whole-effluent tests, survival, growth, and reproduction data from the 7 day cladoceran or fish exposure are often analyzed using hypothesis testing to determine 'acceptable' concentrations. In order to determine the appropriateness of using parametric statistical methods, the data are first tested for normality of distribution and homogeneity of variance, for which the US EPA recommends the use of Shapiro–Wilk's test and Bartlett's test, respectively. Kolmogorov test for normality and Levine's test for homogeneity can be also used for these purposes. Dunnett's ANOVA test is typically used for a parametric data set to compare the treatment mean with the control mean in order to obtain the NOEC and the LOEC for each end point. If either the test for normality or homogeneity fails, then the nonparametric Steel's Many One Rank test may be used when numbers of replicates among treatments are equal. Statistical analyses for sublethal end points such as growth and reproduction are generally performed with treatments of which mortalities are not significantly different from that of control. A distribution-free ICp analysis may be applied for point estimation of effect concentrations for sublethal end points such as IC_{25}. This nonparametric model uses smoothing of test data to fit the assumption of monotonic response.

Test Failures

Whole effluent toxicity tests may 'fail' primarily for two reasons: an aborted test due to a lack of good quality data generated (e.g., organism health or effluent sample exceeded its holding time of 72 h), or whole effluent toxicity is demonstrated to exceed permitted levels.

In terms of the second case, whole effluent toxicity may be the direct result of unidentified and persistent contaminants discharged to a wastewater treatment facility. These influent sources of test failures include refractory substances (pass through toxics) in effluent, such as floc and coagulating agents, or pesticides such as diazinon, which cause observed patterns of strong toxicity to the test organisms. Furthermore, ammonia may be present in concentrations that cause WET test failures even though the effluent discharge meets NPDES permit numerical limitations. Although the federal regulations do not define the 'failure' of an effluent toxicity test as an automatic permit violation (subject to fine or notice of violation), in practice, single-species WET tests have been treated in this manner. For those with permits, it is very important to read proposed or actual permit language for qualitative or quantitative information surrounding what constitutes a reasonable potential to approach exceedance of the limit, a pattern of toxicity, or a 'failure', which might trigger fines or additional testing. In fact, the significance of episodic exceedance of WET limits should depend on a host of additional factors, such as the receiving water conditions, mixing zone, and duration of events.

Often, regulatory requirements are such that test failures trigger additional evaluations. As part of the toxicity-based approach to effluent permitting, toxicity identification and reduction evaluations (TI/RE) schemes were implemented to identify and reduce the toxic components of complex effluents

(e.g., un-ionized ammonia, hexavalent chromium, selenium, and chlorides) and assist managers in controlling toxics. The TIE process involves the stepwise chemical manipulation (pH adjustment, filtration, aeration, C18 solid-phase extraction column, EDTA chelation, and sodium thiosulfate treatments) of the effluent to identify specific compounds causing toxicity and renders them biologically unavailable.

However, before implementing potentially time-consuming and expensive toxicity reduction studies, it is important to understand their capabilities and limitations. For instance, past protocols have had the greatest success with effluent constituents that cause acute toxicity and have questionable usefulness for chronic toxicity. In addition, no method ensures success for very complex waste mixtures (e.g., polar organic compounds), which are exceedingly difficult to analyze.

Furthermore, in the United States a TRE may vary between states. The US EPA's TRE is based on compliance with whole effluent toxicity, but a specific state's toxic reduction evaluation may address compliance with chemical-specific water quality standards and implement action-oriented programs that provide technological solutions for pollutant removal (e.g., pretreatment facilities). Thus, various toxicity reduction options may be available and should be evaluated before initiating time-consuming and expensive studies.

Alternative Test Methods

There are major efforts in support of replacing *in vivo* vertebrate tests, refining existing approaches, and reducing test organism mortality. In terms of the adequacy or inadequacy of current *in vivo* designs, questions have arisen as to their accuracy in predicting hazards to humans and aquatic life, inter- and intra-laboratory variation, and ethical concerns on how animals are used. For instance, in the United Kingdom, fish are protected under the Animal Scientific Procedures Act of 1986 as soon as they are capable of independent feeding. Furthermore, there has been a general evolution in toxicology studies from a merely descriptive science to one describing actual mechanisms of action.

The bacterial luminescence toxicity assay Microtox (*Vibrio fischeri*) has achieved international recognition for its usefulness in detecting acute and chronic aqueous toxicity that correlates with traditional invertebrate and fish species. Moreover, microalgae (*Selenastrum*), brine shrimp (*Artemia*), plants (*Lemna*), mysid shrimp (*Mysidopsis*), and fish cells (both primary and lineages) have gained acceptance as useful alternatives for measuring aquatic toxicity. In conjunction with chemical-specific analysis, a general consensus is that a battery of biological tests should be utilized to broadly characterize toxicity because each test organism and system responds and characterizes toxicity uniquely. Overall, water quality biomonitoring must harbor goals of cost-effectiveness, sensitivity, relevance, and precise results.

See also: Biomonitoring; Clean Water Act (WA), US; EDTA (Ethylenediaminetetraacetic Acid); Good Laboratory Practices (GLP); Statistics.

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Electromagnetic Fields

C F del Pozo

This entry gives a brief introduction to the current knowledge on the potential health effects of human exposure to electromagnetic fields, in the frequency range of 0–300 GHz. The principal characteristics of these fields are that they are of relatively weak intensity, and are at frequencies well below the range of the ionizing radiation (extreme ultraviolet, X- and γ-rays). **Table 1** shows the frequency spectrum of the electromagnetic field radiation in the frequency range of 0–10^{20} Hz. The table also includes some examples of broadly used applications, and shows the separation between nonionizing and ionizing radiation as a function of the frequency range.

The pace in the development of electromagnetic field (EMF) technologies and the ever-increasing number of commercial applications in all areas and activities of our life is breathtaking. This is particularly true of radio frequency wireless technologies and telecommunications. Nowadays, especially in urban environments, exposure to human-made EMFs is an unavoidable fact of life, and understanding its potential health effects and possible environmental impacts and risks, a priority.

Public concern, and fears, about potential adverse health effects of human exposure to electromagnetic radiation from extremely low frequency (ELF) and radio frequency (RF) sources, in particular, has grown significantly in recent years. Apart from scientific uncertainties in establishing any potential health risks, which induces the public debate on the validity of limit values, this problem is also caused by the roll out of the mobile telephone networks, and the high visibility of increasing number of base stations. This number will have to rise even more in order to implement the latest developments on mobile phone technology and this expected growth makes it imperative for public authorities to ensure transparency, trust, and confidence in decision-making.

General Definitions

The EMF has two components, the electric field (**E**) and the magnetic field (**H**), which vary in time and propagate together in space. These quantities are vectors having both magnitude and direction. The electric field exists whenever an electric charge is present, and the magnetic field arises from the motion of electric charges (an electric current). Electric fields and magnetic fields are strongest close to their

Table 1 Frequency spectrum of the electromagnetic field radiation in the frequency range of 0–10^{20} Hz. It includes some examples of broadly used applications and also shows the separation between nonionizing and ionizing radiation as a function of the frequency range

Radiation type	*Frequency range*	*Application examples*
Nonionizing		
Static fields	0 Hz	Static electricity
ELF (extremely low frequencies)	50 Hz to 3 kHz (1 kHz = 10^3 Hz)	Electric power transmission, domestic power supply and appliances (50–60 Hz)
VLF (very low frequencies)	3–30 kHz	TV, video display units, induction heaters
RF (radio frequencies) 30 kHz to 300 GHz	30 kHz to 3 MHz (1 MHz = 10^6 Hz)	Induction heaters, electronic surveillance
	200 kHz to 900 MHz	Radio AM, FM, and TV broadcasting
	300 MHz to 3 GHz	Mobile telephony
	900 and 1800 MHz	GSM (European standard)
	1900 MHz to 2.2 GHz (1 GHz = 10^9 Hz)	UMTS (standard for enhanced telephony services including mobile internet)
	2.5–300 GHz	Microwave ovens, civil and military radars, satellite links
Infrared	300–10^5 GHz	Intruder detectors, remote controls
Visible	10^5–10^6 GHz or wavelength 0.8–0.4 μm (1 μm = 10^{-6} m)	Light, lasers
Ultraviolet	0.4–0.1 μm, 0.1–0.01 μm (EUV)	Sun, phototherapy
Ionizing		
X-rays	0.03 μm to 0.3 nm (soft), $<$0.3 nm (hard) (1 nm = 10^{-9} m)	Radiology
γ-rays	0.03 nm and less	Nuclear physics

sources and diminish rapidly away from them. Both fields exert physical forces on electric charges, but the magnetic field only does so when the charges are in motion. Electric fields are shielded very effectively by metal conductors, and building materials and trees also provide some shielding capability. Magnetic fields, on the other hand, are not blocked by common materials such as walls of buildings.

EMFs are wave motions characterized by their frequency or wavelength, strength (intensity), or power density. Frequency is the number of variations of the field per second, and it is given in hertz (Hz) or cycles per second. The wavelength is the distance between two consecutive maximum (or minimum) amplitudes in the wave, and the product between frequency and wavelength is the velocity of propagation of the wave, which is equal to the speed of light ($\sim 300\,000\,\mathrm{km\,s^{-1}}$ in air). The field strength is the amplitude of the wave, and it is generally expressed in volts per meter ($\mathrm{V\,m^{-1}}$) for the electric field and in ampere per meter ($\mathrm{A\,m^{-1}}$) for the magnetic field. The magnetic field can also be specified as magnetic flux density ($\mathbf{B}$) expressed in tesla (T).

The electromagnetic field strength is also expressed as an equivalent power density (S), in watts per square meter ($\mathrm{W\,m^{-2}}$). The quantity S is proportional to the product of the electric and magnetic field strengths ($\mathbf{S} = \mathbf{E} \times \mathbf{H}$). Away from the radiating source, the far-field plane-wave model gives a good representation of the EMF propagation and the power density is the power per unit area normal to the direction of propagation with amplitude given by: $S = E^2/377 = 377 \times H^2$. Under these conditions, exposure levels and safety limits can be specified by the power density of the electromagnetic field at a given location, or by its electric field intensity. Near the source, however, the relationship is more complicated and the spatial distributions of both the electric and the magnetic fields are highly variable. In this case, it is more appropriated to specify exposure levels in terms of the specific absorption rate (SAR), which is the power per unit mass that is absorbed by the human body. Limits have been prescribed for whole-body-averaged SAR and SAR for 1 or 10 g of tissue anywhere in the body.

Exposure to Weak-Intensity Nonionizing EMF Radiation in the 0–300 GHz Frequency Range

Common sources of low-level exposure to electromagnetic fields are electric and magnetic powered transport (static fields), overhead power lines, domestic electric appliances (ELF), antitheft electronic devices, or video display units (in very low to low frequencies). Low-frequency electric and magnetic fields interact with the electric charges in the biological tissues and induce electric currents in the body. The magnitude of these currents is a function of the electric properties of the body and of the intensity of the interacting field. Exposure to low-frequency fields normally results in negligible energy absorption and no measurable temperature rise in the body.

At higher frequencies (RF, above 100 kHz), on the other hand, energy absorption and tissue heating effects may be significant. Exposure to radio waves and the resulting energy absorbed by the body depends on many factors, such as intensity of the field (which varies with the position of the device in relation to the base stations and on the position and type of antenna), field modulation and length of exposure. In addition to the 'passive environmental exposure' from telecommunications and wireless technologies, RF emissions are absorbed from handsets by the head when in use. When the tissue is subjected to an RF field, part of the field is reflected, and part penetrates the tissue. Heat is generated inside the tissues to dissipate the currents induced by the associated RF electric fields.

Possible Health Effects

The harmful effects (thermal) of exposure to high levels of EMF radiation are both proven and acknowledged. With regard to low-level exposure, however, scientific research has not been able to establish, in a consistent and consensual manner, the existence of any causal links between exposure to weak EMF radiation sources, below the accepted limits, and possible adverse health effects. Moreover, there is no established evidence of potential harm from long-term exposure to radio frequencies. The argument is different on exposure to ELF, in this case possible connection between quasistatic magnetic fields and childhood leukemia cannot be ruled out completely. According to the International Agency for Research on Cancer classification, this connection is possible but more evidence is needed ("limited and still inconclusive evidence on causation coming from serious research and supported by consistent epidemiological studies").

Nonthermal Effects of RF

Nonthermal effects may result from the possible interactions between RF fields and the various components of the biological material. Established effects include: (1) The interference of radio frequencies with cardiac pacemakers is possible, however, new models of pacemakers are currently equipped with electronic filters making them immune to fields from

telephones. (2) The microwave auditory effect (or 'microwave hearing') at radar frequencies which is indeed an extremely low level thermal effect. With mobile telephones, the energy in the pulses is too weak to produce a hearing effect. (3) Indirect effects such as induced currents from touching a metallic structure exposed to an electric field.

Other nonthermal effects have also been reported. They involve possible interactions of EMF with genetic material (genotoxic and carcinogenic effects, e.g., DNA damage, chromosome aberration, gene mutation, etc.), effects on cell membranes, effects on the thermoregulatory system, behavioral disorders and various effects on physiological systems or organs, etc. They are at the center of the debate on health effects of EMF at radio frequencies but it appears to be a general agreement among experts that supporting evidence for such reported effects is still insufficient and inconclusive, or even contradictory in a number of cases. There is also a broadly shared opinion on the necessity to support further research in this area and to keep the International Commission on Non-Ionizing Radiation Protection (ICNIRP) recommendations on exposure limits under review according to the available scientific evidence.

Risk Management and Preventative Measures

Misrepresentation of scientific uncertainties in identifying and quantifying health risks – particularly in the long term – may be contributing to public fears. It may also strengthen the feeling that any risk is unacceptable and should be banned. Public perception of technological risks, and EMF in particular seems to be distorted by the lack of a common knowledge base and of straightforward contextual references, and not least by the presence of often competing and conflicting sources of information. This makes very difficult for both citizens and decision makers to understand the actual facts and to reach an accurate view of the potential risks as well as the benefits of the EMF technologies. It also makes the task of managing and communicating risk a complex process that requires full transparency and the active participation of all parties concerned.

Preventative measures may be taken in order to avoid or mitigate possible harm from uncertain risks and this will depend on the degree of importance and indeed harm of such risks. The 'prudent avoidance' concept is defined as the full set of voluntary measures that can be taken by private individuals to minimize any unnecessary and/or easily avoidable exposure. The 'precautionary approach' is, on the other hand, a risk management tool allowing decision makers to take action when scientific indications of possible 'serious and irreversible' health hazards are judged to be sufficient to establish 'reasonable doubt'. Preventative measures, however, cannot be based on a hypothetical risk.

Exposure Limits

Guidelines on maximum exposure levels and reference values established by ICNIRP (1998) are endorsed by many countries (including the United States and European Union member states) and are generally accepted and increasingly applied across the world. Exposure limits and recommendations are based on peer-review of the scientific evidence; they apply to all devices emitting EMF and are based on established health effects (below which health is not affected, even to repeated exposure). As far as the current understanding and scientific evidence goes, these exposure limits seem to provide common, minimum requirements for health protection of the general population.

Basic restrictions and maximum exposure levels recommended by ICNIRP are listed in **Tables 2** and **3**. Basic restrictions apply equally to workers and to members of the general public. As a function of the frequency, restrictions are based on the potential of low frequency electric and magnetic fields, as well as

Table 2 ICNIRP recommended basic restrictions for exposure to EMF. Specified in units of induced current density in human body including high safety factor

Frequency range	*Current density* ($mA\,m^{-2}$)	*SAR (full body)* ($W\,kg^{-1}$)	*SAR*	
			Head/trunk	*Limbs*
0 Hz	–			
>0–1 Hz	8			
1–4 Hz	8/*f*			
4–1000 Hz	2			
1–100 kHz	*f*/500			
100 kHz to 10 MHz	*f*/500	0.08	2	4
10 MHz to 10 GHz		0.08	2	4
10–300 GHz	($10\,W\,m^{-2}$)	–		

SAR: specific absorption rate (watts per kg of body-mass or tissue).

Table 3 Reference exposure levels for general public, recommended by ICNIRP, expressed in electric and magnetic field intensities, and electromagnetic field power density

Frequency range (f)	*E-field ($V m^{-1}$)*	*B-field (μT)*	*Power density ($W m^{-2}$)*
0–1 Hz	–	4×10^4	
1–8 Hz	10 000	$4 \times 10^4/f^2$	
8–25 Hz	10 000	$5000/f$	
0.025–0.8 kHz	$250/f$	$5/f$	
0.8–3 kHz	$250/f$	6.25	
3–150 kHz	87	6.25	
0.15–1 MHz	87	$0.92/f$	
1–10 MHz	$87/f^{1/2}$	$0.92/f$	
10–400 MHz	28	0.092	2
400–2000 MHz	$1.375 \times f^{1/2}$	$0.0046 \times f^{1/2}$	$f/200$
2–300 GHz	61	0.20	10

RF radiation, to cause illness or injury through respectively the induction of currents, or the heating of body tissues. Across all frequencies they are supported by a solid body of observations and referred studies. The starting point at RF is the observed behavioral changes in experimental animals exposed to radiation levels raising whole-body temperature in excess of 1°C; SAR of 1–4 $W kg^{-1}$ or higher is needed to cause these changes. From this evidence and with the inclusion of a safety or uncertainty factor of 10, the value of 0.4 $W kg^{-1}$ was proposed as the exposure limit for workers under conditions of whole body exposure. An additional safety or uncertainty factor of 5 was introduced for the general public (thus 0.08 $W kg^{-1}$).

See also: Radiation Toxicology, Ionizing and Nonionizing.

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Emergency Response and Preparedness

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Introduction

Everyday throughout the United States millions of tons of hazardous chemicals are produced, transported, stored, used, and disposed. Citizens live and work among a wide variety of what are considered hazardous chemicals. These chemicals are transported by trucks, trains, pipelines, and ships and are used on farms and in fixed facilities such as chemical plants. Hazardous substances also are found in many consumer products and services that we use everyday, including household cleaners, paints, batteries, dry-cleaning processes, pesticides, and many others.

Under normal conditions, these substances are controlled and pose no threat to human life and the environment. But when they enter the environment through an accidental release, they can adversely affect human health, contaminate the land we use, the water we drink, and the air we breathe, with potentially disastrous results. Nobody expects an emergency or disaster in their community or at their workplace. However, the simple truth is that emergencies and disaster happen everyday, at anytime, and in any location. For example, during 2003, there were 15 100 releases of hazmat while in transportation, of which 465 were considered serious. On average, there are more than 41 incidents each day in the United States. The estimated monetary damage from these incidents is exceedingly high.

Since September 11, 2001, emergency response and preparedness for hazardous materials is shifting from hazmat releases being the result of accidents to hazmat releases being used as weapons of mass destruction. Current discussions of hazmat have identified a real potential for hazmat to be used as weapons of mass effect, which takes into account the potential effect on the public consciousness.

The following entry outlines current best practices to prepare for and respond to a release of hazmat, irrespective of whether the release is an act of terrorism or an accident. The basic tenets of response to both are consistent. These basic tenets include the following: the hazards are the same, the equipment employable for material detection and monitoring will still work, the modeling software of plume dispersion remains valid, and the personal protective equipment (PPE) will continue to function as designed. Two important distinctions need to be made. If the release is an intentional act, responders should be aware of the possibility of secondary devices that could be targeted at emergency responders. In addition, if the release is an intentional act, a crime scene will be established to gather and process evidence. The crime scene and secondary devices will not be discussed in this article.

Emergency Preparedness

Emergency Response Plan

An emergency response plan should be developed in order to delineate how a facility responds to an emergency. The first aspect of the plan is an inventory of the chemical, physical, and biological hazards associated with the facility. The list could include the storage, use, or transportation of hazardous materials, hazardous wastes, and hazardous substances. The Occupational Safety and Health Administration (OSHA), the Environmental Protection Agency (EPA), Transport Canada, and the Federal Emergency Management Agency (FEMA) provide regulations and guidance in developing emergency response plans.

OSHA requires that the plan be developed and implemented for anticipated emergencies. The plan must be written and available for OSHA's inspection. However, if the facility's procedures are to evacuate and have no employee assistance during the emergencies, the facility is exempt from having a written plan.

The written plan should include the following: (1) pre-emergency planning and coordination with outside parties; (2) personnel roles, lines of authority, training, and communication; (3) incident command; (4) emergency recognition and prevention; (5) safe distances and places of refuge; (6) site security and control; (7) evacuation routes and procedures; (8) decontamination; (9) emergency medical treatment and first aid; (10) emergency alerting and response procedure; (11) critique of response and follow-up; (12) PPE and emergency equipment; (13) coordination with local fire and police personnel and the Local Emergency Planning Committee; and (14) postincident remediation and recovery.

When developing the emergency response it is imperative to determine the capabilities of the local civil responders, including whether they are full or part-time, have hazardous material training and to what level, and have knowledge of facilities hazards and processes, and what response equipment is available. Upon completion of the emergency response plan, the plan should be evaluated through training including full-scale or tabletop exercises.

Creating the Emergency Response Team

The emergency response plan will establish the need for an emergency response team. The emergency response team can either be staffed by in-house employees or by outside contractors, or a combination of both. When outside contractors are chosen to provide a hazardous materials emergency response, they should be evaluated for their training, regulatory compliance, capabilities, equipment and response times. In order to allow the contractor to provide the best service, they should be provided with the emergency response plan along with a list of the chemical, biological, and physical hazards, including their physical state, temperature, associated process, or package. In-house responders can be full-time, part-time, or additional-duty responders. The emergency response plan will establish the responder's required level of training and expectations.

A medical surveillance program must be developed that complies with OSHA's regulation (CFR 1910.120 (q) (9)), which requires physical exams to be offered when an employee becomes a responder. Physical exams are given yearly, sometimes after exposure or upon termination from the team.

If it is anticipated that responders will need the use of respirators while responding, a respiratory protection program must be developed in compliance with OSHA's regulations (29 CFR 1910.134).

Training the Emergency Response Team

When establishing a hazardous materials response team, OSHA's regulation for hazardous waste operations and emergency response operations and emergency response, also called HAZWOPER, must be followed. Under Title 29 CFR 1910.120, OSHA includes hazardous waste operations and emergency response, which have separate training requirements.

OSHA defines hazardous waste operations as facilities that conduct treatment, storage, and disposal of hazardous wastes, cleanup sites required or recognized by federal, state, and local governments, and cleanup operations at uncontrolled hazardous waste sites. Workers at these defined facilities or locations are either general workers or occasional workers. General workers, such as equipment operators, general laborers, and supervisors, must have 40 h of initial training with 3 days of supervised field experience. Workers who are at the site only occasionally for a specific task, such as groundwater monitoring and land surveying, must have 24 h of initial training and 1 day of supervised field experience. In addition to the initial training, general and occasional workers must be provided with an annual 8 h refresher class. Initial and refresher training should be on aspects of site safety and health plan, hazards present at the site, PPE needed, and work practice that can minimize risks from hazards.

OSHA (1910.120 (q)) defines an 'emergency response to a hazardous substance release' as employees engaged in emergency response no matter where it occurs. OSHA separates individuals who respond to these incidents into six levels, each having its own training requirement. OSHA's responder levels are First Responder – Awareness Level; First Responder – Operations Level; Hazardous Material Technician; Hazardous Materials Specialist; Incident Commander; and Skilled Support Personnel.

A responder at the First Responder – Awareness Level is in the position to witness or discover a release. They can only initiate an emergency response sequence. They cannot take offensive or defensive actions. Their training consists of the following: (1) to identify the hazardous substances and risks associated with the materials; (2) to anticipate the potential outcome of an incident; (3) to recognize the presence of a hazardous substance; (4) to understand their role as being trained to the awareness level; (5) to understand site security and the Emergency Response Guidebook; and (6) to know when additional resources are needed.

A responder at the First Responder – Operations Level can respond to the initial release to protect nearby persons, property, or the environment. They can take defensive actions, without trying to stop the release. They can also contain the release from a safe distance. They should have had at least 8 h of training or have had sufficient experience to objectively demonstrate competency at the First Responder – Operation Level. The employer shall certify that the First Responder – Operations has the following: (1) knowledge of basic hazards and risk assessment; (2) proper PPE selection; (3) understanding of basic hazardous material terms; (4) understanding of basic control, containment, and confinement; (5) knowledge of basic decontamination procedures; and (6) understanding of relevant standard operating and termination procedures.

A Hazardous Materials Technician can take more aggressive action toward hazardous materials incidents than an operations level first responder. They can plug, patch, and stop a release. Their training is of at least 24 h, equal to that of the first responder at the operation level; in addition, the technician must have competency and the employer shall certify that competency in the following areas: (1) function of the Incident Command System (ICS); (2) proper PPE selection; (3) hazard and risk assessment techniques; (4) advanced control, containment, and confinement operations; (5) decontamination procedures – or lack of decontamination; (6) termination procedures; and (7) basics of chemical and toxicological terminology and behavior.

A Hazardous Materials Specialist can respond to and support the hazardous materials technicians. They may have direct or specific knowledge of various substances and can act as a liaison between the federal, state, and local governments. Their training is of at least 24 h, equal to that of the Technician; in addition, the hazardous materials specialists must have competency and the employer shall certify that competency in the following areas: (1) proper PPE selection; (2) specialized control, containment, and confinement operations; (3) decontamination procedure; (4) ability to develop site safety and health plan; and (5) understanding of chemical, radiological, and toxicological terminology and behavior.

The On-Scene Incident Commander (IC) assumes control of the incident. Their training is of at least 24 h, equal to that of the first responder at the operation level; in addition, they must have competency and the employer shall certify that competency in the following areas: (1) knowledge and ability to implement the employer's ICS and emergency response plan; (2) knowledge and understanding of the hazards and risks associated with workers working in chemical protective clothing; (3) knowledge and understanding of the state, federal, or regional emergency response plan; and (4) knowledge and understanding of the importance of decontamination procedures.

Skilled support personnel are proficient in the operation of certain equipment, such as earth-moving or heavy-lifting, which is needed temporarily to provide immediate emergency support which cannot reasonably be provided in a timely fashion by an employer's own employee or a contractor. Skilled support personnel are not required to be trained. However, they must receive an initial briefing at the site on the proper use, function, and limitation of PPE, the chemical and physical hazards involved, and the duties to be performed. All other appropriate safety and health precautions that are provided to the employer's own employees should be used to ensure the safety and health of these personnel.

All hazardous material responders shall receive annual refresher training of sufficient content and duration to maintain their competency or demonstrate competency to the employer yearly.

Trainers who instruct any of the responder levels shall have satisfactorily completed a training course for teaching the subjects they are expected to teach, such as at the US National Fire Academy, and possess training and/or academic credentials, instruction experience necessary to demonstrate competent instructional skills, and good command of the subject matter of the course they are to teach.

Equipping the Emergency Response Team

In order to provide the proper equipment to the emergency response team, a complete review of the chemical and physical hazards and the function to be performed by the hazardous materials responder should be done. This review will allow the selection of the proper PPE and response equipment. OSHA, in 29 CFR 1910.120 appendix A, describes four basic levels of protection for the hazardous materials emergency responder:

- *Level A*: Positive-pressure self-contained breathing apparatus (SCBA) or supplied air, totally encapsulated suit – gas tight, inner and outer gloves, chemical-resistant boots with protective toe, and hard hat.
- *Level B*: Positive-pressure SCBA or supplied air, hooded coveralls or two-piece splash clothing, inner and outer gloves, chemical-resistant boots with protective toe, and hard hat.
- *Level C*: Full-face or half-face air-purifying respirator, splash clothing or chemical-resistant coveralls, inner and outer gloves, chemical-resistant boots with protective toe, and hard hat.
- *Level D*: Coveralls, gloves, boots with protective toe, safety glasses or splash goggles, and hard hat.

When selecting the type and manufacture of the PPE, the purchaser should understand the functions being preformed by the responder and the chemical and physical hazards associated with the operation. They must also know how the operation and chemical will effect the degradation of the suit, gloves, and boots and the tactility and dexterity needed by the responder. Especially when Levels A and B equipment is in use, it is important not to overlook nonchemical hazards, such as heat stress, cold stress, slip, trip and falls, moving equipment, and lifting.

ICS and Structure

The ICS was developed in the 1970s after southern California wildfires caused the destruction of 600 000 acres and 772 structures and 6 fatalities. Congress funded a study to analyze the problems and found a lack of common organization, poor on-scene and interagency communications, inadequate joint planning, lack of valid and timely intelligence, inadequate resource management, and limited prediction capabilities. The ICS was developed as a tool for command, control, and coordination of resources at the scene of an emergency. Incident command consists of procedures for organizing personnel, facilities, equipment, and communication.

One of the most important aspects of incident command is the span of control. In an effort to minimize overtasking and to help ensure good decision making, planning, and execution, no person within the incident command has more than seven direct reports, with the optimum number being five. The IC is responsible for all aspects of the emergency. This includes, but is not limited to, hazard mitigation, remediation, safety, evacuations, and restoration. A public information officer provides press releases and briefings in order to provide information on the incident to the public and to minimize misinformation. A safety officer is responsible for the safety of the incident and always has the authority to shut down

the site due to safety issues. The liaison has the responsibility to work with other agencies working outside the incident command. Operations are responsible for the mitigation of the incident. Teams, sections, or groups that could work under operations are entry, decontamination, environmental remediation, and fire suppression. Planning has the responsibility of developing the operations for the response. The planning group looks hours or days in the future and provides operations with their work plan. Logistics is responsible for obtaining all of the materials needed for the response and staging the resource(s) upon their arrival. Upon receiving the operation plan from the planning section, logistics ensures that the right materials are available to support the response. Finance is responsible to know what has been spent and what will be spent and to start paying for the response.

The incident command should not be a means to obtain control or authority for other agencies or departments, a way to subvert the normal chain of command within a department or agency, too big and cumbersome to be used in small everyday events, or restricted to use by government and departments.

Incident Analysis and Initial Response

Accidents involving hazardous materials must be evaluated and approached with great care. Absence of visible warning labels, placards, etc., does not guarantee that the material is harmless. An incident may present such a high degree of hazard that the only safe course is to evacuate or shelter in place. If you are the first on scene, your first step is to call for help, and make appropriate notifications to local, state, and federal emergency response personnel. Provide as many details as possible, such as name, location, and telephone number, location of the incident, type of vehicle or container involved, wind direction and speed, identification of any injuries, presence of smoke, fire, or fumes, presence of marking, labels, or placards, carrier or facility name, etc. After the initial report, attempt to ensure that all unnecessary people are cleared from the site. Do not smoke, use flares, shut off engine(s), and resist the urge to 'run' to the accident site and rescue injured personnel until after the materials are identified and the nature and severity of the hazard is assessed.

Remain a safe distance upwind. Use binoculars to survey the area. Make notes such as location of the injured, the surrounding hazards, location of threatened people, markings, labels, or placards, note the number and types of vehicles, the containers involved, and any visible damage and/or leakage. Note the accessibility to the site and the possible escape routes along with the weather conditions and any notable topographical features such as water bodies.

To be able to make rapid and sound decisions for appropriate resource allocation, there must be an understanding of the hazards present, location of the incident, availability of equipment, training and capabilities of the personnel, and potential for the incident to 'grow'. To illustrate the interdependencies of these criteria we can look at a couple of scenarios involving the same material and quantity released. In scenario 1, you have a 1000 gal diesel fuel spill in the parking lot of a truck stop, the parking lot is asphalt, and the parking lot runoff is to a storm water collection pond. For scenario 2, you have all the same incident facts, except that the parking lot runoff is to a stream, livestock use the stream for water, and 1000 ft downstream the water utility has an intake for the potable water system. It is evident that analysis of a response to these scenarios would not be the same. However, the initial incident report from the truck stop or truck driver, may only be of a 1000 gal spill and the location of the incident. It is through planning, training, and communication that the additional information that is pivotal to making an appropriate and adequate analysis will be gained.

The IC must analyze the incident based on the facts and be prepared to change the response tactics and allocated resources as the situation reports mandate. The IC is a decision maker and a delegator. To be effective the IC must assign sector officer responsibilities to qualified staff, encourage ideas and opinions to be voiced through the command structure, insist on key stake-holder participation, seek the input of experts and practitioners alike, continually re-evaluate the tactics and strategy, and develop alternate plans.

An incident creates a dynamic environment, the decisions made in the initial analysis and the corresponding responses are critical to a desirable outcome. The continued thoughtful consideration of the interdependencies between the hazards, location, equipment, personnel, and cascading effects will make for sound decision making and appropriate resource allocation.

Response Actions

The method of accident mitigation is directly dependent on the type of material involved and the interaction of that material in the environment. The chemicals must be identified in order to develop a prudent response. Failure to correctly identify a material prior to mitigation may result in injury or exacerbating the incident. To help in the initial stages of an incident, Ludwig Benner developed the DECIDE

Process to guide responders through this stage as follows:

DECIDE Process.
D – Determine if there is a hazard present by looking for placards, signs, labels, or shipping papers.
E – Estimate likely harm without intervention: Identify the possible damage if the incident is allowed to run its course without intervention. There may be less danger to personnel, equipment, and the environment by choosing this option.
C – Choose response objectives: Select the harm you want to prevent (the exposures you want to protect against) before you act.
I – Identify action options: With your objective in mind, identify the options available to accomplish it. You must consider your practical options before you act.
D – Execute the best option: When you have multiple options, you should pick the option that provides a solution with the greatest gain and the least loss.
E – Evaluate progress: Once you have decided on a course of action, you must constantly monitor your progress.

If at all possible, the leak should be contained with dams, dikes, or secondary containment. When applicable the leak may be capped or the container can be patched. In order to minimize secondary contamination, responders' personal protective clothing should be decontaminated prior to doffing. Decontamination is also conducted on response tools and equipment.

Chemistry and Toxicology

Chemistry

When responding to hazmat incidents the general public and first responders often have difficulty in accurately determining the exact chemical(s) released. Confusion occurs because chemicals are often identified by product or trade names, placards, labels, or identification numbers, or have different synonyms. Thus, one must first ensure the exact chemical identification (ID). Product or chemical ID can best be determined by referencing the chemical's Chemical Abstract Services number (CAS#).

After identifying the exact chemical(s) for the incident, the responder needs to obtain critical information about the chemical's physical/chemical properties in order to determine the chemical's principle hazards and environmental fate (e.g., how will it behave and move in the environment).

1. Physical state at 20°C (68°F) is used to determine the nature of the chemical, in other words, if it is a solid, liquid, or gas at a defined, typically ambient temperature. Changing temperature may alter the physical state.
2. Boiling point is the temperature at which a liquid changes to a gas under standard atmospheric pressure and is used to determine if the chemical will become a gas during an incident.
3. Vapor pressure is a measure of the relative volatility of a chemical. A chemical with a high vapor pressure 'gives off' more vapors than a substance with a low vapor pressure at the same temperature and thus would require consideration as gas as well as a liquid or solid in a spill situation.
4. Vapor density is used to determine if a gas is heavier than air and thus will accumulate in low spots. A vapor density of less than 1 indicates that the vapor will be buoyant and rise in air, and vice versa. Heavy vapors present a particular hazard in the way they accumulate. If toxic, they may poison workers or responders; if nontoxic, they may displace air and cause suffocation; if flammable, they represent a fire or explosion hazard. Examples of gases heavier than air include chlorine, hydrogen sulfide, and sulfur dioxide.
5. Water solubility is used to determine if the chemical will mix with water, which is important in the handling and recovery of spilled material.
6. Specific gravity (SG) of a liquid is used to determine whether a spilled material that is insoluble will float or sink. Materials heavier than water have SGs greater than 1 and materials lighter than water have SGs less than 1.
7. Flashpoint is the lowest temperature at which a chemical gives off enough vapor to form an ignitable mixture with air near the surface of the liquid when exposed to an ignition source. Flash point values are used to rate the flammability or combustibility of a substance.
8. Autoignition temperature is the temperature at which ignition occurs without an ignition source and the material continues to burn without further heat input.
9. Flammable or explosive limits are the upper and lower vapor concentrations at which a mixture will burn or explode. LEL is the lower explosive limit and UEL is the upper explosive limit.
10. Odor data or odor threshold values are used to help determine the warning properties of the chemical (e.g., if it can be smelled at concentrations below health guidelines or standards).

Consideration should also be given to chemical incompatibilities (whether it will react with other chemicals), combustion or decomposition products (hazardous gases), and impurities or other chemicals or additives in the product.

Toxicology

Regardless of the specialization within toxicology, or the types of toxicities of major interest to the toxicologist, essentially every toxicologist performs one or both of the two basic functions of toxicology, which are (1) to examine the nature of the adverse effects produced by a chemical or physical agent (hazard identification function) and (2) to assess the probability of these toxicities occurring under specific conditions of exposure (risk assessment function). Ultimately, the goal and basic purpose of toxicology is to understand the toxic properties of a chemical so that these adverse effects can be prevented by developing appropriate handling or exposure guidelines.

Toxicologists typically divide the exposure of animals or humans to chemicals into four categories: acute, subacute, subchronic, and chronic. Of these four types of exposure, the general public, emergency responders, or nearby workers typically involve a situation in which potential exposure exists on an acute (<24 h) or subacute (days to months) basis. Chronic (months to years) exposure scenarios are unlikely because the majority of the release of hazardous materials is typically contained, neutralized, or remediated in a relatively short period of time. For most hazardous materials, the toxic effects that follow a single exposure are quite different from those produced by repeated exposures. For example, the primary acute toxic manifestation of benzene exposure is central nervous system (CNS) depression, but repeated exposures over a long period of time can cause leukemia. Acute exposure to hazardous materials that are rapidly absorbed into the body in most cases is likely to produce immediate toxic effects. Conversely, in chronic exposure scenarios, some immediate effects may occur after each dose received in addition to the long-term, low-level, or chronic effects of the toxic substance. Thus, emergency responders must understand the specific type of exposure scenario they are responding to in order to identify the potential type of toxicity a chemical may cause in humans.

The major routes (pathways) by which hazardous materials solids, liquids, gases, or vapors gain access to the body are the gastrointestinal tract (ingestion), lungs (inhalation), skin (topical, percutaneous, or dermal), and other parental (other than intestinal canal) routes. Toxic agents generally produce the greatest effect in the shortest period of time when given directly into the bloodstream. However, rarely, if ever, does this route of exposure occur during incidents involving hazardous materials. During a hazmat incident, toxic agents most frequently result from breathing contaminated air (inhalation) and/or direct and prolonged contact of the skin with the substance (dermal exposure). Comparison of the lethal dose of a hazardous material often provides useful information about its extent of absorption. For example, if the lethal dose for an agent by the oral or dermal route is similar to the lethal dose after an intravenous dose, the assumption is that the toxic agent is absorbed easily and rapidly into the body. Thus, emergency responders should evaluate the LD_{50} data (lethal dose to 50% of the test organisms) for an agent using available information such as Material Safety Data Sheets, toxicology literature, or the DOT guide book to help understand the most important routes of exposure they want to protect against. Emergency responders should also look for the concentration of the hazardous material they are dealing with and determine whether the hazardous material is a single chemical or a mixture that contains one or more chemicals at various concentrations.

The pathway by which a substance enters the body determines how much of it enters (rate and extent of absorption) and which organs are initially exposed to the largest concentration of the substance. For example, the water and lipid solubility characteristics of a chemical affect its absorption across the lungs (after inhalation), the skin (after dermal application), or the gastrointestinal tract (after oral ingestion), and the effect differs for each organ. Furthermore, the rate and site of absorption (organ) may in turn determine the rates of metabolism and excretion. So, changing the route of exposure may alter the dose required to produce toxicity, and it may also alter the organ toxicity that is observed.

Some toxic effects of hazardous materials are reversible, and others are irreversible. If a chemical produces injury to a tissue or cell, the ability of that tissue to regenerate largely determines whether the effect is reversible or irreversible. For example, most toxic injuries to a tissue such as the liver, which has a high ability to regenerate, are reversible whereas the injury to the CNS is usually irreversible because the cells of the CNS cannot divide and be replaced.

The general site in the body for toxic action of a hazardous material can be local or systemic. Local effects refer to those that occur at the site of first contact between the hazardous material and the biological system. For example, chlorine gas reacts

with lung tissue at the site of contact, causing damage and swelling of that tissue, and depending upon dose may be fatal even though very little of the chemical is absorbed into the bloodstream. Systemic effects of a hazardous material require absorption into the blood, then distribution of the agent from its entry point to a distant site in the body where adverse effects are produced. Most chemicals (except for highly reactive chemicals) cause systemic effects. Further, most hazardous materials that cause systemic effects do not cause a similar degree of toxicity in all organs. These agents typically elicit a toxic response in only one or two organs. These organs or sites are referred to by toxicologists as the target organs of toxicity. The CNS is the most frequently affected by toxicity from hazardous materials followed by the circulatory system, the blood and hematopoietic system, visceral organs such as the liver, kidney, and lung, and the skin. Muscle and bone are the least frequent target tissues for hazardous materials.

Exposure Limits Used by Emergency Responders

Perhaps the greatest danger faced by the public, workers, or emergency responders is inhalation of gases and vapors caused by the release of hazardous materials to the environment. One of the key tasks in responding to an incident involves identifying, measuring, notifying, evacuating, sheltering, or otherwise protecting populations that may be exposed to such gases or vapors. To accomplish this task, the emergency responder must identify, first, what chemical or chemicals are a human health concern from the spilled product and, second, what are the acceptable levels of these chemicals for workers, the public, etc. Thus, the emergency responder must have a working knowledge of the source and nature of available exposure limits for airborne contaminants as well as an understanding of the strengths and limitations of these exposure limits for their intended use. The following are some recognized sources for toxicology data for airborne contaminants:

1. American Conference of Governmental Industrial Hygienists (ACGIH), threshold limit values (TLVs) time-weighted average (TWA)
2. OSHA, permissible exposure limits (PELs) TWA.
3. National Institute for Occupation Safety and Health Administration (NIOSH), immediately dangerous to life or health (IDLH) levels.
4. American Industrial Hygiene Association (AIHA), workplace environmental exposure limits (WEELs).
5. AIHA emergency response planning guidelines (ERPGs) and temporary emergency exposure limits (TEELs).
6. National Academy of Sciences (NAS)/National Research Council (NRC) emergency exposure guidance levels (EEGLs) and short-term public emergency guidance levels (SPEGLs).
7. EPA National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances.

The ACGIH, through its TLV committee, reviews available data on chemicals to establish exposure limits for employees working in the presence of airborne chemicals/substances. The committee publishes a list of several hundred compounds and recommended exposure limits in a booklet entitled *Threshold Limit Values and Biological Exposure Indices*. The primary purpose of the exposure limits is to protect healthy workers in chronic exposure situations. Even though these limits are intended to prevent toxicity from chronic exposures, they nonetheless provide valuable guideposts for identifying exposure limits that will usually be decidedly safe for short-term acute exposures. Exposure limits established and published by ACGIH are of several different types:

- *TLV – TWA*: The TWA concentration for a normal 8 h workday and a 40 h workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effects.
- *TLV – STEL*: The TWA concentration to which workers should not be exposed for longer than 15 min and which should not be repeated more than 4 times per day, with at least 60 min between successive exposures. This limit supplements the TLV – TWA where there are recognized acute effects from a substance whose toxic effects are primarily of a chronic nature. STELs are recommended only where toxic effects have been reported from high short-term exposures in either animals or humans.
- *TLV-Ceiling (TLV-C)*: The concentration in air that should not be exceeded at any point of the workplace exposure. Ceiling limits may supplement other limits or stand alone.

In addition, the ACGIH occasionally enters the notation ‘skin’ after listed substances. This notation indicates the potential for absorption of the chemicals through the skin, eyes, or other mucous membranes and that such exposure may contribute to the overall exposure. For emergency responders, this notation indicates the need for special protective measures.

The OSHA sets safe and healthy workplace standards. When OSHA was formed, they adopted the then current ACGIH TLV – TWAs and TLV-Cs as occupational exposure limits and made them federal standards. However, instead of calling them TLV – TWAs, OSHA called them PELs. OSHA has both TWA and ceiling values for various chemicals. PELs are listed in Title 29 of the Code of Federal Regulations (CFR), Part 1910, Subpart Z, General Industry Standards for Toxic and Hazardous Substances. Emergency responders should understand that ACGIH and OSHA values are not always the same for each chemical.

NIOSH defines IDLH levels as the maximum airborne contaminant concentration "from which one could escape within 30 minutes without any escape-impairing symptoms or any irreversible health effects." Since, IDLH levels are only intended for emergency situations, their concentration for a particular chemical is considerably higher than OSHA and ACGIH values.

AIHA established a committee to develop WEELs for chemicals which have no current exposure guidelines established by other organizations. There are two WEEL limits for most materials. The first is an 8 h TWA value similar to ACGIH TLV – TWA values. The second, which is only available for a limited number of cases, is a short-term TWA for exposure of either 1 or 15 min duration.

AIHA ERPG values were developed for selected chemicals by a task force made up of several major chemical companies. The results of the task force for ERPG values have three limits for each material:

- *ERPG-3*: The maximum airborne concentration below which, it is believed, nearly all individuals could be exposed for up to 1 h without experiencing or developing life-threatening health effects.
- *ERPG-2*: The maximum airborne concentration below which, it is believed, nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible adverse or other serious health effects or symptoms which could impair an individual's ability to take protective action.
- *ERPG-1*: The maximum airborne concentration below which, it is believed, nearly all individuals could be exposed for up to 1 h without experiencing or developing health effects more severe than sensory perception or mild irritation, if relevant.

In addition, TEELs are provided for 676 chemicals. The TEEL is an interim, temporary, or equivalent exposure limit for which official ERPGs have not yet been developed.

NAS/NRC has published a list of EEGLs and SPEGLs as guidance in advance planning for the management of emergencies.

SPEGLS are concentrations whose occurrence is expected to be rare in the lifetime of any one individual. These values "reflect an acceptance of the statistical likelihood of a nonincapacitating reversible effect in an exposed population while avoiding significant decrements in performance." They are considered concentrations acceptable for public exposure during emergencies.

EEGLs differ from SPEGLs in that they are intended to apply to defined occupational groups.

The National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances is developing AEGLs on an ongoing basis to assist federal and state agencies and private sector organizations with their need for short-term hazardous chemical exposure information. AEGLs represent short-term threshold or ceiling exposure values intended for the protection of the general public, including susceptible or sensitive individuals, but not hypersusceptible or hypersensitive individuals. AEGLS represent biological reference values for this defined human population and consist of three biological end points for each of four different exposure periods of 30 min, and 1, 4, and 8 h.

- AEGL-1 is the airborne concentration (expressed as ppm or $mg\,m^{-3}$) of a substance at or above which it is predicted that the general population, including susceptible but excluding hypersusceptible individuals, could experience notable discomfort. Airborne concentrations below AEGL-1 represent exposure levels that could produce mild odor, taste, or other sensory irritations.
- AEGL-2 is the airborne concentration (expressed as ppm or $mg\,m^{-3}$) of a substance at or above which it is predicted that the general population, including susceptible but excluding hypersusceptible individuals, could experience irreversible or other serious long-lasting effects or impaired ability to escape. Airborne concentrations below AEGL-2 but at or above AEGL-1 represent exposure levels that may cause notable discomfort.
- AEGL-3 is the airborne concentration (expressed as ppm or $mg\,m^{-3}$) of a substance at or above which it is predicted that the general population, including susceptible but excluding hypersusceptible individuals, could experience life-threatening effects or death. Airborne concentrations below AEGL-3 but at or above AEGL-2 represent exposure levels that may cause irreversible or other

serious, long-lasting effects or impaired ability to escape.

In addition to the above emergency response toxicity values for various hazardous materials, various states have developed their own values for acute and chronic situations.

Monitoring and Detecting Hazardous Substances

Detecting and quantifying gases, vapors, aerosols, and particulates during a chemical emergency is essential to protect both response workers and members of the community. Proper air monitoring/sampling can be used to determine a multitude of key questions during a chemical incident including: (1) Can workers safely perform their necessary tasks? (2) Does PPE need to be upgraded or can it be downgraded? (3) Do evacuation zones need to be expanded or can they be reduced?

There are essentially two types of methods used to detect and quantify hazardous chemicals – air monitoring and air sampling. Air monitoring involves the use of direct reading instruments or real-time instruments. These instruments can be used to obtain nearly instantaneous concentrations of a hazardous substance in the field. Direct reading instruments generally have a short response time from seconds to minutes, depending on the type of instrument. Results are usually displayed on an LCD screen or a color change can be compared with a calibrated scale as in the case of colorimetric detector tubes. Many direct reading instruments are capable of data logging, which alleviates the need for the user to hand log every reading and allows more detailed statistical analyses downstream. There are many different subclasses of real-time instrumentation such as photoionization detectors (PIDs), flame ionization detectors, combustible gas instruments, infrared spectrophotometers, ultraviolet (UV) spectrophotometers, electrochemical sensors, and colorimetric devices. Direct-reading instruments are powerful tools in the field because important decisions can be made in a relatively short time frame.

Direct-reading instruments can be used to monitor a specific chemical or can be used to monitor multiple chemicals. All direct-reading instruments are designed to operate in a specific detection range, which depends on the manufacturer and the properties of the chemical. All direct-reading instruments should be calibrated prior to performing any type of analysis regardless of the circumstances in which they will be used. It is also important to pay close attention to the particular manufacturer's technical notes regarding the instrument. For example, every PID operates on the same fundamental principle; however, correction factors for a specific chemical could vary greatly between instruments by different manufacturers.

As opposed to air monitoring, air sampling involves collecting an air sample for subsequent analysis by a laboratory using one or multiple methods such as ion chromatography, gas chromatography, or high-performance liquid chromatography. Air sample collection can be performed using several methods and mediums. These mediums include sorbent tubes, filter cassettes, Summa™ canisters or mini cans, and impingers. The method used to collect an air sample depends on the properties of the chemical being studied and the goal of the study. With the exception of the Summa™ canister, a sampling pump is used to pull air through a medium, such as a sorbent tube, at a determined flow rate. The sampling pump is calibrated both before and after the sampling event and an average flow rate is obtained. The flow rate along with the total sampling time is used to determine the volume of air that has passed through the medium and thus a concentration can be determined.

Summa™ canisters are evacuated cylinders that allow the attachment of regulators that collect air over a preset time period. Common regulators are instantaneous (grab), 8 h, and 12–8 h regulators.

Industrial Hygiene Issues

Emergency personnel responding to an incident are generally focused on a variety of concerns such as controlling a leaking or venting container, evacuating the area, or assessing the situation. When performing these activities, they are often exposed to a variety of hazards. These hazards can be classified as chemical, physical, radiological, or biological.

Hazards associated with exposure to chemicals are usually the primary concern. However, other hazards may be more of a concern than the potential effects from short-term exposure to a chemical. Physical hazards include thermal stress, noise, fire and explosion, fatigue, and general safety concerns such as slips, trips, and falls, fall protection, and moving vehicles. Thermal stress (heat more so than cold) is almost always a concern for responders, particularly when working in protective clothing designed to shield them from skin contact with chemicals. In warm weather, moderate work activity in protective clothing can cause a rapid increase in the core temperature. In cold weather, the protective clothing often traps heat and insulates the responder from the cold. If the protective clothing is removed outdoors,

perspiration can lower the core temperature and lead to hypothermia and frostbite. Noise from heavy equipment and remediation activities can exceed occupational levels. When working around chemicals, there are always concerns about fire and explosion. Responders are typically confronted with walking on uneven, wet, and slippery terrain. This coupled with carrying equipment and wearing protective clothing increases the likelihood of a fall. It is common for responders to work at elevated heights or below grade tasks. These conditions increase the chances of injuries to personnel or from falling objects. During most incidents the amount of vehicle and equipment traffic increases and responders must always be concerned about these potential hazards. Additionally, responders often work long hours without adequate rest between shifts and fatigue has the potential to magnify each of these potential hazards.

Air monitoring should be performed on hazmat personnel to ensure that workers are not overexposed to chemicals and to comply with applicable regulations such as the OSHA standard. Note that some chemicals have substance-specific standards that need to be followed during an incident. The OSHA has established substance-specific standards for 30 chemicals that they have identified as unique and in need of specific guidance. Employees potentially exposed to a chemical with a specific standard must be monitored and protected in accordance with that chemical's specific standard. A substance-specific standard may require integrated air monitoring for 8 h TWA exposure, STEL monitoring, real-time air monitoring, or various forms of biological monitoring.

Regulations regarding the use and transport of ionizing radioactive material help to minimize the likelihood of encountering radioactive materials during an incident. However, responders should be familiar with the radiation symbol and ask if there is any potential for exposure to ionizing radioactive materials. A more likely hazard is exposure to nonionizing radiation, in particular UV radiation from the sun that can cause sunburn to unprotected skin and eye damage in a short amount of time. Other nonionizing radiation activities that responders may encounter include welding and the use of devices that use lasers.

Because of recent events, the awareness of responders to biological hazards associated with weapons of mass destruction has increased. These biological agents are certainly a matter of concern, but other biological hazards exist that responders are more likely to encounter. These include poison plants, biting and stinging insects, reptiles, and infections of cuts and scrapes. Responders who administer first aid should be trained in universal precautions for protection against blood-borne pathogens. Additionally, responders should practice good personal hygiene to protect themselves from exposure to infectious agents in water and on surfaces typically transmitted by hand-to-mouth contact.

Overview of Consequence Analyses for Emergency Planning and Response

Mathematical models provide emergency planners and emergency responders a tool to effectively evaluate hazards, plan for emergencies, and respond appropriately to incidents. With constant improvement in computer speed and network access speed, this field is one which has been experiencing tremendous growth over the last 5 years, and one which will likely continue to morph rapidly for years to come.

Consequence analyses for emergency planning generally take the form of risk assessments. Risk assessments utilize time-averaged data or probability curves to determine the likelihood that a situation poses a hazard. For example, to determine the hazard posed by a chemical facility to the surrounding area, one would compile meteorological data for the area, assess the chemical hazards stored at the facility, locate sensitive population zones, identify potential evacuation routes, and so on – defining as many variables as possible based on available data. Variables that fluctuate over time, such as wind speed or direction, can be defined by time-averaging data, or by generating a probability distribution, and utilizing values from the distribution proportionally over a number of model runs. Monte Carlo model simulations combine probability distributions of multiple variables and account for all combinations of those variables by running multiple scenarios, and essentially averaging the result.

Due to the wide range of variables present in emergency planning, computer models used for emergency planning require a great deal of time for development of input parameters. Examples of EPA-approved models which can be used for long-term planning are the Industrial Source Complex (ISC), CAL-PUFF, SCREEN3, ROADS, and MOBILE5. Each of these models is designed to address specific nuances of potential hazards. For example, it is possible to model the dispersion of biological agents (particulate matter) with ISC, while some other models do not have that capability.

Consequence analyses for emergency response address specific issues resulting from an incident. This type of consequence analysis includes what-if scenarios (e.g., what if a railcar experiences a boiling

liquid expanding vapor explosion due to an impinging fire) and hazard assessment during the early minutes of an incident. The goal of emergency response modeling is to aid the first responder in critical decision making, such as when to shut down roads or stand down evacuations.

Unlike modeling for emergency planning, emergency response modeling utilizes on-site information specific to an ongoing incident. On-site information such as maps, meteorological data, topographical data, and others must be readily available to the model, and easy to input. Time is of the essence when modeling during an emergency. Models that are used for this purpose include ALOHA, DEGADIS, and SLAB (dense gas models), SAFER STAR™ (for mobile sources), SAFER REAL-TIME™ (for fixed facilities), SEVEX, and others.

Establishing and Managing Hazard Zones

In establishing a hazardous materials emergency response, three hazard zones should be established, namely, the exclusion, contamination reduction, and support zones. In the exclusion zone, a high level of contamination is present and overexposure, without the use of PPE, is likely. Therefore, PPE is typically required. Personnel, with the exception of skilled support personnel, must be trained. The exclusion zone may be activity-specific for operations that can generate high levels of contaminants.

The contamination reduction zone is the area between the highly contaminated area and the noncontaminated area. Reasonably, the decontamination area is often located in the contamination reduction zone. PPE in the contamination reduction zone is usually one level of protection below that of the worker in the exclusion zone. Because airborne levels within the contamination reduction zone can be unpredictable and can change quickly, evacuations within this area is recommended.

The support zone is where the majority of the incident command structure is located. No exposures are likely in the support zone. No training or PPE is required in the support zone.

The zones are determined by using air monitoring and by identifying the type of operations to be conducted. Natural barriers, such as roads, building, and trees, are a good way to define where the zones should be located. If natural barriers are not available, barrier tape can be used to demarcate the different zones.

Triage and Medical Case Management

Sorting and prioritizing patients for treatment is referred to as triage. Triage establishes the organizational framework to provide the greatest good for the greatest number of casualties. The word triage comes from the French word 'trier' meaning to sort or to cull. Triage is not a static process but one that occurs at every level of medical care and preferably several times. The first triage performed will be at the scene of the incident, the second upon arrival at a medical facility and subsequently with each patient encounter in the medical treatment process.

In order for healthcare providers to triage chemically exposed casualties effectively and appropriately, they must be provided with accurate and timely information about the chemical release. This information should include a description of the chemical of concern including synonyms, physical properties, and applicable exposure standards. In addition, healthcare providers will need pertinent toxicological information on the potential routes of exposure involved, target organ systems, dose–response relationships, potential exposure sequelae, the risk, if any, of secondary exposure to healthcare staff and facilities, and recommended medical procedures to be followed for exposed individuals.

An important component of responding to a significant chemical incident is to prepare and provide a Public Health Statement/Patient Information Sheet to the healthcare facility. The healthcare provider can then disseminate this objective toxicological information to all treated individuals and the community at large to both educate and allay fears within the local population.

Healthcare facilities need to expect that in case of a chemical event, many people are likely to self-evacuate and present to medical facilities. In order to maintain some control, it is recommended that all access and egress at treatment facilities be controlled and monitored to prevent contamination of noncontaminated individuals and facility areas. It is helpful to have law enforcement involved to provide security and crowd control.

Site Remediation

Site remediation is the environmental cleanup of the site after the emergency has been completed. Gross contamination should be removed and properly disposed in accordance with applicable state and federal regulations. Cleanup standards are negotiated with the applicable state or federal agencies.

Postresponse Recovery

Before an incident is stood down and people return to their homes and businesses, incident command

needs to be prepared to address questions such as: What was the chemical(s)? How can it affect my health? Is the air safe? Is my drinking water safe? Is my food safe (there may have been a power outage)? What about food left out on the table? Do I need to clean my house? What about my pet? Is it okay to turn on my air-conditioner and return to normal household activities? This information can be provided in a handout or at a public meeting. Also, consider providing a list of contacts and phone numbers for people to call in case they have questions regarding their health and property. After the emergency incident has been mitigated and people have returned to their homes, it is important also to address medical concerns and public relations, including risk communication. The command's public information team should continue to provide information to the public on the risks associated with the type and quantity of the material released during the incident, the status of the environmental remediation, and the amount of material still in the environment. Following the above guidelines can help restore the community to normal operations with minimal disruption to people's lives.

See also: Chemical Hazard Communication and Material Safety Data Sheets; Environmental Protection Agency, US; Flavor and Extract Manufacturers Association; Hazard Identification; Hazardous Waste; Occupational Safety and Health Administration.

Relevant Websites

http://www.bt.cdc.gov – Emergency Preparedness and Response (from the US Centers for Disease Control and Prevention).
http://www.epa.gov – Emergency Response (from the US Environmental Protection Agency).
http://hazmat.dot.gov – Emergency Response Guidebook (from the Office of Hazardous Materials Safety, US Department of Transportation).
http://bookstore.gpo.gov – Emergency Response Publications (from the US Government Printing Office).
http://www.fema.gov – Federal Emergency Management Agency.
http://www.csb.gov – US Chemical Safety and Hazard Investigation Board.

Endocrine System

Karen Chou

This article is a revision of the previous print edition article by Karen Chou and Barbara Salem, volume 1, pp. 531–535, © 1998, Elsevier Inc.

Overview of the Endocrine System

The life of a multicell organism requires coordination between organs and tissues. The endocrine system, composed of ductless secretory organs and structures, maintains this coordination by producing hormones in response to physiological and environmental changes. Hormones are discharged into the blood and lymphatic system, and transported to other parts of the body, including other hormone-secreting organs, in order to elicit characteristic cellular responses in target organs. The major endocrine organs include the hypothalamus, pituitary, thyroid, parathyroid, pancreas, adrenal, ovary, and testis. Other endocrine organs and tissues include the placenta, liver, kidneys, and cells throughout the gastrointestinal tract (**Figure 1**).

Hormones can be glycoproteins, polypeptides, peptides, steroids, or modified amino acids. They function as messengers traveling through the bloodstream to target tissues and organs, where they bind to surface or nuclear receptors and regulate gene expression, ion channels, or enzyme activities. The major target organs and tissues include mammary glands, reproductive organs, bone, muscle, and the nervous system.

Endocrine organs function to maintain a relative balance of cellular parameters in the body through the hormones they produce. Hormones are produced in response to changes in the external environment and the internal physiological status. Examples of external signals include light cycles, temperature, nutrient availability, and toxicants. Disease, growth, and reproduction are examples of internal factors that accompany changes in hormonal balance. The intensity of the endocrine effect on the target organ depends on the amount of hormones produced and the binding property, amount, and response of receptors in the target cells.

Target cells differ from nontarget cells by the presence of hormone-specific cell receptors. A receptor, therefore, is a signal discriminator capable of binding to a specific hormone and translating its message into specific cellular responses. These responses regulate protein phosphorylation, cell growth, gene expression, enzyme activity, nutrient metabolism, mineral release/retention, and cell death. Hormones also modulate responses of the immune and central nervous systems.

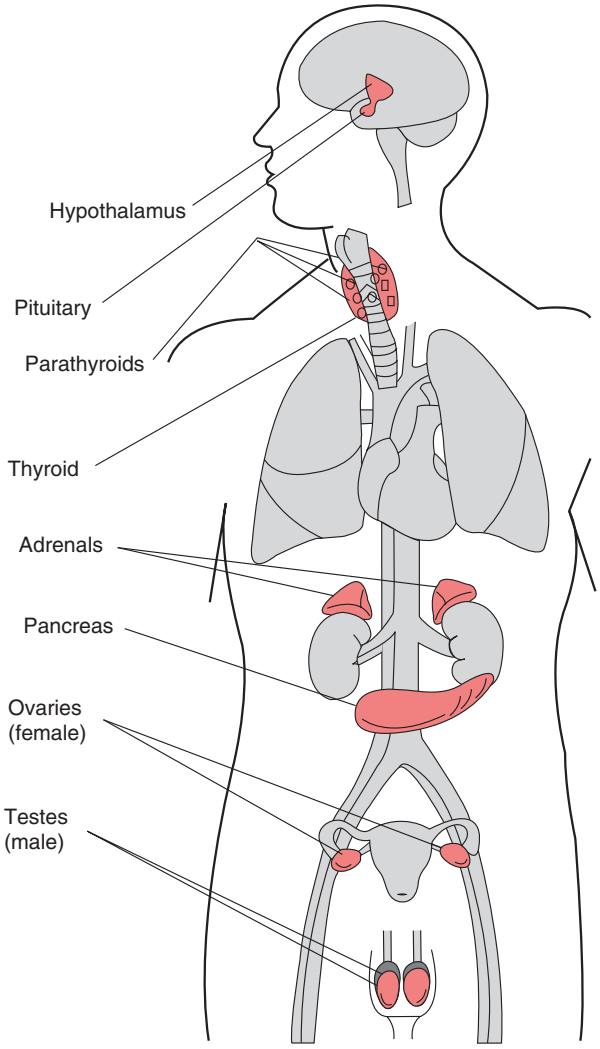


Figure 1 Major endocrine organs in the body.

Maintenance of Homeostasis

Hormones are responsible for maintaining physiological and cellular homeostasis that are essential for reproduction, growth/development and or neurobehavioral functions. The production of any hormone in the endocrine system is the result of an entire chain of events involving precisely choreographed interactions of many other endocrine organs. For example, the initiation of testosterone and estrogen production can be traced to the release of gonadotropin releasing hormone (GnRH) from the hypothalamus in the brain. GnRH stimulates the pituitary gland to produce luteinizing hormone (LH) and follicle stimulating hormone (FSH), which in turn act on the testis and ovary to stimulate testosterone and estrogen production, respectively.

When there has been a sufficient cellular response in the target cell, negative feedback, a control mechanism, relates this information back to the organ that

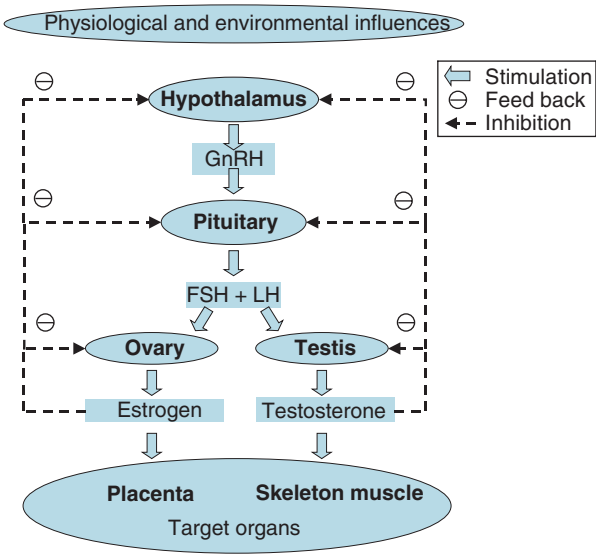


Figure 2 Examples of feedback control mechanisms in hormone homeostasis.

produced the hormone, inhibiting further hormone production (**Figure 2**). This mechanism prevents overstimulation and modulates the stability of the cellular status. For example, in the case of testicular function, sufficient amounts of testosterone in the blood will send feedback signals to the hypothalamus and the pituitary gland. This will reduce the production of GnRH, LH, and FSH, thus decreasing the signals for further testosterone production and release.

Mechanisms of Endocrine Disorders

Substances with the ability to modulate the endocrine system do not necessary pose any health risk for humans and other organisms. In fact, humans and animals are constantly exposed to substances in food and other environmental media that interact with the endocrine system. In general, due to precise yet adaptable control mechanisms, and the intertwined nature of the hormonal balance, moderate amounts of chemical effects on hormones seldom compromise normal physiological functions. Fluctuations of hormone concentration and receptor activities, by design, absorb environmental and physiological challenges in order to maintain functional equilibrium in the body. Only when the equilibrium control mechanisms are overwhelmed do deleterious effects occur.

Another consequence of the interdependent nature of the endocrine system is that manifestation of an endocrine disorder is virtually always associated with changes in synthesis or concentration of multiple hormones. For example, in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-treated rats, the decrease in thyroid

hormone 3,5,3′,5′-tetraiodothyroxine (T_4) is always associated with an increase in the blood concentration of thyroid stimulating hormone (TSH), which is secreted by the pituitary gland in response to the low blood T_4.

Altered hormone concentrations in response to chemical exposure could be an adaptation response. It alone, therefore, is not a sufficient indicator of toxicity. Endocrine toxicity is characterized by disease conditions in the host in addition to hormonal changes.

Endocrine Disruptors

Dysfunction of the endocrine system could be due to either hyperfunction (excessive hormone production or responses) or hypofunction (insufficient hormone production or responses). Environmental chemicals that have the potential to perturb the endocrine system are known as endocrine or, synonymously, hormone disruptors.

The term endocrine disruptors was first used by Theo Colborn and Peter Thomas in 1992. In 1996, the US Environmental Protection Agency (EPA) convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee to make recommendations to EPA concerning endocrine disruptors. The term 'endocrine disruptors' has been used interchangeably with hormonally active agents and endocrine modulators. As the term is used now, endocrine disruptors include any substance that affects the synthesis, secretion, transport, binding, action, inactivation, or elimination of natural hormones in the body.

Excessive amounts of hormones in circulation may be due to overproduction of hormones in the endocrine organs, rapid release of hormones from storage, decreased hormone metabolism, or altered rate of clearance and excretion of hormones. On the other hand, cell injury in hormone-producing tissues, inhibition of synthetic enzymes, and induction of metabolic enzymes are causes of hormone deficiency.

Examples of Alterations in Endocrine System

Hormone Synthesis

Hormone synthesis can be altered by changes in the size and population of hormone producing cells, modification of the activity of hormone synthesizing enzymes, lack of precursors, or interference with enzyme cofactors such as divalent cations. For example, the fungicide fenarimol inhibits the enzyme aromatase, which converts testosterone to estrogen. Exposures to acrylamide monomer induce changes in the volume of cellular components in the thyroid gland, thus resulting in an increase in the thyroid hormone thyroxine (T4) and a decrease in TSH in blood.

Cadmium decreases testosterone production by preventing the synthesis of cholesterol, a precursor of all steroid hormones. Other chemicals that interfere with steroid hormone synthesis include aminoglutethimide, cyanoketone, and ketoconazole. Copper chelating compounds, such as dithiocarbamates, metam sodium, and carbon disulfide, suppress the conversion of dopamine to norepinephrine and subsequently to epinephrine.

Storage and Release

In addition to synthesis, the release of hormones from the storage compartment in cells also controls the amount of hormones in circulation. Reserpine and amphetamine are examples of compounds that affect hormone storage in granular vesicles. Compounds that activate LH receptors could potentially cause hypersecretion of testosterone from the Leydig cells (site of testosterone synthesis in the testes). On the other hand, direct cell injury of secretory cells may cause hyposecretion.

Carrier Proteins

Most lipid-soluble hormones in the blood are bound to specialized carrier proteins. The availability of hormones for physiological functions depends on the total concentration of the hormone as well as the amount of hormone existing in the free state; protein-bound hormones are not readily available for receptor binding. While lack of carrier proteins could impair the transport of hormones to target organs, excessive amounts may decrease the availability of free hormones.

Some estrogenic compounds (compounds with estrogen activity) are known to increase the amount of testosterone–estrogen-binding globulin (TEBG), a sex hormone carrier protein, while high doses of androgens and glucocorticoids may decrease the TEBG concentration in plasma. Salicylates and diphenylhydantoin have been shown to cause changes in thyroxine-binding globulin, a thyroid hormone carrier protein, thus modifying the amount of free circulating thyroxine.

Receptor and Ligand Interactions

Endocrine disruptors often are structural analogs of endogenous hormones (hormones produced naturally in the host). Hormone analogs may act like the endogenous hormone if the analog–receptor complex in the target cell mimics the function of the hormone–receptor complex. Hydroxy metabolites of both *o,p′*-DDT and methoxychlor bind to estrogen

receptors and cause estrogenic effects in birds and reptiles. Alkyl phenols, the biodegradation products of alkyl phenol ethoxylates, bind to estrogen receptors in fish and human cells *in vitro* and induce estrogenic effects in rats. Some hydroxy metabolites of polychlorinated biphenyls (PCBs) are also estrogenic. The receptor-binding property of diethylstilbestrol (DES), a synthetic estrogen, is implicated in the detrimental effects on the reproductive system of men and women exposed to DES *in utero*.

Hormone analogs can also act like antagonists, if they compete with endogenous hormones at the receptor binding site, but elicit no cellular response. An example of such an estrogen antagonist is tamoxifen, which binds competitively to the estrogen receptor and alters the effectiveness of the hormone–receptor complex in regulating gene expression. Vinclozolin, a dicarboximide fungicide, is an androgen antagonist; its metabolite blocks the androgen receptor. Similarly, the major DDT metabolite, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene(*p*,*p*′-DDE), acts as an antiandrogen in rats, causing abnormalities in male sexual development.

Many endocrine disruptors can bind to more than one type of receptor. For example, *o*,*p*-DDT and chlordecone bind to both estrogen and progesterone receptors, while nonylphenol and EPTE, a metabolite of methoxychlor, bind with the same affinity to estrogen, progesterone, and androgen receptors. In addition, environmental estrogenic chemicals, as well as endogeneous estrogens, can exert rapid actions on nonestrogen receptors on cell membranes, such as receptors shared by neural transmitters, dopamine, epinephrine, and norepinephrine.

Endocrine disruptors may also interfere with hormone function by altering the nature of the receptors or interfering with interactions between the hormone–receptor complex and genes or other cellular components. For example, TCDD and some of the PCBs that resemble the chemical structure of TCDD act as antiestrogens by decreasing the sensitivity of estrogen receptors to estrogen. In such cases, despite adequate estrogen production, organisms respond as if in an estrogen-deficient condition. In addition, under normal conditions, the hormone–receptor complex elicits cellular responses by regulating gene expression. Another type of endocrine disruptor interferes with the interaction between this hormone–receptor complex and DNA.

Metabolism and Clearance

Chemicals that cause either induction (increased synthesis) or degradation of hormones are also potential disruptors of the endocrine system. Several liver enzymes, including cytochrome P450 enzymes, inducible by drugs and environmental pollutants, are involved in hormone clearance. For example, DDT and similar compounds are potent inducers of cytochrome P450-dependent monooxygenases, an enzyme system that degrades endogenous androgens. These compounds, therefore, potentially have antiandrogenic activity. Likewise, lindane has been reported to decrease the amount of circulating estrogen by increasing estrogen clearance.

It has also been hypothesized that long-term hormone imbalance can induce cancers in endocrine-sensitive organs, such as gonads, adrenals, thyroid, prostate, and breast. Lipid-soluble compounds are of special concern because they are retained in the body, therefore causing a long-term effect. Some of these chemicals are known cytochrome P450 enzyme inducers, such as PCBs, DDT, and butylated hydroxytoluene, and have been implicated in cancers in the adrenals, uterus, and thyroid.

UDP-glucuronosyltransferase, an enzyme that conjugates UDP-glucuronic acid with T_4 and other steroid hormones, can be induced by TCDD via an aryl hydrocarbon receptor-dependent mechanism. In rats, TCDD exposure has been shown to increase the rate of removal of T_4 from the blood.

Structurally similar compounds can also compete with endogenous hormones for the binding sites of metabolic enzymes and make the enzyme unavailable for normal hormone degradation. This would lead to a decreased clearance rate and prolong the half-life of circulating endogenous hormones.

Cell Differentiation

Other hypotheses suggest that *in utero* and neonatal exposure to estrogenic compounds may affect cell differentiation and alter cellular responses to sex hormones later in life, resulting in cancer. This mechanism is probably involved in the development of vaginal cancer in women whose mothers were given large doses of diethylstilbestrol during pregnancy. In this case, the manifestation of vaginal cancer was not evident until years after exposure. Chronic low-dose exposure to other endocrine disruptors has been associated with thyroid, testicular, and mammary tumors in human populations. The cause-and-effect relationship for most of the associations, however, is yet to be confirmed at the biochemical and molecular levels.

Endocrine Organs as Targets of Radiation

Endocrine organs can also become the target of physical agents, such as radiation. This is especially

true if a radioactive compound is actively taken up through normal mechanisms and concentrated in the organ. For example, the inorganic iodine in the body is largely taken up by the thyroid in connection with the synthesis of thyroid hormone. More than 20% of iodine in the body is found in the thyroid, an organ which weighs less than 0.005% of the total body. Almost 10 years after the radioactive fallout from the 1986 Chernobyl nuclear power plant explosion, a more than 10-fold increase in the incidence of childhood thyroid cancer in Belarus, the Ukraine, and the Russian Federation was observed. These countries received most of the radioactive fallout. Although many toxic and radioactive compounds were released through the explosion, the geographical distribution of the cancers most closely matches the pattern of fallout from the radioactive iodine. It is also evident through this accident that children are much more susceptible to thyroid cancer caused by radioactive iodine than adults.

Radiation can also cause male infertility, impotency, decreases in sperm fertilizing ability, and damage to the process of sperm formation. These disorders have been observed in victims of the Chernobyl accident as well as in patients treated with radiotherapy. In laboratory rats, the damage to sperm formation can be alleviated by treatment with testosterone and estrogen, indicating an etiology of sex hormone imbalance.

Multiple Targets of Endocrine Disruptors

The health effect of endocrine disruptors is further complicated by the fact that an endocrine disruptor or a family of endocrine disruptors may have multiple mechanisms of actions. For example, PCBs may mimic estrogen, prevent binding of thyroid hormone to thyroid binding globulin, and accelerate the metabolism and excretion of several steroid hormones.

See also: Androgens; Diethylstilbestrol; Environmental Hormone Disruptors; Radiation Toxicology, Ionizing and Nonionizing; Reproductive System, Female; Reproductive System, Male; Toxicity Testing, Reproductive.

Further Reading

Chia SE (2000) Endocrine disruptors and male reproductive function – A short review. *International Journal of Andrology* 23(Suppl. 2): 45–46.

Klaassen CD (2001) *Casarett and Doull's Toxicology, The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.

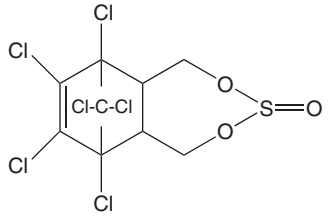
Sonnenschein C and Soto AM (1998) An updated review of environmental estrogen and androgen mimics and antagonists. *Journal of Steroid Biochemistry and Molecular Biology* 65(1–6): 143–150.

Thomas JA and Colby HD (1996) *Endocrine Toxicology*, 2nd edn. Washington, DC: Taylor and Francis.

Endosulfan

Elmar Udarbe Zamora

- Chemical Abstract Service Registry Number: CAS 115-29-7
- Synonyms: α- and β-Endosulfan; Thiodan® (Australia)
- Chemical/Pharmaceutical/Other Class: Organochlorine insecticide
- Chemical Formula: $C_9H_6C_{l6}O_3S$
- Chemical Structure:



Endosulfan

Uses

Endosulfan is a cream to brown-colored solid that may exist in the form of crystals or flakes. Endosulfan is used as an insecticide for food crops such as grains, tea, fruits, and vegetables as well as on cotton and tobacco. It is also used as a wood preservative.

Exposure Routes and Pathways

Humans can be exposed through ingestion, inhalation, and dermal contact. Consuming contaminated food and water, direct skin contact with contaminated soil, smoking cigarettes made from tobacco with endosulfan residues and inhalation of the vapors during its manufacture and spray applications are possible avenues of exposure.

Toxicokinetics

In animals, ~80% is absorbed through the oral route and 20% through the dermal route. Three

weeks after repeated (21 days) dosing in mice, the highest concentrations were found in the liver and spleen. Little residue was found in the kidneys and fat, and there was no accumulation of radiolabeled endosulfan residues. Microsomal enzymes degrade it to polar and nonpolar metabolites including endosulfan sulfate, diol, α-hydroxy endoether, and endolactone. Excretion occurs through both urine and feces, but fecal elimination is predominant. The diol appears to be eliminated primarily via urine. Although a minor elimination route, lactating women can excrete endosulfan through breast milk.

Mechanism of Toxicity

The toxic effects of endosulfan during acute exposure are primarily due to its effects on the central nervous system. In animals, it has been shown to affect membrane permeability and glucose metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs given 200–500 mg kg^{-1} orally exhibited salivation, vomiting and generalized tonic and clonic convulsions. In general, piloerection, salivation, hyperactivity, respiratory distress, diarrhea, tremors, hunching, and convulsions can be elicited. Respiratory arrest and renal failure can occur with high exposures. A decrease in red blood cell count and hemoglobin content were also observed. The oral LD_{50} in rats is 18–220 and 74 mg kg^{-1} through the dermal route. The LC_{50} for rats exposed for 4 h through inhalation is 0.34–0.76 mg l^{-1}.

Human

Endosulfan can be lethal if large amounts are inhaled, swallowed or absorbed through skin. Contact may cause burns to skin and eyes. Depression, disorientation, headache, vomiting, dizziness, and tremors are the first signs seen 20 min to 12 h after ingestion of endosulfan. Respiratory arrest and renal failure appear to contribute to death.

Chronic Toxicity (or Exposure)

Animal

Long-term exposure can potentially damage kidneys, testes and liver. It may also compromise immunological mechanisms against disease and infection. It is not genotoxic and no toxic effects in the reproductive system were seen in animals. It also does not disrupt the endocrine system.

Human

There are no data to verify if the above chronic effects seen in animals are also seen in humans. No epidemiological studies of cancer have been conducted.

In Vitro Toxicity Data

Endosulfan was not mutagenic in the Ames test or mammalian mutagenic assays and was negative in clastogenesis and micronucleus assays *in vitro* and *in vivo*.

Clinical Management

In case of inhalation, the victim should be moved to fresh air and emergency medical care called. If not breathing, artificial respiration should be administered. In case of direct contact, the skin and eyes should be flushed with running water for at least 15 min to remove the chemical as soon as possible. Contaminated clothing and shoes should be removed and isolated at the site. Normal body temperature should be maintained and the victim kept quiet. Vital signs should be monitored as the onset of toxic effects of endosulfan might be delayed.

Environmental Fate

Endosulfan enters the air, soil, and water during its manufacture and during field spray applications. During spraying, endosulfan may travel over long distances before reaching the crops, water, and soil. It takes a few weeks for endosulfan on the crops to be degraded. For residues in soil particles, it may take years for complete degradation.

Ecotoxicology

Endosulfan can accumulate in bodies of animals that live in contaminated water. It is highly toxic to many aquatic fishes. Male, 3–4-month-old Mallard ducks showed tremors, high carriage, wings crossed at the back and tail pointed down. In August 1995, more than 240 000 fish in Alabama, United States, were killed due to a run-off from cotton fields contaminated with endosulfan.

Other Hazards

Endosulfan is a combustible fluid and should be kept away from sources of ignition. It should be kept in a

cool, well ventilated area and not exposed in the sun for prolonged periods. It is incompatible with strong oxidizing agents like chlorine and permanganate. Chemicals like sulfurous oxides and chlorine compounds may be produced after decomposition. Protective clothing should be worn during handling and manufacture.

Exposure Standards and Guidelines

The Environmental Protection Agency sets upper limits of not more than 74 ppb in waterways and not more than 0.1–2 ppm on raw agricultural products.

The Food and Drug Administration sets that not more than 24 ppm should be present on dried tea.

The time-weighted average set by the American Conference of Governmental Industrial Hygienists for endosulfan is $0.1\,\mathrm{mg\,m^{-3}}$.

The Australian and New Zealand Environment and Conservation Council sets the water quality guideline not to exceed $0.01\,\mu\mathrm{g\,l^{-1}}$ in unfiltered water. (Note: Yes, originally, the limit was set at $0.1\,\mu\mathrm{g\,l^{-1}}$. In 1997, they changed it to $0.001\,\mu\mathrm{g\,l^{-1}}$ but detection is only up to $0.01\,\mu\mathrm{g\,l^{-1}}$ so it has been expressed as is.)

See also: Pesticides.

Further Reading

Agency for Toxic Substances and Disease Registry (ATSDR) (2000) Toxicological Profile for Endosulfan. Update. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

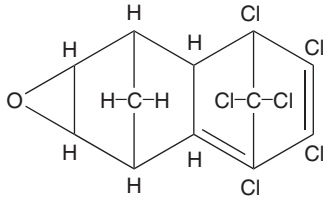
Relevant Websites

http://www.epa.gov – United States Environmental Protection Agency.
http://www.macquarie2100.org.au – Macquarie Valley Landcare Group, Inc.

Endrin

Benny L Blaylock

- Chemical Abstracts Service Registry Number: CAS 72-20-8
- Synonyms: 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-1,4-*endo*,*endo*-5,8-dimethanonaphthalene; Endrex; Hexadrin
- Chemical/Pharmaceutical/Other Class: Organochlorine
- Chemical Structure:



Uses

Endrin is used as an insecticide. The use of endrin has been significantly restricted in the United States and several other countries. There are currently no Environmental Protection Agency registrations for endrin.

Exposure Routes and Pathways

The most important exposure routes for endrin are oral and dermal.

Toxicokinetics

Endrin is absorbed through the gastrointestinal tract, respiratory tract, and through intact skin.

Endrin is metabolized by liver microsomal enzymes. In all species, oxidation of the methylene bridge in endrin to *syn*-, but mostly *anti*-12-hydroxyendrin occurs, followed by dehydrogenation to 12-ketoendrin, a more toxic metabolite than the parent compound. Hydroxylated metabolites are conjugated as glucuronides and sulfates.

Unlike other organochlorine insecticides, endrin has not been found in fat samples taken from general surveys of exposed humans. In addition, it has not been found in the blood of endrin workers with the exception of recent gross accidental exposure.

The parent compound and its metabolites have been identified in both feces and urine.

Mechanism of Toxicity

Like other cyclodienes, endrin resembles picrotoxin, an antagonist of a postsynaptic receptor for the inhibitory neurotransmitter γ-aminobutyric acid

(GABA). The binding of GABA to this receptor, called the $GABA_A$ receptor, stimulates influx of Cl^- ions, which hyperpolarizes the cell and makes it more resistant to depolarization. Thus, these insecticides promote excitotoxicity by blocking the stimulation of Cl^- influx by GABA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal toxicity with endrin is similar to that of other organochlorine cyclodiene insecticides. The oral LD_{50} in rats is 7–43 mg kg^{-1} while in mice it is 1370 μg kg^{-1}. Animal studies confirm most health effects are to the central nervous system (CNS).

Human

Endrin is more highly toxic than other organochlorine insecticides. The major target is the CNS. Major symptomatology is exemplified by rapid onset of violent epileptiform convulsions in severe poisoning cases. The onset may occur as rapidly as 0.5 h or delayed as much as 10 h after ingestion of contaminated food. Other symptoms include headaches, dizziness, nervousness, confusion, nausea, and vomiting.

Chronic Toxicity (or Exposure)

Animal

Groups of three to seven dogs per sex were fed diets containing 0.1, 0.5, 1.0, 2.0, or 4.0 ppm endrin for 2 years. Dogs receiving 2 or 4 ppm experienced occasional convulsions, slightly increased relative liver weights, and mild histopathological effects in the liver (slight vacuolization of hepatic cells). No adverse effects on these parameters or on growth, food consumption, behavior, serum chemistry, urine chemistry or histological appearance of major organs occurred at 1 ppm (no-observed-effect level) or less. The 2 ppm level is the lowest-observed-adverse-effect level.

Animal studies have also shown exposure to endrin can cause birth defects, especially abnormal bone formation.

Human

Endrin is not classifiable as to its carcinogenicity to humans by International Agency for Research on Cancer. No long-term health effects have been noted in workers who have been exposed to endrin by inhalation or skin contact.

Clinical Management

Management of endrin poisoning is symptomatic. Diazepam or phenobarbital is used to control convulsions. In severe cases, mechanically assisted breathing may be necessary as well as administration of succinyl-choline for muscle relaxation and control. Activated charcoal as a slurry has been reported to absorb endrin and increase its rate of excretion after oral exposure. Emesis is not recommended due to potential CNS depression or seizures.

Environmental Fate

Endrin is very persistent, but it is known to photodegrade to delta-ketoendrin (half-life 7 days). Endrin released to soils will persist for extremely long periods of time (up to 14 years or more). Biodegradation may be enhanced somewhat in flooded soils or under anaerobic conditions. Its low water solubility and strong adsorption to soil makes leaching into groundwater unlikely. However, the detection of endrin in certain groundwater samples suggests that leaching may be possible in some soils. Endrin's low vapor pressure suggests only limited evaporation from soil. However, several studies have suggested that moderate to extensive loss of endrin from soils and crops was due to evaporation. Runoff from rain or irrigation of particle-associated endrin will carry particle-associated endrin to water systems.

Endrin released to water systems will not hydrolyze or biodegrade. It will be subject to photoisomerization to ketoendrin. It will extensively sorb to sediment. Evaporation from water will not be significant.

The fate of endrin in the atmosphere is unknown, but it probably will be primarily associated with particulate matter and be removed mainly by rainout and dry deposition.

Ecotoxicology

Endrin is very toxic to fish, aquatic invertebrates, and phytoplankton (96 h LC_{50} for fish, aquatic invertebrates, and phytoplankton mostly below 1 μg l^{-1}). Fish kills were observed in areas of agricultural runoff and industrial discharge; and declining populations of brown pelicans (in Louisiana, USA) and sandwich terns (in the Netherlands) have been attributed to exposure to endrin in combination with other halogenated chemicals.

Endrin has been detected in rainwater from Creek Lake in northern Saskatchewan, and has been reported in a fresh water lake in the Canadian Arctic. It has been found to bioaccumulate in species from

algae, pouch snail, flathead minnow, rainbow trout, Virginia oyster, and sheepshead minnow.

Other Hazards

Endrin is slightly corrosive to metals. As a solid it is not combustible; however, it may be dissolved in a flammable solvent. Toxic hydrogen chloride and phosgene may be generated when a solution of endrin burns. Endrin is incompatible with strong oxidizers, strong acids, and parathion.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.0002\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.
- Maximum contaminant level (MCL) is $0.002\,\mathrm{mg\,l^{-1}}$.
- Reference dose is $0.025\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.
- Permissible exposure level is $0.1\,\mathrm{mg\,m^{-3}}$ (8 h).

See also: Cyclodienes; Dieldrin; Organochlorine Insecticides.

Relevant Websites

http://www.osha-slc.gov – US Department of Labor, Occupational Safety and Health Administration.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Endrin.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Endrin.

Environmental Advocacy in the United States

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In the United States, environmental advocacy groups make up what is known as the 'environmental movement'. The history of this large, diverse social movement can best be understood by analogy to a river created by the confluence of about two dozen rivulets and tributaries. The main stems derive from two distinct nineteenth-century social movements, one promoting the conservation of natural resources, the other public health.

Ancient Sources

Of course even the nineteenth-century conservation and public health movements had ancient sources. Around 530 AD, the Roman emperor Justinian codified the legal basis for natural resource protection – the idea that air, water, oceans, wildlife, and more are owned by all of us together and none of us individually, and that the sovereign has a duty to protect and conserve these resources for present and future generations. The code of Justinian eventually led to the modern 'public trust doctrine' of environmental management and protection, described below.

Roman practices also foreshadowed the main ideas of the modern public health movement. For example, the architect Vitruvius (like the Greek physician Hippocrates before him) understood that human health is dependent upon the natural environment, so he taught his students to site and orient buildings to take advantage of fresh air and sunlight. The Romans also understood that individual health was dependent upon interventions at the scale of the whole population, not merely the individual. So, for example, the Roman aqueducts were built by the state to deliver clean water to entire cities. Public baths made cleanliness possible for everyone. Romans also separated incompatible land uses (without calling the practice 'zoning') to protect public health. For example, the Roman boarium (the cattle market) was separated from the forum (the center of public life) for health reasons. And finally, the Romans were keenly aware that certain occupations, such as silver mining, were dangerous and unhealthful, and those occupations were restricted mainly to slaves.

A fundamental question for people in every culture is the relationship of humans to the rest of the natural world. Are humans part of nature or are they separate from it and superior to it?

From the philosophies of Aristotle, Plato, and Socrates, the Romans inherited a strongly anthropocentric perspective – that nature exists solely to serve human purposes. This view was reinforced in the fourth-century AD when Rome adopted Christianity as its state religion. At that time, the dominant interpretation of the Biblical story of creation held that humans are separate from the natural world and were intended to dominate and exploit it. As time passed, this European view evolved further until the natural

world was considered defective and incomplete until humans had 'developed' and 'improved' it – terms still widely used today. Although religious leaders have reinterpreted the relevant Biblical passages in modern times, it would be difficult to overstate the influence of these early, strongly anthropocentric views upon European and American life, thought, and policy.

The Conservation Movement

When Europeans first arrived in North America, they encountered indigenous people who viewed nature as a community to which humans and all other living things belonged and upon which all humans depended. The native people modified the environment with fire and took from it the plants and animals they needed, but with considerable restraint. We now know that in prehistoric times, large mammals had been driven to extinction at least partly by human agency, but for the most part the indigenous people of North America lived within nature's limits.

For the first 200 years of European advance into North America, natural resources seemed limitless. When one local environment was exhausted by logging or farming, there was always new land to be developed by moving westward. However, by the mid-nineteenth century, despoliation of the landscape in the United States had become evident on a grand scale, as documented by George Perkins Marsh in *Man and Nature* (1864). The movement to preserve natural landscapes in the United States has been traced back to a proposal in 1832 by artist George Catlin to protect the great Midwestern prairies and their inhabitants, buffalo, and indigenous people, from extinction by encompassing them in a large national park. That particular park never materialized and both the buffalo and the native people were subsequently decimated, but the idea of conservation for sustainable yield slowly took hold.

During the period 1830–1930, a national conservation ethic developed, aiming to preserve land and use it wisely for human purposes ranging from logging and hunting to spiritual regeneration. Particularly during the progressive era (roughly 1900–20), scientific management of water, soil, trees, and minerals came to be accepted as a reasonable goal, though by no means a universal practice.

During the same period, another view of nature began to emerge as well – the natural world valued for its inherent beauty, as a place for solace and regeneration of the human spirit. Henry Thoreau had articulated this perspective when he lived in the woods near Walden Pond, 1845–47. John Muir, who founded the Sierra Club in 1892, became a leading proponent of this view. He said, "Everybody needs beauty as well as bread, places to play in and pray in, where nature may heal and cheer and give strength to body and soul alike." As time passed, the Thoreau–Muir tributary of the environmental movement gained a tinge of misanthropy, which naturally limited its political appeal.

Hunters and Fishers

Despite the slow spread of a conservation ethic, by the last quarter of the nineteenth century, the nation's fish and bird populations had declined dramatically. Wearing bird plumage had became fashionable in the United States and Europe, resulting in the slaughter of millions of egrets and other wading birds. The Passenger Pigeon, which had numbered in the billions, was hunted to extinction, followed by the Carolina Parakeet, the Ivory Billed Woodpecker, Bachmans Warbler, and the Heath Hen.

Habitats for native fish were being decimated by deforestation, farming, urbanization, dams, and pollution. By the 1870s, even the Great Lakes (containing one-fifth of the world's fresh surface water) were experiencing severe declines in native fish such as trout and salmon, from habitat destruction and overfishing. In 1871, Congress created the US Fisheries Commission to oversee the nation's fisheries interests, and the following year President Ulysses S. Grant designated the first national park, Yellowstone, in Montana and Wyoming.

Forest and Stream, a weekly founded in 1873, quickly became the premier publication serving recreational hunters and fishers. In 1880, George Bird Grinnell assumed the editorship and began campaigning for the protection of natural resources and habitat for wild game. He became a leading advocate of 'sustainable yield' timbering and led a campaign for the protection of Yellowstone National Park from commercial exploitation. When one of his readers, Theodore Roosevelt, became president of the United States in 1901, many of Grinnell's ideas were incorporated into federal conservation programs.

In 1886, Grinnell founded the first Audubon Society for the Protection of Birds, named after the American naturalist and artist, John James Audubon (1785–1851). Within 10 years Audubon Societies arose in many states and in 1901 a loose national federation of Audubon societies formed. In 1903, the Audubon societies provided support for Theodore Roosevelt as he set aside the first national wildlife refuge for the protection of birds and other wildlife. In 1913 and again in 1918, Congress passed laws, and signed treaties with Canada, protecting migratory birds from wanton slaughter.

In 1874, the US Fisheries Commission issued a report advocating the artificial cultivation of particular fish species. By 1875, several states had developed fish hatcheries, intending to supplement fish populations by artificially stocking streams. This practice is now routine, creating a large commercially successful recreational fishing industry but masking the fact that many of the nation's freshwaters can no longer support large populations of native fish. For example, between 1966 and 1998, 745 million fish were released into the Great Lakes from hatcheries.

Throughout the twentieth century, fishers and hunters organized to protect the habitat of wild game. In 1922, sportsmen formed the Izaak Walton League (named after the seventeenth-century author of *The Complete Angler*) to protect the nation's rivers and streams from industrial dumping, sewage discharges, and soil erosion.

In 1936, President Franklin Roosevelt convened a North American Wildlife Conference to promote conservation, and from that emerged the General Wildlife Federation (later renamed the National Wildlife Federation) which grew to 4 million members composed of hunters and others interested in maintaining populations of wild species. The creation of other wildlife organizations followed – the Wildlife Society (1937), Ducks Unlimited (1937), and Defenders of Wildlife (1947). Still human encroachment into wild habitats continued to displace wild creatures, whose populations continued to decline. Of course the problem was not limited to the United States. The Nature Conservancy was formed in 1951 to take ownership of exemplary endangered ecosystems, to prevent their destruction. In 1961, an international group of scientists, conservationists, and political and business leaders formed the World Wildlife Fund (WWF), which is now a leading advocate for the control of persistent toxic pollutants worldwide.

In recent years, many groups formed to protect wildlife have been emphasizing the connection between human health and environmental deterioration – a connection readily apparent in fish. In many waters of the United States today, fish become moderately toxic as they grow to edible size. Many states now publish book-length lists, suggesting limits on consumption of particular species taken from particular waters. The need to artificially stock fishing waters, and the need to warn the public to curb consumption to avoid toxic exposures, indicate that the status of wildlife in the United States remains precarious and deeply troubled – a view confirmed by numerous reports on loss of biodiversity, poor water quality, and declining ecosystem health published throughout the 1990s.

The Public Health Movement

The public health movement to protect people arose in England during the nineteenth century in response to exceedingly high rates of death and disease amidst appalling environmental conditions in cities and factories (dark satanic mills) as society was reorganized along industrial lines.

Edwin Chadwick and the Poor Law Commission in England in the 1840s documented the fact that disease has both environmental and social determinants. In Chadwick's view, filthy air, water, and soil caused disease (by creating a 'miasma'), but so did poverty. Chadwick's three-volume report in 1842, *Survey into the Sanitary Condition of the Labouring Classes in Great Britain*, offered copious detail demonstrating how environmental and social conditions contribute to disease. Disease arose from filth, Chadwick had no doubt, and it was to be engineered out of existence. As Chadwick saw it, maintaining public health by preventing disease was chiefly an engineering problem, not a medical problem. Chadwick and his colleagues explicitly recognized the limitations of the medical model (one doctor, one patient) as a response to disease. The medical model aimed to cure disease, but the public health model aimed to prevent it – through proper engineering of water supply, drainage, improved sewerage, and waste removal. Society had to create the conditions that made health possible, and a clean environment was crucial.

Spurred by Chadwick's efforts, in 1848 the English Parliament enacted the first Public Health Act and the Nuisances Removal and Diseases Prevention Act. These laws established prevention of disease – employing interventions by public authorities at the scale of the population, not just the individual – as the central tenets of the public health approach.

Long before medical science confirmed (between 1865 and 1890) that germs can cause illness, sovereign powers were imposing preventive measures to protect public health. Mandatory quarantining of plague victims had been practiced in Europe since the fourteenth century. In 1878, the US Congress enacted federal quarantine legislation to forcibly separate the sick from the healthy, to stem cholera, yellow fever, typhoid fever, and other epidemics of contagion. Individual liberties were sacrificed to achieve public health goals.

Vaccination to prevent smallpox was widely adopted throughout Europe in the first quarter of the nineteenth century and by 1905 compulsory vaccination was upheld by the US Supreme Court as a valid exercise of a state's 'police power' to protect public health.

Other preventive measures came into widespread use by the beginning of the twentieth century.

As early as the 1840s, Dr. Oliver Wendell Holmes (father of the Supreme Court justice) advocated that physicians should wash their hands between patient visits. His ideas were considered extreme and slightly mad at the time, but eventually hand washing became routine throughout the world of medical practice and beyond. Today many people are required by law to wash their hands as a condition of employment because frequent hand washing is still considered the single most effective way to prevent the spread of communicable disease.

By the early twentieth century, it was widely understood and agreed that individuals had to yield some of their personal liberty in order to protect public health. As much as citizens of the United States once enjoyed spitting on the sidewalk, it was generally outlawed at the beginning of this century to reduce the spread of tuberculosis. It was not long before the propertied classes were expected to support public water, sewer, and solid waste disposal systems to protect public health. Landlords are today required by law to provide adequate space, light, and air in their rental properties (a triumph for Vitruvius) and property owners must take specific steps to prevent fires. In 1926, the US Supreme Court authorized municipal zoning commissions to limit the uses to which privately owned land could be put, to prevent, for example, industrial and residential uses side by side. These and many other restrictions on personal liberty were instituted by governments to protect public health, for the public good.

Today there is wide agreement that (1) prevention is the first principle of public health; (2) public health requires community action to create conditions that prevent disease and other threats to the health and welfare of individuals and the larger community; and (3) an environment that is free from harm is the starting point of good public health practice.

Since at least the time of Sir Francis Bacon (1561–1626) and Rene Descartes (1596–1650), Europeans have believed that a mathematically based scientific knowledge of the material world is possible, that such knowledge would permit the conquest of nature, and that this conquest was the very definition of progress. The accelerating industrial revolution, based on replacement of human and animal labor by fossil fuels, seemed to prove them right. By 1930, the standard of living of average US citizens would have been unimaginable to people 100 years earlier.

The Main Stems Converging: 1950–70

By the middle of the twentieth century, it seemed as if the conquest of nature was nearly complete. Leading the way in taming nature was the chemical industry, which had learned to manipulate raw materials like coal and petroleum to create an astonishing array of useful molecules that seemed superior to anything that nature had created. Cheap fertilizer was making possible increased crop yields and abundant food. Chemical pesticides were reportedly vanquishing insect pests. An array of antioxidants, emulsifiers, thickeners, dyes, sweeteners, preservatives, and bleaching agents had made processed foods widely available. Synthetic fibers like rayon and nylon, along with synthetic dyes, were making fabrics cheaper, more colorful, and longer-lasting. Automobiles powered by low-cost leaded gasoline, constructed of special steel alloys, with tires of synthetic rubber and windshields of safety glass, gave mobility to millions.

With the help of vaccines, X-rays, radioactive isotopes, antibiotics, synthetic hormones, and vitamins, medical science seemed on the verge of eradicating most, if not all, human ailments. Like yellow fever and cholera before them, polio and tuberculosis were being vanquished. Atomic energy promised to provide cheap electricity to serve civilization's rapidly growing need for power. One corporation's slogan celebrated "Better things for better living through chemistry," and another's said, "Progress is our most important product." Belief in the inevitability of universal progress has perhaps never been stronger than it was in 1950.

But progress as conceived in 1950 depended upon technologies that turned out to have a powerful dark side. Public health hazards from radioactive fallout, pesticides, energy and transportation systems, artificial food additives, and toxic household chemicals all began to attract public attention as post-World War II optimism gave way to the 1960s.

- Atomic weapons tests in the South Pacific in 1946 exposed 40 000 US Navy personnel to radioactivity and an Army doctor's diary describing the incident hit the best seller list in 1948. In April, 1953, Geiger counters in the City of Troy, New York, recorded substantial radioactive fallout from tests that had been conducted in Nevada 36 h earlier. News reports of the incident provoked widespread fear and concern.
- In 1948 in Donora, Pennsylvania and again in London, England, in 1952, air pollution killed and injured large numbers of people – 14 000 injured in Donora and 4000 killed during one weekend in London.
- By the mid-1950s, public health officials were concerned about the toxicity of various modern products. Alarmed by a rise in reported household poisonings, in 1957 the American Public Health Association passed a resolution calling for better

labeling and 'uniform control of hazardous substances', meaning household chemicals.

- Between 1945 and 1966, the US Department of Agriculture licenced ~60 000 individual pesticides at a time when the agency had only one toxicologist on staff, whose job it was to make safety evaluations and judgments based on available health studies (to the extent that any existed) for each of the 60 000 products.
- In 1957, a committee within the American Association for the Advancement of Science wrote, "We are now in the midst of a new and unprecedented scientific revolution which promises to bring about profound changes in the condition of human life. The forces and processes now coming under human control are beginning to match in size and intensity those of nature itself, and our total environment is now subject to human influence. In this situation it becomes imperative to determine that these new powers shall be used for the maximum human good, for, if the benefits to be derived from them are great, the possibility of harm is correspondingly serious."
- In 1958, Rachel Carson – a trained biologist with a literary flair – began writing *Silent Spring*, drawing parallels between the hazards of radioactive fallout and chemical pesticides.
- Just before Thanksgiving in 1959 the federal government issued a public warning, urging people not to eat cranberries, which had been found to be contaminated with amitrole, an herbicide thought to cause cancer in laboratory animals. This created widespread fear and awareness of cancer-causing chemicals in the nation's food.

During the 1960s, there seemed no end to the bad news. In 1961, newspapers featured photographs of entire rivers covered with foam from detergents. Anyone could see that something was amiss.

In June, 1962, chapters from *Silent Spring* began to appear in *The New Yorker* magazine and soon thereafter became a best-selling book. Many would identify this as the single most important event in the history of the modern environmental movement. In *Silent Spring*, Rachel Carson offered a powerful indictment of what she called 'man's war against nature'. "...[C]hemicals are the sinister and little-recognized partners of radiation in changing the very nature of the world," she wrote. "Can anyone believe it is possible to lay down such a barrage of poisons on the surface of the Earth without making it unfit for all life?" Ms Carson raised the specter of chemical and radioactive technologies causing vast and lasting damage to the natural environment and to humans.

At the time, Ms. Carson was excoriated by representatives of chemical corporations, who accused her of being ignorant and hysterical. However, subsequent studies showed that she was correct on all essentials, and that she had underestimated the severity and magnitude of many of the problems she described.

Meanwhile, starting in the late 1950s in St. Louis, Missouri, a group of independent scientists organized by Barry Commoner took it upon themselves to begin studying radioactive fallout and then other dangerous technologies.

In the early 1960s, with colleagues around the country (such as the newly formed Physicians for Social Responsibility in Boston), the St. Louis group collected thousands of baby teeth and demonstrated that radioactive strontium-90 was building up in children as a consequence of testing nuclear weapons above-ground. Partly based on this 'baby tooth survey', in 1963 President Kennedy signed a treaty with the Soviet Union banning the testing of nuclear weapons in the atmosphere and the oceans.

The original Greater St. Louis Citizens' Committee for Nuclear Information soon expanded into a nationwide network that became known as the 'scientific information movement', guided by the idea that scientists have an ethical duty to help the public understand the technical aspects of public issues because an informed electorate is essential for democratic self-governance. They believed scientists have a duty to serve the public good, in return for which society supports the scientific enterprise through universities, government research, and the vast infrastructure of public services (libraries, courts, universities, communication networks, patent offices, standards for weights and measures, standards for accounting, and so forth) that make possible the corporate research and development enterprise. By 1968, the St. Louis group had renamed itself the Committee for Environmental Information.

Throughout the 1960s, the scientific information movement brought a public health perspective to environmental problems – human-centered, prevention-oriented, espousing population-scale interventions by the state (a ban on above-ground testing of nuclear weapons, e.g., to eliminate radioactive fallout), with no reluctance to consider the hazards of the workplace and urban environments. Here we find the beginnings of the modern environmental movement.

As the 1960s unfolded, other serious threats to public health and the workforce were revealed – toxic lead in paint and gasoline, asbestos in building insulation, food contaminated with mercury from pesticides and industrial products.

Inheritable genetic damage from radioactivity had been discovered in 1927, but during the 1960s scientists revealed that common air pollutants could alter genes, as well as cause cancer. It was becoming apparent that advanced technologies were capable of harming future generations.

"Pollution now is one of the most pervasive problems of our society," wrote President Lyndon Johnson in a report titled *Restoring the Quality of Our Environment*, published by the White House in November 1965.

That same report concluded that, "The pollution from internal combustion engines is so serious and growing so fast, that an alternative nonpolluting means of powering automobiles, buses and trucks is likely to become a national necessity." It did become a national necessity, but one that remained unmet almost 40 years later.

That same year – 1965 – Ralph Nader published *Unsafe at Any Speed* – charging that the US automobile industry was knowingly selling unnecessarily dangerous cars to an unsuspecting public. General Motors Corporation (GM) hired a detective to shadow Mr Nader, who then sued GM, winning a monetary settlement. Mr Nader invested the proceeds to form the Center for Study of Responsive Law and the consumer safety branch of the environmental movement was born.

In 1967, the Environmental Defense Fund (EDF, now simply Environmental Defense) was created by a group of attorneys and scientists to bring lawsuits against polluters and to educate lawyers about environmental issues. EDF was instrumental in persuading the federal government to ban DDT in 1972. In 1970 the Natural Resources Defense Council (NRDC) was formed to watchdog federal pollution-control agencies and, when necessary, to take the government to court to enforce the law. The Sierra Club Legal Defense Fund formed in 1971 (with no formal connection to the Sierra Club) to litigate on behalf of the environment; in 1997 the organization changed its name to Earthjustice.

Throughout the 1970s and 1980s, environmental litigation provided a powerful tool for environmental protection, until the federal bench and the appellate courts became less sympathetic to the environment. Since the early 1990s, environmental litigation has become more difficult for plaintiffs than it once was, and less successful at protecting the environment.

This legal tributary of the environmental movement spawned some important new theories of law that have begun to influence decisions in state courts. In 1970, Christopher Stone published *Should Trees Have Standing? Toward Legal Rights for Natural Objects*, and in 1971 Joseph Sax published *Defending the Environment: A Strategy for Citizen Action*. Stone planted the idea that perhaps nonhuman species deserved their day in court just as humans did, and Sax argued that the sovereign state had a legal duty to protect air, water, soil and more, even if it meant limiting some of the prerogatives of private property. Today this ancient 'public trust doctrine' – traceable to the code of the Roman emperor, Justinian – is evolving into an important new principle of environmental protection, and the rights of nonhuman species are the subject of intense debate.

In 1968, Ann and Paul Ehrlich published *The Population Bomb*, warning of dire threats to the future of all living things because of growing human encroachment into all of nature's domains. The book led to the creation of an organization called Zero Population Growth (ZPG), which in 2002 renamed itself The Population Connection. They offer evidence that every environmental problem would be easier to solve if the human population were smaller and growing more slowly than it is.

That same year – 1968 – the first humans circled the moon in a spacecraft and brought back dramatic photographs of 'spaceship earth' – a small blue ball suspended in the vast blackness of space. These photos would forever change the way humans view their home.

In 1969 – the year Greenpeace was founded – the federal government issued the 'Mrak Report' (named for its senior author, Dr. Emil Mrak) which confirmed many of the dangers from pesticides described 7 years earlier by Rachel Carson. That same year the Cuyahoga River caught fire in Ohio, and a huge oil spill occurred off the coast of affluent Santa Barbara, CA, soiling the beaches of southern California.

To many, it seemed as though frail nature was under heavy assault by humans using powerful technologies in pursuit of narrow economic purposes. To many, it seemed that the future itself was endangered.

As a consequence, people began to react and to mobilize. For example, the Sierra Club grew from 15 000 members in 1960 to 113 000 in 1970, and 350 000 in 1983.

In the final years of the 1960s, three other specific responses developed:

- Ralph Nader expanded the new consumer safety movement (whose origins could be traced back to the founding of the National Safety Council in 1913 and Consumers Union in 1936), which came to be known as the 'public interest research movement'.

  Nader hired college students (quickly dubbed 'Nader's raiders' by the media) during the summer

of 1970 to pore through the records of the federal agencies charged with protecting air, water, and food. During the following 3 years Nader issued a series of book-length studies offering evidence that government regulators were failing to protect public health and safety, the workforce, and the natural environment. From Nader's efforts there emerged a network of college-based organizations called public interest research groups (PIRGs). Akin to the earlier 'scientific information movement', PIRGs study public problems, issue reports, and advocate particular solutions.

- Labor activist Tony Mazzocchi of the Oil, Chemical and Atomic Workers organized a series of public forums, giving workers a platform for testifying about hazardous conditions in the industrial workplace. Mazzocchi compiled a formal record of the forums, to pressure Congress to enact the Occupational Safety and Health Act (OSHA). In 1970, OSHA became the first federal law aimed at protecting the health of the nation's workforce.

  Starting in 1972, a national network of 'COSH' groups developed nationwide – Committees/Coalitions on Occupational Safety and Health. Currently there are 22 COSH groups across the country – private, nonprofit coalitions of labor unions, health and technical professionals, and others interested in promoting and advocating for worker health and safety.
- A coalition of activists planned a series of events across the country to be held April 22, 1970 – the first Earth Day celebration.

The Modern Environmental Movement Becomes Visible

The 1970s

Starting in the mid-1950s, the civil rights movement used the nonviolent tactics of boycotts, protest marches, and sit-ins at racially segregated businesses. By 1964 the movement had successfully ended legal discrimination against African-Americans. Soon teach-ins, sit-ins, and then large-scale protest marches against the Vietnam war further revealed the power of nonviolent direct action in ways unheard of since the labor movement's sit-down strikes of the 1930s.

Modeling itself on these contemporary protest movements, the activist movement for environmental protection broke onto the national scene on April 22, 1970 – Earth Day – with hundreds of teach-ins across the country aimed at creating awareness of environmental destruction.

An advertisement in the *New York Times* on January 18, 1970, explained that, "Earth Day is a commitment to make life better, not just bigger and faster; to provide real rather than rhetorical solutions. It is a day to re-examine the ethic of individual progress at mankind's expense. It is a day to challenge the corporate and government leaders who promise change, but who shortchange the necessary programs. It is a day for looking beyond tomorrow. April 22 seeks a future worth living. April 22 seeks a future."

The following year Barry Commoner's best-selling book, *The Closing Circle*, helped millions of newly awakened readers understand something about how ecosystems work, and how certain modern technologies are disrupting them with far-reaching consequences for the future of humankind.

Although initially an upper middle-class phenomenon (like the conservation movement before it), the burgeoning 'environmental movement' began to appeal to a broader spectrum of people as the mass media told the general public that its health and well-being were threatened by pesticides, food dyes and other additives, dangerous household products, and the discharge of industrial toxicants into air and water. This came as no surprise to people of color or the poor, who had borne the brunt of pollution as long as anyone could remember.

Two months after Earth Day, 1970, President Richard Nixon issued an executive order creating the US Environmental Protection Agency (EPA) to administer the nation's environmental laws and programs. The President adopted an apocalyptic tone characteristic of the time when he said, "The 1970s must be the years when America pays its debt to the past by reclaiming the purity of its air, its waters, and our living environment...it is literally now or never."

President Nixon appointed William Ruckelshaus as first administrator of EPA. In an interview years later, Mr. Ruckelshaus explained the engineering perspective he brought to the job:

> I thought that pollution could be solved by mild coercion. Once the federal government set some standards and began to enforce them, people would fall in line and the problem would essentially disappear. I thought we knew what the bad pollutants were, knew at what levels they caused adverse health and environmental effects, and knew the technology needed to combat them. Finally, I thought all of this could be done at a reasonable cost within a reasonable time.

"I was there about 3 months when I began to question every single one of the assumptions I had entered the agency with," Mr. Ruckelshaus said.

Between 1969 and 1990, Congress enacted (or strengthened by amendment) a series of laws aimed at protecting and restoring the quality of the environment: The National Environmental Policy Act (1969); the Clean Air Act (1970); the Water Pollution Control Act ('Clean Water Act', 1972); the Federal Environmental Pesticide Control Act (1972); the Coastal Zone Management Act (1972); the Endangered Species Act (1973); the Safe Drinking Water Act (1974); the Resource Conservation and Recovery Act (RCRA) (1976) – to manage solid and hazardous wastes; The Toxic Substances Control Act (1976); The Surface Mining Control and Reclamation Act (1977); The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) ('Superfund', 1980) creating a tax on the chemical and petroleum industries to pay for cleanup and restoration of chemically contaminated sites; and the Fish and Wildlife Conservation Act (1980). Several of these laws were subsequently amended and strengthened during the 1980s and 1990s.

From the outset, EPA was charged with administering and enforcing parts or all of these laws, but Congress rarely, if ever, allocated sufficient funds for the agency to do a thorough job.

Furthermore, from the outset, EPA personnel defined natural resource damage, and environmental contamination, as science and engineering problems, rather than problems of human behavior.

As a result of inadequate resources and the belief that applied engineering held the key, and seeking to establish a rational and consistent basis for making decisions, EPA personnel soon adopted a judgment-based technique that often relied heavily on mathematical models for calculating risks: quantitative risk assessment. Somewhat later the assessment and management of risks were conceptually separated, though the two activities have always remained interdependent.

The concepts of risk assessment had been developed by the US Food and Drug Administration (FDA) in the late 1930s to establish allowable levels for food additives, to give consumers 'reasonable certainty of no harm when used as intended'.

In its simplest form, risk assessment asks, 'How much exposure can we allow without causing irreparable harm?' Harm to whom? Initially, to a 'maximally exposed individual' and more recently to a 'sensitive' and maximally exposed individual. For example, to keep chemical contamination to 'acceptable' levels in a river, EPA would define 'acceptable risk' to a maximally exposed individual. Acceptable risk would be defined as an exposure to a contaminant below its threshold for causing damage or, more often, a one-in-a-million risk of getting cancer from a lifetime of exposure. With acceptable risk defined, the agency would issue permits to each discharger of contamination along the length of the river, specifying numerical limits on each discharge. The goal was to keep the total discharge into the river from exceeding the acceptable risk for the maximally exposed individual. To a large extent, this approach still prevails today.

This technique has been applied to hundreds of thousands of air and water discharge permits over the past 30 years. In addition, risk assessment has become the standard way of setting safe limits for pesticides in food; determining 'how clean is clean' in the remediation of contaminated sites; judging how much contaminated fish it is safe to eat; decreeing how many logging roads can be cut into a national forest without decimating the bear population, and on and on. Today, in the United States, nearly all resource and contamination issues are decided based, to one degree or another, on risk assessments.

Because data on the hazards of individual chemicals were often incomplete, from the beginning, risk assessment was made manageable by adopting many simplifying assumptions. Initially the maximally exposed individual was assumed to be an 'average' person in good health and the only harm considered was physical manifestation of disease, such as liver necrosis or cancer – ignoring the possibility of behavioral disorders, or of harm to the immune system, the nervous system, the endocrine system, the reproductive system, the metabolic system, or the genes. Another simplifying assumption was that everyone was exposed to one chemical at a time even though in the real world everyone is exposed to low levels of a panoply of exotic chemicals – pharmaceutical products, household cleaners and disinfectants, second-hand smoke, automotive exhausts, food additives, low levels of industrial compounds in drinking water, and so on. In reaching the final number that represents 'acceptable risk', imponderables are typically taken into account by applying imprecise 'safety factors' – more recently called 'uncertainty factors' – which are usually multipliers of 10.

To remedy these limitations of risk assessment, as time has passed government risk assessors have increasingly tried to take into account some of the missing elements. However, limitations on the available data have always forced risk assessors to rely on assumptions, the use of somewhat arbitrary uncertainty factors, and judgments. As a result, despite many advances in the science of toxicology during recent decades, conclusions about risk can still vary dramatically depending upon who is doing the risk assessment. As William Ruckelshaus said in 1984,

"We should remember that risk assessment data can be like the captured spy: If you torture it long enough, it will tell you anything you want to know." Peer review of risk assessments by all stakeholders can reduce the range of disagreement; nevertheless, despite substantial effort and constant improvements in risk assessments, the goal of a rational and reproducible technique for making decisions has eluded decision-makers. As William Ruckelshaus said in 1983, "No amount of data is a substitute for judgment."

Furthermore, because it is a mathematical technique based largely on scientific information and technical judgments, risk assessment tends to mystify the general public. Thus reliance on risk assessment for decision-making has had the effect of discouraging members of the public from participating in decisions affecting their lives, thus weakening democratic institutions that depend on citizen interest and participation.

In addition, as time passed, it became clear that assessing the risks to a maximally exposed individual – even a maximally exposed and sensitive individual – had the unintended consequence of overlooking millions of small discharges that posed acceptable risks to the hypothetical individual but which, taken together, contaminated the entire planet with low levels of industrial toxicants, pesticides, pharmaceuticals, and personal care products. In recent years, government scientists have measured low levels of hundreds of synthetic chemical compounds in the nation's rivers and streams, and even in drinking water: pain killers, antibiotics, dry cleaning fluid, solvents, degreasers, plasticizers, antimicrobials, flame retardants, tranquilizers, contraceptives, antidepressants, perfumes, deodorants, chemotherapeutic compounds, and so on, all of which largely escaped official notice as they leaked into the natural environment.

Despite the limitations of risk assessment as a decision-making technique, in 1983 the National Academy of Sciences (NAS) codified methods for risk assessments conducted by federal agencies. With this stamp of approval from the nation's most prestigious scientific organization, risk assessment spread quickly throughout federal, state and even municipal governments.

The 'Anti-Nuke' Movement As government was responding to environmental threats by passing laws, developing bureaucracies and refining techniques for assessing risks, citizens developed expertise on their own and began to organize around particular problems. The bombing of Hiroshima and Nagasaki in 1945 and radioactive fallout from atmospheric testing in the 1950s provoked a response initially among scientists. The Federation of American Scientists was formed in 1945; the *Bulletin of the Atomic Scientists* began publishing in 1949. As we have seen, the 'scientific information movement' developed in the late 1950s, intending to inform the citizenry.

The early history of atomic weapons and fallout left a residue of fear and distrust of nuclear technologies. The so-called 'anti-nuke' movement developed from this residue. Civilian nuclear power plants use a controlled nuclear fission reaction to boil water to turn a steam turbine to make electricity. The corporate sector was initially reluctant to finance nuclear power plants when President Eisenhower praised them in 1953. However after Congress enacted the Price-Anderson Act in 1957, to limit corporate liability in case of mishap, development of the technology proceeded apace. The first nuclear power plant built without direct government funding went on-line in late 1959.

From the beginning, four separate issues fanned citizen opposition to nuclear power: (1) the possibility that the nuclear fuel might heat up excessively, leading to a 'meltdown' that might release large quantities of radioactivity into the environment; (2) concern about small, continuing releases of radioactivity into local air and water; (3) the difficult technical problem of safely disposing of radioactive wastes that will remain dangerous for 240 000 years, far longer than homo sapiens has walked the earth; and (4) the possibility that radioactive materials from a nuclear power plant might some day be fashioned into a crude but effective atomic bomb or a 'dirty bomb' composed of radioactive materials wrapped around a core of dynamite. After more than 40 years of experience with nuclear power plants, the last two problems have not been solved, and the first two are still the subjects of intense scientific debate.

The Union of Concerned Scientists was formed in 1969. In 1970, nuclear scientists John Gofman and Arthur Tamplin published their estimate that civilian nuclear power plants could cause 24 000 cancer deaths per year in the United States. This ignited citizen opposition to expansion of nuclear power technology and by 1975 the Clamshell Alliance in New England and the Abalone Alliance on the west coast had grown into large coalitions of citizens aiming to stop nuclear power. Their slogan was simple: 'No nukes!' and eventually they achieved their goal in the United States, where there are currently 103 nuclear power plants operating, but the industry ceased to expand in the mid-1970s. After the Three Mile Island nuclear plant in Pennsylvania suffered a partial fuel meltdown in 1979, investors shied away from new nuclear power stations. The Chernobyl disaster in

1986, which produced measurable radioactive fallout across large areas of Europe, only made matters worse for the nuclear power industry. In the early years of the twenty-first century, significant efforts have been made by the US government to revive the industry.

The rise of the civilian nuclear power industry served to spread concern about radioactivity to many communities that might not have otherwise given it a second thought. Proposals to truck many tons of radioactive waste across the nation's highways had similar effect.

Other proposals to develop new nuclear technologies provoked opposition as well. Although it is now legal to irradiate certain foods in the United States, including meat, to kill microorganisms and lengthen the 'shelf life' in stores, irradiated food drew significant opposition from the environmental movement, and growth of the industry has been slow.

The 1980s

The 'Toxics' Movement In 1978, families living in Love Canal, NY, near Buffalo, discovered an unusual number of serious health problems among their children, which they attributed to toxic wastes that they found oozing into their basements and onto the local school playground. They learned that the original canal had been dug for barge transportation, but it eventually had been filled with 20 000 tons of hazardous wastes and covered with a thin layer of soil. Over the next 2 years President Jimmy Carter declared Love Canal a federal emergency and the government helped many families relocate. Subsequent study confirmed that children of families living closest to the canal tended to weigh less than average at birth, and to suffer from various health problems.

One of the leaders of the Love Canal protest was a housewife named Lois Gibbs, who subsequently moved to northern Virginia to establish the Citizens Clearinghouse for Hazardous Waste (since renamed the Center for Health, Environment and Justice), to advise other communities afflicted by toxic wastes. Over the next decade, the dimensions of the chemical waste problem began to emerge and thousands of local groups formed to advocate for the cleanup of contaminated local lands. This 'toxics movement' eventually encompassed many thousands of local groups in all 50 states.

By the late 1980s, EPA acknowledged the existence of 32 000 locations contaminated with toxic chemicals but even then EPA had no formal process for discovering new sites. Congress's Office of Technology Assessment (OTA) in 1989 estimated that the total number of contaminated sites in the United States might run as high as 439 000, including contaminated military properties, mine wastes, leaking underground storage tanks, pesticide-contaminated lands, contaminated nonmilitary federal properties, underground injection wells, abandoned municipal gas manufacturing facilities, and wood-preserving plants.

In 1991, the National Academy of Sciences studied the health effects attributable to toxic waste sites and concluded, "[W]e find that the health of some members of the public is in danger," but "We are currently unable to answer the question of the overall impact on public health of hazardous wastes." The Academy pointed out that "Millions of tons of hazardous materials are slowly migrating into groundwater in areas where they could pose problems in the future, even though current risks could be negligible." The Academy concluded, "...the committee does find sufficient evidence that hazardous wastes have produced health effects in some populations. We are concerned that populations may be at risk that have not been adequately identified, because of the inadequate program of site identification and assessment."

Environmental Justice Meanwhile, in 1982, a pivotal new branch of the modern environmental movement emerged in Warren County, NC, in response to a proposed toxic waste landfill in a predominantly African-American community. Over 500 people were arrested during a series of protests and the term 'environmental racism' came into the language. This was the beginning of the 'environmental justice' (EJ) movement. In 1983, the US General Accounting Office (GAO) published a study showing that waste dumps in the southeastern US were mainly located in communities where the population was predominantly African-American or of low income. In 1987, the United Church of Christ published a study confirming that the pattern revealed in the 1983 GAO study was evident nationwide.

As the 1980s evolved, environmental justice groups developed in many different racial and ethnic communities: African-Americans, Hispanics, Asian-Pacific groups, and the indigenous people of North America. By 1991, the EJ movement had a clear national identify and philosophy, expressed in the 'Principles of Environmental Justice' adopted at the First National People of Color Environmental Leadership Summit in Washington, DC, which was attended by more than a thousand community activists.

It would be difficult to overstate the importance of the combined effects of the toxics and EJ movements. Together, they redirected the environmental movement in the United States. The movement that had

emerged in 1970 – a combination of conservation organizations, plus the new litigators – tended to view issues from the traditional conservation perspective, and it was largely staffed and supported by the middle and upper-middle classes. The toxics and EJ movements introduced a public health perspective, a working class perspective, and the perspectives of people of color and people with low-income, thus creating the diverse blend of viewpoints and interests that defines the environmental movement today.

- Toxics and EJ groups permanently expanded the definition of 'the environment' to include not just wild lands and animals but all the places were people live, work, play, pray and learn. Now for the first time, the 'environmental movement' would focus attention on the cities – and to a lesser extent the workplaces – where most Americans spend their lives. This new perspective meant that the environmental movement could now appeal to huge numbers of people previously overlooked by the earlier focus on wilderness and endangered species.
- They emphasized the cumulative impacts of pollutants on communities – the combined effects of all sources of contamination, not just one particular pollutant or facility. For many years, risk decisions had been made in an artificial vacuum, considering one oil refinery, or one cement kiln, or one hazardous waste incinerator, ignoring all other sources of contamination in the general vicinity. This narrow perspective had burdened certain communities with numerous sources of contamination, each of which individually was deemed 'acceptable' yet in the aggregate created a patently unhealthful environment.
- They emphasized the social determinants of disease – the health consequences of poverty, stress, and the social isolation created by artificial hierarchies based on race, income, wealth, and class – combined with poor nutrition, insufficient recreational opportunities, inadequate health care, and exposure to toxicants. They thus broke down the barrier – which had been created in the original National Environmental Policy Act of 1969 – between environmental issues and socioeconomic issues.
- They emphasized the importance of respect for cultural traditions, local knowledge, and the historical integrity of community and place. The emphasis on risk assessment as a decision-making technique had inadvertently created a great divide between 'experts' who had 'useful' knowledge, and ordinary people who 'only' had common sense, historical understanding of their communities, strong preferences for how things should be, and a well-developed sense of right-and-wrong, fair play, and justice.
- They emphasized the essential importance of democratic participation: a key question in any decision affecting the environment or public health is, who gets to decide? And they emphasized that local communities have the preeminent right of self-determination, to decide what's best: "We speak for ourselves," they said.
- They emphasized that a clean and healthful environment is a basic human right under international law, as well as a civil right under US law.
- They emphasized leadership by women. Most community-based toxics and EJ groups were and are led by women. Perhaps this reflects somewhat the influence of the women's movement that energized women throughout the 1970s to seek equal pay for equal work and to demand other rights and opportunities that had traditionally been denied to them by a society organized along patriarchal lines.
- They emphasized that environmental issues are mainly about justice, fairness, ethical choices, and acceptable behavior, not just acceptable risk. The earlier narrow focus on science and engineering was expanded to give explicit recognition to the importance of ethics and values in decisions. In 2001, the European Union expressed the contemporary view of the proper role of science in environmental protection when it said, "science should be on tap, not on top."

In 1994, President Bill Clinton issued Executive Order 12898, requiring all federal agencies to 'make achieving environmental justice part of their mission'. The federal government's definition of environmental justice has two parts: equal protection from environmental and health hazards, and equal access to the decision-making processes that create a healthy environment in which to live, learn, and work. EJ is about fair treatment, and about expanding democracy to include everyone who is affected by a decision.

US EPA says equal access to decisions means (1) potentially affected community residents have an appropriate opportunity to participate in decisions about a proposed activity that will affect their environment and/or health; (2) the public's contribution can influence the regulatory agency's decision; (3) the concerns of all participants involved will be given serious consideration in the decision-making process; and (4) the decision-makers will seek out and facilitate the involvement of those who will likely be affected.

Toxics Use Reduction and Prevention By the mid-1980s, it was apparent to many people that certain toxic chemicals could not be managed safely and needed to be phased out or 'sunsetted'. In 1987, attorney Sanford Lewis proposed legislation to reduce the use of toxic chemicals in Massachusetts and 2 years later the state legislature passed the Toxics Use Reduction Act (TURA). The Act created the Toxics Use Reduction Institute (TURI) to help Massachusetts firms reduce their use of toxic materials. Today, compared to 1990, Massachusetts firms subject to reporting under TURA are generating 58% less waste per unit of product and have reduced on-site releases of federally reportable toxic chemicals by 90%. In addition, since 1990, quantities of chemicals shipped in product have been reduced by 47% (per unit of product shipped).

Since that time, many other states have created 'pollution prevention' programs of one kind or another, some voluntary, some mandatory. The mandatory programs, which represent a traditional public health approach, have achieved greater success than voluntary efforts.

Despite the growing emphasis on prevention and the avoidance of harmful substances, the US chemical industry currently introduces about 1700 new chemical compounds into commercial use each year, all largely untested for their effects on human health or the environment. In 2003, the European Union's proposal to require pre-market safety testing of chemicals was vigorously opposed by many national governments, including the United States, and by the chemical industry worldwide.

Agriculture Industrial agriculture got an early start in the United States. To avoid the laborious task of manuring soils to supply nutrients, inorganic fertilizers, such as superphosphates, came into use as early as the 1840s. However, a countercurrent quickly developed, the 'humus farming' movement focused on maintaining the humus content of agricultural soils. For the next 150 years, industrial agriculture would expand dramatically, but so would countercurrents stressing the need to maintain a holistic view of farm ecology – the complex relationships between plants, animals, soils, water, and human communities.

Chemical pesticides, such as Paris Green, were introduced for insect control starting in the 1870s. In the 1930s, federal farm policies began rewarding farmers who could increase their per-acre crop yield and, to that end, the US Department of Agriculture aggressively promoted the use of inorganic fertilizers and pesticides, and the development of the rural infrastructure (transportation, communication) needed to support large-scale industrialized farming.

However, as some of the unintended ill consequences of industrial farming technologies came to light, the principles of 'organic' farming became more widely known and practiced. Furthermore, an anti-pesticide movement developed in the 1960s after *Silent Spring* sounded the alarm about long-lived chlorinated compounds.

The chemical industry responded by developing new pesticidal products that did not persist so long in the environment, but were more toxic. Most pesticides do not reach the target organism but enter the environment where they may cause direct and indirect effects in nontarget species.

The Northwest Coalition for Alternatives to Pesticides (NCAP) opened in Eugene, Oregon in 1977, the National Coalition Against the Misuse of Pesticides (since renamed Beyond Pesticides) formed in Washington, DC in 1981, and the Pesticide Action Network North America (PANNA) was organized in San Francisco in 1982 as part of an international network.

As time passed, the organic farming movement shifted into a 'sustainable agriculture' movement with three goals: farming practices compatible with natural systems, using organic fertilizers and few or no chemical pesticides; achieving food security, emphasizing locally grown foods; and maintaining rural economies that could sustain, and be sustained by, relatively small-scale farms. Wendell Berry's *The Unsettling of America* (1978), Wes Jackson's *New Roots of Agriculture* (1980), and the National Academy of Science's *Alternative Agriculture* (1989) offered an ecological and social critique of industrial agriculture and showed that viable alternative models already existed.

The technology-driven corporate industrial model of farming remains dominant today, but energy, chemicals and large-scale equipment have proved expensive to supply and consequently the farm economy has been badly depressed in recent years. Net farm income in 2003 was lower than it had been in 1929.

In recent years, genetic engineering techniques have been used to create proprietary plant cultivars with desirable new characteristics. However, it is not clear that this new technology can substantially reduce industrial agriculture's negative ecological impacts or solve its pressing problems of economic viability.

In the 1990s, the use of genetically modified organisms (GMOs) in agriculture provoked strong controversy on every continent. The expanding uses of biotechnology, and most recently nanotechnology, raise a fundamental question: Does history indicate that humans can gain the knowledge and the wisdom

needed to rearrange the genetic and atom-scale building blocks of nature without causing widespread unintended harm?

Multiple Chemical Sensitivity As the toxics movement expanded during the 1980s, large numbers of people recognized that chemicals encountered in their daily lives negatively affected their health in one way or another.

In various large surveys 15–30% of Americans (40–80 million people) report that they are unusually sensitive or allergic to certain common chemicals such as detergents, perfumes, solvents, pesticides, pharmaceuticals, foods, or even the smell of dry-cleaned clothing. An estimated 5% (14 million people) have been diagnosed by a physician as being especially sensitive. Many people react so strongly that they can become disabled from very low exposures to common substances. Typical symptoms include prolonged fatigue, memory difficulties, dizziness, lightheadedness, loss of concentration, depression, feeling spacey or groggy, loss of motivation, feeling tense or nervous, shortness of breath, irritability, muscle aches, joint pain, headaches, head fullness or pressure, chest pains, difficulty focusing eyes, nausea, and more. This group of symptoms is known as environmental illness or, more commonly, multiple chemical sensitivity (MCS), meaning 'sensitivity to many chemicals'.

Because MCS does not fit any of the three currently accepted mechanisms of disease – infectious, immune system, or cancer – traditional medicine has not yet satisfactorily explained MCS, and so has often labeled it 'psychogenic', meaning originating in the mind. This has left MCS sufferers in limbo. Told they are crazy, or imagining their disease, or making it up, they find themselves passed from physician to physician without satisfactory answers and often without relief from their very real distress. (Some MCS sufferers do have psychological symptoms, but that does not necessarily mean their disease originated in their minds.) Forty percent of MCS sufferers report having seen more than 10 medical practitioners.

MCS came to the attention of mainstream science and medicine forcibly in 1987 when US EPA installed 27 000 square yards of new carpeting and painted and remodeled office space at its Waterside Mall headquarters in Washington, DC. Some 200 agency employees developed symptoms associated with 'sick building syndrome' (physiologic response to exotic chemicals in new construction materials) – and several dozen EPA employees later reported developing MCS. The National Research Council has now accepted that 'sick building syndrome' is a real phenomenon, producing MCS-like symptoms.

In the mid-1980s, Mary Lamielle founded the National Center for Environmental Health Strategies in Voorhees, NJ, and emerged as a leading spokesperson for people suffering from MCS, and the related disorders, chronic fatigue syndrome (CFS), and fibromyalgia (FM). In 1990, Congress acknowledged all three syndromes in the Americans with Disabilities Act (ADA).

In recent years, some of the symptoms of MCS have been reported to afflict two new populations – military veterans exhibiting 'Gulf War syndrome' and women having silicone breast implants for esthetic reasons or for breast reconstruction after cancer surgery.

The 1990s

The Endocrine Disruptor Hypothesis During the 1990s, new scientific and medical studies gave people surprising new perspectives on environment and health problems.

In 1991, Dr. Theo Colborn and Dr. John Peterson Myers invited an international group of scientists to meet and share notes on their own research. The meeting produced a consensus that some industrial chemicals, under some circumstances, can interfere with the endocrine system of laboratory animals, wildlife, and perhaps humans. The endocrine system includes a complex of organs and tissues whose actions are coordinated by chemical signals provided by hormones, neurotransmitters, growth factors, cytokines, and so on. Chemical signaling systems control reproduction, growth, development and behavior in plants, mammals, birds, fish, amphibians and reptiles, strongly influencing the immune, nervous, and reproductive systems. Signaling systems assert control before birth, hatching or sprouting and retain control throughout the remainder of life.

The influence of chemicals on the endocrine system had been the subject of a federally sponsored research conference in 1979, but the issue remained unrecognized by the environmental movement until 1991. Since 1991, the 'endocrine disrupter hypothesis' has profoundly influenced the direction that the environmental movement has taken.

In 1996, the book *Our Stolen Future*, by Colborn, Myers, and Dianne Dumanoski, popularized the endocrine disrupter hypothesis by framing it as a kind of scientific detective story. Critics of the book argued that it distorted the underlying science, but like *Silent Spring* before it, subsequent research has shown it to be correct on all essentials and to have underestimated the severity and magnitude of the problem.

Since 1991, a decade of intensive study of biological signaling systems has revealed (among other things):

- The effects of exposure to signal-disrupting industrial chemicals can vary significantly, depending upon the timing of exposure. The development of an organism may be highly sensitive to disruption by exogenous chemicals during a particular stage of growth, yet be relatively immune to disruption during a different period of time. Therefore, the apparent toxicity or biological effectiveness of a chemical can vary significantly, depending upon the timing of exposure.
- Prenatal exposures appear to be particularly important, perhaps because cell replication and differentiation are occurring most rapidly during this stage of life.
- Individual industrial chemicals present at insignificantly low levels can, under some circumstances, combine together to produce significant effects on signaling systems.
- Low-level exposure to a particular chemical can sometimes produce effects quite different from those caused by higher doses of the same chemical. These differences can include positive effects at low doses (hormesis) and harmful effects at higher doses, as in the case of the essential mineral, chromium. Or, in the case of some chemicals that interfere with biochemical signaling systems, harm can occur at low doses but not at higher doses, exhibiting an inverted U-shaped dose–response curve. The need for low-dose testing is becoming apparent, but in the recent past it has been considered prohibitively expensive in many instances.

These basic findings have resulted in new ways of looking at old problems, including:

- Because very few chemicals have been tested for these recently discovered effects, risk assessments now seem to rest on assumptions that are more uncertain than previously realized. If timing of exposure is sometimes crucial, if complex mixtures must be taken into consideration, and if low levels of exposure can sometimes produce greater interference in signaling systems then higher levels of exposure, then the underlying assumptions of many risk assessments completed to date need to be reexamined.
- The increasing incidence of many kinds of birth defects suggested that prenatal exposures may determine the course of life. Studies of signal-disrupting chemicals have provided evidence that this is the case, which means that personal liberty and lifetime opportunities may be truncated or constrained by chemical exposures before birth. This kind of 'chemical trespass' in the absence of informed consent raises fundamental questions of ethics and human rights. From a public health perspective, it indicates that women who are pregnant, or who may become pregnant – which in principle includes almost all premenopausal women – should be protected from exposure to even low levels of exotic chemicals, to eliminate these potential sources of harm to their offspring.
- As the 1990s unfolded, the environmental movement and US EPA developed a focus on children, for several reasons. Compared to adults, children are undergoing more rapid cell division with more opportunities for interference by signal-disruptors; children breathe more air and ingest more water and food, per unit of body weight; children put their hands in their mouths more than adults do; children absorb chemicals through their digestive tract differently, and detoxify absorbed chemicals differently; children spend more time close to the ground where they may encounter toxicants in dust, soil and carpets as well as pesticide vapors; growth and development create 'windows of vulnerability' during which chemical exposures may cause permanent, irreversible damage; and because they are exposed to toxicants at an earlier age, children have more time to develop environmentally triggered diseases with long latency periods, such as cancer. Children are also among the most dependent members of society and therefore need special protection.

The increasing incidence of autism and attention deficit hyperactivity disorder (ADHD) among children has led researchers to uncover evidence that industrial chemicals may be contributing to these and other intellectual deficits and behavioral problems including aggression and violence. In an 'information age' that emphasizes the importance of intelligence and intellectual skills in nearly every sphere of life, diminishing children's intellectual and emotional capacities by chemical trespass without informed consent is widely regarded as unethical and unacceptable.

At this writing, the endocrine disrupter hypothesis has become very widely accepted for testing. Numerous scientific studies are now published each month, many of them providing evidence that some industrial chemicals, under some circumstances, can interfere with biological signaling systems, producing a wide range of ill effects in many different species, including humans.

Environment and Disease By the 1990s, readers of US government journals (e.g., *Environmental Health Perspectives*, and *Morbidity and Mortality Weekly Reports*), were familiar with evidence that the incidence of many chronic diseases was increasing. Furthermore, a growing body of literature was revealing suggestive links between chemical exposures and familiar problems such as asthma, diabetes, lupus erythematosus, birth defects, infertility and other reproductive disorders, learning and behavior problems, Parkinson's disease, and several cancers (e.g., brain, female breast, prostate, testicular, and childhood leukemia), among others. The rise in childhood cancers seemed particularly worrisome because children's lifestyles had not changed dramatically during the previous 30 years, so researchers sought explanations in the environment, as noted above.

Connections between chemicals and human disease have always been difficult to establish conclusively because of routine exposures to mixtures of chemicals, the absence of an unexposed population to serve as a control, and sometimes long delays between the time of exposure and the manifestation of harm, delays sometimes spanning more than one generation. Many argued that the resulting scientific uncertainty provides grounds for continuing along our present path undeterred. Sir Austin Bradford Hill responded to such an argument in 1965 when he said, "All scientific work is incomplete – whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us as freedom to ignore the knowledge that we already have, or to postpone the action that it appears to demand at a given time."

Many survivors of serious diseases have traditionally formed support groups to share information and experiences among themselves. However, since the early 1990s, these groups have become more politically active, joining the toxics and environmental justice movements. In 2002, the Collaborative for Health and the Environment (CHE) was formed to give voice to the concerns of disease survivors, to disseminate information linking chemical exposures to various illnesses, and to urge a precautionary, preventive approach to chemical exposures.

Global-Scale Environmental Harm

In the searingly hot summer of 1988, global warming finally came to people's attention in a dramatic way when a respected government scientist told Congress that he believed humans were partially responsible for increasing the average temperature of the planet.

Carbon dioxide in the atmosphere had risen 30% during the previous 200 years, from the burning of coal and petroleum. Since 1896 scientists had periodically reminded us that increasing the carbon dioxide level in the atmosphere would eventually warm the planet by a mechanism known as the 'greenhouse effect'. Just as a glass roof warms a greenhouse by trapping solar energy, carbon dioxide in the atmosphere acts like a glass roof over the earth, trapping the sun's energy and warming the planet. Since the 1970s, scientists had wondered whether the earth's noticeable warming trend was caused by humans or was a natural fluctuation in temperature. In the summer of 1988, the public began to be told that global warming was partly caused by humans, though another 7 years would pass before the Intergovernmental Panel on Climate Change (IPCC), which includes 2500 atmospheric scientists, would officially take that position.

In addition to global warming, the 'hole' in the Earth's stratospheric ozone shield made headlines each spring starting in 1985. Human dislocation of the atmosphere revealed just how powerful human technologies had become – and how poorly understood they were. For thousands of years the Earth had seemed enormous and humans had seemed puny though clever. Now suddenly the Earth was starting to look small and humans, armed with modern technologies, were beginning to look like clumsy behemoths flying blind.

By the end of the 1980s, it was becoming evident to many people that governments were not succeeding in bringing destructive technologies under rational control. This led to a focus on another institution – the modern corporation.

Focus on the Corporation

Starting in the early 1970s, the consumer movement set out to modify corporate behavior through economic pressure. In 1973, the Interfaith Center for Corporate Responsibility brought together faith-based institutional investors, to influence corporate behavior through shareholder resolutions. In 1977, a group called Infact organized a consumer boycott of the Nestle Corporation to protest the firm's marketing of baby formula, and numerous boycotts of other corporations followed.

In 1994, a new approach emerged when Richard Grossman and Ward Morehouse formed the Program on Corporations, Law and Democracy (POCLAD), to study the legal entity called 'the corporation', pointing out that it derives all its power from state legislatures that grant 'corporate charters' – pieces of paper granting certain rights and privileges to groups

of individuals while limiting their liability for their actions.

In the United States, in the early nineteenth century, corporations were chartered only for narrow purposes, such as construction of a canal or a toll road, and for a finite period of time (typically 20 years). Investors were held personally liable for corporate actions, and the corporation's total capitalization might be limited by legislative fiat. It is evident from the historical record that up until the Civil War, and even beyond, legislatures were reluctant to give broad authority to corporations. Many legislatures explicitly acknowledged that corporations are subordinate entities that exist only to serve public purposes and can be dissolved if they fail in that duty.

Initially, corporations could only fulfill the specific mandate in their charter. However, in 1886 a decision by the US Supreme Court seemed to give corporations the Constitutional rights enjoyed by individual humans (also known as 'corporate personhood'). From that time forward judges took the position that, like any other individual protected by the Bill of Rights and the Constitution, corporations have broad authority to do anything not specifically prohibited by law. In the following 100 years, corporations grew exceedingly large and powerful.

Now the anticorporate tributary of the environment movement is working through legislatures, courts, city and county councils, and other venues, trying to limit the rights that corporations enjoy. Their argument is that large publicly held corporations should not be protected by the Bill of Rights because such entities are nothing like individuals: corporations can grow without limit; they cannot die; they have limited liability for their actions; they have no built-in conscience analogous to that of an individual; they have a fiduciary duty to return a more-or-less steady profit to investors, a duty they are required by law to uphold before all else; and therefore they have a powerful incentive to externalize their costs, for example, to find ways to avoid paying for environmental damage to which their operations may contribute.

The phenomenon known as 'globalization' has focused a different kind of attention on corporations. In recent years major US corporations have worked hand in glove with the US government to create conditions conducive to 'free' trade – meaning, at base, the unrestricted flow of goods and capital (though not labor) across international borders. Many in the environmental and labor movements fear that globalization will undermine the authority of national governments and will thereby weaken or reverse hard-won environmental and labor standards in the United States and abroad. As a consequence US labor and environmental groups now work more closely together than at any previous time, and both are cooperating with counterparts abroad to an unprecedented degree. Within the larger environmental movement, the international antiglobalization tributary is one of the most significant developments of the past decade.

Animal Rights

The animal rights perspective can be traced to the founding of the American Society for the Prevention of Cruelty to Animals in 1866. The American Humane Associations was founded in 1877 as a network of local organizations aiming to prevent cruelty to children and animals. By the 1890s, several state Antivivisection Societies were founded to oppose the cruel treatment of animals, first in medical and scientific research, and later in education and product testing.

Slowly the animal welfare approach came to be supplemented by an animal rights approach. The Vegan Society was formed in 1944 on the principle that humans should avoid, to the extent possible, the use of animal products for food or for any other purpose. People for the Ethical Treatment of Animals (PETA), founded in 1980, defines animal rights this way:

Animal rights means that animals deserve certain kinds of consideration – consideration of what is in their own best interests regardless of whether they are cute, useful to humans, or an endangered species and regardless of whether any human cares about them at all (just as a mentally challenged human has rights even if he or she is not cute or useful or even if everyone dislikes him or her). It means recognizing that animals are not ours to use – for food, clothing, entertainment, or experimentation.

The animal welfare and animal rights movements can both be seen as part of a centuries-old movement toward pan-species democratization – toward the view that nonhuman species have a fundamental place in nature and a basic right to lead their own lives.

In a sense this approaches the view of nature held by the indigenous people of North America when Europeans first arrived (though indigenous people usually eschew a vegetarian diet because they believe that an omnivorous diet is fundamental to the sacred natural order).

At this writing the most militant branch of the environmental movement includes the Animal Liberation Front (ALF) and the Earth Liberation Front (ELF), both of which are committed to destroying property, though not life, as a way to stop the cruel

exploitation of animals (ALF) and to expose and derail destructive development (ELF).

Appropriate Technology

Questions about the manageability of complex technologies have caused many people to ask whether there were inherently safer ways to provide food, shelter, energy, transportation, and consumer products. And since the United States had put Neil Armstrong on the moon in 1969, the question was phrased, "If we can put a man on the moon, shouldn't we be able to invent nondestructive technologies?"

The 'appropriate technology' branch of the environmental movement had been sparked by E.F. Schumacher, who published the book *Small is Beautiful* in 1973. From 1950 to 1970 Schumacher held an important government position as chief economic advisor to the British Coal Board. In 1955 Schumacher spent time in Burma, where he developed the principles of what he called Buddhist economics: the belief that good work was essential for proper human development and that 'production from local resources for local needs is the most rational way of economic life'. He subsequently became a pioneer of what is now called appropriate technology: earth- and user-friendly technologies matched to the scale of community life.

The appropriate technology branch of the environmental movement remained small and obscure until 1987 when the United Nations' World Commission on Environment and Development (dubbed the Brundtland Commission for its chairperson, Gro Harlem Brundtland, then the Prime Minister of Norway) published the book-length study, *Our Common Future*. In essence, the Brundtland report leveled a fundamental critique at the world industrial system: it was not sustainable because it was incompatible with nature. A sustainable system is one that survives or persists throughout its full expected life span.

The Brundtland Commission identified three steps needed to make the human economy sustainable: (1) improved efficiency (technical improvements, producing more with less, recycling); (2) reducing the human population explosion; and (3) redistributing wealth from overconsumers to the world's poor.

The Commission also said that, in order to achieve sustainability, the global human economy would need to grow by a factor of 5–10 in total size to eliminate poverty and give everyone the wherewithal to protect the environment. This prescription matches that of traditional economists, who have long asserted that the solution to poverty and environmental destruction lies with economic growth (meaning physical growth in the flow of energy and materials, from extraction, through use, to final disposal). In this view, growth in the total size of the global economic pie assures that even those holding a tiny slice will gain something even without intentional redistribution, and nations will then be able to afford to protect their natural environments.

At the historical rate of global economic growth (3.5% year^{-1}), a fivefold increase in the global economy would occur in 47 years and 10-fold in 67 years.

Shortly after publication of the Brundtland report, 'sustainability' was on everyone's lips and the appropriate technology movement suddenly gained new attention. This branch of the environmental movement is clearly on the ascendancy today, as more and more people – including thousands of scientists and engineers – acknowledge that trying to 'conquer' nature has led time after time to unintended consequences that damage ecosystems, harm human health and ultimately are self-defeating. Nature cannot be conquered, at least not in the way people assumed it could in 1950.

This new perspective still has many different names, indicating that it is not yet a fully coherent approach to industrial design. The National Academy of Sciences calls it 'industrial ecology'. Greenpeace calls it 'clean production'. Recycling professionals and activists sometimes call it 'zero waste', others call it 'toxics use reduction'. The United Nations Environment Program calls it 'cleaner production'. Many chemists call it 'green chemistry' and many engineers call it 'green engineering'. Many biologists call it 'biomimicry' after a book of the same name by Janine Benyus published in 1998. Biomimicry means 'a new way of viewing and valuing nature'. Biomimicry 'introduces an era based not on what we can extract from the natural world, but on what we can learn from it'.

To a very real degree these emerging disciplines are informed – consciously or not – by the worldview held by the indigenous people of North America when the Europeans first arrived: If we will observe carefully, and behave respectfully, nature will show us how to live in community with all living things.

The Faith-Based Environmental Movement

Some members of the US Judeo-Christian religious community have long been concerned with public health, sustainable agriculture, water and air pollution, urban land use, and other issues now labeled 'environmental'. However, the 'faith-based environmental movement' traces its roots to January 1, 1990, when Pope John Paul II said, "Men and women

without any particular religious conviction, but with an acute sense of their responsibilities for the common good, recognize their obligation to contribute to the restoration of a healthy environment. All the more so should men and women who believe in God the Creator, and who are thus convinced that there is a well-defined unity and order in the world, feel called to address the problem..."

In a widely circulated 'Open Letter to the American Religious Community' in 1991, a group of 32 Nobel laureates expressed grave doubts about the sufficiency of humankind's response to the environmental crisis. In 1993, the National Religious Partnership for the Environment was formed "to further integrate the mission of care for God's creation throughout religious life, once and for all.... Humankind must better understand its role in the greater web of life. Destruction of habitat requires a change in the values of civilization. Changes of thought and behavior require transformation of heart and spirit. These are the perennial concerns of religion."

In the United States, where 80% of the population professes the Christian faith and 33% attends weekly worship services, this tributary of the environmental movement has the potential to reach large numbers of people with the compelling message that the Earth belongs to God and humans have a duty to care for it.

New Decision-Making Techniques

By 1990, risk assessment had been used for more than 20 years to establish allowable residues of pesticides on food; to assess the dangers of living near toxic waste sites; to determine acceptable levels of air and water pollution; to decide how to prioritize expenditures on environment-related government programs, and on and on.

Just when new information about the effects of chemical exposures was provoking new questions about the scientific bases of risk assessment, community-based environmental groups were coming to realize that risk assessment was not a value-neutral scientific tool, but was in fact quite political, in the sense that it could be used to reach predetermined conclusions through the deliberate choice of assumptions, uncertainty factors, and judgments. Furthermore heavy reliance on risk assessment had the effect of placing decisions in the hands of experts instead of the hands of the people who would be affected by the decisions.

These concerns led to the development of new decision-making criteria, which are still evolving now. Some examples:

- The 'polluter pays' principle, which was adopted by the Organization for Economic Cooperation and Development (OECD) in 1974 and reaffirmed in the US's Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, or 'Superfund') in 1980.
- The 'substitution principle', adopted in Sweden's 1990 Act on Chemical Products, which reads, in part, 'Anyone handling or importing a chemical product must take such steps and otherwise observe such precautions as are needed to prevent or minimize harm to man or the environment. This includes avoiding chemical products for which less hazardous substitutes are available'.
- The principle of reverse onus, or reverse burden of proof, says that when there are scientifically based suspicions of harm about a chemical or product or process, the burden is on the producer or user to convince government authorities, beyond a reasonable doubt, that the product or process should not be restricted and that it is the least-damaging alternative available. This principle was recommended for adoption by the US and Canada in 1990 by the International Joint Commission (IJC, created by the Boundary Waters Treaty of 1909 to oversee international matters in the Great Lakes). To date, the United States and Canada have not acted on the IJC's recommendation, but Sweden adopted the principle of reverse onus for chemicals in 1990.
- In 1993, the World Bank suggested an ethical criterion for choosing among development projects: the least harmful alternative should be selected.
- Though not specifically intended for use in environmental decision-making, the political philosophy of John Rawls, with its focus on benefiting the least well-off and protecting the most vulnerable, has been gaining influence since it emerged in 1971.
- The public trust doctrine has evolved as a foundation stone for environmental protection. This ancient legal doctrine holds that our common heritage – the environment – is held in trust, with government the trustee and present and future generations the beneficiaries. Under this doctrine the trustee has an affirmative and inalienable duty to protect the trust property (nature, human health, the genetic integrity of living things, accumulated human knowledge, and, arguably, more) from the exploitive tendencies of the beneficiaries themselves.

- Biological criteria for environmental decisions:
  - Synthetic chemicals should be eliminated if they are toxic or persistent in the environment. In their joint 1978 Water Quality Agreement, the United States and Canada defined a 'toxic

substance' as 'a substance which can cause death, disease, behavioral abnormalities, cancer, genetic mutations, physiological or reproductive malfunctions or physical deformities in any organism or its offspring, or which can become poisonous after concentration in the food chain or in combination with other substances'. The International Joint Commission defined 'persistent' chemicals as those having a half-life of 8 weeks or longer. The half-life is defined as the time it takes for half of any substance to degrade once it has been released into the environment.

- Synthetic chemicals that bioaccumulate should be eliminated. A substance bioaccumulates if its concentration increases as it moves through the food chain. For example, DDT may be found at 1 part per million (ppm) in fish and at 10 ppm in fish-eating birds. Thus DDT bioaccumulates and therefore would be a candidate for elimination.
- The Natural Step: It was invented by a Swedish pediatric oncologist Karl-Henrik Robert with assistance from physicist John Holmberg. Robert, a respected cancer researcher, concluded in the mid-1980s that humans are destroying the natural environment because they lack fundamental principles for making decisions about technologies. He said then, "Up to now, much of the debate over the environment has had the character of monkey chatter amongst the withering leaves of a dying tree."

  The Natural Step defines four 'system conditions' that must prevail for a society to become sustainable.

  System condition no. 1: In order for a society to be sustainable, nature's functions and diversity will not be systematically subject to increasing concentrations of substances extracted from the Earth's crust.

  System condition no. 2: Nature's functions and diversity will not be systematically subject to increasing concentrations of substances produced by society.

  System condition no. 3: Nature's functions and diversity must not be systematically impoverished by physical displacement, over-harvesting, or other forms of ecosystem manipulation.

  System condition no. 4: Resources must be used fairly and efficiently in order to meet basic human needs globally.

- The precautionary principle, which originated in Germany in the 1970s in response to damage by acid rain in the beloved Black Forest. The original German concept, 'Vorsorgeprinzip', was developed to guide environmental planning. It translates best as 'the forecaring principle' but it also carries the connotation of foresight and preparation for the future, not merely precaution.

In 1992, the US government ratified the precautionary principle by signing the 'Rio Declaration' of the United Nations Conference on Environment and Development. Article 15 of the Rio Declaration says, "In order to protect the environment, the precautionary approach shall be widely applied by States [nations] according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation." Cost-effective means lowest cost.

In one form or another, the language of precaution has now been adopted in many international treaties and conventions, such as the North Sea Declaration (1987), The Ozone Layer Protocol (1987), the Ministerial Declaration of the Second World Climate Conference (1990), the Maastricht Treaty that created the European Union (1994), the United Nations Fisheries Convention (1995), The London Convention Protocol on ocean dumping (1996) and the Cartagena Protocol on Biosafety (2000), among others.

In the United States, the precautionary principle has evolved since 1992. A 1998 formulation, known as the Wingspread Statement, says,

> When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically.
>
> In this context the proponent of an activity, rather than the public, should bear the burden of proof.
>
> The process of applying the Precautionary Principle must be open, informed and democratic and must include potentially affected parties. It must also involve an examination of the full range of alternatives, including no action.

All formulations of the precautionary principle share three common precepts:

1. where there is reasonable suspicion of harm based on evidence,
2. and we have scientific uncertainty about cause and effect,
3. then we have a duty to take action to prevent harm.

The central feature of the precautionary approach is the way it alters society's traditional response to uncertainty. Since at least the publication on John

Stuart Mill's *On Liberty* (1869), westerners have assumed that individuals enjoyed the fundamental freedom to behave as they saw fit until their behavior harmed another person (or harmed the larger society in some way), at which point society assumed the right to intervene. Traditionally, suspicion of harm accompanied by uncertainty has not provided a sufficient justification for society's intervention. Instead, convincing proof of harm, bordering on certainty as to cause and effect, has been required.

During the 1960s and 1970s, the study of environmental and human health problems revealed that by the time scientific consensus has been achieved regarding the causes of a particular harm, great damage can occur. Diethylstilbestrol (DES) was shown to cause cancer in laboratory animals in 1938 but it was nevertheless prescribed to millions of women until it was banned in 1971 after it was shown that children of DES-treated women were developing cancers and reproductive problems; lead in paint was known to poison children in the 1920s, or even earlier, yet it was not banned until 1976; studies in the 1930s indicated asbestos could cause lung cancer but its use in building materials was allowed to continue into the 1970s. This list could be readily extended with additional instances in which reasonable suspicion of harm was ignored while the search for scientific certainty unfolded – all at enormous cost to individuals and society.

Opponents of a precautionary approach say they fear it will stifle technical innovation, but proponents believe it will spur innovation through the systematic search for least-harmful ways of meeting society's needs.

In 2000, the European Environment Agency formally adopted the precautionary principle to guide all its environmental policies. In the United States the federal government has generally resisted adopting a precautionary approach, but precautionary thinking is beginning to be embraced at the local level. In 2003, the City and County of San Francisco, California adopted the precautionary principle to guide all policies. Many municipalities across the United States have adopted a precautionary approach to pest control, based on the assumption that chemical pesticides may be harmful to nonpest species and should be used only as a last resort.

The essence of the precautionary principle is perhaps best summed up in the time-worn adage, 'Better safe than sorry', and 'A stitch in time saves nine'. This approach seems to be slowly replacing the earlier philosophy of development, which was closer to 'Nothing ventured, nothing gained' and even in many cases, 'Damn the torpedoes, full speed ahead!'

New Ways of Seeing

In 1987, a new academic discipline emerged, called 'ecological economics', based on a new view of the world and the role of humans in it. The new view reveals a world that is no longer empty, but is now full – of humans and their artifacts. In this view, the human economy is now large enough to stress the recuperative powers of the ecosystems upon which the human economy depends. This branch of the environmental movement is thus offering a fundamental challenge to 300 years of economic thinking, which has always assumed that the human economy was in danger of remaining too small to meet all human wants, not that it might grow too large.

In support of their 'full world' hypothesis, ecological economists adduce various kinds of evidence:

- The human economy uses, directly or indirectly, ~40% of the net primary product of terrestrial photosynthesis. This means that, with one more doubling of human population (expected in 40–45 years), humans will be appropriating 80% of net terrestrial primary productivity – leaving little room for other than domesticated species.
- One result of human appropriation of the earth's terrestrial resources is soil degradation, which is widespread; worldwide, rates of soil loss exceed rates of soil formation by at least a factor of 10.
- Another result of human appropriation of terrestrial resources is the rapid loss of species, which is now proceeding at a rate somewhere between 100 and 1000 times the historical rate of extinction.
- The human contribution to atmospheric carbon dioxide (a 30% global increase in 200 years) and methane (which doubled in concentration during the same period) indicates that the human economy is now capable of disrupting ecosystems at a global scale. The US National Academy of Sciences has warned that climate change caused by the accumulation of 'greenhouse gases' (carbon dioxide, methane, nitrous oxide, and others) in the atmosphere may be the most pressing international issue of the present century.
- The buildup of greenhouse gases in the atmosphere, and the rupture of the earth's stratospheric ozone shield by chlorofluorocarbons (CFCs), indicate that the human economy has already exceeded the assimilative and regenerative capacities of the biosphere to absorb some human wastes. There is considerable evidence to support this general proposition. For example, persistent synthetic toxicants are now measurable from the peaks of the highest mountains to the floors of the deepest oceans and everywhere in between. It is

evident that nature is unable to degrade certain synthetic compounds as rapidly as humans are able to produce them.

Obviously if this 'full world' view is correct, then relying on global economic growth to solve problems of poverty and environmental degradation could be self-defeating. Indeed, ecological economists assert that the Brundtland Commission's proposed solution to poverty and environmental destruction – expanding the size of the global economy by a factor of 5 to 10 – is ecologically impossible. In this new view, poverty will have to be alleviated – and environmental degradation checked – by economic growth in poor regions matched by a reduction in the size of the economy in wealthy regions.

Unceasing growth is not observed in nature, giving support to the ecological economists' view that the human economy must eventually achieve a steady state. In this view, 'growth' must cease but 'development' can continue indefinitely. Growth is defined as increase in material throughput whereas development is qualitative improvement, such as gains in the efficiency of resource use. This distinction between 'growth' and 'development' is central to ecological economics. In the 'full world' view, there are definite ecological limits to growth, but not to development.

Ecological economists argue that the 'full world' hypothesis calls for new scientific approaches, new solutions for economic problems long ignored by traditional economists, new emphasis on conserving nature's bounty, and new 'incentives based' policies for managing and protecting the environment.

New Scientific Approaches

Ecological economists say the 'full world' hypothesis will require humans to re-organize their intellectual activities, shifting the fundamental scientific paradigm from Newtonian physics to ecology. Newtonian physics views the world as linear, separable, reducible to its component subsystems, which can be readily aggregated to model the behavior of the whole system. In contrast, an ecosystem perspective develops a worldview adapted to complex living systems – constantly interacting and evolving, nonlinear, and not scalable by simple aggregation.

In the ecological view, human knowledge of the evolving world is fraught with fundamental uncertainties that are large and likely to remain so, spawning a scientific approach that has less confidence in its predictions and prescriptions than was common in an earlier era. The ecological approach has produced a generation of scientists – among them, conservation biologists – who advocate greater humility and a more precautionary approach than was common in the past, with an orientation toward learning from nature for the purpose of working with it rather than subduing it.

Problems Traditionally Ignored by Economists

The full-world hypothesis, combined with the desire to design sustainable economic systems, confronts ecological economists with three economic problems – scale, distribution, and allocation. The problems of scale and distribution have traditionally been ignored by economists.

The scale problem requires economists to ask how large the human economy can become in relation to the biosphere from which it draws raw materials and to which it returns wastes. Neoclassical economists have never addressed this problem of scale, believing as they do that scarcities will always generate price signals that cause new technical solutions to emerge, making everyone better off – the very definition of progress. However, for reasons given above, ecological economists believe that the first priority must be to establish the ecological limits of sustainable scale and then establish policies to assure that the material throughput of the economy remains within those limits. Without limits on the scale of the human enterprise, damage to ecosystems may deprive us of essential ecosystem services (such as regulation of atmospheric temperature, or protection from ultraviolet radiation from the sun) making us all worse off. Traditional market mechanisms take no notice of scale, so limits must be determined by informed choices, then translated into explicit policies, ecological economists believe. Discovering ecological limits will require urgent scientific effort in coming decades, they say.

Distribution has to do with the fair and equitable apportionment of economic goods and opportunities. Solutions to sustainability that are seen as unfair may be rejected or dismissed out of hand by decision-makers. As with scale, traditional market mechanisms take no notice of fair or equitable distribution, so a desirable distribution must be consciously chosen and implemented through explicit policies. Ecological economists say that the clearest implication of the full world hypothesis is that the current level of per capita resource use in wealthy countries cannot be generalized to poor countries, given the present global population, because present global resource use already appears to be unsustainably large. If the scale of the total global economy is bounded by ecological limits and resource scarcities, then poverty

can be alleviated by distribution policies that promote growth in poor regions balanced by economic shrinkage in wealthy regions.

After questions of scale and distribution have been settled, then markets and prices can be relied upon to achieve their traditional purpose – an efficient allocation of resources: how many shoes will be produced at a particular price, versus how many bicycles, and so forth. Allocative efficiency by itself does not ensure sustainability, which is why questions of scale and distribution must be settled first, as ecological economists see it.

New Emphasis on Conserving Natural Capital

One consequence of a full world, ecological economists say, is that the human economy has evolved from one in which human capital was the limiting factor to one in which remaining natural capital is the limiting factor. Natural capital is the stock that produces a flow of natural resources – forests that yield lumber, petroleum reserves that yield petrochemicals, and so on. If the pattern of scarcity has fundamentally evolved to a new condition – as ecological economists believe it has – then economic policies should aim to preserve, and where possible increase, natural capital and enhance the efficiency of its use, rather than merely continuing to liquidate natural capital to accumulate human capital.

Traditional economists teach that human capital is a nearly perfect substitute for natural capital – as we exhaust one natural resource, human ingenuity will always find a substitute. Ecological economists emphasize that human capital is far more likely to complement natural capital than to substitute for it, a view that highlights the importance of preserving our remaining natural capital and using it more efficiently. Fishing boats complement, but do not substitute for, fish, and sawmills complement, but do not substitute for, trees.

Neoclassical economists take it on faith that technological advances, driven by higher prices generated by scarcities, will always be able to overcome resource limits, and that new technologies can substitute for ecosystem services that become degraded. Ecological economists are not so optimistic because they believe human activity is ultimately constrained by ecological limits.

Herman Daly offers three criteria for the maintenance of natural capital:

1. For renewable resources, the rate of harvest should not exceed the rate of regeneration.
2. The rates of waste generation should not exceed the assimilative capacity of the environment.
3. The depletion of a nonrenewable resource should require comparable development of renewable substitutes for that resource.

Ecological economists believe that traditional economists have, so far, not taken into account the fundamental shift that has occurred in the pattern of scarcity – from human capital as the limiting factor to natural capital as the limiting factor – perhaps partly because the changes wrought by exponential growth appear so quickly. With a constant rate of growth, in the same time it took the world to move from 1% full to 2% full, the world will move from half full to completely full – a change that ecological economists see occurring in the present era.

New Policies

The environmental movement developed in response to the perceived failure of government to protect our common heritage, the natural environment, from harmful human activities and technologies. Ecological economists trace this failure to government's reliance on a limited set of policy mechanisms – chiefly regulation.

State and local governments have proved to be ineffective regulators because they compete with each other to achieve economic growth, creating a 'race to the bottom'. In principle, this defect could be remedied by federal regulations to which all states must conform. However, even federal standards suffer lapses in compliance and enforcement so, for example, many major municipalities have failed to meet federally mandated air quality standards.

Perhaps the most fundamental criticism of the regulatory system is that it assumes that emissions are harmless until violation of a regulation can be proved, or harm can be shown beyond a reasonable doubt. Since the vast majority of emissions are not subject to any regulations, the public bears the burden of proving harm, a very difficult burden to meet, for reasons discussed above.

Although the regulatory system has unquestionably prevented much harm, relevant questions remain: Has regulation adequately protected the public trust? Can regulation provide adequate protection against potential harms of current and emerging technologies such as pesticides, nuclear proliferation, biotechnology and nanotechnology? Can regulation provide sufficient protection for public health and the environment at least cost?

Ecological economists would likely answer all three questions in the negative. They – and many

traditional economists – favor new 'incentives based' environmental management techniques to supplement or replace regulation, including some or all of these:

- taxing emissions;
- issuing a limited number of marketable permits to pollute;
- adding 'product charges' to the prices of products that pollute;
- in come cases, subsidizing polluters who abate;
- creating property rights in open-access resources;
- labeling products with their contents;
- educating consumers;
- imposing deposit-refund systems on manufacturers of products; and
- prohibiting the discounting of future benefits.

Emission fees, or taxes, are fairly straightforward. They require government to set a fee per unit of emission, monitor emissions, and collect the fees. Emission fees must be set high enough to provide a continuous incentive for emitters to abate, yet not so high as to be perceived as grossly unfair or unreasonable. This requires knowledge of the costs of abatement and of the harms caused by various emissions – a difficult problem inherent in the regulatory system as well. Exceptionally toxic materials – for example, highly radioactive elements, lead, mercury, and polychlorinated biphenyls (PCBs), to name a few – would not be candidates for control by emission fees alone, but would remain under strict regulatory limits.

Marketable permits to pollute offer an economically efficient way to reduce pollution from numerous sources in a region, at least total cost to the economy. This is so because the cost of pollution abatement varies from firm to firm. Firms that can abate cheaply can be expected to do so, and can then sell their unused pollution permits to firms having higher abatement costs. A total cap on allowable pollution in a region would be set by the total number of pollution permits issued. As time passed, the total amount of permitted pollution could be ratcheted down. Difficulties with this scheme arise from the obvious injustices that can occur when firms with obsolete plants in poor neighborhoods find it cheaper to buy pollution permits than to upgrade and abate, potentially creating dangerous 'hot spots' of pollution in communities of color or low income.

Ecological economists are quick to point out that the economic efficiencies to be gained by 'incentives based' approaches should never be allowed to trump other critical, deeply held values such as fairness, justice, scientific integrity, and political acceptability achieved through democratic participation.

The ecological economists' vision summarized:

1. Earth is materially finite and not growing; therefore, there are limits to the size of the human economy that can be supported. The human economy is the product of human population and per-capita resource use and it is the total product that must be limited, not merely population.
2. A sustainable future is possible, offering a high quality of life to all creatures (human and nonhuman), subject to the limits inherent in a materially closed system, but achieving the vision will require new ways of doing things, many of which remain to be discovered.
3. In ever-changing complex systems, such as human economies interacting with ecosystems, fundamental uncertainty is large and likely to remain so, and some processes are irreversible – which counsels humility and a precautionary approach.
4. Because human capital and natural capital complement each other, and hardly ever substitute for one another fully, new emphasis must be given to retaining and enhancing natural capital, finding ways to make it more productive without destroying it. In sum, we must learn to live off the interest derived from natural capital while preserving the capital stock itself.
5. Management should be proactive, experimental and adaptive, using incentives to change human behavior, relying less on regulation than in the past. In other words, more carrots, fewer sticks, and much experimentation to discover what works.

Ecological economists believe that good environmental management must be experimental and adaptive because ecosystems are ever-changing and therefore our knowledge of them is fraught with uncertainties. Rather than relying solely on scientists to determine the best approach, resource managers must experiment, observe, and adapt their management techniques to what they are learning. Ecological economists expect local communities and the interested public to play an important role in experimentation, monitoring, and learning. This is quite a different approach from older resource management techniques in which scientists were expected to determine the truth about a problem, managers to apply the scientific information, and the public to sit passively by.

Summary

The US environmental movement is large, diverse, and growing. However, it has not yet achieved the

coherence of previous social movements like the nineteenth-century crusade to end slavery, or the 100-year struggle that won women the right to vote. Toxics activists, cancer survivors, animal rights groups, traditional conservationists, and workers focused on job safety all know of each other's existence but do not often coordinate their efforts, and they do not yet share a few simple goals toward which they are all striving.

As environmentalism has developed over the past 50 years, major shifts in emphasis have become apparent:

- The overarching goal is slowly shifting from managing problems after they appear to avoiding problems before they occur. This incorporation of the first principle of public health – prevention – into the industrial enterprise has begun, but it has also met implacable opposition from some who are committed to business as usual. It remains to be seen to what extent humans can develop the capacity to foresee and forestall while retaining the corporate structure that presently provides so many of the benefits of industrial production.
- There is no 'away', so none of us can any longer claim to throw anything away. Everything must go somewhere, which has spawned attempts at comprehensive materials management – cradle to cradle thinking, not cradle to grave. This new approach has generated a host of innovative ideas including biomimicry, green chemistry, zero waste, clean production, and so forth. Presently these ideas reside at the fringe of industrial innovation, but how long will they remain there?
- An earlier focus on preserving wild lands and protecting endangered species has been expanded to include the need to protect all living things. Two basic arguments support this expanded view:
- In the modern era, humans have learned anew that their well-being is dependent upon the mosaic of biological systems that make up the biosphere. And because the biosphere is constantly evolving and therefore may never be fully understood in its totality of relationships, no one today can reliably discern which parts of it are expendable without sacrificing long-term stability. Therefore it seems only prudent to conserve all the parts.
- The reemergence of an ancient ethical perspective, that all living things have an inherent right to lead their own lives, beyond any instrumental value they may have for humans.
- Human health requires more than the control of infectious diseases. As the ancients knew, it first requires relatively clean and safe places in which to live, grow, play, learn, and work. And it requires active community attention to the social determinants of health including income, education, living conditions, and the corrosive dynamics of social isolation, racism, inequality, and injustice.
- For 100 years or more the conservation and public health movements relied almost exclusively on experts to design and implement solutions to problems that were viewed through the twin lenses of engineering and the physical sciences. In the last quarter of the twentieth century, citizens began to insist that other valid ways of knowing must be brought to bear as well – perspectives of local knowledge, spiritual values, history, ethics, environmental justice, fairness, and democratic participation in decisions by those who are affected.

Still, despite heroic efforts by all concerned, a basic conflict persists between the needs of the natural environment and the needs of the human industrial enterprise. Although in principle nothing prevents humans – and all other creatures – from sharing the Earth and enjoying 'the good life' without undermining the biological integrity of the planet, in the modern era that goal has so far remained elusive and unmet. Nevertheless, rapidly growing recognition of the urgent nature of the problem – and new ways of conceptualizing, understanding, and responding to it – give reason for hope.

See also: Clean Air Act (CAA), (US); Clean Water Act (CWA) (US); Environmental Protection Agency (US); Food and Drug Administration (US); Genetically Engineered Foods; Lead; Mercury; Occupational Safety and Health Act, US; Organisation for Economic Cooperation and Development; Polychlorinated Biphenyls (PCBs); Risk Assessment, Ecological; Risk Assessment, Human Health; Toxic Substances Control Act.

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Environmental Change *See* Global Environmental Change.

Environmental Health

Chris Theodorakis

Environmental health is a discipline that examines human health effects from exposures to harmful agents in the environment. 'Environment' may include the outdoors, home, workplace, or public buildings. This field incorporates aspects from many diverse fields, including: (1) environmental, occupational, and food toxicology; (2) environmental and occupational medicine; (3) food safety; (4) occupational health and safety; (5) industrial hygiene; (6) public health; (7) epidemiology; (8) environmental policy and law; and (9) psychology and sociology.

Potentially harmful agents in the environment include biological, physical, and chemical agents. Harmful biological agents, or pathogens, may include bacteria, viruses, and parasites. Pathogens spread by person-to-person contact are sometimes included in the field of environmental health, but more often they are limited to those spread by environmental contamination as a result of human activities, such as sewage disposal and livestock production. Environmental health often addresses diseases caused by noncontagious pathogens and biotoxins. Noncontagious diseases are contracted directly from the environment rather than spread person-to-person, such as Anthrax and *Escherichia coli* infections. Biotoxins are poisons produced by bacteria or fungi, and may be taken up by ingestion or inhalation. Examples include botulinum toxin, aflatoxins (produced by fungi), and toxins produced black mold growing in houses. Diseases spread by arthropod vectors (mosquitoes, flies, ticks, mites, and fleas) and zoonoses may also be included under environmental health. Zoonoses are diseases that are contracted from animals, either by direct contact (Ebola, hanta virus, rabies, monkey pox, etc.) or via arthropod vectors (e.g., west Nile virus, St. Louis encephalitis). In this case, wild or domestic animals may provide a reservoir for the disease. Within the context of environmental health, interest in these diseases is often focused on anthropogenic (man-made) environmental disturbances, for example, global warming, agriculture, affect the spread of such diseases.

Physical agents in the environment that may cause illness include solar ultraviolet (UV) radiation, ionizing radiation (produced by radioactive materials and X-rays), extreme temperatures, noise, vibrations, and particulates. The most famous particulates inducing adverse health effects include asbestos and silica dust. Other physical agents, such as electric or magnetic fields and microwaves, may also cause adverse health effects, but there is of yet not enough solid evidence to support or refute this hypothesis.

Effects of environmental chemical agents on human health perhaps represent the bulk of environmental health research. People may be exposed to harmful chemicals in the outdoor air, surface water (lakes, rivers, oceans, etc.), soil, indoor air, at the workplace, in food, or from consumer products. Exposures to chemicals (or other agents) in the workplace are called 'occupational exposures'. Food exposure to chemical or biological agents may occur as a result of agricultural applications, environmental pollution, or are formed when foods are

cooked. Effects of food additives, chemicals produced by plants, and organisms associated with spoilage usually come under the subject of food safety or food toxicology. Consumer products include objects that may give off gases or vapors or leach chemicals into the water (e.g., carpeting, upholstery, plastics), items that may contain hazardous materials (e.g., batteries), or chemicals used in the home, yard, garden, or garage. Also, exposure to biological or chemical agents as a result of warfare or terrorist attacks has recently become a prime concern of environmental health workers.

Exposure to environmental agents may be through the skin, lungs, or digestive system. Effects from environmental exposures to harmful agents depend upon duration, frequency, and severity of exposure, as well as susceptibility of the individual. Susceptibility may depend upon age, nutritional status, sex, or genetic makeup (heredity). The duration, frequency, and severity or exposure depend upon many factors including locality, occupation, behavior, socioeconomic status, and population density.

Effects of exposures may encompass environmentally induced diseases and syndromes such as cancer (including leukemia), birth defects, neurobehavioral disorders, autoimmune diseases, acquired allergies, multiple chemical hypersensitivity, infections, and 'chronic fatigue syndrome'. Other effects of particular concern include effects on the immune, reproductive (including sterility and fertility), and endocrine (hormone) systems, nerve toxins, and brain development. Disorders of various organs that are routes or exposure or function in detoxification of chemicals – including lungs, skin, digestive tract, liver, and kidney – are also common. Organs with high rates of cell division, such as the skin, bone marrow, gonads, and developing embryo/fetus, are often highly susceptible to environmental agents. Also, some organs tend to accumulate ('bioaccumulate') toxic chemicals. For example, fat-soluble chemicals may bioaccumulate in fatty organs such as liver, brain, and breasts, while certain metals and radioactive materials may accumulate in bone.

Monitoring

In cases where people may exposed to hazardous agents on a regular basis, environmental surveillance may be carried out. This may consist of regular medical check-ups, environmental monitoring, biomonitoring, and dosimetry. Environmental monitoring includes collection of environmental media (air, water, soil) for chemical analysis, or may include real-time monitoring using devices that detect exposures to hazardous agents immediately or nearly so. Biomonitoring includes collection of biological samples, usually fluids or expired air, for determination of chemical concentrations or biomarker analysis. Finally, dosimetry is often determined using small devices worn on the person that give an indication of the dose of hazardous agents to which people were exposed.

Protecting the public from hazardous agents depends upon knowledge of the health effects of such exposures. One method of gathering information on the health effects of environmental chemicals is by using toxicity tests on animals' (usually rodents) *in vitro* systems (cultured cells or cellular components in flasks or test tubes). A second method of determining health effects of hazardous agents is by clinical challenge studies, clinical observations, and case studies. Challenge studies are when volunteers are exposed to low levels of chemicals or other agents under carefully controlled clinical settings. Clinical observations involve identifying clusters of disease associated with toxic exposures. Case studies are indices where individuals or a small group of people are exposed to high doses of a contaminant as a result of accidental poisonings or industrial accidents.

Another tool used by environmental health professionals to determine health effects of environmental agents is the field of epidemiology, which studies the incidence and progression of diseases in populations. Epidemiological studies may be prospective or retrospective. Prospective studies predict the scope and magnitude of diseases that have not yet been manifested, while retrospective studies assess the cause and/or magnitude of disease that already occur. Epidemiological studies may also be descriptive or analytical. Descriptive epidemiology uses vital statistics (birth and death rates), patterns of disease, or incidence of disease in exposed and unexposed populations at a single point in time ('cross-sectional' studies). Analytic epidemiology calculates risk factors for hazardous agents by either identifying exposed and unexposed segments of the population and comparing their disease frequencies ('cohort' or 'longitudinal' studies), or by identifying diseased and healthy individuals and determining their exposure histories ('case–control' studies). Another branch of epidemiology is molecular epidemiology, which incorporates biomarkers into descriptive or analytical studies in order to determine individual susceptibility to environmental hazards.

Unfortunately, however, assigning a cause to environmentally induced diseases is often difficult for the following reasons: (1) there is often a latency period of months, years, or decades between exposure and onset of disease; (2) there are often multiple causes of each disease; (3) there are few diseases that

are specific to any one agent; and (4) there are usually a host of confounding factors (e.g., age, gender, socioeconomic status, and behavioral factors such as smoking and consumption of caffeine or alcohol) that may influence incidence of disease. For these reasons, epidemiological studies often employ an established set of criteria for establishing causality for any environmental disease.

Determination of exposure and toxic effects of chemicals also requires knowledge of toxicokinetics. Toxicokinetics is the study of changes in the levels of toxic chemicals and their metabolites over time in various fluids, tissues, and excreta of the body, and determines mathematical relationships to explain these processes. These processes depend upon uptake rates and doses, metabolism, excretion, internal transport, and tissue distribution. Methods for determining these processes include studies with laboratory animals, volunteer human subjects, persons accidentally exposed to high doses of chemicals, and experiments with tissue or organs cultured in the laboratory. Computer simulations of such processes are often formulated using complex mathematical equations.

Protection of the public from exposures or effects of chemicals involves constructing safety standards, regulations, and exposure limits. This process usually relies upon human health risk assessment, which is a process of quantifying the likelihood, magnitude, and duration of human health effects from hazardous environmental agents. Human health risk assessment of hazardous chemicals consists of the following steps: (1) hazard definition and identification: establishing cause and effect relationships using animal or *in vitro* toxicity studies, clinical studies, epidemiology, and quantitative structure activity relationships (QSARs: prediction of a chemical's toxicity from its molecular structure); (2) establishing dose–effect relationships (i.e., what is the magnitude and duration of an effect for a given degree of exposure); (3) exposure assessment: includes environmental chemistry and surveillance, mathematical and computer simulation modes of environmental behavior of chemicals, toxicokinetics, and identification of all likely exposure pathways (intentional ingestion of contaminated food or water, accidental ingestion of soil, inhalation of gases, vapors, and particles, and absorption through the skin); and (4) risk characterization, which involves integration of the above three steps. Like epidemiology, risk assessments may be prospective (or predictive) or retrospective. Prospective studies assess possible occurrence and severity of health risks in which environmental exposure is hypothetical, but likely to occur. In retrospective studies, humans are environmentally or occupationally exposed to hazards and the potential for health risks (and how to mitigate them) is assessed.

Environmental health professionals, policy makers, and government officials use the outcome of risk assessments for risk communication (informing the public of possible risks and how to avoid them) and risk management (weighing policy alternatives and selecting appropriate regulatory action). Regulatory actions may include establishing exposure limits for certain chemicals, setting emissions standards for environmental pollutants, or taking protective actions. Protective actions may include: (1) isolation: prohibiting the public from, or advising against, entering certain areas; (2) shielding: using physical shields or protective clothing to prevent exposures; (3) time: limiting the time people may spend in hazardous areas; (4) treatment: treating environmental media to reduce or eliminate toxic or infectious agents; and (5) prevention strategies, such as vaccination against infectious agents or eating antioxidants to prevent toxic effects of certain chemicals.

To an increasing degree, environmental management programs are integrating protection of the environment and protection of human health. This may include integrating human health and ecological risk assessments. This also includes integration of effects of pollutants on ecosystem and human health. For example, an algal bloom caused by fertilizer pollution may deplete dissolved oxygen in the water and affect the natural ecosystem, and produce chemicals that are toxic to humans. Such integrated assessments are a practical means of reducing effort and money used in environmental management and protection.

See also: Asbestos; Biomarkers, Human Health; Biomonitoring; Botulinum Toxin; Chemicals of Environmental Concern; Ecotoxicology; Epidemiology; Pharmacokinetics/Toxicokinetics.

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Environmental Hormone Disruptors

Lorenz Rhomberg and Mara Seeley

The idea that exposure to small amounts of certain chemicals in the environment might disrupt normal endocrine function in wildlife and humans garnered widespread attention in the mid-1990s, and debate over the importance of this phenomenon continues today. Effects that have been potentially linked to these chemicals include feminization of males in various aquatic and avian species; declines in sperm quality; increased incidence of abnormal or incomplete genital development in human males; and increases in certain endocrine-mediated cancers such as breast, testicular, and prostate cancer. Concern for compounds that could cause such adverse effects initially focused on chemicals with estrogenic activity, and thus they were commonly referred to as environmental estrogens, or xenoestrogens. The initial focus on xenoestrogens has since expanded to include compounds with androgenic activity, as well as thyroid-active chemicals. Thus, today these compounds are commonly referred to as endocrine disrupting chemicals (EDCs). Compounds that have been implicated as EDCs include certain chlorinated organic compounds, principally some pesticides such as DDT and kepone, as well as polychlorinated biphenyls; some plasticizers and breakdown products of polycarbonate plastic, such as pthalates, and bisphenol A (BPA); and some pharmaceutical agents such as diethylstilbestrol (DES).

The endocrine system comprises glands located throughout the body, hormones synthesized by these glands, and specialized receptor molecules in target tissues that recognize and bind with these hormones. Naturally occurring hormones in the human body, such as the sex hormones estrogen and testosterone, thyroid hormone, and insulin (among others) are involved in a variety of processes including growth and development, sexual differentiation and behavior, metabolism, and reproductive function. Appropriately timed changes in the circulating concentrations of hormones exert control over these processes by binding with specific receptor molecules in target tissue, and thus eliciting the appropriate biological effects. At least in the laboratory, some EDCs, in sufficient concentrations, act as agonists, by binding with hormone receptors to trigger the receptor's biological actions. Other EDCs act as antagonists, by binding with hormone receptors in a way that prevents access of the naturally occurring hormones to their receptor, without triggering the receptor's biological actions. EDCs can also alter either the production or metabolism of certain hormones. The concern with EDCs is that they could potentially produce hormonal stimuli that are of inappropriate timing, magnitude, or biological context, which could thus result in unwanted biological effects such as feminization of males or increases in endocrine-mediated cancers. Because only very small amounts of naturally occurring hormones in the body are required to elicit their effects, some scientists believe that likewise only very small quantities of environmental EDCs would be required to elicit inappropriate endocrine effects. There is particular concern for exposures occurring during development, when timing and magnitude of hormone signals are critically important for many developmental processes.

Several lines of evidence helped to focus attention on the phenomenon of endocrine disruption. One was the observation, beginning in the 1950s, of reproductive problems in various wildlife populations, including reptiles, birds, and mammals. Some decades later, it was proposed that a common factor underlying these effects might be that environmental chemicals were affecting sex hormone-controlled processes. Then, in the late 1980s, there was the discovery that very low concentrations of the chemical *p*-nonylphenol, which is used in the manufacture of plastics, was leaching out of plastic test tubes and causing extensive cell proliferation in laboratory cultures of estrogen-sensitive breast cancer cells, even in the estrogen-free control cultures. At about the same time, *in vivo* studies in mice indicated that traits such as aggressiveness in females and prostate gland size in males could be influenced by intrauterine position.

In these studies, female mice that as fetuses were situated *in utero* between two males were more likely to be aggressive, while male mice that had been situated *in utero* between two female fetuses were more likely to have enlarged prostate glands. These studies demonstrated that even small variations in concentrations of hormones in the uterus could affect subsequent development.

Observations of modestly enlarged prostate glands, similar to those observed in male mice situated *in utero* between two female mice, were subsequently observed in male mice exposed *in utero* to low doses of DES, BPA (which is widely used in food and beverage containers), and the pesticide methoxychlor. These effects, which were observed at environmentally relevant doses, also included increased weight of preputial glands, decreased daily sperm production, and decreased epididymal weight. Not all studies, however, have shown effects with *in utero* exposure to low doses of EDCs. For example, additional studies with DES and BPA, several of which used the same study design and strain of mice as the initial studies, did not show any effect on the prostate gland, sperm production, or other organs. Based on a review of both positive and negative studies regarding low-dose *in utero* effects of BPA, an National Institute of Environmental Health Sciences (NIEHS)/Environmental Protection Agency (EPA) Endocrine Disruptors Low Dose Peer Review Panel concluded that these low dose effects have not yet been established as a 'general and reproducible finding'. Possible reasons for the discrepancies among studies include differences in intrauterine position, diet, living conditions (e.g., type of bedding and type of housing, and animals per cage), within strain differences, seasonal variation, and differences in body weight and prostate weight for control animals.

In addition to the question of whether *in utero* exposure to low doses of environmental EDCs is actually associated with specific adverse effects in laboratory animals, there is also the question of whether effects observed in laboratory animals, such as rats and mice, are relevant for humans. Although there are definite similarities between laboratory animals and humans in terms of the key hormones that are involved during gestation (e.g., estrogens and progestins), there are several differences that could play a role in relative susceptibility to environmental EDCs. One key difference relates to both the types and levels of estrogens observed in the fetus. Whereas pregnant rats and mice produce estradiol and estrone, pregnant humans also produce estriol, in addition to estradiol and estrone. Moreover, estrogen concentrations are greater in the human fetus as compared to the mouse and rat, and the proportion of unbound (i.e., biologically active) estrogen is also greater in the human fetus. As a consequence of the greater concentration of biologically active estrogens, the human fetus may be less sensitive to low concentrations of EDCs than rats or mice.

Although many chemicals are suspected of being potential EDCs, based, for example, on their ability to bind to hormone receptors, very few chemicals have been clearly established as endocrine disruptors *in vivo*. This is because currently there is insufficient data to establish causal relationships between exposure to many suspected EDCs, particularly at the low concentrations typically found in the environment, and effects potentially associated with endocrine disruption. In humans, the only chemical that has been clearly established as causing endocrine-mediated adverse health effects is the synthetic estrogen DES. DES was given to pregnant women in the 1940s–1960s to prevent miscarriages, thus resulting in relatively high exposure to the developing fetus. The primary effect of *in utero* DES exposure was abnormal development of the uterus and vagina as well as vaginal cancer in daughters whose mothers had taken DES. These effects, which were clearly associated with DES exposure, are not indicative of effects for most environmental EDCs, as the magnitude of exposure to DES far exceeded levels of concern for environmental EDCs. In addition, most other estrogenic substances, including the naturally occurring estradiol, are much less potent than DES. In fact, most estrogenic EDCs are far less potent than naturally occurring estradiol, thus requiring much greater concentrations of EDCs as compared with estradiol to elicit an effect.

Outside of the experience with DES, there is no direct link between exposure to environmental EDCs and specific endocrine-mediated effects in humans, although some observers have attributed several temporal trends to exposure to EDCs. These include reports of declines in sperm quality (generally referring to sperm quantity, but also to morphology and motility in some cases), as well as increases in hypospadias (abnormal location of the urethral opening in males), cryptorchidism (undescended testes), and testicular cancer. Many of these studies lack information on actual exposure to environmental EDCs, however, and associations between chemical exposure and effect are only speculative. For many of these end points, there are alternative and equally plausible factors that could explain the observed trends, including changes in diet, a more sedentary lifestyle, changes in diagnosis, or changes in collection methods (for declines in sperm quality). In some cases, the observed trend is not temporally consistent with exposure to EDCs. For example, incidence of

testicular cancer began to increase after World War I, while exposure to environmental EDCs increased most dramatically after World War II. Thus, exposure to environmental EDCs cannot necessarily be implicated as the primary cause for the observed increase in rates of testicular cancer. In other cases, such as for breast cancer, most studies indicate that there is no correlation with actual tissue concentrations of EDCs.

When likely exposure concentrations are taken into account, the most important EDCs to consider are not industrial chemicals but rather phytoestrogens, such as the isoflavones that are abundant in dietary soy products. Exposure to EDCs in the environment (e.g., through exposure to contaminated soil, water, or air) is typically dwarfed by our intake of these naturally occurring phytoestrogens. Soy products, which have long been consumed as part of the typical Asian diet, are increasingly consumed in Western diets. Few data exist for humans regarding potential effects of phytoestrogens, particularly for *in utero* exposures, although data in animals indicate that exposure to phytoestrogens at high enough levels may be associated with developmental abnormalities. Most notably, infertility has been observed in sheep grazing on clover with high concentrations of isoflavone precursors and in captive cheetahs following addition of soy meal to their diet. On the other hand, isoflavones may offer protection against breast cancer, particularly for postmenopausal women. Studies in animals also suggest that early exposure to soy products protects against formation of breast tumors later in life.

Evidence linking exposure to EDCs with specific effects is perhaps more convincing in wildlife than in humans, particularly for aquatic wildlife. For example, reduced genitalia in male alligators in Florida's Lake Apopka has been attributed to a massive spill of DDT in the late 1980s. There is also evidence that natural and synthetic estrogens in effluent from wastewater treatment plants may be causing male fish to produce elevated levels of the reproductive protein vitellogenin, which is ordinarily produced at elevated levels only in females. While such observations are certainly important in terms of their significance for aquatic populations, their significance for humans and other mammals is less certain, due to differences in physiology and metabolism. For example, although there is a high degree of similarity of the hormones and their receptors between aquatic species and mammals, there are also significant differences in hormone function and regulation. In addition, most effects that have been well established in wildlife are generally associated with localized exposure to relatively high concentrations of chemicals, such as DDT in Lake Apopka, or effluent from sewage treatment plants.

In response to heightened concern for exposure to potential endocrine disruptors in the environment, the US EPA chartered the Endocrine Disruptors Science and Technical Advisory Committee (EDSTAC), with the charge of recommending a screening and testing strategy for potential endocrine disrupting chemicals. EDSTAC proposed an Endocrine Disruptors Screening Program (EDSP) focusing on disruption of endocrine effects related to estrogen, androgen, and thyroid hormones for both humans and wildlife. The EDSP consists of a tiered approach involving sorting and prioritization of chemical substances and mixtures for further screening and testing, based on existing data. 'Screening' of prioritized chemicals identifies chemical substances and mixtures that are potentially capable of affecting endocrine control, while subsequent and more sophisticated 'testing' studies confirm, characterize, and quantify the nature of endocrine disrupting properties. US EPA is currently in the process of establishing screening priorities, and ensuring that Tier 1 Screening and Tier 2 Testing assays are scientifically validated. The overall aim of the EDSP is to facilitate identification of EDCs, so that ultimately the US EPA can take appropriate action, if necessary. At present there are 87 000 chemicals or chemical mixtures that are slated at least for screening in the EDSP.

Apart from the number of chemicals that need to be evaluated, evaluating potential EDCs for their ability to cause endocrine disruption presents several unique challenges to toxicologists. As illustrated by conflicting results from studies with BPA, the ability to detect effects at very low doses may depend on a variety of factors seemingly unrelated to exposure, such as diet, living conditions, and specific animal strain. In addition, it will be important to consider cross-species physiological differences in order to determine the relevance of findings in laboratory animals for humans or wildlife. Thus, one challenge will be to define the precise conditions under which laboratory responses can predict the existence and magnitude of true risks for humans or wildlife exposed to low levels of environmental EDCs. Another challenge is the possibility that EDCs may display either a U-type or inverted U-type dose–response curve (i.e., a dose–response curve for which low doses may be more potent than high doses). This means that the dose range for studies evaluating EDCs may need to be extended below doses at which no adverse effects are observed. Considering these challenges, it will likely be some time before there is a general consensus among scientists as to whether the

endocrine disruption phenomenon is real, particularly for low-dose exposures.

See also: Developmental Toxicology; Endocrine System; Estrogens I: Estrogens and Their Conjugates; Estrogens IV: Estrogen-Like Pharmaceuticals; Reproductive System, Female; Reproductive System, Male; Toxicity Testing, Developmental; Toxicity Testing, Reproductive.

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Environmental Processes

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Environmental processes include fate and transport of pollutants in the environment, which are components of the field of environmental chemistry. The fate of pollutants in the environment depends upon the 'compartment' in which they occur: air, surface water, ground water, soil, sediment (the 'mud' at the bottom of water bodies), or living organisms. The water compartment may include ground water, surface water (water bodies such as rivers, streams, lakes, ponds, oceans, etc.), or pore water (water in between soil or sediment particles). Within each compartment, the chemical may remain unaltered, but more often is altered by biotic (living) or abiotic (nonliving) components of the environment.

Alterations may include transformation, degradation, and changes in speciation or ionization. Transformation occurs when a contaminant is chemically altered by the addition of oxygen, hydrogen, or nitrogen, or is combined with or bound to another chemical. Abiotic transformations may include chemical oxidations or reductions in aerobic or anaerobic environments, respectively. Biotic transformations may be carried out by bacteria and fungi in the environment, or may take place within the bodies of plants and animals. Transformations may either make the chemical more or less toxic, depending on the reaction involved. If the chemical is broken down into smaller molecules, this process is called degradation. Abiotic degradation may occur through reactions of the chemical with oxygen, acids, alkalis, other chemicals, or by exposure to sunlight ('photolysis' or 'photodegradation'). Biotic degradation ('biodegradation') may be carried out by plants or animals, but bacteria and fungi accomplish the bulk of the biodegradation in natural systems. If a chemical resists biotic and abiotic degradations, it is termed a 'persistent' chemical. Some especially persistent chemicals, DDT, for example, may remain in soils and sediments for decades.

Many chemicals can also exist as various species or states of ionization. For example, nitrogen can exist as nitrate, nitrite, or ammonia, arsenic can exist as arsenate or arsenite, and lead can exist as lead nitrate or lead chloride. The species or ionization state may depend upon abiotic variables such as soil or water pH, amount of dissolved oxygen in the water, and presence of other chemicals. Alternatively, bacteria and fungi may change the species or ionization state of a chemical. For example, bacteria can convert arsenite to arsenate, and add methyl groups to ionic mercury to produce methylmercury.

Chemicals may also be taken up by plants and animals from the environment. Plants can take up pollutants through roots or leaves, while animals can take in pollutants by ingestion of contaminated food or water, absorption through skin or gills, or inhalation into the lungs. Chemicals can be absorbed from the lungs by inhaled vapors or gases, particulate matter (e.g., dust), or aerosols (tiny droplets suspended in the

air). Animals may also ingest from soil or sediment, and absorb chemicals through the skin from air, water, soil, or sediment. Chemicals may also be passed along in the food chain ('trophic transfer'). Certain chemicals that are lipophilic (i.e., accumulate in fat) may reach greater and greater concentrations in animals higher and higher in the food chain. Such a process is called 'biomagnification'.

The degree to which a pollutant is taken up, which also determines its potential toxicity, is determined by its bioavailability. Bioavailability refers to the ability of a chemical to move from the environment into a living organism. Bioavailability depends upon the ionization state and speciation of a chemical. Because certain organic compounds and clays can strongly bind various hydrocarbon chemicals and metals, the amount of organic carbon and clay in the soil, sediment, and water determines the bioavailability of these compounds. Bioavailability of metals is also dependent on the amount of sulfur precipitates of other metals in soils and sediments.

The fate of chemicals in the environment depends not only on processes taking place within compartments, but also by chemical partitioning between compartments. For example, there may be exchange of chemicals between air and water or soil. Movement from the water or soil into the air is accomplished by volatilization and evaporation of volatile or semivolatile compounds. Movement of chemicals from the air to water or soil is accomplished by deposition or diffusion into the water. Chemicals can also move from water to soil or sediment and vice versa. If a solid chemical in the soil or sediment dissolves into the water, this is called 'dissolution', while the opposite is called 'precipitation'. If a chemical dissolved in water attaches to a soil or sediment particle, this is called 'adsorption', while the opposite is called 'desorption'. The fugacity of a chemical, that is, its tendency to remain within a compartment, is affected by the properties of that chemical, as well as the chemical and physical properties of the environments such as temperature, pH, and amount of oxygen in water and soil. Wind or water currents, wave action, water turbulence, or disturbance of soil or sediment (through the action of air or water currents, animals, or human activities) may also affect partitioning of chemicals.

Chemicals can also be transported within and between compartments. Transport may be via convection, diffusion, or bulk transport. Convection occurs when environmental contaminants dissolved or dispersed in air or water are carried along by air or water currents. Diffusion through air or water occurs relatively slowly, and is of importance only over small distances. However, diffusion (as well as evaporation and volatilization) may be enhanced by convection, and diffusion, convection, and volatilization may act in concert to promote transport of contaminants between compartments. The third type of transport, bulk transport, occurs when pollutants are adsorbed to soil or sediment particles and are carried along by wind or water currents. Bulk transport of contaminated dust by the wind may transport the contaminants from the soil to the air compartments. Bulk transport from air to water or soil compartments may also occur when rain forms around contaminated dust particles or suspended particulate pollutants, or when gaseous pollutants dissolve in rain droplets and fall onto the soil or water surfaces. Chemicals may also be transported from water to air or soil compartments when strong waves or waterfalls produce aerosols that are carried away by the wind. Flooding may also transport contaminants from the water to the soil, either due to contaminants dissolved or dispersed in the water or from bulk transport of contaminated sediment particles. In addition, when rainwater falls on contaminated soil, it may flow overland and carry dissolved or dispersed contaminants or contaminated soil particles to surface waters, or percolate through the soil to carry pollutants to ground or surface waters. Finally, plant growth and activities of animals and humans may facilitate transport or contaminants within and between compartments.

Transport and fate of pollutants also depends upon the source of pollutants. Contaminants that originate from a definable source, for example, a smokestack or effluent (wastewater discharge) pipe, are known as 'point sources'. Sometimes, however, the source of the pollutant is more diffuse; for example, when pesticides are carried by runoff from a large area into a river. These sources are called 'nonpoint sources'. Often, point and nonpoint sources may be combined or interconverted; for example, if nonpoint runoff from a city is channeled into a discharge pipe via storm sewers.

There are also several methods to determine patterns of fate and transport of pollutants in the environment. In some cases, microcosms and mesocosms are used to study fate, biodegradability, bioavailability, and transport within compartments. Field surveys may also be used to study fate and transport of pollutants in contaminated environments. Such studies involve collection and analysis of biota, water, air, soil, or sediment. In some cases, radioactively labeled contaminants ('tracers') may be introduced in mesocosms or noncontaminated environments in order to determine their fate and patterns of transport. Finally, mathematical models are often used to produce computer simulations to

study fate and transport on a large scale. Often, these models rely on the 'mass balance' concept, which asserts that the total mass of a contaminant introduced into an ecosystem must be accounted for by summing the masses present in each compartment.

See also: Ecotoxicology.

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Environmental Toxicology

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Environmental toxicology can be broadly defined as the effects of environmental contaminants ('pollution') on living organisms. Such contaminants are also known as anthropogenic (man-made) or 'xenobiotic' (foreign to living organisms) chemicals. As a discipline, environmental toxicology is more of an applied science rather than a basic science, but environmental toxicological investigations may be slanted more toward basic research or applied research. Studies that have more of a basic research slant may include, for example, investigations of the mechanism of action of a particular chemical on a particular organism. Research in the more applied direction may include biomonitoring, toxicity tests, or ecological risk assessments. The diverse field of environmental toxicology may include effects on individual organisms, effects on humans, or effects at higher levels of biological organization (population, community, or ecosystem). The study of the latter effects is a subdiscipline known as 'ecotoxicology'. Toxic effects of xenobiotics on humans are also covered elsewhere in this encyclopedia. Therefore, the discussion below will focus on individual-level effects on nonhuman organisms. Such effects may include overt toxic responses (e.g., acute and chronic toxicity) or sublethal effects.

Acute and Chronic Toxicity

Toxicity tests examine the effects of xenobiotics on living organisms under controlled laboratory conditions. Acute toxicity tests are typically short-term tests (most commonly, 96 h in duration) and measure such endpoints as growth and mortality. In species with short generation times, reproductive endpoints may also be used. For terrestrial and aquatic plant species, the rate of photosynthesis or chlorophyll content may also be measured. Toxic effects are typically reported as LD_{50} for terrestrial species and LC_{50} for aquatic species (lethal dose or lethal concentration 50, the dose or concentration that is lethal to 50% of the test organisms). Nonlethal endpoints, such as growth or reproduction, may be expressed as ED_{50} or EC_{50} (effective dose or concentration, e.g., the concentration required to inhibit growth or reproduction by 50%). Toxicity tests are commonly determined using fish, crustaceans, insects, birds, algae, aquatic and terrestrial plants, and rodents. Alternative tests may use microorganisms such as bacteria. The duration of exposure is usually included in reporting acute toxicity, for example, 96 h LC_{50}.

Chronic toxicity tests may span the entire life-cycle of the organism (i.e., from zygote to age of first reproduction). However, chronic tests are often difficult to perform because of their long duration (9–30 months for fish tests) and are very expensive, which makes their routine use prohibitive. For this reason, three alternatives to full life-cycle tests include partial life-cycle tests, early life-stage tests, and short-term chronic tests. Partial life-cycle tests were developed for organisms that require >12 months to reach reproductive maturity. They are typically long enough to span a period from gonadal maturation until the first reproduction. Early life-stage tests expose organisms from the embryonic through juvenile stages, because these life stages are thought to be

most sensitive to effects of environmental toxicants. Short-term chronic assays last from 4 to 7 days. Results of the chronic tests are usually expressed as lowest-observed-effects level or concentration (LOEL and LOEC, respectively) and the no-observed-effects level or concentration (NOEL and NOEC). The LOEL and LOEC are defined as the lowest toxicant level or concentration causing an effect that is statistically significantly different from the control (no toxicant present), while NOELs and NOECs are the highest toxicant levels concentrations for which the effect is not significantly different from control.

There are a variety of factors that can affect the toxicity of chemicals to living organisms. These include intrinsic factors of the organism such as species, genetic constitution, age, sex, nutritional status, and overall health. This can also include extrinsic factors such as temperature, photoperiod, and, for aquatic or soil organisms, pH, water hardness, and oxygen availability. The presence of other chemicals can also affect the toxicity of a particular chemical. These toxic interactions can include additivity (toxicity of chemical A + B = toxicity of chemical A + toxicity of chemical B), synergism (toxicity of chemical A + B > toxicity of chemical A + toxicity of chemical B), or antagonism (toxicity of chemical A + B < toxicity of chemical A + toxicity of chemical B). In fact, humans and other organisms are usually exposed to complex mixtures of chemicals in the environment, and the effects of such mixtures on living systems has yet to be completely understood.

Sublethal Effects

Except in extreme cases (oil or chemical spills, overapplication of pesticides, etc.), environmental contamination does not result in acute toxicity in field situations. Thus, in the natural environment, pollution generally induces sublethal responses. Sublethal responses may include growth and reproductive indices, embryo/fetal development, larval metamorphosis, hormone and endocrine function, immune function, bioenergetics and metabolic rate, and overall health of the organism. Such sublethal responses can be determined during chronic or subchronic toxicity tests in the laboratory or in field surveys of indigenous organisms. Reproductive and developmental toxicity tests have also been developed using sea urchins, frogs, algae, chickens, rodents, and the aquatic organisms known as Hydra.

Although they may not directly affect survival, such responses may affect the fitness of organisms and eventually growth and sustainability of populations. Another type of sublethal effect includes so-called 'biomarkers of environmental contamination', which can be defined as alterations of physiological, cellular, biochemical, or molecular structures or processes that are indicative of contaminant exposure and effects.

Sublethal effects may also include behavioral traits of exposed organisms. Such altered behaviors may include simple behaviors or more complex behaviors. Simple behaviors include general activity level, abnormal or disoriented behavior, and avoidance of contaminated media (air, water, soil, etc.). These may occur at earlier or at lower exposure levels than overt mortality, and may be used as an early warning of toxic effects. More complex behaviors may include alterations or feeding, foraging, or predator–prey relationships, schooling in fish, migration, and homing, or reproductive behaviors. In general, simple behaviors are easier to standardize in laboratory tests, but may be less relevant to effects on fitness components (survival, growth, and reproduction) or ecological endpoints (effects on populations, communities, and ecosystems) that are more complex behaviors.

Toxicokinetics

Toxicokinetics refers to the uptake, excretion, metabolism, and distribution of environmental contaminants within the body of organisms. This discipline was derived from pharmacokinetics, which focuses on pharmacologic drugs rather than toxicants. Processes involved in toxicokinetics include uptake, elimination, and biotransformation. Uptake of contaminants can occur from ingestion of contaminated food, water, soil, or sediment, by absorption from air and water through respiratory surfaces such as lungs or gills, or absorption through skin when organisms come into contact with contaminated water, soil, or sediment. Elimination from various organs can occur through excretion of the toxicants, metabolic conversion into other chemicals, or total metabolic breakdown of the contaminants ('biodegradation'). Elimination from all possible organs and tissues is known as 'depuration'. Metabolic conversion of contaminants results in a less toxic product and the process is known as 'detoxification', but sometimes metabolic conversion results in a more toxic product, a process known as 'bioactivation'. Toxicokinetics can be determined with living organisms, but can also be simulated using computer simulation models (mathematical equations describing movements of chemicals). Such models rely on real data, however, as input and for determination of accuracy.

The rates of uptake, excretion, and metabolism and the equilibrium (or steady state) concentration of

chemicals in the body ('body burden') rely on characteristics of the chemical, the environment, and the animal. (*Note*: equilibrium or steady state refers to a condition when body burden does not change over time.) Characteristics of the chemical include its water or fat solubility, its volatility, stability, and how rapidly it can be metabolized. Characteristics of the environment include temperature, soil or water pH, and presence of other chemicals or contaminants. Characteristics of the animal include age, sex, breeding condition, species, and health condition. Because a chemical must be taken up to be toxic, and because body burden affects toxicity, characteristics of the chemical, organism, and environment also affect the toxicity of chemicals.

These characteristics also determine whether or not a chemical bioconcentrates (body burden concentration > environmental concentration due to absorption from skin or respiratory organs) or bioaccumulates (body burden concentration > environmental concentration due to all routes of uptake). Similar to toxicity tests, bioaccumulation tests are laboratory exposures designed to assess the potential for bioaccumulation or bioconcentration for a chemical.

See also: Biomonitoring; Ecotoxicology; Environmental Health.

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Eosinophilia–Myalgia Syndrome

Ken Kulig

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Introduction

During the summer and fall of 1989, an epidemic of an apparently new disease occurred throughout the United States. The illness was characterized by blood eosinophilia (greatly increased numbers of the type of white blood cell usually associated with allergic reactions), myalgias (severe muscle pain), fever, joint pain, rash, itching, and generalized swelling and was termed the eosinophilia–myalgia syndrome (EMS). It was initially recognized in October 1989 when physicians in New Mexico identified three women with similar clinical findings; all three had consumed L-tryptophan prior to onset of illness. Soon after, additional cases were recognized throughout the United States and in several other countries. Some cases ultimately resulted in death or severe disability.

Epidemiological studies initiated in early November 1989 by the health departments of New Mexico and Minnesota demonstrated a strong association between antecedent tryptophan consumption and EMS. A national surveillance program to investigate the new disease was initiated by the US Centers for Disease Control (CDC). On November 11, 1989, the US Food and Drug Administration (FDA) issued a nationwide warning that advised consumers to discontinue use of tryptophan food supplements. Six days later, the agency requested a nationwide recall of all dietary supplements that would provide a daily dose of more than 100 mg of tryptophan. The recall was expanded on March 22, 1990, to include all products containing tryptophan at any dose (with the exception of protein supplements, infant formulae, and intravenous solutions that incorporated small amounts of tryptophan for nutritional requirements).

With the removal of tryptophan from the consumer markets, the number of new EMS cases diminished

rapidly. Nevertheless, over 1500 persons were affected by the illness in the United States, with 37 known deaths. While the epidemiological and chemical investigations indicate that the epidemic of EMS was caused by contaminated L-tryptophan, the precise contaminant(s) or metabolites causing the disease is still uncertain.

Prevalence and Reasons for L-Tryptophan Usage

L-Tryptophan usage was widespread in the United States in 1989. In Oregon and Minnesota, ~2% of the household members surveyed had used tryptophan at some time between 1980 and 1989. The most common reasons for tryptophan use were insomnia, premenstrual syndrome, and depression; other reasons included anxiety, headaches, behavior disorders, obesity, and smoking cessation. Although most consumers purchased tryptophan for therapeutic use, it was marketed as a food supplement and widely available in the United States without a prescription. This product was not approved or regulated by the FDA.

L-Tryptophan is an essential amino acid; however, sufficient quantities are present in the diet of most US citizens without the need for supplements. The typical daily US diet contains 1–3 g of tryptophan, which satisfies the recommended daily dose of $3\,mg\,kg^{-1}$ body weight (or $210\,mg\ (70\,kg)^{-1}$ individual). It is metabolized to serotonin and therefore theoretically might have sedative and antidepressant properties.

Eosinophilia–Myalgia Syndrome National Surveillance Data

As of June 1993, 1511 EMS cases had been reported to the US CDC, including 37 deaths. The case definition developed by the CDC for epidemiological surveillance included (1) blood eosinophil count greater than $1000\,ml^{-1}$, (2) generalized debilitating myalgia, and (3) no evidence of infection or neoplasm that would explain the clinical findings. National surveillance data of July 1990 revealed that 84% of patients were female, 97% were non-Hispanic white, and 86% were over 34 years old (median age, 49 years). One-third of the patients required hospitalization. Ninety-seven percent of the patients with EMS had reported tryptophan use before onset of the disease, in doses ranging from 10 to $15\,000\,mg\ day^{-1}$ (median, $1500\,mg\,day^{-1}$). The prevalence of EMS was higher in the western United States than in other parts of the country, apparently paralleling the higher rate of tryptophan consumption in those states. The true prevalence of EMS was most likely underestimated by surveillance reports because persons with mild disease were excluded by the surveillance case definition. In addition, some cases may not have been reported to state or federal health agencies because the syndrome was not recognized by the patient or their physician.

Cases of EMS were also reported in Canada and Europe. In Germany, more than 100 persons became ill with EMS (as delineated by the CDC case definition). Unlike in the United States, tryptophan was available only by prescription in Germany, and thus case histories on German patients were well documented. As in the epidemiological investigations in the United States (see below), all of the tryptophan associated with EMS in Germany was traced back to a single manufacturer in Japan.

Epidemiologic Studies

After initial studies implicated the consumption of tryptophan as a major risk factor for EMS, US state and federal health agencies began investigations to further examine this association. Consumers of tryptophan were classified as either case (EMS patients) or control (non-EMS tryptophan users), and the lots of tryptophan consumed by each group were traced back to determine the tryptophan source. Before the epidemic, L-tryptophan had been manufactured by six companies, all in Japan. Analysis of the tryptophan sources for case patients and controls demonstrated a strong association between EMS and consumption of tryptophan manufactured by a single company, Showa Denko K. K. (Tokyo), a large petrochemical company.

In the Oregon study, 98% of case patients had consumed tryptophan manufactured by Showa Denko compared to 44% of controls. In the Minnesota study, 29 (97%) of the 30 case patients consumed tryptophan that was traced back to Showa Denko compared to 21 (60%) of the 35 controls (odds ratio (the ratio of the odds of the disease occurring in exposed individuals relative to the odds of the disease occurring in unexposed individuals), 19.3; 95% confidence interval, 2.5–844.9). High-performance liquid chromatography (HPLC) analysis (see below) of the tryptophan ingested by the one case of EMS in Minnesota that was not traced back to Showa Denko showed a chromatogram that was characteristic of the company's product, revealing that the tryptophan was, in fact, produced by Showa Denko. All later trace-back studies support the association between tryptophan manufactured by Showa Denko K. K. and the occurrence of EMS.

Data from a cohort of tryptophan users in a South Carolina psychiatric practice provide an estimate of the rate of occurrence of EMS (attack rate) in persons exposed to the etiologic agent. Of 157 people who consumed a single brand of tryptophan (comprising only three lots of tryptophan manufactured by Showa Denko), 29% were diagnosed as definite cases of EMS, and an additional 23% were classified as 'possible cases' because they had some clinical findings of EMS (such as eosinophilia without myalgia) but did not meet the strict CDC surveillance case definition. Thus, the pooled attack rate was 52% among persons exposed to the etiologic agent. Among those taking more than 4 g of this brand of tryptophan per day, the definite EMS attack rate was 59% and the pooled (definite and possible EMS) attack rate was 84%. Therefore a dose–response relationship appears to have been established. These data also suggest that most if not all individuals are susceptible to EMS if exposed to sufficient quantities of the etiologic agent.

Risk Factors

Two risk factors for EMS, other than consumption of implicated tryptophan lots, have been identified: the amount of tryptophan consumed and the age of the individual. The risk of developing EMS increased with larger dosages of tryptophan and with increasing age. The tryptophan dosage most likely reflects the degree of exposure to the etiologic agent: Persons who consumed larger doses of tryptophan probably had higher amounts of toxic metabolites formed, or had a greater probability of encountering tryptophan tablets that were contaminated with the causative chemical(s). The reason for the increased risk of EMS with age is unclear; it may be due to age-dependent physiologic changes in renal or hepatic function that delay the metabolism or clearance of a toxic substance, or to age-dependent changes in the immune system. No other host factors were found to alter significantly the risk of developing EMS.

Clinical Features

EMS is a syndrome with multiple clinical presentations and variable severity. The clinical course of EMS consists of an early (acute) phase and a late, long-lasting (chronic) phase. During the early phase, most patients developed severe myalgias, and perhaps in conjunction with weakness, joint pain, rash, shortness of breath, cough, headache, swelling, or paraesthesias (numbness and tingling) these symptoms prompted a visit to their physician. A complete blood count (CBC) would then reveal profound eosinophilia (sometimes up to $30\,000\,\text{cells ml}^{-1}$; normal $< 5\,\text{cells ml}^{-1}$). In different groups of EMS patients, the median eosinophil count has been reported to be $4000–6000\,\text{cells ml}^{-1}$.

The majority of patients also had an elevated leukocyte count with modestly elevated levels of aldolase, a marker of muscle injury; however, creatine phosphokinase, another indicator of muscle injury, was normal in most patients. This inconsistency between the levels of these two muscle-associated enzymes, previously described in some patients with systemic sclerosis and the toxic oil syndrome (TOS) (see below), is helpful in differentiating EMS from other myopathies (muscle diseases) and from eosinophilic fasciitis (EF) (see below). Approximately one-half of patients had abnormal liver function tests, although the changes were mild. The erythrocyte sedimentation rate, rheumatoid factor, and levels of IgE, complement, and cryoglobulin (all markers of immune dysfunction) were normal in most patients tested.

For some patients, cessation of tryptophan ingestion led to resolution of the symptoms; in other patients the use of high dose corticosteroids appeared to be helpful. However, for some patients, the disease evolved into a chronic phase, with cutaneous, neuromuscular, pulmonary, cardiac, and cognitive involvement. The most common features of chronic EMS are fatigue, muscle cramping, myalgia, paraesthesias with objectively demonstrated hypesthesias (lessened sensitivity to touch), chronic joint pain, scleroderma-like skin changes, and proximal muscle weakness. In one study, 88% of EMS patients continued to manifest more than three of these clinical symptoms after 3 years.

Pathologic studies have demonstrated a perivascular, lymphocytic infiltrate with eosinophils in the dermis, fascia, and skeletal muscle, with variable numbers of eosinophils. The perivascular infiltrate was accompanied by thickening of the capillary and arteriolar endothelium in dermal, fascial, and muscle vessels. The frequent occurrence of microangiopathy (disease of the small blood vessels) in biopsy specimens suggests that ischemia (deficiency of blood supply) may contribute to tissue injury. Deposition of major basic protein (an eosinophil-specific protein) in affected tissue of some patients suggests that cytotoxic eosinophil degranulation products may also play a role in the pathogenesis of EMS.

The histopathologic examination of affected skin showed thickening of the fascia, deep dermal fibrosis, and accumulation of mononuclear cells and eosinophils. *In situ* hybridization and immunohistochemical studies have demonstrated increased production of type I and type VI collagen in the extracellular

matrix of the affected fascia. Thus, the dermal and fascial fibrosis of patients with EMS is likely due to stimulation of collagen synthesis by fibroblasts.

Most patients with EMS reported paraesthesias, and in some patients severe peripheral neuropathy was the most prominent clinical feature. In a few, persistent paraesthesias have been accompanied by axonal and demyelinating abnormalities on electrophysiologic testing. Muscle biopsies showed a characteristic histopathologic picture, with extensive inflammation (fasciitis) and fibrosis in the connective tissue surrounding the muscle, but little evidence of muscle fiber damage. Perineural inflammation and type II muscle fiber atrophy with denervation features have been observed, but muscle fiber necrosis was uncommon. The severe myalgias may be related to inflammation of nerves in the fascia or muscle, peripheral nerve injury caused by granule proteins, possibly eosinophil-derived neurotoxin, or ischemia of nerves caused by occlusive microangiopathy.

Lung biopsies performed in a small number of patients revealed a vasculitis and perivasculitis with a chronic interstitial pneumonitis. Disturbances of cardiac rhythm and conduction have also been documented. Examination of cardiac autopsy specimens has demonstrated neural lesions throughout the conduction system, similar to the neuropathology seen in skeletal muscle. Inflammatory lesions of the small coronary arteries were also present. The prevalence of cardiac abnormalities among all patients with EMS is unknown, although life-threatening rhythm disturbances appear to be uncommon.

The most commonly observed disease process leading to death of patients with EMS was progressive polyneuropathy (disease involving the peripheral nerves) and myopathy (disease of muscles) that produced complications of pneumonia and sepsis or respiratory failure due to weakness. Two-thirds of EMS patients died of these complications. Other causes of mortality were cardiomyopathy (disorder affecting the muscles of the heart), primary pulmonary disease, arrhythmia (deviation from the normal rhythm of the heart), and stroke.

The response to therapy has been disappointing. Multiple therapeutic interventions have been suggested, but no clearly effective treatment has been identified. In the early phase, glucocorticoid treatment (usually prednisone) was generally helpful in treating pneumonitis, myalgias, and edema and in reducing the eosinophil count. However, some patients have not responded to high doses of prednisone, and others have had an exacerbation of symptoms when the dose was tapered. There is no evidence that prednisone therapy alters the natural history of the disease or the risk of neuropathy. Other treatments that have been used include nonsteroidal anti-inflammatory drugs, cyclophosphamide, hydroxychloroquine, D-penicillamine, methotrexate, octreotide (a somatostatin analog), and plasmapheresis. Many of these therapies have been tried in patients with severe illness, but insufficient information is available to assess efficacy.

The clinical and histopathologic findings of EMS overlap those of EF, a scleroderma-like syndrome characterized by tender swelling and induration (hardening) of the subcutaneous tissue, primarily in the arms and legs. Some cases of EF, in retrospect, were associated with tryptophan ingestion. However, EF is probably triggered by additional factors because few, if any, cases of EF occurring before 1986 can be attributed to the ingestion of tryptophan. Several clinical and laboratory features distinguish EMS from EF. Patients with EMS had greater frequency and severity of myalgias, fever, peripheral neuropathy, and other visceral organ involvement than patients with EF. Moreover, positive antinuclear antibody and a dichotomy between elevated serum aldolase and nonelevated creatine phosphokinase were features of EMS, but not of EF. EMS appears to be a more severe disease than EF in terms of hospitalization rate, duration of symptoms, and mortality.

Taken together, the epidemiological and clinical findings in patients with EMS could be explained by changes in the manufacturing process of L-tryptophan from 1985 to 1988 which resulted in contamination of the product, with a likely increase in the quantities of contaminants in 1989.

Manufacture of L-Tryptophan

The L-tryptophan produced by Showa Denko K. K. was manufactured by fermentation using the bacterium *Bacillus amyloliquefaciens*. The biosynthetic pathway of L-tryptophan is shown in **Figure 1**. Several new strains (I–V), each modified slightly to increase the biosynthesis of tryptophan, were introduced sequentially during the years preceding the outbreak of EMS (**Table 1**).

In December 1988, the company introduced a new strain of *B. amyloliquefaciens* (strain V), which had been genetically modified to increase the synthesis of 5-phosphoribosyl-1-pyrophosphate, an intermediate in the biosynthesis of tryptophan (see **Figure 1**). After fermentation, tryptophan was extracted from the broth and purified using a series of filtration, crystallization, and separation processes. The purification procedures included contact with powdered activated carbon and then granulated activated carbon. The amount of powdered activated carbon in each batch was usually

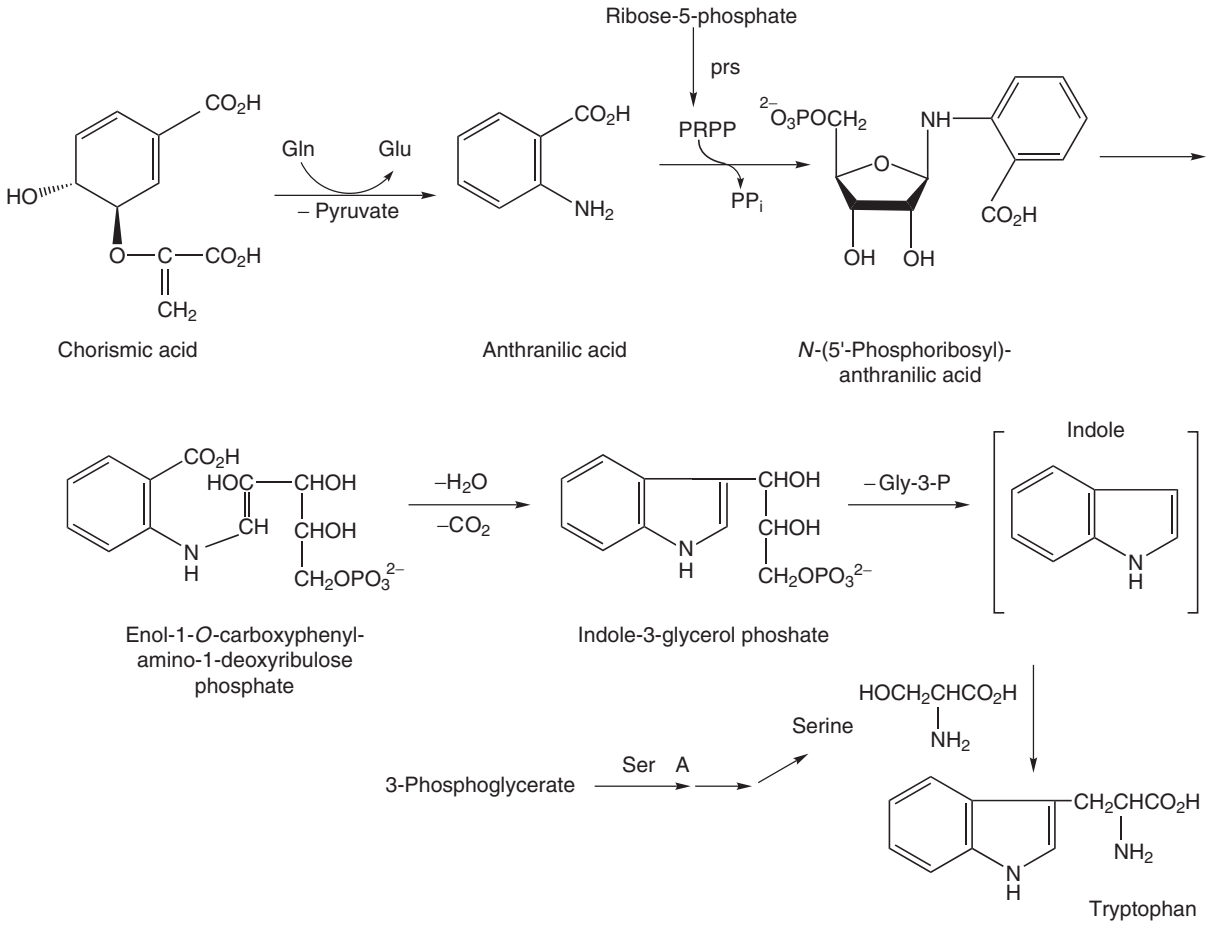


Figure 1 Biosynthesis of L-tryptophan. (Reproduced from Mayeno AN and Gleich GJ (1994) Eosinophilia–myalgia syndrome and tryptophan production: A cautionary tale. *Trends in Biotechnology* 12: 346–352, with permission from Elsevier.)

Table 1 Genetic modifications of the different strains of *Bacillus amyloliquefaciens* used to manufacture L-tryptophan

Strain[a]	*Modification*
I	Original strain of *B. amyloliquefaciens* IAM 1521
II	The tryptophan operon (coding for all enzymes catalyzing reactions from chorismate to L-tryptophan, as well as for those involved in the biosynthesis of serine and 5-phosphoribosyl-1-pyrophosphate (PRPP)) of strain I was duplicated through chromosomal integration
III	The isolated tryptophan operon was attached to a more efficient promoter prior to integration into chromosomal DNA of strain II
IV	The *ser A* gene (coding for phosphoglycerate dehydrogenase[b]) was amplified using a plasmid vector with strain III
V	The *prs* gene (coding for ribose phosphate pyrophosphokinase[c]) was isolated and integrated into the chromosome of strain IV

[a] Strains II–V were derived by successive modifications of strain I.
[b] Phosphoglycerate dehydrogenase catalyzes the conversion of 3-phosphoglycerate to 3-phosphohydroxypyruvate, an intermediate in the biosynthesis of serine.
[c] Ribose phosphate pyrophosphokinase catalyzes the phosphorylation of ribose-5-phosphate to give PRPP.
Reproduced from Mayeno AN and Gleich GJ (1994) Eosinophilia–myalgia syndrome and tryptophan production: A cautionary tale. *Trends in Biotechnology* 12: 346–352, with permission from Elsevier.

20 kg through 1988. In 1989, the amount of powdered activated carbon used to purify some batches of tryptophan was reduced to 10 kg. From October 1988 to June 1989, a portion of some fermentation batches also bypassed a filtration step that employed a reverse-osmosis membrane (ROM) filter to remove chemicals with a molecular weight of more than 1000 Da. According to the company, these changes did not significantly alter the purity of the tryptophan powder, which was maintained at 99.6% or greater.

Univariate analysis of retail lots of tryptophan consumed by case patients and controls demonstrated an association between development of EMS and the ingestion of tryptophan processed with 10 kg of powdered carbon per batch (odds ratio, 9.0; 95% confidence interval, 1.1–84.6; $p = 0.014$) and the use of *B. amyloliquefaciens* strain V (odds ratio, 6.0; 95% confidence interval, 0.8–51.8; $p = 0.04$). Thus, both a reduction in the amount of powdered activated carbon and use of *B. amyloliquefaciens* (strain V) were significant manufacturing changes, but the independent contribution of each manufacturing change could not be assessed because of the high correlation between them. Bypass of the ROM filter was not significantly associated with the case lots. Studies carried out by Showa Denko suggested that the 'biochemical and physiological characteristics' of *B. amyloliquefaciens* (strain V) did not differ from those of earlier strains.

Contaminants Associated with EMS

Once the link between EMS and manufactured L-tryptophan had been established, chemical analyses of bulk tryptophan lots were performed by researchers at the Mayo Clinic (Rochester, MN), FDA (Washington, DC), CDC (Atlanta, GA), and the Japanese National Institute of Hygienic Sciences (Tokyo) to determine if any contaminants were associated with EMS. HPLC was used to separate the contaminants in tryptophan and revealed that each manufacturer's tryptophan produced a unique chromatographic pattern, or 'fingerprint', that was distinctive for the product from each company, as shown in **Figure 2**. The chromatographic pattern consisted of multiple peaks, each of which represented a trace chemical constituent other than tryptophan, which eluted as a large, broad peak between 11 and 15 min. L-Tryptophan manufactured by each of the six companies contained impurities. The chromatogram for Showa Denko tryptophan included five 'signature' peaks that were present in all tryptophan manufactured by this company (see **Figures 2** and **3**). Initial comparison of individual peaks in case and control lots of Showa Denko tryptophan revealed a single peak (called 'peak E' or 'peak 97') that was significantly associated with case lots (**Figure 3**). The chemical structure of peak E was subsequently determined to be 1,1′-ethylidenebis [L-tryptophan], or EBT (**Figure 4**). Two other contaminants were subsequently reported to be associated with case lots of tryptophan manufactured by Showa Denko. One of the peaks, labeled UV-5, eluted before tryptophan (**Figure 3**) and was determined to be 3-(phenylamino)-L-alanine (PAA) (**Figure 4**). The other peak (UV-28) eluted much later than EBT and is as yet uncharacterized. Recent HPLC studies revealed more than 60 trace contaminants in Showa Denko tryptophan, six of which are associated with EMS. The structures of three are known (EBT, PAA, and 'peak 200' (2[3-indolylmethyl]-L-tryptophan)), but the other three have not yet been characterized. One of the uncharacterized contaminants, called 'peak AAA', was the contaminant most significantly associated with EMS and was recommended for characterization.

The amount of EBT present in Showa Denko tryptophan varied markedly in the period 1987–89 (**Figure 5**), presumably reflecting alterations in the manufacturing conditions. It is likely that levels of all of the contaminants varied with time. These data are consistent with the hypothesis that a contaminant(s) in tryptophan is responsible for EMS and for the sporadic cases of EF between 1986 and 1988. Recent statistical analyses of EBT, adjusted for serial autocorrelation (to take into account that sequential lots of tryptophan may be related), revealed that higher levels of EBT are still associated with EMS, but the association ($p = 0.120$) did not achieve statistical significance. Nonetheless, the results do not vindicate EBT as a cause of EMS because misclassification of lots as case or control could weaken the association and the methods used to account for the lack of independence of observations over time probably reduce the power of the statistical analysis.

Investigation into the origin of contaminant PAA reveals that it can be formed from aniline and serine by heating at 80°C for 6 h under alkaline conditions (pH 11). Although aniline was not used in the biosynthesis of tryptophan, small amounts of aniline are formed from anthranilic acid, a biosynthetic precursor of tryptophan (see **Figure 1**), after heating at 80°C for 6 h under acidic conditions (pH 2). The industrial process used by Showa Denko to purify tryptophan from the fermentation broth consisted of several steps, including anion exchange at pH 10.5, cation exchange at pH 11, and heat treatment at 80–90°C. Thus, the fermentation and purification processes used to produce tryptophan may have led to the formation of PAA as a by-product.

Connection with Toxic Oil Syndrome

The clinical and pathologic findings of EMS bear a striking resemblance to those of the TOS, which occurred as an epidemic in Spain during the spring and summer of 1981. Over 20 000 persons were affected, and several hundred deaths have been attributed to TOS. The similarities between EMS and

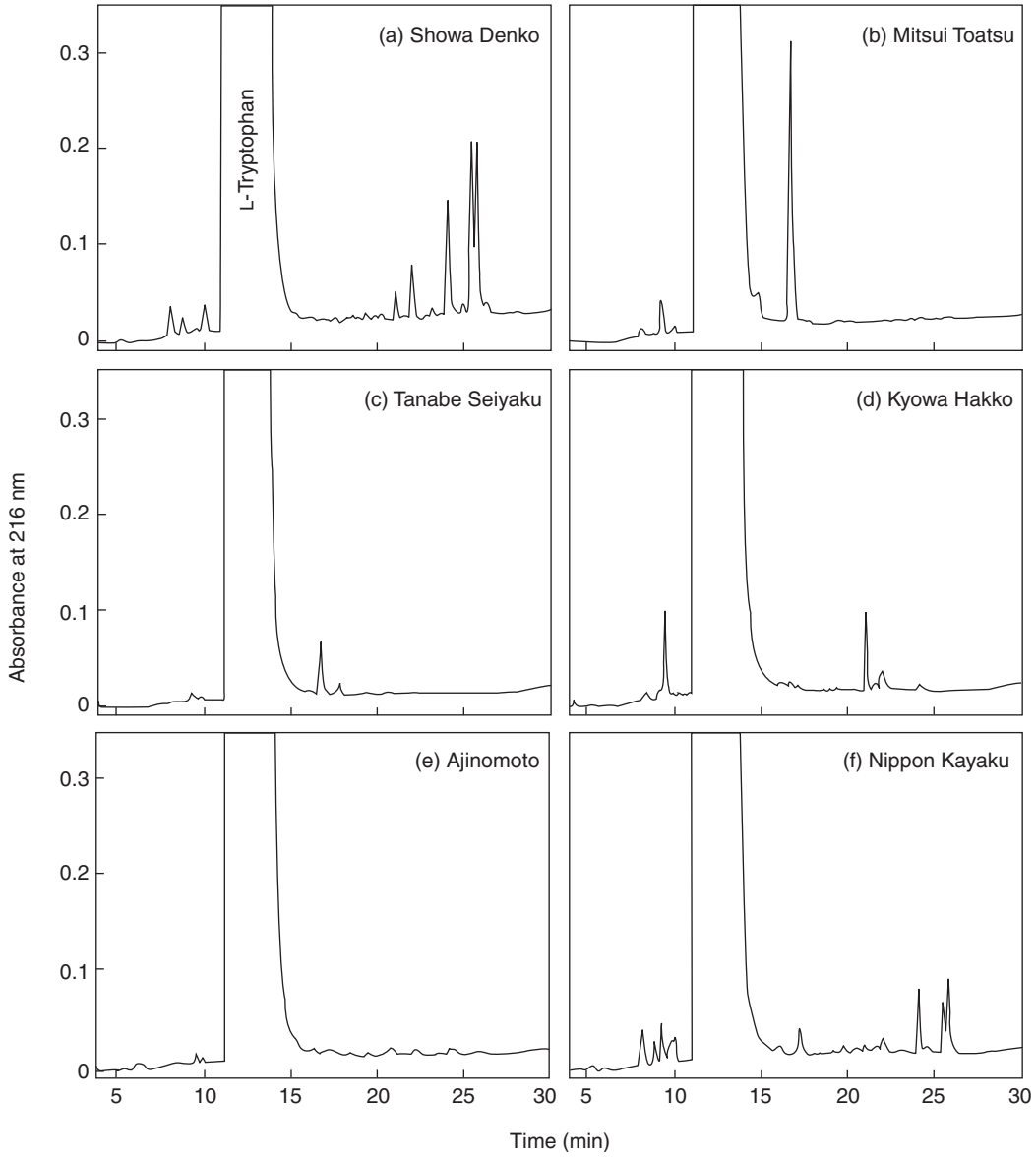


Figure 2 Typical HPLC chromatograms of L-tryptophan manufactured by the six different companies. (Reproduced from Mayeno AN and Gleich GJ (1994) Eosinophilia – myalgia syndrome and tryptophan production. A cautionary tale. Trends in Biotechnology 12: 346–352, with permission from Elsevier.)

TOS are summarized in **Tables 2** and **3**. Unlike EMS, respiratory symptoms (cough or dyspnea) were prominent and severe in TOS during the first week of illness (acute phase). Other early symptoms included fever, malaise, headache, nausea, and pruritic (itchy) rash. In some patients, the disease progressed to an intermediate and chronic phase that resembled EMS more closely. The intermediate phase (2–8 weeks after onset) was characterized by eosinophilia and leukocytosis (raised numbers of leukocytes). Patients whose illness progressed to the late phase developed muscle cramps and severe myalgias, peripheral edema, scleroderma-like skin changes, and polyneuropathy. The histopathological changes of skin, nerve, and skeletal muscle are remarkably similar between EMS and TOS.

The pathophysiology of both TOS and EMS involves an immunological component. Generally, early skin biopsies in both TOS and EMS showed edema and inflammatory infiltrates. Inflammatory lesions of arteries and cardiac neural structures in both EMS and TOS patients were primarily composed of lymphocytes. Persistent elevated levels in the serum level of the soluble fraction of IL-2 receptor were noted in both EMS and TOS patients, suggesting chronic immune activation.

Epidemiologic investigations implicated ingestion of adulterated rapeseed oil that had been imported

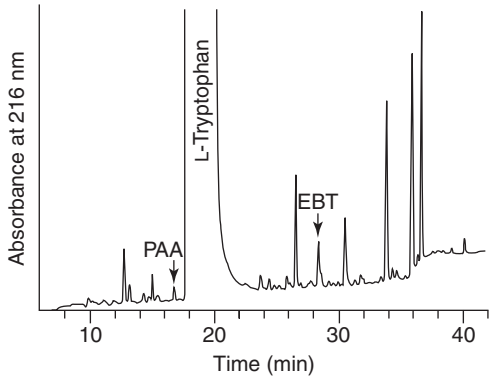
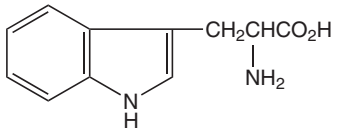
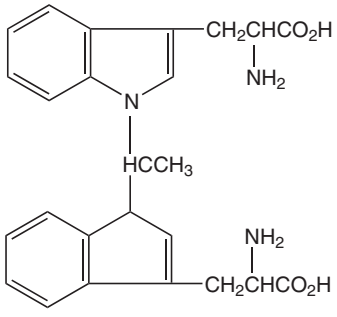


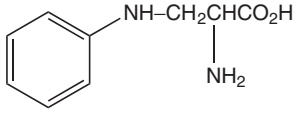
Figure 3 HPLC chromatogram of EMS-associated L-tryptophan. HPLC conditions differ from those used in **Figure 2**. (Reproduced from Mayeno AN and Gleich GJ (1994) Eosinophilia–myalgia syndrome and tryptophan production: A cautionary tale. *Trends in Biotechnology* 12: 346–352, with permission from Elsevier.)



L-Tryptophan



1,1′-Ethylidenebis[tryptophan] (EBT)



3-(Phenylamino)alanine (PAA)

Figure 4 Chemical structures of tryptophan and of contaminants EBT and PAA associated with EMS.

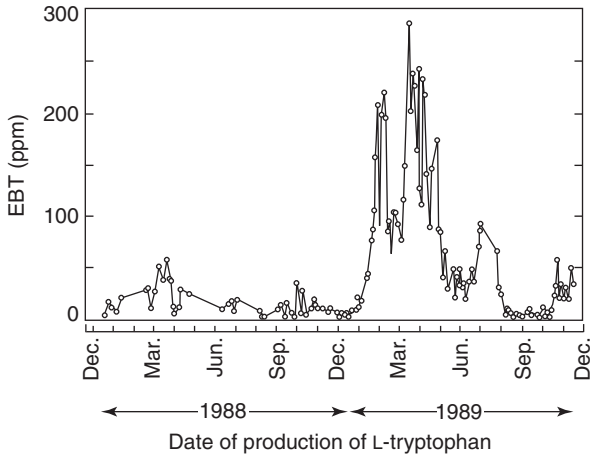


Figure 5 Levels of 1,1′-ethylidenebis[L-tryptophan] (EBT) in lots of L-tryptophan produced by Showa Denko K. K. during 1988 and 1989. (Reproduced from Mayeno AN and Gleich GJ (1994) Eosinophilia–myalgia syndrome and tryptophan production: A cautionary tale. *Trends in Biotechnology* 12: 346–352, with permission of Elsevier.)

Table 2 Comparison of the clinical features of eosinophilia–myalgia syndrome (EMS), toxic oil syndrome (TOS), and eosinophilic fasciitis (EF)

	EMS	*TOS*	*EF*
Female (%)	80	90 (late)	50
Myalgia	+++	++	±
Dyspnea/cough	+	+++ (early)	−
Pruritus	++	+	−
Rash	+	+	−
Swelling, edema	++	++	++
Muscle weakness	+	+	−
Scleroderma-like lesions/ fasciitis	++	++	+++
Heart involvement	±	+	−
Axonal polyneuropathy	++	++	−
Arthritis	+	+	+

+, Occasional; ++, common; +++, very common.
Reproduced from Varga J (1993) L-Tryptophan-associated eosinophilia–myalgia syndrome: Clinical and pathological features of an evolving new disease and current concepts of etiology. *Journal of Intensive Care Medicine* 8: 229–242.

from France. At the time, rapeseed oil could not be legally imported into Spain as a food substance, only as an industrial lubricant after denaturation with aniline, a toxic chemical. The oil had been denatured with aniline (to give a concentration of 2% aniline by weight) as required by law. However, the oil was then illegally de-denatured in Spain by a refining process that removed almost all of the aniline and was subsequently mixed with 10–30% of other seed oils, about 30% of animal fats, and up to 5% of a poor quality olive oil or, alternatively, chlorophyll to produce the desired color. The resulting adulterated oil was sold as pure olive oil, typically in unlabeled 5 l containers by street vendors and itinerant salesmen.

Chemical analyses of implicated oil samples and 'control' oil samples demonstrated that free aniline and aniline derivatives were significantly associated with case-related samples. Fatty acid anilides, in particular oleylanilide (**Figure 6**), have been reported to be markers of TOS-causing oil. Another

Table 3 Comparison of the laboratory features of EMS, TOS, and EF

	EMS	*TOS*	*EF*
Eosinophilia	+++	++	+
Elevated IgE	–	±	–
Elevated aldolase	++	+	–
Antinuclear antibody	++	+	–
Lymphocytes in lesion	+	+	–
Eosinophils in lesion	±	±	±
Vasculitis	+	+	±

+, Occasional; ++, common; +++, very common.
Reproduced from Varga J (1993) L-Tryptophan-associated eosinophilia–myalgia syndrome: Clinical and pathological features of an evolving new disease and current concepts of etiology. *Journal of Intensive Care Medicine* 8: 229–242.

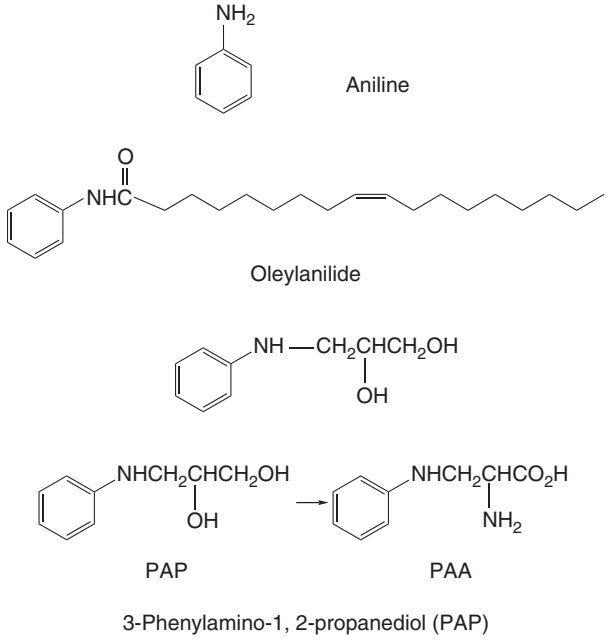


Figure 6 Chemical structures of oleylanide and 3-phenylamino-1,2-propanediol (PAP) associated with TOS.

contaminant, 3-phenylamino-1,2-propanediol (PAP) (**Figure 6**), has been isolated from implicated oil and is chemically similar to the tryptophan contaminant PAA. Efforts to evaluate the biologic activity of the aniline contaminants have been limited by the absence of an animal model for TOS.

The striking similarities between EMS and TOS suggest that they may share the same final pathway that leads to neuromuscular damage. The recent discovery of a chemically related aniline derivative in tryptophan preparations implicated in causing EMS suggests of a related etiology. Recently, PAP has been demonstrated to undergo biotransformation to PAA by both rat hepatocytes and human liver tissue *in vitro*, linking the two diseases to a common chemical, namely PAA (see below). This finding is the first reported chemical link between TOS and EMS.

EMS Not Apparently Associated with L-Tryptophan

Some patients with EMS reported no history of tryptophan ingestion. An EMS-like syndrome has been associated with use of L-5-hydroxytryptophan (5-HTP). HPLC analysis of the 5-HTP that might have caused the symptoms revealed the presence of an impurity not present in 5-HTP preparations that did not cause symptoms. The structure of the impurity has not been reported. In addition, a recent pharmacoepidemiological study in Canada identified several EMS patients with no history of tryptophan ingestion. These reports suggest that factors other than tryptophan ingestion can lead to the induction of EMS or EMS-like diseases.

Investigations of the Etiology and Pathogenesis

Animal Models

Several studies using animals have been performed; however, the results of studies have been inconclusive, with no animal model tested replicating all of the clinical features of the disease. Initially, the Lewis rat showed promise as a model for EMS. Muscle biopsies of Lewis rats given either implicated tryptophan (containing EBT) or US Pharmacopoeia (USP) grade tryptophan (without EBT) demonstrated perimysial inflammation in seven of nine animals receiving implicated tryptophan compared to 0 of 10 receiving USP grade tryptophan. A significant increase in fascial thickening was also observed in rats receiving implicated tryptophan. However, leukocyte counts and eosinophil counts remained normal in both groups. Gastrointestinal changes were also noted with an increased number of degranulating inflammatory cells in the lamina propria of the rats that received case-implicated tryptophan. Recently, however, control L-tryptophan alone was observed to cause mild myofascial thickening, alterations in peripheral blood mononuclear cell (PBMC) phenotypes, and pancreatic pathology in Lewis rats, suggesting that tryptophan itself may play a role in EMS and other fibrosing diseases. Another recent study using C57BL/6 mice found that EBT caused inflammation and fibrosis in the dermis and subcutis, including fascia and perimysial tissues, mimicking some of the clinical features of EMS. Eosinophilia was not observed.

These findings, however, may not be reproducible. F-344 and Lewis rats, as well as BALB/c mice, were treated with one of the following substances: feed or food-grade L-tryptophan, tablets containing L-tryptophan, isopropanol extracts from L-tryptophan, synthetic EBT or PAA, and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (a breakdown product of EBT). No EMS-like symptoms were observed in any of the animals tested. The tryptophan preparations were manufactured by Showa Denko; however, neither the specific lots numbers nor their association with EMS were indicated. Thus, it is difficult to interpret the results of this study in light of the fact that only certain lots of L-tryptophan manufactured by Showa Denko were linked to EMS. A recent toxicologic study using PAA supports the negative findings; PAA (1, 10, and 100 mg $kg^{-1} day^{-1}$) was administered by gavage to Sprague–Dawley rats for up to 13 consecutive weeks. No EMS-like symptoms were observed. Overall, the animal studies suggest that Lewis rats and C57BL/6 mice may be useful in replicating only certain aspects of EMS.

In Vitro Models

In vitro investigations have attempted to clarify the mechanism of immune activation but so far have provided limited data. Studies testing the hypothesis that implicated tryptophan or EBT can trigger PBMCs to release cytokines have been equivocal, although one study found that EBT activates eosinophils and induces IL-5 production from T cells. Another recent study found that certain lots of L-tryptophan could stimulate PBMCs to release granulocyte-macrophage colony-stimulating factor (GM-CSF); this response, however, was caused by endotoxin contamination and not associated with case lots of tryptophan. The mechanism of immune activation is clearly complex and may be difficult to reproduce with an *in vitro* assay. Similar difficulties have been encountered in the study of immune system activation in TOS.

Despite the negative findings of *in vitro* studies, there is evidence that cytokines may play a role in the pathogenesis of EMS. It is known that IL-3, IL-5, and GM-CSF can each induce eosinophil production and enhance *in vitro* survival. In one study, EMS patients had significantly elevated serum levels of IL-5 and a higher proportion of hypodense eosinophils compared to normal controls. Elevated levels of IL-3 and GM-CSF were not observed. Their results suggest that IL-5 is the cytokine that triggers eosinophilia and converts peripheral blood eosinophils to the hypodense phenotype. The mechanism responsible for the elevation of IL-5 levels in the blood is undefined.

Possible Pathogenetic Mechanisms

Although the sequence of events leading to the pathologic changes of EMS is undoubtedly complicated, a framework for possible mechanisms can be advanced. One hypothesis involves a direct effect of the etiologic agent on mononuclear cells, leading to production of cytokines, including IL-5. This cytokine could then activate tissue eosinophils and convert them to a hypodense phenotype. Effector functions would be augmented with release of cytotoxic molecules from eosinophils. Once activated, eosinophils can release additional cytokines such as IL-3, GM-CSF, IL-5, and transforming growth factor-α. A cascade of interacting cytokines could then lead to recruitment of additional inflammatory cells and increased collagen synthesis by fibroblasts.

The predominance of inflammatory changes in the fascia suggests that mediators produced by mesenchymal cells (fibroblasts and endothelial cells) may also play a role in the pathogenesis. For example, fibroblasts augment IL-5-dependent eosinophil survival and stimulate conversion to the hypodense phenotype. Fibroblasts can also produce IL-8, which recruits neutrophils and lymphocytes when injected *in vivo*. Thus, one can speculate that the etiologic agent interacts with these cells to stimulate release of inflammatory mediators and increase collagen synthesis.

Another general hypothesis involves incorporation of the etiologic agent into metabolic or biosynthetic pathways that utilize chemically related compounds. EBT and PAA are amino acids with structural similarities to tryptophan and phenylalanine, respectively, and might function as an analog with adverse immunologic effects. If EBT, PAA, or one of their metabolites is recognized by an analogous transfer RNA, it might be incorporated into a nascent protein molecule, stimulating an autoimmune response.

The biotransformation of the toxic oil contaminant PAP to the L-tryptophan contaminant PAA may link TOS and EMS to a common chemical agent, namely PAA. Both PAP and PAA are metabolized further to the *p*-hydroxylated forms, HPAP and HPAA (**Figure 7**). Such compounds readily autoxidize to the benzoquinoneimine, which is reactive toward nucleophiles such as the sulfhydryl and amino moieties present on many biological molecules. Thus, upon oxidization, HPAA and HPAP may react with macromolecules as a hapten to form immunogenic targets. HPAA possesses some chemical properties

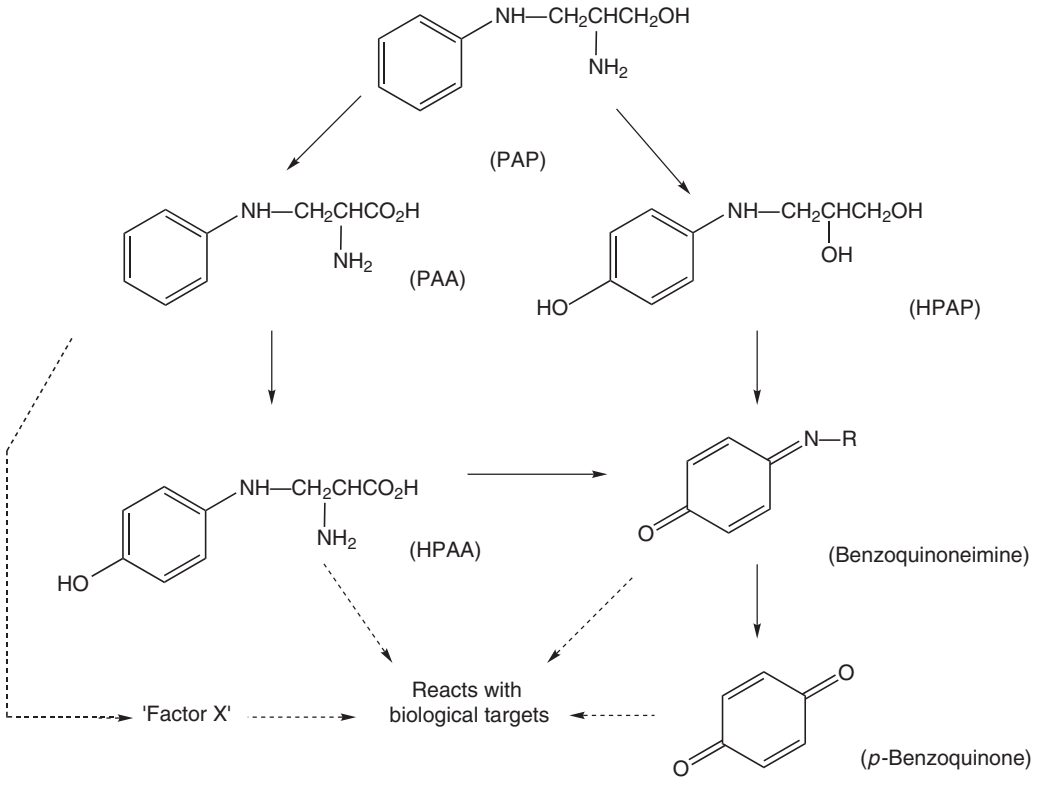


Figure 7 Hypothetical scheme for the bioactivation of PAP and PAA.

similar to that of homogentisic acid (HGA), a hydroquinone derivative implicated in the causation of alkaptonuria, a connective tissue disorder resulting from an inherited abnormality in phenylalanine and tyrosine metabolism. HGA interacts with connective tissue reversibly or is oxidized enzymatically by an enzyme (polyphenol oxidase) present in connective tissue to benzoquinoneacetic acid, which covalently bonds to macromolecules.

One may hypothesize that HPAA reacts similarly, as shown in **Figure 7**. PAP is initially metabolized to PAA or HPAP. PAA is then converted to HPAA. Both HPAP and HPAA can undergo oxidation to the benzoquinoneimine. Benzoquinoneimines can hydrolyze to *p*-benzoquinone, and both benzoquinoneimines and benzoquinone readily react with nucleophilic molecules. It is also possible that PAA is metabolized to another undetermined molecule, shown as factor X (**Figure 7**), that reacts with biological targets. Thus, HPAA may haptenize proteins, and T-cell activation could result from hapten recognition.

The oxidation of HPAA and HPAP to benzoquinones may require an enzyme with properties similar to polyphenol oxidase. The inflammatory pattern and a lack of an animal model for EMS may be explained if the enzyme is localized to the connective tissue (fascia) and is specific to humans. In addition,

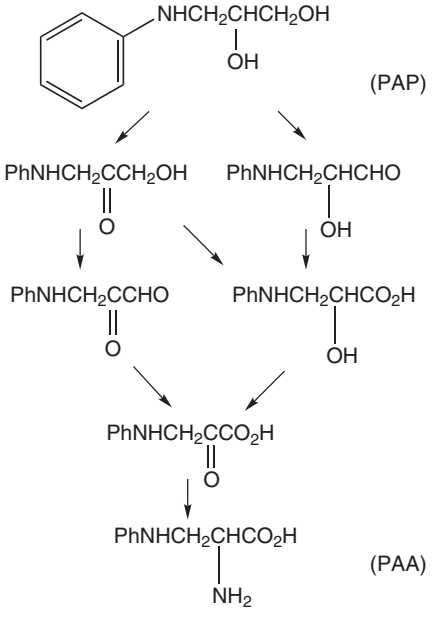


Figure 8 Hypothetical metabolic pathway for the biotransformation of PAP to PAA.

the observation of greater respiratory symptoms during the early phase of TOS in comparison with EMS may result from accumulation and metabolism of PAP or HPAP in the lung.

The biotransformation of PAP to PAA must proceed through various intermediates (**Figure 8**). The first steps most likely involve stepwise oxidation of the diol to an α-keto acid or some other keto intermediate, which then undergoes transamination to give PAA. It is likely that any one of these intermediates, in the presence of hepatocytes, can be metabolized to PAA. Thus, many molecules related to PAP (e.g., phenylamino compounds) may be channeled down this pathway to give PAA. This model suggests that numerous molecules with similar chemical structures to PAP will give rise to PAA and, if PAA is indeed responsible for EMS and TOS, the model predicts that an entire class of molecules, as shown in **Figure 8**, can cause EMS/TOS-like diseases, consistent with the reports of EMS cases not associated with tryptophan ingestion.

Summary

EMS occurred as an epidemic in the United States during 1989–90, with isolated cases occurring before and after the major epidemic. EMS is a multisystemic disease that resulted from the ingestion of L-tryptophan manufactured by one company. The illness is clinically and pathologically similar to EF and the TOS. The syndrome is likely triggered by one or more contaminants in tryptophan. Contaminants currently studied include EBT and PAA, although other uncharacterized contaminants have recently been discovered and may likewise be responsible. One or more of these chemicals may cause EMS by an undefined mechanism, or they may be surrogate markers for another unidentified substance that triggers the syndrome. Consumption of high tryptophan doses and increased age have been identified as risk factors. Patients who ingested tryptophan and were diagnosed with EF during 1986–88 had most likely EMS. The recent demonstration of the biotransformation of PAP to PAA suggests that both EMS and TOS share a common etiology, namely PAA. Ongoing research is focused on identification of contaminants in implicated tryptophan and on establishing an animal model of the diseases. Success in these endeavors would greatly increase our understanding of eosinophilic diseases in general and hopefully prevent the outbreak of future epidemics.

See also: Blood; Epidemiology; Immune System; Neurotoxicity.

Further Reading

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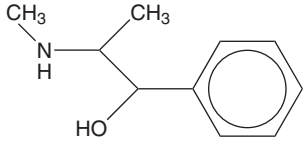
Ephedra

Vishal S Vaidya and Harihara M Mehendale

- Chemical Name: Ephedra
- Chemical Abstracts Service Registry Number: CAS 299-42-3 (Ephedrine)
- Common Names: Ephedra sinica family (ephedraceae); Chinese ephedra, Ma Huang
- Related Species: *Ephedra distacha* (European ephedra); *E. trifurca* or *E. viriditis* (desert tea); *E. nevadensis*; *E. americana* (American ephedra); *E. gerardiana* (Pakistani ephedra)

- SYNONYMS (Ephedrine): Benzenemethanol, α-(1-(Methylamino)ethyl)-, (*R*-(*R**,*S**)); Biophedrin; Eciphin; Efedrin; Ephedral; Ephedrate; Ephedremal; Ephedrin; Ephedrine; (–)-Ephedrine; (L)-Ephedrine; Ephedrital; Ephedrol; Ephedrosan; Ephedrotal; Ephedsol; Ephendronal; Ephoxamin; Fedrin; α-Hydroxy-β-methyl amine propylbenzene; 1-Hydroxy-2-methylamino-1-phenylpropane; α-Hydroxy-β-methylaminopropylbenzene; Isofedrol; Kratedyn; Ma Huang; Manadrin; Mandrin; α-(1-(Methylamino)ethyl)benzenemethanol; α-(1-(Methylamino)ethyl)benzyl alcohol; 1-α-(1-Methylaminoethyl)benzyl alcohol; 1-2-Methylamino-1-phenylpropanol; 2-Methylamino-1-phenyl-1-propanol; Nasol; Nci-C55652; Norephedrine, *N*-Methyl-1-phenyl-1-hydroxy-2-methylaminopropane; 1-Phenyl-2-methylaminopropanol; Sanedrine; 1-Sedrin; Vencipon; Zephrol
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Herbal supplements; Ephedra alkaloids; Adrenergic agents; Natural products
- CHEMICAL FORMULA: $C_{10}H_{15}NO$
- CHEMICAL STRUCTURE:



Uses

The medicinal use of *Ephedra sinica* in China dates from ~2800 BC. Ma Huang (the stem and branch) was used primarily in the treatment of common cold, asthma, hay fever, bronchitis, edema, arthritis, fever, hypotension, and urticaria. Ephedra has been used to treat bronchoconstriction for centuries, because of its activity at β_2-adrenergic receptors. It contains pseudoephedrine, ephedrine, and other similar alkaloids. These are sympathomimetics that either directly or indirectly stimulate α- and β-adrenergic receptors. It has become less extensively used with the advent of more selective agonists.

Background Information

Ephedra plants are erect, branching shrubs found in desert or arid regions throughout the world. The 1.5–4 ft shrubs typically grow on dry, rocky, or sandy slopes. The many slender, yellow-green branches of ephedra have two very small leaf scales at each node. The mature, double-seeded cones are visible in the fall.

Ephedra is one of the plants that are a source of ephedrine alkaloids, including ephedrine and pseudoephedrine. Chemically synthesized, ephedrine, and pseudoephedrine are regulated under the Federal Food, Drug, and Cosmetic Act as drugs. In contrast, the Dietary Supplement Health Education Act (DSHEA)-regulated dietary supplements that contain ephedrine alkaloids, the safety and effectiveness of drug products containing ephedrine alkaloids in drug products have to be proven by the manufacturer.

Within the last 10 years, the use of dietary supplements containing ephedrine alkaloids was extensively promoted in the United States for aiding weight control and boosting sports performance and energy (**Table 1**). Drinks like Ripped Fuel claimed to give athletes a quick jolt of energy and gained substantial popularity not only in just athletes but also in those wanting to get a better workout in health clubs. Ephedra contains a natural alkaloid ephedrine, similar to the hormone epinephrine (adrenaline), a stimulant that acts on the central nervous system (CNS), dilates the bronchial tubes in the lungs, elevates blood pressure, and increases heart rate thereby giving a feeling of jolt of energy. The use of ephedra can also increase feelings of alertness and reduce or suppress appetite.

Since 1994, the Food and Drug Administration (FDA) and Centers for Disease Control collected reports of over 100 deaths and 500 reports of adverse events associated with ephedrine-containing dietary supplements over a 2 year period. The NCAA banned the use of ephedra-containing products since 1997, and the Olympics banned the use of ephedra for over a decade.

Scientists have conducted several studies and the totality of the available data showed little evidence of the effectiveness of ephedra except for modest, short-term weight loss without any clear health benefit, while confirming that the substance raises blood pressure and otherwise stresses the circulatory system. These effects are linked to significant adverse health outcomes, including heart attack and stroke. On February 6, 2004, the FDA issued a final rule prohibiting the sale of dietary supplements containing ephedrine alkaloids (ephedra) because such supplements present an unreasonable risk of illness or injury (**Table 2**).

Exposure Routes and Pathways

Herbal medicines are widely perceived by the public as being healthful and innocuous. An estimated three billion servings of ephedra are reportedly consumed

Table 1 Facts about ephedra

Proposed indications	*Popular products containing ephedra extracts*	*Label ingredient indicating ephedra compounds*	*Adverse effects*
Weight loss	Metabolife	Ephedra	Nervousness
Asthma	Ripped Fuel	Ma huang	Dizziness
Common cold	Diet Fuel	Ephedrine	Tremor
Hay fever/allergies	Stacker 3	*Ephedra sinica*	Alternations in blood pressure or heart rate
Increasing energy	Natural TRIM	Sida cordifolia	Headache
Congestion	Hydroxycut	Epitonin	Gastrointestinal distress
Weight lifting formula	Xenadrine RFA-1	Pseudoephedrine	Chest pain
	Metab-O-Lite	Methyl ephedrine	Myocardial infarction
	Metabolift		Hepatitis
	Up Your Gas		Stroke
	Truckers Luv It		Seizures
	Yellow Jackets		Psychosis
			Death

Table 2 Federal and State regulatory actions against ephedra and ephedrine-containing alkaloids

Date	*Action*	*Authority*
November 1989	Ephedrine and pseudoephedrine placed on a list of controlled substances used in the manufacture of illegal drugs on a list of controlled substances used	Drug Enforcement Agency (DEA)
1991–94	State regulations controlling sale of ephedrine and/or ephedra	Arizona, Arkansas, California, Florida, Hawaii, Idaho, Missouri, Nevada, New Mexico, Ohio, Oklahoma, Oregon, Texas, Virginia, Washington
1993	Exemptions for Ma Huang products with less than 25 mg of total ephedrine alkaloids	Arizona, Nevada, and Washington
August 1994	Ephedrine placed in Schedule V of Ohio's Controlled Substance Act. Sale of ephedra banned in Ohio	Ohio's Drug Laws Board
June 1996	Proposed warning and dose limitations on dietary supplements containing ephedra	Food and Drug Administration (FDA)
January 1997	Texas withdraws proposed regulations to ban ephedra	Texas Board of Health
March 1997	Ban on ephedra sales amended in Ohio. Bill permits natural products stores to sell the herb containing limited alkaloid levels	Ohio's Drug Laws Board
February 2000	Withdrawal of proposed ephedra rules	FDA
February 2004	Final rule prohibiting the sale of dietary supplements containing ephedrine alkaloids (ephedra) because such supplements present an unreasonable risk of illness or injury	FDA

yearly, making it an extremely popular stimulant contained in diet pills and sports drinks. The quantity of ephedrine in dietary supplements, as reported on package labels, is typically about 20 mg per serving, and the usual dose frequency is two to three times per day. However, these products may contain larger or smaller amounts of ephedra alkaloids that are listed on the product label. For example, 11 of 20 supplements tested either failed to list the alkaloid content on the label or had greater than a 20% difference between the amount listed on the label and the actual amount. Therefore, even in the absence of simultaneous ingestion of other known or unknown stimulants, consumers of ephedra are often overdosed.

Toxicokinetics

Ephedrine is rapidly absorbed after oral, intramuscular, or subcutaneous administration. It is strongly bound to human saliva, and binding is independent

of pH. L-Ephedrine-(14)c injected intraperitoneally in rats was metabolized to l-norephedrine and 4-hydroxy-l-ephedrine plus minor products. The average half-life is 6 h, although acidifying the urine will decrease the half-life considerably and alkalinization will increase the half-life. The major route of elimination of ephedrine is as the unchanged drug in the urine.

Mechanism of Toxicity

The basic pharmacological action of Ephedrine is that of a sympathomimetic. It does not contain a catechol moiety and is effective after oral administration. The drug stimulates heart rate and cardiac output and variably increases peripheral resistance; as a result, ephedrine usually increases blood pressure. Stimulation of the α-adrenergic receptors of smooth muscle cells in the bladder base may increase resistance to the outflow of urine. Activation of β-adrenergic receptors in the lungs promotes bronchodilation. Ephedrine stimulates the cerebral cortex and subcortical centers to produce its effects in narcolepsy and depressive states.

Ephedra can produce the same side effects as ephedrine, that is, increased blood pressure and heart rate, insomnia, and anxiety. A highly potent CNS stimulant, ephedrine may even induce toxic psychosis at high dosages. The FDA advisory review panel on nonprescription drugs recommended that ephedrine not be taken by patients with heart disease, high blood pressure, thyroid disease, diabetes, or difficulty in urination due to the enlargement of the prostate gland. Nor should ephedrine be used in patients on antihypertensive or antidepressant drugs. Ephedrine administered to chick embryos has resulted in cardiovascular teratogenicity and embryotoxicity at doses as low as 1 mmol per egg. The teratogenic effect of ephedrine is potentiated by caffeine.

Acute and Short-Term Toxicity (or Exposure)

Animal

There are very few animal studies conducted investigating the toxic effects of ephedra. Studies using ephedrine in animals suggest that if it is given in excessive amounts, it produces symptoms of sympathetic stimulation, manifested by anxiety and restlessness. If the dosage is large, muscular tremors and even convulsions may occur.

Human

Probable lethal dose in man is 50 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

The only chronic carcinogenecity data available is for ephedrine sulfate which states that there was no evidence of carcinogenicity for F344/N rats or B6C3F1 mice of either sex receiving 125 or 250 ppm ephedrine sulfate in the diet for 2 years. Ephedrine sulfate was not mutagenic in four strains of *Salmonella typhimurium* (TA100, TA1535, TA97, or TA98), with or without Aroclor 1254 induced male Sprague–Dawley or Syrian hamster liver S9 activation. Ephedrine sulfate did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells.

Human

Many cases of serious adverse effects and even fatalities have been reported that were linked with ephedra or ephedrine administration over the last 10 years. Haller and Benowitz published a review of 140 reports of adverse events related to the use of ephedra alkaloids that were submitted to the FDA between June 1997 and March 1999. Using standardized rating system for assessing causation, 31% of the cases were considered to be definitely or probably related to the use of ephedra alkaloid-containing supplements, and another 31% were deemed to be possibly related. Among these adverse events, 47% involved in cardiovascular symptoms and 18% involved the CNS. Hypertension was the most frequent adverse effect, followed by palpitations, tachycardia, or both stroke and seizures. Ten events led to death and 13 cases produced permanent disability.

The central nervous-simulating effects of ephedrine may result in nervousness, anxiety, apprehension, fear, tension, agitation, excitation, restlessness, weakness, irritability, talkativeness, or insomnia. Dizziness, lightheadedness, and vertigo may occur, especially with large doses. Tremor or tremulousness, and hyperactive reflexes have also been reported. Large parenteral doses of ephedrine may cause confusion, delirium, hallucinations, or euphoria. Ephedrine may deplete norepinephrine stores in sympathetic nerve endings and tachyphylaxis to the cardiac and pressor effects of the drug may develop. In addition, after several doses of ephedrine are administered, hypotension more severe than that

originally being treated may result from direct cardiac depression and vasodilation.

The overall conclusions drawn from all these studies are that the adverse effects of ephedra may be amplified, sometimes culminating in death, due to the following reasons:

1. *Individual susceptibility*: Individuals undergoing very stressful situations such as athletes/football players who practice in very hot temperatures, extensive workout for muscle building or people who fast to achieve weight loss are particularly susceptible. Ephedra puts an undue stress in these individuals by increasing the blood pressure and causing additional stress on the cardiovascular system, blood supply in the brain, which may result in heart attack or stroke.
2. *Additive stimulant effects of caffeine*: Caffeine is present in many products that contain ephedra alkaloids, and those who take these products might also be consuming considerable quantities of caffeine in coffee, tea, and soft drinks. Caffeine can enhance the undesirable effects of ephedrine on the heart, blood supply system, and brain function.
3. *Variability in contents*: The quantity of ephedrine in dietary supplements, as reported on package labels, is typically $\sim$20 mg per serving, and the usual dose frequency is two to three times per day. However, these products may contain larger or smaller amounts of ephedra alkaloids that are listed on the product label. For example, 11 of 20 supplements tested by Bill Gurley, PhD, an associate professor in the College of Pharmacy at the University of Arkansas for Medical Sciences, either failed to list the alkaloid content on the label or had greater than a 20% difference between the amount listed on the label and the actual amount. Therefore, even in the absence of simultaneous ingestion of other known or unknown stimulants, consumers of ephedra are often overdosed.
4. *Preexisting medical conditions*: The likelihood of adverse effects of ephedrine is heightened in individuals with a history of high blood pressure, heart or thyroid disease, diabetes, kidney disease or difficulty urinating, glaucoma, a seizure disorder, depression, prostate enlargement, history of stress, or are involved in stressful activities.
5. *Taking ephedra along with other drugs*: If taken with other drugs simultaneously, ephedra may cause serious complications. Antidepressants, allergy, asthma, or cold medications containing ephedrine, pseudoephedrine or phenylpropanolamine, caffeine-containing drugs or soft drinks are known examples of substances that exaggerate the adverse effects of ephedra.

Clinical Management

Vital Signs

The clinical effects following overdose depend on the receptor selectivity (alpha and/or beta effect). Most patients require only observation for a period of 4–8 h. Pharmacologic intervention is required only in severely symptomatic patients (cardiac arrhythmias, hypertensive crisis, seizures, hyperthermia). Severe overdose effects may most commonly result in hypertension, tachycardias, followed by bradycardia, and arrhythmias, seizures, cerebral hemorrhages and ischemia or vasoconstriction, psychosis, and hyperthermia.

Antidote and Emergency Treatment

This includes protecting the patient's airway and supporting ventilation and perfusion, monitoring and maintaining, within acceptable limits, the patient's vital signs, blood gases, and serum electrolytes, besides monitoring electrocardiogram continuously. In alert patients, removing ephedrine from the stomach by inducing emesis with ipecac, followed by activated charcoal (as long as ileus is not present); in depressed or hyperactive patients, removing ephedrine by airway-protected gastric lavage. For supraventricular or ventricular tachycardia, administering a β-adrenergic blocker, such as propranolol by slow intravenous administration, is necessary to control cardiac arrhythmias; however, in asthmatic patients, a cardioselective β-adrenergic blocker (e.g., acebutolol, atenolol, metoprolol) may be more appropriate. The β-blocker should be used with caution in asthmatic patients because it could induce severe bronchospasm or an asthmatic attack. Nitroprusside or phentolamine infusion may be used for marked hypertension, if necessary. For 'true' hypotension, administration of intravenous fluids, elevation of legs, or administration of an inotropic vasopressor, such as norepinephrine, should be considered. To control convulsion, administer diazepam. For refractory seizures, general anesthesia with thiopental or halothane and paralysis with a neuromuscular blocking agent may be necessary. Pyrexia can be controlled by cool applications and by slow intravenous administration of 1 mg dexamethasone per kilogram of body weight.

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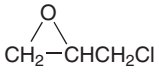
Ephedrine *See* Speed.

Epichlorohydrin

Brad Hirakawa

- Chemical Abstracts Service Registry Number: CAS 106-89-8
- Synonyms: 1-Chloro-2,3-epoxypropane; 3-Chloro-1,2-epoxypropane; Epi-chlorohydrin; Chloromethyloxirane; Chloropropylene oxide; Glycerol epichlorohydrin; Glycidyl chloride; NCI-C07001; Propane, 1-chloro-2,3-epoxy-; SKEKhG; Gamma-chloropropylene oxide; 3-Chloro-1,2-propylene oxide; (DL)-α-Epichlorohydrin; Glycerol epichlorohydrin; ECH; RCRA waste number U041
- Chemical/Pharmaceutical/Other Class: Solvent
- Chemical Formula: C_3H_5ClO
- Chemical Structure:



Uses

Epichlorohydrin is usually prepared from propene and is mainly used in the manufacture of glycerol and epoxy resins. It is also used: in the manufacture of elastomers, glycidyl ethers, cross-linked food starch, surfactants, plasticizers, dyestuffs, pharmaceutical products, oil emulsifiers, lubricants, and adhesives; as a solvent for resins, gums, cellulose, esters, paints, and lacquers; and as a stabilizer in chlorine-containing substances such as rubber, pesticide formulations, and solvents.

Exposure Routes and Pathways

Inhalation and dermal exposure are the most common routes of exposure.

Toxicokinetics

Epichlorohydrin is absorbed rapidly into the body through the skin, after ingestion or inhalation. Epichlorohydrin is itself a reactive epoxide and is metabolized by binding to glutathione and by hydration via epoxide hydrolase. The same hemoglobin adduct has been detected in humans and rats. Epichlorohydrin is distributed widely throughout the body. The highest tissue concentrations in rodents were found in the nose after inhalation and in the stomach after ingestion. In rats, regardless of the route of exposure, most absorbed epichlorohydrin is metabolized rapidly, part being excreted as carbon dioxide via the lungs and part as water-soluble compounds via the urine.

Mechanism of Toxicity

Epichlorohydrin is an alkylating agent (mutagenic, affects DNA). Epichlorohydrin is also an irritant, sensitizer, and corrosive.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} is $90\,mg\,kg^{-1}$ in rats and $236\,mg\,kg^{-1}$ in mice. The inhalation LC_{50} is 250 ppm per 8 h in rats. Epichlorohydrin caused reproductive toxicity (testicular damage) in male rats exposed by inhalation to concentrations as low as 50 ppm per 6 h day^{-1} over 50 days.

Human

The lowest published single toxic dose is 20 ppm via inhalation. The kidneys and respiratory tract are the target organs after acute epichlorohydrin exposure. Symptoms include nausea, vomiting, and abdominal distress. It can also cause facial swelling, eye and nasal mucosal irritation, respiratory tract irritation, bronchitis, dyspnea, central nervous system depression, hepatomegaly, and kidney lesions.

Chronic Toxicity (or Exposure)

Animal

There is sufficient evidence in experimental animals for the carcinogenicity and teratogenicity of epichlorohydrin exposure via oral and inhalation routes. Epichlorohydrin was tumorgenic in rats exposed (inhalation) to concentrations as low as 100 ppm per 6 h day^{-1} over 30 days.

Human

Epichlorohydrin has been shown to cause chromosomal aberrations in humans, and is classified as a probable human carcinogen (group 2A).

Clinical Management

Emesis is not recommended. Activated charcoal slurry with or without saline cathartic and sorbitol can be used after oral ingestion. In case of inhalation exposure, good ventilation should be maintained. Skin decontamination should be performed with repeated washing with soap. Exposed eyes should be irrigated with copious amounts of room-temperature water for at least 15 min. Liver and kidney function should be monitored. Consult a physician as soon as possible.

Environmental Fate

Environmental contamination by epichlorohydrin mainly occurs via air ducts and waste disposal of heavy ends in industries that produce or use epichlorohydrin. Epichlorohydrin can also be lost to the environment via industrial water, during transport and storage, by volatilization during use, and by inadvertent industrial production. Epichlorohydrin is relatively volatile and would therefore readily evaporate from near-surface soils and other solid surfaces. If released into water it will be lost primarily by evaporation (half-life is 29 h in a typical river) and hydrolysis (half-life is 8.2 days). It will not adsorb appreciably to sediment. If spilled on land, it will evaporate and leach into the groundwater where it will hydrolyze. In the atmosphere, epichlorohydrin will degrade by reaction with photochemically produced hydroxyl radicals (estimated half-life is 4 days). It will not bioconcentrate appreciably in aquatic organisms.

Exposure Standards and Guidelines

Threshold limit value, 2 ppm; 7.6 mg m^{-3} (skin) (American Conference of Governmental Industrial Hygienists, 1992–1993).

See also: Pollution, Water; Respiratory Tract.

Further Reading

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Relevant Websites

http://ntp-server.niehs.nih.gov – National Toxicology Program.

http://www.inchem.org – INCHEM website.

http://www.epa.gov – US Environmental Protection Agency website.

Epidemiology

Shayne C Gad

Epidemiology looks at the association between adverse effects seen in humans and a selected potential 'cause' of interest, such as the use of or exposure to a chemical, a disease agent, radiation, a drug, or a medical device. Epidemiology is sometimes simply defined as the study of patterns of health in groups of people. Behind this deceptively simple definition lies a surprisingly diverse science, rich in concepts and methodology. For instance, the group of people might consist of only two people, such as the case of a father suffering from rheumatoid arthritis and his daughter with vertigo. In both father and daughter, the pattern of affected areas was remarkably similar, which might suggest that the distribution of joint lesions in rheumatoid arthritis is genetically determined. At the opposite extreme, studies of the geographic distribution of diseases using national mortality and cancer incidence rates have provided clues about the etiology of several diseases such as cardiovascular disease and stomach cancer. The patterns of health studied are also wide-ranging and may include the distribution, course, and spread of disease. The term 'disease' also has a loose definition in the context of epidemiology and might include ill-defined conditions, such as organic solvent syndrome and sick-building syndrome, or consist of an indirect measure of impairment such as biochemical and hematological parameters or lung function measurements.

Epidemiology and toxicology differ in many other ways, but principally in that epidemiology is essentially an observational science, in contrast to the experimental nature of toxicology. The epidemiologist often has to make do with historical data that have been collected for reasons that have nothing to do with epidemiology. Nevertheless, the availability of personnel records such as lists of new employees and former employees, payrolls and work rosters, and exposure monitoring data collected for compliance purposes has enabled many epidemiological studies to be conducted in the occupational setting. Thus, the epidemiologist has no control over who is exposed to an agent, the levels at which they are exposed to the agent of interest, or the other agents to which they may be exposed. The epidemiologist has great difficulty in ascertaining what exposure has taken place and certainly has no control over lifestyle variables such as diet and smoking.

Despite the lack of precise data, the epidemiologist has one major advantage over the toxicologist. An epidemiological study documents the actual health experiences of human beings subjected to real-life exposures in an occupational or environmental setting. The view has been expressed that uncertainty in epidemiology studies resulting from exposure estimation may be equal to or less than the uncertainty associated with extrapolation from animals to humans. Regulatory bodies such as the US Environmental Protection Agency (EPA) are starting to change their attitudes toward epidemiology and recognize that it has a role to play in the process of risk assessment. However, there is also a complementary need for epidemiologists to introduce more rigor into the conduct of their studies and to introduce standards akin to the Good Laboratory Practices standards under which animal experiments are performed.

Measurement of Exposure

Epidemiologists have placed much greater emphasis on the measure of response than on the measure of exposure. They claim that this is because most epidemiologists have been trained as physicians and are consequently more oriented toward measuring health outcomes. It is certainly true that a modern textbook of epidemiology says very little about what the epidemiologist should do with exposure assessments. However, this is probably as 'much a reflection of the historical paucity of quantitative exposure information as a reflection on the background of epidemiologists. Nevertheless, it is surprising how many epidemiological studies do not contain even a basic qualitative assessment of exposure. The contrast between epidemiology and toxicology is never more marked than in the area of estimation of dose response. The toxicologist can carefully control the conditions of exposure to the agent of interest; moreover, the toxicologist can be sure that the test animals have not come into contact with any other toxic agents. An industrial epidemiologist conducting a study of workers exposed to a hepatotoxin will certainly have to control for alcohol intake and possibly for exposure to other hepatotoxins in the work and home environments. Nevertheless, it can be argued that epidemiological studies more accurately measure the effect on human health of 'real-life' exposures.

If an exposure matrix has been constructed with quantitative estimates of the exposure in each job and time period, then it is a simple matter to estimate cumulative exposure. It is a more difficult process when, as is common, only a qualitative measure of exposure is available (e.g., high, medium, and low). Even when exposure measurements are available, it

may not be sensible to make an assumption that an exposure that occurred 20 years ago is equivalent to the same exposure yesterday. The use of average exposures may also be questionable, and peak exposures may be more relevant in the case of outcomes such as asthma and chronic bronchitis. Noise is a good example of an exposure that must be carefully characterized and where the simple calculation of a cumulative exposure may be misleading.

Study Designs

This section provides a brief introduction to the most important types of studies conducted by epidemiologists. It is an attempt to briefly describe the principles of the major types of epidemiological studies in order to provide insight into the reporting of epidemiological studies and the assumptions made by epidemiologists. The next section will discuss the similarities and differences between the methodologies of toxicology and epidemiology.

Cohort Studies

Historical Cohort Study When the need arises to study the health status of a group of individuals, there is often a large body of historical data that can be utilized. If sufficient information exists on individuals exposed in the past to a potential workplace hazard, then it may be possible to undertake a retrospective cohort study. The historical data will have been collected for reasons that have nothing to do with epidemiology. Nevertheless, the availability of personnel records, such as registers of new and former employees, payrolls, work rosters, and individuals' career records, has enabled many epidemiological studies to be conducted, in particular, mortality studies.

The principles of a historical cohort study can also be applied to follow a cohort of workers prospectively. This approach will be discussed further in the next section, although it should be emphasized that many historical data studies have a prospective element in so far as they are updated after a further period of follow-up. The discussion of historical cohort studies in this section will concentrate on mortality and cancer incidence studies. However, there is no reason why hearing loss, lung function, or almost any measure of the health status of an individual should not be studied retrospectively if sufficient information is available.

Mortality and cancer incidence studies are unique among retrospective cohort studies in that they can be conducted using national cancer and mortality registers even if there has been no medical surveillance of the work force. A historical cohort study also has the advantages of being cheaper and providing estimates of the potential hazard much earlier than a prospective study. However, historical cohort studies are beset by a variety of problems. Principal among these is the problem of determining which workers have been exposed and, if so, to what degree? In addition, it may be difficult to decide what an appropriate comparison group is. It should also be borne in mind that in epidemiology, unlike animal experimentation, random allocation is not possible and there is no control over the factors that may distort the effects of the exposure of interest, such as smoking and the standard of living.

The principles of historical cohort studies are described in the following subsections.

Cohort Definition and Follow-Up Period A variety of sources of information are used to identify workers exposed to a particular workplace hazard, to construct an occupational history, and to complete the collection of information necessary for tracing (see below). It is essential that the cohort be well defined and that criteria for eligibility are strictly followed. This requires that a clear statement be made about membership of the cohort so that it is easy to decide whether an employee is a member or not. It is also important that the follow-up period be carefully defined. For instance, it is readily apparent that the follow-up period should not start before exposure has occurred. Furthermore, it is uncommon for the health effect of interest to manifest itself immediately after exposure, and allowance for an appropriate biological induction (or latency) period may need to be made when interpreting the data.

Comparison Subjects The usual comparison group for many studies is the national population. However, it is known that there are marked regional differences in the mortality rates for many causes of death. Regional mortality rates exist in most industrialized countries but have to be used with caution because they are based on small numbers of deaths and estimated population sizes. In some situations the local rates for certain causes may be highly influenced by the mortality of the patients being studied. Furthermore, it is not always easy to decide what the most appropriate regional rate for comparison purposes is, as many employees may reside in a different region from that in which the plant is situated.

An alternative or additional approach is to establish a cohort of unexposed workers for comparison purposes. However, workers with very low exposures to the workplace hazard will often provide similar information.

Analysis and Interpretation In a cohort study the first stage in the analysis consists of calculating the number of deaths expected during the follow-up period. In order to calculate the expected number of deaths for the cohort, the survival experience of the cohort is broken down into individual years of survival, known as 'person years'. Each person year is characterized by the age and sex of the cohort member and the time period when survival occurred. The person years are then multiplied by age-, sex-, and time period-specific mortality rates to obtain the expected number of deaths. The ratio between observed and expected deaths is expressed as a standardized mortality ratio (SMR) as follows:

$$\text{SMR} = \frac{\text{observed deaths}}{100 \times \text{expected deaths}}$$

Thus, an SMR of 1.25 represents an excess mortality of 25%. An SMR can be calculated for different causes of death and for subdivision of the person years by factors such as the level of exposure and time since the first exposure.

Interpretation of cohort studies is not always straightforward; there are a number of selection effects and biases that must be considered. Cohort studies routinely report that the mortality of active workers is less than that of the population as a whole. It is not an unexpected finding since workers usually have to undergo some sort of selection process to become or remain workers. Nevertheless, this selection effect, known as the 'healthy worker' effect, can lead to considerable arguments over the interpretation of study results, particularly if the cancer mortality is as expected but the all-cause mortality is much lower than expected. However, even an experimental science such as toxicology is not without a similar problem of interpretation, namely, the problem of distinguishing between the effects of age and treatment on tumor incidence.

Proportional Mortality Study

There are often situations in which one has no accurate data on the composition of a cohort but does possess a set of death records (or cancer registrations). In these circumstances a proportional mortality study may sometimes be substituted for a cohort study. In such a mortality study the proportions of deaths from a specific cause among the study deaths is compared with the proportion of deaths from that cause in a comparison population. The results of a proportional mortality study are expressed in an analogous way to those of the cohort study with follow-up corresponding to the observed deaths from a particular cause; it is possible to calculate an expected number of deaths based on mortality rates for that cause and all causes of death in a comparison group and the total number of deaths in the study. The ratio between observed and expected deaths from a certain cause is expressed as a proportional mortality ratio (PMR) as follows:

$$\text{PMR} = \frac{\text{observed deaths}}{100 \times \text{expected deaths}}$$

Thus, a PMR of 125 for a particular cause of death represents a 25% increase in the proportion of deaths due to that cause. A proportional mortality study has the advantage of avoiding the expensive and time-consuming establishment and tracing of a cohort but the disadvantage of little or no exposure information.

Prospective Cohort Study Prospective cohort studies are no different in principle from historical cohort studies in terms of scientific logic, the major differences being timing and methodology. The study starts with a group of apparently healthy individuals whose health and exposure are studied over a period of time. As it is possible to define in advance the information that is to be collected, prospective studies are theoretically more reliable than retrospective studies. However, long periods of observation may be required to obtain results.

Prospective cohort studies or longitudinal studies of continually changing health parameters, such as lung function, hearing loss, blood biochemistry and hematological measurements, pose different problems from those encountered in mortality and cancer incidence studies. The relationships between changes in the parameters of interest and exposure measurements have to be estimated and, if necessary, a comparison made of changes in the parameters between groups. These relationships may be extremely complicated, compounded by factors such as aging, and difficult to estimate because there may be relatively few measurement points. Furthermore, large errors of measurement in the variables may be pre1sent because of factors such as within-laboratory variation and temporal variation within individuals. Missing observations and withdrawals may also cause problems, particularly if they are dependent on the level and change of the parameter of interest. These problems may make it difficult to interpret and judge the validity of analytical conclusions. Nevertheless, prospective cohort studies provide the best means of measuring changes in health parameters and relating them to exposure.

Case–Control Study

In a case–control study (also known as a case–referent study) two groups of individuals are selected

for study, of which one has the disease whose causation is to be studied (the cases) and the other does not (the controls). In the context of the chemical industry, the aim of a case–control study is to evaluate the relevance of past exposure to the development of a disease. This is done by obtaining an indirect estimate of the rate of occurrence of the disease in an exposed and an unexposed group by comparing the frequency of exposure among cases and controls.

Principal Features Case–control and cohort studies complement each other as types of epidemiological study. In a case–control study the groups are defined on the basis of the presence or absence of a given disease and, hence, only one disease can be studied at a time. The case–control study compensates for this by providing information on a wide range of exposures that may play a role in the development of the disease. In contrast, a cohort study generally focuses on a single exposure but can be analyzed for multiple disease outcomes. A case–control study is a better way of studying rare diseases because a very large cohort would be required to demonstrate an excess of a rare disease. In contrast, a case–control study is an inefficient way of assessing the effect of an uncommon exposure, when it might be possible to conduct a cohort study of all those exposed.

The complementary strengths and weaknesses of case–control and cohort studies can be used to advantage. Increasingly, mortality studies are being reported which utilize 'nested' case–control studies to investigate the association between the exposures of interest and a cause of death for which an excess has been discovered. However, case–control studies have traditionally been held in low regard, largely because they are often poorly conducted and interpreted. There is also a tendency to overinterpret the data and misuse statistical procedures. In addition, there is still considerable debate among leading epidemiologists themselves as to how controls should be selected.

Analysis and Interpretation In a case–control study it is possible to compare the frequencies of exposures in the cases and controls. However, what one is really interested in is a comparison of the frequencies of the disease in the exposed and the unexposed. The latter comparison is usually expressed as a relative risk (RR), which is defined as

$$\mathrm{RR} = \frac{\text{rate of disease (exposed group)}}{\text{rate of disease (unexposed group)}}$$

It is clearly not possible to calculate the RR directly in a case–control study since exposed and unexposed groups have not been followed in order to determine the rates of occurrence of the disease in the two groups. Nevertheless, it is possible to calculate another statistic, the odds ratio (OR), which, if certain assumptions hold, is a good estimate of the RR. For cases and controls the exposure odds are simply the odds of being exposed, and the OR is defined as

$$V = \frac{\text{cases with exposure/controls with exposure}}{\text{cases without exposure/controls without exposure}}$$

An OR of 1 indicates that the rate of disease is unaffected by exposure of workers to the agent of interest. An OR greater than 1 indicates an increase in the rate of disease in exposed workers.

Matching Matching is the selection of a comparison group that is, within stated limits, identical with the study group with respect to one or more factors (e.g., age, years of service, and smoking history), which may distort the effect of the exposure of interest. The matching may be done on an individual or group basis. Although matching may be used in all types of study, including follow-up and cross-sectional studies, it is more widely used in case–control studies. It is common to see case–control studies in which each case is matched to as many as three or four controls.

Nested Case–Control Study In a cohort study, the assessment of exposure for all cohort members may be extremely time-consuming and demanding of resources. If an excess of incidence of death has been discovered for a small number of conditions, it may be much more efficient to conduct a case–control study to investigate the effect of exposure. Thus, instead of all members being studied, only the cases and a sample of noncases would be compared with regard to exposure history. Thus, there is no need to investigate the exposure histories of all those who are neither cases nor controls. However, the nesting is only effective if there are a reasonable number of cases and sufficient variation in the exposure of the cohort members.

Other Study Designs

Descriptive Studies

There are large numbers of records in existence that document the health of various groups of people. Mortality statistics are available for many countries and even for certain companies. Similarly, there is a wide range of routine morbidity statistics, in particular, those based on cancer registrations. These health statistics can be used to study differences between geographic regions (e.g., maps of cancer mortality and incidence presented at a recent

symposium), occupational groups, and time periods. Investigations based on existing records of the distribution of the disease and of possible causes are known as descriptive studies. It is sometimes possible to identify hazards associated with the development of rare conditions from observation of clustering in occupational or geographical areas.

Cross-Sectional Study

Cross-sectional studies measure the cause (exposure) and the effect (disease) at the same point in time. They compare the rates of diseases or symptoms of an exposed group with an unexposed group. Strictly speaking, the exposure information is ascertained simultaneously with the disease information. In practice, such studies are usually more meaningful from an etiological or causal point of view if the exposure assessment reflects past exposures. Current information is often all that is available but may still be meaningful because of the correlation between current exposure and relevant past exposure.

Cross-sectional studies are widely used to study the health of groups of workers who are exposed to possible hazards but do not undergo regular surveillance. They are particularly suited to the study of subclinical parameters such as blood biochemistry and hematological values. Cross-sectional studies are also relatively straightforward to conduct in comparison with prospective cohort studies and are generally simpler to interpret.

Intervention Study

Not all epidemiology is observational, and experimental studies have a role to play in evaluating the efficiency of an intervention program to prevent disease (e.g., fluoridation of water). An intervention study at one extreme may closely resemble a clinical trial with individuals randomly selected to receive some form of intervention (e.g., advice on reducing cholesterol levels). However, in some instances it may be a whole community that is selected to form the intervention group. The selection may or may not be random.

Veterinary Epidemiology

Humans are in close association with their pets and other animals (e.g., local wildlife and animals on a farm). Veterinary epidemiology, like human epidemiology, looks at the association between adverse effects and a selected potential 'cause' of interest, such as exposure to a chemical or a disease agent. For example, veterinary epidemiology can play a key role in emerging and global disease outbreaks, helping in the understanding and prevention of infections and other emerging diseases, including those transmitted from an animal to other animals, and those possibly transmitted from animals to humans. An example of a veterinary epidemiological study was one investigating the transmission of *Salmonella typhimurium* from cattle which had received no growth-promoting antibiotics to humans who had direct contact with the sick animals. Another example is severe acute respiratory syndrome (SARS). In the investigation of the origins of the SARS outbreak in China, viruses associated with SARS were isolated from Himalayan palm civets found in a live-animal market in Guangdong, China, and evidence of virus infection was also detected in other animals and in humans working at the same market. The detection of these viruses in small, live wild mammals in a retail market helped identify at least one means of the interspecies transmission, that is, infected animals sold in that market to human customers.

Conclusion

Epidemiological studies can be the most powerful and persuasive tools for establishing the hazards associated with chemical exposures or personal actions (such as cigarette smoking). However, due to all the factors discussed previously, such studies also tend to be somewhat insensitive. Unless one can clearly establish the symptoms and signs of a disease for which there is a causal connection, such studies lose the desired specificity.

See also: Analytical Toxicology; Carcinogen Classification Schemes; Carcinogenesis; Exposure; International Agency for Research on Cancer; Medical Surveillance; National Institute for Occupational Safety and Health.

Further Reading

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Relevant Websites

http://www.cvm.uiuc.edu – The Association for Veterinary Epidemiology and Preventive Medicine (AVEPM).
http://www.pitt.edu – Toxicology and Epidemiology (Online Supercourse). (More than 9000 faculty from 118 countries have contributed to an online library of more than 700 lectures with quality control and adherence to accepted pedagogic principles. The goal is to improve teaching and research in epidemiology and public health worldwide.)

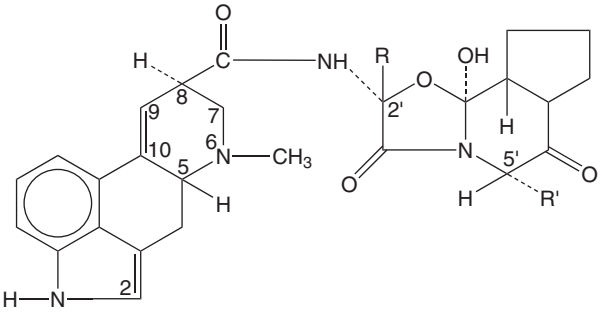
Epinephrine *See* Catecholamines.

Ergot

Christopher P Holstege

This article is a revision of the previous print edition article by Michael J Brabec, volume 1, pp. 560–561, © 1998, Elsevier Inc.

- Representative Chemical: Ergotamine
- Synonyms: Bromocriptine; Dihydroergocornine; Dihydroergocristine; Dihydroergosine; Dihydroergotamine; Dihydroergotaxime; Ergobasine; Ergocornine; Ergocristine; Ergocryptine; Ergometrine; Ergonovine; Ergosine; Ergotamine; Ergotamine tartrate; Ergotaxime; Lergotrile; Lisuride; Lysergol; Metergoline; Methylergonovine; Methysergide
- Chemical/Pharmaceutical/Other Class: Alkaloid
- Chemical Structure:



Alkaloid §	R(2')	R(5')
Ergotamine	—CH_3	—CH_2—phenyl
Ergosine	—CH_3	—$CH_2CH(CH_3)_2$
Ergostine	—CH_2CH_3	—CH_2—phenyl
Ergotoxine group:		
Ergocornine	—$CH(CH_3)_2$	—$CH(CH_3)_2$
Ergocristine	—$CH(CH_3)_2$	—CH_2—phenyl
α-Ergocristine	—$CH(CH_3)_2$	—$CH_2CH(CH_3)_2$
β-Ergocristine	—$CH(CH_3)_2$	—$CHCH_2CH_3$ | CH_3
Bromocriptine	—$CH(CH_3)_2$	—$CH_2CH(CH_3)_2$

Uses

Ergot was used as early as the sixteenth century to strengthen uterine contractions. Currently, ergotamine tartrate is combined with caffeine and administered to relieve migraine headaches. Ergonovine has been used to treat postpartum hemorrhage. Derivatives of ergots are used to manage amenorrhea and as an adjunct in the treatment of Parkinson's disease. Hydrogenated ergot alkaloids have been used for symptoms of idiopathic mental decline in elderly patients.

Background Information

The ergot alkaloids are found within the sclerotium of the fungus *Claviceps purpurea*. The sclerotium is the hard tuber-like resting stage of this fungus and is a dark gray, purple, or black cylindrical structure measuring 1.5 cm in length and 0.5 cm in width. *C. purpurea* may be found on a number of different grains, with rye contamination most often reported. A cold winter followed by wet spring favors germination. If the sclerotia are not removed from contaminated grain by beating or sieving, humans or animals may accidentally ingest them.

Exposure Routes and Pathways

Historically, exposure occurred by consumption of contaminated grain, especially rye flour. Acute poisonings in humans are rare and are generally associated with overdosage with ergotamine tartrate medication. Poisoning by ergot-containing mixtures has been associated with attempts to induce abortion. Animal poisonings result from consumption of contaminated pasture grasses and grains. The last diagnosed human fatalities associated with consumption of ergot-containing grains occurred in a French village in 1951.

Toxicokinetics

The degree of oral absorption of ergots varies depending on the specific agent. For example, ergotamine

is poorly absorbed orally and a considerable amount is eliminated by first-pass metabolism in the liver. On the other hand, ergonovine is rapidly and more completely absorbed following ingestion. Suppositories increase ergotamine bioavailability ~20 times that of ingested doses. Peak plasma levels are reached within 2 h. Symptoms typically appear within 4 h after intake. The volume of distribution is estimated to be $2\,l\,kg^{-1}$. Ergots are primarily metabolized by the liver and 90% of metabolites are eliminated in the bile. The elimination half-life varies depending on which ergot is ingested: ergotamine's half-life is 3 h whereas dihydroergotamine's half-life is 13 h. However, these elimination half-life values were determined at therapeutic doses. In overdose, the half-life of these agents would be expected to prolong.

Mechanism of Toxicity

The pharmacological mechanisms associated with ergot toxicity are complex and have not been fully delineated. Ergotamine interacts with serotonergic, dopaminergic, and α-adrenergic receptors. In the central nervous system, ergots have a sympatholytic effect. They also stimulate serotonergic receptors that contribute to its hallucinogenic activity. Peripherally, ergots act as α-adrenergic agonists resulting in peripheral vasospasm. The vasoconstrictive action of ergots can produce widespread arterial spasm. Endothelial injury associated with arterial spasm may cause local thrombosis and subsequent gangrene.

Acute and Short-Term Toxicity (or Exposure)

Animal

Peripheral vasoconstriction, particularly of the hind limbs and forelimbs, can produce hemorrhagic vesiculations that may progress to gangrene. Ergot induced embryotoxicity has been reported with associated fetal malformations and growth retardation. Ergotism may also result in miscarriages.

Human

Acute early symptoms of toxicity include nausea, vomiting, diarrhea, skin paresthesias, and chest pain. Headache, fixed miosis, hallucinations, delirium, hemiplegia, and convulsions may occur. Historically, 'ergotism' was divided into gangrenous ergotism and convulsive ergotism. St. Anthony's Fire was one descriptive name given to ergotism in Europe during the Middle Ages due to extremity burning pain associated with ergot toxicity. Peripheral ischemia most commonly manifests in the lower extremities, although ischemia of cerebral, mesenteric, coronary, and renal vascular beds may also occur. Legs may become pulseless, pale, and cyanotic. Hemorrhagic vesiculations, pruritus, and gangrene can occur.

Chronic Toxicity (or Exposure)

Animal

Pigs have demonstrated chronic ergot toxicity as blockade of lactation only. No other effects were noted.

Human

Studies of dihydroergotamine nasal spray have described increased incidence of allergic rhinitis and some gastrointestinal complaints. Patients taking ergot derivatives chronically for the management of headache may develop rebound headache (headache that develops from lack of drug, which can lead to continued drug use and worsening of headache symptoms).

In Vitro Toxicity Data

Studies in rat brains have shown that ergot alkaloids do not bind to brain benzodiazepine binding sites.

Clinical Management

Acute poisoning may be treated with decontamination using oral activated charcoal. Arterial spasms may be relieved by administration of vasodilators, such as nitroprusside, nitroglycerin, and phentolamine. Benzodiazepines should be administered to halt seizures and may be used to alleviate agitation associated with hallucinations. Anticoagulants such as heparin may be administered in cases of extreme vasoconstriction. Reaction to the ergot alkaloids is highly variable and clinical progress should be monitored carefully.

See also: Mold.

Further Reading

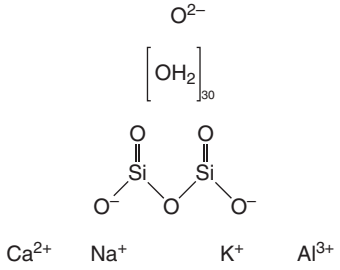
Edwards RJ, Fulde GWO, and McGrath MA (1991) Successful limb salvage with prostaglandin infusion: A review of ergotamine toxicity. *Medical Journal of Australia* 155: 825–827.

Goldfischer JD (1960) Acute myocardial infarction secondary to ergot therapy: Report of a case and review of the literature. *New England Journal of Medicine* 262: 860–863.

Erionite

A Umran Dogan, Meral Dogan, and Salih Emri

- Chemical Name: Erionite refers to a group of minerals: erionite-Ca, erionite-K, and erionite-Na
- Chemical Abstracts Service Registry Numbers: CAS 66733-21-9; CAS 12510-42-8
- Synonyms: Erionite-Ca; Erionite-Na; Erionite-K
- Classifications:
  - Erionite-Ca
    International Mineralogy Association (IMA) status: Approved
    Strunz ID: 8/J.26-94
  - Erionite-Na
    IMA status: Approved
    Strunz ID: 8/J.26-90
  - Erionite-K
    IMA status: Approved
    Strunz ID: 8/J.26-92
    where 8 = Silicates, J = Tectosilicates, 26 = Zeolite Group, Wilhendersonite–Chabazite–Perlialite series
- Chemical Properties: Si/Al ratio (used to be Si/Al > 2.4; Si/Al > 3.0; Si/(Al + Fe) > 2.9 for erionite) is no longer a criteria for discrimination between erionite and offretite, because of the extensive compositional overlap that exists between the two species. However, the Si–Al content in the tetrahedra framework is the major control on the unit cell volume dimensions in erionite. In addition, Mg cation is a major factor in controlling the crystallization of the mineral species.
- Chemical Formulas: Erionite-Ca – Composition of the type specimen is from Maze, Niigita Prefecture, Japan: $(Ca_{2.28}K_{1.54}Na_{0.95}Mg_{0.86})Al_{8.83}Si_{26.90}O_{72} \cdot 31.35H_2O$ (Mg > 0.80). Erionite-Na – Composition of the type specimen is from Cady Mountains, CA, USA: $(Na_{5.59}K_{2.00}Ca_{0.11}Mg_{0.18}Fe_{0.02})Al_{7.57}Si_{28.27}O_{72} \cdot 24.60H_2O$. Erionite-K – Composition of the specimen from Ortenberg, Germany: $(K_{3.32}Na_{2.31}Ca_{0.99}Mg_{0.06}Ba_{0.02})Al_{8.05}\ Si_{28.01}O_{72} \cdot 31.99H_2O$
- Chemical Structure:



Relationship to Other Species

Belbergite, Chabazite-Ca, Chabazite-K, Chabazite-Na, Chabazite-Sr, Gmelinite-Ca, Gmelinite-K, Gmelinite-Na, Levyne-Ca, Levyne-Na, Mazzite, Offretite, Perlialite, Tschernichite, Wilhendersonite, and other Erionite species.

Associated Minerals

Analcime, Chabazite, Clinoptilolite, Heulandite, Mordenite, Phillipsite, Offretite, Levyne, Smectite, Cristobalite, Quartz, Opal, Pyrite, Thenardite, Celandonite, Herschelite, Calcite, Dolomite, Halite, Gypsum.

Erionite Characterization Rules

The toxicity of the mineral is such that quantitative characterization of erionite is extremely important. Samples should be characterized by using one or more of the following techniques: (1) powder X-ray diffraction, (2) electron probe microanalysis or inductively coupled plasma-mass spectroscopy, (3) scanning electron microscopy equipped with wavelength dispersive spectroscopy (WDS) and/or energy dispersive spectroscopy (EDS), (4) transmission electron microscopy equipped with WDS and/or EDS and selected area electron diffraction, and (5) similar or better analytical techniques.

Crystal chemistry of erionites should be computed based upon the guidelines of the IMA Zeolite Report of 1997. The reliabilities of the crystal chemistries of these erionites should be evaluated using the balance error formula of $E = ((Al + Fe^{3+}) - (Na + K) + 2(Ca + Mg + Sr + Ba))/(Na + K) + 2(Ca + Mg + Sr + Ba) \times 100$. The results of chemical analyses of erionite are only considered to be reliable if the balance error (E%) is equal to or less than 10%.

In the crystal structure of erionite, Mg cations can be present up to 0.8 atom per cell. $Si + Al\ (+Fe^{3+})$ should be approximately equal to 36 atoms based upon 72 oxygen atoms in the erionite formula, although the Si/Al ratio alone cannot be used for identification.

Often the erionite specimens were incompletely or incorrectly characterized throwing doubt on the results of the work. Such experiments should only be performed with erionite minerals that have passed the quantitative characterization tests (both E% and Mg-content) and the type of erionite ($-$Ca, $-$Na, $-$K) must be identified properly. Failure to do so makes the results of the experiments problematic.

Because of this correct identification of the mineral is imperative and the characterization guidelines described above must be followed.

Erionite Group Minerals

Erionite occurs in different types of rocks, rarely in pure form. It occurs in two major morphotypes, a short fiber form. Erionite's name came from 'erion', the Greek work for wool, because of its white, fibrous, wool-like appearance.

The basic structure of erionite is aluminosilicate tetrahedra. The oxygen is shared between two tetrahedra. The structure of erionite is chainlike, with six tetrahedra on each edge of the unit forming part of a chain of indefinite length. Erionite is not known to occur in other than fibrous form, in single needles or in clusters. In 1997, erionite was elevated to group status and individual members of erionite-Ca, erionite-Na, and erionite-K have been redefined.

Erionite-Ca

Ca is the most abundant extra framework cation. T_{Si} in the range of 0.68–0.79. Erionite-Ca from Shourdo, Georgia; Durkee, OR, USA; Beach Creek, OR, USA; Montresta, Nuoro, Italy; British Columbia, Canada; Montecchio Maggiore, Italy; Jindivick, Australia; Phillip Island, Australia; and Faedo, Vicenza, Italy passed the balance error and Mg-content test and re-classified as erionite-Ca.

Erionite-Na

Na is the most abundant extra framework cation. T_{Si} in the range of 0.74–0.79. Erionite-Na from Durkee, OR, USA; Cady Mountains, CA, USA; Lake Natron, Tanzania; Crooked Creek, OR, USA; Phillip Island, Australia; Campbell Glacier, Antarctica; Mt. Adamson, Antarctica; Dunseverik, Northern Ireland; Montecchio, Maggiore, Italy; and selected samples from Cappadocian region of Turkey passed the balance error and Mg-content test and re-classified as erionite-Na.

Erionite-K

K makes up 58% of extra framework cation; significant Na, Ca, and Mg are also present. T_{Si} in the range of 0.74–0.79. Erionite-K from Durkee, OR, USA; Rome, OR, USA; Yaquina Head, OR, USA; Reese River, NV, USA; Ortenberg Quarry, Germany; Rome, OR, USA; and selected samples from Cappadocian region of Turkey passed the balance error and Mg-content test and re-classified as erionite-K.

Undifferentiated Erionites

Some erionite data from the literature did not pass the balance error test or Mg-content test or both. Some data could not be re-classified as single erionite mineral. Italian samples reported from Montecchio were re-calculated as erionite-Ca and erionite-Na, respectively. Two sets of data from Phillip Island, Australia were re-calculated as both erionite-Ca and erionite-Na. Data from Durkee, OR, USA by three different authors were re-calculated and found as erionite-Ca, erionite-Na, erionite-K, respectively.

Localities

Type Localities

- Erionite-Ca: Maze, Niigita Prefecture, Chubu Region, Honshu Island, Japan.
- Erionite-Na: Cady Mountains, San Bernandino County, CA, USA.
- Erionite-K: Rome, Marion County, OR, USA.

Other Localities

Large number of erionite deposits have been reported from many countries including Antarctica (North Victoria Land), Australia (New South Wales), Bulgaria, Canada (British Columbia), China, France (Pays de la Loire), Germany (Baden-Wurttemberg, Bavaria, Hesse), Hungary, Iceland (Breidhdalur-Brufjordur, Hofsa, Hvalfjordur), Italy (Latium, Sardinia), Japan, Korea, Mexico, New Zealand (South Island), Romania, Russia (Eastern Siberia), South Africa, Turkey (Cappadocia), UK (Northern Ireland, Scotland), USA (Arizona, California, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming).

Uses of Erionite and Zeolite

Erionite is not known to be currently mined or marketed for commercial purposes. Natural erionite has been replaced by synthetic nonfibrous zeolites. However, erionite was used as a noble metal impregnated catalyst in a hydrocarbon-cracking process, and erionite-rich blocks was also used for house building materials. Its use to increase soil fertility and to control odors in livestock production has been studied.

Since 1978, the use of zeolites to solve environmental pollution and energy conservation problems showed promise and was expected to increase. Natural zeolites have many commercial uses in coal gasification and natural gas purification, selectively adsorb molecules from water or air, purify sludge effluents to potable standards, extract trace amounts

of heavy metals so that existing water supplies may be reused, extract radioactive species from nuclear plant wastes, retain their ion-exchange capacities, and are resistant to nuclear degradation. In addition, they are used in agriculture to decrease ammonia released from animal wastes and retain the nitrogen in the solid wastes, which increases the fertilizer value of the solid material, reduce noxious fumes of ammonia and hydrogen sulfide when spreading in chicken houses, increase egg production when used in the chicken houses, absorb liquid waste and reduce odors when spreading on the floors of pig farms, feed supplements to increase the feed conversion value, control aquaculture environments and fish culture recirculating water systems, appear to exhibit antibiotic properties that reduce illness and death rates among farm animals. Further, zeolites are used in air/water/soil pollution to remove sulfur dioxide from coal and oil burning power plant emissions, are especially suited to low pH and high temperature exhaust systems, absorb oil spills, neutralize low pH soils; in oxygen production for enclosed and poorly ventilated spaces, river and pond aeration, reoxygenate downstream waters of paper and pulp plants, secondary sewage treatment. Zeolites also have miscellaneous uses in paper products, construction products, fluoride toothpaste, recycle-dialysis systems, solar energy collection, dehydration and rehydration resulting in the exchange of several hundred BTUs per pound.

Production of Erionite

Current commercial production and marketing of erionite is not known. Erionite was first described in 1898, but reports of occurrences were not published until 1959. Commercial mining of zeolites, including ores containing erionite, began in the 1960s. In the 1970s, two US companies mined erionite at two of six mineable deposits in the United States. Erionite was one of the four commercially important zeolites in the 1970s.

Background Information

Erionite was first described in 1898. The carcinogenity of erionite was called to the attention of the rest of the world from experiences in the Cappadocia region of Turkey where the cancer rate is about 1000 times greater than that observed elsewhere. Most cases of mesothelioma were found to occur in the villages of Tuzkoy, Sarihidir, Karain, and in neighboring villages. Erionite was first listed for its carcinogenicity in the Seventh Annual (1987) Report on Carcinogens from the International Agency for Research on Cancer (IARC) because there was sufficient evidence for the carcinogenicity in experimental animals.

Exposure Routes and Pathways

Inhalation is the exposure route for humans. Animal studies have used inhalation exposures and intrapleural or intraperitoneal injections. Current potential occupational exposure to erionite appears to be as the result of mining and producing other natural zeolites, some of which may contain erionite fibers. Environmental and residential exposure can occur via dusts containing erionite.

Toxicokinetics

Erionite has been shown to be an agent responsible for malignant mesothelioma. Mesothelioma is an aggressive tumor of the mesothelial cells lining the body cavities for which there is no cure and for which current therapies to reduce the effects of the disease are unsatisfactory. The source of exposure may be occupational or environmental and 2000–3000 cases are diagnosed per year in the United States, many of which are associated with (amphibole) asbestos and erionite.

Erionite induced peritoneal mesotheliomas in male rats when administered by intraperitoneal injection, induced pleural mesotheliomas in rats of both sexes when administered by intrapleural injection or inhalation.

Erionite is also listed as a group-I known human carcinogen because the IARC Working Group reported that there is sufficient evidence for carcinogenicity based upon descriptive studies of three villages from Turkey. Erionite fibers were found in lung tissues of pleural mesothelioma cases of Karain, Tuzkoy, and Sarihidir villages of Turkey, where there was very high mortality from malignant mesothelioma. Therefore, erionite in the Cappadocian region of Turkey is believed to be responsible for the majority of the mesothelioma cases observed in that country.

Mechanism of Toxicity

The tumorigenesis of mineral fibers is governed by fiber dimensions and inherent differences in the physicochemical properties of the fibers (intrinsic fiber potencies), for example, studies of carcinogenic fibers have found that fiber geometry and length seem to be the most important factors, with long thin fibers having a greater proliferative and tumorigenesis capability than short fibers. Although the biological

mechanisms are complex and there is little understanding of how durable mineral fibers cause mesothelioma, the association of erionite with mesothelioma is well established. Recently, a more complex relationship of erionite plus a genetic component to cause mesothelioma has been postulated. Work in progress may yet give a greater understanding of the biological mechanisms involved. What is clear is that erionite is one of the most carcinogenic minerals in the world and it requires the utmost care in handling.

Acute and Short-Term Toxicity (or Exposure)

Animal

There is sufficient evidence for the carcinogenecity of erionite to experimental animals.

A number of experiments have been conducted on the intrapleural and intraperitoneal administration of erionite in mice and rats. These experiments have all been positive, showing a very high mesothelioma yield (90% or above) for amounts of erionite above 0.5 or 1 mg. For higher doses, the time of appearance of tumors was decreased. Other tumors at the site of inoculation as well as lymphomas have been occasionally reported.

Natural erionite, synthetic nonfibrous zeolite with the composition of erionite, and crocidolite type asbestos were tested at a concentration of 10 mg m^{-3} inhalation in rats. Pleural mesotheliomas were found in 27 of 28 rats exposed to erionite; one pulmonary and one pleural tumor were found in 28 rats exposed to synthetic zeolite, and one lung carcinoma was reported in rats exposed to crocidolite.

The relative carcinogenic potency of erionite and asbestos have been compared. In experiments based upon intrapleural inoculation, erionite was 300–800 times more active than chrysotile, and 100–500 times more active than crocidolite. In intraperitoneal experiments, erionite was 20–40 times more active than chrysotile and 7–20 times more active than crocidolite.

Human

No human studies of acute and short-term exposures are available.

Chronic Toxicity (or Exposure)

Animal

No animal studies of chronic exposures are available; the long-term effects of short-term exposures have been studied as part of the evaluation of the carcinogenic potential of erionite.

Human

There is sufficient evidence for the carcinogenecity of erionite to humans. Most of the data on the carcinogenicity of erionite in humans come from the experience of the inhabitants of the erionite-contaminated villages in Cappadocia region of Turkey. It is reported that 25 malignant pleural mesothelioma (MPM) in population of 575 inhabitants of Karain village between 1970 and 1974; 28 MPM in Karain village between 1975 and 1979; 15 MPM, 12 malignant peritoneal mesothelioma (MPEM), and eight lung cancer in Tuzkoy village between 1978 and 1980. The incidence or mortality from mesothelioma was above 1% $year^{-1}$, a rate which is over 10 000 times higher than those seen among populations of nonoccupationally exposed to asbestos from Western Europe or North America.

Several mesothelioma cases were reported for persons who immigrated to Sweden but used to live in one of the Cappadocian villages (Karain). In this group of $\sim$100 men who presently live in Sweden, seven cases of mesothelioma were observed. The incidence was approximately to 1% $year^{-1}$. In another study, 14 deaths due to MPM among 162 Turkish emigrants from Karain. In addition, there were five patients with mesothelioma (four MPM and one MPEM) who were still alive, at the time of the study performed. Thus it is calculated that risk of mesothelioma is for men 135 times and for women 1336 times greater than for the same sex and age groups in Sweden.

In Vitro Toxicity Data

Various *in vitro* studies have examined erionite in noncellular systems and the effects of erionite on isolated cells, mainly to attempt to understand how erionite induces chromosomal aberrations and carcinogenicity via cytotoxic effects and other possible mechanisms. One example is a study of the ability of erionite to initiate hydroxyl radical formation from hydrogen peroxide in a noncellular system. Other examples are the study of the possible mechanisms of reactive oxygen intermediate (ROM) production by human blood cells stimulated with erionite, and the release of superoxide as a biologic response of alveolar macrophages, hamster tracheal epithelial cells, and rat lung fibroblasts exposed to erionite. In *in vitro* mutagenicity studies, erionite induced unscheduled DNA synthesis and morphological transformation in cultured mammalian cells. No data

were available to evaluate the reproductive or prenatal toxicity of erionite in experimental animals.

Clinical Management

It takes 20–30 years for erionite to induce mesothelioma. However, there is no cure for the malignant mesothelioma. Surgery may extend the patient's life 9–12 months.

Environmental Fate

Most of the nonoccupational data on exposure to erionite refer to an agricultural area of Central Anatolia, Cappadocia, Turkey. Limited data are available from the USA, suggesting little exposure to fibers. People and animals must be prevented from contacting erionite; responsible person/group should take necessary precaution and must not permit people work or live in an area contaminated with erionite.

Ecotoxicology

Since erionite has been used in laboratory animals for carcinogenecity experiments and tests positive, it could be harmful to other animals if it is inhaled.

Other Hazards

Erionite is not known as flammable or explosive.

Exposure to Erionite

Airborne erionite fibers are generally respirable. Erionite is reported to be a minor component in some commercial zeolites.

Production and use of the erionite-contaminated zeolites may result in potential exposure for the workers and the general population who use zeolites in a variety of processes and products. All workers involved in the production or use of these zeolite-containing products are potentially exposed to erionite.

Fibrous and nonfibrous zeolites are common minerals in the western United States; there are 10 trillion tons of reserves and 120 million tons near the surface of the ground. Erionite fibers have been detected in samples of road dust in Nevada. United States residents of the Intermountain West may be potentially exposed to fibrous erionite in ambient air. Total dust exposures in an open pit zeolite (containing erionite) mine in Arizona for miners ranged from 0.1 to 13.7 $mg\,m^{-3}$; respirable dust in the mining area was 0.01–1.4 $mg\,m^{-3}$.

Exposure Standards and Guidelines

Erionite is listed as a group-I known human carcinogen by the International Agency for Research on Cancer (IARC) because there is sufficient evidence for the carcinogenicity in experimental animals. The US Occupational Safety and Health Administration (OSHA) regulates erionite under the Hazard Communication Standard, and as a chemical hazard in laboratories. The US Environmental Protection Agency (EPA) regulates erionite under the Toxic Substances Control Act (TSCA) as a chemical substance for which there are significant new uses, and specifies procedures for manufacturers, importers, or processors to report on those significant new uses.

See also: Carcinogen Classification Schemes; Carcinogenesis; Respiratory Tract; Toxicity Testing, Carcinogenesis.

Further Reading

Dogan AU (2003) Mesothelioma in Cappadocian villages. *Indoor Built Environment* 12: 367–375.

Dogan AU (2003) Zeolite mineralogy and Cappadocian erionite. *Indoor Built Environment* 12: 337–342.

Dogan AU (2005) Malignant mesothelioma and erionite. In: Pass H (ed.) *Malignant Mesothelioma*, Chapter 5, pp. 242–258. New York: Springer.

Emri S, Demir A, Dogan M, *et al.* (2002) Lung diseases due to environmental exposures to erionite and asbestos in turkey. *Toxicology Letters* 127: 251–257.

Relevant Websites

http://un2sg4.unige.ch – Athena Mineralogy.

http://www.mindat.org – Mindat.org: The Mineral Database.

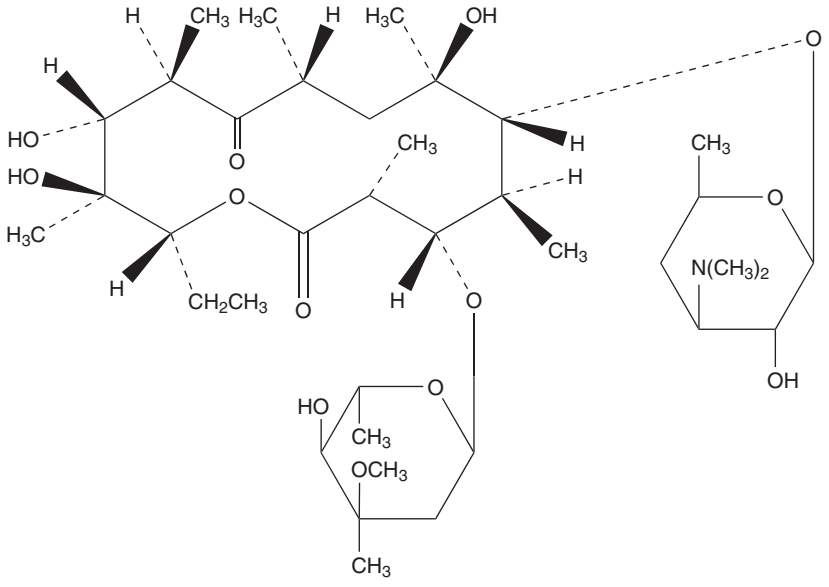
http://webmineral.com – Webmineral.

Erythromycin

Michael D Reed

This article is a revision of the previous print edition article by Carole Wezorek, volume 1, pp. 561–562, © 1998, Elsevier Inc.

- SYNONYMS: Numerous salts and brand names available. E-Mycin; EES; Ilosone; and many others
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Antibiotic; Macrolide
- CHEMICAL FORMULA:



Uses

Erythromycin is indicated for the treatment of infections caused by erythromycin susceptible bacteria. The drug binds to the 50 S ribosomal subunit inhibiting bacterial RNA-dependent protein synthesis. Susceptible bacteria include most Gram-positive bacteria and the "atypical" pathogens.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to erythromycin. Numerous erythromycin salt preparations are available in tablet, capsule, or liquid preparations for oral administration. Other available forms include intravenous, topical (acne), and ophthalmic preparations.

Toxicokinetics

The ester and ester salts of erythromycin are variably absorbed (18–45%) from the small intestine into the systemic circulation. Overall, erythromycin bioavailability depends upon the formulation administered with greatest bioavailability observed with the estolate salt (Ilosone brand) and the least is observed with the base formulation. The bioavailability of the ethylsuccinate salt is highly variable. The majority of erythromycin formulations are more completely absorbed when administered in the fasting state whereas the ethylsuccinate salt is better absorbed when given with food. As would be expected, peak serum erythromycin concentrations are variable and occur ~2–4 h after administration and are dependent upon which formulation ingested. Peak plasma concentrations after intravenous administration of the lactobionate or gluceptate salts occurs within 1 h of administration.

Once in systemic circulation, erythromycin is widely distributed in the body with an estimated V_d ~0.6–0.7 l kg^{-1}. The drug is ~70–90% bound to plasma proteins. The vast majority of the drug is found in the intravascular space, reflecting its widespread clinical use for the treatment of intracellular pathogens. The drug undergoes extensive metabolism with only 5–10% excreted unchanged in the urine; the majority of the drug is excreted via the bile. Erythromycin's half-life is ~2–3 h.

Erythromycin is a substrate for the cytochrome P450 (CYP 450) 3A4 isoenzyme. Once bound to CYP 3A, erythromycin stimulates its own metabolism via demethylation and oxidation to nitroalkane metabolites. These nitroalkane metabolites appear to form stable complexes with the iron of the CYP 3A4 isoenzyme, decreasing its functional capacity. Depending upon the erythromycin dose and duration of therapy, CYP 3A4 activity could be inhibited to undetectable activity and serves as the basis of erythromycin-associated metabolic-based drug–drug interactions.

Mechanism of Toxicity

The gastrointestinal hormone, motilin, is responsible for maintaining the normal, rhythmic peristaltic activity of the intestines. Erythromycin possesses high affinity for the motilin receptor which accounts for the drug's poor tolerability with routine clinical dosing (abdominal cramping, diarrhea) and accounts for the primary toxicity observed after oral overdose. In contrast, very high plasma erythromycin concentrations, which may be obtained after rapid administration of the intravenous formulation or after aggressive erythromycin dosing in any patient who cannot metabolize/excrete the drug (i.e., patient with poor hepatic and renal function) leading to the accumulation of high plasma concentrations, can experience life-threatening cardiac arrhythmias. In a concentration dependent manner, erythromycin will interfere with the potassium rectifier channels within the myocytes of the heart leading to QT prolongation on ECG and arrhythmia. The delayed potassium rectifier (Ik) channels are the potassium channels involved in repolarization of cardiac cells. Blockade of the cardiac Ikr (delayed potassium rectifier-rapid) channel produces a depressed peak in the voltage and a decrease in potassium cellular outflow predisposing the myocardium to early after repolarization, which when sizeable, results in dysrhythmia and most notably, Torsades de Pointes. (Similar to what is observed with astemizole, cisapride, and terfenidine.)

Acute and Short-Term Toxicity (or Exposure)

Animal

Erythromycin is regularly used in veterinary practice and seems to be tolerated well by many animal species. Like humans, animals can develop hypersensitive reactions to erythromycin. Also like humans, acute gastrointestinal effects are the most commonly seen adverse effects.

Human

Patients presenting with acute erythromycin overdose are usually asymptomatic or experiencing minor to moderate gastrointestinal side effects/discomfort. Serious cardiac effects, including prolongation of the QT interval, arrhythmias (i.e., ventricular tachycardia, Torsades de Pointes, ventricular fibrillation, and heart block), may be observed after rapid intravenous administration and coincident with high, peak erythromycin plasma concentrations. The occurrences of these QT prolongation-associated arrhythmias are rare.

Chronic Toxicity (or Exposure)

Animal

Animals have been reported to develop similar symptoms to those seen in humans when larger doses are used. Horses are particularly sensitive to erythromycin induced gastrointestinal effects. At doses of $> 5\,\mathrm{mg\,kg^{-1}\,day^{-1}}$, dogs have been reported to develop ventricular arrhythmias.

Human

Patients undergoing routine antibiotic use with erythromycins orally, generally tolerate the drug well. Gastrointestinal complaints are common. However, more serious effects have been noted, including profound cardiovascular toxicity (e.g., arrhythmias such as Torsades do Pointes), hepatic damage, neurologic effects (e.g., ototoxicity) associated with high serum levels and intravenous use, although one case describes toxic effects without elevated serum erythromycin concentrations or evidence of impaired renal or hepatic drug clearance.

In Vitro Toxicity Data

Mutagenicity assays of bacterial DNA repair in *Escherichia coli* have been negative with erythromycin.

Clinical Management

Though the clinical need for such measures would be expected to be rare, basic and advanced life-support measures as well as aggressive decontamination should be instituted as clinically necessary. Gastric decontamination may be performed dependent on the symptomatology of the patient and history of the exposure. Activated charcoal will effectively adsorb erythromycin. Gastrointestinal discomfort may be treated symptomatically or by reducing the dosage,

if appropriate. Specific attention to maintaining normal fluid and electrolyte homeostasis should be addressed in significant overdoses involving young infants. Liver function tests should be monitored if hepatotoxicity is suspected. Erythromycin blood levels are not clinically useful.

See also: Cardiovascular System; Gastrointestinal System.

Further Reading

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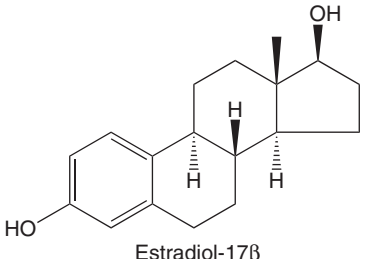
Washington JA and Wilson WR (1985) Erythromycin: A microbial and clinical prospective after 30 years of clinical use. *Mayo Clinic Proceedings* 60: 271–278.

Estrogens I: Estrogens and Their Conjugates

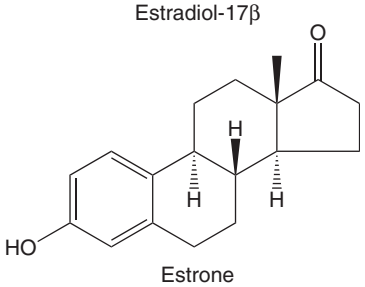
James L Wittliff and Sarah A Andres

Estrogens

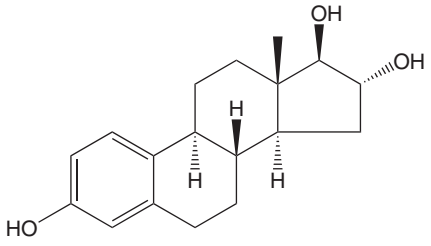
- Representative Chemicals: Estradiol-17β; Estrone; Estriol
- Chemical Abstracts Service Registry Numbers: Estradiol-17β (CAS 50-28-2); Estrone (CAS 53-16-7); Estriol (CAS 50-27-1)
- Synonyms:
  - Estradiol-17β: 1,3,5(10)-Estratriene-3,17β-diol;
  - Estrone: 1,3,5(10)-Estratrien-3-ol-17-one; and
  - Estriol: 1,3,5(10)-Estratriene-3,16α,17β-triol.
- Chemical/Pharmaceutical/Other Class: Steroid hormones
- Chemical Formulas:
  - Estradiol-17β: $C_{18}H_{30}O_2$;
  - Estrone: $C_{18}H_{22}O_2$; and
  - Estriol: $C_{18}H_{24}O_3$.
- Chemical Structures:



Estradiol-17β



Estrone



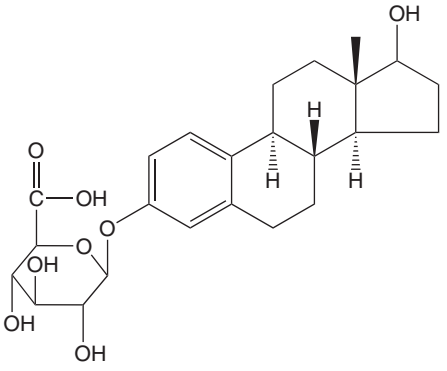
Estriol

Estrogens Conjugates, Physoplogical

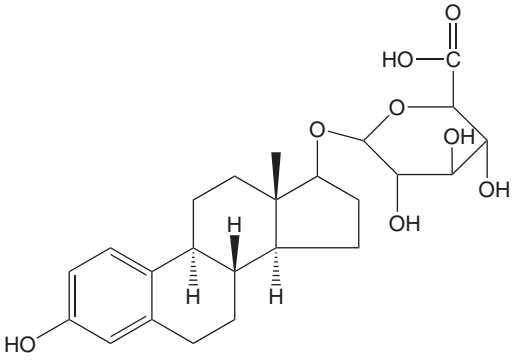
- Representative Chemicals: 17β-Estradiol-3-glucuronide; 17β-Estradiol-17-glucuronide; Estradiol-3,17-disulfate; Estradiol-3,17-diglucuronide; Estrone-3-sulfate
- Chemical Abstracts Service Registry Numbers: 17β-Estradiol-3-glucuronide (CAS 15270-30-1); Estradiol-17-glucuronide (CAS 1806-98-0); Estradiol-3,17-disulfate (CAS 17046-60-5); Estradiol-3,17-diglucuronide; Estrone-3-sulfate (CAS 481-97-0)
- Synonyms:
  - 17β-Estradiol-3-glucuronide: 1,2,5(10)-Estratrien-3,17β-diol 3-glucosiduronate;
  - Estradiol-17-glucuronide: 1,2,5(10)-Estratrien-3,17β-diol 17-glucosiduronate;
  - Estradiol-3,17-disulfate: 1,3,5(10)-Estratrien-3,17b-diol disulfate;
  - Estradiol-3,17-diglucuronide: 1,3,5(10)-Estratrien-3,17β-diol diglucosiduronate; and
  - Estrone-3-sulfate: 1,3,5(10)-Estratriene-3-ol-17-one sulfate.
- Chemical/Pharmaceutical/Other Class: Steroid hormones
- Chemical Formulas:
  - 17β-Estradiol-3-glucuronide: $C_{24}H_{32}O_8$;
  - Estradiol-3-glucuronide sodium salt: $C_{24}H_{31}NaO_8$;

- Estradiol-17-glucuronide: $C_{24}H_{32}O_8$;
- Estradiol-3,17-disulfate: $C_{18}H_{22}O_8S_2$;
- Estradiol-3,17-diglucuronide: $C_{30}H_{40}O_{14}$; and
- Estrone-3-sulfate: $C_{18}H_{22}O_5S$.

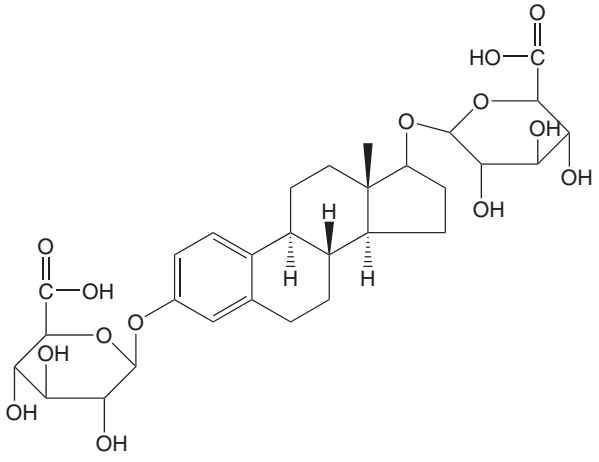
- CHEMICAL STRUCTURES:



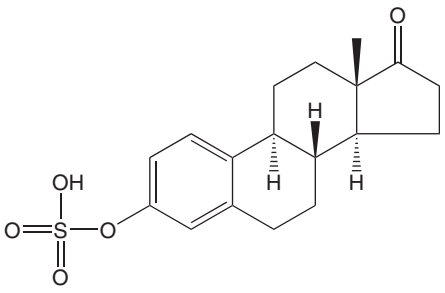
Estradiol-3-glucuronide



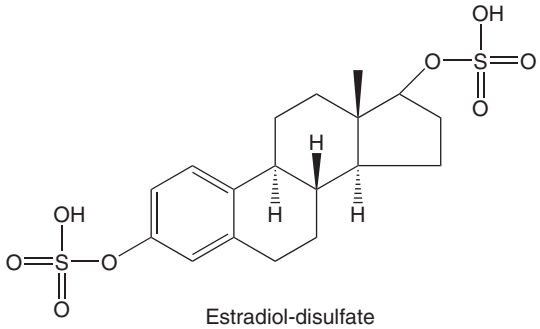
Estradiol-17-glucuronide



Estradiol-3,17-diglucuronide



Estrone-3-sulfate



Estradiol-disulfate

Uses

Estrogens have been used for many therapeutic purposes, including as a contraceptive when in conjunction with a progestin. Naturally occurring estrogens, including those of equine origin (Premarin™), are also employed in hormone replacement therapy to prevent osteoporosis, hot flushes, sweating, atrophic vaginitis and provide some protection against cardiovascular disease by increasing HDL and decreasing LDL cholesterol levels in blood. Estrogens have been used in the treatment of developmental delays or hypogonadism, as well as of some estrogen-dependent neoplasms in women, such as breast carcinomas, and prostate cancer in men in the past. An increasing number of cosmetic creams and preparations contain micronized natural estrogens. Certain types of estrogenic substance (e.g., mycoestrogens) have been used in animal feeds to increase muscle mass in livestock for greater meat production.

Background Information

In the early 1920s, the studies of Allen and the team of Long and Evans demonstrated that removal of

ovaries from prepubertal rodents terminated development of the uterus, vagina, and mammary gland. Following this observation, the influence of administered estrogen on stratification and subsequent quantification of vaginal epithelium was developed as a highly sensitive measure of female sex hormone action. From conception and the earliest stages of embryonic development, through adolescence, to other stages of growth and aging, hormones play a vital role in human life. Estrogens are essential for differentiation, development, and functioning of many target organs in women; however, recent evidence indicates that female sex hormones are also important in male growth, particularly of bone and related tissues. Cardiovascular development and health are also influenced by estrogen in males and females.

At various stages of a woman's reproductive cycle, different types of estrogens are synthesized in distinct organ sites, beginning with the formation of estradiol-17β in the ovary. However in pregnancy, the fetal–placental unit produces the less potent substance, estriol, as a major estrogen. After menopause, the androgens, androstenedione, and dehydroepiandrosterone, are synthesized by the adrenal glands, and serve as precursors that are converted to estrone in peripheral tissues by the action of the enzyme aromatase.

Throughout a woman's life, the naturally occurring estrogens are conjugated with either sulfate or glucuronide (see Structures), which increases their solubility for excretion in urine. These conjugates are weakly estrogenic. Interestingly, a preparation of similar compounds from pregnant mares' urine called Premarin® is used in the treatment of perimenopausal symptoms. Other naturally occurring estrogens in humans include the catechol estrogens discovered in the central nervous system. Although the concentrations of these highly active estrogenic substances are low in relation to those of the ovarian estrogens, they appear to play an important role in the evolvement of sexual behavior and possibly in the development of cancer.

Physiological Roles and Levels

Estrogens provoke their characteristic responses in hormone-target tissues such as breast and uterus by first associating with specialized intracellular proteins called estrogen receptors. These receptor molecules reside in nuclei and bind estrogen with high affinity and specificity. Upon activation, they associate with defined sequences called estrogen response elements located in the 5′-flanking regions of responsive genes and enhance transcription. The estrogen receptor protein is a cellular prerequisite for response to an estrogen or its mimic. If these receptor proteins are not expressed in a target cell or if their structures are severely altered due to gene mutations, female sex hormones will be unable to promote developmental responses required for normal reproductive processes. Two types of estrogen receptors, α and β, have been described.

Although many of the steps involved in the physiological roles of estrogen are well understood, current investigations are directed in diverse areas such as (1) the development of therapeutic estrogen mimics for management of osteoporosis, cardiovascular disease and cancer and (2) a greater understanding of the molecular basis by which certain environmental substances may disrupt normal hormone response mechanisms (so-called endocrine disruptor compounds).

Premenopausal, nonpregnant women produce 100–300 μg of estradiol-17β and 100–200 μg of estrone per day, while postmenopausal women exhibit diurnal fluctuations in estradiol-17β blood levels. Blood levels of estradiol-17β in prepubescent females are 4–12 pg ml^{-1} while those of women in the early follicular phase of the menstrual cycle range from 30 to 100 pg ml^{-1}, the late follicular phase the levels are 100–400 pg ml^{-1} estradiol with concentrations of 50–150 pg ml^{-1} luteal phase. Although postmenopausal women exhibit circulating levels of 5–18 pg ml^{-1} of estradiol, the principal circulating estrogen is estrone formed in peripheral adipose tissues. Prepubertal males have 2–8 pg ml^{-1} circulating estradiol, while adult males exhibit blood levels of 10–60 pg ml^{-1} estradiol.

The excretion profile of estrogens in menstruating women with normal cycles is reflected in either plasma estradiol-17β or urinary levels. However, with clinical problems such as polycystic ovarian disease, the extraovarian production of estrogens is best evaluated using urine specimens.

Exposure Routes and Pathways

In most mammals, estrogens (female sex steroid hormones) are synthesized from cholesterol using the parent ring structure, cyclopentanoperhydrophenanthrene of the estrane series. The steroidogenic pathway includes the production of the androgenic precursors dehydroepiandrosterone and androstenedione, the latter of which is converted to testosterone, then to estradiol-17β. This requires aromatization of these andogenic precursors by an aromatase enzyme complex. The major source of estrogen in postmenopausal women is the conversion of androstenedione to estrone by aromatase activity

in adipose tissue, liver, and skin. The A-ring is aromatic and *cis–trans* isomerism is not possible at carbon 5 and carbon 10. Natural estrogens are produced in the ovaries, placenta, and in small amounts in the central nervous system of mammals, affecting the reproductive system and secondary sexual characteristics, as well as the health of bone and vascular systems.

Naturally occurring or synthetic estrogens are prescribed to many women for use as oral contraceptives or hormone replacement therapy. Oral contraceptives (birth-control pills) are used to prevent pregnancy. Estrogen and progestin are two female sex hormones. Combinations of estrogen and progestin work by preventing the release of eggs from the ovaries (ovulation) and changing the cervical mucus and the lining of the uterus. Oral contraceptives are a very effective method of birth control, but they do not prevent the spread of AIDS and other sexually transmitted diseases. Plant estrogens (phytoestrogens) are also ingested at relatively low levels with certain foods such as soy. Additional environmental estrogens (xenoestrogens) are found in various pesticides and herbicides, as well as industrial pollutants.

Toxicokinetics

Ovarian thecal cells are stimulated by LH to increase the conversion of cholesterol to androstenedione. The enzyme aromatase catalyzes the conversion of androstenedione to estrone and testosterone to estradiol-17β, which is then released into the bloodstream where it is bound to the serum proteins, sex-hormone-binding globulin (SHBG, TeBG), and albumin. In cycling females, ovarian secretion rates of estrogens reach a peak in the late follicular phase. In the liver, estrogens are conjugated to sulfate or glucuronate to make them more water-soluble for urinary excretion.

Mechanism of Toxicity

In vivo, estrogen suppresses ovulation by feedback inhibition at the hypothalamus and pituitary. Additionally, estrogens and progestins induce cervical mucosa thickening altering the success of implantation. Abnormal levels of estrogens binding to the estrogen receptors can lead to an increased transcription of target genes, such as that for progestin receptor. This increase in target gene products can lead to symptoms associated with overexposure to estrogens (e.g., feminization). The distribution of estrogen receptors in organs throughout the body in the reproductive tract, neuroendocrine system, and visceral organs clearly suggest the broad possibilities for altering physiologic responses by hyper-estrogenization or administration/consumption of estrogen mimics.

Toxicity (or Exposure)

Accumulating evidence indicates major roles for estrogens either in diminished or elevated levels in a wide variety of human diseases, such as cardiovascular disease, breast carcinoma, and osteoporosis. For every 10 000 individuals, the average annual mortality of women ages 65–74 due to cardiovascular disease is 59, to breast carcinoma is 10, and to osteoporotic hip fractures is six. The risks associated with estrogen use include carcinoma of the uterus and breast, gallbladder disease, and abnormal blood clotting. Some of the side effects of overexposure to estrogens include nausea, vomiting, migraine headaches, and the exacerbation of endometriosis. Other clinical conditions associated with estrogen in balance include anovulation, hiroutism, and primary amenorrhea. In addition, estrogens and estrogen mimics can interfere with normal embryonic development.

In Vitro Toxicity Data

The National Institute of Environmental Health Sciences was directed by Public Law 103-43 to develop and validate alternative methods for acute and chronic toxicity testing. To implement this, they established an Interagency Coordinating Committee on the Validation of Alternative Methods in 1997.

Relative binding affinities for the estrogen receptor vary considerably based on the source of the estrogen receptor. Generally, if the RBA for estradiol-17β is set at 100, that of estrone is 15–60 and that of estriol is 0.2–30.

Environmental Fate

Both natural estrogens, their conjugates and those used as therapeutics result in their release into the wastewater supply due to it being excreted from the body in urine. Although most wastewater is highly filtered before it is released into the water supply, some estrogens and their mimics are not completely removed. Estrogenic compounds are also present in various pesticides and herbicides. Increased use of these chemicals and xenoestrogens can lead to an increased amount of estrogens present in the crops themselves as well as in the ground water supply.

Ecotoxicology

Reproductive and developmental abnormalities in certain wildlife and the occurrence of compounds which mimic hormones appear to be related. For example, between 1995 and 1997 more than half of the counties in Minnesota reported the presence of malformed frogs in sites with no prior history of mutations. Using a *Xenopus* (FETAX) assay and an evanescent field fluorometric biosensor, substances exhibiting estrogenic activity were detected in pond water samples from which frogs with malformations were collected. Furthermore, downstream of pulp paper mills, the sex ratio of certain fish was altered, presumably due to released agents. Although these compounds are unlikely to be of human origin, they appear to be exhibiting estrogen mimicry.

See also: Estrogens II: Catechol Estrogens; Estrogens III: Phytoestrogens and Mycoestrogens; Estrogens IV: Estrogen-Like Pharmaceuticals; Estrogens V: Xenoestrogens.

Further Reading

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Erb J, *et al.* (2001) Data from an estrogen receptor-based biosensor correlates with evidence of frog malformation and demonstrates a differential response of hERα and β to beneficial and harmful estrogenic compounds. In: *Proceedings of the 2nd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water*, pp. 203–217. Westerville, OH: The National Ground Water Association.

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Smith E, *et al.* (1994) Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *New England Journal of Medicine* 331: 1056–1061.

Wittliff J and Raffelsberger W (1995) Mechanisms of signal transduction: Sex hormones, their receptors and clinical utility. *Journal of Clinical Ligand Assay* 18: 211–235.

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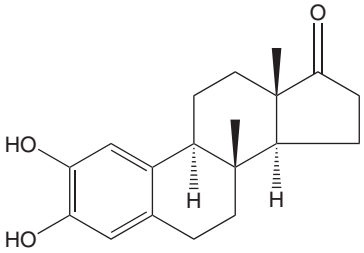
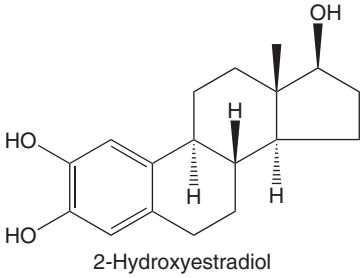
Estrogens II: Catechol Estrogens

James L Wittliff, Sarah A Andres, and D Alan Kerr II

- Representative Chemicals: 2-Hydroxyestrone; 2-Hydroxyestradiol; 4-Hydroxyestrone; 2-Methoxyestrone; 2-Methoxyestriol
- Chemical Abstracts Service Registry Numbers: CAS 362-06-1 (2-Hydroxyestrone); CAS 362-05-0 (2-Hydroxyestradiol); CAS 3131-23-5 (4-Hydroxyestrone); CAS 362-08-3 (2-Methoxyestrone); CAS 1236-72-2 (2-Methoxyestriol)
- Synonyms:
  - 2-Hydroxyestrone: 1,3,5(10)-estratrien-2,3-diol-17-one
  - 2-Hydroxyestradiol: 1,3,5(10)-estratriene-2,3, 17β –triol
  - 4-Hydroxyestrone: 1,3,5(10)-estratrien-3,4-diol-17-one
  - 2-Methoxyestrone: 1,3,5(10)-estratrien-2,3-diol-17-one 2-methyl ether
  - 2-Methoxyestriol: 1,3,5(10)-estratrien-3,16α, 17β-triol 2-methyl ether
- Chemical/Pharmaceutical/Other Class: Steroid hormones
- Chemical Formulas:
  - 2-Hydroxyestrone: $C_{18}H_{22}O_3$
  - 2-Hydroxyestradiol: $C_{18}H_{24}O_3$
  - 4-Hydroxyestrone: $C_{18}H_{22}O_3$
  - 2-Methoxyestrone: $C_{19}H_{24}O_3$
  - 2-Methoxyestriol: $C_{19}H_{26}O_4$
- Chemical Structures:



2-Hydroxyestrone



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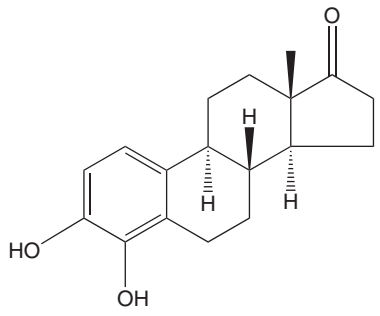
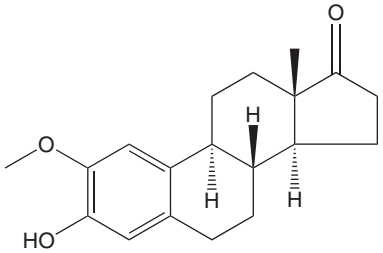
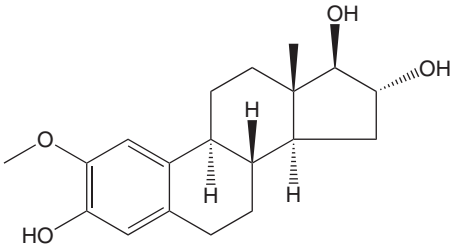


4-Hydroxyestrone



2-Methoxyestrone



2-Methoxyestriol

Uses

Although the majority of studies on catechol estrogens have focused on their activities *in vivo*, the therapeutic potential of the methoxyestrogens have been explored with preliminary results suggesting an antiproliferative effect that appears to involve disruption of microtubule function, induction of apoptosis and inhibition of angiogenesis. Catechol estrogens act as potent inhibitors of the methylation of catecholamines apparently by competitive binding to catechol-*O*-methyltransferase (COMT). Furthermore, 2- and 4-hydroxy catechol estrogens are readily *O*-methylated by COMT. It is suggested that catechol estrogens influence the secretion of luteinizing hormone (LH) and prolactin in rodents and man, as well as sexual behavior in lower mammals.

Background Information

Existence of catechol estrogens was postulated as early as the late 1930s from observations such as that made by Dingemanse and Laqueur who reported the rapid disappearance of estrogen biologic activity *in vivo*. In 1940, Westerfield suggested this resulted from the hydroxylation of the aromatic A ring of the estrogen cyclopentanoperhydrophenanthrene nucleus which formed an *O*-dihydroxyphenol (a catechol). Catechol estrogens are derivatives of the parent estrane series. Chemical synthesis was completed in the 1950s allowing the first studies of catechol pharmacology.

Catechol estrogens are formed from the naturally occurring estrogens (estradiol-17β and estrone) in the central nervous system (e.g., hypothalamus and cerebral cortex) and the anterior pituitary and to a lesser extent in other organs. 17β-Oxidoreductase and cytochrome P450 (CYP1A1 and CYP1B1) are enzymes that convert estrogens to catechol metabolites.

Exposure Routes and Pathways

Various catechol estrogens are secreted in high amounts in urine due to formation in measurable quantities in different organs, such as the adrenal, heart, hypothalamus, kidney, liver, pituitary gland, placenta, prostate, and testes, implying the presence of both 2-hydroxylase and 4-hydroxylase. Estrone is metabolized by two alternative pathways of which the 2-hydroxylation pathway leads to the formation of the catechol estrogens, primarily 2-hydroxyestradiol, 2-hydroxyestrone, 2-hydroxyestriol, and their corresponding methoxy derivatives. The 16α-hydroxylation pathway leads predominantly to estriol. Direction of estradiol-17β metabolism is dependent upon the pathophysiologic state of the individual. Although catechol estrogens may play an antiestrogen role under some circumstances, they evoke a variety of pharmacologic activities.

Physiological Roles and Levels

Because catechol estrogens are highly unstable in solution, being easily decomposed by oxidation especially under alkaline conditions, accurate measurements in sera, urine and tissue extracts have been difficult. In the second half of human pregnancy, concentrations of 2-hydroxyestrone range from 110 to 2100 µg per 24 h, those of 2-hydroxyestradiol are in the range 20–180 µg per 24 h, and those of 2-hydroxyestriol are in the range 35–240 µg per 24 h. Levels of catechol estrogens in post-menopausal women, children, and men are below that required for conventional assays and more sensitive procedures such as a double isotope derivative method based on enzymatic methylation of the catechol estrogen must be employed.

Although the half-lives of catechol estrogens in sera appear to be too short (less than a minute) for consideration as circulating hormones, it appears that they are locally active in certain tissues. It is known that they exhibit a potent influence on gonadatropins in that they induce ovulation and an LH surge in immature rats. Neurophysiologic data suggest that 2- and 4-hydroxylated estrogens also play an important role in the activation of lordosis behavior in rats.

Binding Affinities

Although a wide variety of tissues contain estrogen receptors, it has been proposed that there are neuronal cell receptors that bind catechol estrogens, allowing them to function as neurotransmitters. Affinities of catechol estrogens are highly dependent on the source of the receptor proteins. Relative binding affinities (RBAs) of catechol estrogens compared to that of estradiol-17β (RBA of 100) were 100–150 for 2-hydroxyestradiol and 4-hydroxyestradiol, and 0.1–0.7 for 4-methoxyestradiol, 2-methoxyestrone and 2-methoxyestradiol using human estrogen receptor. Using rat uterine estrogen receptors, 4-hydroxyestradiol had an RBA of 45, 2-hydroxyestradiol of 24, 4-hydroxyestrone of 11, and 2-hydroxyestrone of 2, while 4-methoxyestradiol and 2-methoxyestrone and 2-methoxyestradiol were less than 0.1–1.

There is growing evidence that catechol estrogens bind to membrane-associated receptors in responsive tissues. Investigations suggest that catechol estrogens may exhibit competitive inhibition of catecholaminergic agonists and antagonists binding to dopaminergic and noradrenergic receptors in brain tissues. Other studies suggest catechol estrogens react directly with specific estrogen receptors. There is also evidence that catechol estrogens associate with sex-hormone binding globulin (SHBG, TeBG) with relative binding affinities of 200 for 2-hydroxyestradiol, 75 for 2-hydroxyestrone, and 1 for 2-hydroxyestradiol compared to testosterone.

Toxicokinetics

Catechol estrogens are metabolized by three principal reversible reactions including methylation and demethylation, catalyzed by the enzyme COMT, and conjugation with either glucuronic or sulfuric acid (see structures). Two irreversible reactions involved in metabolism to products with diminished activities, include additional hydroxylations leading to tetrahydroxylated, highly water-soluble compounds and the irreversible binding to amino acids and proteins, including the formation of thioesters.

Mechanism of Toxicity

Carcinogenesis induced by estrogens has been proposed to be mediated by activated catechol metabolites, which are known to react covalently with protein and nucleic acids. Although the reactive 16α-hydroxy, 2-hydroxy and 4-hydroxy estrogens are inactivated by COMT to species that are excreted, these compounds can also be oxidized to semiquinone and *o*-metabolites, in particular estrogen-3,4-quinone which depurinate DNA. Studies have now shown that the apurinic region of the DNA sequence is often left unchanged by DNA repair enzymes. Mutagenic potential of both oxidative activities and estrogen DNA adducts have been implied. Results such as these suggest the complexity of the mechanism of estrogen carcinogenesis and assessing the risks of estrogen therapy.

Toxicity (or Exposure)

The phenolic A-ring and the oxygen function at C-17 are essential for biological activity and substitutions at other positions in the molecule reduce their estrogenicity (feminizing potency). Thus, 2-methoxyestrone and 2-methoxyestradiol exhibit very little activity. Actions of catechol estrogens include (1) 2-hydroxyestradiol appears to interact with dopamine receptor, (2) interference of catecholamine synthesis at high local concentrations of the compounds, (3) inhibition of catecholamine degradation via competitive inhibition of COMT, and (4) ability to bind to estrogen receptors in brain and act either as an agonist or antagonist depending upon the pathway.

Chronic alcoholism is associated with alterations with sex-steroid hormone metabolism and clearance. Symptoms of hyperestrogenism in males include gynecomastia, palmar erythema, and spider angiomata. Furthermore, catechol estrogen metabolites are genotoxic in that they appear to cause DNA damage. Metabolites of equine estrogens, such as those administered in therapeutics such as Premarin™, have been implicated in breast cancer development by associating with the estrogen receptor, which stimulates cell proliferation and gene expression.

In Vitro Toxicity Data

Catechol estrogens are potent inhibitors of tyrosine kinase activity *in vitro*. High concentrations of catechol estrogens are necessary to cause measurable inhibition, but the physiologic role of this interaction remains uncertain. Catechol estrogens have also been shown to be potent inhibitors of the COMT-mediated inactivation of catecholamines.

Environmental Fate

Because of the chemical instability of catechol estrogens, it is unlikely they exist in an active form after excretion. However, this has not been evaluated conclusively.

See also: Estrogens I: Estrogens and Their Conjugates; Estrogens III: Phytoestrogens and Mycoestrogens; Estrogens IV: Estrogen-Like Pharmaceuticals; Estrogens V: Xenoestrogens.

Further Reading

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Merriam G and Lipsett M (eds.) (1993) *Catechol Estrogens*. New York: Lippincott Williams and Wilkins.

Van Aswegen C, Purdy R, and Wittliff J (1989) Binding of 2-hydroxyestradiol and 4-hydroxyestradiol to estrogen receptors from human breast cancers. *Journal of Steroid Biochemistry* 32: 485–492.

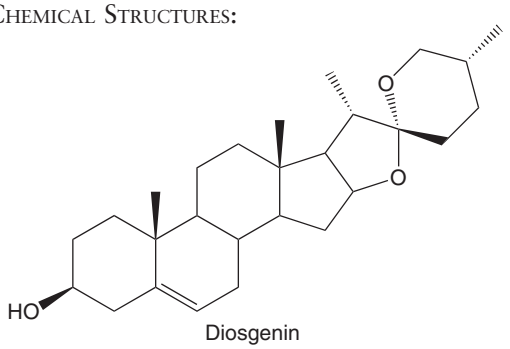
Relevant Website

http://iccvam.niehs.nih.gov – ICCVAM/NICEATM Final Report, Expert Panel Evaluation of the Validation Status of *In Vitro* Test Methods for Detecting Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays, 2002.

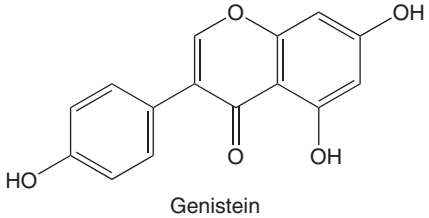
Estrogens III: Phytoestrogens and Mycoestrogens

James L Wittliff, Sarah A Andres, and D Alan Kerr II

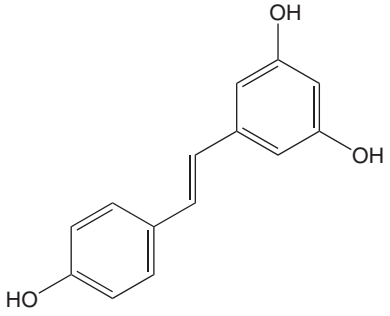
- Representative Chemicals: Diosgenin; Genistein; Resveratrol; Zearalenone
- Chemical Abstracts Service Registry Numbers: Diosgenin (CAS 512-04-9); Genistein (CAS 446-72-0); Resveratrol (CAS 501-36-0); Zearalenone (CAS 17924-92-4)
- Synonyms:
  - Diosgenin: (25*R*)-Spirost-5-en-3beta-ol
  - Genistein: 4′,5,7-Trihydroxyisoflavone
  - Resveratrol: *t*-3,4′,5-Trihydroxystilbene
  - Zearalenone: (*E*)-3,4,5,6,7,8,9,10-Octahydro-14,16-dihydroxy-3-methyl-7-oxo-1*H*-2-benzoxacyclotetradecin-1-one
- Chemical/Pharmaceutical/Other Class: Coumestans; Dihydroxychalcones; Isoflavones and Resorcylic Acid Lactones
- Chemical Formulas: Diosgenin, $C_{27}H_{42}O_3$; Genistein, $C_{15}H_{10}O_5$; Resveratrol, $C_{14}H_{12}O_3$; Zearalenone: $C_{18}H_{22}O_5$
- Chemical Structures:



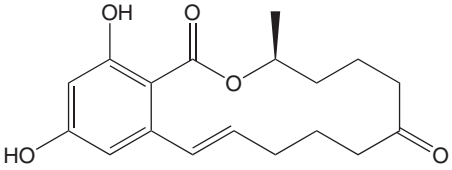
Diosgenin



Genistein



Resveratrol



Zearalenone

Uses

In general, xenoestrogens in the environment are separated into those occurring naturally (produced by either plants or fungi) and those produced commercially (e.g., insecticides, herbicides, estrogen-like therapeutics, and industrial by-products). Phytoestrogens and mycoestrogens are found in various

plants, such as soy (genistein), yams (diosgenin) and grapes (resveratrol), and in fungus species such as *Fusarium molds* (zearalenone). The compounds associated with these classes of molecules bind to estrogen receptor proteins with a variety of affinities, sufficient to label them as estrogen mimics. Various compounds are present in common foodstuffs and are ingested by humans and animals on a frequent basis. Preliminary epidemiological studies suggest protective effects of certain isoflavones and to a smaller degree as a result of consuming foodstuffs containing lignans (i.e., cereals, flaxseed, and fruits) in altering the development of osteoporosis, cardiovascular disease, and certain cancers. These investigations, as well as traditional and alternative medicine practices, have elevated the interest in using dietary supplements containing these extracts or the purified compounds as part of a healthy diet and for problems associated with menopause, prevention of bone loss, and cardiovascular disease. Furthermore, certain mycoestrogens (e.g., zearalenone) and chemical derivatives have been used as additives to livestock feeds to increase muscle mass for greater meat production.

Background Information

For decades, the consumption of soybean-derived products by Asians living in China, Japan, and Korea has been thought to be related to their lower incidence of certain cancers and coronary artery disease compared to that of western populations. Observations such as the appearance of higher plasma and urine concentrations of isoflavanoids of individuals eating a largely vegetarian diet compared to that of omnivores suggested a role for phytoestrogens in lowering disease incidence, but the contributions of red meat and fat consumption were unclear in these types of comparisons.

More than 20 different compounds have been identified as either phytoestrogens or mycoestrogens in at least 300 unique plant and fungus species, based on their abilities to either bind to estrogen receptors and/or stimulate estrogen-like activities in cell cultures or *in vivo*. There are numerous chemical classes of phytoestrogens, including coumestans, dihydroxychalcones, isoflavones, prenylated flavonoids, stilbenes, and resorcylic acid lactones. Representative examples are coumestrol for coumestans, phloretin for dihydroxychalcones, genistein for isoflavones, 8-prenylnaringenin for prenylated flavonoids, resveratrol for the stilbenes, and zearalenone for resorcylic acid lactones. A list of representative chemicals is shown below with source and relative binding affinities for the estrogen receptors:

Phytoestrogens and mycoestrogens with suspected endocrine-associated effects

Compound	*Common source*	[a]*RBA for estrogen receptor*
Artemisinin	Wormwood	
Biochanin A	Soy	0.004
Coumestrol	Red clover and alfalfa	0.7–5
Curcumin	Turmeric	
Daidzein	Soy	0.02–1
Diosgenin	Yams	
Diosmin		
Formononetin	Soy	NA
Genistein	Soy	0.5–45
Glycitein	Soy	0.001
Naringenin	Grapefruit and citrus	0.01–0.2
Phloretin		0.07–0.7
Prunetin	Soy	0.0001–0.002
Resveratrol	Muscadine grapes	0.001–0.05
Sarsasapogenin	Sarsaparilla root	
Zearalanone	Fusarium molds	2–15
Zearalenone	Fusarium molds	5–18
α-Zearalanol	Fusarium molds	25–36
α-Zearalenol	Fusarium molds	36–70
β-Sitosterol	Soy	NA
β-Zearalanol	Fusarium molds	0.6–16
β-Zearalenol	Fusarium molds	0.2–23

[a]RBA, relative binding affinity (% of estradiol-17β activity).
Refer to ICCVAM report for most RBAs (see report for various estrogen receptor sources).

Exposure Routes and Pathways

Human and animal exposure to phytoestrogens usually occurs by two routes, ingestion of food products containing the agents or as over-the-counter nutritional supplements. However, a number of cosmetic preparations contain certain of these natural estrogens which may be absorbed through the skin. Medical problems associated with administration of hormone replacement therapy, as described in the report of the Women's Health Initiative, have motivated many women to seek nonmedical means of post-menopausal endocrine replacement, such as phytoestrogen supplements.

Most exposures to mycoestrogens result from consumption of food products that have been contaminated with *Fusarium* molds. Studies of the activities of phytoestrogens clearly indicate that these compounds bind to the estrogen receptor proteins displacing native estradiol-17β with variable affinities. Details of the signaling transduction pathways are poorly understood, but appear to involve alterations in target gene expression.

Toxicokinetics

It is generally accepted that the assessment of actual risks or benefits of phytoestrogens and mycoestrogens

to humans and animals is controversial. The complexity of the estrogen signaling network, involving multiple forms of the receptor proteins and the cross talk with the other pathways (e.g., growth factor-induced), limits our current understanding regarding the mechanisms by which these estrogen mimics act as agonists or antagonists. Furthermore, the biological role for the presence of phytoestrogens in plants or mycoestrogens in fungi is unknown. Although many of these compounds are metabolized in the mammalian gut by enzymes such as β-glucosidases, significant quantities appear in urine and feces. For example, lignans may be metabolized to enterodiol and enterolactone while isoflavones may be metabolized to equol and *O*-desmethylangolensin by intestinal microflora prior to urinary excretion.

Mechanism of Toxicity

Ingestion of phytoestrogens and mycoestrogens may result in alterations in physiological processes controlled by estrogens, since these types of compounds either mimic or compete with natural estrogens for the estrogen receptors with relatively low affinity. While these structurally diverse compounds collectively exhibit estrogen mimicry, they also share common properties such as retention in adipose tissues due to their lipophilicity and ability to cross the placental barrier. These properties, particularly estrogen receptor association, may lead to an up-regulation or down-regulation of expression of target genes (e.g., progestin receptor, prolactin receptor) containing an estrogen response element (ERE) sequence. Since estrogens are essential for normal mammalian differentiation, development and function, primarily in females of the species, interference with or enhancement of related pathways may adversely affect or enhance the health of the host and offspring. For example, some phytoestrogens at concentrations consumed in the American-style diet appear to be associated with a reduced risk of endometrial carcinoma.

Certain phytoestrogens have also been suggested to inhibit enzymes involved in estrogen biosynthesis and metabolism, as well as thyroid biosynthesis. Preliminary studies suggest they inhibit protein kinases and topoisomerases, as well as influence the cell cycle and subsequent proliferation, differentiation and apoptotic pathways. Interest in phytoestrogens, such as isoflavones, has increased since they are reported to exhibit some nonhormonal effects, such as antioxidation.

Chronic Toxicity (or Exposure)

Livestock ingestion of large quantities of phytoestrogens in certain clovers, alfalfas, and in moldy grains adversely affects fertility, leading to concerns that similar effects could occur in humans, particularly with infants fed soy-based formulas. Studies on populations that traditionally consume diets rich in phytoestrogens (i.e., Japanese and Chinese) suggest that they may have a beneficial effect regarding the prevention and development of osteoporosis, cardiovascular disease, and some cancers.

In Vitro Toxicity Data

The National Institute of Environmental Health Sciences was directed by Public Law 103-43 to develop and validate alternative methods for acute and chronic toxicity testing. To implement this, they established an Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in 1997.

In summary, the body of experimental and epidemiological evidence suggesting phytoestrogens may alter human health continues to expand to cover a wide range of exposures. Effects of transgenerational exposure to unrecognized agents that may be present in foodstuffs, drinking water, and other consumables including medications and cosmetics are of particular concern. Using hormone receptor-based technologies (i.e., recombinant human estrogen receptors) and highly purified preparations of these compounds as standards, exposure and risk assessment may be improved for environmental estrogen mimics, and the quantitative analysis of their occurrence in the environment.

Environmental Fate

Similar to the routes of release of mammalian estrogens, ingested phytoestrogens and mycoestrogens may appear in the environment as a result of intestinal metabolism and excretion in urine and feces. In addition decomposition of botanicals containing these compounds may be released in soil and water. Little is known regarding their biotransformation by soil and water organisms. It is generally accepted that the concentrations of these compounds existing free in the environment pose few health concerns.

Exposure Standards and Guidelines

Dietary supplements containing various combinations of phytoestrogens are neither controlled by federal agencies such as the Food and Drug Administration nor have exposure standards and guidelines been established. This is due to the paucity of data regarding their clinical safety and efficacy.

See also: Estrogens I: Estrogens and Their Conjugates; Estrogens II: Catechol Estrogens; Estrogens IV: Estrogen-Like Pharmaceuticals.

Further Reading

Blair R, *et al.* (2000) The estrogen receptor relative binding affinities of 188 natural and xenochemicals: Structural diversity of ligands. *Toxicological Sciences* 54: 138–153.

Davis S, *et al.* (1999) Phytoestrogens in health and disease. *Recent Progress in Hormone Research* 54: 185–210.

Fang H, *et al.* (2001) Structure–activity for a large diverse set of natural, synthetic and environmental estrogens. *Chemical Research in Toxicology* 14: 280–294.

Mäkelä S, *et al.* (1994) Dietary estrogens act through estrogen receptor-mediated processes and show no antiestrogenicity in cultured breast cancer cells. *Environmental Health Perspectives* 102: 572–578.

Ososki A and Kennelly E (2003) Phytoestrogens: A review of the present state of research. *Phytotherapy Research* 17: 845–869.

Wittliff J and Raffelsberger W (1995) Mechanisms of signal transduction: Sex hormones, their receptors, and clinical utility. *Journal of Clinical Ligand Assay* 18: 211–235.

Relevant Websites

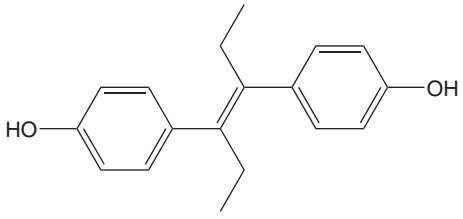
http://e.hormone.tulane.edu – e.hormone website.

http://iccvam.niehs.nih.gov – ICCVAM/NICEATM Final Report, Expert Panel Evaluation of the Validation Status of *In Vitro* Test Methods for Detecting Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays, 2002.

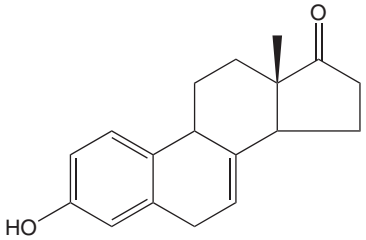
Estrogens IV: Estrogen-Like Pharmaceuticals

James L Wittliff, D Alan Kerr II, and Sarah A Andres

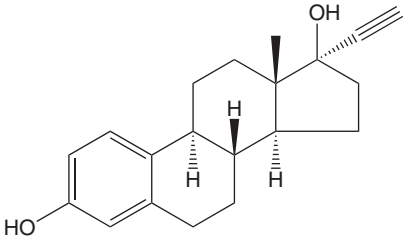
- Representative Chemicals: Diethylstilbestrol; Equilin; Ethynyl estradiol; Raloxifene; Tamoxifen
- Chemical Abstracts Service Registry Numbers: Diethylstilbestrol (CAS 56-53-1); Equilin (CAS 474-86-2); Ethynyl estradiol (CAS 57-63-6); Raloxifene (CAS 84449-90-1); Tamoxifen (CAS 10540-29-1)
- Synonyms:
  - Diethylstilbestrol: 3,4-Bis(4-hydroxyphenyl) hex-3-ene
  - Equilin: 1,3,5(10),7-Estratetraen-3-ol-17-one
  - Ethynyl estradiol: 17α-Ethynyl-1,3,5(10)-estratriene-3,17β-diol
  - Raloxifene: 6-Hydroxy-2-(*p*-hydroxyphenyl)benzo[*b*]thien-3-yl-*p*-(2-piperidinoethoxy)phenyl ketone hydrochloride, Evista™
  - Tamoxifen: (*Z*)-2-[4-(1,2-Diphenyl-1-butenyl) phenoxy]-*N*,*N*-dimethylethanamine, Nolvadex™
- Chemical/Pharmaceutical/Other Class: Steroid hormones
- Chemical Formulas:
  - Diethylstilbestrol: $C_{18}H_{20}O_2$
  - Equilin: $C_{18}H_{20}O_2$
  - Ethynyl estradiol: $C_{20}H_{24}O_2$
  - Raloxifene: $C_{28}H_{27}NO_4S$
  - Tamoxifen: $C_{26}H_{29}NO$
- Chemical Structures:



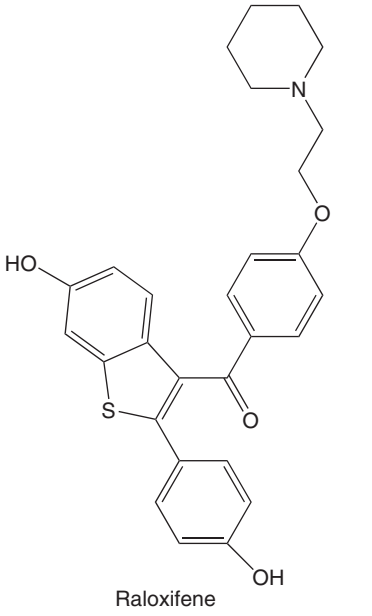
Diethylstilbestrol



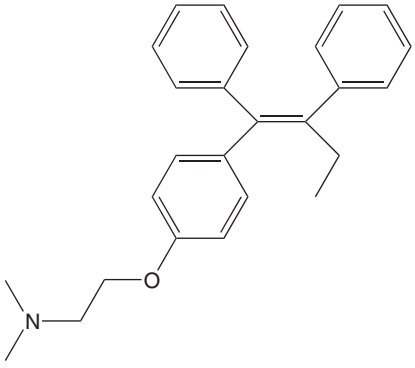
Equilin



Ethynyl estradiol



Raloxifene



Tamoxifen

Uses

Estrogen-like pharmaceuticals have a variety of clinical uses including as fertility therapeutics (clomiphene), contraceptives (ethynyl estradiol), prevention and treatment of osteoporosis (equilin, Raloxifene) and cancer therapeutics (Tamoxifen). These pharmaceuticals have a variety of affinities for estrogen receptor proteins α and β, and may either activate (agonist) or inactivate (antagonist) estrogen receptor-induced signaling pathways. Representative examples of these pharmaceutics with their relative binding affinities (RBAs) for estrogen receptors are listed in **Table 1**; however, the RBA values are highly dependent upon the source of the receptor protein used in the *in vitro* binding assay.

Background Information

Oral contraceptives have been used widely by women since the 1960s and most contraceptive pills contain various ratios of estrogenic and progestomimetic substances. Hormone replacement therapy (HRT) has evolved considerably in the composition and doses utilized in the treatment of postmenopausal women, during the past four decades. While pharmaceutical compounds exhibiting estrogen mimicry are structurally diverse, they share common properties such as their retention in body fat deposits, their ability to cross the placental barrier, their transport in blood usually bound to serum proteins, and their affinity for the estrogen receptor protein.

Table 1 Common estrogen-like pharmaceuticals

Compound	*Clinical use*	**RBA for ER*
Clomiphene	Fertility therapeutic	**0.1–12
Diethylstilbestrol	Sustaining pregnancy (discontinued) and cancer therapeutic (discontinued)	400
Droloxifene	Cancer therapeutic	**0.2–15.2
Ethynyl estradiol	Oral contraceptive and reproductive medicine therapy	100–200
Equilenin	Osteoporosis prevention and therapeutic (HRT)	**8
Equilin	Osteoporosis prevention and therapeutic (HRT)	**24
17α-Dihydroequilenin	Osteoporosis prevention and therapeutic (HRT)	
17β-Dihydroequilenin	Osteoporosis prevention and therapeutic (HRT)	
ICI 164,384	Cancer therapeutic	14.5
ICI 182,780	Cancer therapeutic	37.5
Idoxifene	Cancer therapeutic	
Nafoxidine	Oral contraceptive and cancer therapeutic	0.7
Norethynodrel	Oral contraceptive	0.2
Raloxifene	Osteoporosis prevention and therapeutic (postmenopausal women) and cancer therapeutic (under investigation)	**16–69
Tamoxifen	Cancer therapeutic and cancer chemoprevention	0.06–16
Toremifene	Cancer therapeutic and HRT (under investigation)	1.4

Refer to Blair *et al.* and Fang *et al.* (see the Further Reading section) for RBAs (using rat uterine estrogen receptor); and to the ICCVAM report for RBAs listed with ** (see report for various estrogen receptor sources).
*RBA, relative binding affinity (% of estradiol activity); ER, estrogen receptor.

When they associate with estrogen receptor proteins, they either disrupt native hormone action or communicate activities similar to those of estrogen.

Exposure Routes and Pathways

Estrogen therapeutics, prescribed to patients for cancer treatment, menopausal symptom relief and osteoporosis, are ingested orally. Most estrogen-like substances used in therapy are absorbed easily through the gastrointestinal tract, mucous membranes, and the skin.

Toxicokinetics

Overdoses of either contraceptives or hormone replacement therapeutics are uncommon. A variety of dermatologic effects have been observed including photosensitivity, alopecia, and bullous eruption following oral contraceptive overdoses. Neurologic effects of estrogen-containing contraceptives in the presence of a progestin include increased risk of ischemic stroke in generally healthy postmenopausal and occasional exacerbation of migraine headaches. Well-documented serious hematologic effects include increased risk for venous thromboembolism. The primary mode of clinical management is essentially symptomatic and supportive. The primary concern is the ingestion of large doses of estrogen-like pharmaceuticals by children, although few acutely serious ill effects have been reported.

HRT, which reaches a peak level in 4–5 h after oral absorption, is strongly bound to serum proteins. As certain estrogen-like pharmaceuticals enter breast milk, breast-feeding is not recommended and the use of HRT in pregnancy is also not recommended.

Because of the wide chemical diversity of estrogenic pharmaceutics, it is difficult to categorize the pathways for metabolic conversion and excretion. However, the majority of these reactions occur in the liver where they are inactivated by various hydroxylation and oxidation reactions, although the major pathways involve conjugation and excretion in the urine and feces as sulfates and glucuronates.

Mechanism of Toxicity

These compounds have relatively high affinities for estrogen receptors, which can lead to altered regulation of estrogen-responsive genes, thereby altering the proliferation and differentiation of cells in target organs. Detailed studies of their binding specificity for estrogen receptors alpha and beta suggest alternative mechanisms in different tissues.

Toxicity (or Exposure)

Animal

In both rats and mice, the possible toxic side effects of ethynyl estradiol include convulsions/seizure activity, ataxia, and changes in kidney and bladder function. The oral lethal dose is $950\,mg\,kg^{-1}$ for mice and $960\,mg\,kg^{-1}$ for rats. The intraperitoneal lethal dose is $250\,mg\,kg^{-1}$ for mice and $471\,mg\,kg^{-1}$ for rats.

Human

Since estrogen-like pharmaceuticals exhibit potent activities, many of which are related to naturally occurring estrogens (agonistic), they also exhibit antagonistic activities on a variety of physiologic pathways. Their ability to recognize a diverse group of compounds represents another example of ligand binding promiscuity by the human estrogen receptor proteins. Risks associated with these compounds include endometrial carcinoma, breast carcinoma, gall bladder disease and abnormal blood clotting, as well as a variety of others more specifically related to the agent. Because of the structural diversity of these compounds and their pharmacology, representative examples are described.

Hexestrol and diethylstilbestrol (DES) are synthetic, nonsteroidal estrogens derived from stilbene, which have been used earlier in the treatment of breast cancer in women and prostate carcinoma in men. Furthermore, DES was used in the 1950s and 1960s as treatment of pregnant women who threatened premature delivery. However, a serious medical complication arose in the progeny of these mothers in that daughters were at high risk of developing clear cell adenocarcinoma of the vagina, as well as cervical and uterine deformities. Male offspring of DES-treated mothers also developed genital tract abnormalities. Surprisingly, there appears to be an increase in the incidence of hypospadias in grandsons of DES-treated women. Clinical symptoms of DES administration include arterial and venous thrombosis, fluid retention and nausea in women, as well as gynecomastia and impotence in men.

Currently, ethynyl estradiol represents the most common estrogenic component in combination oral contraceptives (i.e., an estrogen and a progestin). Ethynyl estradiol, a derivative of estradiol-17β with the substitution of the ethynyl group at C-17, is one of the most potent analogs with estrogenic activity equal to or greater than that of the parent compound. Similar to other compounds in this class, ethynyl estradiol is orally activity being rapidly absorbed by the gut mucosa and liver. Detoxification involves

hydroxylation and conjugation to form the glucuronate or sulfate forms prior to excretion in the urine or feces.

Ethynyl estradiol can cause nausea or vomiting, body temperature increase and other menopausal symptoms, and blood clotting. The lowest published toxic dose is $21\,mg\,kg^{-1}$ $(21\,day)^{-1}$ intermittent.

Raloxifene (Evista™) is another example of a selective estrogen receptor modulator (SERM) that belongs to the triphenylethyene-based group of therapeutics. Its primary pharmacologic activity is as an agonist used in the prevention and treatment of osteoporosis with complementary activities on the liver and serum lipid profiles. The SERM increases bone mineral density, decreases total and low-density lipoprotein (LDL) cholesterol while acting as an estrogen antagonist in uterine and breast tissue via interaction with the estrogen receptor. Raloxifene is primarily absorbed upon oral administration and rapidly metabolized by conjugation with glucuronic acid prior to excretion in the urine and feces.

Raloxifene side effects include bloody or cloudy urine; painful urination; pain in chest, arm or leg (rare); coughing blood (rare); sore/dry throat; trouble in swallowing; body aches; cramping; skin rash; vaginal itching; migraine headache (rare) and loss of speech, vision or coordination (rare). There is also an increased risk of blood clot formation (i.e., deep vein thrombosis, pulmonary embolism, and retinal embolism) in patients with a history of these conditions if treated with Raloxifene.

A recent major clinical trial, published by the National Institutes of Health Women's Health Initiative, was terminated in 2002 because an increased risk of invasive breast cancer was observed from HRT with estrogen and progestin. Furthermore, an increased risk of ischemic stroke was noted. Women participating in this study took either a placebo or a combination hormone therapy that contained conjugated equine estrogens and medroxyprogesterone acetate, most commonly prescribed as Premarin™ or Prempro™. These pharmaceutics contain estrogens extracted from pregnant mare's urine, of which almost one-half are equilin and equilenin. The increased risk of breast cancer was first observed after 4 years of HRT; and after 5 years, the risk of breast cancer showed a 26% increase. Although early results suggested a protective effect of HRT in lowering risk of coronary artery disease, later, more thorough studies have revealed an increased risk of heart disease, stroke, and venous thromboembolism. As a result of the Women's Health Initiative, HRT with a conjugated estrogen alone or an estrogen/progestin combination, previously considered first-line therapy for osteoporosis, is now considered a second-line agent, which should be used only when benefits outweigh risks. In general, acute and chronic toxicity of HRT is uncommon.

Tamoxifen, prescribed clinically as Nolvadex™, is an estrogen receptor antagonist used in the treatment of estrogen receptor-positive breast cancer, and to a lesser extent in other cancers of the female reproductive system. A therapeutic dose of Tamoxifen is in the range of $0.5–0.6\,mg\,l^{-1}$, although the concentration associated with acute toxicity is unknown. Historic studies from the National Surgical Adjuvant Breast & Bowel Project established Tamoxifen as a chemopreventive therapy for breast cancer in women at high risk for the carcinoma. One of the significant side effects of Tamoxifen therapy is an increased risk of endometrial carcinoma.

Tamoxifen side effects include headaches, depression, stomach irritation, vaginal discharge, constipation, shortness of breathe (rare), loss of vision (rare), and swelling of the hands and lower legs.

Clomiphene citrate (Clomid™ or Serophene™) is a fertility drug used in the treatment of ovulatory failure in women desiring pregnancy. Clomiphene, a nonsteroidal antiestrogen, is a triphenylethylene-derived drug related to Tamoxifen and Raloxifene. Representative clinical doses of $50\,mg\,day^{-1}$ for 5 days (day 3–day 7 of the cycle), administered orally, are used to induce ovulation by acting upon the hypothalamus. Women with either abnormal or irregular uterine bleeding should not be treated with Clomiphene unless the absence of endometrial or cervical abnormalities has been confirmed. Furthermore, women with either liver disease or ovarian cysts should not be given this therapeutic. Although there is a paucity of information regarding fetal damage, estrogen-like pharmaceuticals should not be administered to women who may be pregnant.

In Vitro Toxicity Data

The National Institute of Environmental Health Sciences was directed by Public Law 103-43 to develop and validate alternative methods for acute and chronic toxicity testing. To implement this, they established an Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in 1997. RBAs for the estrogen receptor vary considerably based on the source of the estrogen receptor proteins and isoforms (α or β). Generally if the RBA for estradiol-17β is set at 100, that of estrone is 15–60 and that of estriol is 0.2–30.

Clinical Management

Fluid replacement may be necessary after nausea/vomiting. There are few published results indicating

benefit of gastric lavage, in the case of a massive overdosage. Therapy to enhance elimination will most likely not be effective due to the tissue distribution of these compounds.

Environmental Fate

Wastewater from pharmaceutical industries producing these therapeutics may allow introduction of these endocrine disruptors into the ecosystem if proper filtration is not employed. Moreover, the conjugated products excreted in urine and feces from individuals receiving these pharmaceuticals also can be introduced into the wastewater supply. There are few published results regarding bioaccumulation and biotransformation of estrogen-like pharmaceutics released into the ecosystem.

Exposure Standards and Guidelines

For clinical use, 20–40 mg day^{-1} of Tamoxifen is a safe range for patients, as is 60 mg day^{-1} Raloxifene. Skin exposure is not dangerous, though some estrogens can be absorbed through the skin. When handling these chemicals in powder form, gloves, eyewear, and facemasks should be worn to avoid contact.

See also: Estrogens I: Estrogens and Their Conjugates; Estrogens II: Catechol Estrogens; Estrogens III: Phytoestrogens and Mycoestrogens; Estrogens V: Xenoestrogens.

Further Reading

Blair RM, Fang H, Branham WS, *et al.* (2000) The estrogen receptor relative binding affinities of 188 natural and xeno chemicals: Structural diversity of ligands. *Toxicological Sciences* 54: 138–153.

Dart RC (ed.) (2004) *Medical Toxicology.* Philadelphia: Lippincott Williams and Wilkins.

Fang H, Tong W, Shi LM, *et al.* (2001) Structure–activity relationships for a large diverse set of natural, synthetic and environmental estrogens. *Chemical Research in Toxicology* 14: 280–294.

Henningsen B, Linder F, and Steichele C (eds.) (1980) *Recent Results in Cancer Research, Endocrine Treatment of Breast Cancer: A New Approach*. Berlin: Springer-Verlag.

Jordan VC, *et al.* (1993) Update: breast cancer treatment and prevention with antiestrogens in the 1990s. *Endocrine Reviews* 1: 82.

Relevant Websites

http://e.hormone.tulane.edu – e.hormone website.

http://iccvam.niehs.nih.gov – ICCVAM/NICEATM Final Report, Expert Panel Evaluation of the Validation Status of *In Vitro* Test Methods for Detecting Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays, 2002.

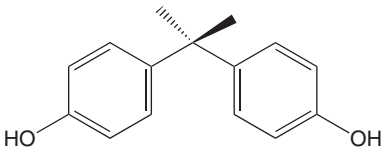
http://www.whi.org – Women's Health Initiative.

Estrogens V: Xenoestrogens

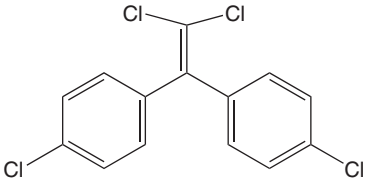
James L Wittliff, D Alan Kerr II, and Sarah A Andres

- Representative Chemicals: Bisphenol A; DDE; DDT; Dibutyl phthalate; Methoxychlor; Simazine
- Chemical Abstracts Service Registry Numbers: Bisphenol A (CAS 80-05-7); DDE (CAS 72-55-9); DDT (CAS 50-29-3); Dibutyl phthalate (CAS 84-74-2); Methoxychlor (CAS 72-43-5); Simazine (CAS 122-34-9)
- Synonyms:
  - Bisphenol A: Bis(4-hydroxyphenyl)propane
  - DDE: 2,2-Bis(4-chlorophenyl)-1,1-dichloro ethylene
  - DDT: 1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethane
  - Dibutyl phthalate: 1,2-Benzenedicarboxylic acid dibutyl ester
  - Methoxychlor: 2,2,2-Trichloro-1,1-bis(4-methoxyphenyl)ethane
  - Simazine: 1-Chloro-3,5-bisethylamino-2,4,6-triazine
- Chemical/Pharmaceutical/Other Class: Steroid hormones
- Chemical Formulas:
  - Bisphenol A: $C_{15}H_{16}O_2$
  - DDE: $C_{14}H_8Cl_4$
  - DDT: $C_{14}H_9Cl_5$
  - Dibutyl phthalate: $C_{16}H_{22}O_4$
  - Methoxychlor: $C_{16}H_{15}Cl_3O_2$
  - Simazine: $C_7H_{12}ClN_5$

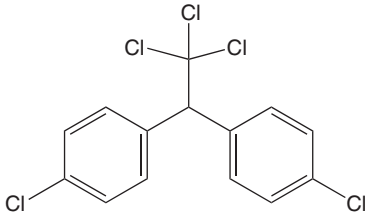
- Chemical Structures:



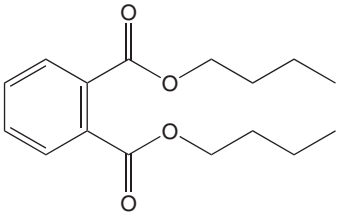
Bisphenol A



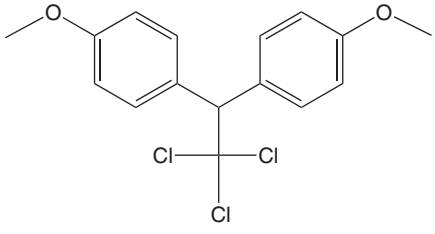
DDE



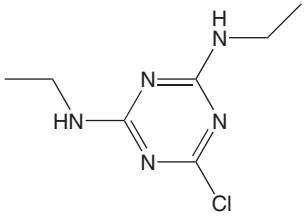
DDT



Dibutyl phthalate



Methoxychlor



Simazine

Uses

Xenoestrogens, exhibiting a wide molecular diversity, are found in a number of cosmetic products, such as plasticizers, perfume fixatives, and solvents (e.g., dibutyl phthalate), industrial chemicals and pollutants such as insecticides (e.g., methoxychlor, DDT, and DDE), epoxy resins, and polycarbonate (e.g., bisphenol A), and herbicides (e.g., simazine). This group of chemicals has been classified as environmental endocrine disruptor compounds (EDCs), defined as exogenous agents that interfere with the synthesis, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior. A list of representative chemicals is shown in **Table 1** based on commercial usage.

Background Information

While those compounds exhibiting estrogen mimicry are structurally diverse, they share common properties such as retention in body fat deposits (highly lipophilic), ability to cross the placental barrier, transport in blood usually unbound to specialized serum proteins (e.g., steroid hormone binding globulin, SHBG/TeBG), and their affinity for the estrogen receptor protein. If the environmental compound impersonates estrogen sufficiently, it associates with the estrogen receptor protein and either disrupts the action of the native hormone or communicates activities similar to estrogen (i.e., antagonistic or agonistic activities). In addition to the phenotypic expression of gender, estrogens and their mimics may influence development and physiological processes in many organs of the body, particularly the reproductive tract, as well as the central nervous system and skeleton. It is obvious that fragile, biological events occurring during ovulation, pregnancy, fetal development, and lactation could easily be influenced by EDCs, which mimic naturally occurring hormones.

With a variety of sensitive, rapid assays, EDCs now may be recognized by estrogen receptor proteins. The range of techniques available includes:

1. cell-free preparations of receptor proteins using both ligand titration and ligand competition assays;
2. antibody-based assays in enzyme immunoassay (EIA) or ELISA formats;
3. electrophoretic mobility shift assays, conducted in the presence (supershift) and absence of monoclonal antibodies to estrogen receptors; and

Table 1 Examples of environmental chemicals with suspected endocrine-disrupting effects

Enviromental Chemical	*RBA for ER**
Pesticides	
Herbicides	
2,4-D	
2,4,5-T	
Alachlor	N/A
Amitrole	
Atrazine	0.0003**
Metribuzin	
Nitrofen	
Simazine	N/A
Trifluralin	
Fungicides	
Benomyl	
Hexachlorobenzene	
Mancozeb	
Maneb	
Metiram-complex	
Tributyl tin	
Zineb	
Ziram	
Insecticides	
β-HCH	
Carbaryl	N/A
Chlordane	N/A
Dicofol	
Dieldrin	0.0005**
DDT and metabolites	0.001
α-Endosulfan	0.012**
Heptachlor and H-epoxide	N/A
Lindane (γ-HCH)	
Methomyl	
Methoxychlor	0.01–0.1 (0.001 – Blair)**
Mirex	N/A
Oxychlordane	
Parathion	
Synthetic pyrethroids	
Toxaphene	0.00032**
Transnonachlor	
Nematocides	
Aldicarb	
DBCP	
Industrial chemicals	
Bisphenol A	0.008
Cadmium	
Dioxin (2,3,7,8-TCDD)	
Lead	
Mercury	
PBBs	
PCBs	0.0002–0.228
Pentachlorophenol (PCP)	
Penta- to nonylphenols	0.019–0.037 (4-nonylphenol)
Phthalates (dimethyl, diethyl, and dibutyl)	
Styrenes	

Refer to Blair *et al.* and Fang *et al.* (see Further Reading Section) for RBAs (using rat uterine estrogen receptor) and to the ICCVAM report for RBAs listed with

*RBA = relative binding affinity (% of estradiol activity), ER = estrogen receptor.

**See report for various estrogen receptor sources.

4. cell-based bioassays using either intact mammalian target cells or yeast cells containing a two-plasmid system with a reporter gene.

Additionally, certain investigations are focused on differential recognition of EDCs by estrogen receptor isoforms separated by high-performance liquid chromatography.

In summary, the body of experimental and epidemiological evidence suggesting many substances in the environment may disrupt human health continues to expand to cover a wide range of exposures. Of greatest concern are the effects of transgenerational exposure to unrecognized agents, which may be present in food stuffs, drinking water, and other consumables including medications and cosmetics. Using hormone receptor-based technology and highly purified preparations of EDCs as standards, there is an opportunity to improve exposure and risk assessment for environmental estrogen mimics, as well as the quantitative analysis of their occurrence in the environment.

Exposure Routes and Pathways

Xenoestrogens are particularly dangerous to animal and human health because they are persistent, ubiquitous chemicals in the environment that bioaccumulate and may even be activated further as a result of biotransformation. An environmental endocrine disruptor is defined as a man-made compound that interferes with one or more steps in the signal transduction pathway of natural hormones in the body responsible for maintenance of homeostasis, reproduction, development, and/or behavior. The enormous chemical complexity of xenoestrogens (e.g., more than 200 possible congeners of polychlorinated biphenyls (PCBs)) as well as variations in the degree of modification (e.g., extent of chlorination) preclude the establishment of common routes of accumulation and mechanisms of both biotransformation and biodegradation. Exposure to xenoestrogens occurs mainly by ingesting contaminated foods and liquids, although small amounts may be inhaled or absorbed through the skin and mucous membranes in the body.

Toxicokinetics

Because of the broad chemical diversity of compounds in this group, metabolism, detoxification, and excretion pathways are quite variable; therefore, the reader should refer to information for a particular compound. As an example, methoxychlor and bisphenol A in low doses have been shown to be

rapidly eliminated from the body as conjugated forms by the liver and have efficient metabolic clearance. Only excessive doses may lead to accumulation if the detoxification pathways are saturated. Once absorbed, they are readily distributed via the lymph and blood to all body tissues and are stored in these tissues generally in proportion to organ tissue lipid content. Excretion of DDT in the form of its metabolites (e.g., DDE and its conjugates) is largely via the urine, regardless of route of exposure, but DDT excretion may occur via feces, semen, and breast milk.

Mechanism of Toxicity

With regard to estrogen-associated toxicity, the primary mechanism appears to be via association with the estrogen receptor and subsequent alteration in the signal transduction pathway. Many studies of toxicokinetics suggest the difficulty in extrapolating structure–activity relationships of particular compounds with their influence on biological responses (e.g., reproduction, neuroendocrine behavior).

Acute and Short-Term Toxicity (or Exposure)

Due to variability in toxicity of this large group of compounds, no pattern of exposure symptoms has been observed. In animal studies, short-term exposure to large amounts of DDT in food affected the nervous system and may affect reproduction (e.g., embryonic survival in bald eagles). Exposure to endocrine-disrupting chemicals has also been shown to affect thyroid function in birds and fish. Humans exposed to simazine at high levels for a relatively short period of time can experience weight loss and changes in liver enzymes in the serum.

Chronic Toxicity (or Exposure)

The large number of compounds with highly diverse molecular properties precludes listing individual symptoms of chronic toxicity. As an example, animals exposed to high levels of methoxychlor experience tremors, convulsions, and seizures. Exposure to large doses of DDT had a negative affect on the metabolic function in the animal's liver. High doses of methoxychlor may cause damage to the human nervous system. High exposure to simazine can cause tremors, gene mutations, cancer, and damage to testes, kidneys, liver, and thyroid. Exposure to high PCB concentrations may increase heart size and blood pressure, two factors known to elevate the risk of heart disease. Laboratory tests have been developed to detect DDT and DDE in fat, blood, urine, semen, and breast milk. These tests may indicate low, moderate, or excessive exposure to these compounds, but cannot provide results assessing extent of exposure or whether there will be adverse biological effects in subjects.

In Vitro Toxicity Data

The National Institute of Environmental Health Sciences was directed by Public Law 103-43 to develop and validate alternative methods for acute and chronic toxicity testing. To implement this, they established an Interagency Coordinating Committee on the Validation of Alternative Methods in 1997.

Environmental Fate

Xenoestrogens, regardless of their molecular diversity, have the ability to leach into ground water and contaminate the water supply if not removed by various methods of water purification. Before 1973 when it was banned, DDT entered the air, water, and soil during its production and use as an insecticide. DDT is present at many waste sites, and releases from these sites may continue to contaminate the environment. Most of the DDT in the environment is a result of past use, but it still enters the environment because of its current use in other areas of the world where it is not banned. DDE is only found in the environment as a result of contamination or breakdown of DDT.

DDT and DDE are rapidly degraded when exposed to sunlight with a half-life of 2 days, but in soil they are biodegraded much more slowly with a half-life of 2–15 years, depending on the type of soil organisms. Small amounts of DDT and DDE leach into the ground water supply as well as pollute plants and accumulate in the fatty tissues of fish, birds, and other animals.

Exposure Standards and Guidelines

No general set of guidelines has been established for exposure to the general category of xenoestrogens. Therefore, exposure assessment of a particular class of xenoestrogens (e.g., phthalates) requires determinations of the parent compound and derivatives. As an example, the US Environmental Protective Agency (EPA) has set a reference dose for methoxychlor at $0.005\,\mathrm{mg\,day^{-1}}$. This is the highest daily oral exposure humans can be exposed to without resulting

in harmful side effects. The EPA has also set a limit of 0.04 parts per million (ppm) of methoxychlor in water. Children should not drink water containing more than 0.05 ppm for more than 1 day, while adults should not drink water containing more than 0.2 ppm for up to 7 years. The Occupational Safety and Heath Administration (OSHA) has established a work place exposure limit for methoxychlor at 15 mg m^{-3} for an 8 h work day and 40 h work week.

The EPA has set a maximum contaminant level for simazine at 4 parts per billion (ppb), because it is believed that exposure to this level of herbicidal compound has not induced health problems.

The OSHA has set a limit of 1 mg m^{-3} of DDT in the workplace for an 8 h shift, 40 h work week.

See also: Estrogens I: Estrogens and Their Conjugates; Estrogens II: Catechol Estrogens; Estrogens III: Phytoestrogens and Mycoestrogens; Estrogens IV: Estrogen-Like Pharmaceuticals.

Further Reading

Blair R, Fang H, Branham WS, *et al.* (2000) The estrogen receptor relative binding affinities of 188 natural and xenochemicals: Structural diversity of ligands. *Toxicological Sciences* 54: 138–153.

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Davis D, *et al.* (1993) Medical hypothesis; xenoestrogens as preventable causes of breast cancer. *Environmental Health Perspectives* 101: 372–377.

Fang H, Tong W, Shi LM, *et al.* (2001) Structure–activity relationships for a large diverse set of natural, synthetic, and environmental estrogens. *Chemical Research in Toxicology* 14: 280–294.

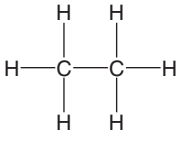
Relevant Website

http://iccvam.niehs.nih.gov – ICCVAM/NICEATM Final Report (2002) Expert Panel Evaluation of the Validation Status of *In vitro* Test Methods for Detecting Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays.

Ethane

Stephen R Clough

- Chemical Abstract Service Registry Number: CAS 74-84-0
- Synonyms: Bimethyl; Dimethyl; Ethyl hydride; Methyl methane; Ethane, copressed (UN1035, DOT); Ethane, refrigerated liquid (UN 1961, DOT)
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon
- Chemical Formula: C_2H_6
- Chemical Structure:



Uses

Ethane is used as a fuel and as a raw material in the manufacture of synthetic organic chemicals (e.g., pharmaceutical and chemical industry).

Exposure Routes and Pathways

Because ethane exists as a gas at normal temperature and pressure, exposure occurs by inhalation. Concentrations of ethane in natural gas range from 5 to 10%. It is also found in the exhaust of diesel (~1.8%) and gasoline (1.3–2.0%) engines. Small amounts of ethane, along with other C1 and C4 alkanes and alkenes, have been detected in mined coal samples. Ethane emissions from cigarettes have been measured at 1600 μg per cigarette. Typical background air concentrations in major US cities range from 0.05 to 0.5 ppm. Because it is lighter than air, a major spill would not be expected to migrate and affect adjacent properties or neighborhoods. It is possible to spill liquid ethane from a refrigerated tank, causing frostbite upon contact with the skin due to rapid evaporation and loss of heat.

Mechanism of Toxicity

Ethane acts as an asphyxiant at concentrations that are high enough to displace oxygen.

Acute and Short-Term Toxicity (or Exposure)

Animal

Guinea pigs exposed to 2.2–5.5% of the gas for 2 h have shown slight signs of irregular respiration that were readily reversible on cessation of exposure. As in humans, ethane acts as a simple asphyxiant at high concentrations.

Human

Ethane is not toxic to humans; studies have shown no adverse effects at air concentrations of up to 50 000 ppm. Ethane is, however, a simple asphyxiant. Concentrations that are high enough to displace oxygen would be expected to cause lightheadedness, loss of consciousness, and possibly death.

Chronic Toxicity

No information could be found on the chronic toxicity of ethane.

Clinical Management

Persons who are exposed to high concentrations should vacate or be removed from the source of the gas and seek fresh air.

Environmental Fate

Ethane is unlikely to undergo photolysis or hydrolysis or to be bioconcentrated in both soil and water. Volatilization is the most important fate process in these environmental media. Ethane is most likely to be found in the atmosphere.

Other Hazards

Ethane is highly flammable and is therefore an explosion and/or fire hazard (lower explosion limit is 3–12.5% by volume). Extreme care must be taken to keep areas of high concentration free from ignition sources, such as sparks from static electricity. Explosion-proof equipment should also be used in these areas.

Exposure Standards and Guidelines

Industrially, ethane is handled similarly to methane, and a threshold limit of 1000 ppm is commonly assumed. Many states regulate ethane as a hazardous substance based on its flammable properties (typically over 10 000 lbs).

Miscellaneous

Ethane has been shown to be a product of lipid peroxidation. Some studies have shown that certain microorganisms are able to use ethane as a nutrient while other types of bacteria are inhibited by its presence.

See also: Lipid Peroxidation.

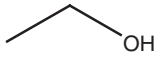
Further Reading

Snyder R (ed.) (1987) Ethel Browning's toxicity and metabolism of industrial solvents. *Hydrocarbons*, 2nd edn., vol. 1, p. 260. Amsterdam: Elsevier.

Ethanol

Bradford H Strohm and Leonard I Sweet

- Chemical Abstracts Service Registry Number: CAS 64-17-5
- Synonyms: Ethyl alcohol; Grain alcohol; Methyl carbinol; Ethyl hydrate; Cologne spirit; EtOH; Potato alcohol
- Chemical/Pharmaceutical/Other Class: Alcohol
- Chemical Formula: C_2H_6O
- Chemical Structure:



Uses

Ethanol is one of the largest volume organic chemicals used in industrial and consumer products. The primary industrial uses of this aliphatic alcohol are as an intermediate in the production of other chemicals and as a solvent. Ethanol is used in the manufacture of drugs, plastics, lacquers, polishes, plasticizers, and cosmetics. Ethanol is used in medicine as a topical anti-infective, and as an antidote for ethylene glycol or methanol overdose. Commercial products containing ethyl alcohol include beverages, perfumes, aftershaves and colognes, medicinal liquids, mouthwashes, liniments, and some rubbing alcohols.

Exposure Routes and Pathways

Most exposures to ethanol for the general population are through ingestion. Occupational exposure to ethanol occurs principally via inhalation and dermal contact. Ethanol is not well absorbed

through intact skin, but is well absorbed via inhalation.

Toxicokinetics

Ethanol is readily absorbed upon inhalation or ingestion. Absorption from the gastrointestinal tract is by simple diffusion with ~80% of an oral dose being absorbed in the small intestine. About 80–90% of ethanol is absorbed within 30–60 min, although food may delay complete absorption for 4–5 h. Inhalation of ethanol vapors in the range of 5000–10 000 ppm by human volunteers indicates absorption from lungs to be ~62%.

Ethanol is both water- and lipid-soluble, and therefore distributes into total body water and readily penetrates the blood–brain barriers and placenta. Ethanol has been found in the amniotic fluid of animals after a single oral dose.

Once peak blood ethanol levels are reached, disappearance is linear, with a 70 kg man metabolizing 7–10 g of alcohol per hour.

The metabolism of ethanol occurs predominantly in the liver. The metabolism of ethanol is carried out in the liver by several enzymes, including alcohol dehydrogenase, aldehyde dehydrogenase, microsomal ethanol-oxidizing system or CYP2E1, and peroxisomal catalase. Initially, ethanol is broken down into acetaldehyde by alcohol dehydrogenase, and then it is further broken down to acetic acid by aldehyde dehydrogenase. Acetic acid is released into the blood where it is further oxidized through normal intermediary metabolism in peripheral tissues to carbon dioxide and water.

Normally, 90–98% of the ethanol that enters the body is completely oxidized, predominantly in the liver, eventually entering the citric acid cycle or utilized in anabolic synthetic pathways. The kidney and lungs excrete only 5–10% of an absorbed dose unchanged. The rate of ethanol metabolism varies between individuals, by age, and may be under genetic control.

Mechanism of Toxicity

Ethanol is a central nervous system (CNS) depressant that initially and selectively depresses some of the most active portions of the brain (reticular activity system and cortex). The mechanism of action most likely involves interference with ion transport at the axonal cell membrane rather than at the synapse, similar to the action of other anesthetic agents. Ethanol can bind directly to the gamma-aminobutyric acid (GABA) receptor in the CNS and cause sedative effects. Ethanol may also have direct effects on cardiac muscle, thyroid tissue, and hepatic tissue.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral, inhalation, and dermal toxicity of ethanol in animals is low. Oral LD_{50}s in rats, mice, guinea-pigs, rabbits, and dogs range from ~6 to 18 g kg^{-1}. Inhalation LC_{50}s range from 12 000 to ~50 000 ppm in studies of mice, guinea pigs, and rats. Animals exposed to ethanol in air may manifest the following signs of intoxication including irritation of the mucous membranes, drowsiness, CNS depression, and possibly respiratory failure. Ethanol is not significantly irritating to the skin of rabbits, although it does produce eye irritation in rabbits. A drop of concentrated ethanol placed on the eyes of rabbits causes reversible injury, whereas repeated applications to rabbit eyes may cause loss of corneal tissue function.

Human

Ethanol is an irritant of the eyes and mucous membranes and causes CNS depression at very high levels of exposure. The major acute toxic effect of ethanol is neuronal dysfunction. Ethanol acts principally on the brain whether ingested or inhaled, first as an inhibitor of the higher functions and then as an anesthetic. Animals as well as humans develop tolerance.

In general, inhalation concentrations up to 3500 ppm cause neither irritation nor any subjective symptoms. Exposures of humans to ~5000–10 000 ppm cause transient eye and nose irritation as well as cough. Exposures at 15 000 ppm produce continuous lacrimation and cough, and levels of 25 000 ppm and above were judged as intolerable.

Mild ethanol intoxication is observed at blood alcohol levels in the range of 0.05–0.15%. Symptoms of exposure include impairment of visual acuity, muscular incoordination, decreased reaction time, and changes in mood, personality, or behavior. At blood alcohol levels of 0.15–0.3%, visual impairment, sensory loss, muscle incoordination, slowed reaction time, and slurred speech is observed. At levels of 0.3–0.5% blood alcohol, there is severe intoxication characterized by muscular incoordination, blurred or double vision, and sometimes stupor, hypothermia, vomiting, nausea, and occasionally, hypoglycemia and convulsions. At 0.4% and above, symptoms include coma, depressed reflexes, respiratory depression, hypertension, hypothermia, and possibly death from respiratory or

circulatory failure, often as a result of aspiration of stomach contents in the absence of a gag reflex. The fatal concentration in whole blood is usually considered to be $>400\,mg\,dl^{-1}$. The lethal dose for man is 8–10 $ml\,kg^{-1}$ body weight.

Chronic Toxicity (or Exposure)

Animal

Subchronic and chronic toxicity testing in animals indicate that the liver is the primary site of action. Effects upon the liver observed in animals parallel those observed in humans and include fatty degeneration, focal necrosis, inflammation, and fibrosis leading to cirrhosis.

Ethanol has been studied in rats, mice, and hamsters for carcinogenicity. While some results are inconclusive, there are data from animals indicating that ethanol consumption may enhance the carcinogenic activity of other known carcinogenic agents. However, studies on male and female mice conducted by the National Toxicology Program (NTP) indicate that the evidence for carcinogenicity is inadequate. According to the American Conference of Governmental Industrial Hygienists (ACGIH), ethanol is 'not classifiable as a human carcinogen'.

Ethanol has been investigated for reproductive toxicity in male mice and rats, and while producing effects upon testes and other reproductive tissues, has generally not been shown to affect reproductive outcome or performance.

Rats, mice, and rabbits have been tested for developmental toxicity upon ethanol exposure. Inhalation of ethanol by pregnant rats at up to 20 000 ppm for 7 h per day on gestational days 1–19 produced no treatment-related effects on uterine implantation or embryonic development. Similarly, 15% ethanol in drinking water of rats, mice, and rabbits, while eliciting maternal toxicity and reducing fetal weights, failed to elicit teratogenic effects. Effects were noted in the offspring of female mice maintained on liquid diets containing 15–35% ethanol dry calories for at least 30 days before and during gestation until day 18.

Human

Alcohol consumption and its relationship to the occurrence of human cancers has been the subject of numerous epidemiological investigations. From these studies, the International Agency for Research on Cancer (IARC Volume 44) has concluded that there is sufficient evidence for the carcinogenicity of alcoholic beverages in humans. Malignant tumors of the oral cavity, pharynx, larynx, esophagus, and liver have been causally related to the consumption of alcoholic beverages. Alcohol ingestion during pregnancy has been found to lead to congenital malformations that have been collectively termed 'fetal alcohol syndrome'. Fetal alcohol syndrome is characterized by mental deficiency and microcephaly. Affected infants typically are small, demonstrate poor muscle coordination, have impaired immune systems, and exhibit various other abnormalities. These abnormalities may be due, at least in part, to a direct action of ethanol that inhibits embryonic cellular proliferation early in gestation. The severity of the effects is related to the extent and timing of alcohol consumption by the mother during pregnancy. This syndrome has been associated with alcoholic women who drink heavily and chronically during pregnancy. There have been no reports of fetal alcohol syndrome resulting from industrial exposure.

Chronic exposures to ethanol vapors can result in irritation of mucous membranes, headache, and symptoms of CNS depression, such as lack of concentration and drowsiness.

Chronic ethanol ingestion has also been shown to produce liver damage, which can eventually lead to cirrhosis of the liver and possibly death. Signs include enlarged liver, elevated serum enzymes, and jaundice.

Infants and toddlers have a clinical course different from that of adolescents and adults. Ethanol ingestion and intoxication can lead to a marked hypoglycemic state, respiratory depression, and hypoxia, in infants and young children.

Clinical Management

The mainstay of medical treatment of patients with ethanol toxicity is supportive care. In general, a conservative approach is recommended for ethanol intoxication. Supportive therapy for overdose may include treatment for respiratory depression, hypotension, and altered glucose or thiamine levels. If the ingestion occurred within one hour of presentation, placing a nasogastric tube and evacuating the stomach contents can prove helpful. In patients with chronic ethanol abuse, therapy may include administration of thiamine to prevent neurologic injury. The administration of medications to cause emesis is not recommended because of the rapid onset of CNS depression as well as aspiration risks.

Pathologic effects of ethanol on hematopoietic tissue can result directly from alcohol ingestion or from secondary nutritional deficiencies or hepatic disease. The clinician will often confront an array of overlapping syndromes in the alcoholic patient, which involves abnormalities of immune system cells. Hemodialysis efficiently clears ethanol from the

blood (removing ~50–100%) but as an invasive procedure its use is not routinely recommended unless the patient's condition is deteriorating, or the patient has impaired hepatic function, or is nonresponsive to standard therapeutic intervention.

Environmental Fate

If released to the environment from natural or anthropogenic sources, ethanol is expected to preferentially partition to the soil, water, and air. Bioconcentration and bioaccumulation potential is expected to be low, based on the estimated bioconcentration factor and experimental octanol water partition coefficient. If released into water, it is expected to have a half-life of less than 10 days. When released into the air, it is expected to have a half-life of less than 5 days, and is expected to be removed from the air by wet deposition. Biodegradation and volatilization are expected to be important fate and transport processes for ethanol.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for ethanol include the following:

- American Conference of Governmental Industrial Hygienists (1000 ppm TWA);
- Argentina (1000 ppm TWA);
- Australia (1000 ppm TWA);
- Belgium (1000 ppm TWA);
- Brazil (780 ppm TWA; for a 48 h work week);
- Canada (1000 ppm TWA);
- Chile (800 ppm TWA);
- Denmark (1000 ppm TWA);
- Finland (1000 ppm TWA);
- Germany – DFG (1000 ppm peak limitation);
- Mexico (1000 ppm TWA);
- Sweden (500 ppm TLV; LLV);
- United Kingdom (1000 ppm TWA); and
- USA OSHA permissible exposure limit (1000 ppm TWA).

Miscellaneous

Ethanol is a colorless, flammable, volatile liquid that has a characteristic odor and burning taste. Odor is generally detected at concentrations ranging from 100 to 180 ppm.

See also: Developmental Toxicology; Neurotoxicity; Poisoning Emergencies in Humans.

Further Reading

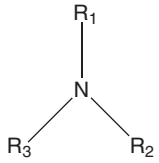
National Toxicology Program (2002) *Toxicology and Carcinogenesis Studies of Urethane, Ethanol, and Urethane/Ethanol (Urethane, CAS No. 51-79-6; Ethanol, CAS No. 64-17-5) in B6C3F$_1$ Mice (Drinking Water Studies)*. Research Triangle Park, NC: US Department of Health and Human Services, Public Health Service.

Ethanolamine

William Stott

This article is a revision of the previous print edition article by Ralph Parod, volume 1, pp. 565–566, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: Ethanolamine (CAS 141-43-5); Diethanolamine (CAS 111-42-2); Triethanolamine (CAS 102-71-6)
- Synonyms: Monoethanolamine, 2-Aminoethanol; Diethanolamine, 2,2′-Iminodiethanol; Triethanolamine, 2,2′,2″-Nitrilotriethanol
- Chemical/Pharmaceutical/Other Class: Alcohol amines
- Chemical Formulas: $C_2H_7NO_2$ (Monoethanolamine); $C_4H_{11}NO_2$ (Diethanolamine); $C_6H_{15}NO_3$ (Triethanolamine)
- Chemical Structure:



Monoethanolamine: $R_1 = CH_2CH_2(OH)$; $R_2 = H$; $R_3 = H$
Diethanolamine: $R_1 = CH_2CH_2(OH)$; $R_2 = CH_2CH_2(OH)$; $R_3 = H$
Triethanolamine: $R_1 = CH_2CH_2(OH)$; $R_2 = CH_2CH_2(OH)$; $R_3 = CH_2CH_2(OH)$

Uses

Ethanolamines are variously used in the synthesis of ethyleneamines, to remove carbon dioxide and

hydrogen sulfide from natural gases and other gas streams, as corrosion inhibitors in metal removal fluids; to produce surfactants used in a variety of industrial, consumer and personal care products, as dispersing agents for agricultural chemicals, and in the manufacture of cosmetics and pharmaceuticals.

Exposure Routes and Pathways

The uses and relatively low vapor pressures of di- (0.00473 Torr, 0.63 Pa) and tri- (0.00018 Torr, 0.0239 Pa) ethanolamines result in primarily dermal exposure of humans. However, the somewhat higher vapor pressure of monoethanolamine (0.75 Torr, 100 Pa) indicates the potential added exposure for this ethanolamine via inhalation. The inhalation of any of the ethanolamines may also occur when respirable aerosols are generated during the use of consumer products or in occupational settings.

Toxicokinetics

Absorption may occur via the oral, inhalation or dermal routes resulting in systemic toxicity. Dermal absorption can vary greatly dependent upon species and whether applied neat or as a component of a formulation. Acute toxicity data suggest that monoethanolamine, a potentially corrosive chemical, exceeds that of the less irritating and higher molecular weight ethanolamines. *In vitro* skin studies have demonstrated a relatively high rate of absorption of mono- and diethanolamines through mouse skin relative to rabbits (2–6-fold), rats (13–15-fold), or humans (18–23-fold). Other studies have shown that less than 2% of di- or triethanolamines as aqueous solutions or complex mixtures are absorbed through rat or human skin. Once absorbed, the active uptake of mono- and diethanolamines by tissues, primarily liver, and their metabolic incorporation into phospholipids dictates the pharmacokinetics of these compounds. Monoethanolamine is a naturally occurring precursor of phospholipid metabolism and diethanolamine competes for the same metabolic pathways. More than 50% of monoethanolamine underwent metabolic incorporation into lipids of treated rats while ~12% was excreted as CO_2 in 8 h. Likewise, ~70–75% of diethanolamine was retained in tissues, primarily liver and kidneys and remaining carcass, with only 22–35% excreted unchanged via urine in rats 96 h postdosing. In contrast, triethanolamine was excreted by mice almost entirely via the urine unchanged within 24–48 h postdosing. Reflective of this, di- and triethanolamines were eliminated from blood with terminal-phase half-lives of ~170 and 10 h, respectively.

Mechanism of Toxicity

The mechanism of toxicity differs significantly between the ethanolamines. The toxicity of monoethanolamine is primarily dictated by its irritant properties as a base ($pK_a = 9.7$), which limits toxicity primarily to portal-of-entry tissues. Systemic effects of diethanolamine appear related to competition with ethanolamine for incorporation into phospholipids and with cellular choline uptake processes which together may result in severe choline deficiency in treated rodents. Chronic choline deficiency is believed to be responsible for diethanolamine-induced tumor formation in mice via this relatively well-characterized nutrition-based mode of tumorigenicity, to which humans are relatively refractory. As a secondary amine, diethanolamine also has the potential to undergo nitrosation to form the carcinogen *N*-nitrosodiethanolamine in the acidic stomach if ingested with high levels of a nitrosating agent such as nitrite. In contrast, effects of triethanolamine upon tissues appear more related to adaptation to high dosages than frank toxicity. However, triethanolamine may also inhibit cellular uptake of choline resulting in choline deficiency in liver and resultant toxicity, including tumor formation, when administered to mice. Though sharing a choline deficiency mode of action, triethanolamine is less potent than diethanolamine and does not appear to compete with ethanolamine for metabolic incorporation into phospholipids.

Acute and Short-Term Toxicity (or Exposure)

Animal

In general, skin and ocular irritation potential of ethanolamines is directly related to their strength as bases and inversely with molecular weight. Neat monoethanolamine can cause a chemical burn within a few hours while triethanolamine is a relatively weak irritant after prolonged contact. Oral and dermal lethal dosages also vary considerably. Oral LD_{50} values reported for mono-, di- and triethanolamine in rats are 1.1–2.7, 0.7–2.8, and 5.5–11.3 g kg^{-1}, respectively. Dermal LD_{50} values reported for mono-, di- and triethanolamine in rabbits also vary widely; 1.0–2.5, 8.1–12.2, and greater than 20 g kg^{-1}, respectively. Inhalation acute lethality data (LC_{50}) are not reported and no lethality has been reported at saturated atmospheres of any of the ethanolamines, ~520, 0.37 and 0.0047 ppm for mono-, di- and triethanolamine, respectively. Short-term repeated dosing of animals with relatively high dose levels of ethanolamines via inhalation, oral, and/or dermal

routes result in effects generally reflected in longer-term testing discussed below.

Human

No reports of significant acute toxic responses to the ethanolamines were noted; however, it would be expected that severe skin and eye irritation could result from contact with concentrated monoethanolamine and, to a much lesser extent, diethanolamine.

Chronic Toxicity (or Exposure)

Animal

The toxicity of monoethanolamine, a product of normal metabolism, is largely limited to its irritant effects, and their sequelae, at the site of contact. Exposure of rats, guinea pigs and dogs to vapors in excess of ~ 50 ppm resulted in significant skin irritation, respiratory distress, and changes in respiratory tract, liver, and kidney tissues. The toxicity of diethanolamine is primarily dictated by its disruption of phospholipid synthesis and an induced deficiency in the nutrient choline. Administration of diethanolamine to rats and mice via oral or dermal route resulted in a species-dependent spectrum of effects, generally at dosages of ~ 150–$200\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ or higher. Organs affected in rats included liver, kidney, central nervous system, and testes and in mice liver, kidneys, heart, and salivary glands were affected. The most sensitive systemic effect was a microcytic, normochromic anemia, which occurred in female rats at dosages as low as $15\,\mathrm{mg\,kg^{-1}\,day^{-1}}$. Repeated inhalation of diethanolamine aeorosol by rats resulted in laryngeal irritation at $\sim 8\,\mathrm{mg\,m^{-3}}$ or higher concentration. Lifetime dermal administration of $40\,\mathrm{mg\,kg\,day^{-1}}$ or higher diethanolamine to mice resulted in an increased incidence of liver tumors in males and females and an increased incidence of kidney tumors in $160\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ males. No tumors were observed in lifetime dermal rat bioassays at up to $250\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ or in a dermal transgenic mouse (TG.AC) bioassay at ~ 1000–$1200\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.

Administration of triethanolamine to rats and mice via dermal application resulted in only minimal effects upon body weights at 250–$2000\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ and changes in liver and/or kidney weights with or without histopathologic changes at 500–$1000\,\mathrm{mg\,kg^{-1}\,day^{-1}}$. Aerosolized triethanolamine has also been reported to cause irritation of the larynx of rats exposed to 20–$100\,\mathrm{mg\,m^{-3}}$ or higher. Lifetime oral administration of up to 1000–$2000\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ triethanolamine to rats and up to $3000\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ to mice or dermal administration of up to $250\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ triethanolamine to rats and $2000\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ to male mice have revealed no evidence of carcinogenic potential. However, lifetime dermal administration of $100\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ triethanolamine to female mice resulted in an increased incidence of liver tumors. No tumors were observed in a dermal transgenic mouse (TG.AC) bioassay at a dose of ~ 1000–$1200\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.

Ethanolamines have not been found to be developmental toxins in standard or screening assays nor was diethanolamine found to be neurotoxic in a standard neurotoxicity test following subchronic inhalation exposure up to $400\,\mathrm{mg\,m^{-3}}$.

Human

Standardized animal and human tests of allergic sensitization to the ethanolamines have been negative, but sporadic case reports of human sensitization and a low incidence of sensitization in specific work groups of dermatitis patients have been reported.

In Vitro Toxicity Data

None of the ethanolamines has been found to be genotoxic in a variety of assays in the absence of added nitrosating substances.

Clinical Management

Clinical management involves removal from exposure and treatment of symptoms.

Environmental Fate

Ethanolamines released to the environment will partition primarily to the water due their relatively low volatility and high water solubility. Bioconcentration factors range from -1.23 to -1.56 reflecting the very low potential for ethanolamines to bioaccumulate in the environment. All three ethanolamines reportedly undergo near complete degradation in the presence of 'acclimated' microflora under standardized testing conditions and microbial degradation pathways have been elucidated. Environmental persistence and bioaccumulation data do not identify any of the ethanolamines as potentially either persistent or bioaccumulative environmental contaminants under criteria used by the United Nations, European Union and Canadian EPA.

Ecotoxicology

The toxicity of the ethanolamines has been extensively studied in aquatic species. LC_{50} values for

several species of fish range from 150 to 2100 mg l^{-1} for monoethanolamine, from greater than 100 to 47 000 mg l^{-1} for diethanolamine, and 1800 to greater than 10 000 mg l^{-1} for triethanolamine. LC_{50} values for the invertebrate *Daphnia magna* were in the range of 140 mg l^{-1} for monoethanolamine, 55–306 mg l^{-1} for diethanolamine, and 1390 mg l^{-1} for triethanolamine in one set of tests. The most sensitive assay system studied appears to be algae and cyanobacteria whose growth has been reportedly inhibited in the range of 1–10 mg l^{-1} for monoethanolamine, 3–20 mg l^{-1} for diethanolamine, and 2–715 mg l^{-1} for triethanolamine.

Exposure Standards and Guidelines

International occupational exposure limits for most major industrialized regions list 2–3 ppm as an 8 h time-weighted average (TWA) and 6 ppm in Sweden as a short-term exposure level (STEL) (15 min) for monoethanolamine; 15 mg m^{-3} (United Kingdom is 3 mg m^{-3} while United States does not list a value) as a TWA and 30 mg m^{-3} as an STEL for diethanolamine; 5 mg m^{-3} as a TWA and 10 mg m^{-3} in Sweden as an STEL for triethanolamine. Additional occupational exposure values for monoethanolamine include a US Occupational Safety and Health Administration permissible exposure limit of 3 ppm or 6 mg m^{-3}, a National Institute of Occupational Safety and Health (NIOSH) recommended exposure limit (REL) of 3 ppm or 8 mg m^{-3}, a German MAK level of 2 ppm or 5.1 mg m^{-3}, and an American Conference of Governmental Industrial Hygienists (ACGIH) 8 h TWA value of 3 ppm or 7.5 mg m^{-3} and an STEL of 6 ppm or 15 mg m^{-3}. Additional values for diethanolamine include an ACGIH threshold limit value (TLV) of 0.46 ppm or 2 mg m^{-3} and a NIOSH REL of 3 ppm or 15 mg m^{-3}. The only additional value for workplace triethanolamine exposure is an ACGIH TLV of 5 mg m^{-3}. Neither an IRIS value nor other ambient, nonworkplace guidance values have been established. The US Food and Drug Administration advises cosmetics manufacturers to avoid using any secondary amines, including diethanolamine, along with nitrosating agents due to the risk of nitrosamine formation. The US Environmental Protection Agency has promulgated a rule prohibiting the use of nitrites in diethanolamine-containing metal removal fluids for this same reason. Mono- and triethanolamines are listed as potential indirect additives in foods.

Di- and triethanolamines are listed by the International Agency for Research on Cancer (IARC) as Group 3, "not classifiable as to its carcinogenicity to humans." None of the ethanolamines is identified as a carcinogen by the US EPA, National Toxicology Program Report on Carcinogens, ACGIH or the MAK Commission. ACGIH and the MAK Commission provide a notation that significant skin absorption is possible while MAK Commission identifies all three ethanolamines as potential dermal sensitizers.

See also: Carcinogenesis; Eye Irritancy Testing; Nitrosamines; Skin.

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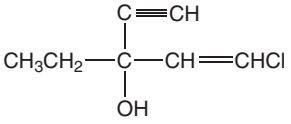
Ethchlorvynol

S Rutherfoord Rose

- Chemical Abstracts Service Registry Number: CAS 113-18-8
- Synonyms: β-Chlorovinyl ethyl ethinyl carbinol; 1-Chloro-3-ethyl-pent-1-en-4-yn-3-ol; Placidyl®; Arvynol®; Serenesil®
- Chemical/Pharmaceutical/Other Class: Tertiary acetylenic alcohol
- Chemical Formula: C_7H_9ClO
- Chemical Structure:



Uses

Ethchlorvynol is used as a sedative/hypnotic. It also possesses some anticonvulsant and muscle relaxant properties. As with most sedative/hypnotics, ethchlorvynol has abuse potential.

Exposure Routes and Pathways

Ethchlorvynol is marketed as a liquid-filled capsule with a polyethylene glycol diluent. Toxicity has resulted from oral overdose and from intravenous injection of the liquid contents of a capsule.

Toxicokinetics

Ethchlorvynol is highly lipid soluble and rapidly absorbed with peak plasma levels occurring within 1–1.5 h. Approximately 90% of a dose undergoes hepatic hydroxylation and glucuronidation, and several metabolites have been identified, including hydroxyethchlorvynol. Both the parent compound and metabolites undergo enterohepatic recirculation. Binding to plasma proteins is ~35–50%. The distribution volume is 2.5–4 l kg^{-1}, with significant amounts distributed to, and slowly released from, adipose tissue. The elimination of ethchlorvynol appears to be biphasic, with a distribution half-life of 1–5 h, and an elimination half-life of 10–25 h. The elimination phase is prolonged (up to 100 h) following large overdoses. Less than 10% is excreted unchanged in the urine.

Mechanism of Toxicity

The pharmacology of ethchlorvynol is much like that of the barbiturates; thus, an interaction that results in γ-aminobutyric acid-like activity is likely involved. Toxicity results in dose-dependent depression of the central nervous system (CNS).

Acute and Short-Term Toxicity (or Exposure)

Animal

Pulmonary edema and pleural effusions have been reported following intravenous injection in experimental animals.

Human

Overdosage results in dose-dependent depression of the CNS, ranging from fatigue and lethargy to respiratory depression and coma. Coma may be profound, with a flat EEG, and has been reported to last as long as 17 days. CNS depression may be potentiated by the presence of ethanol or other CNS depressants. Hypothermia is a frequent finding, and hypotension with either tachycardia or bradycardia is common with large doses. Ataxia, nystagmus, and headache may occur. Delayed-onset (24–48 h) noncardiogenic pulmonary edema has occurred following large overdoses and is usually associated with deep coma; however, onset may be rapid following intravenous exposure. Overdoses have also been reported to cause paradoxical excitement and pancytopenia and hemolysis. As with other sedative/hypnotic drugs, bullous lesions and pressure necrosis have been found on comatose patients, and seizures have occurred during withdrawal. Death has occurred following ingestion of 2.5 g ethchlorvynol plus alcohol, but the usual fatal dosage range is $\geqslant$10 g. Postmortem blood concentrations ranged from 14 to 400 mg l^{-1} in one study and from 22 to 213 mg l^{-1} in another. Blood levels, however, are not used clinically to guide treatment.

Chronic Toxicity (or Exposure)

Human

Patients chronically using ethchlorvynol may be able to tolerate larger doses and higher serum concentrations compared to drug naive patients.

In Vitro Toxicity Data

Ethchlorvynol has been studied in cultured endothelial cells in order to help explain clinical findings of pulmonary edema seen in some ethchlorvynol

overdose patients. Endothelial cells demonstrated retraction within 10 min of addition of ethchlorvynol 1 mg ml^{-1} to cell culture.

Clinical Management

Treatment is generally supportive. All patients should have intravenous access, cardiac monitoring, and should be observed for hypothermia and hypotension. Gastrointestinal decontamination procedures should be used as appropriate based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used to adsorb ethchlorvynol if used within an hour of the exposure. A complete blood count should be obtained to assess for anemia or thrombocytopenia. Hypotension should initially be treated by elevating the feet and administering an intravenous fluid bolus, followed by administration of vasopressors such as norepinephrine or dopamine if necessary. Pulmonary edema should be managed with positive end expiratory pressure (e.g., not diuretics or inotropic agents) if needed. There are no antidotes. Drug clearance may be enhanced by resin (15–50% removal) or charcoal (5–10% removal) hemoperfusion, but an affect on morbidity has not been demonstrated. Hemodialysis has not proven to be of benefit.

See also: Barbiturates, Long-Acting; Barbiturates, Short-Acting; Drugs of Abuse.

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Ethene

Patricia J Beattie

- Chemical Abstracts Service Registry Number: CAS 74-85-1
- Synonyms: Ethylene; Acetene; Bicarburetted hydrogen; Elayl; Olefiant gas
- Chemical/Pharmaceutical/Other Class: Aliphatic alkene
- Chemical Formula: C_2H_4
- Chemical Structure: $H_2C=CH_2$

Uses

Ethene is used primarily as a feedstock in the production of polymers and industrial chemicals. Approximately 80% is used for production of polyethylene, ethylene oxide/ethylene glycols, and ethylene dichloride/vinyl chloride. Additionally, ethene is used for the controlled ripening of citrus fruits, tomatoes, bananas, other fruits, vegetables, and flowers.

Exposure Routes and Pathways

Because ethene is a gas, inhalation exposure is the primary route of entry.

Toxicokinetics

The inhalation toxicokinetics of ethene have been investigated in human volunteers at atmospheric concentrations of up to 50 ppm (57.5 mg m^{-3}). The majority (94.4%) of ethene inhaled into the lungs is exhaled unchanged without becoming systemically available via the bloodstream. The remaining ethene is metabolized to ethylene oxide, which then reacts to form complexes with hemoglobin, *N*-(2-hydroxyethyl)histidine, and *N*-(2-hydroxyethyl)valine. The biological half-life is $\sim$0.65 h, with ethene being excreted in urine and feces and exhaled as CO_2. The toxicokinetics of ethene in humans and experimental animals appears to be similar.

Mechanism of Toxicity

Ethene is classified as a simple asphyxiant. In sufficient concentrations, ethene causes central nervous system depression and unconsciousness by displacing oxygen in air, which reduces the oxygen available to support cell function.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of inhaled ethene is low, with very high concentrations causing asphyxia due to oxygen

displacement. The LC_{50} for mice is estimated to be 950 000 ppm (1093 g m^{-3}). Ethene has been tested in both rats and dogs in short-term inhalation exposure studies. Exposure to skin and eyes does not cause irritation. The anesthetic properties reported in humans have also been observed in experimental animals. Ethene is not a cardiac sensitizer in dogs.

Human

In humans, ethene is a relatively nontoxic gas. No adverse effects are observed at concentrations of less than 2.5%. At higher concentrations, ethene exhibits the anesthetic properties associated with oxygen deprivation. Humans exposed to ethene may experience subtle signs of intoxication, resulting in prolonged reaction time. Exposure to 37.5% ethene for 15 min resulted in memory disturbances, and concentrations at 50% resulted in unconsciousness. If oxygen is deprived for a sufficient amount of time, death can occur. Ethene has been used as an anesthetic and has some advantages over those more typically used in that its effects are rapid in onset and recovery with minimal effect on other organ systems.

Chronic Toxicity (or Exposure)

Animal

The toxicity and carcinogenicity of inhaled ethene was studied in Fischer 344 rats. The animals were exposed to 300, 1000, or 3000 ppm for 6 h day^{-1}, 5 days $week^{-1}$ for 24 months. These exposures resulted in no toxicity or carcinogenicity. Rats exposed by inhalation to ethene 6 h day^{-1}, 5 days $week^{-1}$ for 13 weeks at 0, 300, 1000, 3000, or 10 000 ppm exhibited no adverse effects, with 10 000 ppm considered to be the no-observed-effect level (NOEL). Ethene was tested in rats for reproductive effects as well as impacts on growth and development of the offspring following head-only inhalation exposure to 200, 1000, or 5000 ppm for 6 h day^{-1} 2 weeks prior to mating, during the mating period, and until the day prior to necropsy of the males or until day 20 of gestation for the females. No adverse effects were observed on male or female reproductive performance, fertility, pregnancy, maternal and suckling behavior, and growth and development of the offspring. The highest dose was determined to be the NOEL for reproductive and developmental effects in rats.

Human

The International Agency for Research on Cancer has concluded that there is inadequate evidence in humans and experimental animals for the carcinogenicity of ethene.

In Vitro Toxicity Data

Ethene at atmospheric concentrations up to 20% was not mutagenic to one strain of *Salmonella typhimurium* with and without liver metabolic activation system (S9). In other strains of *Salmonella* in the presence and absence of S9, ethene was also negative. Ethene has shown no genotoxic activity in *Escherichia coli*. In nonbacterial tests, ethene did not induce chromosome aberrations in cultured Chinese hamster ovary cells exposed to 280.5 mg l^{-1} in the presence and absence of S9, and did not induce micronuclei formation in bone marrow cells of rats or mice exposed up to 3000 ppm for 6 h day^{-1}, 5 days $week^{-1}$ for 4 weeks.

Clinical Management

Overexposure to ethene is treated by simply moving the victim to fresh air. Recovery is usually rapid and complete.

Environmental Fate

Emitted ethene is distributed primarily into the atmosphere and reacts with photochemically reactive hydroxyl radicals, ozone, and nitrate radicals, with half-lives ranging from 1.9, 6.5, and 190 days, respectively. Biodegradation in water occurs with half-lives in the range of 1–28 days, or under anaerobic conditions, 3–112 days. Bioaccumulation in aquatic organisms is not expected to occur, based on ethene's high vapor pressure and log octanol/water partition coefficient.

Ecotoxicology

Ethene is a natural plant hormone and plays a role in flowering, fruit ripening, senescence, and abscission. Exposure to high concentrations, however, can adversely impact photosynthesis and growth, resulting in leaf curling and shedding of flowers and leaves. Commonly impacted plants are peas, potatoes, and oats where retardation effects were observed at concentrations ranging from 8 to 50 mg m^{-3}. Other sensitive plants include African marigolds and Cattleya orchids. Aquatic plants and algae do not exhibit similar sensitivity. Calculated LC_{50} values for various fish species following 4 days of exposure range from 50 to 120 mg l^{-1}. The calculated no-observed-effect concentration for fish (fathead minnow) after 28 days of exposure is 13 mg l^{-1}.

Exposure Standards and Guidelines

American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value: simple

asphyxiant – inert gas or vapor. A4: Not classifiable as a human carcinogen.

Switzerland: time-weighted average: 11 500 mg m^{-3}.

Miscellaneous

The primary hazard associated with use of ethene, however, is its flammability and explosivity.

See also: Polymers; Propene.

Further Reading

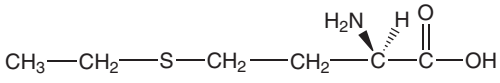
Goodman LS and Gilman A (eds.) (1975) *The Pharmacological Basis of Therapeutics*, 5th edn., p. 84. New York: Macmillan.

Ether *See* Diethyl Ether.

Ethionine

Fu-Min Menn

- Chemical Name: 1-2-Amino-4-(ethylthio)butyric acid
- Representative Chemical: L-Ethionine
- Chemical Abstracts Service Registry Numbers: L-Ethionine (CAS 13073-35-3); D-Ethionine (CAS 535-32-0); DL-Ethionine (CAS 67-21-0)
- Synonyms: *S*-Ethyl-L-homocysteine; α-Amino-γ-(ethylmercapto)butyric acid
- Chemical/Pharmaceutical/Other Class: Carcinogen
- Chemical Formula: $C_6H_{13}NO_2S$
- Chemical Structure:



Uses

There is no known commercial use for this compound.

Exposure Routes and Pathways

There are multiple routes of entry including skin contact, inhalation, and ingestion.

Toxicokinetics

Ethionine can be absorbed readily through the gastrointestinal tract. This compound can transmit through the placenta from pregnant mother to embryo in rats.

Mechanism of Toxicity

Ethionine is the ethyl analog of the amino acid methionine and has proven to be a useful compound for studying the disturbance in the metabolism of methionine and altered gene expression during cancer development. L-Ethionine is the active form and is highly toxic to most organisms. It acts as an antagonist of methionine that competes with methionine for adenosyl groups from ATP (adenosine triphosphate) to yield *S*-adenosylethionine instead of *S*-adenosyl-L-methionine, a universal methyl donor in prokaryotes and eukaryotes. As a consequence of the formation of the *S*-adenosylethionine, proteins, and RNA become ethylated instead of being methylated. The deficiency of *S*-adenosylmethionine results in impairing all transmethylation reactions in the various cellular processes of organism, including DNA, RNA, protein, and phospholipid methylation, and causes damage on cellular growth, differentiation, and function. The decrease of *S*-adenosyl-L-methionine level in animals can cause hypomethylation and DNA damage, which may lead to the development of cancer. However, the L-ethionine induced ATP depletion and protein synthesis impairment can be reversed in rats by the administration of methionine and adenine, the ATP precursor. Ethionine is also known to be a carcinogen and a teratogen.

Acute and Short-Term Toxicity (or Exposure)

Ethionine is an acutely toxic compound that targets the liver and pancreas in animals, and possibly humans. Ethionine inhibits intracellular *S*-adenosylmethionine mediated methylation, and cause widespread liver and pancreatic necrosis.

Animal

Induction of acute hemorrhagic pancreatitis has a 100% mortality rate in young female mice when fed with a choline-deficient diet containing 0.5% ethionine. However, neither mortality nor pancreatitis was reported when ethionine was eliminated from the diet.

Ethionine was detected in the liver, plasma, kidney, small intestine, and red blood cells (in the order of decreasing concentration) of rats after 8 h oral administration with radiolabeled L-(1-^{14}C ethyl)-ethionine.

Chronic Toxicity (or Exposure)

Ethionine-induced teratogenesis has been reported in rats and chicks. Both mice and rats demonstrate significant strain difference to the carcinogenic effects that are caused by ethionine. In addition to cancer, the ethionine-induced abnormal methylation may also have pathological effects leading to birth defects, neurological disorder, and liver and pancreatic toxicities.

Animal

Tumors were formed in the lung, thorax, respiratory tract, and liver in mice study after 2 year oral administration with high dosages. Low chronic doses of ethionine exposure result in irreversible testicular lesions in rats.

Ethionine-induced hepatocellular carcinoma was reduced from 89% to 36% by adding phenobarbital to the 0.1% ethionine diet in F344 rats during an 18-month carcinogenicity study. A different study showed vitamin E protects rat liver mitochondria from ethionine toxicity.

In Vitro Toxicity Data

Ethionine has been demonstrated to inhibit amylase secretion from the AR42J pancreatic cell line *in vitro*, and it also inhibits amylase secretion *in vivo* from freshly isolated rat acini. Ethionine has been shown to significantly increase sister chromatid exchange frequency in human lymphocytes.

Clinical Management

Treatments for skin exposure include removal of contaminated clothing; the exposed area should be washed with soap and water. For eye exposure, the eyes should be immediately rinsed with plenty of running water for at least 15 min. If swallowed, the mouth should be washed; plenty of water should be taken to induce vomiting. For inhalation exposure, remove victim to fresh air area and provide oxygen or artificial respiration as necessary.

Other Hazards

Ethionine may cause heritable genetic damage.

Exposure Standards and Guidelines

The aerosol should not be breathed and prolonged or repeated exposure avoided. All laboratory work should be conducted in a fume hood, glove box, or ventilated cabinet. Use water to dissolve ethionine if a spill occurs.

Miscellaneous

Ethionine is a white crystalline solid. It is very soluble in water and ethanol, and insoluble in nonpolar solvents. The melting point is 280°C (L-form).

D-Ethionine has been used as an antiinflammatory compound in albino rats.

See also: Butyric Acid; Carcinogen–DNA Adduct Formation and DNA Repair.

Relevant Website

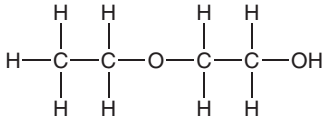
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Ethionine.

Ethoxyethanol

Brad Stanard

- Chemical Abstracts Service Registry Number: CAS 110-80-5
- Synonyms: Ethanol; 2-Ethoxy; β-Ethoxyethanol; Cellosolve; 2-Ethoxyethanol; Ethyl cellosolve; Ethyl glycol; Ethylene glycol ethyl ether; Hydroxy ether
- Chemical/Pharmaceutical/Other Class: Glycol ethers

- Chemical Formula: $C_4H_{10}O_2$
- Chemical Structure:



Uses

2-Ethoxyethanol is a stable, colorless, flammable liquid, synthetically produced throughout the world. It is produced by the reaction of ethylene oxide with ethanol. A large portion is used in the coatings industry (paints, stains, lacquers) and as solvent for printing inks and dyes, and home and industrial cleans. In 1998, the consumption in Western countries was 571 000 tons. The major function of glycol ethers is to dissolve various components of mixtures to keep them in solution until the last stages of evaporation. The glycol ethers are miscible in polar and nonpolar solutions, which make them unique and add to the quality as a solvent and a cleaner.

Exposure Routes and Pathways

The primary route of exposure occurs via inhalation, ingestion, and eye and skin contact. The potential indoor exposures include paints and other coatings, inks, adhesives, nail polishes, cosmetics, and household cleaning supplies. Exposures can also occur through accidental industrial releases.

Toxicokinetics

2-Ethoxyethanol is readily absorbed through the skin, lungs, and gastrointestinal tract. Once absorbed, it is rapidly metabolized. The metabolic pathway involves dehydroxylation by alcohol dehydrogenase. The resulting aldehyde is quickly reduced to form ethoxyacetic acid, the primary metabolite. Further conjugation occurs in rodents to form the secondary metabolite, ethoxyacetyl glycine; however the glycine; conjugate has not been observed in humans. Ethoxyacetic acid is detected in blood and mucous membranes within minutes. Addition of ethanol will slow the process as a result of competitive inhibition of alcohol dehydrogenase. Finally, the ethoxyacetic acid is dealkylated by P450 monooxygenase and eliminated. Excretion is relatively slow and appears to be biphasic, suggesting evidence of accumulation.

Mechanism of Toxicity

The toxicity associated with 2-ethoxyethanol appears to be caused by the metabolites ethoxyaldehyde and ethoxyacetic acid. The metabolites have a longer half-life implying a higher accumulation following repeated exposures. Both *in vitro* and *in vivo* studies have shown toxic effects from administration of the metabolites that were not seen at higher doses of the parent.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rats, mice, dogs, and rabbits have been exposed by the inhalation, dermal, and oral routes. Hepatic changes have been observed at inhalation exposures above 300 ppm. Reduced cytoplasmic density, disruption of lobular structure, elevated plasma fibrinogen, reduced serum proteins, and elevated liver weights have been reported in rats, mice, and rabbits. Most effects seen after acute exposures were reversible.

Human

Acute exposure to high levels of 2-ethoxyethanol results in narcosis, pulmonary edema, and severe liver and kidney damage. Low-level exposure causes conjunctivitis, upper respiratory tract irritation, headache, nausea, and temporary corneal clouding. There are limited human data available in the public domain.

Chronic Toxicity (or Exposure)

Animal

2-Ethoxyethanol is a potent reproductive toxicant. Oral exposure of male rats for six weeks resulted in testicular atrophy, as well as significant decreases in testicular weight, spermatid count, and epididymal sperm count at doses 300 mg kg^{-1} or less. The specific altered sperm morphologies indicate that pachytene spermatocyte is the most sensitive target cell. Inhalation studies in pregnant rats have shown complete resorption of fetuses and reduced fetal weight, as well as skeletal and cardiovascular abnormalities following 733 mg m^{-3} for 7 h per day throughout gestation. Increased resorption rates and fetal deaths, decreased viable fetus weights, and increased cardiovascular defects and skeletal malformations were seen following 0.25 ml applied to skin four times per day. Increased resorptions, incidences of major malformations, minor anomalies, and skeletal variants have been observed following high inhalation doses in both rats and rabbits.

There have been no adequate longer term animal studies to date. There are only minimal data on animal carcinogenicity.

Human

Repeated exposures have resulted in increased oligospermia (low sperm counts) and azoospermia (dead sperm) in male shipyard workers, but there has been some skepticism about these reports due to small sample size. One case controlled study found significant ethoxyacetic acid presence in infertile men. An increased risk of spontaneous abortion and lowered fertility was shown in an epidemiological study of women exposed to mixtures of ethylene glycol ethers.

In Vitro Toxicity Data

Studies on cultured Sertoli cells and germ cells showed both reproductive and genotoxicity following ethoxyacetic acid exposure while lower concentrations of 2-ethoxyethanol showed no morphological changes. Metabolites of 2-ethoxyethanol have been shown to cause chromosomal aberrations and micronuclei in genetic toxicity studies and to induce perturbations in the cell cycle, suggesting DNA lesions as observed on germ cells. Accumulation of other glycol ethers in cell nuclei suggests possible effects on gene regulation, although such studies have yet to be conducted specifically on 2-ethoxyethanol.

Environmental Fate

The environmental fate of ethoxyethanol is relatively short-lived due to degradation by microorganisms in soil, sewer sludge, and water. In the absence of degradation, accumulation in water could occur due to the solubility of 2-ethoxyethanol in water and its relatively low vapor pressure. Degradation to carbon dioxide and water occurs under aerobic conditions, while anaerobic degration yields methane and carbon dioxide. The environmental half-life under aerobic conditions is an estimated 1–4 weeks. It is somewhat more persistent under anaerobic conditions. Atmospheric emissions from use as evaporative solvents result in the greatest environmental exposure, although rapid photolytic degradation occurs. 2-Ethoxyethanol reacts with hydroxyl radicals in the air with a half-life of about 0.2–4 days. The majority remains suspended, although a proportion would partition to water and to soil. 2-Ethoxyethanol has a very low octanol/water partition coefficient and is therefore not expected to bioaccumulate to any significant degree. Data on toxicity exist for aquatic organisms, including microorganisms, invertebrates, and fish. 2-Ethoxyethanol is not very toxic to these organisms; in a number of studies, the LC_{50} was above the highest concentration tested.

Exposure Standards and Guidelines

- National Institute for Occupational Safety and Health (NIOSH) immediately dangerous to life or health value is 500 ppm ($1843\,mg\,m^{-3}$).
- NIOSH recommended exposure level is 0.5 ppm ($1.8\,mg\,m^{-3}$).
- Occupational Safety and Health Administration permissible exposure level is 200 ppm ($740\,mg\,m^{-3}$).
- American Conference of Governmental Industrial Hygienists threshold limit value is 5 ppm ($18\,mg\,m^{-3}$) (with skin notation).
- Environmental Protection Agency (EPA) reference concentration is 0.05 ppm ($0.2\,mg\,m^{-3}$)
- EPA reference dose is $0.4\,mg\,kg^{-1}\,day^{-1}$ (provisional).

See also: Ethylene Glycol Monoethyl Ether; Ethylene Glycol Mono-*n*-Butyl Ether; Methoxyethanol.

Further Reading

American Conference of Governmental Industrial Hygienists (ACGIH) (2003) *2003 TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents. Biological Exposure Indices*. Cincinnati, OH: ACGIH.

National Institute for Occupational Safety and Health (NIOSH) (2003) *Pocket Guide to Hazardous Chemicals*. Cincinnati, OH: US Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention.

Relevant Websites

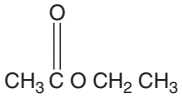
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Ethoxyethanol.

http://www.inchem.org – World Health Organization, Environmental Health Criteria 115, Search for 2-methoxyethanol, 2-ethoxyethanol, and their acetates.

Ethyl Acetate

Dale J Marino

- Chemical Abstracts Service Registry Number: CAS 141-78-6
- Synonyms: Acetic acid ethyl ester; Acetic ether acetidin; Acetoxyethane; Ethyl acetic ester; Ethyl ethanoate; Vinegar naphtha
- Chemical/Pharmaceutical/Other Class: Aliphatic ester
- Chemical Formula: $C_4H_8O_2$
- Chemical Structure:



Uses

Ethyl acetate is used primarily as (1) a coatings solvent for paints, lacquers, and varnishes; (2) an extraction solvent for various processes, including decaffeination of coffee and tea; (3) a process solvent in the pharmaceutical industry; and (4) a carrier solvent for printing inks, adhesives, and nail polish. It is also used in the manufacture of artificial leather and perfumes, and in certain household products including airplane glue, and paint and nail polish removers.

Ethyl acetate finds particular use as a flavor enhancer in foods and pharmaceuticals because of its fruity taste when diluted. It is included on the US Food and Drug Administration's Generally Recognized As Safe list for use as a synthetic flavoring agent. Ethyl acetate is also approved for use as an indirect food additive in certain packaging materials.

Background Information

Ethyl acetate is a naturally occurring constituent in various fruits, including apples, pears, oranges, and grapefruit; and, because it is a by-product of fermentation, ethyl acetate is found in alcoholic beverages including beer and wine. Given its natural occurrence in fruits and use as a flavor enhancer, ethyl acetate is found in foods, including baked goods, frozen dairy products, fruit juices, candy, beverages, and gum.

Exposure Routes and Pathways

Exposure to ethyl acetate can occur via inhalation, ingestion, or dermal contact. Occupational exposures primarily involve inhalation exposure, given ethyl acetate's use as a solvent. Dermal contact would also be expected.

The general population is primarily exposed to low concentrations of ethyl acetate in food. Higher exposures could occur as a result of inhalation of vapors from lacquers, varnishes, nail polish remover, paint remover, and airplane glue. Dermal exposure to ethyl acetate from use of these products is also possible.

Toxicokinetics

Ethyl acetate is readily absorbed following oral, dermal, or inhalation exposures. However, it is rapidly hydrolyzed to ethanol and acetic acid prior to absorption in the gastrointestinal tract following oral administration, and in the upper respiratory tract following inhalation exposure. Absorbed ethyl acetate is also quickly hydrolyzed in blood to ethanol and acetate. Blood concentrations of ethanol can increase if the production of ethanol, following absorption and hydrolysis of ethyl acetate, exceeds metabolism and elimination of ethanol.

Mechanism of Toxicity

Local effects are thought to be due to the formation of acetic acid resulting from hydrolysis of ethyl acetate. Central nervous system (CNS) depressive effects are thought to be due to a combination of absorbed ethyl acetate and formation of ethanol that results from hydrolysis.

Regional deposition of ethyl acetate in the upper respiratory tract and biochemical differences (higher carboxyesterase activity) is believed to be responsible for damage observed to the olfactory mucosa in laboratory animals following inhalation, compared to that noted in the respiratory epithelium.

Acute and Short-Term Toxicity (or Exposure)

Ethyl acetate is considered to have a low order of acute toxicity by all exposure routes. Its primary effects are sensory irritation and, at higher levels, CNS depression.

Animal

Acute oral LD_{50} values in rats have been reported to range from $5.6\,g\,kg^{-1}$ to $11.3\,ml\,kg^{-1}$ ($10.2\,g\,kg^{-1}$).

In mice, rabbits, and guinea pigs, reported acute oral LD_{50} values are 4.1, 4.94, and 5.5 g kg^{-1}, respectively. The dermal LD_{50} in rabbits is >20 ml kg^{-1} (18.0 g kg^{-1}). Inhalation LC_{50} values in rats have been reported to be 1600 ppm (8 h exposure) and >6000 ppm (6 h exposure). Ethyl acetate is mildly irritating to the eye and minimally irritating to skin.

Exposure of mice to nonlethal concentrations of 2000 ppm ethyl acetate for 20 min produced CNS effects during exposure, including decreased locomotor activity, decreased arousal, and delayed righting reflex. Exposure of rats to either 3000 or 6000 ppm for 6 h decreased motor activity and resulted in CNS effects indicative of sedation at 1 h following exposure. In high-dose animals, decreased motor activity was evident the day following exposure. Body weight loss was also noted in all test groups, that is, 600, 3000, and 6000 ppm.

Human

The most common effects in humans exposed to ethyl acetate are ocular and respiratory tract irritation. Exposures in excess of 400 ppm have been associated with irritation of the eyes and respiratory passages. Subjects have reported mild irritant effects at 400 ppm. At higher concentrations, ethyl acetate exposure is associated with CNS depression, potentially causing headache, nausea, vomiting, drowsiness, and dizziness. Very high concentrations of ethyl acetate as might occur in enclosed spaces with no ventilation, for example, tanks have been reported to cause death from narcosis and anoxia.

Chronic Toxicity (or Exposure)

Animal

A 90 day rat oral gavage study resulted in depressed body and organ weights and depressed food consumption in high dose males (3600 mg kg^{-1} day^{-1}). No effects were noted at 900 mg kg^{-1} day^{-1}. From these results, the US Environmental Protection Agency derived an oral reference dose of 0.9 mg kg^{-1} day^{-1} for ethyl acetate (uncertainty factor = 1000).

Degeneration of the nasal olfactory mucosa was observed in rats exposed to 350, 750, or 1500 ppm ethyl acetate, 6 h day^{-1}, 5 days week^{-1} for 90 days. In the mid- and high-dose groups, mild, transient sedation was observed during exposure, and decreased bodyweight, bodyweight gain, and food consumption were observed postexposure. No treatment-related changes were evident in clinical observations, behavioral observations, motor activity, schedule controlled-operant behavior, ophthalmic examinations, urinalysis, organ weights, or the number concentration, motility, or morphology of sperm.

Human

Repeated inhalation exposure of humans to airborne concentrations exceeding 400 ppm is expected to produce irritation of the eyes, nose, and throat. Ethyl acetate can potentially cause drying and cracking of the skin with repeated dermal exposures because of its ability to defat the skin.

In Vitro Toxicity Data

Short-term studies of ethyl acetate in bacteria yielded equivocal results in a *Bacillus subtilis* rec assay for DNA damage/repair and negative (not mutagenic) results in the *Salmonella*/microsome reverse mutation assay.

Ethyl acetate induced aneuploidy in *Saccharomyces cerevisiae*. It yielded positive results in an *in vitro* sister chromatid exchange assay in Chinese hamster ovary cells. *In vitro* chromosomal aberration assays were positive in Chinese hamster fibroblast cells and negative in Chinese hamster ovary cells. (An *in vivo* bone marrow micronucleus study in Chinese hamsters yielded negative results by both intraperitoneal and oral administrations.)

Clinical Management

There is no specific antidote for ethyl acetate exposure. Treatment should be symptomatic and supportive. Inhalation and ingestion exposures often do not require treatment given the low acute toxicity and rapid hydrolysis of ethyl acetate. If large quantities are ingested, the individual should be monitored for CNS depression, respiratory function, and cardiac contractility. For inhalation exposure, the individual should be moved to fresh air and monitored for respiratory distress. A cough or breathing difficulties may indicate respiratory irritation, bronchitis, or pneumonitis. Artificial respiration should be administered if the individual is not breathing. If ethyl acetate comes in contact with skin or eyes, flush the affected areas with water and monitored for persistent irritation or pain. Contaminated clothing should be removed following dermal exposure. Dermal irritation or dermatitis caused by defatting of the skin from repeated dermal contact should be treated symptomatically.

Environmental Fate

Given its vapor pressure of 73 mmHg at 20°C, ethyl acetate will remain in the vapor phase if released to the atmosphere where it will react with photochemically produced hydroxyl radicals. It is expected to be quite mobile if released to soils ($\log K_{ow} = 0.73$). Volatilization from both dry and moist soils is also expected. If released to water, ethyl acetate will not adsorb to suspended or bed sediments. Volatilization from water is anticipated to be an important loss process (Henry's law constant $= 1.34 \times 10^{-4}$ atm $m^3\,mol^{-1}$). Biodegradation is also expected to be an important loss process in both soil and water. Ethyl acetate will not bioconcentrate in aquatic biota.

Ecotoxicology

The reported 96 h, no-observed-effect concentration in algae (*Selenastrum capricornutum*) is 2000 mg l^{-1}. The 48 h EC_{50} in *Daphnia pulex* is 262 mg l^{-1}, and the 96 h LC_{50} in fathead minnows (*Pimephales promelas*) is 230 mg l^{-1}.

Other Hazards

High airborne concentrations of ethyl acetate can form in poorly ventilated spaces with the potential for eye, nose, and respiratory tract irritation; CNS depression; and escape impairment. Ethyl acetate is a flammable liquid with a flash point of –4.4°C. Because its vapor density is heavier than air (density = 3.04), vapors can travel a considerable distance to an ignition source and flash back. Explosive concentrations of ethyl acetate can form in air, especially in enclosed spaces (lower explosive limit (LEL) = 2.2%).

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (ACGIH TLV), the National Institute for Occupational Safety and Health recommended exposure limit (NIOSH REL), and the Occupational Safety and Health Administration permissible exposure limit (OSHA PEL) for ethyl acetate are 400 ppm. Both the ACGIH TLV and OSHA PEL are 8 h time-weighted average (TWA) while the NIOSH REL is a 10 h TWA.

See also: Acetic Acid; Carboxylesterases; Ethanol; Fragrances and Perfumes; Volatile Organic Compounds (VOC).

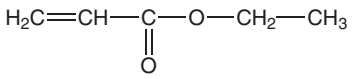
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Ethyl Acetate.

Ethyl Acrylate

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 140-88-5
- Synonyms: Ethyl 2-propenoate; 2-Propenoic acid ethyl ester; Acrylic acid ethyl ester
- Chemical/Pharmaceutical/Other Class: Ester
- Chemical Formula: $C_5H_8O_2$
- Chemical Structure:



Uses

Ethyl acrylate monomer is used to make acrylic resins as well as emulsion (water-based) and solution (solvent-based) polymers. Water-based ethyl acrylate polymers are used in latex paints, caulks, leather-treating base coats, floor polishes, and textile finishes. Solvent-based ethyl acrylate polymers are used in lacquers, enamels, and lubricating oils.

Exposure Routes and Pathways

Exposures to ethyl acrylate monomer are most likely to occur in an occupational environment via skin contact and inhalation. However, the closed systems used during manufacture and transportation will limit worker exposures to those that may occur during routine process maintenance, periodic plumbing leaks, and the collection of quality control samples. Under these conditions, exposures are further limited by the use of industrial hygiene controls and personal protective equipment. The acrid odor of ethyl acrylate, which can be detected at 0.001–0.005 ppm, also serves to limit exposure. Studies of monomer production workers have indicated that

mean exposures to ethyl acrylate are typically <1 ppm. The general population does not receive a significant exposure to ethyl acrylate due to low concentrations of residual monomer in consumer products.

Toxicokinetics

Data in experimental animals indicate that ethyl acrylate is readily absorbed from the gastrointestinal tract and the respiratory tract. Absorption of ethyl acrylate through the skin occurs less readily and may be limited by evaporation of ethyl acrylate if the applied dose is unoccluded.

The primary route of ethyl acrylate metabolism is its rapid hydrolysis by tissue and circulating carboxylesterases to acrylic acid and ethanol that undergo further metabolism to CO_2. In the rat, it has been estimated that $\sim$50% of the ethyl acrylate that passes through the upper respiratory tract is hydrolyzed by carboxylesterase in the nasal mucosa before entering the blood. Another route of ethyl acrylate metabolism is conjugation with the sulfhydryl group of glutathione. Both pathways serve to detoxify ethyl acrylate.

Ethyl acrylate is rapidly distributed throughout the body. Ethyl acrylate and/or its metabolites can be detected in all organ systems, with the highest concentrations being present in the urine, expired air, and organ of entry (i.e., stomach, upper respiratory tract, and skin). Metabolites of ethyl acrylate are excreted primarily via the lungs (as carbon dioxide) and the kidneys. Approximately 60% of an orally administered dose of ethyl acrylate is eliminated from the body as CO_2 within 8 h.

Mechanism of Toxicity

Pretreatment of rats with a carboxylesterase inhibitor enhances the respiratory irritation and lethality produced by the inhalation of ethyl acrylate. This and other observations suggest that the toxicity of ethyl acrylate becomes manifest when local detoxification/defense mechanisms become overwhelmed.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicological studies in animals indicate that ethyl acrylate exposures do not generally result in systemic toxicity at sublethal doses. Although ethyl acrylate concentrations approaching lethal doses may cause histopathological changes in the liver and kidneys, ethyl acrylate toxicity is largely limited to irritant effects, and their sequelae, at the site of application. Ethyl acrylate can produce an allergic contact dermatitis that may cross-react with other acrylic esters. The acute oral and dermal LD_{50} values in rats are 1120 and 3049 mg kg^{-1}, respectively. The 4 h LC_{50} for ethyl acrylate vapor is 2180 ppm (rat). Ethyl acrylate was not clastogenic in *in vivo* mouse micronucleus assays.

Human

Ethyl acrylate can be highly irritating to the skin, eyes, gastrointestinal tract, and the respiratory tract. Ethyl acrylate may cause an allergic contact dermatitis and may cross-react with other acrylate esters.

Chronic Toxicity (or Exposure)

Animal

In a 13 week gavage study, rats were exposed to ethyl acrylate at doses of 0, 7, 14, 28, 55, and 110 mg kg^{-1} for 5 days week^{-1}. Toxicity was limited to the high dose and consisted of changes in the stomach and duodenal mucosa. The histopathology of other organs, including the sex organs, was normal. The study of no-observed-adverse-effect level (NOAEL) was 55 mg kg^{-1}. In an inhalation study, rats and mice were exposed to ethyl acrylate concentrations of 0, 25, 75, and 225 ppm for 6 h day^{-1}, 5 days week^{-1} for either 6 months (high dose) or 27 months (mid and low doses, and control). The high dose was terminated due to significant loss in body weight. In both species, body weight was reduced at 75 ppm and dose-dependent degenerative changes to the olfactory epithelium and nasal turbinates were noted at all three concentrations. The histopathology of the sex organs was normal. Tumor incidences were not increased. The National Toxicology Program (NTP) studies reported forestomach tumors in rats and mice gavaged for a lifetime with 100 or 200 mg kg^{-1} ethyl acrylate. The NTP subsequently determined that these tumors resulted from localized irritation and cellular proliferation produced by high tissue concentrations of ethyl acrylate and did not provide an adequate basis for classifying ethyl acrylate as a potential human carcinogen.

In a developmental study, pregnant rats were exposed to ethyl acrylate at concentrations of 0, 25, 50, 100, or 200 ppm on days 6–20 of gestation. The highest dose produced a decrement in maternal body weight gain. Decrements in fetal body weight were observed only at 200 ppm; decreases in embryonic survivals and increases in fetal malformations were not observed at any concentration. The NOAELs for

maternal toxicity, developmental toxicity, and teratogenicity were 100, 100, and 200 ppm, respectively.

The normal sex organ histopathology noted in animals combined with the occurrence of rat fetotoxicity only in the presence of maternal toxicity suggests that ethyl acrylate does not pose a significant reproductive and developmental hazard to humans.

Human

Limited epidemiology data exist for exposure to ethyl acrylate. Mortality from cancer of the colon and rectum was elevated in workers from plants manufacturing and polymerizing ethyl acrylate; however, the findings were confounded by coexposure to other chemicals. Currently, there is inadequate evidence to link human exposures to ethyl acrylate with cancer.

In Vitro Toxicity Data

Data on the *in vitro* mutagenicity (*Salmonella* reverse mutation assay) are negative; positive responses in some *in vitro* assays occurred only in the presence of significant cytotoxicity.

Clinical Management

Clinical management involves removal from exposure and treatment of symptoms.

Environmental Fate

Ethyl acrylate is a volatile (38 hPa at 20°C) liquid under normal environmental conditions. At equilibrium in the environment, ethyl acrylate will partition primarily to air (94%) with lesser amounts to water (5.6%), soil (<1%), and sediment (<0.1%). In air, ethyl acrylate will be removed by reaction with photochemically produced hydroxyl radicals (11.8 h half-life) and ozone (33 h half-life). When released to water, ethyl acrylate will volatilize to air (Henry's law constant of 25 $Pa\,m^3\,mol^{-1}$) or be biodegraded (57% removal in 28 days). Based on its relatively low octanol–water partition coefficient (log K_{ow} of 1.18), ethyl acrylate does not pose a significant bioaccumulation hazard.

Ecotoxicology

Ethyl acrylate is acutely toxic to aquatic organisms. In a series of studies with analytically measured concentrations, ethyl acrylate exhibited a 96 h LC_{50} of 4.6 $mg\,l^{-1}$ in freshwater fish (rainbow trout), a 48 h LC_{50} (immobilization) of 7.9 $mg\,l^{-1}$ in an aquatic invertebrate (*Daphnia magna*), and an 96 h EC_{50} (growth rate) of 5.5 $mg\,l^{-1}$ in algae (*Selenastrum capricornutum*).

Other Hazards

Ethyl acrylate is flammable with lower explosive limit of 1.8% by volume in air.

Exposure Standards and Guidelines

International occupational exposure limits (OELs) for ethyl acrylate range from 5 to 20 ppm as an 8 h time-weighted average (TWA), with 5 ppm being the predominant value as in the case of the TWA OEL established by the American Conference of Governmental Industrial Hygienists (ACGIH). International short-term exposure limits (STELs) range from 10 to 80 ppm, with 15 ppm being the predominant value as in the case of the STEL established by the ACGIH. The US Occupational Safety and Health Administration lists a permissible exposure limit of 28 ppm for ethyl acrylate (TWA). The National Institute of Occupational Safety and Health indicates 300 ppm ethyl acrylate is immediately dangerous to life or health. Ethyl acrylate is classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer. Ethyl acrylate has been delisted by the US NTP as reasonably anticipated to be a human carcinogen because (1) rat forestomach tumors were seen only when ethyl acrylate was administered by gavage at high concentrations that resulted in marked local irritation and cellular proliferation, (2) animal studies by other routes of exposure, including inhalation, were negative, and (3) chronic exposure of humans to such high concentrations of ethyl acrylate is unlikely.

See also: Carboxylesterases; Respiratory Tract.

Further Reading

Sweeney LM, Andersen ME, and Gargas ML (2004) Ethyl acrylate risk assessment with a hybrid computational fluid dynamics and physiologically based nasal dosimetry model. *Toxicological Sciences*.

Relevant Websites

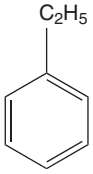
http://www.epa.gov – Ethyl Acrylate (from the US EPA's Air Toxics Website).

http://www.bibra.co.uk – Toxicity Profile for Ethyl Acrylate (from BIBRA International Ltd., Carshalton, Surrey, UK).

Ethyl Benzene

William S Utley

- Chemical Abstracts Service Registry Number: CAS 100-41-4
- Synonyms: Phenylethane
- Chemical Formula: C_8H_{10}
- Chemical Structure:



Uses

Ethyl benzene is used as an industrial solvent, and as a component in automotive and aviation fuels. The majority of ethyl benzene is used in the production of styrene.

Exposure Routes and Pathways

The primary exposure route for ethyl benzene is via inhalation and the skin. Ethyl benzene is known to cross the placental barrier but has not been established as a reproductive hazard.

Toxicokinetics

Ethyl benzene distributes to the adipose tissues. It is metabolized to mandelic acid (64%) and phenylglyoxylic acid (25%). The percentage of metabolites may vary according to the route of exposure with mandelic acid formation being favored with inhalation. The primary route of excretion is via the urine. Experimental evidence indicates that the percutaneous absorption rate of ethyl benzene is $37\,\mu g\,cm^{-2}$.

Mechanism of Toxicity

Ethyl benzene is an inducer of the cytochrome P450 and cytochrome *c* reductase enzyme systems. Ethyl benzene acts as a mitochondrial-uncoupling agent. It is believed that ethyl benzene metabolites are capable of interfering with dopamine catabolism in the brain. The tuberoinfundibular dopaminergic system may be a target for these metabolites.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ethyl benzene is extremely irritating in animal studies. Repeated skin application has caused blisters. Inhalation or ingestion of high concentrations has led to central nervous system (CNS) depression with death attributed to depression of the respiratory center. Pathological observations include pulmonary edema and generalized visceral hyperemia. The oral LD_{50} for ethyl benzene is $3500\,mg\,kg^{-1}$. The dermal LD_{50} in rabbits is $\geqslant 5000\,mg\,kg^{-1}$ and the LC_{50} is 4000 ppm (4 h exposure).

Human

Ethyl benzene is irritating to the eyes and skin. Concentrations of 200 ppm ethyl benzene are known to be irritating to the eyes of humans. Dermal application has led to erythema and inflammation of the skin. Ethyl benzene is the most irritating of the benzene series of compounds tested. Inhalation of high concentrations may cause CNS excitation followed by depression.

Chronic Toxicity (or Exposure)

Animal

Exposure to high concentrations has led to increase liver and kidney weights in experimental animals.

Human

Prolonged exposure may lead to functional pulmonary changes. These may be expressed as an increase in deep reflexes and irritation to the upper respiratory tract. Prolonged exposure has also led to hepatbiliary complaints. While there have been complaints of leukopenia and lymphocytosis, unlike benzene, ethyl benzene does not appear to cause bone marrow problems.

Clinical Management

General life support should be maintained, symptoms treated, and decontamination undertaken if necessary. Persons at special risk are those with impaired pulmonary functions, particularly obstructive airway diseases. The irritant properties of ethyl benzene may exacerbate these preexisting respiratory conditions. Additionally, predisposed groups include persons with liver, nervous system disorders, blood

and hemopoietic disorders, and women with ovulation and menstrual cycle disorders.

Environmental Fate

Ethyl benzene is expected to volatilize from surface water and soils where it can undergo degradation via photooxidation. With a K_{oc} of 520 there will be only moderate binding to the soil giving ethyl benzene a tendency to migrate in the soil column. Ethyl benzene will degrade in an aqueous environment with reported values ranging from 10 to 16 days. Ethyl benzene has a low potential to bioconcentrate with reported bioconcentration factors (BCFs) ranging form 0.67 to 15.

Ecotoxicology

Ethyl benzene is only moderately aquatically toxic expressing LC_{50} values in the range of 40–100 mg l^{-1} for bluegill, fathead minnow, and grass shrimp.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 100 ppm (435 mg m^{-3}) (8 h time-weighted average), based on irritation. The National Institute for Occupational Safety and Health short-term exposure limit (15 min exposure limit) is 125 ppm (543 mg m^{-3}), based on irritation. The odor threshold is 8.7 ppm. Hazardous waste number F003. The acceptable daily intake (US Environmental Protection Agency (EPA)) is 1.6 mg day^{-1}. The EPA oral reference dose (Rfd) for ethyl benzene is 0.1 mg kg^{-1} day^{-1}. The oral Rfd is based on liver and kidney toxicity observed in a subchronic rodent experiment.

Ethyl benzene is listed under section 111/112 of the Clean Air Act and is listed under section 304/307/311 of the Clean Water Act.

International Agency for Research on Cancer has classified ethyl benzene as a category 2B (possibly carcinogenic to humans, based on inadequate human data and sufficient animal data).

American Conference of Governmental Industrial Hygienists classifies ethyl benzene as an A3-confirmed animal carcinogen.

The comprehensive environmental response, compensation and liability act (CERCLA) reportable quantity is 1000 lb.

Ethyl benzene is listed under emergency planning and community right to know act (EPCRA) under section 313.

The European Union classifies ethyl benzene as Xn: R-20 (harmful by inhalation).

See also: Diesel Fuel; Jet Fuels.

Further Reading

Ethylbenzene. *IARC Mongr Eval Carcinog Risks Hum* 2000; 77: 227–266.

Tang W, Hemm I, and Eisenbrand G (2000) Estimation of human exposure to styrene and ethylbenzene. *Toxicology* 144(1–3): 39–50.

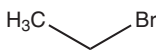
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Ethyl Benzene.

Ethyl Bromide

Kathryn A Wurzel

- Chemical Abstracts Service Registry Number: CAS 74-96-4
- Synonyms: Bromoethane; Bromic ether; Halon 2001; Hydrobromic ether; Monobromoethane
- Chemiical/Pharmaceutical/Other Class: Halogenated aliphatic hydrocarbons
- Chemcial Formula: C_2H_5Br
- Chemical Structure:



Uses

Ethyl bromide is used as a refrigerant, an ethylating agent in organic synthesis, a solvent in the chemical and pharmaceutical industries, a grain and fruit fumigant, and in ethylation of gasoline. It was earlier used as an anesthetic, both topically and by inhalation.

Exposure Routes and Pathways

Exposure to ethyl bromide may occur through oral, dermal, and inhalation exposures.

Toxicokinetics

Ethyl bromide may be hydrolyzed to a significant degree resulting in the formation of inorganic bromides. Glutathione *S*-alkyl transferase catalyzes conjugation in which the halogen group is replaced. Further metabolism then occurs to alkyl mercapturic acid sulfoxides. Ethyl bromide crosses the placenta. Unchanged ethyl bromide accounted for $\sim$70% of the dose in the expired air of rats dosed via gavage.

Mechanism of Toxicity

Ethyl bromide causes irritation and has a tendency to cause fatty degeneration of the liver, renal tissue, and the heart.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ethyl bromide is moderately toxic by ingestion and intraperitoneal routes and mildly toxic by inhalation. It is an eye and skin irritant.

Guinea pigs exposed to 2.4% ethyl bromide in air for 30 min experienced some delayed deaths and pathological changes in lungs, liver, spleen, and kidneys. Ethyl bromide is a central nervous system (CNS) depressant causing pulmonary congestion, centrilobular necrosis of the liver, and diffuse nephritis.

Human

Ethyl bromide is markedly irritating to the respiratory tract and the eyes. It is moderately toxic by ingestion but bromide poisoning following acute ingestion is rare. Acute effects of ethyl bromide include CNS depression, coma, hypotension, tachycardia, respiratory distress, nausea and vomiting, headache, and vertigo. Ethyl bromide can produce acute congestion, edema, and liver and kidney damage. Fever may also occur. Serum bromide concentrations >50–$100\,\mathrm{mg\,dl^{-1}}$ are usually associated with signs of toxicity; bromide levels greater than $200\,\mathrm{mg\,dl^{-1}}$ are uniformly associated with signs of toxicity. There is significant interpatient variation in severity of symptoms at a given bromide level. Aftereffects of severe exposure may occur up to 30 h after the exposure has ceased.

Acute or chronic exposure may result in redness of face, dilation of pupils, and a rapid pulse. The former use of ethyl bromide as a human anesthetic produced respiratory irritation and caused some fatalities, either immediately due to respiratory or cardiac arrest or from delayed effects as evidenced at autopsy by pulmonary edema and fatty degeneration of the liver, kidney, and heart.

Chronic Toxicity (or Exposure)

Animal

A 2 year study indicated some evidence of carcinogenicity in male rats (pheochromocytomas of the adrenal gland and neoplasms of brain and lung) and equivocal results in female rats (neoplasms of brain and lung) and male mice (lung neoplasms). Female mice experienced an increase in uterine cancer.

Human

Chronic ingestion of excessive amounts of ethyl bromide may produce a toxic syndrome known as 'bromism'. The symptoms are behavioral changes, irritability, headache, confusion, anorexia, weight loss, lethargy, muscular weakness, and slurred speech. Incontinence may develop with chronic intoxication. Chronic intoxication usually develops over 2–4 weeks or longer and the condition is of long duration with symptoms disappearing slowly.

Individuals with certain medical conditions may be at increased risk from ethyl bromide exposure: skin disease, liver disease, kidney disease, chronic respiratory disease (particularly obstructive airway diseases), and cardiac disease with arrhythmias.

Dermal exposure to ethyl bromide can result in bromoderma, which is an erythematous, nodular, or acneform rash over the face and possibly the entire body.

Bromides cross the placenta and may be detected in the milk of nursing mothers. Case reports suggest that prenatal exposure may cause growth retardation, craniofacial abnormalities, and developmental delay.

No epidemiological data relevant to the carcinogenicity of ethyl bromide are available.

Clinical Management

Acute oral exposure is generally treated by emesis. Emesis is most effective when initiated within 30 min of ingestion.

Chronic overexposure is treated by rehydration and administration of sodium chloride (NaCl; salt) intravenously until symptoms are alleviated. Bromide clearance may be increased by rehydrating in conjunction with administration of diuretics. Severe cases may require hemodialysis.

Environmental Fate

Ethyl bromide is likely to be a vapor in the atmosphere where it may be degraded by reaction with

photochemically produced hydroxyl radicals. It is moderately mobile in soil. Volatilization is expected to be the most important fate process in soil and water although ethyl bromide is susceptible to hydrolysis in wet soil. It is not expected to adsorb to soil or sediment, or bioaccumulate to any great extent.

See also: Bromine; Gasoline.

Further Reading

Bromoethane. Inter-Organization Programme for Sound Management of Chemicals (IOMC) (2002) Available from the World Health Organization, Distribution and Sales Service, 1211 Geneve 27, Switzerland, 2002. iv, 26P. 71 ref.

IARC (1999) *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 71, p. 1307. World Health Organization, International Agency for Research on Cancer, Geneva.

Ethylamine

Dale J Marino

- Chemical Abstracts Service Registry Number: CAS 75-04-7
- Synonyms: Ethanamine; Monoethylamine; Aminoethane; 1-Aminoethane
- Chemical/Pharmaceutical/Other Class: Amine
- Chemical Formula: C_2H_7N
- Chemical Structure: $C_2H_5NH_2$

Uses

Ethylamine is primarily used in the production of triazine herbicides. Ethylamine has also found use in resin chemistry, oil refining, and solvent extraction; as a stabilizer for latex rubber; and in the manufacture of dyestuffs, medicinals, corrosion inhibitors, urethane foam catalysts, and agents used in wash-and-wear fabrics.

Background Information

Ethylamine has been reported to occur in various fresh fruits and vegetables, grains, coffee, various cheeses, and fish. Ethylamine also occurs in the environment as a result of amino acid decomposition.

Exposure Routes and Pathways

Exposure to ethylamine primarily occurs in occupational settings. Given ethylamine's high vapor pressure of over 1 atm at 25°C, such exposures typically occur via inhalation, although dermal contact (and to a lesser extent ocular contact) with aqueous solutions of ethylamine would also be possible. The general population is potentially exposed to low concentrations of ethylamine by ingestion from food and by inhalation from releases to air.

Toxicokinetics

Ethylamine is not as readily metabolized as methylamine, and portions are excreted unchanged from the lung and in the urine. The metabolism of ethylamine is believed to occur in two stages. The amino group is initially dehydrogenated to an intermediate imine (ethyl imine), which reacts spontaneously with water, forming the corresponding aldehyde (acetaldehyde) and ammonia. The final metabolic products of acetic acid and urea are excreted in the urine. Ethylamine is a normal constituent of mammalian and human urine.

Mechanism of Toxicity

The toxic effects of ethylamine are due primarily to its corrosive action on tissues.

Acute and Short-Term Toxicity (or Exposure)

Ethylamine is severely irritating to the eyes, skin, and respiratory tract.

Animal

The acute rat oral LD_{50} is 400 mg kg^{-1} and the acute dermal LD_{50} in rabbits is 390 μg l^{-1} (270 mg kg^{-1}). The reported 1 h inhalation LC_{50} in rats is 5540 ppm (3010 mg m^{-3}). A 70% aqueous solution reportedly produced prompt skin burns, leading to necrosis when applied dermally to guinea pigs. Severe skin irritation with extensive necrosis and deep scarring were noted after a 2 h exposure. Severe eye irritation was observed in rabbits following instillation of 0.5 ml of 1% aqueous ethylamine.

Human

Exposure to elevated concentrations of various amines, and presumably ethylamine, is known to cause transient visual disturbance called glaucopsia,

which is often referred to as blue haze or halovision. Exposure to elevated levels of ethylamine is expected to potentially cause severe eye and respiratory tract irritation. Dermal contact with aqueous ethylamine is expected to cause severe skin irritation with possible skin burns. Ingestion of aqueous ethylamine is expected to cause burns of the mouth, throat, esophagus, and stomach with possible perforation of the esophagus and stomach.

Chronic Toxicity (or Exposure)

Animal

Repeated exposure of rabbits to 50 ppm ethylamine for $7\,h\,day^{-1}$, $5\,day\,week^{-1}$ for 6 weeks produced corneal erosion and lung irritation. Pulmonary irritation and kidney effects were noted at 100 ppm.

Ten exposures of rats to 250 or 1000 ppm ethylamine for $6\,h\,day^{-1}$ produced slight or moderate necrotizing irritation of the respiratory tract, respectively.

Repeated exposure of rats to 500 ppm ethylamine for $6\,h\,day^{-1}$, $5\,days\,week^{-1}$ for 24 weeks produced inflammatory necrosis and squamous metaplasia in anterior portions of the nasal cavity. No lesions were observed in the nasal cavities of rats in 0, 10, or 100 ppm exposure groups.

Human

Repeated exposures to higher concentrations are expected to produce irritation of the eyes, nose, and throat. Repeated exposures could potentially aggravate existing respiratory diseases.

In Vitro Toxicity Data

In vitro assays with ethylamine yielded negative (not mutagenic) results in the *Salmonella*/microsome reverse mutation assay, and an increase in sister chromatid exchanges in Chinese hamster V79 cells.

Clinical Management

Exposed skin and eyes should be irrigated with copious amounts of water. After inhalation exposures, the victim should be moved to fresh air and monitored for respiratory distress. Humidified, supplemental oxygen (100%) should be administered, with assisted ventilation as required. If coughing or breathing difficulties are noted, the patient should be evaluated for irritation, bronchitis, or pneumonitis, including chest X-rays and determination of blood gasses. If pulmonary edema is present, positive-end expiratory pressure ventilation and steroids should be considered. For ingestion exposures, emesis or lavage should be avoided. Use of diluents is controversial. Delayed abdominal pain and tenderness or shock may indicate gastric or esophageal perforation.

Environmental Fate

Given its high vapor pressure of over 1 atm at 25°C, ethylamine will remain in the vapor phase if released to the atmosphere where it will react with photochemically produced hydroxyl radicals ($T_{1/2}$ of ~ 14 h). Dissolution into rain droplets is also thought to be an important removal process. Other atmospheric removal processes, for example, photolysis and hydrolysis, are not significant.

The predominant form of ethylamine under environmental conditions is the ionized (protonated) species, which is expected to bind to soil constituents, suspended sediments, and bed sediments to a greater degree than the neutral form. As such, migration from soil to groundwater is expected to be less than would be anticipated for the neutral form. Volatilization from moist soils or surface water is not expected to be an important fate process. Biodegradation is expected to be an important loss process in both soil and water. The potential for bioconcentration in aquatic biota is low.

Ecotoxicology

The reported no-observed-effect concentration (NOEC) in algae (*Scenedesmus*) is $10\,mg\,l^{-1}$. The 24 h NOEC and EC_{50} values in crustaceans (*Daphnia magna*) are 31–52 and 94–110 $mg\,l^{-1}$, respectively. A nonlethal concentration of $30\,mg\,l^{-1}$ has been reported in fish (creek chub) with a 96 h LC_{50} of 40–240 $mg\,l^{-1}$ (goldfish).

Other Hazards

High airborne concentrations of ethylamine can form, given its vapor pressure, with the potential for severe eye, nose, and respiratory tract irritation; escape impairment; and possible death. The immediately dangerous to life or health (IDLH) concentration for ethylamine is 600 ppm. Anhydrous ethylamine is a flammable gas; aqueous ethylamine is a flammable liquid. Vapors can travel a considerable distance to an ignition source and flash back because ethylamine vapor density is heavier than air.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for ethylamine include the following:

- USA: Occupational Safety and Health Administration permissible exposure limit (Table Z-1) is 10 ppm (18 mg m^{-3}).
- USA: National Institute for Occupational Safety and Health recommended exposure limit is 10 ppm (18 mg m^{-3}).
- USA: American Conference of Governmental Industrial Hygienists threshold limit value is 5 ppm (9 mg m^{-3}), with a 15 min short-term exposure limit of 15 ppm (27 mg m^{-3}) (skin notation).
- Australia: 10 ppm.
- Federal Republic of Germany: 10 ppm (skin notation).
- Sweden: 10 ppm, with a short-term value of 15 ppm (skin notation).
- United Kingdom: 2 ppm with a short-term exposure level of 6 ppm.

See also: Corrosives; Respiratory Tract.

Relevant Websites

http://www.osha.gov – Ethylamine (Health Guidelines from the US Occupational Safety and Health Administration).

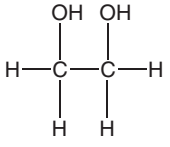
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Ethylamine.

Ethylene Glycol

Christopher P Holstege

This article is a revision of the previous print edition article by Edward Kerfoot, volume 1, pp. 573–575, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 107-21-1
- Synonyms: 1,2-Dihydroxyethane; 1,2-Ethanediol; Ethane-1,2-diol; Ethylene alcohol; Glycol alcohol
- Chemical/Pharmaceutical/Other Class: Glycols
- Chemical Formula: $C_2H_6O_2$
- Chemical Structure:



Uses

Ethylene glycol has numerous industrial and commercial applications. A major use is in antifreeze-coolant mixtures. It is also used in heat-transfer fluids, airport deicing fluids, hydraulic brake fluids, printers' inks, wood stains, adhesives, pesticides, and as a solvent in various other chemicals.

Exposure Routes and Pathways

The primary risk for ethylene glycol toxicity is through gastrointestinal exposure. Exposure can also occur by dermal, ocular, and inhalation routes.

Toxicokinetics

Following ingestion, ethylene glycol is rapidly absorbed and distributed. The volume of distribution is 0.5–0.8 l kg^{-1}. The ethylene glycol elimination half-life ($t_{1/2}$) is 3–9 h. This $t_{1/2}$ is prolonged to 11–18 h during ethanol and fomepizole therapy. Metabolism occurs primarily in the liver and kidneys. Ethylene glycol is first metabolized by alcohol dehydrogenase (ADH) to glycol aldehyde. Glycol aldehyde is then metabolized by aldehyde dehydrogenase to glycolic acid. Glycolic acid is converted to glyoxylic acid, which is the rate-limiting step in the metabolism. Glyoxylic acid is then metabolized to oxalic acid, α-hydroxy-β-ketoadipic acid, or glycine. Ethylene glycol and its metabolites are subsequently excreted in the urine.

Mechanism of Toxicity

Ethylene glycol and glycoaldehyde have an intoxicating effect on the central nervous system that can lead to ataxia, sedation, coma, and respiratory arrest. The metabolic acidosis reported in toxicity is due to the acidic metabolites, especially glycolic acid. Ethylene glycol itself may result in a large osmolar gap. Oxalic acid may combine with calcium to form calcium oxylate crystals. The precipitation of these crystals in tissue may result in renal failure and hypocalcemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ethylene glycol causes toxicity in animals similar to the toxicity seen in humans. The clinical management is similar to that described above.

Human

Acute exposure to ethylene glycol usually occurs when single large doses are ingested either accidentally or after intentional suicide attempts. Ingestion of ethylene glycol may initially lead to a state of intoxication similar to ethanol intoxication. This is associated with ataxia, slurred speech, nystagmus, somnolence, coma, and apnea. Ethylene glycol intoxication may lead to an elevated osmol gap due to ethylene glycol itself. A low or normal osmol gap does not rule out ethylene glycol toxicity. As metabolism occurs, the osmol gap begins to diminish and an anion gap metabolic acidosis becomes more pronounced. Renal effects include oliguria, acute tubular necrosis, and renal failure. Other reported clinical effects include mydriasis, focal nerve paralysis, seizures, cardiac disturbances, and cerebral and pulmonary edema. Hypocalcemia may result in tetany, hyperreflexia, and dysrhythmias. Inhalation or eye contact with ethylene glycol vapor may cause upper respiratory tract and eye irritation.

Chronic Toxicity (or Exposure)

Animal

Rats fed 1–2% ethylene glycol in their diets over 2 years demonstrated decreased life span, calcium oxalate bladder stones, plus renal and hepatic injuries.

Human

Human volunteers exposed to ethylene glycol for 20–22 h a day of 1.4–27 ppm developed only mild symptoms of throat irritation, mild headache, and low backache.

In Vitro Toxicity Data

No mutagenic activity was demonstrated in Ames *Salmonella* tests of ethylene glycol.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Because of the rapid absorption of ethylene glycol, gastrointestinal decontamination procedures are rarely indicated. Charcoal does not bind ethylene glycol and should not be administered. Administration of ethanol or fomepizole should be considered in any symptomatic patient or any patient with a history of significant ingestion. Both ethanol and fomepizole competitively inhibit ADH and prevent metabolism of ethylene glycol to its toxic metabolites. Careful correction of fluid and electrolyte abnormalities is essential. The clinician should insure adequate urine output, but forced diuresis should be avoided. Administration of sodium bicarbonate infusions should be considered in patients manifesting significant acidosis. Administration of thiamine and pyridoxine should be considered as these agents potentially enhance metabolism of ethylene glycol to α-hydroxy-β-ketoadipic acid and glycine, respectively, both of which are less toxic than oxalic acid. Hemodialysis effectively increases clearance and improves fluid/electrolyte balance. This extracorporeal method of elimination should be considered in patients with acute mental status changes, renal failure, significant metabolic acidosis, or serum levels over 50 mg dl^{-1}. Inhalation exposures to ethylene glycol mist should be monitored for respiratory tract irritation. Humidified supplemental 100% oxygen should be administered. Exposed skin and eyes should be treated with irrigation and supportively.

Environmental Fate

Release of liquid ethylene glycol into the environment would be expected to result in volatilization of the substance. In the atmosphere, ethylene glycol is broken down photochemically to produce hydroxyl radicals with $\sim$2 day half-life. Release into soil results in near complete aerobic biodegradation within 4 days. Under anaerobic conditions, complete degradation is expected within 7 days.

See also: Ames Test; Ethanol; Pyridoxine; Thiamine.

Further Reading

Barceloux DG, Krenzelok EP, and Olson K (1999) American academy of clinical toxicology practice guidelines on the treatment of ethylene glycol poisoning. *Journal of Toxicology – Clinical Toxicology* 36: 537–560.

Jacobsen D, Sebastian CS, and Barron SK (1990) Effects of 4-methylpyazole, methanol/ethylene glycol antidote, in healthy humans. *Journal of Emergency Medicine* 8: 455–461.

Ethylene Glycol Monoethyl Ether

Christopher P Holstege

This article is a revision of the previous print edition article by Rhonda S Berger, volume 1, pp. 575–576, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 110-80-5
- Synonyms: 2-Ethoxyethanol; Cellosolve; Dowanol EE; Ektasolve EE; Ethyl cullosolve; Ethylene glycol ethyl ether; Glycol ethyl ether; Glycol monoethyl ether; Hydroxy ether; Jeffersol EE; Oxitol; Poly-solv EE
- Chemical/Pharmaceutical/Other Class: Glycol
- Chemical Formula: $C_4H_{10}O_2$
- Chemical Structure: $HOCH_2CH_2OC_2H_5$

Uses

Major uses include its use as a component of natural and synthetic resins; metal solvent for formulation of soluble oils; solvent for lacquers, lacquer thinners, dyeing, textiles, and varnish removers; carrier for printing ink; wafer fabrication process for semiconductor manufacturing; and anti-icing additive for aviation fuels.

Exposure Routes and Pathways

Exposure may occur by inhalation of the vapor, ingestion, and dermal contact.

Toxicokinetics

Ethylene glycol monoethyl ether (EGEE) is a colorless and nearly odorless liquid. It is miscible in aqueous and organic solutions, has a low vapor pressure, and is readily absorbed through skin, lungs, and gastrointestinal tract. It is metabolized by alcohol dehydrogenase to alkoxyacetic acids, primarily ethoxyacetic acid. The metabolites of EGEE are renally excreted. Less than 1% of absorbed EGEE is eliminated unchanged through the lungs. In one study, peak ethoxyacetic acid levels occurred 4 h after inhalation exposure with a terminal half-life of $\sim$24 h.

Mechanism of Toxicity

The potential toxicity of EGEE is believed to be due to its metabolites. The exact mechanism has not been clearly defined.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals acutely exposed to large quantities of EGEE developed dyspnea, weakness, paralysis, acute nephrosis, pulmonary edema, gastrointestinal hemorrhage, and intrauterine demise. Studies suggest a teratogenic potential of EGEE, predominantly affecting the cardiac, neurologic, and skeletal systems. The oral LD_{50} was $3\,g\,kg^{-1}$ in rats, $3.1\,g\,kg^{-1}$ in rabbits, and $1.4\,g\,kg^{-1}$ in guinea pigs.

Human

Reports of EGEE toxic exposures in humans are limited. EGEE is low in oral toxicity. It is not significantly irritating to the skin. It is potentially irritating to the eyes and mucous membranes depending upon the concentration. Potential central nervous system effects are similar to effects manifested by other solvents and include headache, drowsiness, weakness, staggering gait, tremor, and blurred vision. Aspiration pneumonitis may occur if EGEE is ingested. Unlike ethylene glycol, metabolic acidosis and urine oxalate crystals do not typically develop. Preliminary studies have suggested a potential for reproductive toxicity. No studies have definitively found carcinogenic activity of EGEE in humans.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies in mice over 2 years demonstrated almost no effects in doses up to 5%.

Human

Studies of painters exposed to EGEE have demonstrated increased presence of oligospermia, azospermia, and increased odds ratio for lower sperm count.

In Vitro Toxicity Data

Mutagenicity and carcinogenicity studies using the Ames *Salmonella* and *Escherichia coli* tests have been negative for EGEE.

Clinical Management

Exposure victims should be moved immediately to fresh air. Standard emergency supportive care

should be provided. Exposed eyes and skin should be rapidly and copiously flushed. Contaminated clothing should be removed. Charcoal and syrup of ipecac should be avoided as their efficacies in EGEE toxicity have not been demonstrated and these agents may increase the risk of pulmonary aspiration. The role of ethanol or of fomepizole as competitive inhibitors of alcohol dehydrogenase for EGEE toxicity has not clearly delineated. Theoretically, either agent may be of benefit to prevent formation of EGEE metabolites. Hemodialysis may be considered for substantial exposures in patients who have developed profound symptoms (e.g., metabolic acidosis).

Environmental Fate

EGEE is produced in large quantities for industrial purposes. Release of EGEE into soil and water should result in rapid degradation, based on biodegradation studies.

See also: Ethanol; Ethylene Glycol; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50).

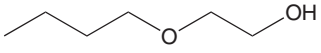
Further Reading

Johanson G (2000) Toxicity review of ethylene glycol monomethyl ether and its acetate ester. *Critical Reviews in Toxicology* 30: 307–345.

Ethylene Glycol Mono-*n*-Butyl Ether

Bradford H Strohm, Leonard I Sweet, Sharmilee P Sawant, and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: 111-76-2
- Synonyms: 2-Butoxyethanol; Butyl cellosolve; EGBE; Butyl glycol; Butyl oxitol
- Chemical/Pharmaceutical/Other Class: Glycol ether
- Chemical Formula: $C_6H_{14}O_2$
- Chemical Structure:



Uses

Ethylene glycol mono-*n*-butyl ether (EGBE) is a widely used solvent present in many surface coatings, lacquers, enamels, varnishes, varnish removers, inks, and latex paints. It is also used in metal cleaning formulas, commercially available household cleaners, and herbicide components.

Exposure Routes and Pathways

Exposure to EGBE can occur via inhalation, ingestion, or skin absorption. Inhalation is the primary route of human exposure. Exposure of the general population can also occur from dermal absorption during the use of products containing EGBE.

Toxicokinetics

EGBE is rapidly absorbed, distributed, metabolized, and eliminated. The uptake and elimination of EGBE is expected to be linear. The urine is the primary route of excretion followed by expiration in the form of the metabolite CO_2. The carboxylic acid, 2-butoxyacetic acid (BAA), is the major urinary metabolite of EGBE. Butoxyacetic acid is formed by oxidation of the alcohol moiety of EGBE through alcohol dehydrogenase and aldehyde dehydrogenase sequentially. Alternate pathways include *O*-dealkylation to ethylene glycol and conjugation to EGBE glucuronide and/or EGBE sulfate. In human, but not animal studies, the amino acid conjugate of EGBE, *N*-butoxyacetylglutamine, has been identified.

Rats administered EGBE in drinking water excrete 50–60% of the consumed dose in the urine as BAA and exhale 8–10% as CO_2. As dose increases, the proportion of EGBE conjugated with glucuronic acid also increases. Similarly, oxidative dealkylation of EGBE to form ethylene glycol also becomes a more prevalent route of metabolism as dose increases.

The relatively high clearance rate and low mean residence time of EGBE suggests that accumulation potential in the body is low. Elimination of EGBE and BAA following repeated inhalation exposure or dermal uptake appears to be dependent upon species, age, time of exposure, sex, and exposure concentration.

Mechanism of Toxicity

The principal toxicological effect observed upon overexposure to EGBE is the destruction of red blood cells (i.e., hemolysis). BAA, the predominant oxidative metabolite of EGBE, appears responsible for this hemolytic activity. It has been speculated that BAA may interact with red blood cell membranes disrupting erythrocyte osmotic balance, leading to cellular swelling, loss of deformability, and eventually

hemolysis. In studies with male rats, treatment with alcohol dehydrogenase inhibitors protected against EGBE-induced hematotoxicity and inhibited EGBE metabolism to BAA. Another event in the mechanism of action is compensatory erythropoiesis, where as a response to the loss of erythrocytes, the bone marrow increases production of young red blood cells.

In vitro studies have indicated that the red blood cells of rats, mice, rabbits, and baboons are susceptible to hemolysis by BAA, whereas blood from pigs, dogs, cats, guinea pigs, and humans are resistant. A number of other studies have confirmed these results *in vitro*, in red blood cells from a large cross section of the human population, including those with hereditary red cell disease (i.e., sickle cell and spherocytosis) and the aged. These studies indicate that human cells are not as susceptible to hemolysis as rat cells tested under similar conditions. These findings suggest that humans exposed to equivalent doses of EGBE would not be expected to exhibit the same spectrum or severity of hematotoxic-related effects as those produced in rats. *In vitro* experimental results also suggest that red blood cells are more sensitive to hemolysis by BAA than to hemolysis by EGBE.

Acute and Short-Term Toxicity (or Exposure)

Animal

EGBE acute toxicity has been studied in numerous species via all routes of exposure. In general, overexposed animals exhibited inactivity, weakness, and difficulty breathing, while autopsies revealed congested lungs and kidneys. The principal effect observed leading to death in these acute toxicity studies was damage to the kidneys. Kidney, hematologic, and central nervous system effects have been observed in experimental animals exposed to EGBE, with hemolysis as an initial and sensitive indicator of overexposure. Rats are the most sensitive species and older rats are more sensitive than younger animals to the hemolytic effects of EGBE and its metabolites.

Oral LD_{50} values ranged from $900\,mg\,kg^{-1}$ in rabbits to $250\,mg\,kg^{-1}$ in rats; dermal LD_{50} values were $\sim 1500\,mg\,kg^{-1}$ in rabbits; and inhalation LC_{50} values (4–8 h exposures) were in the vicinity of 500 ppm for cats, guinea pigs, and rats.

Human

EGBE is of low to moderate acute toxicity in humans. Clinical tests and reports from occupational exposures indicate EGBE is an irritant when inhaled. EGBE is considered a skin absorber, with the possibility of significant uptake through the skin. EGBE has not been found to be a sensitizer in clinical tests. Metabolic acidosis, hypokalemia, and hemoglobinuria paralleled by progressive erythropenia, have been reported in individuals poisoned through ingestion of materials containing EGBE. Human volunteers exposed to 98–200 ppm EGBE for 4–8 h reported nasal and ocular irritation and disturbed taste (e.g., metallic taste). No abnormalities were detected in blood pressure, pulse rate, erythrocyte fragility, urinary glucose, or albumen. The estimated immediately dangerous to life or health air concentration is 700 ppm. Severe toxicity has been described in adults who ingested 30–60 ml of pure EGBE; and the estimated lethal oral dose is $\sim 1.4\,ml\,kg^{-1}$.

Chronic Toxicity (or Exposure)

Animal

The subchronic toxicity of EGBE has been examined in animals via oral, inhalation, and dermal routes of exposure. The lowest no-observed-effect level (NOEL) in an oral subchronic study was $80\,mg\,kg^{-1}$ for rats administered EGBE in feed over a 90 day period. Inhalation exposure of rats for 13 weeks, $6\,h\,day^{-1}$, 5 days $week^{-1}$ to EGBE vapors at 25–77 ppm indicated an NOEL of 25 ppm. In a 90 day dermal study of rabbits, EGBE was applied $6\,h\,day^{-1}$, 5 days $week^{-1}$ at doses up to $150\,mg\,kg^{-1}$. There was no evidence of systemic toxicity or skin irritation at the site of application at any of the dose levels tested.

Studies on male and female rats and mice conducted by the National Toxicology Program have yielded mixed results on carcinogenic potential: male rat (no evidence of carcinogenicity); female rat (equivocal evidence); male mice (some evidence); and female mice (some evidence). The relevance of these studies to humans is uncertain. The American Conference of Governmental Industrial Hygienists (ACGIH) classifies EGBE as a confirmed animal carcinogen with unknown relevance to humans.

The reproductive and developmental toxicity of EGBE in both male and female animals has been the subject of numerous investigations. EGBE has been found to have no-adverse-effects upon the male reproductive systems of mice or rats exposed orally at doses ranging from 222 to $2000\,mg\,kg^{-1}$, 5 days $week^{-1}$ for 5 or 6 weeks. Similarly, inhalation exposure of rats for 3 h produced no-observed-effects upon gross macroscopic postmortem examination.

EGBE has also been tested for effects upon the female reproductive system and the developing

embryo. In general, fetal toxicity has only been observed in animals at maternally toxic doses. Mice, rats, and rabbits have been exposed during gestation at doses of 4000 mg kg^{-1} day^{-1} (oral), 424 mg kg^{-1} day^{-1} (dermal), and 25–200 ppm 6 h day^{-1} (inhalation). No teratogenic effects were observed in the litters of dams exposed to EGBE. Signs of maternal toxicity, including decreased body weight and body weight gain, were observed. At the maternal LD_{20}, EGBE did induce fetal deaths in rats. BAA, the metabolite of EGBE, was also studied and found to have no adverse effect upon the developing embryo *in vitro*.

Human

Long-term or repeated exposure may have effects on the hematopoietic system, resulting in blood disorders. The hematotoxicity in humans is characterized by decreased hemoglobin content, progressive erythropenia and hemoglobinuria.

Clinical Management

Management of individuals overexposed to EGBE begins with removing those individuals from the source of exposure, flushing eyes and skin with water, and removing contaminated clothing. The treatment of choice for acute and severe hemolytic anemia, which may result from overexposure to EGBE, is exchange transfusion. If renal failure develops as a consequence of red blood cell hemolysis, hemodialysis is the treatment of choice. In general, monitoring of blood counts, electrolytes, urine hemoglobin, urinary BAA levels, and blood gases may prove useful in assessing overexposure. Administration of ethanol as well as charcoal as a slurry have proven useful in therapeutic intervention after EGBE overexposure.

Environmental Fate

If released to the environment, EGBE is expected to preferentially partition to the soil and water. Bioconcentration and bioaccumulation potential are expected to be low, based on the estimated bioconcentration factor and experimental octanol water partition coefficient. If released to soil or water, aerobic degradation is expected to occur rapidly. Volatilization may be an important fate and transport process based on the Henry's law constant and vapor pressure. When released into the water, EGBE is expected to have a half-life of <10 days. When released into the air, it is expected to have a half-life of <1 day, and is expected to be removed from the air by wet deposition.

Ecotoxicology

Based on the available data, risk to aquatic organisms is low.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for EGBE include the following:

- ACGIH (20 ppm time-weighted average (TWA));
- Argentina (50 ppm TWA);
- Australia (25 ppm TWA);
- Belgium (25 ppm TWA);
- Brazil (39 ppm TWA; for a 48 h work week);
- Canada (25 ppm TWA);
- Chile (20 ppm TWA);
- Denmark (20 ppm TWA);
- Finland (20 ppm TWA);
- Germany – DFG (80 ppm peak limitation);
- Mexico (26 ppm TWA);
- Sweden (10 ppm threshold limit value; lowest limit value);
- United Kingdom (25 ppm TWA); and
- United States Occupational Safety and Health Administration permissible exposure limit (50 ppm TWA).

Miscellaneous

EGBE is a colorless liquid with a mild ether odor. Odor is generally detected at concentrations from 0.1 to 0.35 ppm.

See also: Glycol Ethers.

Further Reading

Agency for Toxic Substances and Disease Registry (1998) *Toxicological Profile for 2-Butoxyethanol and 2-Butoxyethanol Acetate*. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

Boatman R, Corley R, Green T, Klannig J, and Udden M (2004) Review of studies concerning the tumorigenicity of 2-butoxy-ethanol in B6C3F1 mice and its relevance for human risk assessment. *Journal of Toxicology and Environmental Health, Part B: Critical Reviews* 7(5): 385–398.

Gualideri JF, deBoer L, Harris CR, and Corley R (2003) Repeated ingestion of 2-butoxyethanol: Case report and literature review. *Journal of Toxicology: Clinical Toxicology* 41(1): 57–62.

National Toxicology Program (2000) Technical Report on the Toxicology and Carcinogenesis Studies of 2-Butoxyethanol in F344/N Rats and B6C3F$_1$ Mice. Research Triangle Park, NC: US Department of Health and Human Services, Public Health Service.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for EGBE.

Ethyleneimine

Shayne C Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 1, pp. 577–578, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 151-56-4
- Synonyms: Azocyclopropane; Dimethylenimine
- Chemical/Pharmaceutical/Other Class: Alkyl amines
- Chemical Formula: C_2H_3N
- Chemical Structure:

Uses

Ethyleneimine has a broad range of applications that stem from its high reactivity. Ethyleneimine is used in the manufacture of triethylenemelamine (i.e., a precursor in plastic synthesis) and the polymer polyethyleneimine. It is also used in textile chemicals, adhesives, binders, petroleum refining chemicals, fuels, lubricants, coating resins, varnishes, lacquers, agricultural chemicals, cosmetics, ion exchange resins, photographic chemicals, surfactants, and as an alkylating agent.

Exposure Routes and Pathways

Contact with skin or mucous membranes (eyes and nasal) and inhalation are the routes of exposure.

Toxicokinetics

Ethyleneimine is absorbed readily by the oral, dermal, and inhalation routes. It penetrates the skin so quickly that its toxicity is not decreased even if the area of contact is washed 1 min after contact. Urinary excretion accounts for ~50% of administered doses.

Mechanism of Toxicity

Ethyleneimine is an extremely reactive alkylating agent that undergoes ring opening reactions with cellular nucleophiles.

Acute and Short-Term Toxicity (or Exposure)

Animal

The inhalation LC_{50} (1 h exposure) was 185 ppm in rats, 150 ppm in mice, and 170 ppm in guinea pigs. The oral LD_{50} was 15 mg kg^{-1} in rats and mice. Ethyleneimine exposure can result in extensive degeneration of the tissues but not sensitization. *In vivo* studies in mammalian systems have shown that ethyleneimine is strongly mutagenic to murine bone marrow cells *in vivo*.

Human

Breathing vapors causes nausea and vomiting, accompanied by a characteristic swelling of the face (mouth, eyelids, and throat). These symptoms disappear when the exposure ceases. Ethyleneimine can be very irritating to the skin, eyes, or mucous membranes. It is mildly corrosive to skin and mucous membranes. Fatal intoxication caused mainly by skin absorption has been observed.

Chronic Toxicity (or Exposure)

Animal

A study of rats receiving subcutaneous injections of ethyleneimine over 540 days found local (i.e., site of injection) sarcomas in some animals. Studies of mice receiving ethyleneimine by stomach tube for 2–4 weeks after birth, and then in the diet for 77–78 weeks found heptatomas and pulmonary tumors in many of the mice, and lymphomas in a small percentage of the mice. Another subcutaneous injection study in mice over 48 weeks of injections found sarcomas at the site of injection, along with some hepatomas, pulmonary tumors, and Harderian gland tumors over the 2 years of total observation.

Human

Exposure may cause cancer. The (US) Occupational Safety and Health Administration has categorized ethyleneimine as a carcinogen. The (US) National Institute for Occupational Safety and Health considers ethyleneimine to be a potential occupational carcinogen.

In Vitro Toxicity Data

Studies in viruses, prokaryotes, fungi, and algae were discussed. These systems have shown ethyleneimine to be strongly mutagenic. For example, studies in *Drosophila* have shown that ethyleneimine induces mitotic or meiotic chromosome aberrations and dominant or recessive lethal mutations. Ethyleneimine is strongly mutagenic to Chinese hamster ovary cells *in vitro*. Further, ethyleneimine has been shown to induce large numbers of chromatid type aberrations *in vitro* in human WI-36 cells and leukocytes. The DNA in cultivated lymphocytes is degraded indicating that ethyleneimine inhibits DNA repair system.

Clinical Management

In acute situations, the skin should be washed thoroughly with soap and water. If ingested an emetic should be administered or gastric lavage performed. Oxygen should be provided if breathing is difficult. If severe blood poisoning occurs, 1% methylene blue solution should be given at 1 ml kg^{-1} intravenously.

Environmental Fate

Ethyleneimine may be released to the environment as emissions or in wastewater connected with its manufacture and use. It is a reactive molecule; however, there are no data on its fate in environmental media. In the atmosphere, it should react with hydroxyl radicals (the estimated half-life is 1.5 days). If released in water, it will hydrolyze at neutral pH in about 5 months but it is apt to be lost much faster by evaporation or chemical reactions with metal ions. While it should rapidly evaporate from soil, it may also leach into the soil or complex with metal ions in the soil.

Ecotoxicology

There is no bioconcentration or bioaccumulation; however, it would not be expected to bioconcentrate in fish.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration (OSHA) has categorized ethyleneimine as a carcinogen, and has stated that worker exposure to ethyleneimine is to be controlled through the required use of engineering controls, work practices, and personal protective equipment, including respirators. Ethyleneimine is listed by the US Environmental Protection Agency as a hazardous air pollutant generally known or suspected to cause serious health problems.

See also: Carcinogenesis; Toxicity Testing, Mutagenicity.

Further Reading

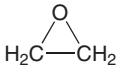
Bingham E, Corhssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, 5th edn., vol. 4, pp. 1108–1112. New York: Wiley.

International Agency for Research on Cancer (IARC) (1999) Aziridine. *IARC Monographs on Evaluation of Carcinogenetic Risks in Humans* 71: 337–344.

Ethylene Oxide

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 75-21-8
- Synonyms: 1,2-Epoxyethane; Oxirane
- Chemical/Pharmaceutical/Other Class: Epoxides
- Chemical Formula: C_2H_4O
- Chemical Structure:



Uses

Ethylene oxide is typically manufactured by the catalytic oxidation of ethylene. Most ethylene oxide is produced and consumed captively in the production

of ethylene glycol. It is also used in the production of nonionic surfactants, polyester resins, and specialty solvents. A small percentage of ethylene oxide consumption is attributed to its use as a sterilant for medical devices and pharmaceuticals.

Exposure Routes and Pathways

Ethylene oxide is a gas at room temperature and pressure; therefore, inhalation is the primary route of exposure. Dermal exposures may occur to liquid ethylene oxide that exists at temperatures below 11°C; however, rapid evaporation minimizes the opportunity for absorption. Background exposures to ethylene oxide occur due to its presence in cigarette smoke and automobile exhaust as well as its conversion from ethylene normally present in the body as the result of metabolic processes and the consumption of plants where it is a natural hormone.

Toxicokinetics

Ethylene oxide is rapidly absorbed through the respiratory tract. Due to its high solubility in blood, uptake is largely dependent on the ventilation rate and the concentration of ethylene oxide in the inspired air. Studies in mice indicate that at $\sim$0.5 ppm, $\sim$100% of the inspired ethylene oxide is absorbed. At higher concentrations, the percentage absorbed decreases from 90% (10 ppm) to 68% (100 ppm) and falls to 36% at 1000 ppm. Humans exposed to ethylene oxide at levels ranging from $\sim$0.1 to 10 ppm absorb 75–80% of the inspired ethylene oxide.

Absorbed ethylene oxide is rapidly distributed throughout the body. In mice exposed by inhalation to radiolabeled ethylene oxide, distribution was immediate, with the highest concentrations of ethylene oxide or its metabolites in the lungs, liver, and kidneys. After 4 h, levels in the liver and kidney had decreased and were comparable to those detected in the lungs, testes, spleen, and brain.

Ethylene oxide is metabolized by either conjugation with glutathione or hydrolysis by epoxide hydrolase. Metabolites from both pathways are excreted primarily in the urine, although some are further metabolized to CO_2 and exhaled via the lungs along with a small amount of unmetabolized ethylene oxide. While metabolism of ethylene oxide is qualitatively similar among species, the glutathione pathway appears to predominate in mice and rats while the epoxide hydrolase is the primary metabolic pathway in larger species, including man. The half-life of ethylene oxide in the blood of mice (3–12 min), rats (9 min), and humans (42 min) is relatively short.

Mechanism of Toxicity

The mechanisms of toxicity are not yet understood; however, it is likely that, in general, the toxic effects of ethylene oxide are due to its ability to react with cellular molecules, altering function. The carcinogenicity of ethylene oxide noted in experimental animals is probably due to its direct alkylation of nucleic acids.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute 4 h inhalation LC_{50} values range from 835 ppm in mice to 1460 ppm in rats, with an intermediate value of 960 ppm in dogs. Oral exposures of rats and mice to ethylene oxide in water or corn oil have LD_{50} values of 250–350 mg kg^{-1}. For both routes of exposure, the dose–response curve for mortality is steep. Effects associated with overexposure via inhalation include eye and respiratory tract irritations, central nervous system (CNS) depression, salivation, vomiting, incoordination, and convulsions. Deaths shortly after exposure are typically associated with pulmonary edema, while later deaths are thought to result from secondary lung infections with potential contribution from systemic toxicity. Study survivors may exhibit bronchitis, pneumonia, dyspnea, and muscle paralysis, particularly of the hindlegs. Histopathological examinations show damage to the lung, liver, kidney, spleen, and brain. Aqueous solutions of ethylene oxide are irritating to the skin but have not been associated with sensitization. Corneal opacities have been noted in some species.

Human

At high concentrations, ethylene oxide acts as an eye and respiratory irritant as well as a CNS depressant. Symptoms of overexposure include nausea, vomiting, and neurological effects. Pulmonary edema may result. Contact with liquid ethylene oxide or its solutions may result in irritation and burns as well as frostbite from evaporative cooling.

Chronic Toxicity (or Exposure)

Animal

Cancer is generally considered the critical endpoint for chronic exposures. In lifetime studies, rats exposed to airborne concentrations of 10, 33, or 100 ppm for 6 h day^{-1}, 5 days week^{-1} exhibited several treatment-related tumors including mononuclear cell leukemia, peritoneal mesothelioma, and

brain tumors. Ethylene oxide was also carcinogenic in mice. Greater exposures (>50 ppm) can cause reproductive toxicity (changes in sperm count, motility and morphology, increased postimplantation losses, decreased litter size) and neurotoxicity (abnormal gait, paralysis, axonal degeneration). Lung damage (edema, pneumonia) is associated with exposures in the range of 100–300 ppm. Very large exposures (>900 ppm) can produce teratogenicity.

Human

Studies of chronically exposed populations suggest that ethylene oxide may cause allergic contact dermatitis and cataracts. Neuropsychological, peripheral, and central nervous system deficits have been reported in hospital workers thought to be exposed to ethylene oxide in the range of 15–250 ppm. There is suggestive but inconclusive evidence from epidemiological studies that ethylene oxide exposures are associated with hematological cancers or reproductive toxicity (i.e., spontaneous abortions).

In Vitro Toxicity Data

Ethylene oxide is regarded as a direct acting mutagen and/or clastogen in a wide range of organisms from bacteria to mammalian cells.

Clinical Management

If contact with the liquid or its solutions occurs, affected areas should be flushed thoroughly with water for at least 15 min. The areas should be observed for burns or resulting irritation. In case of inhalation of ethylene oxide, the victim should be moved to fresh air, an airway should be established, and respiration should be maintained as necessary. The victim should be monitored for irritation, bronchitis, and pneumonitis. If excessive exposure occurs, hospitalization and monitoring for delayed pulmonary edema is recommended.

Environmental Fate

Ethylene oxide released to the environment will partition primarily to the atmosphere due to its high volatility. Although the high water solubility of ethylene oxide indicates it can be extracted from air by rainfall, its rapid volatilization from water (half-life of 1 h) argues against this process being a significant factor in its environmental fate. In the atmosphere, ethylene oxide reacts with hydroxyl radicals resulting in a half-life of 1–12 months. The hydrolysis half-life for ethylene oxide in water and soil is ~1 week. In fresh water, ethylene oxide is hydrolyzed to ethylene glycol; in salt water, it is hydrolyzed to ethylene glycol and ethylene chlorohydrin. In unacclimated aqueous media, ethylene oxide is also subject to biodegradation with estimated half-lives of 1–6 months (aerobic) and 4–24 months (anaerobic).

Ecotoxicology

The 24, 48, and 96 h LC_{50} values for fish are 84–90 $mg\,l^{-1}$. For the aquatic invertebrates, the 48 h LC_{50} values are 137–300 $mg\,l^{-1}$ (water flea) and 490–1000 $mg\,l^{-1}$ (brine shrimp). The 16 h IC_{50} value for ethylene oxide on activated sludge organisms is 10–100 $mg\,l^{-1}$.

Other Hazards

Ethylene oxide vapor is extremely flammable at concentrations ranging from 3% to 100% and subject to explosive decomposition. Although liquid ethylene oxide is relatively stable, contact with acids, bases, or heat, particularly in the presence of metal chlorides and oxides, can lead to a violent polymerization.

Exposure Standards and Guidelines

International occupational exposure limits (OEL) generally range between 0.1 and 39 ppm as an 8 h time-weighted average (TWA), with 1 ppm being the most common value. The US Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) have established an 8 h TWA OEL for ethylene oxide of 1 ppm. OSHA has also established a 15 min excursion limit of 5 ppm as well as an action level of 0.5 ppm, which if met or exceeded as an 8 h TWA for 30 or more days per year triggers additional requirements. Ethylene oxide has been judged a potential/suspected (ACGIH, National Institute for Occupational Safety and Health (NIOSH)) or known (International Agency for Research on Cancer, National Toxicology Program) human carcinogen. For this reason, NIOSH recommends workplace exposure is maintained below 0.1 ppm as an 8 h TWA. NIOSH also lists a concentration of 800 ppm ethylene oxide as immediately dangerous to life or health.

See also: Respiratory Tract; Sensory Organs.

Further Reading

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glycidyl ethers and aromatic monoglycidyl ethers. In: Bingha E, Cohrssen, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 6, pp. 993–1085. New York: Wiley.

Relevant Website

http://www.inchem.org – Concise International Chemical Assesment Document 54, search for Ethylene Oxide

EU *See* European Union and Its European Commission.

Excretion

Jules Brodeur and Robert Tardif

Excretion is the process by which chemicals are eliminated from the body. When chemicals gain access to the body, they usually do so as lipid-soluble molecules. In order to be eliminated, most of them must first undergo biotransformation to become more water soluble and, consequently, more easily excreted. Biotransformation and excretion are therefore the main processes involved in the elimination of chemicals.

The most important routes for excretion are urine, feces, and exhaled air; others include milk, sweat, saliva, tears, and hair.

The mechanism responsible for excretion of chemicals is usually (but not exclusively) passive diffusion; lipid-soluble, electrically neutral molecules find a passage through cellular membranes by solubilizing within the lipids of the membrane. The concentration gradient of the chemicals between each side of the membrane acts as a driving force for this process, directing the movement of the molecules from the side of the membrane with a high concentration to the side with a low concentration. Other mechanisms for excretion include filtration through pores in cell membranes and active transport provided by specialized carrier proteins.

Urinary Excretion

This is the most important route for excretion. The kidney is made of several functional units called nephrons. The initial segment of the nephron is a tuft-like structure called the glomerulus, which acts as a filter for plasma; once filtered under the driving force of circulating blood pressure, plasma fluid becomes diluted urine. During the process of filtration, some of the endogenous substances that are dissolved in the plasma can also filter freely altogether with plasma water. The next segment of the nephron is a long tubular structure that allows exchange of water and solutes between the newly formed urine and the blood circulating in the kidney.

Chemicals behave as endogenous solutes; they may filter through the glomerulus and then undergo exchange along the tubular segment to be partially reabsorbed into the blood or excreted. Electrically neutral molecules are subject to reabsorption from urine into blood by simple passive diffusion, moving along a concentration gradient. For drugs that are weak electrolytes, as it is the case for many therapeutic agents, urinary pH has a considerable influence on excretion. At a moderately alkaline pH, weak organic acids are present mostly as ionized or electrically charged molecules; this prevents their diffusion from urine back into blood and facilitates their elimination with the voided urine. The same occurs with weak organic bases at a moderately acidic pH. Altering the pH of urine is currently used to enhance elimination of chemicals in certain drug poisonings (e.g., salicylates and phenobarbital).

Passive diffusion is not the sole mechanism by which chemicals are exchanged between urine and blood in the tubular segment of the nephron. For some agents, active transport, even against a concentration gradient, is another means by which molecules may be transferred from urine to blood, but much more often from blood to urine. There are at least two types of specialized transport systems by which chemicals can be actively secreted in the urine. One is for weak organic acids like penicillin and certain diuretics; another is for weak organic bases like quinine. Like most active transport systems, these systems can be saturated at high concentrations of the transported chemicals and can also be blocked by other chemicals sharing the same transport system; for example, the transport of penicillin into urine can be prevented by concomitant administration of a drug known as probenecid.

In patients with severe renal impairment, kidney function can be effectively substituted by using an

artificial kidney. The latter exploits the properties of semipermeable membranes to allow elimination of endogenous waste materials. The same principle can be used to help remove certain freely diffusible chemicals in severe cases of poisoning (e.g., bromides, ethylene glycol, isopropyl alcohol, and lithium). This procedure is called hemodialysis.

Fecal Excretion

This is the second most important route for excretion. Chemicals present in feces are mainly those excreted in the bile but also, to a much smaller extent, those diffusing passively through the intestinal wall. Of course, chemicals that are not completely absorbed during their passage in the gastrointestinal tract are also found in the feces.

All chemicals absorbed in the gastrointestinal tract first reach the liver, where they normally undergo biotransformation to new, more water-soluble molecules (metabolites). Some of these will eventually be excreted in the bile. Thus, in addition to playing an important role in the digestion and intestinal absorption of fats, bile is also involved in the elimination of chemicals from the body.

Bile is formed by liver cells and is collected and transported in the biliary system, which comprises a series of ducts, from extremely small ones to larger ones, branching like a tree throughout the liver. In contrast to what happens in the kidney, the driving force for bile secretion is not the pressure of the circulating blood. It is rather a drawing pressure that is generated within the system of ducts by the presence of various solutes in the bile, creating a passive movement of fluids from liver cells and intracellular spaces (osmotic pressure). Solutes that contribute to create such pressure are bile acids and also smaller molecules like sodium, chloride, and bicarbonate ions. Bile collected at the very smallest ducts, next to each single liver cell, is later modified in larger ducts by processes of reabsorption or secretion of electrolytes and water. Ultimately, bile empties into the first segment of the small intestine, the duodenum.

Some endogenous and foreign chemicals, usually molecules with a molecular weight larger than 325 Da, will appear in bile at concentrations exceeding that in plasma by a factor of 10–1000. Biliary excretion is thus an important route of elimination for such chemicals. Bilirubin is an example of an important end-product of endogenous metabolism of red blood cells that is normally excreted in bile. The excretion of foreign chemicals is supported by various active, carrier-mediated, and saturable transport systems, thus enabling selective removal of organic acids (dyes like sulfobromophtalein and indocyanine green and various glucuronide conjugates of chemicals), organic bases, and neutral substances (ouabain, a cardiac stimulant). Lead (the metal) is also actively transported.

A significant portion of the more water-soluble metabolites secreted in bile is ultimately excreted in the feces. Some metabolites, however, may undergo further enzymatic modification by the intestinal bacterial flora to a state of greater lipid solubility. This metabolic step facilitates reabsorption of such chemicals and extends their life in the body. The process is known as the enterohepatic cycle.

Exhaled Air

Chemicals present in blood that are gases or possess a high degree of volatility diffuse passively into the alveolar air of the lung until they reach equilibrium. The concentration of these chemicals in the air phase is directly proportional to their concentration in blood, and the latter in turn is in equilibrium with the concentration of the chemicals in the tissues. This phenomenon can be applied to noninvasively monitor the presence and the concentration of gases and volatile substances in blood. A practical example of such application is the indirect measurement of alcohol present in blood by analyzing for ethanol in exhaled air with an instrument known as the Breathalyzer.

In industrial settings, exhaled air is used to monitor exposure to volatile organic solvents. A major drawback of this approach is the very high sensitivity of the analysis to rapid changes in exposure concentrations; such changes are rapidly reflected by parallel fluctuations in the concentrations of exhaled air. Under these conditions, point measurements represent exposure poorly over an entire work shift. When exposure concentrations fluctuate, as is usually the case during a work shift, it is recommended to analyze exhaled air the morning after exposure. At this time, blood concentrations of solvents are in equilibrium with concentrations in fatty tissues. The latter present the advantage of slowly and progressively taking up and releasing solvents, thus integrating the previous day exposure independently of the pattern of exposure. Point measurements of solvents in exhaled air the morning after exposure are therefore proportional to exposure during the entire previous work shift.

Milk

Human milk is essentially a solution of sugars and minerals forming a suspension medium for other important nutrients like fat globules and proteins.

Normal milk components are derived from maternal blood. Any extraneous chemical that enters blood circulation may also eventually appear in milk.

The transport of foreign chemicals from maternal blood into breast milk can proceed by a number of different mechanisms. Uncharged lipid-soluble molecules may diffuse passively through membranes, whereas small water-soluble and small charged molecules may cross membranes through minute pores or water channels. In addition, lactating cells may secrete nutrients like proteins and fat droplets; both can carry foreign chemicals, either bound to proteins or dissolved into fat droplets.

Nursing mothers taking medications can expect to transfer minute amounts of drugs to their child. However, at maternally therapeutic doses, the amounts transferred are too low to produce pharmacological effects. Over-the-counter analgesics like aspirin and acetaminophen, at usually recommended doses for the mother, should not represent a risk for the nursing infant. The same holds true for nonsteroidal anti-inflammatory agents (like ibuprofen) that are frequently used in self-medication for common aliments like arthritic conditions and musculoskeletal pain. For all prescription drugs, it is strongly recommended that nursing mothers ask a physician or pharmacist about the compatibility of medication with breastfeeding.

Caffeine, a central stimulant found in commonly consumed beverages, like coffee, tea, and certain soft drinks, is excreted in breast milk. Although newborn infants eliminate caffeine very slowly, normal consumption of caffeine is not contraindicated during nursing. Heavy coffee drinking, of course, is not recommended.

Ethanol diffuses readily in the water fraction of breast milk. Nursing mothers should refrain from chronic consumption of alcoholic beverages since such action is conducive to adverse effects on the intellectual and psychological development of the infant.

Drugs of abuse, like cocaine and heroin, are excreted in breast milk in amounts that may be clinically effective; such exposure is formally contraindicated during breastfeeding. Although no adverse effects in the infant have been reported in the case of mothers using marijuana, caution should be exercised.

Finally, lipid-soluble chemicals like the insecticide DDT, polychlorinated biphenyls, and methylmercury are excreted readily as dissolved chemicals into milk fat droplets. Lead is secreted into milk using the same transport system as calcium. Nursing mothers may therefore transfer environmental contaminants to their infants – not to the point, however, of negating the well-established benefits of breastfeeding, provided the milk is not too heavily contaminated with these chemicals.

Saliva

Saliva is not an important route of excretion since most of the chemicals present in saliva will eventually reach the gastrointestinal tract to be reabsorbed or eliminated in the feces. The unbound fraction of several therapeutic drugs may diffuse passively from plasma into saliva. This provides a noninvasive means of indirectly monitoring plasma concentrations of drugs like lithium, phenytoin, and theophylline. Metals like lead, cadmium, and mercury are also present in saliva.

Hair

Hair is an unexpected and only minor route of elimination for certain chemicals, especially metals. However, the presence of metals in hair has been used as a practical means of monitoring exposure to such chemicals.

Hair is formed from matrix cells present in a bulb-shaped follicle located in the dermis. During growth, hair is exposed to circulating blood and extracellular fluids; certain chemicals can then diffuse into cells producing the hair root and eventually the hair strand, where they will be fixed. The interesting aspect about monitoring exposure to metals using hair is the fact that metals will distribute along the hair strand exactly in the sequence of deposition while hair is growing. Knowing that human hair grows at a rate of $\sim 1\,\text{cm}$ month^{-1} makes it possible to monitor retrospectively exposure to certain toxic elements, like arsenic, cadmium, mercury, and lead, during the past several months and to establish the duration of exposure.

Part of the evidence that Napoleon Bonaparte suffered poisoning during his exile at St. Helena Island rests upon finding increased concentrations of arsenic in hair samples taken from the emperor's scalp.

In 1971, consumption of homemade bread prepared with flour containing a mercurial fungicide in Iraq led to severe intoxication. The degree of exposure to mercury was monitored using hair as the biological sample. A threshold for neurotoxic effects in children born to exposed pregnant mothers was established at values slightly above $10\,\mu\text{g}\,\text{g}^{-1}$ of maternal hair.

Currently, hair is used routinely to monitor exposure to methylmercury in fish-eating native populations of northern Canada. The objective is to adjust consumption of fish, an important, yet contaminated nutritional source, so as not to exceed concentrations of $30\,\mu\text{g}\,\text{g}^{-1}$ of hair in the general adult population and $15\,\mu\text{g}$ in fertile women.

Analysis of metals in hair is of limited practical value for monitoring exposure to metals in occupational settings due to the very distinct possibility of hair contamination with exogenous metals present in the ambient air.

See also: Absorption; Biotransformation; Distribution; Kidney; Liver; Metals; Pharmacokinetics/Toxicokinetics.

Further Reading

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Exposure

Gary Whitmyre and Sam Kacew

Introduction

The biosphere is composed of air, water, and soil media. These media contain chemicals of natural and man-made origin to which people can be exposed. In the broadest sense, the term exposure is defined as the condition of being in contact with or exposed to a chemical, physical, or biological agent. Exposure can be quantified as the amount of chemical available at the exchange boundaries of the organism ('external exposure' or 'potential exposure'). Exposure can also be quantified as an absorbed dose or internal exposure that accounts for the fraction of material that can pass through a biological barrier (e.g., skin, lung surface, gut). The physical course that a chemical takes from the source to the exposed individual is known as the exposure pathway. The manner in which an individual comes into contact with an exposure media (e.g., drinking water, food, air, soil) or a toxic agent is referred to as the route of exposure. Exposure or contact with a toxic agent does not necessarily mean that a harmful or toxic outcome will result (e.g., in the case of exposure to very small amounts of chemicals that are only moderately toxic). In certain situations, a given exposure does not produce an adverse effect, while under other conditions an adverse effect can occur (e.g., in the case of concurrent exposure to a different chemical with the same toxic mechanism of action). There are conditions under which exposure to some chemicals produces a beneficial effect (e.g., dietary exposure to trace metals that are important micronutrients or administration of therapeutic drugs such as antibiotics). Exposure becomes a concern only when contact with a toxic chemical or agent occurs at a level or amount that results in a harmful consequence to the individual. The agents to which an individual may be exposed include (1) gases (e.g., nitrogen oxide, carbon monoxide, methyl bromide); (2) nonvolatile inorganic chemicals (e.g., cadmium, mercury, lead); (3) biological agents and allergens (e.g., mold, mildew, dust mite allergens); (4) organic chemicals (e.g., solvents); and (5) radiation (e.g., γ-emitters, α-emitters, electromagnetic frequencies (EMF)). Consideration of exposure takes into account the routes of exposure, the dose, the duration of exposure, and the characteristics of the exposed population.

Exposure Routes and Pathways

Individuals may be exposed to toxic agents via a number of possible exposure routes. Exposure to a chemical in the process of the normal breathing of air is termed 'inhalation' exposure. Exposure to a toxic agent in water or food in the diet is termed 'ingestion' exposure. Exposure to a chemical through contact of a media with the skin is referred to as 'dermal' exposure; this exposure route can also be referred to as 'topical' (as in the case of a material intentionally applied to skin) or 'percutaneous' exposure. It should also be noted that the physiological conditions of pregnancy and lactation introduce two other routes of exposure. During pregnancy, there is an exchange of constituents and nutrition from mother to fetus, and removal of wastes from the fetus to the mother through the placenta. Because the placenta is porous to many toxic agents, they may pass from the mother's blood supply into the fetus, resulting in 'transplacental' exposure of the fetus. In addition, because the newborn infant is dependent on maternal milk for nutrition, certain agents may accumulate in the mammary tissue and be passed on to the fetus via human breast milk. Thus, the nursing infant can be

exposed to a variety of environmental chemicals that accumulate in the breast milk as well as directly to chemical in the biosphere and contaminants in environmental media (e.g., through inhalation of air, bathing).

Although exposure can occur normally through various routes, an artificial route of exposure allows a toxic agent to reach the blood directly. The administration of certain pharmaceuticals to humans and exposure in some laboratory animal studies involves introduction of the agent via injection through a needle penetrating the skin. This route of exposure is referred to as 'injection route' exposure. A route of exposure involving injection of a chemical or other agent directly into a vein is termed 'intravenous'. 'Subcutaneous' and 'intramuscular' exposure routes involve the injection of a chemical or agent below the skin layer, or into muscle tissue, respectively.

Certain consumer products or medical devices result in unique exposure routes by which individuals may be exposed to chemicals. For example, certain feminine hygiene products result in 'intravaginal' exposure to trace amounts of dioxins and other chemicals. Exposure route possibilities for chemicals in medical devices range from intravenous (e.g., for plasticizers such as DEHP that are leached from intravenous administration tubing sets) to local tissue exposure (e.g., in the case of chemicals leaching from endotracheal tubes and various types of implants). Some complex exposure situations, such as full immersion during swimming, may result in other minor exposure routes such as nose (nasal) and ear canal (aural) exposure. These latter two exposure routes may also be relevant to administration of some pharmaceuticals, such as nasal sprays and eardrops. Another exposure route relevant to ophthalmic formulations and certain irritation testing of chemicals involves administration to the cornea of the eye (occular). The major normal routes of exposure of humans to environmental chemicals and agents typically involve oral, inhalation, and/or dermal exposure routes. Because of the differences in the effectiveness of different biological barriers (e.g., the skin, gut, and lung) a given external exposure may result in a different internal exposure or absorbed dose depending on the route of exposure. Thus, a given external exposure may result in the following descending order of magnitude of absorbed dose for various exposure routes, as an example: injection > inhalation > oral > dermal.

A single individual may be exposed to a given chemical through multiple exposure routes. For example, a worker who is occupationally exposed to a pesticide (e.g., through inhalation and dermal contact during spraying of crops) can be exposed orally to the same pesticide through ingestion of food and contaminated well water, and through dermal contact with contaminants in water during bathing. Another example of multiple exposure routes is for cadmium, whereupon an individual who is occupationally exposed via the dermal and inhalation routes (e.g., smelter worker) can also be exposed via the diet and via inhalation of tobacco smoke (cadmium is a chemical found in tobacco smoke).

Dose

In considering the dose of a toxicant resulting from exposure, 'external' exposure at the biological barriers (e.g., skin) should be distinguished from 'absorbed dose' (e.g., amount of chemical reaching the blood reflecting partial absorption of the chemical), and from the dose at the target tissue (e.g., micrograms per gram organ tissue). The extent to which an exposed individual displays an adverse effect from exposure to a toxic agent depends, in part, on the dose or amount of chemical that reaches the target site. In an industrialized society, individuals are continuously exposed to numerous chemicals by several routes. If one took a sample of the exposed population, different individuals in the population would be receiving widely different doses. If a given exposure is insufficient to produce a toxic dose at the target site, then no adverse effect occurs. In addition, because of interindividual variation, it is likely that different individuals receiving the same external exposure will have different levels of response to the toxicant.

Various factors affect the concentration of a toxicant at the target site. The ability of a substance to be absorbed through the biological barriers (e.g., skin, gut, lung) to reach the blood and then to be distributed to tissues followed by excretion ultimately affects the dose at the target tissue. External exposure to a chemical is, thus, the first step in a multistep chain resulting in target tissue exposure. For example, with oral exposure, the chemical may be absorbed into the blood from the gastrointestinal tract, followed by transit to the liver and other tissues where conversion to a more toxic or less toxic metabolite may occur. The substance, or its metabolites, may be eventually removed from the body by excretion into the urine, feces, or breath (in the case of gases and solvents).

The units of dose are typically expressed in terms of amount of chemical per unit time (e.g., milligrams per day) or amount per unit time normalized to body weight (e.g., milligrams per kilogram per day). Because an individual can be exposed to a given

chemical from multiple sources (occupational, drinking water, food, air), the concept of 'aggregate exposure' was developed. In the United States and Canada, the aggregate exposure for a chemical is the sum of all exposures to that chemical by all relevant routes and from all relevant sources. This is different from 'cumulative exposure', which is the combined exposure of two or more chemicals that have the same mechanism of toxic action, summed across all relevant exposure pathways and all relevant sources. The concepts of aggregate exposure and cumulative exposure are widely used in the United States and Canada, for example, in considering exposures to pesticides.

As a first step to estimating exposure or dose, the concentration of a chemical in environmental media (e.g., drinking water, food), in biological fluids (e.g., urine, blood), or in biological tissues (e.g., adipose tissue) can be determined. A number of large-scale human exposure surveys have been conducted that can provide a starting point for estimating chemical exposures to the general population. The design of these studies can be generally characterized by probability-based selection of human subjects, and direct measurement of exposure to multiple chemicals in multiple environmental media and/or multiple body fluids/tissues. The first large-scale study of human exposures to chemicals was the Total Exposure Assessment Methodology (TEAM) study of the early 1980s, conducted by the US Environmental Protection Agency (US EPA). This study conducted personal monitoring of inhalation exposures to 25 volatile organic compounds (VOCs) for 550 individuals. Subsequent US EPA TEAM studies have addressed carbon monoxide, pesticides, and airborne particles. The Non-Occupational Pesticide Exposure Survey (NOPES) focused specifically on pesticide exposures in the United States not involving pesticide exposures in the workplace. Major European studies on indoor levels of VOCs and pesticides have taken place in the Netherlands and Germany. The German Environmental Survey (GerESII) measured selected metals and pesticides in blood urine, hair, house dust, drinking water, and indoor air. In the United States, a major multimedia study of human exposures to chemicals, known as the National Human Exposure Assessment Survey (NHEXAS) was initiated in 1993 by the US EPA and is ongoing. NHEXAS includes measurements of VOCs, pesticides, polycyclic aromatic hydrocarbons (PAHs), and heavy metals in indoor air, outdoor air, drinking water, food, soil, and house dust. Besides measuring chemical concentrations in these environmental media, NHEXAS also measures chemical levels on skin (via wipes), and in urine and blood. Further, in a recently updated study by the US Centers for Disease Control and Prevention called the National Health and Nutrition Examination Survey (NHANES), selected VOCs, lead dust, phthalates, pesticides, metals, PAHs, and dioxins were measured. Because NHANES has been conducted periodically over the last two decades, the overall data set provides time profiles from which long-term trends in exposures for some of the chemicals may be determined (e.g., declining exposures to for pentachlorophenol).

Duration and Frequency

An exposure is defined not only in terms of dose, but also in terms of the duration and frequency of exposure. Toxicologists usually think of exposure duration in four categories: acute (typically <24 h), subacute (repeated exposure for a month or less), subchronic (1 month to 1 year), and chronic (several years up to a lifetime of repeated exposures). While this general classification scheme was developed primarily in the context of toxicity testing of chemicals in laboratory animals, the same concepts apply to humans who may be exposed to environmental chemicals. Human exposures to many chemicals (e.g., for common air pollutants) may be chronic in nature, occurring daily over a lifetime (although the actual daily dose may vary from day to day and trend down over time as environmental controls improve). Some categories of human exposures (such as professional applicator exposures to specific pesticides over the growing season) may be more subchronic in nature, involving repeated exposures over several months. Some exposures (e.g., to toluene in spray paint) may be episodic, reflecting infrequent exposure; such exposures are more acute in nature. These defined exposures may be in addition to the daily background exposures to very small amounts of the same chemicals from other environmental sources.

Frequency is the temporal characterization of exposures. For humans, frequency can range from daily for air pollutants, to several times weekly for chemicals in certain household cleaning products, to infrequent or intermittent exposures where there are long periods measured in days, weeks, or months between exposure events (e.g., for exposure to an insecticide once a month during monthly treatment of pets for fleas). For chronic toxicants, it is the long-term time-amortized or time-averaged exposure that will determine whether an adverse effect will occur from repeated exposures. For some toxicants, the toxic effects following a single high exposure are quite different from the effects of repeated low exposures. For example, the primary toxic

manifestation of a single high exposure to benzene is central nervous system depression, whereas more moderate repeated exposures to benzene can result in leukemia. For some acute toxicants, dividing an exposure into repeated increments over a period of days generally elicits less of a toxic effect than if the entire amount is given as a single dose, because there is adequate time for biotransformation, excretion, and recovery, resulting in partial or complete reversal of effects prior to the next exposure.

Residential, Occupational, and Other Types of Exposures

It is evident that different members of the population are not exposed to identical doses of a given chemical because all individuals in a population are not exposed to the same environmental conditions. Concentrations of chemicals in environmental media vary throughout the biosphere, and individual differences in lifestyle, employment, housing characteristics, and geographic location result in different exposures to the same chemicals. Human time-activity studies in the US have shown that people on average spend about 16 h day^{-1} at home, 30 min of which may be in the backyard on average. Exposures in this environment are generally referred to as 'residential exposure'. Exposures to chemicals in the workplace, which can include for example, worker exposures during and after application of pesticides to crops, factory worker exposures to solvents, and office worker exposures to chemicals released from office supplies and equipment, are referred to as 'occupational' exposures. Some exposures that occur not in the home environment or at work can be referred to as 'commuting', 'recreational', 'child daycare', 'school place', or by other terms depending on the location of exposure or the type of exposure being studied or reported.

Sensitive Subpopulations

Sensitive populations may include individuals who, because of predisposing conditions, may be more sensitive to the effects of a toxicant. Sensitive populations may also include individuals who, because of their age, physiological characteristics, lifestyle, location, or dietary habits, may actually receive a higher exposure than typical exposures to the general population. Sensitive populations may include individuals who, because of asthma or prior lung damage (e.g., from smoking or industrial exposures), may be predisposed to respiratory effects being induced by much smaller amounts of respiratory irritants (such as aldehydes in urban air) than individuals who are not predisposed. Another example of a sensitive population is children who, because of eating paint chips contaminated with lead and their low body weight, may receive a significantly higher dose in milligrams per kilogram body weight per day than an average adult. Because of the age of children and their continuing physiological development, they are also more prone to learning deficits from small amounts of lead compared to adults. Subsistence and Native American fishermen may have higher exposures to PCBs, dioxins and other lipophilic chemicals because of bioaccumulation in fish and the higher fish consumption rate (grams per day) of this subpopulation compared to other individuals in the general population.

See also: Exposure Assessment; Exposure Criteria; Medical Surveillance.

Further Reading

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Exposure Assessment

Gary Whitmyre and Jeffrey H Driver

Exposure assessment is one of the four major steps in the risk assessment paradigm, as defined by the National Academy of Sciences in the United States. Thus, exposure assessment is a key element in any quantitative risk assessment. Exposure assessment is defined as the qualitative or quantitative determination or estimation of the magnitude, frequency, duration, and rate of exposure. The quantitative amount of exposure usually refers to the amount or intake rate (e.g., mg kg^{-1} body weight day^{-1}) of a chemical or physical agent at biological exchange boundaries (e.g., lungs, skin, and gastrointestinal tract); whereas absorbed dose refers to the amount or intake rate of a chemical or physical agent that has

penetrated biological exchange boundaries. Thus, exposure can be defined as either an external or internal (absorbed) metric.

The magnitude, frequency, duration, and rate of exposure may be evaluated using measurement or modeling approaches. The determination of which approach is taken, and what level of complexity is appropriate, depends upon a number of factors, such as the purpose of the specific assessment, the quality and quantity of available data, and the experience of the exposure assessor. There are three basic types of exposure assessment: direct, reconstructive, and predictive.

Direct exposure assessment consists of monitoring approaches to actually measure the amount of a chemical in environmental media (e.g., air, water, food, surfaces). Examples of direct monitoring approaches include the radiation dosimeter badge, personal air monitoring approaches to measure breathing-zone concentration of chemicals in air, measurements of chemical residues in foods as consumed, and passive dermal dosimeters (e.g., cotton clothing) for measuring consumer or occupational exposure during the application of products containing pesticides.

Reconstructive exposure assessment uses biological monitoring data, in conjunction with pharmacokinetic data and models, to estimate the levels of absorbed dose (e.g., systemic levels in plasma or whole blood), and in some cases, external exposure to a chemical that resulted in the measured levels in biological tissues and/or fluids. Biological monitoring consists of the measurement of the concentration of a chemical and/or its biotransformation products in biological tissues or fluids (e.g., adipose tissue, blood, urine) or the measurement of the amount of chemical bound to a target molecule (e.g., DNA-bound chemical).

Predictive exposure assessment, which involves estimation of potential exposure using mathematical models, is perhaps the most widely used approach to exposure assessment, and for many exposure assessors is synonymous with the term 'exposure assessment'. Predictive exposure assessments can be of varying sophistication, ranging from simplistic screening-level algebraic equations using worst-case assumptions to more complex models involving random-number based simulations (stochastic or probabilistic models) to define the likely distribution of exposure in a human population of concern. Predictive exposure assessment usually consists of a number of steps, including determination of exposure pathways, construction of likely exposure scenarios, estimation of environmental concentrations (sometimes through more complex environmental fate modeling), and calculation of the frequency and magnitude of human exposures as a function of time.

Important to the concept of exposure assessment is the profile of temporal exposure that a person experiences. Key elements of a temporal exposure profile include:

- *Exposure duration*, which is the length of time involved in each discrete exposure event (e.g., minutes to days) and, if applicable, the length of time over which two or more discrete exposure events occur (e.g., days to years).
- *Exposure frequency*, which is a measure of how often a discrete exposure event occurs over a defined time period (e.g., a few hours per use for many consumer product exposures, 5 days $week^{-1}$ for several years in the case of some occupational exposures, or continuous exposure over a lifetime in the case of ambient air pollutants).
- *Exposure chronology*, which may provide a measure of timing (e.g., age interval, such as 13–50 years of age) of the exposure event(s) relative to a time period of toxicological relevance (e.g., life stage, such as females of reproductive age) for a particular adverse (toxic) effect of interest (e.g., fetal developmental toxicity). Thus, the exposure metric should be consistent with the time frame of the dose–response that is particular to the toxicological endpoint of concern. For example, because exposure of a pregnant woman to a teratogen (i.e., a birth-defect causing agent) on a given day of gestation can cause adverse effects in the fetus, the focus is on estimation of daily 'per-event' exposure. Thus, exposure chronology, in combination with knowledge about the toxicological endpoint, can help determine the most appropriate statistical metric for the exposure or absorbed dose estimate and the associated time-averaging period or integration interval (e.g., maximum daily absorbed dose, versus 90 day moving average absorbed dose) that should be using for calculating an exposure metric for a given chemical.
- *Exposure patterns*, which usually reflect the time (e.g., hours day^{-1}, days per calendar year) and location (e.g., geographical, microenvironment) relationship between sources of exposure (e.g., motor vehicles, home appliances, consumer products, and drinking water) and human activity patterns. Human activity patterns include where and how much time people spend in defined microenvironments during a given day. Further, it can be important to characterize their specific microactivities within a given microenvironment, e.g., the frequency of a 1–2-year-old child's hand-to-mouth

events h^{-1} while playing on a residential lawn. This provides for characterization of exposure patterns that have temporal, spatial, demographic, and behavioral specificity, and allows the exposure assessor to differentiate the relative contribution of different sources (and routes) of exposure (e.g., residential indoor air, versus ambient air, versus inside vehicles such as cars, buses, trains, and airplanes). Thus, a given individual (defined demographically) can be exposed to the same chemical, through multiple routes, and a variety of microenvironments, over the course of a single day or multiple weeks, as a function of their time–activity profile.

Screening-level or initial tier predictive exposure assessment methods typically involve the use of an algebraic equation that expresses exposure or absorbed dose as a function of the concentration of a chemical in relevant media (e.g., air, food, and water) and other important factors. For example, inhalation exposure (E_{inh}) to an airborne chemical can be estimated using some form of the following equation:

$$E_{inh} = (C_a \times IR \times ED \times EF)/(BW \times AT)$$

where C_a is the concentration of chemical in air ($mg\,m^{-3}$), IR is the inhalation rate ($m^3\,h^{-1}$), ED is the exposure duration (h per day), EF is the exposure frequency (day per year), BW is the body weight of the exposed individual (kg), and AT is the averaging times, or period over which exposure is amortized (days $year^{-1}$).

In contrast to acute or short-term estimates of exposure, for purposes of chronic or long-term exposure and risk assessments, such as evaluation of potential lifetime excess cancer risk, regulatory agencies typically set the averaging time at that of an average lifetime (e.g., 70 or 75 years times 365 days $year^{-1}$) to obtain the lifetime average daily dose.

Different methods of human exposure assessment vary with respect to the 'input' data or information required and the degree of uncertainty associated with resulting estimates. For example, the film-thickness approach to dermal exposure assessment is a screening-level methodology that assumes a uniform layer of material (e.g., a liquid consumer product) is on the skin, and that a portion of the material in this layer is absorbed, per the dermal absorption characteristics of the chemical. In contrast, dermal exposure assessment and percutaneous absorption methods can include metrics that account for time-dependent exposure and absorption processes. For example, in the case of secondary dermal contact with chemicals on surfaces (e.g., transfer of pesticide residues from treated carpet to skin or clothing), dermal transfer coefficient methods have been developed from studies involving concurrent measures of transferable residues (e.g., amount per unit time of chemical transferring from a treated surface to a collection medium such as gauze wipe or a cotton cloth rolled with a cylinder exerting a known force) and time-based human passive dosimetry (e.g., amount per unit time of chemical transferred from the treated surface to cotton clothing worn by person during choreographed activities). The resulting dermal transfer coefficient estimates are typically expressed as a 'contact rate' in units of $cm^2\,h^{-1}$. Secondary dermal exposure assessment approaches can also be used to estimate dermal exposure to hands (transfer of chemical residues from a surface to hands as a function of time and specific behaviors) and subsequent hand-to-mouth-based incidental ingestion exposures.

Exposure assessment is complicated by the fact that chemical or physical agents can move dynamically, via various pathways, from the source of contamination to human receptors. Historically, these pathways and associated exposure routes have been characterized separately. However, because many human exposures can occur across time through a variety of environmental pathways and by different routes (e.g., inhalation, ingestion, and dermal contact), more recently exposure assessors are using an integrated 'total human exposure assessment' approach. Probabilistic models have been developed to quantify potential multipathway, multiroute exposure distributions to chemicals via multiple sources including diet (food, drinking water), and the residential environment (e.g., exposures during consumer product use and postproduct use or postapplication via the inhalation, dermal, and/or incidental ingestion routes) and to discern source contribution and uncertainty, for specified time periods, geographical locations, and demographic subpopulations. Chemical-specific multipathway/route assessments are also referred to as aggregate exposure assessments. Aggregate exposure assessments have been developed in the United States for various purposes; for example, including pesticides registration and reregistration.

Compilations of exposure factor values and distributions are available through the US Environmental Protection Agency (US EPA) and other organizations. Examples of exposure factors include individual physiological factors (e.g., body weight, inhalation rate, and skin surface area), exposure-related factors (e.g., time–activity data), and building factors (e.g., air exchange rate, room volume, and house volume). In Europe, collections of and recommended values for various exposure factors are available through

the German Exposure Standards document (Standards zur Expositionsabschätzung), Netherlands National Institute for Public Health and the Environment (RIVM) guidance (e.g., 'fact sheet') documents, and through the work of the European Commission's European Information System on risks from chemicals released from consumer products/articles (EIS-ChemRisks). In addition, experts from many organizations have collaborated via leadership from the Finnish National Public Health Institute (KTL) and funding from Cefic (the European Chemical Industry Council) to develop a multicountry collection of exposure factors called the Exposure Factors Sourcebook for Europe (ExpoFacts).

Because every person in a given population is likely to experience a different exposure from a given source due to, for example, different inhalation rates, different skin surface areas contacted, and different frequencies and durations of exposure due to different time–activity patterns, the recent trend has been to integrate exposure assessment with quantitative methods of uncertainty analysis. Estimates of exposure are bounded by a range of possible values, as a function of inherent variability and uncertainty in exposure parameters, and across alternative methods or modeling approaches. Using probabilistic methods, one can obtain an exposure distribution curve, where each given exposure level has a specific probability of occurring in an exposed population under the defined assessment methods used. In such a distribution, the central tendency value for exposure to a chemical in a population (e.g., 50th percentile) may be orders of magnitude less than the theoretical upper bound estimate of exposure obtained by assuming worst-case values for some exposure parameters (e.g., concentration in air, frequency of exposure, duration of exposure). Thus, probabilistic methods of exposure assessment hold the potential to yield a more realistic cross-section of exposures for the subject population because such methods make full use of all available information, and disclose variability and uncertainty in a manner that informs the risk assessment process.

See also: Exposure; Exposure Criteria; Monte Carlo Analysis; Risk Assessment, Human Health; Uncertainty Analysis.

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Relevant Websites

http://ihcp.jrc.it and http://www.jrc.cec.eu.int – European Commission, Joint Research Centre, Institute for Health and Consumer Protection. Websites for accessing the European Information System on risks from chemicals released from consumer products/articles (EIS-ChemRisks.

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Exposure Criteria

Andrew Maier

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A central concept in toxicology is that all chemicals have the potential to cause adverse effects at sufficiently high doses. Exposure criteria (exposure limits, toxicity, or risk values) define a dose or exposure level that is likely to be below the level at which adverse effects are expected to occur under particular conditions or that minimize risk to an acceptable level. Most commonly, criteria are expressed either as a dose (e.g., milligram of chemical per kilogram of body weight per day), a chemical concentration in a media (e.g., milligrams of chemical per cubic meter of air, per kilogram of soil, or per liter of water), or as an internal or absorbed dose (concentration in

blood or amount excreted), or other units based on the nature of the exposure (e.g., chemical versus physical agents).

Types of Exposure Criteria

Exposure criteria are established by diverse organizations with different areas of responsibility. For example, different federal government agencies in the United States establish exposure criteria for food ingredients and drugs (Food and Drug Administration – FDA), environmental exposures to commodity chemicals and pollutants (Environmental Protection Agency – EPA), and workplace exposures (Occupational Safety and Health Administration – OSHA). Similar separations of activity occur among numerous international organizations and other country governments. The laws that set the statutory authority of these agencies differ and they often regulate different environments, activities, and commodities. Exposure criteria developed for each of these areas are often based on different assumptions and use different procedures and may be designed to protect different populations. For this reason, the exposure criteria developed for the same chemical may differ across the various organizations. **Table 1** provides examples of different types of exposure criteria.

Certain exposure criteria serve as health-based guidelines; others are legally binding standards. For example, OSHA Permissible Exposure Limits are enforceable regulatory standards that define acceptable concentrations in air in the work environment. However, National Institute for Occupational Safety and Health (NIOSH) recommended exposure limits (RELs), American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit values (TLVs), and American Industrial Hygiene Association (AIHA) workplace environmental exposure levels (WEELs) are health-based guidelines for occupational exposure. Similarly for environmental exposure, US EPA establishes reference doses (RfDs) as guidelines for oral exposure from contaminated media, while establishing different regulatory limits for contaminant concentrations in drinking water. In general, enforceable regulatory standards are based on risk management considerations such as technical feasibility for meeting the standard and cost implications, as well the underlying toxicology.

Table 1 Examples of different types of exposure criteria

Organization	*Criteria*	*Effect*	*Route*	*Target population*
US EPA	RfD	Noncancer – chronic	Oral ($mg\,kg^{-1}\,day^{-1}$)	Public
US EPA	RfC	Noncancer – chronic	Inhalation ($mg\,m^{-3}$)	Public
US EPA	MCL	All	Drinking water ($mg\,l^{-1}$)	Public
US EPA	NAAQS	All	Air ($mg\,m^{-3}$)	Public
ATSDR	MRL	Noncancer – acute, intermediate, chronic	Oral ($mg\,kg^{-1}\,day^{-1}$) and inhalation ($mg\,m^{-3}$)	Public
Health Canada	TI	All	Oral ($mg\,kg^{-1}\,day^{-1}$) and inhalation ($mg\,m^{-3}$)	Public
WHO	TD/TC	All	Oral ($mg\,kg^{-1}\,day^{-1}$) and inhalation ($mg\,m^{-3}$)	Public
US OSHA	PEL	All	Inhalation (ppm or $mg\,m^{-3}$)	Workers
US NIOSH	REL	All	Inhalation (ppm or $mg\,m^{-3}$)	Workers
ACGIH	TLV	All	Inhalation (ppm or $mg\,m^{-3}$)	Workers
AIHA	WEEL	All	Inhalation (ppm or $mg\,m^{-3}$)	Workers
EU SCOEL	Binding Limit	All	Inhalation (ppm or $mg\,m^{-3}$)	Workers
US EPA	AEGL	Noncancer	Inhalation (ppm or $mg\,m^{-3}$)	Public
AIHA	ERPG	Noncancer	Inhalation (ppm or $mg\,m^{-3}$)	Public
US NIOSH	IDLH	Noncancer	Inhalation (ppm or $mg\,m^{-3}$)	Workers
ACGIH	BEI	All	Concentration in biological media	Workers
ACGIH	TLV	All	Various (acoustical energy, temperature, nonionizing radiation, or ergonomic stresses)	Workers
US OSHA		All	Various (noise, ionizing radiation)	Workers
ICRP		All	Various (nonionizing and ionizing radiation)	Public

ACGIH – American Conference of Governmental Industrial Hygienists; AEGL – acute emergency guidance level; AIHA – American Industrial Hygiene Association; BEI – biological exposure indices; EPA – Environmental Protection Agency; ERPG – emergency response planning guideline; EU SCOEL – European Union Scientific Committee for Occupational Exposure Limits; ICRP – International Commission for Radiological Protection; IDLH – immediately dangerous to life or health; MCL – maximum contaminant level; MRL – minimal risk level; NAAQS – National Ambient Air Quality Standard; NIOSH – National Institute for Occupational 'Safety and Health'; OSHA – Occupational Health and Safety Administration; PEL – permissible exposure limit; REL – recommended exposure limit; RfC – reference concentration; RfD – reference dose; TD/TC – tolerable dose or tolerable concentration; TI – tolerable intake; TLV – threshold limit value; WEEL – workplace environmental exposure level; WHO – World Health Organization.

Exposure criteria also vary based on the route of exposure. Depending on the anticipated exposure patterns, route-specific exposure criteria can be very valuable for health protection. Many organizations establish exposure criteria for inhalation and oral routes of exposure. Dermal exposure criteria are less common, but are often critical where skin contact is the primary exposure of concern. Care must be taken in applying route-specific criteria for other applications, and should include a thorough evaluation of the underlying toxicological considerations. For example, an exposure criterion for oral dosing may not adequately protect against respiratory effects of a potent respiratory irritant, or an agent that induces dermal sensitization.

The length of time over which the exposure is likely to occur should be considered in evaluating the potential for adverse effects. Many organizations develop exposure criteria for different durations of interest, and multiple criteria for the same chemical may be relevant based on the anticipated duration of exposure. For example, most organizations that establish occupational exposure limits such as the ACGIH or OSHA have procedures for recommending criteria for maximum peak exposures (i.e., ceiling limits), short-term exposure (i.e., 15 min STEL), and full-shift exposure criteria (e.g., 8 h time-weighted average). Acute emergency exposure guidelines also are established with a range of durations from as little as 10 min to 8 h. Longer-term environmental exposure criteria also differ by duration of exposure. For example, the US Agency for Toxic Substances and Disease Registry (ATSDR) establishes acute, intermediate, and chronic minimal risk levels (MRLs). Typically, a higher exposure can be tolerated for a shorter period than a lower exposure, and therefore, criteria developed to protect against acute exposure are often higher than for long-term or chronic exposure.

Most exposure criteria are derived to prevent any adverse effect from occurring in the population of interest. However, in some cases, particularly for emergency exposures, thresholds or criteria are needed to evaluate the potential for adverse effects of differing severity. For example, the AIHA Emergency Response Planning Guidelines (ERPGs) provide separate exposure criteria to minimize the potential for minimal, intermediate, or severe effects. NIOSH establishes immediately dangerous to life or health (IDLH) values to prevent severe effects that might impair escape or cause serious irreversible effects.

Target populations of interest also differ among organizations that establish exposure criteria. Many criteria (e.g., EPA RfDs, ATSDR MRLs) are established for the general public and account for potential sensitive populations such as young children, the elderly, or individuals with existing medical conditions. Occupational exposure levels often assume a healthy worker population. Exposure criteria for some drugs consider specific populations of patients who are candidates for the drug. The exposed population intended to be protected by an exposure criterion often impacts underlying assumptions regarding variability in response among the exposed individuals as well as acceptable levels of risk.

Notwithstanding these differences in the underlying assumptions and intended uses among exposure criteria, some general methods and approaches are commonly applied. For many organizations the basic methods differ between noncancer and cancer effects. However, in some cases noncancer effects are the primary effect of concern (e.g., emergency and acute exposure criteria). For most longer-term criteria, an assessment is generally made of both noncancer and cancer effects. Approaches for developing noncancer and cancer criteria are discussed separately below.

Noncancer Exposure Criteria

In general, criteria developed to protect against noncancer effects are based on the assumption that there is a threshold below which no adverse health effects will occur. A critical evaluation of available human health and animal toxicity studies is performed to identify the most sensitive adverse effect relevant to humans. Noncancer exposure criteria are often based on an experimentally defined dose at which no adverse effects were observed (i.e., the no-observed-adverse-effect level – NOAEL). If no adequate NOAEL is available, the lowest dose at which adverse effects were observed (lowest-observed-adverse-effect level – LOAEL) is used. Another commonly used approach is to fit study data to dose–response models to identify appropriate values (e.g., dose corresponding to the upper bound of the 10% response level or $BMDL_{10}$) as the basis for deriving the exposure criteria.

The resulting critical effect level (NOAEL, LOAEL, or BMDL) is generally divided by additional factors to account for uncertainty in extrapolating from the selected critical effect level to the safe exposure level in human populations. The application of uncertainty factors varies among organizations and on the intended application of the exposure criterion. For example, lower factors are generally used for occupational settings than for environmental exposures intended to protect the general public. Recent efforts have seen increased use of chemical-specific data to replace default applications

of uncertainty factors. For example, data on the toxicokinetic differences among species for a specific compound may be used to replace default factors in extrapolating from animal toxicity data to humans. The following general areas of uncertainty are often weighed in developing a composite uncertainty factor:

- Extrapolation from animal toxicity studies to humans.
- Variability in human sensitivity.
- Extrapolation from subchronic to chronic duration.
- Use of a LOAEL rather than a NOAEL as the critical effect.
- Other uncertainties in the overall database.

The factors that apply to a given dataset will vary. Some organizations apply default values for these or other considerations (e.g., US EPA in deriving RfDs), while others do not (e.g., ACGIH in deriving TLVs). For example, the US EPA RfD is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. It is defined in terms of dose in milligrams per kilogram body weight per day ($mg\,kg^{-1}\,day^{-1}$). A critical effect is determined following a thorough evaluation of the human health and toxicology literature, and uncertainty factors are applied to this value to derive the RfD as follows:

$$(\text{NOAEL, LOAEL, or BMDL})/(\text{Composite UF}) = \text{RfD}$$

RfDs are commonly used to evaluate whether exposures to contaminants in environmental media are acceptable. Exposure may occur via drinking water or contact with soil (separate criteria called reference concentrations or RfCs are established for inhalation by US EPA). Exposure must be estimated in terms of $mg\,kg^{-1}\,day^{-1}$ and is then compared to the RfD. If the estimated exposure exceeds the RfD, then the exposure may not be acceptable. While details vary, similar principles are used in deriving and evaluating exposure criteria established by other US organizations, organizations that establish emergency or occupational criteria, and international organizations.

Exposure Criteria for Carcinogens

Many organizations establish exposure criteria for carcinogens in a manner similar to noncarcinogens, by identifying effect levels for the tumorigenic response and accounting for uncertainties by applying uncertainty factors. Other organizations have traditionally applied alternative methods that rely on linear extrapolation from tumorigenic dose levels to low dose exposures. More recently, many organizations determine which of these approaches is most appropriate based on the underlying carcinogenic mode of action. The weight of evidence for alternative modes of action is judged and the implementation of dose–response approaches is then based on the underlying biology.

As an example of evaluating weight of evidence, a number of organizations have developed weight of evidence classification schemes to evaluate the likelihood that a chemical is carcinogenic. Such organizations include the International Agency for Research on Cancer (IARC), the National Toxicology Program (NTP), US EPA, OSHA, and ACGIH, among others.

Depending on the mode of action, some carcinogens may act via threshold mechanisms and, therefore, exposure criteria can be reasonably developed using the same approach as for noncarcinogens. However, other compounds may act through mechanisms such as direct DNA reactivity that may not have determinable thresholds. In these cases, most organizations employ methods that assume that any exposure carries some risk of effect even at low doses. A dose–response curve is generated to identify exposure levels associated with a specified level of excess risk. A number of dose–response models are used to extrapolate from high doses to low doses, often associated with environmental exposure. Such models include the one-hit, multistage, gamma multihit, probit, and Weibull models among others. Software is available that provides these modeling tools (e.g., Benchmark Dose Modeling software available from the US EPA). The slope of the dose–response curve in the low-dose region is used as an indicator of the potency of a carcinogen. Some models calculate this slope directly as part of the fit to the data. Alternatively, the slope can be calculated from the line extrapolated from a defined tumor response level as determined using Benchmark Dose modeling (e.g., the upper bound estimate of the tumorigenic dose associated with a 10% response level) to zero. An estimate of the upper bound of the slope is often used to define the slope factor. The slope factor can be used to estimate cancer risk as shown below:

$$\text{Cancer risk} = \text{dose}\ (mg\,kg^{-1}\,day^{-1}) \times \text{slope factor}\ (mg\,kg^{-1}day^{-1})^{-1}$$

The unit risk is defined as the upper bound additional lifetime cancer risk associated with exposure to either $1\,\mu g\,l^{-1}$ in water or $1\,\mu g\,m^{-3}$ in air. The dose or exposure concentration associated with a given risk can also be calculated by rearranging terms in the slope factor equation shown above to solve for the dose term. The result is termed the risk specific dose (or concentration). The risk specific dose is often used as the basis for the exposure criteria for carcinogens.

The level of risk that is considered acceptable varies, and may be defined by law, regulation, or policy. In general, only very low risks are used in setting criteria for environmental exposures (e.g., between 1 in 10 000 and 1 in 1 000 000). For occupational settings a higher risk level (e.g., 1 in 1000) is often used in deriving the exposure criteria.

See also: Carcinogen Classification Schemes; Dose–Response Relationship; Emergency Response and Preparedness; Exposure Assessment; Occupational Exposure Limits; Risk Assessment, Human Health.

Relevant Websites

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http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Exposure Criteria.
http://www.hc-sc.gc.ca – Health Canada.
http://www.inchem.org – World Health Organization.
http://www.osha.gov – Occupational Safety and Health Administration.
http://www.cdc.gov – National Institute for Occupational Safety and Health.
http://www.acgih.org – American Conference of Governmental Industrial Hygienists.
http://www.aiha.org – American Industrial Hygiene Association.
http://europe.osha.eu – European Union Scientific Committee for Occupational Exposure Limits.
http://www.icrp.org – International Commission for Radiological Protection.

Exxon Valdez

Michael A Kamrin, Pallavi B Limaye, and Harihara M Mehendale

Introduction

Spills of oil into the world's oceans are common occurrences although very large releases are rare. The spills from the *Torrey Canyon* in the English Channel in 1967 and from the *Amoco Cadiz* off the coast of Brittany, France in 1978 are two of the largest and most serious spills in recent decades. Significant but somewhat smaller spills continue to occur. For example, in 2002, the tanker *Prestige* sank off the northern coast of Spain covering ~350 miles of rocks and coves with oil sludge. Public reactions to these spills have mainly been local and the environmental impacts of the spills have been appreciated most by citizens in the regions and countries affected.

The size of the spill is not the only factor that affects the impact. The seriousness of the problems that these spills can cause is also a function of its location and the type and amount of the various chemical constituents in the oil. Those spills closest to shore generally have the greatest adverse effects on the environment since the oil does not have time to disperse before reaching shore and the higher concentrations can significantly impact the sensitive habitats of a variety of organisms. Very large spills farther from the shore can also have serious impacts since a longer stretch of coastline may be affected as the oil spreads out and, even with dilution, levels may remain high enough to have serious effects on aquatic and shoreline ecosystems. With regard to the composition of the oil, one important consideration is the presence and amount of polycyclic aromatic hydrocarbons, which appear to produce toxic effects on some marine species at low concentrations.

Exxon Valdez

The Spill

The ship called *Exxon Valdez* transporting crude oil grounded on March 24, 1989 at Bligh Reef resulting in the rupture of eight of its 11 cargo tanks spilling ~11 million gallons of crude oil into Prince William Sound, Alaska. Approximately 1300 miles of pristine shoreline was contaminated with oil to varying degrees, and Prince William Sound was most severely affected. A majority of the spill appears to have been recovered as the result of the cleanup and the natural evaporation process. However, pockets of crude oil remain in some locations, where there is evidence of continuing damage. **Figure 1** shows the spread of the spill in the months following the event.

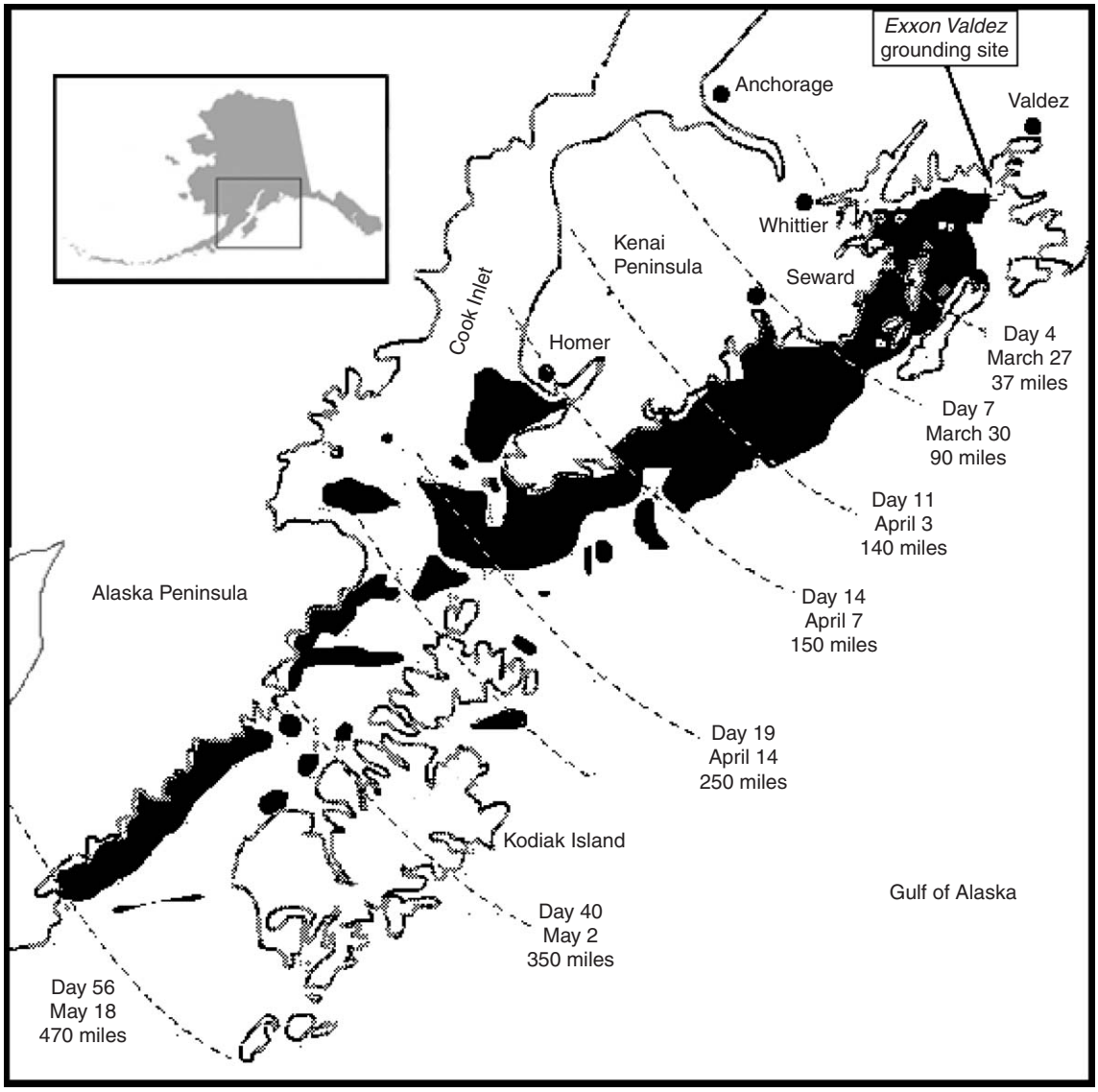

Figure 1 Spread of the spill in the months following the event.

This spill off the coast of Alaska generated the most media attention in the United States of any spill anywhere in the world. It also led to the most comprehensive scientific investigation of such incidents although much less oil was spilled than in many other tanker accidents. For example, the *Exxon Valdez* spilled only one-sixth as much oil as the *Amoco Cadiz* and about one-half as much as the *Prestige*. It is estimated that the *Amoco Cadiz* spilled ~220 000 tons of oil, probably the largest amount spilled by a tanker in history.

Attempts to clean up the *Exxon Valdez* spill were made by a combination of government agencies and the industry. The main methods employed were burning the oil, mechanically removing it from the water and the shore, use of chemical dispersants, and application of hot water to the shore. In addition, many beaches were fertilized to promote growth of microscopic bacteria that degrade hydrocarbons, a process known as bioremediation. However, it was later found that some of the cleanup methods were damaging (e.g., hot water treatment turned out to be harmful since small organisms were literally cooked by the hot water).

Ultimately, the cleanup crews collected ~14% of the oil that was spilled while ~13% sunk to the sea floor. A major portion of the remaining oil evaporated and another portion was naturally degraded over the years. About 2% (~216 000 gal) remained on the beaches. The most recent survey of lingering oil was conducted in the intertidal zone of Prince William Sound in the summer of 2001 by the

National Oceanic and Atmospheric Administration. The survey results indicate that oil was present at 58% of the sites assessed and that a total shoreline area of ~20 acres in Prince William Sound is still contaminated.

Toxicological Impacts

During the 15 years since the spill, there has been a continuing research effort to evaluate the initial and long-term impacts of the spill, how they were ameliorated by remedial actions and the passage of time, and the status of various organisms affected by the spill. Extrapolation from the data that have been collected suggests that the *Exxon Valdez* spill led to the deaths of ~250 000 seabirds, 3000 sea otters, 300 harbor seals, 250 eagles, and a number of killer whales. Monitoring data indicate that populations of some of these creatures, such as the sea otter, had not recovered as of 2002 – more than a dozen years later. However, other animal populations, such as those of the bald eagle, recovered fully in the intervening years. The fate of sea otters falls into an intermediate category, since populations are recovering but were not fully recovered as of 2002.

There are four general causes of toxicity in animals exposed to oil spill residuals. The first is the adverse impact of the oil on the insulation value of the fur and feathers of animals. The second is acute toxicity from ingesting oil products, often while animal is trying to clean the oil off fur or feathers. The third is long-term or delayed toxicity due to oil residue exposures that are not lethal but which decrease the hardiness or reproductive fitness of the exposed animals. The last is the brain lesions and disorientation caused by inhalation of toxic fumes. In addition, populations of animals can be severely affected if oil toxicity adversely impacts the creatures that they feed on or greatly decreases available habitat. Both of these problems can affect the organism's ability to survive and reproduce.

In addition to effects of the spill on larger animals, there were also impacts on smaller organisms resulting from the spill and the cleanup. Mortality in microalgae and benthic invertebrates occurred due to a combination of chemical toxicity and their physical displacement from natural habitat by pressurized wash-water that was applied after the spill to clean up the contaminated area.

Postspill Research

One of the aims of the research undertaken after the *Exxon Valdez* spill was to understand the impacts of oil spills on the environment. However, assessing the toxicity of oil spills is complicated by a number of variables, such as the presence of oil from other sources; for example, natural seeps, and by the absence of baseline prespill data with which to compare postspill environmental levels and effects. In addition, it has been difficult to determine the natural factors that affect the persistence of the oil in various environmental locations near the spill since the persistence was strongly influenced by the steps taken to clean the oil. Further, the *Exxon Valdez* spill occurred in the arctic environment and it is not clear how valid it is to extrapolate the data gathered in this environment to other climates. These considerations suggest that any conclusions drawn about persistence would be situation-specific and hard to apply to other spills. A further complicating factor is that in a number of cases, scientific studies were designed to address very specific concerns related to litigation rather than to answer broad environmental toxicology questions which might be applicable to a variety of other locations and situations.

Research into the impact of the spills has been aided by the passage of the US Oil Pollution Act of 1990, which included a provision establishing the Oil Spill Recovery Institute (OSRI). OSRI provides funding to support oil-spill related research as well as education and technology development for dealing with oil spills in the Arctic environment. The results of research it supported and other research, such as that funded as part of litigation activities, has been summarized in the 2002 National Research Council report Oil and the Sea: Inputs, Fates and Effects. This report also puts into perspective the small contribution of tanker and pipeline spills as compared to other sources of ocean oil such as land-based runoff, polluted rivers, small boats and water craft, as well natural seeps from the sea floor.

Summary

Tanker spills are dramatic examples of the adverse impacts of large amounts of oil on aquatic and coastline ecosystems. The *Exxon Valdez* spill led to great public awareness of this problem and subsequent legislation aimed at decreasing the probability and impact of oil spills, improving our understanding of the impacts of such spills, and increasing our capabilities for dealing with spills of oil from tankers and other sources. The continuing occurrence of tanker spills and the difficulties in dealing with the consequences of such incidents suggest that additional research emphasis be placed on ways to prevent such incidents in the future.

Further Reading

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Eye Irritancy Testing

Samantha E Gad

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Introduction

Virtually all man-made chemicals have the potential to end up in the eyes of people. In fact, many (e.g., cosmetics and shampoos) are intended to be used in such a manner that ocular exposure is inevitable in a large number of cases.

In the early 1930s, an untested eyelash dye containing *p*-phenylenediamine ('Lash Lure') was brought on the market in the United States. This product (as well as a number of similar products) rapidly demonstrated that it could sensitize the external ocular structures, leading to corneal ulceration with loss of vision and at least one fatality. This occurrence led to the revision of the Food, Drug and Cosmetic Act of 1938. To meet the new provisions of this act, a number of test methods were proposed. Latven and Molitor and Mann and Pullinger were among those to first report on the use of rabbits as a test model to predict eye irritation in humans. No specific scoring system was presented to grade or summarize the results in these tests, however, and the use of animals with pigmented eyes (as opposed to albinos) was advocated. Early in 1944, Friedenwald *et al*. published a method using albino rabbits in a manner very similar to that of the original (1944) Draize publication but still prescribing the description of the individual animal responses as the means of evaluating and reporting the results. Although a scoring method was provided, no overall score was generated for the test group. Draize (head of the Dermal and Ocular Toxicity Branch at the US Food and Drug Administration (FDA)) modified Friedenwald's procedure and made the significant addition of a summary scoring system.

During the 40 years since the publication of the Draize scoring system, it has become common practice to call all acute eye irritation tests performed in rabbits 'the Draize eye test'. However, since 1944, ocular irritation testing in rabbits has significantly changed. Clearly, there is no longer a single test design that is used, and there are different objectives that are pursued by different groups using the same test. This lack of standardization has been recognized for some time and attempts have been made to address standardization of at least the methodological aspects of the test (such as how test materials are applied and scoring performed), if not the design aspects (such as numbers and sources of test animals). For the purposes of the remainder of this entry, the term Draize test has been replaced with eye irritancy testing.

Ocular irritation tests are significantly different from the other local tissue irritation tests on a number of grounds. For the pharmaceutical industry, eye irritation testing is performed when the material is intended to be put into the eye as a means or route of application for ocular therapy. There are a number of special tests applicable to pharmaceuticals or medical devices that are beyond the scope of this discussion since they are not intended to assess potential acute effects or irritation. In general, however, it is desired that an eye irritation test be both sensitive and accurate in predicting the potential to cause irritation in humans. Failing to identify human ocular irritants (lack of sensitivity) is to be avoided, but of equal concern is the occurrence of false positives.

The primary eye irritation test was originally intended to predict the potential for a single splash of chemical into the eye of a human being to cause reversible or permanent damage. The common core design of the test, as currently utilized, consists of instilling either 0.1 ml of a liquid or 0.1 g of a powder (or other solid) into one eye of each of six rabbits. The material is not washed out, and both eyes of each animal (the untreated eye acting as a control)

are graded according to the Draize scale (**Table 1**) at 24, 48, and 72 h after test material instillation. The resulting scores are summed for each animal. A variation of the test involves the use of three additional rabbits which have their eyes irrigated shortly after instillation of test material. There are, however, many variations of these two major design subsets (i.e., with and without irrigation groups).

Table 1 Scale of weighted scores for grading the severity of ocular lesions

Cornea	
A. Opacity – degree of density (area which is most dense is taken for reading)	
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
B. Area of cornea involved	
One-quarter (or less) but not zero	1
Greater than one-quarter, less than one-half	2
Greater than one-half, less than the whole area	3
Greater than three-quarters up to the whole area	4
Iris	
A. Values	
Folds above normal, congestion, swelling, circumcorneal ingestion (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is possible)	1
No reaction to light, hemorrhage; gross destruction (any of these)	2
Scoring equals A × B	
Total possible maximum = 10	
Conjunctivae	
A. Redness (refers to palpebral conjunctival only)	
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
B. Chemosis	
Any swelling above normal (includes nictating membrane)	1
Obvious swelling with partial eversion of the lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4
C. Discharge	
Any amount different from normal (does not include small amount observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hair just adjacent to the lids	2
Discharge with moistening of the lids and considerable area around the eye	3
Scoring (A + B + C) × 2	
Total maximum = 20	

Note: The maximum total score is the sum of all scores obtained for the cornea, iris, and conjunctivae.

Reproduced from Draize JN, Woodard G, and Calvery HO (1944) Methods for the study of irritation and toxicity of substances applied to the skin and mucous membranes. *Journal of Pharmacology and Experimental Therapeutics* 82: 377–390.

Even though the major objective of the Draize scale was to standardize scoring, it was recognized early that this was not happening; instead, different people were 'reading' the same response differently. To address this, two sets of standards (also called training guide) have been published by regulatory agencies through the years. In 1965, the US FDA published an illustrated guide with color pictures as standards. In 1974, the Consumer Product Safety Commission (CPSC) published a second illustrated guide which provided 20 color photographic slides as standards. The US Environmental Protection Agency (EPA) also supported the development of a guide with color plates/slides.

A second source of methodological variability has been in the procedure utilized to instill test materials into the eyes. The general consensus is that the substance should be dropped into the cul-de-sac of the conjunctiva formed by gently pulling the lower eyelid away from the eye, then the animal should be allowed to blink and the material should be spread across the entire corneal surface. In the past, however, there were other application procedures (such as placing the material directly onto the surface of the cornea).

There are also variations in the design of the 'standard' test. Most laboratories observe animals until at least 7 days after instillation and may extend the test to 21 days after instillation if any irritation persists (in fact, US EPA labeling requires such an extension). These prolonged postexposure observation periods are designed to allow for evaluation of the true severity of damage and for assessing the ability to repair the ocular damage. The results of these tests are evaluated by a descriptive classification scale (**Table 2**) such as that described in National Academy of Sciences (NAS) publication No. 1138, which is a variation of that reported by Green *et al*. This classification is based on the most severe response observed in a group of six non-irrigated eyes, and data from all observation periods are used for this evaluation.

Different regulatory agencies within the United States have prescribed slightly different procedures for different perceived regulatory needs. There have also been a number of additional grading schemes, but these will not be reviewed here.

Current *In Vivo* Test Protocols

Any discussion of current test protocols (or of any proposed *in vitro* alternatives) must start with a review of why tests are performed. What are the

Table 2 Severity and persistence of irritation

Inconsequential or complete lack of irritation: Exposure of the eyes to a material under the specified conditions caused no significant ocular changes. No staining with fluorescein can be observed. Any changes that do occur clear within 24 h and are no greater than those caused by normal saline under the same conditions.

Moderate irritation: Exposure of the eye of the material under the specified conditions causes minor, superficial, and transient changes of the cornea, iris, or conjunctivae as determined by external or slit-lamp examination with fluorescein staining. The appearance at the 24 hr or subsequent grading of any of the following changes is sufficient to characterize a response as moderate irritation: opacity of the cornea (other than a slight dulling of the normal luster), hyperemia of the iris, or swelling of the conjunctivae. Any changes that are seen clear within 7 days.

Substantial irritation: Exposure of the eye to the material under the specified conditions causes significant injury to the eye, such as loss of the corneal epithelium, corneal opacity, iritis (other than a slight injection), conjunctivitis, parmus, or bullae. The effects clear within 21 days.

Severe irritation or corrosion: Exposure of the eye to the material under the specified conditions results in the same types of injury as in the previous category and in significant necrosis or other injuries that adversely affect the visual process. Injuries persist for 21 days or more.

objectives of eye irritation testing, and how are these different objectives reflected not just in test design and interpretation but also in the regulations requiring testing and in the ways that test results are utilized?

There are four major groups of organizations that are required to perform eye irritation studies. These are the pharmaceutical, cosmetic and toiletries, consumer product, and industrial chemical groups. There are also minor categories of use (which we will not consider here) such as for military agents.

In the pharmaceutical industry, eye irritation testing is performed when the material is intended to be put into the eye as a means or route of application or for ocular therapy. There are a number of special tests applicable to pharmaceuticals or medical devices which are beyond the scope of this discussion because they are not intended to assess potential acute effects or irritation. In general, however, it is desired that an eye irritation test that is utilized by this group be both sensitive and accurate in predicting the potential to cause irritation in humans. Failing to identify human ocular irritants (lack of sensitivity) is to be avoided, but of equal concern is the occurrence of false positives.

The cosmetics and toiletries industry is similar to the pharmaceutical industry in that the materials of interest are frequently intended for repeated application in the area of the eye. In such uses, contact with the eye is common, though not intended or desirable. In this case, the objective is a test that is as sensitive (as that in the preceding paragraph), even if this results in a low incidence of false positives. Even a moderate irritant would not be desired but might be acceptable in certain cases (such as deodorants and depilatories) in which the potential for eye contact is minimal.

Consumer products which are not used for personal care (such as soaps, detergents, and drain cleaners) are approached from yet a different perspective. These products are not intended to be used in a manner that either causes them to get into the eyes or makes that occurrence likely. However, because of the very large population that uses them and the fact that their modes of use do not include active measures to prevent eye contact (such as the use of goggles and face shields), the aim is to accurately identify severe eye irritants. Agricultural chemicals generally fit in this category, though many of them are covered by specific testing requirements under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

Finally, there are industrial chemicals. These are handled by a smaller population (relative to consumer products). Eye contact is never intended and, in fact, active measures are taken to prevent it. The use of eye irritation data in these cases is to fulfill labeling requirements for shipping and to provide hazard assessment information for accidental exposures and treatment information. The results of such tests do not directly affect the economic future of a material. It is desired to accurately identify moderate and severe irritants (particularly those with irreversible effects) and to know if rinsing of the eyes after exposure will make the consequences of exposure better or worse. False negatives for mild reversible irritation are acceptable.

To fulfill these objectives, a number of basic test protocols have been developed and mandated by different regulatory groups. **Table 3** gives an overview of these protocols. Historically, the philosophy underlying these test designs made maximization of the biological response equivalent to having the most sensitive test.

One widely used study design, which begins with a screening procedure as an attempt to avoid testing severe irritants or corrosives in animals, is described in the following section.

Rabbit Eye Irritancy Testing: Widely Used Study Design

Test Article Screening Procedure

1. Each test substance will be screened in order to eliminate potentially corrosive or severely irritating

Table 3 Regulatory guidelines for irritation test methods

Agency	*Draize*	*FHSA*	*NAS*	*OECD*	*IRLG*	*CPSC*	*TOSCA*	*FIFRA*[g]
Test species	Albino rabbit	Same	Same[a]	Same	Same	Same	Same	Same
Age/weight	NS[b]	NS	Sexually mature/ less than 2 years old	NS	Young adult/2.0	NS	NS	NS
Sex	NS	NS	Either	NC	Either	NS	NS	NS
No. of animals/group	Six	6–18	Four (minimum)	Three (minimum)	Three (preliminary test)[c]; six	6–18	Six	Six
Test agent volume and method of instillation – liquids	0.1 ml in the eye	Same as for Draize	Liquids and solids: two or more different doses within the probable range of human exposure[d]	Same as for Draize	Same as for Draize	Same as for Draize	Same as for FHSA	Same as for FHSA
Solids	NS	100 mg or 0.1 ml equivalent when this volume weighs less than 100 mg; direct instillation into conjunctival sac	Manner of application should reflect probable route of accidental exposure	Same as for FSHA	Same as for FSHA	Same as for FSHA	Same as for FSHA	Same as for FSHA
Aerosols[e]	NS	NS	Short burst of distance approximating self-induced eye exposure	1 s burst sprayed at 10 cm	1 sec burst sprayed at ~4 in.	NS	Same as for OECD	Same as for OECD

Irrigation schedule	At 2 s (three animals) and at 4 s (three animals) following instillation of test agent (three animals)	Eyes may be washed after 24 h reading	May be conducted with separate experimental groups	Same as for FHSA; in addition, for substances found irritating; wash at 4 s (three animals) and at 30 s (three animals)	Same as for FHSA	Same as for FHSA	Same as for FHSA	Same as for FHSA
Irrigation treatment	20 ml tap water (body temp.)	Sodium chloride solution (USP or equivalent)	NS	Wash with water for 5 min using volume and velocity of flow which will not cause injury	Tap water or sodium chloride solution (USP or equivalent)	Same as for FHSA	NS	NS
Examination times (postinstillation)	24, 48, and 72 h; 4 and 7 days	24, 48, and 72 h	1, 3, 7, 14, and 21 days	1, 24, 48, and 72 h[f]	24, 48, and 72 h	24, 48, and 72 h	Same as for OECD	Same as for OECD
Use of flourescein	NS	May be applied after the 24 h reading (optional)	May be used	Same as for FHSA	Same as for FHSA	Same as for FHSA	Same as for FHSA	Same as for FHSA
Use of anesthetics	NS	NS	NS	May be used	May be used	NS	May be used	May be used
Scoring and evaluation	Draize *et al.*	Modified Draize *et al.* (1944) or a slit-lamp scoring system	CPSC (1976)	CPSC (1976)	CPSC (1976)	CPSC (1976)	CPSC (1976)	CPSC (1976)

Note: FHSA, Federal Hazard Substance Act; NAS, National Academy of Sciences; OECD, Organization for Economic Cooperation and Development; IRLG, Interagency Liaison Group.

[a] Tests should be conducted on monkeys when confirmatory data are required.

[b] NS, not specified.

[c] If the substance produces corrosion, severe irritation, or no irritation in a preliminary test with three animals, no further testing is necessary. If equivocal responses occur, testing on at least three additional animals should be performed.

[d] Suggested doses are 0.1 and 0.05 ml for liquids.

[e] Currently, no testing guidelines exist for gases or vapors.

[f] Eyes may be examined at 1 h and at 7, 14, and 21 days (at the option of the investigator).

[g] Office Pesticide Assessment.

materials from being studied for eye irritation in the rabbit.
2. If possible, the pH of the test substance will be measured.
3. A primary dermal irritation test will be performed prior to the study.
4. The test substance will not be studied for eye irritation if it is a strong acid (pH of 2.0 or less) or strong alkali (pH of 11.0 or greater) and/or if the test substance is a severe dermal irritant (with a primary dermal irritation index (PDII) of 5–8) or causes corrosion of the skin.
5. If it is predicted that the test substance does not have the potential to be severely irritating or corrosive to the eye, continue to Rabbit Screening Procedure.

Rabbit Screening Procedure

1. A group of at least 12 New Zealand White rabbits of either sex are screened for the study. The animals are removed from their cages and placed in rabbit restraints. Care should be taken to prevent mechanical damage to the eye during this procedure.
2. All rabbits selected for the study must be in good health; any rabbit exhibiting sniffles, hair loss, loose stools, or apparent weight loss is rejected and replaced.
3. One hour prior to instillation of the test substance, both eyes of each rabbit are examined for signs of irritation and corneal defects with a hand-held slit lamp. All eyes are stained with 2.0% sodium fluorescein and examined to confirm the absence of corneal lesions. Fluorescein staining: Cup the lower lid of the eye to be tested and instill one drop of a 2% (in water) sodium fluorescein solution onto the surface of the cornea. After 15 s, thoroughly rinse the eye with physiological saline. Examine the eye, employing a hand-held long-wave UV illuminator in a darkened room. Corneal lesions, if present, appear as bright yellowish-green fluorescent areas.
4. Only 9 of the 12 animals are selected for the study. The nine rabbits must not show any signs of eye irritation and must show either a negative or a minimum fluorescein reaction (due to normal epithelial desquamation).

Study Procedure

1. At least 1 h after fluorescein staining, the test substance is placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup (conjunctival cul-de-sac) into which the test material is dropped. The upper and lower lids are then gently held together for 1 s to prevent immediate loss of material.
2. The other eye remains untreated and serves as a control.
3. For testing liquids, 0.01 ml of the test substance is used.
4. For solid or pastes, 100 mg of the test substance is used.
5. When the test substance is in flake, granular, powder, or other particulate form, the amount that has a volume of 0.01 ml (after gently compacting the particles by tapping the measuring container in a way that will not alter their individual form) is used whenever this volume weighs less than 10 mg.
6. For aerosol products, the eye should be held open and the substance administered in a single, 1 s burst at a distance of $\sim$4 in. directly in front of the eye. The velocity of the elected material should not traumatize the eye. The dose should be approximated by weighing the aerosol can before and after each treatment. For other liquids propelled under pressure, such as substances delivered by pump sprays, an aliquot of 0.01 ml should be collected and instilled in the eye as for liquids.
7. The treated eyes of six of the rabbits are not washed following instillation of the test substance.
8. The treated eyes of the remaining three rabbits are irrigated for 1 min with tap water at room temperature, starting 20 s after instillation.
9. To prevent self-inflicted trauma by the animals immediately after instillation of the test substance, the animals are not immediately returned to their cages. After examination and grading of the eyes of the control animals at 1 h postexposure, the animals are returned carefully to their respective cages.

Observations

1. The eyes are observed for any immediate signs of discomfort after instilling the test substance. Blepharospasm and/or excessive tearing are indicative of irritating sensations caused by the test substance, and their duration should be noted.
2. Blepharospasm does not necessarily indicate that the eye will show signs of ocular irritation.
3. Grading and scoring of ocular irritation are performed in accordance with **Table 1**. The eyes are examined, and grades of ocular reactions are recorded.
4. If signs of irritation persist at Day 7, readings are continued on Days 10 and 14 after exposure or until all signs of reversible toxicity are resolved.
5. In addition to the required observation of the cornea, iris, and conjunctiva, serious effects (such as parmus, rupture of the globe, or blistering of the

conjunctivae) indicative of a corrosive action are reported.
6. Whether or not toxic effects are reversible depends on the nature, extent, and intensity of damage. Most lesions, if reversible, will heal or clear within 21 days. Therefore, if ocular irritation is present at the 14 day reading, a 21 day reading is required to determine whether the ocular damage is reversible or nonreversible.

Limitations

Commonly used methodological variations to improve the sensitivity and accuracy of describing damage in these tests are inspection of the eyes with a slit lamp and instillation of the eyes with a vital dye (very commonly, fluorescein) as an indicator of increases in permeability of the corneal barrier.

To assess the adequacy of the currently employed eye irritation tests in fulfilling the objectives behind their use, we must evaluate them in terms of (1) their accuracy (how well they predict the hazard to humans); (2) whether comparable results can be obtained by different technicians and laboratories; and (3) what methods and designs have been developed and are being employed as alternatives to rabbit eye irritation tests. Assessing the accuracy of rabbit eye irritation tests – or indeed, of any predictive test of eye irritation – requires that the results of such tests be compared to what happens in humans. Unfortunately, the human database for making comparisons is not large. The concerns, however, have been present almost as long as the tests have been performed.

Rabbit Eye Irritancy Testing: Alternative Methods

A number of alternatives have been proposed and adapted for the performance of rabbit eye irritation tests. These alternatives have been directed at the twin objectives of making the tests more accurate in predicting human responses and reducing both the use of animals and the degree of discomfort or suffering experienced by those that are used.

Alternative Species

Dogs, monkeys, and mice have all been suggested as alternatives to rabbits that would be more representative of humans. Each of these, however, also has shown differences in responses compared to those seen in humans and poses additional problems in terms of cost, handling, lack of database, and so on.

Use of Anesthetics

Over the years, a number of authors have proposed that topical anesthetics be administered to the eyes of rabbits prior to their use in the test. Both OECD and IRLG regulations provide for such a use. However, numerous published and unpublished studies have shown that such use of anesthetics interferes with test results, usually by increasing the severity of eye irritation findings.

Decreased Volume of Test Material

An alternative proposal (one which a survey showed has been adopted by a number of laboratories) is to use a reduced volume/weight of test material.

In 1984, Freeberg *et al*. reported a study in which they evaluated 21 different chemicals at volumes of 0.1, 0.03, 0.01, and 0.003 ml. These are materials on which human data were already available. It was found that the volume reduction did not change the rank order of responses, and that 0.01 ml (10 µl) gave results which best mirrored those seen in humans.

In 1985, Walker reported an evaluation of the low-volume (0.01 ml) test which assessed its results for the correlation with those in humans based on the number of days until clearing of injury and reported that 0.01 ml gave a better correlation than did 0.1 ml.

While it must be pointed out that there may be some classes of chemicals for which low-volume tests may give results less representative of those seen in humans, it seems clear that this approach should be seriously considered by those performing such tests.

Use of Prescreens

This alternative may also be considered a tier approach. Its objective is to avoid testing severely irritating or corrosive materials in many (or in some cases, any) rabbits. This approach entails a number of steps which should be considered independently.

First, a screen based on physicochemical properties should be used. This usually means pH, but also should be extended to materials with high oxidation or reduction potentials (e.g., hexavalent chromium salts).

Although the correlation between low pHs (acids) and eye damage in the rabbit has not been found to be excellent, all alkalis (pH 11.5 or above) tested have been reported to produce opacities and ocular damage. Many laboratories now use pH cutoffs for testing of 2.0 or lower and 11.5 or 12.0 and higher. If a material falls outside these cutoffs (or is so identified due to other physicochemical parameters), then it is (1) not tested in the rabbit eye and is assumed to be corrosive; (2) evaluated in a secondary screen such as an *in vitro* cytotoxicity test or primary dermal irritation test; or (3) evaluated in a single rabbit before a full-scale eye irritation test is performed. It should be kept in mind that the correlation of all the

physicochemical screen parameters with acute eye test results is very concentration-dependent, being good at high concentrations and marginal at lower concentrations (where various buffering systems present in the eye are meaningful).

The second commonly used level of prescreen is the use of PDI test results. In this approach the PDI study is performed before the eye irritation study, and if the score from that study (PDII ranging from 0 to 8) is above a certain level (normally 5.0 or greater), the same options already outlined for physicochemical parameters can be exercised.

Table 4 *In vitro* alternatives for eye irritation tests

Morphology
Enucleated superfused rabbit eye system
BALB/c 3T3 cells/morphological assays (HTD)
Cell toxicity
Adhesion/cell proliferation
BHK cells/growth inhibition
BHK cells/colony-formation efficiency
BHK cells/cell detachment
SIRC cells/colony-forming assay
BALB/c 3T3 cells/total protein
BCL/D1 cells/total protein
Primary rabbit corneal cells/colony-forming assay
Membrane integrity
LS cells/dual dye staining
Thymocytes/dual fluorescent dye staining
LS cells/dual dye staining
RCE-SIRC-P815-YAC-I/Cr release
L929 cells/cell viability
Bovine red blood cell/hemolysis
Mouse L929 fibroblasts/erythrocin C staining
Rabbit corneal cell cultures/plasminogen activator
Agarose diffusion
Cell metabolism
Rabbit corneal cell cultures/plasminogen activator
LS cells/ATP assay
BALB/c 3T3 cells/neutral red uptake
BALB/c 3T3 cells/uridine uptake inhibition assay
HeLa cells/metabolic inhibition test (MIT-24)
MDCK cells/dye diffusion
Cell and tissue physiology
Epidermal slice/electrical conductivity
Rabbit ileum/contraction inhibition
Bovine cornea/corneal opacity
Proptoses mouse eye/permeability test
Inflammation/immunity
Chorioallantoic membrane (CAM)
CAM
HET-CAM
Bovine corneal cup model/leukocyte chemotactic factors
Rat peritoneal cells/histamine release
Rat peritoneal mast cells/serotonin release
Rat vaginal explant/prostaglandin release
Bovine eye cup/histamine and leakotriene C4 release
Recovery/repair
Chorioallantoic membrane
Other
EYTEX assay
Computer-based structure–activity
Tetrahymena/motility

In Vitro Tests

Sustained efforts to develop a true alternative (*in vitro*) to current ocular irritancy tests have been undertaken during the past 6 years. A complete review of this work is beyond the scope of this entry, and it continues to be in a high state of flux. However, at least a brief outline or summary of the approaches being pursued is appropriate.

As summarized in **Table 4**, there are six major categories of approaches to alternative eye irritation tests. The first five of these aim at assessing portions of the irritation response (alterations in tissue morphology, toxicity to individual component cells, alterations in cell or tissue physiology, inflammation or immune modulation, and alterations in repair and recovery processes). These methods have the limitation that they assume that one of these component parts can or will predict effects in the complete organ system. A more likely case is that, while each may serve well to predict the effects of a set of chemical structures which have that component as a determining part of the ocular irritation response, a valid assessment across a broad range of structures will require the use of a collection or battery of such tests.

The sixth category contains tests which have little or no empirical basis, such as computer-assisted structure–activity relationship models. These approaches can only be assessed in terms of how well (or poorly) they perform.

See also: Analytical Toxicology; Consumer Product Safety Commission; Federal Insecticide, Fungicide, and Rodenticide Act; Food and Drug Administration, US; Good Laboratory Practices (GLP); *In Vitro* Test; *In Vivo* Test; Sensory Organs; Toxicity, Acute; Toxicity Testing, Alternatives.

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F

FAO *See* Food and Agriculture Organization of the United Nations.

Federal Insecticide, Fungicide, and Rodenticide Act, US

Chris F Wilkinson and Michael A Kamrin

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) is the main statute under which all pesticides are distributed and sold in the United States. Federal regulation of pesticides started with the Insecticide Act of 1910, which was directed primarily toward protecting consumers from fraudulent pesticide products; it remained the major law governing pesticide products for 37 years. The Insecticide Act was essentially a labeling statute and did not require registration of products or establish any significant safety standards.

The Insecticide Act was replaced by FIFRA in 1947. FIFRA required that pesticides be registered by the Secretary of Agriculture before being distributed or sold in interstate or foreign commerce and required label warnings and instructions for safe use of highly toxic products. Since that time, there have been numerous amendments to FIFRA to bring the statute to its current form, and a number of these have been linked with major federal reorganizational changes. In particular, on December 2, 1970, President Nixon created the US Environmental Protection Agency (EPA). EPA assumed the pesticide regulatory functions of United States Department of Agriculture (USDA) and with the 1972, 1975, 1978, 1980, 1988, and 1990 amendments as well as the passage of the Food Quality Protection Act in 1996, FIFRA has become an increasingly complex statute with greatly increased authority to regulate pesticide products. The regulations governing pesticides are administered by EPA's Office of Pesticide Programs.

FIFRA requires that every pesticide to be sold or distributed in interstate and intrastate commerce be registered. A registration is equivalent to a license to sell or distribute a pesticide in commerce. Registration is based on submission to EPA, by the registrant, of data 'demonstrating that the pesticide will not cause unreasonable adverse effects on human health or the environment when it is used according to approved label directions'; FIFRA is a risk-balancing statute that does not state that pesticides must be free of all potential risk. It also considers economic and social costs and benefits. A pesticide must also be registered with the appropriate agency in each state in which it is to be used and, in some states such as California, pesticide registration requirements may be even more stringent than those of EPA.

An application for an EPA pesticide registration must be accompanied by data establishing that it is efficacious and can be used without causing unreasonable adverse effects. In registering new products, pesticide manufacturers have the responsibility of providing the data necessary to demonstrate that a material will not present unreasonable risks to humans or the environment. This requires the manufacturer to conduct a comprehensive battery of tests to determine acute and chronic mammalian toxicity, potential adverse effects on nontarget species (birds and fish), environmental fate and transport, and other factors. The likelihood that the material will leave residues in food crops or might leach into groundwater is also evaluated. The tests required to get a single new pesticide product on the market may cost as much as $40 million and the process may take from 6 to 8 years.

Toxicology data are among the most time-consuming and costly to generate. They are designed to establish the potential adverse effects of the pesticide by different routes of exposure (oral, inhalation, and dermal) and include a complete series of acute, subchronic, and chronic animal studies; metabolism and pharmacokinetic studies; and a battery of tests to determine potential mutagenic activity. While certain exemptions for specific uses may be granted, the tests typically required under FIFRA include acute oral, dermal, and inhalation toxicity; skin and eye irritation; skin sensitization; subchronic (90 day feeding); developmental toxicity (teratology); two-generation reproductive toxicity; and chronic oncogenicity. Several

of these, such as the developmental toxicity and oncogenicity studies, are usually conducted with two different species. The primary objective of toxicology testing under FIFRA is to establish no-observed-effect levels (NOELs) or lowest-observed-effect levels for noncarcinogenic endpoints and cancer potency factors (q1* values) for pesticides classified as carcinogens. The NOEL values are used to calculate a reference dose (RfD) (once termed the acceptable daily intake). The RfD is considered to be the daily dose of the pesticide that could be consumed by humans each day, for a lifetime, without causing any adverse effects. The RfD is obtained by applying a 'safety factor' or 'uncertainty factor' to the NOEL. The uncertainty factor reflects the degree of uncertainty in the data; if the data are good, the factor may be relatively small (perhaps 10) but if the toxicology data are uncertain, it may be as great as 1000. A typical safety factor is ~100, a factor of 10 being used to express uncertainty in extrapolating from animals to humans and an additional factor of 10 to cover possible differences in susceptibility in the human population.

All tests required for FIFRA must be conducted according to FIFRA Good Laboratory Practices (GLP) that specify the minimum practices and procedures that must be followed to ensure the quality and integrity of the data submitted in support of a pesticide registration. GLP regulations were initially promulgated in 1983. Compliance with GLP standards is monitored through a program of laboratory inspections and study audits coordinated through the EPA.

If, after a pesticide product has been registered and in commerce for some time, new data become available that suggest that criteria for determining unreasonable adverse effects have been exceeded, a special review (formally called a Rebuttable Presumption Against Registration) can be initiated. The special review may result in no change in the registration status of the pesticide (if the criteria for unreasonable risk are found not to have been exceeded) or may lead to restrictions in the use of a pesticide or the complete suspension or cancellation of the registration.

See also: Delaney Clause; Food, Drug, and Cosmetic Act, US; Food Quality Protection Act, US; Good Laboratory Practices (GLP); Levels of Effect in Toxicological Assessment; Pesticides; Pharmacokinetics/Toxicokinetics; Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Toxicity Testing, Alternatives; Uncertainty Analysis.

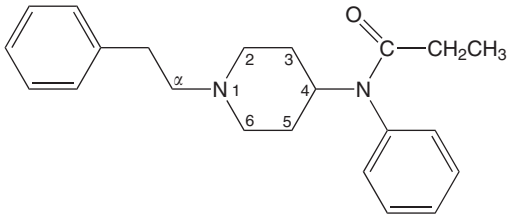
Female Reproductive System *See* Reproductive System, Female.

Fentanyl

Amanda Lofton

This article is a revision of the previous print edition article by Denise A Kuspis, volume 2, pp. 2–3, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 437-38-7
- Synonyms: Actiq; Phentanyl; Sublimaze; Duragesic
- Chemical/Pharmaceutical/Other Class: Synthetic opioid analgesic
- Chemical Formula: $C_{22}H_{28}N_2O$
- Chemical Structure:



Uses

Fentanyl is a potent analgesic. Intravenous or intramuscular fentanyl is used in the management of acute or postsurgical pain, as a component of balanced anesthesia, or as a preoperative analgesic. Fentanyl is administered via the epidural route in combination with bupivacaine for analgesia. Transdermal fentanyl is indicated for the treatment of chronic pain. Oral transmucosal fentanyl is used for breakthrough acute pain in patients concurrently receiving transdermal therapy. Fentanyl and its derivatives have high dependence liability.

Background Information

In October 2002, the Russian military released a mystery gas to incapacitate Chechen rebels in a theater in Moscow. Hundreds of hostages suffering from 'sleeping gas' poisoning were taken to local hospitals. According to physicians in Moscow, many patients exhibited the classic triad of symptoms

consistent with opioid intoxication: miosis, decreased level of consciousness, and respiratory depression. Investigation into the incident suggested that the gas was a mixture of a potent aerosolized fentanyl derivative, like carfentanil, and an inhalational anesthetic, such as halothane.

Exposure Routes and Pathways

Exposure may occur through the parenteral, oral, transmucosal, and dermal routes.

Toxicokinetics

Parenteral fentanyl is rapidly absorbed and effects are observed within minutes. Transdermal absorption is temperature dependent. Febrile patients will more rapidly absorb transdermal fentanyl. Skin in contact with the fentanyl patch absorbs drug and becomes a depot of fentanyl. A concentration gradient between the drug in the patch and the skin layers drives the continued absorption of drug. Steady-state serum concentrations are achieved ~2–24 h after initial patch application in the naive user. Peak serum concentrations of transmucosal fentanyl are achieved within 30–60 min of administration. Epidural concentrations peak after ~30 min. The duration of action of fentanyl varies according to the drug's route of administration. The effects of an intravenous dose last ~30–60 min. Transdermal fentanyl patches steadily release drug for more than 72 h. The volume of distribution of fentanyl ranges from 3 to 6 $l\,kg^{-1}$. The drug is ~80–86% protein bound in plasma. Fentanyl is primarily metabolized by the liver. Metabolism occurs via *N*-dealkylation to norfentanyl and other inactive metabolites. Approximately 75% of the drug is renally metabolized with 10% excreted unchanged in the urine. The elimination half-life varies with route of administration. An intravenous dose exhibits a half-life of ~219 min, a transmucosal dose 7 h, and transdermal administration 17 h. The half-life may be increased in patients with hepatic dysfunction.

Mechanism of Toxicity

Fentanyl stimulates mu-opioid receptors in the central nervous system (CNS), altering the body's response to pain. Fentanyl may alter the release of different neurotransmitters, such as β-endorphin, sensitive to pain. Fentanyl can produce profound CNS and respiratory depression through mechanisms common to other opioids. Respiratory depression is mediated through action on the medullary respiratory center. Fentanyl is ~50–100 times more potent by weight than morphine. However, unlike morphine, fentanyl appears to cause minimal histamine release. Fentanyl may induce chest wall rigidity, even when administered at therapeutic doses.

Acute and Short-Term Toxicity (or Exposure)

Animal

Fentanyl produces excitatory effects in cats, pigs, and horses. Its effects on dogs mimic its human toxicity. Naloxone can be administered to animals.

Human

Fentanyl overdose leads to the classic triad of symptoms consistent with the opioid intoxication syndrome: miosis, respiratory depression, and CNS depression. Additional toxic effects of fentanyl include bradycardia, hypotension, decreased gastrointestinal motility, euphoria, and acute lung injury.

Chronic Toxicity (or Exposure)

Animal

Studies in rats have demonstrated that fentanyl used in large doses can produce limbic system brain damage.

Human

Fentanyl is used chronically in the management of major pain in humans. One of the common side effects of therapy with opioids is constipation. However, a recent cohort analysis of a large California HMO looking at the incidence of constipation in patients receiving opioid analgesics showed a low incidence of constipation in the patients receiving fentanyl patches (3.7%).

In Vitro Toxicity Data

In an *in vitro* model of opioid dependence using rat pheochromocytoma cells, fentanyl produced an upregulation of adenylate cyclase-cAMP dependent protein kinase.

Clinical Management

Treatment is based on the patient's clinical presentation. Basic and advanced life support measures should be performed as needed. Activated charcoal may be utilized to adsorb orally administered fentanyl, such as the ingestion of a fentanyl patch. Whole bowel irrigation should be considered to speed the

elimination of an ingested transdermal patch. Naloxone is the specific pharmacologic antagonist for fentanyl. Naloxone displaces fentanyl at the opioid receptor and reverses its clinical effects.

See also: Charcoal; Morphine.

Further Reading

Arvanitis ML and Satonik RC (2002) Transdermal fentanyl abuse and misuse (letter). *American Journal of Emergency Medicine* 20: 58–59.

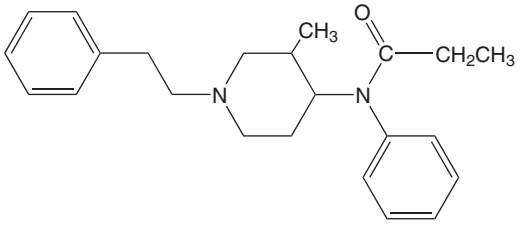
Chaturvedi AK, Rao NGS, and Baird JR (1990) A death due to self-administered fentanyl. *Journal of Analytical Toxicology* 14: 385–387.

Fentanyl Derivatives, Illicit

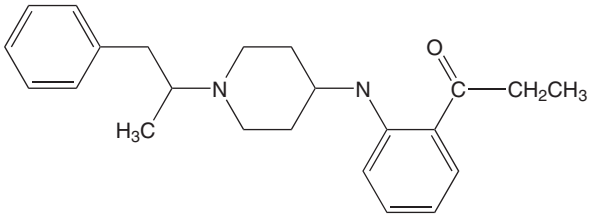
Amanda Lofton

This article is a revision of the previous print edition article by Denise A Kuspis, volume 2, pp. 3–4, © 1998, Elsevier Inc.

- Synonyms: 3-Methylfentanyl (3MF); α-Methylfentanyl; China White; Designer fentanyl; *p*-Fluorofentanyl; Street fentanyl; Synthetic heroin; Tango and Cash
- Chemical/Pharmaceutical/Other Class: Synthetic opioid
- Chemical Structures:



3-Methylfentanyl



α-Methylfentanyl

Uses

Illicit fentanyl derivatives are synthesized in clandestine laboratories solely for substance abuse. In the United States, these agents are classified as restricted Schedule I substances.

Background Information

In 1979, epidemic deaths among heroin users in Orange County, California, were traced to overdoses of α-methylfentanyl, sold under the name China White. Similar events occurred in Pittsburgh in 1988 and again in Philadelphia in 1992 with the sale of 3-methylfentanyl. In later years, another epidemic took place in New York City when 3-methylfentanyl reappeared on the illicit drug market under the name Tango and Cash.

Exposure Routes and Pathways

Illicit fentanyl derivatives may be nasally insufflated as powder or solubilized and injected intravenously.

Toxicokinetics

The exact kinetics of illicit fentanyl derivatives is uncertain. Kinetics may vary with each manufactured product batch. Fentanyl derivatives are rapidly absorbed across mucous membranes. Addicts report an onset of action or 'rush' within 90 s of administration. Illicit derivatives may be up to 6000 times more potent than morphine. Like their pharmaceutically manufactured counterparts, illicit fentanyl derivatives are likely metabolized by the liver.

Mechanism of Toxicity

Illicit fentanyl derivatives act as agonists at the opioid receptor. Unsuspecting heroin users typically administer their usual 'dose' of heroin, and receive variable amounts of the more potent fentanyl analog. These agents produce profound central nervous system and respiratory depression through mechanisms common to other opioids.

Acute and Short-Term Toxicity (or Exposure)

Animal

The effects of illicit fentanyl derivatives in many animals may be similar to the actions of fentanyl. Swine

develop stimulant effects when exposed to fentanyl. Naloxone can be administered to animals as needed.

Human

The toxic effects of illicit fentanyl derivatives include rapid onset respiratory and central nervous system depression. Patients often present comatose and apneic. Other signs and symptoms consistent with opioid intoxication such as bradycardia, hypotension, miosis, and decreased gastrointestinal motility also occur.

Chronic Toxicity (or Exposure)

Animal

Studies in rats have demonstrated that fentanyl used in large doses can produce limbic system brain damage.

Human

Fentanyl is used chronically in the management of major pain in humans. One of the common side effects of therapy with opioids is constipation. However, a recent cohort analysis of a large California HMO looking at the incidence of constipation in patients receiving opioid analgesics showed a low incidence of constipation in the patients receiving fentanyl patches (3.7%).

In Vitro Toxicity Data

In an *in vitro* model of opioid dependence using rat pheochromocytoma cells, fentanyl produced an upregulation of adenylate cyclase-cAMP dependent protein kinase.

Clinical Management

Basic and advanced life support measures should be initiated immediately. Activated charcoal may be utilized to adsorb illicit fentanyl derivatives following ingestion. Naloxone is the specific pharmacologic antagonist for fentanyl derivatives. Naloxone displaces these agents at the opioid receptor and reverses their clinical effects; however, higher than customary doses may be needed to successfully overcome the opioid receptor.

See also: Charcoal; Fentanyl; Heroin; Morphine.

Further Reading

Hardwick WE, King WD, and Palmisano PA (1997) Respiratory depression in a child unintentionally exposed to transdermal fentanyl patch. *Southern Medical Journal* 90: 962–964.

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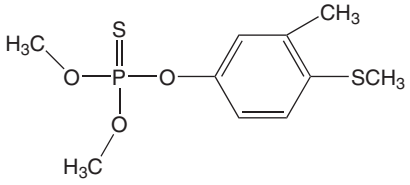
Fenthion

Andrew M Geller

Published by Elsevier Inc.

- Chemical Name: *O*,*O*-Dimethyl *O*-[4-(methylthio)-*m*-tolyl]phosphorothioate
- Chemical Abstracts Service Registry Number: CAS 55-38-9
- Synonyms: Baytex; Baycid; Bay 29493; Dalf; DMTP; Entex; Lebaycid; Tiguvon; Mercaptophos; Prentox Fenthion 4E; Queletox; S1752; Spotton; Talodex
- Chemical/Pharmaceutical/Other Class: Organophosphorothioate pesticide
- Chemical Formula: $C_{10}H_{15}O_3PS_2$
- Chemical Structure:



Uses

Fenthion is a broad spectrum insecticide. When applied as a surface spray, it controls adult mosquitoes and other insect pests and spiders in agricultural, horticultural, and home garden use. Recent reports indicate that resistance to fenthion has developed in some species of mosquitoes. Fenthion is used in dermal application for treatment of swine and cattle for control of lice, flies, and ticks, and in flea and tick treatments for pets. It is also used in aqueous applications to kill dragonfly larvae in ornamental fish production ponds. Fenthion is an effective avicide, and has been marketed to control birds considered to be pests.

While fenthion is currently available worldwide, use of fenthion in the United States has greatly decreased. Its use as an avicide was canceled in March, 1999. Its use as a livestock treatment was voluntarily withdrawn by the registrant (Bayer) over a 2 year period from March, 2000. In May, 2003, the registrant requested voluntary cancellation for all of their products containing fenthion. This will likely take effect in the United States in November, 2004.

Exposure Routes and Pathways

When fenthion is used in mosquito control operations that involve adulticide applications over wide areas, exposure to adults and children can occur. Health risk is considered low for homeowners performing yard work or for adults or children taking part in other recreational activities in treated areas because typical applications use ultra-low volumes (0.023–0.046 kg active ingredient (a.i.) per acre aerial, 0.014 kg a.i. per acre ground-based application). There is, however, concern for children if they are exposed to repeated levels at the maximum allowed rate. There is also concern for workers who mix, load, and/or apply fenthion for both aerial and ground mosquito adulticide applications.

Risk of dietary exposure to fenthion is largely due to potential residues in beef meat and fat; while it can be excreted in cow's milk, US Department of Agriculture (USDA) analyses of close to 1300 samples yielded no detections. The US Environmental Protection Agency's (US EPA's) most recent re-registration documents contain data on potential dietary exposure, but these estimates have not been refined because of the change in use of the pesticide.

Drinking water is not considered to be a significant source of exposure because only minor exposure to surface water is likely due to ultra-low volume application rates.

Toxicokinetics

Fenthion is quickly absorbed into the bloodstream through the digestive tract, lungs, and skin. It is eliminated through excretion in the urine and the feces. Studies in rats reported 14 urinary metabolites. The major urinary metabolites are the sulfoxide and sulfone forms of the compound. These are rapidly eliminated primarily via the urine. Four desmethyl metabolites were also identified with the oxygen analogue sulfoxide, constituting a minor component.

Mechanism of Toxicity

The major mechanism of toxicity for fenthion is inhibition of acetylcholinesterase by the oxon metabolite of fenthion. Following extensive cholinesterase inhibition, acetylcholine accumulates in synaptic regions and disrupts cholinergic transmission in the central and peripheral nervous systems. This can result in cholinergic signs and symptoms (see section Clinical Management, for a list of relevant signs and symptoms). Fenthion toxicity does not cause organophosphorus-induced delayed neuropathy (OPIDN) but has been reported to lead to the intermediate syndrome.

Recent work with models both *in vivo* and *in vitro* shows that fenthion also has antiandrogenic activity and can produce oxidative stress. More research is needed to establish whether these mechanisms are responsible for significant toxic effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values range from $\sim$150–550 mg kg^{-1} body weight (BW) in mouse, rat, and rabbit. Intraperitoneal and dermal LD_{50} values range from 125 to 330 mg kg^{-1} BW and from 330 to 800 mg kg^{-1} BW, respectively, in these species. An acute no-observed-adverse-effect level (NOAEL) of 0.07 mg kg^{-1} day^{-1} for dietary exposure for monkeys was derived from a 2 year exposure study.

Human

A 28 day study was conducted using male human subjects. No clinical cholinergic signs (described in the section Clinical Management) were noted during this study. Significant cholinesterase inhibition was noted at a dosage of 0.02 mg kg^{-1}day^{-1} one week after initiation of the study.

Chronic Toxicity (or Exposure)

Animal

Chronic toxicity studies on fenthion are available in the rat, dog, and monkey. The dog and monkey studies did not reveal any systemic signs of toxicity due to prolonged exposure to fenthion with the exception of cholinesterase inhibition. In the rat study, however, pathology was noted in the epididymis, the nasolacrimal duct, and in ocular tissue. Fenthion is not considered to be a carcinogen. While one test of carcinogenicity in mice indicated that fenthion may be a carcinogen in male mice, further studies in mice and rats did not support this.

Developmental toxicity studies in rat and rabbit do not show signs of enhanced sensitivity of the developing fetus to fenthion. Dams exhibited clinical signs and decreased body weights at the same dosages that induced fetal effects. In addition, plasma, erythrocyte, and brain cholinesterase inhibition was seen in dams at doses lower than those causing fetal effects. For this reason, the Food Quality Protection Act safety factor for fenthion was reduced from a default 10$\times$ to 1$\times$.

Human

Studies of pesticide applicators, who sprayed mainly fenthion showed some adverse effects on cognitive function and a higher incidence of retinal (macular) degeneration than in controls or in applicators who

sprayed other pesticides. Findings of ocular toxicity were also noted in rats dosed subcutaneously with fenthion for a year. This ocular effect may be related to long-lasting effects of fenthion on biochemical function in the retina.

In Vitro Toxicity Data

Fenthion did not show evidence of mutagenicity in the bacterial reverse mutation test or in the chromosome aberration test in Chinese hamster ovary cells. A test of unscheduled DNA synthesis in rat hepatocytes was also negative. Fenthion showed a weakly positive response in 2 of 5 assays for sister chromatid exchange.

Clinical Management

If poisoning is suspected, one should not wait for symptoms to develop. A physician, the nearest hospital, or the nearest Poison Control Center should be contacted immediately. Signs and symptoms of fenthion toxicity include:

- *Mild:* Headache, dizziness, weakness, anxiety, pupillary contraction, blurred vision, and nausea.
- *Moderate:* Nausea, salivation, lacrimation, abdominal cramps, diarrhea, vomiting, sweating, slow pulse, muscular tremors and respiratory compromise.
- *Severe:* Respiratory difficulty, pinpoint and nonreactive pupils, pulmonary edema, cyanosis, loss of sphincter control, muscle spasms, convulsion, coma, and eventual death due to respiratory failure.

Treatment

Eye and Skin Exposure For exposure to the eyes, the eyelids should be held open and the eyes flushed with copious amounts of water for at least 15 min. In the case of skin contact, the affected areas should be washed immediately with soap or shampoo and water, as appropriate.

Inhalation If fenthion is inhaled, the victim should be removed from the source of contamination to fresh air. If the victim is not breathing, artificial respiration should be administered and medical attention sought as soon as possible.

Ingestion If the victim is alert and respiration is not depressed, vomiting should be induced. Gastric decontamination should be performed within 30 min of ingestion to be most effective.

Atropine sulfate, in conjunction with pralidoxime (2-PAM), can be administered as an antidote. Atropine should be administered by intravenous injection. Intramuscular injection can be used if IV injection is not possible. Atropine dosage: Adults: 0.4–2.0 mg repeated every 15 min until atropinization is achieved: tachycardia (fast pulse), skin flushing, dry mouth, clearing of bronchial secretions. Atropinization should be maintained by repeated doses for 2–12 h or longer depending on severity of poisoning. Children under 12 years: 0.05 mg kg^{-1} BW, repeated every 15 min until atropinization is achieved. Maintain atropinization with dosage of 0.02–0.05 mg kg^{-1} BW.

2-PAM should be administered in conjunction with atropine in cases of severe poisoning in which respiratory depression, muscle weakness, and twitching is severe. Adults: 1.0 g IV at no more than 0.5 g min^{-1}. Children under 12 years: 20–50 mg kg^{-1} (depending on severity of poisoning) IV, injecting no more than half the total dose per minute. This may be repeated at 2–4 h.

Environmental Fate

The persistence of fenthion in the environment is dependent on several factors, including photolysis, metabolism in plants and insects, and microbial degradation. Estimates of the half-life of fenthion in soil vary from <1 day in studies cited by the US EPA for aerobic soil metabolism to 3–6 weeks, cited by Extoxnet. Half-lives for aquatic degradation range from 2.9 to 21.1 days for various ocean, river, swamp, or lake aquatic conditions. Sunlight accelerates degradation of fenthion 20-fold in river water and fivefold in seawater.

The fenthion parent compound is fairly insoluble in water and binds tightly to soil particles. It is, therefore, relatively immobile in most soil types. Its transformation products have higher mobility through the soil.

Fenthion has an octanol:water partition coefficient ($\log K$ O:W) of 4.8, which indicates that it has the potential to bioaccumulate in fish and nontarget organisms.

Ecotoxicology

Fenthion is very highly toxic to birds. The use of fenthion for control of mosquitos has been implicated in several bird kills, including recent incidents on Marco Island, Florida. The major metabolites, fenthion phenol sulfoxide and fenthion phenol sulfone, have very low toxicity to birds.

Fenthion is also very highly toxic to freshwater, estuarine, and marine invertebrates, and moderately to highly toxic to fish. Fenthion is reported to be toxic to American linden, hawthorn and sugar maple trees, and to certain rose varieties. Germination and vegetative-vigor tests showed that fenthion had little effect on a wide variety of food plants. Toxicity testing using aquatic plants indicated that fenthion is not particularly toxic to these plants.

Exposure Standards and Guidelines

Fenthion is classified as a Toxicity Category II chemical for acute oral, dermal, and inhalation toxicity. It is classified in Toxicity Category III for eye irritation and Category IV for dermal irritation. It is not considered by the US EPA to be a carcinogen, and is therefore classified as a Group E chemical, that is, not likely to be carcinogenic in humans via relevant routes of exposure.

The US EPA Acute Dietary population adjusted dose (PAD) is $0.0007\,mg\,kg^{-1}\,day^{-1}$ fenthion. This standard is based on plasma cholinesterase inhibition in monkey (NOAEL $= 0.07\,mg\,kg^{-1}\,day^{-1}$), divided by a composite uncertainty factor of 100 (10× interspecies, 10× intraspecies), and a 1× factor for Food Quality Protection Act (FQPA).

The US EPA Chronic Dietary PAD of $0.000\,07\,mg\,kg^{-1}\,day^{-1}$ is also based on the monkey NOAEL/LOAEL $= 0.02\,mg\,kg^{-1}\,day^{-1}$, with a composite uncertainty factor of 300 (as above, with an additional 3× for lack of a true NOAEL).

See also: Organophosphate Poisoning, Intermediate Syndrome; Organophosphates; Pesticides.

Further Reading

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Relevant Websites

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http://www.inchem.org – International Programme on Chemical Safety (ICPS). Fenthion, Pesticide residues in food: 1995 evaluations Part II Toxicological & Environmental, 1995.

http://www.hc-sc.gc.ca – Pest Management Regulatory Agency, Health Canada. Re-evaluation of Fenthion. PACR2003-05, March, 2003.

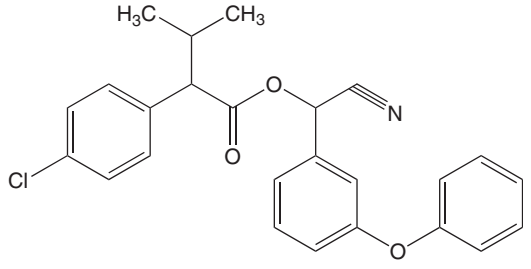
http://www.epa.gov – US Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances. Interim Reregistration Eligibility Decision for Fenthion. EPA 738-R-00-013, January, 2001.

Fenvalerate

Betty J Locey and Janice Reeves

This article is a revision of the previous print edition article by Carey N Pope and Janice Reeves, volume 2, pp. 5–6, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 51630-58-1
- Synonyms: Sumicidin; Pydrin; Phenvalerate; Cyano(3-phenoxyphenyl)methyl-4-chloro-α-(1-methylethyl) benzeneacetate; (*RS*)-α-Cyano-3-phenoxybenzyl (*RS*)-2-(4-chlorophenyl)-3-methylbutyrate. Esfenvalerate is an isomer of fenvalerate with its own common name
- Chemical/Pharmaceutical/Other Class: Type II synthetic pyrethroid insecticide
- Chemical Formula: $C_{25}H_{22}ClNO_3$
- Chemical Structure:



Uses

Fenvalerate is a restricted-use pyrethroid pesticide used to control insects on food crops (both leaves and fruit), on animal feed, and on cotton. Food crops include peanuts, soybeans, sugarcane, and sunflowers. It is also used to control flies and ticks in stables and barns.

Exposure Routes and Pathways

Fenvalerate may be absorbed through the skin, through the respiratory tract if inhaled, and through the digestive tract if ingested. Dermal contact is the main route of exposure during application.

Toxicokinetics

Fenvalerate is absorbed readily following ingestion, dermal exposure, or inhalation. Plasma levels of pyrethroids are not clinically useful. Fenvalerate undergoes ester cleavage to alcohol followed by rapid hydroxylation. Fenvalerate is distributed to lipid-rich tissues including the brain. Elimination from fatty tissues is slow, with a half-life of 2–7 days. Fenvalerate is eliminated through urine.

Mechanism of Toxicity

Fenvalerate has low toxicity in mammals due to its rapid metabolic breakdown. It acts directly on nerve axons by prolonging sodium channel opening in cell membranes. Insects exposed to fenvalerate are quickly paralyzed: exposure causes quick insect knockdown. In small animals, type II pyrethroids cause salivation, chewing, burrowing, choreoathetosis, and seizures. They also cause lower action potential amplitude, marked membrane depolarization, and eventual total neural activity blockade. Fenvalerate is likely to act both on peripheral and central nervous system. It is also a potent inhibitor of calcineurin (protein phosphatase 2B).

Acute and Short-Term Toxicity (or Exposure)

Animal

Fenvalerate has moderate mammalian toxicity, with an oral LD_{50} in rats $>400\,mg\,kg^{-1}$. The dermal LD_{50} in rabbits was $>2\,g\,kg^{-1}$. It is practically nontoxic by inhalation, with an LC_{50} in rats of $>2.9\,mg\,l^{-1}$. Animals exposed to fenvalerate may exhibit choreoathetosis (abnormal body movements), salivation (CS-syndrome), restlessness, tremors, and piloerection (hair of the skin stands up).

Human

Ingestion commonly results in headaches, dizziness, weakness, nausea, and vomiting. The solvent appears to markedly affect toxicity. A more concentrated dose may cause seizures and coma. Common adverse effects of inhalation are runny nose and scratchy throat. Hypersensitivity reactions that may be noted include wheezing, sneezing, shortness of breath, pneumonitis, pulmonary edema, bronchiospasm, and chest pain. Contact with eyes may cause mild to severe corneal damage. Dermal exposures cause tingling and burning sensations and numbness of the skin. Excitability, tremors, incoordination, numbness, seizures, and coma may result from massive exposure.

Chronic Toxicity (or Exposure)

Animal

Six of 50 male rats exposed to $1000\,mg\,kg^{-1}$ in a 2 year feeding study showed transient muscular incoordination of the hind limbs, abnormal gait, and ataxia during the third and fourth weeks of the study.

Mice exposed for 12 months or longer reportedly showed evidence of an inflammatory response in the liver, lymph nodes, or spleen. The response was characterized by giant cell infiltration and/or multifocal microgranulomata, typical of a 'foreign-body' type of response. A similar response has been reported in dogs exposed to 1000 mg fenvalerate per kg in the diet for 6 months. There is little or no evidence to suggest that fenvalerate is carcinogenic, mutagenic, or a reproductive/developmental toxicant in animals. Demyelination in peripheral nerves has been reported in experimental animals. Teratogenicity tests have been negative.

Human

Fenvalerate does not pose significant chronic hazard potential.

In Vitro Toxicity Data

Mutagenicity tests with and without metabolic activation have been negative. Testing included *Salmonella typhimurium* assays, testing in *Bacillus subtilis*, and testing in V79 Chinese hamster cells. In addition, bone marrow evaluated after oral exposure was not reported to contain chromosomal damage. It was not reported to cause dominant lethal mutations in mice at $100\,mg\,kg^{-1}\,day^{-1}$.

Clinical Management

There is no antidote for fenvalerate. Treatment is primarily supportive. The victim should be monitored for the development of respiratory distress, seizures, and hypersensitivity reactions. Convulsions are often treated with diazepam. Prevention of absorption may be accomplished by gastric lavage followed by activated charcoal. Some formulations include solvents so care should be taken to protect against pulmonary effects during lavage. Basic and advanced life-support measures should be used as necessary.

Environmental Fate

Fenvalerate has low water solubility ($<300\,\mu g\,l^{-1}$). Its solubility in surface waters is increased with organic matter. Fenvalerate is moderately persistent in soil, with a half-life of 0.5–3 months. Fenvalerate and its degradation products are relatively immobile and not expected to pose leaching problems. Because of this and low water solubility, it has not been found in groundwater sampling. Fenvalerate undergoes photodegradation in water.

Ecotoxicology

Fish are extremely sensitive to fenvalerate. A 48 h LC_{50} of $<0.1\,mg\,l^{-1}$ was reported in carp and a 96 h LC_{50} of $3.6\,\mu g\,l^{-1}$ was reported in rainbow trout. Fenvalerate is highly toxic to *Daphnia*, with an LC_{50} of $1\,\mu g\,l^{-1}$. Fenvalerate is only slightly toxic to birds. Oral LD_{50} values reported in hens, bobwhite quail, and mallard ducks were all $>1\,g\,kg^{-1}$. Fenvalerate is highly toxic to honey bees with a contact LD_{50} of 0.23 μg per bee.

Other Hazards

Avoid contact with acids and bases. In a fire, thermal decomposition will occur, and may produce toxic gases such as carbon monoxide and carbon dioxide.

Exposure Standards and Guidelines

The US Environmental Protection Agency IRIS developed an oral reference dose of 2.5×10^{-2} mg $kg^{-1}\,day^{-1}$ based on a 13 week feeding study in rats. The no-observed-effect level was identified as 125 ppm. The critical effect was identified as neurological dysfunction.

The American Conference of Governmental Industrial Hygienists threshold limit value, time-weighted average, is $5\,mg\,m^{-3}$. The minimal lethal dose is probably in the range of 10–100 g.

Maximum residue limits have been recommended by the Joint FAO/WHO Meeting on Pesticide Residues. An acceptable daily intake of 0–0.02 $mg\,kg^{-1}$ body weight was established for fenvalerate by JMPR in 1986.

Further Reading

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IARC (1991) Fenvalerate. In: *Occupational Exposures in Insecticide Application, and some Pesticides*, vol. 53, pp. 309–328. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Lyon, France: International Agency for Research on Cancer.

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Relevant Website

http://www.inchem.org – WHO/FAO (1996) Data Sheets on Pesticides. No. 90. Fenvalerate. WHO/PCS/DS/96.90.

Fetal Alcohol Syndrome

Kartik Shankar and Harihara M Mehendale

Fetal alcohol syndrome (FAS) is a set of birth defects caused by maternal consumption of alcohol during pregnancy. The term FAS was coined in 1973 by Jones and Smith in their seminal article that described the constellation of birth defects that characterize FAS in children born to mothers known to have consumed alcohol during pregnancy. FAS is considered the most common preventable cause of mental retardation. The annual cost of FAS according to the 10th Special Report to the US Congress on Alcohol and Health estimated the annual cost of FAS in 1998 to be $2.8 billion.

Clinical Assessment of FAS

Most experts agree on three diagnostic criteria for the clinical assessment of FAS: (1) prenatal or postnatal growth restriction; (2) central nervous system (CNS) effects including insufficient development of the brain such as microcephaly, agenesis of corpus callosum; and (3) specific craniofacial dysmorphic features. However, it is more difficult to diagnose more subtle effects of fetal alcohol effects in which the more pronounced effects of FAS are not apparent. FAS is estimated to occur at a rate of 5–10 per 10 000 live births in the United States, but it may be higher in certain populations. However, the true incidence of FAS and fetal alcohol effects (FAE) are probably greater because it is difficult to recognize the symptoms and to obtain reliable history of alcohol ingestion. The overall incidence of FAS among infants born to mothers who have a history of drinking alcohol during pregnancy is only 2–3%.

Risk factors for FAS

Several risk factors determine the toxic outcome of *in utero* alcohol exposure. Risk factors suggested include genetic predisposition, marital status, smoking, use of prescribed or over-the-counter drugs and medications, concomitant usage of other drugs of abuse, occupational or environmental exposure to chemicals, socioeconomic status, and adequate nutrition.

Models of FAS

Research using animal models has shown that each of the major characteristics of human FAS, including craniofacial abnormalities, growth deficiency, and abnormalities of the CNS occurs in one of these animal models including mice, rats, chicks, and primates. Because different species and even strains within species show different degrees of vulnerability to alcohol, experimental results must be interpreted with caution. However, most common animal models of FAS (rats and mice) are very similar to humans and their biochemical processes are virtually the same. Research from animal models has also revealed critical time periods of vulnerability that leads to certain FAS abnormalities. For example, in the mouse and chicken embryos exposure to alcohol during cranial neural crest cells development corresponding to the first 3–4 weeks of human gestation resulted in patterns consistent with observed dysmorphologies of cranial structures and craniofacial defects.

How Much Alcohol is Safe in Pregnancy?

There is no safe drinking level of alcohol during pregnancy. Significant controversy surrounds the amount of alcohol that presents a risk to the fetus and whether a single exposure is of greater consequence than a pattern of exposure during development. Results from clinical and animal studies demonstrate that lower levels of alcohol are needed to produce behavioral anomalies than are needed to produce physical effects and that some brain regions are more susceptible than others. FAS is completely preventable by abstinence to alcohol. The American Academy of Pediatrics recommends counseling women of childbearing age about the effects of alcohol on the fetus. In addition, government-required warning labels about the health effects of alcohol are displayed on alcoholic beverages. A recent study found almost 80% of 7334 women interviewed were aware of the detrimental effects of alcohol, including the high-risk drinkers. However, the warning labels had only a modest effect on personal risk perceptions and drinking behaviors, clearly stressing the need for other effective strategies to decrease alcohol consumption during pregnancy. Considering the cost in economic and human suffering imposed by FAS on the child and the family, prevention via abstinence seems to be the most effective way in reducing incidence of FAS. Identifying high-risk drinkers is an important first step in this process.

Mechanisms of Induction of FAS

The mechanisms leading to FAS remain elusive. A single mechanism for the entire spectrum of FAS is unlikely. Influences of genetic susceptibility and nutrition may be of critical importance. Clearly simultaneous or prior exposure to other chemicals may influence the mechanisms and the nature and extent of effects. Several mechanisms involving the direct effects of alcohol on neural development and organogenesis have been explored in animal models. Among them are alcohol-induced changes in neural cell proliferation, reduced growth and neurotropic factors, inhibition of cell adhesion molecule L1, increased oxidative stress and production of free radicals, fetal zinc deficiency, altered vitamin A and folate function, impaired placental function, and disruption of retinoic acid. Of special note is the mechanism of alcohol-induced inhibition of the cell adhesion molecule L1, which is involved in neural cell migration. Recent animal studies have shown that NAPVSIPQ, an active fragment of the glial-derived activity-dependent neuroprotective protein, which antagonizes the alcohol-induced inhibition of L1 also protects from alcohol-induced fetal growth retardation and demise. These studies open an exciting area of potential pharmacological intervention for FAS.

See also: Alcoholic Beverages and Alcoholism.

Further Reading

Randall CL (2001) Alcohol and pregnancy: Highlights from three decades of research. *Journal of Studies on Alcohol* 62: 554–561.

Tenth Special Report to the US Congress on Alcohol and Health. US Department of Health and Human Services.

Relevant Website

http://www.niaaa.nih.gov – 10th special report – National Institution on Alcohol Abuse and Alcoholism.

Fexofenadine

Stephen R Clough

- Chemical Name: (±)-*p*-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl) piperidino]butyl]-a-methylhydratropic acid, carboxyterfenadine, terfenadine carboxylate, a-dimethyl benzeneacetic acid hydrochloride
- Chemical Abstracts Service Registry Number: CAS 83799-24-0
- Synonyms: Fexofenadine hydrochloride (generic name); ALLEGRA; ALLEGRA-D
- Chemical/Pharmaceutical/Other Class: Pharmaceutical; Antihistamine (H1-receptor antagonist)
- Chemical Formula: $C_{32}H_{39}NO_4 \cdot HCl$

Uses

Fexofenadine hydrochloride is a white to off-white crystalline powder. It is soluble in methanol and ethanol, slightly soluble in chloroform and water, and insoluble in hexane. It is the active ingredient of ALLEGRA and acts as a histamine H1-receptor antagonist. Fexofenadine is a selective antihistamine used to relieve allergic rhinitis (seasonal allergy) symptoms including sneezing, runny nose, itching, and watery eyes that come with hay fever. Its effect begins in 1 h and lasts 12 h, peaking around the second or third hour. ALLEGRA is one of the new types of antihistamines that rarely cause drowsiness.

In addition to the antihistamine in ALLEGRA, ALLEGRA-D also contains the nasal decongestant pseudoephedrine.

Exposure Routes and Pathways

ALLEGRA is a drug that is prescribed to be taken in pill form, so exposure would only be expected to occur orally.

Toxicokinetics

In laboratory animals, no anticholinergic, α1-adrenergic, or *β*-adrenergic-receptor blocking effects were observed. No sedative or other central nervous system effects were observed. Radiolabeled tissue distribution studies in rats indicated that fexofenadine does not cross the blood–brain barrier.

Fexofenadine hydrochloride was rapidly absorbed following oral administration of a single dose of two 60 mg capsules to healthy male volunteers with a mean time to maximum plasma concentration occurring at 2.6 h postdose. After administration of a single 60 mg capsule to healthy subjects, the mean maximum plasma concentration was 131 ng ml^{-1}. Following single-dose oral administrations of either the 60 or 180 mg tablet to healthy, adult male volunteers, mean maximum plasma concentrations were 142 and 494 ng ml^{-1}, respectively. The tablet formulations are bioequivalent to the capsule when administered at equal doses. Fexofenadine hydrochloride pharmacokinetics is linear for oral doses up to a total daily dose of 240 mg (120 mg twice daily). The administration of the 60 mg capsule contents mixed with applesauce did not have a significant effect on the pharmacokinetics of fexofenadine in adults.

Fexofenadine hydrochloride is 60–70% bound to plasma proteins, primarily albumin and α_1-acid glycoprotein. The mean elimination half-life of fexofenadine was 14.4 h following administration of 60 mg, twice daily, in normal volunteers. Human mass balance studies documented a recovery of ~80% and 11% of the [^{14}C] fexofenadine hydrochloride dose in feces and urine, respectively. Because the absolute bioavailability of fexofenadine hydrochloride has not been established, it is unknown whether the fecal component represents unabsorbed drug or the result of biliary excretion. Approximately 5% of the total oral dose was metabolized.

Special population pharmacokinetics (for geriatric subjects, renal, and hepatic impairment), obtained after a single dose of 80 mg fexofenadine hydrochloride, were compared to those for normal subjects from a separate study of similar design. While subject weights were relatively uniform between studies, these adult special population patients were substantially older than the healthy, young volunteers. Thus, an age effect may be confounding the pharmacokinetic differences observed in some of the special populations.

In older subjects ($=65$ years old), peak plasma levels of fexofenadine were much greater than those observed in normal volunteers (<65 years old). Mean elimination half-lives were similar to those observed in normal younger volunteers.

Cross-study comparisons indicated that fexofenadine hydrochloride distribution in the body of 7–12-year-old pediatric allergic rhinitis patients following oral administration of a 60 mg dose was 56% greater compared to healthy adult subjects given the same dose. Plasma concentrations in pediatric patients given a dose that is one-half of what an adult would receive (30 mg fexofenadine hydrochloride in child versus 60 mg for adult) is comparable to adults.

In patients with mild to moderate and severe kidney impairment, peak plasma levels of fexofenadine were 87% and 111% greater, respectively, and mean elimination half-lives were 59% and 72% longer, respectively, than observed in normal volunteers. Peak plasma levels in patients on dialysis were 82% greater and half-life was 31% longer than observed in normal volunteers. Based on increases in bioavailability and half-life, a dose of 60 mg once daily is recommended as the starting dose in patients with decreased renal function. The pharmacokinetics of fexofenadine hydrochloride in patients with hepatic disease did not differ substantially from that observed in healthy patients.

Acute and Short-Term Toxicity (or Exposure)

Most drugs are proved to be safe through a series of short- (acute) and long-term (chronic) tests. No drugs that show adverse toxic affects when tested as single-dose study will make it into the long-term study programs.

Animal

In acute toxicity studies with laboratory animals, clinical signs of toxicity and effects on body weight or food consumption were not observed in several animal species administered fexofenadine by oral lavage at doses up to $2000\,mg\,kg^{-1}$.

In *in vitro* (bacterial reverse mutation, CHO/HGPRT forward mutation, and rat lymphocyte chromosomal aberration assays) and *in vivo* (mouse bone marrow micronucleus assay) tests, fexofenadine hydrochloride revealed no evidence of mutagenicity.

No deaths occurred at oral doses of fexofenadine hydrochloride up to $5000\,mg\,kg^{-1}$ in mice (110 times the maximum recommended daily oral dose in adults and 200 times the maximum recommended daily oral dose in children based on $mg\,m^{-2}$) and up to $5000\,mg\,kg^{-1}$ in rats (230 times the maximum recommended daily oral dose in adults and 400 times the maximum recommended daily oral dose in children based on $mg\,m^{-2}$). Additionally, no clinical signs of toxicity or gross pathological findings were observed. In dogs, no evidence of toxicity was observed at oral doses up to $2000\,mg\,kg^{-1}$ (300 times the maximum recommended daily oral dose in adults and 530 times the maximum recommended daily oral dose in children based on $mg\,m^{-2}$).

Human

Most reports of fexofenadine hydrochloride overdose contain limited information. However, dizziness, drowsiness, and dry mouth have been reported. Single doses up to 800 mg and doses up to 690 mg twice daily for 1 month were studied in healthy subjects without the development of clinically significant adverse events.

Chronic Toxicity (or Exposure)

All drug studies require chronic animal tests before they can be tested in primates or humans. Fexofenadine is now on the market and is therefore considered 'safe' by standards set by the US Food and Drug Administration.

Animal

The carcinogenic potential and reproductive toxicity of fexofenadine hydrochloride were assessed using terfenadine studies with adequate fexofenadine hydrochloride exposure (based on plasma area under the concentration versus time (AUC) values). No evidence of carcinogenicity was observed in an 18 month study in mice and in a 24 month study in rats at oral doses up to $150\,mg\,kg^{-1}$ of terfenadine (which led to fexofenadine exposures that were, respectively, $\sim$3 and 5 times the exposure from the maximum recommended daily oral dose of fexofenadine hydrochloride in adults and children).

In rat fertility studies, dose-related reductions in implants and increases in postimplantation losses were observed at an oral dose of $150\,mg\,kg^{-1}$ of terfenadine (which led to fexofenadine hydrochloride exposures that were $\sim$3 times the exposure of the maximum recommended daily oral dose of fexofenadine hydrochloride in adults).

There was no evidence of teratogenicity (category C) in rats or rabbits at oral doses of terfenadine up to $300\,mg\,kg^{-1}$ (which led to fexofenadine exposures that were $\sim$4 and 31 times, respectively, the exposure from the maximum recommended daily oral dose of fexofenadine in adults).

Dose-related decreases in pup weight gain and survival were observed in rats exposed to an oral dose of $150\,mg\,kg^{-1}$ of terfenadine ($\sim$3 times the maximum recommended daily oral dose of fexofenadine hydrochloride in adults based on comparison of fexofenadine hydrochloride AUCs).

Human

There are no adequate and well-controlled studies in pregnant women. Fexofenadine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Clinical Management

Medication is used to cause a desired biological effect. When used, other effects, side effects, may also

occur. These may be mild or serious. If side effects develop a physician should be consulted to determine whether the use of the drug should be discontinued. Side effects of ALLEGRA may include colds or flu, drowsiness, fatigue, indigestion, menstrual problems, and nausea. Side effects of ALLEGRA-D may include abdominal pain, agitation, anxiety, back pain, dizziness, dry mouth, headache, heart palpitations, indigestion, insomnia, nausea, nervousness, respiratory tract infection, and throat irritation.

Generally, ALLEGRA-D should not be used by patients who have glaucoma, urination problems, or severe high blood pressure or heart disease. Use is not recommended for children under 12 years. ALLEGRA-D should not be taken within 2 weeks of using an MAO-inhibitor drug (e.g., Marplan, Nardil, or Parnate). Some individuals may be allergic to ALLEGRA or ALLEGRA-D.

See also: Immune System; Respiratory Tract.

Further Reading

Meeves SG and Appajosyula S (2003) Efficacy and safety profile of fexofenadine HCl: A unique therapeutic option in H1-receptor antagonist treatment. *The Journal of Allergy and Clinical Immunology* 112(Suppl. 4): S69–S77.

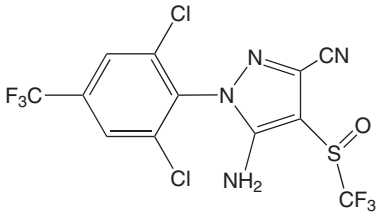
Relevant Websites

http://www.rxlist.com – The Internet Drug Index.
http://www.fda.gov – US Food and Drug Administration.

Fipronil

Xilong Zhao

- Chemical Name: 5-Amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((1,*R*,*S*)-(trifluoromethyl)sulfinyl)-1-*H*-pyrazole-3-carbonitrile
- Chemical Abstracts Service Registry Number: CAS 120068-37-3
- Synonym: Fiprole
- Chemical/Pharmaceutical/Other Class: Fiprole; Phenylpyrazole insecticide; Chloride channel blocker
- Chemical Formula: $C_{12}H_4Cl_2F_6N_4OS$
- Chemical Structure:



Uses

Fipronil is a highly active, broad-spectrum insecticide from the phenylpyrazole family and has been on the market since 1993 under different brands. It is widely used for crop protection against major lepidopterous and orthopterous pests on a wide range of field and horticultural crops. Fipronil is used against coleopterous larvae in soils and on golf and commercial turf grass. Fipronil is also commonly used as a public hygiene insecticide to control fleas, ticks and mites on domestic animals under the trade name Frontline, Topspot, or Combat, for control of cockroaches and ants under the brand name Maxforce FC Baits, Goliath, and Nexa, to control termites under the name Termidor, and for control of fire ants under the name Over'n Out!.

Exposure Routes and Pathways

The potential routes for occupational exposure to fipronil are through inhalation, skin contact, and ingestion during production, packaging, and application of fipronil products. Potential for nonoccupational exposure to fipronil, its major metabolite fipronil sulfone or its major photodegradation product desulfinyl fipronil is expected to be very low. Since fipronil has an extremely low vapor pressure and low dermal penetration, nonoccupational exposure to fipronil through inhalation and skin is minimal. Exposure through contacting pet animals applied with Frontline fipronil product is also expected to be low.

Toxicokinetics

Fipronil is well absorbed from the gut but relatively poorly absorbed from the skin. Once absorbed, fipronil is rapidly oxidized to the sulfone derivative by the cytochrome P450 NADPH-dependent monooxygenases in liver and other tissues. Fipronil is also metabolized via hydrolysis of nitrile to yield an amide metabolite. Both fipronil and its metabolites

are well distributed in tissues where significant amounts of residues can remain, particularly in fatty tissues. The long half-life (150–245 h in some cases) of fipronil in blood may reflect slow release of residues from fat and might suggest potential bioaccumulation of metabolic products of fipronil. The major routes of elimination of fipronil and its metabolites are feces (45–75% in rat) and urine (5–25% in rat).

Mechanism of Toxicity

Fipronil has a higher toxicity to insects than to mammals. The putative mode of insecticidal activity of fipronil is to block the GABA- and glutamate-regulated chloride ion channels, which are responsible for inhibition of normal neural activity. When the inhibitory function is suppressed by fipronil, neural overexcitation ensues leading to death of the insect. The mammalian toxicity of fipronil is related to its blocking action on $GABA_A$ receptor chloride channels in the nervous system. The high selective toxicity of fipronil to insects may be due to its higher potency to block the insect GABA-regulated chloride channel than the mammalian GABA-regulated chloride channel, and its potent inhibitory action on invertebrate-specific glutamate-regulated chloride channels not present in higher organisms.

Acute and Short-Term Toxicity (or Exposure)

Animal

Fipronil is classed as a World Health Organization Class II moderately hazardous pesticide and is less toxic to mammals than to some birds, fish and most invertebrates. Fipronil has moderate acute toxicity by the oral and inhalation routes in rats with acute LD_{50} values of $97\,mg\,kg^{-1}$ and $0.39–0.68\,mg\,l^{-1}$, respectively. The acute oral LD_{50} of fipronil in mouse is $95\,mg\,kg^{-1}$. Dermal absorption in rats is less than 1% after 24 h and acute toxicity is considered to be low with an LD_{50} of more than $2000\,mg\,kg^{-1}$. In contrast, it has moderate dermal toxicity to rabbits with an LD_{50} of $354\,mg\,kg^{-1}$. Fipronil may cause mild irritation to the eyes and slight skin irritation, but it is not a skin sensitizer in guinea pigs. Signs of toxicity in rats include reduced food consumption, anuria, increased excitability and seizures. Affected organs may include the liver, thyroid and kidney. Desulfinyl fipronil is ~10 times more toxic to mammals than fipronil itself.

Human

No confirmed human intoxication cases have been reported.

Chronic Toxicity (or Exposure)

Animal

Fipronil is neurotoxic in both rats and dogs. Rats receiving 300 ppm fipronil in males ($12.68\,mg\,kg^{-1}\,day^{-1}$) and females ($16.75\,mg\,kg^{-1}\,day^{-1}$) showed an increased incidence of thyroid follicular cell tumors. Similar studies in mice and dogs did not show an increased incidence of thyroid tumors, however. Fipronil can induce reproductive toxicity in rats at high dietary exposure levels (300 ppm). Clinical signs include decreased fertility, decreased body weights in litters, and resorptions. Fipronil may cause a delay in body development at high doses, but there is no evidence of teratogenicity.

Human

Fipronil is classified as a group C (possible human) carcinogen based on findings in rats. Human data on cancer, reproductive and development toxicity are not available.

In Vitro Toxicity Data

Fipronil was not mutagenic in the *Salmonella* or HGPRT gene mutation assay at concentrations up to 500 μg per plate and $386\,\mu g\,ml^{-1}$, respectively. No evidence of a clastogenic or aneugenic effect of fipronil was found in the micronucleus assay or human lymphocyte cytogenic assay *in vitro* at concentrations up to $300\,\mu g\,ml^{-1}$.

Clinical Management

Fipronil can be harmful or fatal with overexposure through skin, ingestion, or inhalation routes. It may cause skin irritation, rash, edema, shortness of breath, drowsiness, excitement, involuntary shaking, and convulsions. Eye contact may cause redness and tearing. Inhalation of fipronil may aggravate existing chronic respiratory problems such as asthma, emphysema, or bronchitis.

If exposed on skin or clothing, the contaminated clothing should be removed and the skin rinsed with copious water for 20 min. Exposed eyes should be immediately irrigated with a steady, gentle stream of water for 20 min. In case of inhalation or ingestion exposure, move the person to fresh air and call a poison control center or doctor immediately for treatment advice.

There is no specific antidote for fipronil intoxication. Clinical treatment should be based on observed signs and symptoms of distress in the patient. Recommendations for treatment of overexposure cases

are based on routine anticonvulsant therapy. Phenobarbital or diazepam may be useful in controlling convulsions induced by fipronil. For phenobarbital, start with 10–20 mg kg^{-1} of phenobarbital in rapid intravenous perfusion and continue according to the patient's response. For diazepam, start with 10–30 mg diazepam by intravenous injection according to body weight. This dose is to be repeated every 10–30 min according to the patient's response. Even when symptoms of fipronil intoxication are rapidly reversed by treatment, the treatment must be continued for several days, gradually decreasing the dose of anticonvulsant based on the patient's clinical response. This is necessary due to the slow elimination of the compound.

Environmental Fate

Fipronil is photolyzed into several degradates represented by desulfinyl fipronil and fipronil sulfone in the field. Fipronil is also rapidly metabolized to fipronil sulfone in plants. The half-life of fipronil on treated vegetation ranges from 3 to 7 months depending on the substrate and the habitat where it is applied. Its photodegradation, volatilization and hydrolysis are the contributors to fipronil field dissipation. Fipronil has low soil mobility. It binds to the soil and has little potential for groundwater contamination. In water and sediment that lack oxygen, fipronil degrades with a half-life of 116–130 days. Fipronil remains stable to breakdown in water at mildly acid to neutral pH and degrades in basic solutions with a half-life of 28 days. In studies where fipronil was exposed to light, fipronil had a half-life of 3.6 h in water and 34 days in loamy soil.

Ecotoxicology

Nontarget toxicity of fipronil to wildlife varies with species. Fipronil is highly toxic to fish with LC_{50} (96 h) of 42 μg l^{-1} for African tilapia, 85 μg l^{-1} for bluegill sunfish, 248 μg l^{-1} for rainbow trout, and 430 μg l^{-1} for European carp. Fipronil is also highly toxic to aquatic invertebrates including oysters, shrimps and other crustacea, and highly toxic to bees and upland game birds, but is almost nontoxic to earthworms, soil microorganisms, aquatic plants, and certain groups of waterfowl and birds such as field sparrow ($LD_{50} = 1120$ mg kg^{-1}) and Mallard duck ($LD_{50} > 2150$ mg kg^{-1}). Its tendency to bind to sediments and its low water solubility may reduce the potential hazard to aquatic wildlife. The metabolites of fipronil have a higher toxicity to birds and freshwater invertebrates than fipronil itself. It is appropriate to use fipronil-based insecticides with accompanying environmental, ecotoxicological, and human health monitoring.

Other Hazards

Flammable properties of fipronil product (e.g., Termidor® SC Termiticide): flash point $>93°C$ (199°F); flammability class: will burn. Closed containers may explode (due to the build-up of pressure) when exposed to extreme heat. Suitable extinguishing media include water spray, foam, CO_2, and dry chemical media. Firefighters should be equipped with self-contained breathing apparatus and turnout gear.

Exposure Standards and Guidelines

The reference dose (RfD) and acceptable daily intake (ADI) for fipronil are both 0.0002 mg kg^{-1} day^{-1}. RfD and ADI values for fipronil-desulfinyl are about an order of magnitude lower, 0.000 03 and 0.0002, respectively.

See also: Neurotoxicity; Pesticides.

Further Reading

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Ikeda T, Zhao X, Nagata K, *et al.* (2001) Fipronil modulation of γ-aminobutyric $acid_A$ receptors in rat dorsal root ganglion neurons. *The Journal of Pharmacology and Experimental Therapeutics* 296: 914–921.

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http://www.fao.org – Food and Agricultural Organization (FAO). Fipronil data sheet (T*).

Fish Consumption Advisory

John L Hesse

Introduction

In the United States, it was discovered in the early 1970s that fish tissue sometimes contains trace levels of environmental contaminants that may be harmful to humans if the fish are eaten too frequently. In response to this discovery, state, territorial, and tribal governments developed fish consumption advisories to protect the public from potentially harmful effects linked to eating sport-caught fish. Advisories are often targeted at the protection of women of childbearing age, the fetus, and young children since these population groups are most sensitive to the adverse effects of many of the contaminants. The goal of advisories is to create public awareness of the potential dangers as well as the benefits of fish consumption while recognizing the cultural importance of regular fish consumption for particular segments of the population.

Fish consumption advisories have historically been aimed at sport fish anglers and people who eat sport-caught fish. Consumers of fish sold commercially in markets or restaurants are protected by the US Food and Drug Administration (FDA) which has the authority to remove fish from the market that exceed FDA tolerance or action levels for chemical contaminants. However, in recent years, the FDA has also issued consumption advice to sensitive populations who may be consuming large amounts of certain commercially purchased fish (e.g., shark, swordfish, king mackerel, and tilefish). Many state, territorial, and tribal governments are now including some commercial fish species, such as these, in their consumption advisories for anglers.

Balancing the risks and benefits of fish consumption is becoming more and more complex as an ever-increasing amount of research has shown that regular fish consumption can play a role in reducing cardiac disease, diabetes, arthritis, asthma, and certain forms of cancer. The benefits are, in large part, due to the high quality protein, omega-3 fatty acids, vitamins, and minerals found in fish. An additional benefit is that fish are very good sources of protein while containing much lower fat levels than many other protein sources in the diet; for example, red meats. Fish consumption advisories, by providing information on risk-reducing behaviors such as fishing less contaminated bodies of water, targeting fish species and sizes lowest in contaminants, and using fish preparation and cooking methods that reduce contaminant levels, can help consumers achieve the health benefits of fish while minimizing any potential health threats from contaminants.

Background and History of Advisories

The first fish consumption advisories were issued in the United States in the early 1970s in response to the discovery of mercury in fish in waters of southeast Michigan downstream of an industrial discharge of mercury. As more and more states tested fish for contaminants over the last 30 years, consumption advisories spread to nearly every state in the nation. In 2002, the US Environmental Protection Agency's National Listing of Fish and Wildlife Advisories included 2800 advisories in 48 states, the District of Columbia, and the US Territory of Samoa. The 2800 advisories in the national listing cover almost 33% of the nation's total lake acreage and 15.3% of the total river miles. Twenty-seven states and the District of Columbia have issued statewide advisories for lakes, streams, or coastal waters.

The US EPA has also been encouraging states to include information about safe sources of fish in their consumption advisories. In response, more and more states are informing the public about specific species of fish and fish from specific bodies of water that have been tested and have been shown to contain very low levels of contaminants. This information promotes the continued enjoyment of recreational fishing and the inclusion of fish as part of a healthy diet.

Bioaccumulative Pollutants

The US EPA's website identifies a total of 39 chemical contaminants that have triggered fish consumption advisories in the United States. Most advisories, however, have involved five primary contaminants: mercury, polychlorinated biphenyls (PCBs), chlordane, dioxins, and DDT. Chemicals of greatest concern are ones that accumulate in fish tissue at levels many times higher than those in the water, in some cases, more than 1 million times higher.

These chemicals are typically ones that are very persistent in the environment and remain biologically available in the sediments where they can be passed up the food chain from bottom-dwelling organisms to fish. As a result of biomagnification, the highest contaminant levels are found in top-predator fish species such as bass, walleye, pike, or muskellunge in freshwater environments and in sharks, swordfish, tuna, king mackerel, or bluefish in marine systems. This is

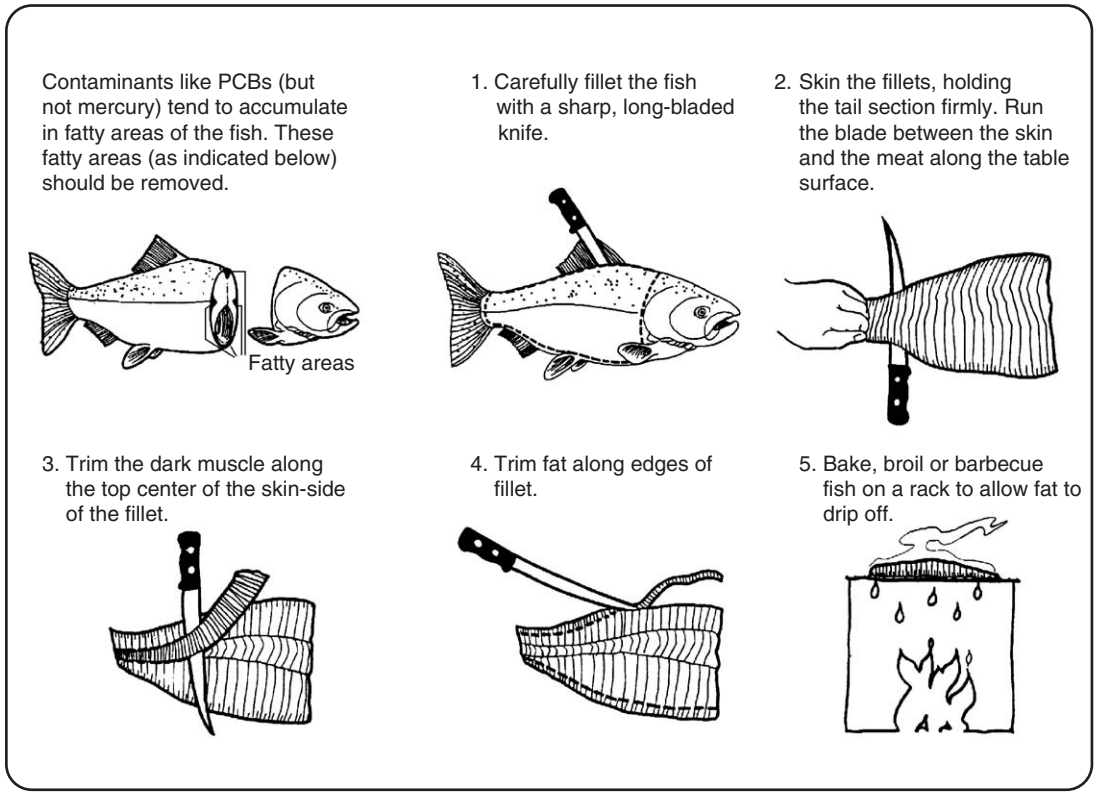

Figure 1 Recommended fish preparation method.

true particularly for mercury. Elemental mercury can be converted by microorganisms in the sediment and water column to its most toxic form, methylmercury, and is predominantly stored in this form in fish tissue.

Salmon, lake trout, and fatty, bottom-dwelling fish species such as carp and some catfish species tend to accumulate high levels of the lipophilic (fat-soluble) organic contaminants like PCBs, chlordane, DDT, and dioxins. Fish consumers can reduce their exposure to these lipophilic compounds by trimming the belly flaps, subcutaneous and dorsal fat, dark muscle, gills, eye, brain, and internal organs before cooking the fish. In addition, cooking the fish in ways that allow the fats to drip away further reduces the potential exposure to these contaminants. **Figure 1** illustrates proper fish cleaning and preparation methods. Studies have shown that proper preparation and cooking can eliminate 50% or more of the organic contaminants. However, these methods do not significantly reduce mercury from the portion eaten since the mercury is not stored predominantly in fat.

Potential Health Effects of Eating Contaminated Fish

Surveys have clearly shown that sport anglers tend to eat more fish (two- to threefold higher on average) than the general US population. Consequently, human body burdens of mercury and persistent organic contaminants that are typically found in fish also tend to be higher in sport anglers than in the general population. Because the body burden level increases with the amount of fish eaten, subsistence anglers (e.g., low income) or populations who eat large amounts of fish for cultural reasons (e.g., native Americans and certain immigrant groups) usually have the highest exposures to contaminants and therefore may be at higher risk of adverse effects. The human fetus, infants, and children are often at greater risk because of their rapid growth and developing organ systems. Elderly people may also be at greater risk because their immune systems may already be compromised.

Epidemiological studies are now looking at effects of persistent contaminants on the immune system, the nervous system, prenatal and postnatal development, fertility, and the development of cancers. Methylmercury is known to be a neurotoxin and the developing fetus is considered to be the most sensitive life stage. The adverse developmental effects of methylmercury were first recognized in the 1960s through studies of a poisoning epidemic linked to fish consumption in Minamata, Japan, and investigation of an incident linked to mercury-contaminated grain in Iraq. In the Japanese poisoning incident, some

children born to mothers who consumed heavily contaminated fish developed infantile cerebral palsy and other neurological deficits. Symptoms of mercury poisoning in adults include impairment of hearing and peripheral vision, numbness in extremities, uncoordinated movements, impaired speech, mental disturbances, and death in extreme cases.

High-level exposure to polychlorinated biphenyls can cause adverse health effects ranging from developmental effects in children to increased risk from cancer. Children born to exposed mothers have exhibited long-term adverse effects on cognitive development.

Sources of Contamination

Mercury and persistent organic pollutants in the environment come from a variety of sources. Mercury, for example, is a toxic metal that comes from both natural and manmade sources. Coal-fired power plants, municipal waste incinerators, medical waste incinerators, and cement kilns that burn hazardous waste or coal are currently among the major anthropogenic sources of mercury. Natural sources of atmospheric mercury include gases released from the Earth's crust by geysers, volcanic eruptions, and forest fires. PCBs are industrial chemicals used widely in the United States from 1929 until 1978 as coolants and lubricants and in electrical equipment. The manufacture of PCBs in the United States stopped in 1977, and use was restricted in 1979. Dioxins and furans are unwanted by-products of various industrial processes, including production of certain pesticides. DDT is an insecticide that was widely used in the United States from 1946 until 1972. DDT is still used in other countries and, by special permit, in the United States. Chlordane is a pesticide that was once used broadly in the United States but was banned in the 1980s. It is still used in some developing countries.

Content and Dissemination of Consumption Advisories

The core of a fish consumption health advisory is a set of fish consumption recommendations based on risk assessment and risk management considerations. There are three basic types of recommendations:

- Advisories for 'unlimited consumption' (no restrictions) are issued to inform the public that fish from specific water bodies have been tested for chemical contaminants and the results have shown that specific species of fish are safe to eat without a consumption limit.
- Advisories for 'no-consumption' are issued when contaminant levels in fish pose a potentially serious health risk to the general population or sensitive subpopulations (such as children and pregnant women). These types of advisories recommend that members of the identified population or sub-populations not eat any amount of certain types of locally caught fish.
- Advisories for 'restricted-consumption' are issued when contaminant levels in fish may pose a health risk to the general population or sensitive subpopulations if too much fish is consumed over a specific time period. Examples of recommended time intervals between fish meals include: no more than one meal per week, one meal per month or six meals a year.

In addition to the specific recommendations in the advisories, as described above, risk communicators generally try to include supplemental information that supports the recommendations and provides alternative consumption options that also can be used to minimize the risk. Information on the potential health effects of the chemicals triggering the advisory is often provided. Similarly, the health benefits of fish consumption are also commonly included. Ideally, advisories will identify ways fish consumers can reduce any potential risks while still gaining the health benefits. These methods include fishing cleaner water bodies, eating smaller fish, avoiding certain fish species, and/or trimming and cooking the fish in ways that remove fatty tissues where some fish contaminants are concentrated.

In the past, the most common method of disseminating sport-caught fish consumption advisories has been in conjunction with fishing regulations issued to sport anglers. Although surveys of anglers have shown a general awareness of advisories in a majority of this population, the messages may not be reaching segments of the population at highest risk, such as women of childbearing age and young children, subsistence anglers, members of tribes and other ethnic groups for which fish has a strong cultural significance, and non-English speaking populations. More effective outreach programs to better educate at-risk populations are being developed cooperatively between regulators and communication partners at the local level who are familiar with and sensitive to the needs of the at-risk populations in specific locales. The US EPA has developed guidance for state, territorial, and tribal governments on the most effective risk communication methodologies.

See also: Chlordane; DDT (Dichlorodiphenyltrichloroethane); Dioxins; Food and Drug Administration, US; Mercury; Methylmercury; Polychlorinated Biphenyls (PCBs).

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Relevant Websites

http://www.epa.gov – Environmental Protection Agency website. For further information about the safety of locally caught fish and shellfish, visit the Environmental Protection Agency's Fish Advisory Website.

http://www.cfsan.fda.gov – For further information about the risks of mercury in commercial fish and shellfish, visit the US Food and Drug Administration's Food Safety Website.

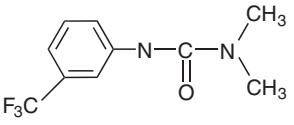
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Fish Consumption Advisory.

Flame Retardants *See* Polybrominated Diphenyl Ethers (PBDEs).

Fluometuron

Elmar Udarbe Zamora

- Chemical Abstracts Service Registry Number: CAS 2164-17-2
- Synonyms: *N*,*N*-Dimethyl-*N*′-[3-(trifluoromethyl)phenyl]urea; C-2059; Ciba-2059; Cotoran; Cotorex; Cottonex; Flo-Met; Higalcoton; Lanex; Pakhtaran
- Chemical/Pharmaceutical/Other Class: Phenylurea herbicide
- Chemical Formula: $C_{10}H_{11}F_3N_2O$
- Chemical Structure:



Uses

Fluometuron is a selective herbicide that acts on susceptible weeds and broadleaf grasses by inhibiting their photosynthesis. In the United States, fluometuron is only used for cotton and sugarcane production. It can be applied before or after planting for the control of weed and grass. Fluometuron is available in liquid, dry-flowable, and wettable powder formulations.

Exposure Routes and Pathways

The major routes of exposure are through the skin and inhalation. Protective clothing must be worn to prevent skin exposure. Animals can also be exposed by eating contaminated cotton leaves or cotton gin trash.

Toxicokinetics

Fluometuron is absorbed slowly through the oral route. Rats given fluometuron orally excreted the chemical unchanged in the feces and urine after 3 days. Fluometuron and its metabolites were detected in liver, kidneys, pituitary, adrenal gland, plasma, red blood cells, and spleen, with highest concentrations noted in red blood cells.

Mechanism of Toxicity

Little is known about the mechanism of fluometuron toxicity in mammals. Fluometuron is a weak inhibitor of cholinesterases.

Acute and Short-Term Toxicity (or Exposure)

Animal

Fluometuron is practically nontoxic to rats and rabbits through the oral and dermal routes. It takes a large dose to cause toxicity in rats and rabbits. There is moderate to low toxicity through inhalation (LD_{50} of $2\,mg\,l^{-1}$). Guinea pigs appear to be more sensitive to fluometuron, however. It caused skin sensitization and cholinesterase inhibition after inhalation exposure ($0.6\,mg\,l^{-1}$ for 2 h) in this species. Fluometuron caused slight eye and skin irritation in rabbits. Clinical signs associated with high dosages of fluometuron in rats include muscular weakness, tearing or watery eyes, extreme exhaustion, and collapse. Healthy sheep (6–9 months old) exhibited grinding of the teeth, ruminal tympany, mydriasis, difficulty in breathing, staggering, paresis of the hindlimbs and forelimbs, and recumbency after drenching with fluometuron.

Human

Signs of poisoning include nausea and vomiting. Fluometuron can irritate skin, and gastrointestinal and respiratory tracts.

Chronic Toxicity (or Exposure)

Animal

Conjunctivitis, skin sensitization, and congestion of major organs like the liver and kidneys as well as the spleen and adrenal glands can occur with repeated exposures. Abnormalities in the red blood cells also occur with repeated exposures. Pregnant rabbits treated orally from days 6 to 19 of gestation with 50, 500, or $1000\,mg\,kg^{-1}\,day^{-1}$ fluometuron showed an increased incidence of resorptions. No carcinogenic response was noted in rats exposed by diet to 125 or $250\,mg\,kg^{-1}\,day^{-1}$ fluometuron.

Human

Repeated exposure to fluometuron may cause conjunctivitis and skin sensitization. Fluometuron is not likely to lead to reproductive, teratogenic, mutagenic, or carcinogenic responses in humans.

In Vitro Toxicity Data

Fluometuron appears to have little genotoxic effect based on Ames assay, micronucleus test, DNA damage, or rat DNA repair inhibition tests.

Clinical Management

If ingested, vomiting is induced or plenty of water or syrup of ipecac is given. Nothing should be given to an unconscious person. Eyes are flushed with plenty of tap water for 15 min. If inhaled, the victim should be moved to fresh air or respiration applied if possible. If exposure on skin, the exposed area should be washed with plenty of soap and water and medical attention provided as soon as possible.

Environmental Fate

Fluometuron adsorbs to soil particles. Half-lives for degradation in soil and water are estimated at 12–17 days and 110–114 weeks, respectively. Breakdown in soil is mainly through photodegradation. Soil microbes also degrade fluometuron. In field studies in agricultural areas in California and Georgia, fluometuron residues were not detected in soil below 12 in. Roots more readily absorb fluometuron compared to leaves. Differences in the plant's ability to break down fluometuron should be considered in using this chemical in weed control.

Ecotoxicology

Fluometuron essentially is nontoxic to birds and bees. The LC_{50} is $30\,mg\,l^{-1}$ in rainbow trout, $48\,mg\,l^{-1}$ in bluegill sunfish, $170\,mg\,l^{-1}$ in carp, and $55\,mg\,l^{-1}$ in catfish. There is low potential for bioaccumulation.

Other Hazards

Fluometuron is stable under normal temperatures and pressures, but it may pose a slight fire hazard if exposed to excessive heat or flame. Containers may explode in the heat of a fire. It poses a fire and explosion hazard in the presence of strong oxidizers. It is incompatible with acids and bases. Thermal decomposition may release highly toxic fumes of fluorides and oxides of nitrogen and carbon. Water runoff during fire control may also give off toxic gases. In case of fire, water should not be used as it has the tendency to spread the flames. In case of spills, the area should be contained with absorbent material. The area should be cleaned with detergent and water.

Exposure Standards and Guidelines

No occupational exposure limits have been established for fluometuron by Occupational Safety and

Health Administration, The National Institute for Occupational Safety and Health, or American Conference of Industrial Hygienists.

The National Fire Protection Association considers fluometuron as moderately hazardous (rating of 2). The reference dose of fluometuron is $13\,\mu g\,kg^{-1}\,day^{-1}$.

See also: Diuron.

Further Reading

US Environmental Protection Agency (1985) *Chemical Information Fact Sheet Number 88: Fluometuron*, pp. 9–12. Washington, DC: Office of Pesticides and Toxic Substances.

Fluoride

Greene Shepherd

- Representative Chemicals: Sodium fluoride; Hydrofluoric acid
- Chemical Abstracts Service Registry Number: CAS 7681-49-4
- Chemical/Pharmaceutical/Other Class: Halogen
- Chemical Formula: F^-

Uses

Fluoride is used in a variety of industries, most notably as an additive in the majority of municipal water supplies in the United States and is used as a mineral supplement for children living in areas that do not have fluoridated water. It is used in hydrofluoric acid, fluoropolymers, and refrigerating agents.

Exposure Routes and Pathways

Ingestion, dermal exposure (most notably via hydrofluoric acid), and inhalation are possible routes of exposure. Fluorine gas (F_2) produces little toxicity unless it reacts with some other chemical to produce fluoride ion.

Toxicokinetics

Chronic ingestion of fluorides causes exaggerated buildup on teeth, bones, and ligaments. Exposure to skin, eyes, and mucous membranes has a corrosive effect.

Mechanism of Toxicity

Fluoride interferes with the metabolism of cells and enzymes. It is a cross-linking agent and rarely occurs in an elemental state in nature. It is a metabolic inhibitor, interfering with calcium metabolism and electron transport. Calcium is essential for maintaining cardiac membrane potentials and in regulating coagulation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Brief exposures (<60 min) to concentrations between 38 and 73 ppm in several animal species showed no effects. The LC_{50} for inhalation in animal models ranged between 150 and $200\,ppm\,h^{-1}$. Higher concentrations will produce lethality in a shorter duration of time. Chronic exposures at 5–10 ppm produced irritation of the eyes, oropharynx mucosa, and respiratory tract.

Human

Chronic over-absorption can cause hardening of bones, calcification of ligaments, and buildup on teeth. Fluoride can cause irritation or corrosion to eyes, skin, and nasal membranes. Large ingestion of fluoride salts or hydrofluoric acid may result in fatal arrhythmias due to profound hypocalcemia. Inhalation may be fatal. The American Conference of Governmental Industrial Hygienists threshold-limit value/time-weighted average for fluorine is 1 ppm. National Institute of Occupational Safety and Health reports that concentrations of 25 ppm are immediately dangerous to life and health. Inhalant abuse of fluoridated hydrocarbon refrigerants like Freon® has been associated with 'sudden sniffing death', which is thought to be a fatal arrhythmia caused by myocardial sensitization to catecholamines.

Chronic Toxicity (or Exposure)

Animal

Sheep fed fluoride 10 ppm in water over 7 years demonstrated decreased wool production. Flourosis, painful stiff gait, lameness, decreased milk production, and dental changes developed in cattle fed 40 ppm fluoride in their diet over 6 months to 1 year.

Human

Fluorosis is a chronic public health problem in many parts of the world. Exposures to fluoride in concentrations greater that 1 ppm result in bone deformities, spinal compressions, and restricted movements of joints. These symptoms are easily prevented by decreasing exposure to fluoride.

In Vitro Toxicity Data

Assessments of mutagenicity using the Ames Salmonella model have not demonstrated toxic effects at concentrations up to 500 μg per plate.

Clinical Management

Small ingestions of low concentration fluoride products (e.g., toothpaste) may generally be managed by dilution with milk. Dermal exposures should be washed for 15–60 min under running water. Burns resulting from contact with high-concentration fluorides should be coated with water-based calcium- or magnesium-based gel. Affected eyes should be irrigated with running water or saline solution for 30–60 min. In cases where systemic toxicity is evident intravenous calcium may be utilized.

See also: Catecholamines; Fluorine; Hydrofluoric Acid.

Further Reading

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Augenstein WL, Spoerke DG, Kulig KW, *et al.* (1991) Fluoride ingestion in children: A review of 87 cases. *Pediatrics* 88: 907–912.

Fluorine

Robert Kapp

- Related Chemicals: Fluorine, hydrogen fluoride and fluorides
- Chemical Abstracts Service Registry Number: CAS 7782-41-4
- Synonyms: Bifluoriden (Dutch); Fluor; Fluor (Dutch, French, German, Polish); Fluorine-19; Fluoro (Italian); Fluorures acide (French); Fluoruri acidi (Italian); Saeure fluoride (German)
- Chemical/Pharmaceutical/Other Class: Halogen group
- Chemical Formula: F^-

Uses

Fluorine is combined either directly or indirectly with other elements to form compounds such as hydrofluoric acid, fluoropolymers and is used in the synthesis of organic fluorine compounds such as fluorides as in the manufacture of Freon (i.e., dichlorodifluoromethane, CCl_2F_2), which is used as a refrigerant. Fluorine is used in the manufacture of uranium hexafluoride that is necessary for the separation of the isotopes of uranium in centrifuges in the production of nuclear weapons. Fluorine and its compounds are used in producing more than 100 commercial fluorochemicals, including many well-known high-temperature plastics. The presence of fluorine as a soluble fluoride in drinking water to the extent of 2 ppm may cause mottled enamel in teeth, when used by children acquiring permanent teeth; in smaller amounts; however, fluorides are added to water supplies to prevent dental cavities. Fluoride is found in a variety of dental products including toothpastes and mouth washes, or as a preventive measure against dental caries. Fluorine has been studied as a rocket propellant since it has an exceptionally high specific impulse value. Although certain fluorine-containing compounds have been identified as being involved in ozone depletion and global warming effects, legislative measures have been, or are being, put in place where necessary to reduce this impact. Fluorine-containing compounds have been said to have a great potential for clean synthesis (i.e., use in 'green chemistry'), and utilization of the high activity that fluorinated groups can impart molecules may help to reduce the quantities required of certain classes of chemicals that are used in the biosphere, including pesticides and pharmaceuticals. Fluorine will react with water or steam to produce heat, and toxic and corrosive fumes.

Background Information

Fluorine is a nonmetallic, pale yellow-green gaseous element with a pungent odor. It is the most electronegative and reactive of all the elements. Fluorine is

an element that is widely distributed in the environment, but because of its high reactivity it is not found naturally in its elemental state. It is found in Cryolite, Fluorspar, and Fluorapatite. Fluorine combines with hydrogen to form hydrogen fluoride, which is a colorless gas. Hydrogen fluoride subsequently can dissolve in water to form hydrofluoric acid. Fluorine gas also combines with some metals such as sodium to form sodium fluoride and with calcium to form calcium fluoride. In 1529, Georigius Agricola described the use of fluorspar as a flux, and as early as 1670, Schwandhard found that glass was etched when exposed to fluorspar treated with acid. Fluorine is a naturally occurring compound that was first identified by Scheele. Many later investigators, including Davy, Gay-Lussac, Lavoisier, and Thenard, experimented with hydrofluoric acid, with some experiments ending in tragedy. Fluorine was first isolated by Moissan in 1886, when he noted the inclusion of fluorine in crystals of Fluorspar.

Exposure Routes and Pathways

Exposure to fluorides can be through contaminated air, food, drinking water, and soil. The exposure pathways for fluorine and fluoride are via dermal, inhalation, or ingestion pathways. Fluorides enter the environment naturally through the weathering of rocks and minerals, and by emissions from volcanoes. The greatest amount of the total volume of fluorides in the environment is released from natural sources, in particular volcanoes and oceans. However, the greatest concentrations are found near anthropogenic point sources. Fluorides are found in soil, air, water, and in most foods, from both natural and anthropogenic sources (though rarely as molecular fluorine). It is highly electronegative and therefore reacts vigorously with other compounds. Anhydrous hydrogen fluoride will react with moisture in the air and form hydrofluoric acid, which will gradually settle to the ground or be dispersed with the wind. Calcium fluoride is most common in alkaline soils, and fluoroaluminate complexes are most common in acidic soils. Thus, exposure to hydrofluoric acid would occur at a hazardous waste site only if someone came in contact with material leaking from a storage container or breathed contaminated air before it was dispersed. Once in a stable form, fluoride persists in the environment for a relatively long time unless transformed to another compound or decomposed by radiation.

The general population is exposed to fluoride through consumption of drinking water, foods, and dentifrices, primarily in the form of sodium fluoride and stannous fluoride. Populations exposed to relatively high concentrations of fluoride include workers in fluoride-processing industries and individuals residing near such industries. These individuals may be exposed to higher than back ground concentrations as a result of the levels of hydrogen fluoride and dusts from fluoride compounds in the air, and from foodstuffs produced within the vicinity that have collected excess fluoride from the environmental media. Populations near hazardous waste sites may be exposed to high levels of fluoride by similar routes, although no information has been obtained to support this.

Toxicokinetics

Fluoride, rather than fluorine, appears to be the agent that is toxicologically active in the body because fluorine is so reactive that it is not absorbed chemically unchanged. Existing data indicate that all common forms of inorganic fluoride are rapidly and quite extensively absorbed. The highest degree of absorption has been noted with aqueous solutions of sodium fluoride resulting in absorption within 30 min of oral exposure. Hydrofluoric acid has been shown to be absorbed across the skin. Rats exposed to fluorine via subchronic inhalation showed marked elevation in teeth and bones as well as in the bloodstream; however, there is no evidence of accumulation or retention of fluoride in soft tissues in humans. Chronic ingestion of fluorides can cause exaggerated buildup in teeth and bones. Cessation of exposure will decrease the fluoride levels in bone slowly; however, the rate of decay is undetermined in humans.

Mechanism of Toxicity

Hydroxyapatite is a mineral phase during bone formation. Apparently fluoride replaces the hydroxyl ion and/or perhaps the bicarbonate ion associated with hydroxyapatite. This results in the formation of hydroxyfluorapatite. The excess fluoride is excreted in the urine, feces, sweat, and saliva within 24 h. Skeletal sequestration and renal excretion appear to be two primary mechanisms of the body, which controls the toxic levels of fluoride. Fluoride appears to interfere with cell metabolism, and fluoride is a cross-linking agent. Fluoride ions carried in human blood exist in two forms, as an inorganic ion (F^-) and in combination with an organic molecule. The toxicological significance of the fluoride ion with the organic molecule is not known. The inorganic fluoride ion forms metal–fluoride–phosphate complexes that interfere with any enzymes that require a metal ion cofactor. The inorganic fluoride ion may interact directly with

the enzyme. In addition, the fluoride ion is thought to be a general inhibitor of the energy production organization of the cell, specifically the oxidative phosphorylation necessary in ATP formation.

present. Acute fluorine ingestion results in four specific categories of symptoms (A–D):

	A	*B*	*C*	*D*
Symptoms	Gastric symptoms: nausea, salivation, abdominal pain	Direct effects of fluoride	Inhibition of metabolism	Persistent abnormal serum fluoride levels
Etiology	$NaF + HCl \rightarrow HF$	Glycolytic pathway, cholinesterase	Hyperkalemia, hypocalcemia	Mineral homeostasis, cellular damage

The acute toxic dose of fluoride ranges from 0.1 to 0.8 mg kg^{-1} of body weight.

Acute and Short-Term Toxicity (or Exposure)

Animal

Five species of laboratory animals were exposed to fluorine for brief periods (5, 30, or 60 min). The LC_{50} values for these animals ranged from 150 ppm for mice (exposure time = 60 min) to 820 ppm for rabbits (exposure time = 5 min). Mice exposed to sublethal concentrations had pulmonary irritation and delayed development of focal necrosis in the liver and kidney. The animals showed no effects from 60 min exposures at levels from 38 ppm (dog) to 73 ppm (guinea pig), as judged by lack of irritation, dyspnea, and pulmonary congestion and hemorrhage. Repeated, short-term exposures (60 min exposures at weekly intervals for 4 weeks) at levels from 55 ppm (mice) to 75 ppm (rats) either showed no effects, or very slight effects grossly in the lung, liver, and kidney. Evidence for a mild degree of tolerance was found by elevated 60 min LC_{50} values in mice, and for decreased lung and kidney weights in the pre-exposed animals compared with previously unexposed controls. No cytogenetic changes occurred in the oocytes of mice given single or repeated treatments of sodium fluoride.

Human

Elemental fluorine and the fluoride ion are highly toxic. Low overdose ingestion produces local gastrointestinal upset, salivation, and a metallic taste that may last 48 h. Acute inhalation of fluorine can cause severe respiratory irritation, dyspnea, and death. High overdose ingestion, in addition to causing more severe local manifestations, may produce systemic symptoms of convulsions, coma, dysrhythmias, hypotension without compensatory tachycardia, acidosis, paresthesias, and coagulation disturbances. Hypocalcemia can develop very rapidly. Coagulopathies can develop as a result of hypocalcemia. Hyperkalemia may be

Chronic Toxicity (or Exposure)

Animal

High doses of fluorine gas and hydrogen fluoride in animal studies have resulted in testicular degeneration. The available animal and human data strongly suggest that the reproductive system is a target of fluoride toxicity at high exposure levels.

Feeding of sodium fluoride to mice at concentrations of up to 50 mg kg^{-1} diet for seven generations did not induce chromosomal aberrations or sister chromatid exchanges in the bone marrow. The (US) National Institute of Environmental Health Sciences, National Toxicology Program (NTP) oral carcinogenicity study on sodium fluoride concluded that there was "equivocal evidence that fluoride is a carcinogen in male rats, but not in female rats or mice of either gender." Another rat carcinogenicity study found no evidence of carcinogenicity of fluoride in rats.

Human

Chronic exposure to very small amounts of fluoride in the drinking water is recognized as being beneficial to the prevention of dental caries. Fluorination of the drinking water allows for the incorporation of fluoride into tooth enamel pre-eruptively, inhibition of demineralization, enhancement of remineralization, and inhibition of bacterial activity in dental plaque formation. Chronic exposure to excessive amounts of fluorine can result in mottled teeth (fluorosis), for example, a chronic fluoride ingestion of 1 ppm of fluoride in drinking water can cause mottling of the teeth, and an exposure of 1.7 ppm will produce mottling in 30–50% of patients. Chronic poisoning may cause osteosclerosis, calcification of ligaments and tendons, bony exostoses, and renal calculi. Chronic inhalation exposure to high levels of hydrogen fluoride and fluoride dusts, or chronic oral exposure to high levels of fluoride can cause skeletal malformations and severe

joint pain, and an increased incidence of nonvertebral skeletal fractures. The existing data do not indicate that fluoride is a carcinogen in humans.

There have been reports of a decrease in fertility in women and decreased serum testosterone levels in men living in communities with high fluoride levels in municipal water.

In Vitro Toxicity Data

Sodium fluoride did not induce reverse mutations in *Salmonella typhimurium*, nor did it induce gene conversion in *Saccharomyces cerevisiae*. A fluoride level of 0.4–1.0 mg l^{-1} inhibited DNA repair after irradiation of mouse spleen cells *in vitro*. Sodium fluoride was not mutagenic in cell cultures of human leukocytes at concentrations of 18 and 54 mg l^{-1}. Little or no effect was noted on chromosomes when mouse oocytes were exposed *in vitro* to a fluoride concentration of 200 mg l^{-1} in media for up to 14 h.

Clinical Management

Skin or mucousal burns should be washed for 15–60 min under running tap water. Burns should be coated with a water-based magnesium dioxide ointment with ~20% glycerin and should contain no oil. Exposed eyes should be washed for 15 min with running tap water, and then irrigated with saline solution for 30 min. If ingested, soluble calcium should be administered. Gastric upset can be treated by ingesting milk or cream every 4 h.

Environmental Fate

Fluorine is not destroyed in the environment, but rather it combines with minerals to form salts, which remain in the soil. Hydrogen fluoride gas is absorbed into the atmosphere to form hydrofluoric acid, which eventually returns to earth in the form of precipitation. Fluorides form strong associations with sediment and soil particles, which eventually accumulate in plants and the bones and/or shells of animals.

In dilute solutions and at neutral pH, dissolved fluorides are usually present as the fluoride ion (F^-). As pH decreases, the proportion of F^- decreases, while hydrogen fluoride (HF^{2-}) and nondissociated hydrogen fluoride increase. Levels of nondissociated hydrogen fluoride also increase in concentrated solutions. In seawater, fluorides exist in equilibrium. Calcium carbonate precipitation dominates the removal of dissolved fluoride from seawater. The next most important removal mechanism is incorporation into calcium phosphates. Undissolved fluoride is generally removed by sedimentation. The residence time of fluoride in ocean sediments has been computed at 2–3 million years.

Several factors influence the level of fluorides in food. These include the locality in which the food is grown, the amount of fertilizer and pesticides applied, the type of processing the food receives, and whether fluoridated water is used in food preparation. Foods characteristically high in fluoride content are certain types of seafood (1.9–28.5 mg kg^{-1}), especially those types in which the bones are consumed, bone products such as bone meal and gelatin, tea, and baby formula processed with fluoridated water.

Ecotoxicology

Considerable differences exist in plant sensitivity to atmospheric fluoride, but little or no injury will occur when the most sensitive species are exposed to about 0.2 μg m^{-3} air, and many species tolerate concentrations many times higher than this.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, 8 h time-weighted average, for fluorine is 1 ppm, and the short-term exposure limit, 15 min, is 2 ppm. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA, is 0.1 ppm (0.2 mg m^{-3}). The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day is 0.1 ppm (0.2 mg m^{-3}), and the NIOSH Immediately Dangerous to Life or Health value is 25 ppm.

See also: Chlorine; Hydrofluoric Acid.

Further Reading

Brush DE and Aaron CK (2004) Hydrogen fluoride. In: Dart RC (ed.) *Medical Toxicology*, 3rd edn., pp. 1353–1357. Philadelphia, PA: Lippincott Williams and Wilkins.

Tavener SJ and Clark JH (2004) Can fluorine chemistry be green chemistry. *Journal of Fluorine Chemistry* 123: 31–36.

Teitelbaum DT (2001). The halogens. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 3, pp. 731–739. New York: Wiley.

Relevant Website

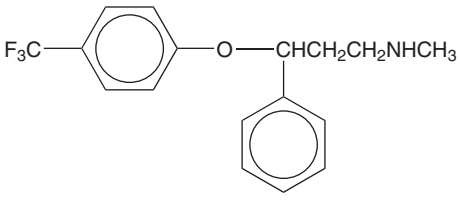
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Fluorine.

Fluoxetine

Rebeca Gracia

This article is a revision of the previous print edition article by Douglas J Borys, volume 2, pp. 7–8, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 54910-89-3
- Synonym: (±)-*N*-Methyl-3-phenyl-3-[(a,a,a-trifluoro-*p*-tolyl)-oxy]propylamine hydrochloride (Prozac)
- Chemical/Pharmaceutical/Other Class: Selective serotonin reuptake inhibitor; bicyclic antidepressant unrelated to tricyclic or tetracyclic compounds
- Chemical Formula: $C_{17}H_{18}F_3NO$
- Chemical Structure:



Uses

Fluoxetine is used to treat depression, anorexia, bulemia, obesity, obsessive–compulsive disorder, premenstrual syndrome, panic attacks, narcolepsy, kleptomania, and diabetic neuropathy.

Exposure Routes and Pathways

Fluoxetine is available orally as capsules or liquid. Ingestion is the most common route of exposure.

Toxicokinetics

Fluoxetine is rapidly and completely absorbed orally, reaching a peak in 6–8 h. Food does not affect absorption. Fluoxetine is *N*-demethylated in the liver to an active metabolite, norfluoxetine, and many other minor inactive metabolites. Both fluoxetine and norfluoxetine are then conjugated prior to excretion. Protein binding is 94%. The volume of distribution is estimated to be 11–88.4 l kg^{-1}. Approximately 2.5% of the drug is renally excreted unchanged and 10% as the norfluoxetine metabolite. A total of 65% of radiolabeled fluoxetine is recovered in the urine after 35 days and 15% is recovered in the feces. The elimination half-life of fluoxetine is 48–72 h, averaging almost 70 h. The half-life of norfluoxetine is 7–9 days. The elimination half-lives for both are prolonged in patients with hepatic disease.

Mechanism of Toxicity

Fluoxetine has been found to cause selective central nervous system (CNS) neuronal uptake inhibition of serotonin. While fluoxetine may bind to adrenergic, muscarinic, and histaminic receptors, it has not been shown to have the profound effects on catecholamines that are common to tricyclic antidepressant overdose patients.

Acute and Short-Term Toxicity (or Exposure)

Animal

Six dogs were given intentional overdoses of oral fluoxetine during preclinical testing. Five of the six dogs had grand-mal seizures. All recovered with standard veterinary doses of intravenous diazepam. Chronic administration of fluoxetine has led to increased phospholipids in mice, rats, and dogs; this increase was reversed when the drug was discontinued.

Human

The therapeutic dose of fluoxetine ranges from 20 to 60 mg day^{-1}. At therapeutic levels, the most commonly reported adverse effects are headache, nervousness, insomnia, drowsiness, tremor, nausea, anorexia, and diarrhea. Patients reported to have overdosed with fluoxetine have generally had a benign course with very little neurologic or cardiovascular toxicity. A decreased level of consciousness is the most common effect noted in overdose patients. Reported neurologic clinical symptoms include tremor, confusion, ataxia, insomnia, and coma. Seizures have been rarely reported following either therapeutic dosing or overdose. Cardiovascular symptoms seldom occur. The most common cardiovascular effects include mild tachycardia, bradycardia, and hypertension. No consistent EKG changes have been noted. Other symptoms reported are a flu-like syndrome, nausea, vomiting, and diarrhea. The estimated lethal dose is 1200–2000 mg. However, most fluoxetine-related fatalities occur in patients who have taken a concurrent tricyclic antidepressant overdose. It has been disputed that therapeutic dosing of fluoxetine has been associated with the development of mania, psychosis, and suicidal ideation. Fluoxetine use may be associated with serotonin syndrome following both therapeutic use and overdose, primarily in combination with other serotonergic agents. Recently, fluoxetine has been implicated in a discontinuation syndrome characterized by neurologic and gastrointestinal disturbances such as dizziness, nausea, lethargy, and headache. It is recommended to slowly taper dosage when stopping therapy to avoid untoward effects.

Chronic Toxicity (or Exposure)

Animal

Genetically obese mice were treated with fluoxetine to assess the ability of fluoxetine to produce weight loss. In this model, fluoxetine did produce initial weight loss but overtime had no positive impact on mouse weight.

Human

Fluoxetine and other selective serotonin reuptake inhibitors (SSRIs) have been associated with increasing suicidal ideation in some populations of patients. Recent studies have led the British Department of Health to warn physicians against using paroxetine off label. Fluoxetine was specifically exempted from this recommendation. Long-term studies of patients with depression who were treated with fluoxetine have shown it to be fairly well tolerated. Primary adverse effects include nausea (23%), headache (21%), and insomnia (20%).

In Vitro Toxicity Data

In assays of mutagenicity and carcinogenicity, fluoxetine and its metabolite norfluoxetine have not demonstrated toxic effects in the Ames *Salmonella* assay, DNA repair assay in cultured rat hepatocytes, and mouse lymphoma assay or sister-chromatid exchange in Chinese hamster bone marrow cells.

Clinical Management

All basic and advanced life-support measures should be implemented. Gastric decontamination should be performed. Fluoxetine is readily adsorbed by activated charcoal and charcoal should be considered for substantial recent ingestions. Aggressive supportive care should be instituted. There is no specific antidote for fluoxetine overdose. Hemoperfusion and hemodialysis are ineffective.

See also: Charcoal; Diazepam.

Further Reading

Riddle MA, Brown N, and Dzubinski D (1989) Fluoxetine overdose in an adolescent. *Journal of the American Academy of Child and Adolescent Psychiatry* 28: 587–588.

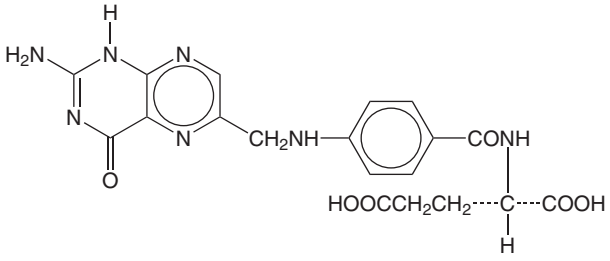
Spiller HA, Morse S, and Muir C (1990) Fluoxetine ingestion: A one year retrospective study. *Veterinary and Human Toxicology* 32: 153–155.

Folic Acid

Diana Ku

This article is a revision of the previous print edition article by Denise L Kurta, volume 2, pp. 8–9, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 59-30-3
- Synonyms: Folacin; Folate; Pteroylmonoglutamic acid; 4-(2-Amino-4-hydroxypteridin-6-yl)methylaminobenzoyl-L-glutamic acid
- Chemical/Pharmaceutical/Other Class: Water-soluble vitamin
- Chemical Formula: $C_{19}H_{19}N_7O_6$
- Chemical Structure:



Uses

Folic acid is a nutritional supplement frequently used during periods of deficiency. Folic acid needs increase during chronic diseases, such as malabsorption liver disease, alcoholism, and anticonvulsant or oral contraceptive use. Folic acid supplementation during pregnancy is strongly recommended to prevent neural tube defects to the unborn child. The active form of folic acid, folinic acid, is used in the management of certain medical diseases (e.g., patients taking methotrexate, and 5-fluorouracil).

Exposure Routes and Pathways

Routes of exposure are oral, intravenous, intramuscular, and subcutaneous. Dietary sources of folic acid are green leafy vegetables, some fruits, legumes, eggs, yeast, whole grain cereals, lean beef, veal, liver, and kidneys. Heat destroys folic acid in cooked foods.

Toxicokinetics

Folic acid is almost completely absorbed from the gastrointestinal tract, mostly in the upper duodenum,

Peak serum levels occur within 30–60 mm. Folic acid is converted in the liver to tetrahydrofolic acid in the presence of ascorbic acid by dihydrofolate reductase. Tetrahydrofolic acid and its derivatives are distributed into all body tissues with approximately half of it in the liver. It is excreted renally almost entirely as metabolite. Excessive amounts of folic acid (beyond the daily needs) are excreted unchanged in the urine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity is not expected.

Human

Toxicity is unlikely even after acute ingestions of 100 times the recommended daily allowance. Allergic reactions have been reported.

Chronic Toxicity (or Exposure)

Animal

It would be unlikely for animals to be given chronic folic acid overdoses.

Human

Chronic large doses may interfere with sleep patterns and cause malaise, irritability, and gastrointestinal symptoms such as anorexia, nausea, bloating, flatulence, and bad taste. Seizure threshold may be lowered in epileptics and progression of neurologic injury in pernicious anemia has also been reported.

In Vitro Toxicity Data

Studies of human epithelial cells failed to demonstrate cytotoxic effects even at concentrations of $200\ \mu g\ ml^{-1}$.

Clinical Management

Acute ingestions seldom require treatment. In cases of chronic excessive use, the patient should be instructed to discontinue the supplement. Any toxic symptoms should be treated symptomatically.

See also: Vitamin A; Vitamin D; Vitamin E.

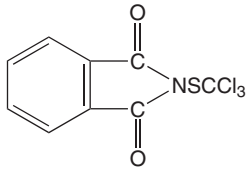
Further Reading

Butterworth CE Jr. and Tamura T (1989) Folic acid safety and toxicity: A brief review. *American Journal of Clinical Nutrition* 50: 353–358.

Folpet

Paul R Harp

- Chemical Abstracts Service Registry Number: CAS 133-07-3
- Synonyms: Acryptan; Faltan; Faltex; Folnit; Folpan; Folpel; Folpex; Ftalan; Fungitrol II; Intercide TMP; Phaltan; Phaltane; Spolacid; Thiophal; Vinicoll; ENT 26539; SHA 081601
- Chemical/Pharmaceutical/Other Class: *N*-[(Trichloromethyl)thio]phthalimide fungicide
- Chemical Structure:



Uses

Folpet is a broad-spectrum contact fungicide used on various fruits, vegetables, berries, flowers, and ornamentals. It is also used on seeds, plant beds, and structural surfaces and is added to some paints and plastics for antifungal purposes. Folpet is effective only for prevention of fungal growth, not for treatment of an existing infection.

Exposure Routes and Pathways

Folpet is available as a wettable powder or suspension concentrate. Eye, skin, or respiratory exposure may occur during production or application of folpet. Ingestion of contaminated food products is also a potential route of exposure.

Toxicokinetics

Folpet is readily absorbed following oral administration in rats. Metabolites of folpet in rats are

tetrahydrophthalimide and phthalimide, which may be further metabolized to phthalic acid and ammonia. Absorption following dermal exposure is limited. Both folpet and certain metabolites are highly reactive with thiol groups, which results in rapid degradation and limits adverse effects to the contact area. Due to the reactive nature, a variety of enzymatic and nonenzymatic reactions are possible and elimination occurs rapidly.

Mechanism of Toxicity

Interaction with thiol groups initiates both the fungicidal activity in target organisms and the toxicological activity in nontarget organisms. An intermediate, thiophosgene, which readily reacts with thiols and other functional groups, is likely involved with the variety of reported mechanisms, which include inhibition of glyceraldehyde-3-phosphate dehydrogenase and *O*-demethylase activity in liver microsomes, uncoupling of oxidative phosphorylation, and activity as a hapten, stimulating the immune system to produce allergic responses against folpet and other structurally similar compounds.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, folpet and the related fungicides captan and captafol have been shown to decrease cytochrome P450 activity and increase serum enzymes, suggesting a hepatotoxic effect. Reported LD_{50} values vary greatly depending on the route of exposure. Folpet is slightly toxic via oral and dermal routes with reported oral LD_{50} values $>10\,000\,mg\,kg^{-1}$ in rats and dermal LD_{50} values $>22\,600\,mg\,kg^{-1}$ in rabbits. In contrast, intraperitoneal LD_{50} values of 40 and $68\,mg\,kg^{-1}$ have been reported in rats. The large differences in LD_{50} values between oral and intraperitoneal routes are most likely due to route-dependent differences in absorption.

Human

Local irritation can result following dermal, ocular, or respiratory contact with folpet. Ingestion of folpet may cause vomiting and diarrhea, leading to dehydration and electrolyte depletion. Exposure to folpet has been linked to contact dermatitis but incidence rates in humans appear low.

Chronic Toxicity (or Exposure)

Animals and Humans

Folpet has been classified by US Environmental Protection Agency (EPA) as a probable human carcinogen (B2) since the mid-1980s based on induction of neoplastic growth in the duodenum of several strains of mice and positive results in multiple *in vitro* mutagenicity assays. EPA has not yet reviewed folpet under their most recent guidelines for carcinogenic risk assessment, which place more emphasis on mechanism of action and evidence for thresholds. Reevaluation under the new guidelines will likely result in a decreased classification of carcinogenic risk to humans. Researchers have thoroughly examined the possible teratogenic effect of folpet due to its structural similarity to the known human teratogen thalidomide. All test results for teratogenicity were negative.

In Vitro Toxicity Data

As indicated above, folpet is mutagenic in a number of assays.

Clinical Management

For eye contact, the eyes should be flushed immediately with generous amounts of water. For dermal exposure, contaminated clothing should be removed and the skin should be washed thoroughly with soap and water. A physician should be contacted promptly if irritation does not subside. For cases of substantial ingestion of folpet within the last few hours and in the absence of significant vomiting, gastric lavage or a combination of activated charcoal and sorbitol may be indicated. Sorbitol should not be administered if diarrhea is present or if only a small amount of fungicide was ingested. In those cases, only activated charcoal should be administered. Acute exposure to folpet is not likely to result in toxicity; treatment, if necessary, is symptomatic and supportive.

Environmental Fate

Degradation is likely similar to that of captan, in which trithiocarbonate, thiophosgene, and phthalimide are produced. Folpet can produce phytotoxicity, in particular in dry conditions.

Ecotoxicology

Folpet is slightly toxic to birds including quail and ducks. The LD_{50} for bobwhite quail is $>2510\,mg\,kg^{-1}$ and the dietary LD_{50} for the mallard duck is >5000 ppm. Folpet is highly toxic to fish including rainbow trout and bluegill sunfish. The LC_{50} (96 h) of Folpet in bluegill sunfish was 675 ppb and for rainbow trout was 185 ppb. Folpet is also highly toxic to aquatic invertebrates, the LC_{50} (48 h)

for *Daphnia magna* was 0.60 ppm. Folpet is relatively nontoxic to honeybees.

Exposure Standards and Guidelines

The reference dose for folpet is 0.09 mg kg^{-1} day^{-1}. The acceptable daily intake is 0.1 mg kg^{-1} day^{-1}.

See also: Captafol; Captan; Pesticides.

Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Food *See* Dietary Restriction; Dietary Supplements; Food Additives; Food Quality Protection Act, US; Food Safety and Toxicology; Food, Drug, and Cosmetic Act, US; Food and Agriculture Organization of the United Nations; Genetically Engineered Foods; Monosodium Glutamate.

Food Additives

James C Griffiths and Joseph F Borzelleca

Food additives are substances or ingredients, which are incorporated into the final food product, either as a result of direct addition to achieve a desired effect (functionality or technical effect) or indirectly as a result of production or processing. Direct additives include antioxidants, leavening agents, texturizing agents, preservatives, colors, and flavors. The levels of these agents in food are usually low; with the levels of colors and flavors usually the lowest. Direct food additives may increase stability and prolong shelf life, improve the organoleptic qualities (appearance, texture or mouth-feel, aroma, and/or flavor) of food and increase market penetration. The number of additives used in processing foods has been increasing due to advances in food technology and consumer expectations. These additives are identified on the food label.

These increases in the use of food additives and/or their improper use may pose potential health hazards to the consumer or introduce an element of deception, disguising unpalatable/unhealthy food. It is appropriate and necessary that food additives be safe and suitable for their intended use. This is assured by governmental oversight and regulations that establish safety and conditions of use.

This section will focus primarily on the regulatory principles and practices in the United States, and these will be compared with the European and the UN's Codex Alimentarius Commission.

Definitions

Definitions of food additives (direct food additives, ingredients added to foods for a specific purpose) vary among government agencies and organizations and include the following:

- *US Food and Drug Administration* (*FDA*): The term 'food additive' means any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and including any source of radiation intended for any such use), if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case as a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use; except that such term does not include: (1) a pesticide chemical in or on a raw agricultural commodity; or processed food; or (2) a pesticide chemical; or (3) a color additive; or (4) any substance used in accordance with a sanction or approval granted prior to the enactment of this paragraph[1] (footnote 2) pursuant to this Act, the Poultry Products Inspection Act (21 U.S.C. 451 and the following) or the Meat Inspection Act of March 4, 1907 (34 Stat 1260) as amended and extended (21 U.S.C. 71 and the following); (5) a new animal drug; or (6) an ingredient described in paragraph (ff) in, or intended for use in, a dietary supplement (US FFDCA §201 (s)).[1]

[1] US FFDCA §201(s) = United States Federal Food Drug and Cosmetic Act (1938 enaction, as amended), section 201 'Definitions'.

- *European Economic Community* (*EEC*): A food additive is any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods. 89/107/EEC.
- *World Health Organization* (*WHO*): Food additive means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result (directly or indirectly), in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities Codex Alimentarius, second edition (revised 1995), volume 1A (General Requirements), p. 11.

Principles

Several principles are common to the use of food additives including:

- A food additive must be safe and suitable, it must present no risk to the health of the consumer at the levels of intended use, it must not be used to deceive the consumer (wholesomeness and stability must not be compromised).
- A food additive must be used in accordance with the principles of current good manufacturing practice (cGMP) which dictates that the lowest possible amount necessary to accomplish the desired technical effect be used and that the preparation and handling of the food additive be to the same quality as food itself.
- A food additive may serve one or more technological functions (functionality) listed in **Table 1**. US Regulations may include broader generalizations and finer subcategories. For example, Section 172 of 21 CFR includes these broad categories:
  - food preservatives,
  - coatings, films and related substances,
  - special dietary and nutritional additives,
  - anticaking agents,
  - flavoring agents and related substances,
  - gums, chewing gum bases and related substances,
  - other specific usage additives, and
  - multipurpose additives.

  Whereas some of the subcategories also listed in Section 172 of 21 CFR on a case-by-case basis include:
  - antigushing agent,
  - bleaching agent,
  - cure accelerator agent,
  - malting agent,
  - pesticide surfactant agent,
  - retard struvite (innocuous magnesium ammonium phosphate crystals) formation agent, and
  - wetting agent.

Regulations – US

United States food law is codified in the Federal Food Drug Cosmetic Act (FFDCA) with amendments and further clarification in the Code of Federal Regulations, (CFR), Title 21. This deals with food and food additives.

Product categorization (e.g., food or drug) determines the nature and extent of testing. More rigorous and restrictive requirements, including premarket approval by the FDA, are applied to a drug than to a 'food'. There may be distinct advantages for classifying a product as a dietary supplement instead of a conventional food and this will be discussed in more detail in the section entitled 'Dietary Supplements Ingredients'. Inconsistent regulatory treatments of similar (or in some cases, identical) products can lead to a blurring of the lines between different 'intended use' FDA categories. Placement in a category depends in part on how the manufacturer positions the product for consumer use. A soluble fiber found in conventional breakfast cereals may be a food or a food additive; it also may be used as a dietary supplement and as an over-the-counter (OTC) drug, with concomitant allowable health benefit claims.

Premarket clearance (clearance prior to marketing) may be required by the FDA in some instances but not in others, The prevailing legal principle in the FFDCA is the prohibition to the use of 'any poisonous or deleterious substance which may render…[the food] injurious to health…'.

The following seven categories will be briefly described: (1) traditional foods used as food ingredients; (2) prior-sanctioned ingredients; (3) generally recognized as safe ingredients; (4) direct food additive ingredients; (5) indirect food additive ingredients; (6) dietary supplement ingredients; and (7) bioengineered food ingredients.

Table 1 Technological functions of a food additive

Technical effect	*Notes*	*Example*	*US*[a]	*JECFA*	*EU*
Acidity regulators	Buffer acid/base	Ammonia solution	U*	J	
Acids	pH change, tartness	Lactic acid		J	E
Adjuvants		Polyvinylpyrrolidone		J	
Adsorbents	Remove moisture	Activated carbon	U**	J	
Anticaking agents	Prevent clumping	Aluminum silicate	U	J	E
Antifoaming agents	Prevent foaming	Polydimethylsiloxane		J	E
Antioxidants	Prevent oxidation	Ascorbic acid	U	J	E
Bulking agents	Add bulk	Ethyl cellulose		J	E
Carrier solvents	Dissolve	Ethyl alcohol		J	
Clouding agents	Add opacity	Brominated vegetable oils		J	
Color retention agents	Preserve color	Cupric sulfate	U***	J	
Colors	Add color	Canthaxanthin	U	J	E
Curing and pickling agents	Add unique color/flavor		U		
Dough strengtheners	Modify starch, improve dough		U		E*
Emulsifiers	Modify surface tension	Lecithin	U, U****	J	E, E**
Enzyme preparations	Improve food processing	Avian pepsin	U	J	E
Extract solvents	Dissolve	Acetone	U	J	
Filtering aids	Remove sediment	Polyvinylpolypyrrolidone		J	
Firming agents	Add firmness	Calcium sulfate	U	J	E
Flavor enhancers	Modify original flavor	Disodium 5′-guanylate	U	J	E
Flavoring agents	Add flavor	Citral	U	J	
Flour treatment agents	Improve milled flour	Stearyl tartrate	U	J	E
Foaming agents	Add foam	Methyl ethyl cellulose		J	
Formulation aids	Produce texture		U		
Freezing agents	Freeze	Nitrogen		J	
Fumigants	Control pests		U		
Gelling agents	Gel	Sodium alginate		J	E
Glazing agents	Glaze, surface-treat	Beeswax	U*****	J	E
Humectants	Add/retain moisture	Xylitol	U	J	E
Lubricants/release agents	Prevent sticking, including molds	Castor oil	U	J*	
Miscellaneous		Helium; Gelatin; Carbon dioxide		J	
Nonnutritive sweeteners	Non caloric sweeteners	Sucralose	U		
Nutrient supplements	Add vitamins/minerals	Potassium gluconate	U	J	E
Nutritive sweeteners	Caloric sweeteners	Sucrose	U		
Oxidizing and reducing agents	Improve stability		U		
Preservatives	Prevent microorganisms growth	Hydrogen peroxide	U******	J	
Processing aids	Enhance food		U*******		
Propellants	Discharge pressurized foods	Argon	U********	J	E
Raising agents	Enable dough to rise	Ammonium carbonate	U*********	J	E
Reduced-energy fat and oil replacement	Low-fat oil	Salatrim		J	
Sequestrants	Form soluble metal complexes	Stearyl citrate	U	J	E
Stabilizers	Improve dispersions	Gellan gum	U	J	E
Sweeteners[b]	Sweetening capacity	Aspartame		J	E
Synergists	React with another ingredient	Sodium percarbonate	U	J	E
Texturizers	Affect mouth feel, appearance		U		
Thickeners	Produce viscosity, body	Carob bean gum	U	J	
Yeast foods		Urea		J	

[a] US 21 CFR170.3(o).

[b] US splits sweeteners into nutritive and nonnutritive sweeteners. (see appropriate spaces in **Table 1**)

U*, pH control agents; U**, drying agents; U**, coloring adjuncts; U***, emulsifiers and emulsifier salts; U****, surface-active and surface-finishing agents; U*****, antimicrobial agents; U******, includes clarifying, clouding, filtering, etc.; U*******, propellants, aerating agents and gases; U********, leavening agents; J*, release agents; E*, modified starch; E**, emulsifiers and emulsifier salts.

Traditional Foods Used as Food Ingredients

Common or traditional foods that have a history of safe use (e.g., most vegetables, fruits, grains, meats, poultry) are recognized as safe for human consumption. They are an integral part of the final food that is consumed.

Prior Sanctioned Ingredients

When the key amendments were made to the FFDCA in 1958, it was recognized that some exceptions were needed to the food additive category, such as ingredients previously established as safe and already listed in FDA regulations. Some of the

prior-sanctioned ingredients include gum guiac, calcium propionate, linseed oil, and sodium nitrate. A complete listing appears in 21 CFR Section 181.

Generally Recognized as Safe Ingredients

Also in 1958, it was recognized that expertise other than that in the FDA could be utilized to determine safety of foods and food ingredients. Statutory language states a substance is generally recognized as safe (GRAS) and thus outside the scope of the food additive definition if it is "recognized, among experts qualified by scientific training and expertise to evaluate its safety, as having been adequately shown through scientific procedures…to be safe under conditions of its intended use…" Over the years, the Agency has continued to modify this process. There are currently two approaches, the GRAS self-determination (usually managed by the interested company) and the GRAS Notification (managed by the FDA). This latter regulatory process allows a company to submit the details of their successful GRAS self-determination to the FDA, which will critically evaluate all aspects within a predetermined time period. The FDA will then issue a letter to the petitioner and post it publicly on the FDA's webpage (see section on Relevant Websites). The letter with the best outcome for the submitter may be interpreted as an affirmation of GRAS but, it is a 'no objection letter', that is, the FDA had no objections to the GRAS self-determination. For example, the public letter may read, 'Based on the information provided by Company X, as well as other information available to FDA, the Agency has no questions at this time regarding the conclusion of Company X that Ingredient Y is GRAS under the intended conditions of use. The Agency has not, however, made its own determination regarding the GRAS status of the subject use of Ingredient Y. As always, it is the continuing responsibility of Company X to ensure that food ingredients that they market are safe, and are otherwise in compliance with all applicable legal and regulatory requirements'. An alternative response from the FDA is an 'objection letter', which clearly identifies deficiencies in the submitted GRAS Notification.

Direct Food Additive Ingredients

If a candidate substance is to be used directly in/on food, and is not already covered under prior sanction or GRAS categorization, and is not bioengineered nor a dietary supplement, then it is most likely a *bona fide* food additive/ingredient and stringent premarket (i.e., FDA approval before marketing) approval is mandated by a petition process. If successful, official regulations will be published and codified in the 21 CFR and there are likely to be specific conditions of use including food categories in which it may be used and appropriate levels of use.

Indirect Food Additive Ingredients

If a substance is found in food but was not directly added to the food and if it does not have a technical effect in the food, it is considered an indirect food additive. This category includes food contact materials such as packaging. Originally these ingredients were subjected to similar, rigorous regulations comparable to direct food additive ingredients, but this has been significantly simplified into a premarket notification instead of premarket approval.

Dietary Supplement Ingredients

Dietary supplement ingredients were removed from the FDA's food additive regulations in 1994 with the passage of the Dietary Supplement Health and Education Act (DSHEA) creating a more favorable, less onerous process (FDA premarket notification) with the advantage that labels and labeling were permitted to provide 'statements of nutritional support' but, these materials are prohibited from being 'represented for the use as a conventional food'.

Bioengineered Food Ingredients

Although controversial, bioengineered foods, genetically modified organisms, and products of biotechnology are not required to undergo food additive scrutiny by the FDA as long as there are no significant changes in composition, nutrient value, allergenicity (beyond the conventional 'version') or, other safety concerns compared to the familiar, conventional, and traditional food. The bioengineered food ingredient should be substantially equivalent to the conventional/traditional food. FDA does expect a premarket consultation to review safety and any other potential concerns.

Regulations – Europe

European Union (EU) countries regulate food additives through a number of key directives that are approved by the members. The legislative processes are very complex and beyond the scope of this discussion. Suffice, the EU is 'run' by a number of institutions, including the European Commission (EC), the Council of Europe, and the European Parliament (EP). The EC initiates proposals for legislation, is guardian of treaties and ensures that EU legislation is applied correctly by member states, and manages EU policies and international trade

relations. The EC is further subdivided administratively into departments referred to as Directorate-Generals (DGs), one of which, the DG for Health and Consumer Protection is the most important in the area of food law. A number of advisory Scientific Committees, most critically, the Scientific Committee for Food (SCF) prepares scientific opinions and risk assessments. The three types of European legislation are (1) directives, (2) regulations, and (3) decisions. Directives express obligatory objectives but do allow the members flexibility in the translation of the directive into their national law. Regulations are inflexible, apply to all members, are binding and circumvent the member's national legislation. Decisions are binding but are more specifically addressed to discrete member states, companies, or individuals.

The key Directive, 89/107/EEC, provides the framework for general regulatory and safety aspects of food additives, including the aforementioned definition of food additive "…any substance not normally consumed as food…addition of which…for a technological purpose…becoming…a component of food". This definition does not include processing aids (similar to the US indirect food additive ingredients), plant health products, flavorings and substances used as nutrients. Additional directives have been promulgated that address several specific subcategories including among others, 'sweeteners', 'colors', and 'flavorings'. Authorized food additives, conditions of use, limitations, purity criteria, etc. are listed in the directive. The process can occur in stages, as there are provisions for temporary use, specific national (member country-specific) marketing of a nonlisted additive as well as suspension or restriction of an authorized food additive in their country on grounds of suspected danger to health or erosion of 'traditional national foods', such as Greek feta cheese, German beer (Reinheitsgebot) and Spanish 'lomo embuchado'. Authorized additives are listed in one of the newer directives (94/35/EC (sweeteners); 94/36/EC (colors); 89/107/EEC (flavors); and 95/2/EC (amended 96/85/EC, 98/72/EC, 2001/5/EC; for all other food additives)).

Regulations – Codex Alimentarius

The Codex Alimentarius (CA; latin for 'food law' or 'food code') Commission (CAC) is an intergovernmental subsidiary to the Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) of the United Nations (UN) with the aim to formulate and implement internationally accepted food safety standards for the protection of consumer health and to ensure fair trade practices in the food industry. The principal result has been the publication of food commodity standards, hygiene and technical codes of practice, guidelines and other recommendations. The CAC professes to take into account the individual concerns of governments, nongovernmental organizations, industry, and the consumer. The CAC issues CA standards, which are requirements aimed at providing consumers with a 'sound, wholesome food product' free from adulteration, correctly labeled and presented, and CA codes of practice as advisories to member nations providing flexibility in translation and implementation.

The adoption of standards is a multistep process requiring the involvement of appropriate expert committees charged with preparing the 'proposed draft standard' which undergoes a series of circulations to governments and other interested international organizations for comment.

Safety – US

Recognizing that the establishment of safe food (and color) additives would be predicated upon appropriate and responsible safety assessments, the FDA issued 'Guidelines' in 1982 entitled 'Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food (termed the "Redbook")' and currently has a web-based dynamic version entitled 'Redbook 2000: Toxicological Principles for the Safety Assessment of Food Ingredients' (see section on Relevant Websites). Safety is defined by the Agency as 'a reasonable certainty that a substance is not harmful under the intended conditions of use'. The Redbook guidelines are an attempt to 'delineate the sensitivity and rigor of toxicological and other information needed to make safety determinations' for these additives. The guidelines outline a 'tiered' approach linking the level (and therefore financial and temporal costs) of effort to the chemical structure of the ingredient and to the potential exposure. Sample protocols are provided to ensure consistency in study design facilitating Agency and/or other expert evaluation. The food additive petition process requires the submission of appropriate data derived from animal and/or human safety studies such as described in the Redbook. The animal toxicity data must supply substantive information that addresses (1) the identification of any hazards inherent to the use of the substance; (2) the indication of a dose–response relationship for those identified hazards; and (3) the extrapolation of these data to establish a safe exposure for consumers (e.g., an acceptable daily intake).

Safety studies that are consistent with other internationally accepted guidelines (e.g., those of the Organization for Economic Cooperation and Development (OECD)) may be acceptable to the FDA.

Safety – Europe

The original and overriding Directive, 89/107/EEC, mandates that food additives can be approved only provided that "…they present no hazard to health of the consumer at the level of use proposed, so far as can be judged on the scientific evidence available…" Further, to assess the possible harmful effects of a food additive "it must be subjected to appropriate toxicological testing and evaluation. The evaluation should also consider, for example, any cumulative, synergistic or potentiate effect of its use and the phenomenon of human intolerance to substances foreign to the body." And the EU did not consider this to be a once and done activity as the regulation further stipulates that "all food additives must be kept under continuous observation and must be re-evaluated whenever necessary in the light of changing conditions of use and new scientific information."

Safety – Codex Alimentarius

The CAC has emphatically stated that all CA standards, codes of practice and other texts shall be firmly anchored in sound scientific analysis and evidence, involving a thorough review of all relevant information so that the issued standards ensure the objective, safe food. An instrumental expert committee, the Joint Expert Committee on Food Additives (JECFA), serves as a scientific advisory body to FAO, WHO, to the UN member country governments and to the Codex Alimentarius Commission. JECFA is charged with evaluating the safety of food additives (as well as contaminants, naturally occurring toxicants and the residues of veterinary drugs in food derived from animals used for human food). JECFA evaluations provide impartial advice and technically rigorous science-based risk assessments. There is another committee, the Joint Meeting on Pesticide Residues (JMPR), which critically evaluates data on pesticides and recommends ADIs and residue levels. It is also an advisory body to FAO, WHO, and to UN member countries.

Evaluation Steps

The evaluation of the safety of food ingredients can be resource-intensive depending on the chemical nature of the ingredient and the extent and conditions of exposure. An outline of the process follows:

1. Initial evaluation
   - 1.1. Clear definition of the issues
   - 1.2. Test material characterization
   - 1.3. Exposure assessments (assume 100% replacement)
   - 1.4. Critical evaluation of the literature
   - 1.5. Initial safety assessment
   - 1.6. Issues to be addressed
     - 1.6.1. Safety testing must simulate human exposure conditions
     - 1.6.2. Absolute versus relative safety
     - 1.6.3. Animal versus human; animal and/or human; sensitive subsets of population
     - 1.6.4. History of use (US and other countries)
     - 1.6.5. Nature of material, extent of exposure (levels (dose), duration)
     - 1.6.6. Initial sensory evaluation ('sip and spit')
2. Primary evaluation
   - 2.1. Acute toxicity test(s)
     - 2.1.1. Oral (dermal, ocular, inhalation)
   - 2.2. Repeated dosing tests (7–30 days)
   - 2.3. Initial genotoxicity tests (short term tests)
   - 2.4. Initial sensory evaluation ('sip and spit')
   - 2.5. Comparative (animal, human) ADMEK (absorption, distribution, metabolism, excretion, kinetics) (single-dose human study)
   - 2.6. Special studies
3. Secondary evaluation
   - 3.1. Subchronic tests ($<1/2$ lifetime of the animal; usually 90 days)
   - 3.2. Repeated human dosing tests
   - 3.3. Reproductive/developmental toxicity tests
   - 3.4. Genotoxicity tests
   - 3.5. Special studies
4. Definitive evaluation
   - 4.1. Chronic toxicity (lifetime) tests (several species; *in utero* exposure)
   - 4.2. Long-term human dosing tests
   - 4.3. Special studies
   - 4.4. ADI (acceptable daily intake)
5. Risk assessment
6. Postmarketing surveillance (PMS)
   - 6.1. Active versus passive

See also: European Union and Its European Commission; Food and Agriculture Organization of the United Nations; Food and Drug Administration, US; Food, Drug, and Cosmetic Act, US; Organisation for Economic Cooperation and Development.

Further Reading

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Food Quality Protection Act, US

Patricia M Nance

In 1996, the US Congress unanimously passed landmark pesticide food safety legislation supported by the Administration and a broad coalition of environmental, public health, agricultural, and industry groups. President Clinton promptly signed the bill on August 3, 1996, and the Food Quality Protection Act (FQPA) of 1996 became law (P.L. 104-170, formerly known as H.R. 1627). The FQPA requires the US Environmental Protection Agency (EPA) to consider new factors when making pesticide regulatory decisions. Registrants, applicants, or petitioners for pesticide product registrations or reregistrations, or for tolerances or tolerance exemptions, whether pending or future, are advised to consider comprehensively the provisions contained in the FQPA, specifically the factors relevant to aggregate exposure assessment, children's exposure, and other issues raised by the new statutory standard.

EPA regulates pesticides under two major federal statutes. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), EPA registers pesticides for use in the United States and prescribes labeling and other regulatory requirements to prevent unreasonable adverse effects on health or the environment. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), EPA establishes tolerances (maximum legally permissible levels) for pesticide residues in food. Tolerances are enforced by the Department of Health and Human Services/Food and Drug Administration (HHS/FDA) for most foods, US Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) for meat, poultry, and some egg products, and the US Department of Agriculture/Office of Pest Management Policy.

For over two decades, there were efforts to update and resolve inconsistencies in the two major pesticide statutes, but consensus on necessary reforms remained elusive. The 1996 law represented a major breakthrough, amending both major pesticide laws to establish a more consistent, protective regulatory scheme, grounded in sound science. It mandates a single, health-based standard for all pesticides in all foods; provides special protections for infants and children; expedites approval of safer pesticides; creates incentives for the development and maintenance of effective crop protection tools for American farmers; and requires periodic re-evaluation of pesticide registrations and tolerances to ensure that the scientific data supporting pesticide registrations will remain up to date in the future.

Effective upon signature, the FQPA significantly amended the FIFRA and the FFDCA. Title IV of the FQPA amended the Federal Food, Drug and Cosmetic Act. The most important aspect of this title is the establishment of a single, health-based standard for setting pesticide residue tolerances. This eliminated the longstanding problems posed by different standards for pesticides in raw and processed foods. The provision removed the requirement of food additive tolerances for processed foods and instead regulates them all under the same tolerance provision. A tolerance (or exemption from tolerance) for a pesticide residue on a raw agricultural commodity (RAC) also applies to residues in a processed food derived from the RAC that are not higher than the RAC tolerance. If the levels in the processed food are higher, a separate tolerance must be set for that processed food. Residue levels in both the RAC and the processed food must be determined by EPA to be 'safe'.

The new safety standard, provided in section 408(b)(2)(A)(ii) of the FQPA, is a 'reasonable certainty of no harm' standard for aggregate exposure using dietary residues and all other reliable exposure information. When setting new or reassessing existing tolerances or tolerance exemptions under the new standard, EPA must now focus explicitly on exposures and risks to children and infants. EPA must explicitly determine that the tolerance, or exemption from tolerance, is safe for children; consider the need for an additional safety factor of up to tenfold to account for uncertainty in the data base relative to children unless there is evidence that a different factor should be used; and consider children's special sensitivities and often unique exposure patterns to pesticides.

In addition, when making a determination as to whether or not there is a reasonable certainty that a pesticide chemical will cause 'no harm', EPA must now consider other nonoccupational sources of pesticide exposure when performing risk assessments and setting tolerances. This includes dietary exposure from drinking water, nonoccupational exposure, exposure from like pesticides that share a common mechanism of toxicity as well as other exposure scenarios. When setting new or reassessing existing tolerances and tolerance exemptions, EPA must also evaluate the potential for endocrine disruption. The new law directs the Agency to use its authority to require specific tests and information on estrogenic effects for all pesticide chemical residues.

EPA began the task of implementing the requirements of the FQPA by explaining its goals and immediate plans in a letter sent in August 1996, to all current pesticide manufacturers, grower and other pesticide user groups, industry, environmental, consumer, and public interest groups. A second letter, containing more detailed information, was sent on September 1996, to all holders of pesticide registrations. In its September 1996 letter, the Agency stressed that work was continuing on many registration and reregistration activities and that interim decisions were being made. However, to ensure compliance with the new law's provisions to protect against pesticide uses that may pose unacceptable risks to children, additional time was needed to adequately review certain applications, especially food use applications.

Highlights of the FQPA

New Safety Standard for all Pesticide Residues in Food

- 'Reasonable certainty of no harm' from exposure to residues.
- Aggregate assessment of all nonoccupational sources of exposure, including drinking water, residential, and dietary exposure.
- Assessment of cumulative exposure to a pesticide and other substances with common mechanisms of toxicity.

Reduced Risk Pesticides

- Streamlined the registration process of reduced risk pesticides, including new active ingredients, new uses of existing active ingredients already found to be reduced risk, and amendments to all uses deemed to reduce risk.
- Adoption of integrated pest management techniques through research, education, and procurement and regulatory policies.

Tolerance Assessment and Reassessment

- Application of the new safety standard to all tolerances issued after August 3, 1996.
- Reassessment, within 10 years, of all tolerances issued prior to enactment of FQPA to ensure they meet the new safety standard.
- Establishment of tolerances for emergency exemptions issued under Section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act.
- Authorization to charge fees for performance of tolerance functions.

Pesticide Reregistration and Periodic Registration Review

- Reauthorization and increase of maintenance fees to complete review of older pesticides first registered prior to November 1984.
- Authorization for a 15 year registration review program.

Right-to-Know

- Development of a simple, understandable consumer brochure on pesticide residues to be distributed to large, retail grocers for public display.
- Publication of an informative statement about the data relating to a tolerance.

Special Protections for Infants and Children

- Consideration of children's special sensitivity and exposure to pesticides.
- Use of an extra tenfold safety factor in addition to the traditional 100-fold safety factor, unless, on the basis of reliable data, a different factor is determined to be safe for children.
- Explicit determination that a tolerance is safe for children.

Endocrine Disruptors Screening and Testing Program

- Development and application of a screening and testing program for chemicals with the potential to disrupt the endocrine process.
- Progress report to Congress by August 2000.

Antimicrobial Pesticides

- Reform of the antimicrobial registration process to meet shortened review period goals while still ensuring efficacy and safety.
- Annual report to two Congressional Committees on progress in meeting the reform goals.

Minor Use Pesticides

- Incentives to maintain existing minor uses and to develop new ones.
- Establishment of minor use offices within EPA and the US Department of Agriculture.

See also: Delaney Clause; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Food, Drug, and Cosmetic Act, US; Pesticides; Risk Assessment, Human Health; Toxic Torts.

Further Reading

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Food Safety and Toxicology

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The Federal Food Safety Program resides in part under the authority of the US Food and Drug Administration. The specific statutory authority is the Federal Food, Drug, and Cosmetic Act (FFDCA). The areas of responsibility include the consideration of the safety or risk of food and color additives, both direct and indirect, and food-borne contaminants, both natural and anthropogenic. The FFDCA proscribes somewhat different standards of safety/risk for intentional food additives that undergo pre-market assessment of safety, specifically, versus those dietary constituents that are found in food as contaminants because they occur naturally or because they arise from anthropogenic sources.

The prototypical safety assessment for food-borne compounds is the acceptable daily intake (ADI) methodology, which was first documented in 1954, and has come to be employed throughout the world. This paradigm has also been codified in the consideration of food (e.g., aspartame) and color additives (e.g., Red Dye No. 2), and pesticides (e.g., atrazine). It is also routinely used in the consideration of incidental food-borne chemical contaminants (e.g., lead), particularly as a tool for screening out trivial incidents of exposure. This procedure specifies that an acceptable dose of a chemical may be calculated with the following equation:

$$\mathrm{ADI} = \mathrm{NOEL}/\mathrm{SF}$$

where ADI is the acceptable daily intake, NOEL is the no-observed-effect level, and SF is a safety factor. In subsequent considerations an identical methodology has been implemented where new terms have been substituted for those originally described for the ADI safety assessment methodology. These terms are the reference dose (RfD), minimal risk level (MRL), and tolerable daily intake (TDI) for the ADI. In these methodologies, NOEL has been further defined to be the no-observed-adverse-effect level (NOAEL) and the SF is called an uncertainty factor (UF). These alternative terms have supplanted the ADI in considerations of food-borne contaminants, because unlike food additives, 'acceptance' is not an applicable term for chemical contaminants that do not have a premarket approval evaluation, and therefore these alternative terms were devised. Since contaminants are not deliberately added, and for some are fairly widespread in the environment, including the food chain, it is often much more difficult to achieve population exposures that are below an RfD/MRL/TDI.

As a general rule the NOEL/NOAEL is a dose from a controlled animal experiment where no adverse effect (i.e., an effect not considered harmful) is noted. The experiment does not establish that no effect can possibly occur at that dose under any conditions – it only denotes that none of the effects looked for in the experiment was observed. Since a statistical significance test is typically used to establish whether or not an effect occurred, the NOEL/NOAEL will tend to diminish as the sensitivity of the measurement or the number of observations increases. Since the burden of proof is on science to show that an effect has occurred, greater uncertainty tends to raise the level of exposure that is deemed acceptable/tolerable. The selection of the effect that is considered adverse is a matter of societal values (i.e., localized, reversible, mild discomfort versus frank, irreversible systemic toxicity). That is, establishing that an effect has occurred is a separate consideration from how much one cares if it will occur or not.

In the original safety assessment paradigm a single safety factor (SF) of 100 was used to derive an ADI from a NOEL. The justification of the SF (also called an uncertainty factor (UF)) was based on scientific considerations as well as a judgment about how to manage the inherent uncertainty associated with the

assessment. Scientific issues raised included the notion that humans may be more sensitive to chemicals than rodents used in a laboratory test, and that there may be substantial variability among individuals in a population. The necessity of managing uncertainty was acknowledged through the recognition that it is impossible to demonstrate that no adverse affect could not occur under any circumstances. As the ADI approach evolved, the single safety factor was replaced by two or more safety/uncertainty factors of 10, with each factor applied as a response to a particular scientific issue. For example, an NOEL/NOAEL from an animal experiment is normally divided by a factor of 10 as a matter of policy in response to the supposition that humans may be more sensitive than laboratory animals and by another factor of 10 to account for variability of response in the population of concern. SF/UFs dictate the impact of uncertainty of some quantity on the decision. Although the magnitude of the uncertainty is not precisely described, the general idea is that the greater the uncertainty, the larger the SF/UF needs to be. Thus uncertainty in SF/UFs and uncertainty in the derivation of the NOAEL push in opposite directions. As a matter of practice, the uncertainty underlying an SF/UF application is not quantified, so that a factor of 10 is almost always employed. Even if the uncertainty were quantified, the magnitude of the uncertainty factor would still depend on some judgment about what degree of risk-adversity is appropriate.

One outcome of the dependence of the NOEL/NOAEL on the statistical significance test is that it tends to penalize chemicals for which there is more or better data. To remedy this problem, the benchmark dose (BMD) concept was introduced as an alternative approach. The BMD depends on the specification of a low level effect that would typically be unobservable. The endpoint may be the specified percentage (5 or 10%) above background of a population for an endpoint deemed to be adverse. Since the endpoint is defined, determinations for different chemicals and different data sets tend to be more comparable.

Other efforts have endeavored to give SF/UFs a scientific basis by assembling a range of observations that are analogous to the occasions for SF/UF application. As indicated previously while the RfD/MRL/TDI methodology is essentially the same as for the ADI derivation, the endpoint has been recast as a threshold estimate. However, the ADI/MRL/RfD/TDI interpretation of the product of a NOAEL as a threshold presents some difficulties. First, since the number generated is partly a product of the management of the uncertainty, it does not make sense to say that threshold of safety itself is uncertain. Second, there is always some uncertainty about whether or not there is a threshold, especially when considering subpopulations of individuals whom may already be ill. Perhaps more importantly, even if the NOAEL/UF procedure is considered as a rough estimate of a threshold dose, it only provides information about what may happen at that particular dose; there is no indication about the likelihood or frequency of an adverse effect at higher doses.

An important part of the safety assessment process lies in establishing the impact of scientific uncertainties on the eventual decision. This is typically done by making conservative estimates that deliberately err on the side of safety. Since scientists are more familiar with the scientific issues, the responsibility for this is often delegated to them. There are two potential difficulties with this. The first problem is that the technical person is entrusted with a decision that is partly a social/political one. This is particularly true if there is an interaction between degree of uncertainty and degree of harm. For example, a larger degree of uncertainty may be tolerated when the adverse effect is relatively trivial than when it is severe. However, if the technical person is not aware of all the competing dietary and nondietary risks that impinge on the decision, then they may not be in a position to judge the relative importance of a particular risk. The second problem is that a technical person may dictate the impact of the uncertainty for only part of the problem, without being aware of the other uncertainties involved. There is therefore no way to judge the 'appropriate' degree of conservativeness for the particular problem. Because the safety assessment process may have many steps, the decision process may be fragmented to a degree that no one can judge whether or not a decision is reasonable.

From an administrative standpoint, a great benefit of the safety assessment process is its relative simplicity. It deals with a broad spectrum of dietary public health issues that may be quite complex both socially and scientifically with a few short rules. For small problems, the benefit of either more carefully considering the scientific issues or encouraging widespread participation in the decision process may not justify the effort that must be expended to do so. The efforts to improve the scientific basis of safety assessment often result in the complexity that may result from a risk assessment without the benefit of clarity of purpose. There are occasions where the safety assessment process may prove to be too simple. The bigger the problem is economically and politically, the less comfortable the general public may be with delegating important social/political decisions to scientists. As a result, the safety assessment process is more useful for identifying small problems, than it is in dealing with large problems. For food

additives, the safety assessment process ensures that any risk from a compound that is intentionally added to food is trivial.

As with food additives, the safety assessment process for contaminants is helpful in identifying chemicals that pose a trivial risk. However, the safety assessment process may not be useful for those contaminants where the level of exposure in a population already exceeds what has been identified as the threshold of safety. The level specified by the safety assessment process may be unattainable in all circumstances, and control may best be directed at reducing exposure in general rather than attaining a particular level. Considering the best options available may require better information than the safety assessment process can deliver. In this case a formal risk assessment is required, where the goal is to provide an estimate of the probability of harm for a public health threat. A key difference between safety and quantitative risk assessment (QRA) is that while the former is a decision process in and of itself, a QRA is intended to provide information as part of a larger decision process – this view of risk assessment is illustrated in **Figure 1**.

Since QRA is an applied analysis, the goal is to describe what is known in response to a particular question. Where matters of degree of exposure and risk are involved, numbers provide more precise descriptions than words, particularly for exposures that exceed the threshold of safety. In QRA, numbers may be used to describe both empirical quantities and, for the purpose of describing uncertainty, degrees of knowledge. As these are two fundamentally different purposes, the benefits of using quantitative tools are somewhat different. For describing empirical quantities (e.g., body weight, heart rate), numbers are useful because they are more accurate, in that they provide a more precise statement of what is known.

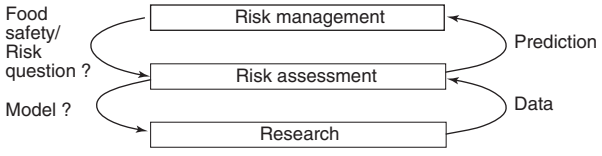

Figure 1 A dietary public health decision paradigm.

On the other hand, when describing uncertainties, the primary advantage of quantitative methods is that they are more transparent, thereby allowing more people to participate in the decision process.

QRA can be depicted as a series of four steps, as illustrated in **Figure 2**. Note that this breakdown of the risk assessment process maintains the relationships to risk management and research that are depicted in **Figure 1**. The interactions with risk management occur at the beginning (formulating the question) and end (making the decision) of the risk assessment process. The interaction with research occurs primarily at the data generation and modeling steps. The design process involves assembling a chain of quantitative inferences that answer the public health question. The end result is the risk assessment model that explicitly states how a particular adverse effect is causally related to a particular dietary exposure to a contaminant.

Uncertainty

Public health risk assessments used in considerations of food safety are intended to make predictions, particularly quantitative, of plausible negative impacts. Obviously depending on the underlying scientific information, some predictions are more reliable than others, and the extent to which predictions are reliable should be taken into account in the public health decision process. In order to accomplish this, the characterization of risk must contain a statement about the degree of uncertainty.

In discussing uncertainty, it is important to distinguish efforts to quantify uncertainty from the related and oft confused endeavor of theorizing about the nature and extent of frequencies in a population or series. Although uncertainties may often be reasonably calculated or based on frequencies (i.e., statistical uncertainty), frequencies may be of interest on their own (e.g., population variability), while some uncertainties are not frequency based (e.g., model uncertainty). As depicted in **Figure 3** the difference between probability and frequency is often a matter of context. The same distribution might be used to describe the frequency of an event when the question is about a population, or serve as the basis

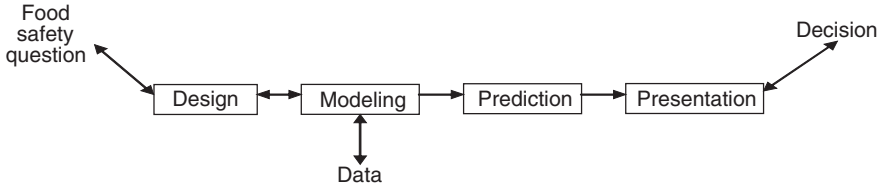

Figure 2 Four steps of quantitative risk assessment.

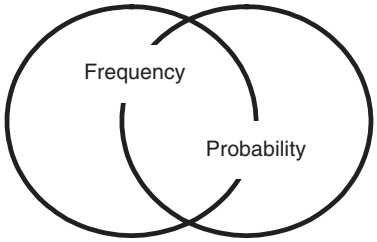

Figure 3 Variability and uncertainty.

for a quantitative probability statement when the question is about an individual. Consider two examples. First, a distribution describing the variability of a scientific instrument over time may be used to describe the uncertainty of individual measurement made by the instrument. Second, a distribution describing body weight could be used to predict the frequency of values expected in a population, or the uncertainty of the value in a particular individual for whom a measurement is unavailable.

Different results may be observed under conditions that are ostensibly the same. To keep track of this variation, we must maintain records or statistics. There are two general strategies that we may employ. First, we may simply store the results. That is, if we have a thousand observations, we can maintain access to all the individual values. The record may then be employed as an empirical distribution function, in which particular percentiles may be identified on demand. Second, we may use a mathematical model to summarize the distribution. There are two very different reasons for doing this. First, a statistical model may be used to provide a concise summary. The facility with which an analyst can store and retrieve data makes this motivation less compelling than it once was. Second, when a sparse data set is not considered representative of a large population, a model may also be used to infer or predict values that are not represented in the data set.

When mathematical models are used to draw inferences, the values of the model parameters may be a source of uncertainty. As the values of model parameters are not measured by direct observation (they are estimated as part of the model fitting process), the uncertainty of a parameter cannot be characterized by simply recording variability in a series of measurements. However, once the best model criterion has been established, the variability associated with a parameter can be linked to the variability in the data. If standard statistical assumptions are employed, the variability of and correlations among the parameters may be calculated directly.

Model uncertainty arises from the availability of multiple equations with different parameters with

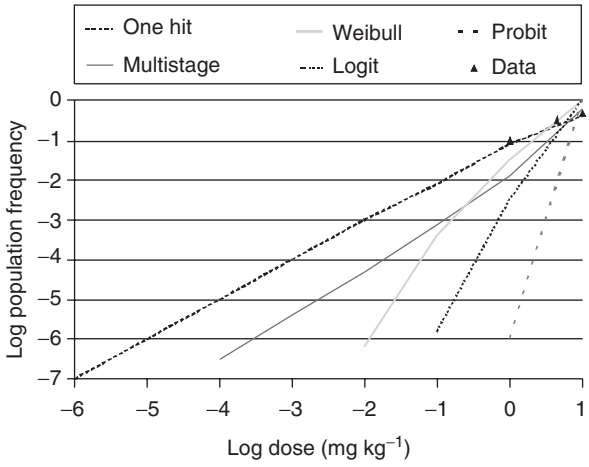

Figure 4 Model uncertainty in cancer risk assessment.

which an inference or prediction might be made. As a practical matter, model uncertainty is often not an important issue. In particular, so long as there are a number of observations to guide the model that come from similar circumstances as the problem that the analysis is concerned with, then any plausible model should yield very similar results. However, when models are used to extrapolate values, model uncertainty can be an important problem. Prime examples are the use of models to predict outcomes at low doses (e.g., **Figures 3** and **4**), and the use of distributions to estimate the frequency of occurrence of rare events.

Model uncertainties are generally represented by constructs that are commonly referred to as probability trees. The basic idea behind a probability tree is that the sum of the probabilities of all the models under consideration is one. Selecting and assigning probabilities to theories can be resolved by resorting to expert opinion. If a more formal process is necessary or desired – possibly to enable the evaluation to be carried out by a computer, an algorithm may be used. Since presumably the same considerations are involved, the same criteria that are used to select a 'best' model may also be used to weight them.

Frequency Distributions

Frequency distributions may be used to represent or draw inferences about the variation in a particular value among a population or series (i.e., a single set of values such as body weight). There are a number of ways of fitting or estimating the parameters for a frequency distribution. The popularity of the normal distribution may be at least partly attributed to the fact that estimates for the parameters (with a particular set of assumptions about the relationship

between the model and the data) can be calculated directly. There are a large number of frequency models to choose from. Although there is some theoretical basis for many of them, the theory is at best only loosely applicable to describing variability in biological populations – the biology is almost certainly more complicated than the model. It is often a good idea to use several models, and to use a probability tree to represent model uncertainty.

Dose–Response Models

Dose–response models describe a cause–effect relationship. There are a wide range of mathematical models that have been used for this purpose. The complexity of a dose-response model can range from a simple one-parameter equation to complex multicompartment pharmacokinetic/pharmacodynamic models. Many dose–response models, including most cancer risk assessment models, are population models that predict the frequency of a disease in a population. Such dose–response models typically employ one or more frequency distributions as part of the equation. Dose–response may also operate at an individual level and predict the severity of a health outcome as a function of dose. Particularly complex dose–response models may model both severity of outcome and population variability, and perhaps even recognize the influence of multiple causal factors.

Making Predictions

Once the overall risk assessment model is constructed, it may be used to make predictions. Running a model and collecting the results is often referred to as a simulation. If there are statistical components to the model, the model may be run repeatedly using different random numbers to select values from the statistical distributions each time. This process is known as Monte-Carlo simulation. In public health models, distributions can be used to describe variability in populations or the uncertainty in a value, parameter, or model. Since uncertainty is ever present, the presence of distributions in the model that are intended to describe variability usually results in a two-dimensional (2D) distributional model, where one dimension represents population variability and another represents uncertainty in the outcome. To use the Monte-Carlo method to assimilate the results of the model, a 2D simulation may be used. A program written to accomplish this task will look something like this:

- Begin Uncertainty Loop.
- Randomly Select New Values from Uncertainty Distributions.
- Begin Variability Loop.
- Randomly Select New Values from Variability Distributions.
- Calculate Output using Selected Values.
- Collect Output values into 2D Array.
- Repeat Variability Loop.
- Repeat Uncertainty Loop.

Because the total number of iterations is the product of the variability and uncertainty iterations, a 2D Monte-Carlo procedure is very calculation intensive. Even with longer calculation times, fewer iterations will generally be performed for each dimension than for a 1D simulation – with a concomitant decrease in the reliability of the estimates at the tails. The results of a 2D simulation will be a 2D array, rather than a 1D array. In order to reduce the number of values that need to be stored, it may be desirable to calculate summary statistics for each variability distribution as the simulation progresses.

QRA Output

Whether it is 1D or 2D, Monte-Carlo simulation produces many individual estimates – one per iteration. While these numbers are the end result of the risk assessment, no one can look at all these numbers and make sense of them. Therefore, they need to be sorted and tabulated and summarized. However, deciding how to do this involves considering how the results are going to be used and by whom. To use the classic example, reporting a distribution as a mean entails that the mean is what will be used in making a decision. It is important to inform the decision maker on the summary process so that they understand that a distribution lies behind the single number. The simplest way to do this is to simply display a list of percentiles along with the summary statistics. The results of a 2D simulation will necessarily be more complicated. If simulations are being run for a number of different scenarios (e.g., expected values with and without public health intervention), it is preferable to generate a table of summary statistics such as shown in **Table 1**.

The units reflect a scale constructed for the assessment, which ranges from 0 to 4. The mean and standard deviation for the uncertainty distribution are given for the mean, median, 95th percentile, and 99th percentile population values.

Although they may allow for quick comparisons, tables inherently compare one value at a time. Graphing or visualization is in some ways a better means of digesting the entire distribution. A 1D simulation will produce a frequency (when simulating variability) distribution or a likelihood distribution

Table 1 Methylmercury exposure scenarios – consumption vs. level limits

Scenario	*Average*	*Median*	*95th Percentile*	*99th Percentile*
No tuna	0.447±0.079	0.370±0.143	0.963±0.026	1.362±0.117
2.0 ppm	0.447±0.079	0.370±0.143	0.963±0.026	1.362±0.117
1.0 ppm	0.447±0.079	0.370±0.143	0.962±0.026	1.362±0.117
0.5 ppm	0.447±0.079	0.370±0.143	0.962±0.026	1.362±0.117
0.2 ppm	0.447±0.079	0.369±0.143	0.962±0.027	1.361±0.117
No limit	0.446±0.079	0.368±0.142	0.961±0.026	1.359±0.117

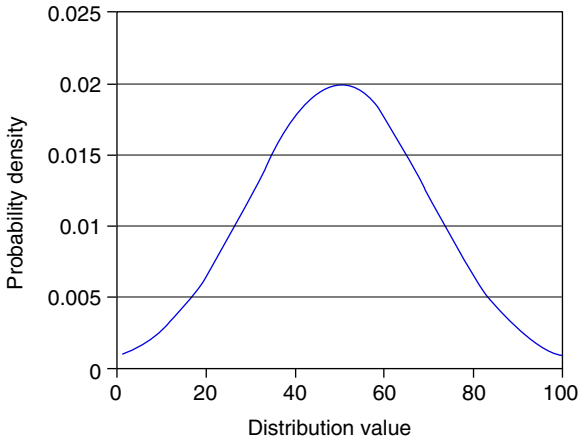

Figure 5 Frequency/likelihood curve: density vs. value.

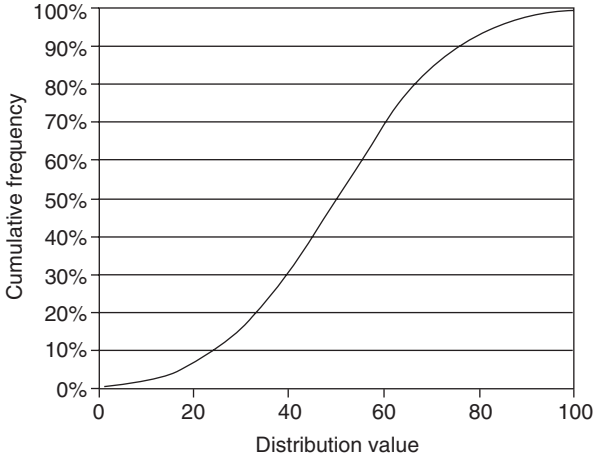

Figure 6 Frequency/likelihood curve: cumulative frequency/percentile vs. value.

(when representing uncertainty). There are two ways of plotting frequency or likelihood curves; the first is to plot density versus value (**Figure 5**), which emphasizes the values which are the most common or likely and the second is to plot cumulative percentiles versus value (**Figure 6**), which allows the percentile corresponding to a particular value to be read from the plot.

Two-dimensional results are more difficult to display. Two strategies for adding an extra dimension

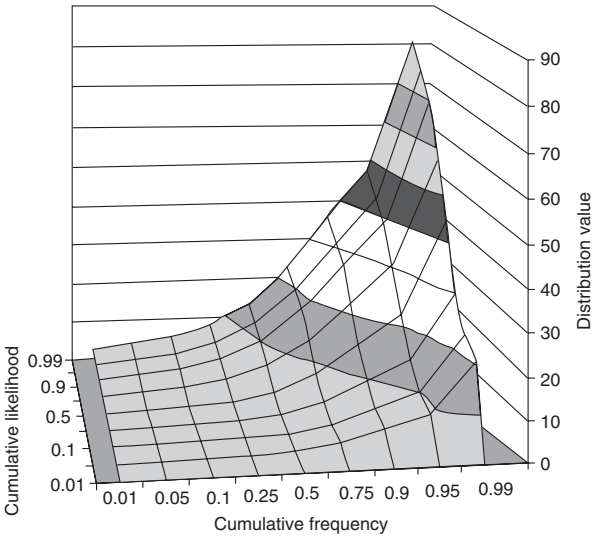

Figure 7 Three-dimensional representation of risk: severity (distribution value), frequency and uncertainty (cumulative likelihood).

are illustrated below. The first uses 3D perspective to portray the third dimension (**Figure 7**). The second uses shading, where darker hues are used to represent either higher density or more central values (**Figure 8**). This is particularly useful for displaying uncertainty, as the less well-defined parts of a curve appear fuzzy and unclear.

Identifying Data Gaps and Planning Research

Putting a formal QRA together will inevitably raise areas of uncertainty that can be addressed through further research. Since it has identified the issues that are important for the decision, a risk assessment can be useful in planning the research that will have the most impact on future decisions. Research proposals are justified on the basis of an expectation that they will reduce uncertainty. There are three general purposes that additional research may be geared toward:

1. Measurement of values used directly in risk assessment (e.g., in empirical distribution functions).

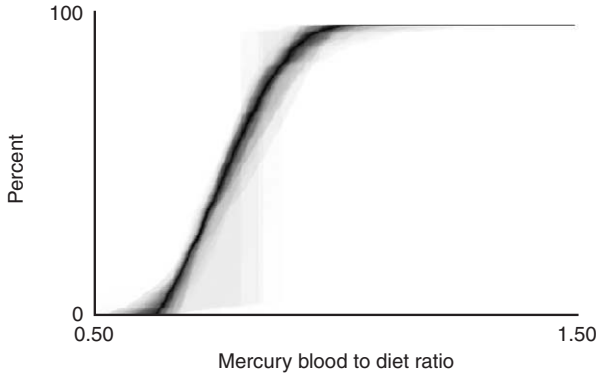

Figure 8 Variability in mercury blood to diet ratios.

2. Collection of data that will allow more accurate estimates of model parameters.
3. Collection of data that will allow discrimination among models.

Summary

Although contaminants are not intentionally added, the assessment of contaminants typically begins with the application of the same safety assessment process that is used for the evaluation of food additives. In most instances, such an assessment is sufficient in providing the assurance of safety of the potential exposure to many dietary contaminants. There are, however, other instances where the environmental contaminant is so ubiquitous, and therefore difficult to avoid, that a more informative analysis needs to be considered. The output of such an assessment is intended to describe the degree of harm expected in the population and the degree of uncertainty associated with the estimates. Specific areas of uncertainty can also be identified that will inform and suggest meaningful areas of research.

See also: Cumulative Risk Assessment; Monte Carlo Analysis; Safety Pharmacology; Uncertainty Analysis; Uncertainty Factors.

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http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Food Safety and Toxicology.

http://www.cfsan.fda.gov – US Food and Drug Administration, Toxicological Principles for the Safety Assessment of Food Ingredients.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Food Safety and Toxicology.

http://foodsafe.msu.edul – National Food Safety and Toxicology Center (at Michigan State University).

Food, Drug, and Cosmetic Act, US

Robert Kapp

- Agency: US Food and Drug Administration (FDA)
- Year of Initial Enactment: 1938, followed by numerous amendments

Background Information

The first federal legislation dealing with oversight of food and drug distribution in the United States included the Pure Food and Drugs Act of 1906 (Public Law 59-384) and the Meat Inspection Act, both of which were precipitated, in part, by the Upton Sinclair Novel entitled the *Jungle* (Doubleday, Page & Company, New York, 1906). This book was an exposé of unsanitary conditions in meat packing plants in America. Dr. Harvey W. Wiley of the Bureau of Chemistry, whose laboratory experiments and persistent demands led to this initial legislation regulating the food and drug industries, championed the legislation. This early legislation prohibited interstate commerce in misbranded and adulterated foods, drinks, and drugs and permitted inspections of

meat packing facilities. The disclosures of unsanitary meat packing plants' conditions, the use of poisonous dyes and preservatives in foods, and the 'cure-all' claims for ineffective and toxic medicines set the stage for this early food and drug legislation. The Sherley Amendment was passed in 1912 to prohibit labeling of medicines with false therapeutic claims intended to defraud the public. In 1913, the Gould Amendment was passed which required that food packages be "plainly and conspicuously marked on the outside of the package for weight, measure or numerical count." In 1914, the Supreme Court issued its first ruling on food additives (US vs. Lexington Mill and Elevator Company). The ruling found that the mere presence of a toxic ingredient was not sufficient to render the food illegal. The government now had to prove a relationship between the chemical additive and the harm it allegedly caused in humans. The Harrison Narcotic Act was passed that required increased record keeping for physicians and pharmacists who prescribed medications that exceeded the allowable limit of narcotics.

The Food, Drug and Insecticide Administration was renamed the Food and Drug Administration (FDA) in 1931. The FDA recommended a complete revision of the 1906 Food and Drugs Act in 1933 launching a 5 year legislative battle. Finally, Senator Royal S. Copeland of New York sponsored and engineered the passage of the Federal Food, Drug, and Cosmetic Act of 1938 (Public Law 75-717) after several sulfanilamide fatalities.

Overview of the Food, Drug, and Cosmetic Act (FDCA)

This was originally passed in 1938 and modified the 1906 Pure Food and Drugs Act to extended control to cosmetics and therapeutic devices; prohibited false advertising; required informative labeling; authorized definitions and standards for foods and drugs; required that new drugs could not be introduced into interstate commerce without documentation that the drug was safe before marketing – starting a new system of drug regulation; provided that safe tolerances be set for unavoidable poisonous substances; authorized the operation of plants under federal permit; and increased criminal penalties and authorized seizures where necessary.

Numerous amendments have been passed since 1938, including the following critical items:

- 1939 First Food Standard issued for canned tomatoes.
- 1941 Insulin Amendment required the FDA to test and certify purity and potency of insulin.
- 1945 Penicillin Amendment required FDA to test and certify safety and effectiveness of penicillin products.
- 1948 Miller Amendment affirmed that the FDCA applies to goods regulated by the agency that have been transported from one state to another and have reached the consumer.
- 1949 FDA published the 'Black Book' containing industry guidance entitled *Procedures for Appraisal of the Toxicity of Chemicals in Food*.
- 1951 Durham–Humphrey Amendment defined the types of drugs that could only be used with medical supervision and restricted their sale by prescription of a licensed physician.
- 1953 Factory Inspection Amendment required FDA to give manufacturers written reports of conditions noted during inspections.
- 1954 Miller Pesticide Amendment clarified procedures for setting safety limits for pesticides on raw agricultural commodities.
- 1958 Delaney Food Additives Amendment required manufacturers of new food additives to establish safety and prohibited the approval of any food additive shown to induce cancer in animals.
- 1958 Publication of the first list of Substances Generally Recognized as Safe (GRAS). This list contained about 200 substances.
- 1960 Color Additive Amendment required manufacturers to establish safety of color additives in foods, drugs, and cosmetics and prohibited the use of any color additive shown to induce cancer as per the Delaney Amendment noted above.
- 1962 Kefauver–Harris Drug Amendments passed to ensure drug efficacy and greater drug safety in response to the thalidomide birth defects disaster.
- 1965 Drug Abuse Control Amendments are enacted to deal with problems of abuse of depressants, stimulants, and hallucinogens.
- 1968 Animal Drug Amendments consolidates all regulations of new animal drugs under Section 512 making approvals more efficient.
- 1970 FDA requires the first patient package insert with oral contraceptives that provides patients with risk/benefit information.
- 1973 Consumer Product Safety Commission was created and took over administration of the Federal Hazardous Substances Labeling Act duties originally assigned to the FDA.
- 1976 Medical Device Amendments required manufacturers with the FDA and follow quality control procedures with some products needing premarket approval and others needing to meet performance standards before marketing.
- 1976 Vitamins and Minerals Amendments known as the Proxmire Amendments stopped FDA from

establishing standards limiting potency of vitamins and minerals in food supplements or regulate them as drugs based upon potency.
- 1982 FDA publishes the 'Redbook' entitled *Toxicological Principles of the Safety Assessment of Direct Food Additives and Color Additives Used in Food*. This document was the successor to the 1949 'Black Book'.
- 1984 Fines Enhancement Laws of 1984 and 1987 amended the code to increase penalties for federal expenses to a maximum of $100 000 for each offense and $250 000 if the violation is a felony or causes death.
- 1988 Food and Drug Administration Act established FDA as an agency of the Department of Health and Human Services with a Commissioner of Food and Drugs appointed by the President.
- 1995 FDA declares cigarettes to be 'drug delivery devices' and restricts the marketing and sales to reduce smoking by adolescents.
- 1996 Food Quality Protection Act amended the Food, Drug, and Cosmetic Act to eliminate the application of the 1958 Delaney amendment to pesticides.
- 1997 Food and Drug Administration Modernization Act mandated many of the reforms in agency practices since 1938 including accelerated reviews of devices, regulated advertising of unapproved drug and device uses, and regulated health claims for foods.

The FDCA as directed by the FDA has the authority to control the introduction of human and animal drugs, direct and indirect food additives, and the components of cosmetics. Both the safety and efficacy of any new drug must be clearly established before FDA approvals can be obtained to market the drug in the United States. Both animal and human clinical data must be submitted as part of the new drug application. There are no formal testing guidelines upon which industry can rely to perform adequate animal studies; however, there are informal guidance documents that indicate what types of studies should be conducted at various stages of the clinical investigations. The FDA subsequently issued the Good Laboratory Practice Regulations in 1976 that govern the conduct of animal studies.

Industry must also show that any chemical intended to be added to the food (i.e., as a preservative, coloring, or flavoring agent) or any material used in packaging (i.e., plastic wrapping or can coating) that could possibly leach into the food must be documented as safe for its intended use. These study results are submitted to the FDA as part of a food additive petition which the FDA reviews and, if the data sufficiently demonstrate the additive is safe, a regulation is published in the Federal Register that states that the direct and/or indirect additive is approved for a particular purpose. The FDA has published guidelines for the types of toxicity studies that must be conducted to support a food additive petition in the 'Redbook' (see above).

The FDA currently has no specific testing guidelines or requirements for cosmetic formulations for safety or efficacy prior to marketing. The FDCA states that the cosmetics must be free of 'poisonous and deleterious' substances.

See also: Delaney Clause; Food Additives; Food and Drug Administration, US; Generally Recognized as Safe (GRAS); Good Laboratory Practices (GLP); Investigative New Drug Application; Toxic Torts.

Relevant Websites

http://www4.law.cornell.edu – Federal Food, Drugs and Cosmetics Act (from the US Code).
http://www.fda.gov – The US Food and Drug Administration.

Foreign Body Response

Shayne C Gad

The foreign body reaction to biomaterials (such as implanted medical devices) results in foreign body giant cells and the components of granulation tissue. These consist of macrophages, fibroblasts, and capillaries in varying amounts, depending upon the form and topography of the implanted material. Relatively flat and smooth surfaces such as those found on breast prostheses invoke a foreign body reaction of a layer of macrophages one to two cells in thickness. Relatively rough surfaces such as those found on the outer surfaces of expanded poly(tetrafluoroethylene) vascular prostheses have a foreign body reaction of macrophages and foreign body giant cells at the surface. Fabric materials typically generate a surface response of macrophages and foreign body giant cells, with varying degrees of granulation tissue subjacent to the surface response.

Thus, the form and topography of the surface of the biomaterial determines the composition of the foreign body reaction. With biocompatible materials, the composition of the foreign body reaction in the

implant site may be controlled by the surface properties of the biomaterial, the form of the implant, and the relationship between the surface area of the biomaterial and the volume. Implants such as fabrics or porous materials will have higher ratios of macrophages and foreign body giant cells in the implant site than smooth surface implants, which will cause fibrosis at the implant site.

The foreign body reaction consisting mainly of macrophages and/or foreign body giant cells may persist at the tissue implant interface for the lifetime of the implant. Generally, fibrosis (i.e., fibrous encapsulation) surrounds the biomaterial or implant with its interfacial foreign body reaction, isolating the implant and foreign body reaction from the local tissue environment. Early in the inflammatory and wound healing response, the macrophages are activated upon adherence to the material surface.

While is it generally considered that the chemical and physical properties of the biomaterial are responsible for macrophage activation, the subsequent events regarding the activity of macrophages at the surface are not clear. Tissue macrophages, derived from circulating blood monocytes, may coalesce to form multinucleated foreign body giant cells containing large numbers of nuclei on the surface of biomaterials. While these foreign body giant cells may persist for the lifetime of the implant, it is not known if they remain activated, releasing their lysosomal constituents, or become quiescent.

The end-stage healing response to biomaterials is generally fibrosis of fibrous encapsulation. However, there may be exceptions to this general statement (e.g., porous materials inoculated with parenchymal cells or porous materials implanted into bone). As previously stated, the tissue response to implants is in part dependent upon the extent of injury or defect created in the implantation procedure.

Repair of implant sites can involve two distinct processes: regeneration, which is the replacement of injured tissue by parenchymal cells of the same type, or replacement by connective tissue that constitutes the fibrous capsule. These processes are generally controlled by either (1) the proliferative capacity of the cells in the tissue or organ receiving the implant and the extent of injury as it relates to the destruction, or (2) persistence of the tissue framework of the implant site.

The regenerative capacity of cells allows them to be classified into three groups: labile, stable (or expanding), and permanent (or static) cells. Labile cells continue to proliferate throughout life; stable cells retain this capacity but do not normally replicate; and permanent cells cannot reproduce themselves after birth. Perfect repair with restitution of normal structure can theoretically only occur in tissues composed of permanent cells and may give rise to fibrosis and fibrous capsule formation with very little restitution of the normal tissue or organ structure. Tissues composed of permanent cells (e.g., nerve cells, skeletal muscle cells, and cardiac muscle cells) most commonly undergo an organization of the inflammatory exudates, leading to fibrosis. Tissues composed of the stable cells (e.g., parenchymal cells of the liver, kidney, and pancreas); mesenchymal cells (e.g., fibroblasts); and vascular endothelial and labile cells (e.g., epithelial cells and lymphoid and hematopoietic cells) may also follow this pathway to fibrosis or may undergo resolution of the inflammatory exudates, leading to restitution of the normal tissue structure.

The condition of the underlying framework or supporting stroma of the parenchymal cells following an injury plays an important role in the restoration of normal tissue structure. Retention of the framework may lead to restitution of the normal tissue structure while destruction of the framework most commonly leads to fibrosis. It is important to consider the species-dependent nature of the regenerative capacity of cells. For example, cells from the same organ or tissue but from different species may exhibit different regenerative capacities and/or connective tissue repair.

Following injury, cells may undergo adaptations of growth and differentiation. Important cellular adaptations are atrophy (decrease in cell size or function), hypertrophy (increase in cell size), hyperplasia (increase in cell number), and metaplasia (change in cell type). Other adaptations include a change by cells from producing one family of proteins to another (phenotypic change), or marked overproduction of protein. This may be the case in cells producing various types of collagens and extracellular matrix proteins in chronic inflammation and fibrosis. Causes of atrophy may include decreased workload (e.g., stress-shielding by implants) and diminished blood supply and inadequate nutrition (e.g., fibrous capsules surrounding implants).

Local and systematic factors may play a role in the wound healing response to biomaterials or implants. Local factors include the site (tissue or organ) of implantation, the adequacy of blood supply, and the potential for infection. Systematic factors may include nutrition, hematologic derangements, glucocortical steroids, and preexisting disease such as atherosclerosis, diabetes, and infection.

Finally, the implantation of biomaterials or medical devices may be best viewed at present from the perspective that the implant provides an impediment

of hindrance to appropriate tissue or organ regeneration and healing. This reflects our current inability to control the sequence of events following injury in the implantation procedure, and restitution of normal tissue structures with function is rare. Current research is directed toward developing a better understanding of the modification of the inflammatory response, the stimuli providing for appropriate proliferation of permanent and stable cells, and the appropriate application of growth factors. This may provide keys to the control of inflammation, wound healing, and fibrous manipulation of biomaterials or implanted devices. For example, one area of recent research is understanding the functions of extracellular matrix (ECM) proteins as modulators of cell–matrix interactions. ECM proteins include thrombospondin (TSP)-1, TSP-2, SPARC, tenascin (TN)-C, and osteopontin, and they have been shown to participate in a number of processes related to tissue repair. Specifically, studies in knockout mice have indicated that a deficiency in one or more of these proteins can alter the course of wound healing, and TSP-1, TSP-2, and SPARC have also been implicated in the foreign body response.

See also: Biocompatibility; Implant Studies.

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Forensic Toxicology

Felix K Adatsi

Introduction

Forensic toxicology is the branch of science that applies the principles and knowledge of toxicology to issues and problems in the field of law. To achieve this, techniques of analytical chemistry are combined with principles of toxicology to address issues related to the toxic effects of substances on humans that are germane to judicial proceedings. Analytical chemistry deals with the techniques and methods for determining the identity and relative amounts of unknown components in a sample of matter. Toxicology has been defined as the study of poisons. A frequently cited definition of a poison is one provided by the physician/alchemist, Paracelsus (1493–1541). He noted that "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy." For this reason, forensic toxicology involves the use of proper chemical or analytical techniques to identify and characterize any unknown substances in biological systems and examine the adverse effects of these substances on humans. Today, the practice of forensic toxicology encompasses three major subdivisions: postmortem toxicology, forensic drug testing, and human performance toxicology. Of these three subdivisions, postmortem toxicology probably bears the closest relationship to the historical perception of forensic toxicology; one that is replete with vivid imagery of intricate homicidal poisonings. In spite of this expanded application of forensic toxicology, the basic responsibility of the forensic toxicologists still remains one of assisting the judicial system in deciding whether a particular substance could have a clinical or toxicological impact on the outcome of a legal matter. To this end, the forensic toxicologist must first establish the presence and exact identity of that chemical substance (prescription or illicit drug or poison) in an individual and establish a relationship between exposure to that chemical and the occurrence of an injurious effect or death.

Forensic drug testing arose primarily as a method of detecting drug use among individuals and as a way to curb or deter contemplated or further use. At the present time, drug use is a major medical and socioeconomic problem worldwide. In the United States, large amounts of financial and human resources are expended to combat the problem. This subdivision of forensic toxicology has wide application in areas such as workplace testing, testing of athletes, compliance with drug-related probation, and screening of new job applicants. Human performance toxicology is

concerned with the relationship between the presence of a drug in an individual and changes in behavior or performance on assigned tasks. This subdivision of forensic toxicology forms the scientific cornerstone upon which law enforcement agencies build on to enforce laws dealing with driving under the influence (DUI) of drugs. Frequently, the forensic toxicologist is called upon to analyze biological specimens from individuals suspected of drug use and to connect the results to complex human activities such as driving. For some drugs, notably alcohol, pharmacological data exist on the principal target organs affected and the generally accepted performance impairing effects in humans. The forensic toxicologist can apply some of these generally accepted effects, vis-à-vis the analytical results obtained, to assist law enforcement agencies when human performance toxicology issues arise.

Poisoning is usually an act contrived of evil intentions. The poisoner seeking the death of another individual is often very discreet about the manner in which a poison is introduced into the body of a victim. Great precaution is often taken to conceal the steps or events leading to the completion of this activity. Therefore, establishing that the cause of death of an individual is due to poisoning, whether by accident, suicide, or homicide, is a difficult task requiring the application of a vast amount of knowledge. For the forensic toxicologist, this may involve knowledge of a number of factors and reliance on the expertise of other professionals. The forensic toxicologist must have an inquisitive mind, be familiar with a wide variety of chemicals and poisons, and be conversant with current knowledge regarding the 'drug culture', including the flow, distribution, and patterns of use of illicit drugs. In the normal course of their duties, forensic toxicologists work closely with other professionals. For example, when called upon to assist in establishing the cause of a sudden death, the forensic toxicologist would probably work with a medical examiner, a team of scientists, nurses, police officers, and other law enforcement personnel. The knowledge and information provided by each of these professionals can assist, either collectively or individually, the forensic toxicologist in resolving the mysteries surrounding the death.

Collection of Forensic Specimens

A typical forensic toxicological investigation to determine a cause of death or to determine the presence or absence of a drug or poison in an individual begins necessarily with the collection of an appropriate biological specimen. The biological specimens usually collected from living individuals are peripheral blood and urine. Blood is of particular usefulness to forensic toxicologists because the presence of a drug or poison in blood indicates that absorption has taken place. Additionally, good correlations exist between the blood levels of most drugs and poisons and their pharmacological and/or behavioral effects on humans. Urine drug levels, on the contrary, only indicate that a subject had past exposure to the drug in question without specifying the exact time of exposure or possible physiological effects. Nevertheless, the acquisition of a urine sample from an individual provides a noninvasive method of gaining valuable information regarding the presence or absence of a drug in the body of an individual. Additional advantages in the use of urine as a toxicological specimen include ease of testing, the presence of high concentrations of parent drug and/or metabolites and the relatively low cost of testing. In the United States, for example, the mandatory guidelines for workplace drug testing require the use of urine as the specimen of choice.

Occasionally, gastric contents, saliva, and hair samples may also be collected from living individuals. In one case, an arresting police officer saw a man suspected of possessing and distributing a white powder believed to be cocaine. However, before an arrest could be made the suspect stuffed the white powder and its plastic wrapper into his mouth and swallowed the crucial evidence. Upon finally arresting the man, part of the forensic investigation involved toxicological analysis of available body fluids. In this instance, acquisition of gastric or stomach contents from the suspect is indicated and despite the risks involved, a gastric lavage must be performed. The forensic toxicologist should request and analyze the gastric contents from this living individual as part of the battery of tests to be performed. By analyzing a sample of the stomach contents the toxicologist may be able to answer questions about the chemical identity of the white powder. Finally, a biological specimen that has received prominence in certain parts of the world in forensic toxicology testing is human breath. It is sampled to determine the bodily alcohol content of an individual and the result is compared with the legal definition of intoxication in driving-related offences. It may also be sampled for the presence or absence of inhalants, most of which are volatile organic solvents that are not easily detected in blood.

For deceased subjects, the specimens should be collected before embalming the body. Embalming may cause a dilution, destruction, or false indication of the presence of a drug or chemical in the decedent's body. The specimens collected from a decedent will include samples from a number of body fluids and organs since drugs and other chemicals distribute themselves in body fluids and organs with varying

affinities. Whenever possible, a portion of the liver and whole kidneys should be obtained from deceased persons suspected of dying from a drug overdose or chemical toxicity. The liver is the organ primarily responsible for the detoxification of foreign substances such as drugs and chemicals in the body and tends to sequester most of them in high concentrations. The kidneys are the major organs responsible for the excretion of most drugs and poisons, particularly the heavy metals, and are also expected to contain high concentrations of these poisons.

Additionally, depending on the case history presented, the forensic toxicologist should request and analyze samples from specific tissues and organs collected from a decedent. For instance, the eye fluid or vitreous humor may be the preferred specimen to be analyzed for the presence of alcohol in the driver of a fatal accident whose blood or other bodily fluids become contaminated by stomach alcohol as a result of injuries received. The vitreous humor is preferred because it is anatomically well isolated from the stomach and is well protected from microbiological degradation. Certain toxicants, such as the organometallic compounds (methylmercury and trimethyltin), and drugs, such as the anticancer drug doxorubicin, have great affinities for the nervous system, and sampling of brain tissue may be indicated if these toxicants are suspected in a death investigation. For example, if called upon to investigate the cause of a sudden death of a cancer patient being treated with doxorubicin, the toxicologist should also analyze a sample of the brain tissue for the presence and concentration of the drug, in addition to analyzing the usual biological specimens, since the drug is selectively toxic to brain cells and tissues. If doxorubicin is found to be present in high concentrations in the brain, the forensic toxicologist may be able to ascribe the cause of death to doxorubicin toxicity rather than to the effects of cancer, with a high degree of certainty.

The specimens should be collected by qualified personnel and each container into which a specimen is placed must bear a label with the name of the subject, the type of specimen in the container, the date and time of the collection of the specimens, and the signature of the person collecting the specimen. Forms and labels are usually developed to facilitate inventory of the specimens collected and to document the activities at the collection site. Frequently, a police officer is at the scene of the collection of the specimen and that officer should also append his or her signature to the labels and form. The specimens collected should be properly packaged with the proper documentation and case history if available and transferred to a forensic laboratory for analysis. From a legal perspective, the specimens are part of the evidence that can be introduced in legal proceedings, as is any specimen analysis performed by a forensic toxicologist. For this reason, the processes involved in the transfer of the specimens from the collection site to the forensic laboratory must be carefully documented to establish a 'chain of custody'. The chain of custody ensures that only authorized personnel handle the specimens and thereby ensure their integrity.

Analysis of Forensic Specimens

Once in the laboratory, the types of toxicological analyses to be performed on the specimens will depend on several factors. In fact, the types of drugs or poisons to which any population of people are exposed will vary with the prevailing social, political, economic, and religious climate. Sometimes a specimen may arrive in a toxicology laboratory with a request for the analysis of a specific type of drug or poison. Other times, however, the type of analysis to be performed will be determined largely by the case history or other factors associated with the specimen. For example, the analysis performed on biological specimens taken from the driver of a vehicle involved in a traffic accident may involve first and foremost the determination of the presence or absence of alcohol and/or other commonly abused drugs such as marijuana or cocaine, primarily because of the overwhelming involvement of these drugs in traffic fatalities. However, the type of analysis to be performed on a decomposed body will involve searching for drugs and poisons other than alcohol. During decomposition, certain drugs initially present at death may be destroyed and others produced either by virtue of bacterial activity or by changes in the ambient environment.

To the untrained individual, determining both the presence and amount of an unknown drug or poison in an individual is a daunting task. However, systematic and well-standardized methods aimed at detecting the largest possible number of commonly encountered toxic substances have been developed over the years to assist the forensic toxicologist. Generally these methods have focused on the type of biological matrix being analyzed and the chemical class to which a drug or poison belongs. Thus, the method used in analyzing for a poison such as arsenic in hair will be different from that used in analyzing for alcohol in blood.

The type of procedure or instrument used for the detection of a particular drug or analyte will depend on the type of analyte or drug sought. Usually, however, the first line of tests performed includes a

protocol of immunoassay screening tests designed to determine the presence or absence of a class or group of drugs. If a positive result is obtained with these tests, a second test using a different procedure based on physicochemical principles different from the first is performed to identify and confirm the particular drug. Some of the instruments that are currently used for the unequivocal identity of most drugs or chemicals are gas chromatography/mass spectrometry, atomic absorption spectrometry, and high-performance liquid chromatography.

A classification scheme that is commonly employed involves placing poisons in the following groups: corrosive agents, gases and volatile agents, metallic poisons, nonvolatile organic agents, and miscellaneous poisons. The corrosive agents include mineral acids and bases. This group of poisons also includes a number of household products formulated with caustic compounds. These poisons can be analyzed using basic chemical and clinical techniques which take advantage of physical properties such as solubility, acidity, or basicity, and observable color changes of the poisons. Gaseous and volatile poisons include several compounds such as acetone, acetaldehyde, carbon monoxide, cyanide, ethanol, methanol, and several other organic solvents. This class of poisons can generally be determined using gas–liquid chromatography techniques. Metallic poisons include arsenic, mercury, lead, and other heavy metals. The method of choice in analyzing for metallic poisons is atomic absorption spectrometry. The nonvolatile organic group contains by far the largest number of prescription and illicit drugs. Drugs such as the antipsychotic agents, antidepressants, amphetamines, central nervous system (CNS) stimulants, and hallucinogens belong to this group. Extraction techniques which take advantage of the acidic, basic, neutral, or amphoteric nature of these drugs are combined with appropriate instrumental methods to analyze for these compounds. Miscellaneous poisons will include agents such as plant and animal toxins and any other chemical substance whose detection from biological specimens will involve the application of some or all of the techniques described, including immunoassay techniques.

Regardless of the type of toxicological analysis eventually performed on a biological specimen, it is essential to follow specific and well-established scientific and good laboratory procedures. Needless to say, the quality of the analytical result is only as good as the quality of the overall process governing the analysis. A clean laboratory environment should be maintained and only chemicals of the highest grade and purity should be used for analysis. Instruments and equipment should be properly calibrated and maintained in proper working condition. Following the analysis, a written report detailing the outcome of the test must be prepared and submitted to the agency or party requesting the analysis. The results should be presented and interpreted in accordance with the definitions and framework established by the legal system of the particular country or state. Usually, this report will conclude any further involvement of the toxicologist in the issues surrounding the specimens or case. For example, a test result of '0.08 g of ethanol per 100 ml of blood' that is reported in a case of a motorist suspected of operating his vehicle under the influence of alcohol may be all that is needed by the arresting agency to sustain a charge of 'operating under the influence of alcohol' against the motorist. This is because some jurisdictions adopt *per se* limits for alcohol in blood and an individual is presumed to be DUI at or above this level.

Interpreting Specimen Analysis

However, instances arise when the forensic toxicologist will have to provide detailed interpretation of the result of the analysis. The relevance and importance of the toxicological analysis to the overall forensic investigation resides in the correct interpretation of the test results. To this end, the forensic toxicologist must bring his or her knowledge of human anatomy, biochemistry, pathology, pharmacology, physiology, general toxicology, and concepts in other basic sciences to bear on the test results. Generally, the objectives of the forensic toxicologists are to answer the following questions. Are drugs or poisons present in the subject? When did exposure to the drug occur? How much did the subject take? Is the drug responsible for a specific type of behavior or prompt a particular type of behavior? In fatal cases, was the drug the cause of death?

Ethanol, the active ingredient in most alcoholic beverages, is perhaps the most widely studied drug in terms of its effects, disposition and fate in humans. It is so common in many societies that it is usually considered a social beverage and its classification as a drug comes as a surprise to some when it is implicated in legal issues. For this reason, forensic alcohol analysis is the most frequently performed analysis in many forensic laboratories. Similarly, it is often in the support of a blood or breath alcohol analysis that the forensic toxicologist comes face to face with the legal community. In the majority of cases involving alcohol and driving, the judiciary is concerned with establishing the following facts: (1) Whether the driver's alcohol result exceeded an established statutory alcohol level of intoxication. (2) Whether the

measured alcohol level may have been responsible for the bad driving or accident. (3) Whether at the time of the incident the alcohol level may have been higher or lower than that legally established.

The disposition, fate, and intoxicating effects of alcohol have been extensively studied and well documented. A large body of scientific evidence exists on the correlation between the effects of alcohol on humans and the blood alcohol levels. Alcohol is a CNS depressant and is expected to adversely influence the correct execution of tasks, such as driving an automobile, requiring the proper functioning of the CNS when it is present. The forensic toxicologist may rely on these and other data and factors to provide an interpretation of the results presented; however, bearing in mind that different individuals may respond differently to different levels of alcohol. In some instances, the scientific expectation of the outcome may be different from that which is actually observed. In order to establish whether the alcohol level of a driver may be higher or lower at the time of driving than that measured when an arrest is made, the forensic toxicologist may rely on concepts of retrograde extrapolation. Retrograde extrapolation, or relating back, requires the use of basic principles of the pharmacokinetics of alcohol in humans to arrive at an estimate of the alcohol level of an individual at a time in the past, when knowledge of the alcohol level at the current time exists. The acceptability of this type of calculation has been legally challenged because of different scientific opinions on the subject. However, the forensic toxicologist, using proper assumptions, can still provide an estimated value of the alcohol level and use of the result then goes to the weight of the evidence presented.

The interpretation of postmortem toxicology results probably constitutes the greatest challenge to the forensic toxicologist. This is because of the many factors that affect drugs in postmortem cases. For example, many drugs are unstable *in vivo* and *in vitro* and a search for a particular drug during an investigation of a drug-related fatality using information from the case history presented may be futile. An important factor affecting the interpretation of postmortem drug concentration is the phenomenon of postmortem redistribution. Postmortem redistribution is a complex phenomenon that is believed to account for the observation that blood concentrations of a drug may be higher at autopsy than those immediately after death. For this and other reasons, the forensic toxicologist attempting to interpret postmortem toxicology results should do so after gaining a thorough understanding of every available aspect of the circumstances surrounding the case. Mere reliance on tables of therapeutic, toxic, and lethal concentrations of a drug may result in misinterpretation.

Another complicating factor in the interpretation of postmortem toxicology results is the phenomenon known as postmortem production. This phenomenon is most applicable to blood alcohol levels after a fatality has occurred. Postmortem production can account for measurable blood ethanol levels after a fatality that may have no connection to prior exposure to alcohol. Postmortem ethanol production can result from a number of sources that include the existence of large numbers of appropriate microorganisms in improperly preserved bodies, or from bodies that suffered severe trauma at death. In any case, the forensic toxicologist attempting to offer an interpretation of these results should carefully consider these facts.

Should the presence of the metabolite or biotransformation product of a drug be detected in the body of an accused individual, the forensic toxicologist will have to rely on several factors such as age, weight, gender, and health status of the accused as well as relevant concepts in toxicology to aid in the interpretation of that result. A 45-year-old female was charged with operating her vehicle under the influence of drugs, causing the death of another individual. The accused reportedly failed to obey a traffic signal and drove her vehicle through a red light into an oncoming vehicle, killing its occupant. She fled the scene of the accident but was later apprehended. In her defense, she stated that she was being treated by her physician for depression and had consumed her medication after the accident but prior to her arrest. Toxicological analysis showed the presence of the prescribed drug in addition to two major metabolites of the parent drug. In this case, simply sending out a toxicology report without interpretation or a summary of what the results mean is unlikely to assist the court in adjudicating the matter fairly. Because there is an admission by the defendant in this case to the consumption of the drug, it is in answering the question of whether the presence of the drug may have been responsible for the defendant's behavior at the time of operating her vehicle that the toxicologist's expert knowledge can assist the court in an impartial ruling on this case. If the prescribed medication is a short- or long-acting drug, the toxicologist may be able to use information on the relative half-life (time required to break down half of the original dose) of the medication and amount of metabolite detected upon analysis to ascertain the approximate time of drug intake. The toxicologist should be ready to provide this type of information to assist in the resolution of the matter.

The Forensic Toxicologist as Witness

Because he or she may be called as a witness, the forensic toxicologist must be aware of the constraints and demands imposed by the judicial system and ensure that the techniques and procedures used in the laboratory are based on a firm, well-established, and generally accepted scientific foundation as well as satisfying the criteria of admissibility established by the courts. Historically, in the United States, most courts deferred to the landmark ruling of Frye versus United States of America in 1923 as a criterion for judging whether a scientific principle or method is 'generally accepted' by those expected to be familiar with its use. Recently, the 'Frye test' has undergone a change in US federal courts in order to allow the introduction of valid scientific data or information gathered from rapidly advancing scientific techniques or novel tests into evidence. In 1993, the US Supreme Court held that the general acceptance test was too restrictive and incompatible with modern rules of evidence in the case involving Daubert v. Merrell Dow Pharmaceuticals, Inc. The Daubert ruling was later expanded to include expert testimonies from engineers, scientists, and other experts who are not scientists. Although the new rules were initially applied in federal courts, some state courts have adopted the general requirements of the Daubert ruling and expect expert witnesses to satisfy the vital elements of Daubert.

A forensic toxicologist may be subpoenaed as a witness to offer two distinct types of testimony pertaining to the results of an analysis. First, he or she may testify only to the results of the analysis. This type of testimony is known as objective testimony. Objective testimony usually involves furnishing the court with information such as the identification and description of the specimen analyzed, the manner in which the specimen was received in the laboratory, the location of the laboratory, a description of the methods used for analysis, and education and training which qualify the toxicologist to perform the tests used. The second type of testimony offered by the toxicologist is known as expert testimony. For this type of testimony, the toxicologist is presented as an expert witness who can offer interpretive opinions on his or her own results as well as those obtained by other scientists. To be accepted by the court as an expert witness, the forensic toxicologist must be qualified, usually in the presence of a jury. As an expert witness, the forensic toxicologist should be very well prepared in his or her area of expertise and be aware that every trip to court is an engagement in a potentially hostile arena. Opposing counsels will seek to present differing points of view on the same subject and attempt to reach different conclusions through their own experts. All conclusions must be based on sound scientific knowledge and the information presented to the court with impartiality, integrity, and honesty. It is only by providing the court with scientific knowledge in this manner that the forensic toxicologist truly functions in his or her role as one who applies the principles and knowledge of toxicology to the resolution of problems in the field of law.

Summary

The role of the forensic toxicologist continues to be pivotal to society, particularly when it comes to the administration of justice. Because many drugs, chemicals, or poisons do not always produce characteristic and clinically observable tissue or organ damage to the medical examiner, the contribution by a forensic toxicologist is invaluable if a cause of death is probably due to a drug or poison. The findings from a forensic toxicological analysis can be combined with those from a medical examiner or forensic pathologist to establish the cause or causes of death and the information used in a judicial proceeding. It is a truism that the administration of justice has become a multidisciplinary mosaic of law, science, and modern technology. In this regard, because of its long tradition of evolving according to new legislation and advances in science, toxicology as a whole and forensic toxicology in particular, will continue to offer exceptional value to the truth-seeking goal of the judicial process. As long as society strives to ensure that justice is properly carried out for all and sundry, reliance on the activities of the forensic toxicologist in cases involving human exposure to chemicals and their possible role in causing injury or death will be expected to continue. A society in which the unfortunate reliance on drugs (prescription and illicit) has become a way of life for some is bound to have its share of sudden unexplained deaths, traffic accidents, and other serious outcomes of drug exposure and toxicity. The forensic toxicologist will continue to contribute to the overall knowledge gained about drugs as society continues to grapple with the identity and toxicity of new drugs, particularly 'designer drugs' and their analogs.

See also: Analytical Toxicology; Law and Toxicology; Toxicology, Education and Careers; Toxic Torts.

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Formaldehyde

Kathryn J Kehoe

- Chemical Abstracts Service Registry Number: CAS 50-00-0
- Synonyms: Formalin; Formic aldehyde; Formal; Methaldehyde; Methanal; Methyl aldehyde; Methylene oxide; Oxomethane; Oxymethylene
- Chemical/Pharmaceutical/Other Class: Aldehyde
- Chemical Formula: CH_2O
- Chemical Structure:

Uses

Formaldehyde is compound that is ubiquitous in the environment. It is a gaseous contaminant of emissions from power plants, manufacturing sites, and automobiles. It is also present in cigarette smoke, produced in wood fires and in photochemical smog. It is a normal metabolic intermediate found in all cells. It has several commercial, industrial, and medical uses. It is utilized in the production of cosmetics, paint pigments, and in the processes responsible for generating wrinkle-free, crease-resistant fabrics. It was used as a component of urea–formaldehyde insulating foam. In medical specialties, it may be used as a disinfectant, antiseptic, deodorant, and a tissue fixative. It is also an embalming agent.

Exposure Routes and Pathways

Occupational and residential exposures to formaldehyde are not uncommon. Due to a very high vapor pressure formaldehyde readily vaporizes and is a gas at room temperature. The most likely route of exposure is by inhalation. Exposure may also occur by ingestion or dermal absorption; however, permeability is low through the skin.

Toxicokinetics

Cells in the lungs rapidly and nearly completely absorb inhaled formaldehyde. At the site of contact it will quickly undergo conversion to formate. The resulting formate is either oxidized to carbon dioxide and exhaled, or excreted as formic acid in the urine. Higher doses may overwhelm the metabolic capabilities of the site of exposure and will result in formaldehyde remaining unchanged. In humans exposed to 1.9 ppm formaldehyde, blood levels of the compound remained stable for 40 min. Under these conditions it may then undergo reactions characteristic to the aldehyde carbonyl group. In addition to oxidation to acid (formic acid), it may be reduced to alcohol (methanol) or undergo conjugation with glutathione (*S*-acyl glutathione). The conjugation product forms rapidly and is the direct substrate for oxidation reactions that may follow. Unmetabolized formaldehyde may enter the one-carbon pool and subsequently be incorporated into purines, thymidines, and amino acids.

Mechanism of Toxicity

At high exposure levels the carbonyl group of formaldehyde can react with nucleophilic sites on amino acids and DNA. At the site of contact, the primary metabolic products will contribute to the toxicity of formaldehyde. The formation of formic acid generally will cause an acidosis, corrosion of the

gastrointestinal tract, and other systematic effects. Formaldehyde also serves as an allergen due to its ability to combine with protein in the epidermis. This combination results in a hapten–protein complex that sensitizes T lymphocytes. Exposure can result in sensitization and contact dermatitis upon subsequent exposures.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the oral LD_{50} ranges from 0.2 to 0.8 g kg^{-1}, the subcutaneous LD_{50} is 0.42 g kg^{-1}, and the inhalation LC_{50} is 250 ppm per 4 h and 815 ppm per 0.5 h.

Human

The toxicity of formaldehyde is related to its metabolic products and, as a result, individual variability in metabolism will determine toxic outcomes. Human ingestion of 118 ml of formaldehyde was fatal in some cases but not others. Systemic acidosis may appear upon ingestion along with corrosion and hemorrhaging of the digestive tract. Allergic sensitization may occur after exposure. This may lead to contact dermatitis after subsequent skin exposure, as well as asthmatic attack upon inhalation exposure. Inhalation may also result in irritation of the respiratory tract and pulmonary edema.

Chronic Toxicity (or Exposure)

Animal

Formaldehyde is an animal carcinogen and a mutagen. High levels (14 ppm) have been associated with nasal cancers in rats and mice.

Human

In humans, long-term, high exposures to formaldehyde are linked to lung cancer.

In Vitro Toxicity Data

Formaldehyde has been shown to significantly inhibit the viability and proliferation of mouse lymphocytes. IC_{50} values in a 3 h exposure ranged from 1.19×10^{-5} to 8.20×10^{-4} mol l^{-1}. At 1–3 mmol l^{-} formaldehyde concentrations, dissociated rat thymocytes showed a dose-dependent decrease in cell viability. This may have been associated with an observed reduced cellular content of glutathione or an increased concentration of cellular calcium ion.

Clinical Management

There is no specific antidote for formaldehyde exposure. Contact with skin should be followed by a soap and water wash for a minimum of 15 min. For inhalation exposure, the victim should be moved to fresh air and, if not breathing, given artificial respiration. If breathing difficulties are apparent, oxygen may be administered. After ingestion, decontamination with milk or water should be followed with a bolus of charcoal (1 g kg^{-1}) and a mild saline cathartic. Dialysis may be started if severe acidosis or deteriorating vital signs are apparent. Electrolytes and blood methanol levels should be monitored.

Environmental Fate

Atmospheric formaldehyde is rapidly degraded by photolysis and photooxidation. It will undergo significant biodegradation in the soil or in water and shows no evidence of bioconcentration in several types of fish and shrimp.

Ecotoxicology

Formaldehyde is highly toxic to algae, protozoan, and other unicellular organisms. Fish show slight toxicity (guppies have TLm = 50–200 mg l^{-1}).

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 0.75 ppm. The short-term exposure limit is 2 ppm.

See also: Pollution, Air Indoor; Respiratory Tract; Sensory Organs; Skin.

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Formamide

Gerald L Kennedy

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- Chemical Abstracts Service Registry Number: CAS 75-12-7
- Synonyms: Methanamide; Carbamaldehyde; Formimidic acid
- Chemical/Pharmaceutical/Other Class: Aliphatic amide
- Chemical Formula: $HCONH_2$

Uses

Formamide is a good solvent for proteins and salts owing to its high dielectric constant. Its main applications are as a solvent in the chemical industry, as a softener for paper, as an intermediate for the manufacturing of formic acid and esters and hydrocyanic acid, and as a reaction medium.

Exposure Routes and Pathways

Occupational exposure to formamide may occur through inhalation and dermal contact with this compound at workplaces where formamide is produced or used. Formamide, a physiological product of *N*,*N*-dimethylformamide, was detected in the urine of synthetic leather factory workers. Formamide may be inhaled, swallowed, or absorbed through the skin. The chemical is moderately irritating to the skin and can produce from mild to severe irritation to the eye. In its usual application, inhalation is the most common route of exposure; although dermal contact is always possible.

Toxicokinetics

Formamide is reported to be a minor metabolite from demethylation of the solvent dimethyl formamide. The molecule is relatively difficult to metabolize with the amide group hydrolyzed to a slight extent by liver extracts at pH 7.4. The dog, cat, and rat excrete a large proportion of an oral dose of formamide unchanged in the urine.

Mechanism of Toxicity

The mechanism of toxicity of formamide is not known; the response profile is quite different from the better studied dimethyl derivative.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in rodents ranges from 3.2 to 6 g kg^{-1} with intravenous LD_{50} in rodents ranging from 5.1 in mice to 5.6 in rats. The dermal lethal dose in rabbits is 6 g kg^{-1} and the inhalation lethal concentration for rats exposed for 6 h is 1500 ppm. Fetal malformations and fetotoxicity were induced when laboratory animals were treated by the oral, dermal, and injection routes during pregnancy.

Human

No reports could be found in the literature concerning the potential acute human health effects of formamide.

Chronic Toxicity (or Exposure)

Animal

Repeated oral administration to rats caused tissue changes at a number of sites including the gastrointestinal tract, spleen, testes, and blood. Multiple dermal or inhalation exposures induced blood effects, changes in organ weights, and testes damage in rats. Chromosome damage was reported in rats treated with formamide.

Human

No reports could be found in the literature concerning the potential human health effects of chronic exposure to formamide.

In Vitro Toxicity Data

No evidence of mutagenicity was seen in Ames bacterial tests.

Clinical Management

Exposed persons should be removed to fresh air and get medical attention as needed for any breathing difficulty. If swallowed, several glasses of water should be given to dilute the chemical and again medical attention is needed if large amounts are ingested. Formamide is moderately irritating to skin and mucous membranes. For skin contact, the exposed area should be washed with soap and water, and medical attention should be sought if irritation develops. For eye contact, the eyes should be flushed

with water for at least 15 min by lifting the lower and upper eyelids occasionally and immediate medical attention should be obtained.

Environmental Fate

If released to air, formamide will exist solely as a vapor in the ambient atmosphere. Vapor-phase formamide will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals. The half-life for this reaction in air is estimated to be 8.0 days. If released to soil, formamide is expected to have very high mobility. Volatilization from moist soil surfaces is not expected to be an important fate process. If released into water, formamide is not expected to adsorb to suspended solids and sediment. Several biodegradation screening studies have observed significant biodegradation of formamide, which suggests that biodegradation may be important. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's law constant. An estimated bioconcentration factor of 3 suggests that the potential for bioconcentration in aquatic organisms is low. Hydrolysis is expected to be slow.

Exposure Standards and Guidelines

Occupational Safety and Health Administration (OSHA) standards: Vacated 1989 OSHA permissible exposure limit time-weighted average (TWA) 20 ppm ($30\,mg\,m^{-3}$); short-term exposure limit 30 ppm ($45\,mg\,m^{-3}$) is still enforced in some states. American Conference of Governmental Industrial Hygienists threshold limit values (TLVs): 8 h TWA 10 ppm, skin. Excursions in worker exposure levels may exceed three times the TLV-TWA for no more than a total of 30 min during a work day, and under no circumstances should they exceed five times the TLV-TWA, provided that the TLV-TWA is not exceeded.

Atmospheric Standards

There is a standard of performance for equipment leaks of formamide and other volatile organic compounds (VOCs) in the Synthetic Organic Chemical Manufacturing Industry (SOCMI). The intended effect of these standards is to require all newly constructed, modified, and reconstructed SOCMI process units to use the best demonstrated system of continuous emission reduction for equipment leaks of VOCs, considering costs, nonair quality health and environmental impact and energy requirements.

Food and Drug Administration Classification

Formamide is an indirect food additive for use only as a component of adhesives.

Further Reading

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Thiersch JB (1962) Effects of acetamides and formamides on the rat litter *in vitro*. *Journal of Reproduction and Fertility* 4: 219.

Warheit DB, Kinney LA, Carakostas MC, and Ross PE (1989) Inhalation toxicity study of formamide in rats. *Fundamental and Applied Toxicology* 13: 702.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Formamide.

Formic Acid

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 64-18-6
- Synonyms: Aminic acid; Formylic acid; Methanoic acid; Myrmicyl
- Chemical/Pharmaceutical/Other Class: Organic acid
- Chemical Formula: CH_2O_2

Uses

Formic acid is used as an additive to silage to improve its nutritional value. It is also used as an animal feed additive, food preservative, and in flavor enhancer formulations. In the manufacturing industry it is used as an acidulating agent in dyeing and finishing textiles, in leather tanning, wool dyeing, preparation of organic esters, pesticide manufacturing, electroplating, as an antiseptic in wine and beer brewing, and as a coagulating agent for rubber latex. In nature, formic acid is produced by bees, wasps, and ants.

Exposure Routes and Pathways

In industry, exposure to formic acid can occur through the oral, dermal, and inhalation routes. Formic acid can also be produced in the mouth and stomach from ingested formaldehyde. Formic acid can also be produced in the liver and other organs from the metabolism of methanol and formaldehyde. Stings by bees, wasps, and ants may result in the subcutaneous injection of formic acid.

Toxicokinetics

Formic acid can be readily absorbed from the digestive tract and the respiratory system. Systemic absorption produces acidosis, neuropathy, and visual and mental disturbances. Acidosis can also be produced when formic acid is produced by liver aldehyde dehydrogenase from formaldehyde. Formaldehyde in turn can also be produced metabolically by alcohol dehydrogenase from methanol. Formic acid is oxidized to carbon dioxide by the folate-dependent pathway. Some formic acid is excreted unchanged in the urine.

Mechanism of Toxicity

Exposure to formic acid may produce irritation and acid burns at the site of contact. Oral exposure may produce salivation, vomiting (may contain blood), diarrhea, gastritis, and pain. Dermal contact produces dermal irritation, dermatitis, and ulceration of membranes. Accidental splashes in the eyes may result in irritation, lacrimation, and pain. Inhalation of vapors, mists, or aerosols may result in increased nasal discharge, cough, throat discomfort, and pulmonary edema. Systemic absorption of large doses of formic acid may result in damage to the liver, kidneys, and eyes. Acute ingestion of high doses may result in shock, breathing difficulties, circulatory collapse, and death.

Acute and Short-Term Toxicity (or Exposure)

Animal

Formic acid is slightly toxic by the inhalation route. The LC_{50} for the rat and mouse has been estimated to be $15\,g\,m^{-3}$ per 15 min and $6.2\,g\,m^{-3}$ per 15 min, respectively. Rats consuming a diet containing 0.5–1.0% formic acid for 6 weeks experienced a reduced organ and total body weight compared with controls. The same response was noted when rats were given formic acid in their drinking water at a concentration of 0.5–1.0%. The oral LD_{50} for mice and rats has been reported to be 1076 and $1830\,mg\,kg^{-1}$, respectively.

Human

The main target organs for formic acid poisonings are respiratory and gastrointestinal systems as well as the skin, eyes, liver, and kidneys. Direct contact with formic acid may result in severe tissue damage (burns), ulceration, and permanent scarring. Systemic absorption can result in severe acidosis. Signs and symptoms of overexposure include eye irritation, lacrimation, throat irritation, coughing, severe osmolar gap, hypotension, renal failure, apnea, ocular damage, circulatory collapse, and death.

Chronic Toxicity (or Exposure)

Human

It has been reported that chronic intake may result in albuminuria and hematuria.

Clinical Management

If ingested, the formic acid should be diluted with milk or water in alert patients. Careful gastric aspiration with a nasogastric tube may be attempted to limit systemic absorption. The goal of the clinical management is to correct the acidosis. Acidosis may be treated with sodium bicarbonate or by hemodialysis. Immediate hemodialysis may remove formic acid from systemic circulation. Acid–base balance, electrolytes, and kidney function should be monitored closely.

Environmental Fate

Formic acid is found in nature as it is produced by plants, insects, and bacteria. However, it is also used in industry for the manufacture of numerous consumer products. Therefore, the chemical may be released to the environment as a waste product or from unintentional, accidental releases. If released to soil it is expected to biodegrade and has a short half-life. If released to water, it is expected to biodegrade and hence not likely to bioaccumulate in aquatic organisms. If released to air, it is expected to react with hydroxyl radicals contained in water vapor.

Exposure Standards and Guidelines

Food

Formic acid is a food additive permitted for direct addition to food for human consumption as a synthetic flavoring substance and adjuvant in accordance with the following conditions: (1) the quantity added to food does not exceed the amount reasonably required to accomplish its intended physical,

nutritive, or other technical effect in food, and (2) when intended for use in or on food it is of appropriate food grade and is prepared and handled as a food ingredient.

Formic acid may be safely used as a preservative in hay crop silage in an amount not to exceed 2.25% of the silage on a dry weight basis or 0.45% when direct-cut. The top foot of silage stored should not contain formic acid and silage should not be fed to livestock within 4 weeks of treatment.

Occupational Safety and Health Administration Standards

Permissible exposure limit: Table Z-1 8 h time-weighted average (TWA): 5 ppm ($9\,mg\,m^{-3}$). Threshold limit values: 8 h TWA: 5 ppm; 15 min short term exposure limit: 10 ppm. National Institute for Occupational Safety and Health recommendations: recommended exposure limit: 10 h TWA: 5 ppm ($9\,mg\,m^{-3}$).

Further Reading

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Formic Acid.

Foxglove

Fermin Barrueto Jr.

This article is a revision of the previous print edition article by Lanita B Myers, volume 2, pp. 36–37, © 1998, Elsevier Inc.

- SYNONYMS: *Digitalis purpurea*, *Digitalis obsura*, *Digitalis lanata*, *Digitalis ferruginea* – Scrophulariaceae family; Digitalis; Fairy bells; Fairycap; Fairy glove; Fairy thimbles; Rabbit flower; Lady's thimbles; Lion's mouth; Throatwort; Witch's thimbles; Folks glove; Willow-leaves foxglove

Uses

Foxglove contains approximately a dozen different cardioactive steroids, the most prominent being digitoxin. An extract of foxglove has been used medicinally for decades. The pharmaceutical drugs digoxin and digitoxin are contained within foxglove and are used to treat atrial fibrillation with a rapid ventricular response and for congestive heart failure.

Background Information

Foxglove is an erect biennial herb with simple toothed leaves and a central stalk of pink, purple, yellow, or white tubular, bell-shaped pendent flowers ~3 in. long and numerous in number. This herb grows up to 4 ft high. Foxglove is a cultivated plant in the western United States and Hawaii; it is native to Britain and Europe. It is found in open land, roadsides, waste areas, and is commonly grown in gardens.

Exposure Routes and Pathways

The most common route of exposure is ingestion of any part of the plant or of any material or drug derived from the plant.

Toxicokinetics

Limited useful data are available for plants containing cardiac glycosides; however, some reference will be made to digitoxin, which is a major constituent of *Digitalis purpurea*.

Digitoxin is readily and completely absorbed (90–100%) from the gastrointestinal tract and the aglycones or genins of the plant-derived cardioactive steroids are even more rapidly absorbed. Peak absorption occurs between 4 and 12 h postingestion, but in overdose, peak amounts and absorption can be delayed. Plasma protein binding is extensive (97%) and there is a relatively small apparent volume of distribution ($0.6\,l\,kg^{-1}$). The elimination half-life is very long and variable with this mix of cardioactive steroids, ~100 h or 4–6 days. It is slowly eliminated, with 60–80% of the dose appearing as metabolites in the urine.

Mechanism of Toxicity

Foxglove contains approximately a dozen cardioactive steroids, most prominently digitoxin. There are also other physiologically active chemical constituents

including digoxin, digitonin, digitalin, antirhinic acid, digitalos, and digitoflavone. These toxins are cardiotonic or cardioactive steroids. They consist of a steroid backbone with a five-membered lactone ring attached to it, forming an aglycone or genin. Most plant-derived cardioactive steroids have a five-membered lactone, except red squill; whereas almost all animal-derived steroids have a six-membered lactone. The addition of sugar residues to the aglycone at C3 then creates a cardiac glycoside. The aglycones are derivatives of cyclopentenophenanthrene, and the sugars are unusual methylpentoses. These cardioactive steroids influence the heart in two ways: stronger cardiac contractions due to increased intracellular calcium from inhibiting Na^+,K^+ ATPase and slower contractions through vagal stimulation, prolonging diastole. These cardioactive steroids inhibit the Na^+,K^+ ATPase pump mechanism, which disturbs the sodium gradient increasing intracellular sodium. There is a corresponding increase in extracellular potassium and intracellular calcium. This leads to electrical conduction impairment and reduction of the normal resting membrane. With a decreased ability of the myocardial cells to act as pacemakers, the myocardium becomes sensitized. This leads to premature ventricular contractions, ventricular dysrhythmias, and virtually any dysrhythmia except for a supraventricular tachycardia with a rapid ventricular response.

Acute and Short-Term Toxicity (or Exposure)

Human

Foxglove plant poisoning is fairly uncommon but has occurred from unintentionally making a tea out of the plant's leaves and from eating the plant. Some references state that as little as two or three leaves can produce serious toxicity, although no direct observations have been found to support these statements. Foxglove toxicity resembles digoxin or digitoxin toxicity. Gastrointestinal symptoms develop within several hours and include mouth and throat pain, nausea, vomiting, cramping, abdominal pain, and diarrhea. This is followed by central nervous system changes (e.g., severe headache, drowsiness, vision disturbances, confusion, hallucinations, tremors, and convulsions), hyperkalemia, dysrhythmias, and heart block. Yellow haloes are classic symptoms of chronic digitalis leaf poisoning but usually are not seen with pharmaceutical grade digoxin. Severe overdose results in hyperkalemia, myocardial sensitization, and dysrhythmias. There is often marked difficulty in managing these cases due to the sensitized myocardium. Placing an intravenous pacemaker has been associated with an increase in mortality due to the mechanical stimulation of the sensitized myocardium. The myocardium can lose its ability to respond to electrical pacing. Foxglove tea poisoning has been associated with ventricular tachycardia, junctional rhythms, and atrial fibrillation with high-grade atrioventricular block requiring 6 days to revert to normal. One report described a patient suffering from confusion and visual disturbances lasting 5 days and EKG changes for 10 days. Elevated digitoxin and digoxin levels, hyperkalemia, and the electrocardiogram will help confirm toxicity (in the absence of simultaneous consumption of digitalis preparations).

Therapeutic digitoxin levels range from 18 to 22 $ng\,ml^{-1}$ (23–28.18 $nmol\,l^{-1}$). Toxicity in most patients is above 25 $ng\,ml^{-1}$ (32 $nmol\,l^{-1}$). For digoxin, therapeutic serum concentrations range between 1.5 and 2.5 $ng\,ml^{-1}$. The serum concentration cannot be used to guide management or determine level of exposure as it is merely a cross-reaction of the mix of cardioactive steroids with the assay and not an accurate quantization. Qualitatively, if there is any level, besides undetectable, it indicates the presence of cardioactive steroid. In combination with the electrocardiogram, hyperkalemia, and gastrointestinal symptoms, these findings are enough to determine toxicity and need for treatment with digoxin-specific Fab.

Chronic Toxicity (or Exposure)

Animal

Signs and symptoms of toxicity in animals and livestock would be similar to those in humans. Livestock symptoms include diarrhea, bloody stools, anorexia, weakness, urge to urinate, and dysrhythmias. Treatment should consist of symptomatic and supportive care.

Human

Chronic toxicity develops following the use of herbal products or teas that contain foxglove. The development of toxicity is unpredictable since the digitalis glycoside content of these products is not standardized. This is now extremely rare.

In Vitro Toxicity Data

Studies of digoxin in an *Escherichia coli* model of genotoxicity were inconclusive.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Activated charcoal should be considered for all patients that present within 1 h of exposure. Treatment with digitoxin- or digoxin-specific Fab should be considered in those with severe symptomatology who fail to respond to conventional therapy, with ECG evidence of digoxin/digitoxin toxicity, or potassium greater than 5.0 $mEq\,l^{-1}$. Digoxin-specific Fab should be administered over any antidysrhythmic as this is a polyvalent antibody that will bind digoxin, digitoxin, and other cardioactive steroids. There is marked variability in response to ingestion of cardiac-glycoside-containing plant parts (e.g., leaves and stems) depending on various factors (e.g., the season, age of plant, and humidity). Therefore, all patients with a history of ingestion should have decontamination with activated charcoal, a baseline ECG, and electrolyte monitoring and replacement (if necessary) and should be observed for 4–6 h. Patients presenting with any sign of toxicity should be admitted to a monitored setting for a least a 24 h observation.

See also: Charcoal; Digitalis Glycosides; Red Squill.

Further Reading

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Rich SA, Libera JM, and Locke RJ (1993) Treatment of foxglove extract poisoning with digoxin-specific Fab fragments. *Annals of Emergency Medicine* 22(12): 1904–1907.

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Fragrances and Perfumes

Anne Marie Api and Pertti J Hakkinen

Introduction

Fragrances, like color and music, can play a major part in feelings about what is experienced in life. Scent is present in flowers, rain, fresh air, the sea, and many other natural sources. Scents can also been created by those skilled in the art of perfumery. Perfumers can create scents that mimic those that occur naturally. The use of fragrances dates back at least 10 000 years to the Egyptians, who used scented oils to clean and soften the skin, and to mask body odor. Today, fragrances enhance the quality of life, and researchers claim that the use of fragrance can have a strong psychological impact, boosting moods, keeping people alert, providing a feeling of calmness, and may even enhance the learning process. Repeated exposure to a scent can trigger a conditioned response, for example, how ones feels when smelling a favorite food.

The terms fragrances and perfumes can generally be used interchangeably as the mixtures of chemicals artfully assembled by a perfumer. Fragrances and perfumes are used in a wide variety of consumer products, ranging from the 'fine fragrances' applied directly to the skin, to perfumes used in creams, lotions, detergents, and many other personal and household products. In addition to enhancing the use of many products, fragrances can be used to neutralize the unpleasant odors associated with many cleaning agents.

The Safe Use of Fragrance Ingredients

Fragrances today are much different than those of olden days. A single fragrance or perfume might contain several hundred or more natural and synthetic materials. The natural ingredients come from materials found in roots, bark, flowers, and from other parts of plants from many regions of the world. They are obtained through physical processes such as distillation or extraction. The synthetic ingredients used in fragrances and perfumes are manufactured through chemical processes. In the past, some important natural ingredients were from animal sources such as whales and the civet cat; however, synthetic replacements made of individual chemicals or mixtures of natural plant and/or synthetic chemicals providing the same or nearly the same smell as the animal-derived chemicals are available to perfumers as replacements. More and more synthetic ingredients are being used today due to conservation and availability issues and time and cost efficiencies.

Because of the widespread use of a large variety of consumer products ranging from perfumes to

cosmetics to skin products and other personal and household hygiene products as well as air fresheners, scented oils, and candles, it is important to examine the dermal effects, systemic toxicity, and environmental consequences of the use and exposure to fragrance materials. It can be argued that the natural and synthetic chemicals used in fragrances and perfumes are among the most studied chemicals used in consumer products. The D-limonene entry in this book provides an example of a fragrance and perfume chemical that has undergone extensive toxicology testing, exposure assessment, and risk assessment. This was done to understand the reasons behind D-limonene's skin sensitization potential (oxidation of D-limonene is necessary for its sensitizing potential). In addition, it was done to understand the human relevance of carcinogenicity observed in male rats (the tumorigenic activity of D-limonene has been concluded to be nonrelevant to humans because of the role that α2u-globulin plays in the nephrotoxicity and carcinogenicity in male rats).

The fragrance industry has maintained a strict system of safety assurance for more than 30 years. Originally designed to be self-regulatory, it is based primarily on a scientific assessment of potential hazards and exposure to fragrance materials by the scientific staff of the Research Institute for Fragrance Materials, Inc. (RIFM). RIFM, an independent nonprofit institute, was founded for the purpose of obtaining and evaluating safety data on fragrance ingredients. RIFM maintains a comprehensive scientific program that covers human health methodology, environmental methodology, respiratory safety, fragrance allergy, group health and environmental testing, and use level testing.

All of RIFM's test results are evaluated by the RIFM Expert Panel (REXPAN), an independent, international group of scientists. The REXPAN includes experts in dermatology, pharmacokinetics, toxicokinetics, toxicology, pathology, environmental science, and other experts. The experts have no commercial ties to the fragrance industry. Outside experts and RIFM scientists provide consultation as needed. The evaluations by the REXPAN are based on existing data or, where insufficient data exist, on testing performed by RIFM itself. REXPAN's findings and conclusions are published in peer-reviewed journals. RIFM also maintains the most extensive technical database of human health effects, environmental fate, and product regulations on fragrance ingredients available worldwide.

The overall RIFM process results in well-documented conclusions that are provided to the International Fragrance Association (IFRA) as the basis for consideration of a new or existing Fragrance Material Standard, and to industry for appropriate product risk assessment and risk management. The IFRA standards regarding use restrictions (e.g., the maximum allowable level of a particular chemical or related class of chemicals in a type of consumer product) are carefully reviewed by the IFRA Scientific Committee. IFRA then disseminates the information worldwide to its national and regional associations for subsequent distribution to member companies. These determinations reflect industry's stewardship and are interpreted to have the same legal value as other sources of law, such as legislation.

The IFRA Code of Practice and Standards is comprehensive and applies to the manufacture and handling of all fragrance materials for all types of application. It formulates the basic principles, which are the standards of good operating practice by the fragrance industry. Compliance is encouraged throughout the supply chain by a system of notifications and potential enforcement action. When IFRA is informed about a suspected infringement to the code, its staff investigates the facts and contacts the parties in question, as needed. In the few cases that have arisen in the past, a satisfactory resolution has been achieved. The application of IFRA's Code of Practice and Standards does not dispense individual manufacturers from the obligation to comply with all national or international regulations relevant to their operations. IFRA also analyzes and reviews pending regulation applicable to fragrances, as well as legislative trends in related areas such as cosmetics, intellectual property, chemicals, and occupational health and safety.

Both RIFM and IFRA develop and maintain open communication and cooperation with national and international government bodies, concerned members of the medical and scientific community, the industry customers using fragrances, and other stakeholders.

See also: International Fragrance Association (IFRA); Limonene; Research Institute for Fragrance Materials (RIFM).

Further Reading

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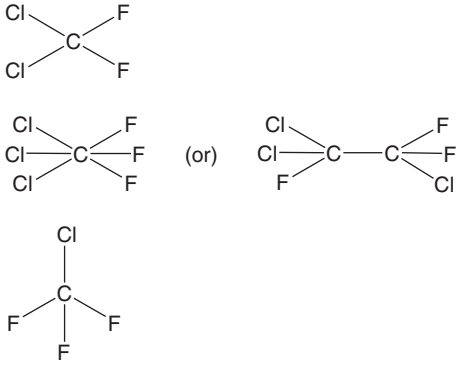
Freons

Kathryn A Wurzel

- Preferred Name: Chlorofluorocarbons (CFCs)
- Chemical Abstracts Service Registry Number: The CAS registry number is dependent on the specific freon compound
- Synonyms: Halons; Halocarbons; Freon 12 (CAS 75-71-8); Freon 13 (CAS 75-72-9); Freon 22 (CAS 75-45-6); Freon 113 (CAS 76-13-1); Freon 114 (CAS 76-14-2); CFC-12; CFC-13; CFC-22; CFC-113; CFC-114
- Chemical/Pharmaceutical/Other Class: Halogenated solvent
- Chemical Formulas: Freon 12, CCl_2F_2; Freon 113, $C_2Cl_3F_3$; Freon 22, $CHClF_3$
- Chemical Structure:



Uses

Freons are commonly used as refrigerants and propellants in many types of aerosols. They are also used as selective solvents for degreasing.

Exposure Routes and Pathways

Inhalation (pulmonary route) is the main source of toxic exposures to chlorofluorocarbons. Dermal exposure may also occur. Ingestion would be intentional.

Toxicokinetics

Chlorofluorocarbons have low dermal absorption characteristics. Absorption by the lungs is slow (based on data collected from animal studies). The main factor affecting distribution of chlorofluorocarbons in an individual is the amount of body fat. Chlorofluorocarbons are concentrated in the body fat and are slowly released into the blood at concentrations that do not present a risk of cardiac sensitization.

Loss of CFC-113 from tissues is rapid during the postexposure period with virtually 100% clearance within 24 h of exposure. Freons are eliminated entirely by the respiratory tract. Chlorofluorocarbon compounds partition preferentially into lipid-rich tissues and are poorly metabolized. Significant accumulation occurs in brain, liver, and lung tissues compared to blood levels.

Mechanism of Toxicity

The exact mechanism of central nervous system (CNS) depression has not been determined, but the most plausible hypothesis is change in membrane fluidity that alters neural transmission. No significant histological damage has been noted in the brains of animals exposed to lethal concentrations.

Chronic skin irritation occurs as a result of defatting of the skin. Ventricular fibrillation is due to the direct sensitization of the myocardium to endogenous catecholamines.

Acute and Short-Term Toxicity (or Exposure)

Animal

The main effects observed in animals following exposure to chlorofluorocarbons are CNS depression, respiratory tract irritation, rapid breathing, lung congestion, and microscopic liver changes. Cardiac dysrhythmias and mild chemical conjunctivitis have

also been noted. Chlorofluorocarbons are skin irritants and slight eye irritants, but are not skin sensitizers in animals.

Chlorofluorocarbons are more acutely toxic to rabbits than to mice via the oral route of exposure. Dogs have demonstrated vomiting, lethargy, nervousness, and tremors following inhalation exposure to chlorofluorocarbons. High-concentration exposures to dogs, monkeys, and rats resulted in cardiac arrhythmias.

Human

Eye and skin irritations have been observed following exposure to chlorofluorocarbons. No corneal opacity has been noted as a result of exposure to chlorofluorocarbons but frostbite of the eyelids may be severe.

Chlorofluorocarbons are very toxic when inhaled at high concentrations and/or for extended periods of time. Lower concentrations or brief periods of exposure result in transient eye, nose, and throat irritations. Temporary CNS depression, dizziness, headache, confusion, and incoordination are associated with exposure to high concentrations ($\geq$2500 ppm in air). Gross overexposure may lead to abnormal liver function, refractory ventricular dysrhythmias, and sudden death. Intentional sniffing of aerosols has resulted in sudden death. There is significant individual variability in response to chlorofluorocarbons.

Chlorofluorocarbon compounds are cardiac sensitizing agents. Pulmonary edema, bronchial constriction, and lung irritation may also occur following inhalation exposure to high chlorofluorocarbon concentrations.

Chronic Toxicity (or Exposure)

Animal

A 2 year inhalation exposure to rats did not result in any hepatotoxic effects. Animal testing indicates no carcinogenic, mutagenic, embryotoxic, or reproductive effects. Generally, no changes in offspring were noted when doses of chlorofluorocarbons were below those associated with maternal toxicity (both oral and inhalation exposures).

Human

Chlorofluorocarbons are acutely toxic but do not appear to induce chronic toxicity.

Clinical Management

Oral exposures to liquid chlorofluorocarbons are rare but have resulted in severe frostbite to the upper respiratory system and gastrointestinal tract. Necrosis and perforation of the stomach have been reported. Thus, emesis, activated charcoal, and gastric lavage are not recommended.

Following inhalation overexposure, a calm environment with no physical exertion is imperative to avoid an endogenous adrenaline surge. Exogenous adrenergic drugs should not be used to prevent induction of sensitized myocardial dysrhythmias. Diphenylhydantoin and countershock may be effective for ventricular dysrhythmias.

Exposure of the eyes to liquid chlorofluorocarbons or significant air concentrations should be treated by irrigating the eyes with tepid water for at least 15 min. Cryogenic dermal injuries should be treated by water bath rewarming until vasodilatory flush has returned.

See also: Catecholamines.

Relevant Websites

http://www.inchem.org – Fully Halogenated Chlorofluorocarbons; Environmental health Criteria 113 from the International Programme on Chemical Safety.

Fuel Oils

Richard D Phillips

- Representative Chemicals: Fuel oils can be grouped into three categories: kerosene, gas oils, and heavy fuel oils.
- Chemical Abstracts Service Registry Numbers[1]: CAS 64747-41-9 (Kerosene); 68335-30-5 (Diesel fuel); 68476-30-2 (No. 2 Heating oil)

[1] This is a sampling of CAS numbers for products and refinery streams considered to be in the fuel oil category. For a more complete list, refer to the CONCAWE reports given in the 'Relevant Websites' section.

- SYNONYMS: Jet fuel; Middle distillate; Light cycle oil; Gas oils; Marine diesel fuel; Bunker fuel are a sample of synonyms. See 'Relevant Websites' section for more information.

Uses

Kerosenes are used in blending aviation fuels and can be tailored to meet very strict specifications. Kerosenes are also used as domestic and industrial heating fuels. Kerosenes may be used in a wide range of products including insecticides, solvents and, mould releasing agents. Gas oils are used primarily as fuels in diesel engines and for both industrial and domestic heating. Heavy fuel oils are used in medium to large industrial plants, marine applications, and power stations in combustion equipment such as boilers and furnaces.

Exposure Routes and Pathways

Fuel oils may enter the respiratory system as a vapor or an aerosol. However, the heavier the oil, the lower the vapor pressure and the less likely that one would be exposed to vapor. Exposure to aerosol would be a concern in certain spray applications of kerosene type production. If fuel oils contact skin, this could be a pathway for exposure. Finally, drinking contaminated water or food could result in ingestion of fuel oils.

Toxicokinetics

Fuel oils may be absorbed through the respiratory tract, the gastrointestinal tract and percutaneously. The higher the molecular weight of the hydrocarbons in the oils, the less likely that absorption will occur. Metabolism via oxidation is also likely to occur since the components of fuel oils are hydrocarbons. The degree of metabolism would, of course, be dependent on the nature of the hydrocarbon (i.e., aliphatic, aromatic, etc.), the molecular weight and any other associated molecule (e.g., sulfur, nitrogen). Excretion from the body would also be dependent on the above and could occur via exhalation, in the urine or feces. These molecules are not likely to accumulate in the body.

Mechanism of Toxicity

A primary risk from ingestion of lighter gas oils, such as kerosene, is aspiration during vomiting, which can result in pneumonitis. Like most hydrocarbons, significant exposure may result in central nervous system (CNS) depression. However, fuel oils may have high amounts (10–20%) of three- to seven-ring aromatics which can be carcinogenic.

Acute and Short-Term Toxicity (or Exposure)

Gas oils have a low order of toxicity following acute oral, dermal, or inhalation exposure. Signs observed following high doses are indicative of CNS depression. Skin irritation may result from repeated or prolonged contact with the skin. This, of course, varies depending on the molecular weight (i.e., lower, more irritating), percentage of aromatics and substituted hydrocarbons. Potential for eye irritation varies from slight to mild. Gas oils are typically not skin sensitizers. Ingestion of large quantities of fuel oils can cause vomiting, diarrhea, gastrointestinal disorders, difficulty breathing, and even convulsions, coma, and death.

Heavy fuel oils may contain significant concentrations of hydrogen sulfide (H_2S), which may accumulate in the headspaces of storage tanks. Hydrogen sulfide is neurotoxic.

Chronic Toxicity (or Exposure)

Animal

Heavy fuel oils may have significant amounts of aromatics produced from cracked petroleum stocks. If so, these products may be carcinogenic and have produced tumors in mice. Gas oils and kerosenes have also produced tumors in mice but generally under conditions of severe skin irritation. If skin contact and particularly skin irritation is minimized, the tumor response does not occur.

A long-term inhalation study was conducted with jet fuel vapor (JP-4). Rats and mice were exposed to 0, 1, or 5 $mg\,l^{-1}$ for 6 h per day, 5 days per week for 12 months. At exposure termination, 10% of the rats were sacrificed, and the remainder held for an additional 12 month observation period. There were no toxicologically significant signs observed during the exposures. Body weights for male rats were reduced.

The only consistent pathological change was evidence of a mild progressive kidney effect only in the male rats. This is believed to be a rat specific phenomena and not applicable to humans.

Human

Very little is known about the long-term effects of low level exposure to fuel oils. For example, it is not known whether chronic exposure can cause cancer, birth defects, or reproductive impairment in humans.

In Vitro Toxicity Data

A number of *in vitro* genotoxicity studies have been conducted on fuel oils. Typically, kerosenes are negative in these studies. Gas oils range from inactive to weakly active in a number of *in vitro* assays.

Clinical Management

If symptoms arise from inhalation of fuel oil vapor or aerosol, the individual should be removed to fresh air as quickly as possible. If ingestion occurred, the airway should be protected. Vomiting should not be induced.

Where significant skin contact has occurred, the affected areas should be washed thoroughly with water, using soap if available. Contaminated clothing should be removed as soon as possible.

Eyes should be flushed gently with water for up to 10 min.

Environmental Fate

Most hydrocarbon constituents of kerosene will evaporate and be photodegraded in the atmosphere. This will be true for the lower molecular weight components of other fuel oils as well. The higher molecular weight component will persist in the aqueous environment for longer periods and will biodegrade slowly.

Ecotoxicology

The ecotoxicology of fuel oils is complicated by the complexity of the constituents and by the variety of methodologies used in testing. Sublethal effects have been observed in fish in response to kerosene. For example, lesions in tissues – gill, pseudobranch, kidney, and nasal mucosa.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value for kerosene is $200\,mg\,m^{-3}$, and for diesel, it is $100\,mg\,m^{-3}$. Oil mist from heavier fuel oils would be covered by the oil mist standard, which is currently $5.0\,mg\,m^{-3}$ but has a notice of intended change to $0.2\,mg\,m^{-3}$.

See also: Diesel Fuel; Jet Fuels; Kerosene; Otto Fuel II.

Relevant Websites

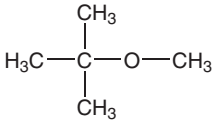
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Fuel Oils.

http://www.concawe.be – Conservation of Clean Air and Water in Europe (CONCAWE, 1995, 1996, 1998) Product Dossier nos. 94/106 Kerosene/Jet Fuels, 95/107 Gas Oils, 98/109 Heavy Fuel Oils.

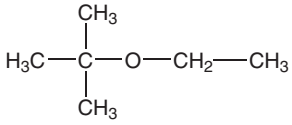
Fuel Oxygenates

Ann de Peyster

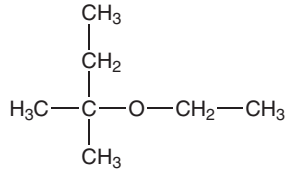
- Chemical Abstracts Service Registry Numbers: Methyl *t*-butyl ether (MTBE) (CAS 1634-04-4); Ethyl *t*-butyl ether (ETBE) (CAS 637-92-3); *t*-Amyl methyl ether (TAME) (CAS 994-05-8); Diisopropyl ether (DIPE) (CAS 108-20-3); Ethanol (EtOH) (CAS 64-17-5)
- Chemical Formulas: MTBE ($C_5H_{12}O$); ETBE ($C_6H_{14}O$); TAME ($C_7H_{16}O$); DIPE ($C_6H_{14}O$); EtOH (C_2H_6O)
- Chemical Structures:



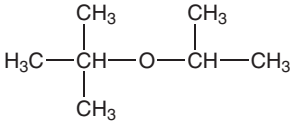
MTBE



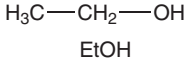
ETBE



TAME



DIPE



EtOH

Uses

Oxygenates have been used in gasoline mainly to reduce emissions of certain air pollutants such as benzene and carbon monoxide. Small quantities may be used as laboratory reagents and as pharmaceutical agents. For example, methyl *t*-butyl ether (MTBE) is a versatile organic solvent that has many applications, including clinical use in dissolving gallstones.

Exposure Routes and Pathways

Exposure to MTBE and other fuel oxygenates can occur at any point in their manufacture, distribution, and use. Opportunities for exposure closely parallel other organic hydrocarbons in gasoline. Exposure can occur by inhalation as oxygenates evaporate from fuel during refueling. Fuel oxygenates are highly water-soluble and contamination of drinking water can occur from spills or leaks.

Toxicokinetics

The toxicokinetics of MTBE have been studied in animal models, primarily rodents. The information available to date on the biological fate of ETBE and TAME indicates that their kinetics are expected to be similar to those of MTBE. This has been confirmed experimentally in rodents, in *in vitro* systems using liver microsome homogenates, and also in studies with human volunteers inhaling these fuel oxygenates while at rest or during light exercise.

Rodent and human studies have shown that MTBE is rapidly absorbed following inhalation exposure. In addition, rodent studies have shown rapid distribution of MTBE after oral and intraperitoneal exposure. Dermal absorption occurs more slowly. Evidence supports metabolic transformation of MTBE by P450 enzymes to the parent alcohol, *t*-butyl alcohol (TBA), and formaldehyde in rodents and humans. Further oxidative metabolism of TBA seems to be slow, and glucuronidation is a major competing pathway. Formaldehyde metabolism to formate is very rapid. The toxicokinetic parameters of MTBE and TBA depend on the dose and route of administration although they appear to be linear following inhalation exposures up to 50 ppm.

Inhaled ETBE and TAME are also eliminated by exhalation or through the urine. Major metabolites of ETBE are TBA and acetaldehyde. TAME breakdown in the body is slightly more complex than that of MTBE or ETBE, producing *t*-amyl alcohol as an initial intermediate of metabolism. Compared to MTBE, ETBE uptake in male human volunteers exposed by inhalation is lower and elimination through the respiratory tract is slightly higher. Like MTBE, ETBE toxicokinetics are linear following exposures up to 50 ppm vapor.

The metabolites of MTBE, ETBE and TAME in humans are qualitatively similar to those observed in rodent studies, and there are no gender differences observed in humans. The half-lives of MTBE, ETBE, and TAME in rodents are less than 1 h, whereas half-lives estimated in human studies suggest a more complex picture with multiple half-lives in blood and urine extending to 28 h for ETBE.

DIPE is rapidly absorbed into the blood from the lungs or GI tract. Elimination through lungs is the most probable route of excretion. Information on DIPE is currently limited.

Ethanol is readily absorbed following inhalation or oral exposure. In the bloodstream, ethanol is rapidly distributed into total body water. Ethanol is removed from the blood primarily by metabolism in the liver. Ethanol is metabolized to acetaldehyde and later to acetic acid by two major pathways: acetaldehyde (ADH) and the ethanol-oxidizing system in the endoplasmic reticulum.

Acute and Short-Term Toxicity (or Exposure)

Animal

The rat LD_{50} for MTBE is $\sim 3.9\,g\,kg^{-1}$ body weight and the LC_{50} is between 18 000 and 40 000 ppm. These are extremely high doses compared to levels occurring in the environment. Death is preceded by ocular and mucous membrane irritation, ataxia, and central nervous system depression.

Systemic effects were observed at concentrations of 3000 ppm for MTBE, 4000 ppm for ETBE, and $0.5\,g\,kg^{-1}$ for TAME when administered to rats for a period of 4 weeks. These effects included increased weights of livers, kidneys, and adrenal glands, and signs of ataxia and hypoactivity. Neurotoxic effects, primarily in the form of activity modification, were observed at 800 ppm MTBE. For ETBE, minor effects were seen only in rats exposed to 4000 ppm for 28 days (the highest concentration tested).

MTBE caused an increase in protein accumulation and cell proliferation in the kidneys of male F344 rats after inhalation exposure of 3000 or 8000 ppm; however, this increase was not accompanied by an increase in the level of α2u-globulin. MTBE administered by gavage also caused an increase in hyaline droplet formation in the kidneys of male Sprague–Dawley rats at doses of 0.44 and $1.75\,g\,kg^{-1}$. Similar effects were observed in males after a 14 day exposure to $1.4\,g\,kg^{-1}$ MTBE. The male kidney appears to be the primary target of MTBE. No

histopathologic changes were noted in the kidneys after exposure to ETBE or TAME, but no detailed analyses of protein changes were conducted.

In rats, MTBE did not seem to be a strong sensory irritant, in terms of inhibiting respiratory function. Such inhibition was noted after exposure to 8000 ppm, but not at lower concentrations. On the basis of this information and the Alarie model, a level for sensory irritation in humans was estimated to be 140 ppm MTBE. Consistent with the study in rats showing that MTBE alone is a weak respiratory irritant, the controlled human exposure studies failed to document significant sensory irritation – either subjective or objective – from MTBE alone.

A 28 day subchronic toxicity study of TAME administered by oral gavage was conducted in Sprague–Dawley rats. Dose levels were 0, 125, 500, or 1000 mg kg^{-1} body weight. Mean body weights were lower in male rats given 1000 mg kg^{-1}, and dose-related increases in adrenal gland and kidney weight were measured in males. There were no treatment-related histopathologic changes.

A 4 week inhalation study was conducted with ETBE in Sprague–Dawley rats. Exposures were at 500, 2000, or 4000 ppm ETBE vapor for 6 h day^{-1}, 5 days $week^{-1}$. There was a statistically significant increase in white blood cells at 2000 and 4000 ppm. In addition, kidney weights were increased in male rats at 4000 ppm; however, there was no histologic evidence of kidney damage.

In teratology studies of MTBE, maternal effects were observed in rats exposed to 3000 or 8000 ppm (but not 400 ppm), and in mice and rabbits exposed to 4000 or 8000 ppm MTBE (but not 1000 ppm). Pregnant rats exposed to 4000 or 8000 ppm MTBE showed a reduced number of viable fetal implantations. Small but statistically significant decreases were observed in the viability of offspring from pregnant rats exposed to 1300 or 3400 ppm MTBE when compared with control animals.

Rat pups exposed *in utero* to 8000 ppm MTBE and, to a lesser extent, those exposed to 3000 ppm, had statistically significant decreases in body weight during lactation compared with control pups and pups exposed *in utero* to 400 ppm. Effects on the central nervous system consisting of hypoactivity, ataxia, and loss of startle reflex were seen in adult rats exposed *in utero* to 3000 or 8000 ppm MTBE. No malformations were reported in the fetuses examined in the three studies in rats described above. Increased frequencies of skeletal malformations were found in fetuses from mice exposed to 4000 or 8000 ppm MTBE. When administered to mice at lower concentrations (1000 ppm), MTBE was neither teratogenic nor toxic to the mother or fetuses.

ETBE has been studied in subchronic inhalation experiments using Fischer 344 rats and CD-1 mice exposed to 0, 500, 1750, or 5000 ppm for 13–14 weeks. Although transient ataxia (uncoordinated gait) was a common observation in rats at the higher concentrations, no lasting neurotoxicity was reported. Few major changes in standard clinical pathology were noted in rats or mice, although liver weight increases and centrilobular hepatocyte hypertrophy suggested that ETBE is mitogenic. Like MTBE, ETBE produced evidence of α2u-globulin droplet accumulation in the male rat kidneys. An unexpected finding was degenerative changes in testicular seminiferous tubules in male rats (but not male mice) exposed to the 1750 and 5000 ppm concentrations. This had not been reported in similar studies with MTBE.

Inhalation effects of TAME were evaluated in a two-generation reproductive toxicity study in CD rats exposed to 0, 25, 1500, or 3000 ppm, and in developmental toxicity studies using timed pregnant CD (Sprague–Dawley) rats and CD-1 mice exposed to 0, 250, 1500, or 3500 ppm. In the reproductive toxicity study, exposure of male rats to high concentrations (1500 or 3000 ppm) 5 days a week for 10 weeks resulted in adult systemic toxicity at 1500 and 3000 ppm, some adult reproductive toxicity at 3000 ppm, and offspring toxicity at 1500 and 3000 ppm. Interested readers should consult 'Further Reading' below for details of experimental findings, which included increased percentages of abnormally shaped sperm in the F_0 generation, and other effects suggestive of hormonal imbalance (delayed preputial separation and vaginal opening, shorter anogenital distance) variously in the offspring. In the developmental toxicity studies, rats were exposed for 6 h a day on gestational days 6–19 and mice were exposed on gestational days 6–16. In rats, the NOAEL (no-observed-adverse-effect level) for maternal toxicity was 250 and 1500 ppm was the NOAEL for developmental toxicity. More severe developmental toxicity was observed in mice, in which NOAELs for maternal and developmental toxicity were both 250 ppm.

Diisopropyl ether (DIPE) has also been evaluated in Sprague–Dawley rats in subchronic inhalation studies with doses administered up to 7100 ppm, 6 h day^{-1} for 90 days. Male and female rats manifested evidence of liver and kidney hypertrophy but few other significant clinical signs. In a standard developmental toxicity evaluation also using Sprague–Dawley rats, the 6745 ppm inhaled dose administered during gestation days 6–17 produced an increase in rudimentary ribs in the offspring. This effect was considered of uncertain significance. The overall conclusion of these studies was that DIPE has a low order of toxicity.

Human

Ethers are odorous compounds that can be detected in air at very low concentrations. The odor detection thresholds are 53 ppb for MTBE, 13 ppb for ETBE, and 27 ppb for TAME. Adding MTBE at a concentration of 15% by volume dramatically lowered subjects' odor detection thresholds for gasoline by 54–80%, depending on the type of gasoline. For ETBE, the effect was even more dramatic, producing an 89% reduction in gasoline odor threshold.

Overall, the available data suggest that most people do not experience unusual symptoms or significant acute medical consequences when inhaling MTBE in fuel. Because some have reported acute symptoms under some circumstances, different individual sensitivity to MTBE has been suggested.

The consequences of acute ingestion of ethanol are overwhelmingly the result of its action on the central nervous system. They range from the easily recognized signs of intoxication, such as slurred speech and ataxia, to subtle impairment of performance detectable only by neurobehavioral testing. Sensitivity varies enormously among individuals even when body weight is accounted for. On the basis of the available literature, it can be projected that blood levels as low as 10 mg% (100 mg l^{-1}) may induce performance deficits in some people under some conditions. Functions such as vigilance and attention seem to be those most affected at low levels.

Chronic Toxicity (or Exposure)

Animal

Results of chronic MTBE exposure studies are the most widely available of all studies on the ether-like fuel oxygenates (MTBE, ETBE, TAME, DIPE). Evidence from animal bioassays demonstrates that long-term, high-level exposures to MTBE by either ingestion or inhalation cause cancer in rodents. Inhalation exposure to MTBE produced an increased incidence of renal and testicular tumors in male rats and liver tumors in mice. Oral administration of MTBE produced an increased incidence of lymphomas and leukemias in female rats and testicular tumors in male rats. Chronic exposure to ethanol also produces cancers (e.g., esophageal) in laboratory animals.

Human

There are no adequate epidemiologic studies of chronic exposure to MTBE or to any of these other ether-like fuel oxygenates that are not confounded by other exposures. Extrapolating effects of chronic animal exposure study findings to humans is questionable for a number of reasons. First, the increased tumor incidences were observed at very high exposure levels of MTBE that were toxic and unlikely to be experienced by human populations. Second, each of the animal bioassays had some notable technical limitations. Third, some of the tumors are of questionable relevance to humans because they may be a species-specific phenomenon involving cytotoxic responses to the high-dose exposure regimen. How and why these tumors arose in animal studies (mode of action) is still not completely understood.

It should be noted that evidence derived largely from animal and cell-based studies indicates that both MTBE and ETBE are oxidatively demethylated to produce *t*-butyl alcohol (TBA). MTBE is also metabolized to formaldehyde. Both TBA and formaldehyde are potentially carcinogenic. In all the studies with rodents, MTBE and TBA increased tumor incidence only at very high oral or inhalation exposures, levels that would not be encountered by humans for prolonged periods of time.

Chronic ethanol exposure by ingestion of alcoholic beverages produces widespread toxicity, ranging from liver and nervous system damage to reproductive impairment and birth defects. People are not likely to be exposed orally to ethanol alone when used as a fuel additive, and systemic effects of chronic exposure to ethanol by respiratory and dermal uptake would be negligible.

In Vitro Toxicity Data

MTBE has been tested for genotoxicity with generally negative results. MTBE was neither toxic nor mutagenic in studies using the *Salmonella* mutation (Ames) assay, nor did exposing primary rat hepatocytes to MTBE in culture cause unscheduled DNA synthesis, an indicator of DNA damage. As part of the inhalation carcinogenicity bioassay study, bone marrow cells from male and female rats were analyzed for chromosomal aberrations. No MTBE-related chromosomal damage or increase in micronuclei was detected in these cells. Oral administration of MTBE (1, 10, 100, or 1000 mg kg^{-1}) to male and female CD-1 mice for 3 weeks did not produce mutations at the *hprt* locus of lymphocytes. In addition, MTBE did not induce sex-linked recessive lethal mutations in the fruit fly (*Drosophila melanogaster*) when administered at 0.03, 0.15, or 0.30% in food. The only report of MTBE-induced genotoxicity is an abstract indicating that it was mutagenic in an S9-activated mouse lymphoma assay, a response that has been attributed to formaldehyde production.

ETBE was negative in mutagenicity assays in bacteria and Chinese hamster ovary cells, in a bone marrow micronucleus test in mice, and in an *in vitro* chromosome aberration assay.

TAME was reported to be nonmutagenic in five standard *Salmonella* strains, either with or without activation, TAME was negative in the micronucleus assay.

DIPE has been tested for genotoxic activity in bacterial mutation assays, a yeast assay for mitotic gene conversion, and in tests using rat liver and Chinese hamster ovary cells with structural chromosome damaging the end point. Negative responses were observed in the bacterial and yeast assays.

Ethanol is negative in mutagenicity assays in bacteria, mouse sperm, cell transformation in hamster and rat embryo cells, and chromosome aberrations *in vitro*. It produces dominant lethal effects in rats and increases sister chromosome exchange *in vitro* in human and nonhuman lymphocytes.

Clinical Management

Individuals overcome by exposure to fuel oxygenates should be moved to fresh air and administered 100% humidified supplemental oxygen. Skin should be thoroughly washed with water. Following ingestion, the potential risk of aspiration outweighs the benefit of inducing vomiting. Following contamination of the eyes, they should be irrigated with copious amounts of water for at least 15 min.

Environmental Fate

The high vapor pressure of MTBE and other ethers leads to partitioning to the atmosphere when released to surface water or soil surfaces. When introduced into subsurface soils or to groundwater, MTBE may be fairly persistent since volatilization is reduced or prevented. The potential for bioconcentration appears to be very minor.

Although MTBE has a reasonably high water solubility, it shows little tendency to degrade from hydrolysis and very little tendency to adsorb to suspended particulates, soils, or sediments. In groundwater, MTBE can be fairly persistent since it shows limited susceptibility to either anaerobic or aerobic biodegradation.

MTBE is not expected to persist in the atmosphere because of its fairly rapid reactions with hydroxyl radicals. Based on what is known about the similar behavior of MTBE and other ether fuel oxygenates, the environmental fate of others is expected to be similar to that of MTBE.

Ethanol volatilizes, photodegrades and biodegrades, and leaches into groundwater. It does not adsorb to sediments or bioaccumlate in fish.

Ecotoxicology

Depending on the time of exposure and endpoint measured, MTBE is acutely toxic to various aquatic organisms at concentrations of 44 to $>1000\,\mathrm{mg\,l^{-1}}$ in invertebrates, and 388 to $>3000\,\mathrm{mg\,l^{-1}}$ in vertebrates. In microalgae, decrease in growth was observed at 2400 and $480\,\mathrm{mg\,l^{-1}}$ within 5 days. MTBE does not appear to bioconcentrate in fish and is rapidly excreted or metabolized. The LC_{50} in *Pimephales promelas* (fathead minnow) is $91.7\,\mathrm{mg\,l^{-1}}$ in a 96 h flow-through bioassay. Ecotoxicological effects of other ether fuel oxygenates have not been well studied but are expected to be similar to the effects of MTBE.

Exposure Standards and Guidelines

- MTBE – 50 ppm is the 8 h time-weighted average (TWA) American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV); $20–40\,\mu\mathrm{g\,l^{-1}}$ is the US Environmental Protection Agency drinking water guideline for MTBE
- ETBE – 5 ppm is the 8 h TWA ACGIH TLV
- DIPE – 500 ppm Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) time-weighted average concentration (TWAC)
- EtOH – 1000 ppm is the OSHA PEL/TWAC

MTBE is currently regarded as an animal carcinogen, but whether these effects seen in laboratory animals have relevance to humans is unknown.

See also: Diesel Fuel; Fuel Oils; Gasoline; Jet Fuels.

Further Reading

Ahmed FE (2001) Toxicology and human health effects following exposure to oxygenated or reformulated gasoline. *Toxicological Letters* 123: 89–113.

de Peyster A, Maclean KJ, Stephans BA, *et al.* (2003) Subchronic studies in Sprague–Dawley rats to investigate mechanisms of MTBE-induced Leydig cell cancer. *Toxicological Sciences* 72: 31–42.

DeKant W, Bernauer U, Rosner E, and Amberg A (2001) Toxicokinetics of ethers used as fuel oxygenates. *Toxicological Letters* 124: 37–45.

Medinsky MA, Wolf DC, Cattley RC, *et al.* (1999) Effects of a thirteen-week inhalation exposure to ethyl *tertiary*

butyl ether on Fischer-344 rats and CD-1 mice. *Toxicological Sciences* 51: 108–118.
Tyl RW, Myers CB, Marr MC, *et al.* (2003) Two-generation reproductive toxicity study of inhaled *tertiary* amyl methyl ether (TAME) vapor in CD rats. *Journal of Applied Toxicology* 23: 397–410.
Welsch F, Elswick B, James RA, *et al.* (2003) Developmental toxicity evaluation of inhaled *tertiary* amyl methyl ether in mice and rats. *Journal of Applied Toxicology* 23: 387–395.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Fuel Oxygenates.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Fuel Oxygenates.

Furan

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 110-00-9
- Synonyms: Axole; Divinylene oxide; 1,4-Epoxy-1,3-butadiene; Furfuran; Oxacyclopentadiene; Oxole; Tetrole
- Chemical/Pharmaceutical/Other Class: Five-membered aromatic heterocyclics
- Chemical Formula: C_4H_4O

Uses

Furan is a solvent used in the organic synthesis of pyrrole, tetrahydrofuran, and thiophene. It is also used as a solvent for resins and in the production of lacquers, agricultural chemicals, and stabilizers.

Background Information

Furan occurs naturally in oils distilled from rosin-containing pinewood. In addition, many natural foods contain the furan ring structure and substituted furans may be formed through cooking of simple carbohydrates. Furan is also found in tobacco smoke as well as in wood smoke and gas emissions from gasoline and diesel engines. Furan has also been detected in industrial effluents and can be emitted to the air from petroleum refineries and coal mining and gasification plants.

Exposure Routes and Pathways

The most significant route of exposure for furan is via inhalation. However, oral and dermal exposure may also occur in industrial settings.

Toxicokinetics

Animal studies suggest that furan is readily absorbed via all routes of exposure. Furan is oxidized by the cytochrome P450 enzymes in the liver and other tissues and may form epoxide intermediates; however, the precise intermediates have not yet been identified. Intermediates of furan may include enedials and dialdehydes, which are metabolized to CO_2 and eliminated through the lungs. Examination of urinary output following furan administration in animals revealed a complex mixture of mercapturate metabolites of furan.

Mechanism of Toxicity

Furan can cause eye, skin, and mucous membrane irritation, a burning sensation, and, in severe cases, corrosion. If inhaled, furan may produce pulmonary edema and bronchiolar necrosis. When absorbed, furan can cause central nervous system (CNS) depression to the point of narcosis and tonic seizures.

Furan is metabolized by the cytochrome P450 enzymes in the liver and other tissues. The furan ring undergoes oxidative cleavage and forms highly reactive furan radical cations or epoxides, which react directly with cellular nucleophiles. These reactive metabolites may react directly with DNA or with cellular proteins to produce disruption of cellular functions and cell death. Chronic cell death and regeneration produced by chronic furan exposure may be a significant factor in the carcinogenicity potential of the chemical. In addition, there is some evidence to suggest that the reactive metabolites of furan may induce mutations in cellular genes.

Acute and Short-Term Toxicity (or Exposure)

Animal

Furan has been shown to be highly toxic by inhalation to laboratory animals. Reported symptoms of

inhalation overexposure include increased respiratory rate, drop in blood pressure, convulsions, anesthesia, and death from paralysis of the medulla and asphyxia. Furan also has an irritating and corrosive effect on mucous membranes and the digestive tract.

The inhalation LC_{50} in mice was reported as $120\,mg\,m^{-3}$ (acute pulmonary edema). The oral LD_{Lo} (lowest published lethal dose) in dogs has been reported as 234 and $140\,mg\,kg^{-1}$ (convulsions or effect on seizure threshold). The inhalation TC_{Lo} (lowest published toxic concentration) in rats has been reported as $200\,mg\,m^{-3}$ per 4 h, $500\,mg\,m^{-3}$, and $5\,mg\,m^{-3}$ per 4 h (CNS disturbance).

Human

Inhalation of furan vapors may produce CNS-depressant effects including headache, nausea, dizziness, drowsiness, and confusion. Acute exposure to high concentrations may produce gastrointestinal congestion, liver and kidney damage, low blood pressure, unconsciousness, and/or death from respiratory arrest. Direct contact with vapors or liquid will irritate or burn the skin and eyes. Oral ingestion may be associated with CNS-depressant effects similar to those following inhalation exposure.

Chronic Toxicity (or Exposure)

Animal

Furan has been shown to be cytotoxic in animal models and may cause cell damage in tissues and organs which show cytochrome P450 oxidase activity. Furan was evaluated for carcinogenicity in rats and mice in a 2 year study by the National Toxicology Program. The primary toxic effects of furan were observed in the liver of treated animals. Cholangiocarcinogma of the liver was observed in all dosed rats and was detected as early as 9 months into the study. The incidence of combined liver carcinomas and adenomas in male rats showed a significant dose-related increase. A significant increase in liver adenomas was observed in treated females. The incidence of combined liver adenomas and carcinomas was also increased for all treated groups of mice. On the basis of animal studies, the International Agency for Research on Cancer has classified furan as being possibly carcinogenic to humans.

Several types of nonneoplastic liver lesions were observed in treated mice and rats. Toxic hepatitis of dose-related severity was noted in all dosed rats, in male mice at doses greater than or equal to $8\,mg\,kg^{-1}$, and in female mice at doses greater than or equal to $15\,mg\,kg^{-1}$. Doses of 2 and $4\,mg\,kg^{-1}\,day^{-1}$ did not produce hepatitis.

Repeated exposure to furan vapors at various concentrations resulted in histopathological liver changes and structural or functional changes in the trachea or bronchi.

Human

Very little information is available concerning the chronic toxicity of exposure to furan. Industrial use of furan is confined to closed systems due to the volatility of the compound; therefore, the potential for direct exposure to furan is limited. The public exposure to commercial furan is minimal.

In Vitro Toxicity Data

Mutagenicity studies conducted using the *Salmonella*/microsome incubation assays gave negative results for furan. The same results were observed when *Salmonella typhimurium* strains were incubated in the presence or absence of rat and hamster liver S-9 fractions.

In vitro treatment of rat or mouse hepatocytes did not induce unscheduled DNA synthesis. However, furan treatment induced gene mutation in mouse lymphoma cells in the absence of metabolic activation. Furthermore, furan was shown to induce sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells both with and without metabolic activation. In a similar study, furan was shown to induce chromosomal aberrations only at comparatively high doses and in the presence of a live activation system.

Clinical Management

If inhalation exposure occurs, the source of conination should be removed or the victim should be moved to fresh air. Artificial respiration should be administered or, if the heart has stopped, cardiopulmonary resuscitation should be administered if necessary. If dermal contact has occurred, contaminated clothing should be removed and the affected area washed with water and soap for at least 5 min or until the chemical is removed. Contaminated eyes should be flushed with lukewarm, gently flowing water for 5 min or until the chemical is removed. If ingestion occurs, vomiting should not be induced. Water should be given to dilute the compound. If vomiting occurs naturally, have the victim lean forward to reduce the risk of aspiration. Aspiration of the compound into the lungs may produce chemical pneumonitis, requiring antibiotic treatment

and administration of oxygen and expiratory pressure.

Environmental Fate

Furan may be released to the environment as a waste industrial product or from unintentional, accidental releases. If released to soil it is expected to volatilize. If released to water, furan is not expected to adsorb to suspended particles and sediment and is likely to volatilize to ambient air. Sulfate-reducing bacteria can degrade furan. However, under non-sulfate-reducing conditions, biodegradation in soil and water is expected to be slow. In the air, furan will exist as a vapor and will be subject to degradation by reacting with hydroxyl radicals.

Ecotoxicology

Feeding or abdominal injection of furan to *Drosophila melanogaster* flies did not induce sex-linked recessive lethal mutations.

TC_{50} for fathead minnow (*Pimephales promelas*) in a flow-through bioassay was reported as follows: 29–31 days, 61 mg l^{-1} per 96 h, at 23.2 °C at a water pH of 8.0 and hardness of 44.5 mg l^{-1}.

Other Hazards

Furan is highly flammable. It can ignite in the presence of flames, heat, and sparks.

Exposure Standards and Guidelines

Given the toxicological properties of furan, the American Industrial Hygiene Association recommends that worker exposure to furan should be minimized to the maximum extent possible.

See also: Tobacco Smoke.

Further Reading

Hamadeh HK, Jayadev S, Gaillard ET, *et al.* (2004) Integration of clinical and gene expression endpoints to explore furan-mediated hepatotoxicity. *Mutation Research* 549(1–2): 169–183.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Furan.

http://ehp.niehs.nih.gov – Furan (Substance Profile from the National Toxicology Program's Tenth Report on Carcinogens, 2002).

Furfural

Richard A Parent

This article is a revision of the previous print edition article by AJ Nordone, DE Sharp, and Richard A Parent, volume 2, pp. 40–41, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 98-01-1
- Synonyms: 2-Furancarboxyaldehyde; 2-Furanaldehyde; 2-Formylfuran 2-furfural; Fural; α-Furaldehyde
- Chemical/Pharmaceutical/Other Class: Five-membered heterocyclic aldehyde

Uses

Furfural is an insecticide, fungicide, and germicide. It has multiple industrial uses, including production of durite, solvent refining of petroleum oils, acceleration of vulcanization, and a component of rubber cements. It is used in the extractive distillation of butadiene. Furfuraldehyde is a synthesis source for furfuryl alcohol, tetrahydrofurfural alcohol, furan, tetrahydrofuran, poly(oxtetramethylene)glycol, and a variety of synthetic resins. It is also used as a constituent of rubber cements, as a synthetic flavoring agent, and as a solvent for synthetic and natural resins.

Exposure Routes and Pathways

Human toxicity can occur as a result of exposure to furfural by ingestion, inhalation, dermally, or via direct eye contact.

Toxicokinetics

Furfural is rapidly absorbed from the gut and lungs and is also absorbed percutaneously following dermal exposure. Metabolism is rapid. The aldehyde

group is oxidized to form furanaryllic acid, the majority of which is conjugated with glycine to produce furoylglycine and furanacryloylglycine. A small portion of the acid is decarboxylated to form CO_2 or condensed with acetic acid.

Furfural has been shown to be distributed throughout the tissues and organs in rats. At 72 h following oral administration, the concentration of labeled furfural and its metabolites was highest in the liver and kidneys and lowest in the brain.

The major route of elimination is via the kidneys as the glycine conjugates, with more than 60% excreted as furoylglycine within the first 24 h after oral administration. Only 3–7% is excreted in the feces and less than 1% is excreted in expired air. The half-life of an oral dose is ~2–2.5 h.

Mechanism of Toxicity

Little is known about the mechanism of action in humans, but some information is available from animal studies. Inhalation exposure in rats is associated with pulmonary irritation, parenchymal injury, and the regenerative proliferation of type II pneumocytes. The activity of acid and alkaline phosphatases and glutamic–pyruvate transaminase is increased, whereas that of arginase and succinate dehydrogenase is decreased. The concentration of lactic acid in the lungs is increased. The activity of glutathione-*S*-transferase is also increased concurrently with a decrease in the concentration of glutathione. After single oral doses given to rats and mice, the effects on the liver are transient and involve scattered eosinophilic globular formation and increased mitotic figures without zonal or massive necrosis. Repeated oral administration results in cirrhotic changes including bridging necrosis and hydropic degeneration of hepatocytes in the parenchyma. Daily intraperitoneal administration to rats caused a time-reversible decrease of respiratory enzyme activity. This results in a degeneration of the processes of reverse resorption in the nephron and may be the cause of the observed functional insufficiency of the kidneys.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal exposures to furfural have demonstrated toxicity via the inhalation and oral routes of administration but little is available relative to its dermal toxicity. Furfural, after ingestion, has been reported to produce central nervous system depression with brain lesions in animals and hepatic cirrhosis in rats. Rabbits exposed by inhalation exhibited hepatic and renal lesions. Inhalation of 260 ppm was fatal to rats but caused no deaths in mice or rabbits. Dogs exposed to 130 ppm furfural in air for 6 h day^{-1} for 4 weeks suffered liver damage but at 63 ppm no such effects were observed.

Ingested furfural has produced liver cirrhosis in rats. Rabbits exposed to vapors for several hours a day manifested hepatic and renal lesions and modifications in blood picture. Administration of a single lethal dose produced a pronounced inhibitory effect on the medullary vegetative centers and brain nuclei with signs of congestion in liver, kidneys, and brain, with degenerative lesions in liver and kidneys.

The inhalation exposure of cats to very high levels of furfural (2800 ppm) for 30 min resulted in death due to pulmonary edema. The oral LD_{50} in rats is between 50 and 100 mg kg^{-1} and the intraperitoneal injection LD_{50} is between 20 and 50 mg kg^{-1}. The symptoms appear to be weakness, ataxia, and unconsciousness. Inhalation exposures (220 mg m^{-3}, 5 h day^{-1}, six times a week for 12 weeks) caused changes in the hypothalamic–hypophyseal–adrenal system of rats which affected bodily adaptation processes. Decreases in brain noradrenaline levels and adrenal gland adrenaline content occurred. Brain acetylcholinesterase concentration increased and urinary 17-hydroxycorticosteroid and 17-ketosteroid excretion decreased. Subchronic inhalation of furfural by Syrian golden hamsters resulted in atrophy and hyperplasia of olfactory epithelium in the nasal cavity, and cats exposed at higher levels demonstrated pulmonary edema.

Human

Furfural can cause skin sensitization and has been shown to cause irritant dermatitis which may become eczematous. It can be absorbed through the skin or by inhalation and it is an irritant to the eyes, skin, and respiratory system. No throat or eye irritation was noted in humans exposed to 10 ppm for 8 h or 20 ppm for 4 h. No data are available relative to reproductive or developmental effects in humans exposed to furfural. When air concentrations reach from 2 to 14 ppm, headaches, itching of the throat, and red/weeping eyes occurred in exposed humans. If exposures are severe, respiratory tract irritation can progress to acute respiratory distress syndrome, which may be delayed in its onset by up to 72 h. The National Institute for Occupational Safety and Health has indicated that 100 ppm in air is a concentration immediately dangerous to life or health.

Chronic Toxicity (or Exposure)

Animal

A 2 year gavage study in F344 rats and B6C3F1 mice by the National Toxicology Program (NTP) indicated some evidence for carcinogenicity. Two male rats showed rare cholangiocarcinomas and two other animals showed bile duct dysplasia with fibrosis. In addition, there was clear evidence of carcinogenic activity in this mouse strain in that there were increased incidences of hepatocellular adenomas and hepatocellular carcinomas in male mice. Hepatocellular adenomas were also increased in female mice. Also, renal cortical adenomas and carcinomas in male mice and squamous cell papillomas of the forestomach in female mice may also have been related to furfural administration.

Human

There is inadequate evidence of carcinogenicity in humans and, consequently, it is characterized by the International Agency for Research on Cancer as a group 3 carcinogen (not classifiable as to its carcinogenicity to humans) whereas the American Conference of Governmental Industrial Hygienists (ACGIH) classifies it as an A3 carcinogen with confirmed evidence in animals but unknown relevance to man.

Workers chronically exposed to the vapor have reported complaints of headaches, fatigue, itching of the throat, lacrimation, loss of sense of taste, numbness of the tongue, and tremor. Occupational exposure is rare due to its low vapor pressure.

In Vitro Toxicity Data

Furfural is definitely genotoxic to mammalian cells. It has been reported to be mutagenic in Salmonella strains TA100 but not TA98 in the Ames test. Chromatid breaks and chromatid exchanges were increased in cultured Chinese hamster ovary cells and was likewise positive for induction of sex-linked recessive lethal mutations in *Drosophila melanogaster* using an NTP protocol. In an assay involving monitoring sister chromatid exchanges in cultured human lymphocytes, both a positive response and a dose–response relationship was reported.

Clinical Management

Gastric lavage may be appropriate if performed soon after furfural ingestion (within 1 h). Administration of activated charcoal should be considered soon after ingestion. For inhalation exposures, the patient should be moved to clean air. Cough, difficulty breathing, bronchitis, and pneumonitis should be checked for. Oxygen should be administered and ventilation assisted as required. Bronchospasm should be treated with inhaled β-2 agonist and oral or parenteral corticosteroids. Severe irritation of the respiratory tract can progress to pulmonary edema with a delayed onset. For direct eye contact, eyes should be irrigated profusely with water at room temperature for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, a physician should be seen immediately. For dermal exposures, contaminated clothing should be removed and the exposed site washed with mild soap. Development of dermatitis or hypersensitivity should be monitored. The affected person should be treated, as appropriate, with corticosteroids or antihistamines.

Environmental Fate

Furfural is a naturally occurring product as well as being a synthetic one. When released into the environment, it volatilizes readily. In the air, furfural is degraded by reaction with hydroxy radicals produced photolytically. If released into the soil, it is highly mobile and has a great potential to leach into groundwater. Volatilization from the soil is expected to be slow. Although data is limited, furfural is expected to biodegrade readily in the soil under both aerobic and anaerobic conditions. Furfural will not bioconcentrate to any great extent in fish or wildlife.

Other Hazards

The flammability of furfural is comparable to kerosene. Its explosive limit potential ranges from 19.3% to 2.1% in air.

Exposure Standards and Guidelines

- Furfural is considered by the Food and Drug Administration to be an indirect food additive related to its use as a component of adhesives (21 CFR 175.105(4/1/88)).
- Occupational Safety and Health Administration permissible exposure limit, 8 h time-weighted average (TWA): 5 ppm with skin designation.
- ACGIH threshold limit value, 8 h TWA: 2 ppm with skin designation.
- ACGIH excursion limit recommendation: 6 ppm for maximum of 30 min.

ACGIH has listed furfural as a confirmed animal carcinogen with unknown relevance to humans (A3).

Miscellaneous

Furfural is a liquid with a pungent almond-like odor. It is found in food items as a natural product. It is soluble in water to the extent of 86 g l^{-1} at room temperature and the log of its octanol/water partition coefficient is 0.41 indicating that it is more soluble in water than in lipophilic solvents. It has a caramel-like taste and boils at 162°C. As a liquid, its density is ~1.16 at room temperature but its vapor density is ~3.3, causing it to settle in low places during an environmental release. Its odor threshold is somewhere between 0.024 and 20 mg m^{-3}.

See also: Pesticides; Sister Chromatid Exchanges.

Further Reading

American Conference of Governmental Industrial Hygienists (ACGIH) (1991) *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th edn. Cincinnati, OH: ACGIH.

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Relevant Websites

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http://toxnet.nlm.nih.gov – GENE-TOX Database.

http://www.osha-slc.gov – Occupational Safety and Health Guideline for Furfural, OSHA, US Department of Labor, online.

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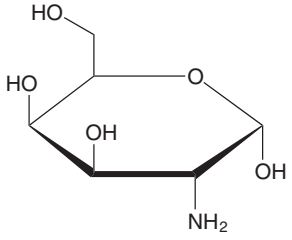
Galactosamine

Udayan M Apte and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 1772-03-8
- Synonyms: 2-Amino-2-deoxy-D-galactose; D-Chondrosamine hydrochloride
- Chemical Formula: $C_6H_{13}NO_5$
- Chemical Structure:



α-Form

Exposure Routes and Pathways

Oral, inhalation, and ocular are possible routes of exposure.

Mechanism of Toxicity

Galactosamine is a model hepatotoxicant, induces hepatitis characterized by neutrophilic infiltration, and kills the animal by fulminant hepatic failure. Galactosamine induces liver injury by interfering with the uridine pool in the cell, which is essential for RNA and protein synthesis. Galactosamine is metabolized via the Leloir pathway of galactoase metabolism, which leads to generation of uridine derivatives of galactosamine. The two enzymes of the Leloir pathway, galactokinase and uridine diphosphate (UDP)-galactose uridyltranserase, convert galactosamine into galactosamine-1-phosphate and UDP-galactosamine, respectively, due to their low substrate specificity. UDP-galactosamine blocks the final enzyme in Leloir pathway, the UDP-galactose-4′ epimerase resulting in accumulation of UDP-galactosamine in the cells. This results in the depletion of uridine triphosphate (UTP), UDP, uridine monophosphate (UMP), and the sugar derivative of uridine such as UDP-glucose and UDP-galactose essential for RNA and protein synthesis. Orotate, a precursor of the hexosamine biosynthesis pathway, has been used as an antidote to galactosamine toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} in mice is 2660 mg kg^{-1} (intraperitoneal exposure). Galactosamine, an amino derivative of sugar galactose, has been used as a model hepatotoxicant since the first reports of hepatotoxicity induced by galactosamine in late 1970s by Keppler and associates. Galactosamine-induced hepatitis has been a model of choice to study various aspects of liver disease including mechanisms of toxicant-induced apoptosis and necrosis, liver tissue repair, neutrophil infiltration and transmigration, and role of endotoxin or lipopolysaccharide (LPS) in initiating liver injury.

Galactosamine induces depletion of UTP, a form of high-energy molecule needed for conducting high-energy requiring procedures, selectively without interfering with other nucleotides such as ATP, CTP, or GTP. Galactosamine treatment results in depletion of important uridine derivatives such as UDP-glucose and UDP-galactose, which play a critical role in glycogen synthesis and RNA production in the cell. Thus, the main mechanism behind galactosamine-induced hepatotoxicity is depletion of cellular uridine uridyl derivatives.

In the last decade, extensive evidence has gathered suggesting involvement of Kupffer cell-mediated inflammatory reactions in Gln-induced liver injury. It is known that Gln leads to activation of Kupffer cells resulting in secretion of proinflammatory cytokines such as tumor necrosis factor (TNF)-α. Increased levels of TNF-α leads to neutrophilic infiltration and cytotoxicity in the liver. Depletion of both Kupffer cells by glycine and gadolinium chloride and of neutrophils by antineutrophil antibodies protect from

galactosamine-induced liver damage. The role of LPS has also been implicated in galactosamine-induced hepatitis. Galactosamine administration has been shown to increase LPS levels in portal circulation, which has been implicated in the neutrophilic infiltration following galactosamine treatment. In recent years, galactosamine in combination with LPS has been extensively used to study inflammatory reactions and neutrophil-mediated liver injury.

Galactosamine and galactosamine + LPS-induced hepatitis are highly reproducible and well-studied models of experimental hepatitis in rodents. A dose of 400–1000 mg kg^{-1} of Gln alone or a dose of 300–700 mg kg^{-1} of Gln in combination with 0.1 mg kg^{-1} of LPS has been successfully employed to induce experimental hepatitis in rodents. In 1950s and 1960s, the galactosamine + LPS-induced hepatitis was considered to represent the experimental model for viral hepatitis but investigations since then have revealed that it is pathologically different from viral hepatitis. In addition to the experimental hepatitis models, galactosamine (300 mg kg^{-1}) has been used in medium-term cancer bioassays as a promoting agent instead of partial hepatectomy.

Galactosamine is one of the hepatotoxicants known to have age-dependent hepatotoxicity (carbon tetrachloride–chlordecone combination, chloroform, and thioacetamide are other examples). It has been reported that neonatal (5 days old) and old rats (24 months old) are less susceptible to galactosamine-induced liver damage as compared to adult rats (5 months old) due to increased liver tissue repair. It is also known that primary hepatocytes isolated from old rats exhibited higher basal levels of UTP, UDP, and UMP in the liver, which may play a role in the protection observed by the old rats. Although the major organ affected by galactosamine is liver, reports of low to moderate kidney and lung injuries are also available in the literature.

Human

No data are available on human toxicity of galactosamine.

Chronic Toxicity (or Exposure)

Animal

Galactosamine has been classified as 'equivocal tumorigenic agent' by Registry of Toxic Effects of Chemical Substances (RTECS) and is a known mutagen in liver cells at high doses. In one study, chronic exposure to galactosamine produced liver tumors after 77 weeks of exposure. Three to four months of exposure to galactosamine induces chronic progressive hepatitis, while 8–10 months of exposure leads to hepatoma and cholangeofibrosis.

Human

No data are available on chronic human exposure to galactosamine.

See also: Liver.

Further Reading

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Gallium

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst and Shirley B Radding, volume 2, pp. 43–44, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-55-3
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Ga^{3+}

Uses

Gallium is the 32nd most abundant element and constitutes 0.0005% of the Earth's crust. It is found most commonly in association with zinc, germanium, and aluminum and is found primarily in the mineral germanite. Gallium(III) is the primary oxidation state for gallium compounds; its chemistry resembles that of aluminum(III). Gallium and gallium compounds have numerous uses in optoelectronics (e.g., LEDs), telecommunication, aerospace, and many commercial and household items, for example, alloys, computers, and DVDs. In addition, gallium is used in special high-temperature thermometers, in place of mercury, and in arc lamps. Medically, gallium alloys are used in dental prostheses, radioactive gallium has been used to locate bone lesions, and nonradioactive gallium has been used as an antitumor agent. Gallium has been used

experimentally as an adjunct to *cis*-platinum cancer chemotherapy. It has also been used to treat hypercalcemia and inhibit bone resorption. Gallium maltolate is under development as a treatment for Paget's disease.

Exposure Routes and Pathways

The most common route of intended exposure to gallium is parenteral injection. Occupational exposure to gallium compounds may occur through inhalation of dust (e.g., gallium arsenide) and dermal contact with these compounds. In semiconductor and solar cell production, indoor gallium arsenide emission losses are relatively high. Because of increased use of gallium compounds in new and developing technologies, exposure to gallium compounds is expected to increase in the future. The many uses of gallium may result in the release to the environment through various waste streams. Gallium is present in parts per million (ppm) concentrations in coal, which may be released into the atmosphere. If released to air, gallium compounds are expected to exist solely in the particulate phase in the ambient atmosphere.

Toxicokinetics

Gallium is not readily absorbed orally, but when administered parenterally it is easily taken up by various tissues. Once absorbed, gallium concentrates in the bone (where it appears to be quite stable). Gallium also concentrates in the liver, kidneys, and spleen but is soon released and excreted in the urine.

There is no information on the effects of gallium on enzymes, either as an agonist or as an antagonist.

Mechanism of Toxicity

Gallium can interfere with the structural integrity of transferrin, the ircin-binding protein that transports iron in the serum. Gallium is believed to bind in the protein methionine. In microorganisms like *Escherichia coli*, gallium suppresses the synthesis of low-molecular weight polypeptides. It also concentrates on the surface of the cell envelope.

Acute and Short-Term Toxicity (or Exposure)

Human

When used as a diagnostic tool, gallium has produced dermatitis in some patients. Gallium can also lead to gastrointestinal distress. There are not any reported cases of gallium toxicity from occupational exposure.

Chronic Toxicity (or Exposure)

Animal

Gallium is a mutagen; in different species of animals, gallium was responsible for renal damage, blindness, and paralysis. Gallium concentrates in tumors in experimental animals.

Human

Reported bone marrow depression may result from radioactivity and not from gallium itself. Gallium nitrate has significant activity as a single agent in the treatment of advanced bladder cancer, especially since it causes minimal myelosuppression, and has activity in patients who did not respond to other treatments. Gallium nitrate is also active in combination regimens for advanced bladder cancer. Evaluation of gallium nitrate in combination with newer agents such as the taxanes or gemcitabine may also be warranted given its activity, different mechanism of action, and nonoverlapping toxicity profile.

Clinical Management

Experimentally, deferoxamine mesylate has been effective in treating gallium toxicity. Ethylenediaminetetraacetic acid and *meso*-2,3-dimercaptosuccinic acid have not been effective.

Environmental Fate

Gallium compounds cannot be oxidized and atmospheric transformations would not be expected to occur during transport. Particulate-phase gallium will be removed from the atmosphere by wet and dry depositions. Gallium compounds are expected to exist as ions in the environment and therefore volatilization from water surfaces is not expected to be an important fate process.

See also: Food Additives; Food and Drug Administration, US; Food, Drug, and Cosmetic Act US

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Gap Junctional Intercellular Communication in Epigenetic Toxicity

James E Trosko and Randall J Ruch

Introduction: "How Does a Physical, Chemical, or Biological Agent Induce 'Toxic' Endpoints in Human Beings?"

The words, toxic, toxicity, toxicology, toxins and toxicants, have been defined many different ways. From the perspective of this short review, we have interpreted the science of toxicology as a multi-disciplinary approach to understand how physical agents (radiation, solid particles), natural (toxins) and synthetic chemicals (toxicants) disrupt critical molecular, biochemical, cellular, physiological, behavioral, and ecological processes that are needed to maintain the normal 'health' of a biological system. Those agents and conditions that irreversibly disrupt the normal health of a biological system would be referred to as 'toxic' agents, and the means by which they bring about toxic effects as their 'toxicity' or 'toxic mechanisms'.

In the case of complex biological systems, such as human beings, health depends on an intricate networking at the molecular (DNA or genes), biochemical (proteins–enzymes; lipids–membranes; carbohydrates; ions, etc.), cellular (neurons, hepatocytes; red blood cells), tissues/organ (liver, brain, skin), organ system (skeletal; respiratory, nervous), physiological (endocrine, immunological), and subconscious and conscious levels. All of these interact with each other and with physical, chemical and environmental factors (dietary, social, and cultural). A toxic agent, by interacting with a complex biological entity, such as a human being, can disrupt that 'cybernetic, hierarchical system' by interacting at any level in such a manner as to cause either immediate (acute) or long-term (chronic) toxicity. Depending on both the genetic background (genetic predisposition or genetic resistance) and the stage of development of the human being (at conception; embryogenesis, fetal, neonatal, sexual maturation, maturation or geriatric stage), the biological or health consequences of the toxic effects will be very different, even though the underlying toxic mechanism could be identical.

Of course, the situation is more complex than just knowing the genetic and developmental status of the individual exposed to the 'toxic' agent. It is also necessary to know how, at the cell level in tissues and organs of an individual, the toxic agent alters the normal behavior of a cell's repertoire, including its ability to divide, differentiate, die by programmed cell death, adaptively respond if it is highly differentiated, or senesce. At this level, cellular exposure to a toxic agent could lead to (1) no discernable effect; (2) mutation (irreversible alteration of the genetic information); (3) death of the cell by either 'necrosis' or programmed cell death (apoptosis), or (4) altered expression of the genetic information. The terms, mutagenicity or genotoxicity, refer to those toxic processes that can lead to either changes at the individual gene level within a chromosome or changes in chromosome morphology (deletions, chromosome exchanges) or chromosome number (polypoidy, aneuploidy) by either damaging the DNA, altering the replication of DNA, or altering the stability of chromosomes.

The terms, necrosis and apoptosis, refer to those toxic process that lead to the death of cells (cytotoxicity). Death of cells can come via many means – by toxic agents that damage DNA (mutagens) or by toxic agents that damage critical functions or structures in cells that have nothing to do with DNA (enzyme inhibitors; membrane disruptors) or by toxic agents that alter expression of genes that are designed to lead to cell 'suicide' or programmed cell death. Some toxic agents can induce apoptosis at nonnecrotic doses but induce necrosis at higher doses. In other words, since most agents at a high enough concentration can lead to cell death via necrosis, there seems to be a relationship between apoptosis and necrosis based on the concentration of the toxic agent. Again, it is not as simple as just the dose of the toxic agent; the cell type within the tissue can determine the cytotoxic consequence of the exposure. All tissues contain a few pluri-potent stem cells (cells capable of giving rise to many cell types of the tissue/organ), many progenitor cells (cells derived from the pluri-potent stem cell but which start to expand the specialized cell type within the tissue/organ), and terminally differentiated cells (highly specialized, non-dividing cells derived from the progenitor cells). These three classes of cells usually have differential sensitivity to cytotoxic agents, as is seen with the stem cells of the small and large intestine.

The term, 'epigenetic toxicology', was coined to refer to processes that alter gene expression after exposure to toxins or toxicants but do not cause mutagenicity or cytotoxicity. These epigenetic toxicants, by altering gene expression, can disrupt the repertoire of a cell's behavior. Cells have a choice of:

(1) staying as is; (2) dividing; (3) differentiating; (4) apoptosing; (5) adaptively responding if terminally differentiated; or (6) senescing. Cells normally make the choice to divide, differentiate, apoptose, adaptively respond or senesce after endogenous (hormones, growth factors) agents trigger intracellular signals within target cells. At the same time these agents alter one of the critical biological structures/functions of most normal cells within tissues/organs, namely that of gap junctional intercellular communication (GJIC).

By either increasing or decreasing this fundamental biological process, gene expression will be altered so the resting cell can alter its state to proliferate, differentiate, apoptose, adaptively respond, or senesce. This can be an adaptive response such as growth, maturation, or wound healing. On the contrary, it can be a maladaptive response if the modulation of GJIC occurs at inappropriate times or for inappropriate durations. To understand the role of GJIC in the field of toxicology, as this is a rather new concept of toxicity, we review the basic biology of gap junctions in the following sections.

Gap Junctional Intercellular Communication: Regulator of Cellular Homeostasis

Humans are composed of ~100 trillion cells, each of which starts out genetically identical but end up unique and organized in subgroups of similar cells (tissues) that perform the functions necessary for the maintenance of the whole organism. The fertilized egg from which each human grows contains a unique set of ~30 000 genes inherited from the parents that provides the foundation on which development and functioning rest. As this egg develops into the complex human organism and this organism matures, cells must respond and adapt to the environment. This requires cells to proliferate, differentiate, repair damage, die, or change in other ways. These response processes must be highly organized for organisms to grow and function properly and for this to happen, the cells that make up that organism must be able to communicate with one another rapidly and effectively. Considering the importance of these processes, it is not too surprising that there is more than one type of cell–cell communication and that the interactions among these types have become increasingly sophisticated over evolutionary time.

There are three different types of cell–cell communication: extracellular, intracellular, and GJIC. Extracellular communication occurs when cells release ions and molecules (growth factors, neurotransmitters, hormones, etc.) into the surrounding medium that are sensed by contiguous cells or into the blood where they are carried throughout the body and sensed by more distant cells. Usually receptors on the cell membranes of cells serve as sensors for these extracellular chemical signals although communication may also occur without a receptor. In such cases, the communication factor can move through the cell membrane into the cytoplasm. The receipt of these molecular signals by a receptor cell activates a cascade of signals inside this cytoplasm causing the cell to modify its activity (adapt), change its function (differentiate), divide, or die. The functioning of this cascade of signals within cells is known as intracellular communication.

Overlying both extracellular and intracellular communication is a third form of cell–cell communication, known as GJIC. This type of communication enables cells to exchange molecular and ionic signals directly through passageways, known as gap junctions, that connect the cytoplasms of contiguous cells. This exchange allows each cell to synchronize its response with that of other adjacent cells.

Figure 1 illustrates the relationships between the three forms of cell–cell communication in a tissue containing a stem cell, progenitor cells coupled by gap junction channels, and a terminally differentiated cell. The stem cell communicates with the progenitor cell through the extracellular substrate triggering intracellular signals inside the latter cell. As the progenitor cell is in surface contact with the stem cell, communication can also occur through cell adhesion molecules. All progenitor cells also communicate intracellular signals to their sister progenitor cells via gap junction intercellular channels. These intracellular signals stimulate the progenitor

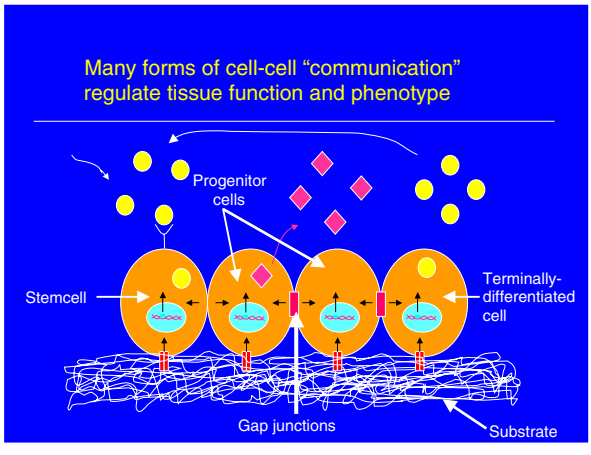

Figure 1 Illustration of how three different kinds of cells within a tissue – the stem cell, progenitor cells coupled by gap junction channels and the terminally differentiated cell – communicate with each other via signals from the substrate, cell-adhesion molecules, soluble secreted factors, and gap junctions.

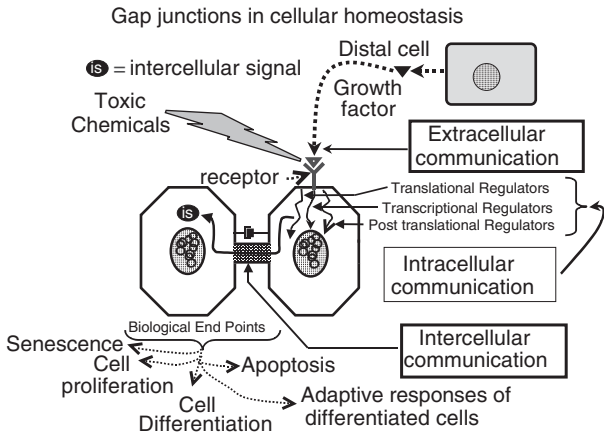

Figure 2 General picture of how 100 trillion cells of the body communicate with each other to maintain homeostatic control of cell functions. (Reproduced from Yamaski H (1990) Gap junction intercellular communication in carcinogenesis. *Carcinogenesis* 11: 1051–1058, with permission from Oxford University Press.)

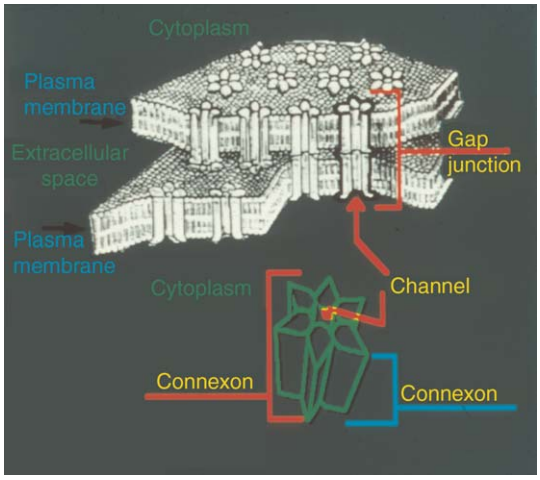

Figure 3 Components of a gap junction linking the cytoplasm of contiguous cells. (Reproduced from Yamaski H (1990) Gap junction intercellular communication in carcinogenesis. *Carcinogenesis* 11: 1051–1058, with permission from Oxford University Press.)

cells to secrete extracellular communication factors that can enter the bloodstream and communicate with distant cells that have receptors for those specific factors; for example, hormones and growth factors. The progenitor cell can also communicate with its terminally differentiated daughter cell via gap junctions. Lastly, the terminally differentiated cell, by receiving signals through both the extracellular substrate and by the GJIC signal, is stimulated to secrete a signal that is sent back to the original stem cell.

Figure 2 provides a general picture of the interplay among the three types of cell–cell communication. The three cells represent gap junctionally coupled cells in a tissue communicating with a cell in a distant tissue via an extracellular-secreted factor; for example, hormones and growth factor. The extracellular signaling molecules then trigger intracellular signals in the target cell. These intracellular signals can subsequently be transferred to the neighboring cell via gap junctions. Depending on the nature of the extracellular stimulus, the intracellular communications can lead to either an increase or decrease in the cell's ability to transfer the signals via gap junctions to a contiguous cell. These increases and decreases in GJIC lead the cell to proliferate, senesce, terminally differentiate or die of a programmed cell death (apoptosis).

The Structure of Gap Junctions

The cells in a human body are organized into tissues; in turn, groups of tissues make up organs. Cells are attached to each other and to the human skeleton by several kinds of cell–cell junctions. These junctions not only hold cells together, but also form barriers to other cells and molecules and, in some cases, act as sensors that detect signals from other parts of the organism. Gap junctions are unique types of cell–cell junctions because they have pores or channels that connect the cytoplasms of contiguous cells; other junctions do not have such channels.

As can be seen in **Figure 3**, gap junctions are made up of 'hemi-channels' or connexons consisting of six proteins called connexins that are coded by an evolutionarily conserved family of genes. When two hemi-channels or connexins unite across the extracellular space between two neighboring cells, they form a complete channel that allows ions and small molecules to move directly from one cell to the other without having to enter the extracellular space. The connexins on opposite cells are attracted to each other in a poorly understood way and connect or 'dock' tightly together. In most gap junctions, several complete channels cluster in one small region of the cell membrane resulting in a gap junction 'plaque'.

Figure 4 is an electron micrograph depicting a portion of a coupled pair of cells in which a cross-section of a 'plaque' or island of hundreds of coupled connexons aggregate to form a 'gap junction'. Each cell can have varying numbers and sizes of these gap junction plaques, depending on the physiological state of the tissue.

Gap junction channels are very small – approximately 1.5–2 nm in diameter. As illustrated in **Figure 3**, two connexons and 12 connexins make up one complete gap junctional channel. The human genome contains genes for ~20 different kinds of

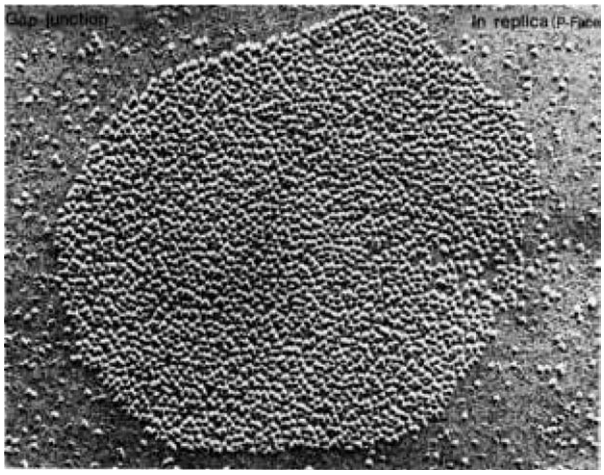

Figure 4 Freeze-fracture micrograph illustrating the gap junction plaque in the membrane between two cells.

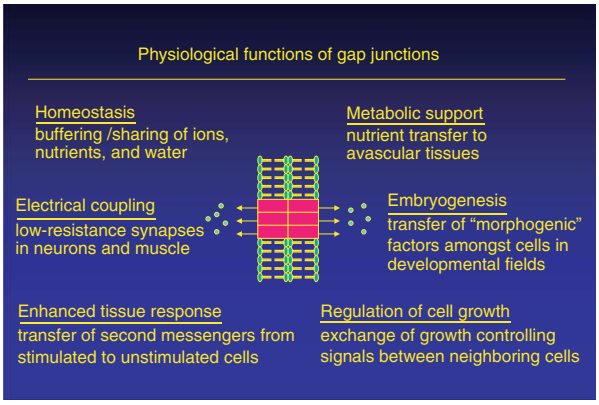

Figure 5 Cellular/tissue functions linked to gap junction function.

connexins so there are many potential combinations of connexins that can make up gap junction channels.

Gap Junctional Intercellular Communication

Once two connexons are docked properly, the complete channel will open and ions and molecules can freely move between contiguous cells. However, gap junction channels allow only very small molecules to pass through. Amino acids, water, simple sugars, and most intracellular signal molecules freely move through gap junction channels but larger molecules, such as proteins and fats, cannot.

This cell–cell movement of ions and molecules through gap junction channels is known as GJIC. It is critical to the health of cells and the entire organism. GJIC helps balance and maintain cellular levels of critical ions and nutrients, helps supply neighboring cells with raw materials needed for synthesis of macromolecules, and helps coordinate cellular functioning. Within most tissues, several gap junction plaques, each composed of hundreds to thousands of channels, connect the cells. In cells that are well connected by gap junctions, ions and molecules move rapidly between cells so that within tissues, groups of cells respond more like a functional unit than individual cells. The GJIC provides for the rapid, direct flow of molecular and ionic information between cells that serves to synchronize the functions of multicellular tissues.

GJIC has been implicated in all of the five basic fates that cells may have: remaining quiescent, proliferating, differentiating, committing programmed cell death (apoptosis), and, in already differentiated cells, adaptively responding. Each of these fates is critical for the development and maintenance of organismal structure and function. Both proliferation and differentiation are needed during growth and repair. Terminally differentiated cells must be able to adapt to changing conditions; for example, insulin cells responding to blood sugar levels. Cells in solid tissues must undergo apoptosis to facilitate tissue modeling or replacement of old cells with new, healthier ones. Lastly, some cells are destined to senesce and become nonproliferating, low activity, end-stage cells. One example of the role of GJIC in determining the fate of cells is the importance of gap junctional communication between sperm cells and Sertoli cells. This communication triggers the differentiation process of the spermatocyte into a mature sperm.

The situation in an organism is even more dynamic than might be suggested by the above summary. Cells uncouple and recouple with each other continuously as part of normal cellular functions. When cells are stimulated to divide by an extracellular growth factor, gap junction channels close. For proliferation to occur, gap junctions must be deactivated so that molecular signals that normally keep the stimulated cell inactive are blocked. Once the cell divides, and there is no longer any growth stimulus, the daughter cells form active gap junctions with contiguous cells.

Figure 5 illustrates a number of the physiological functions that are dependent on GJIC, functions that reflect its role during embryogenesis, development of the fetus and neonate, sexual and adolescent maturation and adult functioning of both electrically coupled tissues; for example, heart, and nonelectrically coupled tissues; for example, liver.

The importance of GJIC in the performance of these physiological functions is a reflection of the impact it has on individual cells. GJIC helps control

the rates of cell births and deaths so that tissue size and activity remain constant. It also helps trigger cellular differentiation. The passage of intracellular signals from stimulated to nonstimulated cells increases the overall response of the tissue to a stimulus. In addition, gap junctions serve as electrical pathways to connect muscle cells; for example, heart, uterus and digestive tract and nervous tissue. Thus, GJIC is critical for normal heartbeat, birth, digestion, brain activity, and other functions. Gap junctions are also important pathways for the interchange of nutrients and waste products in tissues that are not well supplied with blood vessels, such as the lens of the eye and hardened bone.

Epigenetic Toxicity as a Result of Inappropriate Modulation of Gap Junction Function

As might be expected from the range of activities and functions associated with gap junctions, abnormalities in GJIC can lead to a variety of adverse effects. When an individual inherits a mutated form of a connexin gene, the cells that express that gene will be unable to form normal gap junction channels. Human diseases that are linked to the inheritance of a mutant connexin gene include forms of nerve degeneration, deafness, diabetes, cataracts, cancer, birth defects and skin disorders. Adverse effects can also result if exposures to environmental chemicals alter the numbers or functions of gap junctions. **Figure 6** illustrates the variety of conditions associated with defective gap junctions.

Understanding the adverse impacts of gap junction defects has increased with the availability of modern genetic study techniques. For example, mice can be produced that have one of more of their connexin genes permanently inactivated (connexin 'knock-out' mice). Investigations using these mice have shown that inactivation of certain connexin genes leads to the death of the embryo or fetus before birth. When other connexin genes are knocked out, embryonic and fetal development occur normally but, shortly after birth, the mice die because of defects in the organs in which the knocked-out connexins should have been expressed. Finally, when yet other connexin genes are knocked out, the mice survive to adulthood but then develop diseases such as peripheral neuropathy, liver cancer, and cataracts.

Epigenetic alterations of GJIC have also been linked to toxic actions of synthetic and natural agents and human disease. Traditional toxicological studies on adverse effects of a variety of chemicals in the laboratory setting have shown that birth defects can occur from exposure to drugs that affect gap junctions; for example, alcohol and thalidomide. In addition, some carcinogenic agents alter gap junction formation in cell-and connexin-specific ways. These include natural chemicals such as Croton oil; pollutants such as polybrominated biphenyls; drugs such as phenobarbital; nutrients such as unsaturated fatty acids, retinoids, and carotenoids; pesticides such as DDT; metals such as cadmium; hormones such as estrogens; and growth factors such as epidermal growth factor. These toxic agents affect GJIC by inappropriately opening or closing gap junction channels, altering gap junction formation and connexin stability, and changing the expression of connexin genes.

These examples do not prove that chemical alteration of gap junctions is the causative factor of a disease as correlation does not mean causation. But as several known human diseases are due to the inheritance of a mutant connexin gene, it is very likely that chemical alteration of GJIC contributes to non-inherited forms of these and other diseases.

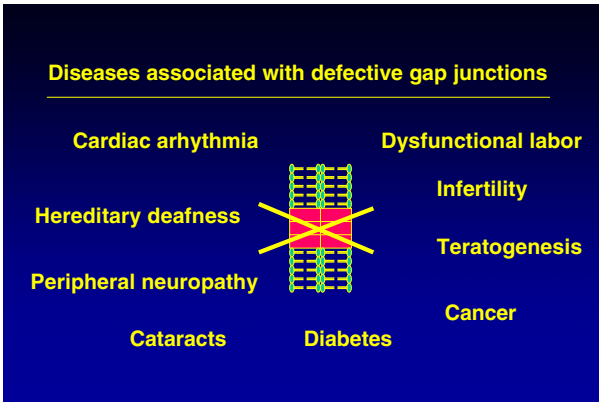

Figure 6 Disease states correlated with defective gap junction function.

Measuring Gap Junctional Communication

Techniques have been developed to determine the degree to which cells are communicating with each other via gap junctions. One of these is called the scrape load/dye transfer assay and it is illustrated in **Figure 7**. The lower right panel is a phase contrast micrograph of normal rat liver cells through which a razor blade cut a path while the cells were immersed in a fluorescent dye solution. When the cells along the cut line were cut, they transiently open and take up the fluorescent dye. The dye then diffuses to neighboring cells through gap junction channels. The panel at the top left shows that cells that were not

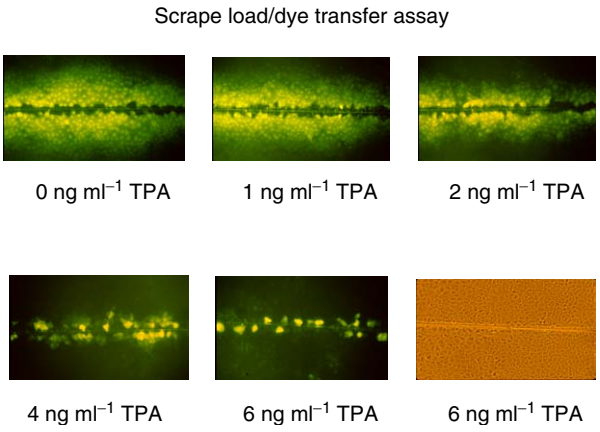

Figure 7 Utility of the scrape loading/dye transfer assay to detect a toxin/toxicant's ability to modulate gap junctional intercellular communication. The last two images (6 ng ml^{-1} TPA) illustrate both the epifluorescent and phase images, respectively, of the same cells to show that the complete inhibition of GJIC was not associated with the death of cells.

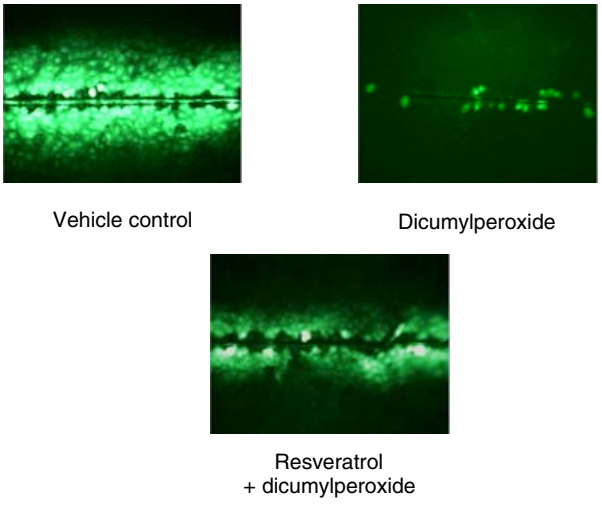

Figure 8 Use of the scrape loading/dye transfer assay to screen for agents that can prevent inhibition of gap junction function.

treated with any chemical could transfer the fluorescent dye to several neighboring cells. In a period of 5 min, the dye went from the cut edge cells to ~10 cells away from the edge. This demonstrates that normal, control rat liver cells communicate extensively via gap junctions. In the subsequent panels labeled with increasing concentrations of TPA (a powerful skin tumor promoter), one sees a dramatic dose-related reduction in the ability of the cell to transfer the fluorescent dye from the cells along the cut edge. This demonstrates that TPA can inhibit GJIC in a threshold, but dose-dependent fashion.

Figure 8 illustrates how this technique can be used to show that some chemicals can protect against the inhibition of GJIC. The top left panel shows that normal, control liver cells transferred the fluorescent dye to about 10 cells away from the cut edge. The top right panel shows that treatment of these cells with noncytotoxic concentrations of another tumor promoter, dicumyl peroxide, completely inhibited the transfer of the fluorescent dye. The bottom panel demonstrates that cells treated with dicumyl peroxide and an antioxidant found in grapes and red wine, resveratrol, had higher levels of GJIC than cells treated only with dicumyl peroxide. This shows that resveratrol protected against damage to gap junctions and suggests that it might be a cancer chemopreventive agent.

Summary

In summary, GJIC enables cells to rapidly share critical ions and molecules that influence whether cells remain active, proliferate, differentiate, commit apoptosis, or adapt in response to external stimuli. In addition to effects on individual cells, this form of intercellular communication synchronizes the activities of cells within a tissue. Thus, when gap junctions are altered in inappropriate ways by endogenous or exogenous factors or conditions, a variety of toxic outcomes and diseases may result.

While extracellular and intracellular communications have long been subjects of study as part of the disciplines of physiology and biochemistry, GJIC is a relatively new area of research. Thus, it is expected that as new research findings are produced, it will be possible to identify the mechanisms by which disease-causing agents alter GJIC and to develop techniques to prevent or correct the resultant adverse effects. It is also likely that in the near future these techniques will be viewed as important tools for improving human health.

This central role of GJIC in normal physiology and disease necessitates that toxicologists understand if and how toxic agents affect intercellular communication to fully understand toxic processes and mechanisms. It is certainly important and appropriate to determine how a toxic agent impinges upon a specific molecule or intracellular process, but one cannot fully understand toxic mechanisms in a multicellular organism without studying GJIC. Fortunately within the past decade, toxicologists have begun to move away from the investigation of single molecules and discrete pathways to study global changes in gene expression, protein activities, and signaling cascades. Advances in genomics, proteomics, and bioinformatics have greatly facilitated this. Still, however, there has been relatively less study of GJIC in toxicology. Clearly, toxic agents alter GJIC, and it is likely that

investigation of such actions will lead to a much greater holistic understanding of toxic mechanisms and risk.

See also: Genetic Toxicology; Toxicity Testing, Mutagenicity.

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Gasoline

Michael A Kamrin

- Chemical Abstracts Service Registry Number: CAS 8006-61-9
- Synonyms: Gas; Motor fuel; Motor spirit; Petrol; Casing head gasoline
- Chemical Formula: Gasoline is a mixed compound that does not contain a fixed ratio of component compounds. However, alkanes constitute the largest percentage of component compounds, followed by aromatics and alkenes

Uses

Gasoline is used almost exclusively as a fuel for automobile and other internal combustion engines.

Background Information

Gasoline is a product of petroleum refining that varies in composition and often includes additives such as antiknock agents, antioxidants, lubricants, and detergents. Tetraethyl lead was one of these additives, and use of leaded gasoline as fuel was responsible for much of the human body burden of this metal for a number of years. However, the phase out of lead from gasoline during the past three decades (in the United States) has led to an over 90% reduction in human blood lead levels. More recently, other additives such as methylcyclopentadienyl manganese tricarbonyl and methyl *t*-butyl ether have been foci of concern because of possible adverse environmental impacts of these compounds.

Exposure Routes and Pathways

The major route of exposure to gasoline components and their combustion by-products is inhalation. Skin

exposure may also occur during handling of gasoline. Ingestion of contaminated groundwater is another potential route of exposure but is generally not toxicologically significant since only very low levels of gasoline components have been found in drinking water.

Toxicokinetics

Since gasoline is a mixture, relevant toxicokinetics information must reflect not only the characteristics of the individual gasoline components but also any interactions among them. Thus, the toxicokinetics of various components of gasoline are not sufficient to understand the mixture. Some information on gasoline, itself, is available; e.g., percutaneous absorption is slow and absorption from the respiratory tract is more efficient than absorption from the gastrointestinal tract. However, many other pieces of toxicokinetic data are lacking so that the toxicokinetics of gasoline is presently poorly characterized.

Mechanism of Toxicity

Little information is available on most of the mechanisms of toxicity of gasoline. It has been suggested, however, that renal effects in rats are mediated by $\alpha 2\mu$-globulin and thus of little relevance to humans who do not produce this compound.

Acute and Short-Term Toxicity (or Exposure)

Animal

Gasoline is an irritant.

Human

Gasoline is an eye irritant and may also cause damage to the skin, lungs, and the intestinal mucosa at high exposure levels. At such levels, it may also cause neurotoxicological effects such as dizziness, nausea, and headache as well as adverse effects on the cardiovascular system. At high enough levels, e.g., adult ingestion of several hundred grams, it may cause coma or even death.

Chronic Toxicity (or Exposure)

Animal

In laboratory studies, chronic gasoline exposure has been linked to renal tumors in male rats and liver tumors in female mice. However, the applicability of these results to humans is questionable.

Human

Although there have been numerous epidemiological studies of workers exposed to gasoline, the results are inconclusive with regard to a link to cardiac toxicity, neurotoxicity, or any form of cancer. However, some of the components of gasoline, e.g., benzene, are classified as known human carcinogens. In addition, intentional excessive exposure, e.g., from gasoline sniffing, can lead to adverse neurological and renal effects.

In Vitro Toxicity Data

Studies of bacterial and mammalian cells in culture indicate that gasoline is not mutagenic.

Clinical Management

Victims exposed only to gasoline vapors are not contamination risks; however, those whose clothing has been contaminated with liquid gasoline are. To decontaminate victims, exposed skin and hair should be flushed with plain water for 2–3 min and then washed with mild soap. Thorough rinsing with water should be undertaken. Exposed or irritated eyes should be irrigated with plain water or saline for 15 min. If gasoline has been ingested, emesis should not be induced or gastric lavage and activated charcoal should not be used. Catharsis with magnesium or sodium sulfate is acceptable.

If vomiting occurs, pulmonary aspiration should be watched for. In cases of respiratory compromise, airway and respiration should be secured via endotracheal entubation. Patients who have bronchospasm should be treated with aerosolized bronchodilators. Epinephrine or related substances should not be administered. There is no antidote for gasoline. Treatment should support respiratory and cardiovascular functions.

Environmental Fate

Since gasoline is a mixture, no simple summary can address the fates of all of the components. However, many of the toxicologically significant components are volatile and so are lost to the atmosphere after being released to surface soil or surface water. These compounds are then subject to photochemical oxidation. In addition, these components can leach through the soil and contaminate groundwater where they may remain for long periods of time. Under aerobic conditions, biodegradation of gasoline components can occur in soil and surface water.

Other Hazards

Gasoline is flammable at room temperature. It also poses a danger from explosion.

Exposure Standards and Guidelines

The permissible exposure limit time-weighted average for gasoline in workplace air is $900\,mg\,m^{-3}$ (300 ppm). Federal limits for gasoline in drinking water and air have not been promulgated although such limits exist for some gasoline components; e.g., benzene. However, some states and municipalities have promulgated acceptable ambient air concentrations for gasoline.

See also: Manganese.

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Automotive Gasoline.

Gastrointestinal System

M Joseph Fedoruk and Tee L Guidotti

Introduction

The gastrointestinal (GI) system or tract begins with the mouth and ends in the anus. The primary function of the GI system is the digestion and absorption of food, including solids and liquids, and the provision of a barrier to many potentially harmful ingested substances. Digestion is the breakdown or hydrolysis of food into smaller molecules in preparation for absorption into the body. Absorption is the transport of nutrients across the intestinal cell. The GI system also includes the exocrine pancreas which secretes proteolytic digestive enzymes into the duodenum, which facilitates the digestion of sugars, protein, and fats. The endocrine functions of the pancreas are considered elsewhere in this encyclopedia. Bile salts produced by the liver also play a critical role in the absorption of fats and fat-soluble vitamins and the effects of xenobiotics on bile metabolism. The liver is considered an integral part of the GI tract but has a broad range of metabolic functions in addition to its role as an organ supporting digestion. With respect to blood flow, the liver is positioned in a circuit between the stomach and small intestine and the rest of the body, receiving venous blood directly from them via the portal system and draining into hepatic veins into the systemic circulation. As a consequence, the liver metabolizes many xenobiotics absorbed by the intestine before they reach the system circulation and is a target organ for toxic or metabolically activated xenobiotics delivered to it from the digestive organs of the GI tract.

The GI tract provides the second largest surface area for direct contact of xenobiotics after the lung. Agents can contact the GI tract directly after oral ingestion and through the swallowing of particles that have been cleared from the respiratory tract by mucociliary clearance. Other potential routes of contact include direct introduction into the rectum, which is a route of administration for certain drugs. Agents metabolized by the liver and excreted in bile come into direct contact with the small intestine and can be continually recirculated because of enterohepatic circulation.

From a toxicological perspective, the GI tract is an important organ system since it is the initial site of contact of many environmental agents including food contaminants which have the potential to produce a broad array of toxicological effects. The GI tract is the target organ for a significant number of poisonings due to either inadvertent ingestion of medications, household products, and other items by children or intentional ingestion of poisons by adults during suicide attempts. GI symptoms are also common manifestations of systemic toxicity from a wide variety of toxic agents. Knowledge and recognition of such symptoms can be essential in identifying a toxic condition.

This entry provides an overview of the anatomy and physiology of the GI system and later describes the type of toxic effects that can be observed with different classes of agents.

Anatomy and Physiology

The GI tract is composed of several segments that have specialized functions with respect to digestion and absorption but share common anatomical and histological features. The GI tract can be considered as, and is embryologically derived from, a tube composed of several layers. The tube consists of (1) an inner mucosal layer that contains mucus-secreting cells and the specialized cells; (2) a submucosal layer that consists of loose connective tissue containing blood and lymphatic vessels, inflammatory cells, and nerve fibers; (3) a muscular layer containing many smooth muscles that is responsible for peristalsis and emesis; and (4) a serosal layer or covering. The specialized cell types vary in each GI tract segment and reflect the specialized function of each GI segment with respect to digestion and absorption. The individual segments are discussed below.

Oral Cavity and Pharynx

This segment of the GI tract includes the mouth and tongue, the pharynx, and oropharynx. The primary functions of the oral cavity are (1) as a processing and storage place for food to enable chewing and mixing of food with salivary enzymes, and (2) as a passage for transport to the esophagus. Salivary amylase helps break down starch. The oral cavity is lined by stratified squamous columnar epithelium, which is keratinized in areas subject to a high degree of mechanical friction such as the tongue and palate. The pharynx is also lined by stratified squamous columnar epithelium, but unlike the oral cavity it has striated muscle that is not under voluntary control. The connective tissue surrounding the cavity is loose and highly vascularized. When presented with antigenic stimuli, this tissue can become edematous quickly and lead to obstruction of the airway, a potentially life-threatening condition.

The oral cavity is also the site of a rich commensual bacterial flora that elaborates ammonia, which up to a limit neutralizes acid-forming gases inhaled through the mouth.

Esophagus

The esophagus is a musculomembranous conduit that extends from the pharynx to the stomach and is approximately 23–25 cm in length in an adult. It is lined by stratified squamous columnar epithelium and surrounded by a muscular layer composed of longitudinally arranged smooth muscle bundles. The submucosa contains many nerve fibers. It also contains submucosal glands, which are mainly present in the lower and upper portions of the esophagus and are thought to be continuations of the minor salivary glands in the oropharynx.

The primary functions of the esophagus are the transport of solid and liquids into the stomach and the prevention of retrograde flow or reflux of gastric contents. Aspiration of gastric contents into the lung can produce serious lung injury. These functions require coordinated esophageal motor activity. Manometric pressure studies have revealed that the esophagus has two areas of increased pressure or sphincters that function to prevent reflux. An upper sphincter at the level of the cricopharyngeal muscle remains closed most of the time due to its elastic properties and the tonic contraction of the cricopharyngeal and inferior pharyngeal muscles. A lower esophageal sphincter just proximal to the gastroesophageal junction also remains closed much of the time. Both sphincters must relax in response to a peristaltic wave and later increase in pressure to prevent gastric reflux.

The control mechanisms for the lower esophageal sphincter are complex and not well understood. The sphincter is innervated by preganglionic parasympathetic fibers of the vagus nerve and postganglionic inhibitory and excitatory neurons of the symptomatic nervous system. However, vagotomy or surgical cutting of the vagus nerve does not abolish sphincter tone. Many agents can decrease lower esophageal tone such as cholinergic muscarinic agonists, gastrin, and α-adrenergic agonists. Other substances, such as nicotine, β-adrenergic agents, nitric oxide, and dopamine, cause a decrease in the sphincter tone. It is not clear whether these agents play a role in the normal functioning of the tone of the lower esophagus or whether the effects are pharmacological. Smoking and high-fat meals lead to a decrease in tone and can produce symptoms of heartburn, which is due to reflux of gastric contents into the esophagus. Chronic reflux is a risk factor for esophageal cancer.

Stomach

The key function of the stomach, a bag-like widening of the digestive tract, is to receive food and to secrete gastric acid and pepsin (an enzyme to digest food); however, very little absorption of food takes place in the stomach, except for some lipid-soluble substances such as alcohol. In humans, the stomach is composed of four main segments: the cardia, the fundus, the body, and the antrum (**Figure 1**). The cardia, a narrow portion just distal to the gastroesophageal junction, is primarily lined with mucus-secreting cells. The fundus is the proximal portion that lies above the gastroesophageal junction and is largely composed of cells that secrete mucus and hydrochloric

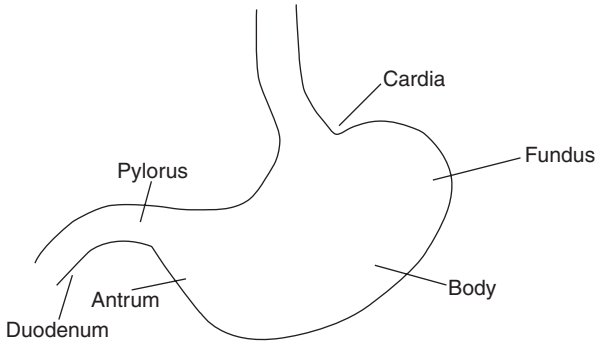

Figure 1 The stomach.

acid. The body is the part that lies proximal to the angle above the lesser curvature and contains similar cells. The antrum is located distal to the angle of the lesser curvature and is demarcated from the duodenum by the pyloric sphincter. The stomach also contains a muscular layer which facilitates mixing of the stomach contents with hydrochloric acid and pepsin. The surface of the stomach has coarse rugae, which are infoldings of the submucosa and mucosa that provide a larger area for digestion.

The stomach is lined with a mucosal surface that is punctuated by gastric pits leading to gastric glands. Foveolar cells, which secrete mucin, line the surface of the stomach and the gastric. These are tall and columnar cells that contain clear mucin-containing granules. Neck cells are located in the gastric pits, which are probably progenitor foveolar cells. Various glands empty into the gastric pits, including cardiac glands in the cardia stomach section, which mainly secrete mucus; gastric glands in the body and fundus, which contain large numbers of parietal cells; and pyloric or antral glands in the antral stomach, which contain large numbers of mucus secretory cells.

The individual cell types reflect specialized gastric digestive functions. The stomach secretes hydrochloric acid and pepsinogen, which not only digest food but can also damage gastric tissues which must be protected from these factors. Hydrochloric acid is secreted by approximately 1 billion parietal cells located in the fundus and body of the stomach secrete. The cells are interspersed along the course of mucous glands and secrete hydrochloric acid at a concentration that is approximately 3 million times that found in blood. Parietal cells contain large numbers of intracellular tubulovesicular structures derived from the endoplasmic reticulum. The endoplasmic reticular membranes contain a hydrogen–potassium ATPase that pumps hydrogen ion across a membrane in exchange for potassium.

Proteolytic proenzymes pepsinogen 1 and 11 are released by chief cells which are located at the base of the gastric glands found also in the body and fundus of the stomach. Chief cells have morphological features of cells that synthesize protein and are characterized by an extensive endoplasmic reticulum and numerous apical secretory granules. Pepsinogen is activated by the stomach's low pH environment and is inactivated by the high pH ($\geqslant 6$) in the duodenum.

Mast cells and enterochromaffin-like cells found in the interstitium and among parietal cells contain histamine, which acts on parietal cell receptors to stimulate the release of hydrochloric acid. The histamine receptor on parietal cells is designated as H2 and is blocked by H2 blockers such as cimetidine which are widely used to treat peptic ulcers.

The factors associated with the regulation of gastric acid secretion are complex and involve chemical, neural, and hormonal influences. The stomach and small intestine contain several endocrine cells that affect the release of gastric acid. The most important factor that stimulates the release of hydrochloric acid is gastrin, a hormone that is released into the circulation by G cells which are located in the epithelial lining of the pyloric glands in the antrum of the stomach, duodenum, and proximal jejunum. Gastrin is released in response to food in the stomach and small intestine. Stimulation of the vagus nerve results in the release of acid via the muscarinic cholinergic receptors located on the parietal cells. Vagal stimulation is also thought to stimulate the release of gastrin into the circulation and lower the parietal threshold for releasing gastrin into the circulation.

Gastric acid secretion can be inhibited by several mechanisms including acid in the stomach (pH 3 inhibits gastrin release), acid in the duodenum, the presence of fat in the pancreas, and hypertonic fluids or hyperglycemia. Somatostatin, a hormone produced by antral mucosal endocrine cells (D cells), inhibits the release of gastrin by directly inhibiting the parietal cells. Somatostatin is also present in other GI tissue and the pancreas. C cells, endocrine cells in the proximal small intestine, secrete secretin in response to mucosal acidification, which also decreases gastric secretion.

The stomach has several protective mechanisms against hydrochloric acid and pepsinogen. The primary defense is the presence of gastric mucus, a large polymeric glycoprotein that is secreted by mucus glands throughout the stomach. Gastric mucous exists in two phases: (1) an insoluble mucus gel layer that coats the stomach and has a low diffusion coefficient for H^+ and (2) in gastric juice as a soluble phase. Mucus secretion is enhanced by cholinergic stimulation of muscarinic receptors and occurs in response to mechanical and chemical irritation of the stomach. Other protective mechanisms exist,

including the secretion of bicarbonate by nonparietal cells via carbonic anhydrase, and are present in foveolar and parietal cells. Gel thickness increases in response to secretion of E series prostaglandins and is decreased by nonsteroidal, anti-inflammatory medications. The other protective mechanism is tight cell junctions between surface epithelial cells, which are almost impermeable, to back diffusion of hydrochloric acid or pepsin. Prostaglandins of the E series which are in the gastric mucosa are thought to play a role by stimulating the secretion of gastric acid mucus and bicarbonate and by maintaining blood flow to the gastric mucosa which is necessary to maintain the integrity of cell surfaces and promote epithelial renewal.

Gastric acid secretion is not a primary cause of peptic ulcer disease except in extreme conditions of overproduction (the Zolinger–Ellison syndrome). However, once defenses against acid are breached, gastric acidity plays a role in perpetuating the ulcer. More significant is infection with *Helicobacter pylori*, a small flagellated gram-negative spiral bacillus that produces urease, which serves to neutralize acidity in the gastric mucus, where it is found. *H. pylori* infection, although associated with an increased risk for peptic ulcer and gastric carcinoma, is so common worldwide as to be considered commensual. Peptic ulcer is now routine treated with antibiotics directed against *H. pylori*.

Small Intestine

The primary functions of the small intestine are digestion and absorption of food. The adult small intestine is approximately 6 m in length and is composed of the duodenum, ileum, and jejunum. Digestion occurs primarily in the upper small intestine and requires the action of pancreatic enzymes such as amylase, lipase, and trypsin, which are released from the pancreas into the duodenum, and bile salts from the biliary system. Absorption largely takes place in lower portions of the small intestine.

The mucosa or lining of the small intestine is enormous. A characteristic feature of the small intestine is the mucosal lining, which is principally composed of enterocytes that contain numerous villi that serve as absorptive areas. The villi extend into the lumen and appear as finger-like projections covered with epithelial cells. The villi also contain microvilli, which are also composed of microfilaments that form a brush border. Absorption of nutrients is also enhanced by motility of the small intestine, which places food in proximity to capillaries and lymphatic lacteals that serve as absorptive channels, and by the direct movement of villi.

Several types of absorptive mechanisms exist for nutrients including active transport, passive diffusion, facilitated diffusion, and endocytosis. Endocytosis occurs when the outer plasma membrane surrounds soluble or particulate nutrients in the GI tract and engulfs the contents. This process is similar to phagocytosis.

Carbohydrates, or starches, which are complex polysaccharides, are hydrolyzed to oligosaccharides and disaccharides by the action of pancreatic amylase. Disaccharides, including lactase, sucrase, and maltase, are enzymatically split by enzymes contained in the microvilli of enterocytes. Glucose and other monosaccharides are absorbed by an active transport mechanism and this action is coupled to energy derived from a sodium pump mechanism.

Proteins are initially broken down in the stomach by pepsins, but completion of digestion occurs in the duodenum by the action of pancreatic trypsin and chymotrypsin. This results in the formation of oligopeptides, dipeptides, and amino acids. Dipeptides are broken down by dipeptidases located on the microvilli and the cell cytoplasm. Amino acids are absorbed rapidly in the duodenum and jejunum by active transport mechanisms, including the generation of sodium ions.

Most dietary fat is composed of long-chain triglycerides, which contain saturated and unsaturated fats. The stomach's churning action acts to reduce the particle size of fats. In the duodenum, hydrolysis of triglycerides occurs through the action of pancreatic lipase, colipase, and bile salts, which form a ternary complex. Bile salts, which are synthesized by the liver, have detergent properties and enable the formation of micelles, which are emulsions of triglycerides or fats with bile salts. The micelles enable pancreatic lipase enzyme to access the water–fat-insoluble phase. Colipase, a pancreatic enzyme, acts to place the pancreatic lipase in close proximity to the surface of a triglyceride droplet and is necessary for the action of lipase. Lipase hydrolyzes the triglyceride to form 2-monoglycerides and fatty acids. Monoglycerides are released from micelles and come into contact with the cell surface, where they are absorbed by diffusion. Once inside the cell, the fate of fatty acid is dependent on chain length. Long-chain fatty acids are esterified to triglycerides by enzymes in the endoplasmic reticulum and interact with cholesterol phospholipids and apoproteins to form chylomicrons and very low-density lipoproteins. Medium-chain fatty acids are not reesterified and enter the portal venous system, where they are transported and bound by albumen. Other nutrients absorbed in the small intestine include fat-soluble vitamins, iron, calcium, water, and sodium.

Bile salts are absorbed from the ileum or terminal portion of the small intestine and are recirculated via the portal vein. If the ileum is diseased, as in Crohn's disease, bile salts may not be absorbed, and fat absorption, including absorption of fat-soluble vitamins, may be impaired.

Endocrine cells are scattered among walls of the small intestine including the villi and crypts. The cells can release a large array of secretory products into the bloodstream. The hormones play a key role in the digestive process and exert actions through neurocrine and paracrine mechanisms. Products released in the small intestine include gastrin, somatostatin, secretin, cholecystokinin, motilin, neurotensin, enteroglucagon, vasoactive intestinal polypeptide, GI polypeptide, and other agents.

The small intestine, in addition to other portions of the mucosa and submucosa of the alimentary tract contains a large number of individual T and B cells, macrophages, and plasma cells. This lymphoid tissue becomes confluent in the ileum and forms unencapsulated nodules, which are macroscopically visible and known as Peyer's patches. The M (membrane) cells are present in the epithelial tissue overlaying the GI tract and can transcytase antigenic macromolecules from the intestine to intact lymphocytes. The tissue with lymphocyte tissue in the appendix and mesenteric lymph nodes constitutes the mucosa-associated lymphoid tissue. This system forms part of the afferent link of the intestinal immune system and is involved in the secretion of IgA, which serves as a defense mechanism against external pathogens. Other mucosal epithelial surfaces in the body (e.g., respiratory tract and genitourinary tract) contain similar populations of lymphocytes that serve to protect pathogenic organisms.

Large Intestine

The large intestine or colon is ~ 1.5 m in length. The principal function of the colon is to reabsorb water and electrolytes that are present inside a liquid luminal stream. In contrast to the small intestinal mucosa, the lining of the colon is composed of columnar absorptive cells that have shorter, flat epithelial cells with no villi, although some absorptive cells have microvilli. The mucosa is punctuated by tubular crypts that extend to the mucosal layer and contain goblet cells, which secrete mucus; Paneth cells, which secrete lysozyme; endocrine cells; and undifferentiated goblet cells. Cellular proliferation is confined to the crypts and cells differentiate and migrate to the surface to replace superficial epithelial cells lost to surface abrasion or degeneration. Lymphoid tissue is found in the mucosa and submucosa.

Cellular Replication

The GI tract has one of the highest rates of cell turnover of mitosis of any organ system. The highest rate of mitosis is in the small intestine, where between 60% and 75% of cells are turned over on a daily basis. Over 50% of the cells in the stomach pylorus are turned over daily and 10% of the cells of the colon are replaced. Agents that are known to interfere with cellular replication, such as alkylating agents or antimetabolites used in cancer chemotherapy, can have potential effects on the GI tract by interfering with normal regeneration of the cells that are undergoing rapid replacement.

Intestinal Flora

The GI tract is not sterile and bacteria are continually swallowed to the stomach with food. Hydrochloric acid limits the concentration of bacteria in the stomach (except for *H. pylori*). In contrast, the large and small intestine contain numerous bacteria. Intestinal bacteria may contain several enzymes including β-glucosidase, β-galactosidase, and β-glucuronidase. These enzymes may play a role in transforming medications to their active form or affecting their excretion. Administration of ampicillin leads to an increase in the excretion of conjugated estrogens. Diets rich in meat, which has been identified as a risk factor for cancer of the colon, also increase β-glucuronidase activity in fecal bacteria. Gastrointestinal bacteria also play a role in metabolizing some compounds into more toxic forms such as reducing azo dyes, which are used in some food additives. Some bacteria, like *Lactobacilli,* may serve to decrease the risk of cancer.

Exocrine Pancreas

The pancreas contains cells that have endocrine and exocrine functions. The gland is largely formed of acinar cells, which secrete digestive enzymes or their precursor into the duodenum. Exocrine function is subject to hormonal and neural regulation. Islets of Langerhans contain strands of cells including B and A cells that secrete insulin and glucagons, respectively, and form the endocrine portion of the gland.

Toxicant Effects on the GI System

Toxic effects can be mediated in the GI system by several mechanisms, which are discussed in the following sections.

Direct Mucosal or Cellular Injury

Xenobiotics that contact the mucosal or other cells of the GI tract produce irritation characterized by

inflammation, degeneration, and/or proliferation. The type of toxic effect that is manifest is dependent on several factors including chemical characteristics of the agent, dose or magnitude of exposure, and type of tissue involved.

Erosions or a superficial ulceration of the mucosa can occur focally or diffusely. Erosions are due to focal necrosis of the epithelium and associated stroma and are restricted to the superficial layers. Diffuse irritation accompanied by an inflammatory reaction is called enteritis. Ulcers, in contrast, are deeper lesions extending beyond the mucosa and penetrating into the adjacent tissue layers. Chronic irritation can produce proliferative lesions including dysplasia, which potentially could become malignant.

Ingestion of strong alkali and acids has the potential to produce severe tissue destruction or liquefaction necrosis. Alkali with a pH of $\geqslant$11.5–12 and acids with a pH <2 can produce significant corrosive injury. Other substances such as phenol may not be highly alkaline but can still produce corrosive injury. Alkali are found in many commercial products, such as household and industrial cleaners, dishwasher soaps and drain openers, and low-phosphate detergents. Factors affecting the degree of tissue injury or destruction include the amount ingested, the duration of contact with tissue, concentration, pH, physical form, titratable alkaline, and acid reserve.

This liquefaction process has four distinct phases:

1. *Inflammatory phase*: This phase lasts 1 or 2 days and consists of marked fibroblastic proliferation. In this stage, perforations may occur.
2. *Necrotic phase*: This phase occurs 1–4 days after injury. Cells die from coagulation of intracellular protein. Vascular thrombosis and bacterial invasion may worsen the underlying injury. The esophagus is especially vulnerable to perforation during this phase.
3. *Granulation phase*: This phase begins 3–5 days postinjury when necrotic tissue sloughs. Granulation tissue begins to fill in tissue defects and connective tissue begins to form in 10–12 days.
4. *Constriction phase*: This phase occurs 2.5–3 weeks following injury and is related to the formation of collagen in the healing lesion. Marked narrowing of the esophageal lumen may occur as the collagen fibers begin to contract.

The most frequent injury following alkali ingestion is esophageal burns. Diffuse circumferential esophageal burns are more common in patients ingesting liquid forms of concentrated alkaline corrosives; granular forms tend to produce more oral burns and esophageal burns that are in patches or streaks. Gastric injuries may also be more common in patients ingesting liquid alkaline corrosives or solids that have been placed in capsules. Intestinal burns, mostly duodenal, have been reported but are much less frequent. Severe duodenal injury may be more common with suicidal ingestions. From a clinical perspective, the absence of visible oral burns does not reliably exclude the presence of esophageal burns. Symptoms of stridor, vomiting, and drooling can indicate serious esophageal injury.

Sequelae associated with ingestions of caustics include a tracheoesophageal and aortoesophageal fistulae; strictures of the mouth, esophagus, and stomach; and esophageal carcinoma. In severe cases, GI bleeding or perforated viscus with mediastinitis or peritonitis may develop. Strictures are more likely to develop after second- or third-degree or circumferential burns.

Several other agents produce GI tract irritation by interfering with the gastric mucosal barrier. Gastric ulcers have been associated with the use of anti-inflammatory medications including aspirin and nonsteroidal anti-inflammatory medications. The mechanism is thought to be the inhibition of cyclooxygenase, which is required for prostaglandin secretion. Prostaglandins play a key role in maintaining mucosal defenses of the stomach. Other agents that can cause severe injury to the GI tract include salicylates, heavy metals, and iron.

Several agents have been associated with producing acute pancreatitis or inflammation of the pancreas. The main causes are alcohol and a disturbance of the bile duct, which account for $\sim$50% of cases. Drugs with a clear association include sulfonamides, thiazide diuretics, tetracycline, azathioprine, estrogens, and valproic acid. The mechanism for the underlying injury is not well understood. Possible associations have been reported with other medications including methyldopa, procainamide, and l-asparaginase. A relationship between corticosteriods has not been established.

The pancreas is uniquely susceptible to high concentrations of ethanol, which can induce an acute inflammatory response characterized by release of amylase into the circulation. If severe or if this evolves into chronic pancreatitis it may lead to autodigestion by exocrine enzymes and ultimately to secondary endocrine dysfunction.

Interaction with Receptors of the GI Tract

Gastrointestinal function can be affected by interaction with cellular receptors. Stimulation of cholinergic muscarinic receptors by agents such as

cholinesterase inhibitors (organophosphate pesticides and carbamates) and nicotine and opioid withdrawal can lead to an increase in motility and secretions of the GI tract. This process can lead to symptoms of abdominal pain, cramps, and diarrhea. Similarly, the administration of drugs that block with cholinergic muscarinic receptor functioning (e.g., atropine, tricyclic antidepressants, opiates, and sedative hypnotic medications) can slow motility and lead to constipation.

Indirect Effect

Vomiting can occur as a consequence of the interaction of a chemical with the central chemoreceptor zone or vomiting center in the fourth ventricle of the brain. This results in GI symptoms caused by an indirect effect. Glycosides, opiates, nicotine, and possibly carbon monoxide may act in this manner. Vomiting can also occur as a consequence of local GI tract stimulation from a wide array of agents including soaps, detergents, solvents, metals (including arsenic and thallium), and toxins associated with several types of food poisonings.

Allergic Reactions

The GI tract can be a site of hypersensitivity reactions. Angioedema of the mouth including the pharynx can occur following use of several medications including ACE inhibitors. The reaction is mediated via IgE.

Carcinogenesis

Cancers of the GI tract comprise a large proportion of malignancies in the USA. Colorectal cancer is the second most common malignancy in the USA. There is strong evidence that GI cancers are affected by environmental factors since there are considerable geographical differences between cancer incidence of the same organ and migration studies have demonstrated that migrants who move to new countries over time will experience the same risks or cancer rate as the people in the host country.

Oral cavity cancers have been associated with cigarettes, alcohol, and chewing tobacco or snuff or betel nut quid (popular in parts of Asia). Cancer of the oral cavity is not common in the Western world but frequently found in some developing countries including India, where it accounts for approximately 8% of all malignancies. Risk factors associated with oral cancers include excessive alcohol consumption, although the effects of alcohol are sometimes hard to differentiate from tobacco use since persons commonly smoke and drink. Chewing of tobacco has been identified as a principal risk factor. Other factors include a history of ionizing radiation exposure and nutritional deficiencies including iron in association with Plummer–Vinson syndrome.

Esophageal cancer has been related to the use of alcohol and nitrosamines and possibly chewing betel nut (popular in parts of Asia). Other risk factors include a history of ingestion of alkaline corrosive agents, including lye. Nutritional deficiencies have also been linked to this type of malignancy.

Gastric neoplasms were among the most frequent malignancies at the turn of the century, but the incidence has decreased in the past 50 years even though there have been no major advances in diagnosis or treatment. Factors that have been linked to gastric cancer include nitrate ingestion as well as other dietary factors. Persons with atrophic gastritis who have hypochlorhydria or a relative lack of stomach acid are at greater risk. This may be secondary to the presence of bacteria, which are normally killed in an acid gastric environment and that transform nitrates to nitrites, which can eventually form carcinogenic nitrosamines.

Colon cancer has been associated with several factors including radiation exposure, limited physical activity, dietary fat intake, high meat intake, and nitrosamines. Fats may increase the risk of cancer by changing the intestinal flora or increasing the concentration of bile acids or because of a secondary effect on metabolism of xenobiotics. Negative associations have been associated with intake of fresh vegetables and meats. Other risk factors include family polyposis, chronic ulcerative colitis, and familial cancer syndrome. Cancer of the rectum not only shares some risk factors with the colon cancer overall but also has distinct characteristics possibly related to sexually transmitted infections, chronic inflammation, and cigarette smoking.

See also: Absorption; Acids; Alkalies; Carbamate Pesticides; Carcinogenesis; Corrosives; Endocrine System; Liver; Metals; Organophosphates; Poisoning Emergencies in Humans.

Further Reading

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Generally Recognized as Safe (GRAS)

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Generally Recognized as Safe (GRAS) is a regulatory category created for a group of food additives that were exempted from the more rigorous regulatory requirements for food additives in the 1958 Food Additives Amendment to the (US) Food, Drug, and Cosmetics (FD&C) Act of 1938. If a substance was accorded GRAS status, it was generally recognized by experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures or experience based on common use in food, to be safe under the conditions of its intended use.

The statutory definition of 'food additive' covers only a substance that "is not generally recognized … to be safe under the conditions of its intended use." Thus, in the peculiar meaning of the term as it is used in the statute, a substance that becomes a component of a food (even as an ingredient) would not be a 'food additive' if it is generally recognized as safe. Congress further defined GRAS as requiring that a substance used in food be generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common used in food) to be safe under the conditions of its intended use.

Thus, GRAS status may exist if some level of scientific agreement about a substance's safety exists based either on appropriate testing or common use in food prior to 1958.

The GRAS exception obviously raises a number of serious interpretive difficulties. The statute provides little further elaboration about the required degree of scientific agreement, the types of scientific procedures that could provide the necessary predicate for such agreement, or, in the case of substances in widespread used before 1958, the required nature and extent of such prior use. For instance, how could a substance be GRAS without experience based on common prior use? Did Congress thereby intend to give manufacturers of new food-use substances the option of submitting test results to non-Food and Drug Administration (FDA) scientists for their evaluation and possible stamp of approval? At least one witness at the 1957 congressional hearings apparently thought so, suggesting that a company could seek the advice of private or academic consultants on the question of whether there was general recognition of safety based on existing data. As discussed below, this has become a common practice.

Because the GRAS exception became a common feature of every one of the numerous bills on the subject considered by Congress, the legislative history sheds some additional light on these and other questions. Although not technically a 'grandfather clause' (which would permanently exempt from coverage all substances used in food prior to the enactment date), the GRAS exception attempts to minimize the potentially significant and unnecessary burden that would otherwise be placed on both the industry and the FDA if the agency had to evaluate and formally approve common substances used in food. In addition, for substances that were not regarded as GRAS and therefore subject to regulation as food additives, Congress initially provided a transitional period of up to 30 months for compliance with the new premarket approval requirements, but it subsequently extended this phase-in period by almost five additional years.

The FD&C Act included similar GRAS language in defining the term 'new drug,' as did the Pesticide Residues Amendment of 1954. Although conceding during the congressional hearings that the language was inherently ambiguous, the agency thought that it could apply this flexible GRAS exception to food additives in a sensible manner. (Interestingly, just 2 years after enacting the Food Additives Amendments.) As subsequently construed by reviewing courts, the exception applicable to drugs is quite narrow, in part because the statute requires that a drug be both GRAS and GRAE (generally recognized as effective). In 1973, the Supreme Court held that the exception in the definition of new drug required an 'expert consensus' of both safety and effectiveness.

Both the FDA and reviewing courts sometimes have struggled to make sense of the GRAS exception. All agree that there must be a fairly high level of scientific agreement. The FDA's implementing regulations, finally promulgated almost two decades after passage of the Food Additives Amendment, provide as follows:

- Generally, recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be

based upon published studies that may be corroborated by unpublished studies and other data and information.

- Evidence of GRAS must relate to the conditions of intended use; general recognition of the safe use of a substance in a different product or at a different level would not suffice to escape the food additive definition. The exception turns not on safety itself so much as on recognition of safety by scientific experts. Testimony of an absence of any evidence of a health hazard would not suffice to establish GRAS status, at least not unless coupled with evidence of common prior use. If GRAS status is premised on common use prior to 1958, then such use must have been fairly extensive.

Originally, the FDA categorically refused to recognize use outside of the United States. This policy did not, however, survive a subsequent judicial challenge. The revised regulations provide that prior foreign use may support GRAS status, but only if the information about such use is readily available and corroborated. In addition, GRAS status based on prior foreign use must satisfy domestic conceptions of safety. If GRAS status is based on prior foreign use, the FDA urges the manufacturer to seek its concurrence. Other sections of the regulations continue to define eligibility for GRAS status by reference to common use in the United States.

During the congressional hearing leading up to enactment of the Food Additives Amendment, the FDA submitted a 'partial' list of what it would regard as GRAS substances including items such as butter, coffee, cream, gelatin, lard, lemon juice, margarine, molasses, mustard, olive oil, paprika, pepper, salt, sugar, vinegar, and wine. During the first several years after enactment of the Food Additives Amendment, the FDA listed in its regulations hundreds of ingredients as GRAS. The original GRAS lists included, for example, ascorbic acid, calcium chloride, caramel, and sodium phosphate.

Because these inventories emerged without any detailed scientific assessment of the original safety data, much less of the data subsequently generated with constantly improving detection and safety assessment methods (as underscored by the discovery of evidence linking an artificial sweetener mixture containing cyclamate to cancer), the FDA initiated a systematic review in 1969 in order to settle the GRAS or food additive status of a number of substances commonly added to food. The agency designated several categories of food ingredients for this review: substances of natural biological origin that were widely consumed as food before 1958 but subsequently were modified in certain respects by new production processes or selective breeding; distillates, isolates, extracts, and reaction products of GRAS substances; and substances not of natural biological origin or intended for consumption for other than their nutrient properties.

The (US) National Academy of Sciences (NAS) undertook ingredient usage surveys, and, in 1972, the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) established a Select Committee on GRAS Substances (SCOGS) to conduct reviews of the available scientific literature. Over a period of 10 years, SCOGS forwarded to the FDA detailed reports on 468 food substances (of which 422 were direct ingredients). The Select Committee first created an array of five standardized recommendations, and it concluded that 72% of the food substances under review should remain GRAS and only 1% should immediately become subject to food additive requirements. Although the FDA planned to review each of these reports and pursue appropriate rulemaking, it has not completed its GRAS list review many years after receiving the last SCOGS report.

A number of substances currently appear on the GRAS affirmation list that emerged from the FDA's comprehensive review. At present, almost 200 separate ingredients are included as GRAS for direct use in food. The FDA concedes, however, that its GRAS lists are not exhaustive: "Because of the large number of substances the intended use of which results or may reasonably be expected to result, directly or indirectly, in their becoming a component or otherwise affecting the characteristics of food, it is impracticable to list all such substances that are GRAS". Thus, a substance "of natural biological origin that has been widely consumed for its nutrient properties in the United States prior to January 1, 1958, without known detrimental effects, which is subject only to conventional processing … will ordinarily be regarded as GRAS without specific inclusion" in one of the GRAS lists. More specifically, "by way of illustration, the Commissioner regards such common food ingredients as salt, pepper, vinegar, baking powder, and monosodium glutamate as safe for their intended use."

GRAS status does not free a substance of FDA controls. At a minimum, a GRAS substance must comply with any applicable food grade specifications appearing in the FOOD CHEMICAL CODEX, and it must perform an appropriate function (and be used at a level no higher than necessary to achieve its intended purpose) in the food or food-contact article in which it is used. In addition, a substance must comply with any specific usage limitations appearing in any GRAS affirmation regulation. If no specific

limitation applies, GRAS status is lost only if the conditions of use differ significantly from those providing the basis for eligibility. Finally, '[n]ew information', and any revision of an existing GRAS regulation would be accomplished by the FDA through notice-and-comment rulemaking procedures. In contrast, any revision of a food additive regulation would require more cumbersome procedures.

Unless the FDA previously has decided otherwise, a person may take the position that a particular food-use substance is GRAS and, therefore, exempt from food additive approval requirements. In fact, there is no present requirement that the agency be advised of such private GRAS determinations. A few manufacturers have commissioned safety reviews by reputable scientific organizations, and FASEB has conducted a handful of private GRAS reviews during the last several years. For example, the Procter & Gamble Company asked the Federation to review the safety of caprenin, a reduced calorie fat substitute; on the basis of FASEB's report, the company determined that this substance was GRAS, filed a GRAS affirmation petition with the FDA, and began selling it to food processors. Similarly, Nabisco Foods sought a FASEB review of salatrim, another fat substitute subsequently brought to market on the basis of a GRAS self-determination. Some have suggested that the National Center for Food Safety and Technology, an organization recently established in the Chicago area with private and government support, might play a similar role in the future.

Whether undertaken for the FDA or a private entity, FASEB's LSRO assembled an ad hoc panel of experts from several different scientific disciplines to conduct the requested reviews. These experts usually are drawn from among FASEB's >more than 40 000 members, and they are hired by LSRO to serve as independent consultants. The expert panels prepare study reports that are "peer-reviewed by an independent internal FASEB committee for clarity, objectivity, and scientific integrity…, [and] the reports of each study are published in scientific journals or are made available publicly."

Similarly, a few industry associations have created their own expert panels to review the possible GRAS status of food ingredients, as the Flavor and Extract Manufacturer's Association (FEMA) has done for the last few decades. FEMA's project began in 1959, initially surveying the industry about the usage of different flavoring substances. The association then established a permanent panel – composed of six to eight recognized and independent experts from various disciplines including toxicology and biochemistry – to evaluate scientific literature reviews (SLRs) assembled for its consideration and then assess the GRAS status of those flavoring substances. Over the last 30 years, the expert panel's reports have been published periodically in the journal '*Food Technology*'. Furthermore, the SLRs underlying the Panel's GRAS determinations were made available to the public and forwarded to the FDA.

In the case of a new flavoring substance, a company seeking an opinion about the flavor's potential GRAS status must submit an application form and literature search to FEMA's staff which, after a preliminary check for completeness, forwards the request and information to the expert panel for consideration at its next regularly scheduled meeting. The available literature is evaluated against FEMA's published criteria, and a GRAS designation requires a unanimous vote by the panel; otherwise, the flavoring substance will be placed in a hold category for further study or be designated as not GRAS.

Since the inception of this project, FEMA has considered >2000 flavoring substances. The expert panel's initial set of reviews identified 1118 substances as GRAS based on prior safe use and six more as GRAS based on the available scientific information. The FDA incorporated only 277 of these flavors in its own GRAS list, but it also designated another 846 of these FEMA-reviewed substances as approved food additives on the strength of the existing safety data and without the need for filing separate petitions. In 1985, FEMA finished a comprehensive reevaluation aimed at updating its original GRAS determinations, dropping three flavoring substances from the list. In 1993, FEMA began a second reevaluation process, coupled with an effort to update and reformat all existing SLRs, which it hopes to complete in 5 years.

The FDA has not challenged the marketing of flavors the FEMA had identified as GRAS, whether or not it has incorporated them into its own lists of GRAS substances or approved food additives. In extending the date by which persons would have to comply with its bulk flavoring requirements, the agency described the FEMA list as one of the "reliable industry association GRAS lists." The FDA also occasionally refers to FEMA's GRAS listing of a flavor to support a GRAS affirmation proposal.

Another potentially important source of food safety expertise resides in the Joint Expert Committees on Food Additives (JECFA), first organized in 1956 by the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) and now associated with these organizations' Codex Alimentarius Commission. JECFA reports have influenced decisions by the FDA and other regulatory bodies, and its recommendations

concerning particular additives might be relied upon by companies in making GRAS self-determinations. Under the FDA's GRAS criteria, a report by JECFA or a comparable group certainly could qualify as 'general recognition' of safety, even if a substance has never before been used in food.

Although nothing prevents such GRAS self-determinations, the strategy carries obvious regulatory risks. On occasion, the agency has pursued enforcement proceedings, disagreeing with a company's belated claim that a substance is GRAS.

FDA has an 'EAFUS' Food Additive Database website. This is an informational database maintained by the FDA Center for Food Safety and Applied Nutrition (CFSAN) under an ongoing program known as the Priority-based Assessment of Food Additives (PAFA). It contains administrative, chemical, and toxicological information on over 2000 substances directly added to food, including substances regulated by the US Food and Drug Administration (FDA) as direct, 'secondary' direct, and color additives, and Generally Recognized as Safe and prior-sanctioned substances. In addition, the database contains only administrative and chemical information on less then 1000 such substances. The more than 3000 total substances together comprise an inventory often referred to as '*Everything*' *Added to Food in the United States* (EAFUS). This list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS. Nevertheless, it contains only a partial list of all food ingredients that may in fact be lawfully added to food, because under federal law some ingredients may be added to food under a GRAS determination made independently from the FDA. The list contains many, but not all, of the substances subject to independent GRAS determinations.

The Research Institute for Fragrance Materials (RIFM) uses an expert of academic dermatologists, toxicologists, and environmental scientists safe use determinations of fragrance materials. The Expert Panel uses a decision tree approach to assessing the dermal, systemic, and environmental endpoints. Conclusions of the Expert Panel on safe use, drawn from critical evaluation of all available hazard data, and exposure information provided by industry, form the basis for industry-wide standards issued by the International Fragrance Association.

See also: Flavor and Extract Manufacturers Association; Food Additives; Food and Drug Administration, US; International Fragrance Association (IFRA); Joint FAO/WHO Expert Meetings (JECFA and JMPR); Research Institute for Fragrance Materials (RIFM).

Further Reading

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Relevant Website

http://vm.cfsan.fda.gov – US Food and Drug Administration (FDA). CFR 21 (part 184) (includes a list of direct food additives affirmed as generally recognized as safe). See also the link to the 'EAFUS' Food Additive Database.

Genetic Ecotoxicology *See* Ecotoxicology, Genetic.

Genetic Toxicology

Joseph R Landolph

Overview of Genetic Toxicology

Genetic toxicology is the study of the toxic effects of chemicals and radiations on the hereditary material, or the deoxyribonucleic acid (DNA), of cells. Genetic toxicology therefore involves the study of DNA single-strand breaks and double-strand breaks, damage to DNA, mutations in DNA, and recombinational events in DNA mediated by exogenous agents in bacteria, yeast, cells of the fruit fly, plant cells, and mammalian cells. In plant cells and mammalian cells, genetic toxicology also encompasses micronucleus formation, chromosomal aberrations, chromosomal aneuploidy, and morphological and neoplastic transformation. In addition, genetic toxicology also encompasses chemical carcinogenesis in lower animals and in humans. The importance of genetic toxicology is that it allows investigators to measure the DNA-damaging, mutagenic, and carcinogenic effects of chemical carcinogens and ultraviolet (UV) and ionizing radiations and it also allows investigators to study genetic damage and mutations in lower animals and humans.

DNA Damage

Many chemicals, and UV and ionizing radiations, can cause damage to DNA bases. This can result in a labilization of the DNA base-sugar phosphate bond. Bases can then depurinate, or dissociate from the sugar phosphate backbone of DNA. This can leave that DNA strand which is lacking a DNA base susceptible to attack by nucleases, leading to cleavage of that strand by endonucleases. This will result in a break in a single strand of DNA. Single-strand breaks in DNA are easily recognized by centrifugation in alkaline sucrose gradients. This separates the DNA strands, and also allows visualization of the separated and broken strands. Using gel electrophoresis and specific plasmids treated with chemical mutagens or radiations, also allows investigators to detect single-strand breaks in DNA. If these DNA single-strand breaks are not repaired correctly by DNA repair enzymes, this can lead to a cytotoxic event.

Similarly, a number of chemical agents and ionizing radiations can lead to one or more breaks in both strands of DNA simultaneously. This is called a DNA double-strand break. Ionizing radiations, such as X-rays and neutrons, are very effective in depositing a sufficient amount of energy into DNA to result in DNA double-strand breaks. DNA double-strand breaks are easily recognized by centrifugation in neutral sucrose. These types of breaks of the DNA strands are difficult to repair by the DNA repair machinery of the cell. If they are not repaired correctly, these DNA double-strand breaks can lead to cytotoxicity.

UV light (254 nm) and certain chemical mutagens can also efficiently induce cross-links in DNA. These can be manifested as either intra-strand DNA cross-links, that is, occurring within one strand of DNA, or inter-strand DNA cross-links, which occur between two strands of DNA. These types of DNA cross-links can inhibit DNA replication and transcription. This can cause the cell to remain in a static state, where it is trapped and cannot proceed through mitosis. In such a state, a cell can become degraded by nucleases and proteases, leading to cell death. Hence, it is very important for the cell to repair these types of DNA cross-links, so the cell can complete DNA synthesis and mitosis, commence transcription, and survive. Examples of agents that are efficient at inducing DNA cross-links are UV light of 254 nm, nitrogen mustard, and psoralen plus UV light.

In addition, ionizing radiations (X-rays and gamma rays) and certain chemicals that generate active oxygen species, such as superoxide, can cause oxidative damage to DNA bases. Bleomycin and adriamycin are examples of chemicals that can generate superoxide. Superoxide can then dismutate in the presence of the enzyme, superoxide dismutase, which can lead to the generation of hydrogen peroxide. The reaction of hydrogen peroxide and additional superoxide in the presence of ferrous iron can then lead to generation of hydroxyl radicals. Hydrogen peroxide and hydroxyl radicals can oxidize DNA bases.

A fifth type of DNA damage results from the covalent binding of chemical mutagens and mutagenic chemical carcinogens to DNA bases. An example of this is the chemical mutagen and mutagenic chemical carcinogen, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG). MNNG is thought to generate methyl carbonium ions, which can bind covalently to the O-6 position of guanine, and to the N-7 position of guanine in DNA. The chemical carcinogen, benzo(*a*)pyrene (BaP), is metabolized to diol epoxide metabolites. One of the metabolites, the anti-benzo(*a*)pyrene 7,8-dihydroxydiol-9,10-epoxide can bind covalently to the exocyclic amine of guanine in

DNA, leading to a covalent adduct. Covalent mutagen/carcinogen-DNA base adducts are very stable, and can lead to cytotoxicity, mutation, or carcinogenesis if they are not repaired properly.

DNA Repair

We now know that in bacteria, yeast, and mammalian cells, there are enzymatic systems that can repair damaged DNA. These systems are known as DNA repair systems.

Much of the repair that takes place in bacteria and in mammalian cells whose DNA has been damaged by chemical mutagens or by ionizing radiations, proceeds with a high degree of fidelity, and repairs the DNA damage correctly. However, a certain fraction of this repair proceeds incorrectly, and this misrepair leads to mutations. While some of this misrepair generates mutations and can be cytotoxic, a fraction of this misrepair is beneficial by generating mutations that can lead to genetic diversity in organisms and hence provides new organisms that can lead to evolution of various species.

In bacteria, we now recognize a number of DNA repair systems. The first repair system, which has been the most intensively studied and the best understood, is the system of photoreactivation repair, or direct repair. This repair system is very efficient at repairing thymine dimers formed between thymine bases in DNA by absorption of UV light of 254 nm by the thymine bases. In this type of repair, an antenna pigment, methylene tetrahydrofolate (MTHF), absorbs near UV light of wavelength 350 nm. MTHF then transfers the energy of this photon by Forster resonance energy transfer to reduced flavin adenine dinucleotide (FADH). FADH then transfers an electron to the thymine dimer, which decomposes it, returning it to its original state of two separate thymine bases in DNA. This repair takes place in the presence of the photoreactivating enzyme, which contains a pocket that binds to and holds the thymine dimer in place. Since this repair system returns the thymine dimer to its original separate thymine bases in DNA, no mutations occur during this process. Hence, this repair is said to be 'error-free', and it does not induce mutations. The photoreactivating enzyme has been cloned and sequenced, and X-ray crystallographic analysis of the photoreactivating enzyme has revealed the structure of this enzyme.

A second DNA repair system in bacteria is designated excision repair. This repair system efficiently repairs DNA strands that have been irradiated with UV light or ionizing radiations, oxidatively damaged, or that have chemical-DNA adducts in them. This system involves the steps of incision by an incision endonuclease proximate to the site of the damage, followed by excision of the damaged DNA bases by DNA polymerase I. Next, DNA polymerase I fills in the resulting nucleotide gaps by adding nucleotides complementary to the undamaged strand, using the undamaged strand as a template. Finally, DNA ligase seals the phosphodiester chain. This repair proceeds with a high degree of fidelity, and therefore only induces a very low frequency of mutations. Some authors refer to this as 'error-free' DNA repair, although a low frequency of mutations are created by this repair system.

A third type of DNA repair is called the SOS response. The SOS response involves the induction of two different types of DNA repair. In this situation, where there are thymine dimers in DNA due to UV irradiation of the DNA, or other DNA damage, a normally quiescent molecule, called the rec A protease, binds to the site of this DNA damage. The binding of rec A to damaged DNA causes the rec A protease to become catalytically active. The rec A protease then binds to various molecules of the lex A repressor that are already bound to the bacterial genome. Lex A repressor molecules normally bind to the SOS boxes of genes in the genome that encode endonucleases, exonucleases, helicases, DNA polymerases, and other molecules important in DNA repair. When the activated rec A protease binds to the Lex A repressors, this causes the Lex A repressors to autocatalytically cleave themselves. This results in the induction of the synthesis of ~ 50 protein molecules involved in DNA repair, among them an error-prone DNA polymerase. This error-prone DNA polymerase causes nucleotide synthesis to occur opposite the thymine dimers, with a low degree of fidelity. This leads to mutations in the DNA. In addition, during the SOS response, the rec A protease also acts as a recombinogenic enzyme. In this case, at a replication fork containing a thymine dimer, rec A-mediated recombination can occur to generate a situation in which there is at least one good template for DNA synthesis on each strand of the replication fork. While allowing DNA repair and hence DNA synthesis to proceed, rec A-mediated recombination is also a process that proceeds with a low degree of fidelity, with error rates of 1/1000, leading also to mutations. Similar types of DNA exist in mammalian cells.

Mutagenesis

Overview of Mutagenesis and Its Biological Significance

What is mutagenesis and why should we be concerned with this process? Mutagenesis is the process

by which mutations are induced in the cells of organisms. Mutations are changes in the hereditary material of cells, their DNA, that cause observable changes in hereditary traits in offspring. These changes are transmitted to the ribonucleic acid (RNA), which is synthesized according to the instructions carried by the DNA, and then to proteins, which conduct chemical (enzymatic) reactions in the cell, or serve as structural materials, giving a cell its shape.

Mutations can have beneficial effects, deleterious effects, or no consequences in organisms. Certain mutations have a positive effect on the organism. The sickle cell mutation in the hemoglobin gene and hence the hemoglobin protein molecule, for instance, is thought to give humans in Africa an ability to survive malaria better. The resulting mutated hemoglobin aggregates in the red blood cells, leading them to assume a sickled shape, which makes it difficult for the malarial parasite to enter and infect the red blood cells. Many mutations are neutral and have no significant effect on the organism at all. However, certain types of mutations can have deleterious consequences in organisms. An example of a deleterious mutation in humans is one that destroys the activity of an enzyme called adenosine deaminase, leading to a deficient immune system and a consequent inability to fight disease, as occurred in the famous 'bubble boy'. Other deleterious mutations, such as mutations in the germ cells (sperm or egg cells), can lead to a predisposition to cancer, such as the Li-Fraumeni syndrome, and still other mutations can be lethal and result in nonviable offspring. Mutation is an inevitable process, and it is occurring all the time spontaneously. Mutations that lead to beneficial traits in an organism will be selected for, and mutations that lead to defects in critical cellular properties or to the death of the organism will be selected against, during the course of evolution.

During the past 100 years, we have learned a significant amount about the nature and effects of mutations on cell growth and survival and on the growth and survival of various organisms. Mutations have been most intensively studied in bacteria because bacteria grow very rapidly, and the mutations are rapidly expressed. From experimental studies, we now know that UV and ionizing radiations and specific chemicals called mutagens can induce mutations in bacteria, in yeast, in plants, in the fruit fly *Drosophila*, in mice, in single mouse cells in culture, in single human cells in culture, and in humans, although the last has been less well studied. We know a significant amount concerning mutations in the fruit fly, *Drosophila melanogaster*, and a significant amount concerning the effects of mutations in mice. Using recently developed assays to detect mutations in humans by analyzing white blood cells, we are beginning to acquire knowledge concerning the induction of mutations in humans caused by ionizing radiations (e.g., the atomic bomb survivors in Hiroshima and Nagasaki), mutations caused by mutagenic cancer chemotherapeutic agents, and mutations caused by cigarette smoke. With current methods using techniques of molecular biology, we can detect the presence of certain mutations believed to be deleterious in humans, and genetic counseling can occasionally help certain families.

Scientists have also learned a substantial amount regarding cancer induction by mutagenic chemical carcinogens and mutagenic UV and ionizing radiations. A very important finding in this field is that UV and ionizing radiations and specific chemical mutagens induce mutations in specific cellular genes called proto-oncogenes, which activate them to oncogenes. Chemical mutagens and ionizing radiations can also cause amplification of the proto-oncogenes, leading to higher steady-state levels of the protein products of proto-oncogenes. These agents can also cause chromosomal breakage and translocation of a part of the chromsome(s) bearing proto-oncogenes to other chromosomes, where the proto-oncogenes can be placed under the control of different promoters of gene expression or fused with other genes, leading to aberrant proto-oncogene products. Mutagens also induce deleterious or inactivating mutations in other genes called tumor suppressor genes, inactivating them, or cause partial or full deletions of these genes, leading to loss of the tumor suppressor gene protein products. Combinations of activating mutations in proto-oncogenes and inactivating mutations in tumor suppressor genes, on the order of five to eight such mutations, in somatic (nongermline) or germ line cells play a key role in carcinogenesis, or the process of cancer induction caused by mutagenic chemical carcinogens, in humans. Specific details of the types of mutations that occur in organisms and their biological significance follow.

Definition and Description of Mutations

As has been well known since the pioneering experiments of Griffith, Avery, MacLeod, McCarty, Watson, and Crick, DNA is the genetic material of bacterial and mammalian cells. DNA encodes information in a triplet code which specifies the sequence of amino acids in proteins. Proteins are the enzymatic and structural polymers of cells. DNA consists of two antiparallel chains of nucleotides. Hydrogen bonds between bases on one strand and bases on the opposite strand constitute in the aggregate sufficient bond

strength to keep the double helix of DNA intact. However, at room temperature, sufficient energy is deposited in the DNA helix that the hydrogen bonds constantly break and reform, leading the DNA base pairs to occasionally separate, and the DNA structure to 'breathe'. DNA is replicated in a semiconservative fashion, as shown by the original experiment of Drs. Meselson and Stahl, such that each original strand serves as a template on which a new complementary strand is replicated. In this replication, complementary DNA bases are added to bases on the original strand, such that guanine pairs with cytosine and thymine pairs with adenine, as accomplished by DNA polymerases. In bacteria, DNA polymerase accomplishes a large fraction of DNA synthesis, aided by DNA polymerase I, which works on the lagging strand.

The sequence of DNA is specified very precisely. A mutation is any change in the sequence of DNA bases from the original sequence of DNA bases. In the simplest form, during replication, the DNA polymerase enzymes can accidentally substitute an adenine for a guanine opposite a cytosine during DNA replication. This simple kind of mutation would be called a transition mutation, in which one purine, guanine, was instead replaced by another purine, an adenine base. Similarly, if during replication a pyrimidine base, such as thymine, was supposed to be inserted opposite an adenine base on the template strand, but instead a cytosine was inserted opposite the adenine, this would also be called a transition mutation. A transversion mutation is one in which a purine base substitutes for a pyrimidine base (guanine for thymidine) or a pyrimidine base substitutes for a purine base (cytosine for adenine). These types of mutations are also called base substitution mutations.

The next more complex type of mutation is referred to as an addition or deletion mutation. In a deletion mutation, one or more bases are removed from the DNA. In an addition mutation, one or more bases are added to the DNA. Addition mutations are also called insertion mutations. Deletion mutations are called small deletions if only a few bases are deleted from the DNA, or large deletions if many bases are deleted from the DNA. The same considerations hold for small-addition and large-addition mutations.

So far, we have only considered mutations in which the genetic code is kept in strict register. As is commonly known, the genetic code is read in triplets, such that three nucleotides are read together to specify one specific amino acid. With base substitution mutations, only one base is changed, so the amino acid specified by the new triplet nucleotide specifies a new amino acid. However, the rest of the nucleotide sequence remains the same, so the protein specified only has one amino acid changed in it. This situation is similar in the case of addition and deletion mutations, provided the addition or deletion is three bases or a multiple of three bases. Of course, in this situation, there is gain or loss of one or more amino acids, and this can have severe consequences for the resultant protein, depending on where in the protein the amino acids are inserted or deleted. However, beyond the site at which the three base addition or deletion is induced, the genetic code remains in register, and the rest of the protein, beyond the addition or deletion mutation of three or a multiple of three nucleotides, will remain normal.

A special circumstance arises when one or two bases, or any multiple of one or two bases, but not three bases, are deleted or inserted into a DNA sequence. In this case, the sequence of bases encoding amino acids is now shifted. The original amino acids in the encoded protein are changed, and the code is shifted out of register at and beyond the site of this type of mutation. Hence, a new or 'scrambled' protein is produced from the site of the mutation onward. Such a special type of mutation is called a frameshift mutation, since the coding frame is shifted out of its original alignment. In this case, the structure of the protein is 'scrambled' from the site of the deletion or insertion on through the rest of the protein. In this case, the protein can have an altered structure, and if the protein is an enzyme, the enzymatic activity of the protein may be decreased or abolished.

Another simple type of mutation that needs to be considered is gene amplification. In this case, a gene is copied into many more replicas of that same gene. The extra copies of this gene are then inserted into the DNA. A further type of mutation is due to a translocation of a gene sequence. In mammalian cells, there is the additional complication that the DNA and its genes are arranged on discrete chromosomes. These chromosomes can be broken, and pieces from one chromosome attached to another chromosome, to form a structure known as a translocation. This can result in a deletion mutation if the sequences are not joined correctly and can also result in the translocated gene being placed next to a strong promoter element, which can cause the gene to be read more frequently, affecting expression of this gene.

Consequences of Specific Types of Mutations

The consequences of transition mutations and transversion mutations depend on where they occur in a gene coding for a protein. If they occur in a site

that does not significantly change the shape of a protein used to maintain the structural integrity of a cell, or in a site that does not affect the structure of an enzyme, then they do not have a significant effect on the structure of the cell or on the enzymatic activity of a protein. If, however, the transition or transversion mutation occurs in a part of the protein that significantly changes its structure or decreases its enzymatic activity, then the mutation can have severe negative consequences for the survival of the cell.

Three base additions and deletions similarly may not have severe consequences if they occur in a region of the protein that does not affect the structure of the protein or its enzymatic activity. Of course, if these deletions and additions occur in critical parts of the protein that affect its structure, or in the active site of an enzyme, they can have significant effects on cell survival and the phenotype of the cell.

In general, the frameshift type of mutation is usually deleterious to protein structure and enzymatic activity. Such mutations 'scramble' the structure of the protein downstream from the mutation, and hence destroy the structural integrity of structural proteins and destroy the enzymatic activity of enzymes.

General Types of Mutagens: The Concept of Metabolic Activation

Broadly speaking, there are six general types of mutagens. First, there are mutagens that are 'fraudulent' DNA bases. These are bases whose structures are similar to but somewhat different in structure than the normal bases. An example of such a base is 5-bromouracil, which is similar in structure to the normal base thymidine and can substitute for thymidine in DNA, but which has different hydrogen properties and hence different base-pairing properties than thymidine. Approximately 33 large base analogs have been synthesized and their mutagenic properties studied.

Second, there is a group of mutagens called frameshift mutagens. These mutagens are, in general, large, planar aromatic molecules that can intercalate into the DNA. In the process of intercalation, the intercalators slip into the DNA and lie flat between two adjacent base pairs, with the plane of the intercalator lying flat upon the planar aromatic rings of the base pairs. New bonds are formed between the II electron clouds of the DNA bases and the intercalating aromatic molecules, which stabilizes the new intercalated structure. Treatment of cells with this type of mutagen increases the frequency of occurrence of frameshift mutations. Examples of such mutagens include acridine orange, acriflavine, and ethidium bromide. These molecules are highly fluorescent, and are commonly used to stain DNA in agarose gels, due to their intercalating and fluorescent properties.

The third group of mutagens are the direct alkylating agents. These mutagens generate methyl and ethyl carbonium ions, which are chemically reactive and readily bind covalently to nucleophilic groups on the bases of DNA, including but not limited to, the O-6 atom of guanine and the N-7 atom of guanine. Examples of these alkylating mutagens are MNNG, methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), and epoxides such as ethylene oxide. There are also alkylating agents that have two reactive groups on the same molecule, such as nitrogen mustard. Nitrogen mustard and similar molecules can bind to both strands of DNA, leading to a cross-link between them, or to two places within one strand of DNA, leading to an intrastrand cross-link. UV light can also cause formation of intrastrand or inter-strand cross-links in DNA.

Fourth, there is a large group of mutagens referred to as premutagens or promutagens. These compounds are chemically inert and usually very hydrophobic. All organisms must metabolize these hydrophobic compounds to make them sufficiently water soluble for excretion from the cell membrane and from the organism. Otherwise, these hydrophobic molecules will bioaccumulate in the hydrophobic regions of membranes of cells, causing inhibition of the functioning of enzymes in membranes, inhibition of membrane transport functions, and damage to the integrity of the membrane, which delineates the cell from its environment. Examples of such hydrophobic molecules are the polycyclic aromatic hydrocarbons (PAHs), such as the ubiquitous environmental pollutant and carcinogen, benzo(*a*)pyrene. In order to remove these hydrophobic molecules from cells, organisms utilize cytochrome P450 enzymes plus atmospheric oxygen, plus reducing equivalents such as reduced nicotinamide adenine dinucleotide phosphate (NADPH) and reduced nicotinamide adenine dinucleotide (NADH) to epoxidate the hydrophobic molecules, in the case of PAHs, activating them to mutagens. This step results in the generation of an active mutagen, in the form of an epoxide. This active mutagen can cause mutation in the organism, although most of this reactive molecule will subsequently react with water via the enzyme epoxide hydrase or with glucose, sulfate, or glutathione via enzymatic processes to form water-soluble conjugates that can be excreted in the urine. Examples of such promutagens or premutagens are the PAHs (e.g., benzo(*a*)pyrene) and the aromatic amines (e.g., β-naphthylamine). An additional unique promutagen is a metabolite of the fungus, *Aspergillus flavus*, called aflatoxin B1. Aflatoxin B1 serves as a biocide

against other microorganisms to preserve the ecological niche of *Aspergillus flavus*. Other examples of promutagens include the large group of nitrosamines. Dimethyl-nitrosamine, or DMN, is one example of a potent mutagenic, carcinogenic nitrosamine.

Fifth, it must be pointed out that the PAHs can intercalate into DNA and also are metabolized to active alkylating agents such as epoxides. Hence, PAH and similar promutagens form a fifth set of complex mutagens that can intercalate into and alkylate DNA bases, therefore inducing base substitution and frameshift mutations. Other compounds that bind specifically to DNA in a physical sense, such as aflatoxin, and are metabolically activated to epoxides that bind covalently to DNA bases, are also included in this group of complex mutagens, which can both bind to DNA and generate alkylating moieties upon metabolism by cytochrome P450 enzymes.

A sixth type of agent that is active at the DNA level is metal salts. These include compounds of the elements nickel, chromium, and arsenic (a metalloid). Some metal salts are carcinogenic when inhaled by lower animals, cause chromosomal damage in cultured murine and human cells, and are carcinogenic to occupationally exposed humans. Soluble hexavalent chromium compounds are mutagenic and clastogenic. Particular insoluble chromium compounds containing hexavalent chromium induce lung tumors when inhaled by lower animals. Specific insoluble chromium compounds can also be inhaled by humans in the chrome plating or chromium-manufacturing industries, and can induce lung cancer. Specific insoluble nickel compounds, such as nickel subsulfide and black and green nickel oxides, are carcinogenic in animals. In nickel refinery operations in the past, humans have inhaled mixtures of soluble and insoluble nickel compounds, and have shown increased incidences of nasal and lung cancer. Specific insoluble nickel compounds are phagocytosed into cultured murine and human cells and cause oxygen radical generation, chromosomal breakage, and inhibition of DNA methylation in these cells. Arsenic compounds are carcinogenic in humans, in the context of copper smelting operations, where arsenic compounds contaminate copper ores. Roasting copper ores leads to generation of arsenic trioxide, which induces lung cancer in humans. Drinking water contaminated with arsenic compounds also leads to lung cancer and other cancers in humans in Taiwan, where the artesian wells are contaminated with arsenic compounds. Arsenic compounds induce lung cancer, urinary bladder cancer, skin cancer, liver cancer, and leukemias. Arsenic compounds are believed to generate oxygen radicals, which can induce DNA damage.

Molecular Mechanisms of Mutagenesis

Mechanistically, the simplest type of mutagenesis occurs when the enzyme DNA polymerase is copying one strand of DNA into its complementary strand and places the incorrect nucleotide into the newly synthesized strand of DNA. Although it is thermodynamically favored that the correct base will be inserted, there is a lesser but real probability that the incorrect base will be inserted during DNA replication. An example would be placement of the wrong base, adenine (A), opposite the DNA base cytosine (C), instead of inserting the correct base guanine (G) opposite the base C. This results in what is described as a G/C to A/T transition mutation, and it is called a spontaneous mutation.

A second type of induced mutagenesis occurs when an alkylating agent, such as MNNG, reacts with the base guanine in DNA and places a methyl group on the oxygen in the 6 position of guanine to form an ether linkage. During the normal replication of the DNA, guanine pairs with cytosine, with which it makes three hydrogen bonds. When guanine is methylated on the oxygen in position 6, due to treatment with MNNG, this methylated guanine now incorrectly pairs with an incoming thymine instead of the cytosine with which a normal guanine would pair. This results in a G/C to A/T transition mutation.

Deletion and addition mutations are caused when the DNA 'breathes' or opens its structure and occasionally a piece of this DNA loops out. This occurs because the energy of room temperature (kT, where k is the Boltzmann constant), is sufficient to break apart some of the hydrogen bonds which hold the two DNA strands together. When a part of a DNA strand loops out, and when an intercalator molecule, such as acridine orange or ethidium bromide, subsequently intercalates into the DNA strand at a looped out structure, it can stabilize this looped out structure. DNA repair enzymes can then recognize this looped out structure as an aberrant structure, and can then excise this structure out of the DNA by use of incision endonucleases and DNA polymerase I. This process would then result in a deletion mutation. Addition mutations may be caused by insertion of extra DNA bases during DNA replication. This can happen spontaneously. It is also thought to happen when a DNA polymerase either slips along runs of GC base pairs, or when the polymerase incorrectly recognizes the intercalator molecule as a DNA base.

As mentioned previously, the planar PAHs are very complex molecules. They can intercalate into DNA. In addition, by virtue of their being metabolized into epoxides, they can also covalently bind to DNA. Hence, they can cause base substitution mutations

(transitions and transversions) and can also cause frameshift mutations when they are copied by the DNA polymerase incorrectly and incorrect nucleotides are inserted into the DNA, leading to mutations in the DNA. PAH bound to DNA also cause a labilization of the DNA base-sugar bond, leading to depurination of the PAH-adducted base. During DNA repair, the DNA polymerase occasionally adds the correct base back at the depurinated site, but often adds an incorrect base, leading to frequent transversion mutations at the depurinated site.

Bacterial Mutagenesis Detection Systems

Two assays in bacteria are commonly used to detect and study the molecular mechanisms of mutation in bacteria and also to screen chemicals to determine whether they are mutagenic to bacteria. The most commonly employed assay is that of reversion of mutant *Salmonella typhimurium* bacteria back to wild type or normal bacteria, developed by Dr. Bruce Ames and colleagues and the University of California at Berkeley. In this assay, suspect mutagens plus a source of metabolic activation in the form of S-9 are added to and incubated with *S. typhimurium* bacteria. S-9 is a preparation of rat liver, in which the rat liver has been homogenized, then centrifuged at 9000 *g*, and the supernatant is utilized. Rat liver S-9 contains cytochrome P450 and other xenobiotic metabolizing enzymes. The specific Ames' strains of *S. typhimurium* contain mutations in the genes encoding enzymes that biosynthesize histidine, such that these mutant bacterial strains cannot grow unless exogenous histidine is provided. In this assay, the investigator counts the number of mutated bacteria that can now grow in medium lacking histidine, and scores these as reverted mutant bacteria. This assay is very effective at detecting the mutagenicity of chemicals. Since reversion is very specific, it is common for investigators to use two Ames' strains to detect base substitution mutations, two Ames' strains to detect frameshift mutagens, and one strain of bacteria to detect mutagens that generate oxygen radicals. This assay also identifies 50% of all chemical carcinogens that are mutagenic carcinogens by detecting their mutagenicity. This assay is commonly used by industrial firms such as pharmaceutical companies, by governmental agencies charged with regulating the containment of carcinogenic substances, and by researchers interested in identifying new chemical mutagens and in understanding the molecular mechanisms of chemical mutagenesis. It is a rapid assay that takes only 2 or 3 days to complete and is relatively inexpensive ($500 or less per assay in the commercial sector).

A second mutagenesis assay, formulated by Dr. William Thilly and colleagues at the Massachusetts Institute of Technology is based on the Ames' assay. In this assay, the Ames' *Salmonella* bacterial strains are used, but the assay is a forward mutation assay in which bacteria are treated with chemical mutagens plus or minus S-9 metabolic activation. Then, the number of bacterial colonies resistant to the toxicity of 8-azaguanine are scored as mutant colonies. This assay has been claimed to be more sensitive than the original Ames assay because the entire genes should be the targets for mutagenesis, as opposed to small parts of histidine-synthesizing genes in the Ames' reversion assay. However, this assay is not employed as frequently as the Ames' assay. A large body of work has been done to characterize and understand the molecular bases by which the Ames' assay functions, and a very large number of mutagens have been detected and studied in the Ames assays. Hence, today, the Ames' assay remains the assay of choice to detect bacterial mutagens.

In both bacterial mutagenesis assays, one plots the number of mutant colonies on the ordinate (*y*-axis) versus the concentration of test compound on the abscissa (*x*-axis). The plots are usually linear up to the point at which cytotoxicity overwhelms mutation induction, and the number of mutants passes through a maximum and then declines. Hence, bacterial mutagenesis studies are usually conducted with low concentrations of chemical compounds or radiations that induce a linear frequency of mutations but do not cause significant cytotoxicity.

Mammalian Cell Mutagenesis Assays

In mammalian cells, it is common to utilize mutagenesis assays that measure induction of mutants that are resistant to the cytotoxicity of toxic drugs. One of the most frequently employed mutation assays in mammalian cells is the assay detecting mutation conferring 6-thioguanine resistance. This assay is most frequently employed in the Chinese hamster ovary (CHO) cell line or in the V79 Chinese hamster lung fibroblast cell line. In this assay, the normal or 'wild-type' cells are killed by the cytotoxic drug 6-thioguanine, or its closely related analog, 8-azaguanine. These drugs enter mammalian cells and react with the cellular metabolite, 5′-phosphoribosyl-pyrophosphate (PRPP), to form a toxic nucleotide which is incorporated into DNA and RNA, leading to cell death. In the mutation assays, the cells are treated with the suspect mutagen plus and minus S-9 (cytochrome P450) metabolic activation. Next, the cells are reseeded into new cell culture medium containing 6-thioguanine or 8-azaguanine, which is

called a mutant-selective agent. 6-Thioguanine or 8-azaguanine (they have similar effects) kills all the wild-type cells at sufficiently high concentrations and therefore selects against the wild-type cells. However, both spontaneous and mutagen-induced mutant cells are resistant to the cytotoxicity of 6-thioguanine and continue to grow and form discrete colonies. These mutant colonies are then fixed to the dishes with methanol or 70% ethanol, stained with the nuclear stain, Giemsa, and then scored with a microscope. One then plots the mutant frequency (number of viable mutants/cell culture dish)/(plating efficiency of cells x number of cells seeded per dish) on the ordinate (*y*-axis) versus the concentration of the mutagen on the abscissa (*x*-axis). For a strong mutagen, there is usually a dose–response effect, that is, the mutant frequency increases, usually linearly, as a function of increasing concentration of mutagen added to cells.

The mutant colonies are resistant to the cytotoxicity of 6-thioguanine because they have been mutated at the gene encoding the enzyme hypoxanthine guanine phosphoribosyl-pyrophosphate (HGPRT), such that the activity of this enzyme is decreased or abolished. This enzyme carries out a reaction between the DNA bases hypoxanthine or guanine and PRPP to form inosine and guanosine, which are then incorporated into DNA and RNA. In mutant cells, the gene encoding the HGPRT enzyme, hence the enzyme itself, is mutated. Therefore, the enzyme has a substantially reduced ability, or no ability, to carry out this condensation. The mutant cells cannot react toxic 6-thioguanine with PRPP to form a toxic nucleotide and are therefore resistant to the cytotoxicity of 6-thioguanine. They therefore survive and form colonies even in the presence of 6-thioguanine. Plotting the mutation frequency versus the concentration of mutagen added yields linear curves. This is a general assay and, as a forward mutation assay, it detects base substitution, addition, deletion, and frameshift mutations. It is one of the most widely used mutation assays in mammalian cells.

A similar assay is one in which L5178Y mouse lymphoma cells containing one active and one inactive gene encoding thymidine kinase are selected in trifluorothymidine. This treatment is lethal to wild-type cells. However, spontaneous mutants, and those induced by mutagens, have mutations in the second copy of the thymidine kinase gene, and hence do not phosphorylate trifluorothymidine and are resistant to the toxicity of this drug. This mutation assay is very valuable, because it detects point mutations, and it also detects mutations involving large amounts of damage to the chromosome, measured as resistant colonies with a small size.

A third assay that has been used to detect mutations in mammalian cells induced by chemical carcinogens and UV and ionizing radiations is that of mutation to ouabain resistance. Ouabain is a cardiac glycoside that binds specifically to the sodium, potassium adenosine triphosphatase ((Na,K) ATPase). The (Na,K) ATPase is an enzyme located in the plasma membrane of mammalian cells. This enzyme hydrolyzes ATP, and uses the resultant energy liberated to drive electrogenic transport of sodium ion out of the cell and transport of potassium ion into the cell. The transport is not equal, and three sodium ions are transported out of the cell, while two potassium ions are transported into the cell. The resultant electrogenic gradient is used to drive the transport of glucose and certain types of amino acids into the cell and to regulate cell volume, which are all crucial for cell survival. When cells are treated with the cardiac glycoside – ouabain, ouabain binds to a specific binding site on the (Na,K) ATPase, and inhibits the activity of this enzyme. As a result of this binding, the enzymatic activity of the (Na,K) ATPase is inhibited, and the transport of glucose and certain amino acids, and the regulation of cell volume, which are dependent on the activity of the (Na,K) ATPase, are inhibited, and cells die. Hence, ouabain at a concentration of 3 mM will kill murine fibroblasts down to spontaneous mutant frequencies (one mutant/one million wild-type cells). Treating murine fibroblasts, such as C3H/10T1/2 Cl 8 mouse embryo cells, with mutagens such as MNNG or BaP, induces mutations in the gene encoding the (Na,K) ATPase, such that the ouabain binding site is mutated. Hence, ouabain will no longer bind to the (Na,K) ATPase, and these mutant cells are resistant to the cytotoxicity of ouabain and will form mutant colonies in the presence of ouabain. This assay detects a restricted set of base substitution mutations which will mutate the ouabain binding site, but otherwise not affect the other enzymatic properties of the (Na,K) ATPase. It is thought that frameshift mutagens produce ouabain-resistant mutants that have a sufficiently damaged (Na,K) ATPase that these mutants are not viable.

Chromosome Breakage and Micronucleus Formation

In mammalian cells, the genes are arranged on discrete chromosomes. There are many assays that have been developed in mammalian cells to measure the ability of specific chemicals and ionizing and UV radiations to induce damage to these chromosomes, referred to as chromosomal aberrations. In these assays, the cells are seeded, treated with the chemical

or radiation of interest, then treated with colcemid to arrest the cells in metaphase. The cells are then swelled in hypotonic potassium chloride. The swelled cells are then dropped onto microscope slides, which bursts the cells and produces what is called a metaphase spread, or display of chromosomes. The chromosomes in these metaphase spreads are then stained with the nuclear stain, Giemsa stain, and examined by microscope to quantitate the numbers of chromosome aberrations present in the treated cells. Typical chromosomal aberrations that are scored include gaps, breaks, dicentrics (where parts of two chromosomes fuse, such that the resultant structure bears two centromeres), satellite associations, and ring chromosomes. Most mutagenic chemical carcinogens and ionizing and UV radiations have the ability to induce chromosome aberrations in mammalian cells. Translocations, in which one chromosome is broken and one or more parts of it are fused to another chromosome, can also be recognized with this assay. At sufficiently low concentrations, where cytotoxicity exerted by a chemical is sufficiently low, the induction of chromosome aberrations by chemical mutagens is linear as a function of the concentration of the chemical mutagen tested. Chemicals and radiations that can induce chromosome aberrations are referred to as clastogens.

A second type of damage that can be induced by chemical mutagens and ionizing and UV radiation is called micronucleus formation. In this assay, cells are treated with the chemical or radiation of interest, and then the cells are treated with cytochalasin B to inhibit cytokinesis. The cells are then visualized by staining with acridine orange, and micronuclei can be visualized under the microscope. Micronuclei are vesicles containing one or more whole chromosomes or pieces of chromosomes. The implication of observing micronuclei is that on further rounds of cell division, these micronuclei containing chromosomes or pieces of chromosomes can be lost from cells. Hence, formation of micronuclei indicates that large amounts of genetic material can be lost from cells, as much as that contained on an entire chromosome.

Induction of Morphological and Neoplastic Transformation in Mammalian Cells: Cell Culture Models for Chemically Induced Cancer Proceeding through Mutagenesis

There is a group of assays that can be utilized to study the ability of chemical carcinogens or UV or ionizing radiations to convert normal cells into cancerous (transformed or tumor) cells when normal cells are grown in cell culture and treated with these carcinogens. One property of normal cells, particularly fibroblasts, is that they divide, grow, and eventually fill a surface such as that of a tissue culture dish, and then stop growing. This is because cells in contact transmit signals through their membranes, on through the cytoskeleton (cell skeleton), and on to the cell nucleus, which triggers a negative feedback signal, which forces cells to stop growing. This property is referred to as contact inhibition of cell division. In cell culture, this property is manifested when the cells grow and fill the cell culture dish with a layer of cells one cell thick. However, when fibroblastic cells are treated with a chemical carcinogen that is already activated to an electrophile, or that can be activated by the specific types of cytochrome P450 enzymes the cell possesses to an electrophile, then 1% or less of the cells are 'transformed', or changed. They have lost contact inhibition and now grow on top of one another in arrays where the cells 'criss-cross' over one another. Since the fibroblastic cells are changed in shape or morphology, we refer to this as 'morphological transformation', or change in cell shape. This change in cell shape manifests itself as an overgrowth of the cells above the monolayer, in small piles, referred to as foci of transformed cells. Foci are easily seen when cells are stained with specific dyes, such as Giemsa stain or crystal violet. The stained foci can then be counted under the microscope. Scoring foci is an assay for chemical carcinogens since it detects one of the five steps that must be accomplished for fibroblastic cells to become able to form tumors when injected into immunosuppressed mice.

A second property of most normal cells is that they need to anchor to a surface, such as that of a tissue culture dish, in order for them to replicate their DNA and divide. This property is referred to as 'anchorage dependence'. Normal cells cannot grow in suspension in liquid media. An exception to this is white blood cells, which can grow in liquid suspension because this is their normal milieu in the circulating blood. However, most cells, particularly connective tissue cells like fibroblasts, cannot grow in liquid suspension and are anchorage dependent. When fibroblasts are treated with chemical carcinogens that are already activated to alkylating agents, or if the cells themselves have the cytochrome P450 enzymes to activate the carcinogens they are treated with, then a small fraction, on the order of 1 in 1 million, of the normal cells will be converted into anchorage-independent cells. Anchorage independent cells can grow and form colonies in liquid suspension, in a semi-solid medium such as 0.5% agar, which has the

approximate consistency of jello. Anchorage independence is another property that must be acquired by fibroblasts before they can become tumorigenic.

Normal cells also have a finite lifespan and undergo ~60 population doublings, then senesce or die through a process of programmed cell death. When cells are treated with chemical carcinogens or UV or ionizing radiations, a small fraction of them (1% in mouse cells and far less in human cells) can become transformed to immortality, such that they now grow forever. Acquisition of cellular immortality is a third step on the road to tumorigenicity, and can be acquired when cells are treated with chemical carcinogens or UV or ionizing radiations. Cellular immortality is relatively easy to induce in cultured murine cells, and much more difficult to induce in cultured human cells, likely due to the greater stability of the chromosomal complement of human cells compared to that of murine cells.

A final step on the route to development of tumor cells involves conversion of fibroblastic cells that have become immortal, morphologically transformed, and anchorage independent into tumor cells. This can occur spontaneously or upon treatment of cells with chemical carcinogens or UV or ionizing radiations.

What changes in the DNA result in the induction of the changes in cell properties (phenotypes) that we refer to as morphological transformation, anchorage independence, cellular immortality, and tumorigenicity? There are two broad classes of genes that control cell growth in a positive way: the proto-oncogenes, which control cell growth and signal transduction, and the tumor suppressor genes, which inhibit cell growth. Chemical carcinogens can react with and cause mutations in, amplification of, or translocation of proto-oncogenes, converting them into active cell-transforming genes known as activated oncogenes. There are ~50 proto-oncogenes that can be converted into activated oncogenes.

There are four broad classes of proto-oncogenes. One class of proto-oncogenes encodes protein products localized in the cell nucleus which act as factors that activate transcription of specific genes. This group is exemplified by genes such as c-*myc* and c-*jun*. A second group is located in the cell membrane or the cytoplasm and transfers biochemical signals in the cell. A prominent example of this group is the c-Ha-*ras* proto-oncogene. The protein product of this gene binds the high-energy molecule guanosine triphosphate, which activates this protein such that it transfers signals to other proteins and eventually toward the nucleus. The protein products of the last two groups of proto-oncogenes are located at the membrane and are growth factors and receptors for these growth factors. The c-*sis* gene is an example of this type of proto-oncogene. The significance of these genes is that mutational or other types of activations of two or more of these genes play a role in inducing transformed phenotypes and contribute to formation of a tumor cell.

There are ~50 tumor suppressor genes that have been identified to date. Chemical carcinogens cause mutations in these genes to inactivate them, or can break the chromosomes on which these genes are located, or can cause loss of an entire chromosome bearing a tumor suppressor gene from the cell. Inactivation of the two copies of a tumor suppressor gene renders it inactive in the cell. Inactivation by chemical carcinogens of two or more tumor suppressor genes, in concert with activation of two or more proto-oncogenes into oncogenes, cumulatively leads to formation of a tumor cell. Examples of tumor suppressor genes include the retinoblastoma (Rb) gene and the *p53* tumor suppressor gene. Inactivations of tumor suppressor genes and activations of specific oncogenes have been found in all human tumors that have been studied intensively to date.

Tier Concept for Screening and for Detecting Chemical Mutagens and Mutagenic Chemical Carcinogens

Genetic toxicology, the science by which chemicals and agents that cause mutation and other changes to DNA are studied, has evolved substantially during the past 50 years. It is now standard practice to detect chemical mutagens by utilizing the Ames' bacterial mutagenesis assay and other bacterial mutagenesis assays, both with and without S-9 metabolic activation, and to utilize assays detecting mutation to 6-thioguanine resistance in CHO or V79 mammalian cells. There are also assays that detect the ability of chemicals to cause chromosome breakage and micronucleus formation in Chinese hamster V79 cells or CHO cells by examining chromosomes of these cells under a microscope after chemical carcinogen treatment.

Assays that detect the ability of chemical carcinogens or UV or ionizing radiations to induce unscheduled DNA synthesis, or DNA repair, are also commonly used. In one of these, autoradiography is used to measure the incorporation of tritiated thymidine into the DNA of cells treated with these carcinogens. All the assays mentioned above are commonly used at the same time to detect the genotoxic properties of chemicals or radiation in what is referred to as a battery of tests. This battery of tests usually constitutes what is termed a primary screen

for mutagens, and is used to determine whether a specific chemical is a mutagen.

In addition to detecting chemical mutagens, these methodologies can also be used to detect chemical carcinogens. The most certain way to detect chemical carcinogens is to paint the skin of animals with solutions or suspensions of carcinogens, to feed carcinogens to animals, or to add carcinogens to the animals' drinking water, and observe whether the animals develop tumors. Unfortunately, treating mice or rats with chemical carcinogens and assaying for tumor induction takes >2 years and costs $\sim\$5$ million per chemical tested. This price is simply too large and the time too long to permit scientists to use animal bioassays to screen widely and routinely for the carcinogenicity of the hundreds of thousands of chemicals that need to be studied. During the past 20 years, an alternate strategy to detect carcinogens has arisen. This strategy is referred to as a tier screening strategy and relies on the fact that $\sim 50\%$ of the known carcinogens are mutagens. Hence, the current strategy relied on by most government, regulatory, and academic laboratories is to use inexpensive, rapid assays to test the large number of chemicals in use today in what is referred to as a primary screen using a battery of genetic toxicology assays, as outlined above. In this screen, one would use the Ames' bacterial mutagenesis assay, an assay for chromosome breakage, occasionally an assay to detect micronucleus formation, an assay to detect induction of DNA repair, and a mammalian cell mutagenesis assay. Chemicals that cause mutagenesis, chromosome breakage, or DNA repair in these assays would be considered suspect carcinogens. Further product development on these chemicals would likely be halted or the chemicals would be modified in their structure. Chemicals negative in this primary screen that were proposed to be used for human applications, such as food additives or cosmetics, would then be tested in cell transformation assays for the ability to induce morphological or anchorage-independent transformation, in what is considered a secondary screen. Chemicals positive in cell transformation assays are then eliminated from further development or chemically modified so they no longer cause cell transformation. Finally, chemicals to be marketed as cosmetics or food additives would then be tested in whole animal carcinogenesis assays. Only those that were not carcinogenic would ideally then be marketed to the public or used in commerce where large numbers of people would be exposed to them.

See also: Ames Test; Analytical Toxicology; Carcinogen Classification Schemes; Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogenesis; Cell Proliferation; Chromosome Aberrations; Dominant Lethal Tests; Host-Mediated Assay; Molecular Toxicology–Recombinant DNA Technology; Mouse Lymphoma Assay; Radiation Toxicology, Ionizing and Nonionizing; Reproductive System, Female; Reproductive System, Male; Sister Chromatid Exchanges; Toxicity Testing, Developmental; Toxicity Testing, Reproductive.

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Genetically Engineered Foods

William Frez

Introduction

Genetically engineered foods are a subset of genetically modified organisms (GMOs). The definition of a GMO may vary depending on the source of the information. However, the World Health Organization (WHO) specifically defines GMOs as "…organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally."

Farmers began using GMOs for the growth of soy-beans, potatoes, and corn in the mid-1990s finding benefits ranging from reduced operating cost to enhanced crop production. However, the use of GMO technology had not been without controversy then and is still debated now. Concern over the use of GMOs became heightened by the inadvertent introduction of a genetically modified corn (StarLink™) into the human food supply. Ostensibly registered by the US Environmental Protection Agency for use as an insect resistant plant pesticide and animal feed, the StarLink™ controversy propelled its producer to voluntarily cancel its registration and cease production. This episode in the evolution of GMO production has since then prompted citizen's watch groups, nongovernmental organizations (NGOs), and governmental agencies worldwide to address concerns regarding the use of GMOs ranging from specific toxicity to humans, plants, fish, and livestock to fear of large scale ecosystem disruption.

Current Technologies

In its simplest terms, the production of GMOs is derived from manipulation of the subject genome in order to achieve some desired trait or end result. Therefore, traditional agricultural practices intended to manipulate breeding or reproduction to select for desired traits over a somewhat protracted period of time can be thought of as genetic modification practiced over thousands of years. While drawing on these long used natural selection methods, current technologies have now been developed to achieve rapid and more dramatic results such as crop resistance to insects, production of novel pharmaceuticals, and increased animal growth and milk production. Thus, a brief outline of the essential gene manipulation technologies is fundamental to further understanding of potential global health concerns.

Transgenic Manipulation

Transgenic manipulation refers to the insertion, removal, and modification of the plant genome for placement into an individual of the same species, or across species to achieve the desired results in a relatively rapid period of time.

Two widely used techniques developed to achieve these ends are termed *in vitro* and vector-based techniques. *In vitro* techniques mechanically insert or inject a specific protein, gene, or genetic sequence into the subject organism. Within that general category, three methods are often defined; microinjection, particle or microprojectile bombardment, and DNA uptake directly into organism. Alternatively, vector-assisted techniques use live viral or bacterial carriers (i.e., vectors) to facilitate transfer of genetic material into the subject organism.

***In Vitro* Methods** Microinjection *in vitro* methods use a fine needle and microscopic manipulation to directly inject the gene material into protoplasts of the subject organism. The difficulty and cost of the microinjection process may be circumvented by DNA transfer via protoplast mixing. In this method, the 'protoplast-associated' DNA of one individual is allowed contact with that of another individual in a polyethylene glycol environment that is favorable to DNA exchange. Subsequent replication processes of the genetic material facilitate incorporation of the desired gene into the subject organism for an increase in copy number and favorable trait selection. Temporary disruption of the cell membrane via electrical stimulation (i.e., electroporation) can be used to enhance the transfer efficiency of this process.

Another often-used *in vitro* technique is 'particle bombardment'. In this method, microparticles of tungsten or gold are coated with the transgenic material and are literally 'shot' into the plant cell with compressed helium gas or an electrical discharge. Recombination and replication of the DNA material transpires within the subject organism DNA prior to manipulations to increase copy number and trait selection.

Vector-Facilitated Methods Vector-facilitated methods depend on the use of nonpathogenic viruses, or sections of bacterial DNA to transfer portions of the transgene or the transgene in its entirety using the biological invasive capabilities of the vector. Essentially, the vector DNA is modified and subsequently allowed to 'infect' the subject DNA to facilitate DNA transfer to the host. These vectors are frequently engineered to eliminate their virulence yet retain the DNA transfer capability of the original pathogen.

Marker Gene Incorporation

The mere delivery of the transgene into the host species or individual does not guarantee that the production of a GMO will be successful. This is due to the relative inefficient transfer process and the somewhat random selection process that must subsequently be used to enhance the number of individual copies of the transgene for further trait development. In order to address this limitation, genes or DNA segments coding for known and readily observable phenotypes (i.e., marker genes) are 'coinserted' with the transgene to better detect successful transformations. Such marker genes are now commonly used to detect color expressions or other visible attributes for better identification and subsequent selection and enhancement of the desired trait.

Commonly Produced GMOs

GMO technologies discussed above and others have been used to create a variety of modified crop, fish, and animal species for crop prey resistance, pharmaceutical development, and increased production from livestock. It is these applications that have attracted public and scientific interest over the years. As will be noted below, several organisms are currently used, though it should be anticipated that additional organisms will be employed in the future.

Genetically Modified Crops

The use of genetically modified corn and cotton has increased over 10-fold from 1992 to 1999 and as of 2002, 50 crop species have been evaluated for uses by the US Food and Drug Administration (FDA). In the development of transgenic crops, genes isolated from several varieties of the bacterium, *Bacillus thuringienses* (Bt) are probably the best known and most often cited example of GMO development.

The use of this bacterial species has been deemed to be ideally suited for GMO use due to possession of toxins known as delta endotoxins. The structure of the delta endotoxin, is complex, containing three major regions or sections that each connote differential characteristics to the ultimate toxin (**Figure 1**).

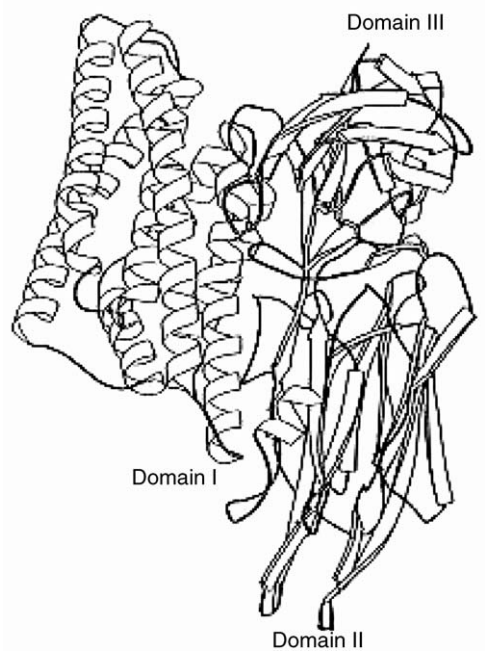

Figure 1 Molecular structure of *Bacillus thuringienses* delta endotoxin. (Reproduced from Li J, Carroll J, and Ellar DJ (1991) *Nature* 353: 815–821, with permission of the Nature Publishing Group.)

The domains of the molecule have markedly different conformations that control key aspects of 'Bt' toxicity to target insects. The α-helical bundles of the domain I region can be inserted into the gut cell membrane of the target species, thus, facilitating ion leakage. The domain II region consists of three β-sheet conformations that are structurally similar to immunoglobulin antigen binding regions, suggesting that the key characteristic section resides in the gut area. Domain III is a densely packed structure that is believed to protect the exposed end of the toxin from cleavage by digestive proteases; thus helping to ensure structural integrity and subsequent toxicity to the target species.

By incorporating these characteristics of the Bt toxin into valued crop species, crop resistance to insects feeding on crop species may be enhanced helping to increase vitality, size, and acreage yields.

Coded genes for the Bt delta endotoxin production have been isolated for insertion into a variety of vegetables including potatoes, field corn, sweet corn, and soybeans.

Genetically Modified Fish

No genetically modified fish have been approved for entry into the US human food supply as of this writing although growing pressure for such use is anticipated. However, as with crop species, the use of GMO technologies in fish has been deemed useful for obtaining an increase in production, size, disease resistance, optimal food consumption, and pharmaceutical development. Common species selected for research into the application of transgenic manipulations include salmon, tilapia, channel catfish, and medaka.

Alternatively, very active research in the use of transgenic fish as research models has been ongoing for several years with ever increasing focus on their use in the investigation of mutagenicity and environmental toxicology. Typical organisms for such applications tend to be small and easily cultured species such as medaka, mummichog, zebra fish, and others displaying favorable genetic attributes.

Transgenic fish were noted as being first introduced into the research community in 1985. Since that time, the use of transgenic fish to refine methods of transgene manipulation and application as potential analytical tools has grown substantially in the assessment of mutation frequencies. Several mutation assays using bacteriophage and plasmid vectors have been developed. Examples are shown in **Table 1**. As can be noted, detection methods may be based on modifications in enzyme systems that become evident in subsequent growth of bacterial species on selective growth media.

In concert with the mutation assays that are shown in **Table 1**, the potential use of transgenic fish in the assessment of environmental health risk assessment offers opportunities to further assess the effects of water- and sediment-associated contaminants in the environment.

Genetically Modified Livestock

The genetic modification of livestock stems from a desire to enhance growth, increase production of high protein milk and cheese, facilitate biomedical research, as well as potentially protect against incidental toxicity via exposure to pesticides that may be associated with food crops. Animal genomes that have been successfully modified include sheep, pigs, cows, rabbits, and chickens. Two key research areas applied to livestock are discussed below.

Enhanced Production Growth, as a primary metric of production, has been and is the subject of much research. Of substantial interest is the potential for use of transgenes in stimulating 'overproduction' of growth hormones to enhance animal growth. Several test protocols have been evaluated with moderate success in various livestock models.

For instance, blood levels of zinc-induced growth hormones have been increased in pigs and sheep. However, such activity was minimal in pigs and negligible via attempted phosphoenolpyruvate carboxykinase induction. Enhanced growth, via increased production of growth hormones also has been used to modify the qualitative nature of the animal such as lean muscle mass. However, such manipulations were not without negative effects such as renal failure and gastric ulcers of the subject animal.

Table 1 Various transgenic fish mutation assays

Assay	*Vector/fish species*	*Detection method*
lacI Mutation assay	Bacteriophage λ LIZ vector/medaka	α-Galactosidase functionality
cII Mutation assay	Bacteriophage λ/medaka	Plaque formation
rpsL Mutation assay	pML4 Plasmid vector/zebra fish	Kanamycin and streptomycin resistance
lacZ Mutation assay	Plasmid pUR288 vector/medaka and mummichog	Galactose-sensitivity

Pharmaceutical Development As of 2001, three companies were engaged in the manufacture of ~30 pharmaceuticals to produce proteins and antibodies derived from transgenic procedures. The transgenic-mediated production of pharmaceuticals is primarily achieved by facilitating construction of proteins that can then be expressed in milk of organisms are often termed 'bioreactors'. Examples of biologically active proteins that have been produced include antitrypsin, tissue plasmogen activator, and human blood clotting factor.

Human Health Risk

Although potentially beneficial, the development of GMOs is fraught with concern over the potential for adverse effects on human health. While human health effects of popular concern consist of increased toxicity, decreased nutritional value of food, and increased antibiotic resistance, possibly the most widely recognized concern centers on the potential increase in allergic reactions of individuals consuming transgenically modified food.

Approximately 1–2% of the population and 5–8% of children experience food allergies that are the result of natural selection processes that have occurred over thousands of years. Elevations in these frequencies may suggest allergic reactions to GMOs. Such allergic reactions are of two general types, immuoglobulin E (IgE)-mediated and non-IgE mediated reactions.

IgE-mediated allergenicity requires that individuals must first be exposed to an allergen in a sensitization dose prior to showing overt signs of the allergy. In this reaction, antigen specific binding of IgE to mast cells and basophils, followed by release of pharmacologically active chemicals such as histamines, cytokines, chemokines, and arachidonic metabolites are ultimately responsible for the classic rapid allergic reaction often termed 'immediate hypersensitivity reactions'. Toxic responses may range from, dermatitis, urticaria, and itching, to fatal anaphylactic shock and may be induced by proteins associated with a variety of foods including peanuts, grains, and fish.

Non-IgE mediated allergic reactions do not require a sensitization exposure and may occur especially in infants and children consuming milk protein and grains, and are characterized by a delayed onset of symptoms after exposure to the food. Food-induced enterocolitic syndromes caused by milk protein ingestion may result in vomiting, diarrhea, and general deterioration of the individual. Celiac disease is a specific example of such a wasting syndrome stemming from a reaction to cereal or grain (i.e., wheat, rye, barley, oats, and spelt) 'glutens'. Individuals experiencing this disease may show weight loss, diarrhea, abdominal cramps, gas and bloating, general weakness, oily stool, and stunted growth in children. Though significant in terms of effects to an afflicted individual, only ~1 in 300–3000 individuals in a population seem to be affected by celiac disease.

To date, no confirmed cases of increased allergic reactions to GMOs have been documented and thus the extent to which genetically modified food contributes to significant allergic reactions in the population is not accurately known. The only reported incident of potential GMO allergenicity occurred after production of soybeans modified with Brazil nut protein. Allergenicity to the genetically modified soybean was detected and the product was not marketed, precluding exposure and toxicity.

The previously discussed StarLink™ episode is perhaps the most widely evaluated incidence potentially leading to potential health effects associated with GMOs. Using enzyme-linked immunosorbent assay (ELISA) in a retrospective study, the US FDA hypothesized that exposure to the Bt Cry9c protein could be cause for allergic responses. However, the results of the FDA research concluded that there was no evidence of GMO-mediated allergenicity subsequent to potential exposure to the StarLink™ corn.

Because the lack of evidence of health effects cannot conclusively show that GMOs are not a human health concern, current global efforts are focused on the development of protocols to proactively and systemically detect and assess potential adverse effects.

Environmental Health Risk

The American Medical Association (AMA) has estimated that in 1999, 200 million acres of land had been planted worldwide with transgenic crops. The AMA further indicated that over 25 000 field trials for environmental effects of GMOs had been performed in 45 countries without noted adverse environmental consequences. Despite these conclusions, the limited geographical size and comprehensiveness of such trials confounds definitive conclusions regarding the potential for adverse effects such as enhanced crop pest resistance, out crossing with weedy relatives of crops, reduced biodiversity, nutritional deficiency of food sources, and toxicity to nontarget species.

Concerns over the safety of transgene introduction into environment was sensitized early in the GMO debate with significant focus on the potential toxicity of Bt endotoxins to monarch butterfly (*Danaus plexippus)* larvae exposed to transgenic pollen. Early data suggested that Bt corn pollen could result in potentially significant reactions in the monarch gut.

However, at that time (mid-1990s), the general regulatory consensus in the United States suggested that the adverse effects of Bt toxins were negligible when actual exposure conditions in the field were considered. In 1999, laboratory tests were conducted to determine if Bt transformed corn pollen consumed by monarch larvae at environmentally significant concentrations could result in adverse effects under well-controlled conditions. The widely published data resulting from this testing, indeed showed that larvae consuming Bt corn pollen experienced a decreased growth rate, expressed as the concentration of protein ($0.76\,\mathrm{ng\,ml^{-1}}$) required to result in growth reduction of 50% of the population (EC_{50}). Mortality, expressed as the LD_{50}, was determined to occur at $3.3\,\mathrm{ng\,protein\,ml^{-1}}$ diet. Estimated pollen densities of ~ 10 and 50–100 $\mathrm{grains\,cm^{-2}}$ were deemed to potentially result in these adverse effects.

As a potentially significant environmental and ecological risk, such Bt pollen toxicity notably stirred the interest and emotions of the scientific and public interest communities. In 2001, a collaborative research effort of Canadian and US scientists developed a weight of evidence risk assessment to address the concern. The risk assessment results suggested that the likelihood of an adverse effect on the monarch population was less than 1 in 10 000 (or less than 1×10^{-4}) when the toxicity information and exposure estimates were integrated into a risk analysis. While these data support the conclusion that an adverse effect on the monarch receptor was unlikely, it should be noted that the absence of data suggesting risk under given assumptions and circumstances should be interpreted carefully as a general conclusion of safety in all instances.

The concern for adverse ecological effects is not limited to plant or insect communities and direct toxicity. For instance, experimental data collected using fish suggests that the incorporation of transgenes may actually benefit the transgenic individual. For instance, mathematical models have been developed and evaluated to assess population effects of the transgenic fish of wild populations. Using the same data that show that the transgene may benefit the individual, models have predicted that the inadvertent release of the transgenically modified fish into the environment may result in significant adverse environmental effects such as invasion by genetically modified fish resulting in increased competition with and displacement of native species, reduced hardiness of the modified fish offspring in the wild, and potential extinction of the naturally occurring population. Although such effects have not been conclusively supported by field evaluations or verifying laboratory data, the concerns for long-term adverse ecosystem effects remains a subject of continued research.

As noted earlier in this article, transgenically modified growth hormones have been administered to cattle to increase milk production. However, it has been noted that the administration of growth hormones can result in adverse effects in the exposed animal including, fertility reduction, changes in bone growth, increase chance of mastitis, and reductions in endocrine function. The long-term effects of inadvertent exposures to the transgene-modified growth hormone on nontreated cattle herds, livestock, or breeding stock is poorly understood.

Safety Evaluation Methodology

The complexity of GMO introduction to the human food chain as well as uncertainties regarding potential ecosystem effects has prompted governmental agencies and NGOs to recommend highly structured and systematic safety evaluation protocols prior to GMO release. While several protocols have been proposed, developed, and are being adopted worldwide, those of the European Union and the WHO provide reasonable and illustrative examples for reference.

Substantial Equivalence

A fundamental concept essential to the currently defined approaches for determining GMO safety is that of 'substantial equivalence' (SE). Used as the basis for establishing a comparative benchmark, SE depends on two key concepts. First, existing traditional foods are assumed to be safe as evidenced by their long-term use. Second, the response to traditional foods can be used as a basis for comparison to transgenically modified foods which are often derived from traditional foods.

Predicated on the definition of substantial equivalence, a decision process for further evaluation of the GMO can be defined according to the general rules shown in **Table 2**.

While the decision matrix shown in **Table 2** suggests a rather simple testing approach, in reality, adherence to the SE concept requires that it be applied to a multitude of characteristic variables including chemical SE, biological SE, SE of potential exposure routes, and the SE of possible overall safe use.

The WHO Decision Tree for Assessment of Potential Allergenicity

Allergenicity is the current overriding concern regarding potential human exposures to GMOs. Thus, the

Table 2 Simplified decision matrix for evaluation of GMO safety according to the concept of substantial equivalence

If the GMO demonstrates	*Then*
Substantial equivalence to traditional food	No further testing or evaluation is necessary
Substantial equivalence to traditional food but also demonstrates evidence of a specific new trait	The assessment must focus on the potential effects of the new trait or gene
No substantial equivalence to traditional food	Comprehensive toxicological and nutritional testing is required

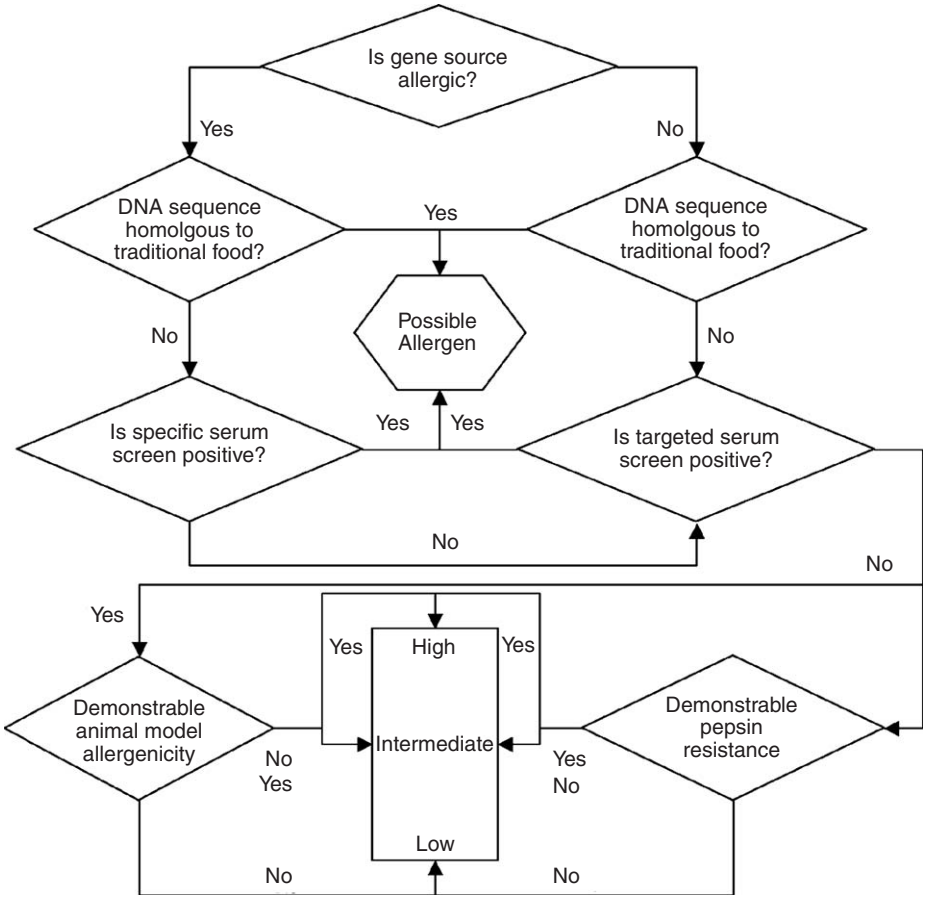

Figure 2 Decision tree for the evaluation of allegenic potential associated with GMO. (Adapted from FAO/WHO (2000) *Safety Aspects of Genetically Modified Foods of Plant Origin.* Geneva, Switzerland: World Health Organization.)

WHO has developed a decision tree approach to determine the human health risk from exposure to GMOs.

The decision tree and associated rationale and documentation is best reviewed in WHO records. However, **Figure 2** shows the general approach (with modifications for presentation purposes) suggested by WHO to determine if a GMO is a potential allergen.

The decision tree is not a prescriptive and mandated method with substantial recommended technical protocols but rather is a conceptual model to help the evaluator determine the potential that the GMO may trigger allergic reactions in greater proportion than the unexposed populations. As noted by examination of **Figure 2**, the incorporation of comparisons with traditional foods coupled with an allowance for graded responses ensures that the risks of allergic reactions are assessed in the context of exposures and the substantial equivalence concept.

Concluding Remarks

The development of GMOs, and genetically modified foods in particular, is, in large part, a direct response to ever increasing global food demands. Current

information suggests that GMOs are unlikely to have substantial adverse near term effects on human health and the environment. However, the assessment of the potential for longer-term adverse effects poses a greater challenge.

As a result of perceived needs, the technology inspired over the last 20 years and continues to develop; both in methodology and application. As a result of increased concerns over health effects, such activities will be scrutinized with growing vigilance on the part of citizens, scientists, regulatory bodies, and advisory commissions; ultimately to assure near- and long-term protection of global human health and the environment.

See also: Food and Drug Administration, US; Toxicity Testing, Mutagenicity; Transgenic Animals.

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Genetically Modified Organisms *See* Genetically Engineered Foods.

Genomics, Toxicogenomics

Kartik Shankar and Harihara M Mehendale

Toxicogenomics refers to the application transcriptonomic (high-throughput analyses of gene expression) techniques to the field of toxicology. Classically, toxicologists examine potential adverse outcomes and putative mechanisms due to xenobiotic exposure by biochemical and histopathological markers of toxicity. In the current healthcare and regulatory environment, chemicals suspected to have potential for significant adverse health effects are selected to undergo subsequent testing for carcinogenicity and chronic toxicity. However, long-term studies are typically labor intensive and time consuming and can cost $2–4 million. As of 2002, the National Toxicology Program had tested or is currently testing 505 chemicals in long-term studies, 66 in short-term studies, and only a single chemical in a subchronic study. Given that almost 70 000–85 000 chemicals are used in commerce today, it is evident that alternative high-throughput methods for screening the toxic potential of chemicals is needed. This would allow for prioritization of untested chemicals in the classical approach of toxicity testing. Concurrent with rapid advances in bioinformatic tools and classification algorithms toxicogenomic analyses is fast becoming a viable option in high-throughput screening of potentially hazardous chemicals.

Applications of Toxicogenomics

Predictive Toxicology

The underlying premise of toxicogenomics is that toxicity is associated with changes in the global gene expression. Since toxicity by itself is resultant due to some form of cellular dysfunction or cell death, it will either be preceded or followed by some level of

changes in gene expression. By monitoring the global gene expression changes a characteristic 'signature' change can be attributed to a particular phenotype of toxicity. Based on known gene expression signatures for established toxicants predictive models can be designed that will judge the toxic potential of untested chemicals based on their gene expression fingerprints. The progress in toxicogenomics as a tool for predictive toxicology is coupled to the advances in bioinformatics. Statistical techniques used to build predictive models range from linear and nonlinear discriminant analysis, Bayesian classification, nearest neighbor approaches, and neural networks.

Understand Mechanisms of Toxicity

The current mainstay for understanding mechanisms of toxicity involve use of *in vivo* model systems, including the rat and the mouse and other *in vitro* approaches involving a wide variety of cell and tissue culture techniques. Molecular mechanisms are explored based on hypothesis-driven experiments that, via appropriate interventional designs, test the involvement of a particular mechanism in mediating toxic outcome. The ability to scan genome-wide changes in expression of thousands of genes *ex vivo* or *ex vitro* now presents the unique ability to create novel hypotheses. It is naive to expect that microarrays and toxicogenomic investigations will replace traditional mechanistic approaches. On the other hand, toxicogenomic approaches complement and enhance the hypothesis generating capacity and allow probing a larger window of potential mechanisms.

See also: Bioinformatics; Microarray Analysis.

Further Reading

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Relevant Website

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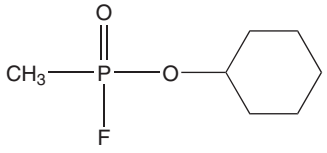
GF

Harry Salem and Frederick R Sidell*

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 329-99-7
- Synonyms: Cyclosarin; Cyclohexyl methylphosphonofluoridate; CMPF; Cyclosin; Methylphosphonofluoridic acid; cyclohexyl ester; Methyl cyclohexylfluorophosphonate; Cyclohexyl ester of methylphosphonofluoridic acid; Cyclohexylmethyl-fluorophosphonate; Methylfluorocyclohexylphosphonate; Nerve gas; Nerve agent
- Chemical/Pharmaceutical/Other Class: GF is a colorless, liquid, organophosphate human-made nerve agent with intermediate persistence. The evaporation rate is ~1/20th that of water. It has a nondescript odor described as sweet, musty, peaches, and shellac
- Chemical Formula: $C_7H_{14}FO_2P$
- Chemical Structure:



Uses

Cyclosarin is a nerve agent used in chemical warfare.

Exposure Routes and Pathways

Exposure can occur by inhalation, skin absorption of liquid or vapor, as well as by ingestion.

Toxicokinetics

Cyclosarin can be absorbed into the body by all routes of exposure. It is usually liquid in its normal

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

state, but will volatilize if heated to form vapor or aerosol. It is considered to have an intermediate persistence with an evaporation rate approximately one-half of water. GF is only slightly soluble in water, and the liquid in large amounts can layer out at the bottom of pools.

Mechanism of Toxicity

GF, an organophosphate, is a lethal cholinesterase inhibitor similar in action to sarin (GB). Limited data suggest delayed neuropathy such as postural sway and impaired psychomotor performance. Miosis has been noted for up to 62 days. Like sarin and the other nerve agents, GF and GB inhibit the enzymes butyrylcholinesterase in the plasma, the acetylcholinesterase on the red blood cell, and acetylcholinesterase at cholinergic receptor sites in tissues. These three enzymes are not identical. Even the two acetylcholinesterases have slightly different properties, although they have a high affinity for acetylcholine. The blood enzymes reflect tissue enzyme activity. Following acute nerve agent exposure, the red blood cell enzyme activity most closely reflects tissue enzyme activity. During recovery, however, the plasma enzyme activity more closely parallels tissue enzyme activity.

Following nerve agent exposure, inhibition of the tissue enzyme blocks its ability to hydrolyze the neurotransmitter acetylcholine at the cholinergic receptor sites. Thus, acetylcholine accumulates and continues to stimulate the affected organ. The clinical effects of nerve agent exposure are caused by excess acetylcholine.

The binding of nerve agent to the enzymes is considered irreversible unless removed by therapy. The accumulation of acetylcholine in the peripheral and central nervous systems leads to depression of the respiratory center in the brain, followed by peripheral neuromuscular blockade causing respiratory depression and death.

The pharmacologic and toxicologic effects of the nerve agents are dependent on their stability, rates of absorption by the various routes of exposure, distribution, ability to cross the blood–brain barrier, rate of reaction and selectivity with the enzyme at specific foci, and their behavior at the active site on the enzyme.

Red blood cell enzyme activity returns at the rate of red blood cell turnover, which is $\sim 1\%$ per day. Tissue and plasma activities return with synthesis of new enzymes. The rates of return of these enzymes are not identical. However, the nerve agent can be removed from the enzymes. This removal is called reactivation, which can be accomplished therapeutically by the use of oximes prior to aging. Aging is the biochemical process by which the agent–enzyme complex becomes refractory to oxime reactivation. The toxicity of nerve agents may include direct action on nicotinic acetylcholine receptors (skeletal muscle and ganglia) as well as on muscarinic acetylcholine receptors and the central nervous system (CNS).

Recent investigations have focused on organophosphate nerve agent poisoning secondary to acetylcholine effects. These include the effects of nerve agents on γ-amino butyric acid neurons and cyclic nucleotides. In addition, changes in brain neurotransmitters such as dopamine, serotonin, noradrenaline, acetylcholine following inhibition of brain cholinesterase activity have been reported. These changes may be due in part to a compensatory mechanism in response to overstimulation of the cholinergic system or could result from direct action of the nerve agent on the enzymes responsible for noncholinergic neurotransmission.

Human Toxicity

The onset of toxicity is usually rapid, occurring within minutes of exposure. Reduced acetylcholine levels are indicators of nerve agent exposure. Signs and symptoms are dependent on the degree of intoxication and may include the following:

- nervousness/restlessness;
- miosis (contraction of the pupil);
- rhinorrhea (runny nose), excessive salivation;
- dyspnea (difficulty in breathing due to bronchoconstriction/secretions);
- sweating;
- bradycardia (slow heartbeat);
- loss of consciousness;
- convulsions;
- flaccid paralysis;
- loss of bladder and bowel control; and
- apnea (breathing stopped).

The LD_{50} in humans has been estimated as $35\,\mu g\,kg^{-1}$ by inhalation, $0.350\,mg\,kg^{-1}$ percutaneously, and 1.0–1.4 mg per person intravenously.

Animal Toxicity

Subcutaneously, the reported LD_{50} values are 56.5–$110\,\mu g\,kg^{-1}$ in guinea pigs, $130\,\mu g\,kg^{-1}$ in hamsters, $100\,\mu g\,kg^{-1}$ in mice, $100\,\mu g\,kg^{-1}$ in rabbits, and $225\,\mu g\,kg^{-1}$ in rats.

Intramuscularly in mice, the LD_{50} was reported as $224\,\mu g\,kg^{-1}$ and in the rhesus monkey as $46.6\,\mu g\,kg^{-1}$.

Clinical Management

The immediate treatment for nerve agent intoxication is intravenous injection of 2 mg atropine sulfate (intramuscular injection should be considered if the patient is hypoxic and ventilation cannot be initiated, as there is a risk of ventricular fibrillation). This should be followed by additional injections of atropine at 10–15 min intervals, continuing until bradycardia has been reversed (e.g., until the heart rate is at 90 beats per minute). If breathing has stopped, a mechanical respirator should be used to ventilate the patient. Mouth-to-mouth resuscitation should not be attempted. If possible, oxygen or oxygen-enriched air should be used for ventilation. If possible, cardiac activity should be monitored.

Oximes (pralidoxime salts, obidoxime) may be of use in restoring acetylcholinesterase activity. Obidoxime may be used to treat GF intoxication; however, it may cause liver damage. Animal studies indicate that the oxime Hi-6 may be significantly superior to other oximes in the treatment of GF intoxication, but it is not widely available. Therefore, pralidoxime salts should be used, with a slow intravenous infusion of 500 mg to 1 g being given initially.

Diazepam should be administered to control convulsions. It also has value in controlling fear on the part of the patient. An initial dose of 5 mg may be followed by additional doses at 15 min intervals up to a total of 15 mg.

Protective equipment (self-contained breathing equipment or gas mask, barrier suit) must be used. Medical personnel treating casualties should avoid direct (skin-to-skin) contact: protective gear including breathing protection should be worn when treating casualties prior to decontamination. Latex gloves are not adequate protection. Casualties should be decontaminated as rapidly as possible. Casualties should be removed from exposure as rapidly as possible, but must not be moved into clean treatment areas where unmasked/ungloved personnel are working until decontamination is complete.

Decontamination of victims is accomplished by removing the victim from the contaminated area, removal of clothing, and removal or neutralization of agent present on the skin (skin decontamination may be unnecessary if the exposure was only to GF vapor). Any visible droplets should be blotted (not wiped) away using an absorbent material (e.g., paper towels and facial tissues); if available, towelettes moistened with a neutralizing solution should be used. Adsorbent powders may also be used for removal of droplets (in the absence of standard adsorbents, field expedients such as flour may be useful). A solution of 0.5% hypochlorite bleach may be used for skin decontamination. Hair should be thoroughly cleaned using soap and water, with care being taken to prevent wash water from contacting skin.

Surface decontamination may be accomplished using hypochlorite bleach slurries, dilute alkalis, or DS2 decontaminating solution. Steam and ammonia may be used for the decontamination of confined spaces.

See also: Acetylcholine; Cholinesterase Inhibition; Nerve Agents.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

GI Tract *See* Gastrointestinal System.

Ginger Jake

Robin C Guy

- Chemical Abstracts Service Registry Number: CAS 78-30-8 (Tri-*o*-cresyl phosphate, TOCP)
- Synonyms: Jamaican ginger paralysis; Ginger Jake paralysis; Ginger Jake walk; Jake leg; Organophosphorus ester-induced delayed neurotoxicity (OPIDN)
- Chemical/Pharmaceutical/Other Class: Organic phosphate compounds, primarily the *o*-isomer of tritolyl phosphate
- Chemical Formula: Example: $C_{21}H_{21}O_4P$

Uses

Tri-*o*-cresyl phosphate (TOCP) is used as a plasticizer in lacquers and varnishes. It is also used in hydraulic fluids, as a flame retardant, gasoline additive, heat exchange medium, waterproofing agent, and as a solvent for nitrocellulose.

Background Information

Discovered in the 1920s prohibition era in the United States, the name coming from the use of Jamaican ginger ('Jake') to flavor batches of cheap ('bath tub') gin. The ginger having been grown (and therefore being contaminated) with the help of pesticides, for example, TOCP, a potent organophosphate. The result was an axonal dying-back neuropathy affecting mainly large muscle groups. Jake poisoning struck thousands of adults in the United States, leaving many crippled by irreversible paralysis. TOCP was also a contaminant in cooking oil in Morocco in the late 1950s.

Exposure Routes and Pathways

TOCP's industrial uses may result in its release to the environment through various waste streams. If released to the atmosphere, TOCP will exist in both the vapor and particulate phases in the ambient atmosphere. Occupational exposure may occur through inhalation of dust particles and dermal contact with TOCP. The general population may be exposed to TOCP via ingestion of contaminated drinking water.

Toxicokinetics

TOCP can be absorbed after ingestion, through the skin, and by inhalation.

Mechanism of Toxicity

TOCP produces a delayed neurotoxicity, by inhibiting a nonspecific neuronal carboxylesterase, neuropathic target esterase. The neuropathic target esterase appears to have a role in neuronal lipid metabolism. Neuropathic target esterase enzymatic activity is highest in nervous tissue.

Acute and Short-Term Toxicity (or Exposure)

Animal

Table 1 lists the LD_{50} and LD_{Lo} values based on the mode of administration of TOCP in different animal species.

Table 1 LD_{50} and LD_{Lo} values, based on mode of administraion of TOCP, for different animal species

	Mode	*Species*	*Amount ($m\,kg^{-1}$)*
LD_{50}	Oral	Rat	1160
LD_{Lo}	IP	Mouse	50
LD_{Lo}	SC	Dog	500
LD_{Lo}	SC	Cat	185
LD_{Lo}	Oral	Rabbit	500
LD_{Lo}	IP	Rabbit	100
LD_{Lo}	SC	Rabbit	100
LD_{Lo}	IV	Rabbit	100
LD_{Lo}	IM	Rabbit	135
LD_{Lo}	SC	Guinea pig	300
LD_{Lo}	SC	Dog	300

LD_{50}, lethal concentration in 50% fatality of those tested; LD_{Lo}, lowest published lethal concentration; Oral, oral administration; IP, intraperitoneal administration; SC, subcutaneous administration; IV, intravenous administration; IM, intramuscular administration.

Human

Inhalation of TOCP may cause headache, nausea, vomiting, and muscle pain. Ingestion may lead to abdominal pain, nausea, and vomiting. Dermal exposure causes redness and pain. Symptoms may be delayed.

Chronic Toxicity (or Exposure)

Animal

Species differ in responses to TOCP. The chicken and cat have been used extensively especially because the responses in those species are very similar to those of man. Rabbit, dog, monkey, and guinea pig react inconsistently while rats and mice are reported to be resistant to paralysis although they still have nervous tissue damage.

Histological examination of hens exposed to TOCP revealed a wallerian 'dying-back' degeneration of the larger diameter axons and myelin sheaths. If the neuropathic target esterase is inhibited by 70%, the typical organophophorus ester-induced delayed neurotoxicity (OPIDN) will follow after an approximate 7–14 day delay.

Human

Main symptoms involve the nervous system. The initial symptoms are characterized by muscle weakness in the arms and legs that may occur days or weeks after exposure. Eventually, symptoms include symptoms of spinal cord injury, including a clumsy shuffling gait, spasticity, hyperreflexia, and permanent damage to the pyramidal tracts and upper motor neuron syndrome. This syndrome is known as OPIDN.

Clinical Management

No specific therapy is known as a treatment for victims presenting signs of Ginger Jake paralysis; as this is a delayed neurotoxic effect. It would be prudent to determine the cause of exposure to ensure that the victim is no longer exposed to TOCP and provide supportive care. The victim should be transported to a hospital for evaluation.

Environmental Fate

An estimated bioconcentration factor value suggests that bioconcentration in aquatic organisms is very high; however, depuration half-lives ranging from 4 to 6 days for TOCP isomers indicate that bioconcentration may not be an important process. TOCP is not expected to volatilize from water surfaces. Biodegradation of TOCP in river water and bottom sediment followed first-order kinetics; the half-life in river water and bottom sediment at 25°C was 10 days. Vapor-phase TOCP is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life in air is estimated to be ~1.2 days. Particulate-phase TOCP may be physically removed from the air by wet and dry deposition.

See also: Food and Drug Administration; Food Quality Protection Act, US; Food, Drug, and Cosmetic Act, US; Organophosphate Poisoning, Delayed Neurotoxicity.

Further Reading

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Ginseng

Michael Wahl

This article is a revision of the previous print edition article by Regina Wiechelt, volume 2, pp. 52–53, © 1998, Elsevier Inc.

- SYNONYMS: *Panax quinquefolium*; American ginseng

Uses

Ginseng is used for many medicinal and rejuvenating reasons domestically and abroad. Traditional Chinese medicine values ginseng as a nerve and cardiac stimulant, a treatment for impotence, to promote metabolism, and to moderate blood pressure and blood sugar levels. Two or three grams are considered therapeutic. Fifty grams has been prescribed but has resulted in adverse side effects.

Background Information

Ginseng is a long-stemmed herb with palmate leaves. Flowers are yellowish green and bloom in groups of two to four. Red drupe berries appear in clusters. This herb is native to the Orient but widely cultivated in the United States for export.

Exposure Routes and Pathways

Exposures occur via ingestion (e.g., teas and soups). Ginseng cigarettes are also available. A topical preparation is used to approximate wound edges. In the United States, it is widely accepted as an effective demulcent.

Mechanism of Toxicity

All plant parts contain 13 capon glycosides. Two of these agents have a prolonged anti-inflammatory action similar to that of nonsteroidal anti-inflammatory medications. Another component is thought to affect corticosteroid secretion in the central nervous system. The fusiform roots are recognized for their vitamin and mineral content.

Acute and Short-Term Toxicity (or Exposure)

Human

Limited information exists for acute ingestions of ginseng. Case reports have described cerebral arthritis following large acute ingestions of ginseng extract.

Chronic Toxicity (or Exposure)

Human

In large therapeutic doses, patients may suffer insomnia, depression, and nervous behavior. When used in chronically excessive doses, patients may suffer from hypertension, insomnia, dermal blemishes, and morning diarrhea. Hypertension and depression may result from abrupt withdrawal after chronic use.

In Vitro Toxicity Data

Studies of the ginseng extract ginsenocide Rb1 in a rat peritoneal mast cell model have demonstrated potent antihistaminic effects.

Clinical Management

Treatment of ginseng exposures is supportive. The use of the herbal preparation is discontinued. Most cases of ginseng overdose do not require aggressive management. Supportive care for symptoms that develop is the mainstay of treatment.

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Global Environmental Change

Thomas Wilbanks

Published by Elsevier Inc.

To say that the global environment is changing is to state the obvious. The global environment always changes, and life on earth has always been affected by those changes. Why, then, has 'global environmental change' become a subject of special interest in recent decades?

Throughout the earth's history, environmental change has reflected the interplay of natural atmospheric and geological forces, with infrequent interventions from outer space. In recent millennia, however, human use of the earth has increasingly reshaped physical and ecological environments locally and regionally through such agencies as fire and irrigation. In recent centuries, human transformations of the earth have come to have truly global impacts, and it is at least possible that some of these impacts – if they continue – could threaten the improved quality of life that is the aim of most human economic development.

The growing dominance of human actions as an aspect of global environmental systems stems from a combination of human population growth, economic growth (which implies increased demands for materials and services), and technological change. Since 1000 BC, the total human population has grown from ~50 million to ~6 billion. Just since 1970, the total output of the global economy has increased from ~$4 trillion (in US dollars) to ~$40 trillion, which means both vastly increased requirements for physical and biological building-blocks to support economic production and also vastly increased waste streams from that production. In the past century, technological change – spurred by economic competition – has changed resource requirements and the composition of the by-products of production, in many cases making the resource requirements more intrusive and the by-products more hazardous to human and ecological health.

The most visible evidence of this transformation is changes in land uses: natural ecosystems being replaced by managed ones, natural landscapes by built ones, uses related to local demands replaced by uses related to distant demands. As a global phenomenon, these changes have been pushed in part by resource extraction for materials and food to support economic growth in centers of global economic and political power, not only in remote source areas but also within the major powers themselves. Results have included land degradation from mining, deforestation for agricultural development, and in some cases desertification in marginal areas from such practices as overgrazing. Associated economic production activities, such as agroprocessing and manufacturing, generate wastes that must be absorbed by environmental systems: the air, water bodies, and/or the land and subsurface.

These activities and impacts arise from a complex interplay of processes that operate at a variety of geographical scales from global to local, and temporal scales from very immediate to very long term. Chains of causality work both from the global scale to the local, as in the case of economic globalization, and from the local to the global, either systemically

(where local actions result in changes in global systems, such as ozone depletion) or cumulatively (where local actions result in local or regional changes of global significance, such as species extinction).

Some observers are convinced that the growing volume and intensity of such activities threaten nature–society balances on which our very survival depends. Waste production and disposal poses hazards from toxic and radioactive substances, which even casual observers can relate to land–surface degradation in countries including the United States and Russia; and many water bodies have been degraded as well. Carbon emissions due to fossil fuel use appear to be the primary cause of global climate change. Deforestation and environmental pollution are reducing global biological diversity, with implications still being studied. Moreover, biological mutations pose the possibility of new dangers to people, animals, and plants, as we find that epidemics such as HIV/AIDS are still imaginable in a modern world.

Other observers argue that it is not environmental 'preservation' that is the primary issue. Sustainable development depends at least as much on economic progress for human societies. The issue is adaptive environmental management, utilizing technological and institutional change to sustain economic growth while reducing adverse impacts on the environment. In order to avoid potentially radical social transformations in the future, economic and environmental progress need to be closely coupled.

Particularly important are issues of technological and institutional change. Technological change offers breakthroughs in environmental monitoring and waste control, prospects for substituting new alternatives for depletable resources, and new tools for analysis and assessment. At the same time, it continues to introduce new substances, some of which could have unintended consequences. Institutional change in this era of the information technology revolution offers new opportunities for information access and participative decision-making, but it raises questions about information control and privacy.

In many cases, progress is being made. In many nations, air and water pollution is being reduced and the degradation of land areas is being reversed. A new awareness of environmental risks and hazards, combined with new tools for monitoring and control, is being reflected in both public policy and stakeholder participation. Even at the international scale, where progress can be complicated by differing national styles and agendas, the Montreal Protocol on substances that deplete the ozone layer shows that action is possible and environmental standards for international trade are being developed and implemented.

But many challenges remain as the world contemplates continued population and economic growth, new risks of unintended consequences from technological developments such as bioengineering, and a tendency for decisions to be shaped by narrow economic and political interests. Unless an 'environmental ethic' becomes widespread, it is likely that global environmental change will remain a pressing concern for both society and science.

See also: Ecotoxicology; Pollution, Soil; Pollution, Water.

Further Reading

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Global Warming *See* Global Environmental Change.

Glutathione

Shayne C Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 2, pp. 53–54, © 1998, Elsevier Inc.

Glutathione, also referred to as GSH, is an endogenous component of cellular metabolism, a tripeptide composed of glycine, cysteine, and glutamic acid. It is normally present in the liver at a concentration of $10\,\text{mmol}\,l^{-1}$. It is an integral part of the biotransformation of xenobiotic substances, and serves to protect the body from reducing agents.

Unlike amino acids, glutathione conjugation involves electrophilic substrates. Some of this is done nonenzymatically. Glutathione *S*-transferases, or ligandin, aids the enzymatic conjugation by catalyzing the reaction, converting GSH to GS^-. Glutathione *S*-transferases comprise $\sim$10% of total cellular protein.

Glutathione can conjugate with xenobiotics in many ways. It may displace an electron-withdrawing group, putting GS^- in its place. It may add itself (GSH) to the substrate. It may also respond to a substrate formed from earlier metabolism. A xenobiotic may stereoselectively conjugate, removing one or more of the peptides.

Glutathione conjugation helps contribute to detoxification by binding electrophiles that could otherwise bind to proteins or nucleic acids, resulting in cellular damage and genetic mutations. Exaggerated presence of glutathione *S*-transferase may indicate resistance to chemical toxicity. Different glutathione *S*-transferase responses to different chemicals between species may add to differences in susceptibility to toxic effects. Glutathione *S*-transferases are a super family of enzymes that provide protection against many electrophilic compounds by catalyzing the conjugation of these compounds with glutathione to excretable water-soluble forms.

On the other hand, glutathione conjugation may activate the toxic moiety within a xenobiotic. Activation mechanisms involving glutathione include:

- Toxic metabolites released from conjugation with haloalkanes, organic thiocyanates, and nitrosoguanides.
- Electrophilic sulfur mustards formed from conjugation with vicinal dihaloalkanes.
- Conjugates of halogenated alkenes activated by enzymatic activity in the kidney.
- Toxic metabolites produced from γ-glutamyltranspeptidase degradation of quiriones, quinoneimines, and isothiocyanates.

A classic example of glutathione-related toxicity is acetaminophen. Phase 1 metabolism of acetaminophen by P450 results in a toxic metabolite. Glutathione conjugation breaks down and detoxifies the metabolite and excretes it as mercapturic acid. Sufficient glutathione is a key player in this protective biotransformation. If as much as 70% of endogenous glutathione is already consumed, toxic activation may take place. It takes only 15.8 g of acetaminophen to reduce glutathione levels to the point where hepatotoxicity may occur.

Glutathione conjugates have two routes of excretion – via bile or via urine. Conjugates eliminated in the urine are first converted to mercapturic acids in the kidney. Mercapturic acid is defined as *N*-acetylated, *S*-substituted cysteine conjugates arising from conjugation of a xenobiotic with glutathione. Its biosynthesis involves conjugation of the GSH itself. Glycine and glutamic acid are removed; then cysteine is conjugated further by interaction with *N*-acetyltransferase. This last step converts the substance to mercapturic acid.

Conjugation with mercapturic acid may also activate hepatotoxins. It may cleave with γ-glutamyltranspeptidase, an enzyme implicated in the degradation quinones, quinoneimines, and isothiocyanates. Acetaminophen also increases the urinary excretion of mercapturic acid and cysteine conjugates, enabling the formation of its own hepatotoxic metabolites. Treatment with mercaptamine, a synthetic mercapturic acid, can reduce acetaminophen intoxication.

It has been reported that binding sites provided with true specificity for GSH exist in the central nervous system, and this satisfies the main requisite for considering GSH as a neuromediator in addition to its functions noted above.

See also: Biotransformation; Kidney; Metallothionein; Oxidative Stress.

Further Reading

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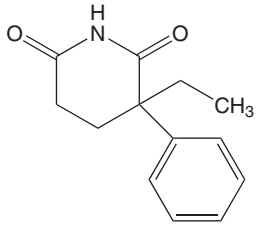
Glutethimide

Rebeca Gracia

- Chemical Abstracts Service Registry Number: CAS 77-21-4
- Synonyms: Doriden; Dorimide; Doridene, Glimid, Elrodorm glutarimide, 2-Ethyl-2-phenylglutarimide; 3-Ethyl-3-phenyl-2,6-piperidinedione; 2-Phenyl-2-ethylglutaric acid imide; α-phenyl-α-ethylglutarimide
- Chemical/Pharmaceutical/Other Class: Piperidinedione hypnotic and sedative
- Chemical Formula: $C_{13}H_{15}NO_2$
- Chemical Structure:



Uses

Glutethimide was once used as a sedative–hypnotic agent. Its use has generally been abandoned because of its acute and chronic toxicity, abuse potential, and the availability of more favorable alternatives.

Background Information

Glutethimide in combination with codeine was commonly abused and was referred to by various slang or street names including: Sets, Loads, Three's and Eight's, Fours and Doors. Glutethimide was changed from a Schedule III to Schedule II Controlled Substance in 1991.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposure to glutethimide. It is available as 125, 250, and 500 mg tablets.

Toxicokinetics

Glutethimide is erratically absorbed from the gastrointestinal tract, but peak serum concentrations generally occur within 1–6 h following a therapeutic dose. Glutethimide is metabolized by the liver to conjugated and unconjugated metabolites, two of which are active. These active metabolizes, 4-hydroxy-2-ethyl-2-phenyl-glutarimide (4-HG) and α-phenyl-γ-butyrolactone, accumulate in overdose patients and contribute to the toxic effects of glutethimide. 4-HG has been found to be twice as potent as the parent compound. Measured plasma levels of glutethimide do not correlate well with toxicity.

Protein binding is 50%. The volume of distribution is $1.7\,l\,kg^{-1}$. Glutethimide is highly lipid soluble and accumulates in the brain and adipose tissue. The elimination half-life for glutethimide is ~10–12 h but is prolonged in overdose.

Mechanism of Toxicity

Glutethimide depresses the central and autonomic nervous systems. The pharmacologic mechanism of glutethimide is not well understood. It produces effects comparable to those of phenobarbital. In addition, it possesses marked antimuscarinic activity.

Acute and Short-Term Toxicity (or Exposure)

Human

Ingestion of a single 500 mg tablet is likely to produce toxicity in a child. The potentially toxic and lethal doses of glutethimide in adults are generally accepted to be 3 and 10 g, respectively. Acute overdose with glutethimide results in central nervous system depression ranging from lethargy to profound coma. Prolonged and fluctuating coma may occur due to redistribution of active metabolites from adipose stores and from enterohepatic recirculation. Hypotension and respiratory depression may develop. Anticholinergic manifestations, such as decreased gastrointestinal motility and urinary retention, may complicate the clinical course. Pulmonary and cerebral edema, cardiovascular shock, and seizures may develop in severe cases.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies in pregnant rats at doses up to 0.4% did not result in fetal toxicity.

Human

Chronic use of high doses of glutethimide may produce psychological and physical dependence. Abrupt discontinuation of therapy may result in withdrawal signs and symptoms such as nausea, vomiting, tremulousness, tachycardia, fever, delirium, hallucinations, and seizures. Unlike opioid withdrawal, glutethimide withdrawal can be life threatening.

In Vitro Toxicity Data

Studies of glutethimide in *Drosophila melanogaster* have not demonstrated genotoxicity.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Activated charcoal can be used to adsorb glutethimide if given within 1 h of exposure. Respiratory support including oxygen and ventilation should be provided as needed. There is no antidote for glutethimide. If hypotension occurs it should be treated with standard measures including intravenous fluids, Trendelenburg positioning, and pressors by intravenous infusion. Standard measures for the management of seizures and cerebral edema should be employed. Hemodialysis and hemoperfusion may be effective for the active removal of glutethimide but should be reserved for severe cases when standard supportive measures are inadequate. The occurrence of withdrawal signs and symptoms indicates the need to reinstitute glutethimide therapy and gradually reduce the dose until it is discontinued. A barbiturate such as phenobarbital or a benzodiazepine may be substituted for glutethimide.

See also: Benzodiazepines; Charcoal; Codeine.

Further Reading

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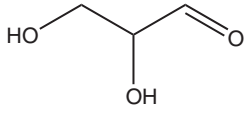
Hansen AR, Kennedy KA, and Ambre JJ (1975) Glutethimide poisoning: A metabolite contributes to morbidity and mortality. *New England Journal of Medicine* 292: 250–252.

Glyceraldehyde

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 367-47-5
- Synonyms: DL-Glyceraldehyde; 2,3-Dihydroxypropanal; α,β-Dihydroxypropionaldehyde; Glyceric aldehyde; Glycerose; 2,3-Dihydroxypropionaldehyde (glyceraldehyde can exist as two different isomers, D-glyceraldehyde and L-glyceraldehyde, which are 'mirror images' of each other)
- Chemical Formula: $C_3H_6O_3$
- Chemical Structure:



Uses

Glyceraldehyde is used: in nutrition; in the preparation of polyesters and adhesives; as a cellulose modifier; and in the tanning of leather. It is also used in biochemical research; the two isomers are used as 'reference' chemicals because each is one of the simplest molecules to compare against other molecules (such as sugars and amino acids). The conformation of the alcohol and aldehyde groups around the central carbon of D-glyceraldehyde helps scientists evaluate the structure and nomenclature (identity) of other simple sugars, such as glucose. This makes glyceraldehyde an important tool and reference standard for the biochemist.

Exposure Routes and Pathways

Only persons involved in the manufacture and production of glyceraldehyde would be expected to be exposed to significant concentrations of this compound. Because it is a solid at room temperature, exposure would be anticipated to occur only through contact with the skin or by inhalation of airborne dust. Skin exposure may also occur from contact with aqueous solutions (40%) of glyceraldehyde.

Acute and Short-Term Toxicity (or Exposure)

Animal

The median lethal dose (LD_{50}) for glyceraldehyde in rats is $2\,g\,kg^{-1}$, which places it in the category of

slightly to moderately toxic. The chemical is also slightly toxic by the intraperitoneal route. No other data are available on animal toxicity.

Human

Because glyceraldehyde is a normal metabolic intermediate in humans, this chemical cannot be readily categorized as a 'toxic' chemical. Given a large enough exposure or dose, any chemical can result in toxic injury. As with any aldhyde/alcohol, very high air concentrations or accidental ingestion of large amounts would be expected to overwhelm the body's natural defenses and produce an adverse effect (e.g., eye, nose, lung irritation from airborne dust; and stomach ache/nausea following ingestion).

Chronic Toxicity (or Exposure)

No information could be found on the chronic toxicity of glyceraldehyde.

Clinical Management

Persons who have been overcome by high concentrations or doses of glyceraldehyde should be removed from the area of high exposure. Medical attention should be sought. Treatment should be similar to first aid following any high-level chemical exposure; irrigation of the eyes with copious amounts of water, washing of exposed skin with soap and water, and supportive therapy following ingestion.

Ecotoxicology

No records are available in the US Environmental Protection Agency ECOTOX database for glyceraldehyde. Because this chemical is a metabolic intermediate and could easily be utilized by microorganisms, a release or spill of this compound into the general environment would not be expected to have any long-term adverse effects as the half-life in soil or water would be expected to be very brief (days).

Miscellaneous

Glyceraldehyde is a chemical that occurs naturally in living organisms, including humans. It is an intermediate in the metabolism of fructose. In the liver, fructose is converted to fructose-1-phosphate by the enzyme fructokinase. Fructose-1-phosphate is then converted to glyceraldehyde and dihydroxyacetone phosphate by the enzyme fructose-1-phosphate aldolase. Glyceraldehyde is then converted to glyceraldehyde-3-phosphate by the enzyme glyceraldehyde kinase. Glyceraldehyde-3-phosphate is a high-energy intermediate that may then move into the glycolysis cycle, which provides the body with a way of extracting energy to make ATP, which can then be used to power other metabolic functions, such as muscle contraction.

Further Reading

British Industrial Biological Research Association (1990) *Toxicity Profile of Glyceraldehyde*. Carshalton, UK: British Industrial Biological Research Association.

Glycerol

Kathryn A Wurzel

- Chemical Abstracts Service Registry Number: CAS 56-81-5
- Synonyms: Glycerin; Glycerine; 1,2,3-Propanetriol; 1,2,3-Trihydroxypropane; Glyceritol; Glycyl alcohol
- Chemical Formula: $C_3H_8O_3$

Uses

Glycerol is used as a solvent for flavors and food colors. It is also used as a humectant, plasticizer, emollient, sweetener, and filler in low-fat food products such as cookies. It is used in the manufacture of dynamite and propellants (nitroglycerol), cosmetics, candy, liqueurs, printing and copying inks, lubricants, pharmaceuticals (suppositories, cough syrups, elixirs, expectorants, and cardiac medications), personal care products (toothpaste, mouthwashes, skin care products, hair care products, and soaps), and antifreeze. Glycerol is also used to keep fabrics pliable and cellophane and special quality papers flexible and tough. Glycerol is a common energy yielding food and is widely distributed in food, both as a natural constituent and as a GRAS (generally recognized as safe) additive. Glycerol is used therapeutically to reduce intraocular pressure due to glaucoma and for cerebral edema.

Exposure Routes and Pathways

Inhalation, dermal contact, ocular contact, and ingestion are the exposure pathways for glycerol.

Toxicokinetics

Oral exposure results in rapid absorption through the gastrointestinal tract with rapid distribution in the blood. Most glycerol is incorporated into the body fat. Seven to 14% is excreted unchanged in the urine within 2.5 h of ingestion with ~80% of metabolism occurring in the liver and 10–20% occurring in the kidneys. Glycerol metabolism in the liver is initiated by glycerokinase, with glycerol further metabolized to carbon dioxide and water or utilized in glucose or glycogen synthesis. Some glycerol may combine with free fatty acid to form triglycerides. The elimination half-life of glycerol is ~30–40 min.

Mechanism of Toxicity

The medicinal action of glycerol as a laxative is a result of an increase in water absorption and irritation effects that cause evacuation of the bowel. Certain medical conditions such as cardiac, renal, or liver disease and/or diabetes may be exacerbated by shifts in body water as a result of exposure to glycerol.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rhabdomyolysis (muscle necrosis) in experimental animals may cause death as a result of acute renal injury. Intramuscular injection in rabbits results in necrosis of muscle fibers and disruption in cell plasma membrane. Extensive regeneration is apparent 7–14 days following exposure: changes are similar to muscular dystrophy. The glycol myopathy may be a good model for pathophysiological studies of Duchennes muscular dystrophy. An oral LD_{50} of $12\,600\,mg\,kg^{-1}$ and dermal LD_{50} of $10\,000\,g\,kg^{-1}$ has been reported in rats and rabbits, respectively.

Human

Glycerol is of a low order of acute oral and dermal toxicity. Toxicity following acute ingestion of excessive amounts of glycerol-based laxatives is generally minimal and limited to the gastrointestinal tract. Aspiration may result in pneumonitis. Adverse effects following oral administration include mild headache, dizziness, nausea, vomiting, thirst, and diarrhea. Glycerol dropped on the human eye causes a strong stinging and burning sensation with tearing and dilation of conjunctival vessels, but no obvious injury. Hemolysis, hemoglobinuria, and renal failure may occur at very large doses and is a function of concentration and route of administration (oral or parenterally). Severe dehydration, cardiac arrhythmias, and hyperosmolar nonketoic coma may be fatal.

Chronic Toxicity (or Exposure)

Animal

Chronic oral exposure to glycerol may cause mild irritation of the gastrointestinal tract. In a 2 year study, no systemic or local effects were reported at a dose of $10\,000\,mg\,kg^{-1}$ body weight. Inhalation of glycerol aerosols may cause irritation of the respiratory tract.

Glycerol did not produce statistically significant effects in chromosome aberrations and dominant lethal assays. It is not thought to be genotoxic.

In Vitro Toxicity Data

Glycerol has not been shown to induce mutations in bacterial assays, chromosomal effects in mammalian cells, or cause primary DNA damage *in vitro*.

Clinical Management

Due to the generally low toxicity of glycerol and potential aspiration hazard, emesis is not generally recommended following ingestion. Activated charcoal should only be used in the event of very large ingestions due to the potential for induction of vomiting. Dehydration, electrolyte imbalance, hyperglycemia, and acidosis or alkalosis require management by the clinician as appropriate. Excessive diarrhea should be treated with high fluid intake and monitoring of fluid and electrolyte status.

Environmental Fate

Glycerol is neither expected to bioconcentrate in fish and aquatic organisms nor expected to adsorb readily to sediment. In soil, glycerol undergoes rapid biodegradation under aerobic conditions, is highly mobile, and demonstrates low volatility. In water, it rapidly degrades under aerobic conditions. Biodegradation in seawater and under anaerobic conditions is expected.

Ecotoxicology

Glycerol has low toxicity to algae and fish. The threshold toxicity is reported as greater than $3000\,mg\,l^{-1}$.

Other Hazards

Glycerol is combustible. Upon combustion, carbon monoxide and carbon dioxide are formed. If heated or in a fire, compressed air or oxygen apparatus and gas-tight suit may be needed.

It may polymerize as temperatures increase. It reacts violently with strong oxidizers and reacts with some acids. Contact with some oxidizers and acids may present an increased risk of fire or explosion.

Exposure Standards and Guidelines

Current data are not sufficient to determine the carcinogenicity of glycerol.

Occupational Safety and Health Administration Standards – permissible exposure limit, 1998: $15\,mg\,m^{-3}$ mist, total dust.

Eight-hour time-weighted average (TWA): $5\,mg\,m^{-3}$ mist respirable fraction.

American Conference of Governmental Industrial Hygienists threshold limit value, 2002: 8 h TWA $10\,mg\,m^{-3}$ mist. Excursion limit three times the TWA for no more than a total of 30 min per workday.

See also: Eye Irritancy Testing; Kidney.

Further Reading

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Relevant Website

http://www.jtbaker.com – Mallinckrodt Chemicals. J.T. Baker (2004) MSDS for Glycerol.

Glycol Ethers

Linda A Malley

- Synonyms: This is a large and diverse group of compounds that can be divided into two general classes: the ethylene glycol ethers and the propylene glycol ethers

Uses

Glycol ethers are extensively used in industrial applications as solvents for the manufacture of lacquers, varnishes, resins, printing inks, and textile dyes; as antiicing additives in brake fluids; and as gasoline additives. In addition, they are used in consumer products such as latex paints and cleaners.

Background Information

Glycol ethers used in industrial applications are generally colorless, and miscible with water and organic solvents. They are produced by reacting ethylene oxide or propylene oxide with anhydrous alcohol in the presence of a catalyst. This process produces mixtures that are separated by fractional distillation.

Exposure Routes and Pathways

Dermal contact and inhalation of vapor, aerosol, and/or mist are the primary routes of exposure. Although the oral route of exposure would not be expected during proper use of the material, there is the potential for accidental ingestion to occur.

Toxicokinetics

Glycol ethers are absorbed readily after oral, dermal, or inhalation exposure. In addition, for the ethylene series, the ratio of the oral LD_{50} to the dermal LD_{50} is ~ 1, indicating that an equivalent amount of material can be absorbed by either route. The differences in toxicity between the ethylene series and the propylene series appears to be due the metabolites produced. The parent glycol ethers are substrates for alcohol dehydrogenase (ADH). Further conversion by aldehyde dehydrogenase produces alkoxyacetic acids. Conversion by ADH can be inhibited by pyrazole, alcohol, and other ADH inhibitors. Higher

molecular weight glycol ethers are also partially metabolized by P450 isozymes. The ethylene glycol ethers are metabolized via the alkoxyacetaldehyde to the respective alkoxyacetic acid. For example, ethylene glycol monomethyl ether (EGME) is metabolized to methoxyacetic acid, which has been shown to produce the same biological effects as the parent compound, EGME. However, in the propylene glycol ether series, propylene glycol monomethyl ether (PGME) is metabolized to propylene glycol, which is further metabolized to carbon dioxide. The alkoxyacid metabolites appear to be responsible for the toxic effects reported in the testes, bone marrow, and embryo.

Mechanism of Toxicity

The alkoxyacid metabolites appear to be responsible for the toxic effects reported in the testes, bone marrow, and embryo. The testes, bone marrow, and embryo contain large numbers of rapidly dividing and differentiating cells, and it is possible that one or more processes of cell division and differentiation are affected. It has been hypothesized that alkoxyacetic acids may be introduced into the Krebs cycle by formation of methoxy- or ethoxyacetyl-CoA and by formation of methoxy- or ethoxycitrate by mitochondrial enzymes.

Acute and Short-Term Toxicity (or Exposure)

Animal

Glycol ethers as a class are not acutely toxic by the oral route. Inhalation exposure to high concentrations of compounds in the ethylene series can cause lethality. However, exposure to compounds in the propylene series was not lethal to rodents even at nearly saturated concentrations.

The monoalkyl ethylene glycol ethers have been shown to possess a wide spectrum of biological activity, with some variation in the range of effects and potency among the individual compounds. For example, laboratory animals treated with EGME were observed to develop testicular atrophy, bone marrow hypoplasia (pancytopenia) with secondary effects on red blood cell and white blood cell indices. EGME was teratogenic, embryotoxic, and fetotoxic in pregnant animals. Ethylene glycol monoethyl ether (EGEE) produced a similar pattern of toxicity, while ethylene glycol monopropyl ether (EGPE) and ethylene glycol monobutyl ether (EGBE) caused hemolysis and embryotoxicity/fetotoxicity without causing teratogenicity or effects on the bone marrow and testes. Therefore, it appears that the testicular and bone marrow effects decrease with increasing size of the alkoxy group, with the maximal effects observed for EGME. In general, the hemolytic effects appear to increase with the size of the alkoxy group. In addition, the hemolytic effects are more pronounced in mice, rats, and rabbits compared to dogs and man, which are less affected. The testicular effects have been observed in mice, rats, rabbits, and dogs, and the hematological effects have been observed in mice, rats, rabbits, cats, dogs, and man. However, propylene glycol ethers do not cause the testicular or hematological effects and are not teratogenic, although fetotoxicity has been reported in some studies at concentrations that also produced maternal toxicity. There are also reports that EGEE and EGBE cause kidney enlargement without functional impairment. EGME has been reported to affect conditioned avoidance behavior in trained rats, and PGME has been reported to cause central nervous system (CNS) depression. In general, the acetates derived from the glycol ethers have the same toxicological activity as the parent glycol ether. However, the acetate of PGME does appear to have teratogenic potential in rabbits, in contrast to the parent compound PGME.

In rats, EGME and its metabolite have also been reported to cause thymic involution in the absence of effects on body weight or spleen weight, reduced response to T cell mitogens, depressed production of interleukin-2, and altered response to primary antibody plaque-forming cells. Mice were not affected.

Human

Acute effects of overexposure include CNS changes (depression, ataxia, dysarthria, somnolence, tremor, personality change, and blurred vision); irritation of the eyes, nose, and throat; renal failure (including albuminuria, hematuria, and oxaluria); hemorrhagic gastritis; metabolic acidosis; and macrocytic anemia. An oral dose reported to cause lethality was $3\,g\,kg^{-1}$. There have been a number of reports in which workers were exposed to glycol ethers in the workplace. Fatigue, weakness, lethargy, anemia, bone marrow hypoplasia, and other abnormalities of hematological parameters (immaturity of neutrophils with some abnormal cells and low platelet concentration) have been reported in workers at exposure concentrations ranging from ~ 60 to 4000 ppm. There does not appear to be an association between exposure to glycol ethers and adverse effects on human testes.

Chronic Toxicity (or Exposure)

Animal

A 2 year inhalation study was conducted in F344/N rats and B6C3F1 mice. Exposure concentrations in rats were 0, 31.2, 62.5, and 125 ppm, and in mice the concentrations were 0, 62.5, 125, and 250 ppm for 6 h day^{-1}, 5 days week^{-1}. No oncogenic effects occurred in male rats. Pheochromocytomas were increased in 125 ppm females; however, the increase was not statistically significant. In male and female mice, forestomach squamous cell papillomas or carcinomas were increased in the 250 ppm group. Hemangiosarcomas were also increased in 250 ppm male mice. However, EGEE was not oncogenic in a 2 year rat feeding study at dietary concentrations of up to 0.9 g kg^{-1} day^{-1}.

Human

Painters at a large shipyard exposed to EGEE and EGME (time-weighted average 0–80.5 mg m^{-3} and 0–17.7 mg m^{-3}, respectively) had an increased prevalence of oligospermia and azoospermia. In addition, a significant proportion of the painters were anemic and granulocytopenic. Workers exposed to EGEE in a metal castings process at concentrations of 0–24 ppm had significantly lower sperm count per ejaculate; however, mean sperm concentrations were similar to unexposed workers.

In Vitro Toxicity Data

A number of the glycol ethers have been evaluated with respect to potential mutagenicity and, in general, they were not mutagenic in a variety of test systems.

Clinical Management

If ingestion has occurred, emesis or gastric lavage may be useful if initiated within 30 min. If acidosis is present, it can be treated with intravenous sodium bicarbonate as needed. Hemodialysis may be indicated in cases of severe acid–base and/or fluid–electrolyte abnormalities or in cases of renal failure. Animal data suggest that ethanol therapy may inhibit the formation of toxic metabolites.

Table 1 Names, molecular formulas, structures, and exposure standards for typical ethylene glycol ethers

Compound	*CAS number*	*Molecular formula*	*Chemical structure*	*Exposure standard*
Ethylene glycol monomethyl ether	109-86-4	$C_3H_8O_2$	$HOCH_2CH_2OCH_3$	TLV = 5 ppm NIOSH = 0.1 ppm IDLH = 200 ppm
Ethylene glycol monoethyl ether	110-80-5	$C_4H_{10}O_2$	$HOCH_2CH_2OC_2H_5$	TLV = 5 ppm OSHA PEL = 200 ppm NIOSH = 0.5 ppm IDLH = 500 ppm
Ethylene glycol monobutyl ether	111-76-2	$C_6H_{14}O_2$	$HOCH_2CH_2OC_4H_9$	OSHA = 50 ppm TLV = 25 ppm NIOSH = 5 ppm Possible human carcinogen
Ethylene glycol monopropyl ether	2807-30-9	$C_5H_{12}O_2$	$HOCH_2CH_2OC_3H_7$	None
Ethylene glycol monophenyl ether	122-99-6	$C_8H_{10}O_2$	$HOCH_2CH_2OC_6H_5$	None
Ethylene glycol monohexyl ether	112-25-4	$C_8H_{18}O_2$	$HOCH_2CH_2OC_6H_{13}$	None
Diethylene glycol monomethyl ether	111-77-3	$C_5H_{12}O_3$	$HOCH_2CH_2O–CH_2CH_2OCH_3$	None
Diethylene glycol monoethyl ether	111-90-0	$C_6H_{14}O_3$	$HOCH_2CH_2O–CH_2CH_2OC_2H_5$	WEEL = 25 ppm
Diethylene glycol monobutyl ether	112-34-5	$C_8H_{18}O_3$	$HOCH_2CH_2O–CH_2CH_2OC_4H_9$	None
Diethylene glycol monopropyl ether	6881-94-3	$C_7H_{16}O_3$	$HOCH_2CH_2O–CH_2CH_2OC_3H_7$	None
Diethylene glycol monohexyl ether	112-59-4	$C_{10}H_{22}O_3$	$HOCH_2CH_2O–CH_2CH_2OC_6H_{13}$	None
Triethylene glycol methyl ether	112-35-6	$C_7H_{16}O_4$	$HOCH_2CH_2O–CH_2CH_2OCH_2CH_2–OCH_3$	None
Triethylene glycol ethyl ether	112-50-5	$C_8H_{18}O_4$	$HOCH_2CH_2O–CH_2CH_2OCH_2CH_2–OC_2H_5$	None
Triethylene glycol butyl ether	143-22-6	$C_{10}H_{22}O_4$	$HOCH_2CH_2O–CH_2CH_2OCH_2CH_2–OC_4H_9$	None

TLV, threshold limit value for an 8 h day; OSHA PEL, Occupational Safety and Health Administration permissible exposure limit; WEEL, workplace environmental exposure level for an 8 h day; NIOSH, National Institute of Occupational Safety and Health exposure level for a 10 h day; IDLH, Immediately Dangerous to Life or Health.

Table 2 Names, molecular formula, structures, and exposure standards for typical propylene glycol ethers

Compound	*CAS number*	*Molecular formula*	*Chemical structure*	*Exposure standards*
Propylene glycol monomethyl ether	107-98-2	$C_4H_{10}O_2$	$CH_3CH(OH)CH_2–OCH_3$	TLV = 100 ppm NIOSH = 100 ppm
Propylene glycol monoethyl ether	1569-02-4	$C_5H_{12}O_2$	$CH_3CH(OH)CH_2–OC_2H_5$	None
Propylene glycol monopropyl ether	1569-01-3	$C_6H_{14}O_2$	$CH_3CH(OH)CH_2–OC_3H_7$	None
Propylene glycol isopropyl ether	3944-36-3	$C_6H_{14}O_2$	$CH_3CH(OH)CH_2–OCH(CH_3)_2$	None
Propylene glycol *n*-butyl ether	5131-66-8	$C_7H_{16}O_2$	$CH_3CH(OH)CH_2–OC_4H_9$	None
Propylene glycol *t*-butyl ether	57018-52-7	$C_7H_{16}O_2$	$CH_3CH(OH)CH_2–OC(CH_3)_3$	None
Propylene glycol butoxyethyl ether	124-16-3	$C_9H_{20}O_3$	$CH_3CH(OH)CH_2–OC_2H_5OC_4H_9$	None
Propylene glycol phenyl ether	770-35-4	$C_9H_{12}O_2$	$CH_3CH(OH)CH_2–OC_6H_5$	None
Dipropylene glycol methyl ether	34590-94-8	$C_7H_{16}O_3$	$CH_3OCH(CH_3)–CH_2OCH(CH_3)–CH_2OH$	TLV = 100 ppm NIOSH = 100 ppm IDLH = 600 ppm
Dipropylene glycol ethyl ether	15764-24-6	$C_8H_{18}O_3$	$C_2H_5OCH_2CH_2–CH_2OCH_2CH_2–CH_2OH$	None
Dipropylene glycol butyl ether	29911-28-2	$C_{10}H_{22}O_3$	$C_4H_9OCH_2CH–(CH_3)OCH_2CH–(CH_3)OH$	None
Tripropylene glycol methyl ether	20324-33-8	$C_{10}H_{22}O_4$	$CH_3OCH_2CH–(CH_3)OCH_2CH–(CH_3)OCH_2CH–(CH_3)OH$	None
Tripropylene glycol ethyl ether	20178-34-1	$C_{11}H_{24}O_4$	$C_2H_5OCH_2CH–(CH_3)OCH_2CH–(CH_3)OCH_2CH–(CH_3)OH$	None
Tripropylene glycol butyl ether	57499-93-1	$C_{13}H_{28}O_4$	$C_4H_9OCH_2CH–(CH_3)OCH_2CH–(CH_3)OCH_2CH–(CH_3)OH$	None
Butylene glycol methyl ether	53778-73-7	$C_5H_{12}O_2$	$CH_3OCH_2CH–(OH)CH_2CH_3$	None
Butylene glycol ethyl ether	111-73-9	$C_6H_{14}O_2$	$HOCH_2CH_2CH_2–CH_2OCH_2CH_3$	None

TLV, threshold limit value for an 8 h day; OSHA PEL, Occupational Safety and Health Administration permissible exposure limit; WEEL, workplace environmental exposure level for an 8 h day; NIOSH, National Institute of Occupational Safety and Health exposure level for a 10 h day; IDLH, Immediately Dangerous to Life or Health.

Environmental Fate

In the aquatic ecosystem, glycol ethers are estimated to remain in the water and are not expected to bioconcentrate in aquatic organisms or adsorb to sediment; however, they are expected to be removed by aerobic biodegradation. In the atmosphere, glycol ethers are expected to react with hydroxyl radicals. In the soil, they are expected to be mobile, and may volatilize from dry soil surfaces. Hydrolysis and photolysis are not expected to significantly contribute to their removal. However, aerobic degradation is expected to occur rapidly in soil.

Ecotoxicology

The 24 h LC_{50} in fish was greater than 5000 mg l^{-1} and the 96 h LC_{50} was greater than 10 000 ppm for EGEE. For EGBE, the 96 h LC_{50} was 1250–1490 ppm for fish and the LC_{50} for shrimp was 800 mg l^{-1}.

Other Hazards

Some glycol ethers are incompatible with strong oxidizers and caustic agents.

Exposure Standards and Guidelines

See **Tables 1** and **2**.

See also: Ethylene Glycol; Ethylene Glycol Monoethyl Ether; Ethylene Glycol Mono-*n*-Butyl Ether; Polyethylene Glycol.

Further Reading

Bioassay Systems Corporation (1983) Determination of the Reproductive Effects in Mice of Nine Selected Chemicals. EPA Document No. FYI-OTS-0483-0240.

Doe JE (1984) Further studies on the toxicology of the glycol ethers with emphasis on rapid screening and hazard assessment. *Environmental Health Perspectives* 57: 199–206.

Miller RR (1987) Metabolism and disposition of glycol ethers. *Drug Metabolism Reviews* 18(1): 1–22.

Toxicology and Carcinogensis Studies of 2-Butoxyethanol in F344/N Rats and B6C3F1 Mice. Technical Report Series No 484 (2000). NIH Publication No. 00-3974.

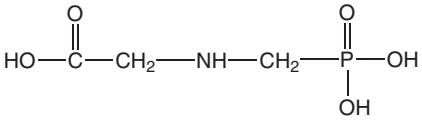
US EPA (1984) Health Effects Assessment for Glycol Ethers. EPA/540/1-86/052.

US EPA (2000) Integrated Risk Information System (IRIS) for Ethylene Glycol Monobutyl Ether (111-76-2), March 15.

Glyphosate

Kevin N Baer

- Chemical Abstracts Service Registry Number: CAS 1071-83-6
- Synonyms: *N*-Phosphonomethyl glycine; Roundup (41%); Accord; Rodeo; Gliialka; Sonic; Glifinox; Glycel
- Chemical/Pharmaceutical/Other Class: Organic phosphate herbicide
- Chemical Structure:



Uses

Glyphosate is the active ingredient in several commercial herbicides for nonselective weed control.

Exposure Routes and Pathways

The primary route of exposure to glyphosate is through accidental or intentional ingestion. Dermal exposure is not typically associated with systemic effects. Most incidents reported in humans have involved skin or eye irritation in workers after exposure during mixing, loading, or application.

Toxicokinetics

The major breakdown product of glyphosate is aminomethyl phosphonic acid (AMPA). The oral absorption of glyphosate and AMPA is low, with both compounds being eliminated essentially unchanged. Dermal absorption is also very low. High concentrations have been found in the kidneys, liver, brain, and blood following intentional oral ingestion. Glyphosate is rapidly excreted in the urine in large amounts. Usually within 24–48 h, glyphosate is undetectable in the urine. Neither glyphosate nor AMPA exhibit any tendency for bioaccumulation.

Mechanism of Toxicity

Several mechanisms have been proposed for glyphosate, such as uncoupling of mitochondrial oxidative phosphorylation, inhibition of aryl hydrocarbon hydroxylase activity, and inhibition of cytochrome P450 activity. However, surfactants present in many commercial preparations (i.e., Roundup), are considered to be responsible, in part, for the observed toxicity. In contrast to organophosphate insecticides, glyphosate is not a significant inhibitor of acetylcholinesterase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Glyphosate is a compound of low mammalian toxicity. Oral LD_{50} values for laboratory rodents are $>4\,g\,kg^{-1}$. The dermal LD_{50} value in rabbits is $\sim 5\,g\,kg^{-1}$. Direct contact with eyes of concentrated solutions of glyphosate can lead to transient irritation.

Human

The primary effects following ingestion include mucous membrane irritation, abdominal pain, vomiting, diarrhea, hypotension, oliguria, and anuria. Esophageal and gastric erosions have occurred after ingestion of concentrated solutions (41% glyphosate). In fatal cases, hypovolemic shock, cardiac arrhythmias, metabolic acidosis, and pulmonary edema have been reported. However, glyphosate has relatively low toxicity, with mortality rates of only 17% in suicidal cases. Ingestions of 150 ml or less have not resulted in deaths.

Chronic Toxicity (or Exposure)

Animal

Glyphosate and AMPA exhibit little potential for chronic toxicity. Multigeneration feeding studies failed to detect any tumorigenic potential. Glyphosate and AMPA were not teratogenic and did not lead to developmental toxicity. Two reproductive toxicity studies failed to detect any significant alterations. Glyphosate and AMPA were negative in endocrine modulation assays.

Human

Dermatitis resembling sunburn has been reported following prolonged skin exposure. There is no evidence of carcinogenicity in humans and only one tumorigenic response has been observed in experimental animals.

Clinical Management

There is no specific antidote; symptoms should be treated. For exposure to the eyes, the eyes should be flushed with plenty of water for at least 15 min. Due to the possibility of esophageal erosion, emesis is not recommended. Activated charcoal and a cathartic should be administered following ingestion of large amounts of glyphosate. Oral irrigation and dilution may be sufficient for smaller ingestions. In severe cases, basic life support, such as fluid replacement for hypovolemic shock, should be provided. Hemodialysis is indicated in patients with renal failure.

See also: Pesticides.

Further Reading

Williams GM, Kroes R, and Munro IC (2000) Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regulatory Toxicology and Pharmacology* 31: 117–165.

Gold

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-57-5
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Au^{1+}, Au^{3+}

Uses

Gold has found many industrial uses because of its excellent electrical and thermal conductivity properties. It is used for plating other metals and as an alloying metal. It is used in the manufacture of jewelry, dental inlays, art, currency, electronic components, and in some medical devices to provide radio opacity. Gold compounds have also found use in medicine in the treatment of certain cancers, rheumatoid arthritis, discoid lupus (a rare skin disease), and in specialized surgical procedures.

Background Information

Gold is probably the first pure metal known to man and is chemically nonreactive.

Exposure Routes and Pathways

The most common exposure pathway is through dermal contact. Inhalation and oral exposure to gold dust may occur in occupational settings.

Toxicokinetics

Gold dust and gold salts are poorly absorbed from the gastrointestinal tract.

Mechanism of Toxicity

The main mechanism believed to be responsible for gold salt toxicity is the formation of gold–protein complexes that elicit immune reactions. That is, gold salts may act as a hapten with subsequent antibody production against the gold–protein complex. The gold–protein–antibody complexes may in turn accumulate in the glomerular subepithelium. A second possible mechanism of gold salt toxicity is that antibodies may be formed against kidney tubular cells damaged by gold.

Acute and Short-Term Toxicity (or Exposure)

Human

Not known to be acutely toxic, though some of its salts are.

Chronic Toxicity (or Exposure)

Animal

Animal experiments have shown that gold dust is not carcinogenic to rats. However, subcutaneous implantation of gold sheets was able to induce tumors.

Human

Oral administration of excessive amounts of gold salts has been found to produce pancytopenia in

certain individuals. In addition, therapeutic doses of gold salts given for the treatment of rheumatic disease may produce adverse side effects such as dermatitis, immune complex hypersensitivity, nephrotoxicity, and peripheral neuropathy. Gold can also cause aplastic anemia and kidney damage.

Clinical Management

If toxicity occurs, further exposure to gold or gold salts should be prevented. Dimercaprol may be used as a chelating agent. A physician should be consulted if gold is being used as a therapeutic agent. Supportive treatment should be provided.

Further Reading

Goyer RA, Klaassen CD, and Waalkes MD (1995) *Metal Toxicology*. San Diego, CA: Academic Press.
Merchant B (1998) Gold, the noble metal and the paradoxes of its toxicology. *Biologicals* 26: 49–59.

Good Clinical Practice (GCP)

Sharmilee P Sawant and Harihara M Mehendale

Good clinical practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording, and reporting the results of clinical trials that involves participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of trial subjects is protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical trial data are credible. The guidance was developed with consideration of the current GCP of the European Union, Japan, and the United States, as well as those of Australia, Canada, and the World Health Organization. The purpose of these guidelines is to set globally applicable standards for the conduct of biomedical research on human subjects.

Traditionally, GCP has been a term used by those in government and industry to identify a collection of related regulations and guidelines that, when taken together, define the clinical study-related responsibilities of sponsors, clinical investigators, monitors, and institutional review boards (IRBs).

Principles of GCP

The principles of GCP are as follows:

1. Before a trial is initiated, risks should be weighed against the anticipated benefits for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits outweigh the risks.
2. The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.
3. The available nonclinical and clinical data on an investigational new drug (IND) should be adequate to support the proposed clinical trial.
4. Clinical trials should be scientifically sound, and described in a clear, detailed protocol.
5. A trial should be conducted in compliance with the protocol that has received prior IRB approval.
6. The medical care given to, and medical decisions made on behalf of, trial subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.
7. Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).
8. Freely given informed consent should be obtained from every subject prior to clinical trial participation.
9. The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality in accordance with the applicable regulatory requirement(s).
10. INDs should be manufactured, handled, and stored in accordance with applicable good manufacturing practice.

Specific responsibilities for investigators, sponsors, and the IRB/Independent Ethics Committee (IEC) detailed in the GCP provisions include the following.

Responsibilities of Investigators

A clinical investigator is the individual who conducts, or who is the responsible leader of a team that conducts, a clinical investigation. Federal regulations

state that "an investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of drugs under investigation."

Investigator responsibilities include:

1. *Control of the product*: The investigator can administer the IND only to subjects under his or her personal supervision.
2. *Record keeping and record retention*: The investigator must maintain adequate drug usage records, and must prepare and maintain, for each subject, adequate and accurate records of all observations and data pertinent to the clinical trial. The records must be kept for a minimum of 2 years till the marketing application's approved or a sponsor has discontinued an IND and notified the Food and Drug Administration (FDA).
3. *The investigator must provide to the sponsor*: (1) progress report of the clinical study; (2) safety reports of all adverse experiences that may reasonably be regarded as caused by, or probably caused by, the drug; and (3) adequate reports shortly after the completion of the investigator's participation in the study.
4. *Assurance of IRB review*: The investigator must assure that an IRB complying with regulatory requirements will be responsible for the initial and continuing review and approval of the proposed clinical trial.
5. *Handling of controlled substances*: The investigator should take adequate precautions to prevent theft or diversion of the product subjected to the Controlled Substances Act.

Responsibilities of Sponsor

Federal regulations define 'sponsor' as "a person who takes responsibility for and initiates a clinical investigation. The sponsor may be an individual or pharmaceutical company, governmental agency, academic institution, private organization, or other organization."

In general, the term 'sponsor' refers to a commercial manufacturer that has developed a product in which it holds the principal financial interest. A sponsor may also be a physician, commonly called a 'sponsor-investigator', which federal regulations define as "an individual who both initiates and conducts an investigation and under whose immediate direction the investigational drug is administered or dispensed."

The FDA defines sponsor responsibilities in Part 312, Subpart D in the Code of Federal Regulations (CFR).

The sponsor responsibilities can be divided into the following general areas:

1. *Selecting qualified investigators and monitors*: The sponsor must select qualified investigators-physicians and other professionals to conduct the clinical trial. According to the guideline, the sponsor may assign one or more appropriately trained and qualified individuals to monitor the progress of the clinical investigation.
2. *Informing investigators*: The sponsor should give complete information to the investigators about the investigational drug.
3. *Trial design and review of ongoing studies*: The sponsor should closely monitor the conduct and progress of clinical trails to determine if the investigator is conducting the trial in compliance with the protocol previously approved by IRB/IEC, applicable federal regulations, and an acceptable standard of GCP and whether the IND study is presenting unreasonable risk to the human subjects.
4. *Record keeping and record retention*: The sponsor should maintain adequate records showing the receipt, shipment to the investigator or disposition of the IND and keep records of the quantity of IND, date of shipment to the investigator.
5. *Ensuring the return or disposition of unused IND and related supplies*.

Institutional Review Board/Independent Ethics Committee

IRB/IEC is an independent body constituted of medical, scientific, and nonscientific members, whose responsibility is to ensure the protection of the rights, safety, and well-being of human subjects involved in a trial by reviewing, approving, and providing continuing review of trials, of protocols and amendments, and of the methods and material to be used in obtaining and documenting informed consent of the trial subjects. IRBs that approve studies of FDA regulated products must be established and operated in compliance with 21 CFR Part 56.

The responsibilities of IRB/IEC are to

1. Safeguard the rights, safety, and well-being of all trial subjects.
2. See if selection of subjects is equitable and informed consent is sought and documented in accordance with federal regulations.

3. Review the investigator brochure (IB), safety information, information about payments and compensation to subjects, the investigator's current curriculum vitae, and/or other documentation evidencing qualifications, and any other documents that the IRB/IEC may require to fulfill its responsibilities.

An IRB must have at least five members, chosen by the institution. FDA regulations allow institutions that do not have IRB/IECs to use 'independent' or other institutions' IRB/IECs to review their studies. IRB/IEC members are often physicians, pharmacologists, toxicologists, and administrative managers from the parent institution. Generally, drug sponsors have limited direct contact with an IRB/IEC. Aside from safety concerns, an IRB/IEC may address several issues including specific standards of the institution, state, and locality in reviewing a study. Any research program that the board approves must meet several criteria specified in FDA regulations:

1. Risk to subjects must be minimized and should be reasonable in relation to anticipated benefits and importance of the knowledge that may be expected to be gained.
2. Subject selection must be equitable.
3. Informed consent must be sought from each prospective subject or the subject's legally authorized representative and should be appropriately documented.
4. The research plan must make adequate provisions to monitor the collected data to ensure safety of subjects.

The sponsor and investigator will prepare the protocol for conducting clinical trials of the IND and the IB and get it approved by the IRB/IEC before the conduct of the clinical trial.

Clinical Trial Protocol

The contents of a trial protocol should generally include the following topics. However, specific information may be provided in separate information sheets such as the IB. The clinical trial protocol briefly contains:

1. *General information*: This includes protocol titles, name, title and address of sponsor, investigator, and qualified physician.
2. *Background information*: Name and description of investigational products, justification for route of administration, dosage and treatment period, description of population studied, and references.
3. *Trial objective and purpose*.
4. *Trial design*: The scientific integrity of the trial and credibility of the data from the trial depends on the trial design (e.g., double-blind, placebo-controlled, parallel design) and can be randomized and/or blinded to minimize/avoid bias.
5. *Selection and treatment of subjects*.
6. *Assessment of safety, efficacy, and statistics*.
7. *Quality control and assurance*.
8. *Ethics*.
9. *Data handling and record keeping*.

Investigator Brochure

The IB is a compilation of the clinical and nonclinical data on the investigational product(s) that are relevant to the study of the products in human subjects. Briefly, it contains:

1. *Summary*: A brief summary highlighting the significant physical, chemical, pharmaceutical, pharmacological, toxicological, pharmacokinetics, metabolic, and clinical information available that is relevant to the stages of clinical development of the investigational product.
2. *Introduction*: Introductory statement about the investigational drug and general approach followed for evaluating the investigational drug.
3. *Physical, chemical, and pharmaceutical properties and formulation*.
4. *Nonclinical studies' data and results should be provided in summary form*.
5. *Effects in humans*.
6. *Summary of data and guidance for the investigator.*

Summary

FDA's GCPs regulations are designed to accomplish two primary goals: (1) to ensure the quality and integrity of the data obtained from clinical studies so that the FDA's decisions based on these data are informed and responsible; (2) to protect the rights and, to the degree possible, the welfare of clinical subjects. To summarize, GCPs are regulations and guidelines that, when taken together, define the clinical study-related responsibilities of sponsors, clinical investigators, monitors, and IRB/IEC for the conduct of clinical trials, and define and monitor the clinical trials as per FDA regulations. Following clinical studies and based on the results of nonclinical and clinical studies, the drug sponsor formally proposes a new

drug application (NDA) to the FDA for approval of a new drug for marketing and sales in the United States. To obtain this government authorization, a sponsor submits in an NDA thousands of pages of nonclinical and clinical test data and analyses, drug chemistry information, and description of manufacturing procedures. Traditionally, the FDA has required that regulatory submissions, such as NDAs, be submitted as paper documents. Regulations in 21 CFR Part 314 provide the requirements and procedures for submitting applications to the Center for Drug Evaluation and Research to obtain approval for the marketing of new drugs. Since August 1997 these applications can be submitted electronically.

See also: Food and Drug Administration, US; Good Laboratory Practices (GLP); Investigative New Drug Application; Safety Testing, Clinical Studies.

Further Reading

Mathieu M (1997) Good clinical practices (GCP). In: *New Drug Development: A Regulatory Overview*, 5th edn., pp. 163–184. Waltham, MA: PARAXEL International Corporation.

Relevant Website

http://www.fda.gov – Food and Drug Administration website.

Good Laboratory Practices (GLP)

Robin C Guy

This article is a revision of the previous print edition article by Shayne C Gad, volume 2, pp. 61–65, © 1998, Elsevier Inc.

Background Information

Good Laboratory Practices (GLPs) are a standard compliance monitoring program that assures the quality and integrity of nonclinical test data submitted to the (US) Food and Drug Administration (FDA) and the (US) Environmental Protection Agency (EPA). These were originally developed by the FDA to develop minimum research standards for laboratories to protect the quality and integrity of studies as a result of procedures conducted in some studies that were not conducted according to conventional laboratory procedures. As a result, FDA promulgated the GLP Regulations, 21 CFR Part 58, on December 22, 1978 (43 FR 59986). The regulations became effective June 1979; sections have since been amended. The EPA adopted GLPs in August 1989. Other countries have also adopted GLPs. While there may be some slight variations, they all have the same central focus. Most countries have regular inspections and data audits to monitor laboratory compliance with the GLP requirements.

Sponsors of FDA-regulated products are required by the Federal Food, Drug, and Cosmetic Act (FFDCA) and Public Health Service Act to submit evidence of their product's safety in research and/or marketing applications. These products include food and color additives, animal drugs, human drugs and biological products, human medical devices, diagnostic products, and electronic products. These data are then used to answer questions regarding the toxicity profile of the article, the observed no-adverse-effect dose level in the test system, the risks associated with clinical studies involving humans or animals, the potential teratogenic, carcinogenic, or other adverse effects of the article, and the level of use that can be approved.

Sponsors of EPA-regulated products are required by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA) to submit evidence that assures the quality and integrity of test data submitted to EPA. These data are used by EPA to regulate pesticides and industrial chemicals.

The importance of nonclinical laboratory studies demand that they be conducted according to scientifically sound protocols and with meticulous attention to quality. GLPs provide that guidance. GLP regulations cover a large part of the aspects of nonclinical research. They include:

- inspection of a testing facility;
- personnel;
- testing facility management;
- study director;
- quality assurance unit;
- animal care facilities;
- facilities for handling test and control articles (EPA also has 'reference substances');
- lab operation areas;
- specimen and data storage;
- equipment design, maintenance, and calibration;
- standard operating procedures (SOPs);
- animal care;

- characterization and handling of articles (EPA also includes this for reference substances);
- protocol;
- reporting results;
- record retention; and
- disqualification of testing facilities.

Details of the GLPs and GLP inspections may be found on government-specific websites. Many are listed in the references below. Briefly, the FDA GLPs, define the scope of the regulation and have a detailed listing of definitions. If the FDA is to consider a nonclinical laboratory study in support of an application for a research or marketing permit, the testing facility, records and specimens must be available for inspection by an authorized employee of the FDA.

Every person who is responsible for any part of any GLP study must have the appropriate education, training, and experience, or combination thereof, to enable that person to perform the assigned functions. Each testing facility shall maintain up-to-date records of training, experience and job description for everyone involved in the conduct of a nonclinical laboratory study. There shall be adequate personnel to conduct the study according to the protocol who shall take appropriate precautions to avoid contamination of the test and control articles and the test systems.

The Study Director is responsible for the aspects of the study, but management also has responsibilities. For every nonclinical laboratory study, the management needs to assign a study director and assure that there is a quality assurance unit. Management must also assure that test and control articles or mixtures have been appropriately tested for identity, strength, purity, stability, and uniformity, as applicable. They need to assure that personnel understand the procedures they are to perform and are available in addition to availability of resources, facilities, equipment, materials, and methodologies. The Study Director has overall responsibility for the technical conduct of the study, as well as for the interpretation, analysis, documentation and reporting of results, and represents the single point of study control. This point has been interpreted in many ways, but the intent is that the study director is responsible for all aspects of the study, including procedures that may take place at another facility. Examples may include analysis of analytical samples for confirmation of concentration or homogeneity of a test article mixture, or for analysis of biological samples (e.g., pharmacokinetic or histology).

The quality assurance unit is responsible for monitoring each study to assure management that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with the regulations in this part. The quality assurance unit shall be entirely separate from and independent of the personnel engaged in the direction and conduct of that study.

The GLPs specify certain requirements for the facilities. In particular, there needs to be dedicated areas for separation of species or test systems, isolation of individual projects, quarantine of animals, routine or specialized housing of animals, safe sanitary storage of waste before removal from the testing facility, feed, bedding, supplies, and equipment. Storage areas for feed and bedding shall be separated from areas housing the test systems and shall be protected against infestation or contamination. There also need to be separate areas for receipt and storage of the test and control articles, mixing of the test and control articles with a carrier, and storage of the test and control article mixtures. In addition, space needs to be provided for limited-access archives.

Requirements for equipment also exist. The equipment must be tested, inspected, maintained, and calibrated on a regular basis, according to written standard operating procedures (SOPs). These procedures need to be documented. Written records also need to be maintained for nonroutine repairs performed on equipment as a result of failure and malfunction.

The testing facility shall have SOPs in writing setting forth nonclinical laboratory study methods that management is satisfied are adequate to insure the quality and integrity of the data generated in the course of a study. All deviations in a study from SOPs shall be authorized by the Study Director and shall be documented in the raw data. Significant changes in established SOPs shall be properly authorized in writing by management. The facility needs to maintain an historical file of SOPs, and all revisions. These SOPs and any appropriate laboratory manuals must be immediately available to the laboratory procedures being performed.

There are many requirements for animal care. Details could be found in the GLPs and also in the (US) Department of Agriculture Guide for the Care and Use of Laboratory Animals. Besides proper husbandry practices, animals need to be identified under certain circumstances.

All of the reagents and solutions used in the laboratory areas need to be labeled to indicate identity, titer or concentration, storage requirements, and expiration date. These must be discarded if the reagents or solutions are deteriorated or outdated. The GLPs have strict guidelines for the test and

control articles (EPA discusses test substances, control substances, and reference substances).

According to the FDA GLPs, a test article is "any food additive, color additive, drug, biological product, electronic product, medical device for human use, or any other article subject to regulation under the act or under Sections 351 and 354-360F of the Public Health Service Act." A control article means "any food additive, color additive, drug, biological product, electronic product, medical device for human use, or any article other than a test article, feed, or water that is administered to the test system in the course of a nonclinical laboratory study for the purpose of establishing a basis for comparison with the test article."

The EPA GLPs for both the TSCA and the FIFRA have basically the same definitions for control substances and reference substances. A control substance means "any chemical substance or mixture, or any other material other than a test substance, feed, or water, that is administered to the test system in the course of a study for the purpose of establishing a basis for comparison with the test substance for chemical or biological measurements." A reference substance means "any chemical substance or mixture, or analytical standard, or material other than a test substance, feed, or water, that is administered to or used in analyzing the test system in the course of a study for the purposes of establishing a basis for comparison with the test substance for known chemical or biological measurements."

Test substances are defined differently. The TSCA GLPs state that a test substance means "a substance or mixture administered or added to a test system in a study, which substance or mixture is used to develop data to meet the requirements of a TSCA Section 4(a) test rule and/or is developed under a TSCA Section 4 testing consent agreement or Section 5 rule or order to the extent the agreement, rule or order references this part." The GLPs for FIFRA state that a test substance means "a substance or mixture administered or added to a test system in a study, which substance or mixture: (1) Is the subject of an application for a research or marketing permit supported by the study, or is the contemplated subject of such an application; or (2) Is an ingredient, impurity, degradation product, metabolite, or radioactive isotope of a substance described by paragraph (1) of this definition, or some other substance related to a substance described by that paragraph, which is used in the study to assist in characterizing the toxicity, metabolism, or other characteristics of a substance described by that paragraph."

The FDA GLPs state that the identity, strength, purity, and composition or any other characteristics that appropriately define the test or control article shall be determined for each batch and shall be documented. In addition, the methods of synthesis of the test and control articles need to be documented. The stability of each test or control article needs to be determined by the testing facility or by the sponsor either before study initiation, or concomitantly. Specific requirements for each storage container for a test or control article shall be labeled by name, chemical abstract number or code number, batch number, expiration date if any, and, where appropriate, storage conditions. Retention samples are needed for any study longer than 4 weeks in duration.

Test and control article handling procedures are addressed. Procedures need to be established for a system for the handling of the test and control articles to ensure that proper handling and storage are utilized; the materials are allocated so that there is no possibility of contamination, deterioration, or damage. At all times, identification of the contents must be maintained throughout the distribution process and all actions are documented. Identification of the contents of a container helps to ensure that the material will be used properly. It is also prudent to label temporary or transport containers to avoid mix-ups.

Documentation and the same type of procedures are important for mixtures of articles with carriers. In addition, procedures for each test or control article that is mixed with a carrier, appropriate analytical methods shall be conducted to determine the uniformity and stability of the mixture and the concentration of the test or control article in the mixture. In GLP studies, these assays should incorporate validated methods. SOPs need to define general ranges for standard parameters used for analytical acceptability.

The protocol for nonclinical studies must address specific concerns. In addition, it must be approved and written so that it clearly indicates the objectives and all methods for the conduct of the study. The nonclinical GLP studies have to be conducted in accordance with the protocol. This includes documentation of all aspects of the study. Over time, personnel leave laboratories; therefore, the only way to reproduce a study is to have original documentation that is adequate and legible. Data need to be signed and dated by the person making the observations. Any change in entries shall be made so as not to obscure the original entry, shall indicate the reason for such change, and shall be dated and signed or identified at the time of the change. With computerized systems that incorporate automated data collection, the individual responsible for direct data input

shall be identified at the time of data input. Any change in automated data entries shall be dated and made so as not to obscure the original entry, the reason for the change needs to be indicated, and the responsible individual needs to be identified.

A final report for each nonclinical laboratory study shall be prepared. Details are provided in the GLPs. The final report summarizes most of the experimental details of the study. The final report needs to include the name and address of the facility(s), objectives and procedures as stated in the approved protocol, including any changes in the original protocol, test, and control article information, information on the preparations used to administer the material, including dosage, dosage regimen, and route of administration. Also needed are duration of the study, a description of the methods, and the test system used. Any circumstances that may have affected the quality or integrity of the data must be addressed. In addition, the locations where all specimens, raw data, and the final report are to be stored, are needed. The quality assurance unit prepares and signs a statement of GLP compliance, and the final report is signed and dated by the study director. If there is any question regarding the GLP compliance to a study, the person(s) responsible should declare this information in the report, as ignoring any issues may lead to legal concerns. The study director addresses any corrections, as in the case of changes to protocols, as an amendment.

The final report and any amendments, all raw data, documentation, protocols and any amendments, and specimens (with the exception of specimens subject to degradation) generated as a result of a nonclinical laboratory study shall be retained in an archive. The archive facility needs to be set up for orderly storage and expedient retrieval. Conditions of storage shall minimize deterioration of the documents or specimens. The archives do not necessarily have to be an in-house facility; the laboratory may contract with commercial archives to store materials in a GLP fashion.

Any off-site data storage locations need to be indexed and documented so that this information is easily obtainable. In either case, the FDA requires that documentation records, raw data, and specimens pertaining to a nonclinical laboratory study shall be retained in the archive(s) for a period of at least 5 years following the date on which the results of the nonclinical laboratory study are submitted in support of an application for a research or marketing permit. With the exception of investigational new drug applications or applications for investigational device exemptions, if an application is approved for a research or marketing permit, for which the results of the nonclinical laboratory study were submitted, data needs to be held for a period of at least 2 years following the date of approval. Other situations (e.g., where the nonclinical laboratory study does not result in the submission of the study in support of an application for a research or marketing permit), data is to be kept for a period of at least 2 years following the date on which the study ends. Appropriate wet specimens, samples of test or control articles, and materials that may be subjected to degradation even under proper storage conditions, shall be retained only as long as the quality of the preparation affords evaluation.

Other documentation targeted for storage in archives include the master schedule sheet, copies of protocols, and records of quality assurance inspections, summaries of training and experience, job descriptions, records and reports of the maintenance and calibration and inspection of equipment. In any case, ensure that the protocol or SOPs address the proper archiving of appropriate materials.

The FDA may find that it needs to disqualify testing facilities if the facility has not complied with the requirements of the GLP regulations. All studies completed after the date of disqualification can be excluded from consideration. It is prudent to ensure that all laboratories used are GLP compliant, or studies and entire projects may be compromised.

See also: Food and Drug Administration, US; Good Clinical Practices (GCP); International Conference on Harmonization; Redbook.

Further Reading

Gad SC (ed.) (2001) *Regulatory Toxicology*, 2nd edn. New York: Taylor and Francis.

Relevant Websites

http://www.mca.gov.uk – Inspection: The United Kingdom Good Laboratory Practice Monitoring Authority.
http://www.epa.gov – US Environmental Protection Agency, Good Laboratory Practice and 40 CFR.
http://www.fda.gov – US Food and Drug Administration, Good Laboratory Practice.
http://www.accessdata.fda.gov – US Food and Drug Administration, Good Laboratory Practice, 21 CFR. and 21CFR
http://www.oecd.org – Organisation for Economic Co-operation and Development (OECD), Good Laboratory Practice.

Grain Incidents and Other Mercury Tragedies: Forms, Fate, and Effects

Sandip Chattopadhyay

Mercury is one of hundreds of toxic substances that people are being exposed to at ever-increasing rates. Incidents such as the outbreaks in Minamata Japan, Iraq, New Mexico, and the Great Lakes should serve as a strong warning of what unchecked industrial pollution and careless handling of toxic substances can do to humans, wildlife, and their surroundings. Strong regulations, education and enforcement are needed to prevent the suffering and tragedies that are commonplace today.

Incidents

Potential sources of human exposure to mercury include food contaminated with mercury, inhalation of mercury vapors in ambient air, and exposure to mercury through water, soil and sediment. Dietary intake is by far the most important source of exposure to mercury for the general population. Fish and other seafood products are the main source of methylmercury in the diet; studies have shown that methylmercury concentrations in fish and shellfish are ~10–100 times greater than in other foods, including cereals, potatoes, vegetables, fruits, meats, poultry, eggs, and milk. As of December 1998, mercury was the chemical contaminant responsible, at least in part, for the issuance of 1931 fish consumption advisories by 40 states, including the US territory of American Samoa. Almost 68% of all advisories issued in the United States are a result of mercury contamination in fish and shellfish. Advisories for mercury have increased steadily by 115% from 899 advisories in 1993 to 1931 advisories in 1998. The number of states that have issued mercury advisories also has risen steadily from 27 states in 1993 to 40 states in 1997, and remains at 40 states for 1998. Advisories for mercury increased nearly 8% from 1997 (1782 advisories) to 1998 (1931 advisories).

In Alamagordo, New Mexico, a farmer worked in a seed store, which supplied local farmers, and he maintained a few pigs at home. He noticed a significant amount of wastage in the form of spilled seed grain (treated with methyl mercuric dicyandiamide) at the store, and he began sweeping it up to feed to his pigs. Within a short time his pigs became ill. Of 18 pigs, 14 developed a neurologic illness and 12 died. Fearful of the loss of his investment, the farmer had rest of the pigs butchered, and froze the meat for the use of his family. Within 2 weeks of eating the poisoned pork, three out of the family of 10 were stricken with brain and spinal cord damage. One girl lay unconscious for 8 months in the hospital before waking totally blind and unable to speak. Twenty-two years after this incident all surviving members of the family were carefully examined and tested. In this interim the two youngest children had died, and autopsy and toxicological findings were available from one of these. Both were left in a vegetative state until their deaths. Some recovery did occur in the older children, but the visual defects, including blindness in one and constricted visual fields in the other, did not improve. Neither parent showed signs of poisoning, although both were exposed.

In 1971–72, a major epidemic occurred in Iraq in which 6530 persons were hospitalized and almost 500 died. In a well-intentioned humane response to famine, several nations shipped wheat grain intended for planting to Iraq. The seeds had been treated with a methylmercury-containing fungicide to hold down mold growth and preserve the viability of the seeds. The seeds were also dyed red to serve as a warning, and attempts were made to inform the natives of the hazards of eating the seeds directly. Unfortunately, the dye washed off readily and the fungicide did not; also, the warnings on the bags were in Spanish, because some of the grain had originated in Mexico. Though the bags were also marked with the skull and crossbones, as meaning poison, in the face of starvation many families milled the seeds directly into flour, and made and consumed the contaminated bread. Average intake was three loaves of bread per day, 80–250 $\mu g\,kg^{-1}\,day^{-1}$. Neurologic syndrome of paraesthesia (peripheral nervous, sensory dysfunction) ataxia, dysarthria, and deafness were observed.

Thermometers contain the less toxic elemental form of mercury and have almost never been a safety issue in peoples' homes. However, in the 1970s and 1980s, workers at the Staco thermometer plant in Poultney, Vermont, began to notice a common series of health problems – headaches, bleeding or sore gums, upset digestive systems, and coordination problems. Upon investigation, mercury was detected in the air of workers' homes, on their clothing and furniture, and most tragically, in the bodies of many workers and their children. This was the first time in which the children of mercury-handling workers were proven to have been affected. The plant closed in 1984. Several plant workers have since settled lawsuits with the company for undisclosed sums.

Two major epidemics of methylmercury poisoning have occurred in Japan (in Minamata Bay and the Agano River in Niigata) between 1953 and 1960. In both cases, mercury was discharged as mercuric chloride, a catalyst for production of vinyl chloride and acetaldehyde. Bacteria methylated the inorganic mercury and the methylmercury bioaccumulated in fish and shellfish. In both cases there was an association between fish consumption and incidence and severity of disease. Mercury was found in concentrations of $\sim 10\,\mathrm{mg\,kg^{-1}}$ of fish. In Minamata Bay, cats were first noted to fall ill, become ataxic, and die. Subsequently, a neurologic syndrome developed in adults and children. 'Fetal Minamata Disease' was the name given to the observed epidemic of 'cerebral palsy' (CP) in Minamata (6% of births in Minamata with CP compared to 0.5% of births elsewhere in Japan): 121 cases and 46 deaths were reported in Minamata from a neurologic disease manifested as paraesthesia, constricted visual fields, ataxia, and deafness (frequently tremor). The clinical presentation varied with the age group. Mortality was 50% in adults, 33% in children, and 12% in fetal exposure. Fetal exposure resulted in CP, involuntary movement, difficulty in chewing, abnormal speech, abnormal deep tendon reflexes, but no deafness or visual deficits.

In 1983, Pomo Indians in California had to stop eating local fish because of high mercury in the fillets. Chippewa Indians in Wisconsin in 1990 were found to have blood levels of mercury high enough to cause developmental problems in fetuses. The Chippewas had a fondness for the walleye that swam in local lakes.

Mercury caused another tragic incident in Hanover, New Hampshire. The story of Dartmouth College Chemistry Professor Karen E Wetterhahn made national headlines when mercury poisoning claimed her life at the age of 48. In August of 1996, Wetterhahn, a specialist in toxic metals, was working under a $7 million federal grant to study toxic metals. She was poisoned in her lab by a drop of an experimental mercury compound dimethylmercury, which accidentally penetrated her latex glove and seeped through to her skin. Symptoms began gradually like a stomach flu, but then she began bumping into doors and suddenly falling down. Words became difficult, her hands tingled, and 5 months after the spill she was taken to the emergency room. Symptoms then progressed rapidly, by the weekend she could not walk, her speech was slurred, and her hands trembled. Diagnosed as mercury poisoning, treatment was started, but little was known about the rare man-made chemical dimethylmercury, a colorless liquid that looks like water but is three times heavier and far more toxic than other forms of mercury. Wetterhahn became ill in January of 1997 and was hospitalized. She rapidly went into a coma and died that June. As a result of her tragedy, safety standards for gloves and other protective equipment were revised, and a movement began to eliminate production and use of this most deadly form of mercury. There was only one other documented case of dimethylmercury poisoning: a Czech chemist in 1972 had suffered the same symptoms as Wetterhahn and died.

Sources of Mercury

Mercury is found in the environment in the metallic form and in different inorganic and organic forms. Most of the mercury in the atmosphere is elemental mercury vapor; most of the mercury in water, soil, sediment, plants, and animals is inorganic and organic mercury (primarily methylmercury). Mercury occurs naturally and is distributed throughout the environment by both natural processes and human activities. Solid waste incineration and fossil fuel combustion processes and human activities contribute $\sim 87\%$ of the emissions of mercury in the United States. Other sources of mercury releases to the air include mining and smelting, which are industrial processes involving the use of mercury such as chlor-alkali production facilities and production of cement. Mercury is released to surface waters from naturally occurring mercury in rocks and soils and from industrial activities, including pulp and paper mills, leather tanning, electroplating, and chemical manufacturing. An indirect source of mercury to surface waters is mercury in the air; it is deposited from rain and other processes directly to water surfaces and to soils. Mercury also may be mobilized from sediments if disturbed (e.g., flooding, dredging). Sources of mercury in soil include direct application of fertilizers and fungicides and disposal of solid waste, including batteries and thermometers, to landfills. The disposal of municipal incinerator ash in landfills and the application of sewage sludge to cropland result in increased levels of mercury in soil. Mercury in air also may be deposited in soil and sediments.

Chemical Speciation of Mercury

The divalent state of mercury (Hg^{2+}) dominates in most natural water; however, elemental Hg^0 is the most stable form in a broad pE/pH range. Hg^{2+} forms very strong complexes with oxygen and sulfur ligands, as well as with chloride. A significant hydrolysis starts at $pH > 1$ and dominates at $pH > 2$, in the absence of other complexing agents. The chloride

complexes $HgCl^+$ and $HgCl_2$ dominate over hydroxide species at pH < 5 and chloride concentrations > 1–3 mg l^{-1}, and $HgCl_2$ dominates in solution up to pH 6 in most groundwater systems under oxic or mildly reducing conditions. A significant fraction of the anionic $HgCl_3^-$ can be expected at high chloride levels and pH < 5–6, as well as of HgOHCl at pH 5–7. The uncharged $Hg(OH)_2$ is the main species at pH > 6 in nonsulfidic water, unless the chloride concentration is very high.

Mercury forms strong complexes with humic and fulvic acids, and these complexes may dominate in humic rich water at pH > 5–6. The strong complexes with humic substances expected to stabilize Hg^{2+}. However, a reduction of the Hg^0 can take place through the reversible quinine–hydroquinone redox couple in the humic substance. Dominating species, governed by the sulfur and organic degradation, would be $Hg(SH)_2$ at pH < 6, HgS_2H^- at pH 6–9, and HgS_2^{2-} at pH > 9.

The three major kinds of organic mercury compounds are phenylmercury (e.g., phenylmercuric acetate), methoxymercury (e.g., methoxymethylmercury acetate), and alkylmercury (e.g., methylmercuric acetate). Of these compounds, by far the most common as well as most dangerous are certain members of the alkylmercury group. Formation of alkylmercury compounds, monomethylmercury (CH_3Hg^+) and dimethylmercury ($(CH_3)_2Hg$), is achieved through microbial processes in soils and lake sediments. The transformation reaction pathways are indicated in **Figure 1**. The precursor Hg^{2+} can be methylated by both aerobic and anaerobic bacteria and subsequently demethylated by other bacteria. Monomethylmercury behaves as a cation capable of forming strong complexes with ligands containing O, S, Cl, etc., and this ion is kinetically inert toward breaking of the C–Hg bond. The formation of organomercury species is affected by parameters such as pH, redox conditions, mercury concentration, microbial population, and temperature.

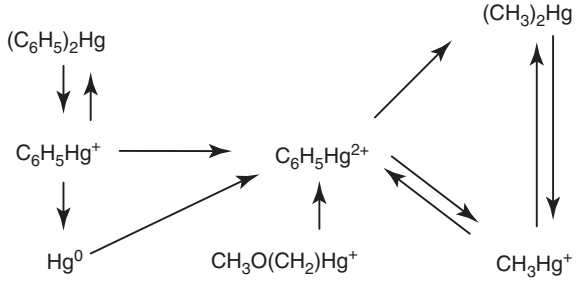


Figure 1 Transformations of mercury leading to alkylmercury.

Fate and Transport of Mercury

The global cycling of mercury is a complex process (**Figure 2**). Mercury evaporates from soils and surface waters to the atmosphere, is redeposited on land and surface water, and then is absorbed by soil or sediments. After redeposition on land and water, mercury is commonly volatilized back to the atmosphere as a gas or as adherents to particulates. Mercury exists in a number of inorganic and organic forms in water. Methylmercury, the most common organic form of mercury, quickly enters the aquatic food chain. In most adult fish, 90–100% of the mercury is methylmercury. Methylmercury is found primarily in the fish muscle (fillets) bound to proteins. Skinning and trimming the fish does not significantly reduce the mercury concentration in the fillet, nor is it removed by cooking processes. Because moisture is lost during cooking, the concentration of mercury after cooking is actually higher than it is in the fresh uncooked fish. Concentrations of total mercury in fish at the top of the food chain, such as pike, shark, and swordfish, are ~10 000–100 000 times higher than the concentrations of inorganic mercury found in the surrounding waters. Bioconcentration factors (BCFs) are simple ratios between the concentration of mercury in an organism and the concentration in the medium to which the organism was exposed. The BCF of methylmercury in fish is of the order of 3 million. Methylmercury levels in predator fish are, on average, ~7 million times higher than the concentrations of dissolved methylmercury found in the surrounding waters.

Mercury's pathway into wildlife primarily begins in the skies, with mercury-loaded rainfall. Sulfate-reducing bacteria, mainly living in sediments and in mats of floating algae, absorb rainwater mercury and turn it into its organic form, methylmercury (CH_3Hg^+). Organisms, which eat such bacteria, feed successive populations of larger organisms in the food web. At each step, methylmercury levels get concentrated. For wetland-dependent animals such as wading birds, raccoons and some panthers, concentrations can reach dangerously high levels. The use of methylmercury as a fungicide has been suspended in the United States, and since this was the only commercial use for the chemical, it is no longer manufactured in this country. It is, however, still found in the environment as a result of bacterial methylation of inorganic mercury.

Toxicology

Depending on the chemical form and the dose received, mercury can be toxic to both humans and

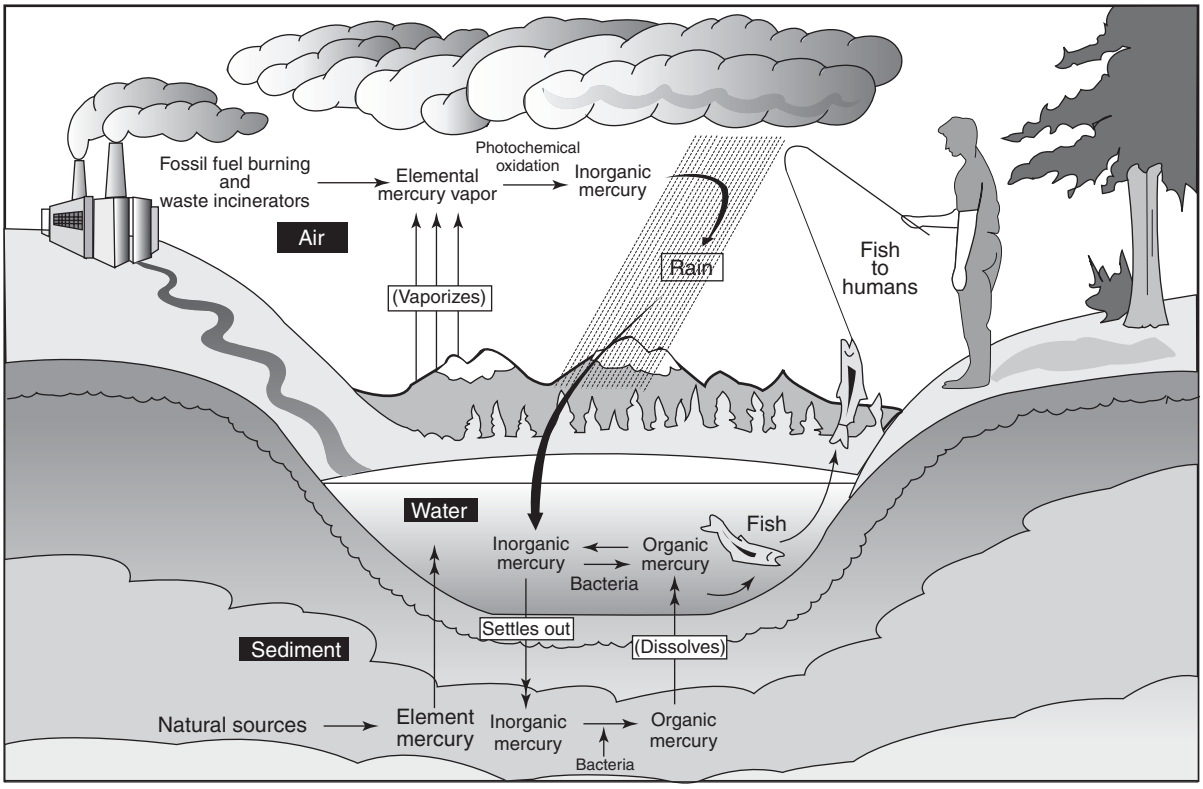


Figure 2 Fate and transport of mercury in water, soil, and sediments.

wildlife. Some chemical forms of mercury are absorbed more effectively by the body than others, and once inside the body, some forms are more likely than others to produce serious damage to internal organs. If inorganic mercury is swallowed, over 98% is excreted rapidly in the urine and feces. Unless ingested repeatedly or in massive amounts, metallic mercury is relatively harmless. Ingestion of the more soluble salts of mercury, however, can cause serious problems. Mercuric chloride is corrosive to the intestinal tract and, like other forms of inorganic mercury, can seriously damage the liver and especially the kidneys. Once absorbed by the body, inorganic mercury may be transported via blood to all parts of the body. Most cases of mercury poisoning from inhalation are chronic. If inhaled, inorganic mercury is initially deposited in the lungs, where in acute cases it may cause irritation and destruction of the lung tissue. The symptoms include inflammation of the gums, metallic taste, diarrhea, mental instability, and tremors. Although ingestion of methylmercury may cause serious damage to organs such as the liver, kidneys, and pancreas, the most serious consequences of methylmercury poisoning cause irreversible damage by attacking the central nervous system. Once absorbed, methylmercury is transported throughout the body by the circulatory system, primarily in the red blood cells. Any effects of mercury on the brain are likely to be permanent, since cells of the central nervous system, once damaged, do not recover.

Pharmacokinetics

Methylmercury is rapidly and nearly completely absorbed from the gastrointestinal tract; 90–100% absorption is estimated. Methylmercury is somewhat lipophilic, allowing it to pass through lipid membranes of cells and facilitating its distribution to all tissues, and it binds readily to proteins. Methylmercury binds to amino acids in fish muscle tissue. The highest methylmercury levels in humans generally are found in the kidneys. Methylmercury in the body is considered to be relatively stable and is only slowly transformed to other forms of mercury. Methylmercury readily crosses the placental and blood/brain barriers. Its estimated half-life in the human body ranges from 44 to 80 days. Excretion of methylmercury is via the feces, urine, and breast milk. Methylmercury is also distributed to human hair and to the fur and feathers of wildlife; measurement of mercury in hair and these other tissues has served as a useful biomonitor of contamination levels.

Acute Toxicity

Acute high-level exposures to methylmercury may result in impaired central nervous system function, kidney damage and failure, gastrointestinal damage, cardiovascular collapse, shock, and death. The estimated lethal dose is 10–60 mg kg^{-1}.

Chronic Toxicity

Both elemental mercury and methylmercury produce a variety of health effects at relatively high exposures, neurotoxicity is the effect of greatest concern. This is true whether exposure occurs to the developing embryo or fetus during pregnancy or to adults and children. A reference dose (RfD) is defined as an estimate of a daily exposure to the human population (including sensitive subpopulations) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD for methylmercury has been determined by the US Environmental Protection Agency (EPA) to be 1×10^{-4} mg kg^{-1} day^{-1} (i.e., a person could consume 0.1 μg methylmercury for every kg of his/her body weight everyday for a lifetime without anticipation of risk of adverse effect).

Developmental Toxicity

Methylmercury causes subtle to severe neurologic effects depending on dose and individual susceptibility. EPA considers methylmercury to have sufficient human and animal data to be classified as a developmental toxicant. Methylmercury accumulates in body tissue; consequently, maternal exposure occurring prior to pregnancy can contribute to the overall maternal body burden and result in exposure to the developing fetus. In addition, infants may be exposed to methylmercury through breast milk.

Mutagenicity

Methylmercury appears to be clastogenic but not to be a point mutagen; that is, mercury causes chromosome damage but not small heritable changes in DNA. EPA has classified methylmercury as being of high concern for potential human germ cell mutagenicity.

Carcinogenicity

Experimental animal data suggest that methylmercury may be tumorigenic in animals. Chronic dietary exposures of mice to methylmercury resulted in significant increases in the incidences of kidney tumors in males but not in females. The tumors were seen only at toxic doses of methylmercury. All of the carcinogenic effects in animals were observed in the presence of profound damage to the kidneys. Tumors may be formed as a consequence of repair in the damaged organs. EPA has classified it as a possible human carcinogen, group C.

Interactive Effects

Potassium dichromate and atrazine may increase the toxicity of mercury, although these effects have been noted only with metallic and inorganic mercury. Ethanol increases the toxicity of methylmercury in experimental animals. Vitamins D and E, thiol compounds, selenium, copper, and possibly zinc are antagonistic to the toxic effects of mercury.

Regulations and Advisories

EPA regulations and advisories on mercury are indicated in **Table 1**.

Correctives and Prospects for the Future

Of ~200 000 tons of mercury emitted to the atmosphere since 1890, ~95% reside in terrestrial soil and sediments, ~3% in ocean surface water, and 2% in the atmosphere. The global atmospheric burden of mercury is continuing to increase. Between 1990 and 1996, atmospheric mercury levels have risen between 5.5% and 17% in the upper Midwest, depending on the season, with an average annual increase of 8%. Even when mercury pollution is detected and halted, the problem of cleaning up the damage remains. Once released to the environment, mercury may continue to cycle between the sediments, water, and biota for tens, hundreds, or even thousands of years before finally being flushed from the system. Mercury in the form of vapor and/or inorganic salts may be transported great distances over several months in the atmosphere before falling out or being deposited by precipitations. It may be emitted back into the atmosphere as a gas or associated with dust particles to be redeposited elsewhere. Mercury in soils has a long retention time, possibly hundreds of years, and may continue to be released into the air and surface water for many years to come. Standfield and Lopez reported that if all mercury releases were stopped today, it could take at least 50 years for the methylmercury levels in fish to return to preindustrial levels. High levels of mercury have been found in fish from several parts of North America, primarily where chlor-alkali plants were discharging mercury-laden wastewater. Many fishes taken from Lake St. Clair (between Lake Huron and Lake Erie) in 1970 contained ~5–7 ppm of mercury. It has been estimated that ~5000 years will be required for the mercury,

Table 1 EPA regulations and advisories

Maximum contaminant level in drinking water	$0.002\,mg\,l^{-1}$
Toxic criteria[a]	
Freshwater	$2.10\,\mu g\,l^{-1}$ (maximum) $0.012\,\mu g\,l^{-1}$ (continuous)
Saltwater	$1.80\,\mu g\,l^{-1}$ (maximum) $0.025\,\mu g\,l^{-1}$ (continuous)
Human health consumption of water and organisms	$0.14\,\mu g\,l^{-1}$
Human health consumption of organisms only	$0.15\,\mu g\,l^{-1}$
Water quality guidance for the Great Lakes System (protection of aquatic life in ambient water)	
Acute water quality criteria for mercury total recoverable	$1.694\,\mu g\,l^{-1}$ (maximum)
Chronic water quality criteria for mercury total recoverable	$0.908\,\mu g\,l^{-1}$ (continuous)
Water quality criteria for protection of human health (drinking water and nondrinking water)	$1.8 \times 10^{-3}\,\mu g\,l^{-1}$ (maximum)
Water quality criteria for protection of human health (mercury including methylmercury)	$1.3 \times 10^{-3}\,\mu g\,l^{-1}$ (maximum)
Emissions from mercury ore processing facilities and mercury chlor-alkali plants	2300 g per 24 h (maximum)
Emissions from sludge incineration plants, sludge drying plants, or a combination of these that process wastewater treatment plant sludge	3200 g per 24 h (maximum)

[a]For those States not complying with Clean Water Act Section 303(c) (2) (B) – criterion concentration for priority toxic pollutants.

stored presently in the Lake St. Clair ecosystem, to effectively flush out by natural processes.

The following approaches can be used for decontaminating mercury-contaminated sediments.

- Dredging of sediments.
- Increasing the pH of the sediments in order to favor demethylation and increased volatilization.
- Introducing oxygen-consuming materials so as to create anaerobic conditions in the sediments and hence reduce mercury methylation.
- Covering the sediments with fresh, finely divided, highly adsorptive materials as reactive cap.
- Covering the sediments with any inorganic, inert material as nonreactive cap (e.g., sand).

None of the above-mentioned methods is without drawbacks, either in terms of cost, performance, effectiveness, or side effects. For example, mercury concentrations in fishes were reported to be higher after dredging of sediments of polluted waterways on two occasions. The burrowing activities of benthic microorganisms can frustrate efforts to seal off contaminated sediments with an overlayer of inert, adsorptive material.

See also: Kidney; Mercury; Methylmercury; Neurotoxicity.

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Great Smog of London

Yvonne R Rodriguez

It had been a cold winter in London of 1952. December 4th of that year seemed to be just as cold as the other days except that in the evening a light fog rolled in. The following day began with a foggy morning. People bundled up and went to work, smoke stacks belched dark smoke out into the air, and coal was being burned in homes and offices to keep warm. As the day progressed, the fog that began in the morning turned into a brown and yellowish smog with an acrid smell. The smog lasted until

December 9th. In the end, the death toll caused by this fog was estimated to be in excess of 4000 people. In addition to the deaths, the smog left a large number of new respiratory illnesses and cardiovascular disease cases among the surviving population.

What Caused the Smog?

The winter of 1952 had been colder than usual. On December 4th the wind began to die down, the ground was cold, the air was moist, and a precipitation formed, more commonly referred to as fog. The cold moist air was trapped beneath a layer of warm air forming a temperature inversion. An anticyclone settled in around the city of London, preventing any wind circulation to occur beneath the temperature inversion.

By 1952, wood was scarce and an expensive commodity in England. Bituminous coal was the primary source of heat for all. The coal being burned for warmth in combination with the industrial smoke stacks pumped pollution into the stagnant air. These pollutants combined with the existing fog created the Great Smog of London. This type of air pollution is known as 'reducing-type' pollution. The burning of the coal gives off a sulfurous gas, plus the industrial particulate matter belched from the smoke stacks mixed with the fog trapped within the temperature inversion results in 'reducing-type' pollution. This type of pollution is capable of causing the corrosion of metals and masonry used in buildings. In 1952, daily recordings of pollutants were taken for only those that were thought to have been the main contributors to pollution, mainly sulfur dioxide (SO_2) and total suspended matter (smoke). On the worst day of the Great Smog in London, the highest pollution levels of SO_2 and smoke reached levels of 1.34 ppm and 4.5 mg m^{-3}, respectively. The average daily levels of exposure during the Great Smog were 0.57 ppm of SO_2 and 1.4 mg m^{-3} for smoke.

Health Effects

Exposure to the air pollution during the 1952 episode could have detrimental effects to the lungs. Sulfur dioxide is water soluble, if inhaled the upper linings of the lungs would absorb it. The inhalation of SO_2 would then cause the lungs to bronchoconstrict. This is a condition that narrows the airways, resulting in difficulty breathing from a lack of airflow. In addition to the sulfur dioxide exposure, particulate matter from the smoke would aggravate and enhance any symptom already appearing with those exposed. Those already suffering from respiratory problems exposed to the Great Smog would have had an extremely difficult time breathing.

The exact number of deaths resulting from this event was difficult to determine. For example, a person with a mild case of bronchitis aggravated by the smog to become a serious case might not have perished until January of 1953. This type of death would not have been included in the final death toll attributable to the smog event. It is estimated that over 4000 deaths resulted from the Great Smog of 1952. In the week ending December 6th of 1952, 945 deaths were recorded in London Administrative County. The following week, the death toll reached 2484. On December 8th and 9th alone the toll peaked $\sim$900 deaths per day. Although the smog lasted 5 days, the death rate in London continued to remain higher than normal through Christmas. Many of the victims were those already suffering from a respiratory illness and the elderly.

The Great Smog of London in 1952 was not an isolated incident. Other such 'reducing-type' killer smogs have occurred in Meuse Valley, Belgium (1930), Donora, Pennsylvania (1948), and again in London (1962). The death toll blamed on the air pollution in these cases was not as high.

Legislation

Air pollution had long been recorded in the past as a nuisance. The Public Health (London) Act of 1891, addressed air pollution and the stacks that emitted it; however, the Act failed to define black smoke, allowing companies to avoid the law by claiming their smoke was not black but another color. Attempts were made to change the wording of the Act but failed due to large companies investing time and money into fighting any new laws. After the Great Smog of 1952, new legislation was enacted addressing both residential and industrial sources of pollution.

On July 5th, 1956, the London Clean Air Act was enacted. This legislation differed from all the other attempts to regulate air pollution by actually defining dark smoke. The law restricted smoke and prohibited the release of dark smoke, which was defined as "anything darker than lattice 2 on the Ringelmann chart." There are 5 different levels noted on the Ringelmann chart, 1 being the clearest and 5 the blackest. The tester would place the lattice chart a distance away so that the shade of the smoke could be compared to the grid. The Clean Air Act of 1956 allowed the government to designate areas free from smoke, by regulating and restricting the emissions from potential sources. From 1952 to 1987, air pollution legislation has worked to reduce 80% of the smoke once emitted over the City of London.

Since the events of December 1952, several advances in air pollution control, legislation, and factors contributing to air pollution have been made. Improvements in air pollution controls have helped lessen the amount of emissions. Combine this progress with an increase of restrictions on air emissions, and advances in understanding conditions that lead to air pollution reduces the likelihood that a situation as devastating as the Great Smog of London could occur again. Nevertheless, burning coal containing high amounts of sulfur in certain areas of the world subject to extreme temperature inversion conditions increases the risk of respiratory problems.

See also: Pollution, Air; Sulfur Dioxide.

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Green Chemistry

Richard E Engler

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Green chemistry (also called sustainable chemistry) is the use of chemistry to prevent pollution. More specifically, green chemistry is the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances. By offering environmentally benign alternatives to the more hazardous chemicals and processes that are often used in consumer and industrial applications, green chemistry promotes pollution prevention at the molecular level:

$$\text{Risk} = f(\text{hazard}, \text{exposure}) \qquad [1]$$

Risk is a function of hazard and exposure (equation [1]). Traditional environmental protection focuses on controlling exposure: minimizing human or environmental exposure to hazardous substances during chemical manufacture, processing, and use. Green chemistry changes the focus to the hazard component of the equation. Reducing hazard is a more fundamental, foolproof way to reduce risk. An exposure control can fail, whether it is a worker's respirator, the lining of a hazardous waste landfill, or the scrubber on an exhaust stack. On the other hand, the risk of a less hazardous chemical will remain low, even if the respirator fails, the filter leaks, or the vat spills. Reducing hazard also increases the safety of chemical manufacturing and may contribute to homeland security.

Green chemistry in the United States grew out of the Pollution Prevention Act (PPA) of 1990. The PPA established a hierarchy that emphasizes source reduction over other methods of dealing with hazardous materials. According to the PPA, source reduction is preferable to recycling, which in turn is preferable to treatment, which in turn is preferable to disposal.

Chemistry provides ways to reduce pollution at the source. The field of green chemistry is developing as chemists begin to use the science of chemistry to reduce the hazard of the chemical products or processes they design, thus minimizing the negative impact of chemicals on human health and the environment. The 12 Principles of Green Chemistry help define green chemistry.

Twelve Principles of Green Chemistry (Adapted from Anastas and Warner, Green Chemistry: Theory and Practice)

1. *Prevent waste*: Design chemical syntheses to prevent waste, leaving no waste to treat or clean up.
2. *Design safer chemicals and products*: Design chemical products that are fully effective yet have little or no toxicity.
3. *Design less hazardous chemical syntheses*: Design syntheses to use and generate substances with little or no toxicity to humans and the environment.
4. *Use renewable feedstocks*: Use raw materials and feedstocks that are renewable rather than depleting. Renewable feedstocks are often made from agricultural products or are the wastes of

other processes; depleting feedstocks are made from fossil fuels (petroleum, natural gas, or coal) or are mined.

5. *Use catalysts, not stoichiometric reagents*: Minimize waste by using catalytic reactions. Catalysts are used in small amounts and can carry out a single reaction many times. They are preferable to stoichiometric reagents, which are used in excess and work only once.
6. *Avoid chemical derivatives*: Avoid using blocking or protecting groups or any temporary modifications, if possible. Derivatives use additional reagents and generate waste.
7. *Maximize atom economy*: Design syntheses so that the final product contains the maximum proportion of the starting materials. There should be few, if any, wasted atoms.
8. *Use safer solvents and reaction conditions*: Avoid using solvents, separation agents, or other auxiliary chemicals. If these chemicals are necessary, use innocuous chemicals.
9. *Increase energy efficiency*: Run chemical reactions at ambient temperature and pressure, whenever possible.
10. *Design chemicals and products to degrade after use*: Design chemical products to break down to innocuous substances after use so that they do not accumulate in the environment.
11. *Analyze in real time to prevent pollution*: Include in-process real-time monitoring and control during syntheses to minimize or eliminate the formation of by-products.
12. *Minimize the potential for accidents*: Design chemicals and their forms (solid, liquid, or gas) to minimize the potential for chemical accidents such as explosions, fires, and releases to the environment.

Some consider green chemistry to be a separate field of chemistry, such as organic or physical chemistry. It is, however, an overarching concept that applies to any of the traditional fields of chemistry. It tends to be multidisciplinary, drawing on organic, inorganic, physical, polymer, and biochemistry, as well as biology, engineering, physics, and other related fields.

Green chemistry is good business. Implementing green chemistry technologies may reduce the costs of raw materials, improve efficiency, and reduce or eliminate waste, regulatory burden, and the need for personal protective equipment. Many companies, large and small, have discovered and implemented green chemistry technologies that not only benefit human health and the environment, but also benefit their corporate competitiveness and profitability. The commercial success of many winners of the US Presidential Green Chemistry Challenge Awards (described below) is perhaps the best example of green chemistry as good business.

In the United States, research, education, awards, and outreach opportunities are available through several organizations and in particular through the US Environmental Protection Agency's Green Chemistry Program and the American Chemical Society's Green Chemistry Institute. International coordinators include the International Union of Pure and Applied Chemistry (IUPAC), and the Organisation of Economic Cooperation and Development (OECD). In addition, many countries have implemented national programs including Australia, Italy, Japan, Spain, and the United Kingdom.

The US Green Chemistry Program recognizes and supports chemical technologies that reduce or eliminate the use or generation of hazardous substances during the design, manufacture, and use of chemical products and processes. The Green Chemistry Program supports fundamental research in environmentally benign chemistry as well as a variety of educational activities, international initiatives, conferences and meetings, and green chemistry tools. The program also administers the prestigious Presidential Green Chemistry Challenge Awards. Each year five technologies win Challenge Awards: one from an academic researcher, one from a small business, and three in specific areas of green chemistry.

See also: Pollution Prevention Act, US; Risk Assessment, Ecological.

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http://www.epa.gov – US Environmental Protection Agency. Green chemistry website.

G-Series Nerve Agents

Harry Salem*

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The G-series and V-series are the two main classes of traditional nerve agents. The G-series consists of GA, GB, GD, GE, GF, and GV.

Although toxic organophosphates were investigated extensively as pesticides during the 1920s and 1930s, their potential as chemical warfare agents was not recognized until 1937. Tabun or GA was the first military nerve agent, and was first examined for use as an insecticide in 1936 by Dr. Gerhard Schrader at the Bayer facility in Germany. At the end of the Second World War, Germany began to produce a second nerve agent, Sarin (GB), and were investigating a third, Soman (GD).

The G-agents are the more volatile of the nerve agents and present respiratory and percutaneous threats. The syndrome of cholinergic effects can be remembered by the acronym SLUDGE representing salivation, lacrimation, urination, diarrhea, gastrointestinal cramping, and emesis, or by DUMBBELLS, representing diarrhea, urination, miosis, bronchorrea, bradycardia, emesis, lacrimation, and salivation.

*The views of the author do not purport to reflect the postion of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

See also: GF; Nerve Agents; Sarin; Soman; Tabun; V-Series Nerve Agents: Other than VX.

Guaifenesin

Brenda Swanson-Biearman

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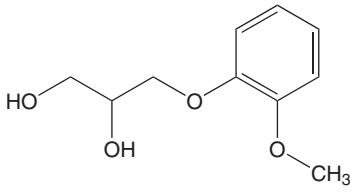
- Representative Chemical: 3-(2-Methoxyphenoxy)-1,2-propanediol
- Chemical Abstracts Service Registry Number: CAS 93-14-1
- Synonyms: Glyceryl guaiacolate; α-Glyceryl guaiacol ether; Guaianesin; Robitussin®
- Chemical Formula: $C_{10}H_{14}O_4$
- Chemical Structure:



Uses

Guaifenesin stimulates receptors in the gastric mucosa, reflexively increasing glandular secretion by the respiratory epithelium promoting lower respiratory tract drainage by thinning bronchial secretions, lubricating irritated respiratory tract membranes through increased mucous flow, and facilitating removal of viscous mucus. As a result, sinus and bronchial drainage is improved, and dry, nonproductive coughs become more productive and less frequent. However, clinical studies documenting the efficacy of this drug as an antitussive or expectorant for patients with upper respiratory infections or chronic bronchitis are lacking and the therapeutic efficacy of this agent is questionable.

Exposure Routes and Pathways

Guaifenesin is available in liquid or capsule form for oral dosing. Accidental or intentional exposure occurs most commonly by ingestion.

Toxicokinetics

Guaifenesin is readily absorbed from the gastrointestinal tract, specifically the intestine, and is rapidly metabolized and excreted in the urine. It is hydrolyzed 60% in the blood within 7 h. Following hydrolysis, urinary metabolites include *β*-(4-hydroxy-2-methoxyphenoxy)lactic acid, *β*-(2-methoxyphenoxy)lactic acid, and guaiacol ether. Following oral administration, no unchanged drug is detected in the urine. Guaifenesin has a plasma half-life of 1 h.

Mechanism of Toxicity

Guaifenesin lacks specific toxicity. Toxicity associated with guaifenesin will generally result from the

presence of antihistamines, decongestants, analgesics, cough suppressants, and/or alcohol in preparations containing a combination of ingredients.

Acute and Short-Term Toxicity (or Exposure)

Animal

Guaifenesin is used as an adjunct in anesthesia for horses. Thrombus formation has been noted in some horses given 10% guaifenesin intravenously.

Human

Guaifenesin is of low order of toxicity. Adverse effects are primarily minor gastrointestinal complaints. Doses larger than those required for expectorant action may produce vomiting, but gastrointestinal upset is rare at therapeutic dosages. Excessive use has been associated with urolithiasis.

Chronic Toxicity (or Exposure)

Animal

Large doses of guaifenesin in some animal models resulted in increased respiratory tract secretions.

Human

Abuse of guaifenesin-containing products has led to the development of urolithiasis secondary to guaifenesin's metabolite, β-(2-methoxyphenoxy)lactic acid.

Clinical Management

Basic and advanced life-support measures should be used as needed. In exposures where guaifenesin is the sole ingestant, treatment is rarely necessary. In exposures involving guaifenesin in multisymptom products containing antihistamines, analgesics, decongestants, and/or antitussives, treatment is directed toward the coingestant(s). Consideration should be given to the alcohol content of liquid preparations of guaifenesin and combination products.

See also: Ethanol; Gastrointestinal System.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Guaifenesin.

H

Hair

Pertti J Hakkinen

True hair is found only in mammals, and there is no such thing as a completely hairless mammal. Hair itself is dead, but is produced in hair follicles by specialized keratinocytes at the base of the hair. The outermost layer of hair is a cuticle, and most hairs have a cortex in which the dead keratinized cells are very densely packed, and an inner medulla in which they are not as densely packed. The pigmentation in hair, like that of skin, comes from melanocytes. Hair exposure to some chemicals may produce hair discoloration, for example, green hair from copper in water or cosmetics, or blue hair in cobalt workers.

The cycle of hair growth involves an active phase of production, anagen, during which the hair grows in length by addition of cells to the bottom end. When this stage ends, it is replaced by catagen, an inactive phase with no new hair cell production. The hair in catagen may detach itself from the underlying matrix that produced it, and be held in the follicle simply by friction. The follicle eventually enters into the transitional stage of telogen, renewing itself for activity, and then returns to the anagen phase. The anagen phase may last up to 2 years, and 86–95% of scalp hair follicles belong to this stage.

Hormones, especially testosterone, are well known as having effects on hair growth. 'Male pattern baldness' in humans is due to the suppressive effect of testosterone on the follicles of the scalp, and is not normally seen in women because of low testosterone levels. Interestingly, testosterone has the opposite effect on hair follicles of the face, for example, the onset of puberty in men and the elevated levels of testosterone stimulate the follicles of the face to make beard hairs. Any physical or chemical agent that affects rapidly dividing cells will affect hair growth, usually by stopping it, and hair loss (alopecia) is a very common side effect of radiation treatment and chemotherapy. Parasites, disease, and poor nutrition can also affect hair growth.

The advantages of analyzing hair samples include the easy and noninvasive collection of the samples, the small sample size required for analysis, and easy storage at room temperature. Scalp hair of a long enough length may be able to provide retrospective information of the previous 5–7 years, and axillary and pubic hair can be utilized if the scalp hair is cut too short to be used. Further, the basic chemical composition of the hair shaft is not influenced by changes in the blood chemistry, or by exposure to chemicals that occurred after hair and nail formation. However, hair analysis can be altered by dyeing, bleaching, and permanent waving, which can decrease the drug or toxic content in hair. In addition, the US Agency for Toxic Substances and Disease Registry (ATSDR) has noted that many questions about sampling and analytical procedures have yet to be answered and procedures have not been standardized. For example, do certain cutting tools introduce contaminants into hair, what part of the scalp should be used for hair sample collection, and what is the influence of washing techniques?

Hair has been used in the biomonitoring of various elements, for example, arsenic, thallium, and zinc, and has been used in the monitoring of drugs and biological substances. The level of mercury in hair is widely used as a biological indicator for exposure to methyl mercury (MeHg). In addition, hair samples have been utilized to evaluate environmental exposure to pollutants such as lead, and occupational exposures to metals such as nickel and chromium. However, the ATSDR has stated:

- That it "believes many scientific issues need to be resolved before hair analysis can become a useful tool to understand environmental exposures."
- "Although hair analysis may answer some questions about environmental exposure to a few substances, hair analysis often raises more questions than they answer."
- Health professionals "have no scientific basis for deciding whether a particular hair analysis result would be associated with adverse health effects. As the exception, scientists have studied how hair concentrations of methyl mercury in pregnant mothers relate to adverse developmental effects in their children."

- "Scientists have not been able to develop models that can use a hair analysis result to predict concentrations of contaminants in other biological media, e.g., blood."
- Health professionals "cannot use a hair analysis result to compute a body burden, an internal dose, or other parameter that would enable a meaningful toxicologic evaluation of a hair analysis result."

Nicotine has been measured in the hair of workers exposed to environmental tobacco smoke (ETS), and a significantly greater level in the level of nicotine in the hair of non-smokers exposed to ETS in the workplace has been observed. However, cotinine (the primary metabolite of nicotine) is a preferred marker of exposure to ETS, particularly as measured in blood, saliva or urine, because up to 80% of a dose of nicotine is metabolically converted to cotinine. Cotinine metabolite has a half-life of 15–17 h, while nicotine has a much shorter half-life and has different clearance rates in smokers and nonsmokers.

Hair samples may be very useful in cases of drug abuse since the detection of the drug together with its metabolites may confirm intake of the substance followed by metabolism, in contrast to exogenous exposure that would not find the metabolites in the hair. Some cases of drug abuse and poisoning can be suggested by examination of hair and/or nail, and can be confirmed by analysis of hair or nail clippings. Further, hair has been used for DNA typing in crime cases.

Hair analysis is also very useful in identifying 'doping' in amateur and professional athletes. Doping substances that can be detected in hair include clenbuterol, corticosteroids, ephedrine, methenolone, nandrolone metabolites, salbutamol, stanozolol, and testosterone. The storage of both nandrolone and its metabolites (norandrosterone and noretiocholanolone) in hair allows for detecting the difference between intake of doping agents and intake of other 19 norsteroids such as norandrostenedione and norandrostenediol, which are available in over-the-counter vitamin supplements.

Hair may also be an important tool for the diagnosis and monitoring of various disease states. For example, the concentrations of polyamines (e.g., putrescine, spermidine, and spermine) in the hair may be helpful in diagnosing and assessing disease activity in women with cervical or ovarian cancer. Assessing the level of polyamines in the hair shaft is preferred to measuring them in plasma and urine because the polyamine levels can vary during the day in plasma and urine. Increased levels of porphyrins in hair have been detected in patients with porphyria cutanea tarda. Hair analysis is useful for monitoring treatment compliance in psychiatric, epileptic, and HIV patients. Further, the levels of androgens in terminal scalp hair may provide a basis for predicting baldness since the ratio of testosterone to epitestosterone is significantly greater in the hair of balding fathers and their sons than in the hair of nonbalding control subjects.

Hair follicle cells possess the enzyme system necessary for the metabolic activation of certain drugs and polycyclic aromatic hydrocarbons (PAHs), and thus can be suitable for use in estimating susceptibility to environmental cancer. Since these cells are readily available and easily obtained, they serve as a good resource for population monitoring studies. A plucked anagen phase hair follicle consists of a hair shaft, inner root sheath, a cuticle layer, and most of the outer root sheath and the upper half of the bulb (but not the dermal papilla). Mitotic cells are located at the periphery of the outer root sheath and can be used for biomonitoring. A nuclear aberration (NA) assay in hair follicles has been developed to assess human exposure to genotoxic agents, and has been used for a study group comprised of cyclophosphamide (CP)-treated multiple sclerosis patients to show a significant increase of NA index in the CP treatment groups compared to the placebo group. CP was also observed to cause a significant drop in the mitotic index of follicle cells at some time points in CP treatment groups. In addition, hair follicles can retain smoke-related DNA adducts, and their use as an alternative to blood lymphocytes have already been recognized in monitoring exposure to PAHs, for example, benzo[*a*]pyrene, which can be activated by follicle cells.

An *in vitro* method using hair follicle cells to investigate unscheduled DNA synthesis (UDS) has been developed. Plucked follicles were exposed to tritiated thymidine and the UDS activities were determined autoradiographically (the number of dark grains in the outer root sheath of nonperipheral cells were used to estimate UDS). Many, but not all chemicals that have been shown to induce UDS in rat liver cells also stimulated UDS in hair follicles.

See also: Biomarkers, Human Health; Biomonitoring; Nails (of the Fingers and Toes).

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Harmonization

Carolyn Vickers

An approach described as 'harmonization' has been employed in a range of national, regional, and international programs aimed at improving the risk assessment and risk management of potentially toxic substances, including pesticides, biocides, food additives and contaminants, industrial chemicals, etc. It is generally used to describe a convergence of approaches which minimizes differences between them, but which may or may not achieve 'standardization' as the final endpoint.

Major areas of harmonization work arose from the United Nations (UN) Conference on Environment and Development (UNCED) held in 1992, and the 2002 World Summit on Sustainable Development. Agenda 21, Chapter 19, the 'blueprint' for the environmentally sound management of toxic chemicals under the principles of sustainable development, has guided most international and national chemical-related activities. Chapter 19 is the agreed upon, endorsed international program of action of governments for developing and implementing national programs for chemicals management within the principles of sustainable development.

One of the major harmonization activities under the UNCED umbrella is the International Programme on Chemical Safety (IPCS) project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals (Harmonization Project). The Intergovernmental Forum on Chemical Safety (IFCS) Forum III in Salvador da Bahia, in October 2000, agreed on Priorities for Action Beyond 2000. Forum III declared that by 2004, IPCS and the Inter-Organization Programme for the Sound Management of Chemicals (IOMC, which comprises seven intergovernmental organizations), should have ensured that recommendations for harmonized approaches are available for terminology, cancer, and reproductive and developmental toxicology, and that common principles for the approach to other specific toxicological endpoints, such as immunotoxicology, endocrine disruptors, and ecotoxicology, should be adopted wherever possible.

The IPCS Harmonization Project, which is ongoing, states that 'harmonization', in the context of risk assessment of chemicals should not simply be equated with standardization. It is not a goal of the project to standardize risk assessments globally, as that is considered to be neither appropriate nor feasible. Instead, harmonization is thought of as an effort to strive for consistency among approaches and to enhance understanding of the various approaches to chemicals risk worldwide. Thus, harmonization is defined, in a stepwise fashion, as an understanding of the methods and practices used by various countries and organizations so as to develop confidence in, and acceptance of, assessments that use different approaches. It further involves a willingness to work toward convergence of these approaches or methods as a longer-term goal.

Achieving harmonization of approaches is considered to provide: a framework for comparing information on risk assessment; understanding of the basis for exposure standards for specific chemicals in different countries; saving of time and expense by sharing information and avoiding duplication of work; and credible science through better communication among organizations and peer review of assessments and assessment procedures. The stated project mission is to ensure better chemical risk assessment and hence management practices that promote the protection of human health and the environment within the framework of sustainable development.

Application of this approach to the targets set by the IFCS has resulted in a range of harmonized products for application in risk assessment and risk management that have been implemented in national and other risk assessment systems. These include:

- The IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis.
- IPCS Guidance Document for the Use of Chemical-Specific Adjustment Factors (CSAF) for Interspecies Differences and Human Variability in Dose/Concentration–Response Assessment.
- IPCS Harmonization of Methods for the Prediction and Quantification of Human Carcinogenic/Mutagenic Hazard, and for Indicating the Probable Mechanism of Action of Carcinogens (This publication is also known as the *IPCS Qualitative Scheme for Mutagenicity*).
- IPCS Glossary of Key Exposure Assessment Terms.
- IPCS/OECD Descriptions of Selected Key Generic Terms Used in Chemical/Hazard Risk Assessment.

This ongoing project is overseen by a geographically representative Harmonization Steering Committee and a number of ad hoc Working Groups that manage the detailed work. Finalization of documents includes a rigorous process of international peer review and public comment.

Another major area of harmonization activity produced the Globally Harmonized System for the Classification and Labeling of Chemicals (GHS). The work on the GHS began with the premise that existing chemical toxicity classification and labeling systems should be harmonized in order to develop a single, globally harmonized system. This built upon the already harmonized system in place for physical hazards and acute toxicity in the transport sector, based on the work of the United Nations Economic and Social Council's Committee of Experts on the Transport of Dangerous Goods (UNCEDTG). Harmonization had not been achieved in the workplace or consumer sectors, however, and transport requirements in countries were often not harmonized with those of other sectors in that country.

As with the IPCS Harmonization Project, Chapter 19 of Agenda 21 provided the international mandate to complete this task. The work was coordinated and managed under the auspices of the IOMC Coordinating Group for the Harmonization of Chemical Classification Systems (CG/HCCS). The technical focal points for completing the work were: the International Labour Organization (ILO) for the hazard communication; the Organization for Economic Cooperation and Development (OECD) for the classification of health and environmental hazards; and the United Nations Sub-Committee of Experts on the Transport of Dangerous Goods (UNSCETDG) and the ILO for the physical hazards.

In 1999, the United Nations Economic and Social Council decided to enlarge the mandate of the Committee of Experts on the Transport of Dangerous Goods by reconfiguring it into a Committee of Experts on the Transport of Dangerous Goods and on the Globally Harmonized System of Classification and Labeling of Chemicals (CETDGGHS), and by creating, in addition to the Sub-Committee of Experts on the Transport of Dangerous Goods (TDG Sub-Committee), a new Sub-Committee of Experts on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS Sub-Committee). The GHS was adopted in December 2002 by the GHS Sub-Committee and endorsed by its parent Committee.

In its Plan of Implementation (para 22.(c)) adopted in Johannesburg on September 4, 2002, the World Summit on Sustainable Development encouraged countries to implement the new GHS as soon as possible with a view to having the system fully operational by 2008.

See also: Inter-Organization Programme for the Sound Management of Chemical; Intergovernmental Forum on Chemical Safety (IFCS); International Labour Organization (ILO); International Programme on Chemical Safety; Organisation for Economic Cooperation and Development.

Relevant Websites

http://www.unece.org – Globally Harmonized System for the Classification and Labeling of Chemicals (GHS).

http://www.who.int – See separate pages for Intergovernmental Forum on Chemical Safety (IFCS); International Programme on Chemical Safety (IPCS); and Inter-Organization Programme for the Sound Management of Chemicals (IOMC).

http://www.un.org – United Nations (UN), Conference on Environment and Development (UNCED) held in 1992, and the 2002 World Summit on Sustainable Development. Agenda 21.

Hazard Identification

Michael A Kamrin

Hazard identification is the first step in the risk assessment process and addresses two questions: (1) Does a given material present a potential hazard? (2) What type of adverse health effect(s) is it likely to cause? Is it, for example, a neurotoxicant, a developmental toxicant, or a potential carcinogen?

Hazard identification depends on a careful scientific evaluation of several different types of information:

- Physical characteristics (e.g., corrosivity, flammability, and reactivity with other substances).
- Results of *in vivo* animal testing studies.
- Results of *in vitro* laboratory tests (e.g., cell cultures or isolated tissues).
- Results of epidemiological studies with human populations.
- Structure–activity analysis (i.e., comparisons with the known toxicity of structurally similar chemicals).

In Vivo Animal Studies

The most commonly used information in hazard identification is obtained from animal bioassays. Although the use of animals in toxicity testing has become a highly controversial topic in recent years, responsible studies with a variety of species of laboratory animals (mainly rats, mice, guinea pigs, rabbits, dogs, and occasionally primates) frequently represent the only sources of information on which to judge the potential adverse effects of a chemical on humans. While always associated with a good deal of uncertainty, the use of animals in toxicology testing rests on the premise that the results observed in animals are applicable to humans.

Chemicals usually can cause multiple effects and the outcome of an exposure is likely to depend on the length of time over which an exposure occurs, the primary route of exposure, the sensitivity of the individual or species, and the resulting dose. Effects can occur as a result of exposure at the point of contact (e.g., skin, eyes, gastrointestinal tract, and respiratory tract) or any internal or systemic target. Animal bioassays are often placed in one of three major groups based primarily on the duration of exposure period; these groups are acute, subacute and subchronic, and chronic.

Acute Studies

Acute studies are designed to evaluate the possible adverse effects that may occur after short-term exposure (e.g., 24 h or less to ~1 week). Exposure may occur from one or possibly a few exposures to the test substance over the time period. In humans, such exposures might result from one-time accidents or other unusual incidents. Acute effects may occur in any organ system and can range from mild irritation of the eyes, skin, and respiratory tract to coma and death.

Subacute and Subchronic Studies

Subacute studies occur over time periods that range from greater than a week to several months. Subchronic studies address exposures that occur for a period of ~90 days. Both subacute and subchronic studies involve repeated, usually daily, exposures to the test substance. These studies have been used to reflect effects that might result from continual occupational exposures over a period of several weeks or months. Subchronic studies in rodents (lifetime of 2 years) have been used as the basis for exposure criteria that are intended to protect against the occurrence of adverse effects in humans resulting from exposures lasting from 1 to 7 years. Animals are observed daily throughout the course of the study and observations made with respect to clinical signs

of toxicity (e.g., loss of body weight, incoordination, and loss of balance), general appearance, and/or unusual behavioral patterns. At the end of the study, all animals are sacrificed and a gross and microscopic pathological examination of selected tissues is conducted. Included with subchronic and subacute studies are special developmental toxicity studies in which pregnant animals are exposed daily to the test chemical throughout the critical stages (organogenesis) of fetal development. Some chemicals (e.g., thalidomide) exert their adverse effects at very specific points in fetal development so that even single acute exposures during that critical period can cause an effect.

Chronic Studies

Chronic studies are typically conducted for periods $\geqslant 90$ days and usually up to the length of the lifetime of the test animal. In some cases, as with bioassays for carcinogenesis, the animals are exposed daily for their lifetimes (usually 18 months in mice and 2 years in rats). Chronic studies focus on identifying effects (e.g., such as cancer or certain reproductive effects) that might occur following continuous exposure over several generations.

The results of animal bioassays for toxicity form the basis of risk assessment and risk management under most regulatory statutes at the federal, state, and international levels. To obtain consistency and to ensure that the studies are designed, conducted, and analyzed in a sound scientific manner, experimental protocols for animal studies are required to meet various US federal statutes. Requirements are all carefully described in the Code of Federal Regulations. The study guidelines consist of a series of Standard Evaluation Procedures that clearly specify experimental conditions such as the number of animals per test group, the number of treatment groups, methods of chemical administration, types of observations that must be made and detailed procedures, and end points for clinical, hematological, ophthalmoscopic, and histopathologic evaluation. A study that does not follow the appropriate guidelines may not be acceptable to the regulatory agency. Currently, attempts are being made to standardize (harmonize) the test guideline requirements in different countries (e.g., United States, Canada, and the European Union).

Depending on the physicochemical nature of the chemical and the route of exposure of particular concern or relevance to expected human exposures, the test materials may be administered orally by incorporation in food or water or by gavage (feeding tube) directly into the stomach, dermally (by application to the skin), or by inhalation in the form of a gas or aerosol. In most cases, the test chemical is administered in a substance that is believed to be a toxicologically inert vehicle (such as saline or corn oil) and the observations in the treated animals are compared with those in control animals receiving only the vehicle. The tests are usually conducted with groups of control animals and three or more groups of experimental animals, each receiving different doses of the test material; groups of each sex are employed. The number of animals in a treatment group varies depending on the nature of the test, ranging from about 6–20 animals for acute and subchronic studies to 50–60 animals in chronic cancer bioassays.

In Vitro Tests in Hazard Identification

In vitro tests are tests in which chemical interactions with any of a wide variety of organs, tissue preparations, cell cultures, enzymes, receptors, etc. are studied 'in the test tube'. Such studies have always been considered important tools in studying the effects of toxic chemicals and identifying the mechanisms through which toxicity occurs and usually complement *in vivo* studies with intact animals. Indeed, with growing concern over the use of animals in laboratory research, there has been a concerted effort in recent years to develop *in vitro* tests that might serve as alternatives for some *in vivo* tests; despite some success, there remains a strong consensus that both types of testing will continue to be required.

The intact animal in a toxicity study is equivalent to a black box. It is possible to make gross observations of toxic signs, symptomology, and clinical effects but these seldom provide the details required to understand the precise mechanisms of toxicity or the primary cause, at the subcellular level, of the adverse effects. The use of *in vitro* systems allows, for example, measurements of the ability of a neurotoxicant to inhibit cholinesterase, identifies potentially reactive metabolites formed in the liver, or demonstrates the ability of a compound to bind to a macromolecular receptor like DNA. *In vitro* studies are also extremely important in metabolic investigations and the identification of reactive intermediates. The use of specific intact organs or a variety of cell or tissue cultures of human, animal, plant, or microbial origin often provides useful laboratory models for studying the potential of a compound to penetrate skin, damage the cornea of the eye, or otherwise interact with living tissues. Adverse effects may be indicated through changes in cell turnover, membrane permeability, or damage to cell organelles (e.g., mitochondria).

The results of *in vitro* studies to determine genetic toxicity have become an important component of assessing the carcinogenic potential of a chemical. Thus, an integral part of the routine testing of all chemicals is a battery of *in vitro* tests to determine each chemical's potential mutagenicity or clastogenicity (potential for DNA damage). These tests include the well-known Ames mutagenicity test with various strains of *Salmonella*, cytogenetic, and unscheduled DNA synthesis assays employing various cell cultures including those of human cells. A positive result with these types of tests suggests genotoxic or mutagenic potential that is taken into account in the total weight of evidence evaluation of carcinogenicity.

Epidemiology in Hazard Identification

Since epidemiology relates directly to the incidence of illness or toxicity in humans, the existence of positive epidemiological data is most relevant to assessing potential human hazard or risk. The major advantages of epidemiological studies are that they are usually based on large numbers of humans exposed to 'real-world' levels of the chemical. Any effects observed are directly relevant to humans and do not require the type of extrapolations used to relate animal studies to humans.

Unfortunately, epidemiological data can only be obtained retrospectively after a chemical has been on the market for a number of years and, obviously, cannot play a role in the prospective hazard evaluation of a new chemical.

Structure–Activity Relationships

In the absence of any other information, the analysis of possible structure–activity relationships (SARs) is often useful as a first attempt at hazard identification. SAR analysis involves a comparison of the structural, physical, and chemical properties of a chemical of unknown hazard with those of similar chemicals having known toxic effects. In some cases, SAR analysis may simply provide a qualitative indicator of a specific type of activity (i.e., if it is a polycyclic aromatic hydrocarbon it is likely to be treated as a suspected carcinogen in the absence of any toxicity data). In other cases, SAR relationships can actually be used to obtain a quantitative estimate of an effect (e.g., with homologous series of compounds or organophosphorus cholinesterase inhibitors). SAR analysis is frequently used by regulatory agencies to develop a series of triggers of concern. Thus, when faced with a new chemical that has never been tested, the presence of certain functional groups may be an indicator of possible toxicological concern.

It is important to recognize, however, that SAR analysis can be misleading and must be used cautiously. With some chemicals, the requirements for exerting an adverse effect are very specific, and small, seemingly insignificant changes in structure (e.g., the *ortho*, *meta*, or *para* position of an aromatic substituent) can have an enormous impact on biological activity.

See also: Ames Test; Analytical Toxicology; Animal Models; Biomarkers, Human Health; Epidemiology; Good Laboratory Practices (GLP); *In Vitro* Test; *In Vivo* Test; Risk Assessment, Human Health; Risk Characterization; Toxicity, Acute; Toxicity, Chronic; Toxicity, Subchronic.

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Hazard Ranking

Andrew Maier and Charley Pittinger

Chemical hazard ranking plays an important role in protecting public health and the environment. Understanding comparative risk profiles early in product development allows for directing resources to substances with better safety profiles. For existing chemicals or products, hazard ranking and screening tools allow for setting priorities in implementing risk management strategies. Hazard ranking is playing an increasing role as a tool in meeting or exceeding regulatory initiatives and satisfying product stewardship goals (e.g., International Organization for Standardization (ISO) 14000, the chemical industry's Responsible Care® programs, 'green certification programs', socially responsible investments, etc.).

In addition, regulatory initiatives such as the European Union's Registration, Evaluation and Authorization of Chemicals (REACH) program, Canada's Domestic Substances List, and the High Production Volume (HPV) chemical programs in the United States and Europe are key drivers for the

large-scale application of hazard ranking and screening tools for safety and risk assessment. Further, occupational and environmental hazard communication methods being standardized under the Global Harmonized System (GHS) for the classification and labeling of chemicals have long required effective assessment and ranking of hazards. Hazard ranking also provides a means for public education about relative risks of chemicals substances or activities. For example, the organization Environmental Defense uses comparative hazard ranking tools as part of its 'Scorecard' for providing information on the relative toxicity of chemicals.

Literally hundreds of hazard/risk databases, models, and algorithms have been developed to address this need for hazard ranking in risk and safety assessment. The available tools are very diverse, having different scientific, geographical, and chemical product focuses, as well as differing levels of sophistication and transparency. For example, some systems evaluate toxicity based solely on acute toxicity potential, while others rely on more comprehensive reviews of the toxicology database. Some systems include a detailed exposure assessment, while others include only minimal or no input for exposure potential. Some systems integrate ecological as well as human health toxicity considerations, while others have a single focus area. In addition, many published approaches include the consideration of toxicity or exposure information, but do not weigh other considerations such as public or regulatory agency risk perception that are critical factors in developing the overall risk profile for the chemical of interest. Because of this diversity in approaches, in many cases, the outcomes of such hazard assessments can vary considerably depending on the tool that is used.

Several authors have provided comparative summaries of hazard and risk ranking tools. One publication organized examples of related hazard ranking tools according to a hierarchy based on the complexity of hazard and risk assessment decisions being supported. This relationship is shown in **Figure 1**.

The diversity in some commonly used hazard ranking tools and approaches is represented in the examples below. The Further Reading and Relevant Website sections include a more comprehensive compilation of available approaches.

- Categorization base on acute toxicity potential – *Hazardous Materials Identification System* (HMIS and HIMS III).
- Ranking based on qualitative exposure potential and longer-term toxicity assessment – *Chemical Hazard Evaluation for Management Strategies: A Method for Ranking and Scoring Chemicals by Potential Human Health and Environmental Impacts*.
- Ranking based on regional and global exposure estimations and qualitative human health and ecological toxicity assessment – *European Union Risk Ranking Method*.
- Ranking based primarily on estimated and modeled exposure and qualitative toxicity data – *Use Clusters Scoring System* (UCSS).
- Recommendations for control strategies based on qualitative exposure and toxicity information – *Control of Substances Hazardous to Health* (COSHH).

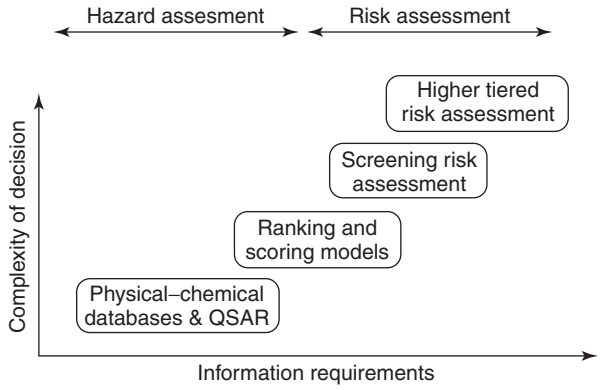

Figure 1 Hierarchy of hazard ranking tools in risk decision making. (Reproduced from Pittinger *et al.* (2003) *Risk Analysis* 23: 529–535, with permission from Blackwell Publishing.)

See also: Exposure Assessment; Exposure Criteria; Hazard Identification; High Production Volume (HPV) Chemicals; Risk Assessment, Ecological; Risk Assessment, Human Health.

Further Reading

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http://www.hse.gov.uk – United Kingdom HSE (Health and Safety Executive) (2004) *COSHH Essentials: Easy Steps to Control Chemicals*.

http://www.epa.gov – US Environmental Protection Agency (USEPA) (2004) *Use Clusters Scoring System*.

Hazardous Chemicals, Import/Export of

Marjorie Collins

Background

Chemicals, many potentially hazardous to health and the environment, are bought and sold in enormous quantities throughout the world. Similarly, hazardous waste often finds its way beyond the borders of the countries which generate it. Largely unregulated for many years, the transfer of chemicals between countries was addressed by voluntary measures beginning in the 1980s, when the procedure that has come to be known as Prior Informed Consent (PIC) took hold. This required exporters trading in hazardous substances to obtain the informed consent of importers before proceeding with any trade. This procedure was strengthened with the adoption of the Rotterdam Convention, making PIC legally binding. Importing countries are provided with the tools and information they need to identify potential hazards and to decline import of chemicals they cannot manage safely. If a country agrees to import chemicals, the Convention promotes their safe use through standards for labeling, technical assistance, and other forms of support. It also ensures that exporters comply with the requirements. The Rotterdam Convention came into force on February 24, 2004.

Related to chemical import and export, but somewhat separate from it, is the issue of the transboundary shipment of hazardous wastes. The international law dealing with it is the Basel Convention, which came into force in 1992. One of its guiding principles is that, in order to minimize the threat, hazardous wastes should be dealt with as close to where they are produced as possible. Movements of wastes must comply with a prior written notification by the exporting country to the importing country. Movement documents are required to track the waste from the beginning of its journey to the point of disposal.

This entry will focus on the United States' interpretation of the regulation of the import and export of hazardous chemicals, specifically with regard to the Toxic Substance Control Act (TSCA). It will not generally address issues related to hazardous waste.

TSCA Basics

The Environmental Protection Agency (EPA) regulates the import and export of chemical substances from the United States under the TSCA. TSCA was first enacted in 1976 and has been amended significantly three times. TSCA gives the EPA broad authority to regulate the manufacture, use, distribution in commerce, and disposal of chemical substances. TSCA is a federally managed law and is not delegated to states.

One of the main objectives of TSCA is to characterize and evaluate the risks posed by a chemical to humans and the environment before the chemical is introduced into commerce. TSCA accomplishes this through the requirement that manufacturers perform various kinds of health and environmental testing, use quality control in their production processes, and notify EPA of information they gain on possible adverse health effects from use of their products. Under TSCA, 'manufacturing' is defined to include 'importing', and thus all requirements applicable to manufacturers apply to importers as well.

TSCA classifies chemicals as either 'existing' or 'new'. Existing chemicals are listed in the TSCA Chemical Substances Inventory (the 'Inventory'). Both existing and new chemicals can be covered by TSCA requirements.

The main regulatory requirements under TSCA which apply to importers and exporters of hazardous chemicals are:

- Premanufacture Notice (PMN)
- Testing Requirements
- Recordkeeping and Reporting Requirements
- TSCA Export Requirements
- TSCA Import Certification

Premanufacture Notice – TSCA Section 5 (40 CFR 700, 720–725, 747)

If a business is importing chemicals or chemical-containing items into the United States, that business must determine whether or not any chemical imported in bulk or as a part of a mixture, is a TSCA chemical substance and/or a 'new chemical substance' prior to its importation for a nonexempt commercial purpose. Under Section 5 of TSCA, persons who intend to manufacture or import a 'new chemical substance' into the United States must seek EPA approval by submitting a premanufacture notice (PMN) to EPA at least 90 days prior to importation to enable EPA to determine whether the new chemical may present an unreasonable risk to human health or the environment. A new chemical substance is one that is not already in commerce in the United States, as determined by inclusion in the TSCA Inventory of Chemical Substances maintained by EPA. New chemical substances include certain genetically modified microorganisms.

In addition, prior to importation of a chemical substance subject to TSCA into the United States, an importer of record must determine whether the substance is subject to a Significant New Use Rule issued under Section 5 of TSCA. Section 5 of TSCA authorizes EPA to designate use of a chemical substance as a 'significant new use', and require the submission of information to EPA prior to the chemical substance being manufactured (including imported) or processed for that use.

The PMN must include information on the manufacturing process, disposal method, and health and environmental effects of the substance. After its review of the PMN, EPA may approve the PMN and/or may limit, restrict, or prohibit the manufacture, use, distribution, and/or disposal of the chemical substance.

If the PMN is approved and the substance is imported, a 'Notice of Commencement' (NOC) is required to be submitted to EPA within 30 days of first importation. After the EPA receives the NOC, the subject chemical substance will be added to EPA's TSCA Inventory of existing chemical substances for future importation and/or domestic production.

There are some exemptions to the PMN requirement, including chemicals such as drugs and pesticides that are regulated by other statutes, as well as chemicals developed under certain special circumstances.

In addition to the PMN requirement an importer of record must determine whether the substance is subject to a Significant New Use Rule issued under Section 5 of TSCA, prior to importation of a chemical substance subject to TSCA. Section 5 of TSCA authorizes EPA to designate use of a chemical substance as a 'significant new use', and require the submission of information to EPA prior to the chemical substance being manufactured (including imported) or processed for that use.

There are certain exemptions to the 90 days review of new chemicals. The TSCA compliance certification is still required to import these chemicals. Some examples of exemptions are as follows:

- Research and Development Exemption (40 CFR 720.36)
- Test Marketing Exemption (40 CFR 720.38)
- Low Volume/Low Release/Low Exposure Exemption (40 CFR 723.50)
- Polymer Exemption (40 CFR 723.250)

Testing Requirements – TSCA Section 4 (Regulations: 40 CFR 766, 790–799)

In addition to the PMN process, chemical manufacturers and importers may be required to perform other testing on health and environmental effects of their products under TSCA. A person who imports or intends to import a chemical substance or mixture subject to a test rule under Section 4 must comply with Section 4 requirements unless the importation qualifies for an exemption included in the regulations at 40 CFR Section 790.42, or under a specific test rule listed under Parts 766 or 799. Following promulgation of a test rule under Section 4 of TSCA, the responsibility to comply with the rule continues for a period of 5 years from the date the data from all required tests have been submitted or an amount of time equal to that which was required to develop the test data, whichever is longer. Importers therefore have a continuing responsibility to determine whether a chemical substance or mixture which they import or intend to import is subject to a test rule.

Recordkeeping and Reporting Requirements – TSCA Section 8 (40 CFR 710, 712, 717, 716)

Section 8 of TSCA authorizes EPA to require chemical manufacturers, importers, processors, and distributors of TSCA-covered chemical substances and mixtures to keep certain records and report certain information to EPA. Specific TSCA Section 8 rules (and implementing policy documents in the case of TSCA Section 8(e)) that apply are:

- Inventory Update Rule
- Preliminary Assessment Information Reporting Rule

- Chemical Specific Recordkeeping and Reporting
- Allegations of Significant Adverse Reactions Recordkeeping and Reporting Rule
- Unpublished Health and Safety Data Reporting
- Substantial Risk Information Reporting Requirement

These requirements are explained in more detail below.

Inventory Update Rule (IUR)

Companies that manufacture or import more than 10 000 lb of certain chemicals that are included on the TSCA Chemical Substance Inventory (primarily organics) are required to report current data on the production volume, plant site, and site-limited status of these chemicals. Reporting under the IUR takes place at 4 year intervals, which began in 1986. Certain small businesses as defined by 40 CFR 710.29 are excluded. The next round of reports will be due in 2006.

Preliminary Assessment Information Rule (PAIR)

Under PAIR, producers and importers of a listed chemical are required to report the following site-specific information on a two page form:

- Quantity of chemical produced and/or imported.
- Amount of chemical lost to the environment during production or importation.
- Quantity of enclosed, controlled and open releases of the chemical.
- Per release, the number of workers exposed and the number of hours exposed.

Exemptions for such reporting are as follows:

- Production or importation for the sole purpose of research and development (R&D).
- Production or importation of less than 500 kilograms during the reporting period at single plant site.

Companies whose total annual sales from all sites owned by the domestic or foreign parent company are below $30 million for the reporting period and who produced or imported less than 45 400 kg of the chemical

- Production or importation of the listed chemical solely as an impurity, a nonisolated intermediate, and under certain circumstances as a by-product.

Allegations of Significant Adverse Reactions Rule

This rule provides a mechanism to identify previously unknown chemical hazards by tracking patterns of adverse effects when they are noticed or detected by requiring companies to record, retain, and in some cases, report to the USEPA 'allegations of significant adverse reactions' for substances/mixtures that they produce, import, process, or distribute.

An 'allegation' is defined as "a statement, made without formal proof or regard for evidence, that a chemical substance or mixture has caused a significant adverse reaction to health or the environment."

'Significant adverse reactions' are defined as "reactions that may indicate a substantial impairment of normal activities, or long lasting or irreversible damage to health or the environment."

TSCA Section 8(c) records must be kept at a company's headquarters or at a site central to the chemical operations and must be retrievable by the alleged cause of the reaction (i.e., specific chemical identity, mixture, article, company process or operation or site emission, effluent, or discharge). The records must be maintained by the company for 30 years (for allegations made by employees) or 5 years for allegations made by plant site neighbors or customers.

The records must contain the original allegation as received, an abstract of the allegation, the results of any self-initiated investigation, and copies of any additional information regarding the allegations (e.g., copies of any reports required to be made to the US Occupational Safety and Health Administration Division).

Verbal allegations must be transcribed either by the company or the individual making the allegation (if transcribed by the individual, they must be signed). To be recordable, allegations must implicate a substance that caused the reaction by naming either the specific substance, a mixture, or article containing the substance; a company process in which the substances are involved; or identifying a discharge from a site of manufacture, processing, or distribution of the substance.

Certain allegations of human health and environmental adverse effects are 'exempt' from the requirements of the TSCA Section 8(c) rule including those that: (1) are made anonymously; (2) identify 'known human effects'; and (3) those that are directly attributable to an incident of environmental contamination that has already been reported to the US Government under any applicable authority.

Unpublished Health and Safety Studies Rule

Businesses may be required to submit to EPA a list and/or copies of unpublished studies that address the health or safety issues of certain listed chemicals.

Businesses that must report under the TSCA Section 8(d) rule include:

- Current as well as prospective producers, importers, and (if specified) processors of the subject chemical(s); and
- Businesses that, in the 10 years preceding the effective date that a substance or mixture is added to the rule, either had proposed to produce, import, or (if specified) process, or had produced, imported, or processed (if specified) the substance or listed mixture.

Once a chemical substance or mixture is added to the rule, reporting obligations terminate (i.e., sunset) no later than 2 years after the effective date of the listing of the substance or mixture, or on the removal of the substance or mixture from the rule.

Unpublished studies on listed substances or mixtures are potentially reportable (i.e., studies may be subject to either copy submission requirements or listing requirements). Generally, copies of studies possessed at the time a person becomes subject to the rule must be submitted, and the following categories of studies must be listed:

- Studies ongoing as of the date a person becomes subject to the rule (copies must be submitted when completed).
- Studies initiated after the date a person becomes subject to the rule (copies must be submitted when completed).
- Studies which are known as of the date a person becomes subject to the TSCA Section 8(d) rule, but not possessed.
- Studies previously sent to US Government Agencies without confidentiality claims.

The term 'health and safety study' is intended to be interpreted broadly and means "any study of any effect of a chemical substance or mixture on health or the environment or on both," including but not limited to:

- epidemiological or clinical studies,
- studies of occupational exposure,
- *in vivo* and *in vitro* toxicological studies, and
- ecotoxicological studies.

Substantial Risk Information Requirement

Businesses are under a duty to report to EPA within 15 days any new information which reasonably supports the conclusions that a substance or mixture the business manufactures, imports, processes, or distributes presents a substantial risk of injury to health or the environment.

The term 'substantial risk' information refers to that information which reasonably supports a conclusion that the subject chemical or mixture presents a substantial risk of injury to health or the environment; however, such information need not and most typically does not establish conclusively that a substantial risk exists.

In deciding whether information is 'substantial risk' information, one must consider (1) the seriousness of the adverse effect, and (2) the fact or probability of the effect's occurrence. In determining TSCA Section 8(e)-applicability/reportability, these two criteria should be weighted differently depending upon the seriousness of the effect or the extent of the exposure, that is, the more serious the effect, the less heavily one should weigh actual or potential exposure, and vice versa. For example, in cases where serious effects such as birth defects or cancer (as evidenced by benign and/or malignant tumors) are observed, the mere fact that the implicated chemical is in commerce (including chemicals at the research and development stage) constitutes sufficient evidence of exposure to submit the newfound toxicity data.

EPA has also received numerous Section 8(e) submissions alerting the Agency that chemical substances already known to be capable of causing serious health and/or environmental effects were detected in significant amounts in environmental media (e.g., soil, surface waters, groundwater, air (including workplace air)) or in products not known previously by the Agency to contain such chemicals. In such cases, the discovery of previously unknown and significant human and/or environmental exposure, when combined with knowledge that the subject chemical is already recognized or suspected as being capable of causing serious adverse health effects (e.g., cancer, birth defects, neurotoxicity) or serious environmental effects (e.g., nontrivial aquatic species toxicity), can provide a sufficient basis to report the new-found exposure data to EPA under Section 8(e) of TSCA.

The decision-making process for Section 8(e)-reportability should focus primarily on whether the toxicity or exposure information offers reasonable support for a conclusion of substantial risk under the criteria described above, but should not focus at all on whether the information is conclusive regarding the risk. A decision to report information to the Agency under Section 8(e) should not involve exhaustive health and/or environmental risk assessments of the subject chemical(s). Further, determining reasonable support for a conclusion of substantial risk should not include any evaluation of either the economic or social benefits of the use(s)

of the subject chemical substance(s). Finally, determining whether reasonable support exists for 'substantial risk' is not synonymous with the determination of an 'unreasonable risk' as that term is used elsewhere in TSCA.

TSCA Export Requirements – TSCA Section 12(b) (40 CFR 707)

Chemical exporters are potentially subject to Section 12(b) of TSCA. EPA's TSCA Section 12(b) export notification requirements apply to chemical substances or mixtures for which data are required under TSCA Section 5(b), an order has been issued under TSCA Section 5, a proposed or final rule has been issued under TSCA Sections 5 or 6, or an action is pending or relief has been granted under TSCA Sections 5 or 7. With regard to Section 4 of TSCA, only those chemical substances or mixtures listed in final TSCA Section 4 test rules and TSCA Section 4 Enforceable Consent Agreements are subject to the export notice requirements under TSCA Section 12(b). Notification of export is generally not required for articles, as provided by 40 CFR section 707.60(b).

Exporters of chemicals that are subject to final or proposed rules and orders under Sections 5, 6, and 7 of TSCA must notify EPA of the country of destination the first time a chemical is shipped to the country during a calendar year. In addition, exporters of chemicals subject to final rules or orders under Section 4 of TSCA must notify EPA of the country of destination the first time a chemical is shipped to the country.

TSCA Import Certification – TSCA Section 13 (40 CFR 707 and 19 CFR 12.118–12.128)

Under EPA and Customs regulations, the importer of a chemical substance or mixture must certify at the port of entry that each shipment is either subject to and in compliance with TSCA (a positive certification); or the shipment is not subject to TSCA (a negative certification). The following series of questions need to be asked when making a determination.

1. Is the material in the shipment to be imported an 'article', or tobacco or tobacco product? If yes, an import certification is not required (positive or negative). If no, continue to the next question.
2. Is the material in the shipment to be imported a pesticide; a source or special nuclear material or by-product; a firearm or ammunition; or a food, food additive, drug, cosmetic, or device? If yes, the material is not subject to TSCA, but a 'negative' TSCA import certification is required unless the shipment is clearly identified as being a pesticide or other chemical not subject to TSCA (e.g., the shipment is accompanied by FDA Form FD701 or EPA (FIFRA) Form 3540-1). If no, continue to the next question.
3. Does the shipment contain any chemical substances or mixtures regulated under TSCA Section 5 (including new chemical substances), TSCA Section 6, or TSCA Section 7? If yes, continue to the next question. If no, a positive TSCA import certification can be made.
4. Are the chemical substances and/or mixture in the shipment compliant with TSCA Sections 5, 6? If yes, a positive TSCA import certification can be made. If no, import certification cannot be provided and the shipment cannot be imported until the chemical substance and/or mixtures is compliant with all applicable requirements under TSCA Sections 5, 6, and 7.

See also: Environmental Protection Agency, US

Relevant Websites

http://www.basel.int – Basel Convention.
http://www.pic.int – Rotterdam Convention.
http://www.epa.gov – Import/Export Requirements (under TSCA, from the US EPA).

Hazardous Waste

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There are two definitions of hazardous waste. The first definition was written into legislation by the US Congress, and the second, which has its basis in the first, was written into regulation by the US Environmental Protection Agency (EPA). In 1976, US Congress defined the term 'hazardous waste' in the Resource Conservation and Recovery Act (RCRA), an amendment to the Solid Waste Disposal Act of 1965, as

> A solid waste, or combination of solid waste, which because of its quantity, concentration, or physical,

> chemical, or infectious characteristics may – (A) cause, or significantly contribute to an increase in mortality or an increase in serious irreversible, or incapacitating reversible, illness; or (B) pose a substantial present or potential hazard to human health or the environment when improperly treated, stored, transported, or disposed of, or otherwise managed.
>
> 42 USC 6903(5)

In Subtitle C of RCRA, Congress created the framework for the 'cradle-to-grave' management of hazardous waste. The broad, subjective statutory definition of hazardous waste, however, did not specify which wastes would be subject to Subtitle C and its accompanying regulatory scheme. A more precise definition of hazardous waste was needed to determine exactly which wastes would be subject to RCRA Subtitle C regulation. Therefore, the US Congress directed the US EPA to promulgate regulations identifying the characteristics of hazardous waste and listing particular hazardous wastes, "taking into account toxicity, persistence, and degradability in nature, potential for accumulation in tissue, and other related factors such as flammability, corrosiveness, and other hazardous characteristics" (42 USC 6921(a)). In 1980, the US EPA fulfilled this statutory mandate by promulgating 40 CFR Part 261, which is titled *Identification and Listing of Hazardous Waste*. Since then, the US EPA has modified Part 261 numerous times to reflect Congressional amendments and trends in waste generation.

In the regulatory sense, hazardous waste identification relies not so much on a definition as on a series of steps that involve checking against lists of waste exclusions and inclusions. The three steps of the hazardous waste identification process are codified in 40 CFR 262.11.

The first step of hazardous waste identification is determining whether a material is a solid waste. A solid waste is any material that is discarded. (The modifier 'solid' is not indicative of the physical state of the material. That is, a solid waste may be in the liquid or gaseous phases as well as the solid phase.) A material is considered discarded when it is abandoned, recycled, or inherently waste-like. Abandonment occurs when a material is disposed of; burned/incinerated; or accumulated, stored, or treated before, or in lieu of, abandonment. Recycling occurs when a material is used in a manner that resembles disposal (e.g., placed on the ground); burned to recover its energy; reclaimed to recover a usable product; or accumulated speculatively. In addition, a few specific materials have been designated inherently waste-like (e.g., certain dioxin-containing wastes) and are considered solid wastes when recycled in any manner. However, a number of materials do not meet the definition of solid waste when discarded because the materials qualify for one of several exclusions from the definition of solid waste that are codified in 40 CFR 261.4(a). For example, in order to avoid duplicative regulations, materials regulated and managed under the Clean Water Act or the Atomic Energy Act are exempt from the definition of solid waste (40 CFR 261.4(a) (2) and 261.4(a) (4), respectively).

The second step in the hazardous waste identification process is determining whether a solid waste qualifies for one of the statutory or regulatory exclusions from the definition of hazardous waste. Several exclusions from the definition of hazardous waste appearing in the regulations have their origins in the statute. For instance, the US EPA incorporated Congress' Subtitle C exclusion for cement kiln dust into the regulations at 40 CFR 261.4(b) (8). In addition, the US EPA has promulgated a number of exclusions independently of Congress, all of which are codified in 40 CFR 261.4(b). For example, the US EPA exempted all solid wastes routinely generated in residences from the definition of hazardous waste (40 CFR 261.4(b) (1)). Waste-specific exclusions may also be obtained on a site-by-site basis by petitioning the US EPA.

The third step of hazardous waste identification is determining whether a solid waste that is not specifically excluded is a hazardous waste. There are two broad categories of hazardous waste: listed and characteristic. The hazardous waste determination process in 40 CFR 262.11 states that the listings are reviewed first. If a solid waste does not meet any of the listing descriptions, then the characteristics are checked. The listings are intended to regulate common hazardous waste streams by specifically listing them by name or by description. The characteristics, on the other hand, are written as broad descriptions, each of which may capture an unspecified number of solid wastes within hazardous waste regulation. All hazardous wastes are assigned a waste code consisting of an initial letter and a number. Listed waste codes begin with an 'F', 'K', 'P', or 'U', while all characteristic waste codes begin with the letter 'D'.

A waste is listed by the US EPA if it meets one of the three criteria codified in 40 CFR 261.11. First, a waste can be promulgated as a listed waste if it exhibits any of the four characteristics (a detailed discussion of the characteristics follows). Although a waste may be listed because it exhibits a characteristic, it is not defined in the regulations in terms of its characteristic(s). Rather, the US EPA defines such wastes in terms of the process by which it is generated or by its chemical name. For example, K044 is listed

because it exhibits a characteristic, but its listing description reads, 'wastewater treatment sludges from the manufacturing and processing of explosives', without reference to the characteristic.

Second, a waste can be listed because it contains any of the toxic constituents listed in 40 CFR Part 261 Appendix VIII, provided the concentration of the constituent in the waste, the persistence of the constituent, any toxic degradation products of the constituent, as well as other factors are taken into account. Hazardous constituents are listed in Appendix VIII if the constituents have been shown in scientific studies to have toxic, carcinogenic, mutagenic, or teratogenic effects on humans or other life forms. The majority of listed wastes have been listed in this manner.

Finally, a waste can be listed if it has been found to be fatal to humans in low doses or, in the absence of data on human toxicity, it has been shown in studies to:

- Have an oral LD_{50} toxicity (rat) $\leqslant 50\,mg\,kg^{-1}$, or
- Have an inhalation LC_{50} toxicity (rat) $\leqslant 2\,mg\,l^{-1}$, or
- Have a dermal LD_{50} toxicity (rabbit) $\leqslant 200\,mg\,kg^{-1}$, or
- Be otherwise capable of causing or significantly contributing to an increase in serious, irreversible, or incapacitating reversible illness. Wastes listed in this manner are classified as acute hazardous wastes that become subject to full hazardous waste regulation in smaller quantities than those for other hazardous wastes. An example of an acute listed hazardous waste is P015, beryllium powder.

Based on the first three criteria, the US EPA has promulgated several hundred listed wastes, dividing the listed wastes into three groupings: wastes from nonspecific sources ('F wastes'), wastes from specific sources ('K wastes'), and (two types of) commercial chemical products ('P or U wastes'). All wastes on the 'P list' are acute hazardous wastes.

Before a characteristic of hazardous waste can be promulgated it must be assessed against two criteria that are codified in 40 CFR 261.10. The first criterion is that a waste exhibiting the characteristic in question must meet the broad statutory definition of hazardous waste. The second criterion is that the characteristic must be able to be measured using standardized test methods or detected through knowledge of the waste. Based on these criteria, the US EPA has identified four characteristics of hazardous waste: ignitibility, corrosivity, reactivity, and toxicity. For each characteristic, the US EPA has promulgated at least two distinct properties. A solid waste need only exhibit one property of one characteristic to be subject to regulation as a characteristic hazardous waste.

The US EPA has identified four properties of ignitibility. One of the four properties pertains to liquids that are not aqueous solutions containing less than 24% alcohol by volume. A liquid meeting this description that has a flash point $\leqslant 60^{\circ}C$ (140°F), as determined by a specified closed cup test, is one example of an ignitible hazardous waste that carries the waste code D001.

Two properties of corrosivity have been identified by the US EPA, either of which can render a solid waste a hazardous waste, identified by the waste code D002. An aqueous solid waste that has a pH $\leqslant 2$ or $\geqslant 12.5$, as measured by a specified test, is a corrosive hazardous waste. Likewise, a liquid solid waste that corrodes steel at a rate $\geqslant 6.35\,mm\,year^{-1}$ at 55°C, as measured by a specified test, is also a corrosive hazardous waste.

A solid waste is hazardous for reactivity if it displays any one of eight separate properties that the US EPA has specified. Unlike the other three characteristics of hazardous waste, several of the properties of reactivity rely on subjective criteria rather than scientifically measured properties. In such cases, a generator of a solid waste must make a judgment about the regulatory status of the waste based on his/her knowledge of the nature of the waste. For example, a solid waste that forms potentially explosive mixtures with water, or a waste that is normally unstable and readily undergoes violent change without detonating, is a D003 reactive waste.

The final characteristic, toxicity, is defined by the concentration levels of 40 hazardous constituents – 26 organics, 8 metals, and 6 pesticides – in an extract of a representative sample of waste. The extract is obtained by subjecting a sample to the Toxicity Characteristic Leaching Procedure, a test designed to estimate the ability of the contaminant to leach if the waste containing it were placed in a landfill. The concentration levels of hazardous constituents measured in a waste extract are compared to maximum allowable concentrations limits established in the regulations. If any of the regulatory concentration limits are equaled or exceeded, the waste stream (not just the extract) is a characteristic hazardous waste for toxicity. Each of the 40 toxicity characteristic (TC) waste codes, D004–D043, corresponds to a different hazardous constituent. For example, if the TCLP leachate of a waste has $\geqslant 5.0\,mg\,l^{-1}$ lead, the waste is TC hazardous for lead and carries the waste code D008. If the leachate has $\geqslant 0.2\,mg\,l^{-1}$ mercury, the waste is TC hazardous for mercury and carries the waste code D009.

It is important to note that the same chemical or compound can have different wastecodes based on the manner in which it is generated. For example, American Petroleum Institute (API) separator sludge from the petroleum refining industry is identified by the waste code K051. Separator sludge generated in a unit other than an API separator would not be K051 because it does not meet the listing description that, as a K listing, specifies the source of the sludge. The same waste generated in another type of separator would most likely be captured by either F037 or F038, neither of which, as F-listed wastes, specifies the source of the waste stream. In addition, if neither listing applies, the sludge could be regulated as a characteristic hazardous waste with the waste code D018 if the TCLP leachate meets or exceeds $0.5\,\mathrm{mg\,l^{-1}}$ benzene, the regulatory limit of the toxicity characteristic for benzene.

According to 40 CFR 262.11, the person(s) who produces a waste (generator) is responsible for carrying out the hazardous waste identification process. First, the generator must review all hazardous waste listings. Only if no listings apply is the generator required to check for characteristics, using testing and/or process knowledge. Although it is not necessary for hazardous waste identification purposes to determine whether a listed waste is also characteristic, it may be necessary to take the added step of identifying characteristics to determine the appropriate treatment for the waste. If a solid waste does not meet a listing description and it does not exhibit any of the four characteristics, it is not a hazardous waste as defined by the federal regulations.

Hazardous waste identification is the first step in determining how a waste must be managed. Wastes that meet the definition of hazardous waste are subject to comprehensive federal regulations (40 CFR 262–279) that govern the generation, transportation, storage, treatment, disposal, and recycling of hazardous waste. The level of regulation varies based on criteria such as the amount of waste generated at a site on a monthly basis, the nature of the waste, and, in some cases, whether the waste is recycled. All hazardous waste regulations are similar in that they are intended to ensure the safe management of hazardous waste from cradle to grave.

See also: Clean Water Act (CWA), US; Environmental Toxicology; Resource Conservation and Recovery Act, US

Relevant Websites

http://www.dtsc.ca.gov – Managing Hazardous Waste; US California Department of Toxic Substances Control.

http://www.osha.gov – Safety and Health Topics: Hazardous Waste; US Occupational Safety and Health Administration.

Health Assessments

C Charles Barton and Alan G Parham

Introduction

The 1986 Superfund Amendments and Reauthorization Act directs the Agency for Toxic Substances and Disease Registry (ATSDR) to perform specific public health activities associated with exposure to hazardous substances released into the environment. Among these activities, ATSDR was mandated to perform a health assessment for each facility listed or proposed to be listed on the National Priorities List. The health assessment has to be conducted within 1 year of being listed (or proposed for listing). In addition, ATSDR may conduct a health assessment for a particular facility or release when petitioned by the public.

A health assessment is the evaluation of data and information on the release of hazardous substances into the environment in order to assess any current or future impact on public health, develop health advisories or other recommendations, and identify studies or actions needed to evaluate and mitigate or prevent human health effects (55 Federal Register 5136, February 13, 1990, as codified at 42 Code of Federal Regulations Part 90).

The health assessment is a mechanism to respond to community health concerns associated with human exposure to hazardous substances at a site. It has three major purposes: (1) evaluating the public health implications of the site; (2) addressing those implications by developing health advisories or making recommendations, including further health or environmental studies; and (3) identifying populations where actions are necessary to mitigate or prevent adverse health effects.

When complete health or environmental data are lacking, it may be necessary to conduct further assessments for a site or facility as the data become available. A major reason for preparing a health assessment is to determine the need for health effects

studies at a site to further assess any current or future risks to public health. The health assessment is an evaluation of relevant environmental data, health outcome data, and community concerns associated with a site where hazardous substances have been released. The health assessment identifies populations living or working on or near hazardous waste sites for which public health actions are needed, such as health studies, health education, or chemical-specific research.

Health assessments are based on factors such as the nature, concentration, toxicity, and extent of contamination at a site; the existence of potential pathways for human exposure; community health concerns; the size and nature of the community likely to be exposed; relevant community-specific, past and current, health outcome data; and any other information available to ATSDR that is relevant to a determination of potential risks to public health.

Thus, health assessments are designed not only to evaluate health effects, but also to identify populations for which additional studies or public health actions are required. Hence, the health assessment is designed to identify: (1) knowledge gaps concerning the toxicity of substances identified at the facility or release under review; (2) communities near facilities or releases where biologic measurements of human exposure or medical investigations (e.g., community-based health outcome parameters) are needed; and (3) the need for additional health information (e.g., pilot studies, epidemiological studies and registries, and site-specific surveillance). A variety of health studies based on the review of the health assessment may then be initiated, including: pilot health effects studies (disease- and symptom-prevalence studies, cluster investigations, exposure studies), epidemiological studies, or disease registries.

Three sources of information build the foundation of the health assessment: (1) environmental characterization data, (2) community health concerns, and (3) health outcome data.

Environmental characterization data for a hazardous waste site includes information on environmental contamination and environmental pathways. Such information is provided in site-specific reports obtained from the Environmental Protection Agency (EPA) and pertinent state and local environmental departments. Site visits are also an important source of environmental characterization data.

Community health concerns associated with a site constitute a key data component of the health assessment. The community associated with a hazardous waste site includes the population living around the site, local public health officials, other local officials, and the local media. In order to acquire information on community health concerns, the health assessor must become an investigator; obtaining that information provides the health assessor with an opportunity to involve the public in the health assessment process. In addition, community health concerns can serve as a guide in evaluating health outcome data.

Health outcome data and parameters are the third major source of data for health assessments. The identification, review, and evaluation of health outcome parameters are interactive processes involving ATSDR, data source generators, and the community involved. Health outcome data are community-specific and may include databases at the local, state, and national level, as well as data from private health care organizations and professional institutions and associations. Databases to be considered include medical records, morbidity and mortality data, tumor and disease registries, birth statistics, and surveillance data. Relevant health outcome data play an important role in assessing the public health implications associated with a hazardous waste site and in determining which follow-up health activities are needed.

Health Assessment Process

To evaluate the public health implications posed by contamination at a site, the assessor must obtain and evaluate data and information on the site's history, the types and levels of contamination, environmental transport mechanisms, routes of human exposure, community health concerns, relevant health outcome parameters, and medical and toxicological implications of the site's contaminants. This evaluation is a dynamic process that considers available data from varying perspectives.

Every health assessment includes six basic steps for acquiring the data and information necessary to evaluate the site's health risks:

1. Evaluating information on the site's physical, geographical, historical, and operational setting. Information should be evaluated to provide a historical perspective on the site, and describe its operations, current status, and the surrounding community. Once the important background details have been presented, the assessor should describe characteristics of populations on or near the site and the use of local land and resources. The text should state the total number of exposed and potentially exposed persons and indicate the characteristics of this population. Population estimates should include persons exposed in the past and present, as well as those at risk for future

exposures. Demographic information may include discussions of specific population groups surrounding the site (e.g., residential, commercial, and occupational populations). If warranted, details about the size, exact location, age distribution, socioeconomic, genetic, and ethnic makeup of populations on and near the site should be discussed based on available information. Ethnic and socioeconomic background information is essential for a full understanding of the health threat a site poses to specific subpopulations. Populations that may be at special risk from exposure to the site, such as children, pregnant women, and the elderly, should receive special note.

2. Identifying health concerns of the affected community. The nature and degree of the residents' health concerns will vary from site to site. However, addressing the health concerns of the community is crucial if the health assessment is to satisfy its purpose of helping the public and health professionals understand the risks posed by a site.
3. Determining contaminants of concern associated with the site. This is the foundation of the health assessment and, therefore, should be composed carefully so that the significant hazards of concern are clearly and concisely presented. In this step, the assessor describes the contaminants that might pose a threat to public health and physical hazards at the site. To determine whether a contaminant is a contaminant of concern based on noncancer end points, the maximum media concentration should be compared to an appropriate health assessment comparison value. Health assessors should use ATSDR's Environmental Media Evaluation Guides (EMEGs). If no EMEG is available, the assessor should use other health guidelines, such as EPA's reference dose (RfD), to back-calculate a medium concentration. The assessor should also evaluate the potential carcinogenicity of contaminants. For carcinogens, comparison values based on a 10^{-6} cancer risk level for exposure to the contaminated media can be calculated from values such as EPA's cancer slope factors. If the maximum medium concentration exceeds a comparison value, the contaminant should be selected for further evaluation.
4. Identifying and evaluating exposure pathways (environmental transport mechanisms and human exposure pathways). In this step, the health assessor evaluates exposure pathways at the site. There are five elements in an exposure pathway: (1) source of contamination (source of contaminant release into the environment), (2) environmental media (this includes groundwater, surface water, air, surface soil, subsurface soil, sediment, and biota. Transport mechanisms serve to move contaminants from the source to points where human exposure can occur), (3) point of exposure (a location of potential or actual human contact with a contaminated medium, for example, residence, business, residential yard, playground, campground, waterway or water body, contaminated spring or hand-drawn well, food services, etc.), (4) route of human exposure (means by which the contaminant actually enters or contacts the body, such as ingestion, inhalation, dermal contact, and dermal absorption), and (5) receptor population (persons who are exposed or potentially exposed to the contaminants of concern at a point of exposure). Completed exposure pathways exist when the five elements of a pathway link the contaminant source to an exposed population. Potential exposure pathways exist when information on one or more of the five elements is missing.
5. Determining public health implications based on available community-specific health outcome databases and other medical and toxicological information. In this step, the health assessor discusses the health effects of site contaminants, evaluates health outcome data, and addresses all questions raised by the community. Accordingly, there are three parts to this step: (1) toxicological evaluation, (2) health outcome data evaluation, and (3) community health concerns evaluation. The health assessment must demonstrate how information in each step of the health assessment relates to the public health discussion.
6. Determining conclusions and recommendations concerning the health threat posed by the site. The final step of the health assessment should address conclusions about the site and the health threat it poses. The first conclusion should be a statement about the site's level of public health hazard. The assessor should assign one of the five public health categories: urgent public health hazard, public health hazard, indeterminate public health hazard, no apparent public health hazard, or no public health hazard. For the categories 'urgent public health hazard' and 'public health hazard', the text should identify the contaminant(s), the completed exposure pathway(s), the health effect(s), and the exposed population(s). The text should also summarize conclusions about the following issues: health effects from exposure to site contaminants, response to community health concerns, results of health outcome data evaluation, and the effect that missing or insufficient information has on analyses and conclusions. Furthermore, every conclusion of the health assessment should have one or more recommendations associated with it.

Information reviewed for each step in the health assessment process is evaluated for adequacy of data and potential health impacts at a hazardous waste site. Consideration is given to known past or expected future contamination and exposures.

Categorizing Hazards

The final section of the health assessment should address conclusions about the site and the health threat it poses. The first conclusion should be a statement about the site's level of public health hazard. The assessor should assign one of the five public health categories:

- *Category A* – Urgent Public Health Hazard: This category is used for sites that pose an urgent public health hazard as the result of short-term exposures to hazardous substances.
- *Category B* – Public Health Hazard: This category is used for sites that pose a public health hazard as the result of long-term exposures to hazardous substances.
- *Category C* – Indeterminate Public Health Hazard: This category is used for sites with incomplete information.
- *Category D* – No Apparent Public Health Hazard: This category is used for sites where human exposure to contaminated media is occurring or has occurred in the past, but the exposure is below a level of health hazard.
- *Category E* – No Public Health Hazard: This category is used for sites that do not pose a public health hazard.

Health Assessments versus Risk Assessments

Deliberate differences exist between ATSDR's health assessments and the EPA's risk assessments. The two agencies have distinct purposes that necessitate different goals for their assessments.

A risk assessment is defined as a qualitative and quantitative process conducted by EPA to characterize the nature and magnitude of risks to public health from exposure to hazardous substances, pollutants, or contaminants released from specific sites. Risk assessments include the following components: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Statistical and biological models are used in quantitative risk assessments to calculate numeric estimates of risk to health by using data from epidemiological investigations and animal toxicity studies. The product of quantitative risk assessment is a numeric estimate of the public health consequences of exposure to a chemical. In preparing a risk assessment for a site, a risk assessor also attempts to include all adverse health effects, characterizing the risk to sensitive populations when the information is available. EPA risk assessments are used in risk management decisions to establish cleanup levels; to set permit levels for discharge, storage, or transport of hazardous waste; and to determine allowable levels of contamination.

ATSDR health assessments are based on environmental characterization information, community health concerns, and health outcome data. Because of the nature of these databases, health assessments use quantitative as well as qualitative data, focusing on medical public health and toxicological perspectives associated with exposure to a site. The health assessment specifically addresses community health concerns (e.g., sensitive populations, possible disease outcomes) and evaluates relevant, community-specific health outcome data. Combined with environmental data, information obtained from those two data sources are used to determine the public health implications of the site guiding the initiation of follow-up health activities when indicated.

Contact Details

The ATSDR Information Center
Mailstop E-29, 1600 Clifton Road
Atlanta, GA 30333, USA
Tel.: +1-404-498-0110

See also: Chemical Hazard Communication and Material Safety Data Sheets; Hazard Identification; Hazard Ranking; Risk Assessment, Human Health.

Further Reading

ATSDR (1992) *Public Health Assessment Guidance Manual*. Chelsea, MI: Lewis Publishers.

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Hearing *See* Sensory Organs.

Heat Shock Proteins

Kartik Shankar and Harihara M Mehendale

Heat shock proteins (HSPs) are a family of proteins expressed in almost all organisms from prokaryotes to humans. HSPs were originally described about four decades ago as proteins that were induced in the *Drosophila melanogaster* in response to a heat stress and hence derive the name HSP. However, research over the years has uncovered these proteins to have a multitude of functions. Primarily, all HSPs act as molecular chaperons and assist in proper folding of naïve proteins. Furthermore, HSPs have important roles in cellular processes including cell survival, inflammation, immunity, ion channel repair, and others. HSPs are also induced by a variety of stressors. Reactive oxygen species, cytotoxic injury, necrosis, ultraviolet radiation, metals, and many others are some examples.

HSPs are named according to their molecular mass in kilodaltons. Major classes include small HSPs, HSP40, HSP60, HSP70, HSP90, and HSP110 families. Most HSPs have both a constitutive and inducible form. Members of the small HSPs family include HSP32 or heme oxygenase-1, HSP27, HSP22, and HSP20. Of these HSP32 is most relevant in toxicology as it is induced by diverse physiological stressors, including hypoxia, ischemia–reperfusion, hydrogen peroxide, heavy metals (selenium, arsenite, cadmium), and other toxicants (acetaminophen, carbon tetrachloride). Upregulation of HSP32 has been suggested to be protective against several insults although the mechanisms thereof remain unclear. HSP70 and HSP90 are the two main chaperon systems. HSP70 in the cytosol binds to nascent proteins before they are released from the ribosomes. Through several different complex steps involving other chaperone interacting or organizing protein folding, assembly or disassembly of target proteins is accomplished. HSP40 for example assists in loading the target substrate molecules on to the HSP70 chaperone complex. HSP70 also has a well-studied role in ischemia–reperfusion organ damage and overexpression of HSP70 negatively correlates with infarct size. HSP90 is believed to act as a component of the cycle involving chaperone HSP70. In addition, HSP90, along with HSP70 and 56, bind some nuclear receptor (estrogen receptor) in an inactive complex. Binding of the nuclear receptor ligand cleaves of HSP90 hence allowing nuclear translocation and estrogen responsive gene expression to occur. Elevated levels of HSP90 can turn off estrogen mediated gene expression by destabilizing the receptor–ligand complex.

Cellular control of HSP expression: All HSPs are regulated by a small family of transcription factors called heat shock factor (HSF1–4). During a stress condition, HSF1 and 2 are hyperphosphorylated in a *ras*-dependent manner by MAP kinases. Binding of these active HSF1 factors to DNA sequences called heat shock elements in the promoters of all stress-inducible genes occurs. This leads to increased transcription of HSP genes and induction of HSP proteins.

HSPs as cellular markers of stress: HSPs are involved in various aspects of cellular function and a lot is being learnt about its role in normal and pathological states. Recent studies from lower animals, especially fish, have revealed the potential use of induced fish HSPs as a biomarker of exposure to environmental stressors. Industrial effluents, polycyclic aromatic hydrocarbons, metals such as copper, zinc, mercury, pesticides, etc. have shown to induce HSP in fish. Further, the HSP response may vary with the stressor, tissue, species of fish, and the family of HSP studied. Hence it appears that a more extensive and probably a high-throughput profiling (using genomic and proteomic) approaches may be necessary to identify patterns of HSP modulation by various stressors.

See also: Mechanisms of Toxicity; Oxidative Stress.

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Helium

Mary Lee Hultin

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-59-7
- Synonyms: Helium, compressed; Helium, refrigerated liquid (cryogenic liquid)
- Chemical/Pharmaceutical/Other Class: Nonflammable gas; Simple asphyxiant
- Chemical Formula: He

Uses

Liquid helium is used to produce low temperatures. The inert, nonflammable gas is used in balloons and in scientific studies (e.g., meteorological). It is also used in inert gas shielding for arc welding, in filling light bulbs, as a carrier gas in chromatography, and as a substitute for nitrogen in air supplies for deep diving.

Exposure Routes and Pathways

Exposure is possible through inhalation of the gas or dermal contact with liquid helium.

Toxicokinetics

Helium is an inert gas that acts in the lungs as an asphyxiant by keeping oxygen from reaching the blood.

Mechanism of Toxicity

Helium may displace oxygen, leading to oxygen deficiency.

Acute and Short-Term Toxicity (or Exposure)

Animal

Studies in animals indicate that helium acts as a simple asphyxiant.

Human

Helium is nontoxic at normal temperature and pressure. The primary concern is its ability to displace oxygen in the air. Oxygen content must remain above 19% by volume in order to prevent symptoms of oxygen deficiency. At extremely low temperatures, a clinical case of quick freeze injury to both hands due to helium was reported. The exposed individual was wearing protective gloves which, upon rapid removal after exposure, reduced the depth and severity of the injury. Skin contact with liquid helium can cause frostbite.

Clinical Management

Rescue workers must wear a self-contained breathing apparatus before entering areas of oxygen deficiency. If a victim is unconscious or does not respond, the victim should be moved to fresh air. If breathing has stopped, trained personnel should begin artificial respiration or, if the heart has stopped, cardiopulmonary resuscitation. Oxygen may be administered by a person trained in its use.

Exposure Standards and Guidelines

The Temporary Emergency Exposure Limits (TEEL) are: TEEL-0 = 60 ppb; TEEL-1 = 145 ppb; TEEL-2 = 280 ppb; TEEL-3 = 500 ppb.

The US Department of Energy classifications for TEELs are:

- TEEL-0 is the threshold concentration below which most people will experience no appreciable risk of health effects.
- TEEL-1 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing other than mild transient adverse health effects or perceiving a clearly defined, objectionable odor.
- TEEL-2 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing irreversible or other serious health effects or symptoms that could impair their abilities to take protective action.
- TEEL-3 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing life-threatening health effects.

See also: Respiratory Tract.

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Hemlock, Poison

Michael Wahl

- SYNONYMS: *Conium maculatum*, Umbelliferae family; Spotted hemlock; Deadly hemlock; Poison parsley; Poison stinkweed

Exposure Routes and Pathways

Ingestion of plant parts is the route of exposure. Toxicity may be experienced by those who ingest animals that have fed extensively on poison hemlock (e.g., quail, robins, skylarks, etc.).

Mechanism of Toxicity

Coniine (piperidine alkaloid), *N*-methyl coniine, conhydrine, λ-coniceine, and pseudconhydrine are the toxins identified. Coniine has a number of pharmacological activities resembling nicotine. It is capable of producing stimulation followed by depression of autonomic ganglia.

Acute and Short-Term Toxicity (or Exposure)

Animal

All animal species (except certain small birds) appear to display toxicity similar to that seen in humans, such as muscle tremors, salivation, dyspnea, vomiting, polyuria, central nervous system (CNS) depression, and death.

Human

Poison hemlock toxicity has effects similar to those of nicotine. The alkaloid content varies significantly between species, plant parts, and geographic location. The alkaloid concentration increases in all parts as the plant matures but remains the highest in the roots. Initial CNS stimulation, nausea, vomiting, and sore throat are followed by cardiorespiratory depression and ascending paralysis.

In Vitro Toxicity Data

Binding studies of congeners of cicutoxin have demonstrated a strong correlation between blocking of a noncompetitive GABA agonist to chloride gated GABA channels and acute toxic effects in a mouse cortex model.

Clinical Management

There is no antidote to hemlock poisoning. Death is generally due to paralysis of respiratory muscles. After assessment of airway, breathing, and circulation with necessary supportive care, decontamination of the gastrointestinal tract should be undertaken for substantial recent ingestions. Oxygen and benzodiazepines should be administered as needed for patients experiencing seizures.

See also: Coniine; Nicotine; Toxicology, History of.

Further Reading

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Hemlock, Water

Michael Wahl

- SYNONYMS: *Cicuta maculata*, Apiaceae (carrot) family, *Cicuta* species; Cowbane; Snakeweed; Wild carrot; Poison parsnip; Spotted hemlock; Masquash root; Beaver poison; False parsley; Fever root; Wild parsnip

Exposure Routes and Pathways

Exposure occurs via ingestion of any part of the plant (especially the root). The roots of this plant are sometimes mistaken for wild carrots or wild turnips. These exposures result in large ingestions and can produce profound clinical effects and death.

Mechanism of Toxicity

The major toxicity results from the central nervous system (CNS) stimulant properties of cicutoxin.

Cicutoxin is concentrated in the roots but also may be found in aboveground parts. A mouthful of the root may be sufficient to kill an adult. Death results from status epilepticus, possibly caused by excessive stimulation of cholinergic receptors in the basal ganglia or brain stem.

Acute and Short-Term Toxicity (or Exposure)

Animal

All species of animals are at potential risk of poisoning with symptoms similar to those found in humans.

Human

All parts of the plant are considered toxic, with the root being the most toxic portion. In a typical case of water hemlock poisoning, severe nausea, vomiting, and abdominal pain begin within 5–90 min postngestion. These symptoms are rapidly followed by seizures and profound CNS depression. Excess salivation, diaphoresis, flushing, and dizziness are also commonly seen. The major toxicity is related to CNS stimulation. Death usually results from status epilepticus and respiratory failure.

Clinical Management

A patient exposed to water hemlock may convulse suddenly and without warning; therefore, it is important to establish intravenous access immediately. For recent ingestions activated charcoal and a cathartic should be considered. Seizures generally respond to benzodiazepines. Care should be symptomatic and supportive.

Survivors may experience long-term effects including changes in sensorium with impaired intellectual function and acute anxiety reactions.

See also: Benzodiazepines; Charcoal; Toxicology, History of.

Further Reading

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Hemocompatibility

Kathleen Rodgers

Introduction

During the use of medial devices, there are situations, such as open heart surgery or vascular grafts, in which the device comes into direct contact with the blood. Blood contacting devices include needles, cannulae, blood containers, extracorporeal circuits, and dialysis components. Each of these uses will have differing concern with regards to hemocompatibility. For example, during open heart procedures, patients' blood must be continuously processed using extracorporeal circuits fitted with pumps and suitable active components (e.g., specific filters, oxygenators). This would involve prolonged interaction with patients' blood, with the additional component of flow rate. On the other hand, a needle would reside in the blood strain only transiently, whereas cannulae would be implanted longer. The primary hemocompatibility parameter of concern with a needle would be hemolysis, the destruction of red blood cells as a result of interactions between the needle and the blood. However, with prolonged implantation with the cannulae, there may arise the additional concern of thrombogenicity or clotting.

As a result of the contact of blood with nonendothelial surfaces, several humoral and cellular systems can be activated. Exposure of blood proteins and cells to blood contacting medical devices can activate plasma proteolytic systems (coagulation (blood clotting system), fibrinolysis (process by which clot is broken down), complement cascade (a system of soluble proteins involved in microbiocidal activity and the release of inflammatory components), Kallekrein-kinin and contact systems) and at least three cellular elements (leukocytes, endothelial cells, and platelets). Contrary to the normal situations whereby these mechanisms are localized and intended to promote wound healing, activation of these systems by medical devices can result in nonlocalized systemic reactions. The preclinical and clinical assessments of hemocompatibility are designed to minimize modification of these systems.

Definitions

The general term 'hemocompatibility' refers to those properties that allow medical devices to maintain contact with flowing blood without causing adverse

reactions, without releasing leachable components and without suffering adverse reactions. The properties that define hemocompatibility include inability to initiate thrombogenic phenomena, to cause any hemolysis and to activate the complement system. The standard tests, as will be outlined below, address broad groups of devices and are not device specific. The exception to this is the addition of pump flow rates to the assessment of extracorporeal devices. The hemocompatibility depends not only on the surface characteristics of the device, but also on extrinsic conditions such as site of placement, duration of contact with blood and local hemodynamic status. Blood is a non-Newtonian fluid, composed of a suspension of cells as a significant fraction of the total. This characteristic results in a non-homogenous fluid that will be modified by blood flow and shear. Blood velocity will determine shear rate, the main parameter of the stress to which blood cells are exposed. A reduction in the response of humoral and cellular components to the introduction of the device into the blood flow is paramount to improving hemocompatibility and assuring the safety of the device.

Inflammatory Response

As a result of exposure to a blood contacting medical device, blood may initiate the production of proteolytic substances. This will result in the production of thrombin, plasmin, and proinflammatory complement cleavage products, C3a and C5a. Although the production of proteolytic substances is initiated by proteins present in plasma (humoral phase), its amplification results mainly from the contribution of cellular elements. As mentioned, the cellular reaction of blood to biomaterials is not limited to platelet activation and aggregation. Products of the activation of the humoral phase, for example fibrin degradation products and C3a/C5a, are chemotactic for polymorphonuclear cells and monocytes. This will lead to more cells to be available for interaction when surfaces are wounded by the introduction of the device. These cellular elements will bind to surfaces (both endothelial and non-endothelial) and adhesion of leukocytes will be followed by degranulation and release of factors that further contribute to reduced hemocompatibility. Endothelial cells will also respond to humoral signals to increase leukocyte adhesion (through expression of integrins and selections) and procoagulant activity. These alterations in cellular activity, if modified by the introduction of the device, will result in an inflammatory response that may have consequences beyond those tested by *in vitro* hemocompatibility tests. For example, the assessment of complement activation has been included in guidelines to lessen the potential for dialysis-induced chronic lung disease.

Testing of Hemocompatibility

Testing of hemocompatibility involves the assessment of hemolysis, cell depletion, and generation of thromboemboli. The conditions under which the assessment is to be done can vary with the medical device to be assessed (e.g., extracorporeal blood devices, and vascular grafts). However, the requirement for the test to reflect the proposed use of the device is not extensive. In one example, guidance for extracorporeal blood devices indicates that hemolysis and cell depletion should be evaluated over a 6 h circulation period. In this testing, blood compatibility parameters at both the maximum and low flow rates should be assessed. To evaluate the ability of a surface to affect clotting mechanisms, this test should also include a visual inspection for thromoemboli. The ability of the materials to cause platelet activation should also be assessed. This is in addition to the standard hemocompatibility/hemolysis testing recommended under the US Food and Drug Administration tripartite guidance on ISO 10993. In this guidance, assessment of hemocompatibility is required for many types of medical devices including external communication devices that are indirectly in contact with the blood path or directly in contact with circulating blood. Further, this testing is required for implantable devices in contact with blood. Standard practice for the performance of these tests is available from the American Society for Testing and Materials (ASTM) and ISO Standards.

In ASTM F78-98 'Standard Practice for Selecting Generic Biological Tests Methods for Materials and Devices', the selection test methods to evaluate medical devices is described. Regarding hemocompatibility tests for blood compatibility, hemolysis, and complement activation are described. Under blood compatibility, hemolysis and thrombosis are described as the most obvious examples of incompatibility with blood. It is suggested that thrombogenicity (formation of thromboemboli or platelet activation) be tested under dynamic conditions that simulate in the use procedures for the device. Complement activation is of concern in some cases and should be tested *in vitro* by assessing the status of various complement components. However, complement activation will probably not represent the only portion of the inflammatory response stimulated by medical devices.

Hemolysis procedures are described in ASTM F756-93 'Standard Practice for Assessment of Hemolytic Properties of Materials' and ASTM F1841-97 'Standard Practice for Assessment of Hemolysis in Continuous Flow Blood Pumps'. The presence of

hemolytic material in contact with blood may produce increased levels of free hemoglobin leading to anemia and stress on kidneys and other organs. In F756-93, the hemolytic properties of the material in contact with blood are assessed. In this test, anticoagulated rabbit blood is collected and exposed under static (countertop) or dynamic (rocker plate) conditions, to the medical device. After a proscribed time, the cellular component of the blood is removed and the amount of free hemoglobin determined. In F1841-97, the integrity of red blood cells in human, cow, or pig blood passed through continuous flow blood pumps is assessed. Again, the blood is exposed to standardized blood flow conditions for a proscribed time and the level of hemolysis then determined.

Within the ISO guidelines, there are guidelines that vary with blood contacting devices with specific uses. For example, blood collection sets are covered under ISO 1135-3, 'Blood Taking Set' and ISO 3826-4, 'Plastic Collapsible Containers for Human Blood and Blood Components'. Both documents list requirements for testing of biocompatibility, including cell culture cytotoxicity, short-term intramuscular implantation, hemolysis *in vitro*, delayed contact sensitization, intracutaneous irritation, pyrogenicity, and sterility. Within this, customary measurements for whole blood containers are total hemoglobin, hematocrit, and cell counts. The preferred hemolysis test is a static assay under the conditions of use (21 days of storage at 4–8°C with citrate phosphate dextrose or 42 days with citrate phosphate dextrose adenine solution). Common measurements on containers for red cell concentration are erythrocyte adenosine triphosphate, lactate, and glucose. Red cells may also be assessed microscopically for changes in morphology.

The need for further definition that evaluates medical devices under conditions that reflect the intended use is evident. For example, under the broad grouping of devices, termed externally communicating devices with the same testing recommendations are percutaneous circulatory support devices, extracorporeal oxygenators, and apheresis equipment. However, in clinical use, the potential of these devices to affect blood parameters varies significantly as they are exposed to blood for differing lengths of time, present different risks for air emboli at blood air interfaces and protein denaturation due to foaming. These differences should be assessed during the assessment of the hemocompatibility of blood contacting medical devices.

New Directions in Development of Hemocompatible Materials

Medical devices that can contact blood during their use utilize a broad spectrum of synthetic materials including: polyethylene, polystyrene, polyurethane, silicone, polysulfone, polyamide, polypropylene, polyvinyl chloride, polyester, and polytetrfluoroethylene, etc.

A great deal of research has been conducted into the increase of biocompatibility and hemocompatibility of various polymers, especially those used in blood purification (hemodialysis), blood circulation and implant materials. Several strategies have been attempted to increase hemocompatibility including modification of material surface properties, structure and addition of drugs to the surface of the device. Structure influences the surface area and level of trauma that the blood encounters. Compact materials with smooth surfaces are typically preferred as both the surface area and possible trauma to cellular elements are minimized. On the other hand, smooth surfaces are not practical for all blood-conducting medical devices, for example, vascular grafts. Polymer based artificial grafts are often used in situations where large caliber vascular grafts (internal diameter of 7 mm or greater). These grafts have controlled surface patterns and porosity. Polyester yarn is knitted or woven into various porous patterns. Polytetrafluorethylene tubes are expanded into porous conduits. This porosity is considered to be critical for proper healing and overall graft patency, but causes the blood to leak through the graft wall and is a serious drawback. Currently, collagen, gelatin and albumin are used as sealants. Hydrogels are being considered and will be modified to maximize sealant properties while minimizing complement- and cell-activation by varying other surface properties by degrees and types of substituents.

Surface tension, the residual binding capability of the exposed surface, can affect the hemocompatibility of a material. Blood cells and vessels are negatively charged an isoelectric point between pH 4.8 and 5. The vessel wall being negatively charged causes platelets to be repelled and helps reduce thrombogenic potential. Distribution of charged sites and surface polarity will affect plasma protein absorption to the material. Modification in one area may not solve all issues with regards to hemocompatibility. For example, hydrophilic substituents have a stimulatory effect on the complement cascade, but simultaneously have negligible effect on platelet activation. However, hydrophobic substituents show a reduced complement activation, but stimulate platelet adhesion. Often, blending or mosaics are used to balance hydrophilic/hydrophobic properties to minimize hemocompatibility.

Modification of the surface of a material with a therapeutic can also improve blood compatibility. For example, to reduce thrombogenesis, blood contacting

materials might be heparinized. Numerous clinical studies have compared heparin-coated versus noncoated medical devices. The coating is thought to improve patient safety by reducing the adherence of blood components and by inhibiting blood clotting. Heparin-bonded devices showed lessened humoral and cellular activation, in particular a reduced complement activation and enhanced platelet protection. Clinical trials demonstrated shortened hospital stays, less drainage bleeding and reduced cerebral complications with heparin-coated oxygenated devices. The failure of the oxygenator due to a significantly reduced pressure gradient was also observed when heparin-coated devices were utilized. This surface modification has led to a decrease in healthcare costs and an increase in patient safety due to increased hemocompatibility of this blood-coating device. As this area progresses, further benefit to healthcare will be evident.

Pharmacological inhibition of the key enzymes responsible for the consecutive activation of cascade of reactions, including aprotinin, tranexamic acid, aminocapron acid, C1-esterase inhibitor, antioxidants, and free radical scavengers are also under evaluation to improve hemocompatibility. Research into the modification of surfaces with integrin (receptors that link cellular components to extracellular matrix proteins) fragments, such as peptides with RGD amino acid sequence, has been conducted to increase hemocompatibility and biocompatibility. Further research into the improvement of device function by the local modification of the device surface is ongoing and may prove to be one of the most exciting areas of research in this field.

Conclusions

Contact of blood with medical devices can result in not only hemolysis, but activation of proteolytic, inflammatory, and thrombogenic responses. Assessment and improvement of hemocompatibility are essential to the formation of an ideal blood-contacting medical device. Standard methods are available to measure hemocompatibility at a gross level and more sensitive/thorough techniques are being developed/validated. As these are available, better materials can be developed through direction given by the results from the conduct of these more sensitive assay methods.

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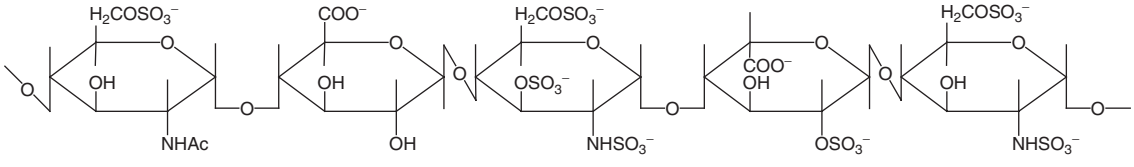
Heparin

David Eldridge and Christopher P Holstege

- SYNONYMS: Heparin sodium (CAS 9041-084); Heparin calcium (CAS 37270-89-6)
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Anticoagulant
- CHEMICAL STRUCTURE:

Uses

Heparin is a heterogeneous mixture of anionic sulfated glycosaminoglycans of 5000–30 000 Da molecular weight commercially derived from bovine lung or porcine intestinal mucosa. Low molecular weight heparins (LMWHs) are specific heparin preparations prepared by chemical or enzymatic cleavage that produce a mixture of products with lower weights of 4000–6000 Da. These mixtures have distinctly different properties from 'unfractionated' heparin.



Heparin is used as an anticoagulant for prophylaxis and treatment of various thromboembolic disease processes. It is used to maintain relatively anticoagulated states in patients on extracorporeal circulation or hemodialysis and to help maintain patency of indwelling vascular catheters.

Exposure Routes and Pathways

Heparin is administered both parenterally and subcutaneously. Oral, rectal, sublingual absorption is poor due to heparin's large size. Intramuscular administration leads to irregular absorption and hematoma development at the site of injection.

Toxicokinetics

Subcutaneous administration leads to a peak heparin level 2–4 h after injection and an onset of anticoagulant effect within 1–2 h. Intravenous administration leads to an immediate peak heparin level with anticoagulant activity within 20–30 min. Heparin binds extensively to a number of plasma proteins. Its volume of distribution is $0.07\,l\,kg^{-1}$ in adults. The pharmacokinetics of heparin is complex and incompletely understood. Heparin metabolism occurs primarily in the reticuloendothelial system by desulfation. The LMWH agents have longer half-lives than standard heparin. Heparin's elimination half-life increases disproportionately with increasing dose, indicating saturable kinetics.

Mechanism of Toxicity

Heparin acts as a catalyst for antithrombin III (AT III), increasing its activity by approximately a thousand times. Antithrombin III is a plasma enzyme that inactivates certain activated serine proteases of the coagulation cascade, most importantly activated factors II (thrombin) and X. The larger heparin species (found in unfractionated heparin) catalyzes the inactivation of activated factors II and X. In contrast, LMWH chiefly inactivates activated factor X. The final effect of both is systemic anticoagulation. Heparin also possesses inherent platelet-aggregating properties and may also induce the production of platelet-aggregating antibodies. Heparin can inhibit aldosterone synthesis.

Acute and Short-Term Toxicity (or Exposure)

Human

Bleeding is the most common complication of heparin therapy and can occur even when dosing is thought to be in therapeutic range. Hemorrhages can occur virtually anywhere and vary in severity from minor to life threatening.

Two forms of heparin-induced thrombocytopenia (HIT) have been observed. The first (HIT I) is a transient, mild, and benign thrombocytopenia seen soon after initiation of heparin therapy (normally within 2 days) and is felt to be due to inherent platelet-aggregating properties of heparin. A second, more severe form of HIT (HIT II) is typically seen later and is immune-mediated. The incidence of HIT II is estimated at 3–5%. The onset is generally 3–14 days after initiation of heparin therapy but may occur sooner with repeat exposure. HIT II may occur with any dose and type of heparin, but the frequency is highest with continuous intravenous infusions of unfractionated heparin. HIT with subsequent thrombosis is a feared complication. These thrombi can form in the venous or arterial circulation. Thrombotic complications include necrotic skin lesions, myocardial infarction, stroke, and gangrene. Hyperkalemia may be seen with heparin therapy due to aldosterone synthesis inhibition.

Chronic Toxicity (or Exposure)

Animal

Heparin is used in veterinary medicine for the management of thromboembolic problems postvascular surgery. It is uncommon for animals to be maintained on heparin for these long term purposes. Heparin is used in a scientific setting in animal dialysis models as well as in models of graft rejection.

Human

Osteoporosis with subsequent rib and vertebral fractures has been reported with long-term use. The mechanism for these abnormalities is not completely understood. Heparin does not cross the placenta and generally is thought to be safe to use in pregnancy.

Clinical Management

The anticoagulant effect of heparin is best monitored by the activated partial thromboplastin time. If an excessive dose of heparin has been administered, careful monitoring for signs of bleeding and hemodynamic instability is indicated. If there is no clinical evidence of bleeding, discontinuation of heparin is normally sufficient. With potentially life-threatening hemorrhage, use of protamine sulfate, a specific, rapidly acting heparin antidote, should be considered. Protamine is given intravenously at a dose of ~ 1 mg per 100 units heparin. Considerable variation can

exist between patients, and dosing should be individualized and monitored. Potential complications of protamine include hypotension, anaphylaxis, and pulmonary vasoconstriction.

Platelet counts should be carefully monitored for any decline. If thrombocytopenia develops, the time course and severity should help differentiate which type of HIT exists. If HIT I is suspected, heparin may be continued with caution. If HIT II is suspected, heparin therapy should be discontinued and an alternate form of anticoagulation therapy begun. If a low platelet count is encountered with a thrombotic complication, heparin should be discontinued immediately. Thrombolytic therapy or embolectomy may be necessary. Lepirudin (recombinant hirudin) is approved by the US Food and Drug Authority for use as an anticoagulant for HIT thromboembolism.

See also: Poisoning Emergencies in Humans; Warfarin.

Further Reading

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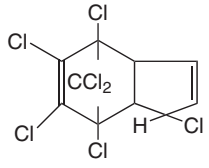
Hepatotoxicology *See* Liver.

Heptachlor

Benny L Blaylock

- Chemical Abstracts Service Registry Number: CAS 76-44-8
- Synonyms: 1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane; Biarbinex; Cupincida; Drinox; E 3314; Fennotox; Heptagran; Heptamul; Heptox; Termide; Velsicol 104
- Chemical/Pharmaceutical/Other Class: Organochlorine cyclodiene insecticide
- Chemical Formula: $C_{10}H_5Cl_7$
- Chemical Structure:



Uses

Heptachlor is used as an insecticide. Its use is banned for most applications.

Exposure Routes and Pathways

The main route of exposure is via ingestion. However, inhalation and dermal contact are also potential exposure routes.

Toxicokinetics

Heptachlor is readily absorbed from the gastrointestinal tract, respiratory tract, and skin. As is the case with other organochlorine insecticides, the rate of absorption from the gastrointestinal tract is affected by fiber and fat content in the diet, with a lack of these favoring increased absorption.

The primary metabolism of heptachlor is in the liver where microsomal enzymes convert the parent compound to the more toxic heptachlor epoxide as well as to the less toxic metabolites 1-chloro-3-hydroxychlordene, 1-hydroxychlordene, and 1-hydroxy-2,3-epoxychlordene. These latter metabolites are also more easily excreted. Both heptachlor and heptachlor epoxide are stored in adipose tissue and in the liver, kidney, and muscle tissues. The epoxide is the primary storage form. Heptachlor is able to cross the placenta and has been found in human milk. Metabolites of heptachlor are excreted in urine and feces.

Mechanism of Toxicity

As with other cyclodiene insecticides, heptachlor blocks the neuronal uptake of chloride ions by blocking the activity of γ-aminobutyric acid. This results in only a partial repolarization of activated neurons leading to an uncontrolled excited condition. Additionally, chlordane inhibits Ca^{2+},Mg^{2+}-adenosine triphosphatase (ATPase) and Na^{+},K^{+}-ATPase functions, leading to increased concentrations of

intracellular free calcium in neurons and the release of neurotransmitters. This neurotransmitter release potentiates depolarization of adjacent neurons in a chain reaction manner, propagating stimuli through the central nervous system (CNS).

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values for rats are reported at 100–220 $mg\,kg^{-1}$ while those for mice are 30–68 $mg\,kg^{-1}$. The toxic effects of heptachlor in animals are the same as those of chlordane. Chlordane toxicity in animals is similar to that of other organochlorine insecticides except tremor is absent. CNS involvement produces hyperexcitability and convulsions.

Human

The toxic effects of heptachlor are the same as those for chlordane. A case report of oral exposure to technical-grade chlordane reported neurological effects including irritability, salivation, dizziness, muscle tremors, and convulsions. However, exposure measurements were not provided in the report, and technical-grade chlordane contains varying amounts of heptachlor. The effects cannot be said to have resulted from exposure to heptachlor only.

Chronic Toxicity (or Exposure)

Animal

Daily oral administrations of 2 or 5 $mg\,kg^{-1}$ body weight heptachlor for 78–86 days to pigs, sheep, and rats induced hepatic necrosis. Results of animal tests show that chronic exposure to heptachlor or its epoxide metabolite adversely affects the liver, kidney, and red blood cells. There is evidence that heptachlor and heptachlor epoxide are associated with infertility and improper development of offspring. Animal studies have shown that females were less likely to become pregnant when both males and females were fed heptachlor. The incidence of liver carcinomas increased in rats receiving doses of approximately 1.2 $mg\,kg^{-1}\,day^{-1}$ of either heptachlor or heptachlor epoxide.

Human

Due to the inconclusive nature of the data, the potential for reproductive effects in humans due to heptachlor is not possible to predict. Also, based on animal data, there is no suggestion that heptachlor is teratogenic in humans.

International Agency for Research on Cancer classifies heptachlor as 2B (possibly carcinogenic for humans). There is inadequate evidence in humans for the carcinogenicity of heptachlor but sufficient evidence in experimental animals for heptachlor carcinogenicity.

Clinical Management

Treatment is symptomatic. Anticonvulsive treatment with diazepam or phenobarbital is usually effective for control of convulsions. Cholestyramine treatment has been shown to increase elimination of heptachlor. Activated charcoal administered as a slurry is recommended. Gastric lavage may be useful if performed quickly after ingestion (within 1 h). Emesis is not recommended due to potential CNS depression or seizures.

Environmental Fate

As with most organochlorine insecticides, heptachlor and its epoxide are highly persistent in soils, with a reported representative field half-life of 250 days. Heptachlor and its epoxide are moderately bound to soils. This should significantly limit their mobility. Due to their persistence, even low mobility may result in appreciable movement. Therefore, heptachlor and heptachlor epoxide may pose a risk of groundwater contamination over time. Heptachlor epoxide exhibits a low susceptibility to biodegradation, photolysis, oxidation, or hydrolysis in the environment.

Due to its insolubility in water, heptachlor enters surface water primarily through run-off and drift. In water, microorganisms readily metabolize heptachlor to the epoxide. The epoxide then undergoes volatilization, adsorption to sediments, and photodegradation. These may be significant routes for disappearance of heptachlor from aquatic environments.

Ecotoxicology

Both heptachlor and the epoxide are very highly toxic to most fish species tested. The reported 96 h LC_{50} values are: 5.3–13 $\mu g\,l^{-1}$ in bluegill sunfish; 7.4–20 $\mu g\,l^{-1}$ in rainbow trout; 6.2 $\mu g\,l^{-1}$ in northern pike; 23 $\mu g\,l^{-1}$ in fathead minnow; and 10 $\mu g\,l^{-1}$ in largemouth bass. Heptachlor is also very highly toxic to freshwater aquatic invertebrates including snails, worms, and crayfish. The toxicity of heptachlor varies significantly from species to species in marine aquatic organisms. Both heptachlor and heptachlor epoxide have been shown to bioconcentrate in fish, mollusks, insects, plankton, and algae.

Heptachlor has been shown to be moderately to highly toxic to several bird species including quail, pheasant, and mallard ducks. Studies have shown that heptachlor decreases the survivability of chicken eggs. Both the parent compound and the epoxide have also been found in the liver, brain, muscle, and eggs of birds.

Other Hazards

Heptachlor is not combustible, but may be dissolved in flammable liquids. Hydrogen chloride fumes gas may form in fire. Heptachlor can react with iron and rust to form hydrogen chloride gas.

Exposure Standards and Guidelines

- Acceptable daily intake is $0.0001\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.
- Maximum contaminant level is $0.0004\,\mathrm{mg\,l^{-1}}$.
- Reference dose is $0.005\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.
- Permissible exposure limit is $0.5\,\mathrm{mg\,m^{-3}}$ (8 h).

See also: Carcinogen Classification Schemes; Charcoal; Chlordane; Diazepam; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Organochlorine Insecticides.

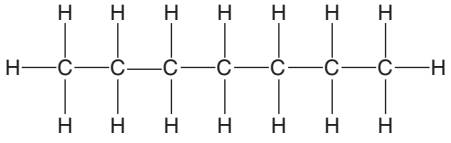
Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Heptachlor.
http://extoxnet.orst.edu – Extension Toxicology Network, a collaborative effort of University of California, Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Heptachlor.
http://www.osha-slc.gov – US Department of Labor, Occupational Safety and Health Administration.

Heptane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 142-82-5
- Synonyms: *n*-Heptane; Dipropyl methane; Gettysolve-C; Heptyl hydride; Heptan (Polish); Eptani (Italian); Heptanen (Dutch); UN1206 (DOT)
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon
- Chemical Formula: C_7H_{16}
- Chemical Structure:



Uses

Heptane is used as the knock-testing 'standard' for octane rating measurements. An isomer, triptane (2,2,3-trimethyl butane), is used in aviation fuel. All isomers are used in organic syntheses and are ingredients of gasoline, rubber solvent naptha, and other petroleum mixtures that are utilized as fuels or solvents.

Exposure Routes and Pathways

Although heptane exists as a liquid at room temperature, most adverse effects observed in people or animals exposed to this solvent occur via inhalation. The most common occupational exposure routes, in order of decreasing importance, are inhalation, dermal contact, and ingestion.

Toxicokinetics

Heptane is converted to hydroxy derivatives (e.g., alcohol) by the cytochrome P450 mixed function oxidase system before being converted to keto forms. It may then be conjugated to the glucuronide and subsequently excreted.

Mechanism of Toxicity

Most of the current toxicological information suggests that heptane is, physiologically speaking, more neurotoxic than other aliphatic hydrocarbons such as pentane, hexane, and octane. However, debilitating peripheral neuropathy, such as that seen on chronic exposure to *n*-hexane, has not been observed in animals or humans. Some cases of polyneuritis, observed in the absence of hexane exposure, might be attributed to the presence of heptane in a solvent mixture. No one to date has discerned a true toxic mechanism for heptane.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rats exposed to heptane showed neurologic signs that are very similar to those seen following exposure

to technical-grade hexane. Righting reflexes in mice are affected at a concentration of 40 mg l^{-1} (~0.96%) while 70 mg l^{-1} (~1.7%) is lethal (isoheptane has the same effect at 50 mg l^{-1}). Narcosis has also been shown in mice exposed to air concentrations of 1–1.5% for 3–50 min. Other permutations of concentration and exposure period caused convulsions, tetany, respiratory arrest, and death.

Human

Heptane is toxic to the human nervous system (neurotoxic). Acute exposure symptoms include distorted perception and mild hallucinations. Humans exposed to 0.1% (1000 ppm) heptane exhibited dizziness in 6 min; higher concentrations caused marked vertigo and incoordination. Humans accidentally exposed to high concentrations showed similar symptoms, as well as mucous membrane irritation, nausea, and lassitude. All these symptoms pass quickly upon recovery in fresh air, but the recovery period is longer than that for pentane or hexane. A gasoline aftertaste has been experienced by people who have been experimentally exposed to heptane.

Chronic Toxicity (or Exposure)

Human

Several authors have noted signs of polyneuropathy or polyneuritis in groups of people exposed to mixtures of solvents that contain significant quantities of heptane.

Clinical Management

People who are exposed to high concentrations should vacate or be removed from the source of the vapor and seek fresh air.

Ecotoxicology

The lowest 24 h LC_{50} reported for hexane was 10 mg l^{-1} using the water flea (*Daphnia magna*) as the test species (US Environmental Protection Agency ECOTOX database). An unpublished 48 h EC_{50} of 1.5 mg l^{-1} was also observed (immobilization) in the same invertebrate species. A 24 h LC_{50} of 4 mg l^{-1} has been reported for goldfish, and a 96 h LC_{50} of 100 mg l^{-1} was reported for Coho salmon.

Other Hazards

Heptane is very flammable and is therefore an explosion and/or fire hazard (lower and upper explosive limits are 1.05% and 6.7%, respectively, by volume). Care should be taken to keep areas of high concentration free from ignition sources, such as sparks from static electricity, and explosion-proof equipment should be used.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit values for heptane are 400 ppm as a time-weighted average (TWA) and 500 ppm as a short-term exposure limit. The National Institute for Occupational Safety and Health recommends a workplace environmental limit (TWA) of 85 ppm for heptane, and a 15 min ceiling limit of 440 ppm. On a weight/volume scale, this is the same limit imposed for pentane, hexane, and octane and is most likely designated to prevent polyneuropathy found following heptane exposure.

Miscellaneous

Heptane is a colorless, flammable liquid that is lighter than, but insoluble in, water. It has a definite petroleum odor that is easily detected at air concentrations of 200 ppm or greater (in air, 1 ppm = 4.10 mg m^{-3}). Naturally occurring heptane is isolated from natural gas, crude oil, or pine extracts.

See also: Neurotoxicity.

Further Reading

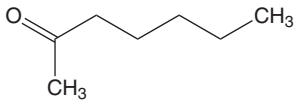
Mackison FW, Stricoff RS, and Partridge LJ Jr (eds.) (1981) *NIOSH/OSHA – Occupational Health Guidelines for Chemical Hazards*. DHHS(NIOSH) Publication No. 81-123. Washington, DC: US Government Printing Office.

Verschueren K (1996) Handbook of Environmental Data on Organic Chemicals. 3rd edn. New York: Van Nostrand Reinhold.

Heptanone

Murali Badanthadka and Harihara M Mehendale

- Chemical Name: 2-Heptanone
- Chemical Abstracts Service Registry Number: CAS 110-43-0
- Synonyms: Methyl *n*-amyl ketone; *n*-Pentyl methyl ketone; Methyl pentyl ketone
- Chemical/Pharmaceutical/Other Class: Ketone
- Chemical Formula: $C_7H_{14}O$
- Chemical Structure:



Uses

2-Heptanone is used as an industrial solvent; as a solvent for synthetic resin finishes; as an inert reaction medium; as a flavor ingredient in foods; and as a fragrance ingredient in creams, lotions, perfumes, soaps, and detergents.

Background Information

2-Heptanone is a naturally occurring compound present in foods and essential oils. It is also used commercially as a solvent in a wide number of industrial applications. 2-Heptanone may be released to the environment as a fugitive emission during its production, formulation, use, or transport, and in the effluent of industrial processes.

Exposure Routes and Pathways

Occupational exposure to 2-heptanone may occur by inhalation or dermal contact during its production, formulation, or transport. Exposure to the general population may occur by ingestion of food in which it occurs naturally, by inhalation during the use of commercial products in which it is used as a solvent or by the ingestion of contaminated drinking water. The principal route of occupational exposure is by inhalation. Skin and eye contact may also occur.

Toxicokinetics

2-Heptanone is absorbed into the bloodstream after ingestion, inhalation, or dermal exposure. Results of tissue distribution studies of ^{14}C-methyl *n*-amyl ketone in rats comparing intraperitoneal and inhalation routes of exposure were similar. Liver tissue had the highest level of radioactivity regardless of the route of administration. However, no liver pathology was evident. Urinary excretion accounted for 25% of the administered dose after 12 h.

When 2-heptanone (950 mg kg^{-1}) was administered orally to rabbits, ~40% of administered dose was excreted as heptyl-2-glucuronide, and traces of the unchanged ketone were also found in the urine. Compound undergoes carbonyl reduction to a secondary alcohol and ω-1 oxidation to a hydroxyketone which is further oxidized to 2,6-heptadione.

A subchronic inhalation study was conducted in which male rats and monkeys were exposed to 0, 131, or 1025 ppm of 2-heptanone for 10 months (6 h day^{-1}, 5 days week^{-1}). Both parent compound and its metabolite methyl *n*-amyl alcohol were detected in the urine and serum of monkeys.

Mechanism of Toxicity

2-Heptanone is known to potentiate the nephrotoxic and hepatotoxic effects of halogenated hydrocarbons.

Acute and Short-Term Toxicity (or Exposure)

Animal

Guinea pigs exposed to 2-heptanone at 2000 ppm for 890 min caused light to moderate congestion of lungs leading to death. Exposure at 1500 ppm caused irritation to mucous membranes. At 4800 ppm concentration, central nervous system (CNS) depression occurred, followed by death in 4–8 h.

2-Heptanone in the undiluted form, ranging in quantities from 5 to 20 ml kg^{-1}, caused slight to moderate skin irritation in guinea pigs after 24 h exposure. No evidence of percutaneous absorption and death.

Male rats and monkeys exposed to subchronic inhalation of 0, 131, or 1025 ppm 2-heptanone for 10 months (6 h day^{-1}, 5 days week^{-1}) did not show any significant alterations in pulmonary function, electrocardiogram, or biochemical parameters.

Oral administration LD_{50} value is 1670 and 730 mg kg^{-1} for rat and mice, respectively.

Human

2-Heptanone may cause mild skin irritation after a single exposure. At concentration of 4% in petrolatum it did not produce any positive reactions.

Inhalation at higher concentration causes CNS depression. Vapor/liquid contact will irritate eyes, nose, throat, and skin. Ketones may potentiate the hepatotoxicity of halogenated hydrocarbons and inhibit aromatic hydrocarbon metabolism.

Chronic Toxicity (or Exposure)

Animal

Oral administration ($20\,mg\,kg^{-1}\,day^{-1}$) for 13 weeks showed ketone bodies in urine of only male rats. Administration at $500\,mg\,kg^{-1}\,day^{-1}$ caused increase of liver weight in both genders and kidney weight only in males, indicating a gender difference in 2-heptanone toxicity.

In Vitro Toxicity Data

A study reported that 2-heptanone binds to DNA spontaneously *in vitro*, to the extent of $400\,pmol\,mg^{-1}$ DNA.

Clinical Management

Exposed individuals should be removed immediately to fresh air after inhalation. Copious dilution is appropriate after ingestion, dermal exposures, or eye exposures. Patients should be treated symptomatically.

Environmental Fate

Terrestrial fate: If released to soil, calculated soil adsorption coefficients ranging from 44 to 285 indicates that 2-heptanone may display moderate to high mobility and it has the potential to leach into groundwater. 2-Heptanone has the potential to biodegrade in soil. The vapor pressure of 2-heptanone is 3.86 mmHg at 25°C.

Aquatic fate: If released to water, 2-heptanone is expected to rapidly volatalize to the atmosphere. The half-life for volatilization from a model river 1 m deep, flowing at $1\,m\,s^{-1}$ with a wind speed of $3\,m\,s^{-1}$ is 8.4 h. The calculated bioconcentration factors ranging from 5.5 to 19 indicate that 2-heptanone is not expected to bioconcentrate in fish and aquatic organisms. The calculated soil adsorption coefficients ranging from 44 to 285 indicate that adsorption to sediment and suspended organic matter is not an environmentally important process. Screening studies indicate that 2-heptanone is likely to biodegrade in aquatic systems under aerobic conditions.

Atmospheric fate: If released to the atmosphere, 2-heptanone is expected to undergo a gas-phase reaction with photochemically produced hydroxyl radicals; the estimated half-life for this process is 1.9 days. 2-Heptanone has relatively high water solubility ($4300\,mg\,l^{-1}$ at 25°C), which indicates that it may undergo atmospheric removal by wet deposition processes. Although 2-heptanone has the potential of being removed from the atmosphere by direct photochemical degradation, the rate of this process is not expected to be able to compete with atmospheric removal by the reaction with hydroxyl radicals.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 100 ppm ($465\,mg\,m^{-3}$) for 8 h time-weighted average (TWA). The threshold limit value is 50 ppm for 8 h TWA. The National Institute for Occupational Safety and Health recommended exposure limit is 100 ppm ($465\,mg\,m^{-3}$) for 10 h TWA.

Further Reading

Albro PW, Corbett JT, and Schroeder JL (1984) Metabolism of methyl *n*-amyl ketone (2-heptanone) and its binding to DNA of rat liver *in vivo* and *in vitro*. *Chemico-biological Interactions* 51: 295–308.

Hewitt WR and Brown EM (1984) Nephrotoxic interactions between ketonic solvents and halogenated aliphatic chemicals. *Fundamentals of Applied Toxicology* 4: 902–908.

Lyman, WJ, *et al.* (1982) *Handbook of Chemical Property Estimation Methods*, pp. 4–33, 1–29. New York: McGraw-Hill.

Lynch DW, Lewis TR, Moorman WJ, *et al.* (1981) Inhalation toxicity of methyl *n*-amyl ketone (2-heptanone) in rats and monkeys. *Toxicology and Applied Pharmacology* 58: 341–352.

Perbellini L, Brugnone F, Cocheo V, De Rosa E, and Bartolucci GB (1986) Identification of the *n*-heptane metabolites in rat and human urine. *Archives of Toxicology* 58: 229–234.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Heptanone.

Herbicides *See* Chlorophenoxy Herbicides.

hERG (Human Ether-a-Go-Go Related Gene)

Jill Steidl

Introduction

Cardiac muscle contraction is an electrical event initiated at the sinoatrial node. Each cardiac muscle cell fires an action potential as a result of excitation propagated from the sinoatrial node, which produces muscle cell contraction. A wave of action potentials spreads across the organ to produce coordinated contraction of the heart and efficient ejection of blood to the body. Excitation and the subsequent return of a cardiac muscle cell to rest (repolarization) during the action potential is dictated by the flow of ions across the cell membrane. Membrane repolarization is produced by the flow of potassium ions through various types of potassium channels.

hERG (human ether-a-go-go related gene; KCNH2) encodes for the ion channel that underlies the rapidly activating delayed rectifier potassium current, I_{Kr}. The *hERG* current (I_{Kr}) is critical for ventricular repolarization in humans. Inhibition of *hERG* currents can induce QT prolongation, which is associated with induction of the potentially fatal ventricular arrhythmia Torsade de Pointes. A wide range of pharmaceutical agents from a variety of chemical classes have been found to inhibit *hERG* currents and produce QT prolongation and/or Torsade de Pointes, resulting in labeling revisions or withdrawal from the market.

Expression of *hERG* Channels

hERG is primarily expressed in human heart, and to a minor extent in hippocampus. In human heart, *hERG* expression levels are highest in the ventricle. *hERG* cardiac expression varies with species, for example, ERG protein levels are higher in rat atria than in the ventricle.

Structure of *hERG* Channels

Ion channels are proteins that span the plasma membrane to allow passage of charged ions into and out of the cell. Four *hERG* subunits coassemble to form an ion channel selective for potassium. Each subunit has six membrane spanning regions (S1–S6) and an intracellular amino and carboxy terminus (**Figure 1a**). An additional hydrophobic region between S5 and S6 dips into the plane of the membrane to contribute to the formation of a central ion channel pore. The S4 α-helix of each subunit is characterized by the presence of positively charged amino acids (arginine or lysine) at every third or fourth position, which are thought to act as voltage sensors and modulate ion channel state.

Function of *hERG* Channels

hERG channels are modulated by membrane potential, and can exist in the closed (C), open (O), or inactivated (I) state (**Figure 1b**). *hERG* channels conduct potassium ions when they are in the open state, but not in the closed or inactivated states. When the membrane potential is hyperpolarized (the interior of the cell is negative in comparison to the outside of the cell), *hERG* channels primarily exist in the closed state. Upon depolarization of the membrane potential (less negative inside the cell), *hERG* channels transition to the open state and then undergo inactivation. Activation (C→O) and deactivation (O→C) are much slower than inactivation (O→I) and recovery from inactivation (I→O). These unique kinetics facilitate late phase cardiac action potential repolarization by *hERG* currents, and suppress premature cardiac beats.

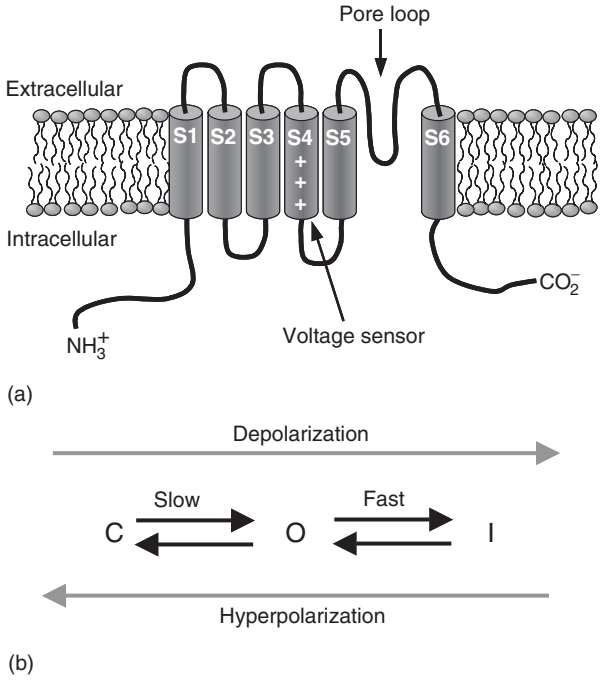


Figure 1 (a) The transmembrane topology of a *hERG* potassium channel subunit is depicted. (b) *hERG* potassium channel state is dependent on membrane potential. Depolarization favors the open (O) and inactivated (I) states, while hyperpolarization induces channel closing (C).

Role of *hERG* Ion Currents in the Cardiac Action Potential

The morphology of an action potential is dictated by the flow of ions across the cell membrane (**Figure 2a**). An inward flow of sodium and calcium ions has a depolarizing influence on the membrane potential, while an outward flow of potassium has a repolarizing effect. The upstroke of the cardiac action potential (phase 0) is due to an inward flux of sodium ions and the plateau phase (phase 2) is maintained by inward calcium currents and outward potassium currents. During the initial phases of the action potential (0 through 2), *hERG* channels open slowly followed by rapid inactivation, resulting in an accumulation of nonconducting inactivated channels by the end of phase 2. As calcium channels inactivate and the membrane potential begins to repolarize in early phase 3, inactivated *hERG* channels rapidly transition from the inactivated to the open state, creating a large outward potassium current that facilitates action potential repolarization. Slow transition of *hERG* channels from the open to the closed state suppresses the propagation of premature beats that may be encountered by the myocyte. Inhibition of *hERG* currents by pharmaceutical agents can delay repolarization of the cardiac action potential, which appears as a prolongation of the QT interval on an electrocardiogram. Delayed repolarization may result in the formation of calcium dependent early after depolarizations (EADs; **Figure 2b**), waveforms believed to trigger initiation of Torsade de Pointes.

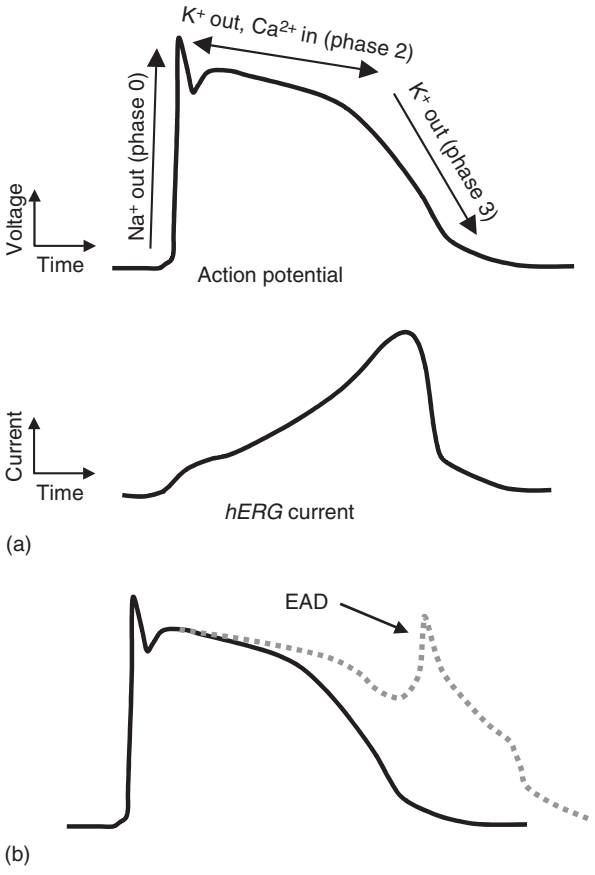


Figure 2 (a) A schematic depiction of a ventricular action potential (top) and relative profile of *hERG* current during such an action potential (bottom). The amplitude of the *hERG* current is small during the early phases of the action potential because channels open followed by rapid transition into a nonconducting inactivated state. In phase 3, *hERG* channels rapidly shift from the inactivated to the open state to facilitate repolarization of the action potential. (b) Inhibition of the *hERG* current can result in prolongation of the action potential duration and may initiate early after depolarizations (EADs).

Assessment of *hERG* Risk

Identification of risk for *hERG* inhibition is an important factor in the development of drugs. Potential for *hERG* inhibition can be assessed using a number of *in vitro* techniques, but the gold standard is whole cell patch clamp. Suitable cell types include isolated animal or human cardiomyocytes, cultured cardiac cell lines, or a heterologous expression system in which *hERG* is expressed in a non-cardiac cell line. Other high throughput techniques such as radioligand binding, rubidium flux or fluorescence may be used for early assessment of *hERG* activity; however, data produced by these techniques is typically not accurate enough for establishing safety margins.

See also: Cardiovascular System.

Further Reading

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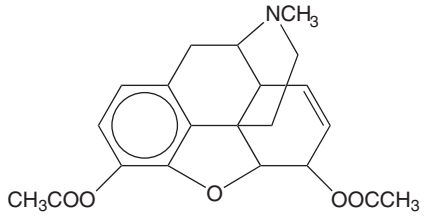
Heroin

Michael Hiotis

This article is a revision of the previous print edition article by Lisa Scheuring-Mroz, volume 2, pp. 79–81, © 1998, Elsevier Inc.

- Representative Chemical: Morphine
- Chemical Abstracts Service Registry Number: CAS 561-27-3
- Synonyms: Acetomorphine; Diacetylmorphine hydrochloride; Diamorphine hydrochloride; Heroin hydrochloride
- Chemical/Pharmaceutical/Other Class: Opioid analgesic
- Chemical Formula: $C_{21}H_{23}NO_5$
- Chemical Structure:



Uses

Heroin is a semisynthetic narcotic that was first synthesized in 1874. It has been used as an analgesic for moderate to severe pain. In the United States, it is a schedule I substance and, therefore, does not have a medicinal use. It is a drug of abuse.

Exposure Routes and Pathways

Administration can be parenteral, sublinqual, oral, rectal, or by nasal insufflation. As a common drug of abuse, heroin is usually present in the street product at concentrations of 2–7%; purer forms of up to 90% are occasionally available.

Toxicokinetics

Heroin is rapidly absorbed from all sites of administration. It has high lipid membrane solubility, thus leading to rapid absorption from the blood and the blood–brain barrier. Heroin undergoes complete presystemic metabolism to morphine following oral administration. Peak morphine serum levels have occurred within 30 min after ingestion. With parenteral administration, peak levels have occurred in 10–15 min. Heroin is rapidly hydrolyzed in whole blood to 6-monoacetylmorphine (6-MAM). The liver then converts most of the 6-MAM to morphine. These two metabolites are the primary contributors to pain relief. It is widely distributed in tissues. Protein binding is 40%. The volume of distribution approximates $25\,l\,kg^{-1}$. The half-life of heroin in blood is less than 20 min, ~3 min after parenteral administration. The elimination half-life is 60–90 min. Urine yields primarily morphine in the free or conjugated form.

Mechanism of Toxicity

Heroin's primary toxic principle is its profound ability to depress the central nervous system (CNS). Opioid analgesics bind with stereospecific receptors at many sites within the CNS. Heroin, similar to other opioids, exerts its pharmacologic effect by acting at mu, kappa, and delta receptors in the brain. Although the precise sites and mechanisms of action have not been fully determined, alterations in the release of various neurotransmitters from afferent nerves sensitive to painful stimuli may be partially responsible for the analgesic effect. Activities associated with the stimulation of opiate receptors are analgesia, euphoria, respiratory depression, miosis, and reduced gastrointestinal motility.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs respond similarly to humans exposed to heroin. Symptoms may include drowsiness, ataxia, vomiting, respiratory depression, miosis, coma, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone may be used at $0.02\,mg\,kg^{-1}$ if needed.

Human

Any amount of heroin can be potentially toxic, especially when the purity of this illicit drug is not known. Heroin depresses the CNS, thereby producing coma and respiratory depression. Pulmonary edema has been described following heroin overdose. Respiratory arrest may occur. Miosis is often present but may be absent in the presence of hypoxia or mixed drug over doses. With depression of the CNS, there is also a decrease in sympathetic tone and an increase in parasympathetic tone. This yields bradycardia and hypotension. Hypothermia may also occur as a result of peripheral vasodilation. Urine can be screened for heroin metabolic products. Blood heroin levels are not clinically useful.

Chronic Toxicity (or Exposure)

Animal

Rats administered heroin chronically demonstrated decreased germinal epithelial thickening. It has also been reported that chronic heroin dosing of rodents disrupts estrous cycles.

Human

Chronic users of heroin may develop tolerance to some of its effects, thereby necessitating larger doses to develop the characteristic 'high'. Cessation of use can result in withdrawal. Classic symptoms are restlessness, insomnia, agitation, hypertension, tachypnea, tachycardia, piloerection, vomiting, and diarrhea.

In Vitro Toxicity Data

Recent studies in rat brains have investigated the role of chronic heroin and other narcotics on apoptosis. Chronic heroin and morphine treatment (as well as heroin/morphine withdrawal) resulted in changes in upregulation of Fas receptors as well as increased levels of dynamin.

Clinical Management

Basic life-support measures should be instituted as necessary. Intensive support therapy may be required to correct respiratory failure and shock. Patients with mild to moderate toxicity may present with lethargy, miosis, decreased blood pressure, heart rate, temperature, and skeletal muscle tone. In patients experiencing severe toxicity, coma, respiratory depression, noncardiogenic pulmonary edema, apnea, and sudden death may occur. If taken orally, administration of activated charcoal is recommended to minimize absorption of heroin. Emesis is contraindicated due to potential significant CNS and respiratory depression. Heroin is often smuggled via 'body packing', whereby an individual swallows receptacles (often condoms) containing heroin to evade customs officials. Most, but not all package types can be visualized on X-ray and flat plate X-ray should be performed to establish diagnosis and location. Whole bowel irrigation (WBI) may be a useful way to facilitate their removal from the gastrointestinal tract. WBI should be continued until rectal effluent is clear and no packets are detected on a contrast study of the bowel. The specific antagonist naloxone hydrochloride is used to counteract respiratory depression and coma. A dose of 0.4–2.0 mg is given intravenously and can be repeated at intervals of 2 or 3 min if necessary. The therapeutic effect of naloxone may be of shorter duration than that of the opiate activity; therefore, a naloxone continuous infusion may be of benefit. Arterial blood gases, vital signs, and level of consciousness should be monitored continuously until cessation of symptoms. Adulteration of street drugs can lead to other toxic effects and should be considered in the overall management.

See also: Drugs of Abuse; Morphine.

Further Reading

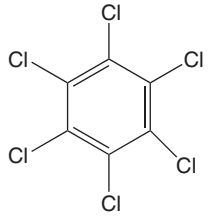
Sporer KA (1999) Acute heroin overdose. *Annals of Internal Medicine* 130: 584–590.

Warner-Smith M, Darke S, and Lynskey M (2001) Heroin overdose: Causes and consequences (review). *Addiction* 96: 1113–1125.

Hexachlorobenzene

Elmar Udarbe Zamora

- Chemical Abstract Service Registry Number: CAS 118-74-1
- Synonyms: HCB; Perchlorobenzene; Pentachlorophenyl chloride; Benzene, hexachloro-; Esaclorobenzene (Italian); Hexachlorobenzol (German); Julin's carbon chloride; Phenyl perchloryl
- Chemical/Pharmaceutical/Other Class: Organochlorine
- Chemical Formula: C_6Cl_6
- Chemical Structure:



Uses

Hexachlorobenzene is a white crystalline solid that is poorly soluble in water. It does not occur naturally in

the environment. It was widely used until 1965 to protect seeds of sorghum, onions, wheat, and other grains. It was also used to make fireworks, ammunition, and synthetic rubber. Currently, hexachlorobenzene is not used commercially in the United States. However, hexachlorobenzene is still produced as a by-product in the manufacture of chlorinated compounds and other chemicals, and in the waste streams of chloralkali and wood preserving plants. Burning of municipal waste also produces hexachlorobenzene.

Background Information

Hexachlorobenzene has been found in at least 84 of the 1430 National Priorities List sites identified by the US Environmental Protection Agency (EPA). The long-range atmospheric transport of hexachlorobenzene to the Arctic and other areas is a well-recognized phenomenon. The substance has been detected in Arctic air, snow, seawater, and flora and fauna. It had also been observed in other remote areas such as the North Pacific Ocean and in the rainfall of two remote islands on Lake Superior. Approximately 4000 persons were poisoned by hexachlorobenzene in Turkey from 1955 to 1959.

Exposure Routes and Pathways

Exposure to hexachlorobenzene occurs primarily from eating low levels in contaminated food, water, fish, and vegetables. Unborn children could also be exposed *in utero* and nursing babies may be exposed from mothers. Direct contact with contaminated soil is also another possible route of exposure. Workers involved in the manufacture or application of hexachlorobenzene also run the risk of exposure through inhalation.

Toxicokinetics

Hexachlorobenzene is slightly absorbed in the gastrointestinal tract but readily distributed in the body, preferentially to fatty tissues. It also readily passes the placenta. Hexachlorobenzene is concentrated in milk. It is metabolized by microsomal enzymes in the liver, kidney, lung, and intestine. Hexachlorobenzene is metabolized slowly by the liver to pentachlorophenol, pentachlorobenzene, tetrachlorobenzene, and some unidentified compounds. In humans, hexachlorobenzene is mainly excreted in the urine as its metabolites, pentachlorophenol and pentachlorothiophenol. In animals, orally absorbed hexachlorobenzene is excreted, mostly unchanged, in the feces.

Mechanism of Toxicity

Hexachlorobenzene affects porphyrin synthesis and consequently the proteins involved in the metabolism and transport of oxygen. Its main target organ is the liver.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hexachlorobenzene has little acute toxicity. Oral LD_{50} values range from 1.7 to $4\,g\,kg^{-1}$ in various species. The primary effect from eating highly contaminated food is hepatotoxicity. Animals exposed to hexachlorobenzene also exhibited acute neurologic toxicity with signs including tremors, paralysis, incoordination, weakness, and convulsions. The ovarian primordial germ cells of nonhuman primates were affected with associated systemic toxicity when exposed to hexachlorobenzene.

Human

Hexachlorobenzene is a skin irritant. In contrast to rodents, humans do not exhibit neurological signs with acute exposure to hexachlorobenzene.

Chronic Toxicity (or Exposure)

Animal

Long-term exposure to hexachlorobenzene caused damage to the liver, thyroid, nervous system, bones, kidneys, blood, and immune and endocrine systems in animals.

Human

The people in Turkey who ate bread contaminated with hexachlorobenzene suffered from a liver disease called porphyria cutanea tarda. This disease can cause red-colored urine, skin sores, changes in skin color, arthritis, and problems of the liver, nervous system, and stomach.

There is no strong evidence that it causes cancer. Babies born from mothers exposed to hexachlorobenzene during pregnancy showed acute illnesses and rashes. Babies nursing from exposed mothers showed porphyria cutanea tarda, poor growth, arthritis, and enlarged thyroids.

In Vitro Toxicity Data

The metabolites of hexachlorobenzene, penta chlorophenol (PCP) and tetrachlorohydroquinone (TCHQ), appear to be capable of altering porphyrin metabolism in *in vitro* systems containing D-ALA

(aminolevulinic acid). In another study, PCP displaced the thyroid hormone, thyroxine (T4), from its receptor by direct competition. This suggests the mechanism involved in hexachlorobenzene induced hypothyroidism in rats. In rat ovary perfused with hexachlorobenzene, increased oxygen consumption suggests a disorder in the respiratory metabolism of ovarian cells after hexachlorobenzene exposure. Hexachlorobenzene was negative in the Ames mutagenicity assay.

Clinical Management

The airway, breathing, and circulation should be monitored and vital functions restored if necessary. All contaminated clothes should be removed. The chemical should be washed out of the eyes with clear water for at least 15 min, and off the skin with soap and water. If hexachlorobenzene has been ingested, milk, fat, oil, or lipid should not be given by mouth. If a very large amount of hexachlorobenzene has been ingested, gastric lavage should be performed. Activated charcoal can be administered. Convulsions should be controlled and treated like any other symptoms.

Environmental Fate

Hexachlorobenzene is degraded slowly and can therefore persist in the environment for long periods of time. It strongly adheres to soil and poorly dissolves in water, so residues can remain in sediments of lakes and rivers.

Ecotoxicology

There is high potential that it can bioaccumulate in bodies of fish, marine mammals, birds, lichens, and other animals and can enter the food chain. It can also accumulate in wheat, grasses, some vegetables, and other plants.

Other Hazards

Hexachlorobenzene could burn when exposed to extreme heat and toxic fumes may be produced. It should not be stored near sources of ignition. In cases of spills, hexachlorobenzene should be taken up with sand or other noncombustible material and then disposed off properly. For large-scale spills, it should be covered with sand/soil taking care to avoid dust formation. Protective clothing, gloves, and eyewear must be worn in handling spill situations.

Exposure Standards and Guidelines

The US EPA has set a maximum contaminant level of 1 ppb.

See also: Organochlorine Insecticides.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hexachlorobenzene.

Hexachlorobutadiene

David Janz

- Chemical Abstracts Service Registry Number: CAS 87-68-3
- Synonyms: Hexachloro-1,3-butadiene; Hexachlorobuta-1,3-diene (HCBD); Perchlorobutadiene
- Chemical/Pharmaceutical/Other Class: Chlorinated hydrocarbon
- Chemical Structure: $CCl_2 = CCl–CCl = CCl_2$

Uses

Hexachlorobutadiene is an industrial by-product of tetrachloroethylene, trichloroethylene, and perchloroethylene production and is used as a solvent for elastomers, heat transfer liquids, transformer fluids, and hydraulic fluids. Hexachlorobutadiene may still be used in certain countries as a fumigant. It is also released during refuse combustion and is found in fly ash.

Exposure Routes and Pathways

Hexachlorobutadiene may be toxic by inhalation, ingestion, and dermal exposures. Occupational

exposure may occur through inhalation or dermal contact. The general population may be exposed via inhalation of ambient air and ingestion of food or water-containing hexachlorobutadiene.

Toxicokinetics

In rabbits, hexachlorobutadiene is absorbed through the skin. In rats, hexachlorobutadiene was found in lungs, blood, liver, brain, kidneys (proximal section of the nephron), spleen, and mesentery after a single injection (unspecified). Glutathione conjugation is the main route of biotransformation in mammals, followed by biliary excretion. Following oral administration of a nephrotoxic dose ($200\,mg\,kg^{-1}$) of hexachloro-1,3-butadiene to male rats, the principal route of excretion was biliary, with 17–20% of the dose being eliminated on each of the first 2 days. Fecal excretion was $<5\%$ of the dose per day, suggesting enterohepatic recirculation of biliary metabolites. Urinary excretion was small, not exceeding 3.5% of the dose during any 24 h period.

Mechanism of Toxicity

Hexachlorobutadiene specifically damages the pars recta portion of the proximal tubule with loss of the brush border. The mechanism involves nonoxidative formation of the glutathione conjugate in liver with subsequent transport to the kidney for mercapturic acid conjugate processing. The resulting cysteine conjugates are substrates for cysteine-conjugate β-lyase, which removes ammonia and pyruvate from the cysteine conjugate to produce thionylacyl halides and thioketenes. These toxic thiol compounds can then bind covalently to proteins and DNA in proximal tubular cells to produce nephrotoxicity. Mitochondrial dysfunction is reported to be the ultimate subcellular toxic lesion. Enterohepatic recirculation of hexachlorobutadiene–glutathione conjugates is believed to play a major role in this mechanism, since cannulation of the bile duct of rats prevents nephrotoxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} (single dose) is $90\,mg\,kg^{-1}$ in guinea pigs, 87–$116\,mg\,kg^{-1}$ in mice, and 200–$350\,mg\,kg^{-1}$ in rats. The dermal LD_{50} at 7 h was $126\,mg\,kg^{-1}$ in rabbits.

Hexachlorobutadiene causes lung, liver, and renal injury (renal proximal tubular dysfunction) in animals. Hexachlorobutadiene and its metabolites were reported to be approximately four times more nephrotoxic to female rats than to males. Eye and nose irritation have been reported in animals exposed to 250 ppm for 4 h and 110 ppm for 6 h.

At dosages high enough to cause maternal toxicity (decreased weight gain) and slight fetal toxicity (decreased fetal weight) hexachlorobutadiene was not teratogenic.

Unscheduled DNA synthesis has occurred in experimental animals. Mutations have been produced in *Salmonella typhimurium* and sister chromatid exchange has occurred in the hamster ovary cell.

Human

Little information is available on the acute toxicity of hexachlorobutadiene in humans. Recent physiologically based pharmacokinetic models suggest an order of magnitude lower activation of reactive nephrotoxic metabolics in humans compared to rats.

Chronic Toxicity (or Exposure)

Animal

In a 30 day study in rats, 30, 65, and 100 $mg\,kg^{-1}\,day^{-1}$ resulted in renal toxicity, increased kidney–body weight ratio, and renal tubular degeneration, necrosis, and regeneration; $100\,mg\,kg^{-1}\,day^{-1}$ resulted in decreased food consumption and body weight and minimal hepatocellular swelling at $100\,mg\,kg^{-1}$; and 10, 30, 65, and $100\,mg\,kg^{-1}\,day^{-1}$ resulted in hemoconcentration. A statistically significant increase of kidney tumors was observed in male and female rats fed diets containing hexachlorobutadiene (99% pure) at $20\,mg\,kg^{-1}$ body weight per day for 22 months.

Human

Hexachlorobutadiene is on the National Institute for Occupational Safety and Health list of suspected carcinogens because it has the potential to cause kidney and lung cancer. A group of 205 vineyard workers who were exposed seasonally to hexachlorobutadiene (0.8–$30\,mg\,m^{-3}$ in air over the fumigated zones) showed multiple toxic effects contributing to the development of hypotension, cardiac disease, chronic bronchitis, disturbances of nervous function, and chronic hepatitis.

The following combination of tests could be useful for detecting renal dysfunction in occupationally exposed workers: examination of urine with reagent strips for the presence of glucosuria and proteinuria and quantitative determination of at least two proteins, one of the high molecular weight for

glomerular function and one of the low molecular weight for tubular function. Some value has also come from the determination of the lysosomal enzyme *N*-acetyl-β-D-glucosaminidase in urine.

Clinical Management

No specific treatment is available. Patients acutely and chronically exposed should be monitored for renal, hepatic, and pulmonary damages. At least some of the renal toxicity appears to be reversible, so supportive care is indicated. Emesis may be indicated and is most effective if initiated within 30 min of ingestion. Charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, should be administered. A saline cathartic should be administered, unless sorbitol–charcoal slurry is used.

In cases of inhalation exposure, the victim should be moved to fresh air and monitored for respiratory distress. If cough or difficulty in breathing develops, the victim should be evaluated for respiratory tract irritation, bronchitis, or pneumonitis. Supplemental oxygen (100% humidified) should be administered with assisted ventilation as required.

Exposed eyes should be irrigated with generous amounts of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should see a doctor. Exposed areas should be washed extremely thoroughly with soap and water. A physician may need to examine the area if irritation or pain persists.

Environmental Fate

If released into air, hexachlorobutadiene will exist solely as a vapor. In soil, hexachlorobutadiene is expected to have low to no mobility, and volatilization is expected to be a significant fate process. If released into water, hexachlorobutadiene is expected to adsorb to particulates and sediment. Volatilization from water may be significant but will depend on the extent of adsorption to particulates and sediment. Disappearance half-lives of hexachlorobutadiene have been estimated to be 3–30 days in river water and 30–300 days in lake and groundwater. Bioconcentration factors of between 5800 and 17 000 indicate a very high potential for accumulation in aquatic organisms.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value is 0.02 ppm.

See also: Glutathione; Pesticides; Sister Chromatid Exchanges.

Further Reading

Birner G, Bernauer U, Werner M, and Dekant W (1997) Biotransformation, excretion and nephrotoxicity of haloalkene-derived cysteine *S*-conjugates. *Archives of Toxicology* 72: 1–8.

Green T, Lee R, Farrar D, and Hill J (2003) Assessing the health risks following environmental exposure to hexachlorobutadiene. *Toxicological Letters* 138: 63–73.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hexachlorobutadiene.

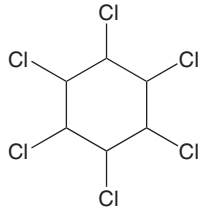
http://www.oehha.ca.gov – Evidence on the Carcinogenicity of 1,3-Hexachlorobutadiene.

Hexachlorocyclohexanes

Guangping Chen

- CHEMICAL NAMES:
  1,2,3,4,5,6-Hexachlorocyclohexane (mixed isomers), CAS 608-73-1
  1α,2α,3β,4α,5β,6α-Hexachlorocyclohexane (α-isomer), CAS 319-84-6
  1α,2β,3α,4β,5α,6β-Hexachlorocyclohexane (β–isomer), CAS 319-85-7
  1α,2α,3β,4α,5α,6β-Hexachlorocyclohexane (γ-isomer), Lindane, CAS 58-89-9
  1α,2α,3α,4β,5α,6β-Hexachlorocyclohexane (δ-isomer), CAS 319-86-8
  1α,2α,3α,4β,5β,6β-Hexachlorocyclohexane (ε-isomer), CAS 6108-10-7
  1α,2α,3α,4α,5α,6α-Hexachlorocyclohexane (ζ-isomer), CAS 6108-11-8
  1α,2α,3α,4α,5β,6β-Hexachlorocyclohexane (η-isomer), CAS 6108-12-9
  1α,2α,3α,4α,5α,6β-Hexachlorocyclohexane (θ-isomer), CAS 6108-13-0
- SYNONYMS: Benzene hexachloride; 1,2,3,4,5,6-Hexachlorocyclohexane; Lindane

- Chemical/Pharmaceutical/Other Class: Organochlorine pesticides
- Chemical Formula: $C_6H_6Cl_6$
- Chemical Structure:



Uses

Hexachlorocyclohexanes (HCHs) are produced and used as insecticide on fruit, vegetables, and forest crops, and for direct application on animals and animal housing. It is also available as a prescription medicine to treat or control scabies and head lice in humans. It is a white solid substance that may evaporate under suitable conditions.

Background Information

HCHs are a group of manufactured chemicals. HCH has eight isomeric forms. The different isomers are named according to the position of the hydrogen atoms in the structure of the chemical. The four common isomers are α-, β-, γ-, and δ-HCHs. The most common of these is γ-HCH (also known as lindane).

Exposure Routes and Pathways

Oral exposure through diet is the primary pathway for the general population. HCHs can also be distributed in air and can be absorbed through inhalation.

Toxicokinetics

HCHs are readily absorbed through the gastrointestinal tract. Inhaling air contaminated with isomers of HCH can also lead to systemic absorption. HCHs can also be absorbed through the skin when used as a lotion, cream, or shampoo for the treatment or control of ectoparasites. In general, HCH isomers and their metabolites can be temporarily stored in body fat. Absorbed HCHs are mainly excreted via the urine. Lesser amounts are excreted in feces. In rats, the highest concentrations have been found in liver, kidneys, body fat, brain and muscles, with substantial deposition occurring in fatty tissue.

Mechanism of Toxicity

HCHs are highly lipophilic molecules exhibiting extended (years) biological half-lives. The γ isoform (γ-HCH; lindane) is a potent neurostimulant and convulsant. γ-HCH mediated neurotoxicity is primarily the result of blockade of Cl^- influx through ionotropic γ-aminobutyric acid receptors, resulting in depolarization and hyperexcitation of the postsynaptic neuronal membrane. γ-HCH has been shown to enhance both spontaneous and evoked release of neurotransmitters from nerve terminals. These actions have been correlated with the ability of γ-HCH to elevate Ca^{2+} in brain synaptosomes. γ-HCH has also been shown to alter contractility in skeletal myocytes. δ-HCH is particularly potent toward disrupting Ca^{2+} homeostasis in a variety of excitable and nonexcitable cells and altering contractility of cardiac muscle. δ-HCH has been shown to stereoselectively mobilize Ca^{2+} from intracellular stores in cultured neural cells, which appears mediated by interaction with ryanodine receptors. γ-HCH and β-HCH have been reported to have estrogenic actions.

Clinical Management

HCH isomers can be measured in the blood, urine, and semen of exposed persons. HCH metabolites can also be measured to determine whether a person has been exposed to HCH. However, this method cannot be used to determine exposure to HCH alone, that is, other environmental contaminants may also produce the same metabolites.

Environmental Fate

HCHs persist in the environment. In air, the different forms of HCH can be present as a vapor or adsorbed to small particles. HCH can remain in the air for long periods of time and residues can travel great distances, depending on environmental conditions. HCH is degraded to less toxic substances by algae, fungi, and bacteria.

See also: Lindane; Organochlorine Insecticides.

Further Reading

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Kylin H and Sjodin A (2003) Accumulation of airborne hexachlorocyclohexanes and DDT in pine needles. *Environmental Science and Technology* 37(11): 2350–2355.

Otero R, Santiago-Silva M, and Grimalt JO (1997) Hexachlorocyclohexanes in human blood serum. *Journal of Chromatography A* 778(1–2): 87–94.

Relevant Websites

http://www.intox.org – International Programme on Chemical Safety.

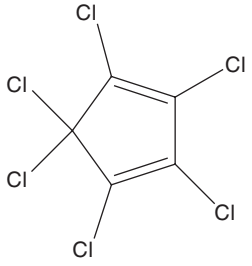
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hexachlorocylcohexanes.

http://www.inchem.org – Folpet, Health and Safety Guide No. 72, IPCS – International Programme on Chemical Safety, World Health Organization, Geneva, 1992.

Hexachlorocyclopentadiene

Murali Badanthadka and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 77-47-4
- Synonyms: 1,2,3,4,5,5-Hexachloro-1,3-cyclopentadiene; 1,3-Cyclopentadiene, 1,2,3,4,5,5-hexachloropentadine; Perchlorocyclopentadiene; Perchloro-1,3-cyclopentadiene
- Chemical/Pharmaceutical/Other Class: Chlorinated cyclic hydrocarbon
- Chemical Formula: C_5Cl_6
- Chemical Structure:



Uses

Hexachlorocyclopentadiene is used as a chemical intermediate for many insecticides, polymer resins, flame-retardant additives, resins, dyes, and in pharmaceuticals. It is also used to make shock-proof plastics, acids, esters, ketones, and fluorocarbons.

Background Information

Hexachlorocyclopentadiene is a light, lemon-yellow liquid with a sharp, musty odor. It is a manufactured chemical that does not occur naturally in the environment. It is used to make a group of related pesticides: aldrin, chlordane, dieldrin, endosulfan, endrin, heptachlor, isodrin, mirex, and pentac. Only endosulfan and pentac are currently registered for use in the United States. Hexachlorocyclopentadiene is also used to make flame retardants, resins that will not burn, shock-proof and unbreakable plastics, acids, esters, ketones, fluorocarbons, and dyes.

Exposure Routes and Pathways

Although occupational exposure appears to be the main source of human contact, certain segments of the population may be exposed through ingestion of contaminated drinking water or contaminated fish. People living in the vicinity of hazardous waste disposal sites containing this compound may be exposed by inhalation of contaminated air. Workers involved in the manufacture or handling of this compound or treatment of wastes containing this compound could potentially be exposed by inhalation or dermal exposure.

Toxicokinetics

Hexachlorocyclopentadiene undergoes chemical alterations in water forming both lipophilic and hydrophilic products. The lipophilic products in fish were extremely volatile. Three days after the intraperitoneal injection of ^{14}C-hexachlorocyclopentadiene, the ethyl acetate extractable radioactivity was ~47% while water-soluble and unextractable radioactivities were 11% and 20% of the injected radioactivity, respectively. Ethyl acetate extracts of fish included at least eight unidentified breakdown products. Water-soluble extract from fish included at least four unidentified products.

Rats given 6 mg kg^{-1} hexachlorocyclopentadiene orally excreted 33% in urine and 10% in feces in 7 days. Most excretion occurred during the first 24 h after dosing. The kidney retained 0.5% and the liver >0.5%. Biliary excretion of only 16% with 66% still voided in the feces of bile duct cannulated rats suggested that the majority of orally consumed was not absorbed. Degradation apparently occurred in the gut since little of the fecal material was of an apolar nature. The kidney, liver, ovaries, and fat were the major sites of deposition of ^{14}C-hexachlorocyclopentadiene equivalents. In rats, the kidney contained the highest levels of residues, whereas in mice the residues in the liver exceeded those in the kidney. Other than this difference, the fate of hexachlorocyclopentadiene in rats and mice, both male and female, was quite similar and in each case the tissue residues reached a plateau after about 2 weeks on the hexachlorocyclopentadiene-containing diets.

In another study, rats were either exposed to ^{14}C-hexachlorocyclopentadiene vapors for 1 h, or were dosed orally with ^{14}C-hexachlorocyclopentadiene in corn oil. Tissue, urine, and feces samples were analyzed, as well as expired air, to assess the fate and retention time. Approximately 84% of the inhaled compound is retained. Inhaled ^{14}C-hexachlorocyclopentadiene was excreted in the urine; orally administered ^{14}C-hexachlorocyclopentadiene was eliminated in the feces. In rats exposed by inhalation, the trachea and lung had the highest residue accumulation. In animals receiving oral doses kidneys and liver were major sites of accumulation. These studies indicate that the route of exposure is critical to the pattern of retention and elimination.

Mechanism of Toxicity

Hexachlorocyclopentadiene's mechanism of toxicity is incompletely understood. Because of its characteristics as a chlorinated hydrocarbon, it would be expected to induce drug-metabolizing enzymes in the liver.

Acute and Short-Term Toxicity (or Exposure)

Animal

Approximate lethal doses for rats and rabbits by single oral administration were between 420 and 620 mg kg^{-1}, respectively. The animals showed diarrhea, lethargy, and decreased respiration. Rabbits were reported to show diffuse degenerative changes in the brain, heart, liver, and adrenal glands; necrosis of the epithelium of renal tubules; and severe hyperemia and edema of the lungs by skin absorption.

Acute range-finding (14 days) and subchronic (90 days) inhalation studies were conducted with Sprague Dawley rats; and subchronic (90 days) inhalation studies were conducted with monkeys. The studies with rats showed steep dose–response curves with male rats being more sensitive than females. The threshold for toxic effects was <0.5 ppm hexachlorocyclopentadiene. Observation of lesions in the olfactory and bronchiolar epithelium as well as inflammatory exudate in the lumen of the respiratory tract was consistent with observed impaired respiratory function, confirming the lungs as the main target organ. Lacrimation, salivation, tremors, and degenerative changes in the brain, heart, liver, adrenal glands, and kidneys have been observed in animal inhalation studies. Hexachlorocyclopentadiene is not carcinogenic based on inhalation studies in rats and mice.

F344 rats and B6C3F1 mice were exposed to the hexachlorocyclopentadiene by gavage at 0–150 and 0–300 mg kg^{-1}, respectively. A dose-related decrease in mean body weight gain occurred in both sexes of rats and mice. Male rats in the 150 mg kg^{-1} dose group and one in 75 mg kg^{-1} group died after exposure. All mice exposed to hexachlorocyclopentadiene at 300 mg kg^{-1} died. Liver:brain weight ratios were significantly increased in the 38, 75, and 150 mg kg^{-1} exposed groups of female rats. Kidney:brain weight ratios in females significantly increased after 75 and 150 mg kg^{-1} exposure. No significant difference in relative weight of other organs was observed. In female mice, the kidney:brain weight ratios were significantly increased at all doses and the lung:brain weight increased after exposure at 300 mg kg^{-1} dose. Clinical signs of toxicity, cysts, and ulceration were seen after exposure. Histopathologically, lesions in the stomach, inflammation in the submucosa, edema, neovascularization and hyperplasia were noted. Toxic nephrosis of the kidney and acute tubular necrosis were also seen in both rats and mice.

Human

Eye and throat irritation has been reported in humans. Independent of the exposure route, the lung appears to be a major target organ resulting in cough, dyspnea, and chest pains. Headache and nausea are common after exposure. Exposed workers have developed reversible subclinical elevations of liver function tests and reversible proteinuria. Skin irritation and blistering may occur from direct contact with liquid hexachlorocyclopentadiene, and skin contact with vapor has been reported to result in skin irritation. Reported human cases have generally been mild.

Chronic Toxicity (or Exposure)

Animal

Pregnant mice and rabbits were administered up to 75 mg kg^{-1} day^{-1} of hexachlorocyclopentadiene by gavage during active oogenesis. Teratogenic effects have not been observed; however, nephrosis and acute tubular necrosis were seen in the dams.

Human

Hexachlorocyclopentadiene is not classifiable as a human carcinogen.

In Vitro Toxicity Data

Hexachlorocyclopentadiene is not mutagenic in the *Salmonella typhimurium* and *Escherichia coli* assay with and without metabolic activation.

Clinical Management

Oral Exposure

Because of potential central nervous system depression, emesis should not be induced. Significant esophageal or gastrointestinal tract irritation or burns may occur following ingestion. The possible benefit of early (within 1 h) removal of some ingested material by cautious gastric lavage must be weighed against potential complications of bleeding or perforation. The victim should be taken to a hospital immediately and should be treated symptomatically.

Inhalation Exposure

Patient should be moved to fresh air. Respiratory distress should be monitored and a healthcare person consulted.

Eye Exposure

Decontamination with copious amounts of room temperature water for at least 15 min should be done. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should consult healthcare facility.

Dermal Exposure

Decontamination should be done by removing contaminated cloth and washing the exposed area thoroughly with soap and water. A physician may need to examine the area if irritation or pain persists.

Environmental Fate

Aquatic Fate

If released to water, hexachlorocyclopentadiene will degrade primarily by photolysis and chemical hydrolysis to form both lipophilic and hydrophilic products. The water soluble substances included at least 11 unidentified breakdown products. Hydrolytic half-lives ranging from several hours to 2–3 weeks are predicted for waters with temperatures in the range of 20–30°C. 2,3,4,4,5-Pentachloro-2-cyclopentenone, hexachloro-2-cyclopentenone, and hexachloro-3-cyclopentenone have been identified as primary photodegradation products of hexachlorocyclopentadiene. Hexachlorocyclopentadiene has the potential to adsorb suspended solids and sediments; nevertheless, adsorption does not significantly affect the rate of hydrolysis. Volatilization from water is expected to be a significant removal mechanism, although in highly turbid waters adsorption to suspended solids and sediments could substantially limit losses via volatilization. The volatilization half-lives from a model river and a model pond with and without adsorption have been estimated to be 5 h, 37 days, and 58 h, respectively. It appears as though hexachlorocyclopentadiene may also be susceptible to biodegradation. Potential bioaccumulation in some aquatic organisms depends upon the organism and the species.

Terrestrial Fate

If released to soil, hexachlorocyclopentadiene will get adsorbed to organic matter and degrades via photolysis on soil surfaces. Volatilization from soil surfaces is expected to be of minor importance. In moist soil, this compound would be subject to chemical hydrolysis (half-life of hours to weeks) and biodegradation under aerobic and anaerobic conditions. A study indicates that loss of hexachlorocyclopentadiene from soil is the result of abiotic and biotic degradation as well as partitioning within the media.

Atmospheric Fate

Organic compounds having a vapor pressure of greater than 1×10^{-4} mmHg at ambient temperature are expected to exist almost entirely in the vapor phase in the atmosphere. Hexachlorocyclopentadiene has a vapor pressure of 0.063 mmHg at 25°C; therefore, it is expected to exist predominantly in the vapor phase in the atmosphere. If released to the atmosphere, direct photolysis is expected to be the dominant removal mechanism. Reaction of hexachlorocyclopentadiene with photochemically generated hydroxyl radicals or ozone molecules is predicted to be too slow and environmentally insignificant.

Exposure Standards and Guidelines

The acceptable daily intake for hexachlorocyclopentadiene is 0.00462 mg day^{-1}. The Occupational Safety and Health Administration 8 h time-weighted

average (TWA) is 0.01 ppm (0.1 mg m^{-3}). The National Institute of Occupational Safety and Health recommended exposure limit is 0.01 ppm (0.1 mg m^{-3}) for 10 h TWA.

See also: Chlorination By-products; Polybrominated Biphenyls (PBBs); Polychlorinated Biphenyls (PCBs).

Further Reading

Abdo KM, Montgomery CA, Kluwe WM, Farnell DR, and Prejean JD (1984) Toxicity of hexachlorocyclopentadiene: Subchronic (13-week) administration by gavage to F344 rats and B6C3F1 mice. *Journal of Applied Toxicology* 4: 75–81.

Dorough HW and Ranieri TA (1984) Distribution and elimination of hexachlorocyclopentadiene in rats and mice. *Drug and Chemical Toxicology* 7: 73–89.

Lawrence LJ and Dorough HW (1981) Retention and fate of inhaled hexachlorocyclopentadiene in the rat. *Bulletin of Environmental Contamination and Toxicology* 26: 663–668.

Mehendale HM (1977) Chemical reactivity-absorption, retention, metabolism, and elimination of hexachlorocyclopentadiene. *Environmental Health Perspectives* 21: 275–278.

National Toxicology Program (1994) NTP Toxicology and Carcinogenesis Studies of Hexachlorocyclopentadiene (CAS No. 77-47-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). National Toxicology Program Technical Report Series 437, pp. 1–308.

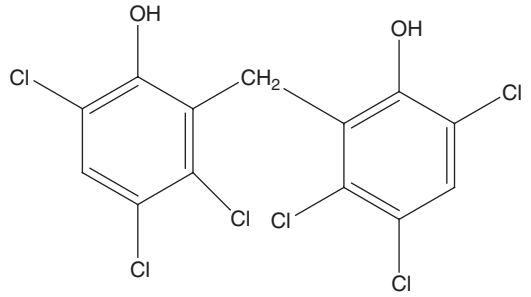
Podowski AA, Sclove SL, Pilipowicz A, and Khan MA (1991) Biotransformation and disposition of hexachlorocyclopentadiene in fish. *Archives of Environmental Contamination and Toxicology* 20: 488–496.

Podowski AA and Khan MA (1984) Fate of hexachlorocyclopentadiene in water and goldfish. *Archives of Environmental Contamination and Toxicology* 13: 471–481.

Hexachlorophene

Cathy Villaroman and Robin C Guy

- Chemical Abstracts Service Registry Number: CAS 70-30-4
- Synonyms: 2,2′-Methylenebis(3,4,6-trichlorophenol); 2,2′-Dihydroxy-3,3′,5,5′,6,6′-hexachlorodiphenylmethane; bis(3,5,6-Trichloro-2-hydroxyphenyl)methane; Hexachlorophane; Hexachlorophen; bis(2,3,5-Trichloro-6-hydroxyphenyl)methane; Acigena; Exofene; Gamophene; HCP; Hexafen; Nabac
- Chemical Structure:



Uses

Hexachlorophene is used as an agricultural chemical, detergent, therapeutic agent, and wood preservative. It is used as a topical antiinfective, fungicide, germicide, bactericide, and disinfectant. It is also used as an antibacterial agent in cosmetics, soaps, shampoos, and deodorants.

Exposure Routes and Pathways

Hexachlorophene can be absorbed into the body by inhalation, through the skin, and by ingestion. Exposure to hexachlorophene is usually dermal as a bactericide. It is sometimes used as a topical treatment for acne vulgaris to suppress associated staphylococci.

Toxicokinetics

Hexachlorophene is well absorbed orally and dermally and through mucosal surfaces. In rats, up to 55% of dermally applied hexachlorophene is absorbed in 24 h. Dermal absorption is enhanced by dimethylsulfoxide and dermatitis or skin abrasions. Placental transfer has been demonstrated in rats. Hexachlorophene is converted to hexachlorophene-β-D-glucuronide in the rat and rabbit. Some hexachlorophene has been found in the blood and adipose tissue. Hexachlorophene was administered intraperitoneally to rats and rabbits; excretion was slow and most (48–83%) was excreted unchanged in the feces. Hepatic function is an important determinant in the removal of hexachlorophene. In a rat study, within 3 h after administration, 50% was excreted in the bile. Rats given intraperitoneal doses excreted ~5% of the dose in the urine and none as CO_2; more than 70% of the material was excreted in feces.

Mechanism of Toxicity

Following skin absorption, hexachlorophene enters the nervous system and results in intramyelinic edema, splitting the intraperiod line of myelin in both the central nervous system (CNS) and the peripheral nervous system. Experimental studies with erythrocyte membranes show that hexachlorophene binds tightly to cell membranes, resulting in osmotic swelling of erythrocyte membranes by altering their permeability to sodium and potassium. Hexachlorophene uncouples oxidative phosphorylation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Pigs fed hexachlorophene for 36 days only exhibited mild neurological signs, with a no-observed-effect limit (NOEL) = 0.1 mg kg^{-1} day^{-1} and a lowest-observed-effect level (LOEL) = 0.5 mg kg^{-1} day^{-1}. A subchronic dog feeding study was conducted by Nationwide Chemical Corporation (1974), where Beagle dogs (four per sex per dose) were fed hexachlorophene (0.75, 1.5, or 3.0 mg kg^{-1} day^{-1}) in the diet for 13 weeks. The principal effects noted were swollen salivary glands, dry mouth, and status spongiosis in the brain, optic nerve, spinal cord, and sciatic nerve at all dose levels tested. An NOEL for this study was not established. However, the LOEL of 0.75 mg kg^{-1} day^{-1} was used to establish an oral reference dose. Applying a total uncertainty factor of 3000 (100 for inter- and intraspecies differences, 10 for the lack of an established NOEL, and 3 to account for subchronic to chronic exposure in the dog = UF of 3000) to the LOEL yielded an oral reference dose of 2.5×10^{-4} mg kg^{-1} day^{-1}.

Human

Acute ingestion of large amounts ($\geqslant$30–60 m for an adult) or repeated ingestion of small amounts of hexachlorophene may cause significant toxicity or death. Exposure may also cause effects on the CNS, resulting in convulsions or respiratory failure. Dermal, gastrointestinal, and neurologic effects are the most common toxic manifestations. Cardiorespiratory arrest may occur most notably following acute ingestion of large amounts. Lethargy frequently occurs as an early manifestation of toxicity.

Chronic Toxicity (or Exposure)

Animal

Rats fed 500 ppm (25 mg kg^{-1} day^{-1}) showed weakness in their hindquarters, which progressed to paralysis. Microscopic examination of the brain and spinal cord revealed a particular edema of white matter resembling spongy degeneration of white matter in infants. When the animals were removed from the hexachlorophene diet, they recovered gradually over a period of weeks; similar signs were noted in the monkey.

Oral administration of hexachlorophene to rats causes degeneration of spermatogenic cells. In sheep, 2500 mg kg^{-1} followed 2 days later by a dose of 50 mg kg^{-1} also caused extensive damage to spermatogonia; after 21 days there was neither sperm in epididymis nor spermatogenesis.

Oral LD_{50}s in rats were 187 and 67 mg kg^{-1}; 56 mg kg^{-1} in the female rat; 120 mg kg^{-1} in the rat weanling; 9 mg kg^{-1} in the rat suckling (10 days old); 63–87 mg kg^{-1} in the female Wistar rat; and 58–87 mg kg^{-1} in the male Wistar rat.

Oral administration of hexachlorophene in rats produced no carcinogenic effects.

Human

Repeated or prolonged dermal contact with hexachlorophene may cause skin hypersensitivity or dermatitis, while repeated or prolonged inhalation exposure may cause asthma. Repeated ingestion may result in tissue lesions or blindness. The estimated lethal dose in humans is 1–10 g. Dermal application, especially in neonates or on damaged skin, of highly concentrated ($\geqslant$3%) preparations on several occasions or repeated applications of less concentrated preparations may result in significant toxicity or death. An erythematous desquamative rash may occur following repeated dermal application, especially in neonates or in high concentrations ($\geqslant$3%).

In Vitro Toxicity Data

Hexachlorophene was not shown to be mutagenic in *Salmonella typhimurium* in tests reviewed. Cytogenetic tests with cultured human lymphocytes were also negative.

Clinical Management

Plasma hexachlorophene levels have not been demonstrated to correlate well with clinical effects. Hexachlorophene may cause seizures. The risk of seizures during emesis may preclude the use of ipecac syrup. Charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, should be administered. The usual charcoal dose is 30–100 g in adults and 15–30 g in children (1 or 2 g kg^{-1} in infants). If seizures cannot be controlled with diazepam or they recur, phenytoin or

phenobarbital should be administered. The patient should be checked for cerebral edema. Other acute symptoms after ingestion exposure include: fever, tremors, absence of light reflex, abdominal cramps, diarrhea, drowsiness, nausea, vomiting, and weakness. Vomiting should be induced in conscious persons. Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. Vigorous washing with soap and water should be followed by washing with 70% isopropanol, olive oil or castor oil, followed by a second vigorous soap and water cleansing, which may increase removal of hexachlorophene. If contact is made via the skin, contaminated clothes should be removed, then the skin is rinse and wash skin with soap and water.

Environmental Fate

Hexachlorophene adsorbs very strongly to soil and is not expected to leach to groundwater. It may undergo slow photodegradation on the surface of soils and water based on its absorption of light (290 nm). No information is available on its biodegradation in soil or surface water. Hexachlorophene released in water adsorbs very strongly to sediments and may bioconcentrate in aquatic organisms. It has an estimated bioconcentration factor (BCF) of 317 000. HCP is not expected to hydrolyze or to significantly evaporate from water. When released into the air, HCP is expected to be mainly in the particle-sorbed state due to its low vapor pressure and high estimated K_{oc}. It is expected to be removed from the atmosphere primarily by dry deposition, but it is also degraded by reaction with photochemically produced hydroxyl radicals, with an estimated vapor phase half-life of 2.5 days.

Ecotoxicology

Hexachlorophene is very toxic to aquatic organisms and may cause long-term effects in the aquatic environment.

Other Hazards

Hexachlorophene is combustible and can give off irritating or toxic fumes (or gases), including hydrogen chloride, if heated or burned in a fire. Hexachlorophene evaporates negligibly at 20°C. However, a harmful concentration of airborne particles can be reached quickly on spraying or when dispersed as dust.

See also: Neurotoxicity.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Hexachlorophene.

http://www.speclab.com – Spectrum Laboratories. Chemical Fact Sheet.

http://vm.cfsan.fda.gov – US Food and Drug Administration. Code of Federal Regulations: Title 21, Volume 4. 21CFR250.250. Revised April 2002.

http://www.state.nj.us – New Jersey Department of Health and Senior Services. Hazardous Substance Fact Sheet. Hexachlorophene.

Hexane

Stephen R Clough and Leyna Mulholland

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- Chemical Name: *n*-Hexane
- Chemical Abstracts Service Registry Number: CAS 110-54-3
- Synonyms: Dipropyl; Esani; Heksan; AI3-24253; Hexanen; Hexyl hydride; NCI-C60571; HSDB 91; Skellysolve B
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon
- Chemical Formula: C_6H_{14}
- Chemical Structure: $CH_3CH_2CH_2CH_2CH_2CH_3$

Uses

Commercial hexane is a mixture of various types of hexane isomers with minor amounts of heptane, pentane, cyclohexane, and cyclopentane isomers. It may contain concentrations of *n*-hexane ranging from 20% to 80%. *n*-Hexane is used for determining the refractive index of minerals, for calibrations, as a paint diluent, and in thermometers. It is also used as a solvent in the extraction of soybean oil, cottonseed oil, flaxseed oil, safflower seed oil, and other oil seeds. It is sometimes used as a denaturant for alcohol, and as a cleaning agent in the textile, furniture, and leather industries although it is slowly being replaced with other less toxic solvents. *n*-Hexane is also a common laboratory reagent and a component of many products associated with the petroleum and gasoline industries

(one study found 1.5% of vapors encountered during gasoline handling were attributed to *n*-hexane).

Exposure Routes and Pathways

The primary exposure pathway for *n*-hexane in humans is via inhalation, and this generally is seen in an occupational setting. Minor exposures may occur when persons fill their automobiles with gasoline. Ingestion or dermal contact would be a less common exposure route, with the former expected to occur during accidental poisoning or suicide attempt and the latter occurring from a spill or the use of a hexane applicator without the proper skin protection (e.g., solvent rag without a chemically resistant glove). Exposure from contact with vapors or emissions from these refined petroleum products is the most widespread form of low-level exposure for the general population. Recent research by Ahearn *et al*. suggests that certain fungi may be able to produce *n*-hexane. These fungi may be common in older buildings, and in some parts of the country may provide exposures from previously unsuspected indoor sources.

Toxicokinetics

Acute exposure usually occurs by inhalation. *n*-Hexane may be absorbed orally or percutaneously. *n*-Hexane has a vapor density of 2.97 and it is heavier than air. *n*-Hexane is believed to be metabolized through the cytochrome P450 system and phenobarbital pretreatment of liver microsomes induced 2- and 3-hydroxylation of *n*-hexane sixfold. 3,4-Benzpyrene suppresses 2-hydroxylation and stimulates 3-hydroxylation of *n*-hexane. For humans, 2-, 3-hydroxyhexane is responsible for various toxicities.

Mechanism of Toxicity

n-Hexane is biotransformed to 2-hexanol and further to 2,5-hexanediol by cytochrome P450 mixed function oxidases by omega oxidation. 2,5-Hexanediol may be further oxidized to 2,5-hexanedione, the major metabolite of *n*-hexane in humans. Identification of 2,5-hexanedione as the major neurotoxic metabolite of *n*-hexane proceeded rapidly after its discovery as a urinary metabolite. 2,5-Hexanedione has been found to produce a polyneuropathy indistinguishable from *n*-hexane. 2,5-Hexanedione is many times more potent than *n*-hexane, the parent compound, in causing neurotoxicity in experimental animals. It appears that the neurotoxicity of 2,5-hexanedione resides in its γ-diketone structure since 2,3-, 2,4-hexanedione and 2,6-heptanedione are not neurotoxic, while 2,5-heptanedione and 3,6-octanedione and other γ-diketones are neurotoxic.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hexane has been reported to be three times as acutely toxic to mice as is pentane. A concentration of 30 000 ppm produced central nervous system (CNS) depression within 30 to 60 min. Concentrations ranging from 35 000 to 40 000 ppm produced convulsions and death. When mice were exposed to an atmosphere containing 2.5–3% of *n*-hexane for 4 days, liver enlargement was observed after 24 h. In another study, mice were exposed to commercial hexane (65–70% *n*-hexane) for 24 h a day, 6 days a week for 1 year. Exposure levels ranged from 100 to 2000 ppm. Atrophy and degeneration of hindleg muscle fibers were present in animals exposed to 1000 and 2000 ppm of *n*-hexane.

Human

Based on a national occupational survey conducted in the mid-1970s, an estimated 643 120 workers are occupationally exposed to *n*-hexane. This commonly used solvent was not regarded as an industrial hazard until the discovery of its neurotoxic potential since its acute toxicity is quite low. Because of its toxicity, the number of people presently exposed to hexane in occupational settings is expected to be much lower than the number in the 1970s. Acutely, vapor concentrations of many hundreds of parts per million are tolerated for several minutes without causing discomfort among workers.

Acute exposure to hexane causes CNS depression. Chronic exposure to an average air concentration of 450–650 ppm for as little as 2 months may result in peripheral neuropathy, characterized by muscular weakness, loss of sensation, and impaired gait. Hexane has been reported to be the most highly toxic member of the alkanes. When *n*-hexane is ingested, it causes nausea, vertigo, bronchial irritation, general intestinal irritation, and CNS effects. It poses an acute aspiration hazard. It has been reported that $\sim$50 g of *n*-hexane may be fatal to humans. An exposure of 880 ppm for 15 min can cause eye and upper respiratory tract irritation in humans. Blurred vision has been mentioned in association with *n*-hexane polyneuropathy. It was concluded that *n*-hexane vapor levels of $<$100 ppm for 8 h per day were not likely to produce a clinical neuropathy, but mild subclinical changes in muscle strength and nerve conduction velocity may occur.

In humans, 2000 ppm of *n*-hexane for 10 min resulted in no effects. However, 5000 ppm caused dizziness, giddiness, slight nausea, headache, and

eye and throat irritation. Three women had motor polyneuropathy following industrial exposure to an adhesive agent containing 80.4% *n*-hexane. In the nerves, there were polymorphous changes in the myelin sheaths and axons of large diameter fibers. Muscles showed denervation changes, with lymphocytic infiltrates and phagocytosis. Three cases of *n*-hexane neuropathy in the shoe industry were reported. In the most severe cases, symptoms consisted of dysarthria, disproportionate ataxia of gait, blurred vision, and sometimes after recovery of peripheral neuropathy, appearance of leg spasticity.

Chronic Toxicity (or Exposure)

Animal

Pregnant Fischer 344 rats were exposed to 1000 ppm *n*-hexane for 6 h per day on days 8–12 of gestation. Postnatal growth of pups born to dams exposed to 1000 ppm on days 8–16 of gestation was significantly depressed compared to controls. New Zealand rabbits exposed in inhalation chambers to 3000 ppm *n*-hexane 8 h per day for 8 days showed changes in the lungs, emphysema, necrotic phenomena in the bronchiolar epithelium, and athelectasis. Epicutaneous administration of *n*-hexane to guinea pigs caused progressing nuclear pyknosis and junctional separation between the basement membrane and the basal cells of the skin.

Male rats were exposed by inhalation to several concentrations of hexane, administered continuously or intermittently. In rats exposed to 1000 ppm hexane 24 h per day, 5 days per week for 11 weeks, the fifth component of the brain stem auditory-evoked response showed an increase in latency and decrease in amplitude, reflecting a brain stem dysfunction. Latency returned to normal within 5 weeks after termination of exposures, but amplitude did not. Latency of the compound action potential of the ventral caudal nerve of the tail of these rats was also increased and this effect was still present 22 weeks after termination of the exposure.

Adult rats were exposed to different concentrations of *n*-hexane and lung tissue was then examined. The direct toxic effect to pneumocytes could be demonstrated as definite regressive alterations, such as fatty generation and change of lamellar bodies of type II pneumocytes as well as increased detachment of cells. After chronic inhalation of solvents, conspicuous aggregation of lamellar discharge material of type II pneumocytes can be seen and, probably as a result of an irritated fat metabolism, there were large lysosome-like bodies with densely packed lipid material in type I pneumocytes.

New Zealand rabbits exposed in inhalation chambers to 3000 ppm *n*-hexane 8 h per day for 8 days showed changes in lungs, emphysema, necrotic phenomena in the bronchiolar epithelium, and atelectasis. The injection of hexane into rabbits caused edema and hemorrhaging of the lungs and tissue, with polymorphonuclear leukocytic reactions. In rabbits, dermal application of 2–5 mg kg^{-1} for 4 h has resulted in ataxia and restlessness. No deaths occurred at 2 mg kg^{-1}; however, some occurred at 5 mg kg^{-1}.

Human

Out of 1662 workers exposed to organic solvents, which consisted mainly of *n*-hexane and a small amount of toluene, 53 were found to have sensory polyneuropathy, 32 had sensorimotor polyneuropathy, and 8 had sensorimotor polyneuropathy with amyotrophy. Cranial nerve involvements, such as visual disorders and facial numbness, were observed. About 50% showed denervation and reinnervation of the nerves. Among 93 cases of *n*-hexane polyneuropathy during a large outbreak in 1968, 44 were studied. Over a few years, most of the cases completely recovered (except for a few with mild sensory impairment) after establishing 100 ppm as the maximal allowable concentration of *n*-hexane and providing well-equipped ventilation systems in individual houses. During rescreening in 1981, 21 cases with mild *n*-hexane polyneuropathy were observed, revealing mostly the same features as in the previous outbreak in 1968. These data suggest that, despite <50 ppm of *n*-hexane concentration in a room, sandal workers have suffered from neurotoxicity from this organic solvent.

In a cross-sectional study, nerve conduction velocities were determined in 59 workers employed in press proofing factories in Taipei. Workers were divided into exposure categories on the basis of air concentrations of *n*-hexane ($\geqslant 100$, 50–99, and <50 ppm) and *n*-hexane concentrations in the cleaning solvent used ($\geqslant 50$, 49–10, and $<10\%$). Fifteen members (25%) of the study group were found to have polyneuropathy. In one factory where all six employees developed polyneuropathy, the air concentration of *n*-hexane was determined to be 190 ppm. In other factories, workers exposed to *n*-hexane at levels of <100 ppm showed significant decreases in motor nerve conduction velocities.

n-Hexane is currently under review for its carcinogenicity; however, it is not classified as a carcinogen at the present time. A US Environmental Protection Agency (EPA) reference concentration of 0.2 mg m^{-3} was calculated based on an epidemiological inhalation study with an uncertainty factor of 300. Critical effects were reported to be neurotoxicity and electrophysiological alterations.

In Vitro Toxicity Data

Hexane was found to be negative when tested for mutagenicity using the *Salmonella* microsome preincubation assay, following the standard protocol approved by the National Toxicology Program. Hexane was tested in as many as five *Salmonella typhimurium* strains (TA1535, TA1537, TA97, TA98, and TA100) in the presence and absence of rat and hamster liver S9 at doses of 0.001, 0.0033, 0.010, 0.033, 0.100, and 0.333 mg per plate. Some cultures exhibited slight clearing of the background bacterial lawn at the two highest doses tested.

Clinical Management

Oral Exposure

In general, gastric emptying is not indicated except in selected cases in which a history of a large ingestion is obtained. An activated charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, can be administered for oral exposure. The US Food and Drugs Administration suggests 240 ml of dilutent per 30 g of charcoal. The usual charcoal dose is 30–100 g in adults and 15–30 g in children. In symptomatic patients (e.g., coughing, choking, and tachypnea), blood gases should be monitored to ensure adequate ventilation and a baseline chest X-ray should be obtained. Ventilation and oxygenation should be maintained for pulmonary edema with close arterial blood gas monitoring. Early use of positive end expiratory pressure and mechanical ventilation may be needed to maintain oxygen pressure.

Inhalation Exposure

The victim should be moved to fresh air to decontaminate. The person should also be monitored for respiratory distress. If cough or difficulty in breathing develops, respiratory tract irritation, bronchitis, or pneumonitis should be evaluated. Supplemental oxygen (100% humidified) should be administered with assisted ventilation as required.

Eye Exposure

Exposed eyes should be irrigated with copious amount of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should be seen in healthcare facility.

Dermal Exposure

The exposed area should be washed thoroughly with soap and water to decontaminate. A physician may need to examine the area if irritation or pain persists.

Environmental Fate

In the environment, *n*-hexane does not absorb UV light ($\geqslant$290 nm); and therefore it will not undergo direct photolysis. Some species of *Pseudomonas* bacteria, which are naturally present in soil and sediment, are able to metabolize hexane, so low-level contamination seen in groundwater is thought to naturally attenuate with time, reducing the risk to humans.

Ecotoxicology

According to the US EPA ECOTOX aquatic toxicity database, the 24 h acute LC_{50} values for saltwater organisms range from 3530 $\mu g l^{-1}$ (brine shrimp) to 154 300 $\mu g l^{-1}$ (rotifer). For freshwater, the lowest respective 24 and 96 h acute LC_{50} values were for *Daphnia magna* (50 000 $\mu g l^{-1}$) and the fathead minnow (2100 $\mu g l^{-1}$). Hexane, in all likelihood, affects aquatic organisms similarly to other volatile alkanes, which is by a narcotic mechanism (i.e., a solvent-like disruption of neuronal membranes). These concentrations are only seen in the laboratory and are many, many times higher than concentrations that would ever be anticipated in natural waters.

Exposure Standards and Guidelines

In recognition of the chronic neurotoxic property of *n*-hexane, the American Conference of Governmental Industrial Hygienists has recommended a threshold limit value of 50 ppm (180 mg m^{-3}), expressed as an 8 h time-weighted average (TWA) (the National Institute for Occupational Safety and Health recommended exposure limit is also 50 ppm). The current Occupational Safety and Health Administration permissible exposure limit is 500 ppm (1800 mg m^{-3} as an 8 h TWA).

See also: Neurotoxicity; Pollution, Water.

Further Reading

Ahearn DG, Crow SA, Simmons RB, *et al.* (1996) Fungal colonization of fiberglass insulation in the air distribution system of a multi-story office building. VOC production and possible relationship to a sick building syndrome. *Journal of Industrial Microbiology* 16: 280–285.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hexane.

High Production Volume (HPV) Chemicals

Pertti J Hakkinen

High production volume chemicals ('HPV chemicals') are existing substances that are produced or imported into countries in high volumes. The definition varies by country and region. For example, in the United States, HPV chemicals are defined as those chemicals produced in or imported in amounts over one million pounds per chemical per year. The European Commission defines HPV chemicals as substances with a production or import volume in excess of 1000 metric tons (2.2 million pounds) per year. The toxicology and exposure assessment data sets available for HPV chemicals have been of increasing interest to regulatory agencies, nongovernment organizations, industry, interest groups, and others globally. A study of all US HPV chemicals found that 8.5% of the chemicals fulfilled a minimum set of data requirements, while another study found that 22% of the US HPV chemicals had 'minimal' toxicity data available. A study of publicly available data by the European Commission found that 14% of European Union HPV chemicals had data at the level of a 'base set', 65% had less than a base set, and 21% had no data. Thus, the focus of attention has been on chemicals that appear to have (1) been untested or unassessed, or (2) have less than a full basic set of data (see below for explanation of SIDS).

Since the late 1980s, governmental authorities in industrialized nations began to focus on these substances because of the presumed widespread exposure based on their large production volume. This focus began with the Organization for Economic Cooperation and Development (OECD) Screening Information Data Set (SIDS) Program in 1991, which has since been joined with similar and parallel activities such as the US HPV Challenge Program and the US Voluntary Children's Chemical Evaluation Program – both in 1998, and the plans to implement a sweeping regulatory change in the European Union (the registration, evaluation, and authorisation of chemicals (REACH) legislation).

OECD SIDS Program

The Organisation for Economic Cooperation and Development's (OECD's) 1987 Council Decision-Recommendation on the Systematic Investigation of Existing Chemicals stated, "Member Countries should establish or strengthen national programmes to systematically investigate existing chemicals." A further OECD Council Decision in 1991 focused on HPV chemicals. These decisions prompted the development of a minimum hazard data set to describe an HPV chemical – the Screening Information Data Set, or SIDS. This includes physicochemical properties (melting point, boiling point, vapor pressure, water solubility, and octanol–water partition coefficient); environmental fate (stability in water, photodegradation, biodegradation, and an estimate of distribution/transport in the environment); environmental effects (acute toxicity to aquatic vertebrates, invertebrates, and plants); and human health effects (acute toxicity, repeated-dose toxicity, toxicity to the gene and the chromosome, and reproductive and developmental toxicity).

In the OECD SIDS Program, member OECD countries are invited to 'sponsor' an HPV chemical, conduct the necessary SIDS-level testing, and present the information at an OECD meeting. The collection/testing of information is called developing a *dossier* of information. Presenting the information at a meeting (a SIDS Initial Assessment Meeting or SIAM) is usually done by developing an assessment report (SIDS Initial Assessment Report or SIAR). The documentation presented at a SIAM also includes an executive summary or SIDS Initial Assessment Profile (or SIAP), which includes one of two recommendations for the meeting participants to consider: either the substance is a priority for further work or it is a low priority for further work. Since 2000, the OECD SIDS Program has been enhanced by the involvement of the Global Initiative on High Production Volume Chemicals of the International Council of Chemical Associations (ICCA) and its affiliated industry associations such as the American Chemistry Council and the European Chemical Industry Council (Cefic).

US HPV Challenge Program

In the United States, 1998 was the year that the HPV Challenge Program was established as part of a voluntary 'Chemical Right-to-Know' (RTK) Initiative between industry and the US Environmental Protection Agency (US EPA). Chemical producers and importers were requested by the US EPA via the HPV Challenge Program to voluntarily provide basic health and environmental toxicity information (the SIDS) on their HPV chemicals. Under this program, a company or consortium of companies (sometimes via a trade association) is sponsoring a chemical or category of chemicals for testing within the period specified by the US EPA.

The United States has ~3200 HPV chemicals and ~2400 have been volunteered for sponsorship in the HPV Challenge Program. The balance have been: (1) determined to be not of concern (i.e., glucose); (2) already sponsored in the OECD SIDS Program; or (3) not sponsored and thus likely targeted for possible rulemaking by the US EPA to require any missing SIDS data. The information generated through the HPV Challenge Program, including the initial testing plans and the results, is being made publicly available, and companies have compiled the existing publicly available or privately held data, and have designed and submitted test plans to address any data gaps. Further, the companies sponsoring a chemical are providing results as they become available and are preparing data summaries. A rationale for not testing a chemical for a specific endpoint can be provided in lieu of testing, for example, the use of structure–activity relationships (SARs) and quantitative structure–activity relationships (QSARs) to compare chemicals and categories of chemicals via their structures, activities, and data sets will be allowed if it meets the available US EPA guidance. Thus, testing one or a few chemicals in a category rather than each chemical in that category will be allowed if judged to be scientifically appropriate. Information on non-SIDS endpoints is also encouraged to be included when available.

In addition to fulfilling the SIDS requirements, the US program will lead to a focus of future efforts on 'priority' chemicals, that is, those that show a likelihood of posing potential harm. Further, there should be greater confidence of the manufacturers, public, and others in the nonpriority chemicals based on their low demonstrated hazard potential. In addition, the US EPA can use the data generated from the program in a risk-based process to make decisions on the need for further information such as additional types of toxicology or environmental testing, or risk management actions. Similar actions are expected outside the United States.

VCCEP

Related to the HPV chemical efforts are other programs such as the Voluntary Children's Chemical Evaluation Program (VCCEP) pilot program in the United States. VCCEP is another part of the US EPA's Chemical RTK Initiative. The goal of the VCCEP program is to enable the US public to better understand the potential health risks to children associated with certain chemical exposures. The US EPA has asked companies that manufacture or import 23 chemicals, which have been found in human tissues and the environment in various monitoring programs, to volunteer for a pilot program and sponsor Tier 1 chemical evaluations. Thirty-five companies and 10 consortia have volunteered to sponsor 20 of the 23 substances. Sponsorship requires the companies to collect or develop health effects and exposure information on their chemical(s) and then to integrate that information in a risk assessment and a 'data needs' assessment. Panels of scientific experts using a peer consultation process are discussing the assessments developed by the sponsors.

HPV Chemical-Related Activities in Europe to Monitor

Finally, major HPV chemical-related activities in Europe to utilize, or to monitor and learn from, include the development of the European Information System on 'Risks from chemicals released from consumer products/articles' (EIS-ChemRisks). EIS-ChemRisks has been designed to be a European-wide expert and stakeholders 'network of networks' to systematically exchange and assess information on risks from chemicals released from consumer products/articles. The overall objective is to develop tools and reference data to enable harmonized exposure assessment procedures in the European Union. These tools and reference data will support the development of a structured stakeholder dialog in the framework of the General Product Safety Directive (GPSD, 2001/95/EC) and progressively in the framework of the European Commission's REACH program as it is established and implemented.

See also: European Union and Its European Commission; Organisation for Economic Cooperation and Development.

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http://ecb.jrc.it – Allanou R, Hansen BG, and van der Bilt Y (European Commission) Public Availability of Data on EU High Production Volume Chemicals.

http://ihcp.jrc.it – European Commission's Institute for Health and Consumer Protection website (e.g., to access information on European Union activities on new and existing chemicals, implementation and harmonization of alternatives to animal testing and other testing methods, quantitative structure–activity relationship efforts, and the EIS-ChemRisks and REACH efforts).

http://www.oecd.org – Organisation for Economic Cooperation and Development (OECD) (e.g., information the Screening Information Data Set (SIDS) program); see also 1987 Council Decision-Recommendation on the Systematic Investigation of Existing Chemicals, and a further OECD Council Decision in 1991 focused on HPV chemicals. Both documents available at the OECD website.

http://www.epa.gov – US Environmental Protection Agency: Chemical Right-to-Know Initiative; HPV Challenge Program; HPV Chemical Human Health Testing: Animal Welfare Issues and Approaches; Voluntary Children's Chemical Evaluation Program (VCCEP).

History of Toxicology *See* Toxicology, History of.

Holly

Ann P Slattery

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- Synonyms: *Ilex* species; *Ilex aquifolium* – Christmas holly, English holly, European holly, Oregon holly, and prick holly; *Ilex cornuta* – Chinese holly; *Ilex opaca* – American holly; *Ilex vomitoria* – black drink, deer holly, emetic holly, and yaupon

Uses

Evergreen shrubs and trees with stiff leathery leaves that may grow from 10 to 50 ft depending on the species. The berries are usually bright red, but some cultivars may have yellow berries. The leaves of the *aquifolium*, *cornuta*, and *opaca* have spiny, prickly leaves, while the leaves of the *vomitoria* are serrated, but spineless. These plants are primarily used in gardening. However, several folk remedies contain plant material or extracts from different *Ilex* species. In addition, *Ilex asprella* contains a variety of cytotoxic compounds that have been tested on several melanoma cell lines.

Exposure Routes and Pathways

Exposure occurs via ingestion of plant material (i.e., leaves and berries).

Mechanism of Toxicity

It is believed the saponins may be responsible for gastrointestinal effects, although the exact mechanism of action is unknown. The toxins involved include ilicin, tannic acid, ilexanthin, ilicic acid, and (in *I. aquifolium*) cyanogenic glycosides. These toxins may be found in the leaves and fruit (berries) of the plant.

Acute and Short-Term Toxicity (or Exposure)

Human

Although symptoms will vary with different types of holly, the predominant toxic effect appears to be gastrointestinal irritation. Ingestion of small amounts of plant material may result in mild to moderate gastritis resulting in nausea, vomiting, abdominal pain, and diarrhea. Mild central nervous system (CNS) depression may be evident. Ingestion of the thorny leaf can cause mechanical irritation. Larger amounts have the potential to cause more severe gastrointestinal irritation along with varying degrees of CNS depression.

Clinical Management

Most unintentional holly exposures result in self-limited gastrointestinal symptoms with no specific treatment needed. The main goal of therapy for holly ingestions is fluid replacement and supportive care. There is no specific antidote available. Activated charcoal may be used for substantial recent ingestions. For patients who are symptomatic with significant gastrointestinal effects, intravenous fluid replacement may be used if oral liquids cannot be tolerated.

See also: Plants, Poisonous.

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Hormesis

Elyisha A Hanniman and Christopher J Sinal

This article is a revision of the previous print edition article by Christopher J Sinal and John R Bend, volume 2, pp. 88–92, © 1998, Elsevier Inc.

Introduction

Hormesis is commonly defined as a beneficial or stimulatory effect caused by exposure to low doses of an agent known to be toxic at higher doses. Conceptually, this is represented by a U-shaped dose–response curve for toxicity where hormetic effects become smaller (after a maximum) with increasing dose up to a threshold, after which toxicity increases with dose (**Figure 1a**). Not surprisingly, proponents of hormesis are frequently at odds with other scientists, particularly those who favor a no threshold, linear dose–response curve (**Figure 1b**) or a threshold dose–response curve (**Figure 1c**). The beneficial effects attributed to low-dose exposure to various toxic chemical and physical agents include increased life span, improved rates of growth and development, decreased tumor incidence, and increased resistance to infection and tolerance to radiation.

Radiation Hormesis

One of the most intensively studied areas of hormesis research is the effects of exposure to low-level ionizing radiation (LLIR). The concept that LLIR can produce beneficial effects in biological systems challenges the conventional radiation paradigm which asserts that (1) radiation exposure is harmful at all dose levels (i.e., there is no threshold); and (2) there are no effects at low doses that cannot be predicted from effects observed at high dose levels (i.e., linear or near-linear dose–response curve). The application of this paradigm extends from single cells to entire populations where it is assumed that the risk of deleterious effects to a population increases directly with the aggregate radiation exposure. In contrast, proponents of hormesis have argued that LLIR exposure produces health benefits that translate into a decreased risk (relative to zero level exposure) of the deleterious effects associated with higher doses of radiation exposure.

There is no doubt that biological systems can respond to exposure to toxic physical and chemical agents in a manner that reduces the severity of the insult. Frequently, these responses are a result of altered gene expression, such as the increased synthesis of heat shock or stress proteins following exposure to thermal or oxidant stress. Such protective effects have also been demonstrated experimentally following exposure to low-level radiation when discrete, sensitive cellular and/or biochemical end points are measured. For example, the level of oxygen free radical scavengers, which protect against the toxic effects of radiation, was shown to increase in bone marrow cells of mice after whole body exposure to as little as 0.1 gray (Gy) of cesium-137 gamma irradiation.

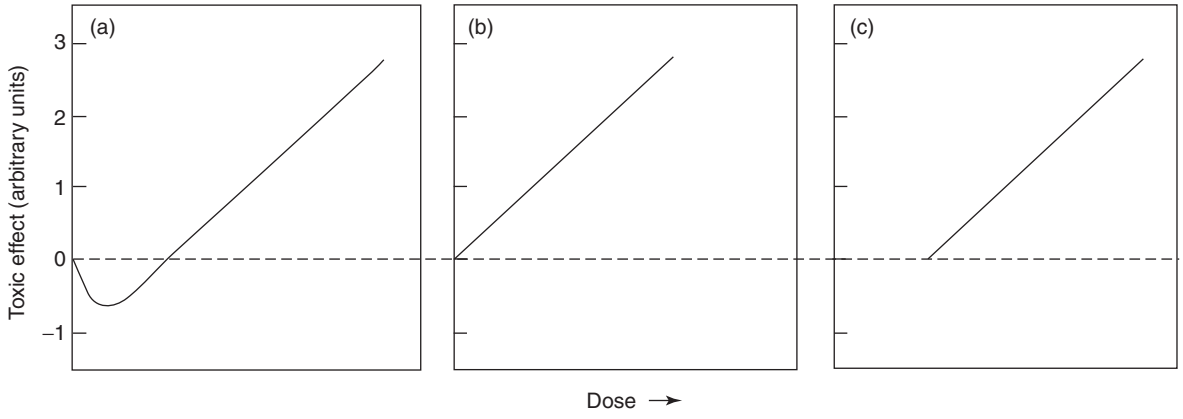

Figure 1 Idealized dose–response curves for the (a) hormesis, (b) linear, and (c) threshold hypotheses. A 'positive' toxic effect is regarded as detrimental, whereas a 'negative' toxic effect is beneficial (hormetic).

(The Gy is a unit of dose absorbed by a material and is equivalent to $1\,J\,kg^{-1}$.) Temporary suspension of DNA synthesis was also observed after 0.01 Gy exposure, an effect that might result in the formation of fewer free radical-mediated DNA lesions during the exposure and facilitate the repair of lesions caused by free radical attack. If a higher concentration of radical scavengers or antioxidants was produced or released than required for neutralization of the excess free radicals produced by irradiation treatment, this would constitute hormesis. The 'excess' radical scavengers available might produce a beneficial effect by protecting against the damage caused by background radiation, by subsequent free radical challenges induced by radiation exposure, or, alternatively, by normal cellular metabolism.

In another example, the growth of lymphocytes in the presence of $0.01–0.10\,\mu Ci\,ml^{-1}$ of [^{3}H]thymidine or very low (0.005–0.01 Gy) doses of X-rays, while not producing deleterious effects, has been reported to confer resistance to chromosomal breakage upon subsequent exposure to 1.5 Gy of X-rays. (The curie (Ci) is a unit of radioactive source activity and is equivalent to 3.70×10^{10} disintegrations per second.) The low-dose radiation-associated decrease in DNA damage could be eliminated by the addition of 3-aminobenzamide, an inhibitor of poly(ADP-ribose) polymerase when added after the high-dose exposure. This indirect evidence suggests that the LLIR exposure induces a DNA repair mechanism involving this enzyme, and thus, hormesis. Critics of these studies have suggested that the small doses of LLIR employed would result in only very small, transient increases above the normal steady-state concentrations of intracellular free radicals and thus would be unlikely to activate repair mechanisms.

Investigations with the protozoan *Paramecium tetraurelia* and the cyanobacterium *Synechococcus lividus* have provided evidence for hormesis using cell growth parameters as a biological endpoints. The growth rate of either organism within chambers shielded from normal background radiation was depressed up to 50% when compared with growth in unshielded chambers. Critical to the hormesis concept, the growth rate in shielded chambers could be restored to unshielded values when the organisms were subjected to LLIR exposure at doses comparable or slightly greater than normal background levels ($7–20\,mGy\,year^{-1}$). This LLIR stimulatory effect disappeared at dose rates $\geqslant 50\,mGy\,year^{-1}$. These data have been interpreted to suggest that LLIR stimulates proliferation of these single-cell organisms, although an exact mechanism(s) has not been demonstrated.

Epidemiological studies involving large populations remain the best means available for the study of potential hormetic effects in humans. A study of atomic bomb survivors from Nagasaki suggested that significantly lower (65% of control) mortality rates from noncancerous diseases occur in males exposed to 50–149 cGy of bomb-related radiation as compared to unexposed, age-matched controls. However, these data conflict with those of other studies that have failed to produce significant evidence for beneficial effects among atomic bomb survivors exposed to low levels of radiation. Some proponents of radiation hormesis have suggested that the lack of hormetic effects among atomic bomb survivors is because the data set represents the effects of acute exposure to radiation and is, therefore, not a valid comparison to the chronic, LLIR exposure ideally associated with hormesis.

The relationship of the annual cancer incidence rate to the intensity of natural background radiation has also been studied. The annual age-adjusted mortality rate from all cancers in five major Indian cities was found to decrease at a rate of $0.3\,mrem^{-1}\,year^{-1}$ increase in the external background radiation dose from a hypothetical incidence level of 79 per 100 000 corresponding to a hypothetical 'zero environmental radiation' level. (The rem is a unit of the biological effects of radiation and is equivalent to the dose absorbed $\times$ a quality factor specific for individual types of radiation.) A similar study from the Guangdong province of China examined two similar population groups of 60 000 people living in adjacent areas but whose exposure to background radiation was 330 and 114 $mrem\,year^{-1}$, respectively. An analysis of total cancer mortality from 1970 to 1986 failed to show a significant beneficial (hormetic) effect in the high background group. When nonleukemic cancers of inhabitants aged 40–70 years from 1970 to 1986 or all cancers within the entire population from 1975 to 1978 were considered separately, however, a significant 14.6% lower cancer mortality rate in the high background group was found. Interpretations of these studies are controversial and have been criticized primarily because of the problems associated with the limited time spans evaluated, lack of an accurate assessment of radiation exposures, and confounding factors such as smoking, diet, and lifestyle.

A number of epidemiological studies have reported that inhalation of naturally occurring radon in the home is associated with an increased risk of lung cancer. In contrast, a large US-wide ecological study performed in 1995 reported an inverse relationship between the average county radon concentration and the average lung cancer rates in the county. This report of an apparent hormetic effect of radon exposure has sparked considerable debate among

investigators in this area. Critics contend that consideration of the mean risk for a county rather than the more accepted practice of using individual risk has biased these results. It has also been suggested that confounding social and geographical factors were not adequately considered in these results. In spite of these criticisms, this study remains a subject of considerable discussion and intrigue within the scientific community.

Chemical Hormesis

Numerous reports of chemical-mediated hormesis in response to exposure of plants or animals to a wide variety of compounds appear in the literature. Chemical hormesis is characterized by a biphasic or U-shaped dose–response curve (**Figure 1a**), where very low doses of toxicants have a beneficial or stimulatory effect, relative to control exposures, and moderate to high doses are obviously toxic. The concept of chemical hormesis also challenges the linear, no threshold dose–response model (**Figure 1b**) and the threshold dose–response model (**Figure 1c**), especially with respect to carcinogens. Metals, in particular, have received much attention with respect to possible hormetic effects. Administration of $22\,mg\,kg^{-1}$ of tetramethyl lead, a known central nervous system toxicant, for 60 days to pregnant rats and subsequently to their offspring was reported to produce an increase in weight gain in the absence of deleterious effects on brain weight or morphology. The authors have presumed this represents a stimulatory effect of low levels of lead on body growth, although critics have maintained that it could also result from stimulation of appetite.

Preexposure of the tidal fish *Fundulus heteroclitus* to $0.05\,mg\,l^{-1}$ concentrations of cadmium prior to partial amputation of the caudal fin resulted in a 5–15% faster rate of regeneration in water containing $0.1\,mg\,l^{-1}$ cadmium compared with unexposed fish allowed to regenerate in 'clean water' assumed to be cadmium free. This effect, however, was observed in only two of three experiments conducted. While the mechanism for this effect was not clear, the increased rate of regeneration was hypothesized to be due to an overcompensation of homeostatic regulation resulting in accelerated growth.

Low concentrations of carcinogenic polycyclic aromatic hydrocarbons (PAHs) have also been evaluated for a potential hormetic effect. Exposure of grunion embryos (*Leuresthes tenuis*; a freshwater teleost) to 7 ppb of benzo[*a*]pyrene resulted in significantly increased respiration rates compared with unexposed controls, whereas at higher concentrations of 24.2, 361, or 868.8 ppb, respiration rates were not significantly different from control. Although no benefit of the increased respiration rate is obvious, it may be that this or similar metabolic responses to low-level PAH exposure represent an overcompensatory response typical of hormesis. It might be argued, however, that an adaptive biological response to low toxicant exposure in the absence of a clearly demonstrated benefit, preferably at the molecular mechanism level, does not constitute hormesis.

Hormesis studies in humans have been performed using populations exposed occupationally to toxic chemicals. Chronic exposure to low levels (5–40 ppm) of the hepatotoxicant, trichloroethylene (TCE), has been associated with changes in cholesterol metabolism rather than causing hepatic cell damage. In particular, elevated levels of serum high-density lipoprotein-associated cholesterol (HDL-C) were noted, while levels of the serum enzymes aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transpeptidase were unaffected. Because HDL-C is thought to be protective against coronary heart disease and to improve longevity, elevated serum levels may have a beneficial effect. However, the significance of the trend for HDL-C to increase with the dose of low-level exposure was only marginal ($p = 0.08$). Furthermore, evidence for decreased incidence of heart disease and/or increased longevity was not part of this epidemiological study; perhaps a follow-up investigation is possible. What we are left with is the demonstration of a biological change that is apparently associated with low-level occupational exposure to TCE and that may be beneficial. However, further epidemiological studies employing definitive biological end points, with careful controls for lifestyle and other variables, are required to definitively demonstrate that chemical hormesis is a common beneficial mechanism resulting from low-level exposure to toxic chemicals in humans.

Conclusions

Adaptations based on changes in gene expression and/or post-translational protein modification are commonly elicited in response to exposure to toxic physical or chemical agents. It is widely believed that these responses act to reduce, but not eliminate the severity of injury caused by all doses (i.e., no threshold) of the agent. In contrast, the theory of hormesis predicts that while higher concentrations of these agents are indeed deleterious, low level exposure elicits a net beneficial response from the organism. While many of the studies that purport to show hormesis suffer from flaws in experimental design and an unimpressive response (the difference

between biological significance and statistical significance is relevant here), the volume of published research material alone suggests that further investigation is warranted. Important aspects that must be evaluated include the dose–response relationship and the specific mechanism or mechanisms of the 'hormetic effect'. Additional insight might also be gained through reevaluation of data sets from previous studies in which appropriate end points have been evaluated and/or from archival databases of epidemiological data. Indeed, a number of recent studies have reexamined the data from previous chemical and radiation toxicity experiments and have identified numerous examples of possible chemical hormesis for a variety of end points studied.

In conclusion, chronic exposure of organisms to physical or chemical stressors can result in changes in gene regulation that confer a protective (detoxication) effect against the stressor in the exposed organism. The issue remains, however, whether chronic exposure to low concentrations of toxic chemicals or radiation can confer long-term beneficial effects to the organism and whether this is a general protective response. However, given the conservative nature of risk assessment, even irrefutable evidence for a hormetic response to a particular toxic agent is unlikely to lead to abandonment of the conservative linear, no threshold dose–response model.

See also: Dose–Response Relationship; Radiation Toxicology, Ionizing and Nonionizing.

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Hormone Disruptors *See* Environmental Hormone Disruptors.

Hormones *See* Anabolic Steroids; Androgens; Estrogens I: Estrogens and Their Conjugates; Estrogens II: Catechol Estrogens; Estrogens III: Phytoestrogens and Mycoestrogens; Estrogens IV: Estrogen-Like Pharmaceuticals; Estrogens V: Xenoestrogens.

Host-Mediated Assay

David A Eastmond

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The host-mediated assay (HMA), developed in the late 1960s by Gabridge and Legator, is an approach for providing the *in vivo* metabolism of a whole animal (the host) for assessing effects on indicator cells that have been placed in the host during chemical exposure and then subsequently removed for *in vitro* measurements of mutagenicity. Due to the limited metabolic capacity of most bacterial and mammalian target cells used for *in vitro* genetic toxicology, a number of chemicals found to be mutagenic and carcinogenic in whole animals were without genotoxic effects *in vitro* as the assayed cells lacked the metabolic systems which could convert the nonreactive test chemicals into the reactive electrophiles that were capable of interacting with nucleic acids and proteins. The HMA was consequently developed to overcome the metabolic limitations of commonly used test cell lines.

The HMA is of historical significance to the field of genetic toxicology primarily for two reasons: (1) its development preceded that of the now widely used exogenous liver homogenates (e.g., the 9000 *g*

supernatant fraction, termed S9) that are added to *in vitro* test systems to approximate *in vivo* metabolic pathways; and (2) in the early 1970s, the HMA was one of three original screening tests recommended for evaluating the mutagenic effects of chemicals, together with the dominant lethal test and the *in vivo* cytogenetic assay.

In the host-mediated mutagenicity assay as initially defined, a microbial indicator organism in which mutation frequencies could be measured, was injected into the peritoneal cavity of the host, and the host was then treated with a potential chemical mutagen. Subsequently, the microorganisms were withdrawn from the host, and the induction of mutants was assessed following growth of the microorganism *in vitro*. Application of the HMA was later expanded to include additional sites of inoculation and recovery of the indicator cells (such as the blood, intestinal tract, liver, spleen, lungs, and testes) and the use of nonbacterial indicator cells, including *Neurospora,* yeast, and various types of mammalian cells.

Since its introduction, the HMA has been used to evaluate several hundred chemicals, with the measured genetic effects including forward and reverse mutations, recessive lethal mutations, mitotic gene conversion, mitotic recombination, differential DNA repair, sister chromatid exchanges, chromosome deletions and aberrations, and alterations in cellular and colony morphology. However, the HMA was not universally successful, in part because in the early 1970s it was initially coupled with mutagenesis systems that were insufficiently defined and validated. Other problems included a failure to allow for animal-to-animal variability, contamination of bacterial indicator cells with intestinal flora, and difficulties in differentiating between the mammalian indicator cells and cells from the host.

As a result, the HMA was no longer recommended for mutagenicity testing for regulatory submissions, when the technically more straightforward and less expensive use of S9 metabolic activation became available. It was also found that very few chemicals were positive in the HMA that were not also positive for mutagenicity when tested with S9, primarily due to the predominance of activating enzymes in this subcellular fraction. It should be remembered, however, that *in vitro* S9 metabolic activation systems cannot address *in vivo* processes such as absorption, storage and excretion, hormonal influences, and tissue-specific metabolism, including preferential enzymatic detoxification.

After a decade of use, host-mediated mutagenicity testing was essentially abandoned for regulatory testing. More recently, a number of the problems initially encountered with the HMA have been resolved or are capable of resolution. *In vitro* mutagenesis systems have now been extensively defined and evaluated; animal-to-animal variability can be minimized by using a sufficient number of animals per dose; and both contamination of indicator cells and difficulties in differentiating between cells from the host and mammalian indicator cells can be overcome by using devices such as diffusion chambers which can be surgically implanted into the host.

Considering these developments, the HMA could be employed more frequently. However, in practice, the HMA continues to be used infrequently in genetic toxicology and is applied primarily for specialized research needs. In addition, the need for the HMA has been supplanted in recent years by the use of metabolically competent cell lines and new *in vivo* mutation detection systems such as the transgenic Big Blue or Mutamouse systems, which in some ways may be viewed as more technologically advanced forms of the HMA.

See also: Ames Test; Carcinogenesis; Chromosome Aberrations; Dominant Lethal Tests; *In Vitro* Test; *In Vivo* Test; Molecular Toxicology–Recombinant DNA Technology; Mouse Lymphoma Assay; Sister Chromatid Exchanges; Toxicity Testing, Alternatives.

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Hydrangea

Brenda Swanson-Biearman

- SYNONYMS: *Hydrangea arborescens*; *Hydrangea macrophylla*; *Hydrangea paniculata*; Seven bark; Wild hydrangea
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Cyanogenic glycosides

Uses

Although there are no accepted therapeutic uses for hydrangea, the dried root has been reportedly used as a diuretic and cathartic.

Exposure Routes and Pathways

Exposure occurs via dermal contact and ingestion.

Toxicokinetics

Small doses of cyanide are converted to thiocyanate by an enzymatic reaction catalyzed by rhodanase. The rhodanase system can detoxify large amounts of cyanide but may not respond quickly enough to avert serious symptomatology.

Mechanism of Toxicity

The leaves and buds contain hydrangin, which has the potential to produce cyanide. When the plant material is ingested, it reacts slowly in the acid pH of the stomach. Once transportation into the alkaline medium of the duodenum occurs, the process is hastened. Hydrocyanic acid is produced forming a stable complex with the ferric iron and cytochrome oxidase, thereby inhibiting the activity of the enzyme and aerobic metabolism. Cells containing cytochrome oxidase become hypoxic because they are unable to utilize available oxygen. However, symptoms of cyanide poisoning from exposure to *Hydrangea* species have not been reported in modern medical literature.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity associated with hydrangins is not known in animals, but ruminal microorganisms have the ability to degrade cyanogenic glycosides causing the release of hydrogen cyanide. However, no recent cases of toxicity have been described.

Human

Cyanide toxicity due to accidental exposure is unexpected. Patients ingesting hydrangea may develop vomiting and epigastric soon after exposure. Allergic contact dermatitis due to the sensitizer, hydrangenol, has been reported.

Clinical Management

In asymptomatic patients, activated charcoal and observation may be all that is necessary. In the unlikely event that patients exposed to hydrangea develop symptoms consistent with cyanide poisoning, ignore gastric decontamination procedures until life-support measures have been instituted. Cyanide antidote kit administration may be necessary.

See also: Cyanide.

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Hydraulic Fluids

Richard D Phillips

Uses

The most common hydraulic fluids are generally of three types: mineral oil, polyalphaolefin (PAO, synthetic oil), or organophosphate ester-based with a small percentage of additives. The majority of hydraulic fluids are mineral oil-based. Their primary function has typically been to transfer pressure from one point to another in machinery or mechanical operations. They also serve as lubricants depending on the specific application. They are used for automobile automatic transmissions, brakes and power steering, as well as heavy machinery.

Exposure Routes and Pathways

Exposure to hydraulic fluids occurs mainly in workers using hydraulic equipment and in maintenance workers on cars, tractors, airplanes, or similar equipment. The components of hydraulic fluids are in other lubricant products as well so exposure is not limited to hydraulic fluids. However, exposure is primarily to the major components (i.e., mineral oil, PAO, or organophosphate ester). Exposures would result from contact with the skin or inhalation of oil mist. In rare instances, oral exposure could occur by accident or eating contaminated food.

Toxicokinetics

The toxicokinetics of hydraulic fluids will be dependent on the type of base oil and the key additives, which in many cases are proprietary. Hydraulic fluids or some of their components are likely to be poorly absorbed from the gastrointestinal (GI) tract or lungs. Accumulation of oil in lungs could occur at high and fairly prolonged exposure. Absorption through the skin would likely be limited and dependent on duration of contact. Hydrocarbons found in mineral oils are not expected to undergo extensive metabolism. The same would be true for PAO-based hydraulic fluids. Some chemical components of organophosphate ester hydraulic fluids can enter the body from the lungs and GI tract.

Mineral oils and PAOs found in hydraulic fluids are not anticipated to undergo appreciable metabolism. There is some evidence that organophosphate esters are oxidized by cytochrome P450 and then form conjugates and are excreted.

Mechanism of Toxicity

Mineral oil and PAO-based hydraulic fluids are generally not toxic. They are expected to be absorbed only to a limited extent by lungs, skin, and the GI tract.

Certain organophosphate esters cause neurotoxicity due to cholinesterase inhibition. Current manufacturing processes for organophosphate esters used in hydraulic fluids are designed to minimize the production of toxic isomers such as tri-*o*-cresyl phosphate.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute toxicity reports on hydraulic fluids are very limited. Fairly high exposure levels would not be expected to be lethal ($LC_{50} > 1000\,mg\,m^{-3}$). Exposure to high concentrations of mist could cause damage to the lungs and may irritate the respiratory tract. Aspiration into lungs following oral exposure could cause severe lung damage and even death.

Organophosphate ester-based hydraulic fluids could cause neurologic effects but only if the toxic isomers are present in the fluid. Specific organophosphate esters have been synthesized to be toxic to insects. These organophosphate esters inhibit neural acetylcholinesterase and at toxic levels can cause increased salivation, watering of the eyes, perspiration, dilated pupils, nausea, vomiting, diarrhea, slowing of heart rate, and frequent urination. Symptoms can also include muscle cramps, weakness, and paralysis. Organophosphate esters can also cause a syndrome referred to as organophosphate-induced delayed neuropathy. This is a syndrome observed in humans and some animal models after exposure to certain organophosphate esters such as tri-*o*-cresyl phosphate.

Chronic Toxicity (or Exposure)

Animal

Hydraulic fluids have not been shown to be carcinogenic in animal cancer bioassays or mutagenic in other test systems.

Human

Hydraulic fluids are not likely to cause chronic toxicity or cancer as currently manufactured and used. Only one epidemiology study has been conducted for a mineral oil-based fluid, and there were no associations between exposure and cancer. The mineral oils used in hydraulic fluids are highly refined and not mutagenic or carcinogenic in animals. The same would be expected for PAOs.

Clinical Management

Gastric emptying by either lavage or vomiting is contraindicated since there is a danger of pulmonary aspiration and subsequent pneumonitis. If a person is overexposed to oil mist, the victim should be moved to fresh air as quickly as possible and monitored to make sure that chemical pneumonitis is not present. If acute effects of central nervous system depression are present, the appropriate treatment may be indicated.

Washing with soapy water is suggested following dermal contact, and ocular washing with water following eye contact.

Environmental Fate

Hydraulic fluids represent a wide range of products which are formulated to conform to performance specifications and not to specific chemical or fate analysis. However, some conclusions can be made based on what's known regarding the major

component (i.e., the base oil). The carbon number present in mineral oil hydraulic fluids ranges from C_{15} to C_{50}. These oils have low water solubility and will tend to partition to sediments if released. These oils will degrade over time and show little tendency to bioaccumulate.

See also: Fuel Oils; Organophosphates.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hydraulic Fluids.

Hydrazine

Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 302-01-2
- Synonyms: Diamine; Hydrazine anhydrous
- Chemical/Pharmaceutical/Other Class: Miscellaneous nitrogen compounds
- Chemical Formula: H_4N_2

Uses

Hydrazine is a chemical intermediate for explosives. It is used in soldering fluxes, as a laboratory reagent, and as a substance to reduce metals of silver, gold, and copper. Hydrazine is also used in the manufacture of certain drugs, dyes, rocket fuel, photographic supplies, insecticides, plastics, corrosion inhibitors, and textiles.

Exposure Routes and Pathways

Exposure to hydrazine may occur by inhalation, dermal contact, ingestion, and injection.

Toxicokinetics

Hydrazine is a colorless oily liquid that fumes and is flammable. Absorption occurs by all routes of administration. Several possible metabolic pathways exist for hydrazine and include hydrolysis, acetylation, and the splitting of hydrazine into two equal amines. Acetylation appears to have a major role in humans and slow acetylators may be more susceptible to toxic effects. Hydrazine and its metabolites are excreted by the kidneys.

Mechanism of Toxicity

Hydrazine has strong caustic action on the skin and mucus membranes. Hydrazine produces a functional pyridoxine deficiency that can lead to seizure activity. Hydrazine causes hepatic necrosis. The gastrointestinal effects are due to local irritation and corrosive effects if ingested.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation exposure to guinea pigs and dogs resulted in rapid weakness, significant liver damage, and injury to the kidneys and lungs. Muscular tremors have been noted in dogs following inhalation exposure. Exposure to pregnant toads to hydrazine resulted in teratogenic effects. Pregnant rats exposed to hydrazine had dose-related embryolethality and maternal toxicity. There was increased tumor incidence in mice (pulmonary tumors), rats (liver tumors), and hamsters (liver tumors). Hydrazine also reduces the latency period associated with the development of tumors in exposed rats. Hydrazine has been shown to be mutagenic in bacteria, viruses, and mammalian assays.

Human

Hydrazine's odor is ammonia-like or 'fishy' and is detectable by smell at 1–10 ppm. Because hydrazine is a marked corrosive, it can cause chemical burns of the skin. Hydrazine vapors may cause irritation of the mucus membranes of the eyes, nose, throat, and respiratory tract. Inhalation of vapors can produce cough, dyspnea, and pulmonary edema. Eye exposure to vapors or liquid can result in conjunctivitis, corneal damage, and blindness. Other clinical effects include nausea, vomiting, tremors, dizziness, hyperreflexia, seizures, hypotension, liver necrosis, methemoglobinemia, and hemolysis. The National Institute for Occupational Safety and Health recommends that the level of hydrazine in workplace air not exceed 0.03 parts of compound per million parts of air (0.03 ppm) for a 2 h period. The Occupational Safety and Health Administration (OSHA) limits the amount of hydrazine in workplace air to 1 ppm for an 8 h workday. The Environmental Protection Agency (EPA) requires that spills or accidental releases into the environment of 1 pound or more of hydrazine be reported to the EPA.

Chronic Toxicity (or Exposure)

Human

Chronic dermal exposure to hydrazine can result in eczema and skin sensitization. The International Agency for Research on Cancer has determined that hydrazine is a possible human carcinogen. The EPA has determined that hydrazine is a probable human carcinogen. The American Conference of Governmental Industrial Hygienists currently lists hydrazine as suspected carcinogens, but has recently recommended that the listing of hydrazine be changed to that of animal carcinogen, not likely to cause cancer to people under normal exposure conditions.

In Vitro Toxicity Data

Hydrazine is mutagenic in several *Salmonella* models as well as in an *H. influenza* model. However, the *H. influenza* model, hydrazine was postulated to have both mutagen and antimutagen effects.

Clinical Management

If dermal or eye contact with the liquid occurs, the affected areas should be flushed thoroughly with water for at least 15–30 min and then observed for resulting irritation. In case of inhalation, the victim should be moved to fresh air and the patient should be monitored for respiratory irritation and pulmonary edema. If ingestion occurs, basic and advanced life-support measures should be utilized as necessary. Due to its potential caustic effects, gastrointestinal decontamination procedures and charcoal should be avoided. The use of methylene blue should be considered in the treatment of hydrazine-induced methemoglobinemia. Seizures should be treated with high-dose pyridoxine (vitamin B_6) at doses of 1–5 g intravenous and treated with benzodiazepines.

See also: Pyridoxine.

Further Reading

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Kirklin JK, Watson M, and Bondoc CC (1976) Treatment of hydrazine-induced coma with pyridoxine. *New England Journal of Medicine* 294: 938–939.

Hydrobromic Acid

Mary Lee Hultin

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 10035-10-6
- Synonyms: Hydrogen bromide; Hydrogen bromide, anhydrous; Anhydrous hydrobromic acid
- Chemical/Pharmaceutical/Other Class: Inorganic acid; Corrosive
- Chemical Formula: HBr
- Chemical Structure: H–Br

Uses

Hydrobromic acid is used in the manufacture of inorganic bromides for use in photography, pharmaceuticals, industrial drying, textile finishing, engraving, lithography, and in fire retardants. It is also used as a reagent in analytical chemistry.

Exposure Routes and Pathways

Exposure may occur by the oral, inhalation, dermal, and ocular routes.

Toxicokinetics

No information is available regarding the toxicokinetics of hydrogen bromide in humans or animals.

Mechanism of Toxicity

The primary mechanism by which hydrogen bromide exerts its toxic effects is via irritation upon contact with tissues. Hydrogen bromide is a potent irritant of the tissues of the mouth, nose, eyes, and respiratory tract.

Acute and Short-Term Toxicity (or Exposure)

Animal

In a comparative toxicity study, hydrogen bromide caused more severe burns to the skin than hydrogen chloride or hydrogen iodide. In another study comparing the acute toxic effects of hydrogen fluoride, hydrogen bromide, and hydrogen chloride, rats were exposed to 1300 ppm hydrogen bromide for 30 min. Rats were separated into two groups. The first group consisted of ordinary mouth-breathing rats and the second group consisted of rats fitted with an apparatus to simulate nose breathing. More than twice as

many rats in the pseudo-mouth-breathing group died as in the nose-only group and none died in the control group. The location of the lesion was found in the nasal passages of the nose-breathing group and in the trachea of the pseudo-mouth-breathing group. The inhalation (LC_{50}) in rats is 2858 ppm for 1 h. The inhalation LC_{50} in mice is 814 ppm for 1 h.

Human

Subjective responses of six human volunteers exposed to levels ranging from 2 to 6 ppm for several minutes were reported as follows: exposure to 5 or 6 ppm resulted in nasal irritation in all volunteers and throat irritation in one volunteer. Eye irritation was not reported at any of the tested concentrations. One individual noted nasal or throat irritation at the 3 ppm level. Odor was detectable at all concentrations tested. Higher levels of inhalation exposure can produce pulmonary edema. If a solution is splashed on the skin or eyes, it will cause a burn. Ingestion can cause burns of the stomach.

Two clinical cases of reactive airways dysfunction syndrome were reported after inhalation exposure in a hot tub where hydrobromic acid and bromine were generated from the disinfectant used.

Clinical Management

Personnel not wearing personal protective equipment should be restricted from areas of spills or leaks until cleanup has been completed. In the case of exposure to the eyes or skin from hydrogen bromide solutions, contaminated areas should be flushed with copious amounts of water for at least 15 min. If an individual inhales large amounts of hydrogen bromide, this person should be moved to fresh air at once. Artificial respiration should be performed if breathing has stopped. In the event of ingestion of hydrogen bromide solution, large quantities of water or milk should be given immediately, provided the individual is conscious. Vomiting should not be induced.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 3 ppm ($10\,mg\,m^{-3}$) as an 8 h, time-weighted average. The American Conference of Governmental Industrial Hygienists and National Institute for Occupational Safety and Health recommend 3 ppm as a ceiling concentration. The Revised (1996) IDLH level is 30 ppm. The temporary emergency exposure limits (TEELs) are: TEEL-0, TEEL-1, and TEEL-2 ($\mu g\,m^{-3}$) 10 000; TEEL-3 ($\mu g\,m^{-3}$) 100 000.

The US Department of Energy classifications for TEELs are:

- TEEL-0 is the threshold concentration below which most people will experience no appreciable risk of health effects.
- TEEL-1 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing other than mild transient adverse health effects or perceiving a clearly defined, objectionable odor.
- TEEL-2 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing irreversible or other serious health effects or symptoms that could impair their abilities to take protective action.
- TEEL-3 is the maximum concentration in air below which it is believed nearly all individuals could be exposed without experiencing or developing life-threatening health effects.

See also: Acids; Corrosives.

Further Reading

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Hydrochloric Acid

Christopher P Holstege

This article is a revision of the previous print edition article by Mary Lee Hultin, volume 2, pp. 97–98, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7647-01-0
- Synonyms: Aqueous hydrogen chloride; Chlorohydric acid; HCl; Hydrochloride; Hydrogen chloride; Muriatic acid; Spirits of salt
- Chemical/Pharmaceutical/Other Class: Inorganic acid; Corrosive

Uses

Hydrochloric acid (HCl) is commonly used for the neutralization of alkaline agents, as a bleaching agent, in the synthesis of dyes and chemicals, and in metal refining.

Exposure Routes and Pathways

Exposure may occur via dermal, ocular, enteral, parenteral, or inhalation routes.

Toxicokinetics

HCl is colorless to light-yellow as both a gas and liquid with an irritating, pungent odor. HCl dissociates in water to hydronium and chloride ions. HCl is highly water soluble.

Mechanism of Toxicity

HCl causes local pH changes and denatures proteins. This leads to edema formation and tissue necrosis. HCl produces a coagulation necrosis characterized by the formation of an eschar. Ingested HCl may give rise to damage of the esophagus and stomach. Gastric damage may occur secondary to pooling of HCl in the antrum as a result of pylorospasm. Patients who survive ingestions of HCl may develop stricture formation, gastric atony, and gastric outlet obstruction. When inhaled, HCl typically deposits in the upper respiratory tract and causes damage. Concentrated HCl can penetrate to the level of the brochioles and alveoli and cause subsequent damage to these regions.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, HCl is a severe irritant of the eyes and respiratory system. The 30 min LC_{50} values in rats and mice are 4701 and 2644 ppm, respectively. Animals exposed to high concentrations of HCl gas developed necrosis of the tracheal and bronchial epithelium, pulmonary edema, atelectasis, emphysema, and damage to the pulmonary blood vessels and liver. Chronic exposure to 10 ppm for 6 h day^{-1} for life did not cause neoplastic lesions or serious irritant effects in the nasal epithelium of rats. In experimental animals, exposure to a concentration of 1350 ppm hydrogen chloride gas caused clouding of the cornea after 1.5 h and exposure to 3000 ppm for 6 h caused slight erosion of the corneal epithelium.

Human

Acute eye exposure to HCl gas or solutions of HCl may cause eye irritation and permanent damage with loss of sight. Dermal exposure may cause burns, with degree depending upon the concentration and duration of the exposure. Inhalation of HCl immediately causes severe irritation with cough and choking sensation. Inflammation and ulceration of the upper respiratory tract may occur. Pulmonary edema can develop if HCl gas is inhaled deeply. Excessive exposures (e.g., 1000–2000 ppm) for a few minutes can cause life-threatening pulmonary edema. Severe breathing difficulties may be delayed in onset. The current Occupational Safety and Health Administration permissible exposure limit (PEL) for hydrogen chloride is 5 ppm (7 mg m^{-3}) as a ceiling limit. Ingestion may cause corrosion of the mucous membranes, esophagus, and stomach with dysphagia, nausea, vomiting, abdominal pain, and hematemesis. Circulatory collapse and death may occur.

Chronic Toxicity (or Exposure)

Animal

Vapor concentrations of hydrochloric acid 100 ppm daily for 50 days in guinea pigs, pigeons, and rabbits resulted in symptoms of only minor irritation.

Human

Chronic exposure to HCl may cause erosion of the teeth, bronchitis, and gastritis. Repeated exposure of the skin to dilute solutions of hydrogen chloride may cause a rash.

In Vitro Toxicity Data

Low concentrations of HCl were required to dramatically reduce the spore concentration of *Bacillus subtilus*. Concentrations as low as 6 mg l^{-1} can inhibit some plant stem growth.

Clinical Management

The first priority in management of patients exposed to HCl is assuring patency of the airway. Direct visualization of the pharynx and vocal cords with fiberoptic devices may be necessary after inhalational and oral exposures to assure lack of injury. If signs of airway edema are present, intubation should be considered as swelling may progress over ensuing minutes and lead to airway obstruction. Fiber-optic intubation or orotrachael intubation with a laryngoscope can be attempted. Emergent surgical airway intervention may be necessary. Vomiting should not be induced. Gastric lavage, syrup of ipecac, activated charcoal, and cathartics should be avoided. There is questionable efficacy in giving water or milk to an awake and minimally symptomatic person who has ingested HCl. The utility of dilution decreases with time and should not be administered to persons with

vomiting, airway compromise, significant abdominal pain, or altered mental status.

Following ophthalmic exposure to HCl, the eyes should be irrigated immediately with water for at least 15 min and continued until pH neutralization. If skin exposure occurs, contaminated clothing should be removed and the exposed areas flushed with water. The exposed person should be moved to fresh air.

See also: Acids.

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Hydrocodone

Christopher P Holstege

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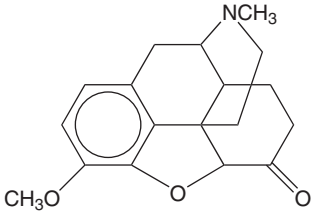
- Chemical Abstracts Service Registry Numbers: CAS 125-29-1 (Hydrocodone); CAS 143-71-5 (Anhydrous hydrocodone tartrate); CAS 34195-34-1 (Hydrocodone tartrate)
- Synonyms: Anexsia; Calmodid; Curadol; Damason-P; Dihydrocodeinone acid tartrate; Dihydrocodeinonum bitartaricum; Duodin; Hycodan; Hydrocodonhydrogentartrat; Hydrocodoni; Hydrocodonum; Hydrocodonium; Hydroconi; Hydroconum; Kolikodal; Lortab; Orthoxycol; Panacet; Procodal; Vicodan
- Chemical/Pharmaceutical/Other Class: Opiate agonist



Uses

Hydrocodone is used as an analgesic and as an antitussive. It has also been diverted as a drug of abuse.

Exposure Routes and Pathways

Ingestion is the most common route of exposure to hydrocodone. It is available as tablets and syrup. Hydrocodone has been solubilized and used parenterally as a drug of abuse.

Toxicokinetics

Hydrocodone is well absorbed from the gastrointestinal tract. At therapeutic doses of regular release products, peak serum levels occur within 1 h after ingestion and peak analgesia occurs within 2 h after ingestion. Extended release products can provide up to 12 h of antitussive effects. Hydrocodone is metabolized in the liver by *O*- and *N*-demethylation and 6-keto-reduction. The primary metabolites of hydrocodone are norhydrocodone, hydromorphone, 6-hydrocodol, and 6-hydromorphal. All metabolites have active pharmacologic activity. The volume of distribution is $3.3–4.7\,l\,kg^{-1}$. The kidneys are the main site of excretion. Unchanged drug ($\sim$12%) and metabolites are excreted in the urine. The elimination half-life is $\sim$4 h.

Mechanism of Toxicity

Hydrocodone is a semisynthetic, centrally acting narcotic analgesic and antitussive agent. It is postulated that the drug's antitussive effects come from its direct depression of the medulla. Analgesic effects are related to the stimulation of opiate receptors in the central nervous system (CNS). Interaction with the opioid receptors mimics the actions of endogenous enkephalins and endorphins. These actions result in analgesia, sedation, euphoria, and decreased gastrointestinal motility.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hydrocodone is used in small animal veterinary practice for the management of pain and for its antitussive properties. Hydrocodone should be used with

caution in cats due to likelihood of the development of stimulant effects (e.g., excitement, muscle spasms, seizures).

Dogs act similarly to humans – symptoms may include drowsiness, ataxia, vomiting, respiratory depression, miosis, coma, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone may be used at $0.02\,mg\,kg^{-1}$ if needed.

Human

CNS depression is the most frequently reported clinical effect. The typical overdose patient may present with extreme somnolence that may progress to frank coma. Miosis is usually present unless the individual is acidotic or has suffered hypoxic brain injury. Respiratory depression can occur and may progress to respiratory arrest. Pulmonary edema may be seen. Bradycardia, hypotension, and hyperthermia can develop. Hydrocodone is often combined in products with acetaminophen; therefore, patients should be evaluated for hepatotoxicity secondary to acetaminophen overdose. Available opiate immunoassays cross-react unreliably with hydrocodone. Peak therapeutic serum levels are $0.024\,mg\,l^{-1}$; toxic levels have been reported to reach $0.1–1.3\,\mu g\,ml^{-1}$, but are of little prognostic or therapeutic value.

Chronic Toxicity (or Exposure)

Animal

Studies have been conducted in animals to attempt to clarify the role of varying rates of drug metabolism in humans and animals and the likelihood for development of addiction/dependence to narcotics. Hydromorphone has been studied in several animal models because it is metabolized in humans by specific cytochrome P450 isoenzymes. So far, studies have looked at administration of agents that either block or promote P450 isoenzymes responsible for hydrocodone metabolism and the impact of enhanced or degraded metabolism of hydrocodone on those animal models. Results in rats and rhesus monkeys have demonstrated little effect from modifying hydrocodone metabolism.

Human

Hydrocodone has the potential for abuse. Chronic users may develop tolerance, thus necessitating larger doses for the desired effect. Abrupt cessation can cause withdrawal, yielding restlessness, insomnia, hypertension, tachycardia, tachypnea, vomiting, and diarrhea. Chronic use of hydrocodone in humans has also been associated with hearing loss.

Clinical Management

In patients presenting with hydrocodone toxicity, the airway should be patent and adequate ventilation assured. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk for subsequent CO_2 narcosis, worsening acidosis, and/or aspiration. If necessary, endotracheal tube intubation should be performed. The initial treatment of hypotension consists of intravenous fluids. Close monitoring of the patient's pulmonary exam should be performed to assure that pulmonary edema does not develop as fluids are infused. The patient should be placed on continuous cardiac monitoring with pulse oximetry. Frequent neurological checks should be made. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,gm\,kg^{-1}$) may be administered with substantial recent ingestions. Syrup of ipecac is contraindicated after overdose with the hydrocodone due to the potential for rapid clinical deterioration. Gastric lavage should be avoided.

Naloxone may be of benefit in reversing the neurological and respiratory depressant effects of hydrocodone. A dose of 0.4–2.0 mg is given intravenously slowly, titrated to resumption of adequate respirations, and can be repeated as needed. The therapeutic effect of naloxone may be of shorter duration than that of hydrocodone activity; therefore, it is imperative that hydrocodone intoxicated patients who demonstrated improvement after naloxone be closely monitored for resedation. Vital sign measurements and neurological checks should be monitored frequently.

See also: Acetaminophen; Drugs of Abuse; Hydromorphone.

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Hydrofluoric Acid

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 7664-39-3
- Synonyms: Hydrogen fluoride; Hydrofluoride; HFA
- Chemical Formula: HF

Uses

Hydrofluoric acid (HFA) is the inorganic acid of elemental fluorine. HFA is used in fluorocarbons, fluoropolymers, aluminum production, stainless steel pickling, uranium processing, glass etching, oil well acidizing, gasoline production, removal of sand and scale from foundry castings, and as a laboratory reagent. Anyone using HFA should understand the safety measures required to protect human health, for example, read the relevant Material Safety Data Sheet (MSDS), call the supplier for additional information if necessary, and confirm that the personal protective equipment (PPE) that has been shown to effectively protect against HFA exposure. In addition, the PPE should be checked carefully before each use, for example, a pinhole-sized hole could cause problems since HFA can penetrate deeply into skin and muscle tissue and simply flushing the area with water is not enough.

Exposure Routes and Pathways

Accidental dermal exposure is the most common route for human exposure; inhalation and ingestion are also possible. Occupational sources include the manufacture of chemicals, photographic film, solvents, and plastics.

Toxicokinetics

HFA rapidly corrodes and penetrates the skin and mucous membranes. Fluoride ions are then readily absorbed but are rapidly almost completely bound to available calcium and magnesium ions. The resulting salts are excreted.

Mechanism of Toxicity

HFA is toxic by ingestion, inhalation, and (most commonly) by dermal exposure. It is highly corrosive to the skin and mucous membranes with very short (5 s or less) exposure concentrations of 0.003% and above, acting by protonation of tissues. It causes a liquefying necrosis at the site of contact. Absorption of fluoride ions leads to systemic fluoride poisoning, in turn leading to hypokalemia and hypomagnesemia potentially resulting in neuromuscular paralysis and cardiac arrhythmias.

Acute and Chronic Toxicity

Animal

Signs of acute systemic fluoride intoxication include increased salivation, lacrimation, vomiting, diarrhea, muscular fibrillation, and respiratory, cardiac, and general depression. The inhalation LC_{50} is 1774 ppm in monkeys, 1276 ppm in rats, 4327 ppm in guinea pigs, and 342 ppm in mice. The intraperitoneal LD_{Lo} in rats is 25 mg kg^{-1}. Repeated inhalation exposure to concentrations in the range of 20–25 ppm produces injury to the lungs, liver, and kidneys. Dermal concentrations as low as 0.001% result in injury. Chronic exposure of guinea pigs and rabbits has caused injury of the cornea and mucous membranes. Repeated inhalation of 17 ppm HFA resulted in damage to the lungs, liver, and kidneys of animals, but inhalation of 8.6 ppm failed to elicit significant pathologic change in these tissues.

Human

HFA toxicity occurs after ingestion, inhalation, or ocular or dermal contact. HFA is one of the strongest and most corrosive acids known, and is highly irritating, corrosive, and poisonous. Therefore, special safety precautions are necessary when using this chemical. Inhalation of anhydrous HFA or HFA mists or vapors can cause severe respiratory tract irritation that could be fatal. The inhalation TC_{Lo} is 100 mg m^{-3} and the LC_{Lo} is 50 ppm. Ingestion of HFA produces pain and corrosion of the oral mucous membranes, esophagus, and stomach, and fatalities have occurred. The oral TC_{Lo} is 143 mg kg^{-1}. Systemic exposure can precipitate cardiovascular collapse quickly, with systemic hypokalemia and prolonged QTc interval. Chronic exposure via inhalation or ingestion can lead to fluorosis, with symptoms such as weight loss, malaise, anemia, leukopenia, discoloration of teeth, and osteosclerosis.

Clinical Management

Most burns are minor if they involve only small parts of the body surface area. When larger parts of the skin are burnt, morbidity and mortality significantly

increase, for example, if more than 20% of the body surface area is burnt with high concentration HFA, mortality approaches 100%. In major HFA burns, death almost always results from severe electrolyte imbalance. *Exitus letalis* (i.e., a fatal outcome) has been reported after a burn with 70% HFA involving as little as 2.5% of the body surface area. Unlike most minor HFA burns, which can be successfully managed by topical and regional therapy as well as close monitoring, major HFA burns require immediate critical care treatment. If the exposure is to the skin, all clothing should be removed from the affected region. The region should be copiously irrigated with water and then treated with a calcium gluconate paste. For exposure by any route, a 10% solution of calcium gluconate should be slowly infused (intravenously) to a total of 0.5 ml kg^{-1}.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists ceiling limit for exposure is 3 ppm. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h time-weighted average, is 3 ppm. The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day, is 3 ppm, the NIOSH ceiling limit for a 15 min exposure is 6 ppm, and the NIOSH 'immediately dangerous to life or health' value is 30 ppm.

See also: Acids; Corrosives; Fluoride; Fluorine.

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Hydrogen Cyanide *See* Cyanide.

Hydrogen Peroxide

David Eldridge and Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 7722-84-1
- Synonyms: Albone; Carbamide peroxide; Hydrogen dioxide; Hydroperite; Hydroperoxide; Inhibine; Perhydrol; Peroxan; Urea hydrogen peroxide; Urea peroxide
- Chemical/Pharmaceutical/Other Class: Antiinfective, topical antiseptic, cleansing agent
- Chemical Formula: H_2O_2

Uses

Dilute concentrations of hydrogen peroxide (up to 6%) are used as an antiseptic. In this capacity, it can be used as a mouthwash and topical wound cleanser. It is useful in loosening cerumen and clearing its impaction from the auditory canal. Dentists use hydrogen peroxide for teeth whitening. When a toxic ingestion is suspected, dilute hydrogen peroxide is commonly given orally by veterinarians to household pets in order to induce vomiting and achieve gastric decontamination.

Exposure Routes and Pathways

Ingestion is the most common route of reported toxic exposure. However, hydrogen peroxide exposure and toxicity can be multifaceted with absorption via inhalation, dermal, or ocular contact. Less common routes include direct instillation into body cavities and intravenous administration.

Toxicokinetics

With any direct exposure, absorption and most subsequent clinical effects are generally rapid.

Mechanism of Toxicity

One mechanism of toxicity by hydrogen peroxide involves gas release. Hydrogen peroxide, as an

oxidizing agent, liberates oxygen upon contact with tissue. This release is considerable as each volume of 3% solution produces 10 volumes of oxygen. This volume is problematic if this gas cannot easily escape. Another major concern, particularly in the case of industrial strength concentrations (>10%), is local, caustic tissue injury. Localized injury can occur with direct exposure to the skin, the eyes, and the gastrointestinal and respiratory tracts.

Acute and Short-Term Toxicity (or Exposure)

Human

Household strength (3%): Ingestion of small amounts usually causes only mild irritation to the mucosa and results in nausea and vomiting. Rarely, ingestions of household hydrogen peroxide has been linked to gastrointestinal erosions and ulcerations in children. Gastrointestinal distention and, in extreme cases, rupture of a hollow viscous can occur in the face of confined gas release. Eye exposure results in immediate but generally transient pain. Dermal exposure causes burning, tingling, and a temporary white discoloration of the skin. Colitis has been documented after use of dilute hydrogen peroxide enemas.

Industrial strength (>10%): More serious complications generally occur at strengths at >10%. Even small amounts of solutions containing >30% hydrogen peroxide have resulted in death when ingested. Ingestions cause corrosive burns to the mouth, throat, and gastrointestinal tract. Inflammation in this setting can also involve airway compromise from direct injury. Again the gas released may lead to viscous distension and rupture. In this setting, gas emboli of blood vessels are well-documented occurrences. These may travel to the brain with subsequent neurological sequelae. Cerebral edema and seizures have been noted with ingestion. Burns to the eyes and skin can be severe. Inhalation of concentrated hydrogen peroxide can potentially cause pulmonary irritation. As hydrogen peroxide is water soluble, its effects generally result in inflammation and irritation of the upper airway when inhaled.

Chronic Toxicity (or Exposure)

Animal

Dogs exposed to 7 ppm of 90% hydrogen peroxide 5 days a week for 6 months developed irritant symptoms on the skin, sneezing, lacrimation, irritated lungs at necropsy, and bleaching of the hair.

Human

A link between interstitial lung disease and chronic exposure to high aerosol hydrogen peroxide levels has been suggested in one case report. Some have suggested that chronic use of dilute hydrogen peroxide mouthwash may cause hypertrophied papillae of the tongue.

In Vitro Toxicity Data

Hydrogen peroxide is mutagenic in *Salmonella* and *Escherichia coli* models.

Clinical Management

Ingestion

No intervention is generally required for asymptomatic patients who have ingested small amounts of household hydrogen peroxide. If the patient has significant symptoms (bloody vomitus, abdominal distension, or discomfort), he/she should seek medical attention. Ingestion of milk or water in an attempt to dilute the hydrogen peroxide has not been proven to be beneficial. Similar exposure to concentrated solutions (>10%) requires more aggressive treatment. These patients are at a greater risk of becoming symptomatic and developing hematemesis, drooling, and stridor. Prompt medical evaluation is a necessity. Syrup of ipecac and activated charcoal are contraindicated. The patient's airway and vital signs should be closely monitored. A careful physical exam is performed initially to assess the extent of injury. This may need to be followed with endoscopic exam to more thoroughly characterize the extent and location of gastrointestinal injury. Hyperbaric oxygen therapy should be considered in cases of gas embolus, especially in patients with proof of cerebral involvement or central nervous system symptoms.

Inhalation

Removal from the source of injury is the primary treatment, and the patient should be promptly moved to fresh air and monitored for respiratory distress. More aggressive support such as supplemental oxygen and assisted ventilation may be used as indicated.

Eye Exposure

The primary intervention consists of irrigation with copious amounts of tepid water for at least 15 min. If symptoms are severe or persist, examination by a physician is indicated.

Dermal Exposure

First, contaminated clothing should be quickly removed. Then, the affected areas should be washed thoroughly with soap and water. If irritation or pain persists, or a chemical burn is present, examination by a physician is recommended.

See also: Mouthwash.

Further Reading

Cina SJ, Downs JCU, and Conradi SE (1994) Hydrogen peroxide – A source of lethal oxygen embolism – Case report and review of the literature. *American Journal of Forensic Medical Pathology* 15: 44–50.

Henry MC, Wheeler J, and Mofenson HC (1996) Hydrogen peroxide 3-percent exposures. *Clinical Toxicology* 34: 323–327.

Hydrogen Sulfide

Betty J Locey

- Chemical Abstracts Service Registry Number: CAS 7783-06-4
- Synonyms: Sulfur hydride; Dihydrogen sulfide; Hydrosulfuric acid; Stink damp; Sewer gas
- Chemical Formula: H_2S
- Chemical Structure: H:S:H

Uses

Hydrogen sulfide is formed naturally as well as produced by a number of commercial methods. It is commonly found in petroleum deposits, volcanic gases, natural sulfur springs, natural gas deposits, and anywhere an organic matter is decaying (e.g., manure and sewage). It may be released into the environment during operations at manufactured gas plants, paper mills, petrochemical plants, at tanneries, and production of heavy water for nuclear reactors. The primary source of hydrogen sulfide is reported to be as a by-product of purification of natural and refinery gases. It may be produced when sulfide or sulfuric acid is used in mixtures or when tanning leather, during glue making, metal recovery, and rubber vulcanizing.

Hydrogen sulfide is used in metallurgy, as a reagent in analytical chemistry, in the manufacture of sodium sulfide, in the production of heavy water, and, at one time, as an agricultural disinfectant. It is a major source of elemental sulfur and sulfuric acid.

Background Information

Hydrogen sulfide gas is highly toxic and can cause death within minutes if exposure is high enough. It is an important cause of morbidity and mortality in the workplace. Workers may be exposed to high levels in certain work environments, including poorly ventilated areas of wastewater treatment plants, in stagnant wells, in certain areas of petroleum refineries, and near fermenting manure. Hydrogen sulfide had been reported at concentrations greater than $700\,mg\,m^{-3}$ ($\sim$500 ppm) at a wastewater treatment plant; greater than $310\,mg\,m^{-3}$ ($\sim$221 ppm) in a stagnant well; and ranging from 70 to $280\,mg\,m^{-3}$ ($\sim$50–200 ppm) at an oil refinery in open maintenance ports.

Hydrogen sulfide has a distinctive and disagreeable odor (often described as smelling like rotten eggs). There is a wide range for reported odor thresholds in air. The American Conference of Governmental Industrial Hygienists (ACGIH) reported a geometric mean for odor thresholds as 0.0045 ppm ($\sim 0.0063\,mg\,m^{-3}$) and an accepted values range from 0.001 to 0.13 ppm ($\sim$0.0014–$0.18\,mg\,m^{-3}$) for those studies reported as reviewed. Odor cannot be used to warn of overexposure. The sense of smell becomes rapidly fatigued and cannot be relied on to warn of the continuous presence of hydrogen sulfide. Loss of sense of smell has been observed to occur after exposure to atmospheres containing between 50 and 150 ppm ($\sim$70 and $\sim 211\,mg\,m^{-3}$) hydrogen sulfide. Hydrogen sulfide levels ranging from 100 to 200 ppm ($\sim$140–$281\,mg\,m^{-3}$) have been reported to cause loss of the sense of smell and lead to olfactory paralysis.

Exposure Routes and Pathways

Hydrogen sulfide is a colorless gas and most human exposures occur via inhalation. However, exposure may also occur via ingestion or skin contact. Exposure to high concentrations is most likely to occur in the workplace. The general public may be exposed from industrial operations, from natural gas wells during drilling operations, and from natural sources. Exposure may be to low-levels, long-term, or high levels during an accidental release.

Exposure to hydrogen sulfide may also occur when precursors enter the body and are changed to produce hydrogen sulfide. Certain sulfhydryl-containing

amino acids (e.g., cysteine) may be metabolized by bacteria in the gut or in the mouth to produce hydrogen sulfide. It may also be produced, and then released after ingestion of soluble inorganic sulfide salts. Soluble salts, administered by injection, have been used in laboratory animals to study the effects of exposure to hydrogen sulfide. Some smooth muscle tissues (e.g., ileum, thoracic aorta, portal vein) and the brain have enzymes that produce hydrogen sulfide.

Toxicokinetics

Hydrogen sulfide gas is quickly absorbed through the lungs. Absorption may also occur through gastrointestinal tract. Absorption through the skin is limited, but does occur. Once absorbed into the body, hydrogen sulfide may be metabolized (changed by the body) or may remain unchanged until eliminated. The major metabolic pathway is oxidation in the liver. However, the kidneys also contain a sulfide oxidizing system. Oxidation may occur by nonenzymatic or enzymatic mechanisms. The primary product of oxidation is sulfate. Hydrogen sulfide may oxidize to the sulfate of thiosulfate in certain tissues. Thiosulfate can bind methemoglobin and form sulfmethemoglobin, which autooxidizes. Hydrogen sulfide may also be metabolized by methyolation and/or reactions with metal-containing proteins (metalloproteins).

Hydrogen sulfide and its metabolites may be eliminated from the body in the urine, through the lungs, or in the feces. The amount excreted by a particular route is influenced by the route of exposure. Hydrogen sulfide that is not metabolized (unchanged) is eliminated either in the volatile form through the lungs or in the feces. After oral exposure, most hydrogen sulfide is eliminated from the body in the urine as sulfate.

Mechanism of Toxicity

Contact with hydrogen sulfide can be irritating to tissues. Systemic effects are generally believed to be caused by changes that occur at the cellular level. Hydrogen sulfide inhibits cellular (mitochondrial) respiration. It binds proteins that are important to mitochondrial electron transport (cytochrome *aa* and cytochrome oxidase (last step in mitochondrial oxidative metabolism) and inhibits the conversion of molecular oxygen to water and the generation of adenosine triphosphate (ATP). ATP provides the energy required for many cellular functions. Hydrogen sulfide stops cellular respiration and leads to hypoxia (decrease of oxygen) at sufficiently high doses. The mechanism of toxicity is believed to be similar to that of cyanide.

Inhibition of respiration on the cellular level inhibits cellular function. Systems in the body with the highest oxygen demand are most vulnerable to its effects. These include the central nervous system, particularly the area that controls breathing, and the heart. High-level acute exposure may cause death due to a depressant effect on the respiratory center in the brain (area that controls breathing). Exposure to high levels leads to respiratory paralysis, pulmonary edema, and death. Chemoreceptors in the carotid body (small body of vascular tissue that is sensitive to changes in the concentration of oxygen in the blood) are believed to be responsive to sulfide in the blood and react to cause changes in breathing (rapid and/or deep breathing), respiratory depression, and apnea (transient cessation of breathing).

The following may contribute or cause the neurotoxicity associated with exposure to hydrogen sulfide.

- Inhibition of cellular respiration in tissues such as lung can lead to hypoxia (decrease of oxygen) and local tissue and cell damage.
- Hypotension (low blood pressure) and ischemia (restricted blood flow) associated with exposure reduces the delivery of oxygen to tissues and cells and may lead to sever damage and/or death of nerve cells (neuronal necrosis).
- High concentrations of hydrogen sulfide gas may alter pulmonary surfactants leading to pulmonary edema. Pulmonary edema can be the primary cause of death.
- Sulfide can irritate certain pulmonary nerves (e.g., afferent endings of the pulmonary vagi) and high doses can paralyze the ventilatory center.
- Sulfides may be selectively taken up by the rat brainstem. Since the brainstem plays a significant role in regulation of respiratory rhythm (catecholaminergic innervation of the brain begins within the brain stem and catecholamines and serotonin affect respiratory rhythm), changes in the levels of neurotransmitters may cause or contribute to loss of central control of breathing.

Acute and Short-Term Toxicity (or Exposure)

Hydrogen sulfide is a highly toxic gas. Inhalation can result in collapse and death in minutes if concentrations are high enough. The likelihood of adverse effects due to exposure to hydrogen sulfide depends on (1) level of exposure (e.g., concentration in the air), (2) how long the individual is exposed, and (3)

the individual's sensitivity to the effects of the chemical. For example, an individual with lung disease (e.g., emphysema or asthma) may exhibit certain symptoms before a healthy individual. Generally, the shorter the duration of exposure, the higher the concentration associated with adverse effects. Effects in animals and humans are generally similar.

Adverse effects may occur at the site of contact (e.g., lungs, eyes, and skin) or systemically. Hydrogen sulfide is a local irritant at low concentrations and may be irritating to all contact surfaces (eyes, skin, and respiratory tract). Contact with the vapor may cause irritation and/or conjunctivitis to the eyes ('gas eye') and lesions in the nasal tract. Prolonged or high-level exposure may cause more serious local effects (e.g., pulmonary edema). Exposure to hydrogen sulfide can cause neurotoxicity.

Systemic effects are serious and can be life threatening. Signs and symptoms of exposure also may include headaches, fatigue, dizziness, confusion, cardiac effects (e.g., tachycardia and bradycardia), cough, respiratory depression, nausea, cyanosis, and shortness of breath. Symptoms may progress to include pulmonary edema, apnea, seizures, coma, and death.

Animal

There is a fairly large body of animal data characterizing the toxicity of hydrogen sulfide. Studies have been conducted in monkeys, dogs, rabbits, mice, rats, and guinea pigs in which animals have been exposed via both inhalation and dermal contact. Inhalation studies have documented responses at different atmospheric concentrations over varying periods of time. Both local and systemic effects have been observed.

Single dose, short-term, and medium-term exposure to hydrogen sulfide via inhalation have caused a number of effects in test animals. These include nasal and respiratory tract irritation and damage, cardiovascular damage, neurotoxicity, changes in the immune system, and developmental toxicity. The respiratory tract has been identified as the most sensitive target organ and nasal lesions have been identified as the adverse effect occurring at the lowest exposure by several regulatory agencies.

Examples of results of animal studies include the following:

- Many studies have been completed to evaluate the concentration that causes death in study animals. Several examples are presented below.
  - Rat (Sprague–Dawley) 2 h lethal concentration in half the study animals (LC_{50} study) was reported as 820 mg m^{-3} (~586 ppm).
  - Rat (Fischer-344) 4 h LC_{50} was reported as 700 mg m^{-3} (~500 ppm).
  - Rat (Long–Evans) 6 h LC_{50} was reported as 470 mg m^{-3} (~336 ppm).
- Rats and mice exposed via inhalation to hydrogen sulfide at concentrations varying from 0.01 to 101 ppm (~0.014–142 mg m^{-3}) for various time periods exhibited irritation of the respiratory tract, anorexia, reduced weight gain, weight loss, changes in the lung, nerve cell abnormalities, changes in certain blood cells (increase in reticulocytes and changes in the mean corpuscular volume), and death.
- Rabbits exposed via inhalation to hydrogen sulfide at 72 ppm (~102 mg m^{-3}) exhibited disturbed liver, brain, kidney metabolism, serum protein, enzyme and mineral changes, decreased myocardial enzymes, cardiac irregularities, and unconsciousness. Dermal exposure resulted in changes in blood chemistry.
- Monkeys exposed via inhalation to hydrogen sulfide at 504 ppm (~710 mg m^{-3}) for various time periods exhibited eye irritation, changes in gray matter (brain tissue), necrosis in certain areas of the brain, moderate changes in the liver, hyperemia, ataxia, anorexia, sudden loss of consciousness, and cardiac arrest.
- Dogs exposed via inhalation to a range of concentrations of hydrogen sulfide from 100 to 1800 ppm (~141–2535 mg m^{-3}) exhibited effects ranging from local irritation to respiratory paralysis and immediate death.
- Rats and mice exposed via inhalation in several subchronic studies report olfactory nasal mucosa as the principal target site affected.

The potential for exposure to hydrogen sulfide to cause developmental effects has been studied. Several studies did not report effects, however, some suggest hydrogen sulfide may be a developmental neurotoxin. *In utero* and postpartum exposure was associated with significant changes in the levels of certain neurotransmitters (e.g., norepinephrine and serotonin) as well as certain amino acids (e.g., aspartate, glutamate) in several regions of the brain.

Human

Effects observed in animals are generally relevant to humans. Exposure to hydrogen sulfide may cause irritation, neurotoxicity, respiratory distress, pulmonary edema, headache, nausea, shortness of breath, dizziness, ataxia, chest pain, collapse, coma, and death.

Certain effects have been observed after acute and/or short-term exposure at ranges of concentrations. Ranges reported in the literature do vary and the

duration of exposure is important to the likelihood of seeing effects. The following provide examples.

- Exposure to very high concentrations (1000–2000 ppm or greater) can cause collapse in seconds (called 'knockdown concentration'). Exposure at 'knockdown' concentrations can cause paralysis of the respiratory center; breathing may stop, leading to collapse, and death within minutes. Another source reported a 'knockdown' concentration range of 500–1000 ppm.
- Exposure to 700 ppm can be rapidly fatal.
- Pulmonary edema may occur after short-term exposure to 250–600 ppm concentration.
- The sense of smell may be lost shortly after exposure to atmospheres ranging from 50 to 150 ppm. Other sources indicated the sense of smell is lost after exposure to 100–200 ppm.
- Prolonged exposure to 50 ppm has also been associated with pulmonary edema.
- Exposure for 1 h to 50–100 ppm may cause eye and respiratory tract irritation.
- Exposure to 14–25 ppm may cause burning eyes, headache, loss of appetite, weight loss, and dizziness.
- Conjunctivitis (eye irritation) has been reported after exposure to atmospheres containing 10–14 ppm hydrogen sulfide.

Data have been collected on how hydrogen sulfide is processed in healthy volunteers (primarily undergraduate and graduate students). Subjects were exposed to 7 or 14 mg m^{-3} ($\sim$5–10 ppm) (mouth breathing) for two 30 min sessions while engaged in aerobic exercise. Blood lactate concentrations increased, and there was a decrease/inhibition of the aerobic capacity of the exercising skeletal muscle. Men were more sensitive to this effect than women.

Hydrogen sulfide was released intermittently from an industrial source in the City of Terre Haute, Indiana, over a period of 2 months. Ambient air concentrations were reported to range from 0.002 to 8 ppm ($\sim$0.0028–11 mg m^{-3}). Twenty-seven residents complained of nausea, headache, shortness of breath, sleep disturbance, and throat and eye irritation during this time.

Chronic Toxicity (or Exposure)

Animal

Long-term animal studies were not identified.

Human

There is some evidence that chronic exposure and/or short-term higher level exposure (e.g., accidental incidents in the workplace) can result long-term effects (e.g., neurophychological and neurobehavorial changes). Long-term exposure to hydrogen sulfide has been reported to cause fatigue, headache, sleep disturbances, irritability, emotional disturbances, diminished memory, dizziness, nausea, vomiting, loss of appetite, weight loss, irregular heartbeat, lung congestion, and nerve damage. There is one report where exposure was believed to have lead to dementia.

Several epidemiological studies have been completed to evaluate the effect of long-term exposure to hydrogen sulfide in humans. In general, these studies did not have good information on the levels and duration of exposure. There is some evidence that exposure in the workplace was associated with increased incidence of spontaneous abortion. There are limited data and information that can be used to determine whether children are more susceptible that adults to the effects of hydrogen sulfide. Most data are anecdotal.

Hydrogen sulfide is not regulated a potentially carcinogenic to humans. None of the generally accepted sources classifies hydrogen sulfide as likely to cause cancer in humans. In 2003, the US Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) assessment described evidence as inadequate for an assessment of the carcinogenic potential. There are several epidemiological studies that address the carcinogenic potential of hydrogen sulfide. A cohort study of residents (Alberta, Canada) living downwind from natural gas refineries did not show an increase in cancer (from 1970 to 1984). A retrospective epidemiological study of residents of Rotorua, New Zealand, a city that uses geothermal energy for industrial and domestic heating, was conducted to study cancer in this population (from 1981 to 1990). The authors concluded it was not possible to evaluate the carcinogenic potential of hydrogen sulfide on the basis of human studies.

In Vitro Toxicity Data

Data indicate hydrogen sulfide is not mutagenic. Hydrogen sulfide was negative when tested in the Ames test with *Salmonella typhimurium* with and without s9 liver fractions. Hydrogen sulfide gas potentiated the mutagenicity of hydrogen peroxide as measured in *S. typhimurium*.

Clinical Management

The victim should be removed to fresh air and away from the source of exposure. Caution should be exercised by rescuers as high concentrations of hydrogen sulfide can cause collapse in seconds.

Oxygen should be provided if there is respiratory distress. Providing life support quickly may be critical to patient survival in certain cases. Administration of naloxone and dextrose may be indicated. For irritation, contaminated clothing should be removed and contaminated skin washed. Medical attention should be sought immediately for all symptomatic exposures. Patients who have had significant exposures should be closely monitored in a hospital.

Symptoms resemble those observed in cyanide poisoning and cyanide antidote kits may be useful for emergency treatment. Amyl nitrate by inhalation and intravenous sodium nitrite may be appropriate for certain patients. In laboratory animals, inducing methemoglobinemia with nitrates provides protection (even antidotal properties) against sulfide poisoning because the hydrosulfide anion (HS^-) can bind methemoglobin and form sulfmethemoglobin. This treatment has been used in some instances in humans.

Measurement of blood sulfide or urinary thiosulfide levels within 2 h can be used to confirm exposure to hydrogen sulfide if needed.

Environmental Fate

Hydrogen sulfide is colorless gas under normal environmental conditions. When released to the environment it will move with, and disperse in, the ambient air. It is estimated to remain in the atmosphere for an average of 18 h. This is generally longer in the winter. Once released to the ambient air, it may become oxidized and form sulfuric acid and/or sulfur dioxide. Oxidation may occur quickly by combination with hydroxide radicals or more slowly by combination with molecular oxygen. Sulfuric acid and sulfur dioxide may contribute to 'acid rain'. Hydrogen sulfide in the air may sorb to soil. Most is then converted to elemental sulfur. Several microorganisms can degrade hydrogen sulfide to sulfate or elemental sulfur (e.g., a heterotrophic fungi, a heterotrophic bacterium (*Xanthomona),* and a marine isopod (*Saduria* (*Mesidotea*) *entomon*).

Hydrogen sulfide is soluble in alcohol, ether, glycerol, gasoline, kerosene, crude oil, and carbon disulfide. It may also be dissolved in water (solubility at 20°C is reported as 1 g per 242 ml of water). In surface water it is generally oxidized by combining with oxygen or hydrogen peroxide. Oxidation is pH dependent. When dissolved in sewage water, sulfur is produced at pH ranging from 6 to 7, however if the pH ranges from 7 to 9 polysulfides, thiosulfates, and ultimately sulfates are formed. In some warm, damp environments (e.g., gravity sewers) it may be oxidized by autotrophic bacteria to sulfuric acid. The potential for evaporation is influenced by temperature and pH. Low pH and high temperature tend to favor evaporation.

As the pH increases, more sulfhydral radical (SH^-) is present. In general, the SH^- is not as biologically available (does not cross biological membranes as easily as hydrogen sulfide) and is therefore considered to pose less of a hazard.

Ecotoxicology

There are locations where hydrogen sulfide concentrations are naturally high (e.g., some geothermal vents, bogs of swamps with local area of decaying matter). In these areas, animal and plant life appear to be able to adapt.

For example, natural hydrocarbon seeps (oil and gas flow out of the ocean floor) have been reported to support dense biological communities. Hydrocarbon seeps produce methane and hydrogen sulfide. Certain bacteria can live on compounds like methane and hydrogen sulfide. Certain species (e.g., tube worms and mussels) can establish a symbiotic relationship with these bacteria and not only survive, but thrive in deep sea seeps. These populations may provide the basis for diverse community in the seep environment. The following are examples of life in areas that have naturally high levels of hydrogen sulfide.

- A seep identified in the Gulf of Mexico (at depths greater than 500 m) was reported to be populated with a worm species, living in a dense network of burrows.
- A sulfurous lake in New Zealand lake that is charged by an active underground geothermal vent reportedly averages hydrogen sulfide concentrations ranging from 5 to 3900 ppb. Studies do not report visible effects on local plant and bird populations.

At higher pH sulfhydral radicals (SH^-) in hydrogen sulfide impacted surface water may be toxic to fish.

Other Hazards

Hydrogen sulfide is a flammable gas. Toxic sulfur oxide fumes are produced when it is heated to decomposition. It can cause a flash fire and is a flash back hazard. In emergency situations full protection (such as positive pressure, pressure-demand, full-face piece, self-contained breathing apparatus (SCBA)) is recommended.

Hydrogen sulfide is heavier than air and tends to sink. Persons closer to the ground (e.g., fallen injured, children) may be exposed to higher concentrations

during a release. Hydrogen sulfide is incompatible with a number of materials, including strong oxidizers, certain metals, and strong nitric acid.

Exposure Standards and Guidelines

Criteria are usually developed for particular populations and are generally based on prevention of an effect that occurs at lower doses ('critical effect'), which, if the levels do not exceed criteria, is expected to prevent the occurrence of more serious effects known to occur at higher doses. Criteria are available that are protective of acute, short-term, and chronic exposure for workers and the general public for hydrogen sulfide. Different agencies have developed exposure criteria and standards for hydrogen sulfide. Selected criteria and guidelines are provided below.

Criteria for workplace exposure:

- The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) ceiling of 20 ppm with a 50 ppm concentration for a maximum of 10 min was set to protect workers against risk of 'eye irritation and conjunctivitis'.
- The ACGIH threshold limit value (TLV) is $14\,mg\,m^{-3}$, with a short-term exposure limit (STEL) of $21\,mg\,m^{-3}$.
- The National Institute of Occupational Safety and Health (NIOSH) recommended exposure limit (REL) ceiling of 10 ppm ($\sim 14\,mg\,m^{-3}$) for 10 min.

Criteria protective of the general public

- The US EPA has developed a chronic reference concentration (RfC) of $2 \times 10^{-3}\,mg\,m^{-3}$ (~ 0.0014 ppm) based on a critical effect of nasal lesions in the olfactory mucosa in rats (updated assessment in 2003). The RfC represents a daily exposure that is expected to be protective of the general public over a lifetime of exposure.
- The Agency for Toxic Substances and Disease Registry (ATSDR) has developed acute and intermediate inhalation minimal risk levels (MRLs) of 0.07 and 0.03 ppm (~ 0.099–$0.042\,mg\,m^{-3}$), respectively.
- The acute MRL was established based on effects reported in humans with the critical effect of respiratory effects-bronchial obstruction (30% change in airway resistance). It is intended to protect for exposure occurring up to 14 days.
- The intermediate MRL was established based on respiratory effects reported in mice. Details on how these values were derived are provided in ATSDR's toxicological profile for hydrogen sulfide.
- The World Health Organization (WHO) air quality guideline is $0.15\,mg\,m^{-3}$ (~ 0.11 ppm) (24 h average concentration). The guideline is based on protection of eye irritation. In addition, WHO recommends the average ambient air concentration, averaged over 30 min, of $7\,\mu g\,m^{-3}$ to avoid odor annoyance (published in 2000).

Acute exposure guideline levels (AEGLs) are airborne emergency exposure guidelines established for the general population (including sensitive populations) for periods ranging from 10 min to 8 h.

- AEGL-1: If exceeded, may cause discomfort, irritation, or effects. Effects are not expected to be disabling. Effects are expected to be short term and reversible.

Table 1 Summary of inhalation exposure criteria commonly used for hydrogen sulfide

Agency	*Standards and guidelines (ppm)*		*Averaging time*
OSHA	Ceiling	20	NA
OSHA	Maximum peak	50	10 min maximum peak
NIOSH	Ceiling	10	10 min
NIOSH	IDLH	100	NA
ACGIH	TLV TWA	10	8 h over a lifetime of work
ACGIH	STEL	15	15 min
NRC	EEGL	50	10 min
NRC	EEGL	10	24 h
ATSDR	MRL	0.03	14–365 days (intermediate exposure)
ATSDR	MRL	0.07	0–14 days (acute exposure)
US EPA	RfC	0.002	Daily dose over a lifetime exposure

NA, not applicable or not available; ppm, parts per million; OSHA, Occupational Safety and Health Act; NIOSH, National Institute for Occupational Safety and Health; ACGIH, American Conference of Governmental Industrial Hygienists; IDLH: immediately dangerous to life or health; EPRGs, Emergency Planning Response Guidelines (2003); NRC, National Research Council; EEGL, Emergency Exposure Guidance Level; MRLs, minimal risk levels; ATSDR, Agency for Toxic Substances and Disease Registry; US EPA, United States Environmental Protection Agency; RfC, reference concentration.

Table 2 US EPA Acute Exposure Guideline Levels (AEGLs) (Interim) as presented on the Internet

Duration of exposure	*AEGL 1 (ppm)*	*AEGL 2 (ppm)*	*AEGL 3 (ppm)*
10 min	0.75	41	76
30 min	0.6	32	59
60 min	0.51	27	50
4 h	0.36	20	37
8 h	0.33	17	31

- AEGL-2: If exceeded, may cause irreversible or serious, long-term adverse health effects. May impair the victim's ability to escape.
- AEGL-3: If exceeded, may cause life-threatening effects or death.

Representative inhalation criteria for hydrogen sulfide are summarized in **Tables 1** and **2**. **Table 2** provides a summary of the US EPA's AEGLs for hydrogen sulfide.

See also: Cyanide; Neurotoxicity; Occupational Exposure Limits; Respiratory Tract; Sensory Organs.

Further Reading

Agency for Toxic Substances and Disease Registry (ATSDR) (1999) Managing Hazardous Materials Incidents. Volume III – Medical Management Guidelines for Acute Chemical Exposures: Hydrogen Sulfide. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hydrogen Sulfide.

http://www.epa.gov – US Environmental Protection Agency. Toxicological review of hydrogen sulfide in support of summary information on Integrated Risk Information System (IRIS). National Center for Environmental Assessment, Washington, DC. EPA/635/R-03/005. June 2003.

Hydroiodic Acid

Mary Lee Hultin

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- Chemical Abstracts Service Registry Number: CAS 10034-85-2
- Synonyms: Anhydrous hydroiodic acid; Hydrogen iodide; Hydrogen monoiodide
- Chemical/Pharmaceutical/Other Class: Inorganic acid; Corrosive
- Chemical Formula: HI

Uses

Hydroiodic acid was formerly used as an expectorant in chronic bronchitis and bronchial asthma. It is used in the manufacture of disinfectants. It is also used for analytical purposes (e.g., as a chemical intermediate for inorganic iodides and organic synthesis).

Exposure Routes and Pathways

Exposure may occur via ingestion, dermal or ocular contact, or inhalation.

Mechanism of Toxicity

Hydroiodic acid is a strong irritant. When used as an expectorant, hydroiodic acid is believed to act by irritating the gastric mucosa, which then stimulates respiratory tract secretion.

Acute and Short-Term Toxicity (or Exposure)

Animal

A study compared the toxicity to rat skin of hydrogen iodide, hydrogen bromide, and hydrogen chloride. Hydrogen bromide caused the most severe burns in the rats. No data are available on animal studies examining effects from inhalation or ingestion.

Human

Inhalation of hydrogen iodide can cause irritation of the upper respiratory tract. A concentration of 35 ppm has been shown to cause irritation of the throat after short exposure. More severe exposures may result in pulmonary edema and laryngeal spasms. As with other acids, oral ingestion may produce oral and esophageal burns with more severe burns occurring in the stomach. Initial signs and symptoms may not reliably predict the extent of injury to the gastrointestinal tract. Tachycardia, hypotension, and circulatory collapse may occur as a result of the ingestion of concentrated corrosive iodine solutions. Severe burns may occur with dermal exposure. Systemic toxicity could result in acute hepatic injury.

Chronic Toxicity (or Exposure)

Human

Repeated exposures to fumes may cause erosion of the teeth. Chronic exposure may result in the development of bronchitis.

Clinical Management

In the case of inhalation exposure, the victim should be moved to fresh air. Trained personnel may administer oxygen and monitor the patient for signs of respiratory distress. Contaminated clothing and shoes should be removed and isolated. In the case of skin or eye contact, the skin or eyes should be flushed with running water for at least 15 min. Contact lenses should not be worn when working with this chemical. In the case of accidental ingestion, vomiting should not be induced. Bicarbonate should not be given to neutralize the acid. From 4 to 8 oz (i.e., 118 to 237 ml) of water or milk should be given to adults (2–4 oz (i.e., 59–118 ml) to children) for dilution. The victim should be kept quiet and normal body temperature maintained.

Exposure Standards and Guidelines

The temporary emergency exposure limits (TEEL) are: TEEL-0 = 35 ppb; TEEL-1 = 10 ppb; TEEL-2 = 500 ppb; TEEL-3 = 5000 ppb. The TEEL-i, i = 0–3, classification is discussed elsewhere in this encyclopedia.

See also: Acids; Corrosives.

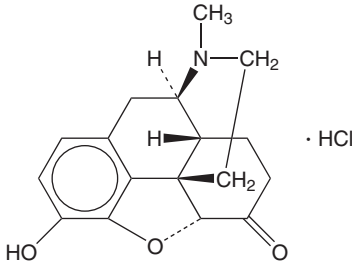
Further Reading

Braker W and Mossman A (1980) *Matheson Gas Data Book*, 6th edn., p. 398. New York: McGraw-Hill.

Hydromorphone

Christopher P Holstege

- Chemical Abstracts Service Registry Numbers: CAS 466-99-9 (Hydromorphone); CAS 71-68-1 (Hydromorphone hydrochloride)
- Synonyms: Dihydromorphinone; Dilaudid; Dimorphone
- Chemical/Pharmaceutical/Other Class: Opiate agonist
- Chemical Structure:



Uses

Hydromorphone is prescribed as an analgesic. It has also been diverted as a drug of abuse.

Exposure Routes and Pathways

Hydromorphone is available in tablet, liquid, suppository, or parenteral formulations. Hydromorphone tablets have been solubilized and used parenterally as a drug of abuse.

Toxicokinetics

At therapeutic doses, hydromorphone has an oral and rectal bioavailability of 51% and 36%, respectively. Peak serum levels occur within 2 h after ingestion and within 1 h after intramuscular administration. Hydromorphone is metabolized primarily in the liver, where it undergoes conjugation with glucuronic acid to form hydromorphone-3-glucuronide. Hydromorphone's volume of distribution is $2.9\,l\,kg^{-1}$. Unchanged drug ($\sim$6%) and metabolites are excreted in the urine. The elimination half-life of the parent compound is $\sim$2.5 h.

Mechanism of Toxicity

Hydromorphone is a semisynthetic, centrally acting opioid analgesic. It is nearly 10 times as potent as morphine on a milligram-to-milligram basis. Analgesic effects are related to the stimulation of opiate receptors in the central nervous system (CNS). Interaction with the opioid receptors mimics the actions of endogenous enkephalins and endorphins. These actions result in the analgesia, sedation, euphoria, and decreased gastrointestinal motility.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs act similarly to humans. Symptoms may include drowsiness, ataxia, vomiting, respiratory depression, miosis, coma, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone may be used at $0.02\,mg\,kg^{-1}$ if needed.

Human

Due to hydromorphone's potency, numerous adverse effects have been reported. CNS depression is the most frequently reported clinical effect. The typical overdose patient may present with extreme somnolence and may progress to coma. Miosis is usually present unless the individual is acidotic or has suffered hypoxic brain injury. Respiratory depression can occur and may progress to respiratory arrest. Pulmonary edema may be seen. Bradycardia, hypotension, and hyperthermia can develop. Available opiate immunoassays cross-react unreliably with hydromorphone.

Chronic Toxicity (or Exposure)

Human

Hydromorphone has the potential for abuse. Chronic users may develop tolerance, thus necessitating larger doses for the desired effect. Abrupt cessation can cause withdrawal, yielding restlessness, insomnia, hypertension, tachycardia, tachypnea, vomiting, and diarrhea.

In Vitro Toxicity Data

Hydromorphone has showed binding affinity to the delta opioid receptor that was equivalent to the binding affinities of etorphine, butorphanol, and lofentanil.

Clinical Management

In patients presenting with hydromorphone toxicity, the airway should be patent and adequate ventilation assured. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk for subsequent CO_2 narcosis, worsening acidosis, and/or aspiration. If necessary, endotracheal tube intubation should be performed. The initial treatment of hypotension consists of intravenous fluids. Close monitoring of the patient's pulmonary exam should be performed to assure that pulmonary edema does not develop as fluids are infused. The patient should be placed on continuous cardiac monitoring with pulse oximetry. Frequent neurological checks should be made. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered to patients who have ingested hydromorphone and present early. Syrup of ipecac is contraindicated after overdose with hydromorphone due to the potential for rapid clinical deterioration. Gastric lavage should be avoided.

Naloxone may be of benefit in reversing the neurological and respiratory depressant effects of hydromorphone. A dose of 0.4–2.0 mg is given intravenously slowly, titrated to resumption of adequate respirations, and can be repeated as needed. The therapeutic effect of naloxone may be of shorter duration than that of hydromorphone activity; therefore, it is imperative that hydromorphone intoxicated patients who demonstrated improvement after naloxone be closely monitored for resedation. Vital sign measurements and neurological checks should be monitored frequently.

See also: Drugs of Abuse; Morphine.

Further Reading

Cook S, Moeschler O, and Michaud K (1998) Acute opiate overdose: Characteristics of 190 consecutive cases. *Addiction* 93: 1559–1565.

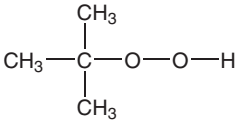
Evans LE, Swainson CP, and Roscoe P (1973) Treatment of drug overdosage with naloxone, a specific narcotic antagonist. *Lancet* 1: 452–455.

Hydroperoxide, *tert*-Butyl

Samantha E Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 2, p. 107, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 75-91-2
- Synonyms: 2-Hydroperoxy-2-methylpropane; 1,1-Dimethylethyl hydroperoxide; TBHP, TBH
- Chemical/Pharmaceutical/Other Class: Peroxides
- Chemical Formula: $C_4H_{10}O_2$
- Chemical Structure:



Uses

tert-Butyl hydroperoxide (TBHP) is an intermediate in the production of propylene oxide and *t*-butyl alcohol from isobutane and propylene. It is primarily used as an initiator and finishing catalyst in the solution and emulsion polymerization methods for polystyrene and polyacrylates. Other uses are for the polymerization of vinyl chloride and vinyl acetate and as an oxidation and sulfonation catalyst in bleaching and deodorizing operations. It is a strong oxidant and reacts violently with combustible and reducing materials, metallic and sulfur compounds.

Exposure Routes and Pathways

Dermal contact and inhalation are primary routes of exposure. Occupational exposure to TBHP may occur through these routes at workplaces.

Toxicokinetics

TBHP can be absorbed into the body by inhalation, through the skin, and by ingestion.

Mechanism of Toxicity

TBHP accelerates oxidation of glutathione and decreases the metabolism of sodium hexabarbitol in rat livers and is a strong oxidation agent.

Acute and Short-Term Toxicity (or Exposure)

Animal

TBHP is a strong irritant to eye and skin. The rat oral LD_{50} is $560\,mg\,kg^{-1}$, and the rat intraperitoneal LD_{50} is $87\,mg\,kg^{-1}$. It is moderately toxic when ingested. The ability of TBHP to cause chromosome aberration was evaluated in bone marrow cells of Sprague–Dawley rats receiving up to 100 ppm inhalation exposure for $6\,h\,day^{-1}$ for up to 5 days. None of the treatments produced chromosomal aberrations or damage in the bone marrow cells.

Human

TBHP causes eye and skin irritation, and its inhalation causes lung damage at high concentrations.

Chronic Toxicity (or Exposure)

Animal

A 45 day oral study found treatment-related changes in the form of tubular nephrosis in male rats receiving up to $30\,mg\,kg^{-1}$ body weight. In a combined repeated dose and reproduction/teratogenic study, male and female rats were given up to $30\,mg\,kg^{-1}$ body weight orally. No effects on male and female reproduction were observed. In an oral dosage study, up to $50\,mg\,kg^{-1}$ body weight was administered to mated female rats on days 6–15 of gestation. Neither embryotoxic nor teratogenic effects were found up to the highest dose.

In Vitro Toxicity Data

TBHP did not produce a genotoxic response in the cell transformation assay, but did produce a mutagenic response in the Ames (*Salmonella*) and mouse lymphoma mutagenesis assays. TBHP was tested for the induction of sex-linked recessive lethal mutations in *Drosophila melanogaster*, and was positive at a dose of 2000 ppm when administered to males by feeding.

Clinical Management

Respiratory therapy should be administered to exposed individuals. Contaminated clothing should be removed. Exposed skin should be washed with soap and water. Exposed eyes should be flushed with water for at least 15 min.

Environmental Fate

TBHP may be released to the environment through various waste streams. Chemical degradation is expected to be the dominant fate process in water because of reaction with organic matter and therefore, it is doubtful that unreacted TBHP would be

biologically available. A bioconcentration factor (BCF) of 3 has been calculated for TBHP, and this BCF suggests the potential for bioconcentration in aquatic organisms is low. If released to air, TBHP will exist solely as a vapor in the ambient atmosphere. Vapor-phase TBHP will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 5 days.

See also: Glutathione; Oxidative Stress.

Further Reading

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Relevant Websites

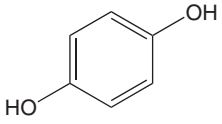
http://www.chem.unep.ch – Organisaton for Economic Cooperation and Development (OECD). *t*-Butyl Hydroperoxide (OECD Screening Information Data Set).

http://www.inchem.org – International Programme for Chemical Safety (IPCS). *tert*-Butyl Hydroperoxide (ICSC 0842) (from IPCS).

Hydroquinone

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 123-31-9
- Synonyms: 1,4-Benzendiol; *p*-Benzendiol; Dihydroquinone; Quinol
- Chemical/Pharmaceutical/Other Class: Hydrocarbon; Ketone; Reducing agent
- Chemical Formula: $C_6H_6O_2$
- Chemical Structure:



Uses

Photographic reducer and developer; reagent for the determination of small quantities of phosphate, dye intermediate; stabilizer in paints and varnishes; motor fuels and oils; antioxidant for fats and oils, and a polymerization inhibitor. Therapeutically used topically for depigmentation to treat skin blemishes, for example, hypermelanosis. Hydroquinone occurs naturally, as a conjugate with β-D-glucopyranoside, in the leaves, bark, and fruit of a number of plants, and its presence may be an important factor in fire-blight resistance in the pear. It may also play an important part in the defense mechanisms of some insects.

Exposure Routes and Pathways

The common exposure routes are dermal, by inhalation, and by ingestion. Hydroquinone exposure is also possible among people developing photographic film. Hydroquinone is not found naturally in the body.

Toxicokinetics

Absorbed from the gastrointestinal (GI) tract and possibly the skin, and appears to act on the body by first being oxidized to quinone. Hydroquinone is excreted in the urine as either a glucuronide or a sulfate.

Mechanism of Toxicity

Benzene, phenol, and hydroquinone are metabolized *in vivo* to benzoquinone and excreted as the mercapturate, *N*-acetyl-*S*-(2,5-dihydroxyphenyl)-L-cysteine. Hydroquinone is a reducing cosubstrate for peroxidase enzymes, and the resultant semiquinone and *p*-benzoquinone may bind to DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values for rats, mice, guinea pigs, cats, and dogs range from 70 to 550 g kg^{-1} of hydroquinone, with the cat having the greatest sensitivity. Hyperexcitability, tremors, convulsions, salivation, and emesis were observed in cats within 90 min of administration of lethal doses, and death occurred after several hours.

Experimental exposure of rabbit eyes to high concentrations of the vapor resulted in conjunctivitis, corneal edema, and necrosis. Acute neurotoxic effects reported in animals include activity increase, hyperactive reflexes, hypersensitivity, convulsions and paralysis.

Human

Ingestion of one g by an adult may cause tinnitus, nausea, dizziness, a sense of suffocation, an increased

rate of respiration, vomiting, pallor, muscle twitchings, headache, dyspnea, cyanosis, delirium, and collapse. The urine is usually green or brownish green in color and continues to darken on standing. Markedly elevated methemoglobin levels may occur with hydroquinone exposure. Increased blood cell fragility, hemolytic icterus, anemia, leukocytosis, reticulocytosis, and hypoglycemia may occur with subacute hydroquinone poisoning. Hydroquinone dust is irritating to eyes, nose, and mucous membranes, and hydroquinone is classified as a strong eye and skin irritant, and can cause hypomelanosis and delayed hyperpigmentation. Further, it is a dermal sensitizer. Ingestion of 5–12 g causes hemolysis, renal and hepatic failure, and death.

Chronic Toxicity (or Exposure)

Animal

In a rabbit study, hydroquinone at 150 mg kg^{-1} day^{-1} produced minimal developmental alterations in the presence of maternal toxicity. The no-observed-effect level for developmental toxicity was 75 mg kg^{-1} day^{-1}. In rat studies, maternal toxic effects from exposure to hydroquinone included changes in the ovaries, fallopian tubes, and menstrual cycle. Postimplantation mortality was also observed in rat studies. Observed paternal toxic effects from exposure to hydroquinone included changes in the testes, epididymis, sperm duct, prostate, seminal vesicle, Cowper's gland, accessory glands, and male fertility index. Further, exposure to hydroquinone produced skeletal malformations in chickens and ocular and skeletal malformations in rabbits. Hydroquinone can induce renal tubule adenomas, bladder carcinomas, hepatocellular neoplasms, and mononuclear cell leukemia in experimental animals.

Human

Humans have been reported to be able to ingest 300–500 mg daily for several months without adverse effects. Several hundred crewmen on a US Navy vessel were reported to have developed GI symptoms (acute onset of nausea, vomiting, abdominal cramps, and diarrhea) due to hydroquinone contamination of the water system from automatic photo developing systems. Vision disturbances are among the chronic toxic effects, for example, discoloration, distortion, and opacification of the corneas of workers exposed long-term to low levels. Workers exposed chronically may develop a reddish discoloration of the hair, and brown and orange-brown nail discoloration. Further, workers exposed chronically to hydroquinone may develop a reddish discoloration of the soles and palms. There is inadequate evidence in humans for the carcinogenicity of hydroquinone, and hydroquinone is in the International Agency for Research on Cancer's group 3 list (not classifiable as to its carcinogenicity to humans).

In Vitro Toxicity Data

Hydroquinone was not mutagenic in *Salmonella typhimurium* strains with or without exogenous metabolic activation. It induced trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells in the presence or absence of metabolic activation. An equivocal response was obtained in tests for induction of sex-linked recessive lethal mutations in *Drosophila* administered hydroquinone by feeding. Hydroquinone induced sister chromatid exchanges in Chinese hamster ovary cells with or without exogenous metabolic activation and caused chromosomal aberrations in the presence of activation.

Clinical Management

Clinical management should be symptomatic and supportive. A benzodiazepine can be administered to control seizures. Methylene blue can be used to treat methemoglobinemia.

Environmental Fate

In the soil, hydroquinone is expected to biodegrade under aerobic conditions. It may be removed from the soil by oxidation processes or by direct photolysis on the surface. Volatilization would be minimal. In the water, it would degrade under either aerobic or anaerobic conditions. In the air, hydroquinone undergoes photochemical degradation. It is listed as undergoing rapid biodegradation in a commercial activated sludge unit under aerobic conditions. The estimated and experimental bioconcentration factors for hydroquinone of 40–65 have been obtained. These data indicate that hydroquinone is not expected to significantly bioconcentrate in fish and aquatic organisms.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average (TWA) is 2.0 mg m^{-3}, and this is also the US Occupational Safety and Health Administration permissible exposure limit, 8 h TWA. Hydroquinone is listed as a US Environmental Protection Agency (EPA) hazardous air pollutant

"generally known or suspected to cause serious health problems." The Clean Air Act, as amended in 1990, directs the EPA to set standards requiring major sources to sharply reduce routine emissions of toxic pollutants. Hydroquinone is a US Food and Drug Administration (FDA) indirect food additive for use only as a component of adhesives.

See also: Benzene; Quinone.

Further Reading

DeCaprio AP (1999) The toxicology of hydroquinone – relevance to occupational and environmental exposure. *Critical Reviews in Toxicology* 29: 283–330.

Devillers J, Boule P, Vasseur P, *et al.* (1990) Environmental and health risks of hydroquinone. *Ecotoxicology and Environmental Safety* 19: 327–354.

International Agency for Research on Cancer (IARC) (1999) Hydroquinone. *IARC Monographs on the Evaluation of Carcinogenic Risks in Humans* 71(Pt 2): 691–719.

Hydroxylamine

Samantha E Gad

This article is a revision of the previous print edition article by Shayne C Gad, volume 2, pp. 107–108, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7803-49-8
- Synonym: Oxammonium
- Chemical/Pharmaceutical/Other Class: Reducing agent
- Chemical Formula: NH_2OH
- Chemical Structure: H–NH–OH

Uses

Hydroxylamine is used as a reducing agent in photography, in synthetic and analytical chemistry, to purify aldehydes and ketones, as an antioxidant for fatty acids and soaps, and as a dehairing agent for hides. In addition, hydroxylamine is used in the production of cyclohexanone oxime or caprolactam. Its potential uses must be carefully evaluated since hydroxylamine has been reported to pose a dangerous fire hazard when exposed to heat and flame, and may ignite spontaneously in air if a large surface area is exposed. Further, it ignites on contact with copper(II)sulfate; metals (e.g., sodium); oxidants (e.g., barium peroxide, barium oxide, lead dioxide, potassium permanganate, chlorine); phosphorus chlorides (e.g., phosphorus trichloride, phosphorus pentachloride).

Exposure Routes and Pathways

Routes of exposure include dermal contact, inhalation, and (potentially) ingestion. The most extensive exposure is occupational, for example, through inhalation of dust particles and dermal contact during production or in loading and unloading of crystallizers and centrifuges, and in the packaging of finished product.

Mechanism of Toxicity

Hydroxylamine acts as a reducing agent when absorbed systemically, producing methemoglobin and the formulation of Heinz bodies in the blood. It can induce hemolytic anemia. It inhibits platelet aggregation and is a 'nitric oxide' vasodilator. Oxylmines such as hydroxylamine and methoxylamine disturb DNA replication and act as potent mutagens, causing nucleotide transition from one purine to another or one pyrimidine to another.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hydroxylamine is a positive dermal sensitizer in guinea pigs and mice. The intraperitoneal LD_{50} is $60\,mg\,kg^{-1}$ in mice and $59\,mg\,kg^{-1}$ in rats. Hydroxylamine is a strong dermal and ocular irritant.

Human

Hydroxylamine produces methemoglobin when systemically absorbed, potentially resulting in cyanosis, convulsions, hypotension, and coma. It is a marked irritant (also reported to be corrosive) to eyes, skin, and mucous membranes.

Chronic Toxicity (or Exposure)

Animal

It is reported to be a teratogen in rabbits but not in rats.

Human

Long-term exposures at lower levels can induce hemolytic anemia. It is a dermal and pulmonary sensitizer.

In Vitro Toxicity Data

It is an *in vitro* mutagen in most test systems, at levels as small as 10–20 mmol l^{-1} in some systems. Industrially used hydroxylamines were studied in human blood cells *in vitro*. The parent compound hydroxylamine and the *O*-ethyl derivative gave very similar results. Both compounds induced a high degree of methemoglobin formation and glutathione depletion. Cytotoxicity was visible as Heinz body formation and hemolysis.

Clinical Management

Treatment consists of administration of methylene blue (1% solution), 0.1 ml kg^{-1} intravenously over a 10 min period.

Environmental Fate

Hydroxylamine's production and use may result in its release to the environment through various waste streams. Hydroxylamine will exist solely as a vapor in the ambient atmosphere, and will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 18 h. Abiotic degradation of hydroxylamine by photochemically produced peroxy radicals is an important environmental fate process in surface waters, with the half-life of this reaction measured at ~2 h. An estimated bioconcentration factor of 3 suggests the potential for bioconcentration in aquatic organisms is low.

See also: Blood.

Further Reading

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http://www.inchem.org – Hydroxylamine (International Chemical Safety Cards – 0661) (October 1995).

Hymenoptera

Gary W Everson

- SYNONYMS: Bees; Hornets; Wasps; Ants

Exposure Routes and Pathways

Hymenoptera envenomation results from the subcutaneous injection of venom through a stinging apparatus.

Mechanism of Toxicity

The constituents of Hymenoptera venom differ among bees, wasps, and ants. As an example, honeybee venom includes phospholipase A, hyaluronidase, and various other allergens. Local skin reactions occur as a result of endogenous inflammatory response to the injected foreign proteins and enzymes. Multiple stings can result in a generalized systemic reaction due to the large volume of venom injected and absorbed. Following multiple stings, large amounts of injected venom may cause the release of vasoactive substances, which can lead to hypotension and shock. Allergic (anaphylactic) reactions may also occur as a result of antibody production from a previous sensitization (previous sting). The resulting IgE mediated reaction triggers the release of such vasoactive substances as histamine and leukotrienes.

Acute and Short-Term Toxicity (or Exposure)

Human

Clinical manifestations following a bite or sting can be classified into three groups. The first and most common reaction is a mild local tissue reaction characterized by minor swelling, redness, itching, and pain at the site of the sting. The second involves a systemic reaction resulting from multiple stings (typically requiring >50–100 stings). Symptoms may include nausea, vomiting, headache, and loss of consciousness. Renal failure and seizures are rare, but have been documented. Lastly, allergic reactions may develop as a result of an individual's prior sensitization to the venom. This may range from a simple urticarial reaction to anaphylaxis. The latter is characterized by laryngeal edema, bronchospasm,

difficulty in breathing, wheezing, hypotension, cardiovascular collapse, and death. Anaphylactic reactions may occur within 15–30 min following a sting. Although unrelated to toxicity, any bite or sting may also result in local infection.

Chronic Toxicity (or Exposure)

Animal

Bees have been used therapeutically in some animal populations for treatment of a variety of disorders. A recent study examined the effects of bee stings on sows with hypogalactia syndrome postpartum. Complications expected from this type of therapy are primarily local effects as well as more systemic hemorrhagic effects.

Human

One small epidemiologic study linked arthritis of the hands with exposure to bee stings in patients occupationally exposed to bee venom (bee keepers).

Clinical Management

Basic and advanced clinical life support may be required for those individuals exhibiting anaphylactic reactions following a bite or sting. As opposed to wasps, honeybees possess barbed stingers that remain imbedded in the skin along with the venom sac. Following a bee sting, the stinger should be removed using a stiff card (e.g., credit card) scraped across the skin at an angle. The stinger should not be grasped since doing so will contract the attached venom sac and force more venom into the skin. Nonallergic local reactions following small numbers of bites or stings can generally be managed outside of the hospital setting. Home remedies such as the application of meat tenderizer to the sting site are not effective. Best results are obtained by washing the site well with soap and water, applying a disinfectant such as iodine or alcohol at the site, and using a cold compress to decrease swelling and pain. Antihistamines, like diphenhydramine, may help relieve itching and swelling. Stings to the oral mucosa have occurred during the process of accidentally swallowing a bee or wasp. Swelling of the oropharynx may occur and impair breathing. These cases represent a medical emergency and should be managed in a health care facility. Patients with multiple stings (>50–100), those with a history of anaphylaxis, and those exhibiting an allergic reaction to a sting should be managed in an emergency department setting. Patients with multiple stings require general supportive care and observation. Urticarial reactions may be managed with antihistamines with or without subcutaneous epinephrine depending on the severity of the reaction. Anaphylaxis must be treated promptly and aggressively. Intravenous fluids should be started immediately. Respiratory status should be evaluated and an airway established with supplemental oxygen, if necessary. Subcutaneous epinephrine 1:1000 should be administered immediately. Intravenous diphenhydramine may be used but it is of secondary importance. A vasopressor is occasionally required in addition to intravenous fluids to manage hypotension.

See also: Animals, Poisonous and Venomous; Diphenhydramine.

Further Reading

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Hypersensitivity, Delayed Type

Leigh Ann Burns-Naas

This article is a revision of the previous print edition article by David E Bice, volume 2, pp. 109–110, © 1998, Elsevier Inc.

In general terms, the purpose of the immune system is to provide protection for the individual against disease, whether infectious, parasitic, or tumorigenic. In doing so, the body must recognize what is 'self' from what is foreign (e.g., nonself). In most situations, the immune system acts appropriately. However, there are times when the immune system acts in an exaggerated manner leading to tissue damage. This is referred to as hypersensitivity. In the classic Coombs and Gell classification system, there are four types of hypersensitivity reactions. The first three (types 1–3) are mediated by antibody (e.g., IgG, IgE). The fourth type (type 4) is mediated by antigen-specific T cells and is also known broadly as delayed-type hypersensitivity (DTH). It is 'delayed' because the reaction appears hours to days after antigen crosses into the skin. Though often thought of as

adverse effects (probably due to the association with contact hypersensitivity), DTH responses are probably important in natural host defense to intracellular infectious organisms, such as mycobacteria, listeria, candida, and certain viruses. DTH reactions are also associated with certain diseases, such as Wegener's granulomatosis and sarcoidosis. The prototypical DTH response is the tuberculin reaction, though the more commonly recognized is cutaneous (contact) hypersensitivity mediated by small molecular weight chemicals. Some DTH responses are also considered to be allergic responses; specifically, chemical allergy that may lead to the development of allergic contact dermatitis.

Contact Hypersensitivity

Of the various DTH responses, contact hypersensitivity possibly affects the greatest number of individuals. It is a common occupational health problem in a variety of industrial settings and it can occur following chemical exposures in the home and in the environment. One of the most recognized contact hypersensitivity reactions affecting many people is that which develops in response to cutaneous exposure to poison ivy (pentadecacatechol). Contact hypersensitivity reactions are initiated by topical exposure to the skin (often by a small molecular weight chemical), and the subsequent response is primarily epidermal.

Contact (or chemical) hypersensitivity reactions develop in two phases: induction and elicitation (**Figure 1**). Induction is the development of the initial sensitization. In this phase, the chemical penetrates the epidermis. Chemicals are haptens because they are, by themselves, unable to elicit an immune response. As such, they must associate with proteins in the skin to stimulate a specific immune response. The hapten–protein complex is then processed by antigen presenting cells in the skin, transported and presented to T cells in the draining lymph nodes. This interaction leads to a proliferative response and the development of memory T cells that distribute systemically within the body.

Elicitation is the clinical response to subsequent challenge with the antigen or hapten. Penetration into the skin leads to encounters with antigen-specific memory T cells. These cells release cytokines such as IFN-γ and IL-17 that recruit other cells to the site and stimulate the production of chemoattractants and proinflammatory cytokines (e.g., IL-8, IL-1, TNF-α, IL-6) from multiple cell types, including T cells and keratinocytes. The resulting effect is an infiltration of inflammatory cells into the tissue and a localized increase in vascular permeability leading to the development of erythema and edema, and/or the appearance of cutaneous vesicles or papules. Although this is typically thought of as a local effect, it may be systemic as well.

In summary, chemical sensitization is dependent upon intact immunological function and the integrity of T lymphocyte responses. It is also dependent upon the ability of the hapten–protein complex to stimulate (in a susceptible individual) an immune response of sufficient vigor and of the right quality such that when that individual is exposed to the inducing chemical for the second time (by an appropriate route) they will mount a more accelerated and more aggressive inflammatory response.

Tuberculin-Type Hypersensitivity

In contrast to contact hypersensitivity, tuberculin-type hypersensitivity reactions are primarily dermal and result from intradermal injections into the skin. In people that have had tuberculosis or have been exposed to the bacterium through infection or BCG immunization, a cell-mediated immune response to the bacterium develops. When small amounts of tuberculin (a complex mixture of antigenic material derived from *Mycobacterium tuberculosis*) are subsequently injected into the skin, a localized T cell-dependent inflammatory response develops in the dermis. Within 24–72 h of injection, individuals with prior exposure to the bacterium display a raised, red, indurated area on the skin at the injection site. The lack of a response suggests no prior exposure to the bacterium.

Methods to Assess Delayed-Type Hypersensitivity

There are several acceptable ways to evaluate DTH responses in nonclinical species. Of these, the most common are the guinea pig assays used to assess contact sensitization. Both the Magnusson and Kligman model (guinea pig maximization test) and the Buehler model measure the elicitation phase of the hypersensitivity response, though the tests vary in their methods of chemical application and utilization of adjuvants. Most recently, the local lymph node assay has been accepted as a stand alone test for chemical hypersensitivity. This assay is conducted in mice and measures the induction phase of sensitization. In humans, the most common methods to assess delayed hypersensitivity are the patch test (contact sensitivity; for diagnostic purposes) and the human repeat insult patch test (contact sensitivity; for predictive purposes). Additionally, intradermal

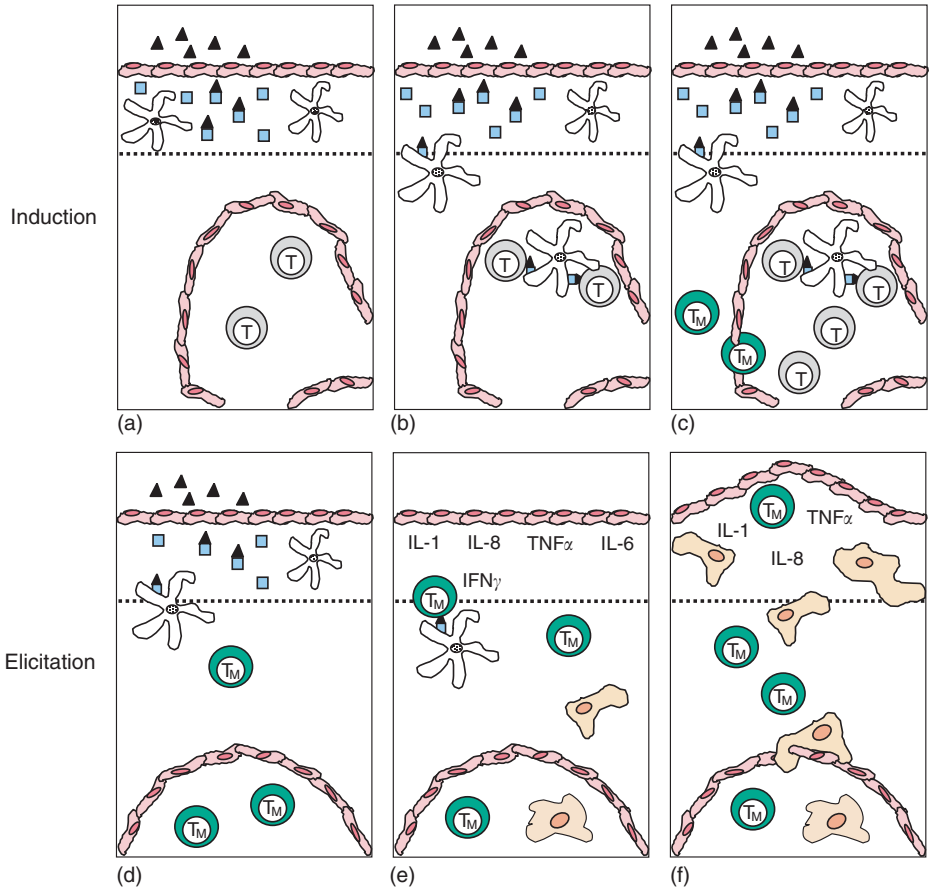

Figure 1 Development of Chemical-Induced (Contact) Hypersensitivity. (a) Following dermal exposure, the chemical (hapten) penetrates the epidermis and complexes with proteins in the skin. (b) The hapten–protein complex is recognized and processed by antigen presenting cells (APCs; Langerhan's cells) in the skin. The APCs then begin to migrate to the draining lymph nodes. (c) Upon entering the draining lymph nodes, the APCs interact with T cells, causing their subsequent activation and proliferation. These antigen-specific T cells develop into memory T cells which then distribute systemically in the body. (d) Upon subsequent exposure to the chemical, APCs in the skin again recognize, process, and present the hapten–protein complex on their surface. (e) Some of these APCs interact with resident, antigen-specific, memory T cells. Activated memory T cells release cytokines causing the release of other proinflammatory mediators and chemoattractants from other cell types in the skin, and beginning the migration of additional memory T cells and proinflammatory cells from the circulation. (f) Memory T cells and inflammatory cells continue to migrate to the epidermis, resulting in erythema and edema at the site of chemical exposure. (Reproduced from Burns-Naas LA, Meade BJ, and Munson A (2001) Toxic responses of the immune system. In: Klaassen C (ed.) *Casarett & Doull's Toxicology. The Basic Science of Poisons*, 6th edn., pp. 419–470. New York: Academic Press.)

injection of an antigen is used to determine if individuals have developed active immunity (e.g., memory T cells) to infectious agents, such as *M. tuberculosis* (e.g., tuberculin skin test).

See also: Immune System; Skin; Toxicity Testing, Sensitization.

Further Reading

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Kimber I and Maurer T (eds.) (1996) *Toxicology of Contact Hypersensitivity*. New York: Taylor & Francis.

Marzulli FN and Maibach HI (1996) Allergic contact dermatitis. In: Marzulli FN and Maibach HI (eds.) *Dermatotoxicology*, pp. 143–146. New York: Taylor & Francis.

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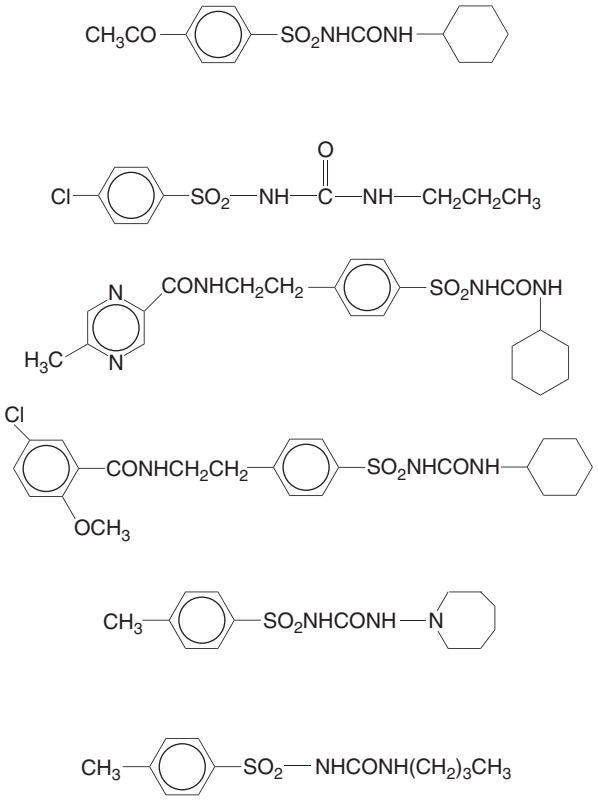
Hypoglycemics, Oral

Henry A Spiller

- Representative Chemicals: Sulfonylureas and glinides
- Synonyms:
  - First-generation sulfonylureas: Acetohexamide, Dylmelor® (CAS 968-81-0); Chlorpropamide, Diabenese® (CAS 94-20-2); Orinase® (CAS 64-77-7); Tolazamide, Tolynase® (CAS 1156-19-0); Tolbutamide
  - Second-generation sulfonylureas: Glipizide, Glucotrol® (CAS 29094-61-9); Glyburide (glibenclamide); Diabeta® (CAS 102.38-2.1-8); Glimepiride, Amaryl®
  - Glinides: Repaglinide, Novonorm® or Prandin®; Nateglinide, Starlix®
- Chemical/Pharmaceutical/Other Class: All sulfonylureas are arylsulfonylureas, with substitutions on the benzene and the urea groups producing the different drugs. Repaglinide is a nonsulfonylurea oral hypoglycemic. Nateglinide is an amino acid derivative oral hypoglycemic agent
- Chemical Structures:



Uses

Sulfonylureas and glinides are used in the treatment of (Type II) noninsulin-dependent diabetes mellitus.

Exposure Routes and Pathways

All sulfonylureas are available as tablets only, with the exception of tolbutamide, which is available as tablets and a sterile solution for injection. The glinides are available as tablets only.

Toxicokinetics

The only significant difference in the sulfonylureas and the glinides is in the toxicodynamic parameters of onset of action and duration of action. Sulfonylureas are readily absorbed from the gastrointestinal tract with the exception of tolazemide, which is absorbed somewhat more slowly. The glinides are rapidly absorbed with peak serum levels in 30–60 min. Peak plasma levels of the sulfonylureas occur within a range of 1 h. However, in overdose the duration of effect is a more important factor than onset of action. There is extensive biotransformation of sulfonylureas and the glinides by the liver. Sulfonylureas with active metabolites are acetohexamide and chlorpropamide. There are no active metabolites for repaglinide or nateglinide. The sulfonylureas and glinides are highly protein bound, from 92% to 99%, except for acetohexamide, which is 65–90%. Glyburide may accumulate in deep body compartments, allowing for later redistribution after withdrawal of the drug. Sulfonylureas and nateglinide are cleared primarily in the urine as metabolites. Repaglinide is cleared primarily via the feces. Glyburide is also cleared to a significant amount as the parent drug in the feces via biliary secretion. The half-lives of sulfonylureas are generally less important clinically than durations of action, which range from 8 to 72 h. Both nateglinide and repaglinide have a short duration of action of $\sim$4 h.

Mechanism of Toxicity

The sulfonylureas and the glinides bind to the sulfonylurea receptor on the cell membrane of the beta cells of the pancreatic islets, which causes the ATP-sensitive potassium channel to close. The closed potassium channel increases membrane potential causing the voltage-sensitive calcium channel to open. The sudden influx of calcium begins a cascade of

events including kinase activation, which results in release of preformed insulin into circulation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Sulfonylureas are not routinely used in animals. Hypoglycemia, lethargy, and seizures can occur.

Human

The cascade of symptoms from a sulfonylurea or glinide overdose will reflect the patient's hypoglycemic state secondary to hyperinsulinemia. Initially, the patient may present with restlessness, diaphoresis, altered mental status, combative behavior, tremors, or confusion. An infant or small child may be difficult to feed. Nausea, vomiting, or abdominal pain may also occur. This will be followed by increasing central nervous system depression, seizures, and coma if the patient's blood glucose continues to fall. Most other effects reflect persistent hypoglycemia. In small children and poorly nourished patients, the onset of hypoglycemia may be sudden. In severe cases of persistent and prolonged hypoglycemia, hypotension, tachycardia, and eventually cardiac arrest may occur. Metabolic acidosis may also occur. In sulfonylurea overdose, for patients without concomitant intravenous glucose supplementation, an 8-h observation period should be sufficient to detect those patients who will become hypoglycemic. Those patients who do show evidence of hypoglycemia should be monitored for 24 h due to the potential for prolonged duration of effect. In a glinide overdose, due to their more rapid onset and shorter duration of action, a 3–4 h period of observation would be sufficient. A 24 h observation period is not expected to be necessary for the glinides. A disulfiram-like reaction may occur with concomitant ethanol and sulfonylurea use.

Chronic Toxicity (or Exposure)

Animal

Rats and mice exposed to chlorpropamide at doses greater than 6000 ppm for 2 years found no evidence of increased rates of tumor development.

Human

The primary toxic effects of oral hypoglycemic agents are related to their effects of decreasing blood sugar. Patients have also reported gastrointestinal effects (nausea, vomiting, diarrhea), rare hepatic toxicity, and hypersensitivity reactions.

In Vitro Toxicity Data

Chlorpropamide was mutagenic in Chinese hamster cells but negative in Ames *Salmonella* assays.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be used as appropriate to the patient's level of consciousness and the history of the ingestion. Activated charcoal effectively binds sulfonylureas. The cornerstone of therapy is glucose replacement or in severe cases inhibition of insulin secretion. Continuous intravenous 10% glucose in water via a peripheral line is usually sufficient to maintain euglycemia. However, patients may require additional boluses of D25W or D50W to maintain adequate blood glucose. In cases of symptomatic sulfonylurea overdose, due to their pronged effects, frequent blood glucose monitoring is recommended. Patients are at greatest risk during or after periods of fasting, such as sleep. Delayed or prolonged effects are not expected in glinide overdose. Glucose therapy should be titrated to the patient's serial blood glucose measurements. Additional oral glucose via frequent snacks and meals will be helpful but usually not sufficient by themselves. In cases of recurrent or refractory hypoglycemia octreotide may be helpful by altering calcium influx in the beta cell and, therefore, reducing insulin secretion. Octreotide, a long-acting somatostatin analog, may be administered subcutaneously or intravenously. Subcutaneous administration may be 50–100 μg every 6–12 h as needed in adults or 1 $\mu g\,kg^{-1}$ every 6–12 h as needed in children. Continuous intravenous administration can be given at a rate of 15–30 $ng\,kg^{-1}\,min^{-1}$.

See also: Diabetes, Effect of Toxicity.

Further Reading

Palatnick W, Meatherall RC, and Tenenbein M (1991) Clinical spectrum of sulfonylurea overdose and experience with diazoxide therapy. *Archives of Internal Medicine* 151: 1859–1862.

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Iatrogenic Disease

Beck Bertine Goldberg

Iatrogenic disease is any disease, sickness, disfigurement, or death that occurs during the practice of acceptable medical care. The word iatrogenic comes from 'iatros', which is Greek for medical or medicinal, and 'genic', which is Greek for 'caused by'. In the United States, death by iatrogenic causes is the third leading cause of death after deaths from heart disease and cancer. These include adverse reactions to prescriptions, medical mistakes, unnecessary surgery, errors in hospitals, and nosocomial infections. It has been estimated in the United States that 106 000 deaths per year occur from nonerror, adverse effects of medications and 7000 deaths per year from medication errors in hospitals.

Three Toxicological Examples

1. Merbromin is a weak antiseptic that contains mercury and is used in the treatment of minor dermal injuries, and in the escharotic treatment of large omphaloceles in neonates. An omphalocele is an abnormality found in neonates where the infant's intestine or other abdominal organs protrude from the infant's navel. Merbromin can be absorbed topically through the omphalocele sac and cause mercury toxicity and/or death. Alternatives lacking the toxicity of merbromin are silver sulfadiazine and gentian violet.
2. Patients taking methotrexate, an antimetabolite used for controlling the symptoms of conditions such as rheumatoid arthritis and psoriasis, can cause suppression in bone marrow. To reduce the incidence of bone marrow suppression, this drug is only given once weekly ($\sim$30 mg a week) along with folic acid supplements.
3. Dental amalgam restorations leak small amounts of elemental mercury vapor into the oral cavity of the mouth. The released mercury can be taken up by the saliva and then distributed to various organs and compartments throughout the body. Daily mercury uptake rates from amalgam are estimated to range from 2 to 25 μg Hg/24 h with the 'worst case' individual estimated to have an uptake of 70 μg Hg/24 h. Mercury is a known neurotoxicant and the off-gassing of mercury over time may cause dementia like conditions in some people.

See also: Interactive Toxicity; Mercury; Monoamine Oxidase Inhibitors.

Further Reading

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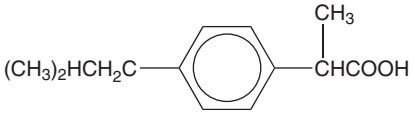
Ibotenic Acid *See* Mushrooms, Ibotenic Acid.

Ibuprofen

Christopher P Holstege

This article is a revision of the previous print edition article by Bonnie S Dean, volume 2, pp. 113–114, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 15687-27-1
- Synonyms: 2-(4-Isobutylphenyl) propionic acid; Advil; Bayer Select; Dayquil Sinus; Dimetapp Sinus; Dristan Sinus; Excedrin IB; Haltran; Medipren; Motrin; Midol 200; Nuprin; Pamprin IB; Profen; Rufen
- Chemical/Pharmaceutical/Other Class: A substituted phenylalkanoic acid; Nonsteroidal antiinflammatory and analgesic agent
- Chemical Structure:



Uses

Ibuprofen is used for analgesic, antipyretic, and antiinflammatory purposes.

Exposure Routes and Pathways

Ibuprofen is available in tablet and liquid dosage forms. Ingestion is the most common route of both accidental and intentional exposures to ibuprofen.

Toxicokinetics

Ibuprofen is rapidly absorbed after ingestion with peak plasma concentrations obtained within 1–2 h. Ibuprofen is highly protein bound (99%) and occupies only a fraction of the total drug binding sites during therapeutic use. The volume of distribution is 0.1–0.2 $1\,kg^{-1}$. Ibuprofen passes slowly into synovial spaces and may remain there in higher concentration as the concentrations in plasma decline. Ibuprofen passes readily across the placenta. Ibuprofen is extensively metabolized, yielding four urinary metabolites formed by hydroxylation. The excretion of ibuprofen is rapid and complete. Ibuprofen's elimination half-life is 1–2 h. Approximately 90% of an ingested dose is excreted in the urine as metabolites or their conjugates, and 10% is eliminated as free drug.

Mechanism of Toxicity

The mechanisms of ibuprofen-induced toxicity have not been clearly defined. Acute renal failure is postulated to result from decreased production of intrarenal prostaglandins via inhibition of the cyclooxygenase pathway. In turn, this will decrease the renal blood flow and glomerular filtration rate. Ibuprofen also interferes with prostaglandin synthesis in the gastrointestinal system that can contribute to its irritating effect on the mucosa of the gastrointestinal tract.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ibuprofen is not recommended for use in animals. Dogs appear to be exquisitely sensitive to the propionic acid group of nonsteroidal anti-inflammatory drugs (NSAIDs) and easily develop gastric ulcers and renal failure. Seizures have been reported in both dogs and cats after ingestion of ibuprofen.

Human

The majority of patients who acutely overdose on ibuprofen remain asymptomatic. In one retrospective study of ibuprofen overdoses, only 19% of patients developed symptoms. Abdominal pain, nausea, vomiting, lethargy, and drowsiness are the most frequently reported symptoms. In rare instances of massive acute overdose, apnea, seizures, hypotension, metabolic acidosis, renal failure, and coma have occurred.

Chronic Toxicity (or Exposure)

Human

The chronic ingestion of excessive amounts of ibuprofen may produce similar toxicity as acute but in a more insidious fashion. Gastritis and renal dysfunction may be seen.

In Vitro Toxicity Data

Studies of ibuprofen and other NSAIDs have produced toxic effects at concentrations 10 times therapeutic in cultured hepatocytes. No adverse effect on cell survival was noted at therapeutic concentrations of ibuprofen in this model, although increases in lactate dehydrogenase leakage were prominent.

Clinical Management

Treatment of acute overdoses of ibuprofen should consist of symptomatic supportive care. Ingestions $>250\,mg\,kg^{-1}$ may require hospital evaluation and treatment. Children ingesting $>400\,mg\,kg^{-1}$ have the greatest risk for serious toxicity. Activated charcoal may be used to adsorb ibuprofen or concomitant ingestants if given within 1 h of the exposure. Adequate hydration should be assured. Serum ibuprofen levels are not readily available and do not influence patient management.

See also: Propionic Acid.

Further Reading

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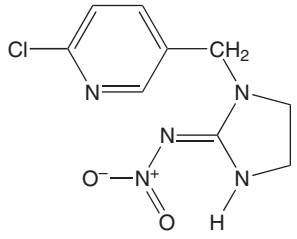
McElwee NE, Veltri JC, and Bradford DC (1990) A prospective, population-based study of acute ibuprofen overdose: Complications are rare and routine serum levels not warranted. *Annals of Emergency Medicine* 19: 657–662.

Imidacloprid

Larry P Sheets

- Chemical Abstracts Service Registry Number: CAS 138261-41-3
- Synonyms: 1-[(6-Chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine; Confidor®; Gaucho®; Admire®
- Chemical/Pharmaceutical/Other Class: Neonicotinoid insecticide
- Chemical Formula: $C_9H_{10}ClN_5O_2$
- Chemical Structure:



Uses

Imidacloprid is a neonicotinoid insecticide that is registered for many uses, including grub and termite control, crop protection, and to control fleas and ticks on companion animals. Its insecticidal activity is attributed to nicotinic activity on post-synaptic receptors.

Exposure Routes and Pathways

Occupational exposure may occur through dermal contact where imidacloprid is produced or used. Exposure to the general population may occur through dermal contact or consumption of food residues. Imidacloprid is not a primary irritant and does not cause damage at the point of contact (i.e., skin, eyes, lungs and gastrointestinal tract). Penetration of the skin is poor but may be facilitated by formulating agents in commercial products.

Toxicokinetics

There are two principal routes of metabolism in mammals. The first involves oxidative cleavage to imidazolidine and 6-chloronicotinic acid, with urinary excretion of imidazolidine moiety. The nicotinic moiety is degraded by glutathione-conjugation to a mercapturic acid derivative and then to methyl mercaptonicotinic acid, which is conjugated with glycine to form a hippuric acid conjugate for excretion. The second involves hydroxylation of the imidazolidine ring, followed by the elimination of water and formation of an unsaturated metabolite. More than 90% of the dose is eliminated within 24 h, with total excretion by 48 h; 80% of the dose is excreted via the urine, with 20% eliminated via the feces. In rats, imidacloprid is absorbed and distributed to organs within 1 h following oral administration. It is not distributed to fatty tissues, the central nervous system (CNS) or bone, so it tends not to accumulate or affect the CNS. Poor penetration of the blood–brain barrier also occurs with other neonicotinoids. Reduced access to receptors in the CNS contributes to its low potential for centrally mediated effects.

Mechanism of Toxicity

Mammalian tissues contain many subtypes of nicotinic receptors that are located in various tissues, including autonomic ganglia, skeletal muscle (neuromuscular junction), spinal cord, and various brain regions. Differences in binding properties to the

various receptor subtypes contribute greatly to the much lower activity of imidacloprid and other neonicotinoids in vertebrate tissues, as compared to insects. The relative specificity for the nicotinic receptor in insects is used to select neonicotinoids for commercial development. This attribute, combined with poor penetration of the blood–brain barrier to access tissues with the most sensitive receptors, contributes to high margins of safety.

The acute toxicity (i.e., lethal potency) of imidacloprid, other neonicotinoids, and related analogs in mammals is most closely related to potency at the α_7 nicotinic receptor subtype, followed in order by potency at α_4, β_2, α_3, and α_1 nicotinic receptors, respectively. However, acute toxicity in mammals involves complex actions (agonist and antagonist) at multiple receptor subtypes and these actions vary greatly with minor changes in chemical structure.

Acute and Short-Term Toxicity (or Exposure)

There are few published studies on the toxicity of imidacloprid or other neonicotinoid insecticides and the ones that are available are generally limited to an examination of acute lethal potency (e.g., LD_{50}). Casida and coworkers reported tremor in mice treated with an acute oral dose of imidacloprid and other neonicotinoids, providing evidence of nicotinic stimulation at near-lethal or lethal dose levels. The limited information on the toxicology of imidacloprid that has been published contrasts with the extensive database generated for the registration of commercial products. These studies were performed in accordance with regulatory guidelines (e.g., US Environmental Protection Agency, Organization for Economic Cooperation and Development, and Japanese MAFF), in compliance with GLP standards.

Animal

Imidacloprid is not an irritant and does not produce evidence of dermal sensitization. Acute exposure to imidacloprid produces minimal toxicity by dermal and inhalation routes of exposure and moderate toxicity by oral administration. The acute (4 h exposure) LC_{50} by inhalation is $>69\,mg\,m^{-3}$ air (droplets) and $>5323\,mg\,m^{-3}$ air (dust). The LD_{50} in rats by oral and dermal exposure is 450 and $>5000\,mg\,kg^{-1}$, respectively. Acute administration of an aqueous suspension by gavage to adult rats had no effect at 50 and $100\,mg\,kg^{-1}$ in males and females, respectively, while higher doses of up to $315\,mg\,kg^{-1}$ produced clinical signs, without mortality. Treatment-related deaths at higher doses generally occurred within 3–7 h following treatment. Signs associated with treatment include tremor, gait incoordination, decreased activity, as well as nasal and urine staining. Signs of intoxication occurred within 15–40 min following oral administration and, except for stains, were reversible within 8–24 h after treatment. This profile is consistent with rapid distribution and metabolism.

Human

Little is known about the acute toxicity of imidacloprid in humans.

Subchronic Toxicity

Imidacloprid was administered through the diet for 13 weeks to young-adult Wistar rats. Clinical signs associated with treatment were not evident at exposures as high as $300\,mg\,kg^{-1}\,day^{-1}$. The liver was the principal target organ, with hypertrophy of hepatocytes and sporadic cell necrosis in high-dose males only. Liver pathology was mild at study termination and fully reversible within the 4 week recovery period. The no-observed-effect level (NOEL) was 14 and $83\,mg\,kg^{-1}\,day^{-1}$ in males and females, respectively.

Imidacloprid was administered through the diet to young-adult beagles for 13 weeks. Tremor was seen in males and females that received 600 and 1800/1200 ppm (dietary level reduced after week 4 due to decreased food consumption and weight loss), however, this finding was not substantiated at comparable doses in other studies. There was no evidence of tissue damage by clinical chemistry, gross necropsy examination, tissue weight or microscopic examination at any dietary level. The NOEL was 200 ppm in both sexes.

Chronic Toxicity (or Exposure)

Animal

Rat and Mouse A chronic toxicity/carcinogenicity study was performed, with imidacloprid administered to male and female Wistar rats for 12 months and 2 years at dietary levels of 100, 300, 900, and 1800 ppm. These dietary concentrations resulted in average daily dosages of 5.7, 17, 51, and $103\,mg\,kg^{-1}$ for males, and 7.6, 25, 73, and $144\,mg\,kg^{-1}$ for females. The thyroid was a target organ, with mineralization of the colloid, fewer colloid aggregation sites, and parafollicular hyperplasia sites at $144\,mg\,kg^{-1}\,day^{-1}$. This lesion did not affect thyroid function (e.g., plasma T3, T4, and TSH levels were normal). Mineralization of the colloid was also evident in males at 300 ppm and in both sexes at 900 ppm. There was no change in liver

morphology at any dietary level. The NOEL was 5.7 mg kg^{-1} day^{-1} and there was no evidence of carcinogenicity.

The oncogenic potential of imidacloprid was investigated in B6C3F1 mice that were exposed through the diet for 12 or 24 months, at concentrations that resulted in average daily doses of 20, 66, 208 or 414 mg kg^{-1} for males and 30, 104, 274 or 424 mg kg^{-1} for females. There were no clinical signs due to treatment and no effect on survival at any level. Metabolic adaptation was apparent at the highest dose only as low-grade periacinary hepatocyte hypertrophy. There were no effects on serum chemistry, tissue weight, or tissue morphology (by gross and microscopic examination) at any dietary level. There was no evidence of carcinogenic potential and the overall NOEL was 66 mg kg^{-1} day^{-1}.

Based on results in both species, imidacloprid is classified in category 'E', which indicates there is evidence of non-carcinogenicity for humans.

Dog Imidacloprid was administered to young-adult beagles for 52 weeks at dietary levels corresponding to dosages of 6.1, 15, and 41/72 mg kg^{-1} day^{-1} (the dietary level was increased at week 17). The tremor that was evident in the aforementioned subchronic canine study was not evident here at any dietary concentration. Effects in high-dose animals included a slight increase in plasma cholesterol (females only) and a slight increase in hepatic cytochrome P-450 activity (both sexes). The liver was the principal target organ, with increased liver weight and induction of cytochrome P-450 enzymes. The NOEL in this study was 15 mg kg^{-1} day^{-1}.

Human

Little is known regarding the chronic effects of imidacloprid in humans.

Mutagenicity

Imidacloprid has been evaluated for mutagenicity using a full complement of *in vitro* and *in vivo* tests required for registration. All tests, including the *in vitro* point mutation tests, *in vivo* chromosomal aberration tests, a mitotic recombination test in yeast, a rec assay with *Bacillus subtilis*, and the unscheduled DNA synthesis (UDS), were negative.

Developmental Toxicity

The potential for imidacloprid to produce developmental toxicity, including teratogenicity, was examined in the rat and rabbit. In the rat, fetal malformations were not evident at any dose, the maternal NOEL was 10 mg kg^{-1} day^{-1} and the fetal NOEL was 30 mg kg^{-1} day^{-1}. In the rabbit, embryotoxicity was only evident at a maternally toxic dose and fetal malformations were not evident at any dose; the maternal NOEL was 8 mg kg^{-1} day^{-1} and the fetal NOEL was 24 mg kg^{-1} day^{-1}. The results in these species indicate that imidacloprid is not a primary embryotoxicant and is not teratogenic.

Reproductive Toxicity

Effects on reproduction and development were examined in a two-generation, two-litter study in Wistar rats (30/sex/dietary level in the parental generation), at dietary concentrations of 100, 250, and 700 ppm. Liver enzymes (cytochrome P-450, *O*-demethylase, and *N*-demethylase) were induced in high-dose maternal animals. Reproduction and development were not affected at any dietary level and there was no evidence of pathology, in the form of malformations, gross lesions, changes in tissue weight or histopathology, at any dose. The NOEL in this study was 6.7 mg kg^{-1} day^{-1} for adults and 12.5 mg kg^{-1} day^{-1} for the offspring.

Neurotoxicity

Acute neurotoxicity was evaluated in adult rats that received a single dose of imidacloprid by gavage as an aqueous suspension at doses of 20 (females only), 42, 151, or 307 mg kg^{-1} (both sexes). The only effect at 42 mg kg^{-1} was a slight decrease in the activity of females in an automated test device. Effects at 150 mg kg^{-1} included tremor (one female), a slight decrease in body temperature and red nasal stain. The high dose produced severe toxicity, including lethality (two males and eight females) within 4–24 h after treatment and 4 h after treatment, tremor was apparent in all surviving animals and body temperature was reduced. Overt toxicity at this lethal dose included motor incoordination (e.g., incoordinated gait and impaired aerial righting), autonomic signs (e.g., perianal and urine stains), and evidence of CNS depression (e.g., minimal activity and a diminished response to stimuli). Clinical signs generally resolved in surviving animals within 8–24 h following treatment. Neuropathology was not evident. The NOEL for males and females was 42 and 20 mg kg^{-1}, respectively.

Neurotoxicity was also evaluated in young-adult rats, with imidacloprid administered via the diet for 13 weeks at dietary concentrations of 150, 1000, and 3000 ppm. These dietary levels resulted in average daily exposures of 9.3, 63, and 196 mg kg^{-1} for males, and 10.5, 69, and 213 mg kg^{-1} for females.

There was little evidence of toxicity at any dietary level. Effects at 1000 and 3000 ppm consisted of decreased food consumption and an associated decrease in body weight gain. Effects were not evident by cage-side observation or automated test of activity at any dietary level. At week 13, there was a modest increase in the incidence of high-dose males with a slightly uncoordinated righting response. There was no evidence of neuropathology. The NOEL was 9.3 and 10.5 $mg\,kg^{-1}\,day^{-1}$ for males and females, respectively.

In Vitro Toxicity Data

As noted above, *in vitro* studies of the selectivity of imadocloprid for nicotinic receptors has been useful in explaining its selective toxicity. *In vitro* mutagenic assays were all negative.

Clinical Management

The recommended treatment in cases of acute poisoning is symptomatic. It is important to monitor and support breathing if signs of respiratory paralysis appear and to monitor blood pressure and pulse rate, since bradycardia and hypotonia may occur. Since imidacloprid does not inhibit acetylcholinesterase activity, treatment with a reactivating oxime (e.g., pralidoxime) is not indicated. Furthermore, treatment with a nicotinic antagonist may be ineffective or potentially harmful since symptoms of poisoning may be mediated by stimulation or inhibition of various nicotinic receptor subtypes or by other possible mechanisms.

Environmental Fate

Numerous laboratory and field studies have been conducted with imidacloprid, providing a comprehensive understanding of its behavior in the environment. Imidacloprid has a low octanol–water partition coefficient ($K_{ow} = 3.72$), which is consistent with it not accumulating in biological tissue or the food chain. Imidacloprid has an extremely low vapor pressure and therefore does not volatilize into the atmosphere. Imidacloprid is generally not persistent in aquatic environments and is quickly degraded by sunlight (4.2 h). In simulated pond studies, imidacloprid quickly degraded, with a half-life of 1.4 days. Imidacloprid also has rather unique soil binding characteristics; the longer imidacloprid ages in the field and the lower the initial residue concentration, the tighter it binds to soil. Field dissipation studies conducted in the USA have shown that the parent compound and its metabolites have limited mobility in soil and are not likely to leach to groundwater.

Ecotoxicology

Avian Species

The LD_{50} values for bobwhite quail and Japanese quail are 152 and 31 $mg\,kg^{-1}$, respectively. Red-winged blackbirds and brown-headed cowbirds have been observed to avoid imidacloprid-treated seeds after experiencing transient gastrointestinal distress (retching) and ataxia (loss of coordination). And so, it was concluded that treated seeds represent a minimal risk to birds.

Aquatic Organisms

Imidacloprid is moderately toxic to fish, with 96 h LC_{50} values of 211 $mg\,l^{-1}$ for rainbow trout, 280 $mg\,l^{-1}$ for carp and 237 $mg\,l^{-1}$ for golden orfe. Imidacloprid is slightly toxic to *Daphnia magna* (48 h $EC_{50} = 85\,mg\,l^{-1}$) but is highly toxic to certain other aquatic invertebrates.

Beneficial Insects

Imidacloprid is highly toxic to bees if used as a foliar application, especially during flowering, but is not considered a hazard to bees when used as a seed treatment.

See also: Acetamiprid; Neonicotinoids; Nithiazine.

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Immediately Dangerous to Life or Health (IDLH) Values

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The immediately dangerous to life or health (IDLH) air concentration values have been recommended by the US National Institute for Occupational Safety and Health (NIOSH) as respirator selection criteria. The current NIOSH definition for an IDLH condition is a situation "that poses a threat of exposure to airborne contaminants when that exposure is likely to cause death or immediate or delayed permanent adverse health effects or prevent escape from such an environment." NIOSH's stated purpose for establishing IDLH values is to "ensure that the worker can escape from a given contaminated environment in the event of failure of the respiratory protection equipment." The complete introduction and documentations to the 398 existing IDLH values can be read or downloaded at the NIOSH Internet website.

At the time of writing, NIOSH is reconsidering several aspects of the IDLH definition and the criteria used in developing IDLH values.

Background

The concept of using respirators to protect workers in IDLH situations was discussed at least as early as the 1940s. The following is from a US Department of Labor bulletin:

> The situations for which respiratory protection is required may be designated as, (1) nonemergency and (2) emergency. Nonemergency situations are the more or less normal ones that involve exposure to atmospheres that are not immediately dangerous to health and life, but will produce marked discomfort, sickness, permanent harm, or death after a prolonged exposure or with repeated exposure. Emergency situations are those that involve actual or potential exposure to atmospheres that are immediately harmful and dangerous to health or life after comparatively short exposures.

The US Occupational Safety and Health Administration (OSHA) defines an IDLH concentration in their hazardous waste operations and emergency response regulation (29 CFR 1910.120) as follows:

> An atmospheric concentration of any toxic, corrosive or asphyxiant substance that poses an immediate threat to life or would cause irreversible or delayed adverse health effects or would interfere with an individual's ability to escape from a dangerous atmosphere.

The OSHA regulation on permit-required confined spaces (29 CFR 1910.146), defines an IDLH condition as follows:

> Any condition that poses an immediate or delayed threat to life or that would cause irreversible adverse health effects or that would interfere with an individual's ability to escape unaided from a permit space.

History

IDLH values were first developed by NIOSH in the mid-1970s based on an earlier concept by the US Bureau of Mines.

In 1974, NIOSH and OSHA jointly initiated development of occupational health standards for substances with then-existing OSHA permissible exposure limits (PELs). This joint effort was called the Standards Completion Program (SCP). The SCP developed 387 substance-specific draft standards with supporting documentations that became the basis for the original 1978 *NIOSH/OSHA Pocket Guide to Chemical Hazards* and the *Occupational Health Guidelines for Chemical Hazards*. As part of the respirator selection process for each draft standard, an IDLH value was established. The purpose for establishing an IDLH value was to determine a concentration from which a worker could escape without injury or without irreversible health effects in the event of respiratory protection equipment failure. At that time, NIOSH did not recommend a respirator for use at concentrations above the IDLH, with the exception of an escape respirator. Respirator selection was based on the reliability of the respirator; the perceived danger was respirator failure.

In determining IDLH values, NIOSH considered a worker's ability to escape without loss of life or irreversible health effects, along with severe eye or respiratory irritation and other deleterious effects that could prevent escape. To provide a safety margin, NIOSH based IDLH values on effects that might result from exposures as long as 30 min.

In 1985, NIOSH changed its respirator recommendations for IDLH environments from 'highly reliable', based on the danger of respirator failure, to 'most protective', based on health effects, and included a recommendation for 'emergency or planned entry in unknown concentration or IDLH conditions'. These highly protective respirators included either:

- self-contained breathing apparatus (SCBA) with a full facepiece and operated in a pressure-demand or other positive-pressure mode, or

- a supplied-air respirator with a full facepiece and operated in a pressure-demand or other positive-pressure mode in combination with an auxiliary SCBA operated in a pressure-demand or other positive-pressure mode.

Criteria for Revised IDLH Values

In 1994, NIOSH used the following hierarchy to develop a preliminary, revised IDLH value for each chemical:

1. Human acute toxicity data were used if sufficient.
2. When sufficient human data were unavailable, mammalian acute lethal concentration (LC) data were considered. The lowest reliable LC data were used, with LC_{50} data preferred. If acute LC data determined from a 30 min exposure were not available, the data were adjusted to an equivalent 30 min value using the following relationship, proposed in 1986 by ten Berge and colleagues:

$$\text{Adjusted } LC_{50}\ (30\ \text{min}) = LC_{50}(t) \times (t/0.5) \times (1/n)$$

   where $LC_{50}(t) = LC_{50}$ determined over t hours and where n is an experimentally derived constant. The adjusted or experimentally derived 30 min LC values were divided by a factor of 10 to determine a preliminary IDLH value.
3. When neither human data nor animal LC data were sufficient, NIOSH considered animal lethal dose (LD) data. NIOSH used the LD data to estimate the equivalent total dose to a 70 kg worker. The 30 min LC was estimated by dividing by $10\ m^3$, even though a worker breathing at a rate of $50\ l\ min^{-1}$ for 30 min would inhale $1.5\ m^3$ of air. NIOSH determined a preliminary IDLH by dividing this estimated LC value by a factor of 10.
4. Chronic toxicity data were considered if no relevant acute toxicity data existed.
5. When relevant toxicity data for the specific chemicals in question were lacking, analogies to substances with similar acute toxic effects were considered.

All preliminary IDLH values derived during this update were checked against the following factors prior to establishing the final revised IDLH value:

1. Ten per cent of the lower explosive limit (LEL). (Note: OSHA considered concentrations in excess of 10% of the LEL to be a hazardous atmosphere in confined spaces.)
2. RD_{50} data: an estimate of severe respiratory irritation measured as the 10 min exposure concentration producing a 50% respiratory rate decrease in rodents.
3. Other short-term exposure guidelines, such as the American Industrial Hygiene Association's emergency response planning guidelines (ERPGs) and the National Research Council's emergency exposure guidance levels (EEGLs); short-term public emergency guidance levels (SPEGLs); and occupational exposure standards or recommendations such as OSHA PELs, NIOSH recommended exposure limits (RELs), or the American Conference of Governmental Industrial Hygienists (ACGIH®) threshold limit values (TLVs®).
4. Based on NIOSH respirator decision logic, the revised IDLH values could not be greater than 2000 times the NIOSH REL or OSHA PEL.
5. The revised IDLH values would not be greater than the original IDLHs derived during the SCP.

See also: National Institute for Occupational Safety and Health; Occupational Safety and Health Act, US; Occupational Safety and Health Administration; Occupational Toxicology; Occupational Exposure Limits.

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Relevant Website

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Immune System

Michael P Holsapple and Norbert E Kaminski

Introduction

The role of the immune system may be stated succinctly as the preservation of integrity. This system is charged with identifying that which is 'self' and that which is 'nonself'. Examples of self are all the tissues, organs, and cells of the body. Examples of nonself are a variety of opportunistic pathogens, including bacteria and viruses, and transformed cells or tissues (i.e., tumors). The great complexity of the mammalian immune system is an indication of the importance, as well as the difficulty, of this task. If the immune system fails to recognize as nonself an infectious entity or the neoantigens expressed by a newly arisen tumor, then the host is in danger of rapidly succumbing to the unopposed invasion. This aspect of immunocompetence is the reason why the immune system is often made synonymous with 'host defense'. Alternatively, if some integral bodily tissue is not identified as self, then the immune system is capable of turning its considerable defensive capabilities against that tissue, and an autoimmune disease may be the end result. This aspect of immunocompetence emphasizes the tremendous destructive potential which is associated with the host defense mechanisms of the immune system. The cost to the host of these mistakes, made in either direction, may be quite high. The fact that mistakes can occur in either direction is discussed below as a continuum. Because the cost of mistakes in immunocompetence can be so high, and since there is tremendous diversity involved in the identification of self versus nonself, a complex array of organs, cells, soluble factors, and their interactions has evolved to regulate this system and minimize the frequency of errors in either direction.

Definition of Immunotoxicology

Studies in animals and humans have indicated that the immune system, like most organs, is a potential target organ, and that damage to this system can be associated with morbidity and even mortality. These studies coupled with tremendous advances made in immunology and molecular biology have led to a steady and exponential growth in our understanding of immunotoxicology during the past 20 years. In addition, recognition by regulatory agencies that the immune system is an important as well as sensitive target organ for chemical- and drug-induced toxicity (i.e., as described in greater detail later in this chapter) almost insures that this subdiscipline of toxicology will continue to flourish and grow in the foreseeable future. Immunotoxicology can be most simply defined as the study of adverse effects on the immune system resulting from occupational, inadvertent, or therapeutic exposure to drugs, environmental chemicals, and, in some instances, biological materials. Collectively, these agents are frequently referred to as xenobiotics, where 'xeno-' means foreign and '-biotic' means anything affecting biology.

Adverse Effects

A critical component of this definition is the term 'adverse'. The need to determine whether an effect is adverse is what differentiates toxicology from other branches of biomedical science. Consequently, immunotoxicology is not merely the demonstration of treatment-related changes in a component of the immune system. Not all treatment-related changes are adverse. Some changes may be beneficial, some may be indifferent, and some are of unknown or uncertain consequence. It is inappropriate to declare an effect to be adverse simply because an adverse consequence cannot be ruled out. The long-term credibility of any scientific discipline depends on involved scientists being forthcoming when there is uncertainty, or when the effects have no known adverse consequence. Some have attempted to classify as adverse any effect which is undesired or unwanted. This definition of adverse effect is social or cultural in nature and has little scientific utility because social definitions may vary as a matter of individual preferences. The term adverse has been defined in classical toxicology as the undesired side effects of a drug, which are deleterious. This definition of an adverse effect differentiates between undesired (unwanted) effects that are deleterious and those that are not, thereby avoiding the quagmire associated with social definitions.

The evolution of immunotoxicology has been based in large part on the design and validation of critical experimental approaches to most clearly answer the question, Is an observed effect adverse or is it not? For the purposes of this entry, an adverse effect in immunotoxicology will be defined as a xenobiotic-induced change in the ability to perform an immune function. A suppression in immune functional ability

would obviously be deleterious if the host were to encounter an opportunistic pathogen. As noted previously, an exaggerated immune response can also be deleterious. The utility of this definition is that it focuses attention on a specific functional ability. This definition does not explicitly address the issue of a xenobiotic-induced change in a structural component of the immune system, that is, such as a change in the weight of an immune organ or a histopathological change in an immune organ. This omission is a deliberate one because a structural change without a concomitant change in a functional component would fall into the category of a treatment-related effect of unknown or uncertain consequence. As such, by this definition, the demonstration of a treatment-related structural change in itself cannot be considered an adverse immunotoxicological effect.

Immunotoxicology as a Continuum

Because the primary role of the immune system is the discrimination of self versus nonself, immunotoxic effects can occur in either direction. As such, immunotoxicology can be thought of as a continuum, which is depicted as the solid dark vertical arrow in **Figure 1**. A xenobiotic-induced change in immunocompetence from the normal range, which is manifest as an underactive immune system, is shown as a 'suppressed immune response', while a xenobiotic-induced change manifested as an overactive immune system is shown as an 'exaggerated immune response'. The adverse (i.e., deleterious) consequences of suppressed immune function would reflect an inability to recognize nonself and are depicted as infections and cancer. The adverse consequences of exaggerated immune function would reflect an inability to recognize self and are depicted as hypersensitivity and autoimmunity. A hypersensitivity response is most simply defined as an acquired immune response which occurs in an exaggerated or inappropriate form, causing tissue damage. Autoimmune disease occurs when the reactions of the immune system are directed against the body's own tissues.

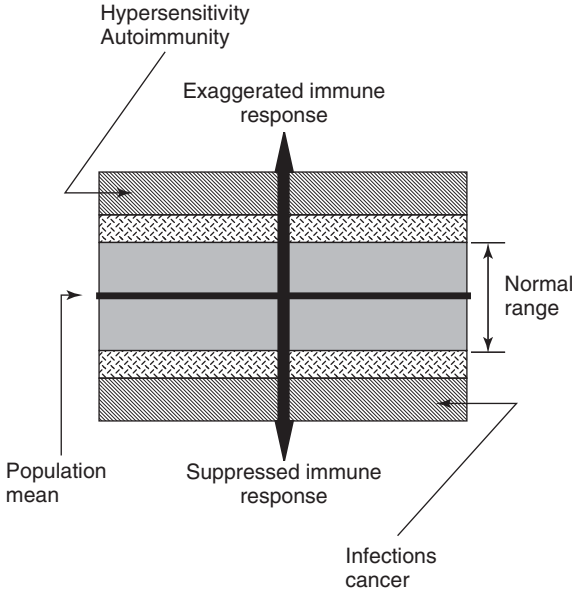

Figure 1 Immunotoxicology is a continuum. There are adverse consequences associated with both suppressed and exaggerated immune responses.

Treatment-related effects which are not clearly manifested as adverse are depicted in **Figure 1**, as the lightly shaded areas both above and below the normal range. The consequences of immunotoxicology are also described in greater detail in later sections of this entry. It is also important to emphasize that whether a treatment-related effect on the immune system is manifest as adverse or not in a given individual is dependent on where that individual is positioned in relationship to the normal range and the population mean. For example, an individual with a suppressed immune system, such as someone with the acquired immunodeficiency syndrome (AIDS) or any of a number of congenital immune deficiencies, could benefit by being exposed to a drug or chemical which causes immunostimulation. Similarly, an individual with an autoimmune disease can be treated therapeutically with an immunosuppressive drug. It is also important to note that, it is routine to treat an individual scheduled for an organ/tissue transplant with an immunosuppressive drug. The goal of this therapy is to purposefully lower that individual's capacity to identify self versus nonself in order to decrease the chances that the transplant (i.e., an example of nonself) will be rejected. However, there are consequences associated with this treatment which are consistent with the continuum depicted in **Figure 1**.

Organization of the Immune System

The mammalian immune system is a complex system which is dependent on the integration of, and orchestrated cooperativity among, several organs, cells, and soluble factors. Components of the immune system are distributed throughout the body. An appreciation of the organization of the various components of the immune system is essential to understand immunotoxicology. By knowing the role played by a given component of the immune system in host defense mechanisms, one can begin to understand the

consequences of a xenobiotic-induced change in the function of that component.

Organs of the Immune System

The organs of the body which comprise the immune system and/or contribute to immune function include the bone marrow, spleen, thymus, lymph nodes, a network of lymphoid tissue along secretory surfaces (i.e., the so-called mucosa-associated lymphoid tissue, MALT), and the skin. Lymphoid organs can be classified in two ways. The first classification is based on the role that organs play in the development of the immune system and/or its ability to elicit a response.

Primary and Secondary Lymphoid Organs

Primary lymphoid organs are those organs in which the production of the cells of the immune system takes place. For example, bone marrow is a primary organ and contains a pluripotent stem cell which serves as the precursor to red blood cells (i.e., erythrocytes) and myeloid progenitors (which ultimately differentiate into granulocytes, mast cells, monocytes, and platelets), in addition to lymphoid progenitors (which ultimately differentiate into the various types of lymphocytes). Hematopoiesis is a general term used to refer to the production of the cells of the blood, and it can be subdivided into erythropoiesis, myelopoiesis, and lymphopoiesis, respectively, based on the cell lineages described previously. Lymphoid progenitors will emerge from the bone marrow and travel to other primary lymphoid organs where the final stages of lymphocyte maturation take place. As described later, mature lymphocytes play a major role in discriminating between self and nonself because they are endowed with surface receptors characterized by tremendous specificity. Lymphoid progenitors which receive their final education in the thymus are called thymus-derived lymphocytes or T cells. The other major subtype of lymphocyte is the B cell, so named because it was originally characterized in the chicken as a lymphoid progenitor which receives its final education in a primary lymphoid organ called the Bursa of Fabricious, an outpocket of the gastrointestinal epithelium. Although there is no Bursa in mammals, fetal liver, spleen, and adult bone marrow are considered the 'bursal equivalents' and function as the primary lymphoid organs for the production of B cells. The process of lymphopoiesis takes place within specific regions of the thymus and bursal equivalents called microenvironments and is regulated by specialized cells (bone marrow stromal cells and thymic epithelial cells) and their soluble factors (including the interleukins IL-3, IL-7, IL-9, and IL-12; see **Table 1**), which comprise the microenvironments. The stages of lymphopoiesis are generally believed to be antigen independent, where antigen is defined as any substance which can stimulate a specific immunological reaction. The surface receptors of lymphocytes, mentioned previously, are directed toward 'antigen'. Although lymphopoiesis is neither antigen dependent nor antigen driven, a role for antigen cannot be excluded because factors secreted during an antigen-specific reaction in the periphery can promote various forms of hematopoiesis in the bone marrow.

Moreover, although antigen is not a driving force for lymphocyte development, the antigen receptors on the surface of lymphocytes play a critical role. Many immature lymphocytes have the potential to respond to self products and therefore pose a threat. During development, immature B cells in the bone marrow and immature T cells in the thymus will have an opportunity to interact with neighboring cells which express surface proteins indicative of self. These self proteins are encoded for by the major histocompatibility complex (MHC). If the antigen receptor on any lymphocyte binds too effectively to these MHC-derived proteins, then that cell will be eliminated. This process of negative selection is thought to be mediated by one of two possible mechanisms. Either this inappropriate recognition of self proteins triggers apoptosis, which is programmed cell death, and the lymphocytes are eliminated, or these cells become anergic, which is an induced lack of responsiveness to antigenic stimulation. This process of negative selection is not limited to self proteins obviously associated with the microenvironments of the bone marrow and thymus. Self proteins from different parts of the body are actually transported to these primary lymphoid organs to probe lymphocytes for reactivity against distant tissues.

Secondary lymphoid organs are those organs in which the antigen-dependent proliferation and differentiation of specific lymphocytes takes place. These organs are responsible for the dissemination of an antigen-specific immune response and include lymph nodes, spleen, and the various types of MALT, which are further defined below. An appreciation for the role that secondary lymphoid organs play in the immune system can be derived from the fact that swollen lymph nodes (i.e., as a consequence of the antigen-specific lymphoproliferation) are a hallmark indicator of certain types of infections.

Table 1 Cytokine network

Cytokine	*Other names*	*Cell source*	*Cell target and actions*
Interferon-α (IFN-α)		Leukocytes	B cells: proliferation and differentiation NK cells: stimulates cytolytic activity T_C cells: increases generation APCs: increases MHC I and II expression Others: increases MHC I and FcR expression; induces antiviral state
IFN-β		Fibroblasts	Similar to IFN-α
IFN-γ		T_H cells	B cells: stimulates IgG2a synthesis and inhibits IL-4-induced IgE/IgG1 synthesis APCs: increases MHC I and II expression Macrophages (macs): activates cytolytic activity NK cells: stimulates cytolytic activity Others: increases MHC I expression; induces antiviral state
Interleukin 1 (IL-1)	Endogenous pyrogen	Monocytes/macs	T_H cells: stimulates production of lymphokines, especially IL-2 and expression of IL-2R B cells: proliferation and differentiation Macs: stimulates production of cytokines, IL-1, IL-6, and tumor necrosis factor-α (TNF-α) Brain: fever response
IL-2	T cell growth factor (GF)	T_H cells	T_H cells: stimulates proliferation and release of lymphokines (especially T_{H1} cells) B cells: proliferation and differentiation NK cells: activates
IL-3	Multicolony stimulating factor (MSF)	T_H cells	Bone marrow (BM): promotes growth of stem cells to granulocytes, macs, and mast cells
IL-4	BCGF; B-cell-stimulating factor (BSF)	T_H cells (B cells)	B cells: stimulates IgE and IgG1 production and increases MHC II expression T_H cells: promotes generation; synergizes with IL-2
IL-5	T-cell-replacing factor (TRF); BCGF II	T_H cells	B cells: proliferation and differentiation; stimulates IgA production
IL-6	IFN-γ_2	T_H cells Monocytes Endothelial cells Fibroblasts	T cells: proliferation and differentiation B cells: proliferation and differentiation Others: similar profile of activity to IL-1; synergizes with IL-1
IL-7	Lymphopoietin	BM stroma	T cells: induces growth of immature cells B cells: induces growth of immature cells
IL-8	Neutrophil-activating factor (NAF)	Monocytes	Neutrophils: chemotaxis; granular exocytosis; respiratory burst
IL-9		T_H cells	BM: stimulates growth of erythroid and megakaryocyte precursors Others: promotes mast cell growth B cells: acts synergistically with IL-4 in production of IgE and IgG1
IL-10		T_H cells (B cells)	T_{H1} cells: inhibits lymphokine synthesis T_{H2} cells: promotes generation Monocytes: inhibits cytokine synthesis T_C cells: stimulates IL-2-dependent growth Mast cells: stimulates growth
IL-11		Fibroblasts BM stroma	BM: stimulates T-dependent antibody response; resembles IL-6
IL-12	NK cell stimulatory factor (NKSF)	Monocytes/macs	NK cells: activates cytotoxicity
		B cells	T_{H1} cells: stimulates proliferation and lymphokine production, especially IFN-γ T_{H2} cells: inhibits generation (negative feedback) T_C cells: activates; synergizes with IL-2
IL-13	P600	T cells	B cells: promotes growth and differentiation macrophages; inhibits inflammatory cytokine production T_{H1} cells; inhibits cytokine release

Table 1 Continued

Cytokine	*Other names*	*Cell source*	*Cell target and actions*
IL-15	T-cell growth factor	T cells	T cells: stimulates growth NK cells; stimulates growth epithelial cells; stimulates growth
IL-16	T cells; mast cells; eosinophils		$CD4^+$ T cells: chemoattractant monocytes; chemoattractant eosinophils; chemoattractant T cells, anti-apoptotic for IL-2-activated cells
IL-17	MCTLA-8	CD4+ memory cells	Epithelial cells: induces cytokine production endothelial cells; induces cytokine production fibroblasts; induces cytokine production
IL-18	Interferon-γ inducing factor		
Lymphotoxin	Tumor necrosis factor-β (TNF-β)	T cells	Target cells: kills
Macrophage-activating factor	MAF	T_D cells	Macs: activates cytotoxicity and proinflammatory actions
Macrophage-inhibiting factor	MIF	T_D cells	Macs: inhibits migration
Transforming growth factor-β (TGF-β)	TGF-β	Lymphocytes	B cells: suppresses growth; inhibits IgM and IgG production; decreases MHC II expression
		Macs	T cells: suppresses growth Monocytes: inhibits TNF production; chemotaxis; induces IL-1 and IL-6 expression
Tumor necrosis factor-α (TNF-α)	Cachectin (TNF-α)	Monocytes/macs	Tumor cells: cytotoxicity Others: similar profile of activity of IL-1; promotes antiviral state

Internal and External Lymphoid Organs

The second classification of lymphoid organs is based on their location, with some being classified as internal organs and others being classified as external organs. The internal lymphoid organs include the bone marrow, thymus, spleen, and some lymph nodes. The external lymphoid organs include all the components of MALT as well as the lymph nodes draining MALT. As indicated previously, MALT is defined as lymphoid tissue associated with mucosa. This tissue can be subdivided into more specific regions based on the anatomical location, and includes gut-associated lymphoid tissue (including Peyer's patches and the appendix) and bronchus-associated lymphoid tissue. The skin is another example of an external organ whose contribution to the immune system is sometimes underappreciated. Although the skin does not contain organized lymphoid tissue, there are immune components associated with the skin that are interconnected with other immune organs, leading to the concept of the so-called skin-associated lymphoid tissue. An appreciation for the important role that skin plays as a 'first line of defense' in the immune system can be derived from the fact that when this barrier is breached, as occurs following an abrasion and especially so after a severe burn, a serious consequence is an increase in the incidence and severity of infections.

The importance of this second classification of lymphoid organs is that the two locations behave somewhat independently in host defense. An immune response mediated primarily by internal lymphoid organs is generally referred to as a systemic immune reaction or systemic immunity, while an immune response mediated primarily by external lymphoid organs is generally referred to as a local immune reaction or local immunity. As will be described in greater detail, there are also differences in the specific effector functions associated with systemic and local immunity.

Cells of the Immune System

The most obvious example of a cellular component of the immune system is the lymphocyte and includes all the various subtypes of T and B cells. As indicated previously, the fundamental distinction between T cells and B cells is based on the specific primary lymphoid organ in which the final stages of lymphopoiesis takes place. As described below, these cell types can also be distinguished based on their respective functions within the immune system as well as by phenotypic characteristics. For the purposes of this entry, a phenotype will be defined as a marker expressed on the surface of a cell which is genetically determined and which is frequently associated with the specific function of that

cell. Other important examples of cellular components of the immune system include monocytes, macrophages, granulocytes, mast cells, and natural killer (NK) cells. As with the lymphocytes, the specific functions of these cells as they relate to the immune system will be described in greater detail. Some of these cells are oftentimes found circulating in the blood. Examples of circulating cells include monocytes, granulocytes, and NK cells.

Other cells important to the immune system are typically tissue bound and include mast cells and macrophages. Macrophages present in tissue constitute the mononuclear phagocytic system, which was formerly known as the reticuloendothelial system. Macrophages located in specific anatomical regions have been frequently given distinct names, including Kupffer cells (liver), Langerhan cells (skin), microglia (brain), osteoclasts (bone), follicular dendritic cells (B-cell regions of lymphoid tissue), and interdigitiating dendritic cells (T-cell regions of lymphoid tissue). The issue of whether a lymphocyte is circulating or noncirculating is a bit more complex than for most of the other cellular components of the immune system. The blood circulation contains only a minor part of the body's total lymphocyte count (estimated at $\sim$1%), which is a relatively select population, the so-called recirculating lymphocyte pool. As such, an assessment of only the blood lymphocyte pool provides an incomplete inventory of the body's immune system as it relates to lymphocytes because it ignores the activities of the nonrecirculating cells. In general, the recirculating lymphocyte pool does not include cells that are in a state of activation, proliferation, or differentiation. As indicated previously, the dissemination of an antigen-specific reaction takes place in secondary lymphoid organs, especially lymph nodes. The lymphatic system represents a second circulatory system of conducting vessels (i.e., lymphatic capillaries and/or lymphatics), loose aggregates of lymphoid tissues (i.e., nodules), and more highly organized and structured organs (i.e., lymph nodes). Through this system passes lymph, a collection of tissue fluids rich in globulins and lymphocytes. As lymph passes through draining lymph nodes, it becomes progressively more enriched with lymphocytes. Lymphocytes within lymph can empty back into the blood circulation via the thoracic duct. After entry into the blood, these lymphocytes will eventually find their way into a lymph node, at which point they may either remain in the circulating blood or reenter the lymphatic circulation. Therefore, lymphocytes and their products are transported between lymphoid organs and throughout the body via the blood and the lymph. However, it is again important to emphasize that the percentage of the total lymphocyte count in the blood is normally very small.

Soluble Products of the Immune System

Soluble products also play an important role in the immune system and are oftentimes the primary mediator of a given effector function of the immune system. Some soluble products of the immune system are secreted by lymphocytes. For example, immunoglobulins (Igs) are secreted by B cells. It is important to emphasize that an antibody is an Ig molecule secreted by a B cell during an immune response that specifically reacts with the antigen. Therefore, 'Ig' and 'antibody' are distinct terms that are often used somewhat interchangeably as they pertain to effector functions by the immune system. A second example of lymphocyte-derived soluble products is the variety of substances secreted by T cells called lymphokines. Although lymphokines have been described as being hormone-like, they are probably more analogous to neurotransmitters because of their very localized sites of action and because of the signal transduction pathways they trigger in responsive cells. Specific examples of lymphokines, including the interleukins, will be provided and are summarized in **Table 1**. It is important to emphasize that lymphokines are a subgroup of a larger family of soluble factors secreted by a variety of cells, called cytokines, which can modulate immune function, and that both lymphokines and cytokines can affect cells, organs, and tissues outside of the immune system.

Some soluble products are secreted by cells of the immune system other than lymphocytes. For example, monocytes/macrophages are also capable of secreting cytokines, which are sometimes referred to as monokines and which include some interleukins, such as IL-1, IL-6, IL-8, and IL-12, and other factors such as tumor necrosis factor (TNF). Some soluble products with important roles in the immune system originate outside of the immune system. One example is interferon (IFN), which is actually a heterogeneous family of proteins of two types. Type I or viral IFNs are induced by infection and consists of IFN-α (interferon-α) and -IFN-β, which are secreted by leukocytes, and fibroblasts or epithelial cells, respectively. Type II or immune IFN, consists of IFN-γ, which is secreted by T cells (therefore, IFN-γ can be classified as a lymphokine) in response to specific antigens. A second example of a soluble product which originates outside of the immune system is complement, which is primarily produced in the liver. Complement is actually a group of $\sim$20

proteins, including several proteases, that activate and split each other in sequential order. The specific functional roles played by complement and the other soluble products of the immune system will be discussed later.

Immune Response

Innate versus Acquired Immunity

Foreign substances, including the various examples of nonself indicated in the Introduction, can provoke two basic types of immune responses, innate (also called nonspecific) immunity and acquired (also called specific) immunity. One of the easiest ways to present and understand the functions of the various cells and soluble products of the immune system is in the context of these two types of immune responses, which each make significant contributions to host defense capability. The principal difference between these two types of immunity is the role of the antigen, which was defined previously as any substance which can trigger a specific immunological reaction. In this context, it is important to note that an antigenic determinant on the surface of a microbe or tumor cell is usually about 10 amino acids in size and can be made up of polypeptides, carbohydrates, or lipids, and that a given type of microbe or tumor cell can express several different types of antigens as well as multiple copies of a given antigen. Innate immunity is considered to be antigen independent and occurs without prior exposure to antigens. Acquired immunity is considered to be antigen dependent and comprises all of the specific immunological reactions alluded to in the definition of antigen. Acquired immunity can be subdivided into humoral immunity and cell-mediated immunity, which are described in greater detail later.

Because innate immunity can be triggered upon the initial encounter with a foreign substance, its components are oftentimes called the first lines of defense. As such, it is appropriate to consider the skin as a component of innate immunity. Similarly, the following bodily functions contribute to host defense and should be considered parts of innate immunity: the lysosomal enzymes found in salivary, lacrimal, and vaginal secretions, which have bacteriastatic properties; the cough reflex, which is an important mechanism to clear the bronchial passages of irritants and potential infectious microbes; and the fever response, which is an important reaction to an infection because of the limited temperature range for the growth of most bacteria.

More traditional components of the innate immune defense system include phagocytic cells such as neutrophils and macrophages, NK cells, and the soluble products, type I IFN and complement. Neutrophils and macrophages are the primary cells involved in inflammatory responses. Their contribution to innate immunity is based on their abilities to phagocytize (i.e., to engulf; literally the foreign particle becomes enclosed by the cell membrane of the phagocyte into a phagosome) and to kill bacteria. The latter mechanism is carried out either by the extracellular release of lysosomal enzymes, oxygen radicals, bactericidal proteins, and proteinases or by the intracellular fusion of phagosomes containing the microbes and lysosomes containing these destructive mediators. Phagocytic cells are attracted to the site of infection or inflammation, a process known as chemotaxis, by a number of factors including some complement components and some cytokines, which are discussed later. Moreover, microbial cells and other foreign particles are also capable of attracting the attention of phagocytic cells directly because of unique properties. For example, bacteria produce peptides with an unusual chemical structure beginning with formyl-methionine sequences that are produced in very small amounts by mammalian host tissue. Therefore, large amounts of formyl-methionine peptides will stimulate neutrophil chemotaxis and phagocytosis. A second example has been identified in macrophages which have receptors for sugars typically found on many microbial organisms, that is, mannose, L-fructose, and galactose. The destructive capabilities of both macrophages and neutrophils can also be modulated by cytokines, primarily lymphokines produced by antigen-specific T cells, as discussed later.

NK cells are leukocytes of lymphoid or myeloid origin with the ability to kill target cells without prior sensitization. NK cells require intimate contact with target cells before lysis can take place. One postulated mechanism for cytolysis involves the production and secretion of a cytolytic protein, perforin, which functions to produce transmembrane channels in the target cell and ultimately leads to porous membrane lesions and cell death. The attachment of NK cells to their target cells is accomplished through an as yet poorly understood chemical means by which these cells seem to recognize certain viral or tumor-associated markers. As with the macrophage, the killing capability of NK cells can be modulated by T-cell-derived lymphokines, most notably IFN-γ.

In addition to their ability to produce and release destructive inflammatory mediators, neutrophils and monocytes/macrophages can contribute to the innate

immune response by the production and release of cytokines. The cell sources, targets, and actions for a number of cytokines are summarized in **Table 1**. IL-1 has been the most studied interleukin because it was the first one to be discovered and because it triggers a wide variety of activities in several organ systems. For the purposes of this entry, the discussion will be limited to actions of IL-1 associated with the innate immune response which include the activation of neutrophils, macrophages, and NK cells; cytostatic and cytotoxic actions for some tumor cells; the induction of the fever response in the brain (IL-1 has been identified as the 'endogenous pyrogen'); and the stimulation of some acute phase-reactive proteins by the liver. IL-6 is produced by a number of different cell types and possesses a profile of activity similar to IL-1, including the following actions: an increase in the synthesis of the major acute phase-reactive proteins by the liver and pyrogenic activity in the brain. IL-1 and IL-6 are known to act synergistically.

IL-8 is produced by activated monocytes and macrophages and acts on neutrophils as a chemotactic factor and as a stimulus for enzyme release and an oxidative burst. IL-12 is also synthesized by monocytes/macrophages in response to bacteria or other parasites and acts on NK cells to activate them. TNF is produced predominantly by activated macrophages and it draws its name from the fact that it was originally isolated as a factor which was capable of triggering a hemorrhagic lesion in transplanted tumors. However, the effects of TNF are now known to extend well beyond that original definition. For example, TNF produces many of the same actions that were identified for IL-1, and these two cytokines can act synergistically. TNF is also known as cachectin because of its association with the wasting syndrome (cachexia) characteristic of chronic diseases, including some malignancies. It is important to emphasize that IL-1, IL-6, IL-12, and TNF also have actions on lymphocytes, and these immunoregulating properties are discussed later. The fact that certain cytokines can contribute to both innate and acquired immunity is an indication of their interdependency. The fact that certain soluble products and cells contribute to multiple components of the immune system is also an indication of the overlapping nature of host defense capabilities, a concept which is sometimes referred to as the redundancy of the immune system.

As noted previously, IFN exists as two types, and it is the type I or viral IFNs—IFN-α and IFN-β, which are produced in an antigen-independent fashion, that contribute to the innate immune response. As with the phagocytic cells, the trigger for the production of type I IFN is a unique feature of the genetic makeup of viruses. Viruses make much greater quantities of double-stranded RNA than do mammalian cells, and the presence of large amounts of double-stranded RNA stimulates the production of viral IFN. Although the sources of IFN-α and IFN-β are different (i.e., leukocytes and fibroblasts or epithelial cells, respectively), their effector functions are similar and include the following actions: stimulation of NK cells, induction of antiviral activity, and cytostasis of some tumor cells.

Complement is not a single soluble factor but a carefully regulated system of $\sim$20 functionally linked proteins. The linkage results because many complement proteins have protease activity, and they interact in an ordered cascade, the so-called complement cascade. In several steps in the cascade, there is the cleavage of small-molecular-weight peptides, which possess most of the biological activities attributed to the complement system. A detailed description of this obviously complicated system is beyond the scope of this entry. Instead, two points will be emphasized, the mechanisms for activating the complement cascade and the biological activities of some of the products of the complement system. The latter point will be discussed as it relates to the relative contributions of innate versus acquired immunity to host defense.

The classical activation of the complement cascade is triggered by Ig complexes with antigen and will be discussed in the following section. The alternative pathway of the complement cascade is triggered by nonimmune-specific activators, most notably polysaccharides associated with the surface of some microbes. Although these two activational schemes differ in the way that nonself triggers the complement cascade, there are common mediators in the two pathways and the biological consequences of the complement components are similar. There are receptors for various components of complement (usually designated CR) located on both neutrophils and macrophages. Some complement peptides (most notably, activated C3b, C5a fragment, and the activated complex of C567) function as chemotactic factors for these inflammatory cells. Other complement peptides (most notably, activated C3b) will attach to the microbe and facilitate the adherence and subsequent phagocytosis of the microbe by neutrophils and macrophages, a process known as opsonization. Finally, the terminal product of the complement cascade, the activated complex of C6789, can form a lytic unit which can attack the cell membrane and directly kill the microorganisms by punching holes in their cell membrane. It is thought that most cells of the host are equipped with surface proteases that inactivate complement and protect them from cytolysis.

Humoral versus Cell-Mediated Immunity

Acquired immunity is antigen dependent and comprises all the specific immunological reactions associated with lymphocytes. In light of the existence of an antigen-specific defense system, a legitimate question arises as to why we have such an elaborate nonspecific immune system. One of the primary reasons may be that an acquired immune response takes time. For example, 5 days are needed to generate a primary antibody response, and the body must rely on the innate immune system to hold the infection in check during this time. As noted previously, acquired immunity can be subdivided into two effector arms, humoral immunity and cell-mediated immunity. The 'humor' (i.e., a bodily fluid) associated with humoral immunity is the secreted form of Ig in the blood. The 'cells' associated with cell-mediated immunity are the various subpopulations of T cells.

Because Ig is the primary effector for humoral immunity, this component of the immune system is associated with the activities of antigen-specific B cells. The steps involved in an acquired immune response by B cells and the regulation of B-cell activity are depicted in **Figure 2** and described later. In this section, the emphasis will be on the specific contributions that Ig makes in the functioning of the immune system. The basic structure of the Ig or antibody molecule consists of four protein chains of two types – that is, two identical light chains and two identical heavy chains. These protein subunits are linked in a fixed and precise orientation to form a 'Y'-shaped molecule. The 'forked' end of the antibody molecule contains two variable regions and is the site which recognizes and binds the specific antigen. To accommodate the many antigens that exist, these variable regions differ from one antibody molecule to another antibody molecule. Each type of antibody molecule is synthesized by a clone or family of identical antigen-specific B cells. The 'closed' end of the antibody molecule is nearly identical among all antibodies and is called the constant region. Although the constant region of the antibody molecule is not involved in the specific binding of antigen, this component of the molecule is critical to the effector functions of Ig, as described later. The remaining feature of the Ig molecular structure that needs to be emphasized is that the heavy chains can vary in type, which gives rise to the various major classes of Ig, namely, IgM, IgG (there are four subclasses of IgG), IgE, IgA (there are two subclasses of IgA), and IgD. The existence of multiple types of Ig adds to the repertoire with which the humoral arm of acquired immunity can add to host defense, as discussed later.

The different types of Ig also provide insights into the complexities associated with B-cell biology. Surface Ig is the hallmark feature of the B cell and is one of the principal phenotypic markers used to identify and enumerate B cells. Surface Ig is also a 'receptor' in the truest sense of the term. The ligand for this

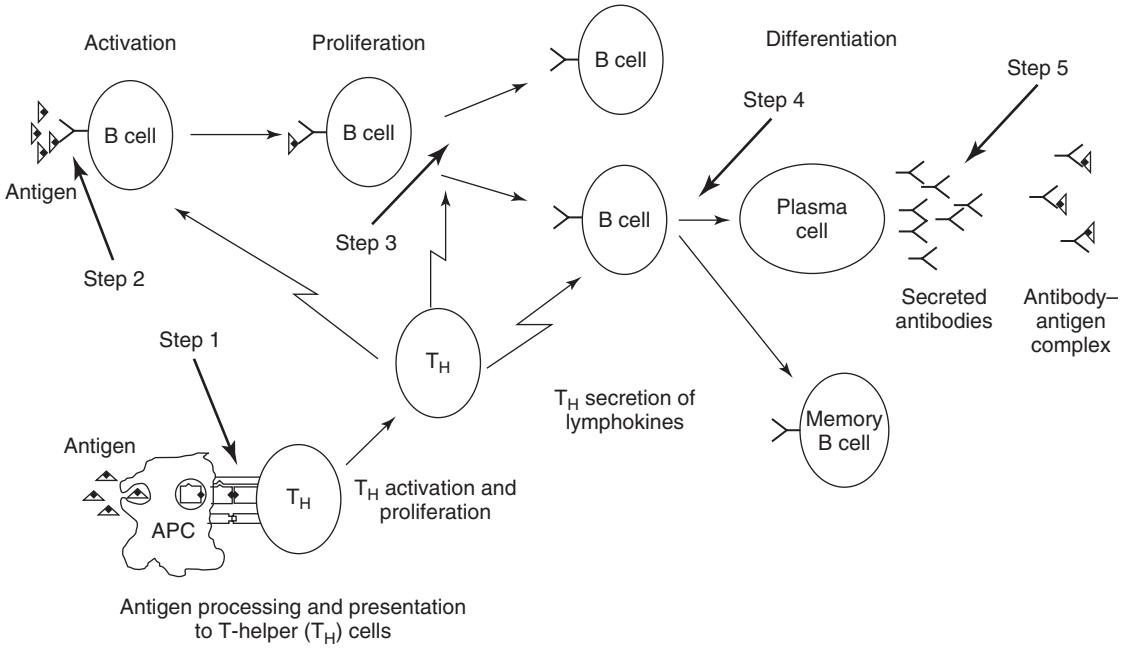

Figure 2 Generation of an antibody response. An antibody response is an example of an acquired immune response and is characterized by a five-step process. The production of memory cells is also depicted.

receptor is the antigen, and because of the molecular structure of Ig, this ligand–receptor (i.e., antigen–antibody) interaction is the driving force for the tremendous specificity associated with humoral immunity. It is important to emphasize that the cytoplasmic region of surface Ig contains only three amino acid residues, which makes it difficult, if not impossible, to conclude signal transduction is being mediated via this domain. However, surface Ig can associate with type I transmembrane glycoproteins to form a B-cell receptor (BCR) complex, which can generate the necessary signal transduction pathways to trigger activation and proliferation in B cells in the presence of the appropriate ligand (antigen). Virgin B cells express either IgM or IgD or both on their surface. The function of IgD is limited to its role as a receptor on B cells, and it cannot mediate any of the effector functions associated with the other classes of Ig. IgM is the first class of Ig to be released by the B cell after antigen challenge during a primary antibody response. In the serum, IgM exists as a pentamer. Depending on the nature of the antigen, B cells can undergo a process known as class switching, which results in the generation of IgG, IgA, or IgE. As described later, the T cell plays a critical role in class switching through the release of a variety of lymphokines. Antigens triggering this type of interaction between B and T cells are therefore called T-dependent antigens. Some antigens can trigger an antibody response in a T-independent fashion and are generally limited to the production of IgM.

Once released, the various forms of Ig possess a number of effector functions to engage the antigen. It is important to emphasize that a given microbe, tumor cell, or foreign protein can express several types of antigens and multiple copies of these antigens. If the antigen to which an antibody is directed is associated with the toxic portion of a molecule, then the antibody can neutralize the toxin. The production of neutralizing antibodies is frequently a problem in the therapeutic application of recombinant proteins generated through biotechnology. This problem can become a rate-limiting step in the preclinical testing of human-derived proteins in animal models because they are perceived as nonself by their hosts. Another example of the ability of antibodies to neutralize occurs when the antibody is directed against an antigen on the surface of an infectious particle which serves as the cellular adhesion site. In the presence of the antibody, the infectious particle cannot attach to the cellular target to initiate infection. Although the neutralizing capabilities of antibodies are known, in actuality, they play a relatively minor role in the effector functions associated with humoral immunity in host defense. In particular, it is important to emphasize that antibody can only bind to an antigen and, in itself, cannot destroy anything.

Most of the effector functions of humoral immunity are mediated by processes activated by antibody. Moreover, the effector processes activated by antibody have already been described as key participants in innate immunity. For example, the classical activation of the complement cascade is triggered by antigen–antibody complexes and is specifically mediated by the constant region of the Ig molecule. Both IgM and IgG can activate the complement system in this manner, which results in all of the biologically active components identified previously, including the lytic unit, the chemotactic factors, and the complement peptides which opsonize the microbe to facilitate its phagocytosis. IgM and IgG can function to opsonize some microbes independent of complement activation in an antigen-dependent fashion because macrophages and neutrophils have receptors on their surface which recognize the constant region of Ig (Fc receptors; FcR). Fc receptors also play a major role in the ability of IgG to participate in a process known as antibody-dependent cellular cytotoxicity, whereby antigen-specific antibody attaches via FcR to certain types of cells, including NK cells, enabling these cells to attach intimately to the target cell and trigger cell death. Finally, Fc receptors are also the primary effector mechanism for IgE, which is the principal immune defense against certain types of parasitic infections (most notably, helminths) and is produced primarily by the external immune system along secretory surfaces. IgE binds to Fc receptors on the surfaces of mast cells and basophilic granulocytes. Once bound to FcR, IgE can serve as an antigen-specific receptor on the surface of these inflammatory cells to trigger the release of a variety of proinflammatory factors, including the vasoactive amines (histamine) and products of the arachidonic cascade (leucotrienes and prostaglandins).

IgA is the principal antibody present in a number of secretions and is the major antibody associated with the external immune system. IgA lacks the effector functions identified previously and acts mainly in immune exclusion (prevention of entry of potentially infectious entities into the body). As noted previously, there are differences in the immune effector mechanisms associated with the internal immune system or systemic immunity and the external immune system or local immunity. Systemic immunity is mediated by IgM and IgG, the latter is the major form of Ig found in the blood. Local immunity is mediated primarily by IgA and IgE. The contribution by the external immune system should not be underestimated because about half of the body's lymphocytes are associated with this system,

and its capacity for Ig synthesis is ~ 1.5 times that of the internal immune system.

The other arm of the acquired immune system is cell-mediated immunity, for which antigen-specific T cells play the primary effector role. The antibody associated with humoral immunity is particularly effective against extracellular pathogens and it is a major constituent of serum. However, Igs are water-soluble proteins which cannot venture across the lipid membranes of cells. Therefore, cell-mediated immunity is needed to defend against intracellular pathogens such as protozoans, fungi (*Candida*), viruses, and certain bacteria (*Mycobacteria* and *Listeria*). Cells infected with these types of intracellular microbes are able to signal the body that they are infected by expressing pieces of the microbe on their surface. Cell-mediated immunity is also an important defense against certain types of malignancies. Central to this component of immune function, which is sometimes referred to as immunosurveillance, is the concept that tumor cells express antigens on their surface that are not found on normal tissue counterparts, so-called tumor-specific antigens.

As with the B cell, the antigen specificity of T cells is derived from a surface receptor. For T cells, the antigen receptor is a heterodimeric molecule (either the α,β heterodimer or the γ,δ heterodimer) which has a constant and a variable region, similar to that previously described for the Ig molecule. Moreover, as with surface Ig, the T-cell receptor (TCR) cannot in itself mediate transmembrane signal transduction, and it is coupled on the cell surface with the CD3 molecule, where 'CD' stands for cluster of differentiation, which has become the standard nomenclature to refer to a multitude of surface markers. CD3 consists of at least four invariant polypeptide chains and is thought to mediate the signal transduction associated with binding of the TCR to antigen. The expression of CD3 has become the hallmark feature of the T cell and is the principal phenotypic marker used to identify and enumerate T cells. Although there are similarities, there are also major differences between the TCR and its counterpart in the B cell. First, it is clear that there are structural differences between the TCR and BCR, and that T cells recognize different antigenic determinants than those recognized by B cells. Second, the TCR complex includes a number of different accessory molecules, namely CD4 and CD8, which play essential roles in the recognition of antigen by T cells. CD4 and CD8 also serve as important phenotypic markers for distinct subpopulations of T cells, T helper cells (T_H; Note: as indicated below, some $CD4^+$ cells are classified as T_D cells by virtue of their primary role in mediating a delayed hypersensitivity response) and T suppressor/T cytotoxic cells (T_S/T_C), respectively. The importance of these various subpopulations is described in greater detail below. Finally, unlike the B cell, in which the secreted form of Ig becomes the primary effector for humoral immunity, neither the TCR nor any component of its complex are secreted for effector function.

Antigen-specific T cells contribute to host defense capabilities by two basic mechanisms. One type of T cell, which is usually designated T_D and expresses the CD4 phenotype, orchestrates an inflammatory response called a delayed hypersensitivity response through the release of a variety of lymphokines. Some T_D-derived lymphokines are capable of causing cell lysis independently of a direct attachment between effector and target cell and are called lymphotoxins. As with the ability of antibody to activate effector functions in humoral immunity, other T_D-derived lymphokines act to markedly stimulate the destructive capabilities of inflammatory cells associated with innate immunity and include macrophage-activating factor, macrophage-inhibiting factor (inhibits the directed movement or chemotaxis of macrophages at the site of infection), and IFN-γ, which stimulates both macrophages and NK cells. The second type of T cell is designated T_C for T cytotoxic and expresses the CD8 phenotype. As the name implies, T_Cs are capable of killing target cells in an antigen-specific fashion through intimate contact. These T cells are thought to play the major role in the rejection of a foreign tissue graft or transplant. Although T_Cs and NK cells have many similarities, including enhanced activity in response to IFN-γ and the postulated role for cytolytic proteins such as perforin, they recognize their targets differently. Only T_Cs are antigen driven and require initial exposure to antigen to become active. Interestingly, T_C cells are capable of producing a protein which interacts with perforin and renders it lytically inactive, thereby providing these cells with some resistance to their killing capability.

Regulation of an Acquired Immune Response

As emphasized throughout this entry, antigen plays the critical role in providing the driving force and the specificity of an acquired immune response. This type of response can be regulated by several general mechanisms including cellular cooperativity, the cytokine network, and genetically determined regulation. Cellular cooperativity can be mediated by both direct cell-to-cell contact and the release of soluble factors, especially the lymphokines. The 'cytokine

network' is a relatively recent term used to emphasize the fact that cytokines can both up- and down-regulate lymphocyte activity. Genetically determined regulation is mediated by two major types of proteins encoded for by the MHC. In humans, MHC-derived proteins are called human lymphocyte antigens. MHC class I proteins are expressed on all cells of a given host and are critically involved in the designation of self versus nonself. These surface markers determine tissue compatibility and are the primary targets (i.e., antigens) in allograft or transplant rejection. MHC class II proteins are expressed on only certain types of immune cells and play a central role in the control of cellular interactions during an immune response.

All three of these mechanisms will be highlighted in the discussion of the five basic steps of an acquired immune response, which are illustrated below and which are depicted in **Figure 2** for the generation of an antibody response. It is important to emphasize that these same five steps are involved in the generation of a cell-mediated immune response:

- Step 1: antigen recognition and presentation
- Step 2: lymphocyte activation
- Step 3: lymphocyte proliferation
- Step 4: lymphocyte differentiation
- Step 5: effector function

Step 1: Antigen Recognition and Presentation

Thus far, antigen has been depicted as the structures associated with a foreign substance that allow it to be recognized as nonself and to therefore provide the driving force for an acquired immune response. It has also been emphasized that both B and T cells are equipped with surface molecules that effectively function as the receptors for antigen, allowing these cells to recognize antigen in a highly specific manner. Generally speaking, the form of the antigen as it appears on the foreign substance when it is introduced into the host is not the form of the antigen which is recognized by the 'antigen receptors' on the surface of lymphocytes. The antigen must be taken up by specialized cells and 'processed' so that the most immunogenic components can be 'presented' to the lymphocytes. As depicted in **Figure 3**, cells carrying out this step of an acquired immune system are called antigen-presenting cells (APCs). In order to present antigen to T cells, the immunogenic components must be presented in the context of MHC molecules, that is, a T cell can only recognize antigen if the APC and the T cell share the same MHC type and antigen recognition by T cells is said to be MHC restricted. The role of MHC in antigen processing and presentation is shown in **Figure 3**. During antigen processing, the microbe or foreign particle is internalized within a vesicle and broken down. The most immunogenic components become associated with MHC-derived proteins, which are produced by the endoplasmic reticulum, and the antigen–MHC protein complex is returned to the surface of the APC. T_H cells, which express CD4 on their surface, can only recognize antigen in the context of MHC class II molecules, and all APCs which can present antigen to T_H cells must express MHC class II molecules. As noted previously, CD4 molecules play an accessory role in the recognition of antigen by T_H cells, and it is the CD4 molecule which recognizes the MHC class II antigens.

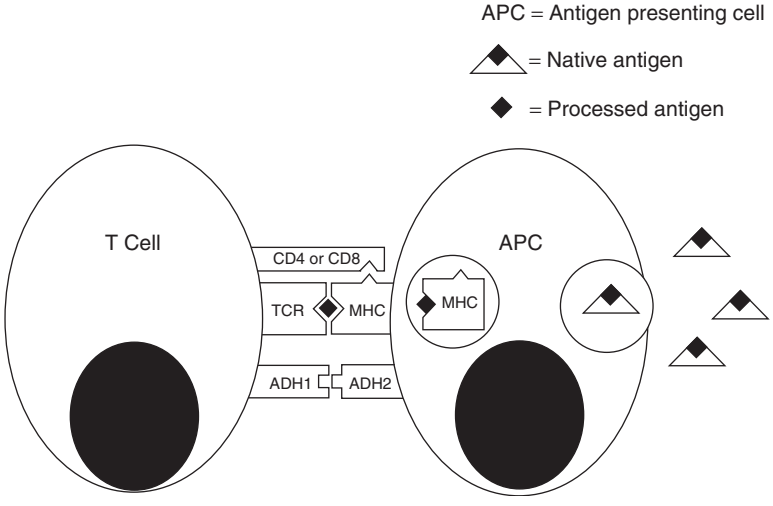

Figure 3 Antigen presentation to T cells. The roles of antigen processing and of MHC-derived proteins in antigen presentation, the first step in the five-step process to generate an acquired immune response, are depicted.

Some examples of APCs include macrophages in the spleen, dendritic cells in lymph nodes, and Langerhan cells in the skin. While it is true that many APCs can also be classified as phagocytic cells, antigen processing and presentation and phagocytosis are two distinct functions. This point can be readily appreciated by the fact that B cells are recognized as efficient APCs under conditions of low antigen concentration. The participation of B cells in antigen processing and presentation occurs by virtue of their ability to complex with antigen via their surface Ig and by the fact that B cells can express MHC class II molecules on their surface. Moreover, because B cells are able to recognize antigen via surface Ig, antigen presentation to B cells is not MHC restricted. Antigen presentation to T_S/T_C cells is also known to be MHC restricted. Because these cells express CD8 on their surface as an accessory molecule to the TCR complex, recognition of antigen can only take place in the context of MHC class I antigens. As noted previously, most cells of the body express MHC class I proteins. When a cell becomes infected, it alerts T cells of the infection by coexpressing some antigenic determinant of the microbe in a complex with MHC class I proteins. This situation makes sense when one considers that T_C cells are $CD8^+$ and are the primary effector cells to engage certain types of virally infected cells and tumors (i.e., those tumors which express tumor-specific antigens) as well as allografts (i.e., a transplant of nonself, with different MHC class I proteins expressed). One important point about MHC proteins that needs to be emphasized is that their expression can be upregulated by certain cytokines, most notably the IFNs, as summarized in **Table 1**. Another point that needs to be emphasized about the process of antigen presentation is that other cell surface molecules, depicted in **Figure 3** as adhesion molecules (ADH 1 and ADH 2), play important roles in the cell-to-cell contact.

Step 2: Lymphocyte Activation

Because both the BCR and TCR are effectively the receptors for the ligand, which is an antigen, once an antigen has been processed and presented to a specific B or T cell in the proper conformation, it can trigger a number of second messenger systems which ultimately initiate transcription. The cell becomes activated and begins to move into the cell cycle toward mitosis and cell division. An important part of this process is the upregulation of surface receptors for cytokines which facilitate the movement of these cells through the cell cycle to proliferate and subsequently to differentiate. Because these receptors are generally expressed in very low levels in a resting cell, only the antigen-activated cells become responsive. In general, as shown in **Figure 2**, antigen-specific T_H cells are the primary source of these lymphokines. The ability of B cells to respond to the majority of antigens is critically dependent on these T_H cell-derived lymphokines, and these antigens are called T-dependent antigens. Other antigens, usually large polymeric molecules with repeating antigenic determinants, are able to trigger B-cell responses without requiring T_H cell-derived lymphokines, and these antigens are called T-independent antigens.

Step 3: Lymphocyte Proliferation

The third step in an acquired immune response is the proliferation of antigen-specific lymphocytes. This step is often referred to as 'clonal expansion', where a clone is a family of cells having an identical antigenic specificity. Therefore, a 'polyclonal activator' is a substance which can trigger the proliferation of several clones, generally not in an antigen-specific fashion. Mitogens, substances which can cause lymphocytes to enter mitosis, are examples of polyclonal activators. A 'monoclonal antibody' is a genetically engineered antibody which has a single antigenic specificity because it is produced by a homogenous clone of cells.

As depicted in **Figure 2** and described previously, the movement of antigen-activated lymphocytes into proliferation is regulated by lymphokines produced predominately by antigen-specific T_H cells. The characteristics of many of these lymphokines are summarized in **Table 1**. IL-2, which was previously known as T-cell growth factor, is of particular importance to T cells, including T_C and T_H cells. In the latter cell type, this lymphokine functions as an autocrine growth factor (i.e., a factor which can stimulate proliferation in the same cells where it is produced). IL-2 can also promote the proliferation of B cells, as can the following T_H cell-derived lymphokines: IL-4, IL-5, IL-6, and IL-1, a cytokine produced by macrophages/monocytes. As indicated in **Table 1**, some cytokines act as negative regulators of cell growth. Of particular importance to both B and T cells is transforming growth factor-β (TGF-β).

Step 4: Lymphocyte Differentiation

The proliferation in step 3 can be repeated several times (i.e., 'clonal expansion'). Eventually, the lymphocytes will stop proliferating and will begin to differentiate into antigen-specific effector cells. As with proliferation, this step in an acquired immune response is under the control of lymphokines produced primarily by T_H cells. One of the critical steps in the differentiation of B cells is the 'switch' to the

specific type of Ig molecule that will ultimately be secreted. As indicated in **Table 1**, IFN-γ stimulates the production of IgG2-α, IL-4 stimulates the production of IgE and IgG1, IL-5 stimulates the production of IgA, and IL-9 can act synergistically with IL-4 to stimulate IgE and IgG. A fully mature antibody-secreting B cell is called a plasma cell. A number of cytokines can also increase the generation of T_C cells including all types of IFN, IL-2, IL-4 (this action is synergistic with IL-2), IL-10, and IL-12.

Step 5: Effector Function

The basis for the effector function by antigen-specific lymphocytes – especially antibody-secreting B cells, T_D cells, and T_C cells – and the participation of other cellular components of the immune system in acquired immunity – including NK cells, macrophages, and neutrophils – have already been emphasized in other sections of this volume. It has also been emphasized that T_H cells play an important effector role in acquired immunity as regulators in the growth and differentiation of B cells and T_C cells. In this regard, it is important to note that both populations of T cells whose participation in an acquired immune response is mediated by the secretion of lymphokines, that is, T_H and T_D cells, are characterized by the expression of CD4.

Recent evidence suggests that there are at least two subpopulations of T_H cells, designated T_{H1} and T_{H2}. To date, the distinction between these two populations has been operationally defined and is based on the respective profiles of lymphokines which are produced: whereas both T_{H1} and T_{H2} cells produce IL-3 and GM-CSF; only T_{H1} cells produce IL-2 and IFN-γ, and only T_{H2} cells produce IL-4, IL-5, IL-6, and IL-10. The importance of this phenomenon can be understood from the discussion of the primary effects of these lymphokines. By virtue of the production of IL-2 and IFN-γ, T_{H1} cells will facilitate the generation of a cell-mediated immune response. In contrast, T_{H2} cells will facilitate the generation of a humoral immune response by virtue of producing lymphokines which support B-cell function. Moreover, as indicated in **Table 1**, T_{H1} cells can downregulate the activity of T_{H2} cells via the production of IFN-γ, and T_{H2} cells can downregulate the activity of T_{H1} cells via the production of IL-4 and IL-10. The basis for this 'cross talk' between the two types of T_H cells has been called the cytokine network. An important outcome of this cytokine network is that under certain conditions, a cell type traditionally characterized as a 'helper' cell can actually 'suppress' an immune functional component (i.e., T_{H1} cells can suppress T_{H2} cells via IFN-γ, and T_{H2} cells can suppress T_{H1} cells via IL-4 and IL-10).

Certain types of T cells expressing CD8 are known to function as antigen-specific T_S cells. T_S cells are activated under conditions designed to induce immunological tolerance, previously referred to as a state of anergy, and their importance can be confirmed in cell transfer experiments. The mode of action of T_S cells is uncertain and may involve the secretion of soluble factors as described for T_H cells. T-cell-derived suppressor factors have been shown to be associated with APCs, such as macrophages. The action of T_S cells is capable of being directed toward T_H cells and B cells in an antigen-specific fashion. Therefore, it becomes obvious that the relative balance between T_H cells and T_S cells can exert tremendous influence over the magnitude of a given immune response and can even dictate if an immune response occurs.

Memory Cells: the Goal of Vaccinations/ Immunizations

One final point about acquired immunity needs to be emphasized. During the five steps of an acquired immune response, some of the antigen-specific and activated lymphocytes will undergo proliferation but will not be stimulated to differentiate and become effector cells, which have a relatively short half-life during a primary immune response. As shown in **Figure 2**, these cells will instead be programmed with the same antigenic specificity and will be returned to the blood and/or lymph circulation as long-lived memory cells. Upon reinfection with the same microbe, the secondary immune response, which is initiated by these memory cells, will occur quicker and with greater intensity. In the case of a humoral immunity, IgM is the predominant Ig released during a primary immune response, whereas IgG is the predominant Ig released during a secondary immune response. The generation of memory cells is the goal of vaccinations and immunizations where the host is injected with nonpathogenic (i.e., inactivated or attenuated) forms of infectious microbes. As children, we are vaccinated to several infectious organisms, including measles, mumps, diphtheria, tuberculosis, rubella, and poliomyelitis. Just prior to the winter months throughout a good portion of the United States, many people elect to receive the latest influenza vaccines, the so-called 'flu shots'. These are all examples of vaccinations and the objective is to clonally expand antigen-specific memory cells. Often, the responses to childhood vaccines,

sometimes called 'recall antigens', are checked as an assessment of human immunocompetence.

Consequences of Immunotoxicity

Having established an appreciation of the immune system, with an emphasis on its functional organization and capabilities, it is now possible to discuss the consequences of immunotoxicity beyond the generalized concept of a continuum, as depicted in **Figure 1**. However, prior to discussing more specific consequences of immunotoxicity, especially the immunosuppressive part of the continuum, the concepts of redundancy and immunological reserve need to be emphasized. While the immune system can be divided into innate and acquired immunity, it is important to emphasize that this classification is based on the role that antigen plays in triggering a response. As described in previous sections, the immune system contains several overlapping mechanisms to cope with a given opportunistic pathogen, regardless of how the response is triggered. Moreover, the same effector mechanisms that are involved in innate host defense capabilities are activated in acquired immunity, and the progression from innate to acquired immunity is generally associated with greater intensity (i.e., greater destructive capability). The concept of overlapping effector function is often called the redundancy of the immune system. For example, phagocytic inflammatory cells, complement-derived peptides, and antibodies are all involved in the defense against bacterial infections. The first two effectors are prime players in innate immunity and are markedly activated by antigen-specific antibody in acquired immunity. Similarly, macrophages, NK cells, IFNs, T_C cells, and T_D cell-derived inflammatory lymphokines are all involved in the defense against viral infections. Again, the first two effectors are prime players in innate immunity and are markedly activated by antigen-specific T cells in acquired immunity.

The importance of redundancy is that the consequences of suppression of a given component of the host defense capabilities are sometimes minimized because of these overlapping systems. This phenomenon is sometimes referred to as immunological reserve. The redundancy and reserve of the immune system are thought to have evolved because of the diverse nature of pathogens with which it must cope to protect self. Moreover, a point about opportunistic pathogens, which is too often ignored, is that they are generally not passive players in their assault on the host. A characteristic of most successful infectious organisms is the ability to elicit different mechanisms to evade detection and/or to minimize the full effects of the host's defense capabilities. Moreover, microbes also evolve rapidly, enabling them to devise new means to evade the inherited defenses of species that evolve much more slowly. Therefore, even if the manifestation of the suppression of a given functional component is not clearly associated with disease, this immunotoxic effect should still be considered adverse because it does represent a loss in functional capabilities.

When the immune system is suppressed, the most severe consequences will be an increase in the incidence and severity of infections and/or an increase in the incidence and progression of malignancies or cancer. The specific types of infections which are increased can provide some clues as to the specific components of the immune system which were suppressed. For example, suppression of humoral immunity (i.e., antibody production) and/or its associated effector systems, such as phagocytic cells (i.e., macrophages and neutrophils) and the complement cascade, will be characterized by infections mediated by extracellular pathogens, including some bacteria and parasites. In contrast, suppression of cell-mediated immunity and/or its associated effector systems, such as NK cells and the IFNs, will be characterized by infections mediated by intracellular pathogens, including protozoans, viruses, and some bacteria, as well as by increased tumor formation. That these are consequences of immunosuppression in man cannot be argued based on a multitude of studies in individuals with congenital or acquired immune deficiencies or in patients undergoing long-term treatment with immunosuppressive drugs subsequent to organ or tissue transplantation. That these are consequences of exposure to suspected immunotoxic (i.e., immunosuppressive) xenobiotics in animal models also cannot be argued, primarily because extensive dose–response characterizations can be conducted under well-controlled experimental conditions and because changes in various immune functional parameters can be correlated with changes in host resistance models. However, this is not the case in humans, where there are few clear-cut examples of, or convincing evidence for, xenobiotic-induced immunosuppression outside of a therapeutic context. What becomes clear (even in the animal studies) is that the effects of immunotoxic drugs and chemicals are generally much more subtle than either the immune dysfunctions associated with congenital conditions or those produced by drugs used for immunosuppressive therapy.

As indicated in **Figure 1**, the other side of the immunotoxicity continuum is manifested either as a hypersensitivity response or as an autoimmune disease. A hypersensitivity response is an acquired immune response (i.e., by definition, it is manifested on second contact with a particular antigen) which

occurs in an exaggerated or inappropriate form to cause tissue damage and which is a characteristic of the individual (i.e., there is a genetic predisposition).

Autoimmune disease occurs when the reactions of the immune system are directed against the body's own tissues and is also characterized by a genetic susceptibility. Examples of autoimmune diseases include myasthenia gravis, in which cholinergic receptors, especially those associated with neuromuscular junctions, are targeted; multiple sclerosis, in which myelin is targeted; and rheumatoid arthritis, in which connective tissue, especially the synovial lining of joints, is targeted. The terms 'hypersensitivity' and 'autoimmunity' are often confused and are certainly interrelated. Based on their definitions, a hypersensitivity response can be a mechanism by which an autoimmune disease is produced. In contrast to the situation regarding suppression, in which the immune system is by and large a passive target for xenobiotic-induced changes, exaggerated immune responses can be mediated by two entirely different types of interactions by the immune system with drugs and chemicals. First, the immune system can again be a passive target for the enhancing effects of drugs and chemicals, such as occurs when a xenobiotic mimics or causes the aberrant production of immunomodulatory cytokines or when a xenobiotic disrupts the regulatory mechanisms which serve to protect self (i.e., suppress a suppressor). Another way that xenobiotics can enhance immune function is by acting as an adjuvant, which is defined as any substance which nonspecifically enhances the immune response to an antigen. The classic adjuvant is complete Freund's adjuvant (CFA), which is a water-in-oil emulsion containing killed mycobacteria. The effectiveness of adjuvants in enhancing immune responses can be demonstrated by the fact that animals are often injected with CFA to increase the production of antigen-specific antibodies and by the desire to develop an adjuvant that is safe in man (i.e., CFA produces severe side effects) which could be used in conjunction with vaccines or immunotherapy. The specific mechanism(s) for the actions of an adjuvant, including CFA, is not known. Moreover, the existence of environmental adjuvants is controversial and/or poorly studied. Therefore, adjuvants will not be further discussed in this entry.

In the second type of interaction, the components of the immune system are active participants in that the xenobiotic or some fraction of a xenobiotic is recognized as nonself and therefore provides the driving force for the response. Drugs and chemicals which are capable of triggering an immune response are generally low-molecular-weight substances possessing some inherent reactivity. For the most part, the xenobiotic cannot be considered an antigen, simply because in itself it is not capable of stimulating an immune response. Instead, these substances are called 'haptens', which are defined as small molecules that can act as antigenic determinants but which cannot stimulate an immune response by themselves. The immune response is triggered when the hapten binds to some tissue of the host, the so-called 'carrier'. This property is called the sensitizing potential of the hapten and is associated with its inherent reactivity. Hapten-specific immune responses are therefore triggered only in the presence of the hapten–carrier complex and can be mediated either by humoral immunity (i.e., antibody), as in an allergic response, or by cell-mediated immunity (i.e., specifically a delayed hypersensitivity response), as in contact dermatitis. The damage associated with either type of hypersensitivity response can be directed against the tissue which is bound by the hapten. Therefore, the morbidity associated with hypersensitivity responses can be manifested in a number of ways reflecting the target tissues, including contact dermatitis, rhinitis, allergy, and anaphylaxis. Animal models have been developed which can clearly demonstrate the sensitizing potential of xenobiotics. Moreover, hypersensitivity disease has become an important human health problem in industrialized societies. One striking example of the consequences of hypersensitivity disease in humans is occupational asthma, which is one of the most common occupational ailments in the Western world. However, it is important to emphasize that the establishment of a cause and effect relationship is more straightforward in the case of hypersensitivity than immune suppression. The onset of hypersensitivity is always a consequence of exposure to an exogenous agent. On the other hand, the repercussions of immune suppression following exposure to a xenobiotic, especially one that produces only modest changes in immunocompetence, in most cases will be subtle. These consequences will likely be manifested as a slightly greater susceptibility to common opportunistic infections, such as those responsible for the common cold or the flu.

Autoimmunity is much more complex than hypersensitivity. Animal models exist for many autoimmune conditions, and autoimmunity has been clearly demonstrated in humans, although it is a relatively infrequent occurrence. Therefore, the existence of autoimmune disease and the expected consequences cannot be denied. However, the ability of drugs and chemicals to exacerbate or trigger autoimmune disease in either animal models or humans is poorly understood. In fact, of all the possible consequences of immunotoxicity, autoimmunity is unquestionably the least understood. Primarily

because of the strong genetic component in the susceptibility to autoimmunity, deciphering the exact role of xenobiotics in the induction of these conditions has proved to be very difficult.

Mechanisms of Immunotoxicity

As described previously, the immune system is a complex, widely distributed and tightly regulated series of overlapping effector functions designed to allow the discrimination between self and nonself. As such, the immune system is characterized by a number of features that make it vulnerable to being targeted by exposure to xenobiotics. Several of these features are briefly highlighted below.

Immune System Features Associated with Vulnerability to Xenobiotics

Many of the effector functions of the immune system are dependent on a multitude of cell types, which all share a common precursor, the pluripotent stem cell. Therefore, any damage to the stem cell would be expected to have devastating consequences, several of which would extend beyond the immune system, most notably involving the red blood cell. Fortunately, the stem cell is refractory to xenobiotic-induced perturbation and is only affected by high doses of radiation. However, subsequent steps of hematopoiesis are affected by exposure to chemicals, with benzene being a classic example. Acute toxicity to benzene is associated with pancytopenia, aplastic anemia, and, at high doses, immunosuppression and leukemia.

The generation of mature lymphocytes with the capability of being programmed to respond against nonself in an antigen-specific manner but without the risk of responding to self is dependent on a complex maturational process that takes place in primary lymphoid organs, such as the bone marrow and thymus. Therefore, xenobiotic-induced damage to these microenvironments can contribute to immunosuppression as well as problems with immune regulatory functions. In addition, the cells that make up the microenvironment within the primary lymphoid organs (e.g., bone marrow stromal cells and thymic epithelium) can also contribute to the mechanism by which xenobiotics alter the differentiation of leukocyte precursor cells. One example of such a mechanism has been the demonstration that the immunotoxic polyaromatic hydrocarbon (PAH), and 7,12-dimethylbenzanthracene (DMBA) induces apoptosis of pro/pre-B cells, precursors of the mature B cell, in a manner that is dependent on direct cell–cell contact with bone marrow stromal cells for delivery of the 'death' signal. In addition to cell–cell contact, the involvement of an unidentified protein derived from the bone marrow stromal cells has been established in the DMBA-induced apoptosis of pro/pre-B cells, which is trypsin-sensitive, greater than 50 kDa in size and is likely a carrier of an immunotoxic DMBA metabolite formed in the stromal cells.

B and T lymphocytes are generally quiescent resting cells requiring appropriate stimulation by an antigen in order to elicit an effector function. Antigenic stimulation of lymphocytes activates multiple signal transduction cascades which alter the profile of gene expression to induce lymphocytes to first clonally expand, by undergoing numerous rounds of proliferation, and then to terminally differentiate into effector cells (e.g., antibody-secreting plasma cells, cytokine-secreting helper T cells or an armed cytotoxic T cell). Xenobiotics that interfere with the signal transduction cascades initiated at the antigen receptor are extremely potent immunosuppressive agents. Two examples of such agents are the transplantation drugs, cyclosporin A and FK506, both of which block T-cell activation by targeting critical components of the T-cell receptor-initiated signal transduction cascade and upregulation of the T-cell growth factor, interleukin-2 (IL-2).

Proliferation (i.e., the clonal expansion of reactive lymphoid cells) is a critical step in any acquired immune response. Therefore, any drug or chemical with antiproliferative properties has the potential to be immunosuppressive. Many types of immunosuppressive drugs are in fact antiproliferative, including cyclophosphamide, methotrexate, and azathioprine. Moreover, many types of anticancer drugs which target highly proliferating cells exhibit immunosuppression as an important side effect. An aspect centered around proliferation, which is rarely considered in immunotoxicology, is the malignant processes of immunocompetent cells. Leukemias and lymphomas are characterized by uncontrolled proliferation of T cells, B cells, or monocytes. However, because these cells are arrested in a specific stage of maturation or differentiation, even though there is tremendous proliferation, these conditions are generally associated with decreased functional capabilities and are manifested as a profile of activity comparable to that associated with exposure to antiproliferative drugs or chemicals (i.e., immunosuppression). As noted previously, benzene is an example of a chemical which can trigger leukemias, and ethylene oxide is an example of a chemical which has been associated with the development of lymphomas. Because these conditions are most appropriately categorized as types of cancers, they will not be further discussed in this entry.

Virtually all aspects of immunity are dependent on recognition of surface structures and can be regulated by a multitude of soluble products. Therefore, any drug or chemical that affects protein synthesis, gene expression, and/or receptor expression has the potential to disrupt immune function.

The immune system is characterized by a unique distribution, and many of its components are located close to the principal sites of absorption, including the gastrointestinal tract, the pulmonary tract, and the skin. Therefore, the immune system is in a position to be exposed to potentially high concentrations of drugs and chemicals. This phenomenon is especially important in hypersensitivity responses where the primary portal of entry of the hapten can determine the specific site of the reaction.

Some components of the immune system have metabolic capability including some forms of the cytochrome P450 system. Therefore, some chemicals, which are inert in their parent form but can be metabolized to reactive intermediates, can be activated by the immune system. However, it is important to emphasize that the metabolic capability of the immune system is minor when compared with other organ systems, most notably the liver, and that it possesses only a small repertoire of metabolizing enzymes. This point is discussed below as a major type of indirect mechanism of immunosuppression.

The immune system is not only regulated by its own products but is also exquisitely sensitive to products generated by other systems. Two examples, that will be discussed below as major types of indirect mechanisms of immunosuppression, are changes in neuroendocrine status and liver damage.

Direct and Indirect Effects of Xenobiotics

The remaining discussion of the mechanisms of immunotoxicity will be presented from the perspective of the following three general consequences of immunotoxicity: immune suppression, hypersensitivity, and autoimmunity.

One of the critical features of any discussion of the mechanisms of immune suppression must be the appreciation that robust changes in immune function can be mediated by either direct or indirect effects (or both) of a xenobiotic. Direct effects can be associated with distinct types of cells. Perhaps the best examples are cyclosporin A and related immunosuppressive drugs, such as rapamycin and FK-506, which specifically target T cells via an interaction with cytosolic and/or nuclear proteins to disrupt antigen-induced activation of transcription. To date, despite the tremendous evolution of the discipline of immunotoxicology, no other xenobiotic associated with occupational or environmental exposure has been as well-characterized from a mechanistic perspective as cyclosporin A. Nonetheless, a few examples are worthy of mention.

Cannabinoids, a family of more than 60 structurally related molecules, which constitute the active ingredients of marijuana, selectively suppress T cell and macrophage function. The mechanism of action, although still superficially understood, appears to be mediated, in part, by specific cannabinoid receptors, termed CB1 and CB2. Both CB1 and CB2 belong to the G-protein coupled receptor superfamily. These receptors are anchored into the plasma membrane by seven transmembrane regions which make up a ligand-binding pore. Agonist binding to G-protein coupled receptors results in a confirmational change in the receptor which in turn induces interactions between the C terminus of the receptor and GTP-binding proteins to initiate signal transduction into the cell. Binding of cannabinoid ligands, such as δ-9-tetrahydrocannabinol, to cannabinoid receptors results in at least two proximal events which occur independently of each other. The first is the inhibition of adenylate cyclase, the enzyme that converts ATP to cAMP. The downstream consequence is an inhibition of the cAMP signaling cascade as evidenced by decreased protein kinase A activity, reduced DNA binding by cAMP response element-binding proteins, and reduced transcription of cAMP responsive genes. The second consequence is a rapid induction of intracellular calcium, through the opening of calcium channels. Elevation of intracellular calcium prior to antigenic stimulation renders T cells anergic (i.e., unresponsive to antigenic stimulation).

Some chemicals selectively affect macrophage function, including asbestos. The effects on macrophage function are frequently manifested as a deficit in antigen-presenting capabilities. The mechanism of action of asbestos is mediated by the fact that asbestos fibers are phagocytized by macrophages to the point where they appear to become engorged.

Perhaps the environmental chemical most studied with regard to effects on the immune system has been 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which is also known as TCDD or 'dioxin'. While it appears that dioxin may affect multiple cell types, it is apparent that the B cell is an especially sensitive target. The mechanism of action for the immunotoxic effects of dioxin remains poorly understood. It is well established that many of the actions of dioxin, including inhibition of the primary antibody response, are mediated by a specific cytosolic receptor, termed the aryl hydrocarbon receptor (AhR). AhR exhibits a profile of activity similar to steroid receptors in that the ligand-activated receptor undergoes a nuclear

translocation, and functions as a transcription factor to regulate the expression of a wide number of genes. Although changes in mRNA expression levels for a number of immunologically relevant genes correlate with dioxin treatment of leukocytes, direct regulation of specific genes via the ligand-activated AhR has been more difficult to establish. There is compelling experimental evidence that genes coding for IL-1, IL-2 and mu Ig heavy chain are regulated, at least in part, by the ligand-activated AhR complex through dioxin response elements present either in the promoter or enhancer regions of the aforementioned genes. In the case of IL-1 and IL-2, TCDD treatment increases expression of both genes. Conversely, TCDD treatment strongly decreases mu heavy chain expression in LPS-activated B cells which is correlated with a marked inhibition of IgM secretion and decreased activity of the Ig heavy chain 3′-alpha enhancer.

Another chemical which affects multiple cell types is the semiconductor, gallium arsenide. Arsenic is the primary immunosuppressive component and both macrophages and T cells are the targets. In macrophages, antigen processing and presentation is identified as a functional target. The effect on T cells manifests as an antiproliferative effect mediated through a decreased expression of surface molecules and could be reversed by the exogenous addition of IL-2, IL-5, and IL-6.

Finally, a group of chemicals which has been widely studied for effects on the immune system includes the PAHs, such as benzo(*a*)pyrene [B(*a*)P] and DMBA. PAHs represent a prototypical class of carcinogens, and this action is mediated by the generation of a reactive intermediate, which is capable of binding directly to macromolecules like DNA. The mechanism of immunotoxicity also appears to be dependent on the generation of the reactive intermediates, and the available evidence suggests that certain types of immunocompetent cells, most notably the macrophage, have enough metabolic capability to activate PAHs such as B(*a*)P and DMBA. The specific macromolecular target that is responsible for the immunosuppression is not known, although it appears that these chemicals can affect multiple cellular targets. Interestingly, there are reports that exposure to PAHs can disrupt the production of immunomodulatory cytokines. Specifically, B(*a*)P decreases the production of IL-1, and the suppression by DMBA can be reversed by the exogenous addition of IL-2. In addition, and as already discussed, DMBA can induce apoptosis in pro/pre-B cells through a mechanism that is dependent on cell–cell contact with bone marrow stromal cells. Present evidence suggests that the stromal cells metabolize DMBA to a form that is then transferred to pro/pre-B cells via a protein carrier, which is greater than 50 kDa in size, that delivers the death signal.

A number of chemicals with demonstrable suppression of immune function produce this action via indirect effects. By and large, the approach that has been most frequently used to support an indirect mechanism of action is to show immune suppression after *in vivo* exposure but no immune suppression after *in vitro* exposure to relevant concentrations. One of the most often cited mechanisms for an indirect action is centered around the limited metabolic capabilities of immunocompetent cells and tissues. A number of chemicals have caused immune suppression when administered to animals but were essentially devoid of any potency when added directly to suspensions of lymphocytes and macrophages. Many of these chemicals are capable of being metabolized to reactive metabolites, including dimethylnitrosamine, aflatoxin B_1, and carbon tetrachloride. Interestingly, a similar profile of activity (i.e., suppression after *in vivo* exposure but no activity after *in vitro* exposure) has been demonstrated with the potent immunosuppressive drug cyclophosphamide. With the exception of the PAHs, few chemicals have been demonstrated to be metabolized when added directly to immunocompetent cells in culture. A primary role for a reactive intermediate in the immune suppression by dimethylnitrosamine, aflatoxin B_1, carbon tetrachloride, and cyclophosphamide has been confirmed in studies in which these xenobiotics were incubated with suspensions of immunocompetent cells in the presence of metabolic activation systems (MASs). Examples of MASs include primary hepatocytes, liver microsomes, and liver homogenates. In most cases, confirmation of a primary role for a reactive metabolite has been provided by *in vivo* studies in which the metabolic capability was either enhanced or suppressed by the administration of an enzyme inducer or a metabolic inhibitor, respectively.

While the demonstration that the immune suppression by a given chemical is mediated by a metabolite, and not the parent compound, is an important observation, it does not in itself account for the mechanism of action. One indirect mechanism of action, which is consistent with a role for metabolism, involves a primary consequence of the generation of many reactive intermediates, that is, liver damage. Among many different types of adaptive responses, the liver is capable of secreting a number of soluble factors. Some of these soluble factors are capable of affecting immune responses. Most notably, as pointed out in **Table 1**, TGF-β is capable of modulating both T- and B-cell effector

functions. Both carbon tetrachloride and cocaine cause immune suppression and liver damage over a comparable dose range. Support for a role by a serum factor was obtained by studies in which serum from either carbon tetrachloride-treated or cocaine-treated mice suppressed the function of immunocompetent cells from untreated mice. Confirmation for a role by TGF-β was obtained by demonstrating its presence in the serum of treated mice and by showing that the suppression by the serum could be reversed by a neutralizing antibody against TGF-β. Significant advances have been made during the past several years concerning the molecular mechanism by which TGF-β modulates B and T cells. TGF-β is one of the most potent immunoregulatory cytokines yet to be identified. It possesses bifunctional activity, enhancing as well as inhibiting a wide variety of immunological responses depending on the context within which immunocompetent cells encounter this cytokine strongly suggesting that its primary role is to maintain immune homeostasis. Concordant with this notion, mice in which the gene for TGF-β has been knocked out rapidly develop a systemic autoimmune response in virtually all the vital organs within the first several months after birth. TGF-β exerts its biological activity through a heterodimeric TGF-β receptor, which in turn propagates signals into the cell via the Smad protein signaling cascade to regulate gene expression.

The liver damage associated with exposure to cocaine is a relatively minor component of its profile of activity. Cocaine is an abused substance that has been studied most extensively for its effects on the brain and on behavior. Cocaine is capable of triggering robust changes in a number of neuroendocrine factors, some of which suppress immune function. In particular, cocaine is able to cause immune suppression at least in part through an elevation in plasma corticosterone as a consequence of the stimulation of the hypothalamic–pituitary–adrenal neuraxis. Ethanol (i.e., at least after acute exposure) and morphine are two other examples of xenobiotics which are suspected of producing their primary actions on the immune system via neuroendocrine effects. In most cases, the evidence is based on a measurable increase in serum corticosterone at doses capable of causing immunosuppression and on the ability of the glucocorticoid antagonist, RU-486, to reverse the suppression.

Hypersensitivity Reactions

Three points about hypersensitivity reactions have already been emphasized. First, the ability of a drug or chemical to trigger a hypersensitivity reaction is due to an inherent property of the xenobiotic (i.e., hapten), its sensitizing potential. Second, in a hypersensitivity reaction, the immune system plays an active role in mediating the response against the hapten. Third, both antigen-specific antibody (i.e., humoral immunity) and antigen-specific T cells (i.e., cell-mediated immunity) can be the effectors which are responsible for the tissue damage associated with hypersensitivity reactions. Traditionally, different types of hypersensitivity have been classified using a scheme originally proposed in 1963. Although the Coombs and Gell classification scheme is still widely used today, it is important to emphasize that the different types of hypersensitivity reactions rarely appear individually and are most often seen as mixed components. Nonetheless, this classification still represents one of the easiest ways to appreciate how the immune system is involved with the tissue damage-associated hypersensitivity reactions. The first three types of hypersensitivity reactions (I–III) are mediated by antigen-specific antibody, while the fourth type of hypersensitivity reaction (IV) is mediated by antigen-specific T cells.

Type I

A type I hypersensitivity reaction is the classic example of an allergic reaction and is also called an immediate hypersensitivity reaction because of its rapid appearance upon challenge in a sensitized individual. The primary mediator for a type I reaction is IgE, which is produced by antigen (hapten)-specific B cells in a T-cell-dependent fashion and which binds by its constant region to the Fc receptors on the surface of mast cells and basophils. As such, hapten-specific IgE can rightfully be considered a true biomarker for exposure in sensitized individuals. Therefore, in the absence of antigen-specific IgE, it is inappropriate to label a condition as an allergic reaction. This situation is true for occupational allergy, where only the presence of antigen-specific IgE should be accepted as a criteria for exposure to a suspected respiratory sensitizer. Upon a second contact with the hapten, the mast cells and basophils will be stimulated to degranulate and release a variety of vasoactive substances, which are the mediators of the inflammatory response and the accompanying tissue damage. The target organs for a type I reaction include the gastrointestinal tract, skin, lungs, and vasculature. Anaphylaxis is a systemic type I reaction which can be life threatening. As noted previously, many hypersensitivity reactions have a genetic component, and atopy is a term used to describe a genetic predisposition toward the development of IgE-mediated reactions against common environmental antigens, such as pollen and dust.

In the section on the effector functions of antibody, it was emphasized that IgE was a primary player in the host defense against a variety of parasites. Therefore, it could be easily argued that individuals endowed with an ability to mount a robust IgE response possessed a distinct survival advantage over individuals without such a defense mechanism against parasitic infections. However, in most developed nations, parasitic infections have been all but eliminated. Interestingly, in most developing nations, while parasitic infections are still a problem, the incidence of allergy or chemically induced hypersensitivity reactions is very low. Taken together, these two observations have prompted the speculation that the incidence of allergy and chemically induced hypersensitivity reactions in industrialized societies is due, at least in part, to the fact that the components of the immune system associated with the production of IgE, in the absence of parasites, are now free to react to other nonparasitic substances in a counterproductive way.

Type II

A type II hypersensitivity reaction is also called a cytolytic reaction because the damage is mediated by hapten-specific antibodies which are capable of triggering cytotoxicity in the target cell. The antibodies involved in a type II reaction are both IgM and IgG, with the latter type predominating. The specific effectors which are responsible for the cell damage include both the complement system and phagocytic cells, and these effectors are activated exactly as described in the section on humoral immunity. The target organs for type II reactions include many cell types circulating in the blood. Examples of type II reactions include xenobiotic-induced hemolytic anemia or agranulocytosis.

Type III

A type III hypersensitivity reaction is also called immune complex disease. Examples of type III reactions include the Arthus reaction and serum sickness. The damage associated with type III reactions is mediated by the generation of hapten-specific antibody, primarily IgG. The basis for the damage associated with type III reactions is that soluble antigen–antibody complexes are deposited in key anatomical locations, such as small capillary beds in the skin or the glomerular regions of the kidney. As described in the section on humoral immunity, the classical activation of the complement cascade is mediated by antigen–antibody complexes. Therefore, the deposition of immune complexes can cause a very localized activation of the complement cascade resulting in the generation of chemotactic peptides as well as the lytic unit. The target organs for type III reactions include blood vessels in the skin, joints, and lungs and the glomerular regions of the kidney.

Type IV

A type IV hypersensitivity reaction is also called a delayed hypersensitivity reaction because of its delayed appearance (i.e., after 24–48 h) following challenge in a sensitized individual. This is the only type of hypersensitivity reaction which is not mediated by antibody and is instead dependent on the generation of hapten-specific T cells, specifically the T_D cells, which contribute to the inflammation and the accompanying tissue damage by the generation and release of a variety of lymphokines. Classic examples of type IV reactions include the response that some individuals have to poison ivy (i.e., again emphasizing the genetic component to hypersensitivity reactions) and contact dermatitis. Target organs besides the skin include the lungs (i.e., the target organ for the well-studied tuberculin reaction), central nervous system (CNS), thyroid, and other organs.

Recent studies characterizing the basis for chemically induced hypersensitivity have uncovered an important interplay between type I hypersensitivity reactions, manifested primarily as respiratory sensitization, and type IV hypersensitivity reactions, manifested primarily as contact sensitization. The most important observation came from studies which showed that a predominantly respiratory sensitizer would still trigger an IgE response when applied topically. This observation can be accounted for by the cytokine network model which was described previously as important for cross talk between humoral immunity and cell-mediated immunity. Basically, a chemical with the capability of being a respiratory sensitizer will trigger an IgE response regardless of its route of exposure because it 'selects' or supports the development of a T_{H2}-dependent response, with the associated cytokine profile, IL-4, IL-5, and IL-10. In contrast, a chemical which lacks the capability of being a respiratory sensitizer; but which can still trigger contact dermititis, will select or support a T_{H1}-dependent response, with the associated cytokine profile, IL-2 and IFN-γ.

Autoimmune Responses

If increased incidence and/or severity of infections represents the critical consequence of a suppressed immune response, then autoimmunity represents the antithesis for an exaggerated immune response. As noted previously, the characterization of the onset

and progression of autoimmune conditions has been complicated by the critically important role that genetics plays in this process. While the exact association to drugs and chemicals as factors is poorly understood, several potential mechanisms have been proposed to account for this association. By definition, autoimmunity is the harmful consequence associated with an immune response which is mediated against self. Therefore, most of the mechanisms which have been proposed are centered around either a xenobiotic-induced change in the antigens associated with self or a xenobiotic-induced change in the recognition of self. Autoimmune responses that are driven by the first mechanism are associated with several possibilities which are identified as follows:

- Immune responses can be directed toward a foreign (i.e., nonself) antigen which has a similar chemical structure to an antigen which characterizes self.
- Immune responses can be directed toward a new nonself antigen that has become nonspecifically absorbed to a cell membrane. This mechanism is essentially the description of the sensitizing potential of a hapten and is one of the reasons why there is some overlap between hypersensitivity reactions and autoimmune conditions.
- Immune responses can be directed against a self antigen which is normally shielded or hidden but becomes available or expressed following exposure to a xenobiotic or during a disease process. Again, this is a potential area of overlap between hypersensitivity reactions and autoimmune disease. As described previously, hypersensitivity reactions can be mediated by the activation of immune effector processes which possess considerable destructive capability. Therefore, it is possible that one of the consequences of the tissue damage caused by a hypersensitivity reaction could be the expression of hidden antigens, which then sets the stage for the initiation of an autoimmune condition.

The second mechanism for autoimmunity is a change in the way that self is recognized. As described previously, the repertoire of antigen-specific immune effector mechanisms must develop with the capability of recognizing a tremendous number of nonself antigens while preserving the ability to recognize an equally vast number of self determinants. There are several regulatory mechanisms which are involved in this critical process that could be targeted to contribute to the onset and/or progression of autoimmune disease. First, T_S cells can play an important regulatory role in preventing an exaggerated immune response. Therefore, the suppression of T_S cells could result in an inappropriate recognition of self antigens. Second, an important step in the maturation of both T and B cells is negative selection, whereby these cells are probed for their recognition of self determinants. Lymphocytes with antigen receptors that can recognize self determinants are either destroyed via the stimulation of apoptosis or these cells are rendered anergic. An important part of the probing process is centered around the transfer and presentation of self determinants of organs or tissues distal to the microenvironments of the thymus or bone marrow. Recent evidence has suggested that this process may be one of the more sensitive targets for triggering an autoimmune mechanism. As such, any xenobiotic-induced changes in the movement of self determinants to the primary lymphopoietic organs and/or any xenobiotic-induced changes in the primary lymphopoietic organs themselves can be a mechanism for autoimmunity.

Autoimmune diseases may be tissue specific, where the damage is associated with a specific type of tissue or a specific organ, or tissue nonspecific, where the signs and symptoms are associated with several organs and tissues. The primary sites of tissue damage in autoimmune disease are many and varied. The following organs, cells, and organelles have all been determined to be the site of autoimmune reactions: nuclei (specifically histones and/or single-stranded DNA – one of the hallmark indicators of certain types of autoimmune disease is the expression of anti-nuclear antibodies), red blood cells, lymphocytes, neutrophils, platelets, Igs (primarily IgG), striated muscle (cholinergic receptors), smooth muscle, mitochondria, skin (basement membranes), thyroid (thyoglobulin), kidney (glomerular and tubular basement membrane), CNS (myelin), connective tissue (synovial lining of joints), lung, and liver. Both cell-mediated immunity and humoral immunity can be involved as effector mechanisms in causing the damage in autoimmune conditions.

Emergence of Regulatory Immunotoxicology Guidelines

The maturity and acceptance of a subdiscipline of toxicology can frequently be directly correlated to the level of interest being demonstrated by the regulatory community. One of the key issues facing immunotoxicology which has scientific, political, and societal implications is the approach to immunotoxicity testing for regulatory purposes. The Office of Prevention, Pesticides and Toxic Substances (OPPTS) of the US Environmental Protection Agency (EPA)

published guidelines entitled, *Biochemicals Test Guidelines: OPPTS 880.3550 Immunotoxicity* in 1996. These guidelines described the preferred study design for an exceptionally thorough evaluation of the potential immunotoxicity of biochemical pest control agents. This guideline described a panel of tests that included standard toxicology tests as well as immune functional tests assessing both humoral and cell-mediated immunity. *OPPTS 880.3550* clearly presented a very comprehensive approach to immunotoxicity; but a second document, *Biochemicals Test Guidelines: OPPTS 880.3800 Immune Response*, was needed to provide the rationale for when these studies should be conducted. The 880 series of immunotoxicity guidelines would arguably detect any type of immunotoxic potential by pesticides. However, the comprehensive nature of these guidelines rendered them prohibitively expensive and time consuming. The EPA released the *Health Effects Test Guidelines: OPPTS 870.7800 Immunotoxicity* in 1998. These guidelines described the approach to immunotoxicology testing for nonbiochemical agents regulated by the EPA. The testing approach in *OPPTS 870.7800* reflected the continued evolution of the science of immunotoxicology and reflected a more limited, case-by-case approach than previously described by the earlier more comprehensive guidelines. The cornerstone of *OPPTS 870-7800* was a functional test, the primary T-dependent antibody response (TDAR), which had been demonstrated in a number of intralaboratory studies to possess the greatest predictivity of known immunotoxicants. Subsequent tests, including the NK cell assay and the phenotypic quantitation of T and B cells, would be conducted on a case-by-case basis depending on the outcome of the TDAR.

The earliest immunotoxicity guidelines from the Food and Drug Administration (FDA) were centered on food additives as the *Draft Redbook II* in 1993. This document, although never finalized, contained an extensive description of immunotoxicology testing. In general, the Redbook guidelines reflected the 'tier' approach to immunotoxicology, as described in greater detail below. Specifically, the Redbook emphasized a step-wise approach that began with expanded studies utilizing data obtained in standard toxicology testing as initial indicators of immunotoxicity. Progressively more complicated immunological tests were prescribed using an approach that was very much case-by-case, with each new level of testing predicated on positive results in the preceding level. The FDA Center for Drug Evaluation and Research (CDER) released its document entitled, *Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs*, in 2002. This document is arguably the most comprehensive description of approaches to immunotoxicology. Not only does the FDA CDER *Guidance for Industry* describe the entire spectrum of adverse events associated with the immunotoxicology continuum, including immune suppression, immunogenicity, hypersensitivity, autoimmunity and adverse immune stimulation, this document also provides approaches at the level of specific methodology for evaluating each event. As with the earlier document from the FDA, the new *Guidance for Industry* advocates the use of information derived from standard repeat-dose toxicity studies to provide the earliest indicators of immunotoxicity.

Approaches to Immunotoxicology

As noted above, several US government agencies with regulatory responsibilities, including the FDA and the EPA, have drafted recommendations for guidelines for immunotoxicity testing strategies. All testing strategies to date recognize the complexity of the immune system as a target organ and recognize that no single immune parameter can be used with sufficient confidence to test for the hazard of immunotoxicity. Therefore, historically, immunotoxicity has been assessed by a battery of assays usually structured in a multitiered approach. However, recent validation studies, most notably studies conducted by the National Toxicology Program, have indicated that immunotoxicity can be assessed with a finite number of assays. Several concepts that have had an impact on the evolution of a testing strategy are highlighted below.

Although the concept of required immunotoxicity testing is a relatively recent development, the toxicity of the immune system has been an important part of routine toxicity testing for some time. The endpoints have included weights and histological evaluation of key immunocompetent organs, including the spleen and thymus, leukocyte counts and differentials, and some parameters in clinical chemistry, including globulin measurements. However, while it is acknowledged that these standard toxicology endpoints are important and can provide some indication of immunotoxicity, it is generally recognized that they are not sufficient as a predictor of immunotoxicity hazard. As many as 30% of known immunotoxic chemicals would be missed if only these endpoints were used. While these endpoints become better predictors when chemicals are assessed at high doses (i.e., at or above the maximum tolerated dose), these types of exposures will also increase the likelihood that indirect mechanisms of immunotoxicity

(i.e., such as neuroendocrine changes, liver damage, or effects on other organ systems) will be involved.

Most experts in the field of immunotoxicology recognize that immunotoxicity can only be measured if the immune system is asked to perform its function. Therefore, a specific functional parameter is now recognized as a critically important component of the first tier of a testing strategy. One of the most sensitive indicators of immune suppression in most animal models has been the primary response to an antigen. An especially sensitive parameter is the antibody response to a T-cell-dependent antigen. The sensitivity of this type of assay is consistent with the description of this response as being dependent on the cooperativity of multiple cell types, including the B cell as the primary effector cell, T cells as important regulatory cells, and APCs. Regardless of the specific immune parameters included in a testing strategy, the interpretation regarding immunotoxicity can only be made in the context of a well-designed study from the perspective of the dose–response relationship.

Because an increase in the incidence and/or severity of infection has been consistently identified as one of the hallmark indicators of immune suppression, a great deal of effort has been put forth into the design and characterization of host resistance models. One of the key features of these characterizations has been the correlation of changes in various host resistance models with changes in specific immune parameters. These results have consistently indicated that changes in specific immune functional parameters are associated with the predicted/anticipated changes in host resistance models. It is now generally accepted that host resistance models are not a feasible choice as an initial predictor of immunotoxicity because of their complexity and cost and that these models are best positioned in the second tier of a testing strategy.

Because immunotoxicity exists in a continuum, it is important to measure xenobiotic-induced changes in immune function in both directions. By and large, more effort has been invested in the validation of studies to address the immunosuppressive part of the continuum. However, recent validation studies have been conducted to address the sensitizing potential of chemicals, including a major effort by the National Toxicology Program.

One of the obvious and most important goals of an experimental immunotoxicity testing strategy is to enable the best extrapolations between the results generated in the animal models and the potential risk of immunotoxicity in humans. One of the recent fallouts of this goal has been the recognition that the historic approaches that have been used in clinical immunology may not have much use in human immunotoxicology. While these end points are sufficient to detect immunodeficiencies associated with either congenital disorders or immunosuppressive drug therapy, they do not possess the necessary sensitivity to detect the more subtle consequences of xenobiotic-induced immunotoxicity. Specifically, many of these end points, including mitogen-induced lymphoproliferation, the analysis of lymphocyte surface markers, and the response to recall antigens, lack appreciable sensitivity in most animal studies. As a result, several recent proposals have been put forth to reevaluate the way that we measure immune function in humans. Most of these testing strategies have incorporated plans to measure the primary response to a new antigen, and several of these testing strategies have recommended using newly developed vaccines as the new antigen.

The immune system has unquestionably been identified as a potential target organ for drugs and chemicals. Therefore, the hazard exists. The assessment of the risk associated with xenobiotic-induced immunotoxicity represents one of the key challenges for this discipline in the immediate future.

See also: Biomarkers, Human Health; Blood; Molecular Toxicology–Recombinant DNA Technology; Multiple Chemical Sensitivities; Polycyclic Aromatic Hydrocarbons (PAHs); Psychological Indices of Toxicity; Resistance to Toxicants; Sensitivity Analysis; Skin.

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Implant Studies

Shayne C Gad

Implant or implantation tests are designed to assess the localized effects of a biomaterial or device designed for therapeutic use inside the human or animal body. These tests are performed to evaluate and establish the safety of a new biomaterial or device, and to meet regulatory requirements. Implantation testing methods essentially attempt to imitate the intended use and likely misuse conditions. Although different tests use various animal species, the rabbit is the species of choice, with implantation performed in the paravertebral muscle. Implantation can be either surgical or nonsurgical: the surgical method involves the creation of a pouch in the muscle into which the implant is placed, while the nonsurgical method uses a cannula and stylet to insert a cylinder-shaped implant. A macroscopic examination may be supplemented with microscopic analysis, and the degree of tissue reaction in the test site is evaluated as a measure of biocompatibility. The implant may be maintained for from 7 days to a year for the study.

The principal regulatory guidelines for implant studies come from the ISO (International Organization for Standardization) and the Ministry of Health, Labour, and Welfare in Japan (MHW). The biological evaluation of medical devices has become more globally harmonized in recent years, concurrently with the publication of the ISO 10993 standard for the testing of medical devices. Some countries still require their national guidelines to be met, but most will accept ISO 10993 as a parallel alternative to their own regulations. Examples of some differences between the ISO and MHW sets of guidelines are summarized below. It should be noted that all guidelines, whether accepted or under preparation, should be regarded as more of a dynamic process than a rigid framework since the various standards are subjected to continuous revision and evaluation. The impact on this will come from authorities, notified bodies, and from national and international expert and working groups (e.g., see the European Centre for Validation of Alternative Methods (ECVAM) working group activity below). Further, the recommended tests must be conducted with consideration for the information available from other sources, with knowledge of the type of material a device is made from, and with awareness of its planned end use and likely misuse (**Table 1**).

Table 1 Differences in ISO 10993 and the MHW guidelines for assessing the effects of device or material implantation

ISO 10993	*MHW 1995*
Time point(s) of assessment: sufficient to achieve steady state; e.g., 2, 4, 6, and 12 weeks	7 days and 4 weeks
Number of animals: at least three per time period of assessment	At least four per time period
Number of samples of evaluation: at least eight per time period for test and control	No minimum number specified
Evaluation criteria: comparative evaluation of responses to test and control materials	If more than two of the four test sites in each animal exhibit a significant response compared to control sites, the test is considered positive

The interactions between intact organisms and implanted devices or biomaterials are complex. Not only do implanted materials affect the organism, but the organism can affect the implanted materials. Most longer-term implant studies incorporate retrieval of the implant at the end of the study to allow evaluation of this latter aspect.

Effects of the implant on its host almost always include evoking a foreign bodily response. The extent of this and other responses are assessed both quantitatively and semiquantitatively, with the latter being done with reference to established grading scales.

Bollen and Harling (see Further Reading section) provide an overview of how to conduct a risk assessment for a medical device and discuss the use of ISO 14971, one of the newest standards in this area, for risk management. This publication also covers ISO 10993, which (as discussed above) covers the biological evaluation of medical devices. A hypothetical risk evaluation of a medical device is also described.

A working group under the auspices of the European Centre for Validation of Alternative Methods has recommended alternative methods that can be used for safer testing of medical devices. These include two *in vitro* tests as potential substitutes for the *in vivo* assays for skin and eye irritation.

Finally, there is a special class of tumorgenesis that is induced by subcutaneously implanted devices only in rodents (induction of fibrosarcomas by what is called the Oppenheimer effect).

See also: Biocompatibility; Foreign Body Response.

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Import and Export of Chemicals *See* Hazardous Chemicals, Import/Export of.

In Vitro Test

Shayne C Gad

In vitro test systems do not employ intact higher organisms as models, and include a large set of alternative models addressed extensively under their own entries in this book. These *in vitro* test systems tend to have the benefit of being low in cost to use and to have well-known mechanisms of action. They can be used for assessing or predicting the toxic effects of chemicals and for elucidating the mechanisms of action. The systems include the use of cell or tissue cultures, isolated cells, tissue slices, subcellular fractions, transgenic cell cultures, and cells from transgenic organisms.

The systems also include *in silico* modeling. For example, *in vitro* methods have been and are being developed for the prediction of toxic effects based on the data from traditional toxicity studies combined with structure–activity comparisons and knowledge

Table 1 Types of *in vitro* models for toxicity testing and research

Level/examples	*Advantages*	*Disadvantages*
Lower organisms (earthworms, fish)	Range of integrated organismic responses	Frequently lack responses typical of higher organisms
Isolated organs	Intact yet isolated tissue and vascular system Controlled environmental and exposure conditions	Donor organism still required Time consuming and expensive No intact organismic responses Limited duration of viability
Cultured cells (such as the hERG assay)	No intact animals directly involved Ability to carefully manipulate system Low cost Ability to study a wide range of variables	Instability of system Limited enzymatic capabilities and viability of system No (or limited) integrated multicell and/or organismic responses
Chemical/biochemical systems	No donor organism problems Low cost Long-term stability of preparation Ability to study a wide range of variables Specificity of response	No *de facto* correlation to *in vivo* system Limited to investigation of a single defined mechanism
Computer simulations (also known as *in silico* modeling)	No animal welfare concerns Speed and low per-evaluation cost	May not have predictive value beyond a possibly narrow range of chemical structures Expensive to establish

of toxicologically important chemical structures. Additional *in vitro* test systems are being developed for use in high-throughput toxicology and pharmacology for the understanding of mechanisms of toxic action and for genomics, transcriptomics, and proteomics applications.

The most famous *in vitro* test system is the Ames mutagenicity assay and, indeed, most mutagenicity tests employ *in vitro* systems. *In vitro* systems are also widely employed for assessing pyrogenicity and cytotoxicity in the medical device industry. Another example is the *hERG* assay, a test required for new pharmaceuticals under ICH S7B to identify drugs with a potential to cause changes in heart electrical function. The term *hERG* is an acronym for human Ether-a- Go-Go Related Gene. This gene encodes the pore-forming unit of one of the cardiac membrane channel that conducts potassium current.

Various *in vitro* test systems are described in **Table 1**.

See also: Ames Test; Analytical Toxicology; *hERG* (Human Ether-a-Go-Go Related Gene); *In Vivo* Test; Microtox; QT Interval; Toxicity Testing, Alternatives; Toxicity Testing, Irritation; Toxicity Testing, Modeling; Toxicity Testing, Mutagenicity; Toxicity Testing, Validation.

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In Vivo Test

Shayne C Gad

Toxicologists are taught to consider and use various methods, including tests on animals, as part of their effort to conduct (as the Society of Toxicology states) "toxicological research to ensure and enhance the quality of human and animal health and the environment." They are also taught about the effective and humane use of laboratory animals in research, and about the ongoing development of valid alternatives to animal testing. The types of animal models and the alternatives to animal testing approaches that are being used in toxicology are typically those that have been accepted by the scientific community and recognized by regulatory bodies.

The major benefit of using an *in vivo* test is that it provides an intact biological system including exposure route-specific absorption, distribution, metabolism, and excretion responses, as well as tissue-specific responses to the parent compound and any metabolite(s) that reach the various tissues. Many of the factors affecting these processes in chemical toxicity are not yet well defined, but an *in vivo* test system provides a tool for evaluating the chemical toxicity in an intact biological system without knowing every aspect of these processes. The tests on animals (also called *in vivo* studies) used by toxicologists are generally considered to be only those performed in intact higher organisms (most commonly, mammals). Eight different species are currently used with any frequency in toxicological research. These are, in approximate numbers of animals utilized (from most to least), rat, mouse, rabbit, guinea pig, hamster, dog, ferret, and monkey. The cat and the frog, while common biological models, have not been used in toxicity testing for some time.

In many cases as the toxicology profile of a test agent begins to be developed, humans are assumed for risk assessment purposes to be at least as sensitive to the effects as the most sensitive species used in evaluating the effects of the chemical or other material, and at least as sensitive as the most sensitive alternatives to animal testing approaches being used. Such assumptions could change as the toxicology profile is developed and understanding is gained about the metabolism, target organs, toxic responses,

etc. The key rationale for using *in vivo* test systems can be summarized as follows:

- They provide evaluation of actions/effects on intact animal and organ/tissue interactions.
- Either neat (i.e., undiluted) chemicals or complete formulated products (complex mixtures) can be evaluated.
- Either concentrated or diluted products can be tested.
- They yield data on the recovery and healing processes.
- They are the required statutory tests for agencies under such laws as the (US) Federal Hazardous Substances Act (unless data are already available), (US) Toxic Substances Control Act, (US) Federal Insecticides, Fungicides, and Rodenticides Act, Organization for Economic Co-operation and Development, and the (US) Food and Drug Administration laws.
- Quantitative and qualitative tests with scoring system are generally capable of ranking materials as to relative hazards.
- They are amenable to modifications to meet the requirements of special situations (such as multiple dosing or exposure schedules).
- They have an extensive available database and cross-reference capability for evaluation of relevance to human situation.
- They involve the case of performance and relative low capital costs in many cases.
- Tests are generally both conservative and broad in scope, providing for maximum protection by erring on the side of overprediction of hazard to humans.
- Tests can be either single endpoint (such as lethality, corrosion, etc.) or multiple endpoint, including such test systems as a 13 week oral toxicity study.

Limitations of *in vivo* testing systems that serve as a basis for seeking *in vitro* alternatives for toxicity tests include the following:

- The complexity of the *in vivo* system and the types of toxic effects (e.g., an effect could be the result of quite different toxic mechanisms) can make it a challenge to difficult to understand what has happened and its relevance to human risk assessment. For example, there could be potential confounding or masking of the findings in an *in vivo* test system.
- *In vivo* systems might be intended to only assess the local effects at the site of application or the immediate structural alterations produced by an agent (however, this may be a purposeful test system limitation).
- Toxicologist and technician training and monitoring are critical (particularly due to the subjective nature of evaluation).
- *In vivo* tests do not always predict results in humans if the objective is to exclude or identify agents creating a high degree of toxicity, for example, skin corrosion.
- Structural and biochemical differences between test animals and humans make extrapolation from one to the other difficult.
- Lack of standardization of some *in vivo* systems.
- Possible variability in correlating the results with those from human exposures.
- Possible considerable biological variability between individual animals.
- Depending on the animal models and toxicity endpoints of interest, the results might be in large, diverse, and fragmented databases that are not readily comparable.

Some test protocols and toxic endpoints are particularly liable to produce misleading or difficult to judge results. Several examples of possibly confusing results obtained in carcinogenesis bioassays are useful in discussing this point:

- Perhaps the first to be recognized in toxicology concerned the induction of sarcomas following the local injection of chemicals subcutaneously in rats. Although there can be little doubt that the injection of small quantities of chemicals such as 7,12-dimethylbenz(*a*)anthracene actually induces these tumors, overloading the tissues with dyestuffs may well lead to cancer because of a mechanism dependent on factors other than the specific interactions of the test chemical.
- Bladder stone formation can lead to bladder cancer in rats and mice, thus making it difficult to be certain whether a chemical that leads to bladder stone formation and turnorigenesis is or is not a true animal and/or human carcinogen.
- Another example is renal toxicity resulting from the accumulation of a protein, α-2u-globulin, in male rat kidney proximal tubule lysosomes. This protein is synthesized exclusively by adult male rats, and the nephrotoxicity of agents such as D-limonene in male rats is attributed to its ability to bind to α-2u-globulin. Other species, including humans, synthesize proteins that share significant homology with α-2u-globulin; however, none of these proteins, including the mouse equivalent of α-2u-globulin, can produce this toxicity. The tumorigenic activity of D-limonene in male rats

has been concluded to be nonrelevant to humans because of (1) the male rat specificity of the nephrotoxicity and carcinogenicity, (2) the role that α-2u-globulin plays in the toxicity, as evidenced by the complete lack of toxicity in other species despite the presence of structurally similar proteins, and (3) the lack of genotoxicity of both D-limonene and D-limonene-1,2-oxide, supporting the concept of a nongenotoxic mechanism, that is, sustained renal cell proliferation. Both D-limonene and *cis*-D-limonene-1,2-oxide (the major metabolite involved in this toxicity) are negative in *in vitro* mutagenicity screens. Therefore, the toxicity-related renal cell proliferation is believed to be integrally involved in the carcinogenicity of D-limonene as persistent elevations in renal cell proliferation may increase fixation of spontaneously altered DNA, or serve to promote spontaneously initiated cells.

Perhaps the greatest cause of confusion in the interpretation of carcinogenicity bioassays occurs when a substantial background incidence of tumors is enhanced. It should be asked whether the test chemical is inducing such tumors or merely enhancing their incidence. Although this problem is clearly recognized with chemicals that enhance the already high incidence of pulmonary tumors in strain A mice, there has been little discussion of the confounding effects of naturally occurring tumors that demonstrate a lower but still appreciable incidence. The B6C3F1 male mouse used in the National Cancer Institute, National Toxicology Program (NCI/NTP) Bioassay Program in the United States demonstrates a 15–60% incidence of hepatic cell tumors by two years of age. However, whether this confounds the interpretation of a bioassay or whether enhancement of the yield of such tumors, as opposed to their direct induction, is relevant to the effects of the chemical in humans is not asked; instead, these chemicals are usually uncritically accepted as carcinogens and generally regulated as such.

There are many tumors that have a naturally high incidence, such as tumors of the endocrine tissues in certain strains of rats. In each case, it is necessary to consider the overall evidence that agents increasing the yield of such tumors may or may not induce cancer in humans. Such considerations require in-depth knowledge of biological and biochemical mechanisms of carcinogenesis and development of new and testable ideas. Increased emphasis on how agents exert their effect, rather than on which agents exert an effect, will move toxicology to the forefront of integrated biological science.

One further problem needs to be addressed regarding human and animal reactions to toxic agents. Although it is possible to control the exposure of a test animal quite precisely in a well-run experiment, humans are exposed to an ever-changing multitude of chemicals because of the food they eat, the drugs they take, or the lifestyles they have chosen. Therefore, single-substance toxicological tests may either overemphasize or underemphasize the significance of the potential hazard to humans, except possibly in the case of massive exposures. There is very limited laboratory evidence on the effects of chemical mixtures because a single chemical assay is so expensive that the assay of mixtures becomes prohibitively costly. However, the coadministration of a carcinogen and a promoting agent may lead to far more tumors, than either agent alone. By contrast, two carcinogens, such as 4-dimethylaminoazobenzene and 3-methylcholanthrene, may fail to produce tumors when given together, yet they do so when given separately. More information on chemical interactions continues to be needed, if animal tests to humans even with qualitative accuracy are extrapolated.

See also: Analytical Toxicology; Animal Models; Toxicity Testing, Alternatives; Toxicity Testing, Modeling; Toxicity Testing, Validation.

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Relevant Website

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Indole

Samantha E Gad

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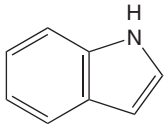
- Chemical Abstracts Service Registry Number: CAS 120-72-9
- Synonyms: 2,3-Benzopyrole; 1-Benzazole
- Chemical Formula: C_8H_7N
- Chemical Structure:



Uses

Indole is used as a chemical intermediate, a perfume fixative, and as a synthetic flavor. In addition, it is possibly a kairomone (a volatile chemical released by a plant to attract phytophagus insects). Indole is also a component of tobacco smoke, and occurs naturally in coal tar, jasmine oil, and orange-blossom oil. It is also a bacterial decomposition product of tryptophan in the gut.

Background Information

The indole nucleus is found in a large number of naturally occurring compounds; the compound constitutes ~2.5% of jasmine oil and 0.1% of orange-blossom oil; in both cases, it contributes to their fragrances. Although synthetic methods have been described for the manufacture of indole, extraction from the 240–260°C coal-tar distillate fraction is the only commercial source. Interesting, given its use in fragrances, is that indole has an intense fecal odor, presumably at higher concentrations.

Exposure Routes and Pathways

The general population may be exposed via inhalation of ambient air or tobacco smoke, ingestion of food, and dermal contact with vapors, food, perfumes, and other products containing indole. Occupational exposure may occur through inhalation or dermal contact at workplaces where indole is produced or used. Indole is one of the odorous components found in sewage and animal wastes, including human feces, and occurs in animal tissues where putrefactive processes have occurred, presumably by the decomposition of tryptophan.

Toxicokinetics

Indole absorbed from the gut is hydroxylated to form indoxyl, which conjugates with sulfate to produce indican (indoxylsulfuric acid) in the liver. Indoxyl and indican are found in human plasma and urine. The daily urinary excretion of indoxylsulfate in normal adults was reported to average 200 mg (range 140–250 mg). Indole was not detected in the blood of rabbits exposed at 10 mg m^{-3} for 3 h.

Mechanism of Toxicity

Indole causes oxidative damage to membranes.

Acute and Short-Term Toxicity (or Exposure)

Animal

The rat oral LD_{50} is 1 g kg^{-1}; rabbit dermal LD_{50} is 790 mg kg^{-1}; mouse oral LD_{50} is 1070 mg kg^{-1}; mouse intraperitoneal LD_{50} is 117 mg kg^{-1}. Indole is a severe (primary) eye and skin irritant.

Human

Indole at a few parts per million has an unpleasant odor and can elicit toxic symptoms, such as nausea. The consistent toxicological property of indole, an aromatic amine, observed in animal studies is its ability to cause the formation of Heinz bodies, which are known to be produced by other aromatic amine compounds, such as aniline.

Chronic Toxicity (or Exposure)

Animal

Rhesus monkeys, rats, and mice were exposed continuously to indole at a concentration of 10.5 ppm (50 mg m^{-3}) for 90 days. Hematological examination of the exposed rodents revealed that numerous Heinz bodies were present in the blood. Pathological studies on some of the exposed mice revealed that 95% of the animals had pigment in the renal tubular cells. However, renal abnormalities were not found in any exposed monkeys or rats that were examined. Pathological examination showed no brain damage in the monkeys, and histopathological studies of the heart, lung, liver, and kidney from the exposed monkeys

revealed no statistical difference from that of control monkeys. In another study, indole at 1.6% in the diet did not induce bladder or liver cancer in hamsters; however, no other organs were examined. Chronic studies by the subcutaneous route have shown that indole might have a weak leukemogenic activity in mice, but not in hamsters. Indole has enhanced the incidence of bladder cancer in some bioassays, for example, addition of indole to a diet containing 2-acetylaminofluorene produced an increased incidence of bladder cancer in hamsters. Other indole compounds have been studied for their carcinogenic and anticarcinogenic effects, for example, indole-3-carbinol, a minor cruciferous vegetable component, inhibited dimethylbenzanthracene (DMBA)-induced mammary tumors in rats.

Human

Indole is a possible carcinogen.

Clinical Management

On exposure, skin should be washed with soap and copious amounts of water.

Environmental Fate

Indole will mainly exist in the vapor phase and is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals, nitrate radicals, and ozone with estimated half-lives of $\sim$2–3 h, $<$1 min, and 6 h, respectively. Indole is expected to readily biodegrade under both aerobic and anaerobic conditions in soil and water. Bioconcentration in aquatic organisms should be low, given an estimated bioconcentration factor value of 25.

Ecotoxicology

Indole is moderately toxic to zooplankton.

Exposure Standards and Guidelines

Indole was granted GRAS (generally recognized as safe) status by the Flavor and Extract Manufacturer's Association (FEMA) in 1965 (FEMA GRAS No. 2593). It was approved by the Council of Europe in 1970 to be included in the list of admissible artificial flavoring materials. For indole, there is no American Conference of Governmental Industrial Hygienists threshold limit value and no US Occupational Safety and Health Administration permissible exposure limit for indole.

See also: Carcinogenesis; Generally Recognized as Safe (GRAS); Phenol.

Further Reading

Anonymous (1974) Fragrance raw materials monographs. Indole. *Food and Cosmetics Toxicology* 12: 925–926.

Bickers DR, Calow P, Greim HA, *et al.* (2003) The safety assessment of fragrance materials. *Regulatory Toxicology and Pharmacology* 37: 218–273.

Lam C-W and James JY (1996) Indole. In: Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants, vol. 2, pp. 235–249. Commission on Life Sciences (CLS). Washington, DC: US National Academy of Sciences (NAS), National Academies Press.

Lewis RJ (2000) *Sax's Dangerous Properties of Industrial Materials*, 10th edn., vol. 3, pp. 2088–2089. New York: Wiley.

Indoor Air Pollution *See* Pollution, Air Indoor.

Industrial Hygiene

Andrew Maier

Industrial hygiene is a professional field with responsibility related to protecting the health of workers, and protecting communities that may be impacted by work-related exposures. Historically, industrial hygiene has been described as the anticipation, recognition, evaluation, and control of workplace health hazards. Industrial hygiene has a broad scope, with practitioners often having responsibility for the prevention of work-related health risks from chemical as well as diverse physical agent exposures (noise, temperature extremes, ergonomic considerations, nonionizing radiation, etc.).

The American Industrial Hygiene Association (AIHA) has developed informational brochures to educate the public on common roles and responsibilities

of a professional industrial hygienist. Typical roles as presented by the AIHA include (with the addition of specific examples of such activities):

- Investigating and examining the workplace for hazards and potential dangers – such as conducting exposure assessments for a chemical or physical agent.
- Making recommendations on improving the safety of workers and the surrounding community – such as developing ventilation system requirements, emission abatement strategies, and developing protective equipment guidelines.
- Conducting scientific research to provide data on possible harmful conditions in the workplace – such as developing new sampling techniques or data evaluation tools.
- Developing techniques to anticipate and control potentially dangerous situations in the workplace and the community – such as development of preventive assessment strategies for newly introduced chemicals or processes or participating in community emergency response planning committees.
- Training and educating the community about job-related risks.
- Advising government officials and participating in the development of regulations to ensure the health and safety of workers and their families.
- Ensuring that workers are properly following health and safety procedures.

Industrial hygienists typically have formal training in disciplines related to engineering, chemistry, physics, biology, or a related physical science from an accredited college or university. Certification is viewed as an important milestone for ensuring the continuing professionalism of the occupation. Through the American Board of Industrial Hygiene a practicing industrial hygienist can become a Diplomate of the American Academy of Industrial Hygiene, which entitles him/her to the designation of Certified Industrial Hygienist. Similar certifications are used to ensure a professional level of practice in other countries. For example, a professional designation as Registered Occupational Hygienist is available to qualified professionals in Canada.

Some Organizations Relevant to Industrial Hygiene

There are a number of professional and regulatory organizations that provide resources on occupational health topics. Key organizations include the American Conference of Governmental Industrial Hygienists, American Industrial Hygiene Association, Canadian Centre for Occupational Safety and Health, the European Union's European Agency for Safety and Health at Work, International Labor Organization, (US) National Institute for Occupational Safety and Health, and the (US) Occupational Safety and Health Administration.

See also: American Conference of Governmental Industrial Hygienists; American Industrial Hygiene Association; Exposure Criteria; International Labour Organization (ILO); National Institute for Occupational Safety and Health; Occupational Toxicology; Occupational Exposure Limits.

Further Reading

DiNardi SR (ed.) (2003) *The Occupational Environment: Its Evaluation and Control*. Fairfax, VA: AIHA.

Harris RL (ed.) (2000) *Patty's Industrial Hygiene*, 5th edn. New York: Wiley.

Relevant Websites

http://www.acgih.org – American Conference of Governmental Industrial Hygienists.

http://www.aiha.org – American Industrial Hygiene Association.

http://www.ccohs.ca – Canadian Centre for Occupational Safety and Health.

http://europe.osha.eu.int – European Union, European Agency for Safety and Health at Work.

http://www.ilo.org – International Labor Organization.

http://www.cdc.gov – (US) National Institute for Occupational Safety and Health.

http://www.osha.gov – (US) Occupational Safety and Health Administration.

Inert Ingredients (in Pesticides)*

Andrew M Geller

Published by Elsevier Inc.

An inert ingredient is a chemical that has been intentionally added to a pesticidal product for reasons other than directly affecting the target pest. Inert ingredients can be contrasted with active ingredients, that is, those that have pesticidal activity. The active and inert ingredients are combined to formulate the final product. Inert ingredients are included in formulations for many reasons. These are generally related to enhancing product performance by, for example, making it easier to apply, helping it to dissolve in water, assisting in pesticide dispersion/adhesion, or stabilizing the product for longer shelf life.

Whether a chemical is defined as 'inert' or 'active' is a function of the product. The term inert in this context does not necessarily mean harmless, nontoxic, or not biologically active. Inert ingredients can range from extremely toxic to compounds of minimal concern. In recognition of this, the US Environmental Protection Agency (EPA) has requested registrants to voluntarily substitute the term 'other ingredients' in lieu of the term 'inert ingredients' to help dispel the impression that inert is equivalent to harmless.

For example, xylene is considered to be an inert ingredient in some formulations, when used as a solvent. Xylene is not harmless; exposure can result in severe health effects ranging from skin irritation to reproductive and nervous system toxicity. Another example is piperonyl butoxide, used in insecticidal formulations. Piperonyl butoxide has clear biological activity; it acts to inhibit metabolic enzymes. It does not, however, directly kill or immobilize insect pests. Piperonyl butoxide potentiates the active ingredient in insecticidal formulations by blocking detoxification pathways in the insect.

The US law (Federal Insecticide, Fungicide, and Rodenticide Act) requires that product labels for pesticides must list active ingredients and their percentages by weight. Similar labeling is not required for inert ingredients except for those defined as being of toxicological concern (see below).

In 1987, the US EPA instituted a policy (52 FR 13305, Inert Ingredients in Pesticide Products) to reduce the potential for adverse effects from pesticide products containing toxic inert ingredients. This policy established data requirements for new inert ingredients and categorized inert ingredients into four lists (**Table 1**).

- *List 1*: Inert ingredients of toxicological concern. The criteria for placing a chemical on List 1 include carcinogenicity, adverse reproductive effects, neurotoxicity, or other chronic effects, or developmental toxicity (birth defects). These effects must be demonstrated in laboratory or human studies and the data subject to peer review. The criteria also include documented ecological effects and the potential for bioaccumulation. List 1 originally contained $\sim$50 chemicals, but many of these were removed from the list because they were no longer used in pesticidal formulations. Future use of de-listed chemicals requires data submission to the US EPA and the guarantee that use of the inert ingredients will not pose an unreasonable risk to human health or the environment. There are 7 compounds currently on this list (**Table 1**). Any formulation containing any of these compounds must list them on the label.
- *List 2*: Potentially toxic inerts, with high priority for testing. These compounds may be structurally similar to chemicals known to be toxic

Table 1 Examples of inert ingredients and category

List 1: Inert ingredients of toxicological concern	Adipic acid, bis(2-ethylhexyl) ester	Ethylene glycol monoethyl ether
	Hydroquinone	Isophorone
	Nonylphenol	Phenol
	Phthalic acid, bis(2-ethylhexyl) ester	
List 2: Potentially toxic inert ingredients/high priority for testing	1,1,1- Acetonitrile	Cresol
	Fuel oil, no. 2	Methyl isobutyl ketone
	Paraffins	Toluene
	Trichloroethane	Xylene
List 3: Inert ingredients of unknown toxicity	Acetone	Boric acid
	Camphor	Hydrogen peroxide
	Piperonyl butoxide	Salicylic acid
	Tea tree oil	Zinc chloride
List 4: Inert ingredients of minimal concern	Beeswax	Ethanol
	Maltodextrin	Nitric acid
	Wintergreen oil	Sodium fluoride

* This document has been reviewed in accordance with the US environmental Protection Agency policy and approved for publication. Mention of trade names and commercial products does not constitute endorsement or recommendation for use.

and/or may have data suggesting a basis for concern.

- *List 3*: Inerts of unknown toxicity: Inert ingredients on this list have not yet been adequately evaluated. These substances will be assessed to determine if they merit reclassification to List 1, 2, or 4.
- *List 4*: Inerts of minimal concern, further broken down into List 4A: minimal risk inerts, including all commonly consumed foods; and List 4B: inerts which have sufficient data to substantiate that they can be used safely in pesticide products.

See also: Pesticides.

Relevant Websites

www.epa.gov – US Environmental Protection Agency, Inert Ingredients in Pesticide Products. This website gives links to regulations, to tables of inert ingredients categorized by list. Last updated January, 2004.

www.oag.state.ny.us – Office of the NY State Attorney General, 1996. The Secret Hazards of Pesticides. This report includes percentages of inert ingredients in some pesticide products, and identifies adverse health effects of some compounds listed as inert ingredients.

Information Resources in Toxicology

Frederick W Berman

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Introduction

The availability of toxicological information dramatically increased during the twentieth century, spurred by an increased awareness of how chemicals and other environmental factors influence biological systems. While during the first half of the twentieth century toxicology was considered a subset of pharmacology, it became a focused discipline largely through public awareness of toxicological issues. Recognition of the need for protection from hazardous chemical exposure, and environmental impact, resulted in legislation designed to safeguard the consumer. This legislation mandated continuing research on the effects of chemicals on humans and the environment. In the past 30 years the major legislation in the United States dealing with controls on medicinals, environmental contaminants, and energy have all included provision for the collection and dissemination of data. Spurred on by these types of regulatory requirements and worldwide concern for the safety of the planet industry, research organizations, government agencies, academic centers, and international groups have all contributed to a huge body of toxicology information.

This entry provides a selection of printed and electronic information resources of use in identifying toxicological information. Technological advances and ways to communicate on a global scale have provided great opportunities for sharing knowledge. It is estimated that scientific information doubles every 4 years; in the vast toxicological arena, this figure is greatly accelerated. Today there are literally hundreds of public databases and databanks that could be consulted for toxicology-related information, thousands of journal articles published each year on the effects of xenobiotics on humans and the environment, and more than a score of organizations whose primary activities are the creation of banks of available files of data and the production of research reports in specialized areas of toxicology.

This huge amount of information, scattered throughout the literature of the scientific disciplines – chemistry, biology, medicine – and present in many forms – raw data, technical reports, articles, monographs, statutes, and regulations – presents an enormous challenge to identify and retrieve relevant information. In the past decades technological developments have provided computerized systems capable of storing and providing access to information on a scale unimagined less than 30 years ago. Some systems provide access to data itself, and some are designed to provide information on sources of data. Some systems determine hazard assessment by applying sophisticated algorithms and use of mathematical modeling, and some systems are designed as interactive, multimedia instructional tools. The development of hypertext and other sophisticated software systems for navigating databases, electronic superhighways such as the Internet linking investigators to potentially valuable electronic repositories of nformation, the creation of local storage and retrieval systems such as those based on CD and DVD technology, and the developments in electronic communication systems provide opportunities, and frustrations, for the investigator.

Approaching the Problem

Although we have access to an enormous amount of information, the quality and reproducibility of data varies considerably. Governmental and regulatory influences have not provided any guarantees as to the accuracy of data related to xenobiotics and the researcher needs to understand the purpose and mandates behind an information resource to fully evaluate the data contained within. For example, the National Institute for Occupational Safety and Health (NIOSH) Registry of Toxic Effects of Chemical Substances (RTECS) criteria for selection of data is not based on the reproducibility of that data. Other sources, such as the National Library of Medicine's Hazardous Substances Databank (HSDB), present information compiled from a wide variety of source materials that are extensively reviewed by experts.

The professional is usually aware of the major texts, journals, organizations, and other standard resources in their own field. However, because of the interdisciplinary nature of toxicology, and the need to access information that may reside in the resources of other scientific areas of study, the user is frequently at a loss. Some types of sources are difficult to find. The so-called 'gray literature' – for example, manufacturer's brochures, internal agency/company reports, conference proceedings, or translations, all excellent sources of potentially useful information – can be extremely difficult to identify. Other obstacles include those related to technology. Hardware limitations, varying search software and operating systems, and telecommunications problems can limit the investigator's ability to identify needed resources. There are also the common problems of interpretation of data-language barriers or varying scientific research conventions.

Valuable assistance in finding information can be obtained by consulting information intermediaries with subject expertise who can identify and access information resources unknown or unavailable locally. These individuals may be in local information centers or may be brokers who specialize in individual information services or creation of compilations of data for a fee. An example of this type of service is the Comprehensive Health and Environmental Monographs (CHEMS) division of Health and Environment International. This service creates detailed reports on the health and environmental effects of a chemical. Professional organizations, database producers, and government agencies can also be sources of experts as well as providers of direct information. An example of the former type of service is UNEP-Infoterra, an international referral and research organization of the United Nations Environmental Program (UNEP). UNEP-Infoterra provides access to an extensive range of information sources, as well as expert consultants, worldwide. The US Environmental Protection Agency (EPA) is the National Focal Point for this organization in the United States, one of over 175 sites around the globe.

Keeping Current

Columns and review sections in journals or newsletters can help the professional keep abreast of new resources or provide comparative information on established resources in selected areas. Collections of reviews are also available online in such sources as *Comprehensive Core Medical Library* (CCML) by Ovid Technologies, Inc., *Book Review Index* (online and in print) by Wilson, and the National Library of Medicine's *TOXLINE* database. Reviews of popular works dealing with consumer health or environmental concerns can also be found in newspapers, popular magazines, and consumer organization newsletters. Summaries and reviews of online databases and databanks can be found in the information science journals, directories of online resources, and producer/vendor documentation.

In addition to journals, texts, and newsletters, resource information can be identified by examining the program reports of government agencies, scientific organizations, or research institutions. Names of experts, identification of programs of interest, and organs for dissemination of information can all be identified in this way. An example of this type of resource is *Access EPA*, which was published by the Information Access Branch, Information Management and Services, Division, US Environmental Protection Agency (US Government Printing Office, Washington, DC) (ISBN 0-160483301). While no longer in print, the information content of this outstanding pathfinder to the resources of the US EPA can be found in more comprehensive form in the EPA's 'Information Sources' website, http://www.epa.gov/epahome/resource.htm, which provides ready access to the EPA clearinghouses, special information dockets, hotlines, records, databases, newsletters, email discussion lists, libraries, publications, directories, and other information resources supported by the US EPA.

Guides to the Literature

There are few guides to the literature of toxicology, but many excellent guides exist in narrower areas. The following is a selection of available tools published within the past several years:

Snow B (1999) *Drug Information: A Guide to Current Resources*, 2nd edn., Lanham, MD: Scarecrow Press (ISBN 0-810833204).

Designed as a self-study guide as well as a reference tool, this volume is considered the best work in the field. Two chapters of particular interest in this context deal with side effects of therapeutic agents, including adverse reactions, poisonings, and drug interactions.

The Toxics Directory (2000). California Environmental Protection Agency, Environmental Health Hazard Assessment, Pesticide and Environmental Toxicology Section, Oakland, CA.

This directory lists agencies and organizations that provide information about or have authority over toxic substances and their health effects. The organizations range from local to national, with emphasis on California-based resources.

Webster JK (1987) *Toxic and Hazardous Materials: A Sourcebook and Guide to Information Sources*. Westport, CT: Greenwood (ISBN 0-313245754).

Although dated, this book still lists useful resources published prior to 1987. It is organized into separately authored chapters, by subject (e.g., radioactive materials, laws and regulations, and transportation), and lists more than 1600 sources (e.g., literature, organizations, audiovisuals, databases, agencies, research centers, and libraries).

Wexler P (2000) *Information Resources in Toxicology*, 3rd edn., San Diego, CA: Academic Press (ISBN 0-127447709).

This is probably the best, most current literature guide available today. Separate sections deal with the US and foreign information resources. Printed and online sources, professional organizations, government agencies, regulations, education centers, and testing laboratories are covered for the United States and for selected countries. A history of toxicology and toxicology information systems developments are also included.

Wexler P (ed.) (2001) Digital Information and Tools. Part I. *Toxicology* 157(1–2).

Wexler P (ed.) (2002) Digital Information and Tools. Part II. Web Resources in Special Toxicology Topics. *Toxicology* 173(1–2).

Wexler P (ed.) (2003) Digital Information and Tools. Part III. Global Web Resources. *Toxicology* 190(1–2).

These three special issues of the journal *Toxicology* provide a comprehensive overview of Web-based toxicology resources that are available to the public. Part I focuses on government resources, including those of the US EPA and various other federal agencies, subnational toxicology information resources (state, territorial, tribal, municipal, and community), resources available from professional toxicology societies and citizen groups, and information on tools available for searching the Web. Part II focuses on information available in specialized areas of toxicology, including alternatives to animal testing, cancer information, food, drug, and pesticide toxicology, developmental toxicity, environmental toxicology, forensic, genetic and veterinary toxicology, and others. Part III looks at information sources provided by agencies from other countries, most notably Canada, Finland, Germany, Italy, Russia, Sweden, and the United Kingdom. All issues are extremely helpful guides to finding toxicology information on the Web.

Texts

This section provides a select list of classic or standard texts, dictionaries, thesauri, glossaries, directories, handbooks, encyclopedias, databooks, and some new works that provide useful toxicology information. Online versions of some of these works are now being offered via site licensing agreements. The primary focus is on works that have been published or revised within the last several years. For information on texts published prior to 1995, consult Wexler's *Information Resources in Toxicology* (listed in the previous section).

Klaassen CD (ed.) (2001) *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 6th edn., New York: McGraw-Hill (ISBN 0-071347216).

A classic, well-documented and detailed text on the general principles, toxic responses by body system, toxic effects of major toxicant classes, and major discipline applications of toxicology. This latest edition features an expanded coverage of risk assessment, contains sections on molecular biology and pharmacogenomics, and includes online references in addition to traditional print journal and review articles.

Ballantyne B, Marrs TC, and Syversen T (eds.) (2000) *General & Applied Toxicology*, 2nd edn., London: Macmillan (ISBN 0-333698681).

This encyclopedic, three-volume set provides an excellent in-depth review of the science of toxicology, its specializations, and practice. This latest edition has been extensively revised and contains 35 new chapters.

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, 5th edn., nine vols. New York: Wiley (set, ISBN 0-471319430; Vol. I, ISBN 0-471319325; Vol. II, ISBN 0-471319333; Vol. III, ISBN 0-471319341; Vol. IV, ISBN 0-47131935X; Vol. V, ISBN 0-471319368; Vol. VI, ISBN 0-471319392; Vol. VII, ISBN 0-471319406; Vol. VIII, ISBN 0-471319414; Vol. IX, ISBN 0-471319422, cumulative index).

Harris RL and Patty FA (eds.) (2000) *Patty's Industrial Hygiene*, 5th edn., four vols. New York: Wiley (set, ISBN 0-471297844; Vol. I, ISBN 0-471297569; Vol. II, ISBN 0-471297542; Vol. III, ISBN 0-471297534; Vol. IV, ISBN 0-471297496 Vol. I, ISBN).

The latest edition of the classic giant compendium, *Patty's Industrial Hygiene and Toxicology*, has now been divided into separate four- and nine-volume sets that cover industrial hygiene and toxicology, respectively. It is one of the most complete in the area of occupational, industrial and general toxicological information. Online versions of both sets are available through licensing agreements with the publisher.

Burczynski ME (ed.) (2003) *An Introduction to Toxicogenomics*. Boca Raton, FL: CRC Press (ISBN 0-849313341).

This text is an excellent primary source of information for students, scientists, and clinicians interested in the new discipline of toxicogenomics. In six sections, this text covers the fundamentals of gene expression profiling analysis, the use of expression profiling in toxicological testing and research, model systems used in toxicogenomic studies, and the areas of mechanistic and predictive toxicogenomics. The last section addresses the future of toxicogenomics, including its use in risk assessment studies and the complex ethical issues that surround the use of toxicogenomics data.

Derelanko MJ and Hollinger MA (eds.) (2002) *Handbook of Toxicology*, 2nd edn., Boca Raton, FL: CRC Press (ISBN 0-849303702).

This is an excellent compendium of practical reference information for the toxicologist. The focus is on providing normal values, reproductive indices, physiological parameters, regulatory requirements, procedures, values, endpoints, recommended sources, animal care, and tables/graphs of use to the practicing toxicologist. Information is presented in a loose chapter arrangement with detailed tables of contents for each.

D'Mello JPF (ed.) (2003) *Food Safety: Contaminants and Toxins*. Cambridge, MA: CABI Publishing (ISBN 0-851996078).

This new text addresses the major toxins and contaminants found in plant and animal products that constitute the staple diets of the world. In four parts: part 1 addresses plant and microbial toxins; part 2 deals with contaminants arising from anthropogenic sources; part 3 addresses contemporary issues, including prion diseases, genetically modified foods, and radionuclides in foods; and part 4 addresses ongoing concerns related to information from the earlier sections.

Dreisbach RH and True B (2002) *Handbook of Poisoning: Prevention, Diagnosis, and Treatment*, 13th edn., Boca Raton, FL: Parthenon Publishing Group (ISBN 1-850700389).

This handbook for the clinical toxicologist is a standard in emergency situations, covering all types of poison situations – agricultural, medical, industrial, and household. The work is organized to facilitate ease of use in emergency situations and as a reference source. Background chapters are on prevention, identification/diagnosis, management, and legal/medical responsibilities.

Duffus JH and Worth HG (eds.) (1996) *Fundamental Toxicology for Chemists*. Cambridge, UK: Royal Society of Chemistry.

This text provides a unique perspective of toxicology specifically for chemists. It addresses general principles as well as emerging issues in reproductive toxicology, ecotoxicology, and behavioral toxicology.

Ellenhorn MJ and Barceloux DG (1997) *Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning*, 2nd edn., Baltimore, MD: Williams and Wilkins (ISBN 0-683300318).

This text provides an excellent reference for clinicians in emergency and occupational health settings. Medical toxicology is defined here as poisoning by overdose of medication and exposure to chemicals and toxins not ordinarily used therapeutically. This is a good companion work to Dreisbach. Also available on CD-ROM and via online subscription.

Genium's Handbook of Safety, Health, and Environmental Data for Common Hazardous Substances. Genium Publishing Corporation (1999). Schenectady, NY: McGraw-Hill (ISBN 0-078531152).

This handbook provides chemical profiles on over 4500 materials. Each profile contains comprehensive listings of physical data, health and toxicity effects, disposal, and regulatory information. Also available on CD-ROM.

Gilman AG, Goodman LS, Hardman JG, and Limbird LE (eds.) (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10th edn., New York: McGraw-Hill (ISBN 0-071354697).

This gigantic textbook is the standard in clinical pharmacology and contains an enormous amount of information of use to the clinician and researcher. It is strong in drug interactions and mechanism of action. It is updated every 5 years. Available on CD-ROM.

Goldfrank LR, Flomenbaum NE, Lewin NA, *et al.* (eds.) (2002) *Goldfrank's Toxicologic Emergencies*, 7th edn., New York: McGraw-Hill (ISBN 0-071360018).

This is a unique text with strength as both a training manual and in clinical management settings (second in popularity to Ellenhorn and Barceloux).

Haddad LM, Winchester JF, and Shannon MW (eds.) (1998) *Clinical Management of Poisoning and*

Drug Overdose, 3rd edn., Philadelphia, PA: Saunders (ISBN 0-721664091).

This book provides in-depth information on the clinical management of chemical poisoning and drug overdose. Chapters provide background, pharmacology, pathophysiology, diagnosis, and clinical management information for each compound listed.

Hamilton A, Hardy HL, and Harbison RD (1998) *Hamilton and Hardy's Industrial Toxicology*, 5th edn., St. Louis, MO: Mosby (ISBN 0-815141815).

This classic provides historical and current reviews on the toxic effects of industrial chemicals, including metalloids, chemical compounds, organic polymers, pesticides, physical agents, dusts, and special topics in industrial toxicology.

Hayes AW (ed.) (2001) *Principles and Methods of Toxicology*, 4th edn., Philadelphia, PA: Taylor and Francis (ISBN 1-560328142).

A textbook and standard reference designed to provide a thorough introduction to toxicology in the broadest sense. Strengths include coverage of methods, techniques, procedures, interpretation of data, and examination of controversial areas.

Herbicide Handbook, 8th edn. (2002) Champaign, IL: Weed Science Society of America.

This work, sponsored by the Weed Science Society of America, contains a wealth of physical, chemical, and toxicological data on more than 150 herbicides. Extensive data are presented on each chemical. Included are producer information and references ($65.00; Weed Science Society of America, PO Box 7050, Lawrence, KS 66044-8897).

Hodgson E and Smart RC (eds.) (2001) *Introduction to Biochemical Toxicology*, 3rd edn., New York: Wiley (ISBN 0-471333344).

Designed as an advanced toxicology textbook and general reference source. Contains well-organized chapters on mechanisms of action, organ systems, interactions, and specific pathways.

Hodgson E, Mailman RB, and Chambers JE (1998) *Dictionary of Toxicology*, 2nd edn., London: Macmillan Reference (ISBN 0333547004).

A condensed and informative guide to the fundamentals and applications of toxicology, this text is suitable for both the novice as well as the expert scientist. The 2nd edition contains ~4000 concise, informative entries on a variety of terms and chemicals in toxicology. Coverage includes biochemical and mechanistic toxicology, environmental and regulatory toxicology, chemical carcinogenesis, risk assessment and risk management, analytical chemistry, and molecular biology. Chemical entries include chemical structures and CAS numbers.

Koren G (ed.) (2001) *Maternal – Fetal Toxicology: A Clinician's Guide*, 3rd edn., New York: Dekker (ISBN 0-824703782) (ISBN 0-585404283 electronic book).

This text contains a series of reviews on the toxic effects of drugs and other compounds, viruses, radiation, and occupational hazards. A chapter on drugs of choice in pregnancy and a section on diagnosis of fetal malformations are included. An online version is available.

Krieger RI (ed.) (2001) *Handbook of Pesticide Toxicology,* 2nd edn., San Diego, CA: Academic Press (Set, ISBN 0-124262600; Vol. I, ISBN 0-124262619; Vol. II, ISBN 0-124262627).

In two volumes, this comprehensive and timely compendium of scientific knowledge covers the toxic effects or pesticides in humans and animals. The 1st edition, edited by Hayes WJ and Laws ER, consists of three volumes that were updated and expanded versions of *Toxicology of Pesticides* (1975) and *Pesticides Studied in Man* (1982), both of which are out of print. Includes information on the diagnosis and treatment of pesticide poisonings. A true classic.

Lewis RJ, Sr. (ed.) (2000) *Sax's Dangerous Properties of Industrial Materials*, 10th edn. (three vols. New York: Wiley (ISBN 0-471354074).

This huge compendium provides properties, toxicity data, and regulatory status (United States) for over 20 000 chemical substances. Extensively indexed by CAS registry number and synonyms. Online and CD-ROM versions are available. Lewis has published a more moderately sized book that contains over 5000 selected entries from this text: Lewis RJ Sr. (2002) *Hazardous Chemicals Desk Reference*, 5th edn. (ISBN 0-471441651).

Lewis RA (1998) *Lewis' Dictionary of Toxicology.* Boca Raton, FL: Lewis (ISBN 1-566702232).

This is an extensively cross-referenced text that covers a broad array of terms commonly used in toxicology and related fields.

Mackay D, Wan YS, and Kuo CM *Illustrated Handbook of Physical – Chemical Properties and Environmental Fate for Organic Chemicals*. Boca Raton, FL: CRC Press.

Volume I: *Monoaromatics, Chlorobenzenes and PCBs* (1991) (ISBN 0-873715136).

Volume II: *Polycyclic Aromatics, Dioxins, Dibenzofurans and Phenols* (1992). (ISBN 0-837315837).

Volume III: *Volatile Organic Chemicals* (1993). (ISBN 0-873719735).

Volume IV: *Oxygen, Nitrogen and Sulfur Containing Compounds* (1995) (ISBN 0-1566700353).

Volume V: *Pesticide Chemicals* (1997) (ISBN 1-566702550).

This collection of volumes provides physical – chemical data on compounds likely to impact the

environment. The emphasis is on structure – activity relationships and prediction of environmental chemodynamics. Calculations are included and explained.

Massaro EJ (ed.) (1997) *Handbook of Human Toxicology*. Boca Raton: FL: CRC Press (ISBN 0-84934493X).

This is a highly detailed compendium of information on a few selected topics in human toxicology: metals toxicology; nutrition and toxicology, inhalation toxicology; immunotoxicology; and reproductive and developmental toxicology. Each section includes state-of-the-art methodology, topics of current interest, difficult to locate data, and complete references. An annual serial version, titled *Human Toxicology Handbook*, is available on CD-ROM through CRC Press.

O'Neil MJ *et al*. (eds.) (2001) *Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 11th edn., Rahway, NJ: Merck & Co. (ISBN 0-911910131).

A compendium of quick information that contains a diverse collection of more than 10 000 monographs organized alphabetically. Approximately 4000 monographs cover pharmaceuticals and drugs, 2000 cover naturally occurring substances and plants, 1000 focus on elements and inorganic chemicals, and almost 1000 pertain to compounds of agricultural significance. Includes nomenclature, physical/chemical properties, patents, uses, literature references, structure, toxicity, and related data. Available online on several public vendor systems and on CD-ROM.

Proctor NH and Hughes JP (1996) In: Hathaway GJ (ed.) *Proctor and Hughes' Chemical Hazards of the Workplace*, 4th edn., New York: Van Nostrand Reinhold (ISBN 0-442020503).

This book contains concise summaries of over 600 of the most common substances found in the industrial setting. Includes CAS numbers, chemical formulas, synonyms, physical properties, exposure sources and routes, and signs and symptoms of acute and chronic exposures.

Rom WN (ed.) (1998) *Environmental and Occupational Medicine*, 3rd edn., Philadelphia, PA: Lippincott-Raven (ISBN 0-316755788).

This text covers basic information on occupational medicine and related areas. This text is suitable for occupational health practitioners and is readable enough for practitioners in other medical disciplines.

Rossoff IS (2002) *Encyclopedia of Clinical Toxicology: A Comprehensive Guide and Reference*. Boca Raton, FL: Parthenon Publishing Group (ISBN 1-842141015).

An excellent and comprehensive guide, this text provides concise information on the clinical toxicology of prescription and over-ther-counter (OTC) drugs, chemicals, herbals, plants, fungi, marine life, reptiles and insect venoms, food ingredients, clothing, and environmental toxins. Organized alphabetically, this book features an extensive appendix that cross-references each compound for alternative nomenclature.

Schardein JL (2000) *Chemically Induced Birth Defects*, Drug and Chemical Toxicology Series, 3rd edn., revised and expanded. New York: Dekker (ISBN 0-824702654) (ISBN 0-585383693 electronic book).

This current and readable text is a comprehensive literature review that addresses mammalian (including human) exposure during organogenesis. Also available as an electronic book online.

Shepard TH (2001) *Catalog of Teratogenic Agents*, 10th edn., Baltimore, MD: Johns Hopkins University Press (ISBN 1-801867223).

A standard text listing the teratogenic potential of hundreds of substances; provides indexes, summaries of literature, and references to source materials.

Sipes IG, McQueen CA, and Gandolfi AJ (eds.) (1997–2002) *Comprehensive Toxicology*, 14 vols. New York: Pergamon (ISBN 0-080423019 set) (Vol. 1, ISBN 0-080429661; Vol. 2, ISBN 0-08042967X; Vol. 3, ISBN 0-080429688; Vol. 4, ISBN 0-080429696; Vol. 5, ISBN 0-08042970X; Vol. 6, ISBN 0-080429718; Vol. 7, ISBN 0-080429726; Vol. 8, ISBN 0-080429734; Vol. 9, ISBN 0-080429742; Vol. 10, ISBN 0-080429750; Vol. 11, ISBN 0-080429769; Vol. 12, ISBN 0-080429777; Vol. 13, ISBN 0-080429785; Vol. 14, ISBN 0-444508686).

This encyclopedic work of 14 volumes addresses general toxicology principles, toxicological testing and evaluation, biotransformation, target organ toxicology, as well as behavioral toxicology, chemical carcinogens, and anticarcinogens. Also available on CD-ROM.

Sittig M and Pohanish RP (2002) *Sittig's Handbook of Toxic & Hazardous Chemicals and Carcinogens*, 4th edn., Norwich, NY: Noyes Publications (ISBN 0-81551459X) (ISBN 1-591243262 electronic book).

This easy to use two-volume handbook presents chemical safety and health information on nearly 1500 toxic and hazardous chemicals. The utility of the 4th edition has been enhanced by addition of eight appendices: five cross index chemicals by CAS number, molecular formula, synonyms and trade names, DOT ID, and RTECS; one is a glossary of terms used; one lists oxidant chemicals; and one contains a list of confirmed and suspected carcinogens. References are included. Also available online.

Spencer PS and Schaumburg HH (eds.) (2000) *Experimental and Clinical Neurotoxicology*, 2nd

edn., New York: Oxford University Press (ISBN 0-195084772).

This comprehensive and useful text provides a broad overview of the neurobiological basis of neurotoxic phenomena in human and veterinary medicine, as well as an alphabetical compendium that describes the neurotoxic properties of over 450 chemicals, drugs, and mixtures, including plants and venoms. It replaces the 1980 edition (available online at http://www.ohsu.edu/research/croet/faculty/spencer/book/first_ed.html), which featured micrographs depicting the neuropathology of neurotoxic agents.

Target Organ Toxicology Series. New York/Washington, DC: Raven Press/Taylor and Francis.

Wallace KB (ed.) (1997) *Free Radical Toxicology.*

Dixon RL (ed.) (1995) *Reproductive Toxicology*, 2nd edn.

Dean JH *et al*. (eds.) (1994) *Immunotoxicology and Immunopharmacology*, 2nd edn.

Kimmel CA and Buelke-Sam J (eds.) (1994) *Developmental Toxicology*, 2nd edn.

Kotsonis FN, Mackey M, and Hjelle JJ (eds.) (1994) *Nutritional Toxicology.*

Plaa GL and Hewitt WR (eds.) (1994) *Toxicology of the Liver*, 2nd edn.

Waalkes MP and Ward JM (eds.) (1994) *Carcinogenesis*.

Gardner DE, Crapo JD, and McClellan RO (eds.) (1993) *Toxicology of the Lung*, 2nd edn.

Hook JB and Goldstein RS (eds.) (1993). *Toxicology of the Kidney*, 2nd edn.

Acosta D, Jr. (ed.) (1992) *Cardiovascular Toxicology*, 2nd edn.

Chiou GCY (ed.) (1992) *Ophthalmic Toxicology.*

Tilson HA and Mitchell CL (eds.) (1992) *Neurotoxicology.*

Hayes AW (ed.) (1985) *Toxicology of the Eye, Ear, and Other Special Senses*.

Irons RD (ed.) (1985) *Toxicology of the Blood and Bone Marrow.*

Thomas JA, Korach KS, and McLachlan JA (eds.) (1985) *Endocrine Toxicology.*

Drill VA and Lazar P (eds.) (1984) *Cutaneous Toxicity.*

Schiller CM (ed.) (1984) *Intestinal Toxicology.*

This excellent, though expensive, series of works focuses on target organ toxicity and disease states. Some of the earlier volumes are out of print.

2004 TLVs and BEIs (2004) American Conference of Governmental Industrial Hygienists (ACGIH), Cincinnati, OH (ISBN 1-882417542).

This pocket-sized handbook provides threshold limit values (TLVs) established by the ACGIH as guidelines for good practices. Main sections provide data for chemical and physical agents. Also includes sections that explain background and tests, biological exposure indices, and the role of the organization. A companion work titled *Documentation of the Threshold Limit Values and Biological Exposure Indices* (available in paper and on CD-ROM) provides the data and references used in developing the TLVs and BEIs.

Tomlin C (2000) *The Pesticide Manual: A World Compendium*, 12th edn., Farnham, UK: British Crop Protection Council (ISBN 1-901396126).

Provides chemical, physical, analytical, use, and toxicity data for nearly 1200 pesticides, herbicides, and other agricultural chemicals. Contains *The Agrochemicals Handbook* from the Royal Society of Chemistry. Environmental fate/transport, resistance information, and lists of manufacturers are also included. A companion tool from the Royal Society of Chemistry is the 3rd edition of the *World Directory of Pesticide Control Organizations* (ISBN 0-85404437X), which gives sources of contacts in over 160 organizations worldwide involved in the control of pesticides.

Tu AT *Handbook of Natural Toxins*. New York: Dekker.

Volume I: Keeler RF and Tu AT (eds.) (1983) *Plant and Fungal Toxins* (ISBN 0-824718933).

Volume II: Tu AT (ed.) (1984) *Insect Poisons, Allergens, and Other Invertebrate Venoms* (ISBN 0-824772075).

Volume III: Tu AT (ed.) (1988) *Marine Toxins and Venoms* (ISBN 0-824776674).

Volume IV: Hardegree MC and Tu AT (eds.) (1988) *Bacterial Toxins* (ISBN 0-824778405).

Volume V: Tu AT (ed.) (1991) *Reptile Venoms and Toxins* (ISBN 0-82478376X); electronic web version available (ISBN: 0585357161).

Volume VI: Keeler RF and Tu AT (eds.) (1991) *Toxicology of Plant and Fungal Compounds* (ISBN 0-824783751); electronic web version available (ISBN: 0585360588).

Volume VII: Tu AT (ed.) (1992) *Food Poisoning* (ISBN 0-824786521).

Volume VIII: Moss J (ed.) (1995) *Bacterial Toxins and Virulence Factors in Disease* (ISBN 0-824793811); electronic web version available (ISBN: 0585345031).

This series of publications describes all aspects of toxins and the consequences of exposure to them. It includes descriptions and categorization of toxins, symptomology with exposure, treatment, and prevention of contact. All volumes in this series are still in print or available as electronic versions on the Web.

Verschueren K (2001) *Handbook of Environmental Data on Organic Chemicals*, 4th edn., New

York: Wiley (ISBN 0-471374903) (ISBN 1-59124482X electronic book).

Provides information on the properties, air pollution factors, water pollution factors, and biological effects of thousands of chemicals. All information is referenced, and the introduction contains a mini review of the ecotoxicologic relevance and determination techniques for the data presented in each monograph. Also available by online subscription.

Wexler P (2005) *Encyclopedia of Toxicology*, 2nd edn., San Diego, CA: Academic Press.

This text is a comprehensive collection of concise and readable explanations of basic principles in toxicology and the potential hazards of chemicals. It contains more than 1000 entries, including entries related to research and clinical toxicology, risk assessment, ecotoxicology, epidemiology, radiation, noise, information resources, organizations, and education. As with the Ist edition, this volume is extensively cross-referenced, contains a detailed index, and provides numerous references to primary and secondary literature.

Periodicals

During the past 30 years, there has been a steady increase in the number of journals transmitting information on toxicity, hazard, and risk. As knowledge has grown, more specialized titles have appeared reflecting the expanding literature of narrower disciplines, that is, cellular toxicology, aquatic toxicology, food toxicology, contact dermatitis, risk analysis, drug/nutrition interactions, molecular toxicology, genomics, and target organ/system toxicity. Also, because of the cross-disciplinary nature of toxicological concerns, relevant information appears not only in the primary toxicology journals but also in those of related disciplines – medicine, epidemiology, food, biology, agriculture, and so on. New journal titles will frequently be distributed directly to professionals and announcements will appear in the review sections of professional journals. Additional titles of journals and series publications can be found in *Ulrich's International Periodicals Directory* (Bowker) or *The Serials Directory* (Ebsco). These directories are available in print and on CD-ROM, as well as online through major vendors.

Generally, information published in a research journal is well known in the scientific community long before it appears in print. The research journal is not an effective rapid communication device but one for quality control, claiming of priority, and as a mechanism of archiving research information. Because the potential sources of toxicological information are so widespread, typical hand scanning issues of journals for newly published information can be overwhelming for the investigator. Also overwhelming is the increasing cost of journal subscriptions. The average cost of a journal has increased ~10% each year since 1990, forcing local collections to judiciously examine journal renewals.

The use of tertiary indexing and abstracting sources can provide an effective alternative to the time-consuming scanning of journal issues and limited subscription resources. These services provide regularly updated title, author, and subject access to the contents of thousands of journal titles. Enlisting the power of computerized systems can automate this process. Profiles of user interest areas are applied against large databases of journal references and results delivered to users on a recurring basis. In some services the user not only can browse the source information (and frequently an abstract) of a newly published article but can also request a full text copy of the original. An example of this type of service geared to rapid communication of journal contents is the *Current Contents* service from the Institute for Scientific Information, Inc., available in both print and electronic form.

Increasingly, scientific journals are now providing direct access to full-text articles online, and many libraries are carrying online subscriptions to journals, either exclusively or in addition to print versions. Many of the larger publishers also provide special services to access their online holdings. An example of this type of service is Elsevier's *ScienceDirect* digital library. Moreover, bibliographic databases, such as CAS's *SciFinder* and *SciFinder Scholar*, ISI *Web of Knowledge* and *MEDLINE*, are now providing full-text access to articles retrieved in bibliographic searches. In a few cases (e.g. MEDLINE via OVID Technologies), links to full-text articles are made directly from the References Cited portion of a journal article search.

Listed below is a sampling of the core journal titles in toxicology. They have been organized into simple subject categories. Sample copies of journals are quite easily obtained from publishers. Addresses and phone numbers for these publishers may be found in *Ulrich's* or *The Serials Directory.*

General

Advances in Modern Environmental Toxicology (Princeton Scientific)

Annual Review of Pharmacology and Toxicology (Annual Reviews)

Archives of Toxicology (Springer)

The Banbury Report (Cold Spring Harbor Laboratory Press)

Chemical Research in Toxicology (American Chemical Society)
Critical Reviews in Toxicology (CRC Press)
Drug and Chemical Toxicology (Dekker)
Food and Chemical Toxicology (Pergamon)
Human and Experimental Toxicology (Macmillan)
Journal of Analytical Toxicology (Preston)
Journal of Applied Toxicology (Wiley)
Journal of Biochemical and Molecular Toxicology (Wiley)
Neurotoxicology and Teratology (Elsevier)
Pharmacology and Toxicology (Munksgaard)
Regulatory Toxicology and Pharmacology (Academic Press)
Toxicity Review (HMSO, London)
Toxicologic Pathology (Society of Toxicologic Pathologists)
Toxicological and Environmental Chemistry (Gordon and Breach Science)
Toxicological Sciences (formerly *Fundamental and Applied Toxicology*) (Academic Press)
Toxicology (Elsevier Science)
Toxicology and Applied Pharmacology (Academic Press)
Toxicology and Industrial Health (Princeton Scientific)
Toxicology in vitro (Pergamon)
Toxicology Letters (Elsevier Science)
Toxicology Mechanisms and Methods (Taylor and Francis)
Veterinary and Human Toxicology (American Academy of Veterinary and Comparative Toxicology)

Alternative Toxicology Testing

Alternatives to Animal Testing and Experimentation: AATEX (Japanese Society of Alternatives to Animal Experimentation)
Alternative Methods in Toxicology (Liebert)
Alternatives to Laboratory Animals: *ATLA* (Fund for the Replacement of Animals in Medical Experiments)
ILAR News (Institute of Laboratory Animal Resources)
Toxicology in Vitro (Elsevier)

Cancer and Carcinogenesis

Cancer Epidemiology Biomarkers & Prevention (American Association for Cancer Research)
Cancer Research (HighWire Press)
Carcinogenesis: A Comprehensive Survey (Raven Press)

Clinical

Adverse Drug Reactions and Toxicological Reviews (Oxford University Press)
Clinically Important Adverse Drug Interactions (Elsevier Science)
Drug and Chemical Toxicology (Dekker)
Emergency Medical Clinics of North America (WB Saunders)
Emergency Medicine (Cahners Publishing)
Human Toxicology (Macmillan)
Journal of the Association of Food and Drug Officials (Association of Food and Drug Officials)
Journal of Toxicology. Clinical Toxicology (Dekker)
Reactions Weekly (ADIS International)
Toxicon (Pergamon)

Environmental

Ambio (Royal Swedish Academy of Sciences)
Aquatic Toxicology (Elsevier)
Archives of Environmental Contamination and Toxicology (Springer)
Archives of Environmental Health (Heldref)
Bulletin of Environmental Contamination and Toxicology (Springer)
Chemosphere (Pergamon)
Developments in Toxicology and Environmental Science (Elsevier Science)
Ecotoxicity and Environmental Safety (Academic Press)
Environmental Health Perspectives (NIEHS)
Environmental and Molecular Mutagenesis (Wiley)
Environmental Science Research (Plenum)
Environmental Toxicology and Chemistry (Pergamon)
International Journal of Environmental Analytical Chemistry (Gordon and Breach Science)
Journal of Environmental Health (National Environmental Health Association)
Journal of Environmental Pathology, Toxicology, and Oncology (Begell House)
Journal of Environmental Science and Health (Dekker)
Journal of Hazardous Materials (Elsevier Science)
Journal of the Air and Waste Management Association (AWMA)
Journal of Toxicology and Environmental Health (Taylor and Francis)
Pesticide and Toxic Chemical News (Food Chemical News)
Reviews of Environmental Contamination and Toxicology (Springer)
Toxicological and Environmental Chemistry (Gordon and Breach Science)

Genomics, Proteomics, and Bioinformatics

American Journal of Pharmacogenomics (Adis)
Bioinformatics (Oxford)
Environmental Health Perspectives – Toxicogenomics (NIEHS)
Genomics (Academic Press)
Journal of Proteome Research (ACS Pubs)
Molecular Genetics and Genomics (Springer)
Pharmacogenomics Journal (Nature Publishing Group)
Pharmacogenetics (Williams and Wilkins)

Mutagenesis

Chemical Mutagens: Principles and Methods for Their Detection (Plenum)
Environmental and Molecular Mutagenesis (Wiley)
Mutagenesis (IRL Press)
Mutation Research (Elsevier Science)

Occupational and Industrial

American Industrial Hygiene Association Journal (American Industrial Hygiene Association)
American Journal of Industrial Medicine (Wiley-Liss)
International Archives of Occupational and Environmental Health (Springer)
Journal of Occupational Medicine (Williams and Wilkins)
Occupational and Environmental Medicine (BMJ)

Radiation

Advances in Radiation Biology (Academic Press)
Annals of the ICRP (Pergamon)
International Journal of Radiation Oncology Biology Physics (Elsevier)
Radiation Research (Academic Press)

Reproduction and Teratology

Advances in the Study of Birth Defects (University Park Press)
Birth Defects Research (Wiley-Liss)
Issues and Reviews in Teratology (Plenum)
Neurotoxicology and Teratology (Pergamon)
Teratology (Wiley)
Reproductive Toxicology (Pergamon)

Series

A type of publication related to the periodical is the report series. While many of the journals listed previously provide monographic reviews (i.e., *Advances in Modern Environmental Toxicity*, *CRC Review Series*, *Methods in Toxicology*, and *Reviews of Environmental Contamination and Toxicology*) the report type of publication issues from government agencies, scientific organizations, or research institutes. They vary considerably in scope and purpose but typically provide excellent summary information compiled by panels of experts. Many of these series are irregularly produced but issue consecutively within a volume/issue framework. Identification of series entries may be found by consulting catalogs such as the National Library of Medicine's *LOCATORplus*. *LOCATORplus*, which replaces NLM's previous online catalogs (*CATLINE, SERLINE, AVLINE*, and *Locator*), is available from the NLM homepage at http://www.nlm.nih.gov. Another useful source is the *OCLC Online Union Catalog,* which contains entries for materials contained in thousands of libraries in North America and the United Kingdom.

Many government agencies are now offering technical reports, journals, newsletters and other series for free online, and in full-text format. Examples include resources from the Agency for Toxic Substances Disease Registry (ATSDR) and Centers for Disease Control and Prevention (CDC). A brief, very select list of major sources of these series and their producers include:

Agency for Toxic Substances and Disease Registry, US Government (ATSDR)
1600 Clinton Road
Atlanta, GA 30333
Tel.: +1-404-498-0110
+1-888-42-ATSDR (Toll-free)
URL: http://www.atsdr.cdc.gov
ATSDR Toxicological Profiles

US Centers for Disease Control and Prevention (CDC)
1600 Clifton Road
Atlanta, GA 30333
Tel.: +1-404-639-3311
+1-800-311-3435 (Toll-free)
URL: http://www.cdc.gov
Morbidity and Mortality Weekly Report and many others

European Commission
Headquarters
200 rue de la Loi
B-1049 Brussels
Belgium
Tel.: +32 2 299 11 11
URL: http://europa.eu.int
EUR Report Series, Reports of the Scientific Committee on Cosmetology, Reports of the Scientific Committee for Food

Food and Agriculture Organization of the United Nations (FAO)
Publishing Management Service
Viale delle Terme di Caracalla

00100 Rome
Italy
URL: http://www.fao.org
Email: Publications-Sales@fao.org
(series include those of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)) *FAO Food and Nutrition Papers, FAO Plant Production and Protection Papers, JECFA Monographs on Toxicological Evaluation of Food Additives, Reports of the FAO Panel of Experts on Pesticide Residues in Food and the Environment*

International Agency for Research on Cancer (IARC)
(part of the World Health Organization)
150 Cours Albert Thomas
69372 Lyon Cedex 08
France
Tel.: +33 (0)4 72 73 84 85
URL: http://www.iarc.fr
IARC Monographs (and supplements) *on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Scientific Publications*

British Industrial Biological Research Association (BIBRA)
Woodmansterne Road
Carshalton, Surrey SM5 4DS
United Kingdom
Tel.: +44 (0)20 8652 1024
URL: http://www.bibra.co.uk/
Email: help@bibra.co.uk
Toxicity Profiles

Environmental Protection Agency, US Government (EPA)
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460
Tel.: +1-202-272-0167
URL: http://www.epa.gov/
Too numerous to list – see EPA's 'Information Sources' website, http://www.epa.gov/epahome/resource.htm

World Health Organization
Avenue Appia 20
CH-1211 Geneva 27
Switzerland
Tel.: +41-22-791-2111
URL: http://www.who.int/en/
Email: inf@who.int
WHO Technical Report Series, Environmental Health Criteria, Health Aspects of Chemical Safety, Food Additives Series, Weekly Epidemiological Record, and others

National Academy of Sciences/National Research Council (NRC)
500 Fifth Street N.W.
Washington, DC 20001
Tel.: +1-202-334-2000
URL: http://www.nas.edu
Medical and Biologic Effects of Environmental Pollutants, Biologic Markers, Drinking Water and Health

National Center for Toxicological Research (NCTR)
3900 NCTR Road
Jefferson, AR 72079
Tel.: +1-870-543-7130
URL: http://www.fda.gov/nctr/
NCTR Quarter, FDA Consumer

National Institute of Environmental Health Sciences (NIEHS)
P.O. Box 12233
Research Triangle Park, NC 27709
Tel.: +1-919-541-3345
URL: http://www.niehs.nih.gov
Email: webcenter@niehs.nih.gov
Environmental Health Perspectives

Newsletters

Newsletters can provide timely reporting of 'hot' information – research findings, regulatory updating, society/organization news – all in brief reports. Most professional organizations support some kind of newsletter to communicate to its membership, but the ones of most interest here focus on current news used to inform and mobilize. Some government and commercial titles in this area include:

BNA Toxics Law Reporter/Daily (Bureau of National Affairs)
Environmental Reporter (Bureau of National Affairs)
Environmental Toxicology Newsletter (UC Davis)
Hazardous Substances and Public Health (ATSDR)
Occupational Safety and Health Reporter/Daily (Bureau of National Affairs)
Pollution Prevention News (P2 News2!, US EPA)
Toxic Materials News (Business Publishers)

Electronic access to newsletters is provided via a number of services. These services vary in scope and how often they are updated. Examples include the *PTS Promt Newsletter* database (IAC) with a focus on business and industry and the *Newsletters in Print* database (Gale Research) available in print and online via several vendor systems. The *McGraw-Hill Publications Online* database carries approximately 60 leading publications on-line in full-text form, many of them newsletters and bulletins.

The need for extremely rapid reporting of information can be filled by specialized, and expensive, electronic 'clipping services' that offer daily feeds of news items from dozens of newspapers, newsletters,

newswires, and other types of information resources directly to the user's workstation or by fax. Examples of these are *NewsDesk* from PR Newswire, *Dow Jones Interactive*, *NewsEdge* from The Thomson Corp., and *AccuClip Express* from CyberAlert, Inc. The Internet has become a very popular mechanism for disseminating timely information via newsgroups and mailing lists (listservs). At least in the United States, an increasing number of associations and interest groups are communicating directly through use of the Internet. Government agencies, commercial producers, and research organizations are using this network as well. It is estimated that more than 18 000 newsgroups and hundreds of thousands of electronic newsletters are available over the Internet, and this figure is growing daily. One such recent effort of interest to the toxicology community is *P2 News2!*, a monthly newsletter that covers pollution prevention activities at the EPA, produced and distributed over the Internet by the Office of Pollution Prevention and Toxics of the US EPA. The US FDA also supports a publicly available server on the Internet covering staff activities, recent regulatory actions, and administrative news. The National Institute of Standards and Technology at the US Department of Commerce sponsors the *Fedworld* system, which provides access to federal government bulletin boards, government files, newsletters, and links to other government computerized systems.

Computerized Information

In the mid-1960s, the first publicly available systems for accessing machine-readable databases appeared. With the explosion of information in the sciences, coupled with the vast number of resources (journals, reports, newspapers, and monographs) in which this information was appearing, the use of computers to store and retrieve information has become an effective means to handle the increasing flow. As a result, computer use has increased exponentially, as evidenced by the growth in number of host computers that store data for use on the Internet. In 1969, four computers held all the information available to users of the nascent 'World Wide Web', whereas today it is estimated that over 132 million host computers now serve in this capacity.

Information files can be accessed through communication with host computers, where data are stored by producers or vendors, or by loading them locally, such as through CD-ROM and DVD storage. No matter what medium is chosen, it is important to recognize the disadvantages of each route: Online may mean subscriber and use charges, but it is convenient and universally available via telecommunications systems; CD-ROM and DVD storage can be very cost-effective for unlimited use, but production invariably necessitates a delay in currency over online. The information science literature contains several articles providing criteria, and checklists help in determining the most appropriate medium for each situation. Transparent bridges between online and CD, rewriteable CD/DVD technology, easier to use interfaces, mass market media platforms, and other developments are increasing the investigator's ability to locate, identify, and retrieve useful information.

Besides the development of data and text information systems, software programs are being designed to provide assistance in predicting the toxicity of substances based on structure – activity relationships, such as the TOPKAT program by Health Designs, Inc., or by an expert system, such as the CASE system by Case Western Reserve University. Computer-assisted instruction programs, incorporating text, audio, graphic, and full-motion video technologies, are an exciting use of computer-assisted instruction. Examples of this last type of CD-ROM product include the environmental health, industrial hygiene, and occupational health and safety titles of the ACTIV Series from ITC (Herndon, VA) and the hazardous materials series training program from Interactive Media Communications (Waltham, MA).

Directories of information on computerized sources, such as the *Gale Directory of Databases* (Gale Research Inc.) and the Mekler directory *CD-ROMs in Print,* give identification, coverage, content, and availability information for publicly available electronic files, as do many of the literature guides listed in the introduction to this entry. An example of a resource tool more limited in scope is the *Environmental Software Directory* (Donley Technologies). Articles and columns appearing in the information science literature (*Online, Searcher, Database, CD-ROM Professional*, and *Information Today*) can provide excellent reviews of new files and comparative studies on resources in specific subject areas.

As the availability of new information technologies has grown, so has the promise of free and equitable access to sources of data and information. Many schools as well as municipal and university libraries now offer free access to computers that are connected to the Internet, and governmental bibliographic and nonbibliographic databases, such as the suite of resources from the National Library of Medicine, are available free online. In addition to these national databases (which are listed later in this entry), a variety of regional, state, tribal, county, and municipal governmental and private sector toxicology information resources have become available on line. These sub-national resources often provide environmental

quality and public/environmental health-related information (or links to information) on subjects such as lead poisoning prevention, fish and wildlife consumption advisories, asthma, air and water pollution and other information that is specific to localized geographic areas. The following are some examples of region-specific online toxicology information sources or guides to available resources (A more comprehensive list of subnational sources of toxicology information can be found in Stoss FW (2001) *Toxicology* **157**:51 – 65.)

Federal and private sector regional resources:

US EPA regional directory (www.epa.gov/epahome/locate2.htm)

EnviroMapper (http://www.epa.gov/enviro/html/em/)

SurfYourWatershed (http://www.epa.gov/surf)

EnviroFacts (http://www.epa.gov/enviro/)

Environmental Defense Scorecard (http://www.scorecard.org/)

Mapcruzin (http://www.mapcruzin.com/)

State, county, and local resources:

Environmental Health and Safety Online (http://www.ehso.com/)

Environmental Organization Web Directory (http://www.webdirectory.com/government/states/)

Library of Congress State and Local Government Directory (http://www.loc.gov/global/state/stategov.html)

GovLinks' (http://governing.com/govlinks/glinks.htm)

Local and regional topic-specific sites:

Lead poisoning prevention (http://www.cdc.gov/nceh/lead/lead.htm)

Cancer registries (http://www.cdc.gov/cancer/npcr/)

Radon, indoor air quality (http://www.ehso.com/ehshome/radon.htm)

Toxic chemical release inventories by state (http://www.epa.gov/tri/statecon.htm)

Emergency planning-state and local (http://rtk.net/lepc/webpage/hotlinks.html and http://www.fema.-gov/library/diz02.shtm)

Poison control centers (http://www.aapcc.org/findyour.htm)

Brownfield programs (http://www.epa.gov/swerosps/bf/glossary.htm#brow)

Fish consumption advisories (http://www.epa.gov/ost/fish/)

Occupational safety and health (http://www.croetweb.com and http://www.osha-slc.gov/fso/osp/)

Selection and Use of Electronic Sources

It is estimated that in 2001 almost 100 million people nationwide, or 75% of adults in the United States, looked for health-related (including toxicological) information online. But anyone can put information on the web. There is no governing body, and information is not screened or standardized in any way to verify its accuracy or usefulness. With the unregulated flow of new sources on the Internet, as well as the thousands of databases available through public and subscription vendors, it becomes increasingly important to evaluate each resource as regards quality of information content. The following are criteria that one can use to determine the value of a resource:

Author Is it clear who writes or is responsible for the material on the site?

Are the author's credentials provided?

Is there a sponsoring institution and, if so, how credible and well known is it?

Is a third party supporting or sponsoring the site?

Is contact information given for the author or sponsoring institution?

Purpose of the Site Is the purpose or mission of the website or sponsoring organization stated?

Is the purpose to inform, persuade, sell, present a viewpoint, or create or change an attitude or belief?

Is there advertising on the site and is it clearly differentiated from the informational content?

Date Is it clear when the site was last updated?

Content Does the site exhibit good grammar, spelling and literary composition?

Does the information consist of documented facts or personal opinion?

Are the sources of factual information provided so they can be verified?

Is there comprehensive coverage of the subject matter?

Are there external links to other sources of information?

Does an editorial board or healthcare professional review the content?

What criteria are used for selecting information displayed on the site?

Using these criteria, there can be a variety of reasons to be skeptical about information that is presented on a website. Lack of authorship or date, vague or sweeping generalizations, overstated significance and extreme tone or language – all mark the information presented as suspect. The credibility of information is also diminished by the absence of source documentation, personal testimonials as the only information sources, and purported 'miracle cures' recommended in lieu of well-established scientific or medical protocols.

One other clue that can provide some information about the source of a website is its uniform resource locator (URL), or web address. Education-related URLs end in '.edu', whereas commercial sites usually contain '.com' in the URL. The URLs of nonprofit organizations usually end in '.org', and government and military organizations end with '.gov' and '.mil', respectively. Despite these guidelines, it is important to note that anyone can register a .com, .org or .net website for any use (including personal).

The following web resources provide good tips for evaluating or presenting information on the web:

Healthfinder: Your Guide to Reliable Health Information (US Federal Govt.)

http://www.healthfinder.gov/(English version)

http://www.healthfinder.gov/espanol (Spanish version)

Usability.gov: National Cancer Institute site for improving the communication of cancer research

http://usability.gov

Tips for Searching the Web

Use More Than One Search Engine There are advantages and disadvantages associated with the method a given search engine uses to locate information. Search engines can be classified into three types: Web Crawlers (or Web Spiders) automatically collect and catalog web pages by looking at the full text on a page, collecting all relevant information, and then following links on the page to locate other relevant sites. Examples include Google (www.google.com) and AllTheWeb (www.alltheweb.com). Web crawlers can potentially access a large portion of the Internet. Directories are search engines that contain only information that web managers have submitted. A website will not appear in a directory unless the web manager has submitted its URL under the most appropriate heading. Examples of directories include Yahoo (www.yahoo.com), MSN Search (www.msnsearch.com) and Open Directory (www.dmoz.org). Meta-search engines automatically submit a keyword search to several other search tools and retrieve results from all their databases. They are convenient time-savers for relatively simple keyword searches of one or two words or phrases in quotation marks. Examples include Profusion (www.profusion.com) and Dogpile (www.dogpile.com).

Use Lower Case Letters Most search tools are not case sensitive or only respond to initial capitals, as in proper names. Thus, it is safe to use all lower case letters, inasmuch as lower case always retrieves upper case letters.

Use Tools Offered by the Search Engine This includes using the Help button. Also, it is helpful to try the 'more like this', 'related searches', or 'narrow your search' options offered by some search engines. Advanced search options often help to eliminate sites that are unlikely to provide the desired information. For example, the advanced search option on Google allows selection or exclusion of specific types of URL, such as '.com' sites.

Use Quotation Marks Around a Search Phrase Without quotation marks, search engines return pages that contain the search terms somewhere in the page, but not necessarily near each other. With quotation marks, most search engines will only provide results in the exact order and way you specify them.

Use Search Engine Math (+/−) Search engine math can increase the specificity of a search, and works for nearly all major search engines. It allows the inclusion or exclusion of documents containing certain words through the use of the + and − symbols. For example, to find information about radiation but not nuclear radiation, type '+radiation −nuclear'.

More information about search engines and searching tips can be found at Search Engine Watch: www.searchenginewatch.com (click on 'Web Searching Tips')

The following is an illustrative, selected list of electronic files containing publicly available toxicology information. Represented here are databases which cover toxicology under a larger discipline – medicine, biology, and chemistry; those devoted to specific areas of toxicological concerns – occupational health, reproductive toxicology, environmental health, hazard assessment; industry-specific files such as those for specific industries – petroleum, nuclear power, agricultural, and engineering; and those that cover specific types of data – regulations, legislation, newsletter, industry or technical reports, and books. In many cases electronic databases are the counterparts to printed indexing, abstracting, or full-text source materials. Increasingly, electronic files are being developed that have no print counterparts, such as the *Hazardous Substances Databank*. In this list, the host system(s) is noted for each file, as is the availability of tape or CD-ROM formats. Many of these databases may also be available through the Internet:

Aquatic Information Retrieval (AQUIRE):

Contains over 220 000 records, which cover acute, chronic, bioaccumulative, and sublethal effects data from experimental assays performed with more than 7000 chemicals on 3700 fresh and saltwater aquatic

species. Produced by the US EPA, ECOTOX Support, Mid-Continent Ecology Division, Duluth, MN (STN, CIS; online at EPA: http://www.epa.gov/ecotox)

Agricultural OnLine Access (Agricola):

This comprehensive bibliographic database contains more than 3.7 million literature citations for journal articles, monographs, proceedings, theses, patents, translations, audiovisual materials, computer software, and technical reports pertaining to all aspects of agriculture. Produced by the US Department of Agriculture (USDA) National Agricultural Library. (OVID, DIALOG, STN; on CD-ROM from Silver Platter; online at http://agricola.nal.usda.gov/)

BioBusiness:

Contains over one million records covering the worldwide periodical literature on business applications of biological and biomedical research. Includes occupational health, biotechnology, bioremediation, pesticides, toxicology, and energy as well as chemical names and CAS Registry numbers. Produced by Biological Abstracts, Inc. (BIOSIS). (Data-Star, DIALOG, STN)

BIOSIS Previews:

Contains more than 14 million citations to the worldwide literature in the life sciences, environmental sciences, and experimental medicine dating back to 1969. One of the largest of the science databases. Produced by BIOSIS. Selected citations contained in a subset of TOXLINE. (Data-Star, CISTI, DIALOG, DIMDI, OVID, STN)

CA File (CAS):

The CA File is a bibliographic database available from CAS (Chemical Abstracts Service). It contains more than 22 million references to the worldwide chemistry literature, including journals, patents, patent families, technical reports, books, conference proceedings, and dissertations from all areas of chemistry, biochemistry, chemical engineering, and related sciences from 1907 to the present. There are also over 600 records for journal articles dated before 1907. A companion Registry File holds records for all the substances cited in the CAS Registry System. The CAS registry number is a widely used identifier for unique chemical substances. (CAN/OLE, Data-Star, DIALOG, ORBIT; collective indexes available from Silver Platter).

CAB Abstracts (CABA):

Contains more than four million records to worldwide literature in the agricultural sciences and related areas of applied biology since 1973. The records in this file contain bibliographic information, abstracts and indexing information, including CAS Registry Numbers®, from the 46 journals published by CAB International, its producer. (CAN/OLE, DIALOG, DIMDI, OVID, STN)

CERCLIS Database of Hazardous Waste:

Contains information on each hazardous waste disposal or spill site nominated or selected for the EPA National Priorities List for cleanup under Superfund (CERCLA) or SARA amendments. Over 44 000 to date. (CIS, WESTLAW)

Chemical Carcinogenesis Research Information System (CCRIS):

This is a scientifically evaluated and fully referenced data bank, developed and maintained by the National Cancer Institute (NCI). It contains over 8000 chemical records with carcinogenicity, mutagenicity, tumor promotion, and tumor inhibition test results. Data are derived from studies cited in primary journals, current awareness tools, NCI reports, and other special sources. Test results have been reviewed by experts in carcinogenesis and mutagenesis. (CIS, DIMDI, TOXNET)

Chemical Evaluation Search and Retrieval System (CESARS):

This database contains toxicological data on over 850 chemicals of particular interest to the United States Great Lakes basin. Each record provides descriptive data on up to 23 topic areas, including physical and chemical properties, toxicity, and environmental fate. Produced by the Michigan Department of Natural Resources and the Ontario Ministry of the Environment. Fully evaluated and referenced data. (CCINFOline; contained in CCINFOdisc; CHEMpendium series from CCOHS)

Chemical Safety Newsbase (CSNB):

Contains over 67 000 records of information on occupational hazards in the chemical industry – identification, storage, handling, transportation, emergency planning, regulations and legislation, standards and practices, and waste management. Only new information on well-known hazards is included. Produced by the Royal Society of Chemistry. (Data-Star, DIALOG, STN, ORBIT/QUESTEL, Data-Star)

ChemIDplus:

This is a free, Web-based search system that provides access to structure and nomenclature authority files used for the identification of chemical substances cited in National Library of Medicine (NLM) databases. It also provides structure searching and direct links to many biomedical resources at NLM and on the Internet for chemicals of interest. The database contains over 367 000 chemical records, of which over 142 000 include chemical structures, and is searchable by Name, Synonym, CAS Registry Number, Molecular Formula, Classification Code, Locator Code, and Structure. (online from NLM at http://chem.sis.nlm.nig.gov/chemidplus).

CHEMLIST (Regulated Chemicals Listing):

Contains information on over 230 000 chemicals subject to legislative and regulatory control. Covers

all US EPA TSCA (Toxic Substances Control Act) and EINECS (European Inventory of Existing Commercial Chemical Substances). Provides citations to regulations, ITC recommendations, rule violations, safety, and health studies, superfund actions, and citizen's petitions. Produced by the CAS. (STN, STN Easy, SciFinder)

DATALOG:

DATALOG was developed through the collaborative efforts of EPA's Office of Toxic Substances and the Syracuse Research Corporation (SRC). It includes bibliographic references to published journal articles on the environmental fate and physical–chemical properties of chemicals released into the environment. References to 18 environmental fate properties (e.g., water solubility, photolysis, hydrolysis, biodegradation, and more) are included for more than 16 000 chemical substances in over 320 000 records. (CIS).

Developmental and Reproductive Toxicology Database/Environmental Teratology Information Center Backfile (DART/ETICBACK):

Together, these databases contain over 75 000 references to the worldwide literature of teratology and some coverage of developmental and reproductive toxicology. DART covers 1989 to the present and ETICBACK covers 1950–1989. Over half the references are scanned into DART from MEDLINE and it includes coverage of technical reports and conference papers. Produced by the National Library of Medicine (both available through TOXNET).

DERWENT Drug File (RINGDOC):

Contains over 1.5 million citations dating from 1964 to the present with lengthy, quantitative abstracts to the worldwide journal literature of drugs and pharmaceuticals. Ability to search by structure–activity is unique to Derwent files. Covers fewer source journals than MEDLINE or EMBASE (1150) but provides more in-depth on-line information through indexing and abstracts. Other Derwent files include Derwent Veterinary Drug File (VETDOC) and Derwent Crop Protection File (PESTDOC) providing similar in-depth analysis of the literature in these areas. The Derwent Drug Registry is the companion nomenclature file giving names, therapeutic class, and structures for chemical compounds. Formerly a subscription service, selected files are now publicly available. (Data-Star, DIALOG, OVID, STN)

ECOTOX:

Provides chemical toxicity information for aquatic life, terrestrial plants, and wildlife. Information is derived from peer-reviewed literature sources. ECOTOX was created and is maintained by the US EPA, Office of Research and Development (ORD), and the National Health and Environmental Effects Research Laboratory's (NHEERL's) Mid-Continent Ecology Division. (available on the EPA website: http://www.epa.gov/ecotox).

EMBASE:

Contains more than 15 million records covering the world's biomedical literature related to human health and medicine. Environmental pollution and health, occupational health, and clinical toxicology are strong areas. Corresponds in part to 46 specialty abstract journals and two literature indexes produced under the *Excerpta Medica* specialty series titles by Elsevier Science. (CDP, Data-Star, DIALOG, DIMDI, OVID, STN; entire database and sections in composite discs available from Silver Platter and DIALOG; online at http://www.embase.com).

Enviroline:

This bibliographic database provides indexing and abstracting coverage of more than 1000 international primary and secondary publications reporting on all aspects of the environment. These publications highlight such fields as management, technology, planning, law, political science, economics, geology, biology, and chemistry as they relate to environmental issues. Enviroline corresponds to the print *Environment Abstracts* and contains over 300 000 records. Produced by the Congressional Information Service (DIALOG, DIMDI; available on CD through Bowker).

ENVIROFATE – Environmental Fate Database

Provides access to information on the environmental fate or behavior of chemicals released into the environment. Data on environmental transformation rates and on physical–chemical properties are included. Records are drawn from papers published around the world involving chemicals that are produced in excess of one million pounds annually. Over 15 400 records cover 1833 different chemicals, predominantly organic compounds. Developed through the collaborative efforts of EPA's Office of Toxic Substances and the Syracuse Research Corporation (SRC) (CIS).

Environmental Mutagen Information Center Data Base (EMIC/EMICBACK):

This database is no longer being updated, but does provide access to the bibliographic information of chemical, biological, and physical agents which have been tested for genotoxic activity, most of which were published since 1950. Contains bibliographic details and keywords of chemicals tested, CAS Registry Numbers, organisms studied, and assay systems used. Users can search by CAS Registry Number, subject terms, title words, and author. Produced by the US Oak Ridge National Laboratory. (CIS, TOXNET; available on CD as a subfile in PolTox I and Toxline, both by Silver Platter).

Food Science and Technology Abstracts:

Covers the worldwide literature of food science and technology. Includes information on occupational toxicology in the food handling and processing areas, toxicology of foods and packaging, and additives information. Produced by the International Food Information Service (IFIS). (Data-Star, DIALOG, DIMDI, ORBIT, STN; available on CD only or with several combined files from a number of producers).

GENE-TOX (Genetic Toxicology):

This database is no longer being updated. Assembled by expert panels at the US EPA Office of Toxic Substances with the cooperation of the NIEHS and the EMIC program at Oak Ridge National Laboratories, this file provides mutagenicity data on over 3200 chemicals from 39 assays systems. The GeneTox program was established to select assay systems for evaluation, review data in the scientific literature, and recommend proper testing protocols and evaluation procedures for these systems (CIS, TOXNET).

Hazardous Substances Databank (HSDB):

This huge data file covers over 4500 potentially hazardous chemical substances. It contains information on human exposure, industrial hygiene, emergency-handling procedures, environmental fate, regulatory requirements, and related areas. All data are referenced and derived from a core set of books, government documents, technical reports and selected primary journal literature. HSDB is peer-reviewed by the Scientific Review Panel (SRP), a committee of experts in the major subject areas within the data bank's scope. (Data-Star, DIMDI, STN Easy, TOXNET; available as part of TOMES PLUS by Micromedex).

Household Products Database:

This database links over 4000 consumer brands to health effects from Material Safety Data Sheets (MSDS) provided by the manufacturers. Information on specific products includes chemical ingredients and their percentages, which products contain specific chemical ingredients, manufacturers of specific brands and their contact information, and acute and chronic health effects information. Available from the National Library of Medicine at http://householdproducts.nlm.nih.gov

HSELINE:

Contains over 180 000 citations to the worldwide literature on occupational health and safety. Includes physical, chemical, and medical hazards. Covers all UK Health and Safety Commission and Health and Safety Executive publications and a wide range of journals, conference papers, reports, and legislation (United Kingdom). Produced by the Health and Safety Executive, United Kingdom (Data-Star, ORBIT; available on CD from Silver Platter; available on CD as part of several products).

International Pharmaceutical Abstracts (IPA):

Contains citations to the worldwide pharmaceutical and pharmacy literature from 1970 to the present, including drug therapy, toxicity, and pharmacy practice as well as legislation, regulation, technology, utilization, biopharmaceutics, information processing, education, economics, ethics and other topics. Covers more than 800 journal sources. Produced by the American Society of Hospital Pharmacists (Data-Star, DIALOG, DIMDI, OVID, STN; available on CD-ROM from Silver Platter).

Integrated Risk Information System (IRIS):

Contains information on hazard identification and dose – response assessment of over 600 hazardous substances. Covers toxicity, carcinogenicity, chemical and physical properties, and applicable regulations. Includes the reference dose as defined by US EPA, unit risk of exposure by oral and inhalation routes. Produced by the US EPA. (CIS, TOXNET; available on CD as part of TOMES Plus by Micromedex and on the EPA Internet website).

International Toxicity Estimates for Risk (ITER):

ITER is a free Internet database of human health risk values for over 600 chemicals of environmental concern from several organizations worldwide. The data are presented in table format with side-by-side comparisons of risk values from the various organizations, below which are synopses that provide explanations for differences among risk values as well as links to more detailed information. ITER currently contains data from the Agency for Toxic Substances Disease Registry (ATSDR), Health Canada, National Institute of Public Health and the Environment, The Netherlands (RIVM), US EPA, and independent parties whose risk values have undergone peer review. Produced by Toxicology Excellence for Risk Assessment (TERA). (TERA, http://www.tera.org/iter; TOXNET, http://toxnet.nlm.nih.gov).

Martindale Online:

This is a full-text electronic version of this standard directory of pharmaceuticals and ancillary substances (Martindale: The Extra Pharmacopoea). Contains reviews, physical/chemical properties, adverse reactions, toxicity, uses, actions, dosages, pharmaceutical properties, contraindications, interactions, and trade and generic nomenclature. Produced by the Royal Pharmaceutical Society of Great Britain. (Data-Star; available on CD-ROM from RPSGB.)

MEDLINE (Medical Literature, Analysis, and Retrieval System Online):

This is one of the largest and most popular international biomedical databases in the world. Strengths include coverage of pre-clinical and clinical aspects of

biomedicine, drug and pharmaceuticals, human and veterinary toxicology, and the practice of medicine. It contains over 12 million references to the journal literature from 1966 to present. Produced by the US National Library of Medicine (CIS, Data-Star, DIALOG, DIMDI, EPIC, OVID, STN; available on CD from a number of producers).

MSDS-CCOHS:

This database contains the complete text of over 130,000 Material Safety Data Sheets compiled by the Canadian Center for Occupational Health and Safety (CCOHS). This information was gathered from over 500 manufacturers and suppliers in the United States and Canada. Each record covers one chemical substance and provides trade and supplier name, description, chemical/physical properties, reactivity, health hazards, storage and disposal, personal protection, cleanup and disposal, and emergency first aid (CCINFOLINE, STN; available on CD from CCOHS).

MSDS-OHS:

This collection contains full text Material Safety Data Sheets, Summary Sheets, and Label Data for more than 59 000 substances, including pure substances and mixtures, 92–96% of which are the most heavily used chemicals in industry. The database originated with Occupational Health Services, Inc. (OHS). The records include occupational, environmental, and regulatory data, as well as names, CAS Registry Numbers, and regulatory list numbers. The OHS online system provides a full file, a summary information file, and a file composed of records to chemicals used in the manufacture of pesticide and other agricultural chemical products (OHS, STN; available on CD from OHS).

NIOSH Technical Information Center (NIOSHTIC):

This bibliographic database contains more than 200 000 references to the literature of occupational health and safety from monographs, journals, and reports. Produced by the US National Institute for Occupational Safety and Health (NIOSH). As of 1997, NIOSH will only add NIOSH publications and articles by NIOSH authors to the database (CCINFOLINE, CIS, DIALOG, ORBIT/QUESTEL, STN, TOXLINE).

Pollution Abstracts:

Contains references to worldwide technical and nontechnical literature on all aspects of pollution, solid waste management, and environmental quality. Covers journals, books, technical reports, conference papers, and government documents. Produced by Cambridge Scientific Abstracts (STN; CD-ROM available from Silver Platter and National Information Services Corporation).

Registry of Toxic Effects of Chemical Substances (RTECS):

Contains toxic effects data (with citations) on over 157 000 chemicals identified by NIOSH and mandated by the Occupational Safety and Health Act of 1970. Acute and chronic effects, selected regulatory information, IARC reviews, TSCA status, GENETOX data, and NTP documents cited. Data selectively included, not comprehensive. This database is compiled, maintained, and updated by MDL Information Systems, Inc., under the authority of the US government (CIS, Data-Star, DIALOG, DIMDI, STN; CD version contained in CCINFOdisc: Core Series C2 from CCOHS and CHEMBANK from Silver Platter).

REPROTOX:

Provides reviewed and summarized information on the reproductive risk of hundreds of chemical substances. Includes coverage of industrial and environmental chemicals, drugs, nutritional agents, and radiation. Effects noted on fertility (male and female), pregnancy, development, and lactation. Produced by the Reproductive Toxicology Center (RTC) of the Columbia Hospital for Women, Washington, DC (available by direct access to the RTC).

SEDBASE:

This database contains current, full-text information from the last 12 years of *Meyler's Side Effects of Drugs*, published every 4 years; *Side Effects of Drugs Annual*, published every year in between; and *Marler's Pharmacological & Chemical Synonyms*. It also contains citations and abstracts from EMBASE (Files 72, 73) that have been referenced by the Meyler's texts. Produced by Elsevier Science (Data-Star, DIALOG, DIMDI; available on CD-ROM from Elsevier).

Toxic Substances Control Act Test Submissions (TSCATS):

Provides over 64 000 citations to unpublished health and safety studies, chemical tests, and substantial risk data on over 8400 chemical substances submitted to the US EPA under the Toxic Substances Control Act (TSCA). Copies of the original submissions are available from the US EPA (CIS, subset of TOXLINE; available on CD-ROM in PolTox I and Toxline from Silver Platter).

Toxics Release Inventory (TRI):

Consists of more than 1 150 000 records containing information on the annual estimated releases of toxic chemicals to the environment. This information is gathered by the US EPA through manufacturers/importers/users of chemicals under the provisions of the SARA amendments of CERCLA. Records contain information on the storage, discharge, waste treatment, and waste transfer of approximately 650 chemicals. RTD Net: The Right to Know Network,

a companion database, contains information from the state of New Jersey's Hazardous Substance Fact sheets collected under New Jersey Right-to-Know Act. This provides information on the safety and ecological effects of most TRI chemicals. Produced by the US National Library of Medicine and the US EPA (CIS, TOXNET; EPA website, RTK Net website).

TOXLINE (National Library of Medicine, Toxicology Information Program):

One of the largest online bibliographic sources for toxicology information, these databases contain over 3 million citations to all areas of toxicology. TOXLINE references are drawn from various sources grouped into two parts – TOXLINE Core and TOXLINE Special. A standard search of TOXLINE retrieves records from both subsets. Users can also limit retrieval to only one. Both files are available on the NLM MEDLARS system (DIALOG; on CD through Silver Platter).

Nomenclature and Locator Files

Many database producers provide controlled vocabulary systems, which assist the user in obtaining specific and comprehensive retrieval. Registry or nomenclature files can be useful in identifying the controlled vocabulary for a compound used in a specific file or to obtain a collection of synonyms identifying that compound (lab code, generic name, chemical name, trade names, government agency control numbers, etc.) to be used in searching other resources. The CAS Registry file provides structure information, provides synonyms, and a chemical identifier code (CAS registry name) used extensively worldwide. Chemical Abstracts started its identification system in the mid-1960s and has established records for over 12.7 million compounds. Derwent, producer of the *Derwent Drug File* and the *World Patents Index* provides a Drug Registry file and establishes its own unique registry number for chemical compounds referenced in its products. Several online files carry extensive nomenclature information embedded in substance records, such as the Merck Index and RTECS, for example. Some files also provide locator information for chemical substance information on files mounted on a particular vendor system along with the nomenclature of the compound. The Chemical Identification File (ChemIdplus), for example, provides nomenclature and locator information for the PubMed and TOXNET systems.

Vendors

Listed below is a selected list of online vendors that focus on providing toxicology information files. The directories listed in the beginning of this section also give contact information for online vendors.

- BIOSIS
  2001 Market Street, Suite 700
  Philadelphia, PA 19103-7095
  Tel.: +1-215-231-7500 (worldwide)
  +1-800-523-4806 (United States and Canada)
  URL: http://www.biosis.org
  Email: info@biosis.org
- CCINFOWeb (Canadian Centre for Occupational Health and Safety)
  135 Hunter Street East
  Hamilton, ON, Canada L8N 1M5
  Tel.: +1-905-570-8094
  1-800-668-4284 (toll free in Canada and the United States)
  URL: http://www.ccohs.ca
  Email: clientservices@ccohs.ca
- Chemical Information System (CIS)
  National Information Services Corporation (NISC USA)
  Wyman Towers, 3100 St. Paul Street,
  Baltimore, MD 21218
  Tel.: +1-410-243-0797
  URL: http://www.nisc.com
  Email: info@nisc.com
- Data-Star
  c/o DIALOG Corporation
  DIALOG Corporation
  11000 Regency Parkway, Suite 10
  Cary, NC 27511
  Tel.: +1-919-462-8600
  1-800-3-DIALOG (North America)
  URL: http://www.dialog.com
  Email: customer@dialog.com
- DIMDI (Deutsche Institut fur Medizinische Dokumentation und Information)
  Waisenhausgasse 36-38a
  50676 Cologne, Germany
  Tel.: +49 (0) 221-47-241
  URL: http://www.dimdi.de
  Email: posteingang@dimdi.de
- European Space Agency – Information Retrieval System (ESA-IRS)
  ESA/ESRIN
  Via Galileo Galilei
  Casella Postale 64
  00044 Frascati (RM), Italy
  Tel.: +39-06-9418-0951
  URL: http://www.esa.int
  Email: franca.morgia@esa.int
- Thomson MICROMEDEX
  6200 S. Syracuse Way, Suite 300
  Greenwood Village, CO 80111-4740

Tel.: +1-800-525-9083
URL: http://www.micromedex.com
Email: mdx.info@thomson.com

- National Library of Medicine
  Specialized Information Services
  2 Democracy Plaza, Suite 510
  6707 Democracy Blvd., MSC 5467
  Bethesda, MD 20892-5467
  Tel.: +1-301-496-1131 (local and international)
  +1-888-FINDNLM (toll free)
  URL: http://sis.nlm.nih.gov
  Email: tehip@teh.nlm.nih.gov
- ORBIT/QUESTEL
  7925 Jones Branch Drive
  McLean, VA 22012, USA
  Tel.: +1-703-873-4700
  1-800-456-7248 (Toll-free)
  URL: http://www.questel.orbit.com
  Email: help@questel.orbit.com
- OVID
  333 7th Avenue
  20th Floor
  New York, NY 10001
  Tel.: +1-646-674-6300
  +1-800-950-2035 (Toll-free in United States)
  URL: http://www.ovid.com
  Email: sales@ovid.com
- SilverPlatter Information, Inc.
  100 River Ridge Drive
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  Tel.: +1-781-769-2599
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CD-ROMS

Computer disc technology has grown to become one of the most popular media for local electronic storage and retrieval. CD recordable technology is widespread, and CDs are now as easy to create as floppy disks. While the most common type of CD-ROM product for bibliographic retrieval is still the single source product, producers are using this powerful storage medium to mix material into subject collection discs, combining various types of resources – handbooks, journal articles, regulations, and directories. Below are examples of these composite CD-ROM products/series of interest to the toxicology professional:

CCINFOdisc Products (CCOHS):

This collection of several CD-ROM products provides a wealth of legislative, regulatory, handbook, directory, numeric, and bibliographic information focusing on occupational health, workplace safety, environmental hazards, regulatory information, and safety topics. There are 12 titles currently available. International in scope. Vendor: Canadian Centre for Occupational Health and Safety.

Environmental/Safety Library:

A comprehensive collection of EPA, OSHA and DOT regulations and state regulations. Contains titles from the CFR (all of Title 40 and portions of 29, 42, and 49), *Federal Register* notices from 1990 to present, and industry standards from OSHA and EPA. Updated monthly. Produced by Information Handling Service, Inc. Comarketed by MICROMEDEX, Inc

ENVIRONMENT ABSTRACTS:

Provides access to journal articles, conference papers and proceedings, and other materials on the environment. Covers air, water, and noise pollution; solid and toxic wastes; radiological contamination; toxicological effects; control technologies; resource management; population; endangered species; and geophysical and climatic change. Produced by the Congressional Information Service; updated quarterly.

OSH-ROM:

Combines six databases with references to the world's literature on occupational health and safety and environmental medicine. Consists of *CISDOC* (CIS Abstracts by the International Labour Office), *HSELine* (from the Health and Safety Executive in the UK), *MHIDAS* (from the United Kingdom Atomic Energy Authority), *NIOSHTIC and NIOSHTIC-2* (from the US National Institute for Occupational Safety and Health), RILOSH Index (from the Ryerson Polytechnic University Library, Canada) and MEDLINE-OEM (occupational and environmental medicine subset from the National Library of Medicine, US). Available from Silver Platter Information, Inc.; updated quarterly.

PolTox:

The PolTox series from Silver Platter, Inc., provides information on pollution and toxicology. PolTox I contains *Aquatic Sciences and Fisheries Abstracts, Ecology Abstracts, Food Science and Technology Abstracts, Health and Safety Science Abstracts, Pollution Abstracts, RISKLINE, Toxicology Abstracts,* and all of *TOXLINE*. PolTox II contains information derived from EMBASE and PolTox III information from the CAB Abstracts database.

REPRORISK:

Contains a collection of reproductive risk information resources, including REROTEXT, a collection of reviews (with hazard ratings) of the health effects of industrial chemicals; REPROTOX from the Reproductive Toxicology Center at Columbia Hospital for Women; the text of Shepard's *Catalog of Teratogenic Agents*; and TERIS, the Teratogen Information System from the University of Washington. Produced by Micromedex, Inc.

TOMES Plus:

This title contains information on toxicology, occupational health, and environmental information with a focus on emergency situations of exposure and hazard control. It also addresses ergonomics and human health risk assessment. It contains bibliographic, full text, and numeric information. Consists of eleven files of information from various government sources – US EPA, OSHA, DOT, Coast Guard, NIOSH, and others. Updated quarterly. Produced by Micromedex, Inc.

Acknowledgments

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Inhalation *See* Respiratory Tract.

Inhalation Testing *See* Toxicity Testing, Inhalation.

Insect *See* Hymenoptera.

Insecticides *See* Permethrin.

Interactions *See* Interactive Toxicity.

Interactive Toxicity

S Satheesh Anand and Harihara M Mehendale

Definition

Interactive toxicity is defined as effects of mixtures deviating from the additive toxic response expected based on the dose–response relationships obtained from individual components.

Background

Humans are exposed to a myriad of chemicals throughout their lifetime in the work place, the environment, and the home. Hence, the human exposure to chemicals is mixtures rather than single chemicals. The real world exposure scenarios are complex in terms of type of chemicals, the exposure situation (duration, routes, rates, and magnitudes), and the existence of confounding socioeconomic factors (ethnicity, diet, alcohol, smoke, age, area of

residence, etc.), which can affect the likelihood or course of toxicity. Approximately, 80 000 chemicals are in use today with more than 1000 added each year worldwide. Toxicity of many of these compounds has not been adequately tested. Some mixtures are intentional (such as pesticide formulations, gasoline, or laundry detergents) and other mixtures are generated (smelting, disinfection by-products, polycyclic aromatic hydrocarbons from fuel combustion). Cigarette smoking generates mixture of large number of chemicals during combustion of tobacco. Many natural chemicals are present in the foods that we eat. The number of chemicals produced by the chemical and pharmaceutical industries in the twentieth century has vastly increased human exposure to chemicals. Unlike the general public, the people living in the vicinity of hazardous waste sites are often subjected to complex and high amounts of chemical exposures. As estimated, 40 million people live within a 4 mile radius of waste sites.

The potential for unusual health effects of chemical mixtures due to the interaction of chemicals or their metabolites (e.g., metabolites of trichloroethylene and benzene) in or with the biosystem constitutes a real issue in the public health arena. However, toxicity testing to predict effects on humans has traditionally studied one chemical at a time for various reasons: convenient to handle, physiochemical properties readily defined, dosage could easily be controlled, biologic fate could easily be measured, and relevant data were often available from human occupational exposures. Chemicals are known to cause disease: for example, arsenic and skin cancer, asbestos and lung cancer, lead and decrements of IQ, and hepatitis B predisposes to aflatoxin-induced liver cancer but the link between the extent of human exposure to even well-defined chemical mixtures and disease formation remains relatively unexplored, but of paramount importance to public health.

Public concern and the interest of scientists and regulators regarding exposure to chemical mixtures have increased. Because of the heightened awareness of public about the unusual toxicity from the mixtures, the regulatory agencies such as the Environmental Protection Agency (EPA), the Agency for Toxic Substances and Disease Registry (ATSDR), and the National Institute of Occupational Safety and Health (NIOSH) are striving to predict the cumulative health risk of mixtures from multiple sources.

Basic Concepts of Interactive Toxicity

Three basic concepts for the description of toxicological action of mixture have been defined.

Simple Similar Action

It is also called as dose addition. This is a noninteractive process, that is, the chemicals in the mixtures do not affect the toxicity of one another. Each of the toxicants in the mixture contributes to the toxicity in proportion to its dose. All chemicals in the mixture act in the same way, by the same mechanisms and differ only in their potencies.

Simple Dissimilar Action

It is also called as response addition. In this case also the components do not interact with each other. However, the mode of action and possibly the nature and site of action differ among constituents. Response addition is referred to if each individual has a threshold and will only exhibit response beyond the threshold. By definition, response addition is determined by summing the responses of each toxicant in a mixture.

Interaction

Compounds may interact with one another, modifying the magnitude and the nature of toxic effect. The interaction may lead to higher (exaggeration) or lower (antagonism) toxicity as compared to the effects of individual compounds.

Exaggeration: This type is further divided as synergism – when the effect of mixture is higher than additivity based on the dose–response relationships of the individual compounds (e.g., asbestos workers who smoke have higher mortality ratio for lung cancer than only smokers or asbestos workers), and potentiation ($PB + CCl_4$ causes increased liver injury, but not resulting in death) and amplification (kepone $+ CCl_4$ causes higher hepatotoxicity and death) – when a component that does not have an effect increases the effect of the other components.

Antagonism: The effect of mixture is less than that estimated for additivity on the basis of the toxicities of the components. The protection against mercury toxicity by selenium is a typical example of this category. This group is further classified into inhibition – a component does not have effect and decreases the effect of another component or other components and masking – one component overrides the effect of other.

Knowledge on the type of interaction is of profound importance. While the knowledge on synergistic interaction is of paramount public health importance, the knowledge on antagonistic interaction is necessary to reduce the unnecessary utilization of resources for cleanups. Because of the potential unexpected toxicity due to interaction of chemicals, toxicology of mixtures is an active area of scientific investigation.

Mechanisms of Interaction

The mechanisms involved in interactive toxicity have been shown to bring changes in the toxicokinetics and/or toxicodynamics of one chemical by another. Toxicokinetic changes affect the absorption, distribution, metabolism, and/or excretion of a chemical and can have profound effects on dose–response relationships. Changes at the toxicodynamic level might involve a competition between chemicals for binding to a target site, such as a receptor or changes in signal transduction pathways and cell cycle control. Presence of one or more chemicals may also interfere with the complex toxicodynamics of tissue repair, thereby permitting continuous escalation of toxic injury, culminating in organ failure, and death. These mechanisms affect the internal concentrations of the toxicants or their active forms and/or the tissue's response to the toxic insult. Information on these aspects is a prerequisite for predicting the toxic effect of mixture. The examples of various interactions and the mechanisms of interactions are presented in **Table 1**.

Challenges in Mixture Risk Assessment

Because many of the components of complex mixtures and their concentrations are unknown, determining the risk such mixtures may pose to human population is a daunting task. The effects of a chemical mixture are extremely complex and may differ for each mixture depending on the chemical composition. Some contaminants may induce differential effects depending on the route of exposure. Simple mixtures may contain two or three chemicals whereas complex mixtures, such as those found at hazardous waste sites, contain hundreds of chemicals, with varying degrees of toxicity and different modes of toxic action. Concurrent exposure to chemicals such as welding fumes, indoor air pollutants, tobacco smoke, alcohol, and drugs makes the health assessment of chemical mixture a more complex task. Clearly, toxicological evaluation of these complex mixtures is difficult but important for hazard assessment and assessment of risk to human health.

Most of the data that do exist on mixtures come from acute or chronic studies of simple and defined

Table 1 Examples of various interactions and the mechanisms of interactions

Basis of interaction	*Examples of interactive toxicities*	
	Synergism	*Antagonism*
Pharmacokinetic		
Absorption	Neurotoxicity of *o*-ethyl-*o*-4-nitrophenyl phenylphosphonothioate enhanced by aliphatic hydrocarbons due in part to increased dermal absorption	Dietary zinc inhibits some aspects of lead toxicity in part by decreasing lead absorption
Distribution	Increased neurotoxicity from increased lead levels in brain after treatment with disulfiram, due to formation of complex that readily distributes lead to brain	Se protects against Cd toxicity by decreasing the concentration of Cd in liver and kidney and by redistributing Cd in the testis from low to high molecular weight Cd-binding proteins
Excretion	Decreased renal excretion of penicillin when coadministered with probenecid, potentiating its therapeutic effect	As antagonizes the effects of Se in part by enhancing the biliary excretion
Metabolism	Ops (profenofos, sulprofos, DEF) potentiate the toxicity of fenvalerate and malathion by inhibiting esterase, which detoxifies many pyrethroid insecticides and malathion	Se inhibits 2-acetylamino-fluorene-induced hepatic damage and tumorogeneis in part by shifting metabolism towards detoxification
Pharmacodynamic		
Interaction at same receptor site or target molecule	Priming doses of a toxicant activates transactivational mechanisms of tissue repair	Atropine antagonizes OP poisoning by blocking acetylcholine receptor sites
Interaction at different sites on same molecule	Tiazofurin and selenazofurin metabolites bind to different sites on ionosine monophosphate dehydrogenase to synergistically inhibit its activity	Antagonism of Cu binding to DNA by other divalent cations
Interaction among different receptor sites or targets	Amplification of hepatotoxicity of chlordecone + CCl_4 by inhibition of hepatocellular repair due to calcium flooding and depletion of cellular energy	Opposing effects of histamine and norepinephrine on vasodilation and blood pressure (functional antagonism)

Source: Adapted from ATSDR (2002) Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures.

mixtures at doses higher than those normally associated in the environment and studies of human occupational exposures. Majority of biologically significant interactions observed at high doses are not detectable at the low doses to which humans are exposed environmentally. Dose is important because interactive effects depend heavily on dose; therefore, characterizing interactions that occur at high dose such as those used in a rodent bioassay is likely to provide very little information about interactions at very low doses generally encountered in the environment. Minimum number of cancer and noncancer studies has been performed on real-world mixtures such as diesel engine emissions, recycled drinking water, urban air samples, tobacco smoke, and incinerator emissions.

Unfortunately, not only is there a lack of knowledge concerning the characterization of real-life mixtures based on human exposure but there are limited experimental strategies available also that focus on understanding the mechanisms of action of chemical mixtures as it relates to human health. As a consequence, one has limited abilities to predict how chemicals in a mixture interact with each other or with biological systems, leading to toxic effects or disease.

The major challenges in the mixture risk assessment are: complex exposure situation, uncharacterized mixtures, extrapolation from high to low doses and animals to humans. Resolving these issues is key in predicting and preventing the risk from mixtures to humans. Testing of all mixtures existing in the real world is virtually impossible. Even with well-studied individual compounds, immense problems exist in extrapolating the findings obtained at high doses in laboratory animals to humans being exposed to lower doses. Individual variability and impact of life style on the toxic outcome further complicate the issue.

Assessing Risk from Chemical Interaction

Mixture risk assessment usually involves substantial uncertainties ranging from inexact descriptions of exposure to inadequate toxicity information. In addition, there are other confounding factors such as life style, exposure to other contaminants, interindividual variation, etc. Because of the uncertainties, many fudge factors are incorporated and there is no single approach for mixture risk assessment. Most risk assessments evaluate the toxicity of individual chemicals and then combine them by simple addition to estimate risk related to chemical mixtures. However, adding risks ignores potential synergistic or antagonistic interactions that could lead to underestimation or overestimation of total risk, respectively. The National Institute of Environmental Health Sciences, EPA, ATSDR, NIOSH, Occupational Safety and Health Administration (OSHA), and American Conference of Governmental Industrial Hygienists (ACGIH) share the common goal of promoting research that will ultimately reduce the extent of adverse human health effects occurring as a consequence of exposure to mixtures of environmental agents. Recently, the Food Quality Protection Act and Safe Drinking Water Act Amendments were passed, raising awareness of chemical mixtures health issues. By and large, regulatory actions and industrial practices are based on use of the default assumption, additivity. The experimental evidence that can be used to infer effects at low doses appears to support the assumption that low-dose additivity does not underestimate, and in most cases probably overestimates, risk. While the simultaneous administration of chemicals of dissimilar mode of action caused no more than additive effects, the chemicals of similar mode of action causes antagonistic effects at no-observed-adverse-effect levels (NOAELs) of individual components.

EPA recommends three approaches: (1) if the toxicity data on mixture of concern are available, the quantitative risk assessment is done directly form these preferred data; (2) when toxicity data are not available for the mixture of concern, data of a sufficiently similar mixture can be used to derive quantitative risk assessment for mixture of concern; and (3) if the data are not available for both mixture of concern and the similar mixture, mixture effects can be evaluated from the toxicity data of components. According to EPA, the dose-additive models reasonably predict the systemic toxicity of mixtures composed of similar (dose addition) and dissimilar (response addition) compounds. Therefore, the potential health risk of a mixture can be estimated using a 'hazard index' (HI) derived by summation of the ratios of the actual human exposure level to estimated maximum acceptable level of each toxicant. A HI near to unity is suggestive of concern for public health. This approach will hold true for the mixtures that do not deviate from additivity and do not consider the mode of action of chemicals. Modifications of the standard HI approach are being developed to take account of the data on interactions.

The ATSDR has established a mixtures program that consists of three components: trend analysis to identify combinations of chemicals of concern, experimental studies to identify data that would be useful in the development and implementation of predictive decision, support methodologies, and development of assessment methodologies and guidance to provide health assessors with the tools to incorporate the evaluation of multiple-chemical exposure into site assessments. ATSDR suggests the weight of evidence

(WOE), which estimates the joint actions (additivity, antagonism and exaggeration) for binary mixtures of chemicals based on the information on individual components. Several factors such as mechanistic interaction, uncertainty factors, route of exposure etc. are taken into account. The better the data set on the individual chemicals is, the more precise the joint action can be predicted. The draw back is the high- to low-dose extrapolation as most of the individual toxicity information is developed at high doses. According to WOE evaluations, considering common mechanisms for simple chemical mixtures can lead to better estimates of the observed toxic responses than the default assumption of dose additivity.

Future Directions

Although progress has been made in recent years by establishing fair risk assessment methodologies and safe concentrations for many individual compounds, related information for chemical mixtures is largely unavailable. No standard methods are yet in place to incorporate interactions because of the lack of understanding of the modes of action, toxicokinetics, and toxicodynamics. Additional research is needed to resolve many unknown and uncertainties concerning toxicity of chemical mixtures. A fuller understanding of biological effects induced by chemical mixtures is essential to the accurate prediction of the hazards and risks for humans and the ecosystem.

Considering the $\sim$80 000 chemicals in commerce, the task of testing these chemicals on individual basis, let alone as a mixture, is not feasible. Two options seem possible: directly investigate the effects of high priority mixtures and develop extrapolation models for remainder. EPA, ATSDR, and NIOSH have organized the Mixed Exposures Research Group, composed of almost 20 federal and state agencies to develop and share regulatory approaches. Systematic toxicity testing of mixtures, using conventional toxicology and carcinogenicity testing methodologies is highly impractical because of the numbers of chemicals and the limited scientific resources. Therefore, the development of unconventional, efficient, and predictive toxicology methods is imperative. These approaches may greatly reduce animal usage, personnel, resources, and time required to evaluate the carcinogenicity of chemicals and chemical mixtures. Using computational technology, mathematical and statistical modeling, mechanistically based short-term toxicology studies, and cellular and molecular biology techniques would greatly enhance the predictability of the methodologies. Significant advances have been made in alternative toxicologic testing methods such as *in vitro* testing, physiologically-based pharmacokinetic/pharmacodynamic modeling, biologically based dose–response modeling, and quantitative structure–activity relationships modeling, and the 'omics' technologies (transcriptomics, proteomics, metabonomics). The genomics evaluates the gene expression, proteomics elicits resulting protein synthesis and metabonomics captures the change in metabolism following toxic insult. The use of 'omics' would map early toxicity-related alterations in cells, tissues, or animals exposed to chemicals, and thus will lead to insights into numerous toxicologically relevant cellular processes simultaneously. These tools can find utility in the decision-making process and the performance of risk characterization. These alternate methods would aid in understanding the mechanistic basis for interactions at a quantitative level and provide realistic risk assessments for chemical mixtures.

Chemical Interaction and Susceptible Population

There is a strong concern and some evidence that sensitive population such as smokers, alcoholics, children, genetically predisposed, etc. are vulnerable to toxicity. Children are found to be more sensitive than adults to some chemicals because of the immature development of the defense system. Recent studies show association between low-level exposures to hazardous chemicals and developmental effects or birth defects. Many chemicals can cross the placenta and concentrate in the fetus. Thus, the developing fetus is extremely vulnerable to chemical exposure. Number of recent epidemiologic studies has demonstrated neurobehavioral impairment at low-dose exposure levels of lead that were once thought to be safe. Susceptibility of an individual to the toxic and carcinogenic effects of a chemical mixture is believed to be affected to a significant degree by genetics. The advent of new gene array technology is expected to allow the analysis of global patterns of gene expression in response to xenobiotic/mixture exposure. These new approaches of pharmacogenomics and toxicogenomics are expected to be of paramount importance in unraveling the complex interactions among environmental toxicants and in determining their effects on human health. Conditions such as diabetes, aging, caloric restriction, etc. alter the outcome of hepato- and nephrotoxicity by interfering with the tissue's ability to respond to injury by compensatory tissue repair. There are reports to claim that low-dose exposure to chemical mixtures may play an important role in developmental toxicology because of possible interactions among

the components of the mixture, but these reports do not consider the maternal occupational exposure, or lifestyle issues such as use or abuse of medications, drinking, smoking, etc. Elevations in the rates of neural tube defects and major cardiac defects have been found in populations residing in the proximity of toxic waste sites and that consumed contaminated public drinking water. However, there is no conclusive scientific evidence behind it.

Conclusions

The unexpected toxicity due to chemical interaction with or in the biosystem is a paramount public health concern and presents immense challenge for risk assessment. Due to the increased awareness on potential interactive toxicity of chemicals, there is a decline in the amount of chemicals released into the environment reports the US EPA. This is a welcome success of primary prevention, but there seems to be little insight into the potential for joint toxic actions of such chemicals at environmental levels. Although the literature on chemical mixture is growing, our knowledge on underlying biological and pathophysiological processes associated with chemical interaction is meager. In addition, the possibility that subpopulation responds differently to the toxic effects of chemicals than general population further complicates the prediction of biological basis of interaction. Understanding what mixtures of chemicals the public is exposed to as well as the mechanisms involved in the interaction of these mixtures and subsequent health effects is essential. Since it is impossible to conduct studies with all mixtures encountered, toxicity testing of complex environmental mixtures of regulatory importance should be performed. A rational approach to studying mixtures includes prioritizing and identifying chemical mixtures that are based on known human exposures, and applying innovative experimental and computational strategies to dissect the mechanistic basis for interactions of chemicals in a mixture. This would aid in developing qualitative and quantitative health assessment methods for assessments of potential risks for developing multiple health effects.

See also: Common Mechanism of Toxicity; Mixtures, Toxicology and Risk Assessment; Modifying Factors of Toxicity.

Further Reading

US Environmental Protection Agency (EPA) (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. EPA/630/R-00/002.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry (ATSDR) (2002) Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures.

Intercellular Communication *See* Gap Junctional Intercellular Communication in Epigenetic Toxicity.

Intuitive Toxicology *See* Toxicology, Intuitive.

Invertebrate Ecotoxicology *See* Ecotoxicology, Invertebrate.

Investigative New Drug Application

Shayne C Gad

- AGENCY: US Food and Drug Administration (FDA)
- YEAR PASSED: 1962 Drug Amendment to Food, Drug & Cosmetic Act with subsequent amendments
- GROUPS REGULATED: Drug and biopharmaceutical industries

Synopsis of Law

The 1962 Drug Amendment to the Food, Drug and Cosmetic Act (with subsequent amendments)

contains the regulations specifically applicable to human drugs in Subchapter D, Parts 30–399. The definition of a new drug is covered in Part 310 (g): "A new drug substance means any substance that when used in the manufacture, processing, or packaging of a drug causes that drug to be a new drug but does not include intermediates used in the synthesis of such substances."

The regulation then goes on to discuss "newness with regard to new formulations, indications, or in combinations." For toxicologists, the meat of the regulations can be found in Section 312 (investigational new drug application (INDA)) and Section 314 (applications for approval to market a new drug or antibiotic drug or NDA). The major focus for a toxicologist working in the pharmaceutical industry is on preparing the correct toxicology 'packages' to be included to 'support' these two types of applications. The exact nature of these packages will be covered below.

Commencement of clinical trials with a new drug substance requires formal notification to FDA. At least 30 days before the drug's sponsor wishes to begin such trials, the sponsor must submit an IND to the agency (basic IND requirements are set out at title 21 CFR part 312 and Section 117 of the 1997 Food and Drug Administration Modernization Act (FDAMA)). If FDA does not object to the IND within 30 days, it automatically becomes effective and clinical trials may begin. If FDA finds a problem with the application, however, it may impose a 'clinical hold', barring commencement of the investigational studies proposed in the application until the problem is resolved to the agency's satisfaction.

In a nutshell, the law requires solid scientific evidence of safety and efficacy before a new drug will be permitted in clinical trials or (later) on the market. The INDA (covered in 21 CFR 310) is for permission to proceed with clinical trials on human subjects. Once clinical trials have been completed, the manufacturer or 'sponsor' can then proceed to file an NDA (covered in 21 CFR 314) for permission to market the new drug.

As stated in 321.21, "A sponsor shall submit an IND if the sponsor intends to conduct a clinical investigation with a new drug … [and] shall not begin a clinical investigation until … an IND … is in effect." (Similar procedures are in place in other major countries. In the United Kingdom, for example, a clinical trials certificate (CTC) must be filed or a clinical trial exemption (CTX) obtained before clinical trials may proceed.) Clinical trials are divided into three phases, as described in 21 CFR 312.21. Phase I trials are initial introductions into healthy volunteers primarily for the purposes of establishing tolerance (side effects), bioavailability, and metabolism. Phase II clinical trials are "controlled studies … to evaluate effectiveness of the drug for a particular indication or disease." The secondary objective is to determine common short-term side effects; hence, the subjects are closely monitored. Phase III studies are expanded clinical trials. It is during this phase that the definitive, large-scale, double-blind studies are performed.

The toxicologist's main responsibilities in the IND process are to design, conduct, and interpret appropriate toxicology studies (or 'packages') to support the initial IND and then design the appropriate studies necessary to support each additional phase of investigation. Exactly what may constitute appropriate studies have varied with time and vary somewhat based on the nature of the drug. The toxicologist's second responsibility is to prepare the toxicology summaries for the (clinical) investigator's brochure (described in 312.23(a)(8)(ii)). This is an integrated summary of the toxicological effects of the drug in animals and *in vitro*. The FDA has prepared numerous guidance documents covering the content and format of INDs. The Guidance for Industry provides an in-depth description of the expected contents of the pharmacology and toxicology sections. The document contains the following self-explanatory passage.

Therefore, if final quality-assured individual study reports are not available at the time of IND submission, an integrated summary report of toxicological findings based on the unaudited draft toxicologic reports of the completed animal studies may be submitted.

If unfinalized reports are used in an initial IND, the finalized report must be submitted within 120 days of the start of the clinical trial. The sponsor must also prepare a document identifying any differences between the preliminary and final reports, and the impact (if any) on interpretation.

Thus, while the submission of fully audited reports is preferable, the agency does allow for the use of incomplete reports.

Once an IND or CTC/X is opened, the toxicologists may have several additional responsibilities. The first is to design, conduct, and report the additional tests necessary to support a new clinical protocol or an amendment to the current clinical protocol (Section 312.20). The second is to bring to the sponsor's attention any finding in an ongoing toxicology study in animals "suggesting a significant risk to human subjects, including any finding of mutagenicity, teratogenicity or carcinogenicity," as described in 21 CFR 312.32. The sponsor has a legal obligation to report such findings within 10 working days. Third, to prepare a "list of the preclinical studies …

Table 1 Composition of standard INDA[a]

1. IND cover sheets (form FDA – 1571)
2. Table of contents
3. General (clinical) investigation plan
4. (Reserved)
5. (Clinical) investigators brochure
6. (Proposed) clinical protocol(s)
7. Chemistry, manufacturing, and control information
8. Pharmacology and toxicology information (includes metabolism and pharmacokinetic assessments done in animals)
9. Previous human experience with the investigational drug
10. Additional information
11. Other relevant information

[a]Complete and thorough reports on all pivotal toxicological studies must be provided with the application.

completed or in progress during the past year" and a summary of the major preclinical findings. The sponsor is required (under Section 312.23) to file an annual report (within 60 days of the IND anniversary date) describing the progress of the investigation. INDs are never 'approved' in the strict sense of the word. Once filed, an IND can be opened 30 days after submission, unless the FDA informs the sponsor otherwise. The structure of an IND is outlined in **Table 1**, though there may be some variation based on the nature of the drug and scope of the proposed trials.

If the clinical trials conducted under an IND are successful in demonstrating safety and effectiveness (often established at a pre-NDA meeting, described in 21 CFR 312.47(b)(2)), the sponsor can then submit an NDA.

Types of INDs

The primary focus of toxicologists is on submissions that are sometimes called 'commercial INDs', which are applications filed principally by companies whose ultimate goal is to obtain marketing approval for new products. There are, however, at least a few types of applications that may be grouped within a second class of filings sometimes referred to as 'noncommercial' INDs. Interestingly, the vast majority of INDs are noncommercial research submissions. These include the following types of INDs.

Investigator IND (also called research IND): The investigator IND is submitted by a physician who both initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. In most cases, an investigator IND proposes clinical studies on previously studied drugs. A physician might submit a research IND to propose studying an unapproved drug, or an approved product for a new indication or in a new patient population. Generally, however, the physician's motivation is not commercial in nature – in other words, the goal is not to develop data to support marketing approval for an unapproved product or to support new labeling for an approved product. For example, the investigator may simply want to treat patients or obtain data to publish a research paper.

Emergency use IND: The emergency use IND is a vehicle through which the FDA can authorize the immediate shipment of an experimental drug for a desperate medical situation. According to FDA regulations, "need for an investigational drug may arise in an emergency situation that does not allow time for submission of an IND … In such a case, FDA may authorize shipment of the drug for a specified use in advance of submission of an IND." Emergency use INDs are generally reserved for life-threatening situations in which no standard acceptable treatment is available, and in which there is not sufficient time to obtain institutional review board (IRB) approval.

Treatment IND: Although the treatment IND has a history dating back to the 1960s and 1970s, the FDA took steps to formalize the treatment IND concept in a 1987 regulation. Through the FDA's treatment IND program, experimental drugs showing promise in clinical testing for serious or life-threatening conditions are made widely available while the final clinical work is performed and the FDA review takes place. The FDA Modernization Act of 1997 codified the treatment IND concept as well as other expanded-use programs (e.g., emergency use) into law, and encouraged the FDA to consider changes that might reduce industry reluctance to participate in expanded drug access programs.

Concurrently with an IND filing (or at any later time), a sponsor can request a 'fast-track product' designation for its drug, provided the therapy addresses unmet medical needs related to a serious and life-threatening condition. This fast-track designation, which was created under the FDA Modernization Act of 1997, makes a product eligible for accelerated approval and other benefits.

The Applicability of the IND

The IND is a requirement for all persons and firms seeking to ship unapproved drugs over state lines for use in clinical investigations. However, the FDA offers exemptions from IND submission requirements for certain types of clinical testing and products, including the following:

- Clinical investigations of a drug product that is lawfully marketed in the United States, provided

that all of the following conditions apply: (1) the investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use, or is not intended to be used to support any other significant change in the drug's labeling; (2) the investigation is not intended to support a significant change in the advertising for a prescription drug; (3) the investigation does not involve a change in the route of administration, dosage level, patient population, or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product; (4) the investigation complies with IRB evaluation and informed consent requirements; and (5) the study's sponsor and investigator do not represent in a promotional context that the drug is safe or effective for the purposes for which it is under investigation, or unduly prolong the study after finding that the results are sufficient to support a marketing application. The FDA has stated that this exemption is intended primarily for practicing physicians.

- Drugs intended solely for testing *in vitro* or in laboratory research animals, provided the drug labels and shipments comply with FDA regulations applicable to investigational drugs.
- Clinical investigations involving the use of a placebo, provided that the investigations do not involve the use of a new drug or otherwise trigger IND submission requirements.
- Certain *in vivo* bioavailability and bioequivalence studies in humans. FDA regulations state, however, that INDs are required for *in vivo* bioavailability or bioequivalence studies in humans if the test product is a radioactively labeled drug product, is a cytotoxic drug product, or contains a new chemical entity. Further, INDs are required for the following types of human bioavailability studies that involve a previously approved drug that is not a new chemical entity: (1) a single-dose study in normal subjects or patients when either the maximum single or total daily dose exceeds that specified in the labeling of the approved product; (2) a multiple-dose study in normal subjects or patients when either the single or total daily dose exceeds that specified in the labeling of the approved product; and (3) a multiple-dose study on a controlled-release product for which no single-dose study has been completed.

In addition to these IND exemptions, FDA regulations provide a mechanism through which individuals and firms can seek an agency waiver from IND requirements. The agency can grant a waiver if certain criteria are met, including that the sponsor's noncompliance will not pose a significant or unreasonable risk to human subjects.

FDA Oversight: Clinical Holds

Through the imposition of a 'clinical hold', FDA may forestall a proposed clinical investigation or suspend an existing one. A clinical hold can be imposed for a number of reasons, including an unreasonable and significant risk to patients, the use of improperly qualified investigators, a deficient or disregarded investigative protocol, or any other serious deficiency in an IND or a particular clinical trial. FDA must communicate the imposition of a clinical hold by telephone or other form of rapid communication, and must provide the drug sponsor, within 30 days, with a written explanation of the basis for the clinical hold. As a general rule, until the agency's consent to lift a clinical hold is obtained, any clinical trial or trials subject to the hold cannot commence or resume. Under FDAMA, a sponsor faced with a clinical hold may submit a written request to FDA that the hold be removed. FDA must respond to such a request in writing within 30 days.

IND Withdrawal

As with the imposition of a clinical hold, FDA can halt further use or distribution of an investigational drug through withdrawal or suspension of an IND. Similar concerns, such as undue patient risk or serious deficiencies in the application or the clinical protocol, trigger both types of agency action, with withdrawal obviously reserved for the more serious cases.

Where the continuation of a clinical study poses, in FDA's judgment, an immediate and substantial danger to human subjects, the agency may order immediate termination of an IND, subject to possible reinstatement. Where no such immediate risk is present, however, if FDA proposes to withdraw an IND, the agency will notify the sponsor in writing and provide 30 days to submit any corrections or explanations. The sponsor's failure to respond within the specified time frame results in the termination of the IND. The sponsor, however, may request a formal hearing if FDA refuses to accept a submitted correction or explanation.

See also: Delaney Clause; Food and Drug Administration, US; Food, Drug, and Cosmetic Act, US; Good Laboratory Practices (GLP); Toxic Torts.

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Relevant Websites

http://www.access.gpo.gov – CFR Title 21 (Food and Drugs) Chapter 1 – Food and Drug Administration, Department of Health and Human Services – Part 312 – Investigational New Drug Application.
http://www.kluweronline.com – *Investigational New Drugs* (an online journal title by Kluwer).

Iodine

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7553-56-2
- Synonyms: Diiodine; Iode
- Chemical/Pharmaceutical/Other Class: Halogen; Halide
- Chemical Formula: I^+

Uses

Iodine was discovered in 1811 by Courtois, and is classed among the rarer elements. Iodine is used as an antihyperthyroid and a topical anti-infective. It is an ingredient in antiseptics and other medicinal preparations and in germicides. The latter use includes udder washes used on cattle in dairy operations; thus iodine is found in cow's milk. Other uses include disinfectants that may be added to swimming pools or drinking water. It is also used as a chemical reagent and is used in dyes (aniline and phthalein dyes), as an alkylation and condensation catalyst, in iodides, iodates, X-ray contrast media, food and feed additive, stabilizers, photographic film, water treatment, and as an unsaturation indicator. Iodine is found naturally in seaweed and is considered and generally recognized as safe substance by the US Food and Drug Administration (FDA). Iodine is a required element by many species, including humans. It has been recognized as preventative against goiter since 1819, and is used in iodized salt for this purpose. Iodine is also used as a dough oxidizer in commercial bread making.

Exposure Routes and Pathways

Exposure may occur via inhalation, ingestion, or dermal or ocular contact.

Toxicokinetics

Iodine is absorbed rapidly and completely as I^- from the gastrointestinal tract. It is also absorbed when applied to the skin. Surgical scrubs containing iodine compounds were found to increase the level of urinary iodine in medical personnel. Iodine compounds are efficiently trapped and concentrated in the thyroid gland. Excretion is primarily via urine, although some iodine is excreted in feces and sweat. There is some salivary recycling. The half-life of iodine in blood is 6–10 h. Prolonged administration of large doses of iodine markedly reduces thyroidal iodine uptake.

Mechanism of Toxicity

Iodine is a powerful oxidizing agent and has a direct action on cells by precipitating proteins. The affected cells may be destroyed. In addition to the primary irritant action of iodine, this compound can act as a potent sensitizer. Iodine is an integral part of thyroid hormones (tetraiodothyronine (thyroxine) and triiodothyronine), and deficiency results in compensatory hyperplasia and hypertrophy of the thyroid gland (endemic goiter). Endemic goiter occurs naturally where soil is deficient in iodine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Iodine is a strong irritant of the mucous membrane, respiratory tract, eyes, and skin application of a 2% solution of iodine in alcohol to rabbit eyes caused reversible damage. Stronger solutions of 7% caused severe damage to rabbit and monkey eyes. The oral LD_{50} for mice is $2\,g\,kg^{-1}$ and the oral LD_{50} for dogs is $200–500\,mg\,kg^{-1}$.

Human

Ingestion of large quantities of iodine may cause burning of the mouth, throat, and stomach and

abdominal pain, nausea, vomiting, and diarrhea. Sufficient exposure may result in progression of symptoms to fever, shock, delirium, and death. Ingestion of 2–4 g has been fatal. The solid element is intensely irritating to eyes, skin, and mucous membranes. Iodine vapor is more irritating than vapors of chlorine or bromine. Occupational reports indicate that concentrations of 0.1 ppm are tolerable, but concentrations of 0.15 or 0.2 ppm are less tolerable. Concentrations of 1 ppm are highly irritating. Vapor concentrations of 0.57 ppm were tolerated for 5 min without eye irritation; but 1.63 ppm caused irritation within 2 min. Symptoms of inhalation exposure include tightness in the chest, sore throat, and headache. High exposures may result in airway constriction, shortness of breath, difficulty in breathing, pulmonary edema (onset may be delayed several hours), and death. Skin contact may result in corrosive tissue destruction at the site of contact. Individual susceptibility to skin reactions varies widely. Application of tincture of iodine to one-third of the body surface was reported as fatal in one case. Iodine solutions are recognized sensitizing agents.

Chronic Toxicity (or Exposure)

Animal

Dogs exposed by injection in the trachea to vapors of iodine demonstrated inflammation of the lungs, breathing problems, and coughing, which persisted for weeks. The lowest doses causing effects were 7–12 mg kg^{-1}. Doses of 14–18 mg kg^{-1} caused pulmonary edema and death within 24 h. In guinea pigs, 0.5 ppm did not cause detectable effects, but 7 ppm caused impaired breathing capacity. Adult female rats fed 500, 1000, 1500, or 2000 ppm iodine (as potassium iodide; KI) from 0 to 35 days prior to giving birth exhibited increased neonatal mortality with increasing dose, and milk secretion was reduced as evidenced by examination of the mammary glands. Reproductive impairment was also noted in studies with rabbits and chickens. Rabbits fed 250 ppm iodine for 2–5 days in late gestation exhibited increased mortality of young, and hens fed 312–5000 ppm KI ceased egg production within 1 week. Clinical signs of excessive dietary iodide in cattle include lacrimation, nasal discharge, conjunctivitis, hair loss, dermatitis, and exopthalmia.

Human

Excessive ingestion in humans can result in iodide goiter resulting from inhibition of the thyroid gland. The resulting lack of thyroid hormone secretion causes compensatory increase in thyrotropin secretion and thyroid enlargement. Patients treated with radioactive iodine (^{131}I) have been studied for chromosome aberrations (dicentrics) in blood samples taken before and at various times after exposure. The increase in aberrations caused by the exposure to iodine was small but statistically significant. In another study, ^{131}I induced clastogenic and age-dependent aneugenic effects in the lymphocytes of exposed patients. The X chromosome was not preferentially involved in the aneugenic effect induced by ^{131}I, and it was concluded that, besides its major clastogenic effect, ^{131}I can also induce an X chromosome-independent aneugenic activity mainly in patients with spontaneous proneness to chromosome loss. A mild toxic syndrome called iodism results from repeated administration of small amounts of iodine. Iodism is characterized by salivation, coryza, sneezing, conjunctivitis, headache, laryngitis, bronchitis, stomatitis, parotitis, enlargement of the submaxillary glands, and skin rashes.

Clinical Management

Rescue workers should avoid direct contact with the chemical. The source of contamination should be removed or the victim should be moved to fresh air. The worker should immediately wash the skin when it becomes contaminated. Oxygen may be administered by a trained professional. A conscious victim who has ingested excess iodine should rinse his or her mouth with water; however, vomiting should not be induced. The victim should drink 8–10 ounces of water. If vomiting occurs, the victim should lean forward to reduce the risk of aspiration. If contact with the eyes occurs, the eyes should be immediately flushed with lukewarm, gently flowing water for at least 15 min, taking care not to rinse contaminated water into the eye. General supportive measures should be provided.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists ceiling limit is 0.1 ppm, and this is also the (US) Occupational Safety and Health Administration permissible exposure ceiling limit. The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (15 min) ceiling value is 0.1 ppm (1 mg m^{-3}) and the NIOSH immediately dangerous to life or health value is 2 ppm. The US recommended daily allowance of iodine is 100–200 mg day^{-1}.

See also: Generally Recognized as Safe (GRAS); Metals; Sensitivity Analysis.

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Ionizing Radiation *See* Radiation Toxicology, Ionizing and Nonionizing.

Iron

Shayne C Gad

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- Representative Compounds: Ferrous sulfate ($FeSO_4$); Iron oxide (Fe_2O_3)
- Chemical Abstracts Service Registry Number: CAS 7439-89-6
- Chemical/Pharmaceutical/Other Class: Metal
- Chemical Formula: Fe^{3+}

Uses

Iron is one of the major essential elements and one of the most important commercial metals. The total number of products made from iron is greater than that of all other metals combined. Iron is the basis for various steels and is used in pigments, fuel additives, catalysts, magnetic tapes, and animal feeds. As an essential element, iron is the central atom in the hemo of hemoglobin. Medicinally, it is administered to anemic patients and to many premenopausal women.

Exposure Routes and Pathways

The primary exposure pathway for iron is ingestion. Iron is present in practically all foods, in dietary supplements, and in drinking water. In some drinking water, iron concentrations may be especially high as iron pipes have been used extensively in transporting potable water. Iron concentration in surface water varies greatly, from 61 to 2680 ppm. In industrial settings, inhalation is a significant exposure pathway (e.g., arc welders are exposed to a high atmosphere of metal fumes and particles). Analyses of urban air samples show that the iron content averages $1.6\,\mu g\,m^{-3}$, with the iron and steel industry probably the most likely source of emission. Dermal contact is not a significant exposure pathway; however, iron is a natural component of soils and its concentration can be influenced by some industries.

Toxicokinetics

The chemical form of iron influences absorption, as do interrelationships with other dietary components. The disposition of iron in the human body is regulated by a complex mechanism to maintain homeostasis. Iron has the capacity to accept and donate electrons readily, and iron concentrations in body tissues must be tightly regulated because excessive iron leads to tissue damage as a result of formation of free radicals. The content of body iron is regulated primarily by absorption since humans have no physiological mechanism by which excess iron is excreted. Iron is absorbed (in a complicated process) through the gastrointestinal tract as the ferrous ion, first into the mucosal cells, where it oxidizes into the ferric state, and then is carried by the plasma. It is bound to the iron protein, transferrin, a globulin which transfers the iron to the various tissues. The enzyme ferroxidase oxidizes the ferrous ion to the ferric state. Most absorbed iron is found bound to hemoglobin (66%), a small amount is found in the protein myoglobin, and a minute amount is found in the iron-dependent enzymes.

Iron is stored in the blood, liver, bone marrow, and spleen. The storage proteins for iron are ferritin and hemosiderin. With 'iron overload', more ferritin is synthesized in the liver to bind this excess iron. Iron is a cofactor for hemoglobin and cytochromes.

The homeostasis mechanism permits up to 15% of ingested iron to be absorbed while the average person only excretes 0.01% of the intake. During periods of increased demand, such as pregnancy or childhood, absorption of iron is greatly increased. Normally, excess iron is excreted and some is contained within shed intestinal cells and in bile and urine. Smaller amounts are excreted in sweat, nails,

and hair. Approximately 0.5 mg of total iron is excreted per day.

Mechanism of Toxicity

In some adults, iron overload can be the result of a genetic defect (idiopathic hemochromatosis) that causes malfunction of the normal homeostasis mechanism and, in turn, excessive absorption of iron. Iron overload can also be caused by too many blood transfusions, which results in too much iron in the various iron-containing organs.

Recently, it has been suggested that the presence of increased transferrin concentrations in males is associated with an increased number of heart attacks. This must be corroborated by further research.

Excess iron can lead to diabetes mellitus, faulty liver functions, and endocrine disturbance. Iron is a catalyst for oxidative damage leading to lipid peroxidation. The latest hypotheses link peroxidation to heart disease, cancer, and accelerated aging. Iron is involved in the Fenton Reaction, which catalyzes the formation of free radicals that cause excessive damage to cells and their components.

Acute and Short-Term Toxicity (or Exposure)

Animal

In a few animal experiments, sarcomas have appeared at the site following the injection of a large dose of the dextran salt or the lactate or gluconate.

Human

Most iron toxicity is found in very young children who ingest iron-containing medicines with candylike coatings. Fatalities have occurred from childhood ingestion of iron. After consuming more than 0.5 g of iron, toxic symptoms can be delayed for up to 6 h. The gastrointestinal tract can be ulcerated, which alters the limiting mechanism of iron absorption. Besides nausea, vomiting of blood (due to ulceration of the gastrointestinal tract), and black stools, acidosis and some liver damage follow; this can, in some cases, lead to cirrhosis of the liver, liver failure, or renal failure.

Chronic Toxicity (or Exposure)

Animal

Iron does not appear to be mutagenic or teratogenic. However, these experimental findings of tumor formation are open to question and may be associated with 'solid-state carcinogenesis' (believed to be a result of an irritation-type effect at the site of injection as opposed to a genetic mechanism).

Human

Iron has been identified as a component of asbestos and other mineral and synthetic fibers. Inhalation of iron and iron oxide fumes or dust may result in deposition of iron particles in lungs, producing an X-ray appearance resembling silicosis. The carcinogenicity of iron is still under debate, for example, for colorectal and liver cancer. An increase in the incidence of lung cancer, as well as in that of tuberculosis and interstitial fibrosis, has been noted in hematite miners. Due to inadequate controls, it is possible that the increased incidence of lung diseases noted in the study is due to smoking or exposure to other carcinogens present in the occupational setting. The American Conference of Governmental Industrial Hygienists (ACGIH) assigns an A4 (not classifiable as a human carcinogen) ranking to iron. Excess free circulating iron damages blood vessels, and hypotension can occur.

Clinical Management

Acute iron poisoning is treated by removal of ingested iron from the gastrointestinal tract using emesis (vomiting) or gastric lavage and by providing therapy for the associated systemic effects of shock and acidosis. The chelation agent, deferrioxamine, is also administered to bind iron that was not successfully removed from the gastrointestinal tract and has been absorbed.

Excess iron can be removed by either phlebotomy (letting of blood from a vein) or administration of the chelating agent, deferrioxamine, for cases of chronic excess iron. Ascorbic acid can accelerate iron excretion about twofold.

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average, for iron oxide dust and fume is $5\,\mathrm{mg\,m^{-3}}$. The Environmental Protection Agency's Federal Drinking Water Guideline for iron is $300\,\mu\mathrm{g\,l^{-1}}$.

See also: Blood; Metals; Poisoning Emergencies in Humans.

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Irritation Testing *See* Toxicity Testing, Irritation.

Islip Garbage Barge

Todd Canedy

On March 22, 1987, an Islip, Long Island garbage barge set sail for Jones County, North Carolina. The Mobro 4000 was loaded with 3168 tons of municipal solid waste (MSW) for an entrepreneurial venture. This barge soon became a martyr of environmentalists, and made a media spectacle of the growing scarcity of landfill space in America. Tipping fees, or fees paid to dispose of waste at landfills are higher where landfill space is more scarce and lower where there is a higher abundance of land suitable for landfills. In a hurried attempt by Lowell Harrelson, a New York businessman, to make a few dollars on the increasing garbage crisis in New York City, the Mobro 4000 was sent south in hopes of cashing in on the lower tipping fees of rural North Carolina. Mr. Harrelson failed to secure a dumping contract prior to setting sail. Rather, he tried to negotiate a contract after the barge was already on its way.

Upon arrival at the North Carolina port, a public outcry began resounding through the media. Concerned citizens spotted the barge offshore, piled high with rotting solid waste. The proper authorities were alerted who soon began an investigation.

In order to save a few dollars, the owners of the barge declined to purchase a tarp to cover the MSW for its trip south. The said purchase may very well have prevented public dissent. The complaining citizens criticized that the stinking mass of out-of-state waste was floating in their ocean rather than being disposed off in its originating state. Due to the lack of a previous-made dumping contract, concerns were raised about medical, toxic, and other illegal contaminants in the waste.

Local officials called the deal off and sent the barge on its way. The barge eventually spent 164 days at sea, and traveled over 6000 miles before returning to New York City. North Carolina, Florida, Alabama, Mississippi, Louisiana, and Texas all declined port to the barge, as did Mexico, The Bahamas, and Belize.

After the media frenzy that followed the barge's voyage, the boat was forced to incinerate the MSW in a Brooklyn facility and bury the ashes in the same landfill from which the waste began its journey. This public spectacle served as a launch pad for environmentalists everywhere. The Mobro 4000 was an icon of America's excessive consumption and waste, and helped to push hundreds of communities to increase recycling efforts (see **Figure 1**). This event, coupled with other environmental issues of the time, served as a springboard for public involvement in environmentalism. Interest in waste stream, air pollution, water pollution, and other environmental health issues were never before on public minds to this extent.

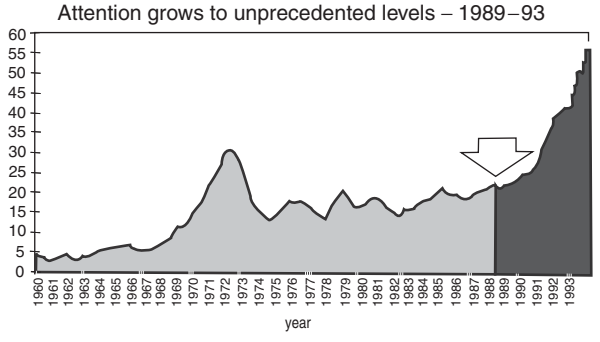

Figure 1 Recycling trends by year.

See also: Resource Conservation and Recovery Act, US.

Further Reading

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Relevant Website

http://www.cfact.org – Committee for a Constructive Tomorrow.

Isocyanates

Robert Kapp

Isocyanates are a group of low molecular weight aromatic and aliphatic compounds containing the isocyanate group (–NCO). The most widely used industrial isocyanates and their applications are listed below. The most notorious isocyanate is methyl isocyanate, involved in one of the worst industrial tragedies recorded in history. In the early morning hours of December 3, 1984, 200 000 people in Bhopal, India were exposed to methyl isocyanate. The 90 min exposure resulted in at least 2500 deaths and countless cases of severe eye and lung damage. Most of the deaths were related to the pulmonary edema.

HDI – Hexamethylene Diisocyanate

- Chemical Abstracts Service Registry Number: CAS 822-06-0
- Synonyms: 1,6-Diisocyanatohexane; HDI; Hexamethylene-1,6-diisocyanate; 1,6-Hexamethylene diisocyanate; HMDI
- Chemical Formula: $C_8H_{12}N_2O_2$

Uses

Hexamethylene diisocyanate (HDI) is used in the preparation of dental materials, medical adsorbents, and contact lenses, and is used as a polymerizing agent in polyurethane paints and coatings.

Background Information

HDI is a colorless liquid with an irritating odor.

Exposure Routes and Pathways

Inhalation and dermal exposure can occur during the manufacture and use of HDI. Workers and individuals in close proximity to an area where spray applications of polyurethane paints may be exposed.

Acute and Short-Term Toxicity (or Exposure)

Acute inhalation exposure may result in pulmonary edema, coughing, and labored breathing in humans. HDI is extremely irritating to the eyes, nose, and throat. Rodent studies revealed that HDI is extremely toxic by inhalation, and moderately to highly toxic by oral ingestion.

Chronic Toxicity (or Exposure)

Chronic inhalation exposure to HDI is thought to cause chronic lung irritation. In addition, chronic inhalation exposure has been reported to cause irritation of the nasal tissues and respiratory tract. Dermal exposure has resulted in sensitization in several animal species. The US Environmental Protection Agency (EPA) has set the reference concentration (RfC) for HDI at $0.000\,01\,mg\,m^{-3}$ based upon the degeneration of the olfactory epithelium in rodents. EPA has not established a reference dose (RfD) for HDI.

Reproductive Toxicity

No information is available in the reproductive or developmental effects of HDI in humans. A rat reproductive study found no effects in any reproductive organs.

Carcinogenicity

No information is available on the carcinogenic effects of HDI in humans. Animals exposed to HDI were reported to show no evidence of carcinogenicity in a 2 year inhalation study. EPA has classified HDI as a group D (not classifiable as to human carcinogenicity.)

Clinical Management

Skin or ocular exposure areas should be generously irrigated with saline. All other treatment is symptomatic.

Exposure Standards and Guidelines

The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level (REL), averaged over a 10 h workday is 0.005 ppm ($0.035\,mg\,m^{-3}$).

MIC – Methyl Isocyanate

- Chemical Abstracts Service Registry Number: CAS 624-83-9
- Synonyms: Methyl ester of isocyanic acid; MIC
- Chemical Formula: C_2H_3NO

Uses

Methyl isocyanate (MIC) is used as a chemical intermediate in the production of carbamate insecticides and some herbicides.

Background Information

MIC is a colorless liquid with a sharp pungent odor.

Exposure Routes and Pathways

Inhalation, ingestion and dermal contact are all possible routes of exposure. Occupational exposure can occur during the use of carbamates produced from MIC. Small amounts of MIC are found in cigarette smoke.

Acute and Short-Term Toxicity (or Exposure)

The acute toxic effects of MIC are essentially similar by either route except for the intensity of the effects. When rats were administered MIC by inhalation or subcutaneous route it produced severe hyperglycemia, clinical lactic acidosis, highly elevated plasma urea, and reduced plasma cholinesterase activity with unaltered erythrocyte acetyl cholinesterase activity. Irrespective of the route of administration, MIC also caused severe hypothermia, which was not ameliorated by prior administration of atropine sulfate. Rats and mice of each sex were exposed by inhalation to MIC at 0, 1, and 3 ppm for 6 h day^{-1} for 4 days followed by up to a 91 day recovery period. Only animals exposed to 3 ppm exhibited exposure related changes. Most of the rats exposed at 3 ppm died within 28 days. A prominent decrease in body weight was observed along with severe lung lesions and thymus atrophy. Lesions of the nasal cavity of rats and mice were characterized by regeneration of the olfactory and respiratory epithelium. By day 28, the respiratory epithelium of rats and mice appeared normal, but olfactory regeneration was still present in the surviving rats. Severe lesions of the trachea extending to the bronchi, bronchioles, and alveoli were seen in rats exposed to 3 ppm. Acute inflammation of the airways and hyaline membranes was observed in high dose animals killed on day 7. In the 3 ppm MIC-exposed animals that died during days 8–14, there was lymphatic necrosis of the thymus, atrophy of the splenic white pulp, coagulative necrosis of the liver, and thrombosis of the left cardiac atrium. Gross examination of exposed mice showed minimal differences between exposure groups. Microscopic observations showed treatment-related changes only in the 3 ppm exposure group of mice. The changes involved primarily the bronchial system and were not as severe as those observed in rats. By day 91, bronchial fibrosis was minimal to mild in mice. One group of five male rats was exposed to 3 ppm MIC for a single 6 h exposure. The lesions in the respiratory system were essentially the same as those observed in the 4 day repeated exposure rats that were killed after 7 days.

In another acute toxicity study, rats were exposed only once to 3.52 and 35.32 ppm of MIC for 10 min; in a subacute study, they were exposed to doses of 0.212, 0.265, and 0.349 ppm for 30 min daily for 6 days and were then observed for 90 days for weight gain. At the end of 90 days, damage to the viscera was evaluated. During exposure, the animals had congestion in eyes, lachrymation, nasal secretion and dyspnea, progressively increasing ataxia, immobility, and uncoordinated movements. MIC exposure greatly inhibited weight gain in the animals in a dose-dependent manner. Upon microscopic examination of the viscera, pathological findings were confined to the bronchial tree, lung parenchyma, liver, and kidneys.

Acute inhalation exposure of humans results in pulmonary edema (most of the Bhopal deaths were due to pulmonary edema and secondary respiratory infections from pulmonary edema). Other acute effects include, blindness, nausea, gastritis, sweating, fever, chills, and liver and kidney damage. MIC was studied in *in vivo* micronucleus test and chromosomal analysis of bone marrow cells. Mice were exposed for 10 min to different concentrations (2.40, 4.80, or 7.20 µl) of MIC at 0 and 24 h. Quantitative analysis failed to exhibit any significant increase in aberration rates in the three treated groups. In another micronucleus assay, mice were exposed to MIC through ip injection for 2 and 5 days in separate experiments, and bone marrow and peripheral blood were sampled 6 and 48 h after the last injection, respectively. MIC did not significantly increase the frequencies of micronucleated erythrocytes in bone marrow and pheripheral blood samples in either twice or multiply treated mice. However, a dose-dependent depression in percentage polychromatic erythrocytes observed was significant. This indicates that MIC exposure led to the cytotoxic effect by inhibition of bone marrow cell proliferation.

Chronic Toxicity (or Exposure)

The long-term carcinogenic and pulmonary effects of a single exposure to MIC were examined in rodents. Rats and mice were exposed to MIC by inhalation at 0, 1, 3, or 10 ppm for 2 h. After 2 years, the animals were sacrificed and tissues and organs were examined microscopically. No differences in survival rates or body weight gains were found in the MIC-exposed animals versus controls. Male and female rats exposed to 10 ppm MIC had 42% and 36% incidence, respectively, of intraluminal fibrosis of secondary bronchi; however, no evidence of this lesion was seen in controls or animals exposed to lower concentrations. For male and female mice and female rats, no neoplastic lesions were significantly associated with MIC exposure. Male rats exposed to MIC had marginally increased rates of pheochromocytomas of the adrenal medulla and adenomas of

pancreatic acinar cells cells. EPA has not established an RfC or an RfD for MIC.

Reproductive Toxicity

Follow-up of exposed humans after the Bhopal incident found a high level of stillborns, spontaneous abortions and increased infant mortality. There was also a high number of survivors with pelvic inflammatory disease, excessive menstrual bleeding and suppression of lactation. Pregnant mice and rats were exposed to 9 or 20 ppm MIC to determine if the chemical was able to cross the placental barrier and directly affect the fetus. The mice were exposed on day 8 and the rats on day 10 of gestation to 9 ppm MIC for 3 h for evaluation of *in vivo* fetal toxicity. In other experiments the animals were exposed for 2 h to 20 ppm and the embryos removed immediately for culture. MIC exposure reduced maternal progesterone levels in mice that lost but not in mice that retained pregnancy. No relationship was observed between fetal toxicity of MIC and maternal plasma corticosterone levels. Fetal toxicity of MIC was not affected by chronic administration of progesterone or the suppression of pulmonary edema with dexamethasone. A concentration dependent decrease in growth in culture was noted in embryos exposed *in utero* or *in vitro* to MIC vapor. No fetal toxicity was noted following exposure to an acute dose, 3 mmol kg^{-1}, of the metabolites. The results indicate that the fetal toxicity of MIC is partly independent of maternal toxicity and may result from the transfer across the placenta and the interaction with fetal tissues.

Monomethylamine, dimethylamine, and trimethylamine are endogenous substances as well as metabolites of MIC. Methylamines exert several toxic effects including inhibition of protein turnover and oocyte RNA synthesis. A study conducted to determine the developmental toxicity of these methylamines using pregnant mice and mouse embryo culture used ip injections (daily from day 1 to day 17 of gestation) of trimethylamine at 2.5 and 5 μmol $kg^{-1}day^{-1}$. Trimethylamine significantly decreased fetal body weight but not the placental weight or maternal body weight gain; however, 5 of 11 mice treated with 5 μmol kg^{-1} trimethylamine died. Similar treatment with dimethylamine or monomethylamine did not exert any obvious maternal or fetal effects. All three methylamines, when added to embryos in culture, caused dose dependent decreases in size, DNA, RNA, and protein content as well as embryo survival; the order of toxicity was trimethylamine > dimethylamine > monomethylamine. The ability of monomethylamines to adversely affect fetal development suggests that these methylamines, especially trimethylamine, may act as endogenous teratogens under certain conditions.

Carcinogenicity

No information is available on the carcinogenic effects of MIC in humans. Animals exposed via inhalation gave mixed results. EPA has classified MIC as a group D (not classifiable as to human carcinogenicity.)

In Vitro Toxicity Data

MIC was nonmutagenic in the Ames (Salmonella), Drosophila sex-linked recessive lethal assays. MIC induced chromosomal aberrations in cultured Chinese hamster ovary cells.

Environmental Fate

MIC may be released to the environment as a result of its manufacture and use as a chemical intermediate. If MIC is released to soil, it will be expected to rapidly hydrolyze if the soil is moist, based upon the rapid hydrolysis observed in aqueous solution. If released to water, it will be expected to rapidly hydrolyze with half-lives of 20 and 9 min at 15°C and 25°C, respectively, calculated from measured overall hydrolysis rate constants. The products of hydrolysis may include *N*-carboxymethylamine, methylamine, carbon dioxide, and *N*,*N*′-dimethylurea. Since it rapidly hydrolyzes, bioconcentration, volatilization, and adsorption to sediment and suspended solids are not expected to be significant processes. No data were located concerning biodegradation, but MIC will probably abiotically hydrolyze significantly faster than it will biodegrade. If released to the atmosphere, it will be expected to exist almost entirely in the vapor phase based upon its vapor pressure. It will be susceptible to photooxidation via vapor phase reaction with photochemically produced hydroxyl radicals. Hydrolysis of MIC in moist air may be significant based upon its rapid hydrolysis in aqueous solution.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, 8 h time-weighted average (TWA) is 0.02 ppm, with a designation that skin exposure is also an important exposure. The (US) Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL), 8 h TWA is 0.02 ppm (with a skin notation). The US NIOSH REL is 0.02 ppm as a 10 h TWA, and the NIOSH immediately dangerous to life or health (IDLH) value is 3 ppm. MIC is listed by EPA as a hazardous air pollutant generally known or suspected to cause serious health problems. The Clean Air Act, as amended in 1990, directs EPA to set standards

requiring major sources to sharply reduce routine emissions of toxic pollutants.

MDI – 4,4′-Methylenediphenyl Diisocyanate

- Chemical Abstracts Service Registry Number: CAS 101-68-8
- Synonyms: 4,4′-Diphenylmethane diisocyanate; Methylene bis(4-phenyl isocyanate); Methylene di-*p*-phenylene ester of isocyanic acid
- Chemical Formula: $C_{15}H_{10}N_2O_2$

Uses

4,4′-Methylenediphenyl diisocyanate (MDI) is used to produce polyurethane foams.

Background Information

MDI is a light-yellow fused solid or it may occur in crystalline form.

Exposure Routes and Pathways

Inhalation and dermal exposure can occur during the manufacture and use of MDI. Workers and individuals in close proximity to the plant may inhale emissions from urethane foam production manufacturing facilities.

Acute and Short-Term Toxicity (or Exposure)

Acute inhalation exposure may result in sensitization and asthma in humans. Dermal contact with MDI resulted in dermatitis and eczema in plant workers. Animal studies revealed skin and eye irritation in rabbits, extreme toxicity by inhalation and moderate toxicity by oral ingestion in rodents.

Chronic Toxicity (or Exposure)

Chronic inhalation exposure to MDI is one of the leading causes of asthma in plant workers. In addition, chronic inhalation exposure can cause dyspnea, immune disorders as well as nasal and lung lesions. EPA has set the RfC for MDI at $0.0006\,mg\,m^{-3}$ based upon irritation of nasal membranes in rodents. EPA has not established an RfD for MDI.

Reproductive Toxicity

No information is available in the reproductive or developmental effects of MDI in humans; however, some effects (decreased placental and fetal weights and increased skeletal variations) were noted in a rat study.

Carcinogenicity

No information is available on the carcinogenic effects of MDI in humans. Animals exposed to polymeric MDI were reported to increase the incidence of pulmonary adenomas. EPA has classified MDI as a group D (not classifiable as to human carcinogenicity).

Exposure Standards and Guidelines

The (US) OSHA PEL 8 h TWA is 0.02 ppm ($0.2\,mg\,m^{-3}$). The (US) NIOSH REL is 0.005 ppm ($0.05\,mg\,m^{-3}$) as a 10 h TWA, and the NIOSH IDLH value is $75\,mg\,m^{-3}$.

TDI – Toluene Diisocyanate

- Chemical Abstracts Service Registry Number: CAS 584-84-9
- Synonyms: 2,4-TDI; 2,4-Toluene diisocyanate
- Chemical Formula: $C_9H_6N_2O_2$

Uses

Toluene diisocyanate (TDI) is commonly used as the 2,4 and 2,6 isomers. It is used as a chemical intermediate in the production of polyurethane materials including foams, coatings and elastomers, as a cross-linking agent for nylon-6, and as a hardener in polyurethane adhesives and finishes. Polyurethane elastomers made from TDI are used in coated fabrics and clay-pipe seals. Polyurethane coatings made from TDI are used in floor finishes, wood finishes and sealers, and in coatings for aircraft, tank trucks, truck trailers, and truck fleets.

Background Information

TDI is a colorless, yellow, or brown liquid with a sharp pungent odor. The major metabolites of TDI in animals and humans are toluenediamines and their acetylated products.

Exposure Routes and Pathways

Inhalation and dermal exposure can occur during the manufacture and use of TDI. Workers and individuals in close proximity to the plant may inhale emissions from urethane foam production manufacturing facilities. It can be present as a unreacted impurity in materials, for example, TDI has been found in a urethane foam fabric coating in a concentration of $<200\,mg\,kg^{-1}$.

Toxicokinetics

The toxicokinetics of TDI (2,4- and 2,6-toluene-diisocyanates) in chronically exposed workers at two

flexible foam polyurethane production plants have been reported. The half-life in urine ranged from 5.8 to 11 days for 2,4- and 2,6-toluenediamines. The differences in exposure were reflected by the plasma toluenediamine concentrations. The mean half-life in plasma was 21 days for 2,4-toluenediamine and 21 days for 2,6-toluenediamine, The study showed that the half-life in plasma of chronically exposed workers for 2,4- and 2,6-toluenediamine was twice as long as for volunteers with short-term exposure. An indication of a two-phase elimination pattern in urine was found. The first phase was related to the more recent exposure and the second, much slower one was probably related to release of toluenediamines in urine from TDI adducts in the body. Two men were exposed to TDI atmospheres in a stainless-steel test chamber. The effective exposure period was 4 h. The isomeric composition of the air in the test chamber was 30% 2,4-TDI and 70% 2,6-TDI. In plasma, 2,4- and 2,6-toluenediamine showed a rapid-phase elimination half-life of ~2–5 h, and that for the slow phase was greater than 6 days. A connection was observed between the concentrations of 2,4- and 2,6-TDI in air and the levels of 2,4- and 2,6-toluenediamine in plasma. The cumulated amount of 2,4-toluenediamine excreted in the urine over 24 h was ~15–19% of the estimated inhaled dose of 2,4-TDI, and that of 2,6-toluenediamine was ~17–23% of the inhaled dose of 2,6-TDI. In another study, five men were exposed to TDI atmospheres for 7.5 h in a stainless-steel test chamber. The urinary elimination of the toluenediamines showed a possible biphasic pattern, with rapid first phases for 2,4-toluenediamine (mean half-life for the concn in urine, 1.9 h) and for 2,6-toluenediamine (mean half-life for the concn in urine, 1.6 h). The cumulative amount of 2,4-toluenediamine excreted in urine within 28 h ranged from 8% to 14% of the estimated dose of 2,4-TDI, and the cumulative amount of 2,6-toluenediamine in urine ranged from 14% to 18% of the 2,6-TDI dose. The average urinary level of 2,4-toluenediamine was $5\,\mu g\,l^{-1}$ in the 6–8 h sample, and the corresponding value for 2,6-toluenediamine was $8.6\,\mu g\,l^{-1}$. Biological monitoring of exposure to 2,4- and 2,6-TDI by analysis of 2,4- and 2,6-toluenediamine in urine is feasible.

Acute and Short-Term Toxicity (or Exposure)

In laboratory animals, TDI has caused inflammation and necrosis when applied directly to the skin, conjunctivitis when applied to the eyes, and rhinitis, laryngitis, tracheitis, bronchitis, and pneumonia when inhaled. All workers develop eye, nose, and throat irritation at 0.5 ppm exposure to TDI. Sensitized individuals may manifest symptoms at levels as low as 0.02 ppm. TDI is a potent respiratory irritant and sensitizer, even at low airborne concentrations. Chronic bronchitis, chronic restrictive pulmonary disease, and hypersensitivity pneumonitis have also been described among TDI-exposed people. The mechanism of TDI-induced asthma is still unknown. TDI may produce a true hemorrhagic syndrome affecting the bone marrow and producing primarily thrombocyte series suppression.

Chronic Toxicity (or Exposure)

Chronic inhalation exposure results in severe lung effects that are characterized by asthma-like reactions characterized by dyspnea, wheezing, and bronchial constriction. The US EPA has neither established an RfC (for inhalation exposure) nor an RfD (for oral exposure) for TDI.

Reproductive Toxicity

No information is available in the reproductive or developmental effects of TDI.

Carcinogenicity

Rats and mice were administered commercial grade TDI (80% 2,4- and 20% 2,6-) in corn oil by gavage at doses of 60 or $120\,mg\,kg^{-1}$ body weight, 5 days per week for 105 or 106 weeks. Other groups of rats and mice received 120 or $240\,mg\,kg^{-1}$ on the same schedule. The results indicated that commercial grade TDI in corn oil was carcinogenic for rats, causing subcutaneous fibromas and fibrosarcomas (combined) in males and females, pancreatic acinar cell adenomas in males, and pancreatic islet cell adenomas, neoplastic nodules of the liver, and mammary gland fibroadenomas in females. TDI was not carcinogenic for male mice, but was carcinogenic for female mice, causing hemangiomas or hemangiosarcomas (combined), as well as hepatocellular adenomas. The International Agency for Research on Cancer (IARC) has judged that TDI is a group B chemical, that is, there is inadequate evidence for the carcinogenicity of TDI in humans, there is sufficient evidence for the carcinogenicity of TDI in experimental animals, and the overall evaluation is that TDI is possibly carcinogenic to humans. The (US) National Toxicology Program (NTP) lists TDI as an anticipated human carcinogen.

In Vitro Toxicity Data

TDI was mutagenic in the Ames Salmonella assay, and induced chromosome aberrations after a 24 h treatment in the absence of metabolic activation in human whole blood lymphocyte cultures. To investigate the role of pharmacological mechanisms in TDI-induced occupational asthma, the effects of TDI

on rat trachea ring and lung parenchymal strip were studied *in vitro*. The most prominent effect observed was a stimulation of metacholine-induced contraction of the tracheal ring by 1 μmol l^{-1} TDI. It was concluded that the pharmacological effect of TDI may result from an autonomic imbalance between cholinergic and B-adrenergic neural control.

Environmental Fate

TDI's production and uses may result in its release to the environment through various waste streams. If released to air, TDI will exist solely as a vapor in the ambient atmosphere and will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 2.7 days. Atmospheric degradation may also occur through contact with clouds, fog, or rain. If released to water or moist soil, toluenediisocyanate is not expected to leach or adsorb to solids due to its rapid degradation reaction with water. It is not expected to bioconcentrate in aquatic organisms.

Exposure Standards and Guidelines

The (US) OSHA PEL, 8 h TWA is 0.02 ppm (0.14 mg m^{-3}).

See also: Bhopal; Carbamate Pesticides.

Further Reading

Cohrssenn BC (2001) Cyanides and nitriles. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 4, pp. 1373–1456. New York: Wiley.

Dart RC (2004) Miscellaneous chemical agents. In: Dart DC (ed.) *Medical Toxicology*, 3rd edn., pp. 1184–1186. Philadelphia, PA: Lippincott Williams and Wilkins.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Isocyanates.

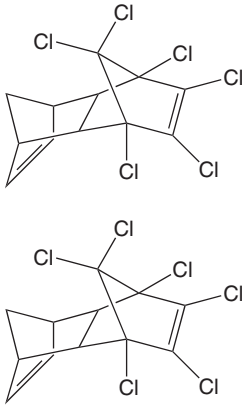
http://www.tc.gc.ca – Isocyanates (from Transport Canada).

http://www.cdc.gov – US National Institute for Occupational Safety and Health (NIOSH). Isocyanates (NIOSH Safety and Health Topic).

Isodrin

K S Rao

- Chemical Abstracts Service Registry Number: CAS 465-73-6
- Synonyms: 1,4;5,8-Dimethanonaphthalene,1,2,3,4,10,10-hexachloro-, 4,4a,5,8,8a hexahydro-endo, Compound 711; Experimental Insecticide 711; SD 3418
- Chemical/Pharmaceutical/Other Class: Cyclodiene; Insecticide
- Chemical Formula: $C_{12}H_8Cl_6$
- Chemical Structure:



Uses

Isodrin, a cyclodiene insecticide has been discontinued and is no longer used in the United States.

Exposure Routes and Pathways

Exposure to isodrin can occur by inhalation, or ingestion; however, the primary exposure is through the dermal route to mixers, loaders, and applicators, during and after normal use.

Toxicokinetics

Isodrin is metabolized by biooxidation to endrin. Isodrin and its metabolite, endrin, have high fat:water partition coefficients and, therefore, tend to accumulate in adipose tissue. At a constant rate of intake, however, the concentration of the insecticide in adipose tissue reaches an equilibrium and remains relatively constant. Following cessation of exposure, it is slowly eliminated from the body. *In vitro* studies have shown that mixed-function oxidase of mouse liver converts isodrin to endrin. Mice excrete 10% of the orally administered dose in urine. Four unidentified metabolites were present in urine, three probably are glucuronide or sulfate conjugates. Feces is

the major route of excretion for isodrin in mice. Five metabolites were present in the organic extracts. Acid hydrolysis of the aqueous phase released four metabolites. None were identified.

Mechanism of Toxicity

Isodrin, a cyclodiene insecticide, is a neuropoison, and the locus of primary toxic action is believed to be sensory and motor nerve fibers and the motor cortex. The underlying mechanism of neurotoxic effect is the slowing of the cessation of sodium conductance across the nerve membrane and inhibition of the initiation of the potassium conductance. A prolongation of the afterpotential results in repetitive firing in the presynaptic nerve membrane, which is due primarily to the slowing of the falling phase of the sodium current and partly to the decrease in the steady state potassium current. Isodrin was among 11 cyclodiene insecticidal compounds tested for the ability to induce detoxifying microsomal oxidase. Isodrin showed a maximum of 30% increase in enzyme activity. The induction of enzymes appeared to be a nonspecific phenomenon.

Acute and Short-Term Toxicity (or Exposure)

Animal

From its acute oral toxicity in animals, isodrin would be considered extremely toxic; the oral LD_{50} in rats is $7\,mg\,kg^{-1}$. Isodrin is one of the extremely toxic chlorohydrocarbon insecticides. It is a skin irritant. Oral administration of isodrin produces central nervous system (CNS) symptoms, and survivors may develop liver and kidney damage.

Human

The probable oral lethal dose for humans is in the range of $5–50\,mg\,kg^{-1}$ (between seven drops to one teaspoon for a 150 lb person). Signs and symptoms of poisoning in humans resulting from high doses of isodrin are due to excitation of the CNS. Acute exposure to isodrin may result in overall discomfort, headache, dizziness, agitation, nervousness, disturbed behavior, tremors, seizures, and/or coma. Convulsive episodes may alternate with periods of severe CNS depression. Seizures may be the first symptom of acute exposure, occurring within minutes to hours of a sufficient exposure to isodrin. Nausea, vomiting, and diarrhea are common side effects. Hypertension (high blood pressure), tachycardia (rapid heart rate), and cardiac arrhythmias (abnormal heart beating) may be noted. Respiratory depression may lead to respiratory arrest. Contact of isodrin with the skin, eyes, and mucous membranes may result in redness and irritation. Victims often have an elevated temperature.

Chronic Toxicity (or Exposure)

Animal

Chronic administration of isodrin to rats results in electroencephalogram abnormalities and seizures.

Human

Epileptiform convulsions and abnormal electroencephalographic patterns have been found in studies of insecticide manufacturing workers suffering from intoxication by isodrin. Fourteen patients with convulsions caused by the insecticide all showed specific anomalies in the electroencephalogram, consisting of bilateral synchronous theta wave activity and occasional bilateral synchronous spike and wave complexes believed to be associated with brain stem injury.

Clinical Management

Acute exposure to isodrin may require decontamination and life support for the victims. Emergency personnel should wear protective clothing appropriate to the type and degree of contamination. Air-purifying or supplied-air respiratory equipment should also be worn, as necessary. Rescue vehicles should carry supplies such as plastic sheeting and disposable plastic bags to assist in preventing the spread of contamination. After acute exposure, vital signs should be evaluated, including pulse and respiratory rate, and any trauma should be noted. If no pulse is detected, cardiopulmonary resuscitation (CPR) should be provided. If the victim is not breathing, artificial respiration should be provided. If breathing is labored, oxygen or other respiratory support should be administered. In case of inhalation exposure, the victims should be moved to fresh air. In case of dermal exposure, the contaminated clothing should be removed as soon as possible. The exposed skin areas should be washed three times. Initially, washing should be done with soap and water, followed with an alcohol wash, and then again with soap and water. If eye exposure has occurred, the eyes must be flushed with lukewarm water for at least 15 min.

Periodic electroencephalographic examination is valuable for the detection of early subclinical intoxication. Persons with a history of convulsive disorders would be expected to be at increased risk from

isodrin exposure. The concentration of isodrin in the blood is helpful in determining the extent of absorption.

Treatment in acute exposure is symptomatic and supportive. Oils should not be used as either cathartics or dermal cleansing agents, as they increase absorption. Gastric lavage and use of activated charcoal and sodium sulfate are indicated for ingestion. Management of seizures is with valium or phenobarbital.

In cases of chronic ingestion exposure, isodrin can accumulate in the adipose tissue, due to its lipophilicity. It is encouraging that a means of hastening the excretion of stored isodrin has been developed. This involves the use of anion exchange resin, cholestyramine, which, when given orally to patients, enhances fecal excretion of isodrin. The rationale for the use of cholestyramine relates to the biliary-enterohepatic circulation, which cycles isodrin; hence, cholestyramine by binding the insecticide, interrupts the reabsorption phase and shifts the equilibrium from reabsorption and storage to fecal excretion.

Environmental Fate

If released into the soil, isodrin may undergo microbial oxidation to endrin. The behavior of isodrin in soil may range from moderately mobile to immobile and isodrin is not expected to hydrolyze since it contains no hydrolysable functional groups. The soil half-life of isodrin has been estimated to be 0.5–1 year. It is absorbed by the roots of plants and is likely to be translocated to above ground parts of plants. If released into water, isodrin may bioconcentrate in aquatic organisms, adsorb onto suspended soils and sediments and undergo very slow microbial transformation. The bioconcentration factor of isodrin was found to be 4500 in molluscs.

See also: Cyclodienes; Organochlorine Insecticides.

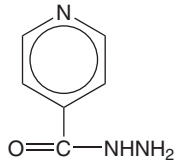
Further Reading

Gosselin RE, Smith RP, and Hodge HC (1984) *Clinical Toxicology of Commercial Products*, 5th edn. Baltimore, MD: Williams and Wilkins.

Isoniazid

Lisa Vivero

- Chemical Abstracts Service Registry Number: CAS 54-85-3
- Synonyms: Isonicotinic acid hydrazide; INH
- Chemical/Pharmaceutical/Other Class: Isonicotinic acid derivative; Antitubercular agent
- Chemical Structure:



Uses

Isoniazid is an antibiotic used for the treatment and prevention of tuberculosis infection.

Exposure Routes and Pathways

Isoniazid is usually ingested orally as a tablet, capsule, or liquid. An injectable formulation is also available.

Toxicokinetics

Isoniazid is rapidly absorbed from the gastrointestinal tract reaching peak plasma concentrations within 1–2 h of ingestion. The extent of absorption may be reduced by oral administration with food or aluminum-containing antacids. Isoniazid binds negligibly to plasma proteins and distributes into all body fluids with a volume of distribution of $0.6\,l\,kg^{-1}$. The metabolism of isoniazid follows Michaelis–Menten kinetics and occurs mainly in the liver by acetylation. The rate of acetylation is genetically determined and is expressed phenotypically as 'fast' (90% of Asians and Inuits) and 'slow' (50% of White and African-Americans) acetylators. Fast acetylators metabolize isoniazid up to 6 times faster, producing plasma concentrations 30–50% lower than in slow acetylators. The mean elimination half-life of isoniazid is about 1 h for fast acetylators and up to 5 h for slow acetylators. Fast acetylators eliminate 11% of isoniazid

unchanged, and slow acetylators excrete 27% unchanged. Approximately 50–70% of an isoniazid dose is renally eliminated within 24 h of ingestion, mainly as metabolites. Metabolites include acetylisoniazid, isonicotinic acid, acetylhydrazine, diacetylhydrazine, and hydrazine.

Mechanism of Toxicity

Isoniazid may cause toxicity directly through toxic intermediates, and immunologic responses, or indirectly through the depletion of pyridoxine (vitamin B_6), and interference with several enzymes and cofactors including those needed to produce γ-aminobutyric acid (GABA), nicotinamide adenine dinucleotide (NAD), and niacin (vitamin B_3).

The exact mechanism of isoniazid-induced hepatotoxicity is unknown. However, the metabolite acetylhydrazine is believed to be responsible for hepatic injury when it is converted to toxic intermediates via cytochrome P-450 (CYP)2E1. Persons with the CYP2E1c1/c1 genotype may be more susceptible to hepatotoxicity. The role acetylator status plays in hepatotoxicity continues to be debated, but it is currently thought that slow acetylators are at greater risk. Other risk factors include increasing age, chronic isoniazid overdosage, comorbid conditions such as malnutrition, pregnancy, diabetes, HIV, renal dysfunction, hepatic dysfunction, alcoholism, and concomitant use of enzyme inducing drugs.

Isoniazid-induced seizures are caused by the depletion of GABA, a primary inhibitory neurotransmitter that requires the cofactor pyridoxal-5′-phosphate for its synthesis from glutamate. Isoniazid-induced GABA deficiency is brought on by at least three processes: (1) metabolites form complexes with pyridoxine increasing its urinary excretion; (2) metabolites block pyridoxine-5′-phosphokinase, the enzyme that activates pyridoxine to pyridoxal-5′-phosphate; and (3) metabolites inactivate pyridoxal-5′-phosphate. Prolonged seizures commonly result in plasma lactic acid accumulation that can lead to a metabolic acidosis. Isoniazid may worsen the severity of acidosis by inhibiting the production of NAD, a cofactor necessary for the conversion of lactate to pyruvate. Long-term exposure to isoniazid therapy commonly causes peripheral neuropathy due to pyridoxine deficiency, and may induce pellagra, a niacin deficiency disorder. Niacin requires the cofactor pyridoxal-5′-phosphate for its production from tryptophan.

Other enzymes inhibited by isoniazid include the cytochrome P450 mixed function oxidases, monoamine oxidase, glutamate decarboxylase, and histaminase. The consequences of these extensive enzymatic disturbances are mood elevation, decreased central nervous system GABA levels, depressed catecholamine synthesis, defects in glucose and fatty acid oxidation, and impaired metabolism of other drugs. Important drug interactions include those with phenytoin, carbamazepine, warfarin, and rifampin.

Acute and Short-Term Toxicity (or Exposure)

Animal

When taken or administered in overdose to dogs, isoniazid produces seizures, metabolic acidosis, and, if untreated, death.

Human

Isoniazid intoxication is characteristically presented by generalized seizures, metabolic acidosis, and coma. Acute ingestions of more than 1.5 g may produce mild symptoms of malaise, nausea, vomiting, dizziness, slurred speech, and tachycardia. Ingestions of more than 2–5 g produce moderate toxicity, and ingestions of >6 g are typically fatal unless there is aggressive intervention. Seizures generally occur within 1 h, but may be delayed up to 5 h postingestion. Status epilepticus may occur with seizures lasting for hours followed by severe anion-gap metabolic acidosis. Coma may ensue after or between seizures.

Chronic Toxicity (or Exposure)

Animal

Rats dosed at 35 mg kg^{-1} isoniazid per day in drinking water for 48 weeks had slightly increased rates of liver and lung tumors compared to controls. Although studies in rats and rabbits have shown that Isoniazid is embryocidal, it has not been shown to be teratogenic in rats, mice, or rabbits.

Human

Chronic therapeutic ingestion of isoniazid is associated with several common adverse effects including rash, fever, and elevated liver function tests (in up to 20% of patients). Isoniazid-induced hepatitis occurs less frequently (0.1–2%) and generally occurs within the first 2 months of therapy. Complete hepatic failure may result if isoniazid therapy is continued. Neurologic symptoms of stocking-glove peripheral neuropathy, optic neuritis, hallucinations, pellagra, and seizures may occur in the absence of an overdose, especially in predisposed persons. Autoantibody production with resulting hemolytic anemia, thrombocytopenia, arthritis, or vasculitis may also develop.

In Vitro Toxicity Data

Mutagenicity studies of isoniazid have yielded mixed results. Some models of sister-chromatid exchange were positive. *Salmonella* assays have been positive and negative. *In vivo* nonhuman primate carcinogenicity studies have been positive.

Clinical Management

In the patient who presents with seizures, airway protection and seizure control are primary goals. Disturbances in cardiac rhythm or function also require immediate attention. Ipecac-induced emesis is contraindicated due to the risk of seizures and the resulting potential for aspiration. Gastrointestinal decontamination via administration of activated charcoal should be considered for substantial recent ingestions. Pyridoxine is administered intravenously to all symptomatic and potentially serious asymptomatic overdoses as it provides rapid relief or prevention of severe toxicity, including seizures. The pyridoxine dosage is equal to the estimated isoniazid dose. If the quantity of isoniazid taken is unknown, 5 g of pyridoxine is empirically given. Repeat doses may be necessary should signs of toxicity persist. Pyridoxine may be supplemented with a benzodiazepine or a barbiturate anticonvulsant. Seizure control usually resolves metabolic acidosis; however, intravenous sodium bicarbonate may be necessary to correct severe acidosis. Hemodialysis is considered for those unresponsive to all other therapy.

See Also: Niacin; Pyridoxine.

Further Reading

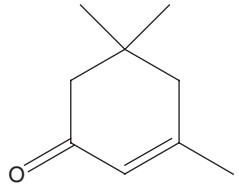
Orlowski JP, Paganini EP, and Pippenger CE (1988) Treatment of a potentially lethal dose isoniazid ingestion. *Annals of Emergency Medicine* 17: 73–76.

Wason S, Lacouture PG, and Lovejoy FH (1981) Single high-dose pyridoxine treatment for isoniazid overdose. *Journal of the American Medical Association* 246: 1102–1104.

Isophorone

Leonard I Sweet

- Chemical Abstracts Service Registry Number: CAS 78-59-1
- Synonyms: Isoacetophenone; 1,1,3-Trimethyl-3-cyclohexene-5-one; 1,5,5-Trimethyl-1-cyclohexen-3-one; 3,5,5-Trimethyl-2-cyclohexen-1-one; 3,5,5-Trimethyl-2-cyclohexenone; 3,5,5-Trimethylcyclohex-2-enone; 3,5,5-Trimethylcyclohexenone; Alpha-isophorone; Isoacetophorone; Isoforon
- Chemical/Pharmaceutical/Other Class: Ketones
- Chemical Formula: $C_9H_{14}O$
- Chemical Structure:



Uses

Isophorone is used as an intermediate in the production of certain chemicals, and as a solvent for resins, polymers, and pesticide formulations. It is used as a solvent for concentrated vinyl chloride/acetate based coating systems, as an adhesive for plastics, and is found in metal paints, nitrocellulose finishes, printing inks for plastics, and some herbicide and pesticide formulations. Isophorone may occur naturally in cranberries.

Exposure Routes and Pathways

Exposure to isophorone can occur via inhalation, ingestion, skin or eye contact, and there is potential for skin absorption. Inhalation and dermal contact is expected to be the primary route of occupational exposure. The general population may be exposed to isophorone via ingestion of contaminated drinking water.

Toxicokinetics

Rapid absorption and elimination is expected after oral or inhalation exposure, though the rate, extent, and relative tissue distribution is not well characterized. Pharmacokinetic studies in rats indicated that the majority of orally administered isophorone was eliminated in the urine (predominantly), expired air, and feces, within 24 h. Studies in experimental animals suggest that isophorone is metabolized to

dihydroisophorone, isophorol, diisophorone glucuronide, and other products after oral exposure; though different metabolic pathways may operate following other routes of exposure.

Mechanism of Toxicity

The toxicological mechanisms of isophorone are not well characterized. Critical effects include irritation, narcosis, malaise, fatigue, and central nervous system (CNS) depression. Isophorone may induce its neurological effects by interference with neuronal impulse transmissions via physical interaction with nerve membrane components. In animal models, isophorone may also act by inducing neuropathy, involving binding to globulin proteins, although this mechanism may not be relevant to humans. Lesions of the liver have been observed after overexposure in mouse models, although it is not clear whether isophorone elicited the lesions directly or by enhancing an age-related process. DNA-binding studies in mice have shown no significant covalent binding of isophorone or its metabolites to DNA from liver or kidney cells, supporting a potential nongenotoxic mechanism of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Isophorone causes irritation of the eyes, skin, mucous membranes, and respiratory tract, and CNS depression. Systemic effects of isophorone toxicity in animals include pulmonary congestion and hemorrhaging, hyperkeratosis of the forestomach, and liver and kidney damage. Short-term overexposure of animals to high levels of isophorone resulted in inactivity and coma. The acute toxicity if isophorone is low, with oral LD_{50} values $>1500\,mg\,kg^{-1}$ in the rat, $>2200\,mg\,kg^{-1}$ in the mouse, and $>2000\,mg\,kg^{-1}$ in the rabbit. Dermal LD_{50} values were $1700\,mg\,kg^{-1}$ in the rat and $>1200\,mg\,kg^{-1}$ in the rabbit. Acute effects from dermal exposure in experimental animals ranged from mild erythema to scabs. Conjunctiva and corneal damage have been reported after direct application to the eye. Skin sensitization potential has been shown to be low.

In acute and short-term studies on rats given high doses of isophorone ($>1000\,mg\,kg^{-1}$) degenerative effects in the liver as well as CNS depression were observed, and there were some deaths. In 90 day oral studies with laboratory animals, no-observed-effect levels ranged from 150 to $500\,mg\,kg^{-1}$ body weight per day. Acute inhalation exposure of test animals to isophorone resulted in irritation, decreased body weights, hematological effects, and pulmonary congestion.

Human

Adverse effects of isophorone reported by people who have been exposed include irritation of the skin, eyes, nose, and throat, as well as dizziness and fatigue. Irritant effects have been reported at air concentrations above $1\,mg\,m^{-3}$, whereas nausea, headache, and dizziness have been reported at above $1142\,mg\,m^{-3}$. The sharp odor of isophorone may induce olfactory fatigue. Dermal exposures have caused irritation including burning. The lowest published toxic concentrations for humans via inhalation are $140\,mg\,m^{-3}$ for eye, nose, and pulmonary system effects. The estimated immediately dangerous to life or health air concentration is 200 ppm.

Chronic Toxicity (or Exposure)

Animal

Isophorone has been investigated for potential genotoxicity, and has generally been shown to lack significant activity in the mouse lymphoma, unscheduled DNA synthesis, and micronucleus assays. Animal models are generally negative with regard to potential reproductive and developmental toxicity of isophorone at high doses.

Isophorone has been investigated for potential carcinogenicity, and in male rats, caused an increase in tumors of the kidney, liver, lymph, and reproductive glands when exposed by ingestion. There was no increase in tumors in female rats or mice.

Human

The US Environmental Protection Agency has classified isophorone as a possible human carcinogen. The National Toxicology Program tested isophorone for evidence of carcinogenicity and found the following: male rat – some evidence; female rat – no evidence; male mice – equivocal evidence; female mice – no evidence. European Risk Phrases suggest there is possible risk of irreversible effects upon repeated overexposure.

In Vitro Toxicity Data

Isophorone induced sister chromatid exchanges but not chromosome aberrations in Chinese hamster ovary cells. Isophorone was positive in L5178Y tk+/tk− mouse lymphoma cell forward mutation assay without metabolic activation. Isophorone was negative in tests with *Salmonella typhimurium* bacterial strains

TA98, TA100, TA1535, TA1537, with or without metabolic activation.

Clinical Management

Management of individuals overexposed to isophorone begins with removing those individuals from the source of exposure, flushing eyes and skin with copious amounts of water, and removing contaminated clothing. If ingested, oral administration of charcoal as a slurry may prove therapeutic in limiting the absorption of isophorone from the intestine. In cases of respiratory overexposure, the victim should be moved to fresh air immediately, breathing should be monitored and oxygen supplied if difficult, and treatment given according to severity of irritation. Medical tests are not well characterized to determine the extent of overexposure to isophorone, although tests for kidney and liver function, as well as examination of the eyes and nose for chronic inflammation may be useful.

Environmental Fate

If released to the environment, isophorone is expected to preferentially partition to the soil and water. Bioconcentration and bioaccumulation potential is expected to be low, based on the estimated bioconcentration factor and experimental octanol–water partition coefficient. Biodegradation is not expected to occur rapidly. Volatilization is expected to be an important fate and transport process based on the Henry's law constant and vapor pressure. When released into the air, isophorone is expected to have a short half-life of much less than 1 day.

Ecotoxicology

The available data suggest that the aquatic toxicity of isophorone is low, with short-term toxicity values for freshwater algae, invertebrates, and fish ranging from 100 to 300 $mg\,l^{-1}$.

Other Hazards

Flammable and explosive when exposed to heat or flame; can react with oxidizing materials.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for isophorone include the following:

- American Conference of Governmental Industrial Hygienists (5 ppm Ceiling);
- Australia (5 ppm Peak);
- Belgium (5 ppm short-term exposure limit (STEL));
- Canada (5 ppm Ceiling);
- China (30 $mg\,m^{-3}$ Ceiling);
- Denmark (5 ppm Ceiling);
- Finland (1 ppm time-weighted average (TWA));
- Germany (4 ppm Peak; 2 ppm TWA);
- Mexico (5 ppm TWA);
- Portugal (5 ppm Ceiling);
- United Kingdom (5 ppm STEL); and
- US Occupational Safety and Health Administration permissible exposure limit (25 ppm TWA).

Miscellaneous

Isophorone is colorless to light yellow liquid with a peppermint or camphor-like odor. Odor is generally detected at concentrations ranging from 0.2 to 2 ppm. It is soluble in water and with most organic solvents.

See also: Neurotoxicity.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Isophorone.

http://ntp-server.niehs.nih.gov – US National Toxicology Program Documents for Isophorone.

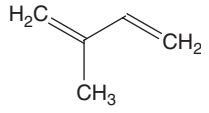
http://www.ecetoc.org – ECETOC Joint Assessment, vol. 10 (1989). Isophorone: CAS 78-59-1. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Isophorone.

Isoprene

Kathryn A Wurzel

- Chemical Abstracts Service Registry Number: CAS 78-79-5
- Synonyms: 2-Methyl-1,3-butadiene; 2-Methylbutadiene; β-Methylbivinyl; Isopentadiene
- Chemical/Pharmaceutical/Other Class: Reactive branched diene
- Chemical Formula: C_5H_8
- Chemical Structure:



Uses

The isoprene unit is the most important building block for lipids, steroids, terpenoids, and a wide variety of natural products. The only chemical reaction of commercial importance (other than polymerization) is its conversion to terpenes. Isoprene is used in the manufacture of 'synthetic' natural rubber, butyl rubber, and as a copolymer in the production of synthetic elastomers.

Exposure Routes and Pathways

Inhalation, ingestion, and dermal exposure are exposure pathways for isoprene.

Toxicokinetics

Hemoglobin adduct formation is linearly related to administered doses of isoprene up to $\sim 55\,\mu mol\,kg^{-1}$; the concentration of hemoglobin adducts may therefore be used as an indicator of previous exposure. Mice exhaled approximately twice as much butadiene as isoprene following exposure to isoprene. The percentage of inhaled isoprene metabolized decreased with increasing exposure concentrations and vapor concentration. Approximately 75% of the total metabolites are excreted in the urine, independent of the inhaled concentration. A higher percentage of metabolites are excreted in the feces following high-concentration exposures.

Saturation kinetics is observed in rats and mice. The half-life in rats and mice are 6.8 and 4.4 min, respectively, following inhalation exposure. The presence of isoprene products in the respiratory epithelium even after short exposure durations suggests that, significant metabolism occurs in this tissue. Human studies have demonstrated 20% isoprene absorption in the upper respiratory tract with 70–99% being retained in the lungs.

Isoprene is metabolized to epoxides and diepoxides. Body fat appears to be a reservoir for isoprene and its metabolites.

Mechanism of Toxicity

A mutagenic metabolite, isoprene dioxide, was tentatively identified in all examined tissues following exposure to isoprene. It is believed that the formation of reactive epoxides following exposure to isoprene results in tumor induction.

Acute and Short-Term Toxicity (or Exposure)

Animal

A 2% isoprene air concentration did not cause central nervous system (CNS) depression in mice but did produce bronchial irritation.

Human

Acute contact with isoprene may irritate skin, eyes, and mucous membranes. Upper respiratory tract irritation is associated with exposure via inhalation. CNS depression is possible with exposure to high concentrations.

Chronic Toxicity (or Exposure)

Animal

Isoprene is nonmutagenic in bacterial test systems. However, isoprene forms adducts of blood hemoglobin in mice and rats. Increases in frequency of sister chromatid exchanges in bone marrow cells and in levels of micronucleated polychromatic erythrocytes were detected. Based on these results, isoprene is expected to induce tumors at multiple sites in exposed mice. Developmental toxicity has been indicated in mice, including decreased fetal body weight and ossification; these impacts were not noted in rats.

Mice exposed to 7000 ppm isoprene via inhalation showed decreased weight gain, testicular atrophy in males, and microscopic lesions. In addition, there is sufficient evidence of the carcinogenicity of isoprene in animals.

Human

There are no epidemiological data relevant to the carcinogenicity of isoprene in humans. It is considered a possible human carcinogen based on sufficient evidence of carcinogenicity in animals.

Clinical Management

There is no information available specifically for isoprene. Exposed skin and eyes should be flushed with copious quantities of water following exposure. Humidified oxygen should be administered after excess inhalation exposure. Induced emesis should be avoided if ingestion has occurred. Measures to decrease gastrointestinal absorption should be instituted (gastric lavage, activated charcoal, or dilution by administration of liquids).

Environmental Fate

Isoprene will exist as a vapor in the atmosphere where it will be degraded by reaction with photochemically produced hydroxy radicals, ozone molecules, and nitrate radicals. Isoprene is moderately mobile in soil and will volatilize from moist soil surfaces. In water, both volatilization and hydrolysis are expected to be important fate processes.

Miscellaneous

Isoprene occurs widely in nature as it is produced by plants during photosynthesis. It is also produced endogenously in humans.

See also: Carcinogenesis.

Further Reading

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Isopropanol

Michael D Reed

This article is a revision of the previous print edition article by Bradford Strohm, volume 2, pp. 191–193, © 1998, Elsevier Inc.

- Synonyms: Numerous derivatives and brand names available. Isopropyl alcohol; 2-Propanol; 'Rubbing' alcohol
- Chemical/Pharmaceutical/Other Class: Alcohol, disinfectant
- Chemical Formula: C_3H_8O
- Chemical Structure: CH_3–CHOH–CH_3

Uses

Isopropanol is used as a solvent in numerous industrial and commercial products including synthetic resins, coatings, lacquers, and paint removers. It is also used in drug and cosmetic formulations, including many toiletries, perfumes, and colognes. It is also found in consumer products such as windshield cleaning fluids and glass cleaners. Because of its widespread availability and low cost, isopropanol has been abused by alcoholics.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to isopropanol. Isopropanol may also enter the systemic circulation via the inhalation, cutaneous, and rectal routes.

Toxicokinetics

Isopropanol is rapidly (within 30 min) and well absorbed ($\sim$70% bioavailability) after oral administration. The V_d of isopropanol is 0.6–0.71 kg^{-1} with minimal to no protein binding. Isopropanol is rapidly metabolized by alcohol dehydrogenase in a first-order, concentration-dependent manner to acetone. This apparent first-order metabolism of isopropanol is probably a result of extensive pulmonary clearance of the acetone. Approximately 80% of systemic isopropanol is metabolized to acetone with the remainder excreted unchanged via the kidneys. A very small amount of isopropanol may be eliminated via the lungs. The presence of ethanol will competitively

antagonize isopropanol metabolism via alcohol dehydrogenase prolonging the isopropanol $t_{1/2}$

Mechanism of Toxicity

Isopropanol is a potent central nervous system (CNS) depressant; it is believed to exert this effect via a similar mechanism as ethanol by modulating ion transport at the cell membrane in excitatory and inhibitory neurons. Ethanol enhances inhibitory or antagonizes excitatory neurotransmission. The metabolite, acetone, may potentiate and lengthen the duration of CNS symptoms observed upon isopropanol exposure. Although early animal studies suggested that the CNS depressant effects of isopropanol is approximately twice that of ethanol, this increased CNS depressant activity is probably a result of the combined effects of isopropanol and acetone.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals develop similar effects as those seen in humans (gastrointestinal effects, CNS depression, confusion, respiratory depression, and death may occur).

Human

Given its widespread availability, use, and cheap cost, isopropanol has resulted in relatively few reports of serious, acute adverse effects in humans. In cases of poisoning or intentional ingestion, the major signs of isopropanol toxicity are those of alcoholic intoxication, including nausea, vomiting, abdominal pain, gastritis, lethargy, weakness, hypotension, ataxia, and hypothermia. In extreme cases, isopropanol depression of the CNS can produce unconsciousness, leading to coma and death due to respiratory depression. Skin absorption of isopropanol in toxic amounts has not been routinely reported, but one case involving a child intoxicated after being sponged with isopropanol suggests that dermal absorption should not be underestimated, particularly in children.

The presence of isopropanol in the blood will result in an increase in the serum osmolality without a concurrent metabolic acidosis. The measured serum osmolality represents the summation effect of both isopropanol and acetone.

Exposure to $\sim$400 ppm isopropanol vapors for 3 min can cause mild irritation of the eyes, nose, and throat.

Chronic Toxicity (or Exposure)

Animal

Several chronic exposure studies in various animal models have been performed to check for increased tumor incidence. Most models (rats, mice) have not shown increased tumor formation. Rats exposed to 0.5–1% isopropyl alcohol in drinking water for 27 weeks developed only decreased weight gain.

Human

Chronic exposure (abuse) to isopropanol will result in more serious gastritis (hemorrhagic gastritis) than is routinely observed with chronic ethanol exposure. Similarly, all of the negative social and physical consequences of ethanol exposure would be observed, probably to a greater extent, with chronic isopropanol exposure.

In Vitro Toxicity Data

Cell multiplication tests in various models have demonstrated inhibition only when concentrations were greater than 1000 mg l^{-1} (e.g., *Pseudomonas putida* 1086 mg l^{-1}, *Microcystis aeruginosa* (algae) 1000 mg l^{-1}, *Scenedesmus guadricauda* (green algae) 1800 mg l^{-1}, *Entosiphon sulcatum* 4930 mg l^{-1}, *Uronema parduczi* 3425 mg l^{-1}).

Clinical Management

Individuals overexposed to isopropanol should be removed from exposure, affected areas of the skin should be washed with soap and water, and the eyes should be irrigated with water. Isopropanol is rapidly absorbed from the gastrointestinal tract. Efforts to decrease absorption are unlikely to be beneficial. Severe isopropanol overdoses have been managed successfully with either peritoneal dialysis or hemodialysis. Since the vast majority of patients respond completely with only supportive therapy, dialysis (hemodialysis much more effective than peritoneal) should be instituted in those patients with a history and physical exam consistent with a very large ingestion (blood isopropyl alcohol $>$400 mg dl^{-1}), those patients with hemodynamic instability (hypotension) and coma.

Environmental Fate

Because of its broad range of uses and large production, isopropyl alcohol can be released into various waste streams. At ambient atmosphere, release

of liquid isopropanol will rapidly change to vapor state. Vapor will react with oxygen radicals in the atmosphere with a half-life of ~3.2 days. Isopropanol has also been identified as metabolic product from aerobic and anaerobic microbes, fungi, and yeast.

See also: Acetone; Ethanol; Fragrances and Perfumes.

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Zaman F, Pervez A, and Abreo K (2002) Isopropyl alcohol intoxication: A diagnostic challenge. *American Journal of Kidney Disease* 40: E12.

Itai-Itai

Rika Shuto

Introduction

Itai-Itai disease is a well-known chronic cadmium poisoning, occurring among inhabitants of the Jinzu River basin in Toyama Prefecture, Japan, during and after World War II. In 1955, Dr. Hagino first reported the disease that is characterized by severe pain resulting from osteomalacia and named the disease, '*Itai-Itai* disease', meaning 'ouch, ouch' or 'painful' in English. According to the various studies, undertaken by many researchers and Toyama Prefecture, the Ministry of Health and Welfare of Japan declared in May 1968 that *itai-itai* disease was caused by chronic cadmium poisoning. Between 1967 and 2003, 187 total cases of *itai-itai* disease (184 women and 3 men) had been officially recognized by the Japanese government, and only four among them were still alive. Most of the victims were middle-aged women who were deficient in calcium due to lactation, multiple pregnancies, and postmenopausal loss of calcium and were living in this community for more than 30 years.

Exposure Routes and Pathways

The Jinzu River basin had been polluted by heavy metals, mainly zinc, cadmium, and lead from the Kamioka mine, located ~20 miles from Toyama Plain. Waste water from upstream mine had been discharged into the river for 50 years between the 1910s and the 1950s. The polluted river water had been used for irrigation in this area; consequently the soil, rice, vegetables, and fish were highly polluted. According to a study by Fukushima, cadmium concentrations in rice paddy soils in this area were higher than those in unpolluted areas. As a result of pollution of the river, the inhabitants living in this cadmium-polluted area for a long time had accumulated high concentrations of cadmium in their bodies through the diet. The intake of cadmium among *itai-itai* patients was $1000\,\mu g\,day^{-1}$, which was about 200 times higher than the normal intake in unexposed populations. High concentrations of cadmium were detected in urine, blood, bone, kidney, and other organs of patients living in or near this area.

Symptoms

In the early stage of the disease, patients suffer from pains in the lumbar areas, shoulders, and eventually the entire body due to renal tubular dysfunction and decrease in the bone mass. In the later, more serious stage, patients may experience difficulty in mobility due to osteomalacia with severe pains, and may further experience spontaneous bone fracture caused by the slightest external pressure, such as coughing. Finally, patients waste away and eventually die due to significant weight loss.

Mechanism of Toxicity

Itai-itai disease is mainly characterized by renal tubular dysfunction, severe osteomalacia, pesudofractures, and anemia. Chronic cadmium exposure induces renal tubular dysfunction resulting in decreased reabsorption of many substances, including calcium, phosphorus, and vitamin D. Chronic cadmium exposure also can lead to excessive urinary excretion of calcium, phosphorus, glucose, amino acids, and low molecular-weight proteins (such as β2-microglobulin, lysozyme, retinol binding protein, and vitamin D binding protein). Hypophosphatemia resulting from excessive excretion of phosphate can lead to decreased serum calcium levels and cause osteomalacia, accompanying pseudofractures and severe bone pains.

Clinical Treatments

Renal tubular dysfunction with *itai-itai* disease is irreversible and progressive even if the cadmium exposure is reduced. There is no specific treatment for renal tubular dysfunction by chronic cadmium poisoning. Long-term administration of vitamin D could be useful in the treatment of osteomalacia; however, its effectiveness is limited, and the reappearance of osteomalacia could be observed due to the latent renal tubular dysfunction.

Resolutions

After the official declaration for the disease by Japanese government in 1968, Agricultural Land Soil Pollution Prevention Law was enacted in December 1970. Maximum concentration of cadmium is regulated as less than $1\,mg\,kg^{-1}$ in rice for agricultural land. Corresponding to this law, Toyama Prefecture had conducted surveys on the cadmium in the polluted area along the Jinzu River and found that cadmium concentration was 1 ppm or more in brown rice and soils in this area. Based on these surveys, Toyama Prefecture declared that the upper soil layer of total 1500.6 ha of paddy fields along the Jinzu should be restored to bring the soil back to normal. Since the restoration work started in 1980, the average cadmium concentrations in soils has reduced to 0.16 ppm from 1.12 ppm (before restoration), and 0.09 ppm from 0.99 ppm (before restoration) in brown rice until 1997. The restoration work is expected to be completed in 2004.

See also: Cadmium; Kidney; Metals; Minamata; Pollution, Water.

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Ivermectins *See* Avermectins.

J

Jequirity Bean

Brenda Swanson-Biearman

- SYNONYMS: *Abrus precatorius*; Deadly crab's eye; Indian bean; Love bean; Lucky bean; Mienie mienie; Prayer bean; Rosary bean; Rosary pea; Seminole bead
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Toxalbumins

Uses

There are no known therapeutic uses for the jequirity bean, but they are used decoratively.

Exposure Routes and Pathways

Ingestion of the bean is the most common route of exposure.

Toxicokinetics

The mature bean is innocuous if the hard outer coat is intact. Any interruption in the integrity of the seed coat (e.g., chewing) or ingestion of the soft-coated immature bean may cause toxicity. The inner core contains the amino acid *n*-methyltryptophan, abric acid, glycyrrhizin, and abrin. Abrin is stable in the gastrointestinal tract where it is slowly, but erratically absorbed. In rats, distribution sites occur primarily in the liver (12%) and spleen. Biotransformation and elimination of toxalbumins are poorly defined.

Mechanism of Toxicity

Abrin exerts its necrotizing toxic action by attaching itself to the cell membranes and direct inhibition of protein synthesis on the parenchymal cells (e.g., liver and kidney cells) and red blood cells. It is responsible for the toxic effects of the bean by causing inhibition of protein synthesis. It has been determined that abrin does not inhibit mitochondrial respiration *in vitro*, but it does interfere with amino acid incorporation in the liver of rats.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute manifestations of jequirity bean toxicity in animals are similar to those found in humans.

Human

Clinical effects include an initial aggregation of red blood cells within 1 h, severe gastroenteritis accompanied by serosal hemorrhage, swelling, inflammation of Peyer's patches, and retroperitoneal lymph nodes. Hepatic and renal necroses have been reported. Retinal hemorrhages may appear. Abrin combines with the cell stroma and agglutinates red blood cells leading to thrombus and embolus formation. Profound endothelial damage and profound capillary hemorrhage may occur in severe cases. Adrenal insufficiency and adrenal failure may also be noted. Symptoms may begin after a delay of up to several days and may persist for as long as 10 or 11 days.

Clinical Management

Supportive measures, including administration of blood products, parenteral fluid, and electrolytes, are recommended. Removal of the seeds from the gastrointestinal tract can be done with charcoal. Whole bowel irrigation may also be considered after a dose of charcoal for patients with voluminous ingestions. Alkalinization of the urine with sodium bicarbonate has been recommended for the prevention of hemoglobin precipitates in the renal tubules.

See also: Ricin and other Toxalbumins.

Further Reading

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Jet Fuels

Udayan M Apte and Harihara M Mehendale

- SYNONYMS: Jet propellant; JP-4; JP-7; JP-8
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Mixture of aliphatic and aromatic hydrocarbons

Uses

Jet propellants are the most widely used aviation fuels used by United States and other North Atlantic Treaty Organization (NATO) militaries.

Background Information

Jet fuels are aviation fuels used mainly by the United States and other North Atlantic Treaty Organization (NATO) nations for military establishments. Other fuels called Jet A and Jet A-1 are closely related fuels used by commercial airlines. JP are a complex mixture of primarily aliphatic (but also aromatic) hydrocarbons, derived from crude oil and/or kerosene by refining and adding various other additives such as fuel icing inhibitors, antioxidants, corrosion inhibitors, metal deactivators, and static dissipaters. Gas chromatographic analysis of JP-8, the most recent JP, indicates that it is made up of complex mixture of 9 to 17 different hydrocarbons, including thousands of isomers and three to six performance additives. They are generally colorless liquids and smell like kerosene.

Exposure Routes and Pathways

Primary exposure routes to JP are inhalation and dermal exposure to the aircraft maintenance personnel working directly with the JP. Additionally, populations residing near air force bases may be exposed to the JP by inhalations route in the form of vapors and aerosol.

Toxicokinetics

No definitive quantitative data are available on the absorption, distribution, metabolism, and excretion of JP in humans or in experimental animals in inhalation, dermal, or oral route of exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Exposure of male Sprague–Dawley rats to as high as $5000\,mg\,m^{-3}$ via inhalation for 4 h did not produce apparent signs of toxicity or mortality in 2 weeks postexposure observation period. Similarly, 5000 and $8000\,mg\,kg^{-1}$ of acute oral administration of JP-4 did not produce any conclusive data on mortality. Dermal exposure to JP-4 and JP-7 resulted in skin irritation, necrosis, and erythema.

Human

Although no studies have been reported with acute exposure to JP in humans, a case was reported where an air force pilot was exposed to high levels of JP fumes and suffered from immediate intoxication but no long-term adverse effects.

Chronic Toxicity (or Exposure)

Animal

Extensive information has been gathered on subchronic and chronic exposures of JP in laboratory rodents, mainly via inhalation and dermal routes of exposure. No mortality has been observed related to chronic exposure to JP in animals but significant systemic effects have been recorded. F344 rats exposed to $500–1000\,mg\,m^{-3}$ of $24\,h\,day^{-1}$ of JP-4 for periods of 90 days to 6 months demonstrated increases in liver and kidney weight along with fatty degeneration in the liver. In the same study, renal tubular hyperplasia, hyaline degenerations, and α-2μ-globulin nephropathy were reported. Subchronic exposure to JP induced decrease in white blood cell counts in rats. A 1 year exposure to JP in mice resulted in nesolacrimal hyperplasia and testicular atrophy at the end of 12 months exposure. Although a 8 months inhalation exposure to JP-4 in F344 rats did induce intestinal tumors no other cancers were reported in any of the species (rats and mice) tested. Therefore, the risk of cancer occurrence by inhalation exposure to JP is considered minimal.

Dermal exposure to JP resulted in irritation, necrosis of skin, and visible separation and sloughing of the skin. A 105 weeks dermal exposure to mice resulted in increased incidence of squamous cell carcinoma and fibrosarcoma.

Human

No data are available on the human exposure to JP via the dermal route.

Ecotoxicology

No data are available on the effects of environmental exposure of JP on any animal species or plants but

considerable evidence exists that JP is biodegradable, mainly by bacteria, in the environment.

See also: Occupational Exposure Limits; Occupational Toxicology.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Jet Fuels.

Jimsonweed

Brenda Swanson-Biearman

- Chemical Abstracts Service Registry Number: CAS 8063-18-1
- Synonyms: *Datura* species; *Datura arborea*; *Datura cornigera*; *Datura folium; Datura suaveolens*; *Datura stramonium*; Angel's trumpet; Downy thorn apple; Horn-of-plenty; Stinkweed; Thornapple; Black henbane
- Chemical/Pharmaceutical/Other Class: Anticholinergic

Uses

Datura species are abused for psychedelic properties. Historically, *D. Stramonium* had been used by the American Indians as a folk medicine and in religious activities. *Stramonium* has been used in homeopathic asthma preparations. *Datura* does not have a therapeutic use.

Exposure Routes and Pathways

Exposure occurs via ingestion of the seeds, tea made from seeds, or smoking leaves.

Toxicokinetics

Studies of the pharmacokinetics of Jimsonweed are incomplete. Decreased gastrointestinal motility may delay or prolong absorption.

Mechanism of Toxicity

The toxins in Jimsonweed are tropane belladonna alkaloids possessing strong anticholinergic properties. They include: hyoscyamine (leaves, roots, seeds); hyoscine (roots); atropine (d,l-hyoscyamine), and scopolamine (l-hyoscine). They act as competitive antagonists to acetylcholine at peripheral and central muscarinic receptors at a common binding site. The peripheral receptors are on exocrine glands, affecting perspiration, salivation, smooth and cardiac muscle. As tertiary amines, there is central nervous system (CNS) absorption, inhibition of CNS receptors, and resultant central anticholinergic syndrome of acute psychosis or delirium.

Acute and Short-Term Toxicity (or Exposure)

Animal

In farm animals, muscle tremors, ataxia, drowsiness, tachypnea, and sudden death have been reported.

Human

Common symptoms of exposure include mydriasis, sinus tachycardia, hypertension or hypotension, anxiety, hallucinations, psychoses, choreoathetosis, delirium, seizures, dry mouth, flushed skin, decreased gastrointestinal motility, ileus, urinary retention, and hyperpyrexia. Anticholinergic agents may be detected in the urine, but this does not direct clinical management. Due to multiple plant variations, the alkaloid content differs greatly.

Clinical Management

Gastric decontamination with activated charcoal up to 36 h after ingestion may be useful. Supportive care is the cornerstone of therapy. Sedation with benzodiazepines may control tachycardia associated with agitation and hallucinations. Esmolol can be considered for the treatment of hemodynamically compromising tachyarrhythmias. Physostigmine may be used in the presence of severe incapacitating or life-threatening anticholinergic effects that are unresponsive to conventional therapies.

See also: Acetylcholine; Anticholinergics; Atropine.

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Joint FAO/WHO Expert Meetings (JECFA and JMPR)

Angelika Tritscher

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History

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) are international scientific expert committees that are administered jointly by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations. Their purpose is to perform the toxicological evaluation of chemicals in food.

JECFA has been meeting since 1956, initially to evaluate the safety of food additives. Its mandate has been expanded to contaminants, natural toxins, and residues of veterinary drugs in food. The Committee has also developed principles for the safety assessment of chemicals in food that are consistent with the current thinking on risk assessment, and take account of recent developments in toxicology and other relevant sciences. These principles were originally published in 1987, as Environmental Health Criteria 70: Principles for the safety assessment of food additives and contaminants in food.

JMPR has been meeting regularly since 1963 to evaluate pesticide residues in food. The Meeting has also developed principles originally published in 1990, as Environmental Health Criteria 104: Principles for the toxicological assessment of pesticide residues in food.

The principles for evaluations have been continuously reviewed and updated to take account of new scientific knowledge. FAO and WHO have recently initiated a project to update and consolidate principles for the assessment of food additives, contaminants, residues of veterinary drugs in food, and pesticide residues in food.

Mission and Purpose

JECFA and JMPR provide independent, international scientific advice on chemicals in food, including their toxicological evaluation.

All countries need to have access to reliable risk assessments of chemicals in food, but relatively few have the expertise and funds available to carry out separate risk assessments on large numbers of chemicals. In this context, JECFA and JMPR serve as scientific advisory bodies to FAO, WHO, to FAO and WHO member governments, and to the Codex Alimentarius Commission.[1] The risk assessments provided by JECFA and JMPR form the basis for food standards, on national, regional, or international level.

Procedures and Membership of the Scientific Committees

The selection of members is made only after a careful consideration of the scientific credentials of the various candidates. Individuals participate as independent scientific experts, and do not represent any country or organization. A balance of scientific expertise and other experience is considered essential. FAO and WHO meet the costs of experts' attendance at JECFA and JMPR meetings.

JECFA

JECFA normally meets twice a year with individual agendas covering either (1) food additives including flavors, contaminants, and naturally occurring toxicants in food, or (2) residues of veterinary drugs in food. The membership of the meetings varies accordingly, with different sets of experts being called upon depending on the subject matter of the meeting. FAO and WHO have complementary functions in selecting members for JECFA. FAO is responsible for selecting members to deal with (1) the development of specifications for the purity of food additives and (2) the assessment of residue levels of veterinary drugs in

[1] The Codex Alimentarius Commission was created in 1963 by FAO and WHO to develop food standards, guidelines, and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. The main purposes of this Programme are protecting health of the consumers, ensuring fair trade practices in the food trade, and promoting coordination of all food standards work undertaken by international governmental and nongovernmental organizations.

food. WHO is responsible for selecting members to perform the toxicological evaluations of the substances under consideration. Both FAO and WHO invite members who are responsible for assessing intakes.

For food additives including flavours, contaminants, and naturally occurring toxicants, the Committee:

- Elaborates principles for evaluating their safety.
- Conducts toxicological evaluations and establishes acceptable daily intakes (ADIs) or tolerable intakes.
- Prepares specifications of purity for food additives.
- Assesses intake.

For residues of veterinary drugs in food, the Committee:

- Elaborates principles for evaluating their safety.
- Establishes ADIs and recommends maximum residue limits (MRLs).
- Determines criteria for the appropriate methods of analysis for detecting and/or quantifying residues in food.

JMPR

JMPR usually meets once every year. FAO and WHO call on experts with complementary responsibilities. The FAO experts are responsible for reviewing the residue and analytical aspects of the pesticides under consideration, including data on their metabolism, fate in the environment, and use patterns, and for estimating the maximum residue levels that might occur as a result of the use of the pesticides according to good agricultural practices. The WHO experts are responsible for reviewing toxicological and related data in order to estimate, where possible and considered necessary, ADIs for chronic intake or acute reference dose (ARfD) for short term (24 h) acute intake of the pesticides under consideration.

In summary, for pesticide residues in food JMPR experts:

- Elaborate principles for evaluating their safety.
- Establish ADIs, ARfDs, and recommend MRLs.
- Determine criteria for the appropriate methods of analysis for detecting and/or quantifying residues in food.

Evaluations

JECFA

To date, JECFA has evaluated more than 1500 food additives, ~40 contaminants and naturally occurring toxicants, and residues of ~90 veterinary drug residues in food.

For food additives, JECFA normally establishes ADIs on the basis of available toxicological and other relevant information. Specifications of purity are also developed for food additives, which help to ensure that the product in commerce is of appropriate quality, can be manufactured consistently, and is equivalent to the material that was subjected to toxicological testing.

For contaminants and naturally occurring toxicants, levels corresponding to 'tolerable' intakes such as the provisional maximum tolerable daily intake (PMTDI) or provisional tolerable weekly intake (PTWI) are established when there is an identifiable no-observed-effect level, that is, a threshold of effect can be assumed based on available data. When a no-observed-effect level cannot be identified, the Committee provides other advice, such as identification of the food(s) that contributes most to intake. This allows for targeted management actions in order to decrease exposure.

With veterinary drugs, data on good practice in the use of veterinary drugs are evaluated and corresponding MRLs in animal tissues, milk, and/or eggs are recommended. Such MRLs are intended to provide assurance that when the drug has been used properly, the intake of residues of the drug present in food is unlikely to exceed the ADI.

JMPR

To date, JMPR has evaluated ~240 pesticides, many of them repeatedly. JMPR establishes ADIs (based on chronic toxicity) and acute reference doses (based on acute toxicity) on the basis of the toxicological data and related information available on the substances that are being evaluated. In addition, JMPR reviews pesticide use patterns, data on the chemistry and composition of pesticides, and methods of analysis of pesticide residues. It recommends MRLs for pesticides that occur in food commodities following their use according to Good Agricultural Practice. The potential intake of pesticide residues is compared with the ADI and acute reference dose to estimate the potential dietary risks associated with the adoption of the MRLs.

In recent years, the scope of the toxicological evaluations has been expanded to include assessment of other routes of exposure that are relevant for public and occupational health. In addition, some environmental hazard assessments have been performed.

Reports and Publications

Summary Reports

A summary report of the meetings is published electronically on the FAO and WHO websites within

2 weeks of the meetings. It provides basic details relating to acceptable or tolerable intakes (ADIs/TDIs) and other toxicological conclusions, and specifications or MRLs.

Reports

The conclusions of JECFA meetings are summarized in reports published in the WHO Technical Report Series. Reports reflect the agreed view of the Committee as a whole, and describe the basis for the conclusions. In the very rare event in which some members cannot accept all of the conclusions, a minority report may be included as an annex. These reports are usually published 6–8 months after the meeting. The conclusions of JMPR meetings are summarized in reports published in the FAO Plant Production and Protection Paper series. Reports reflect the agreed view of the Committee as a whole, and describe the basis for the conclusions. JMPR reports also include the dietary risk assessments.

Monographs

JECFA Toxicological and intake monographs are published after JECFA meetings in the WHO Food Additive Series. These summarize all the data used in the Committee's risk assessments and provide full references to the relevant literature. Specifications for the identity and purity of food additives, which are accessible electronically (see Relevant Websites section), and monographs on veterinary drug residues summarizing the data used for recommending MRLs, are published in the FAO Food and Nutrition Paper (FNP) series. Specifications for food additives are published in a Compendium and addenda to FNP 52, and residues monographs on the assessment of veterinary drugs are published in addenda to FNP 41 (see Relevant Websites section).

JMPR Toxicological evaluations on pesticides residues in food are published by the WHO International Programme on Chemical Safety (IPCS). These summarize all the data used in JMPR's risk assessments and provide full references to the relevant literature.

Residues monographs, which contain information on pesticide use patterns, data on the chemistry and composition of pesticides, methods of analysis for pesticide residues, and information on MRLs, are published in the FAO Plant Production and Protection Paper series.

Most of the JECFA and JMPR toxicological monographs are available online through IPCS INCHEM (see Relevant Websites section).

Further information, including future agenda, call for data, and call for experts, is available at the websites listed in the Relevant Websites section.

See also: Acceptable Daily Intake (ADI); Food Additives; Food and Agriculture Organization of the United Nations; Food and Drug Administration, US; Pesticides.

Relevant Websites

http://www.who.int – The Chemicals in Food Programme focuses on principles and methods for the assessment of chemicals in food.

http://www.fao.org – JECFA at FAO is within the Food and Nutrition division which publishes specifications for food additives in a Compendium and addenda to FNP 52, and residues monographs on the assessment of veterinary drugs are published in addenda to FNP 41. JMPR is within the Plant Production and Protection Division.

http://www.inchem.org – Most of the JECFA and JMPR toxicological monographs are available online through IPCS INCHEM.

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Kava

Molly Broderick and Teresa Dodd-Butera

- SYNONYMS: Kava-Kava; Kawa; Ava; Awa; Intoxicating pepper; *Piper methysticum*

Uses

Kava is used in the South Pacific islands as a beverage to induce relaxation prior to ceremonies. It may also be used as a sedative and to treat anxiety. The herb has been postulated to have anticonvulsant, antifungal, aphrodisiac, and antiseptic properties.

Background Information

Kava is a dried black pepper root from the *Piper methysticum* species found in Polynesia and Micronesia but widely available in Europe and the United States as a herbal medicine.

Exposure Routes and Pathways

Kava is available as a powder, capsule, tincture, extract, or root. Concentrations of active ingredients vary. The root extract has a higher available distribution than single compounds.

Mechanism of Toxicity

The active constituents are resinous compounds called kava lactones. The exact mechanism of action is unclear though it is thought to act on GABA receptors resulting in the sedative effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animal studies, kava lactones decreased muscle action potentials and disrupted voltage-gated Na^+ channels. *In vitro*, kava was found to block norepinephrine intake. In addition, inhibition of certain types of cDNA P450 isoforms was demonstrated.

Human

Toxic effects can include sedation, shortness of breath, alteration in blood pressure, conjunctival redness, visual disturbances, ataxia, and oral paresthesias. Isolated cases of psychotic episodes and dystonic reactions have occurred.

Chronic Toxicity (or Exposure)

Animal

Due to a lack of sufficient scientific evidence regarding safety, further tests are needed to evaluate genotoxicity, reproductive toxicity, neurotoxicity, chronic and carcinogenicity.

Human

Scientific reports address a risk of liver and renal dysfunction. Hepatic failure, hepatitis, and cirrhosis have been reported with chronic use. The most common side effect from chronic, large doses of kava is a scaly skin rash called kava dermopathy.

Clinical Management

Liver and renal function tests should be monitored in symptomatic patients or patients on chronic usage of the herb. Individuals using kava regularly should be cautioned about the potential for adverse interactions with other pharmacologically active substances, including ethanol, barbiturates, and some benzodiazepines. A toxic dose has not yet been established.

Further Reading

Der Marderosian A (ed.) (1996) Kava monograph: *The Lawrence Review of Natural Products*. St. Louis, MO: Facts and Comparisons.

Goldfrank L, Flomenbaum N, Lewin N, *et al*. (eds.) (2002) *Goldfrank's Toxicologic Emergencies*, 7th edn. New York: McGraw-Hill Medical Publishing Division.
Heiligenstein E and Guenther G (1998) Over-the counter psychotropics: A review of melatonin, St. John's wort, valerian, and kava-kava. *Journal of American College of Health* 46: 271–276.
Olson K (ed.) (1999) *Poisoning and Drug Overdose. Appleton and Lange*. CT: Stamford.
Piper methysticum (kava kava). *Alternative Medicine Review* 1998; 3(6): 458–460.

Relevant Websites

http://www.cfsan.fda.gov – Consumer advisory: Kava-containing dietary supplements may be associated with severe liver injury, 3/25/02.
http://www.cdc.gov – Centers for Disease Control.

Kerosene

Shayne C Gad

This article is a revision of the previous print edition article by Gary P Bond, volume 2, pp. 197–198, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 8008-20-6
- Synonyms: Straight-run kerosene (petroleum); Range oil; Fuel oil no. 1; Deobase
- Chemical/Pharmaceutical/Other Class: Petroleum hydrocarbon
- Chemical Formula: Kerosene is a mixture of petroleum hydrocarbons, chiefly of the methane series having from 10 to 16 carbon atoms per molecule. It constitutes the fifth fraction in the distillation of petroleum. C_xH_{2x+2}

Uses

Kerosene, originally used for lighting and heating, is also used as a diesel fuel, as a component in blending aviation fuels, as a solvent and carrier for a wide range of products (including cleaning compositions and pesticides), and as a mold-release agent in the ceramic and pottery industry.

Exposure Routes and Pathways

Kerosene may enter the water or soil environment as a result of regular use (e.g., evaporation of pesticide solvent), from spills during use or transportation, or from leaking storage facilities. The relatively low vapor pressure of kerosene makes inhalation exposure unlikely under ordinary occupational conditions unless conditions of poor ventilation exist. The combustion product of burned kerosene, carbon monoxide, is of real concern when kerosene heaters are not vented. Exposure to kerosene mist can occur as kerosene is often applied in the form of a spray. Eye and skin contact with kerosene and kerosene mists and vapors can occur. The exposure pathway usually of most concern is ingestion because this is the most common means of acute poisoning, especially in children.

Toxicokinetics

No or little quantitative data are available concerning the absorption, distribution, metabolism, and excretion of kerosene. Indirect evidence suggests that kerosene may be absorbed through the respiratory tract, the gastrointestinal tract, and percutaneously.

Mechanism of Toxicity

The specific mechanism of toxicity of kerosene has not been completely determined. The primary risk from ingestion of kerosene is aspiration during emesis, which may cause pneumonitis. The biochemical mechanism of lung response to large concentrations of aerosolized kerosene (resulting in bronchoconstriction and asthma-like symptoms) may involve the parasympathetic nervous system via a direct effect on the vagus nerve or by inhibition of acety1cholinesterase. The mechanism(s) of central nervous system (CNS) depression from kerosene exposure has not been elucidated, but undoubtedly includes disruption of the membranes of nerve cells.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicities (LD_{50}/LC_{50}) for kerosene are $>5\,g\,kg^{-1}$ (oral; rats), $>2\,g\,kg^{-1}$ (dermal; rabbit), and $>5\,mg\,l^{-1}$ per 4 h (inhalation; rats). Skin exposure for more than 4 h resulted in mild to severe irritation in rabbits and other experimental animals. Prolonged eye exposure caused mild irritation in rabbits. Skin sensitization did not occur in guinea pigs when treated with kerosene.

Human

Kerosene is of low-order toxicity following oral, dermal, or inhalation exposure. Symptoms from exposure to high levels of kerosene may include hypoactivity, ataxia, and prostration, consistent with CNS depression, including coma and death. Pneumonia (chemical pneumonitis) is the major lethal complication after ingestion of kerosene due to aspiration into the lungs after vomiting or emesis. Tachycardia, nausea, abdominal cramps, and diarrhea have also been associated with the ingestion of kerosene. Skin irritation, which can be severe, can occur especially after prolonged or repeated exposure. Respiratory tract irritation may occur after inhalation of mists or aerosols. Slight eye irritation may occur if exposure is prolonged. Kerosene was not identified as a skin sensitizer in experimental animals.

Chronic Toxicity (or Exposure)

Animal

Chronic dermal applications of kerosene caused skin carcinoma in mice. Kerosene has been reported to have weak cancer-promoting activity but no cancer-initiating activity. Repeated applications of kerosene to the skin of laboratory animals caused moderate to severe skin irritation with an increase in skin tumors after long latency periods. The increase in skin tumors was considered to be the result of the severe skin damage. This explanation is consistent with the general lack of activity of kerosene in genotoxicity assays.

Human

Kerosene can cause chemical pneumonia. Prolonged or repeated contact of the skin with kerosene may result in drying of the skin and dermatitis, which may lead to severe skin damage with degenerative changes. Repeated inhalation of kerosene vapors may cause symptoms consistent with CNS depression such as headache and vertigo. Other symptoms reported in experimental animals and/or humans after repeated exposure to kerosene include neuralgia, loss of memory, blood changes, kidney effects, and respiratory disturbances.

Clinical Management

Asymptomatic individuals should be observed for 48 h after exposure for the development of symptoms. Respiratory and cardiovascular functions should be supported in symptomatic individuals. If there is suspicion of aspiration, breathing should be observed and the patient should be treated symptomatically.

If kerosene has been ingested, nothing should be administered by mouth and vomiting should not be induced. Gastric decontamination is not usually indicated after ingestion due to the possibility of aspiration. If necessary, gastric lavage must only be performed after cuffed endotracheal intubation. Treatment should be symptomatic. Antibiotic and corticosteroid therapy may be considered for treatment of possible chemical pneumonitis resulting from aspiration. If aspiration is expected to have occurred (i.e., a coughing symptomatic individual), arterial blood should be monitored to ensure adequate ventilation. The development of pulmonary edema may be delayed in onset up to 24–72 h after exposure.

For skin contact, affected areas should be washed thoroughly with soap and water. For eye contact, the eyes should be gently flushed with copious amounts of water for at least 10 min.

Environmental Fate

Kerosene is biodegradable in soil although some components of the mixture adhere strongly to the soil. Kerosene is also biodegradable in surface water. However, some components of the mixture may bioconcentrate in fish and other aquatic organisms.

Exposure Standards and Guidelines

The recommended exposure limit, 10 h time-weighted average, is $100\,\mathrm{mg\,m^{-3}}$.

See also: Petroleum Hydrocarbons; Pollution, Water.

Further Reading

Gerarde HW (1959) Toxicological studies and recommended treatment of kerosene poisoning. *Toxicology and Applied Pharmacology* 1: 462–490.

Ritchie G, Still K, Rossi J, *et al.* (2003) Biological and health effects of exposure to kerosene-based jet fuels and performance additives. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews* 6(4): 357–451.

Segev D, *et al.* (1999) Kerosene-induced severe acute respiratory failure in near drowning: Reports on four cases and review of the literature. *Critical Care Medicine* 27(8): 1437–1440.

Kidney

Gary O Rankin

Introduction

The kidney is an organ that performs several important functions essential to sustain life. These functions include the regulation of volume and electrolyte homeostasis, control of acid–base balance, and the excretion of waste products. The kidney also has endocrine functions including renin secretion, stimulation of erythropoietin formation, and activation of vitamin D. Numerous disease states (e.g., infections, shock, diabetes, gout) can affect the ability of the kidney to perform its normal functions, and if these diseases are not properly treated, serious illness or death can result.

The kidney is also a major target organ for the toxic effects induced by numerous chemical and physical agents. Renal toxicity or nephrotoxicity can be the result of a direct toxic effect of an agent on renal tissue (e.g., mercuric salts, cyanide ion) or a secondary event following toxicity or tissue damage at a site other than the kidney (e.g., carbon monoxide poisoning, crush injury). The kidney may also be damaged by toxic metabolites that originate at renal or extrarenal sites. In addition, the nephron, the functional unit of the kidney, is composed of several distinct anatomical segments. Some compounds or their metabolites induce toxicity to only one segment of the nephron and are considered to be site-selective nephrotoxicants, while other agents may induce widespread renal damage and are considered to be nonsite-selective toxicants.

An understanding of how and why chemicals induce nephrotoxicity requires some familiarity with the anatomy and physiology of the kidney. In addition, interpretation of renal toxicology studies will require a working knowledge of the various techniques used for evaluating renal function. It is also important to be aware of which nephrotoxicants require biotransformation before they induce nephrotoxicity, nephrotoxic mechanisms when known, and the site(s) of renal damage for the various nephrotoxicants.

Renal Anatomy and Physiology

In mammalian species, the two kidneys carry out the normal physiological and endocrine functions described above. The kidney receives blood via the renal artery, and blood leaves the kidney by way of the renal vein. In adult humans, the rate of blood flow through both kidneys is $\sim 1.2\,\mathrm{l\,min^{-1}}$ or $\sim 20\%$ of the cardiac output for a 70 kg individual. Urine formed within the kidneys is transported from kidneys through the ureters to the bladder, a reservoir for the urine until it is excreted.

Each kidney is subdivided anatomically into three zones: (1) an outer zone called the cortex, (2) an intermediate zone called the medulla, and (3) an innermost zone called the pelvis. The cortex and medulla have important characteristics that help facilitate the formation of urine and control the composition of waste products, electrolytes, and water.

Each kidney also contains over one million nephrons, which are the functional units of the kidney. Nephrons originate in the cortex where an afferent arteriole forms a specialized capillary bed known as the glomerulus (**Figure 1**). Some nephrons originate near the surface of the kidney and are called superficial nephrons, while other nephrons originate near the cortical-medullary region and are called juxtamedullary nephrons.

The glomerulus, which forms within Bowman's capsule, is a special capillary bed with large pores, and substances that are not bound to plasma proteins and have molecular weights less than albumin ($\sim 69\,000$) can be filtered at the glomerulus and enter Bowman's capsule. This glomerular filtrate is essentially protein-free plasma, although a small amount of low molecular weight protein is also filtered by the glomerulus. The rate at which the glomerular filtrate is formed, the glomerular filtration rate (GFR), is $\sim 125\,\mathrm{ml\,min^{-1}}$ in humans and serves as an important measure of renal function.

The glomerular filtrate flows from Bowman's capsule into the proximal tubule. The proximal tubule can be subdivided into three segments (S_1, S_2, and S_3). The S_1 segment is closest to the glomerulus and is localized in the cortex, while the S_3 segment is furthest from the glomerulus and is found in both the cortex and the outer portion of the medulla. The S_1 and S_2 segments comprise the pars convoluta, while the latter portion of the S_2 segment and the S_3 segment comprise the pars recta or straight portion.

The proximal tubule cells contain numerous finger-like projections on the luminal surface, which markedly increase the luminal surface area of the cells and help promote reabsorption of substances filtered at the glomerulus. Under normal conditions,

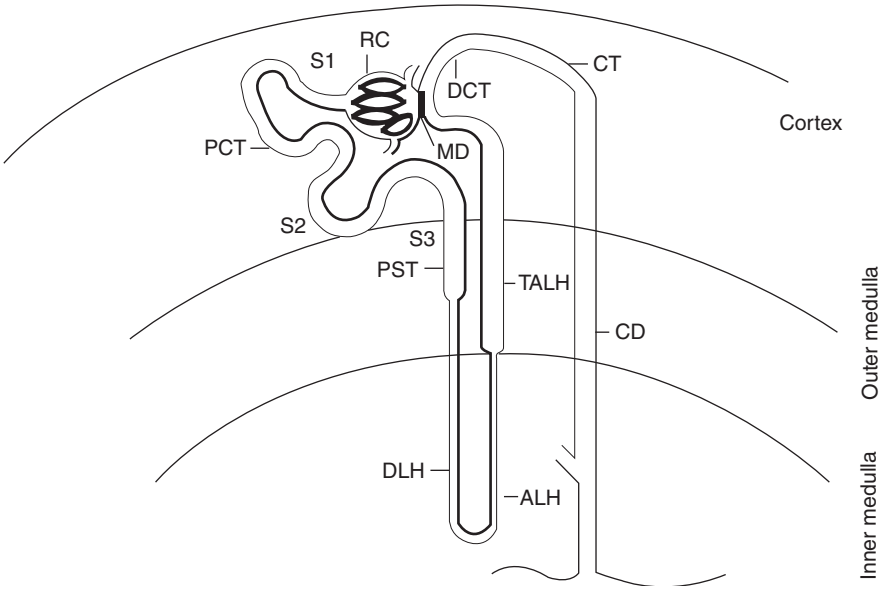

Figure 1 Anatomy of the nephron. RC, renal corpuscle (includes glomerulus and Bowman's capsule); PCT, proximal convoluted tubule; PST, proximal straight tubule; DLH, descending limb of the loop of Henle; ALH, ascending limb of the loop of Henle; TALH, thick ascending limb of the loop of Henle; MD, macula densa; DCT, distal convoluted tubule; CT, connecting tubule; CD, collecting duct.

~65–70% of filtered sodium, chloride, calcium, and water; 80–90% of filtered bicarbonate, phosphate, and urate; and essentially all of the filtered amino acids, glucose, and low molecular weight proteins are reabsorbed from the glomerular filtrate as the filtrate passes the length of the proximal tubule. Reabsorption occurs without a major change in the osmolality of the filtrate such that the fluid leaving the S_3 segment of the proximal tubule has essentially the same osmolality as the fluid entering the S_1 segment. However, the quantity of the glomerular filtrate is markedly reduced during its transit through this nephron component.

Proximal tubule cells also contain active transport systems on the basolateral or antiluminal side of the cells, which are capable of transporting weakly acidic or weakly basic compounds from the interstitial space into the proximal tubular cells. These transport systems are distinct systems and transport either organic anions (weakly acidic compounds) or organic cations (weakly basic and quaternary ammonium compounds). As a result of transport into proximal tubule cells, weak acids and bases can achieve intracellular concentrations that are hundred times higher than the corresponding plasma concentration for the compound. The prototypic organic anion for studying the organic anion transporter is *p*-aminohippurate (PAH), and the prototypic organic cations for examining organic cation transport are *N*-methylnicotinamide (NMN) and tetraethylammonium (TEA). Accumulated material can then enter luminal fluid via either passive diffusion or facilitated transport.

The location of the organic anion and cation transport systems is not homogeneous along the entire proximal tubule. In the rabbit, PAH transport is greatest in the S_2 segment and lower in the S_1 and S_3 segments. In contrast, organic cation transport is highest in the S_1 segment and lowest in the S_3 segment. In superficial nephrons, the transport of organic cations in the S_2 segment is intermediate between the S_1 and S_3 segments, but is about equal to the capacity of S_1 segments in juxtamedullary nephrons. Although the organic anion and cation transporters are clearly localized in the proximal tubules for all mammalian species studied to date, the segmental localization of these transporters has not been studied in great detail in species other than the rabbit. Nevertheless, the ability of many nephrotoxicants to induce nephrotoxicity to proximal tubular segments is dependent on the compound or its metabolites being accumulated in proximal tubule cells via one of these organic transport systems. The function of these systems also serves as a sensitive measure of renal function and is routinely used to monitor renal function in animal models.

Luminal fluid leaving the proximal tubule enters the descending limb of the loop of Henle. This nephron segment passes deeper into the medulla than the proximal tubule, and the high medullary interstitial osmolality causes water to move from the luminal fluid and into the medullary interstitial

space. As a result, the luminal fluid becomes more concentrated as it passes through the descending limb. Organic compounds (e.g., sulfonamides, methotrexate), which have poor water solubility, can precipitate in this nephron segment once their water solubility limit is reached, and they block luminal flow and glomerular filtration.

When the luminal fluid reaches the thick ascending limb of the loop of Henle, water no longer can freely move from the luminal fluid into the medullary interstitial space. Instead, this portion of the nephron is impermeable to water reabsorption and actively reabsorbs sodium, chloride, and potassium ions. Approximately 20–25% of filtered sodium and calcium ions are reabsorbed at this location. In addition, most, if not all, of the potassium ions reaching the thick limb of the loop of Henle are reabsorbed as well. Thus, as the luminal fluid passes through the ascending limb, the luminal fluid becomes more dilute.

Although the ascending limb of the loop of Henle begins in the medulla, it ends in the cortex where it joins the distal tubule. Sodium chloride is also reabsorbed in the early segment of the distal tubule, and the combination of this segment with the cortical portion of the thick ascending limb of the loop of Henle is referred to as the cortical diluting segment of the nephron. In the late distal tubule, sodium ions are reabsorbed in exchange for potassium or hydrogen ions. The secretion of hydrogen ions in exchange for sodium ions results in the acidification of the urine. The process of sodium exchange is partially under the control of mineralocorticoids (e.g., aldosterone) and accounts for the reabsorption of 2–3% of filtered sodium ions.

The kidney also has the ability to respond to changes in the GFR through the action of specialized distal epithelial cells called the macula densa. These cells are in close contact with the glomerular apparatus of the same nephron and can detect even small changes in the flow of luminal fluid. Increases in the flow rate activate the macula densa cells to communicate with the granular cells and vascular components of the juxtaglomerular apparatus and stimulate the release of renin. Renin release results in the formation of the vasoactive peptide angiotensin II and subsequent vasoconstriction that leads to a decrease in the GFR and the luminal flow rate. Thus, the distal tubule is not only important for urine formation, but also plays a role in regulating the GFR.

Another important urinary regulatory mechanism involves the effects of vasopressin (AVP) also known as antidiuretic hormone (ADH). A decrease in blood pressure or an elevation in plasma osmolality will result in the release of ADH from the posterior pituitary. ADH is carried by the blood to the nephron where ADH increases the reabsorption of water from the collecting tubule. Thus, in the presence of ADH, water reabsorption is increased and urine becomes more concentrated, while decreased ADH secretion will result in diuresis and a more dilute urine.

Mechanisms of Nephrotoxicity

The kidney is a target organ for many chemicals, in large part because of the physiology of the kidney described above. The substantial blood flow through the kidneys results in the kidneys being exposed to significant amounts of chemicals and their metabolites. The ability of the proximal tubular cells to transport organic anions and cations can lead to the intracellular accumulation of weakly acidic or basic chemicals as well as amino acid conjugates and quaternary compounds (e.g., paraquat) in this renal nephron segment. Such accumulations can eventually lead to toxic levels of the chemical in proximal tubular cells. In addition, the large demand for energy to support the reabsorption and secretion processes makes the kidney particularly susceptible to compounds (e.g., cyanide ion) which inhibit the production of cellular energy and/or oxygen utilization.

There are several mechanisms by which renal toxicity can be induced (**Table 1**). Direct tubular toxicity is one of the most common mechanisms by which chemicals or their toxic metabolites induce nephropathy. Proximal tubule cells are especially susceptible to toxicity via this mechanism because (1) the proximal tubule is one of the first segments of the nephron to be exposed to toxicants and (2) these cells can accumulate toxicants by actively transporting the

Table 1 Mechanisms of nephrotoxicity

Direct tubular toxicity
Examples: amphotericin B, aminoglycosides, cephaloridine, cisplatin, heavy metals, cysteine conjugates, 4-aminophenol, many others
Altered renal hemodynamics
Examples: angiotensin converting enzyme (ACE) inhibitors, cyclosporine, nonsteroidal antiinflammatory drugs (NSAIDs)
Tubular obstruction
Examples: sulfonamides, methotrexate, oxalic acid, acyclovir
Tubulointerstitial nephritis
Examples: analgesics, penicillins, cephalosporins, heavy metals, cisplatin, nitrosoureas, NSAIDs, cimetadine, many others
Glomerular injury
Examples: heavy metals, D-penicillamine, captopril, methimazole, heroin, puromycin aminonucleoside
Fluid/electrolyte imbalance
Examples: chlorpropamide, lithium, captopril, NSAIDs, fluoride, diuretics, ACE inhibitors

toxicants into this tubular segment and achieving high intracellular concentrations of toxic chemical species or their precursors. The exact mechanism of direct tubular toxicity or the critical cellular target is not known with certainty for most toxicants. However, nephrotoxicants can induce direct toxicity by several mechanisms including (1) alkylation of cell macromolecules (e.g., phosgene formed from chloroform), (2) complexation with cellular sulfhydryl groups and other ligands (e.g., heavy metals), (3) generation of free radials and/or initiation of lipid peroxidation (e.g., cephaloridine, paraquat), or (4) disruption of mitochondrial function and energy production (e.g., cysteine conjugates, cyanide).

Nephrotoxicity can also occur following an alteration in renal hemodynamics. Since renal blood flow (RBF) is important for maintaining a steady supply of oxygen and nutrients to renal cells, a reduction in blood flow to the kidney can result in decreased oxygen delivery (ischemia), decreased energy production, and decreased renal function. The vasodilatory prostaglandins PGE_2 and PGI_2 can be important in maintaining proper renal perfusion. Inhibition of renal cyclooxygenase by administration of nonsterodial antiinflammatory drugs (NSAIDs), such as indomethacin, can result in a decrease in production of the vasodilatory prostaglandins, overriding renal vasoconstriction, and ultimately renal failure. In disease states, such as congestive heart failure and cirrhosis with ascites, where renal perfusion may be augmented by the synthesis of PGE_2, NSAIDs can cause reversible acute renal failure.

The obstruction of renal tubules can occur following the precipitation of compounds with low water solubility. The obstruction of tubules prevents the filtration of blood at the glomerulus and can lead to oliguric acute renal failure characterized by a decrease in urine volume and a rise in the blood urea nitrogen (BUN) concentration. Precipitation of the toxicant occurs as the luminal fluid passes through the descending limb of the loop of Henle and the fluid becomes more concentrated. When the solubility limit of the toxicant is exceeded, the toxicant begins to precipitate and obstruct flow. Tubular obstruction was occasionally seen following the use of some of the early sulfonamide antimicrobial agents, but is less of a problem with the currently used drugs. High-dose methotrexate therapy or rapidly infused acyclovir can also result in tubular obstruction. The use of cancer chemotherapeutic agents can lead to rapid cell killing and the delivery of large amounts of uric acid to the kidney. Uric acid is particularly prone to deposit in acidic urine so that therapy to prevent uric acid deposition can include alkaline diuresis, hydration of the patient with intravenous fluid administration, and allopurinol, an inhibitor of xanthine oxidase, which decreases uric acid formation. Oxalic acid, which is formed from the biotransformation of ethylene glycol and other compounds, can also deposit in renal tubules and may contribute to the nephrotoxicity induced by these agents.

Tubulointerstitial nephritis can be either acute or chronic in nature. Acute interstitial nephritis is characterized by an acute renal interstitial inflammatory response with urinary eosinophils and nonoliguric acute renal failure. The more common drugs that induce acute interstitial nephritis include penicillins, rifampicin, sulfonamides, and cimetadine. Chronic tubulointerstitial nephritis is most commonly associated with the long term use of large amounts of analgesics and antiinflammatory agents (e.g., NSAIDs).

Glomerular toxicity is frequently seen as a 'leaky sieve' effect. Normally, the glomerulus serves as a barrier to high molecular weight ($>50\,000$–$60\,000$) proteins, however, when the glomerulus is damaged, proteinuria can be observed. Chemically induced glomerular disease is frequently immune mediated with the observation of immunoglobulin and complement deposits in renal biopsies noted in some cases. Heavy metals such as mercuric salts may induce their glomerular effects via an immune mediated pathway, but the exact mechanism of this effect is unclear.

Many drugs and other chemicals can adversely affect renal function by directly or indirectly affecting the reabsorption of electrolytes and water in the kidney. Chlorpropamide can enhance the secretion of ADH and promote the water conservation actions of the hormone, while lithium use can lead to a nephrogenic diabetes insipidus. NSAIDs block the formation of renal prostaglandins, which can result in hyperkalemia. Hyperkalemia may also result from the use of beta blockers, potassium-sparing diuretics, and cyclosporine.

Methods for Evaluating Nephrotoxicity

There are numerous methods to determine the nephrotoxic potential of a chemical or to study the mechanism(s) by which a chemical induces nephrotoxicity. In humans, the concern is most often related to either drug-induced or occupationally associated nephrotoxicity. Evaluation of nephrotoxicity in humans is limited primarily to the measurement of urinary changes (e.g., volume, enzymes, protein, etc.), BUN or serum creatinine concentrations, creatinine clearance, or renal biopsy. The measurement of an increase in urinary *N*-acetyl-β-D-glucosaminidase (NAG) or alanine aminopeptidase (AAP) levels,

enzymes localized primarily on proximal tubule cells, has been used as an early marker for chemotherapy-induced nephrotoxicity. However, in laboratory animals, many techniques and models are available for monitoring renal function both *in vivo* and *in vitro*.

In Vivo Techniques

There are two general models for evaluating the nephrotoxic potential of chemicals that utilize whole animals. In one model, conscious animals are administered the test compound and renal functional parameters (**Table 2**) evaluated over a period of hours or days. Some of the urinary parameters routinely monitored using *in vivo* nephrotoxicity studies include volume, osmolality, and contents. Urine volume can increase (polyuria), decrease (oliguria), or approach a zero value (anuria). Urinary osmolality is a measure of the ability of the kidney to concentrate urine. In polyuric states, urinary osmolality usually decreases from control levels, while in oliguric states urine tends to be more concentrated and urinary osmolality values rise above the control level.

The urinary contents also can provide important information concerning the presence of nephrotoxicity. The presence or elevated levels of enzymes, protein, amino acids, glucose, blood, or casts in the urine can signal renal injury. Enzymuria results primarily from the loss of the brush border (microvilli) of proximal tubular cells or from the rupture of necrotic tubular cells with the release of cytosolic enzymes and organelles. The appearance of significant enzymuria has been used as an early indicator of nephrotoxicity and for predicting the injured nephron site. Proteinuria is also an index of chemically induced nephrotoxicity. The amount of protein present in the urine can be used as a measure of the relative degree of renal damage, while the nature of the protein can provide information on the site of the lesion. Low molecular weight proteins such as β_2-microglobulin ($\sim$12 000 Da) are freely filtered at the glomerulus and almost completely reabsorbed by the proximal tubule, while high molecular weight proteins (e.g., albumin, $\sim$69 000 Da) are not normally filtered at the glomerulus. Thus, the appearance of increased amounts of albumin can indicate glomerular damage, and an increased excretion of β_2-microglobulin or a decreased ratio of albumin to β_2-microglobulin can be indicative of proximal tubule damage.

The appearance of amino acids and/or glucose in urine can also indicate proximal tubule toxicity. Both nutrients are almost entirely reabsorbed in the proximal tubule and proximal tubular damage can lead to the increased excretion of both compounds. However, reabsorption of these materials is dependent on membrane transport systems which can become saturated and lead to the increased excretion of the amino acid or glucose (e.g., uncontrolled diabetes). Recent studies have suggested that examination of urine using nuclear magnetic resonance technology might be useful in characterizing the degree and type of nephrotoxicity present (i.e., tubular versus glomerular) following exposure to a renal toxicant.

Creatinine, an endogenous end product of muscle metabolism, is often measured in plasma and urine to determine creatinine clearance. Since, creatinine is freely filtered at the glomerulus and is not reabsorbed or secreted by the proximal tubule of most species, creatinine clearance provides a good measure of the GFR. Another endogenous compound, urea, is also cleared mainly by renal filtration and excretion. Increases in the blood or serum concentration of urea are indicative of decreased GFR. However, increases in BUN concentration occur only after substantial renal damage has been established. Thus, BUN concentration is not a sensitive indicator of nephrotoxicity and changes usually occur later than changes in other parameters (e.g., enzymuria).

At the end of a screening protocol in animal models, the kidneys can be removed and examined for morphological changes (light or electron microscopy) or used in an *in vitro* model as described below to further evaluate renal function following *in vivo*

Table 2 Common parameters of renal function monitored in *in vivo* studies

Clearance of organic compounds; creatinine, urea, inulin, PAH, phenol red, TEA
Comment: allows for the determination of GFR or RBF (PAH)
Urinary volume
Comment: reflects absorption capability of nephron or altered GFR
Urinary free water/osmolality
Comment: represents the ability of the kidney to concentrate urine
Enzymuria/proteinuria
Comment: changes reflect cellular toxicity and/or altered glomerular function
Glucosuria/amino aciduria
Comment: increasing amounts of either in urine suggest proximal tubular damage, extrarenal effects also possible (e.g., diabetes)
Electrolyte excretion; pH
Comment: may be influenced by many factors
Kidney weight
Comment: can increase (edema and hypertrophy) or decrease (atrophy)
Morphological changes
Comment: provides information on the sight and nature of the lesion

PAH, *p*-aminohippurate; TEA, tetraethylammonium; GFR, glomerular filtration rate; RBF, renal blood flow.

exposure to the test agent. Kidney weight can also be easily measured at the end of a screening period as one index of nephrotoxicity. Some nephrotoxicants induce an increase in kidney weight, while exposure to other nephrotoxicants results in decreased kidney weight.

A second *in vivo* model involves the use of anesthetized animals. The animals used in these studies are either untreated or have received the test agent or procedure before anesthesia. Changes in GFR are frequently measured by determining the urinary clearance of inulin, an exogenously administered polysaccharide that is freely filtered at the glomerulus but is not secreted or reabsorbed by the nephron. Changes in RBF, a measure of renal hemodynamics, can be monitored by measuring the renal clearance of PAH or other organic compounds that are essentially 100% extracted from the peritubular fluid but poorly reabsorbed from the luminal fluid or by the use of electromagnetic flow probes placed on one or both renal arteries. In addition, to determining changes in GFR and RBF, the excretion patterns of electrolytes, protein, enzymes, glucose, water, and other urinary components can be determined both before and after exposure to a nephrotoxicant to provide information on the relative renal toxicity of test compound, the temporal aspects of toxicity and the nephron segment where toxicity occurs. Kidneys may be perfused with fixative solutions at the end of the experiment to allow for histological examination of tissue.

In Vitro Techniques

There are a large number of *in vitro* techniques available for evaluating the nephrotoxic potential of an agent or examining potential mechanisms by which an agent induces nephrotoxicity (**Table 3**). These techniques employ various levels of tissue organization including whole kidneys, tubules or tubule segments, cortical slices, cells, and isolated cellular components. *In vitro* techniques offer the advantage of allowing the investigator to study the direct effects of a compound on renal function without the contribution of extrarenal or indirect mechanisms (e.g., extrarenal biotransformation or altered renal hemodynamics). However, many *in vitro* systems remove the integral nature of the kidney, and in other *in vitro* models the *in vivo* cellular characteristics change over time (e.g., cell culture techniques). The relative importance of these factors for the induction of nephrotoxicity by a test compound will determine the toxic potential of that agent in the various *in vitro* systems. Therefore, the use of *in vitro* systems may not always provide the same results as will be obtained in whole animals. Nonetheless, valuable information can be obtained once the toxicant species has been identified and an appropriate *in vitro* model is selected for use. In the remainder of this section, a general description of the more common *in vitro* systems for examining nephrotoxicity is provided.

Table 3 Common *in vitro* models for examining nephrotoxicity

Isolated perfused kidney/tubule
Renal cortical slices
Nephron segments
Isolated renal epithelial cells
Renal cell cultures
Isolated renal organelles (e.g., mitochondria)
Membrane vesicles (basolateral or luminal)

The isolated perfused kidney allows the investigator the ability to monitor the effects of chemicals on the intact kidney without the effects of extrarenal systems. Transport of chemicals occurs via normal mechanisms as vasculature and tubular lumen remain open in the perfused state, and renal handling and biotransformation of chemicals can be evaluated using this technique. However, to utilize an isolated perfused kidney preparation, a special apparatus must be used.

Renal cortical slices offer a convenient and sensitive model for determining the induction of nephrotoxicity following either *in vivo* or *in vitro* exposure to a nephrotoxicant. Slices can be prepared freehand or by the use of a mechanical tissue slicer. When isolated from different depths of the cortex, renal cortical slices can provide one method for examining cell- or site-specific damage. Superficial slices contain S_1 and S_2 segments of the proximal tubule, while deeper slices can be prepared which contain mainly the S_3 segment. Thus, effects of nephrotoxicants on S_1,S_2 segments versus S_3 segments can be performed. Renal function parameters monitored in renal slices can include organic ion (PAH, TEA) accumulation, gluconeogenesis, LDH release, oxygen consumption, electrolyte levels, ATP production, and morphology. In addition, the order of nephrotoxic potential of several agents (mercuric chloride > potassium dichromate > hexachloro-1,3-butadiene > cephaloridine > gentamicin) is the same *in vivo* and in renal cortical slices from Fischer 344 rats.

Renal tubules or tubule segments can be isolated from the kidneys of a variety of species and either perfused with or suspended in an appropriate medium. The rabbit is commonly used as the model for tubule perfusion studies, although renal tubules from many species, including humans, have been used. Tubule segments allow the renal cells to remain in contact, but tubule collapse may occur in nonperfused segments, which could hinder absorption of

toxicants from the lumen. If the normal route of entry into renal tissue for a toxicant is via absorption at the luminal surface, then a reduction in the nephrotoxic potential of the toxicant might be observed using nonperfused tubule segments. However, the perfused tubule allows ready access to the luminal surface when the toxicant is added to the perfusate. Also, addition of a toxicant to the bathing media provides exposure of the basolateral membrane to the toxicant for the measurement of transport and toxicological parameters as well following exposure of this cell surface to the test compound. Thus, the perfused tubule segment can provide an *in vitro* model with many of the characteristics of the *in vivo* model.

Isolated renal cortical epithelial cells and cultured cell lines have become standard *in vitro* model systems for examining the nephrotoxic potential of toxicants, toxicant bioactivation and direct mechanisms of toxicity. Enriched populations of freshly isolated proximal and distal tubular cells can be obtained to examine the effects of toxicants on these distinct cell populations. Growing freshly isolated cells for several days can provide primary cultures of renal cells that can be used to study transport and toxicity of compounds. Such primary cultured cells can be obtained from proximal tubule or distal tubule/collecting duct cells. There are also several cell lines (e.g., LLC-PK_1, MDKC, HK-2) to study the effects of chemicals on renal tissue. In addition, cell lines have been developed from specific segments of the proximal tubule. Overall, these cell systems allow for the rapid screening of chemicals, exposure to apical and/or basolateral surfaces, and more detailed studies of specific cell populations. However, cells in culture can exhibit reduced functional and metabolic characteristics with time. These changes might impact on the potential effects of some chemicals in cell culture systems. Therefore, toxicity in the specific cell line to be used should be validated prior to mechanistic studies.

Isolated renal cell components (cytosol, organelles, membranes) are also commonly used *in vitro* systems. Renal microsomes and cytosol are useful in examining the renal biotransformation and bioactivation of nephrotoxicants. Since mitochondria are frequently targets for nephrotoxicants, isolated renal mitochondria are also an important model system for determining the toxic mechanism(s) of some compounds. Also, the direct effects of toxicants on renal cell membranes can be studied in vesicles prepared from either the luminal (brush border) or basolateral (antiluminal, peritubular) membrane of renal cortical cells. The use of isolated cell components is helpful in answering specific questions about mechanisms of nephrotoxicity and bioactivation of nephrotoxicants.

Nephrotoxicants

Nephrotoxicants can be classified in many different ways including chemical class (e.g., heavy metals, halogenated hydrocarbons), intended use (e.g., drugs, agricultural agents), site of toxicity (e.g., glomerular toxicants, proximal tubule toxicants), and mechanism of toxicity (e.g., crystalluria, interstitial nephritis, direct tubular toxicity). Nephrotoxicants can also be considered either primary or secondary nephrotoxicants. Primary nephrotoxicants (e.g., heavy metals) are capable of inducing nephrotoxicity as the parent compound, while secondary nephrotoxicants (e.g., trichloroethylene) require bioactivation to the ultimate nephrotoxicant species. In the following sections on specific nephrotoxicants, nephrotoxic agents will be divided into therapeutic and environmental nephrotoxicants. Sites of toxicity, mechanisms of toxicity, and the ultimate nephrotoxicant species, if known, will be discussed.

Therapeutic Nephrotoxicants

Many drugs have the potential to alter renal function. In the case of the diuretic drugs, such as furosemide and hydrochlorothiazide, the increased urine flow rate and sodium excretion which these drugs induce is a desirable, therapeutic response. However, for most therapeutics, drug-induced effects on renal function are not desirable and constitute a toxic response. Examples of some drug classes whose members induce nephrotoxicity include the antimicrobials, nonsteroidal antiinflammatory agents, analgesics, cancer chemotherapeutic agents, immunosuppressives, and radiocontrast media. In addition, there are several miscellaneous drugs within other pharmacological classes which affect renal function. The nature and magnitude of the nephrotoxic response varies greatly among these drugs, and a single drug may induce more than one type of nephrotoxic response depending on dose, duration of therapy, age of the patient, and other patient variables.

Antimicrobials Several different classes of antimicrobial agents contain members that induce nephrotoxicity. The primary groups of drugs which induce nephrotoxicity include the β-lactams (penicillins, cephalosporins, carbapenems), aminoglycosides, the antifungal agent amphotericin B, vancomycin, and the tetracyclines. The spectrum of nephrotoxic effects and mechanism(s) of nephrotoxicity vary among these agents, and the potential for a nephrotoxic

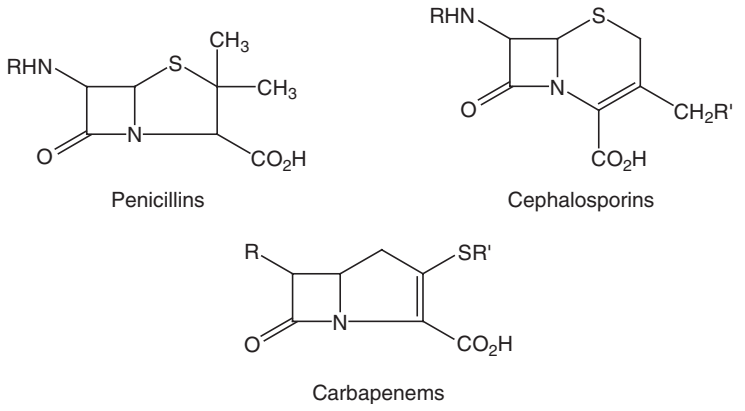

Figure 2 Structure of β-lactam antimicrobials.

response can be increased by combining two antimicrobial drugs that can induce renal toxicity or by combining a nephrotoxicant antimicrobial with other nephrotoxicant drugs.

The β-Lactam Antimicrobials The β-lactam antimicrobial drugs (penicillins, cephalosporins, carbapenems; **Figure 2**) are related chemically by the presence of a four-membered ring containing a nitrogen atom adjacent to a carbonyl group (a β-lactam ring). This β-lactam ring also confers antimicrobial activity to these drugs, since hydrolysis of the ring results in inactive drugs. Because these drugs are weakly acidic drugs, they are actively secreted by the organic anion transport system of the proximal tubule and can concentrate in this nephron segment during the secretory process. The nephrotoxicity observed following administration of a β-lactam antibiotic can occur by different mechanisms depending on the antibiotic used and patient variables.

Penicillin-induced renal toxicity is most commonly seen as allergic acute interstitial nephritis (AIN). Methicillin is the most common penicillin to induce AIN, but the use of penicillin G, ampicillin, amoxacillin, oxacillin, and carbenicillin also can lead to the development of AIN. Typically, acute renal failure follows 1 or 2 weeks of treatment with fever or rashes sometimes occurring before overt renal dysfunction. Removal of the penicillin generally allows renal function to return to normal within a few days or weeks. AIN can also be induced by certain cephalosporins (e.g., cephalothin, cephalexin, cephradine, cefoxitin, cefotaxime) and non-β-lactam antimicrobials (e.g., sulfonamides, rifampicin, tetracyclines, erythromycin).

In addition to inducing AIN, several of the cephalosporins (e.g., cephaloridine, cephaloglycine, cefaclor, and cephalothin) are directly toxic to the proximal tubule. Accumulation in proximal tubular cells via the organic anion transporter is an important step in cephalosporin nephrotoxicity, since inhibition of cephalosporin transport by probenecid also attenuates cephalosporin nephrotoxicity. In addition, the site of the cephalosporin-induced renal lesion correlates with the proximal tubular segment having the greatest capacity for organic anion transport for a particular species.

The cellular mechanism of direct cephalosporin-induced nephrotoxicity may include several possible actions of the cephalosporins. Nephrotoxic cephalosporins are known to induce lipid peroxidation and cellular membrane damage, acylate cellular proteins, and/or interfere with mitochondrial respiration. Mitochondrial respiration appears to be inhibited due to acylation of mitochondrial transporters for metabolic substrates, thereby depriving mitochondria of the necessary intermediates to utilize oxygen. Ultimately, the formation of adenosine triphosphate (ATP), needed to supply cellular energy, also declines to inhibit energy-dependent cellular functions.

Imipenem, a carbapenem antimicrobial, also possesses nephrotoxic potential. In animal models, nephrotoxicity is dose dependent and characterized by tubular necrosis. Interestingly, imipenem nephrotoxicity is markedly attenuated by co-administration of cilastatin, an inhibitor of the cytosolic and brush border enzyme dehydropeptidase I (DHP). Although DHP is responsible for hydrolyzing imipenem to inactive metabolites, the major protective effect of cilastatin appears to be due to inhibition of renal imipenem accumulation rather than DHP inhibition.

Aminoglycosides The aminoglycosides are important antimicrobial drugs used alone or in combination with β-lactam antibiotics for the treatment of certain serious gram-negative infections. Chemically, the aminoglycosides consist of various sugars

containing amino groups and linked by glycosidic bonds. The amino groups are ionized at physiological pH and give the aminoglycosides polycationic character and a highly polar nature.

Aminoglycosides concentrate in the S_1 and S_2 segments of the proximal tubule via a high-capacity, adsorptive endocytotic mechanism following binding to cellular membrane acidic (anionic) phospholipids. This endocytotic process occurs primarily at the brush border membrane. Following adsorption, aminoglycoside-containing vesicles bind to secondary lysosomes where the drug becomes sequestered. Lysosomes become early targets for aminoglycoside-induced effects with inhibition of phospholipid degradation and subsequent myeloid body formation being characteristic of aminoglycoside nephrotoxicity. Some lysosomes may rupture to release lysosomal enzymes into the cytosol of renal cells. Changes in brush border microvilli, alterations in rough endoplasmic reticulum, and increased numbers of cytoplasmic vacuoles also occur. Mitochondrial swelling, decreased mitochondrial respiration, and inhibition of basolateral Na^+,K^+-ATPase activity precede tubular necrosis. Ultimately, nonoliguric renal failure results, which may not completely reverse following cessation of aminoglycoside administration.

Although all aminoglycosides possess the ability to induce nephrotoxicity, differences exist in the nephrotoxic potential of the various drugs. Neomycin and gentamicin are the most potent nephrotoxicant aminoglycosides, while amikacin and netilmicin are the least nephrotoxic aminoglycosides. Other aminoglycosides are intermediate in nephrotoxic potential.

The exact mechanism of aminoglycoside nephrotoxicity is unclear. As discussed above, release of lysosomal enzymes can contribute to altered cellular function. Hydroxy radicals may also play a role in aminoglycoside mitochondrial effects, since catalase inhibits *in vitro* alterations of mitochondrial function by gentamicin and the use of hydroxy radical scavengers or iron chelators reduces gentamicin nephrotoxicity *in vivo*. Additionally, aminoglycoside-induced decreases in cellular pyridoxal 5′-phosphate may contribute to nephrotoxicity by removing an important cellular cofactor.

Amphotericin B The drug of choice for treating many serious systemic fungal infections is the broad spectrum antifungal agent amphotericin B. Unfortunately, nephrotoxicity is a common side effect of amphotericin B administration with up to 50–80% of amphotericin B-treated patients experiencing adverse renal effects. Amphotericin B nephrotoxicity is characterized by a distal renal tubular acidosis, potassium wasting and a defect in urinary concentration. In some cases, nephrotoxicity may progress to renal failure with azotemia.

Amphotericin-induced renal effects are due to the ability of the drug (1) to induce renal vasoconstriction which leads to a decrease in RBF and GFR, and (2) to cause an increase in tubular permeability, particularly in the distal segments of the nephron. Tubular permeability changes are the result of the association of amphotericin B with the sterols in the cell membrane to form a pore or channel. Chemically, amphotericin B is a cyclic molecule composed of lipophilic (multiple conjugated double bonds) and hydrophilic regions (**Figure 3**) such that insertion of amphotericin B into the cell membrane facilitates passive movement of water, potassium ions, hydrogen ions, and other small molecules through the newly created pore and across the membrane. These fluxes appear to account for the decrease in urinary concentrating ability, potassium wasting and renal tubular acidosis. Although it is not clear why the distal segments of the nephron are the major targets for amphotericin B, one explanation may be that the greater level of sterols found in distal membranes might facilitate the binding of amphotericin B to these sites.

Vancomycin Vancomycin is an antibiotic produced by *Streptococcus orientalis*. It is bactericidal, but vancomycin-induced toxicity has primarily limited its use to the treatment of serious infections (e.g.,

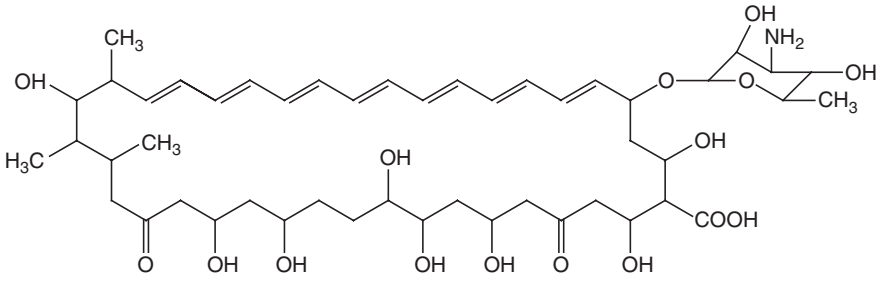

Figure 3 Structure of the antifungal drug amphotericin B.

methicillin-resistant staphalococci). Nephrotoxicity is manifest as proximal tubular toxicity. Elimination of impurities from early preparations and careful monitoring of vancomycin blood levels have reduced the number of cases of nephrotoxicity. However, vancomycin use may potentiate aminoglycoside nephrotoxicity when the two agents are used concurrently.

Tetracyclines Tetracyclines are broad spectrum antibiotics obtained from *Streptomyces* strains or prepared semisynthetically. Use of tetracyclines has resulted in three types of renal effects. First, the use of outdated tetracyclines results in direct proximal tubular toxicity characterized by the increased excretion of amino acids, glucose, and phosphate (Fanconi syndrome). The mechanism of this response is unclear, but appears to be due to the formation of the degradation product anhydro-4-epi-tetracycline. Second, administration of some tetracyclines, particularly demeclocycline, can result in a dose-dependent, reversible nephrogenic diabetes insipidus, which appears to result from an inhibition of ADH effects on water reabsorption. Lastly, in patients with preexisting compromised renal function, tetracyclines can induce increased sodium excretion and azotemia. The mechanism of the naturesis may be due to an effect of tetracyclines on luminal membrane sodium conductance, while the azotemia appears to result from the antianabolic effects of the tetracyclines.

Nonsteroidal Antiinflammatory Drugs The NSAIDs as a class possess the ability to induce renal failure characterized by a rapid decrease in urine volume and significant sodium and water retention which is also rapidly reversed when the drug is removed. Conditions that decrease renal perfusion (e.g., congestive heart failure, decreased blood volume, chronic renal disease, etc.) also predispose individuals to the renal effects of NSAIDs. Under these conditions, RBF and GFR are maintained by a balance of the vasoconstrictor actions of angiotensin II and the vasodilatory effects of prostaglandin E_2 (PGE2). NSAID-induced renal failure results as a consequence of NSAID inhibition of renal cyclooxygenase with a subsequent inhibition of PGE2 synthesis. Renal vasoconstriction predominates under these conditions resulting in acute oliguric renal failure. While all NSAIDs have the potential to induce acute renal failure, sulindac appears to have less of an effect on renal PGE2 synthesis and may be the drug of choice in patients with preexisting conditions which would predispose them to NSAID-induced renal effects.

Nonnarcotic Analgesics Nonnarcotic analgesic drugs are widely used for the relief of minor pain, to reduce fever and/or as antiinflammatory agents. Acetaminophen (paracetamol) is perhaps the most commonly used agent in this class of drugs which also includes aspirin and phenacetin. Analgesic use can result in acute or chronic nephrotoxicity depending on the amount of drug ingested and patient variables. Normally, acute nephrotoxicity results from acute overdose, while chronic use of single or combination products can result in renal papillary necrosis and chronic interstitial nephritis.

Acute overdose with acetaminophen ($>300\,\text{mg}\,\text{kg}^{-1}$) results in hepatotoxicity and/or nephrotoxicity. Although hepatotoxicity is frequently the predominant toxicity, acetaminophen nephrotoxicity can occur in the absence of marked hepatic toxicity. In these cases, liver function returns to normal or near normal levels before the onset of nephrotoxicity. Acute acetaminophen nephrotoxicity is generally characterized as oliguric acute renal failure with acute tubular necrosis. Acetaminophen can also induce acute nephrotoxicity in therapeutic doses, but chronic alcohol intake usually accompanies renal toxicity in these patients.

The mechanism of acute acetaminophen nephrotoxicity is related to the bioactivation of acetaminophen and/or its metabolites to highly reactive species which are capable of arylating renal macromolecules or generating reactive oxygen species. Acetaminophen hepatotoxicity is the result of conversion of acetaminophen to the reactive intermediate *N*-acetyl-*p*-benzoquinoneimine (NAPQI), which can covalently bind to hepatic macromolecules. It is less clear what role formation of NAPQI in the kidney plays in acetaminophen nephrotoxicity. In some species (e.g., the Fischer 344 rat) deacetylation appears to be an important biotransformation step in acetaminophen nephrotoxicity, while in other species (e.g., the CD-1 mouse), bioactivation does not appear to require deacetylation of acetaminophen before the ultimate nephrotoxicant species is produced. Therefore, the role of NAPQI in acute acetaminophen nephrotoxicity might be species dependent.

Biotransformation of acetaminophen by deacetylase enzymes in liver or kidney produces the metabolite 4-aminophenol (**Figure 4**). Evidence suggests that acetaminophen nephrotoxicity may result from 4-aminophenol formation. In animal studies, 4-aminophenol is a more potent nephrotoxicant than acetaminophen and inhibition of deacetylase enzymes also attenuates acetaminophen nephrotoxicity. Deacetylase enzymes are also present in higher levels in renal cortex, the target for acetaminophen nephrotoxicity, than in liver or renal medulla and there is a positive correlation between renal cortex

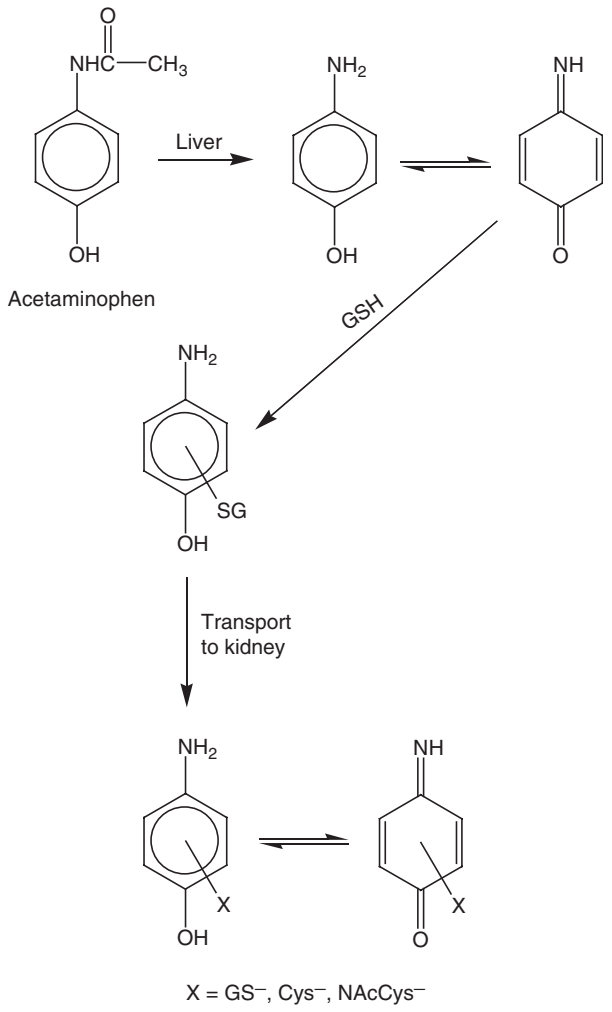

Figure 4 Bioactivation of acetaminophen.

deacetylase activity and susceptibility to acetaminophen nephrotoxicity in various animal models.

The mechanism of 4-aminophenol nephrotoxicity remains to be determined with certainty. The current hypothesis suggests that 4-aminophenol is oxidized by cytochrome P450 isozymes or peroxidases to *p*-benzoquinoneimine which can arylate renal macromolecules and/or redox cycle between 4-aminophenol and *p*-benzoquinoneimine to form reactive oxygen species. Recent studies have suggested that 4-aminophenol might be converted to a glutathione conjugate in the liver prior to transport to the kidney (**Figure 4**), and that the glutathione conjugate or one of its metabolites is the form that accumulates in kidney from extrarenal sources. Thus, acetaminophen nephrotoxicity could result from production of more than one reactive intermediate.

Chronic analgesic nephrotoxicity is characterized by renal papillary necrosis and interstitial nephritis rather than the proximal tubular necrosis observed in acute nephrotoxicity. In most cases, chronic nephropathy results from abuse of combination analgesic preparations (phenacetin, acetaminophen and/or a salicylate) over a long period of time. In these situations, the primary nephrotoxicant appears to be acetaminophen, which concentrates more in the renal medulla than in renal cortex or blood. Within the medulla, acetaminophen can be converted to the reactive intermediate NAPQI by the prostaglandin hydroperoxidase component of the prostaglandin H synthase complex. NAPQI interactions within medullary tissue result in a depletion of the cytoprotective tripeptide glutathione. As glutathione becomes depleted, arylation of medullary macromolecules by NAPQI occurs. In addition, acetaminophen in therapeutic doses can increase prostaglandin hydroperoxidase activity and therefore, stimulate formation of its own reactive metabolite NAPQI.

Aspirin also has the potential to increase acetaminophen nephropathy. Aspirin inhibits the cyclooxygenase component of prostaglandin H synthase without effect on the prostaglandin hydroperoxidase component, while salicylic acid (the deacetylated metabolite of aspirin) decreases renal glutathione concentrations. Thus, coadministration of aspirin with acetaminophen (or phenacetin) results in a synergistic nephrotoxicity.

Table 4 Cancer chemotherapeutic drugs capable of inducing nephrotoxicity

Alkylating agents
Cisplatin
Nitrosoureas
Cyclophosphamide
Antibiotics
Mitomycin C
Mithramycin
Doxorubicin
Antimetabolites
Methotrexate
5-Fluorouracil
6-Thioguanine
Cytosine arabinoside
5-Azacytidine
Miscellaneous
Interferon
Celiptinium

Cancer Chemotherapeutic Drugs Cancer chemotherapeutic drugs can be life saving in the treatment of certain cancers. Unfortunately, host systems are also a target for these agents with several antineoplastic drugs possessing the potential to induce nephrotoxicity (**Table 4**). In some cases, nephrotoxicity normally occurs only at high doses (e.g., methotrexate, 6-thioguanine) or is a low risk (e.g., 5-fluorouracil, interferon). However, nephrotoxicity can be a

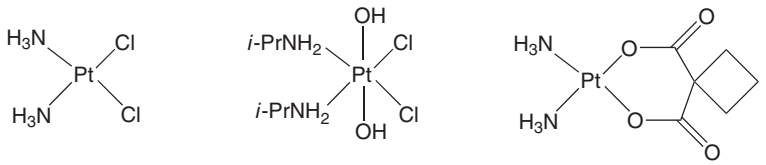

Figure 5 Platinum cancer chemotherapeutic drugs.

significant toxicity following administration of certain antineoplastic drugs (e.g., cisplatin) which can require additional efforts to minimize the development of renal failure.

Alkylating Agents Perhaps the cancer chemotherapeutic drug which is most commonly associated with the induction of nephrotoxicity is cisplatin (**Figure 5**). Cisplatin is a member of the platinum complex class of antineoplastic drugs which also includes carboplatin and iproplatin (CHIP). However, the nephrotoxic potential of cisplatin appears to be greater than the nephrotoxic potential of the other clinically useful platinum agents.

In rat models of cisplatin nephrotoxicity, cisplatin nephrotoxicity is observed as acute proximal tubular necrosis with the primary target being the S_3 segment. However, in humans, cisplatin nephrotoxicity is characterized by tubular necrosis with the distal tubules and collecting ducts affected along with proximal tubules. Early changes in renal function include enzymuria (e.g., *N*-acetyl-β-D-glucosaminidase) and β_2-microglobinuria, which suggests that the proximal tubule is an initial target for cisplatin. Diuresis, increased BUN concentration, decreased creatinine clearance and magnesium wasting soon develop, indicating the presence of renal failure.

The mechanism of cisplatin nephrotoxicity is unclear, although numerous hypotheses have been proposed. Cisplatin is primarily excreted via the kidneys by both filtration and secretion using the organic cation transporter, which indicates that the kidney will be exposed to a large percentage of the administered dose. It is believed that the chloride groups are replaced by water molecules *in vivo*, ultimately forming a hydrated or hydroxylated platinum species which might interact with renal macromolecules (e.g., DNA) to lead to nephrotoxicity. Other postulated targets for cisplatin are renal ATPase enzymes and renal mitochondria.

The nitrosoureas include streptozotocin, an agent useful in treating pancreatic (islet cell) tumors, and the carmustine, lomustine and semustine group, useful in treating brain and gastrointestinal tumors. Streptozotocin induces a reversible, mild nephropathy characterized by proteinuria in 50–70% of patients and decreased creatinine clearance in 20–30% of patients. Renal phosphate wasting and proteinuria are early signs of nephrotoxicity. The primary target in the kidney for streptozotocin is the proximal tubule with glomerular abnormalities also noted. Removal of the drug results in return to normal renal function within weeks.

Semustine is the most common agent to induce nephrotoxicity among the second group of nitrosoureas. Semustine nephrotoxicity results from bioactivation of the nitrosourea to an alkylating metabolite which mainly attacks proximal tubular cells. Carbamoylating metabolites of semustine are also formed *in vivo* but do not appear to contribute to renal toxicity. Renal failure occurs most often following high dose ($>1200\,\mathrm{mg\,m^{-2}}$, total dose) administration. Onset of nephrotoxicity (glomerulosclerosis, renal interstitial nephritis, proximal tubular degeneration) can be delayed for over one year following therapy and may progress to irreversible renal failure.

Cyclophosphamide, a nitrogen mustard alkylating agent, is a widely used cancer chemotherapeutic drug to treat lymphomas, leukemias, multiple myeloma and a numerous solid tumors. Cyclophosphamide can induce nephrotoxicity characterized as decreased water excretion and an inappropriate concentration of urine. These effects are due to a direct effect of one or more alkylating cyclophosphamide metabolites at distal tubules and collecting ducts. Special caution is warranted to avoid water-induced diuresis or diuretic therapy in these patients as hyponatremia can become a problem.

Antimetabolites Nephrotoxicity is generally not a major toxicity of antimetabolite therapy, except when these drugs are administered in high doses or in susceptible patients. Acute renal failure is the most common type of nephropathy induced by the antimetabolites with methotrexate treatment possessing the greatest risk. Acute renal failure has also been reported as a potential toxicity for 5-fluorouracil, 6-thioguanine, cytosine arabinoside, and 5-azacytidine.

Methotrexate is an antimetabolite of folic acid useful in combination therapy for a wide range of cancerous conditions. When methotrexate is administered

in high doses (>50–$250\,mg\,kg^{-1}$ to 1–$7\,g\,m^{-2}$), solubility limits may be exceeded with a resultant precipitation of methotrexate and its 7-hydroxy metabolite within the renal lumina. Tubular obstruction can reduce GFR by as much as 50%. Methotrexate might also have a direct effect on proximal tubular function, since proximal tubular necrosis may be seen in the absence of precipitated material within the renal lumina. In addition, a direct effect of methotrexate on glomerular hemodynamics has been postulated. Renal toxicity induced by methotrexate also can enhance other methotrexate toxicities (e.g., myleosuppression) by decreasing the excretion of the drug from the body.

Antibiotics The clinical use of three antibiotic cancer chemotherapeutic drugs (mitomycin C, mithramycin, and doxorubicin) has been associated with the development of nephrotoxicity. Each of these drugs is commonly used in combination chemotherapy which in some cases might result in additive or enhanced nephrotoxicity.

Mitomycin C is isolated from *Streptomyces caespitosis* and is used in the treatment of solid tumors. Renal failure (elevated BUN and serum creatinine concentration, proteinuria) induced by mitomycin C is dose dependent and cumulative. When administered alone, the incidence of nephrotoxicity is less than 1%, but in combination with 5-fluorouracil nephrotoxicity occurs more frequently and can be marked. The chemical species responsible for mitomycin C nephrotoxicity appears to result from the formation of alkylating metabolites.

Mithramycin is an antibiotic antineoplastic drug isolated from *Streptomyces tanashiensis*. In early studies, treatment with mithramycin (25 or $50\,mg\,kg^{-1}\,day^{-1}$ for 1 week or three times per week each month) resulted in decreased GFR in up to 40% of patients and proteinuria in 78%. Morphological changes included proximal and distal tubular necrosis, atrophy, or swelling. Single dose administration of mithramycin to treat the hypercalcemia sometimes associated with cancer generally does not induce renal toxicity. However, a few isolated case reports suggest that in patients with compromised renal function, nephrotoxicity may occur following single dose therapy.

Nephrotoxicity associated with doxorubicin use is also dose dependent and occurs at the same time as doxorubicin-induced cardiotoxicity. Studies in animal models reveal glomerular effects, renal interstitial fibrosis, and vacuolization of tubules. However, clinical evidence of nephrotoxicity in the absence of cardiotoxicity is limited suggesting that dose reduction efforts to minimize cardiotoxicity also reduce the risk of nephrotoxicity.

Miscellaneous Cancer Chemotherapeutic Agents The lack of curative treatments for most malignancies has stimulated the search for newer, more efficacious cancer chemotherapeutic agents. Interferon-α has recently been obtained using molecular biology techniques in sufficient quantities to begin clinical testing against various cancerous conditions including hairy cell leukemia, non-Hodgkin's lymphoma and Karposi's sarcoma. A few reports suggest that reversible acute renal insufficiency associated with proteinuria may occur following continued interferon-α administration. Morphological changes are consistent with the presence of acute interstitial nephritis.

Celiptinium is useful in the treatment of metastatic breast cancer and is useful in combination therapy because of minimal hematotoxicity. Acute and chronic renal failures have been detected in patients treated with celiptinium. Acute renal failure is dose dependent, while chronic effects appear to be cumulative in nature. The primary manifestation of celiptinium nephrotoxicity is tubular necrosis with celiptinium-induced lipid peroxidation in proximal tubular cells proposed as the mechanism of toxicity.

Immunosuppressive Drugs The modern development of drugs to suppress the immune system has made organ and bone marrow transplants possible and saved countless lives. Two important drugs in this class of agents are cyclosporine (cyclosporin A) and tacrolimus (FK506), fungal products with immunosuppressive properties. Cyclosporine acts primarily by inhibiting helper T-cell activation following the binding of cyclosporine to a cytoplasmic receptor protein, cyclophilin. Other effects on the immune system are also observed, but appear to be less important than T-cell effects. Tacrolimus also inhibits T-cell activation but via interaction with a different cytoplasmic receptor than cyclosporine. A newer drug OKT_3 is a monoclonal antibody that can destroy effector T-cells to act as an immunosuppressive drug.

Nephrotoxicity is a common toxicity and significant problem associated with the use of cyclosporine in humans. Three types of nephrotoxicity have been observed in patients receiving cyclosporine: (1) an acute, reversible renal failure; (2) acute vasculopathy or thrombotic microangiopathy; and (3) chronic renal failure with interstitial fibrosis that may not be reversible.

Cyclosporine-induced acute renal failure is characterized by decreased GFR and urine volume, and

elevated BUN and serum creatinine concentrations. These effects are dose dependent and rapidly reverse when cyclosporine therapy is discontinued. The mechanism of the acute renal failure appears to be related to cyclosporine-induced renal vasoconstriction to reduce glomerular filtration. The precise mechanism responsible for the resultant vasoconstriction remains to be determined with certainty. However, stimulation of the renin–angiotensin system, alteration of renal prostaglandin status (increased vasoconstrictor and/or decreased vasodilatory prostaglandin levels), and stimulation of adrenergic nerves have been proposed as possible mechanisms.

A second form of cyclosporine-induced nephrotoxicity is acute thrombotic microangiopathy. The mechanism for induction of this toxicity is unclear but may be due to a direct toxic effect of cyclosporine on renal arterioles and glomerular capillaries. Histologically, arterioles exhibit protein deposits while glomeruli show thrombosis and endothelial cell damage. These effects are similar in nature to transplant rejection thrombotic microangiopathy, but arcuate and interlobular arteries rather than arterioles are primarily affected with transplant rejection.

Chronic cyclosporine nephrotoxicity can develop in patients receiving the drug for 1 year or longer. In these patients, there is a gradual decline in renal function. While GFR may not be markedly reduced, significant morphological changes including vascular changes, glomerular sclerosis, interstitial fibrosis, and tubular atrophy have been reported. Chronic effects may not be reversible upon discontinuation of cyclosporine, and the renal effects may progress to end-stage renal failure. The mechanism underlying cyclosporine-induced chronic renal failure is unclear. However, it has been proposed that the renal vasoconstriction induced by cyclosporine results in both acute and, ultimately, chronic renal failure.

Tacrolimus nephrotoxicity can occur as proximal tubular vacuolization, proximal tubular necrosis, or glomerular capillary/arteriolar thrombi. Although there are reports that tacrolimus may be less potent as a nephrotoxicant than cyclosporine, tacrolimus potentiates cyclosporine nephrotoxicity in humans.

OKT_3, an immunosuppressive monoclonal antibody, can induce systemic vascular changes (leaky syndrome) and prerenal azotemia, presumably by stimulating the release of cytokines (e.g., tumor necrosis factor). These effects are seen more often in poorly hydrated patients. There is also evidence that OKT_3 may induce a direct tubular toxicity, since significant numbers of patients developing renal insufficiency also exhibit enzymuria.

Radiocontrast Media The use of radiocontrast media to visualize organs in the body has been a common practice for over 50 years. However, the use of these agents is now recognized as a significant cause of hospital-acquired acute renal failure with up to 10% of all cases due to the administration of a radiocontrast agent. Numerous factors may increase the risk of acute renal failure developing following a diagnostic procedure with a radiocontrast agent including existing renal insufficiency, diabetes mellitus, dehydration, anemia, cardiovascular disease, age, and many others.

Typically, radiocontrast-induced acute renal failure is diagnosed when oliguria or a $>50\%$ rise above baseline in serum creatinine develops within 24–48 h following the radiocontrast procedure. The most predominant morphological change seen in the kidney is extensive vacuolization of proximal tubular cells.

The agents currently used as radiocontrast media are triiodinated benzoic acid derivatives and may be ionic (e.g., sodium diatrizoate) or nonionic (e.g., iotrol, iopamidol). The mechanism of nephrotoxicity induced by radiocontrast media involves both renal hemodynamic changes and direct tubular injury, and these effects are related to the high osmolarity of the drugs (up to $1965\,\mathrm{mOsm\,l^{-1}}$).

Radiocontrast agents can enter the nephron by filtration and/or secretion, depending on the agent administered. The changes in renal hemodynamics induced by radiocontrast media result primarily from the large osmotic load delivered to the distal segment of the nephron. At the level of the macula densa, this osmotic load is detected and the tubuloglomerular feedback system is activated to stimulate vasoconstriction and decrease GFR. While evidence exists for direct tubular toxicity (enzymuria, naturesis, diuresis greater than an osmotic effect), the mechanism is unclear. However, tubular injury may be exacerbated by the production of reactive oxygen species generated from mesangial cells and polymorphonuclear leukocytes and macrophages which migrate into glomerular and tubular sites following radiocontrast administration.

Environmental Nephrotoxicants

Exposure to nephrotoxicants not only occurs in the clinical setting, but also occurs from environmental sources. Environmental nephrotoxicants are defined as nephrotoxicants found in the natural, home, and/or work environment which have no therapeutic utility. Unlike therapeutic nephrotoxicants, which are administered to obtain a beneficial health effect, exposure to environmental nephrotoxicants usually

Table 5 Nephrotoxicant metals

Aluminum	Iron
Antimony	Lead
Arsenic	Lithium
Beryllium	Manganese
Bismuth	Mercury
Cadmium	Molybdenum
Chromium	Nickel
Cobalt	Platinum
Copper	Silver
Gallium	Thallium
Germanium	Uranium
Gold	Vanadium
Indium	

occurs accidentally or in suicide/intentional poisoning cases.

Although environmental nephrotoxicants differ markedly in their chemical nature, there are several distinct classes of environmental nephrotoxicants. These classes include the metals, halogenated solvents, agricultural agents, and natural products. A diverse array of miscellaneous nephrotoxicants also exists and will be discussed briefly at the end of this section.

Metals A wide range of metals induce nephrotoxicity in humans and/or in animal models (**Table 5**). Some of these metals (e.g., iron, cobalt, copper) are essential elements required for normal body function, while others can be useful in treating diseases. For example, gold salts are useful in treating rheumatoid arthritis; lithium salts are indicated for the treatment of manic-depressive illness; and aluminum and bismuth salts are available to treat indigestion and stomach aches. However, exposure to these and other metals can occur from environmental sources and in excessive concentrations, can lead to nephropathy.

Many of the metals are potent nephrotoxicants, inducing marked renal effects at concentrations far lower than many other classes of nephrotoxicants. The proximal tubule is a major target for metal toxicity and, in some cases, renal hemodynamic changes are also important for initiating and/or maintaining the renal damage. Although metal-induced nephrotoxicity has been studied for many years, the precise cellular mechanisms that underlie the potent nature of this class of toxicants are not fully elucidated. However, there are several reasons why the kidney is susceptible to metal-induced effects.

The large reabsorptive nature of the kidney insures that filtered proteins, electrolytes, and water will be conserved and homeostasis maintained in the body. Metals make use of these reabsorptive processes to gain entry into renal cells. For example, chromate can enter proximal tubular cells via the sulfate transporter, while cadmium binds to metallothionein, a low molecular weight metal-binding protein, and enters proximal tubular cells along with the protein. Once inside kidney cells, the metals may substitute for endogenous molecules. Arsenate can substitute for phosphate in oxidative phosphorylation within mitochondria to cause a decrease in ATP synthesis. Also, lead can substitute for calcium to alter a large number of calcium-mediated events within cells. Metals also have a high affinity for sulfhydryl and amino ligands and form complexes or chelates with these organic functional groups. Formation of these chelates with essential functional groups of cellular macromolecules can markedly alter cell function to eventually lead to cell death. In addition to direct interaction of metals with cellular targets, radioactive metals that accumulate in renal tissue can release radiation to initiate the formation of cellular free radicals to disrupt cell function and membrane integrity.

The chemical form of a metal that accumulates in the kidney may vary among the metals. However, the ionic form of a metal is normally much more potent as a nephrotoxicant than the elemental form. Once in the body, metal ions interact with numerous molecules (albumin, metal-binding proteins, glutathione, amino acids, etc.) and move around the body primarily as reversible complexes. Unfortunately, little information is available on the chemical form of most metals that actually enters proximal tubular cells and additional research is needed in this area.

Mercury The effects of various forms of mercury on renal function have been known for centuries. Therapeutically, the first class of highly efficacious diuretic drugs was the organomercurials (e.g., mersalyl), but these agents have been now replaced by the loop or high-ceiling diuretics. Toxicological interest has centered on inorganic mercury salts, primarily in the form of mercuric chloride ($HgCl_2$), and organic mercury, mainly methylmercury salts (CH_3Hg^+). In the natural environment, elemental mercury can be converted by microorganisms to both inorganic mercury salts and organic mercurials which can find their way into the food chain. Mercurials are also used agriculturally as insecticides and fungicides so that exposure to these agents can occur occupationally, from industrial wastes or agricultural runoff.

Acute exposure to mercuric salts targets the S_3 segment of the proximal tubule to induce severe necrosis. Acute renal failure develops rapidly characterized by decreased RBF and GFR, oliguria,

glucosuria, proteinuria, and elevated BUN concentration. Renal vasoconstriction contributes to the developing nephrotoxicity and may be due to activation of the renin–angiotensin system. At higher doses or later time points, S_1 and S_2 segments of the proximal tubule are also damaged.

The mechanism of proximal tubule toxicity following administration of mercuric chloride has been extensively studied. However, the key sequence of events leading to cell death remains to be determined with certainty. Mercuric ions induce mitochondrial toxicity, alter cell membrane function, disrupt cell calcium homeostasis and cause changes in membrane phospholipid composition. Binding of mercuric ions to enzymatic sulfhydryl groups and ischemia-induced mitochondrial toxicity have been proposed as the basic mechanisms leading to these cellular effects and ultimately to cell death.

Another aspect of mercuric ion-induced tubule toxicity relates to the nature of the chemical species responsible for proximal tubule damage. Recent studies have suggested that, *in vivo*, mercuric ion forms diconjugates with thiol-containing molecules such as glutathione or homocysteine. When the kidney is exposed to these diconjugates (e.g., glutathionyl–Hg–glutathionyl or cysteinyl–Hg–cysteinyl), the conjugates are accumulated via amino acid transporters or processing enzymes to increase the proximal tubule cell concentration of mercury. The accumulated mercury then interacts with critical intracellular targets to induce toxicity. Thus, the conjugates provide a mechanism for facilitating mercuric ion entry into renal cells which leads to toxicity.

In addition to tubule effects, glomerular changes are also noted. These effects are due to the formation of an autoantibody which localizes along the glomerular basement membrane. Glomerular damage then occurs from complement-mediated events or circulating lymphocytes. Chronic exposure to low levels of mercuric salts or mercury vapor can also induce immune-mediated glomerular toxicity which is more common clinically than necrosis.

Organic mercurials are capable of inducing nephrotoxicity in S_2 and S_3 segments of the proximal tubule. Part of the S_3 damage results from the biotransformation of the organic mercurial to release mercuric ions. Methylmercury (CH_3Hg^+) readily concentrates in renal proximal tubular cells and alters mitochondrial function and lysosomes. At least part of methylmercury-induced nephrotoxicity may be due to homolytic scission of methylmercury to release methyl radicals and to lipid peroxidative toxicity.

Cadmium Cadmium has a variety of uses including electroplating, galvanizing, as a color pigment and in the manufacture of batteries. Industrial exposure is a major source of cadmium in humans, but cadmium is also found in food. Concentrations of cadmium in food varies widely, with shellfish (e.g., oysters, mussels, scallops) being a major dietary source of cadmium ($100–1000\,\mu g\,kg^{-1}$). Cigarettes also contain cadmium and it has been estimated that smoking one or more packs of cigarettes per day may double the body burden of cadmium.

Cadmium nephrotoxicity occurs when renal accumulation of cadmium exceeds 200 μg Cd^{2+} per gram tissue. Nephrotoxicity is characterized by low molecular weight proteinuria (e.g., β_2-microglobulinuria in humans), glucosuria, calciuria, aminoaciduria, phosphaturia, and interstitial inflammatory reactions and fibrosis. These effects are due primarily to damage of the S_1 and S_2 segments of the proximal tubule. Glomerular damage may also occur, since albuminuria is also occasionally observed.

Exposure to cadmium results in hepatic stimulation of the metal-binding protein metallothionein, a small protein with a molecular weight of ~6500–6800 Da. Metallothionein is composed of 20 cysteine residues whose sulfhydryl groups readily complex with seven metal ions such as cadmium. The metallothionein–cadmium complex is released from damaged hepatocytes and is carried via the blood to the kidney. The low molecular weight of the metallothionein–cadmium complex allows it to be readily filtered at the glomerulus and enter the tubular lumen. The complex is absorbed from luminal fluid via a pinocytotic mechanism in the S_1 and S_2 segments and degraded by lysosomal proteases to release free cadmium. The free cadmium can then bind to renal metallothionein or attack targets such as calmodulin within proximal tubular cells.

Lead Lead is the most ubiquitous of the nephrotoxicant metals in the environment. Like mercury, the health effects of lead have been recognized for centuries with the nervous system as well as the kidney being a target for certain forms of these metals. Sources of exposure to lead include food (~100 μg or less per day for adults), lead-based paints, industrial emissions, lead dusts and lead-glazed pottery.

Clinically, lead nephropathy is seen as either reversible tubular dysfunction or as an irreversible interstitial nephropathy. Tubular toxicity occurs most commonly in children following acute exposure and is characterized by glucosuria, phosphaturia, aminoaciduria, and occasionally proteinuria. One unique morphological feature of lead exposure is the formation of nuclear inclusion bodies within renal tubular cells. These bodies are complexes between lead

and an acidic protein aggregate. Recently, it has been suggested that proteins in humans similar to rat α_{2u}-globulin bind lead in the liver (or other tissues) and transport lead to the kidney where the lead:lead-binding protein complex is absorbed by an endocytotic mechanism. Within the renal cells, most of the lead is associated with the nuclear inclusion bodies. However, the lead–protein complex can be reversed with ethylenediaminetetraacetic acid (EDTA) and lead excretion promoted.

The cellular mechanism of lead nephrotoxicity appears to be due to an alteration of calcium homeostasis. Lead (Pb^{2+}) competes with calcium (Ca^{2+}) for transport, binding to calmodulin and at other cell calcium regulatory sites. Lead can accumulate in mitochondria using the calcium transporter and disrupt respiration. Interactions of lead with calmodulin can result in a disruption of the calcium messenger system to adversely affect normal cell function. The nuclear inclusion bodies may also alter the cellular function of DNA, although this interaction has not been fully elucidated.

Chronic exposure to low levels of lead results in lead accumulation within the body. Workers who have been chronically exposed to lead develop interstitial fibrosis, vascular and glomerular sclerosis, and tubular atrophy and/or hypertrophy. Although acute lead nephropathy is reversible with chelator therapy and/or removal from exposure, chronic effects may be irreversible. In addition, chronic exposure to lead may result in a gouty nephropathy as lead reduces uric acid excretion and elevates blood uric acid levels.

Halogenated Hydrocarbons Halogenated hydrocarbons encompass a large group of chemicals with a wide range of applications. Many of these compounds are organic solvents (e.g., chloroform, trichloroethylene) or chemical intermediates (e.g., bromobenzene, chloroanilines) used in laboratory, industrial, or commercial applications. Halogenated hydrocarbons are also used in agriculture as pesticides (e.g., 1,2-dibromoethane). The majority of nephrotoxicant halogenated hydrocarbons contain chloro and/or bromo groups with only a few nephrotoxicants substituted with fluoro or iodo groups.

The site and severity of the nephrotoxic lesion also varies widely among the halogenated hydrocarbons depending on chemical class, number and position of halogen groups, age, sex, species, dose, and preexisting conditions. Unlike the metals, halogenated hydrocarbons are usually not direct nephrotoxicants and require bioactivation before the ultimate nephrotoxicant species is produced. Several mechanisms of bioactivation have been identified for the halogenated hydrocarbons including oxidation, free radical formation, intramolecular cyclization, and conjugation with glutathione (**Table 6**). In the following sections, specific examples of halogenated hydrocarbons and their mechanisms of bioactivation will be discussed.

Chloroform Chloroform (trichloromethane, **Figure 6**) has been used in the past as an anesthetic and as an additive in pharmaceutical preparations. Today, chloroform is used primarily as an organic solvent. Chloroform-induced nephrotoxicity is primarily seen as proximal tubular toxicity with minimal changes in distal tubular function and no evidence of glomerular effects. Nephrotoxicity is characterized by proteinuria, glucosuria, elevated BUN concentration, and kidney weight, decreased accumulation of organic ions by renal cortical slices, and a fatty degeneration of proximal tubular cells. Species and sex differences exist in the susceptibility to the nephrotoxic effects induced by chloroform with certain strains of male mice (e.g., DBA/2J) being particularly sensitive.

Table 6 Mechanisms of bioactivation of halogenated hydrocarbons

Oxidation
Example: Chloroform
Free radical formation
Example: Carbon tetrachloride
Intramolecular cyclization
Example: 2-Bromoethylamine
Glutathione conjugation
Intramolecular cyclization
Example: 1,2-Dibromoethane
Cysteine conjugate β-lyase activation
Example: Trichloroethylene
Facilitated transport
Example: Bromobenzene

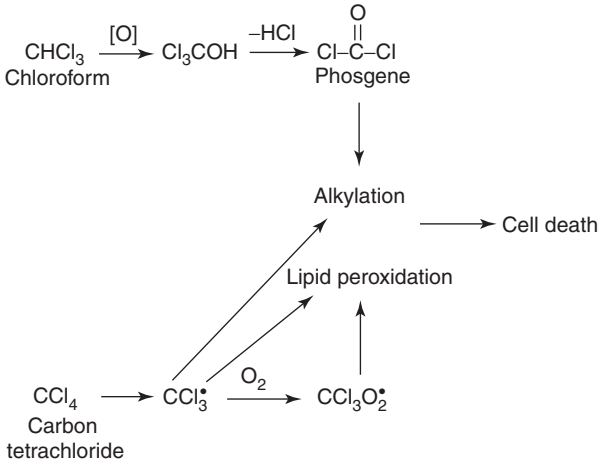

Figure 6 Bioactivation of chloroform and carbon tetrachloride.

The mechanism of chloroform nephrotoxicity involves the oxidation of chloroform to trichloromethanol by renal cytochrome P-450 isozymes (**Figure 6**). The trichloromethanol readily eliminates HCl to form the highly reactive toxicant phosgene ($COCl_2$). The phosgene can (1) be detoxified by conjugation with two molecules of glutathione, (2) react with water to form two molecules of HCl and one molecule of CO_2, or (3) covalently bind to renal macromolecules to disrupt cellular function and induce nephrotoxicity.

There are several lines of evidence that support the formation of phosgene as the ultimate nephrotoxicant species following exposure to chloroform in mice and rabbits. First, susceptible strains of male mice oxidize chloroform faster than resistant strains. Secondly, deuterium labeling of chloroform, to form $CDCl_3$, results in the formation of a chloroform derivative which is oxidized much slower than chloroform and is less potent as a nephrotoxicant. In addition, trapping experiments with cysteine have documented the formation of phosgene as a product of chloroform biotransformation. Although these results support phosgene as the toxicant species in mice and rabbits, it is not known with certainty if the same mechanism for nephrotoxicity is operating in humans. In humans, chloroform nephrotoxicity has been documented in both males and females, while only male mice exhibit nephrotoxicity following chloroform administration. Thus, additional or alternate mechanisms may be contributing to chloroform nephrotoxicity in humans.

Carbon Tetrachloride Carbon tetrachloride (CCl_4) was widely used as a dry cleaning solvent until its potential as a hepatotoxicant, nephrotoxicant, and carcinogen was recognized. Currently, carbon tetrachloride is used as an organic solvent. Nephrotoxicity associated with dermal or inhalation exposure to carbon tetrachloride is seen as acute tubular necrosis which is delayed in onset. Death occurs from acute renal failure, usually within three weeks of intoxication. Interestingly, humans appear to be more sensitive to acute carbon tetrachloride-induced nephrotoxicity than most animal models.

The mechanism of carbon tetrachloride nephrotoxicity involves the initial homolytic cleavage of carbon tetrachloride by cytochrome P450 to form the trichloromethyl and chlorine free radicals (**Figure 6**). The trichloromethyl free radical can then alkylate renal macromolecules or interact with membrane unsaturated fatty acids to initiate lipid peroxidation. The trichloromethyl free radical may also combine with molecular oxygen to form a peroxy free radical that is more reactive than the trichloromethyl free radical. The peroxy radical could also interact with unsaturated fatty acids in membranes to induce lipid peroxidative damage. Recently, it has been proposed that under anaerobic conditions, carbon tetrachloride is converted to a carbene metabolite (Cl_3C:), which can covalently bind to cell macromolecules in the liver. However, the role of the carbene metabolite in carbon tetrachloride nephrotoxicity is less clear.

N-(3,5-Dichlorophenyl)succinimide During the 1970s, a large number of *N*-(halophenyl)succinimides were synthesized and evaluated as agricultural fungicides. The most promising agent in this class of compounds was *N*-(3,5-dichlorophenyl)succinimide (NDPS), which had a broad spectrum of antifungal activity. NDPS, marketed as Ohric, also proved to be a nephrotoxicant and to promote the carcinogenic activity of several nephrocarcinogens. As a result of potential health hazards NDPS production was halted for many years. However, recently NDPS manufacture and sales have begun again in China for use as an agricultural antimicrobial agent.

Acute NDPS nephrotoxicity is characterized by diuresis, proteinuria, glucosuria, hematuria, and elevated BUN concentration and kidney weight, decreased organic ion accumulation by renal cortical slices and marked proximal tubular necrosis. Sex differences exist for NDPS nephrotoxicity with female Fischer 344 rats being twice as sensitive as males. Interestingly, the primary site of the renal lesion in males is the S_1 and S_2 segments of the proximal tubule, while the S_3 segment is the most affected segment in females. Chronic NDPS nephrotoxicity is seen as marked renal interstitial nephritis.

The ultimate nephrotoxicant species responsible for acute or chronic NDPS nephrotoxicity is related to the formation of sulfate and possibly glucuronide conjugates of NDPS metabolites. Oxidation of the succinimide ring in the liver via phenobarbital-inducible cytochrome-P450 isozymes forms *N*-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS, **Figure 7**), an essential bioactivation step in acute nephrotoxicity. Both NDHS and its hydrolysis product, *N*-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (NDHSA), are four times more potent as nephrotoxicants than NDPS, while the decarboxylation metabolite of NDHSA is a nonnephrotoxicant. However, neither NDHS nor NDHSA appears to be directly toxic to renal cortical slices, proximal tubule suspensions or renal mitochondria. Studies also suggest that NDHSA can cyclyze *in vivo* to form NDHS which further clouds the identity of which NDPS metabolite ultimately gives rise to the toxicant species.

Recent reports suggest that the sulfate conjugate of NDHS (NSC) may be the ultimate or penultimate

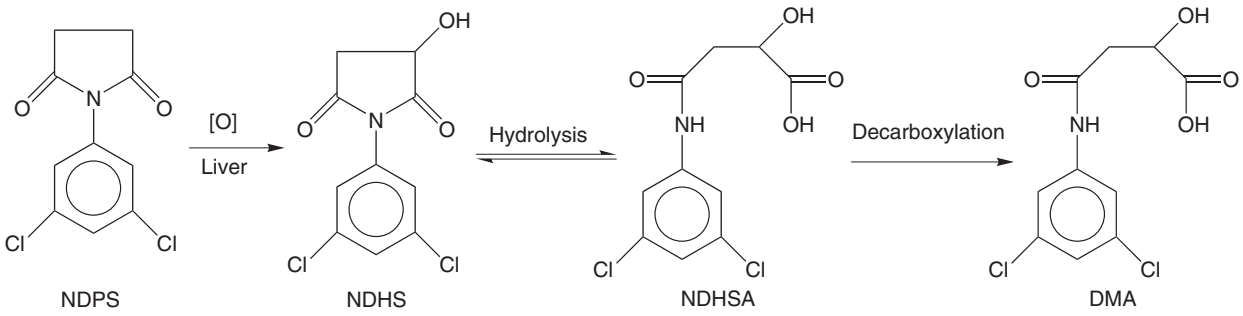

Figure 7 Oxidative biotransformation of *N*-(3,5-dichlorophenyl) succinimide (NDPS).

toxic metabolite. NSC is formed in the liver and is directly toxic to proximal tubule cells *in vitro*. Whether NSC directly interacts with intracellular targets or breaks down to release *N*-(3,5-dichlorophenyl)maleimide (NDPM), a highly reactive chemical species, as the ultimate toxic species remains to be determined. However, NSC and NDPM exhibit similar nephrotoxic potential *in vitro* with freshly isolated renal cortical cells.

2-*Haloethylamines* The 2-haloethylamines are model nephrotoxicants that target different segments of the nephron depending on the halogen atom. The bromo derivative, 2-bromoethylamine (BEA), concentrates in the renal medulla and induces renal papillary necrosis. The renal effects of BEA are dependent on the urinary concentrating ability of antidiuretic hormone (ADH). In the absence of ADH, urine is not concentrated in collecting ducts and BEA nephrotoxicity is diminished. The chemical species responsible for BEA nephrotoxicity is believed to be ethyleneimine formed by the intramolecular cylization of BEA which then alkylates renal macromolecules (**Figure 8**).

The chloro derivative, 2-chloroethylamine (CEA), is less potent as a papillitoxin than BEA, presumably due to the fact that the bromo group is a better leaving group than the chloro group. Thus, the reactive intermediate, ethyleneimine, would form faster from BEA than CEA. The fluoro derivative, 2-fluoroethylamine (FEA), is more lethal than BEA, but at nonlethal doses is toxic to the S_3 segment of the proximal tubule rather than the renal papilla. Since the fluoro group is a poorer leaving group than chloro or bromo groups, it is not clear if FEA nephrotoxicity is due to FEA, ethyleneimine or a FEA metabolite.

Glutathione Conjugates Glutathione (GSH) is a tripeptide (gamma-Glu-Cys-Gly), which forms bonds between the sulfhydryl group of cysteine and electrophilic sites in xenobiotics or their metabolites. The

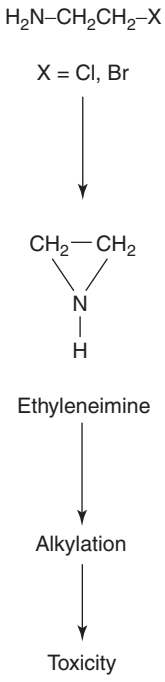

Figure 8 Intramolecular cyclization of haloethylamines.

formation of these glutathione conjugates is catalyzed by one or more of the glutathione *S*-transferase enzymes. The α forms of glutathione *S*-transferase can also catalyze detoxification of organic peroxides to protect cells against free radical toxicity as well as electrophilic attack. Thus, interactions with glutathione serve as an important mechanism for detoxifying reactive chemical species within cells.

Although interactions between glutathione and electrophilic molecules normally leads to detoxification, there are also several examples of bioactivation of chemicals to nephrotoxicants following glutathione conjugation. Glutathione conjugates can be formed in most segments of the kidney with glutathione *S*-transferase activity being highest in proximal tubular cells. However, glutathione conjugates of many electrophiles are formed primarily in the liver and transported to the kidney either as the

glutathione conjugate or as a glutathione conjugate metabolite.

Glutathione conjugates formed in the liver can leave hepatocytes and enter the blood or the bile. Biliary excretion of the glutathione conjugate can result in degradation of the glutathione conjugate to the cysteine conjugate within the biliary tract and small intestine. This degradation is catalyzed by gamma-glutamyl transpeptidase (removes glutamate) and peptidases (remove glycine). The *N*-acetylcysteine conjugate, also known as a mercapturate, can be formed in the intestinal tract or liver following absorption of the cysteine conjugate from the small intestine by action of an *N*-acetylase enzyme. The relative amounts of the three conjugates that reach the kidney from extrarenal sites depend primarily on the chemical nature of the halogenated hydrocarbon and the animal species studied.

The kidney also can process and transport glutathione, cysteine, and *N*-acetylcysteine conjugates. Glutathione conjugates can be converted to the cysteinylglycine conjugate by gamma-glutamyl transpeptidase, which is located in kidney primarily at the brush border membrane. The action of brush border dipeptidase enzymes (e.g., aminopeptidase M) then removes the glycine residue from the dipeptide conjugate to release the cysteine conjugate, which is usually accumulated within proximal tubular cells. Glutathione conjugates may also be directly transported into renal cells at the basolateral membrane via a sodium dependent uptake mechanism. Once inside the cell, the glutathione conjugate may be a substrate for gamma-glutamyl cyclotransferase and peptidases to release the cysteine conjugate. Cysteine conjugates can also be substrates for amino acid uptake systems and accumulate in proximal tubular cells via the amino acid transporters, while mercapturates accumulate in proximal tubular cells via the organic anion transporter at the basolateral membrane. Accumulated mercapturates may then be secreted into the urine or deacetylated to release the corresponding cysteine conjugate. Thus, the kidney can be exposed to metabolites formed from glutathione conjugation originating at renal or extrarenal sites.

Several mechanisms have been identified for the generation of nephrotoxicants following conjugation of halogenated hydrocarbons with glutathione (**Table 6**). These mechanisms include intramolecular cyclization, activation by cysteine conjugate β-lyase, and facilitated renal accumulation of the toxicant. Examples of each of these bioactivation mechanisms will be discussed in the following sections.

Intramolecular Cyclization The 1,2-dihaloethanes (XCH_2CH_2X) are used as pesticides, lead scavengers, industrial solvents and/or grain fumigants. Both 1,2-dichloroethane and 1,2-dibromoethane are hepatotoxicants, nephrotoxicants, and potential carcinogens. Acute renal effects induced by these alkyl halides are characterized as proximal tubular necrosis, primarily in the juxtaglomerular regions. The mechanism of the nephrotoxicity induced by the 1,2-dihaloethanes is believed to initially involve conjugation of the halogenated hydrocarbon with glutathione (**Figure 9**). Both 1,2-dichloroethane and 1,2-dibromoethane form glutathione conjugates in liver, but only 1,2-dibromoethane forms a glutathione conjugate in the kidney.

The glutathione conjugate formed from 1,2-dibromoethane is relatively unstable and quickly undergoes intramolecular cyclization via displacement of the second bromo group by the glutathionyl sulfur to form an episulfonium ion (**Figure 9**). The episulfonium ion then readily forms adducts with renal cell macromolecules, including DNA, which leads to altered cell function and toxicity. Because of the highly reactive nature of the glutathione conjugate formed from 1,2-dibromoethane, it is likely that only the renally formed glutathione conjugate contributes to nephrotoxicity.

The glutathione conjugate formed in the liver from 1,2-dichloroethane is more stable than the conjugate

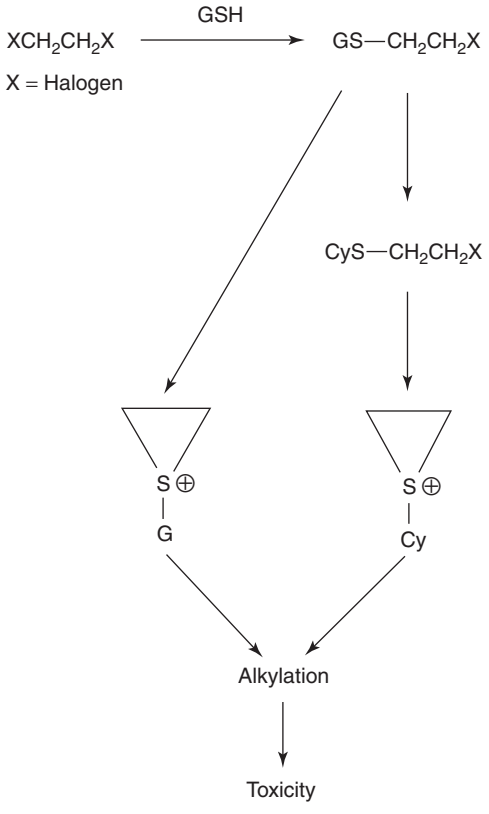

Figure 9 Glutathione conjugation with 1,2-dihaloethanes.

formed from 1,2-dibromoethane, and may be transported to the kidney as the glutathione or related conjugate. Within the kidney, both glutathione and cysteine conjugates appear to contribute to 1,2-dichloroethane nephrotoxicity by forming episulfonium ions which can interact with cellular macromolecules (**Figure 9**). Reactivity of the glutathione and cysteine conjugates toward DNA is greatest for the cysteine conjugate, but the relative contribution of these conjugates to the carcinogenic mechanism for 1,2-dichloroethane remains to be determined.

Two additional compounds containing 1,2-dihaloethyl groups are also nephrotoxicants. The nematocide and soil fumigant 1,2-dibromo-3-chloropropane and the flame retardant tris(2,3-dibromopropyl)phosphate both induce acute tubular necrosis. Although conjugation with glutathione may play a role in the toxicity induced by these agents, the mechanism responsible for the nephrotoxicity induced by these compounds is not known.

Cysteine Conjugate β-Lyase Activation There are numerous glutathione and/or cysteine conjugates of unsaturated halogenated hydrocarbons that are nephrotoxicants and/or nephrocarcinogens (**Table 7**). These conjugates induce nephrotoxicity following cleavage of the cysteine conjugate by the enzyme cysteine conjugate β-lyase to form pyruvate, ammonia and a reactive thiol. Acute nephrotoxicity induced by these conjugates is characterized by diuresis, proteinuria, glucosuria, elevated BUN concentration, and proximal tubular necrosis. The site of the lesion appears to be species dependent with most rodent models exhibiting the initial lesion in the corticomedullary region (S_3 segment of the proximal tubule). However, the initial lesion in dogs appears in the S_1 and S_2 segments of the proximal tubule. In addition, age, gender and strain differences exist for susceptibility to the nephrotoxicity induced by these conjugates.

Table 7 Some halogenated hydrocarbons whose glutathione and/or cysteine conjugates are nephrotoxicants and/or nephrocarcinogens

1-Chloro-1,2,2-trifluoroethylene
1,1-Dibromo-2,2-difluoroethylene
Dichloroacetylene
1,1-Dichloro-2,2-difluoroethylene
Hexachloro-1,3-butadiene
Hexafluoropropene
Tetrachloroethylene
Tetrafluoroethylene
Trichloroethylene
1,1,2-Trichloro-3,3,3-trifluoro-1-propene

The nature of the ultimate nephrotoxicant species formed following the action of cysteine conjugate β-lyase is determined by the halogen substitution on the parent haloalkene. When the haloalkene is a geminal difloroalkene (e.g., tetrafluoroethylene), then the resulting glutathione conjugate and subsequent cysteine conjugate is a saturated or alkyl conjugate (**Figure 10**).

However, if the haloalkene is a geminal dichloroalkene (e.g., trichloroethylene), then unsaturated conjugates are formed (**Figure 10**). The saturated cysteine conjugates are bioactivated by β-lyase to form a thionoacyl fluoride which can rapidly acylate renal macromolecules to induce toxicity, while the unsaturated cysteine conjugates are bioactivated by β-lyase to the highly reactive thioketene metabolites (**Figure 11**). Primary targets for these reactive intermediates appear to be the renal mitochondria which contain a portion of cellular cysteine conjugate β-lyase.

The reasons why the kidney is a major target for cysteine conjugate toxicity are not completely understood, particularly since cysteine conjugate β-lyase is present in organs other than the kidney. However, the ability of the kidney to (1) accumulate metabolites formed by the glutathione conjugate pathway from blood, (2) convert glutathione and *N*-acetylcysteine conjugates to cysteine conjugates, and (3) rapidly activate nephrotoxicant cysteine conjugates to their reactive intermediate may explain the susceptibility of the kidney to these agents.

The toxicity induced by nephrotoxicant glutathione, cysteine, and *N*-acetylcysteine conjugates can be modified by a variety of compounds. Probenecid, an

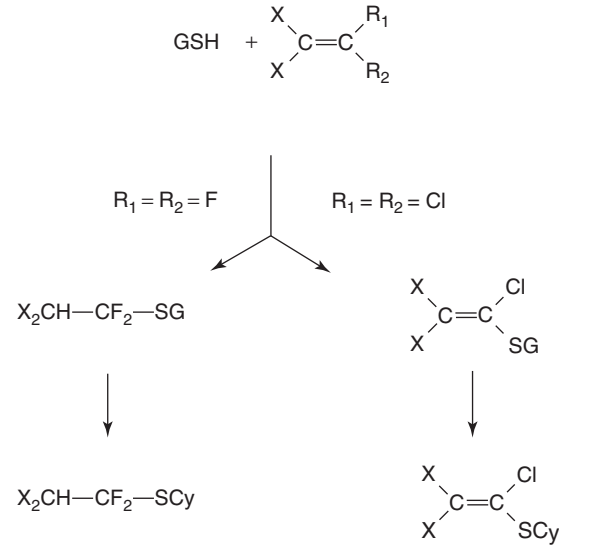

Figure 10 Glutathione conjugation with geminal difluoro- or dichloroalkenes.

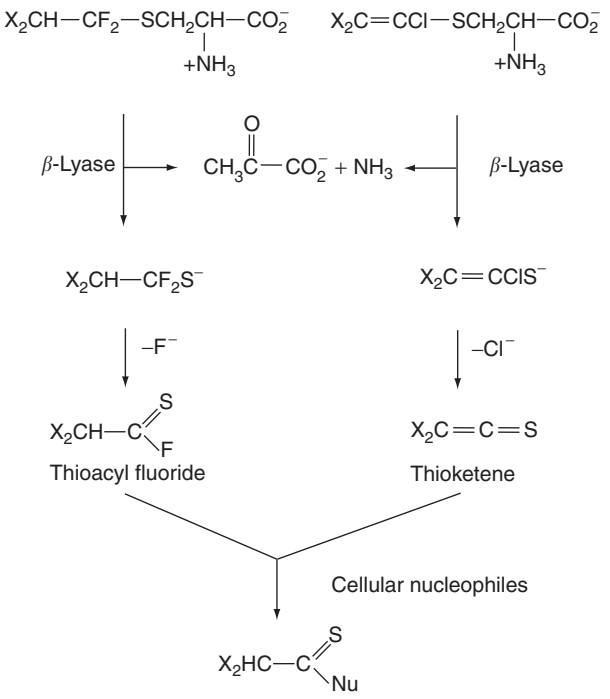

Figure 11 Cysteine conjugate β-lyase bioactivation of cysteine conjugates of haloalkenes.

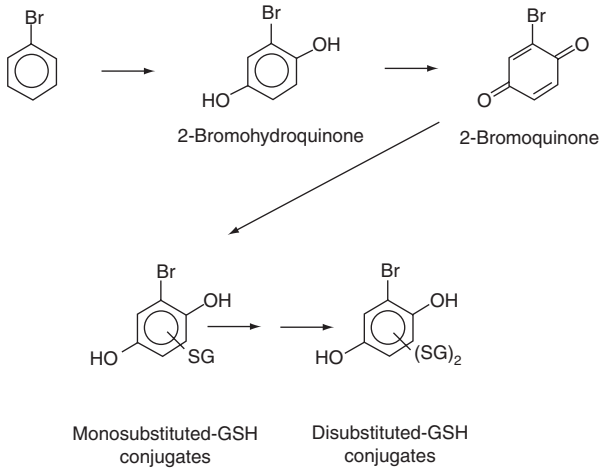

Figure 12 Bioactivation of bromobenzene to nephrotoxicant metabolites.

inhibitor of the organic ion transporter, can reduce the nephrotoxicity induced by glutathione conjugates, presumably by decreasing the renal accumulation of the mercapturate. Acivicin (AT-125), an anticancer agent, is an irreversible inhibitor (>97%) of gamma-glutamyl transpeptidase and can attenuate the toxicity of nephrotoxicant glutathione conjugates by inhibiting conversion of the glutathione conjugate to the cysteine conjugate. In addition, aminooxyacetic acid (AOAA) inhibits the action of pyridoxal-dependent enzymes (e.g., β-lyase) and blocks the conversion of cysteine conjugates to their ultimate nephrotoxicant species. These inhibitors are useful tools in the study of glutathione and cysteine conjugate nephrotoxicity, but they may not always give clear results. Probenecid has biological effects unrelated to inhibition of organic ion transport, and acivicin pretreatment can fail to protect against haloalkene (e.g., hexachloro-1,3-butadiene) nephrotoxicity even when it is known that glutathione and/or cysteine conjugates of the parent haloalkene are nephrotoxicants. Also, AOAA not only inhibits β-lyase but can inhibit oxidative enzymes and other pyridoxal-dependent pathways as well.

Facilitated Accumulation of Nephrotoxicants

Bromobenzene, a chemical intermediate, is both a heptotoxicant and a nephrotoxicant. Bromobenzene-induced nephrotoxicity is characterized by glucosuria, enzymuria, proteinuria, elevated BUN concentration and kidney weight, and proximal tubular necrosis with the S_3 segment exhibiting the greatest damage. Bromobenzene hepatotoxicity is due to cytochrome P450 mediated formation of a 3,4-epoxide (arene oxide) metabolite of bromobenzene that can arylate hepatic tissue. However, the formation of the ultimate nephrotoxicant species appears to require multiple biotransformation steps and possibly multiple toxicant species.

The first step in the bioactivation of bromobenzene to a nephrotoxicant is the hepatic cytochrome P450 mediated oxidation of bromobenzene to 2-bromophenol, which is further oxidized in the liver to 2-bromohydroquinone. 2-Bromohydroquinone can be activated by a renal quinol oxidase to 2-bromoquinone (**Figure 12**) which can directly arylate renal macromolecules. Although 2-bromoquinone can undergo redox cycling to potentially generate oxidative stress and reactive oxygen species, arylation appears to be more important for the cellular toxicity induced by 2-bromoquinone.

Recent studies have also demonstrated that mono- and di-glutathione conjugates of 2-bromohydroquinone can be formed *in vivo* in rats (**Figure 12**). The diglutathionyl conjugate of 2-bromohydroquinone (a quinol-thioether) induces nephrotoxicity which is indistinguishable from bromobenzene, 2-bromophenol, or 2-bromohydroquinone nephrotoxicity, but occurs at a dose 10–15 times lower than with 2-bromohydroquinone. The ability of acivicin but not AOAA to attenuate nephrotoxicity induced by the diglutathionyl conjugate has suggested that gamma-glutamyl transpeptidase but not β-lyase is important in activating the conjugate. Thus, entry into renal tissue via gamma-glutamyl transpeptidase

is a key step for diglutathionyl conjugate nephrotoxicity. Partial protection by acivicin against 2-bromohydroquinone nephrotoxicity *in vivo* suggests that the mono- and/or diglutathionyl conjugates of 2-bromohydroquinone may be formed extrarenally, and that glutathione conjugation may play a role in the transport and selective accumulation of 2-bromohydroquinone in renal tissue.

The cellular mechanism of nephrotoxicity induced by the quinol-thioethers appears to be related to the ability of the conjugates to undergo redox cycling with the concomitant formation of reactive oxygen species and oxidative stress. The cysteine conjugates of 2-bromohydroquinone are more readily oxidized than the corresponding glutathione conjugates or mercapturates, and therefore, are probably responsible for most of the conjugate-induced oxidative stress and resultant nephrotoxicity. However, the exact nature of the subcellular targets and relative contributions of the various bromobenzene metabolites to bromobenzene nephrotoxicity remains to be determined with certainty.

Mycotoxins The mycotoxins are secondary products of fungal metabolism. Numerous mycotoxins have been identified as toxicants in humans and/or animal models with several organ systems, including the kidney, being targets for these fungal products. Perhaps the two mycotoxins that have received the most attention as nephrotoxicants are citrinin and ochratoxin A. These two mycotoxins have received particular interest due to their possible role in endemic Balkan nephropathy.

Citrinin is produced by several *Penicillium* and *Aspergillus* species which may be found associated with grains (e.g., wheat, oats, etc.). Humans and animals eating the grain can experience citrinin-induced nephrotoxicity which is characterized by diuresis, decreased urinary osmolality, glucosuria, proteinuria, and elevated BUN concentration. Morphological changes included cytoplasmic vacuolization of proximal tubular cells, mitochondrial swelling and ultimately, proximal tubular necrosis. The exact site of the renal lesion may vary depending on the species studied.

The mechanism of citrinin-induced nephrotoxicity has not been completely elucidated. However, it appears that citrinin accumulates in proximal tubular cells via the organic anion transporter, and that the parent compound is the nephrotoxicant species. Mitochondria are early targets for citrinin with multiple effects on mitochondrial function observed following exposure of mitochondria to citrinin, including uncoupling of mitochondrial respiration. The subsequent lost of cellular ATP content may eventually lead to cell death.

Table 8 Miscellaneous nephrotoxicants

Petroleum components	Paraquat
Carbon disulfide	Lindane
Oxalic acid	Diquat
Venoms	3-Chloropropane
Crotalus venom	Glycols
Brown recluse spider venom	Maleic acid
Decalin	Ethylene glycol
Tetralin	Propylene glycol
Mushroom poisoning	Glycerol
D-Limonene	Radiation
Carbon monoxide	Physical agents
1,4-Dichlorobenzene	Crush injuries

Ochratoxin A is also produced by *Aspergillus* species and is one of the most widely occurring mycotoxins in food and grains. Ochratoxin A nephrotoxicity is similar to citrinin nephrotoxicity but can also include renal interstitial fibrosis and glomerular changes. Like citrinin, ochratoxin A accumulates in proximal tubular cells via the organic anion transporter and appears to induce nephrotoxicity without bioactivation to a toxic metabolite. Mitochondria are also a target for ochratoxin A with proximal tubular ATP content significantly decreased in the presence of as little as 10^{-8} mol l^{-1} ochratoxin A. However, ochratoxin A also inhibits renal gluconeogenesis and lowers mRNA levels, and it has been suggested that these events may also contribute to ochratoxin A nephrotoxicity. While each of these cellular effects might contribute to the renal toxicity induced by ochratoxin A, the precise cellular mechanism of toxicity remains to be determined.

Miscellaneous Nephrotoxicants There are numerous additional agents that have been identified as nephrotoxicants, and some of these chemical and physical agents are shown in **Table 8**. In addition to the compounds listed, there are hundreds of chemicals whose effects on the kidney are either unknown or are poorly characterized, and unquestionably this list will continue to grow with the ongoing development of newer drugs, agricultural agents, and industrial intermediates.

See also: Carbon Tetrachloride; Cephalosporins; Chloroform; Cisplatin; Glutathione; Metals; Penicillin.

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Killer Lakes

Pertti J Hakkinen

Killer Lakes is the name given to the disasters involving rapid releases of massive volumes of carbon dioxide (CO_2) from two lakes in Cameroon. Humans and other animals were asphyxiated, with reports of 37 victims in the Lake Monoun disaster in 1984, and 1800 victims in the Lake Nyos disaster in 1986. The lakes occupy the crater of a supposedly extinct volcano, in a region known for numerous gaseous water springs, a common feature of old volcanic areas. These disasters began when a cloud of dense gas erupted from each lake, covering the surrounding areas under a layer of gas many meters thick, for an unknown amount of time. The source of the gas was determined afterwards, with the evidence including damage to the shores by waves and strong winds, and having the normally clear waters turn reddish.

Investigations found indications that much, if not all, of the CO_2 released was stored in the lakes prior to the events, and that a volcanic eruption was not thought to be associated with either disaster. Accumulation of CO_2 in the lakes started from CO_2-rich gas of magmatic origin rising to the earth's surface and contacting groundwater; the CO_2-charged groundwater is then discharged into the bottom of the lakes in springs. Before the gas events, these lakes were strongly stratified, that is, the surface and bottom waters did not mix, which allowed the gas that was being discharged from the groundwater to build up in the bottom waters of the lakes. The sudden release to the surface of CO_2 trapped at the bottom of the lakes has been called 'lake overturn'.

The triggers responsible for the gas releases from either lake are unknown, but one cause could have been a large landslide entering the lake and causing the lake stratification to be disrupted and allowing local oversaturation to initiate the gas release. Both disasters occurred in August, when stratification is weakest as the surface water loses heat during the monsoon season. Further, that both disasters occurred in the mid-1980s could be related to lower than normal temperatures and higher than normal rainfall during those years in Cameroon.

Both lakes Nyos and Monoun still contain appreciable levels of CO_2. Steps to reduce the hazards since the 1984 and 1986 disasters have included the use of pipes inserted into each lake to pump the gas-rich bottom waters to the surface, where the gas can be discharged harmlessly to the atmosphere. An international advisory committee is coordinating this effort, with major funding from the Cameroonian Government, The US Office of Foreign Disaster Assistance (part of the US Agency for International Development), and The French Embassy in Cameroon. Researchers involved in the degassing project have been funded in part by their home institutions. Further, private donations have helped to support CO_2 early-warning systems.

A third lake, Kivu in Rwanda, has been highlighted in a survey of deep lakes in Africa and Indonesia as another location where this type of disaster could happen from a massive geological event, that is, an earthquake or volcanic eruption. Lake Kivu was found to have high concentrations of dissolved CO_2 in its bottom water, and is one of the largest and deepest lakes in Africa with two million people living on its shore.

See also: Carbon Dioxide.

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Lanthanide Series of Metals

Charles E Lambert

- REPRESENTATIVE CHEMICALS: Cerium (Ce); Dysprosium (Dy); Erbium (Er); Europium (Eu); Gadolinium (Gd); Holmium (Ho); Lanthanum (La); Lutetium (Lu); Neodymium (Nd); Prometheum (Pm); Praseodymium (Pr); Samarium (Sa); Terbium (Tb); Thulium (Tm); Ytterbium (Yb); Yttrium (Y)
- CHEMICAL ABSTRACTS SERVICE REGISTRY NUMBERS: Cerium (CAS 7440-45-1); Dysprosium (CAS 7429-91-6); Erbium (CAS 7440-52-0); Europium (CAS 7440-53-1); Gadolinium (CAS 7440-54-2); Holmium (CAS 7440-60-0); Lanthanum (CAS 7439-91-0); Lutetium (CAS 7440-94-3); Neodymium (CAS 7440-00-8); Prometheum (CAS 7440-12-2); Praseodymium (CAS 7440-10-0); Samarium (CAS 7440-19-9); Terbium (CAS 7440-27-9); Thulium (CAS 7440-30-4); Ytterbium (CAS 7440-64-4); Yttrium (CAS 7440-65-5)
- SYNONYMS: Rare earths, rare-earth metals
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Transition metals
- CHEMICAL FORMULAS: Common compounds:
  - Cerium oxide: Ce_2O_3
  - Gadolinium oxide: Gd_2O_3
  - Lanthanum oxide: La_2O_3
  - Yttrium oxide: Y_2O_3
  - Cerium nitrate: $Ce(NO_3)_3$
  - Cerium chloride: $CeCl_3$

Uses

Most of the industrial uses of the lanthanides require compounds (e.g., oxides) rather than pure elements. Most of these are cerium compounds or mixtures of lanthanides as they occur in ores (e.g., lanthanum concentrate). Some of the major uses are:

- *Carbon-arc lighting*: The US Army, Navy, and Coast Guard searchlights all use lanthanide-cored carbons.
- *Lanthanide alloys*: These alloys are used in cigarette lighter flints, magnesium alloys, and ferrous alloys.
- *Glass industry*: Important uses for the coloring and decoloring of glass, the polishing of spectacle and optical instrument lenses, the surface preparation of mirror glass and other glass specialties.
- *Medicine*: Gadolinium diethylenetriamine pentaacetic acid (DTPA) is routinely used as an intravenous contrast agent for magnetic resonance imaging.

Other applications for the lanthanides include phosphors for X-ray screens and television tubes, catalysts, lasers, powerful magnets, and high-temperature superconductors.

Background Information

The lanthanide series of metals includes the 15 elements with atomic numbers 57–71, plus yttrium (atomic number 39). The lanthanides occur in the earth's crust at concentrations exceeding some commonly used industrial elements making the term 'rare earths' something of a misnomer. For example, yttrium, cerium, lanthanum, and neodymium are present in the earth's crust at higher concentrations than lead. Of the 15 lanthanides, only promethium does not occur in nature – it is a man-made element. All of the lanthanides have similar physical and chemical properties. Because of similarities in their chemistry and toxicity, the characteristics of the lanthanides are often described as a group. Within the lanthanide group, however, there are differences between the toxicity of the individual lanthanide elements and their compounds.

Exposure Routes and Pathways

Direct eye or skin contact with the powder or liquid forms of the soluble lanthanide compounds including the chlorides and acetates can cause irritation. These forms of the lanthanides are the most likely to cause damage at the point of contact. The highly insoluble lanthanide oxides and carbonates may cause mild abrasive irritation upon dermal contact.

Occupational exposure may occur through inhalation of dust and dermal contact. The general population may be exposed to naturally occurring

concentrations of the oxides in soil or through medical procedures such as the intravenous administration of magnetic resonance imaging (MRI) contrast agents.

Toxicokinetics

Different forms of lanthanide differ in their toxicity. There are three forms of lanthanides: soluble (chlorides, nitrates, acetates), insoluble (oxides, carbonates), and chelated compounds (DTPA). Most of the available information on lanthanide absorption and toxicity comes from the soluble lanthanide salts. In one study, rats given DTPA (chelating agent) 1 or 2 days after oral administration of cerium chloride were found to have significantly reduced whole body retention of soluble cerium (from 40% to 2%).

Different forms of lanthanide have different organ distribution and excretion rates. Intravenously injected chelated lanthanide is transiently accumulated in the kidney and most of the injected dose is excreted in the urine. However, intravenously injected soluble salt is taken up by the reticuloendothelial cells, with most of the dose accumulating in the liver and spleen. The result of this intravenous exposure is liver necrosis.

The lanthanide oxides and carbonates have been shown in *in vitro* bioaccessibility studies to have a very low gastrointestinal bioaccessibility of $\sim$6%.

Mechanism of Toxicity

The soluble lanthanide salts (e.g., chlorides, nitrates, and acetates) can be severely irritating to the skin, eye, and mucous membranes. The irritation appears to be a result of exposure to the anion (e.g., nitrate) and not the lanthanide cation.

Effects from oral exposure to the soluble lanthanides include eosinophil infiltration of the submucosa, hyperkerotosis of the stomach, and gastric hemorrhages. As with the irritation that occurs after skin or eye exposure, the hyperkerotosis and gastric hemorrhages seen in the stomach appears to be the result of the acidic environment produced by the anion.

Acute and Short-Term Toxicity (or Exposure)

The lanthanides have historically been characterized as low toxicity metals and therefore have not been the subject of significant toxicological investigation. The data that have been gathered are primarily from acute and chronic animal studies. Because human exposures have rarely reached toxic levels, few instances of human toxicity have been observed, despite their widespread industrial use. The following discussion of toxicity focuses on the soluble forms and the oral route of exposure.

Animal

Most of the LD_{50}s for the lanthanides are high. For example, the most recent data for lanthanum oxides, carbonates, and concentrates tested show LD_{50}s in excess of 5000 mg lanthanum per kg animal body weight. This LD_{50} range is generally regarded as 'practically nontoxic'. However, some lanthanides appear to be more toxic than the oxides and carbonates – with both compound solubility and the form of the anion playing a role in toxicity. For example, the lanthanum chlorides, nitrates, and acetates have LD_{50}s in the 1600–5000 mg kg^{-1} body weight range, putting them in the 'slightly toxic' range. Common symptoms of acute toxicity seen after these very high doses included writhing, ataxia, slightly labored and depressed respiration, arched back, stretching of limbs on walking, and lacrimation. **Table 1** includes a summary of current oral LD_{50}, eye and skin irritancy data for individual lanthanide compounds and some commonly used mixtures.

Subchronic A number of studies have been completed in which the soluble lanthanide chlorides are given orally to animals over the course of a month. In several studies rats were given the hydrated chloride forms of lanthanum, yttrium, and europium by oral gavage at doses of 0, 40, 200, or 1000 mg kg^{-1} day^{-1} for consecutive 28 days. Those animals administered lanthanum chloride demonstrated a slight decrease in body weight due to decreased food intake at the 200 and 1000 mg kg^{-1} doses. Changes in serum transaminase activity were also observed at the higher dose (1000 mg kg^{-1}). This activity is suggestive of liver toxicity, though no corresponding histopathological changes were observed in the liver. At the higher dose, stomach lesions were also observed. This is not surprising given the chloride form of the lanthanides

Table 1 Acute toxicity of select lanthanides and common mixtures

Compound	*Eye irritation*	*Skin irritation*	*Oral LD_{50} (g kg^{-1})*
Cerium concentrate	Moderate	Nonirritant	>5
Cerium chloride	Severe	Severe	2.8
Cerium nitrate	Severe	Mild	4.2
Lanthanum concentrate	Minimal	Nonirritant	>5
Lanthanum oxide	Mild	Nonirritant	>5

and its potential for irritation. Similar effects were observed for the other lanthanides tested.

In another series of studies of the soluble lanthanide chlorides, rats were fed gadolinium, samarium, terbium, thulium, ytterbium, praseodymium, neodymium, lutetium, europium, dysprosium, holmium, and erbium chloride in their diet at doses of 0, 5, 50, and 500 mg kg^{-1} day^{-1} for 12 weeks. Only ytterbium chloride caused any significant effect, with the 500 mg kg^{-1} dose causing gastric hemorrhages. The other lanthanides caused no adverse effects at the maximum 500 mg kg^{-1} dose.

Human

In one of the best documented human lanthanide exposure studies to date, the toxicity of gadolinium in patients with impaired kidney function was assessed. One hundred fifty-one patients with compromised kidney function were assessed after a dose of 0.1 mmol gadolinium DTPA per kg body weight was administered as a contrast agent for MRI examinations. A retrospective analysis of physician and nursing records, radiology reports, laboratory data, and autopsy records for 3 days prior to the MRI and 30 days after was completed. No significant adverse effects were observed after intravenous gadolinium exposure in this sensitive subpopulation.

Chronic Toxicity (or Exposure)

Animal

In one of the only drinking water studies of the lanthanides, mice consumed yttrium nitrate at 5 ppm for 18 months. Based on this 5 ppm drinking water concentration, the calculated dose was 0.95 mg kg^{-1} day^{-1}. A decrease in body weight was observed in the animals over the course of the study. However, survival of the animals compared to controls was not affected following lifetime exposure.

In a reproductive toxicity study, a diet containing a number of heavy metals including the lanthanides (as oxides) was fed to mice over three generations. The highest calculated lanthanide dose in the diet was a combination of the following: 156 mg dysprosium kg^{-1} day^{-1}, 5 mg europium kg^{-1} day^{-1}, 52 mg lanthanum kg^{-1} day^{-1}, 104 mg samarium kg^{-1} day^{-1}, 156 mg terbium kg^{-1} day^{-1}, 16 mg ytterbium kg^{-1} day^{-1}, and 10 mg thulium kg^{-1} day^{-1}. After three generations of exposure no reproductive or other health effects were observed in the treated animals.

None of the lanthanide toxicity data gathered to date indicates that the lanthanides cause long-term health effects such as reproductive or carcinogenic toxicity.

Human

Current literature on long-term (20 year) occupational exposure to high levels of lanthanide dust suggests that the lanthanide oxides may cause benign pneumoconiosis (the deposition of material in the lung, visible on X-ray, without any impairment of lung function). This conclusion is further supported by animal studies.

Clinical Management

The soluble forms of the lanthanides may cause severe eye and skin irritation. If direct eye contact immediately hold eyelids apart and flush the affected eye(s) with clean water for several minutes. If skin contact, cleanse affected area(s), thoroughly washing with mild soap and water.

Ingestion of the soluble lanthanides may cause gastric irritation. The stomach contents can be diluted by drinking copious amounts of water. Because of potential gastrointestinal irritation, vomiting should not be induced.

The lanthanide oxides and carbonates have a low degree of toxicity by ingestion; however, dusts may be abrasive and irritating to the eyes and skin.

Lanthanides, because of their high density, may produce striking abnormalities on chest X-rays. However, lanthanides are generally not believed to be fibrogenic and the lesions typically have little or no clinical significance. Occasional cases of suspected pneumoconiosis have been reported.

Environmental Fate

The lanthanides can be found in the earth's crust at a wide range of concentrations. For example, thulium is present at only 0.5 ppm, whereas lanthanum and cerium are present at 30 and 60 ppm, respectively. The mineralized forms of the lanthanides that are of greatest commercial and mining interest are monazite, bastnaesite, and cerite. The most common commercial forms of the lanthanides are the oxides and carbonates, which have low solubility and mobility. In contrast, the lanthanide chlorides, nitrates, and acetates, because of their high solubility, are more likely to leach into groundwater and surface water.

Ecotoxicology

There is little information available on the ecotoxicology of the lanthanides. Lanthanides do not appear to be essential elements for plants and animals. In general, plants do not absorb lanthanides from soil due to discrimination against their absorption by the

roots. This negligible accumulation of lanthanides by plants effectively blocks the dietary transfer of lanthanides from the soil to wildlife. In mammals the gastrointestinal absorption of simple lanthanide salts is poor.

One study assessed the toxicity of lanthanides in soil invertebrates. In this study native microflora were exposed to soils treated with 57 ppm lanthanum chloride for 23 days. Reduced respiration, which can be directly related to reproductive success, was observed at 57 ppm lanthanum chloride.

No water quality objectives or other water quality standards were found for the lanthanide metals. Aquatic toxicology data were only found for the lanthanide soluble salts. These soluble salts are known to have high chronic toxicity in fish, moderate chronic toxicity in green algae, and low acute toxicity in daphnids based on exposures in moderately hard water in terms of lanthanide per liter.

Other Hazards

Always refer to the appropriate Material Safety Data Sheet (MSDS) for detailed information on handling and disposal. The soluble lanthanides may be corrosive. The insoluble lanthanide oxides and carbonates are expected to be stable indefinitely under most conditions.

Exposure Standards and Guidelines

Of the lanthanides, only yttrium has occupational exposure standards. The other lanthanides have low levels of toxicity similar to or less toxic than yttrium and therefore the exposure limits set for yttrium are generally used for the other lanthanides.

See also: Kidney; Metals.

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Law and Toxicology

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Introduction

Courts, legislatures, and administrative agencies in the United States frequently rely upon toxicologists to assist them in legal proceedings. The role of the toxicologist varies with the forum. When agencies develop regulations, for example, toxicologists typically are asked only to review documents and advise regulators on technical issues, either as a matter of personal knowledge, or as a matter of expertise. Similarly, in court cases, the toxicologist's role most often is limited to reviewing documents and advising litigants. Occasionally, however, both in regulatory and in judicial proceedings, the toxicologist contributes further by offering oral or written testimony on behalf of the parties, or at the request of the agency or tribunal. Rarely, toxicologists may be granted opportunities to testify before committees of Congress or state legislatures.

Thoughtful toxicologists seeking to make significant contributions to legal process should develop a basic understanding of the law of evidence as it applies to 'experts'. At the outset, toxicologists should appreciate that many of the rules governing their participation in legal proceedings reflect a compromise between two fundamental principles that determine the competency of scientific or medical evidence. One principle holds that problematic or deficient evidence should never be admitted; the other holds that any problem or deficiency in evidence should influence only the weight, and not the admissibility, accorded that evidence.

Recent Developments in American Law of Experts

Historically, the law of experts in the United States has focused on two fundamental questions. First, is the subject matter of the expert's opinion appropriate to the matter at hand? Second, is the expert sufficiently qualified to render the proffered opinion? During the last century, three watershed events – a

federal court decision in 1923, a federal enactment taking effect in 1975, and a federal court decision in 1993 – provided basic answers to these questions.

From 1923 to the present, a decision from the US Court of Appeals for the District of Columbia in *Frye v. United States*, 293 F. 1013, has provided the most recognized standard for admissibility of expert evidence and testimony. In an opinion refusing to admit the results of a 'lie detector' test, the court announced that "just when a scientific principle or discovery crosses the line between the experimental and demonstrable stages is difficult to define. Somewhere in this twilight zone the evidential force of the principle must be recognized, and while courts will go a long way in admitting expert testimony deduced from a well-recognized scientific principle or discovery, the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field in which it belongs." By the early 1970s, *Frye* had been approved not only in the federal courts but also in 46 states.

From 1975 to the present, Rules 403 and 701 through 706 of the Federal Rules of Evidence (FRE) have provided an alternative touchstone for determining the requirements of admissibility of expert testimony. As of 2004, at least 41 states pattern their evidence codes directly after the Federal Rules.

Revised FRE 702 states "if scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise, if (1) the testimony is based upon sufficient facts or data, (2) the testimony is the product of reliable principles and methods, and (3) the witness has applied the principles and methods reliably to the facts of the case." By contrast, Revised FRE 701 states "if the witness is not testifying as an expert, the witness' testimony in the form of opinions or inferences is limited to those opinions or inferences which are (a) rationally based on the perception of the witness and (b) helpful to a clear understanding of the witness' testimony or the determination of a fact in issue, and (c) not based on scientific, technical, or other specialized knowledge."

Revised FRE 703 states "the facts or data in the particular case upon which an expert bases an opinion or inference may be those perceived by or made known to the expert at or before the hearing. If of a type reasonably relied upon by experts in the particular field in forming opinions or inferences upon the subject, the facts or data need not be admissible in evidence in order for the opinion or inference to be admitted. Facts or data that are otherwise inadmissible shall not be disclosed to the jury by the proponent of the opinion or inference unless the court determines that their probative value in assisting the jury to evaluate the expert's opinion substantially outweighs their prejudicial effect."

FRE 704 states "(a) except as provided in subdivision (b), testimony in the form of an opinion or inference otherwise admissible is not objectionable because it embraces an ultimate issue to be decided by the trier of fact; (c) no expert witness testifying with respect to the mental state or condition of a defendant in a criminal case may state an opinion or inference as to whether the defendant did or did not have the mental state or condition constituting an element of the crime charged or of a defense thereto. Such ultimate issues are matters for the trier of fact alone." (Importantly, FRE 704 does not permit expert witnesses to offer legal conclusions, or to directly express opinions about the credibility of other witnesses.)

FRE 705 states "the expert may testify in terms of an opinion or inference and give reasons therefore without first testifying to the underlying facts or data, unless the court requires otherwise. The expert may in any event be required to disclose the underlying facts or data on cross examination."

FRE 706 states "the court may on its own motion or on the motion of any party enter an order to show cause why expert witnesses should not be appointed, and may request the parties to submit nominations. The court may appoint any expert witnesses agreed upon by the parties, and may appoint expert witnesses of its own selection. An expert witness shall not be appointed by the court unless the expert witness consents to act. An expert witness so appointed shall be informed of duties by the court in writing or at a conference in which the parties shall have the opportunity to participate. A witness so appointed shall advise the parties of the witness' findings, if any; the witness' deposition may be taken by any party; and the witness may be called to testify by the court or any party. The witness shall be subject to cross-examination by each party, including a party calling the witness."

FRE 403 provides "although relevant, evidence may be excluded if its probative value is substantially outweighed by the danger of unfair prejudice, confusion of the issues, or misleading the jury, or by considerations of undue delay, waste of time, or needless presentation of cumulative evidence."

In 1993, an opinion from the Supreme Court of the United States in *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 US 579, held that the adoption of the Federal Rules impliedly overturned the decision in *Frye*. Importantly, the text of the FRE does

not mention the *Frye* test or any need for scientific evidence to be generally accepted as a precondition to admissibility. Rather, the FRE replaced the general acceptance test with a validation (reliability) standard derived from the language of FRE 702. According to the *Daubert* Court, to be admissible, the subject of an expert's testimony must be based on reliable 'scientific…knowledge'. To qualify as 'scientific knowledge', a theory or technique must be validated by the scientific methodology of research and experimentation. However, the approach to scientific knowledge is 'flexible', and "its overarching subject is the scientific validity – and thus the evidentiary relevance and reliability – of the principles that underlie a proposed submission." The *Daubert* Court also observed that inquiry must be directed to the principles and methodology used by the expert in reaching his or her conclusions, and not to the conclusions themselves.

Factors to be considered in assessing the adequacy (reliability) of the methodology include whether: (1) the theory can be falsified by empirical testing, (2) the documentation supporting a theory or technique has been subjected to peer review and publication, (3) a known or potential rate of error has been determined, and (4) the theory or technique has been accepted in the relevant scientific community. Regarding the modern role of the general acceptance test in determinations of reliability of evidence, the *Daubert* Court stated "a reliability assessment does not require, although it does permit, explicit identification of a relevant scientific community and an express determination of a particular degree of acceptance within that community. Widespread acceptance can be an important factor in ruling particular evidence admissible, and a known technique that has been able to attract only minimal support within the community may properly be viewed with skepticism."

Standards governing the role of the toxicologist as 'expert' most likely will continue to be defined by the dynamic and evolving interplay of *Frye*, *Daubert*, and the FRE. Federal courts currently must rely on the language of the FRE and the teachings of *Daubert*, with 'general acceptance' providing only one of several factors that may be considered in determining whether the subject matter of the expert's testimony is appropriate for the matter at hand. Confirmation of this point can be found in *Kumho Tire Co. v. Carmichael*, 526 US 137 (1999), where the Supreme Court stated that the factors enunciated in *Daubert* are not meant to be exhaustive and do not necessarily apply in every case. The *Kumho* Court also said that "whether Daubert's specific factors are, or are not, reasonable measures of reliability in a particular case is a matter that the law grants the trial judge broad latitude to determine."

Potential toxicology experts should note that predictions of the demise of the *Frye* "general acceptance" test have yet to be realized. As of the fall of 2002, state courts in at least 17 jurisdictions (Alabama, Arizona, California, Colorado, Florida, Illinois, Kansas, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nevada, New Jersey, New York, Pennsylvania, and Washington) remain committed to *Frye*. Importantly, three-quarters of these jurisdictions fall within the 25 most populated states, and two-thirds fall within the 25 most litigious states. Consequently, a majority of state trials are conducted in *Frye* jurisdictions that may or may not recognize or incorporate *Daubert* indicia (factors) of validity and reliability into their *Frye* jurisprudence.

In retaining the 'general acceptance' standard, some state supreme courts have explicitly or implicitly refused to follow *Daubert*. Proffered explanations for this phenomenon include: (1) simple coincidence with random distribution of case outcomes, (2) lack of perceived need, especially in more populous states, to follow the lead of federal courts, (3) satisfaction with the status quo, (4) desire to prevent 'inappropriate relaxation' of the standards for introducing scientific testimony, (5) perception that *Frye* 'general acceptance' is a more rigorous, cautious, conservative, or higher standard of admissibility than the more liberal, lenient, or relaxed standard of 'validity-reliability' articulated in *Daubert*, and (6) perception that the *Daubert* standard requires state trial judges to make scientific judgments that exceed the typical judge's competence.

Critics of *Frye*, however, maintain that the scope of application of the 'general acceptance' standard is severely limited in many states. These critics argue that *Frye* applies (and often is applied) only to novel theories and techniques of 'hard science', and does not permit scrutiny of traditional techniques, 'soft science', and nonscientific expertise. By contrast, most *Daubert* Courts are compelled to examine all types of expert testimony, especially after *Kumho* made it clear that whether the proponent characterizes the proffered expertise as scientific, technical, or specialized, the proponent nevertheless must make a showing of the reliability of the expertise.

Critics of *Frye* also maintain that most state and federal trial judges can and do marshal the resources needed to increase their competence and confidence in performing the 'gatekeeping' responsibilities mandated by the *Daubert* decision. Examples of such resources include use of court-appointed experts under FRE 706, use of 'special masters' under Rule 53 of the Federal Rules of Civil Procedure, use of the

Federal Judicial Center's *Reference Manual on Scientific Evidence* (2nd edn, 2000), and attendance at forensic science and continuing legal education courses, such as those offered by the National Judicial College in Reno, Nevada.

Finally, critics of *Frye* point out that whether the evidence sought to be admitted has gained general acceptance in the appropriate field can depend on whether the 'field' is defined narrowly or broadly. Although courts have recognized that *Frye* does not require unanimity of view, courts have not provided functional definitions of 'general acceptance'. Consequently, a clear standard has not emerged for measuring 'general acceptance' in the relevant scientific community.

Subject Matter of the Expert's Opinion

Most American courts insist that three basic requirements be met before an individual will be permitted to offer testimony as an 'expert' witness. First, the testimony must be composed of scientific, technical, or other specialized knowledge. Second, the testimony must assist the factfinder in understanding the evidence or in resolving a factual dispute in the case. Third, the witness must be qualified to render the opinion.

Regarding scientific knowledge, the *Daubert* Court explained that "the adjective 'scientific' implies a grounding in the methods and procedures of science. Similarly, the word 'knowledge' connotes more than subjective belief or unsupported speculation. The term applies to any body of known facts or to any body of ideas inferred from such facts or accepted as truths on good grounds. Of course, it would be unreasonable to conclude that the subject of scientific testimony must be 'known' to a certainty; arguably, there are no certainties in science. But, in order to qualify as 'scientific knowledge,' an inference or assertion must be derived by the scientific method. Proposed testimony must be supported by appropriate validation (i.e., 'good grounds') based on what is known. In short, the requirement that an expert's testimony pertaining to 'scientific knowledge' establishes a standard of evidentiary reliability."

Regarding assistance to the factfinder, the expert's specialized knowledge must be 'helpful'. Courts do not agree, however, on the meaning of 'helpful'. Some courts believe that "[w]here the subject matter is within the knowledge or experience of laypeople, expert testimony is superfluous," and therefore not helpful. Others, by contrast, hold that there is no requirement that expert testimony be 'beyond the jury's sphere of knowledge' before that testimony can be deemed helpful.

Regarding qualifications, many courts attempt to characterize the nature of an expert's opinion before deciding on admissibility. Opinions offered by physicians, for example, may address causation, diagnosis, treatment, identity, prognosis, standard of care for diagnosis, and standard of care for treatment. Importantly, American courts are split on the issue of the competency of nonphysicians to testify against physicians regarding diagnosis, treatment, prognosis, or standards of care. Conversely, American courts also are split on the competency of physicians to testify against nonphysician practitioners. By contrast, nonphysicians, as well as physicians, frequently are permitted to testify regarding causation and identity.

In malpractice cases, most courts do not require physician experts to practice in, or to be board-certified in, precisely the same specialty as the defendant practitioner. Furthermore, in jurisdictions that rely on local or state-wide standards of care, it is not necessary that an expert actually live and practice in the locale where alleged substandard care was provided. Some courts allow experts to assert knowledge of local practice through professional contacts, while others allow experts to assert that national standards of care apply equally in every location.

Foundation of the Expert's Opinion

Prior to enactment of the Federal Rules of Evidence in 1975, American courts held that the facts underlying an expert opinion had to be *admitted into evidence* before the expert could state an opinion. As noted previously, however, in jurisdictions that have adopted FRE 703, an expert now can base an opinion on personal knowledge, on facts made known or admitted into evidence, *and* on facts that have not been admitted into evidence and that are themselves inadmissible. FRE 703 "is designed to broaden the basis for expert opinions beyond that current in many jurisdictions and to bring the judicial practice into line with the practice of the experts themselves when not in court. Thus a physician in his own practice bases his diagnosis on information from numerous sources and of considerable variety, including statements by patients and relatives, reports and opinions from nurses, technicians, and other doctors, hospital records, and X-rays. Most of them are admissible in evidence, but only with the expenditure of substantial time in producing and examining various authenticating witnesses. The physician makes life-and-death decisions in reliance upon them. His validation, expertly performed and subject to cross-examination, ought to suffice for judicial purposes."

Regarding the level of scrutiny of facts or data 'reasonably relied upon', by experts in a particular discipline, "courts have adopted two judicial approaches to Rule 703: one restrictive, one liberal. The more restrictive view requires the trial court to determine not only whether the data are of a type reasonably relied upon by experts in the field, but also whether the underlying data are untrustworthy for hearsay or other reasons. The more liberal view…allows the expert to base an opinion on data of the type reasonably relied upon by experts in the field without separately determining the trustworthiness of the particular data involved." *In re* "*Agent Orange*" *Product Liab. Litig*., 611 F.Supp. 1223, 1244 (E.D.N.Y. 1985), *aff'd*, 818 F.2d 187 (2d Cir. 1987), *cert. denied*, 487 US 1234 (1988).

In the Agent Orange cases, the court had to determine the trustworthiness of symptom checklists completed by plaintiffs in preparation for litigation. Are these checklists a type of 'data' reasonably relied upon by physician and nonphysician experts in offering conclusions about diagnosis and causation in the fields of toxicology and epidemiology? The court said 'no', such checklists "are not material that experts in this field would reasonably rely upon and so must be excluded under Rule 703." According to Judge Weinstein, "the court may not abdicate its independent responsibilities to decide if the bases meet minimum standards of reliability as a condition of admissibility. If the underlying data is so lacking in probative force and reliability that no reasonable expert could base an opinion on it, an opinion which rests entirely upon it must be excluded."

Toxicologists should understand that modern courts can, and frequently do, analyze expert opinions from two perspectives. Under FRE 702, courts determine whether an opinion is derived from scientific knowledge. Under FRE 703, courts determine whether an expert opinion has an adequate factual foundation. Whether one or both perspectives are applied, in courts that follow *Daubert*, the critical focus of inquiry is 'reliability'. By contrast, in courts that follow *Frye*, the focus remains 'general acceptance'.

Unfortunately, the Supreme Court in *Daubert* could not, and did not, resolve all the difficult issues regarding admissibility of expert testimony. One question left unanswered by *Daubert* was: does the validation (reliability) standard apply only to traditional 'scientific' evidence, or does it also apply to other 'technical', 'specialized', or 'social science' evidence? A second question was: does the validation (reliability) standard apply only to the *methodology* underlying the expert's evidence and opinion, or does it also apply to the *reasoning process* used by the expert in extrapolating or drawing inferences from the underlying scientific evidence to reach his or her conclusion?

In the *Kumho Tire* decision, 526 US 137 (1999), the Supreme Court held that the 'reliability' standard does apply to 'less scientific' or 'nonscientific' evidence. In *General Electric Co. v. Joiner*, 522 US 136 (1997), the court told federal judges that both methodology and reasoning should be scrutinized. According to the court, "[n]othing in either Daubert or the [FRE] requires a district court to admit opinion evidence which is connected to existing data only by the ipse dixit of the expert. A court may conclude that there is simply too great an analytical gap between the data and the opinion proffered." In other words, reliability and consequent admissibility requires a 'good fit' between the expert's methodology and conclusion.

In *Downs v. Perstorp Components, Inc*., No.00-5507, 01-04-02, the United States Court of Appeals for the Sixth Circuit affirmed summary judgment for the defendant and approved a federal district court's extensive scrutiny of a toxicologist's methodology and reasoning. The facts indicate that in 1995, defendant purchased Rubiflex SI 30690, a chemical product used in the production of foam insulation. Plaintiff, who was contracted to deliver the Rubiflex, found that the packaged product was too large for transport by chartered airplane. Defendant's representative suggested repackaging the Rubiflex in smaller containers. During repackaging, Rubiflex splashed out of the containers and onto plaintiff's arms and face. Plaintiff experienced a burning sensation but was told by defendant's representative that exposure to the chemical was safe. Plaintiff experienced neurological symptoms, and a physician toxicologist diagnosed chemical encephalopathy caused by exposure to Rubiflex.

The federal magistrate judge excluded the toxicologist's expert testimony, finding it failed to meet the *Daubert* admissibility standard. Although the expert identified Rubiflex as an epoxy and determined that it contained two toxic substances, he did not identify the components of Rubiflex that were responsible for plaintiff's condition. Furthermore, the expert did not know the amount of Rubiflex to which plaintiff was exposed and did not attempt to independently identify what dose of Rubiflex is necessary to cause the conditions, he observed in the plaintiff. Moreover, the expert could not point to any scientific literature suggesting that Rubiflex could lead to neurologic problems, and he did not conduct any testing to determine the potential effects of exposure to Rubiflex. Consequently, the Sixth Circuit agreed with the lower court that the expert's opinion should not be admitted because his "methodology

primarily involved reasoning backwards from [plaintiff's] condition, and through a process of elimination, concluding that Rubiflex must have caused it."

As the twenty-first century unfolds, the toxicologist expert witness still needs to distinguish *Frye* jurisprudence from *Daubert* jurisprudence. *Frye* jurisdictions remain divided on whether the 'general acceptance' test applies to technical, specialized, psychological, or other social science types of evidence. *Frye* Courts also disagree on whether 'general acceptance' applies not only to an expert's general methodologies, but also to his or her conclusions.

The future, however, may bring a melding of analytical principles. Recent state appellate decisions suggest a desire by some courts to adopt a '*Frye* plus reliability' standard for admission of some types of scientific evidence. For example, in *Harris v. Cropmate*, 706 N.E.2d 55 (1999), the Illinois Court of Appeals said "Illinois utilizes a '*Frye* plus reliability' standard for admission of novel scientific evidence…in applying the *Frye* standard the trial court must determine that (1) the scientific test is reliable; and (2) the test's reliability is generally accepted in the particular scientific field to which the test belongs." Furthermore, state trial courts "must not delegate their authority to the scientific community…in serving as gatekeepers to keep out scientific evidence that constitutes nothing more than 'junk science' or mere speculation, trial courts should constantly be asking, does the proffered witness have sufficient information, based upon the evidence in this case, to render a *reliable* opinion? Courts should remember that they need not – and should not – accept an expert's opinion on the basis of ipse dixit, that is, such a thing is so because I say it is so."

In *DuPont v. Castillo*, the Florida District Court of Appeals (Fifth District) said "it is the function of the court to not permit cases to be resolved on the basis of evidence for which a predicate of reliability has not been established. Reliability is fundamental to issues involved in the admissibility of evidence… Novel scientific evidence must also be shown to be reliable on some basis other than simply that it is the opinion of the witness who seeks to offer the opinion." Finally, in *Slay v. Keller Industries, Inc*., No. 1001091, Ala. (2001), the Alabama Supreme Court concluded that "mere assertion of belief, without any supporting research, testing, or experiments, cannot qualify as proper scientific testimony under either the 'general acceptance' standard enunciated in *Frye* or the 'scientifically reliable' standard of *Daubert*."

Regarding the admissibility of clinical medical testimony, courts have taken different approaches under *Daubert*. In *Moore v. Ashland Chemical Company* (5th Cir. 1998) (en banc), the Fifth Circuit excluded the conclusion of the plaintiff's pulmonary and environmental medical specialist that the cause of his reactive airways distress syndrome was his workplace exposure to toluene. The 'expert's' opinion was based on his training and experience, the physical examination, laboratory test results, an MSDS warning that inhalation of toluene could cause lung injury, onset of symptoms shortly after 'exposure' to toluene, and existing scientific studies on the association between toluene and respiratory illness. Nevertheless, the court of appeals held that the district court reasonably concluded that this basis was insufficient to meet the requirements of *Daubert*. The court said the studies contained self-doubts and qualifiers, and the MSDS was of limited value because the types of tests underlying the MSDS were unknown and there was no information regarding the airborne concentrations required to sustain the injuries described in the warning. The court also discounted the proximity of onset of the injuries to exposure as inadequate without other studies to demonstrate a scientifically validated causal connection. Furthermore, the court concluded that the plaintiff's lifestyle and medical history were inconsistent with the specialist's opinions because the plaintiff was a smoker, experienced asthma as a child, and had recovered from pneumonia shortly before his alleged inhalation of toluene. Three judges in dissent stated that the majority had improperly interpreted the Daubert factors to require all expert testimony to meet a standard within the 'hard science community'. According to the dissenting judges, 'generally accepted clinical medical methodology' could not meet this standard.

By contrast, in *Westberry v. Guslavad Gummi AB* (4th Cir. 1999), the Fourth Circuit was less hostile in its assessment of clinical medical methodologies. The plaintiff alleged that he developed severe sinus infections following industrial exposure to talc. The plaintiff's physician concluded that the plaintiff's condition was caused by his inhalation of talc. The defendant argued that plaintiff's physician could not and did not rely on epidemiological studies, peer-reviewed published studies, animal studies, or laboratory data to support his conclusions. Moreover, plaintiff's expert could not show that tissue samples from the sinuses contained talc. According to the defendant, plaintiff's expert merely relied on a differential diagnosis and the temporal proximity of the 'exposure' to the onset of symptoms.

The court of appeals recognized that the technique of differential diagnosis includes physical examination, review of the medical history, and review of various laboratory tests prior to any determination of the most probable cause of an illness or condition. The differential diagnostic process includes generation

of a list of possible causes for the symptoms, and elimination of those that can be ruled out. The court viewed as irrelevant not only the physician's lack of knowledge of the precise amount of talc that may have been inhaled by the plaintiff, but also the physician's lack of any means to assess the intensity of exposure that may have been sufficient to produce plaintiff's sinus irritation. According to the court, while such information may be 'beneficial', it was not essential to a determination of causation. On the other hand, the court found the MSDS to be highly relevant because it stated that inhalation of talc dust 'in high concentrations irritates mucous membranes'. Importantly, the court found sufficient evidence of anecdotal proof of 'high concentrations' in the plaintiff's workplace. Furthermore, the court stated that evidence of temporal proximity of the exposure to the onset of the plaintiff's symptoms 'can provide compelling evidence of causation'.

The juxtaposition of Moore and Westberry highlights the significant differences in interpretation of Daubert and its progeny by lower courts. Westberry has been frequently cited for the proposition that clinical medical differential diagnosis, if properly undertaken, can satisfy the standards of Daubert independently. The Fourth Circuit stated that while "[a] differential diagnosis that fails to take serious account of other potential causes may be so lacking that it cannot provide a reliable basis for an opinion on causation," no requirement exists that the physician must rule out each and every possible alternative cause.

Use of Literature as Evidence

Traditionally, learned treatises and articles could not be admitted as substantive evidence because they were viewed as prohibited forms of hearsay. Such literature could be used, however, during cross-examination to impeach or contradict expert testimony. Modern courts typically require that the treatise or article: (1) must have been relied upon by the expert in reaching his or her conclusions, or (2) must be acknowledged by the witness to be an 'authoritative source' or a 'recognized authority' in the relevant field. Some courts also permit treatises and articles to be used for impeachment even if the witness does not acknowledge the source as a recognized authority, as long as authoritativeness can be established by judicial notice or through testimony of other witnesses. FRE 803(18), which is best read in conjunction with FRE 703 (discussed above), states "the following are not excluded by the hearsay rule, even thought the declarant is available as a witness: (18) Learned Treatises – To the extent called to the attention of an expert witness upon cross-examination or relied upon by him in direct examination, statements contained in published treatises, periodicals, or pamphlets on a subject of history, medicine, or other science or art, established as a reliable authority by the testimony or admission of the witness or by other expert testimony or by judicial notice. If admitted, the statements may be read into evidence but may not be received as exhibits."

In both federal and state forums, the proponent of substantive admissibility of medical and scientific literature will argue one or more of the following points: (1) the author of an article or treatise does not have an interest in the outcome of a particular case; (2) the scrutiny of the peer review process increases the reliability of opinions or conclusions published in peer-reviewed literature; (3) treatises may be more 'up to date' than live, testifying experts; (4) attorneys can attempt to prevent confusion, selective presentation, or presentation out of context; and (5) cross-examination is not necessary when a live expert is available to explain the article or treatise.

By contrast, the opponent of substantive admissibility of medical and scientific literature will argue one or more of the following points: (1) the author is not available for cross-examination; (2) treatises quickly outdate because medical and scientific knowledge change rapidly; (3) the trier of fact may be unable to understand complex technical passages that may be presented out of context; and (4) the literature is unnecessary as substantive evidence when live expert witnesses are available.

Conclusion

Toxicologists can make meaningful and significant contributions to legal proceedings. To function effectively in judicial, legislative, or regulatory forums, however, the toxicologist must be willing to do the following: (1) dress appropriately; (2) prepare properly and extensively; (3) leave his or her ego at the door; (4) resist the temptation to elaborate, pontificate, or volunteer information; (5) frequently answer 'yes', 'no', 'I don't know'; 'I don't recall'; and 'I don't understand the question'; (6) avoid bringing documents unless specifically asked to do so; (7) expect to be verbally 'attacked'; (8) think and react calmly under pressure; (9) recognize the 'hypothetical' question; (10) avoid overstatement and use of the words 'always' and 'never'; (11) avoid hasty answers, so as to enable objections; (12) listen to objections carefully; (13) avoid argument with the examiner; (14) refuse to answer if counsel instructs not to answer; (15) appreciate that, in legal forums, one is an expert only if the tribunal so states; (16) ask

to review entirety of a document before answering questions about parts of it; (17) assert the right to read a transcript of testimony before signing it; and (18) use only those methodologies and state only those conclusions that can be defended before peers in the field of toxicology.

See also: Toxic Torts.

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LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50)

Shayne C Gad

Introduction

The 50% lethal dose (LD_{50} or LD50) is the statistically calculated dose (or concentration) of a material (generally expressed as the amount of material per unit of body weight) that would be expected to cause the death of half the members of the target species receiving it. The 50% lethal concentration (LC_{50}) is the equivalent statistical projection for inhalation. Until the mid-1980s, these figures were considered perhaps the basic component of a toxicity profile for any chemical or drug. However, after many years of controversy and debate on a number of fronts, including objections from animal rights advocates, three alternative animal tests have been developed to replace the LD_{50}. They are the fixed-dose procedure, the acute toxic class method and the up and down (or 'up/down') method, and their use has led to significant improvements in animal welfare. These new tests have undergone revision and refinement to improve their scientific performance and to increase their regulatory acceptance. Further, research into replacements for test animals, such as cellular cultures, organs harvested from slaughterhouses, *in silico* (computer) modeling, and physical/chemical systems, has been extensive. While these approaches will not be able to completely replace the use of animals in the foreseeable future, they have a bright future.

The *in vitro* cytotoxicity tests that have been developed can already help reduce the number of animals used in acute oral toxicity testing. For example, cytotoxicity data are being used to determine the starting dose for *in vivo* testing by applying a standard regression between cytoxicity and acute oral LD_{50} values. A database of correlations between cytotoxic responses and the acute oral LD_{50} of rats or mice has been determined for hundreds of chemicals. Using this approach, it has been proposed that a tiered *in vitro/in vivo* testing process will reduce animal use in the up/down method. For example, the *in vitro* cytotoxicity of a new chemical is determined as the first step, and the LD_{50} value ($mg\,kg^{-1}$) is predicted from the cytotoxicity data. The predicted LD_{50} dose is then used as the starting dose in the up/down protocol.

In silico models (or 'expert systems') have also been developed. These are computer software-based structure–activity relationship and quantitative structure–activity relationship analyses of data libraries of acute toxicity data developed for use in evaluating and predicting the acute oral and inhalation toxicity potential of a chemical or drug.

While most of the focus has been on the potential for oral toxicity, *in vitro* testing and computer modeling for the evaluation and prediction of respiratory toxicity are also being developed. For example, one strategy consists of checking the existing data available for the test material itself, or on related substances, followed by acquiring knowledge on the physicochemical properties of the test material. These steps are followed by the use of computer modeling techniques to try to predict the likely toxic effects and target sites. *In vitro* tests could then follow to identify likely target cells and evaluate the specific effects on the cells (e.g., morphology could be determined and assessments of the cellular energy status). A further phase of *in vitro* tests could then be conducted on the basis of results obtained in the first phase of *in vitro* testing, choosing from tests using various types of respiratory tract cells.

When did modern Western society become concerned with lethality testing? For what reasons were protocols developed for describing lethality in animals in quantitative terms for the purposes of making scientific, regulatory, or marketing decisions? Interestingly, in this age of genetic engineering, few people realize that biologically derived materials were the subject of regulations well before the passage of the Pure Food and Drug Act in the United States in 1906. In 1901, a diphtheria epidemic broke out in St. Louis, MO, because of improperly manufactured antidiphtheria toxin. In response to

the resulting public outcry, the US Congress passed the Virus Act of 1902. It regulated all viruses, serums, toxins, antitoxins, and other such products sold for the prevention or cure of disease in man. Among other things, the bill eventually established consistent potency criteria. In fact, by World War II the US FDA was requiring batch-to-batch certification and release for biologicals, a policy that remains in effect for certain drugs. Hence, the earliest lethality testing was for the purpose of establishing consistent potencies of biologicals, such as diphtheria toxin, and not for evaluating synthetic chemicals.

One of the earliest publications discussing lethality testing was an investigation into the lethality of diphtheria toxin in guinea pigs. The publication described lethality empirically in terms of percentage of dead animals at each dosage because methods for calculating lethality curves and the median lethal dosage had not yet been developed. The authors reported that lethal response to a given dosage of toxin varied with the time of the year. Hence, years before the term LD_{50} came into parlance, supposedly as an exact indicator of toxicity, data had been published attesting to the volatility and imprecision of this calculated parameter.

Because the first use of lethality testing was in describing the potency of biologicals, it only makes sense that the same methods were soon applied to extracted botanicals. (Note: There is no doubt that both the Germans and the English tested in animals the various poison gases employed during World War I. Little of this work, however, appears to have been published in the open scientific literature, although portions of it have recently been made public.) In 1926, de Lind van Wijngaarden published on the lethality of digitalis extracts. Interestingly, he did not plot his data as mortality versus dose. He delivered his extracts intravenously and titrated the dosage until he achieved complete heart stoppage. He was thus able to determine the precise lethal dosage for each animal and noted that these followed a bell-shaped or Gaussian distribution. His experiments took 5 years and used more than 500 cats, an effort that would have been excessive and expensive by today's standards. However, he did conclude that no more than nine cats would normally be required to 'calibrate' an extract of digitalis. Trevan, in a pivotal paper (1927), described the lethality of strophanthin, cocaine, and insulin. Modern reviews have focused a great deal of attention on the large number of frogs used by Trevan. Most of the data he discussed, however, were derived from experiments in mice using cocaine or insulin. Perhaps so little attention was given to this aspect of Trevan's paper, even though it comprised the major portions of his work (which ran to 31 pages and contained 11 figures and six tables of data), because it has never been replicated. For some of the lethality curves reported by Trevan, well over 900 mice were dosed. Again, such efforts would be excessive and expensive by today's standards but were necessitated, in part, by the less rigorous method of deriving lethality curves and calculating the median lethal dosage (LD_{50}). Modern methods of data transformation and statistical analysis were, at that time, still in their infancy. He also recognized that it was not necessary to describe an entire dosage–response curve to calculate an LD_{50}. He, in fact, recommended that lethality determinations start with small groups of two or three animals each and that larger groups be used for confirmatory purposes.

Behren confirmed the observations of both de Lind van Wijngaarden and Trevan. It is clear from his article that the use of animals for standardizing digitalis extracts was accepted to the point of being incorporated into the German and Dutch pharmacopoeias. The objective of his paper was to compare the cat and frog methods and develop a basis for using fewer animals. He concluded that the frog method was superior and that no more than 44 frogs needed to be used, which was considerably less than the 100–200 frogs prescribed in the German pharmacopoeia of that period. Interestingly, these early papers are often criticized with regard to the numbers of animals used, but the objectives and conclusions are often ignored.

Both Trevan and Behrens noted that when the percentage of animals that died at specific dosages was plotted against the logarithm of the dosage, the resulting curve (the lethal dosage curve) had a sigmoidal shape slope and range that was 'characteristic' for the species and the test substance. Shackell (1925) first pointed out that such curves are integrated or cumulative frequency curves (or ogives) and coined the term 'dose–response ogive' (curve). Trevan noted that these curves owe their shape to the fact that different individual animals require different quantities of poison for death to occur. It was also Trevan who identified the midpoint on this curve as being the dosage that would kill 50% of the animals exposed. He designed that point as the median lethal dose, or LD_{50}, and, thus, is widely credited with having developed the classical LD_{50}. Trevan and Behrans essentially read the LD_{50} directly from their mortality dose–response curves.

Lethality testing of biologicals and botanicals was essentially a response to governmental regulation. It was only natural that similar methods would be applied to synthetic chemicals. Major chemical companies started establishing toxicity or industrial

health laboratories during the 1930s; the lethality testing of synthetic chemicals was established by the 1930s. However, there were no regulatory requirements for such tests. In fact, there was no premarketing toxicity testing of synthetic chemicals required at all. In 1937, an elixir or sulfanilamide dissolved in ethylene glycol was introduced into the market. Over 100 people died as a result of ethylene glycol toxicity. The public response to this tragedy helped prompt the US Congress to pass the Federal Food, Drug, and Cosmetic Act of 1938. It was this law that mandated the premarket testing of drugs for safety in experimental animals. By the mid-1940s, most chemical and pharmaceutical companies were routinely testing new chemicals for lethality. In fact, until the 1960s, preclinical or premarketing toxicity data packages normally consisted of little more than acute lethality data. Recently, new laws, increased scientific sophistication, and greater societal concern over sublethal chronic toxicity has led to more extensive and expensive preclinical or premarketing toxicity testing packages, where acute lethality is a small, but still real, concern.

The protocols used to assess lethality have changed considerably since the 1920s. While the principles originally described by Trevan have never been questioned, the methods for calculating the LD_{50} have become more sophisticated and the need for a high degree of precision has been questioned. The practical result is that by using modern protocols, relatively few animals are species (generally rats and mice) are employed and only two routes of administration are used. At least one route must be the intended or the most probable human exposure route. Hence, such protocols generally result in the generation of eight lethal dosage curves (one/route/sex/species). In the drug industry (where this approach is common), the two routes are generally oral and intraperitoneal for an oral drug and oral and intravenous for an intravenous drug.

Protocol Design Considerations

Whatever type of experimental protocol one chooses to use in a lethality test, there are certain principles and criteria that should be universally applied. The principles are especially relevant in studies in which small numbers of animals are used.

First, a wide variety of intrinsic and extrinsic factors can influence the outcome of a lethality test. These include species, strain or substrain, age, weight, and sex of the animals; husbandry practices (e.g., type of bedding and cage population); environmental conditions; feed and water quality; nutritional state; and volume and vehicles of test substance delivery. The point to be made here is that the criteria for all these factors should be specified in detail in the protocol and strict adherence to the protocol observed. Otherwise, intrastudy comparisons are invalid. Small differences in protocols can cause large differences in the LD_{50} and are probably the major cause of the considerable laboratory-to-laboratory variation in the LD_{50}s.

Second, because the animals will generally receive a single exposure, great care must be given to the preparation and delivery of the test articles. In a chronic study, occasional miscalculations or misdelivery of the dosage would not generally greatly affect the study outcome but would clearly have a greater effect on the conclusions of a lethality screen. One should always include appropriate safeguards.

Third, one must make sure that all animals are successfully dosed and that accidental deaths are identified as such. In acute rodent studies, we routinely assign spare animals to a dosing group. Permanent numbers are not assigned until we are certain that the dose has been successfully delivered (e.g., Was the supposedly intraperitoneal dose accidentally delivered intravenously? Was there reflux from the site?). Spare animals not dosed are returned to the pool of animals available for the study. Animals found dead should be examined for evidence of accidental trauma. For example, it is not uncommon for a rat to suddenly move while being gavaged. This may result in a torn esophagus that may take 1 or 2 days to become evident. Depending on the administration route, one must pay close attention to dosing techniques and the volume limitations imposed by these techniques. For example, $20\,ml\,kg^{-1}$ is the maximum volume that should be given orally to a rodent. Deaths that are clearly accidental should not be considered in the final conclusions.

Fourth, lethality protocols, by the nature of the question they address, do not specify all dosages. This can sometimes result in a study in which absurdly high dosages are administered. Hence, all protocols should clearly state what the ceiling or limit dosage will be and the reasons for selection.

Classical (Traditional) Designs

The classical or traditional methods of determining the lethality of a substance have been established since the 1920s. In discussing this type of study design, it is assumed that what are desired are an LD_{50} and the slope of the lethality curve. In general, these are only necessary for meeting specific regulatory guidelines. If less precise information will suffice (which is generally the case), other protocols can be used. Briefly, this type of protocol specifies that

animals (of the same species/strain, sex, and age) be divided into groups. All the animals are treated via the same route. The animals are then held and observed for a set and consistent period of time, usually 14 days.

Mortality in each group is calculated on the basis of the number of animals that die during the observation period and is normally presented in percentage terms: (number dead/number dosed) × 100. If mortality at each dosage is plotted against dosages, a sigmoidal dose–response curve is obtained. The LD_{50} is simply the dosage, either observed or calculated, that yields 50% mortality. Seldom are such curves reported as such because the LD_{50} is difficult to read off a curvilinear plot and the small number of dosages normally used makes drawing an accurate lethal dosage curve difficult. It is most common to prohibit transforming the data to obtain a rectilinear plot.

Traditionally, because of US FDA and foreign regulatory guidelines, protocols have frequently been designed as batteries, including both sexes of two species (generally rats and mice) and two routes of administration. At least one route must be the intended or the most probable human exposure route. Hence, such protocols generally result in the generation of eight lethal dosage curves (one/route/sex/species).

While such extensive data packages may still be required for regulatory purposes, scientifically they are of little value. First, there is no reason to assume that either the rat or the mouse is the better predictor for humans – or for each other. The only general correlation between the rat and mouse LD_{50} is that when one is high, so is the other. Obtaining lethality data from two different rodents, rather than a single species, does not generally change our conclusions or improve our understanding of the toxicity of a drug or chemical or the potential hazard to humans. It is recommended that a simple preliminary screen be performed to pick out the most sensitive species and a rigorous protocol applied only to that species. Because the slope of the fitted line in these assays has a very large uncertainty, in relation to the uncertainty of the LD_{50} itself (the midpoint of the distribution), great caution must be used with calculated lethal doses other than LD_{50}s. It is quite possible to calculate values for other points along the lethality curve, such as the LD_{35}, a value close to the LD_{50}, but these are not precise or statistically 'stable' values due to the shape of the curve in areas away from the center point.

Note: The Registry of Toxic Effects of Chemical Substances (http://www.cdc.gov/niosh/rtecs.html) is among the largest single collections of LD_{50} and LC_{50} values.

See also: Animal Models; Dose–Response Relationship; Food, Drug, and Cosmetic Act; Levels of Effect in Toxicological Assessment; Maximum Allowable Concentration (MAC); Maximum Tolerated Dose (MTD); Toxicity Testing, Alternatives; Toxicity Testing, Modeling.

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Lead

Shayne C Gad

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- Chemical Abstracts Service Registry Number: CAS 7439-92-1
- Synonym: C.I. Pigment Metal 4
- Chemical/Pharmaceutical/Other Class: Heavy metals
- Chemical Formulas: Pb^{2+}, Pb^{4+}

Uses

Lead and its compounds are widely used throughout industry. They are found in lead acid storage batteries, paints, sheet metal, bearings, solder, piping, and ammunition. Lead arsenate is used in insecticides and herbicides. Lead chromate is used as a yellow pigment in paints, rubber, plastics, and ceramic coatings. House paints must have less than 0.05% lead.

Various arts and hobbies involve lead-containing materials. Lead is found in artist's paints (certain pigments), ceramic glazes (particularly reds), solder used in stained-glass windows, and linings in containers used for distilling homemade whiskey. Certain Mexican, Middle Eastern, and Asian folk remedies and cosmetics contain lead.

In the past, lead solder was used to seal canned foods and lead pipes were used to carry drinking water. Tetraethyl lead was once routinely added to gasoline as an antiknock agent; certain vehicles may still use leaded gasoline (e.g., agricultural vehicles). Lead was commonly used in paint, with certain formulations containing up to 50% lead.

Background Information

Lead was one of the first metals used by man. It has a wide variety of uses, but its well-established bioaccumulation and chronic toxicity with low exposure levels has led to the enactment of strict limits on use and potential exposure.

Exposure Routes and Pathways

Exposure to lead and its compounds may occur through ingestion, inhalation, or dermal contact. The specific characteristics of a lead compound influence how exposure is to occur through a particular route and the degree of absorption into the body through that route. Most inorganic forms of lead are not well absorbed through the skin, whereas organic forms (e.g., tetraethyl lead) are more likely to be absorbed through the skin.

For the general population, ingestion of contaminated water and food is the primary source of exposure to lead. The average adult ingests $\sim 300\,\mu g$ of lead each day in food. Inhalation is the most significant route of exposure to lead in the workplace.

Respirable particulate or gaseous forms of lead may be inhaled. Sources include cigarette smoke; vehicle exhaust; emissions from municipal waste incinerators, iron and steel plants, smelting and refining operations, lead acid battery manufacturing facilities, and sandblasting and burning of surfaces coated with lead paint. Particulate air emissions may eventually deposit and contaminate the soil.

Direct release of lead-containing industrial wastewater into surface water or ground-water may ultimately impact drinking water. Lead may also be present in drinking water because of leaching from old pipes, solder, water coolers, or faucets. Some historians attribute the fall of the Roman Empire to the effects of lead leaching from drinking-water pipes and wine casks.

Food may contain low levels of lead due to uptake from the environment or higher levels due to lead leaching from containers (e.g., lead crystal or lead-containing glazes on earthenware). An important cause of lead poisoning in young children is ingestion of peeling and chipping lead-based paint in older homes.

Toxicokinetics

Lead is readily absorbed through the digestive tract. Absorption becomes less efficient with age. Children absorb between 30% and 50% of ingested lead, whereas adults absorb less than one-third of that amount (between 5% and 15%). This absorption is enhanced by diets deficient in iron, zinc, and calcium. Absorption is generally greater for organic forms. Lead is well absorbed from the lung (from 50% to 70% of respirable lead particulate). Generally, inorganic forms of lead are not absorbed through the skin, while organic forms (e.g., tetraethyl lead) can be absorbed.

Once absorbed, lead is distributed throughout the body tissues via the blood. Almost all ($\sim 95\%$) of the lead in the blood is found in red blood cells. There are two primary sites in the red blood cell

where lead forms complexes: the membrane and the hemoglobin. The concentration of lead in the blood is used as an indicator of recent lead exposure.

Lead tends to accumulate in the kidneys, the brain (i.e., the gray matter and various nuclei), and the skeleton. Lead can cross the placenta and has been shown to accumulate in the developing child. Prolonged exposure to lead (>4 weeks) in young children can lead to the accumulation of lead in the growth plates at the end of the long bones.

The total body burden of lead is a function of the balance between the amount being taken in (all routes combined), the amount distributed throughout the tissues, and the amount being excreted. Most of the body burden of lead is sequestered in the bones and teeth: over 70% in children and over 90% in adults. The remainder of the body burden is distributed between soft tissue and the blood. Lead is stored in the bone for the greatest length of time. The estimated half-lives of lead range from 10 to 30 years in bone, are 40 days in soft tissues, and range from 28 to 36 days in blood (in adults). Children tend to retain approximately five times more absorbed lead than adults.

In young children (<3 years) the blood–brain barrier (an anatomical barrier that limits access to the brain) is not fully developed. Inorganic lead circulating in the blood is much more likely to reach the brain in an infant or a very young child.

Chronic high-level exposure to lead can result in the accumulation of large stores of lead in the bone, which can be slowly released over many years after the initial exposure has stopped. The release of lead from bone may be accelerated under certain conditions, including high stress, certain metabolic fluctuations, and pregnancy.

Lead is excreted from the body in bile (into feces), and in urine, sweat, sloughed-off skin cells, and lost hair.

Mechanism of Toxicity

Lead can affect most organs and systems in the body. It can interfere with certain cellular signaling processes, the generation of action potentials in certain nerve cells, and the function of a number of enzymes. Lead interferes with the sodium–potassium ATPase pump on cell membranes, the metabolism of vitamin D, heme synthesis, certain enzymes involved in oxidative phosphorylation (cytochromes), and calcium uptake and metabolism. In addition, lead can interfere with signal transmissions in nerve cells, including dopaminergic transmissions and signaling processes at the postsynaptic and presynaptic junctions. Lead can depress the function of the adrenal glands and the thyroid.

Lead binds certain active groups on protein (e.g., sulfhydral groups) and therefore may change the structure and function of certain proteins and enzymes. Lead interferes with the biosynthesis of heme in at least two steps in the multistepped process. Heme proteins are important to the structure and function of hemoglobin in red blood cells. Lead binds with 8-aminolevulinic acid dehydratase and depresses its activity. This biochemical block explains the occurrence of anemia found in chronic lead poisoning. Measurement of the blood levels of this enzyme is used as a test for lead intoxication. Lead also interferes with the incorporation of ferrous iron into the porphyrin ring. If iron is not attached to heme, then zinc will occupy the iron-binding site. The concentration of zinc protoporphyrin also can be used as a diagnostic tool for lead poisoning.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute lead exposure can lead to renal toxicity. The acute intraperitoneal LD_{50} for lead acetate in rodents is 100–200 $mg\,kg^{-1}$. Lead acetate is considerably less toxic by the oral route ($LD_{50} > 4\,g\,kg^{-1}$ in rats). The acute oral LD_{50} of tetraethyl lead in rodents is 10–100 $mg\,kg^{-1}$. Acute organolead exposure can sensitize dopaminergic neurotransmission in the central nervous system.

Human

Anorexia, vomiting, malaise, and convulsions (due to increased intracranial pressure) are most commonly seen in children. Sources of childhood exposures are typically environmental such as to paint chips, pottery, drinking water, and dust. Acute exposure in adults may cause gastrointestinal effects, pain in arms and legs, and hypertension. Exposure to very high levels may cause tremor, memory loss, confusion, stupor, renal failure, convulsions, and coma.

Chronic Toxicity (or Exposure)

Animal

Lead is a tumorigen, mutagen, reproductive and developmental toxicant. Neural, renal, and hematologic toxicity are all possible with chronic exposures. Oral exposure to certain lead compounds has been shown to induce tumors in kidneys of rats and mice in more than 20 studies. Based on such animal data, lead is classified as a probable human carcinogen

by several agencies including the US Environmental Protection Agency (EPA).

In dogs, sheep, goats, and cattle, there have been reports of toxicity resulting from contact with environmental lead. Sources of exposure may include lead salts, lead-based paints, and waste oils.

Lead can disrupt learned behavior in certain adult animals and has been shown to disrupt learning and memory in certain young animals. Young animals tend to be more susceptible to the effects of lead than older animals. Studies on monkeys have shown the abnormalities and effects after lead exposure. Effects included encephalopathy and offspring that exhibited neurological and behavioral symptoms at maturity.

Human

Lead can disturb cellular and molecular processes in the body and affect many organs and physiological functions. The probability of adverse health effects occurring is related to the level of exposure, duration of exposure, and total body burden. The adverse effects associated with exposure to lead are a function of dose and are usually the same regardless of the route of exposure. The primary targets for toxicity are the nervous system, the blood, and the kidneys. Reproductive effects can also occur and include male infertility, abortion, and neonatal morbidity and mortality. Lead may damage sperm and parts of the reproductive tract. Chromosomal effects have been observed in lead-exposed workers. Other effects include impairment of the immune system, which has been associated with joint pains (lead arthralgia) related to gout, myocarditis, cardiac fibrosis, weight loss, and anemia.

The most sensitive and vulnerable target for lead appears to be the nervous system. Exposure to high concentrations of lead can cause either encephalopathy or peripheral neuropathy. Encephalopathy is rare in adults but is more likely to occur in significantly exposed children. It has been observed in young children (1 and 3 years) following chronic lead poisoning due to ingestion of significant amounts of lead-based paint. Typically, there is gastrointestinal distress (e.g., colic), disorientation, stupor, seizures, and coma.

Significant early childhood exposure (above 0.05 mg%, and arguably lower) has been associated with certain neuropsychiatric changes, including learning disorders, decreased IQ, behavioral abnormalities (e.g., hyperactivity), and deficits in vocabulary. In addition, decreased growth, loss of hearing acuity, deficits in reaction time, fine-motor dysfunction, developmental abnormalities, deficits in hand/eye coordination, anemia, and death have been associated with exposure in children. Symptoms of acute exposure in young children include anorexia, vomiting, and irritability. In cases of very high levels of exposure, symptoms may also include slurred speech, peripheral neuropathy, paralysis, convulsions, and coma.

Exposure to lead in adults has been associated with hypertension, nephropathy, decreased hearing acuity, anemia, peripheral neuropathy, and encephalopathy. Onset of symptoms may be slow with chronic exposure. Anemia, common in chronically exposed adults and children, tends to be more severe in children. The life span of red blood cells decreases when lead concentrations in blood increase. In the past, the morphology of various blood cells was used to diagnose lead poisoning. Zero content is allowed in food (Food and Drug Administration).

Clinical Management

The decision to actively treat a patient exposed to lead is made based on a number of criteria, including patient history, symptomology, blood lead levels, and other indicators of level of exposure. It is common to screen for exposure to lead based on blood lead levels ($\mu g\,dl^{-1}$). Certain exposure criteria are expressed in terms of an acceptable blood lead level. The Centers for Disease Control and Prevention in Atlanta defines above $9\,\mu g\,dl^{-1}$ as a trigger of concern in young children. The concentration of erythrocyte protoporphyrin (EP; a heme-containing protein) in red blood cells is also used to indicate exposure levels. The higher the concentrations of EP, the higher the exposure. X-ray techniques may be used to estimate concentrations of lead in bones and teeth.

Chelation therapy is usually the treatment of choice. Both $CaNa_2$-EDTA (calcium disodium salt of ethylenediaminetetraacetic acid) and British Antilewisite compound (BAL; 2,3-dimercaptopropanol) are commonly used to remove lead from the body. Both are administered via intramuscular injection. BAL binds lead to sulfhydral groups and chelates metal from both inside and outside the cellular space. Lead removal through the bile and urine is increased within 30 min of administration. BAL is the common choice when there is known toxicity to the kidney, but it is contraindicated if there is liver failure or glucose-6-phosphate dehydrogenase deficiency. BAL treatment has produced a number of adverse reactions, including nausea, vomiting, tachycardia, and fever.

$CaNa_2$-EDTA binds extracellular lead. After administration, excretion of lead through the kidneys may be increased 20- to 50-fold. If there is renal dysfunction, use of $CaNa_2$-EDTA may enhance toxicity. Blood lead levels may rise after the administration of $CaNa_2$-EDTA alone. BAL is usually given

with $CaNa_2$-EDTA to reduce toxicity associated with the mobilization of lead stored in soft tissues. $CaNa_2$-EDTA is usually not used for patients with known low zinc stores. Sodium-EDTA is not used to treat lead poisoning because it will also chelate and reduce calcium in the body.

D-Penicillamine, a chelating agent that can be administered orally, is currently used to chelate lead on an experimental basis. Individuals who are allergic to penicillin may experience adverse reactions to this agent; toxic effects have been reported in as many as 20% of the patients treated with this compound.

2,3-Dimercapto-1-propanesulfonic acid and dimercaptosuccinic acid have mechanisms of action similar to BAL. Both are water-soluble analogs of BAL that can be administered orally, are less toxic and have fewer unpleasant side effects than BAL. They have been found to be effective in removing lead via the kidneys. Treatment regimens may also include removal from the source of exposure and changes in the patient's diet.

Environmental Fate

Lead occurs naturally in the environment. However, most of the lead dispersed throughout the environment comes from human activities. Before the use of leaded gasoline was limited, most of the lead released into the US environment came from car exhaust. Since the EPA has limited the use of leaded gasoline, the amount of lead released into the air has decreased. Other sources of lead released into the air include burning fuel, such as coal or oil, industrial processes, and burning solid waste.

The release of lead to air is now less than the release of lead to soil. Most of the lead in inner city soils comes from landfills and leaded paint. Landfills contain waste from lead ore mining, ammunition manufacturing, and other industrial activities such as battery production. Very little lead goes directly into water.

Higher levels of lead from car exhausts can be measured near roadways. Very low levels of lead from car exhausts are found at distances of 25 m ($\sim$80 ft) from the road edge. However, once lead goes into the atmosphere, it may travel thousands of miles if the lead particles are small or if the lead compounds are volatile. Lead is removed from the air by rain as well as by particles falling to the ground or into surface water. Once lead deposits on soil, it usually sticks to soil particles. Small amounts of lead may enter rivers, lakes, and streams when soil particles are displaced by rainwater. Lead may remain stuck to soil particles in water for many years. Movement of lead from soil particles into underground water or drinking water is unlikely unless the water is acidic or 'soft'.

Some of the chemicals that contain lead are broken down by sunlight, air, and water to other forms of lead. Lead compounds in water may combine with different chemicals depending on the acidity and temperature of the water. The lead atom cannot be broken down.

The levels of lead may build up in plants and animals from areas where air, water, or soil are contaminated with lead. If animals eat contaminated plants or animals, most of the lead that they eat will pass through their bodies. It is the small amount absorbed that can cause harmful effects.

The amount of lead in paints sold for consumer use may not exceed 0.06%.

Releases from lead-based paints are frequently confined to the area in the immediate vicinity of painted surfaces, and deterioration or removal of the paint can result in high localized concentrations of lead in indoor air and on exposed surfaces. Sandblasting procedures to remove paint may disperse lead in the local environment.

The largest volume of organolead vapors released to the atmosphere results from industrial processes such as primary and secondary nonferrous metal smelting, and from the use of leaded gasoline which contains tetraethyl lead as an antiknock additive. These vapors are photoreactive, and their presence in the local atmosphere is transitory. Halogenated lead compounds are also formed and, ultimately, oxides and carbonates. Tetraalkyl lead compounds have been found to contribute 5–10% of the total particulate lead present in the atmosphere. Organolead vapors are most likely to occur in occupational settings (e.g., gasoline transport and handling operations, gas stations, and parking garages) and high-traffic areas.

Although aquatic releases from industrial facilities are expected to be small, lead may he present in significant levels in drinking water. In areas receiving acid rain (e.g, northeastern United States) the acidity of drinking water may increase, thus increasing the corrosivity of the water, which may, in turn, result in the leaching of lead from water systems, particularly from older systems during the first flush of water through the pipes. Fish in more acidic waters accumulate more lead than fish in a more alkaline environment.

The grounding of household electrical systems to the plumbing can increase corrosion rates and the subsequent leaching of lead from the lead solder used for copper pipes. Areas where the pH of the water is less than 8.0 may have higher lead drinking water levels as well.

Canning foods in lead-soldered cans may increase levels of lead by 8- to 10-fold; however, the impact of canning appears to be decreasing as a result of a decrease in the use of lead-soldered cans. Additional exposure to lead through dietary intake by people living in an urban environment is estimated to be $\sim 28\,\mathrm{mg\,day^{-1}}$ for adults and $91\,\mathrm{mg\,day^{-1}}$ for children, all of which can be attributed to atmospheric lead (dust). Atmospheric lead may be added to food crops in the field or garden (through uptake from soil and from direct deposition onto crop surfaces), during transport to market, processing, and kitchen preparation.

Lead may leach from lead crystal decanters and glasses into the liquids they contain. Flaking paint, paint chips, and weathered powdered paint, which are most commonly associated with deteriorated housing stock in urban areas, are major sources of lead exposure for young children residing in these houses, particularly for children with pica (i.e., the compulsive, habitual consumption of nonfood items). Lead concentrations of $1000–5000\,\mathrm{mg\,cm^{-2}}$ have been found in chips of lead-based paint, suggesting that consumption of a single chip of paint would provide greater short-term exposure than any other source of lead.

Ecotoxicology

The impact of environmental lead on wildlife and ecosystems has been a subject of study and concern. Water fowl may become poisoned from ingesting lead shot. Poisoning may lead to anorexia, lethargy, coma, and death. Other birds have also been shown to be impacted by environmental lead. Water fowl can also be contaminated with lead by swallowing fishing sinkers, in particular the small 'split shot' type.

Exposure Standards and Guidelines

Most adult exposure is occupational. The Immediately Dangerous to Life or Health value for lead is $100\,\mathrm{mg\,m^{-3}}$. The recommended exposure limit is $0.1\,\mathrm{mg\,m^{-3}}$ and the permissible exposure limit/threshold limit value for lead is $0.05\,\mathrm{mg\,m^{-3}}$.

See also: Behavioral Toxicology; Developmental Toxicology; EDTA (Ethylenediaminetetraacetic acid); Kidney; Metals; Neurotoxicity; Occupational Toxicology; Pollution, Air; Pollution, Water; Psychological Indices of Toxicity; Reproductive System, Male; Skeletal System; Toxicology, History of; Veterinary Toxicology.

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Relevant Websites

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http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Lead.

Lethal, Dosage or Concentration *See* LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50)

Levels of Effect in Toxicological Assessment

Michael Dourson

Level of Effect terminology varies somewhat from country to country, and from one organization to another. Terms are used to identify specific locations along a severity spectrum, moving from doses with no toxicologically recognizable effect, to negligible effects, to doses with more profound toxicity. A list of relevant terms is given below.

- *Acceptable daily intake (ADI)*: The daily intake of a chemical, which, during a lifetime, appears to be

without appreciable risk on the basis of all the known information at the time.

- *Acute toxicity*: The older term used to describe immediate toxicity. Its former use was associated with toxic effects that were severe (e.g., mortality) in contrast to the term 'subacute toxicity' that was associated with toxic effects that were less severe. The term 'acute toxicity' is often confused with that of acute exposure.
- *Adaptive effect*: An adaptive effect enhances an organism's performance as a whole and/or its ability to withstand a challenge. An example of an adaptive effect is an increase in hepatic smooth endoplasmic reticulum, but only if hepatic metabolism reduces the chemical's toxicity.
- *Adverse effect*: A biochemical change, functional impairment, or pathological lesion, which impairs performance and reduces the ability of the organism to respond to additional challenge.
- *Allergic reaction*: An adverse reaction to a chemical resulting from previous sensitization to that chemical or to a structurally similar one.
- *Chronic toxicity*: The older term used to describe delayed toxicity. However, the term 'chronic toxicity' also refers to effects that persist over a long period of time whether or not they occur immediately or are delayed. The term 'chronic toxicity' is often confused with that of chronic exposure.
- *Compensatory effect*: This effect maintains overall function without enhancement or significant cost. Increased respiration due to metabolic acidosis is an example of a compensatory effect.
- *Critical effect*: A chemical often elicits more than one toxic effect, even in one species, or in tests of the same or different durations. The critical effect(s) is the first adverse effect(s) or its known precursor(s) that occurs as dose rate increases. The critical effect(s) may change among toxicity studies of different durations, may be influenced by toxicity in other organs, and may differ depending on the availability of data on the shape of the dose–response curve.
- *Idiosyncratic reaction*: A genetically determined abnormal reactivity to a chemical.
- *Immediate versus delayed toxicity*: Immediate effects occur or develop rapidly after a single administration of a substance, while delayed effects are those that occur after the lapse of some time. These effects have also been referred to as acute and chronic, respectively.
- *Local versus systemic toxicity*: Local effects refer to those that occur at the site of first contact between the biological system and the toxicant; systemic effects are those that are elicited after absorption and distribution of the toxicant from its entry point to a distant site.
- *Lowest-observed-adverse-effect-level (LOAEL)*: The lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.
- *Minimum risk level (MRL)*: An estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure.
- *No-observed-adverse-effect level (NOAEL)*: An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control; *some effects may be produced at this level, but they are not considered as adverse, nor precursors to specific adverse effects*. In an experiment with several NOAELs, the regulatory focus is primarily on the NOAEL seen at the highest dose. This leads to the common usage of the term NOAEL to mean the highest exposure without adverse effect.
- *Reference dose (RfD)*: An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime.
- *Reversible versus irreversible toxicity*: Reversible toxic effects are those that can be repaired, usually by a specific tissue's ability to regenerate or mend itself after chemical exposure, while irreversible toxic effects are those that cannot be repaired.
- *Tolerable concentrations* (TCs, often expressed in $mg\,m^{-3}$): TCs are generally airborne concentrations to which it is believed that a person can be exposed continuously over a lifetime without deleterious effect. They are based on noncarcinogenic effects.
- *Tolerable daily intake (TDI)*: An estimate of the quantity of a chemical contaminant in food or water, which can be ingested daily over a lifetime without posing a significant risk to health. 'Contaminants' are different from 'Residues' in this context: a contaminant is a chemical whose presence in food or water does not serve, and never has served, any useful purpose. TDIs are thus distinct from ADIs, which relates to residues of chemicals that have been deliberately added to a product, for example, residues of pesticide sprays or antifungal agents.
- *Uncertainty factor (UF)/safety factor (SF)*: One of several, generally 10-fold, factors used in

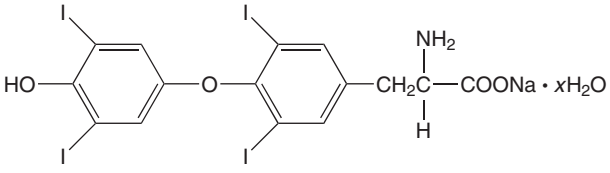
operationally deriving an RfD or ADI from experimental data. Among other things UFs are intended to account for (1) the variation in sensitivity among the members of the human population; (2) the uncertainty in extrapolating animal data to the case of humans; (3) the uncertainty in extrapolating from data obtained in a study that is of less-than-lifetime exposure; (4) the uncertainty in using LOAEL data rather than NOAEL data; and (5) the inability of any single study to address adequately all possible adverse outcomes in man.

See also: Acceptable Daily Intake (ADI); Dose–Response Relationship; Reference Dose (RfD); Toxicity, Acute; Toxicity, Chronic; Uncertainty Factors.

Levothyroxine

Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 51-48-9
- Synonyms: Eltroxin; L-Thyroxine; Levo-T; Levotec; Levothyroid; Levoxyl; Synthroid; T4; Thyroxine
- Chemical/Pharmaceutical/Other Class: Synthetic thyroid hormone
- Chemical Structure:



Uses

Levothyroxine is used for thyroid hormone replacement.

Exposure Routes and Pathways

Ingestion is the most common route of accidental and intentional exposure to levothyroxine (T4). It is also available in an intravenous dosage form.

Toxicokinetics

T4 oral bioavailability varies from 30% to 90%. Peak serum T4 levels occur 2–6 h after therapeutic dosing. Approximately 99% of T4 is protein bound. T4 has a volume of distribution of 8–10 l kg^{-1}. From 75% to 85% of T4 is deiodinated in the liver, kidney, muscles, heart, and brain. Half of this is converted to active T3. Approximately 20% of T4 is excreted in the feces intact after oral dosing and 10% of conjugated T4 is excreted in the urine. The half-life of T4 is 5–9 days in euthyroid patients taking therapeutic doses.

Mechanism of Toxicity

Thyroid compounds are necessary for metabolism, growth, and development. T4's primary action is related to calorigenesis and protein synthesis. Thyroid hormones potentiate the effects of catecholamines. About half of T4 is converted to T3. T3 is three to five times more potent than T4.

Acute and Short-Term Toxicity (or Exposure)

Animal

Both dogs and cats are at risk for thyroid toxicity. Signs of toxicity in animals include vomiting, diarrhea, tachycardia, tachypnea, decreased level of consciousness, and restlessness.

Human

In general, adults and children can tolerate acute overdoses of T4. Ingestion of less than 4 mg of T4 is unlikely to produce symptoms. In acute exposures that become symptomatic, clinical effects are generally mild. Symptoms may develop days after ingestion when T4 is converted to the more potent T3. In cases of intentional overdose with large amounts of T4 ingested, acute clinical effects may include tachycardia, hypertension, tachydysrhythmias, flushing, diaphoresis, nausea, vomiting, diarrhea, restlessness, confusion, headache, mydriasis, and fever that can persist for days.

Chronic Toxicity (or Exposure)

Animal

When administered to young, pregnant rats during the 9th to 20th day of pregnancy, cataracts developed in the offspring.

Human

Chronic exposure to high doses of T4 may cause thyrotoxicosis. The development of thyrotoxicosis in an acute exposure is rare. Thyrotoxicosis is characterized by tachycardia, cardiac arrhythmias, hypertension, hyperpyrexia, tremors, and seizures. In patients with severe toxicity, coma and circulatory collapse can result.

In Vitro Toxicity Data

Studies of *in vitro* and *in vivo* models of hyperthyroidism have documented substantial impact on rat liver function. Recent developments have suggested that these findings are likely due to induction of apoptosis via a mitochondrial mediated pathway or pathways.

Clinical Management

Most patients after acute overdose can be managed on an outpatient basis. Gastric decontamination may be considered in patients presenting early after large ingestions. The absence of clinical effects within the first 24 h does not preclude the later development of significant toxicity. In patients manifesting toxicity, cardiac and blood pressure monitoring should be performed. Cooling methods should be employed to decrease hyperpyrexia. Intravenous fluids should be administered in dehydrated and/or hypotensive patients. Adrenergic hyperactivity can be treated with propranolol. Propylthiouracil (PTU) may be administered to decrease conversion of T4 to T3. Forced diuresis and extracorporeal methods are not effective in levothyroxine overdose.

See also: Endocrine System; Thyroid Extract.

Further Reading

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Tunget CL, Clark RF, and Turchen SG (1995) Raising the decontamination level for thyroid hormone ingestions. *American Journal of Emergency Medicine* 13: 9–13.

Lewisite

Harry Salem*

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- Chemical Abstracts Service Registry Numbers: CAS 541-25-3 (Lewisite 1: 2-chlorovinyldichloroarsine); CAS 40334-69-8 (Lewisite 2: (2-chlorovinyl)chloroarsine); CAS 40334-70-1 (Lewisite 3: Tris(2-chlorovinyl)arsine)
- Synonyms: Arsine; Arsonous; Dichloride; Arsine, L
- Chemical/Pharmaceutical/Other Class: Blister agent/Vesicant class of chemical warfare agents
- Chemical Formula: $C_2H_2AsCl_3$
- Chemical Structure: $ClCH=HC–AsCl_2$

Uses

Lewisite was synthesized in 1918 by Dr. Wilford Lee Lewis as a vesicant for chemical warfare. Its production was too late to use in World War I. It can be used with mustard to lower the freezing point of the mixture for ground dispersal and spraying.

Background Information

Other organic arsenical chemical warfare agents are methyldichlorarsine (MD), phenyldichloroarsine (PD), and ethyldichloroarsine (ED). These plus lewisite (L), mustard agents, and phosgene oxime make up the vesicant class.

Exposure Routes and Pathways

Lewisite is an oily, colorless liquid that can appear amber to black in its impure form. It has the odor of geraniums. It is more volatile than the mustard agents. Lewisite in the air can cause damage to the eyes, skin, and airways by direct contact. Lewisite in water can lead to exposures from drinking the water or from skin contact, and lewisite-contaminated food can be ingested. Lewisite remains as a liquid under a wide range of environmental conditions, from below freezing to very hot temperatures.

*The views of the author do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Toxicokinetics

Although the exact mechanism of biological activity is unknown, the trivalent arsenic in lewisite combines with the thiol groups in many enzymes.

Mechanism of Toxicity

Lewisite is readily absorbed from the skin, eyes, and respiratory tract, as well as after ingestion and through wounds. It causes blistering on the skin and mucous membranes on contact. After absorption, it causes an increase in capillary permeability, which produces hypovolemia, shock, and organ damage. Unlike the mustard agents, lewisite vapor or liquid causes immediate pain or irritation although lesions require up to 12 h to become full-blown cases.

Human Toxicity

Nasal irritation by lewisite begins at $\sim 8\,\mathrm{mg\,min\,m^{-3}}$ and its odor is detected at $\sim 20\,\mathrm{mg\,min\,m^{-3}}$. Vesication and death from lewisite inhalation is caused at the same Ct as mustard, which is $1500\,\mathrm{mg\,min\,m^{-3}}$. The immediately dangerous to life health (IDLH) value of lewisite is $0.003\,\mathrm{mg\,m^{-3}}$. Lewisite causes vesication at ~ 14 mg and the LD_{50} is 2.8 g on the skin.

Within 5 min after contact with liquid lewisite, a grayish area of dead epithelium is produced. Erythema and blister formation follows more rapidly that it does with mustard even though the full-blown lesion does not develop for 12–18 h. The lesion has more tissue necrosis and tissue sloughing than does a mustard agent lesion.

On the eyes, lewisite causes pain, tearing, and blepharospasm on contact. Edema of the conjunctiva and lids follows and the eyes may be swollen shut within an hour. Iritis and corneal damage may also occur. Within minutes, liquid lewisite causes severe eye damage on contact. Upon inhalation, the airway mucosa is the primary target and the damage progresses down the airways with pseudomembrane formation. Pulmonary edema may complicate exposure to lewisite. Runny nose, sneezing, hoarseness, bloody nose, sinus pain, shortness of breath, and cough also occur on inhalation. Lewisite causes an increase in permeability of systemic capillaries resulting in intravascular fluid loss, hypovolemia, shock, and organ congestion. This has been termed 'Lewisite shock' or hypotension. This also leads to hepatitis or renal necrosis with more prominent gastrointestinal effects of diarrhea, nausea, and vomiting.

The long-term effects of lewisite exposure do not include extensive skin burning as is seen with the mustard agents, but chronic respiratory disease may occur. Also, unlike the mustard agents, suppression of the immune systems does not occur, but extensive eye exposure may cause permanent blindness.

Animal Toxicity

Vapor exposure – Inhalation

Species	LCt_{50} ($mg\,min\,m^{-3}$)
Mouse	900
Rat	1500
Rabbit	1000
Guinea pig	1200
Dog	1400
Goat	1250

Liquid percutaneous

Species	LD_{50} ($mg\,kg^{-1}$)
Mouse	15
Rat	20
Rabbit	5
Guinea pig	12
Dog	70
Goat	10

Clinical Management

To prevent or lessen lewisite damage, early decontamination within minutes after exposure must be instituted. Unlike mustard, lewisite does not cause damage to the hematopoietic organs, but fluid loss from increased capillary permeability necessitates careful attention to fluid balance.

British antilewisite (BAL) or dimercaprol was developed as an antidote for lewisite. It is used in medicine as a chelating agent for heavy metals. Although BAL can cause toxicity itself, evidence suggests that BAL in oil administered intramuscularly will reduce the systemic effects of lewisite. BAL skin and ophthalmic ointment decrease the severity of skin and eye lesions when applied immediately after early decontamination, but neither of these ointments is currently manufactured.

See also: BAL (British Antilewisite); Blister Agents/Vesicants.

Relevant Websites

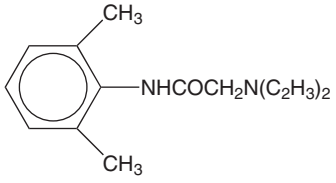
http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Lidocaine

Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 137-58-6
- Synonyms: Dilocaine; Lidoderm; Lidoject-1; Lignocaine; Nervocaine; Nulicaine; Octocaine; Solarcaine; Xylocaine; Xylocard
- Chemical/Pharmaceutical/Other Class: Amide-type local anesthetic; Class IB antiarrhythmic
- Chemical Structure:



Uses

Lidocaine is used for local anesthesia and in the management of ventricular arrhythmias.

Exposure Routes and Pathways

Lidocaine is administered either topically or by the parenteral route. Ingestion of topical lidocaine products can occur and result in toxicity.

Toxicokinetics

Lidocaine is absorbed from the gastrointestinal tract but undergoes significant first-pass metabolism (60–70%). Absorption from local sites is dependent on the dose and vascularity of the site. It is well absorbed from mucosa. Lidocaine is widely distributed to tissues. The volume of distribution is $\sim 1\,l\,kg^{-1}$. Protein binding is 60–80%. Lidocaine is dealkylated by hepatic CYP3A4 to the active metabolite monoethylglycinexylide, which is then inactivated to glycine xylidide. These metabolites and ~5% of unchanged lidocaine are renally excreted. The elimination half-life is 1–2 h.

Mechanism of Toxicity

Lidocaine combines with fast voltage-gated sodium channels and inhibits recovery after repolarization. As a result, cellular conduction is blocked by lidocaine's inhibition of permeability of excitable membranes to sodium that normally is produced by membrane depolarization.

Acute and Short-Term Toxicity (or Exposure)

Animal

The clinical effects of lidocaine in animals are similar to those observed in humans.

Human

Therapeutic lidocaine serum concentrations range from 1 to $5\,mg\,ml^{-1}$. Signs of toxicity are usually seen above $6–10\,mg\,ml^{-1}$, and death has been associated with serum concentrations above $15\,mg\,ml^{-1}$. Nausea, vomiting, lightheadedness, euphoria, dizziness, drowsiness, confusion, visual changes, increasing agitation, and muscle fasciculations may be seen with lidocaine toxicity. Severe lidocaine toxicity can result in seizures and coma. Cardiovascular effects of lidocaine include a variety of arrhythmias and hypotension. Arrhythmias include sinus arrest, sinus bradycardia, heart block, and asystole. Hypotension may be due to vasodilation or decreased cardiac output.

Chronic Toxicity (or Exposure)

Animal

Chronic dosing of rats starting 2 weeks before mating and continuing through delivery at $100–250\,mg\,kg^{-1}\,day^{-1}$ by continuous intravenous infusion resulted in no serious adverse effects.

Human

Small children treated with topical lidocaine 2% for teething five to six times daily for a week developed seizures. Patients being managed for several days with lidocaine for control of acute arrhythmias may accumulate lidocaine and its metabolites if they have changes in blood flow (e.g., shock, circulatory collapse). Decreased clearance and accumulation of lidocaine and desmethyllidocaine may result in the development of drowsiness, tinnitus, muscle twitching, and may eventually lead to seizures, coma, and arrhythmias.

In Vitro Toxicity Data

Ames *Salmonella* assays of the mutagenicity of lidocaine have been inconclusive.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Decontamination with syrup of ipecac is contraindicated due to the likelihood for developing rapid onset seizures. Gastric decontamination with activated charcoal may be considered for substantial recent ingestions. Fluid and electrolytes need to be frequently monitored and replaced as necessary.

Treatment is symptomatic and supportive after decontamination. Bradycardia is usually responsive to atropine. For hypotension, intravenous fluids should be administered and if unsuccessful, vasopressor therapy should be initiated. Arrhythmias can be refractory to drug management; however, treatment should be guided by electrocardiographic changes. Sodium bicarbonate may be used to reverse the effects of sodium channel blockage caused by lidocaine. There are case reports of patients developing methemoglobinemia secondary to lidocaine. Those with elevated methemoglobin concentrations should be managed with intravenous methylene blue.

See also: Poisoning Emergencies in Humans.

Further Reading

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Mofenson HC, Caraccio TR, and Miller H (1983) Lidocaine toxicity from topical mucousal application. *Clinical Pediatrics* 22: 190–192.

Life Cycle Assessment

David W Pennington and Tomas Rydberg

The provision of goods and services (collectively, products) contributes to environmental impacts. Life cycle assessment (LCA) is a tool for comparing product options and for identifying opportunities for reducing related impacts. LCA provides insights that are complimentary to those of many regulatory and more site- or process-oriented risk and impact assessments.

The focus of an LCA is typically on contributions to regional or global scale impacts, including the consumption of resources. All stages in a product's life cycle can result in the generation of wastes, in emissions, and in resource consumption. These environmental exchanges contribute to impacts, such as climate change, stratospheric ozone depletion, photooxidant formation (smog), eutrophication, acidification, toxicological stress on human health and ecosystems, the depletion of resources, and noise, among others. An LCA helps decision makers take into account the contributions to these impacts, as well as the trade-offs, that occur at the many stages in a product's life cycle, that is, during the extraction of raw materials, energy acquisition, production, manufacturing, use, reuse, recycling, through to ultimate disposal.

Structure of LCA

LCA practitioners tabulate the wastes, the emissions, and the resources consumed, for example, at every relevant stage in a product's life cycle, from its 'cradle to grave'. The compilation, tabulation, and preliminary analysis of these data are termed life cycle inventory (LCI). After LCI, it is generally necessary for practitioners to calculate indicators of the contributions to (potential) impacts that are associated with these inventory data. This is life cycle impact assessment (LCIA).

The standards and reports in the International Organization for Standardization (ISO) 14000 series provide, in general, an accepted framework and terminology for LCA (although not practical insights).

ISO 14040:1997	Life cycle assessment – Principles and framework
ISO 14041:1998	Life cycle assessment – Goal and scope definition and inventory analysis
ISO 14042:2000	Life cycle assessment – Life cycle impact assessment
ISO 14043:2000	Life cycle assessment – Life cycle interpretation
ISO/TR 14047	Life cycle assessment – Examples of application of ISO 14042
ISO/TS 14048:2002	Life cycle assessment – Data documentation format
ISO/TR 14049:2000	Life cycle assessment – Examples of application of ISO 14041 to goal and scope definition and inventory analysis

Figure 1 distinguishes the three phases within an LCA. Interpretation is vital at each stage and an assessment will typically be refined iteratively.

In the goal and scope definition of an LCA, the practitioner defines the product system in terms of the system boundaries of the study and a functional

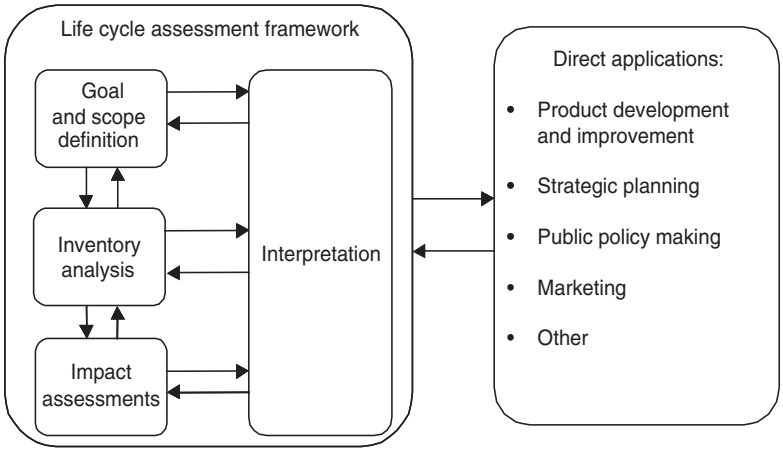

Figure 1 Phases and applications of an LCA. (Based on ISO14040, 1997.)

unit. The functional unit is vital. The functional unit facilitates the direct comparison of alternative goods or services.

A functional unit is not just a quantity of material. Practitioners may compare, for example, alternative types of packaging on the functional unit basis of $1m^3$ of packed and delivered product. The comparison is then in terms of the service that the packaging provides. The quantity of packaging material required to provide this functional unit, termed the reference flow, will vary in the studies depending on the packaging option selected (e.g., paper, plastic, metal, and composite).

LCI is the methodology for estimating the consumption of resources, the quantities of wastes, the emissions, the traffic accidents, the noise, etc., that are associated with each stage in a product's life cycle. The material and energy flows are modeled between the processes within a life cycle. The overall models provide mass and energy balances for the product system, its total inputs and outputs into the environment, on a per functional unit basis.

The design of the product system model, especially with respect to the system boundaries and what processes are included within these boundaries, can be decisive for what data are appropriate and the outcome of an LCA study. As one example different types of life cycle models can be designed to describe:

- a product system and its environmental exchanges considering, for example, the average energy mixes needed to generate electricity (termed an attributional model), or
- how the environmental exchanges within the system might be expected to change as a result of actions taken, for example, accounting for which type of fuel source could be reduced as a result of reduced electricity requirements due to product design modifications (termed a consequential model).

LCIA provides indicators for the interpretation of the inventory data in terms of contributions to different impact categories. The indicator results of an LCIA facilitate the evaluation of a product, and each stage in its life cycle, in terms of climate change, toxicological stress, noise, land use, water consumption, etc. The scope of the evaluation is, with some exceptions, limited to impacts at a regional and global scale.

The overall indicator results of an LCIA reflect cumulative contributions to different impact categories that are summed over time and space. Unlike some other assessment approaches, these indicator results usually do not reflect risks or impacts at any particular location or point in time. The consumption of resources and the generation of wastes, emissions, etc., often occur in a product's life cycle:

- at multiple sites and in multiple regions around the world,
- as different fractions of the total emissions at any one site (the fraction required to provide the specified functional unit, with allocation amongst related and unrelated coproducts in a facility such as a refinery, for example),
- at different times (the use phase of a car compared to dismantling), and
- over short and long time periods (e.g., multiple generations in the case of emissions of persistent chemicals and from landfills).

Interpretation occurs at every stage in an LCA. If two product alternatives are compared and one alternative has a higher consumption of each resource,

for example, an interpretation purely based on the LCI can be conclusive. In other studies, drawing conclusions will require at least an LCIA, a sensitivity analysis, and consideration of the statistical significance of differences in each impact category.

Some category indicators can be further cross-aggregated and compared on a natural science basis. Further aggregation can be to calculate the overall sum of years of human life lost, for example, the years of life lost that are attributable to climate change, potential carcinogenic effects, noise, traffic accidents, etc.

A practitioner may also want to compare across other impact categories, particularly when there are trade-offs between product alternatives or if it is desirable to prioritize within a product's life cycle. This is often termed valuation or weighting. For example, emissions of CO_2 equivalents in one life cycle may result in a higher climate change indicator than for another, but the alternative involves the use of more pesticides. These pesticides may result in a higher contribution to the risks of regional toxicological impacts. A stakeholder may therefore want more information to help guide which difference is of a higher priority.

Resolving such trade-offs between impact categories draws not only on natural sciences but also often relies on social science and economics. In some applications, particularly for policy support and as one example, this results in the monetization of externalities (the impact indicators) to provide results for different impact categories in terms of Euros, Yen, or similar.

Assessing Impacts in LCA

LCIA consists of both mandatory and optional elements, as described, for example, in ISO 14042:

- Selection of the impact categories of interest, the indicators for each impact category, and, although often implicitly considered by most practitioners, the choice of the underlying models (a procedure also considered in the initial goal and scope phase of an LCA study).
- Assignment of the inventory data to the chosen impact categories (*classification*).
- Calculation of impact category indicators using characterisation factors (*characterisation*).
- Calculation of category indicator results relative to reference value(s) (*normalisation*; optional).
- *Grouping* and/or *weighting* the results (optional, weighting not being allowed when strictly adhering to ISO14042 in comparative assertions disclosed to the public).
- *Data quality analysis* (mandatory in comparative assertions disclosed to the public, according to ISO 14042, but receiving little attention in current practice).

In practice, these elements are often supported using available databases and tools.

Toxicological Impact Characterization in LCA

Equation (1) provides a simple example of how impact indicators can be calculated from inventory data using generic *characterization factors*. These factors are generally the output of *characterization model*s. The factors are often available to practitioners in a precalculated format in the literature, in the form of databases, as well as in available support tools.

$$\text{Category Indicator} = \sum_{s} \text{Characterization Factor(s)} \times \text{Emission Inventory(ies)} \quad (1)$$

where the lower limit of the summation, s, denotes the chemical.

For wastes and emissions, the inventory data in eqn (1) are in terms of the mass of each substance that is released into the environment associated with the functional unit. For example, the mass of the different chemicals released into the environment that are associated with all of the life cycle stages related to providing packaging for 1 m^3 of packaged and delivered product. The characterization factors in eqn (1) therefore linearly express the contribution to an impact category of releasing a unit mass (e.g., 1 kg) of an emission into the environment.

As one example of a characterization factor, the relative contributions of different gases to climate change are commonly compared in terms of carbon dioxide equivalents using global warming potentials (GWPs). A GWP_{500} of 100 implies that 1 kg of the substance has the same cumulative climate change effect as 100 kg of carbon dioxide during, in this case, a 500 year time period.

A number of methodologies and associated interpretations have been proposed for calculating characterization factors for toxicological risks and the potential impacts in LCA. Score-based factors initially helped to rank emissions in terms of selected fate, exposure, and toxicity parameters. These were often similar to the ordinal persistence, bioaccumulation, and toxicity (PBT) scores used in other applications. Approaches now rely to a greater extent on the use of mechanistic models and, to a lesser extent, on epidemiological data.

To be consistent, but to provide broad chemical coverage, there has been a common reliance in LCIA

on the use of multimedia chemical fate models, human exposure correlations for organic chemicals, as well as the toxicological methodologies/data that were designed for chemical risk screening in a regulatory context. The results of these early modeling approaches can be interpreted in terms of 'policy-based hazard equivalents'. For example, indicators are presented in terms of 'kg equivalents of benzene' for cancer effects. The characterization factors reflect the ratio of the hazard (exposure/regulated effect level) of a unit emission (kg or $kg\,day^{-1}$) of one chemical relative to the hazard ratio for the same emission of benzene.

Some methodologies are emerging to estimate characterization factors for toxicological effects that are designed more specifically for use in relative comparison applications like LCA. These are more consistent with the underlying basis of commonly adopted approaches for the other impact categories, as well as for the assessment of substances such as radionuclides. The emerging factors reflect the cumulative risk attributable to a unit emission (e.g., 1 kg) of a chemical into the environment. Cumulative risk, in this case, is defined as the risk integrated over time and space, as well as over the entire exposed population, that is associated with the unit mass of a chemical emitted. This definition, and the associated data, required differ from those adopted in many regulatory contexts for chemical screening assessments in the context of toxicological effects.

In estimating the cumulative risk of a chemical in LCA, dose–response extrapolations can be based on toxicological benchmarks. Such a benchmark approach is considered more appropriate for use in comparative assessment contexts, such as in an LCA study. Benchmarks are an exposure measure associated with a consistent change in response, such as the 10% or even the 50% effect level. Regulatory-based measures do not necessarily provide a consistent risk basis for comparison, as they were often never developed for use in such a comparative context or to facilitate low dose-response extrapolation. Other data differences include the use of median, rather than extreme, data in the fate and exposure modeling, as well as the consideration of safety factors only as part of the uncertainty assessment and not as an integral part of the toxicological effects data.

It is important to additionally account for differences in potential toxicological consequences (severity, damage, or impact) in the comparison. Characterization factors can be expressed in terms of metrics such as DALYs (disability adjusted life years) for human health, for example. The results for toxicological impacts can be directly cross-compared with those of DALY-based indicators for other impact categories, such as for climate change.

Metrics such as DALYs are derived from statistics for mortality. These are dominant for cancer effects. Equivalent numbers of DALYs, calculated using social science techniques, are provided for the years of life lost for morbidity (nonfatal) effects. Further developments remain necessary for noncancer toxicological effects. Although some DALY-based characterization factors are available from epidemiological data for respiratory illness, including for secondary particulate matter (nitrates and sulfates).

Similar approaches exist for impacts on ecosystems, although at this time the consequences of ecotoxicological effects are primarily addressed in terms of cumulative risks or hazard indicators multiplied by the area or volume affected.

Concluding Remarks

LCA provides insights that are complimentary to those of many regulatory and more site, or process, orientated risk and impact assessments. LCA is a tool for comparing product options and for identifying opportunities for reducing related impacts.

An LCA study can include the consideration of contributions to potential regional and global scale toxicological impacts. These contributions are calculated using characterization factors. Characterization factors linearly express the cumulative contribution to the risk of a potential toxicological impact and the relative consequences that are attributable to releasing a unit mass (e.g., 1 kg) of a chemical into the environment.

A number of researchers continue to assess the statistical importance of the influences of many of the underlying modeling options on the characterization factors and on the overall results of LCA studies. These include when, and how, differentiation is required in terms of the modes of entry and the times/locations of chemical releases when considering cumulative regional and global effects, the duration over which these effects are likely to occur, as well as the influence of distinguishing between short- and long-term effects by using time horizons like 50 or 500 years.

See also: Risk Assessment, Ecological; Risk Assessment, Human Health.

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Relevant Website

http://www.iso.ch – International Organization for Standardization (ISO).

Lily of the Valley

Amanda Lofton

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- Synonyms: Convallaria; *Convallaria majalis*; Convall-lily; Jacob's Ladder; Ladder-to-Heaven; Liljekonvall; Lily Constancy; Lily convalle; Male Lily; May Lily; Our Lady's Tears
- Chemical/Pharmaceutical/Other Class: Cardiac glycosides

Uses

Lily of the valley is used in bouquets or cut flower arrangements and as a garden perennial.

Background Information

A member of the family Liliaceae, lily of the valley is an herbaceous perennial arising from upright root stocks with pairs of simple broad oval leaves and a flowering stock. The stock bears a one-sided row of scented, waxy, bell-shaped flowers. These fragrant flowers are usually white; however, pale pink, pink, and yellow varieties also exist. The plant also bears a red-orange berry, ~0.5 in. in diameter, which is filled with seeds. Lily of the valley is commonly cultivated in North America, the United Kingdom, and throughout Europe.

Exposure Routes and Pathways

Ingestion of the fragrant flowers or bright berries is the most common route of exposure.

Toxicokinetics

Limited pharmacokinetic data are available on these plants. It is expected that absorption from plant extracts (e.g., tea brewed from the plant) would be faster and greater than absorption from the raw plant.

Mechanism of Toxicity

The whole plant contains convallarin and convallamarin, both cardiac glycosides. With significant exposure, the sodium–potassium ATPase pump in the heart is disrupted, which results in markedly high-grade atrioventricular (AV) conduction block. As intracellular potassium concentrations decline, progressive electrical changes occur. These events may progress to complete loss of normal myocardial electrical function and asystole. The myocardium may lose its ability to respond to electrical pacing.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals are susceptible to toxicity following *Convallaria majalis* exposure. In one case report, a dog that ingested an unknown quantity of lily of the valley leaves suffered seizures and death. Autopsy findings revealed severe hepatic congestion and caudal vena cava distention. Gross and microscopic lesions consistent with cardiac shock were also evident. Leaves of the plant were found in the middle section of the jejunum. Animals exposed to

Convallaria majalis may receive activated charcoal, digoxin-specific immune Fab fragments, and supportive care as required.

Human

Poisoning by *Convallaria majalis* is clinically indistinguishable from digoxin toxicity. Following an acute exposure, patients experience nausea and vomiting within a few hours. Clinical effects later worsen and progress to lethargy, dizziness, hyperkalemia, cardiac conduction delays, hypotension, bradycardia, and tachydysrhythmias. After chewing on a leaf from lily of the valley, a 4-year-old boy was administered activated charcoal and admitted to the hospital for observation and cardiac monitoring. He experienced four episodes of grade II AV-block. A 5-year-old developed only vomiting after ingesting 15 berries from lily of the valley.

Chronic Toxicity (or Exposure)

Human

Chronic ingestion of *Convallaria majalis* may also result in clinically significant cardiac effects, although such exposures have rarely been reported. Patients chronically exposed to the plant may be less likely to present with gastrointestinal distress as their initial symptom of toxicity.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. With significant ingestion, decontamination is advised. Activated charcoal will adsorb plant toxins. Patients should be managed similar to those with digoxin poisoning. Continuous EKG monitoring is indicated. Diagnostic digoxin radioimmunoassays may cross-react with convallarin and convallamarin, but these tests cannot specifically quantify or rule out exposure. Monoclonal antibody assays will not detect convallarin or convallamarin. The administration of digoxin-specific immune Fab fragments may be beneficial in severe intoxications; however, the required dose is unknown. Digoxin-specific immune Fab fragments have been administered to patients poisoned by oleander and foxglove, both cardiac glycoside-containing plants.

See also: Aminoglycosides; Digitalis Glycosides.

Further Reading

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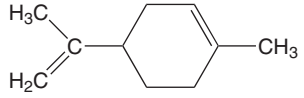
Limonene

Samantha E Gad and Pertti J Hakkinen

- Chemical Name: (*R*)-1-Methyl-4-(1-methylethenyl) cyclohexene
- Chemical Abstracts Service Registry Number: CAS 5989-27-5 (Closely related to D-Limonene are: L-Limonene (CAS 5989-54-8), Chemical Name: (*S*)-1-Methyl-4-(1-methylethenyl) cyclohexene; D,L-Limonene (commonly known as Dipentene) is a mixture of the above two isomers. The isomers are chemically identical except that their molecular structures are mirror images of one another (optical isomers) (CAS 138-86-3), Chemical Name: 1-Methyl-4-(1-methylethenyl) cyclohexene)
- Synonyms: D-Limonene; 4-Isopropenyl-1-methylcyclohexene; (*R*)-(+)-Limonene; (+)-Limonene; D-(+)-Limonene; (D)-Limonene; Limonene; (*R*)-1-Methyl-4-(1-methylethenyl)cyclohexene; D-*p*-Mentha-1,8-diene
- Chemical/Pharmaceutical/Other Class: Terpene, closely related to isoprene
- Chemical Formula: $C_{10}H_{16}$
- Chemical Structure:



Uses

The monoterpene D-limonene is a naturally occurring chemical which is the major component in oil of orange and other natural oils including lemon, grapefruit, berry, leaf, caraway, dill, bergamot, peppermint, and spearmint oils. D-Limonene may be obtained by steam distillation of citrus peels, from pulp resulting

from the production of juice, as cold-pressed oils, and from deterpenation of citrus oils. Any citrus-based cleaning product, air freshener, perfume, essential oil, or fragrance product will likely contain D-limonene.

D-Limonene is also synthetically produced, including as a by-product in the manufacture of terpineol. It is used in flavors and fragrances, as a solvent (e.g., as a natural replacement for petroleum-based solvents in paints and cleaning products), and for numerous other commercial uses. D-Limonene is also used as an inert ingredient in pesticides, and is used in resin manufacture.

Terpenes such as D-limonene have been used as penetration enhancers (also called sorption promoters or accelerants) for improving transdermal drug delivery and work by penetrating into skin to reversibly decrease the barrier resistance.

Commercial mixtures of the limonene molecules can also contain other terpenes, and related compounds such as *p*-cumene.

Exposure Routes and Pathways

Inhalation

D-Limonene may be inhaled when products such as citrus-scented air fresheners, perfumes and candles, cleaners and paints, are used indoors, especially without adequate ventilation. Terpenes are used in products for their solvent properties and, in some cases, for their odor. Limonene is a commonly identified indoor pollutant with time-averaged indoor concentrations in the range of 5–10 ppb, and much higher concentrations (>80 ppb) can occur during the use of limonene-containing products.

Dermal

D-Limonene can be absorbed through the skin after application of citrus oils, perfumes, soaps, and other fragranced personal care products, and through skin contact with citrus-based cleaning products.

Oral

D-Limonene is a compound found in many natural oils, including orange, lemon, grapefruit, berry, leaf, caraway, dill, bergamot, peppermint, and spearmint, and is used as a flavor additive in some foods, beverages, and chewing gum.

Workplace and Occupational Exposures

Inhalation and dermal contact may occur during production, formulation, transport, or use.

Natural Environmental

Exposure to the general population may occur by inhalation due to its presence in the atmosphere as a result of its release from natural sources. Studies have measured levels in both outdoor air and in the indoor air of residences. D-Limonene emissions to the environment have been associated with many plants, for example, wax myrtle, sweet acacia, oranges, tomatoes, grasses, and California western sagebrush.

Environmental Pollution

Found in cigarette mainstream smoke. D-limonene may be released to the environment as a fugitive emission during its production, use, or transport. Limonene has been measured in 'open burning' emissions, and studies have shown that gas phase reactions between ozone and terpenes can be a significant source of secondary organic aerosols. The simultaneous occurrence of ozone and terpenes in an indoor environment is relatively common. Ozone is routinely transported from outdoors to indoors, and indoor sources include photocopiers, electrostatic precipitators, and ozone generators. Limonene has been found as a contaminant in washed and dried shredded polyethylene terephthalate (PET, flake) obtained from curbside collection.

Toxicokinetics

The toxicokinetics of D-limonene were studied in human volunteers exposed by inhalation in an exposure chamber. The relative pulmonary uptake was $\sim 70\%$ of the amount supplied. About 1% of the total uptake was eliminated unchanged in the expired air after the end of exposure, while $\sim 0.003\%$ was eliminated in the urine. A long half-life in blood was observed in the slow elimination phase, which indicates accumulation in adipose tissues.

The major urinary metabolites of D-limonene were identified as perillic acid-8,9-diol in rats and rabbits, perillyl-β-D-glucopyranosiduronic acid in hamsters, *p*-menth-l-ene-8,9-diol in dogs, and 8-hydroxy-*p*-menth-1-ene-9-yl-β-D-glucopyranosiduronic acid in guinea pigs and humans. D-Limonene in male rat kidney is associated with a protein fraction, α-2u-globulin. The major metabolite associated with α-2u-globulin was D-limonene-1, 2-oxide, and parent D-limonene was also identified as a minor component in the α-2u-globulin fraction.

Mechanism of Toxicity

The renal toxicity of D-limonene results from the accumulation of a protein, α-2u-globulin, in male rat

kidney proximal tubule lysosomes. This protein is synthesized exclusively by adult male rats, and the nephrotoxicity of D-limonene in male rats is attributed to its ability to bind to α-2u-globulin. Other species, including humans, synthesize proteins that share significant homology with α-2u-globulin; however, none of these proteins, including the mouse equivalent of α-2u-globulin, can produce this toxicity. This indicates a unique specificity for α-2u-globulin. With chronic exposure to D-limonene, the hyaline droplet nephropathy progresses and the male rat kidneys show tubular cell necrosis, granular cast formation at the corticomedullary junction, and compensatory cell proliferation.

The tumorigenic activity of D-limonene in male rats has been concluded to be nonrelevant to humans because of the (1) male rat specificity of the nephrotoxicity and carcinogenicity, (2) role that α-2u-globulin plays in toxicity, as evidenced by the complete lack of toxicity in other species despite the presence of structurally similar proteins, and (3) lack of genotoxicity of both D-limonene and D-limonene-1,2-oxide, supporting the concept of a nongenotoxic mechanism, that is, sustained renal cell proliferation. Both D-limonene and *cis*-D-limonene-1,2-oxide (the major metabolite involved in this toxicity) are negative in *in vitro* mutagenicity screens. Therefore, the toxicity-related renal cell proliferation is believed to be integrally involved in the carcinogenicity of D-limonene as persistent elevations in renal cell proliferation may increase fixation of spontaneously altered DNA, or serve to promote spontaneously initiated cells.

D-Limonene has chemopreventive and chemotherapeutic activity against many rodent solid tumor types. The chemopreventive activity of limonene during initiation is attributed to the induction of phase I and phase II enzymes, with resulting carcinogen detoxification. The chemopreventive activity of limonene during promotion/progression may be due in part to inhibition of the post-translational isoprenylation of growth-controlling small G proteins, such as p21ras. The complete regression of mammary carcinomas by limonene appears to involve tissue redifferentiation. The multiple antitumorigenic effects of limonene are attainable at a high therapeutic ratio, suggesting that limonene and related monoterpenes may be efficacious in the chemoprevention and chemotherapy of human malignancies.

The dermal allergenic potential of D-limonene oxidation products has been studied. Air-exposed D-limonene was a strong sensitizer in guinea-pig studies and oxidation of D-limonene is necessary for its sensitizing potential, producing potent allergens such as limonene oxide and carvone.

Acute and Short-Term Toxicity (or Exposure)

Animal

The mouse oral LD_{50} is $5600\,mg^{-1}$ (males) and $6600\,mg\,kg^{-1}$ (females), and the rat oral LD_{50} is $4400\,mg\,kg^{-1}$ (males) and $5200\,mg\,kg^{-1}$ (females). The rabbit dermal LD_{50} is greater than $5000\,mg\,kg^{-1}$. Application of undiluted D-limonene, under a covering, to intact or abraded rabbit skin caused moderate irritation after 24 h; in other studies, application of 0.5 ml of D-limonene caused mild to moderate irritation.

Abnormalities in bone formation were seen in offspring of rats fed 591–2869 $mg\,kg^{-1}\,day^{-1}$ and mice fed 591–2363 $mg\,kg^{-1}\,day^{-1}$ of D-limonene, respectively, over several days during pregnancy. Maternal toxicity was seen at high doses. Teratogenic effects were not observed in offspring of rabbits fed 250–1000 $mg\,kg^{-1}\,day^{-1}$ for several days during pregnancy. The high dose was toxic to the females. Repeated administration of high concentrations of D-limonene adversely affected female reproductive organs in rats and male reproductive organs in dogs.

As discussed above, D-limonene causes nephropathy in male rats characterized by hyaline droplet formation with degenerative intracellular changes.

Strong upper airway irritants can be found in reaction mixtures of limonene, other terpenes, and ozone. The identified products included aldehydes, ketones, and carboxylic acids. These identified chemicals and some unidentified reaction products have been studied in upper airway irritation studies in mice, with reduction of the respiratory rate as a key end point.

Limonene and essential oils containing limonene have been tested for anti-inflammatory activity in the mouse model of pleurisy induced by zymosan and lipopolysaccharide (LPS), and assayed for immunoregulatory activity by measurement of the inhibition of nitric oxide (NO) and production of the cytokines, interferon-γ and IL-4. Limonene and the oils, when administered orally, were able to inhibit the LPS-induced inflammation including cell migration. Limonene was also effective in inhibiting production of NO, and produced a significant inhibition of interferon-γ and IL-4 production.

Inhibition of cholesterol biosynthesis occurred in the small intestine of rats after administration of D-limonene for 7 days, but no significant effect on the secretion of radiolabeled cholesterol into bile and feces was observed. D-Limonene increased the perfusion pressure of the sphincter of Oddi in dogs when injected IV or directly into the common bile duct.

Human

Limonene is a mild skin and eye irritant. Ingestion of 20 g of D-limonene caused diarrhea and a temporary increase in protein in the urine (proteinurea) in five male volunteers. These data, in addition to the low acute toxicity in animal tests, suggest that D-limonene is not very toxic by ingestion.

Air levels of D-limonene may irritate the eyes and airways of some people, especially when the levels build up indoors (see above for discussion about gas phase reactions between ozone and terpenes which can be a significant source of secondary organic aerosols).

D-Limonene has been used successfully for the postoperative dissolution of retained cholesterol gallstones.

Chronic Toxicity (or Exposure)

Animal

D-Limonene causes skin sensitization; however, see sections on Mechanism of Toxicity and Chronic Toxicity – Human for discussion and data useful for assessing human sensitization risks.

In 2 year gavage studies, there was clear evidence of carcinogenic activity of D-limonene for male rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney. There was no evidence of carcinogenic activity of D-limonene for female rats. There was no evidence of carcinogenic activity of D-limonene for male or female mice. The nephrotoxicity of D-limonene was studied in rats and mice. Kidney sections taken from male rats that had been part of a 91 day oral dosing study of limonene in rats and mice were examined by light microscopy. The study showed that renal alterations were induced only in male rats. As discussed above, the mechanism by which D-limonene caused the tumors in male rats is irrelevant to humans.

Two studies have been reported where a dog and cats were exposed dermally to insecticidal dips containing 78.2% D-limonene. Severe dermatitis was observed in the dog and, at five and ten times the recommended dose, excess salivation, incoordination, and muscle tremors (signs typical of organophosphate poisoning) were observed in the cats. The effects were reversible within 5 h; in the absence of complete ingredient information, these effects cannot be completely attributed to D-limonene.

The effects of D-limonene and citrus oils on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced neoplasia of the lungs and forestomach of female A/J mice has been investigated. D-Limonene and the citrus fruit oils given orally 1 h prior to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, also administered orally, inhibited pulmonary adenoma formation and the occurrence of forestomach tumors. In an additional experiment, D-limonene given orally 1 h prior to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone administered IP again showed pronounced inhibition of pulmonary adenoma formation. The anticarcinogenic effects of monocyclic monoterpenes such as limonene have also been demonstrated when given during the initiation phase of 7,12-dimethylbenz(*a*)anthracene-induced mammary cancer in rats. The regression of rat mammary tumors caused by limonene has been associated with a marked increase in the mannose-6-phosphate (M6P)/insulin-like growth factor II receptor (M6P/IGF-II receptor) and TGF-β level. TGF-β becomes active when it is released from its latent complex mediated by the M6P/IGF-II receptor.

Human

Low levels of D-limonene in the diet have not been reported to cause adverse effects in humans. D-Limonene can be a dermal sensitizer (see above for discussion about D-limonene oxidation products); however, an 8% solution of D-limonene in petrolatum did not cause an allergic skin response in any of 25 volunteers tested. D-limonene is also a recommended 'quencher' in that it can decrease the sensitizing effect of cinnamic aldehyde when used at a 1:1 ratio with cinnamic aldehyde (International Fragrance Association, that is, IFRA, guidelines developed and used by the fragrance and consumer product industries). There is inadequate evidence in humans for the carcinogenicity of D-limonene, and the overall conclusion by experts is that, as discussed above, D-limonene produces renal tubular tumors in male rats by a non-DNA reactive α-2-globulin-associated response. Therefore, the mechanism by which D-limonene increased the incidence of renal tubular tumors in male rats is not relevant to humans.

In Vitro Toxicity Data

D-Limonene was not mutagenic in four strains of *Salmonella*, did not significantly increase the number of trifluorothymidine-resistant cells in the mouse L5178Y/TK$^{+/-}$ assay, and did not induce chromosomal aberrations or sister chromatid exchanges in cultured CHO cells. All assays were conducted in the presence and absence of exogenous metabolic activation.

Limonene has been among the known chemopreventive substances tested as a reference compound in a battery of cell- and enzyme-based *in vitro* marker systems relevant for prevention of carcinogenesis

in vivo. This battery of tests included systems assessing modulation of drug metabolism, determination of radical scavenging and antioxidant effects, anti-inflammatory mechanisms, and antitumor-promoting activities (e.g., inhibition of phorbol ester-induced ornithine decarboxylase (ODC) activity in murine keratinocytes).

Monoterpenes such as limonene are effective in both preventing and treating a large variety of organ-specific rodent cancers. Based on this, clinical testing of the monoterpene-perillyl alcohol (POH) has been conducted in advanced cancer patients. Mechanistically, similar cellular and molecular mechanisms form the basis of both the cancer-preventive and therapeutic activities of the monoterpenes. These include the inhibition of proliferation and the induction of apoptosis, and the effects are confined to premalignant and malignant tissue and do not affect normal tissue.

Clinical Management

Mild irritation and skin sensitization may occur from dermal exposures. Hematuria and albuminuria might occur from ingestion of large amounts, and other symptoms following limonene ingestion could include a burning pain in the mouth and throat, abdominal pain, nausea, vomiting, diarrhea, transient excitement, ataxia, delirium, stupor, coughing, choking, dyspnea, cyanosis, fever, and tachycardia. In addition, pulmonary edema and pneumonitis may occur with limonene aspiration or systemic absorption, and dizziness and suffocation may be observed following limonene inhalation.

Milk should be given to mitigate gastric irritation for acute ingestions. Fluids should be given to maintain maximum urinary output to expedite elimination of limonene from the body. For inhalation exposures, patients should be moved to fresh air and monitored for respiratory distress. Exposed eyes should be treated with copious amounts of room temperature water. For skin exposures, any contaminated clothing should be removed and the exposed skin washed thoroughly with soap and water.

Environmental Fate

Terrestrial

D-Limonene is expected to have low to slight mobility in soil. It will rapidly volatilize from both dry and moist soil to the atmosphere.

Aquatic

It may bioconcentrate in fish and aquatic organisms, and it may significantly adsorb to sediment and suspended organic matter. It is expected to rapidly volatilize from water to the atmosphere.

Atmospheric

If released to the atmosphere, D-limonene is expected to rapidly undergo gas-phase oxidation reactions with photochemically produced hydroxyl radicals, ozone and, at night, with nitrate radicals. Limonene can react with ozone, forming submicron particulates that could impact asthmatics and those with other respiratory ailments.

Ecotoxicology

Calculated bioconcentration factors based on the water solubility of D-limonene and its estimated log octanol/water partition coefficient indicate that D-limonene may bioconcentrate in fish and aquatic organisms. In addition, D-limonene has been studied for general lethality as well as neurotoxic effects in earthworms. Generally, the chronic and acute intoxication of earthworms involved a rapid and predictable cascade of behavioral and morphologic symptoms. Chronic D-limonene exposures induced significant weight loss, but there was no effect on median giant nerve fiber and lateral giant nerve fiber conduction velocities. Acute exposures, however, induced significant decreases in conduction velocity that were reversible once D-limonene exposure ceased.

Exposure Standards and Guidelines

D-Limonene is regulated as an air pollutant in Florida when released from citrus processing plants, because it can combine with NO to form ozone, contributing to smog, on hot days. The legislation establishes that 65% of the citrus oil (D-limonene) be captured for all plants. Most plants now capture between 40% and 45% of the oil.

A time-weighted average (TWA) or other occupational exposure limit is unavailable for D-limonene. The Workplace Environmental Exposure Level (WEEL) for limonene (terpenes) is 30 ppm as an 8 h TWA.

See the discussion above about International Fragrance Association (IFRA) guidelines that D-limonene is a recommended 'quencher' in that it can decrease the sensitizing effect of cinnamic aldehyde when used at a 1:1 ratio with cinnamic aldehyde. IFRA guidelines also state that limonene and natural products containing substantial amounts of it should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production. Such

products should have a peroxide value of less than 20 mmol peroxide per liter. The entire set of IFRA guidance and restrictions should be consulted by developers and users of perfume materials, and by interested toxicologists, physicians, and others.

See also: Carcinogen Classification Schemes; Carcinogenesis; Fragrances and Perfumes; International Fragrance Association (IFRA); Risk Assessment, Human Health; Toxicity Testing, Sensitization.

Further Reading

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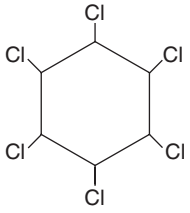
Lindane

Benny L Blaylock

- Chemical Abstracts Service Registry Number: CAS 58-89-9
- Synonyms: 1,2,3,4,5,6-Hexachlorocyclohexane; Agrocide; Ambrocide; Aparasin; Aphitiria; Benesan; Benexane; Benhexachlor; Benzene hexachloride; BHC; BoreKil; Borer-Tox; Exagama; Gallogama; Gamaphex; Gamma-BHC (γ-BHC); γ-Col; γ-HCH; Gammex; Gammexane; Gamasan; Gexane; Hexachlorocyclohexane; HCH; Isotox; Jacutin; Kwell; Lindafor; Lindagronox; Lindaterra; Lindatox; Lintox; Lorexane; New Kotol; Noviagam; Quellada; Steward; Streunex; Tri-6 (BHC stands for benzene hexachloride, which has been used historically but erroneously. This should never be confused with hexachlorobenzene or HCB)
- Chemical/Pharmaceutical/Other Class: Organochlorine insecticide
- Chemical Formula: $C_6H_6Cl_6$
- Chemical Structure:



Uses

Commercially, lindane is used as an insecticide although its use is restricted. Therapeutically, it is used to treat human lice and mite infestations.

Exposure Routes and Pathways

Lindane is readily absorbed from the gastrointestinal tract, the respiratory tract, and skin. The most important human exposure routes are oral and dermal.

Toxicokinetics

Lindane is absorbed from the gastrointestinal tract, the respiratory tract, and skin. The metabolism of lindane is complex and involves a number of pathways depending on which isomer of hexachlorocyclohexane (HCH) is involved (lindane is the gamma (γ) isomer). It is nonetheless rapid. Lindane is metabolized in the liver by microsomal enzymes. The main pathways include stepwise elimination of chlorines to form tri- and tetrachlorophenols and conjugation with sulfates or glucuronides and subsequent elimination. Other metabolic pathways involve the production of mercapturates. These water-soluble products are eliminated in the urine. Lindane is bound by serum proteins in the blood. Storage is in adipose tissue and other

fat-containing tissues. The γ isomer is stored in fat at a much higher rate than the other isomers.

Mechanism of Toxicity

The main site of action of lindane, unlike that of DDT, appears to be at the synapse with both excitatory and inhibitory effects. The effect of inhibition of Na^+,K^+-ATPases, which is slightly greater than on Mg^{2+}-ATPase, is related to cation transport in nerve axons, perhaps to Ca^{2+} extrusion and therefore to the toxic action of the compound. These effects occur at concentrations of lindane greater than required for antagonism of the $GABA_A$ receptor–chloride channel complex at the picrotoxin-binding site. The resulting accumulation of intracellular free calcium ions promotes calcium-induced release of neurotransmitters from storage vesicles and the subsequent depolarization of adjacent neurons and the propagation of stimuli throughout the central nervous system (CNS).

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the acute oral LD_{50} for lindane is 88–190 mg kg^{-1} while for mice it is 59–562 mg kg^{-1}. It is also moderately toxic via the dermal route, with reported dermal LD_{50} values of 500–1000 mg kg^{-1} in rats. The effects on the nervous system are similar to that of DDT. There are also reports of liver, kidney, and immune system toxicity.

Human

Most instances of lindane poisoning following therapeutic use to control scabies or lice have involved gross misuse of the insecticide. Acute toxicity includes CNS stimulation (usually developing within 1 h), mental/motor impairment, excitation, convulsions, increased respiratory rate and/or failure, pulmonary edema, and dermatitis. Nausea and vomiting were common.

Pulmonary edema has been reported after lindane powder was aspirated into the lungs. An acute dermal poisoning of a 2-month-old infant exposed to a whole body application of 1% γ-HCH lotion resulted in death.

Chronic Toxicity (or Exposure)

Animal

Doses of 2.6–5 mg kg^{-1} day^{-1} have been reported to cause convulsions and liver lesions in rats. Reproductive toxicity includes a report in rats that doses as low as 0.5 mg kg^{-1} day^{-1} over 4 months caused observable disturbances in the rat estrus cycle, lengthened gestation time, decreased fecundity, and increased fetal mortality.

Human

Aplastic anemia was documented in a man who applied γ-HCH to his skin for 3 weeks for treatment of scabies. After 1–30 years of exposure, 60 male workers in a factory producing lindane showed no signs of neurological impairment.

International Agency for Research on Cancer lists HCHs as 2B (possibly carcinogenic for humans).

Clinical Management

Management of lindane poisoning is symptomatic. Diazepam or phenobarbital is used to control convulsions. Cholestyramine or activated charcoal has been utilized to inhibit lindane uptake after ingestion. In more severe poisonings, the serum levels of lindane may be lowered by hemoperfusion over Amberlite XAD-4.

Environmental Fate

Lindane has been shown to have a low soil binding affinity. Therefore, it may be mobile in soils with especially low organic matter content or subject to high rainfall and pose a risk of groundwater contamination. Lindane is highly persistent in most soils, with a field half-life of $\sim$15 months.

In both fresh and salt waters, lindane has demonstrated high stability. It is resistant to photodegradation but will disappear from the water by secondary mechanisms such as adsorption on sediment, biological breakdown by microflora and fauna, and adsorption by fish through gills, skin, and food.

Ecotoxicology

The toxicity of lindane to bird species is very low to practically nontoxic. Eggshell thinning and reduced egg production has occurred in birds exposed to lindane.

Lindane is highly to very highly toxic to fish and aquatic invertebrate species. Ninety-six-hour LC_{50} values have been reported to range from 1.7 to 90 μg l^{-1} in trout (rainbow, brown, and lake), Coho salmon, carp, fathead minnow, bluegill, largemouth bass, and yellow perch. The bioconcentration factor for the compound is 1400 times ambient water concentrations, indicating significant bioaccumulation.

Other Hazards

Lindane is corrosive to metals. Lindane itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes. At 102°C in steam, 0.13% hydrogen chloride was produced.

Exposure Standards and Guidelines

Acceptable daily intake	$0.008\,mg\,kg^{-1}\,day^{-1}$
Maximum Contaminant Level	$0.0002\,mg\,l^{-1}$
Reference dose	$0.0003\,mg\,kg^{-1}\,day^{-1}$
Permissible exposure limit	$0.5\,mg\,m^{-3}$ (8 h)

See also: Carcinogen Classification Schemes; Diazepam; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Organochlorine Insecticides.

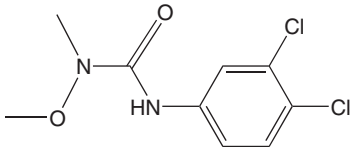
Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Lindane.
http://extoxnet.orst.edu – Extension Toxicology Network, a collaborative effort of University of California, Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Lindane.
http://www.osha-slc.gov – US Department of Labor, Occupational Safety and Health Administration.

Linuron

Guangping Chen

- Chemical Name: 3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea
- Chemical Abstracts Service Registry Number: CAS 330-55-2
- Synonyms: Afalon; Garnitan; Linex; Linorox; Linurex; Lorox; Premalin; Sarclex; Sinuron; *N*′-(3,4-Dichlorophenyl)-*N*-methoxy-*N*-methyl urea
- Chemical/Pharmaceutical/Other Class: Phenylurea herbicide
- Chemical Formula: $C_9H_{10}Cl_2N_2O_2$
- Chemical Structure:



Uses

Linuron is a substituted urea. It is an herbicide used to control germinating and emerging annual and perennial broadleaf and grassy weeds on both crop and noncrop sites. Linuron inhibits photosynthesis in target weed plants. It is labeled for field and storehouse use in a variety of crops. Most of the linuron applied in the United States is in soybean production. Formulations include granules, wettable powders, flowable concentrates, and emulsifiable concentrates/liquid suspensions. Direct application to water is prohibited.

Exposure Routes and Pathways

Oral exposure is the primary route from dietary sources.

Toxicokinetics

Linuron is rapidly absorbed with oral dosing. Linuron is efficiently metabolized in rat liver and does not accumulate in mammalian systems. With intravenous dosing, linuron distributes quickly and widely to peripheral tissues with rapid elimination. Primary metabolites were identified as *N*′-(3,4-dichlorophenyl)-*N*-methoxyurea, *N*′-(3,4-dichlorophenyl) urea, and *N*′-(6-hydroxy-3,4-dichlorophenyl) urea. Linuron is a liver enzyme inducer in rats.

Mechanism of Toxicity

As a reproductive and developmental toxicant, linuron works via androgen receptor antagonist activity, that is, it competes with testosterone for binding to the androgen receptor.

Acute and Short-Term Toxicity (or Exposure)

Animal

Linuron is slightly toxic by dermal, oral, or inhalation route of exposure (US Environmental Protection Agency Toxicity Category III).

Human

Linuron has little acute toxicity potential except for skin, eye, and respiratory tract irritations.

Chronic Toxicity (or Exposure)

Animal

In chronic toxicity studies with beagle dogs, linuron caused red blood cell destruction and changes in liver weight. Testicular tumors, red blood cell damage, and growth retardation were noted in long-term studies using rats. Statistically significant increases in liver tumors, reductions in body weight, and increased liver weights were noted in mice with chronic bioassays. Linuron interfered with the transmission of male hormones in a reproductive toxicity study. Linuron exposure induced malformations of the epididymis and the vas deferens. Developmental toxicity was selective to males.

Human

Relatively little is known of the long-term effects of linuron in humans. Linuron is classified in the United States as a possible (group C) human carcinogen.

In Vitro Toxicity Data

In mouse tissues, linuron competitively blocked transcription through androgen receptor induced by dihydrotestosterone in a concentration-dependent manner. Linuron is not mutagenic.

Environmental Fate

Linuron is moderately persistent (half-life ranging from 57 to 100 days) and relatively immobile. Increased mobility may occur in coarse soils and soils with low organic content. Linuron is effectively degraded by biotic processing including microbial degradation. Processes of adsorption and microbial degradation limit its migration to ground water. Runoff to surface waters can occur. Linuron has been detected in ground water in Georgia, Missouri, Virginia, and Wisconsin.

Ecotoxicology

In acute studies, linuron was slightly to moderately toxic to cold and warm water fish.

Technical linuron is highly toxic to aquatic invertebrates, while the formulated product is less toxic. Linuron was highly toxic to sheepshead minnow and moderately toxic to eastern oyster and mysid shrimp. Linuron is practically nontoxic to honeybees. Reproductive deficits have been noted in birds treated with linuron.

Exposure Standards and Guidelines

The US reference dose for linuron is $0.008\,\mathrm{mg\,kg^{-1}\,day^{-1}}$. No acceptable daily intake has been established.

See also: Diuron; Pesticides.

Further Reading

Anfossi P, Roncada P, Stracciari GL, *et al.* (1993) Toxicokinetics and metabolism of linuron in rabbit: *In vivo* and *in vitro* studies. *Xenobiotica* 23(10): 1113–1123.

Scassellati-Sforzolini G, Moretti M, Villarini M, *et al.* (1994) *In vivo* studies on enzymatic induction activity of linuron. *Journal of Environmental Pathology, Toxicology and Oncology* 13(1): 11–17.

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Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

http://www.epa.gov – US Environmental Protection Agency.

Liothyronine

F Lee Cantrell

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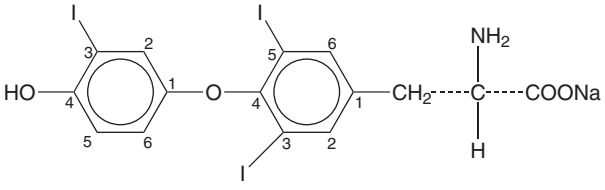
- Chemical Abstracts Service Registry Number: CAS 6893-02-3
- Synonyms: L-Triiodothyronine; T3; Cytomel; Triothyrone
- Chemical/Pharmaceutical/Other Class: Synthetic form of triiodothyronine
- Chemical Formula: $C_{15}H_{12}I_3NO_4$
- Chemical Structure:



Uses

Liothyronine is used in the treatment of hypothyroidism, nontoxic goiter, cretinism, and myxedema.

Exposure Routes and Pathways

Ingestion is the most common route of exposure for liothyronine, although a parenteral form is available.

Toxicokinetics

Liothyronine (T3) is 88% absorbed. Congestive heart failure can reduce absorption by half. T3 is not firmly bound to serum protein. It has a volume of distribution in the range of 41–45 l kg^{-1}. T3 is metabolized to deiodinated and conjugate metabolites. From 75% to 85% of T3 is deiodinated. Conjugation takes place in the kidneys. Approximately 20% of T3 is excreted in the feces and up to 10% in the urine. T3 has a half-life of 2.5 days.

Mechanism of Toxicity

The exact mechanism of action is not well understood. Thyroid hormones are needed for metabolism, growth, and development. Most organ systems are affected by thyroid hormones. Thyroid hormones cause an increase in the basal metabolic rate, in oxygen consumption, and in the metabolism of carbohydrates. T3 increases aerobic mitochondrial function causing an increased rate of utilization of high-energy phosphates. This stimulates myosin adenosine triphosphatase and reduces tissue acetic acid. Toxicity is an extension of the pharmacologic effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs and cats are at risk for thyroid toxicity. Signs of toxicity include vomiting, diarrhea, tachycardia, tachypnea, decreased level of consciousness, and restlessness. Toxic doses have not been established.

Human

Ingestion of small amounts of T3-containing products is unlikely to result in significant toxicity. Symptoms of toxicity typically occur 4–12 h following large overdoses and include tachycardia, nausea, vomiting, diarrhea, restlessness, and fever.

Chronic Toxicity (or Exposure)

Human

Thyrotoxicosis is a concern with chronic exposure. Thyrotoxicosis is characterized by tachycardia, cardiac arrhythmias, hypertension, tremors, and seizures. In severe cases, coma and cardiovascular collapse can result.

Clinical Management

Most patients after acute overdose can be managed on an outpatient basis. Gastric decontamination may be considered in patients presenting early after large ingestions. The absence of clinical effects within the first 24 h does not preclude the later development of significant toxicity. In patients manifesting toxicity, cardiac and blood pressure monitoring should be performed. Cooling methods should be employed to decrease hyperpyrexia. Intravenous fluids should be administered in dehydrated and/or hypotensive patients. Adrenergic hyperactivity can be treated with propranolol. Propylthiouracil may be administered to decrease conversion of T4 to T3. Forced diuresis and extracorporeal methods are not effective in levothyroxine overdose.

See also: Endocrine System; Levothyroxine; Thyroid Extract.

Further Reading

Dahlberg PA, Karlsson FA, and Wide L (1979) Triiodothyronine intoxication. *Lancet* 2(8144): 700.

Gorman RL, Chamberlain JM, and Rose SR (1988) High anxiety-low toxicity: A massive T4 ingestion. *Pediatrics* 82: 666–669.

Lipid Peroxidation

Zhengwei Cai

The word 'lipid' is referred to a chemically heterogeneous group of substances having in common the property of insolubility in water, but solubility in nonaqueous solvents such as chloroform, hydrocarbons, or alcohols. Numerous compounds, which may have little or no chemical relationship between each other, are classified as lipids. Vegetable oil and animal fat in daily life, fatty acids, triacylglycerols, phospholipids, and sterols are good examples of lipids. It has been known since antiquity that at high temperature or under light exposure fats and oils in storage may develop unpleasant tastes and odors. This is a typical characteristic of rancidity of lipids and the consequence of lipid peroxidation.

What is Lipid Peroxidation?

Lipid peroxidation is an oxidative chain reaction in which one lipid molecule after another becomes oxidized to the maximum possible extent or so as to form a lipid peroxide (i.e., a lipid molecule containing one or more O–O bonds). At high temperatures, lipid peroxides decompose to produce a range of unpleasant-tasting and foul-smelling products such as epoxides, ketones, acids, and aldehydes. Most biological membranes are extended bilayers of amphiphilic lipids with hydrophobic moieties directed to the center and hydrophilic head groups at the two surfaces. Biological cell membranes are packed with polyunsaturated fatty acids (PUFAs), such as arachidonic and docosahexaenoic acid, in either the isolated form or the incorporated form in triacylglycerides and phospholipids. PUFAs are particularly susceptible to peroxidation. With increasing concerns about the potential adverse effects of lipid peroxidation in cellular membranes, the relevance of lipid peroxidation to biology and human diseases has been extensively explored since the 1950s.

Mechanism of Lipid Peroxidation

Lipid peroxidation is a free radical-initiated chain oxidation of lipids. Electrons in atoms occupy regions of space known as orbitals. Each orbital can hold a maximum of two electrons. A free radical is defined as any chemical species possessing one or more unpaired electrons and capable of independent existence. Hydroxyl ($OH^{\bullet}$) and superoxide ($O_2^{-\bullet}$) are examples of oxygen-centered radicals. There are also other types of radicals such as thiyl ($RS^{\bullet}$), trichloromethyl ($CCl_3^{\bullet}$), and nitric oxide ($NO^{\bullet}$). A free radical is marked by a dot, which designates the presence of one or more unpaired electrons.

Lipid peroxidation can be divided into three separate processes – initiation, propagation, and termination. During initiation a very small number of radicals (e.g., transition metal ions or a radical generated by photolysis or high-energy irradiation) can abstract hydrogen from lipid molecules to yield free radicals of lipids

$$X^{\bullet} + RH \rightarrow R^{\bullet} + XH$$

Propagation then allows a reaction with molecular oxygen to form lipid peroxyl radicals

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

This peroxide radical can then react with the original substrate, yielding one hydroperoxide and one new radical

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$

Thus, the events form the basis of a chain-reaction process. The lipid hydroperoxide decomposition produces more radicals and noxious aldehydes

$$ROOH \rightarrow RO^{\bullet}, ROO^{\bullet}, \text{aldehydes}$$

When the substrate is depleted, termination reaction begins. Two radicals combine the unpaired electrons to form a nonradical product

$$R^{\bullet} + R^{\bullet} \rightarrow R{-}R$$

The chain reactions are also terminated when antioxidants (A–H), which provide easily donatable hydrogen for abstraction by peroxyl radicals, combine

with lipid radicals to halt further propagation

$$A{-}H + ROO^{\bullet} \rightarrow ROOH + A^{\bullet}$$

The antioxidant-derived radical ($A^{\bullet}$) could be dimerized harmlessly into A_2, or it could be converted back to A–H by reaction with another molecule; it might also react with another $ROO^{\bullet}$ to become a nonradical. The most important chain-breaking antioxidant in human lipids is α-tocopherol

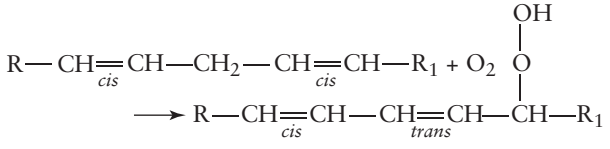
$$ROO^{\bullet}\,(\text{lipid peroxyl radical}) + \alpha\text{-tocopherol}{-}OH \rightarrow ROOH + \alpha\text{-tocopherol}{-}O^{\bullet}$$

The resultant tocopheroxyl radical is relatively stable and, in normal circumstances, insufficiently reactive to initiate lipid peroxidation itself. It has been demonstrated *in vitro* that α-tocopherol radical can be converted back to α-tocopherol by reduction with ascorbic acid at the surface of biological membranes

$$A^{\bullet} + \text{ascorbic acid} \rightarrow \text{ascorbic acid}^{\bullet} + A{-}H$$

However, it is uncertain that this reaction actually happens *in vivo*.

There are two types of lipid peroxidation: chemical (nonenzymic) peroxidation and enzyme-catalyzed peroxidation. The nonenzymic peroxidation has been described above. In the enzyme-catalyzed peroxidation, lipoxidases (or lipoxygenases), which are present in both plants and animals, catalyzed the following reaction:



That is, the addition of molecular oxygen to a 1,4-*cis,cis*-pentadiene moiety to produce a 1-hydroperoxy-2,4-*trans,cis*-pentadiene unit. The product of the enzymic reaction, a hydroperoxide, is similar to the products of purely chemical reaction, but the lipoxidase reaction has a number of distinguishing features. The activation energy is smaller than that for the chemical reaction, and the enzyme has very specific substrate requirements. To be a substrate, the fatty acid must contain at least two *cis* double bonds interrupted by a methylene group. Thus, linoleic and α-linolenic acids are good substrates for the plant enzymes while arachidonic acid, the major PUFA in mammals, is the target for the animal lipoxygenases in their tissues when it is released from complex lipids.

Reactive Oxygen Species (ROS) that Initiate Lipid Peroxidation in Cells

Initiation of lipid peroxidation is still not fully understood. Its promotion by oxygen, singlet oxygen, hydroxyl radical, superoxide anion, or some form of perferryl ion has been proposed. High-energy irradiation of aqueous solutions produces highly reactive hydroxyl radicals ($OH^{\bullet}$) that can attack all biological molecules, including membrane lipids. This is probably a mechanism accounting for initiation of lipid peroxidation in irradiated organisms. With the exception of such an unusual circumstance, ROS that initiate lipid peroxidation in cells are produced during normal metabolism and generally produced by electron transfer reactions.

Superoxide free radical ($O_2^{-\bullet}$) and hydrogen peroxide are continuously produced *in vivo*. In normal circumstances, electron 'leakage' from electron transport chains, such as those in mitochondria and the endoplasmic reticulum, to molecular oxygen can generate the superoxidation radical

$$O_2 + e^{-} \rightarrow O_2^{-\bullet}$$

Superoxide can also be produced by other enzymes, such as the range of flavin oxidases located in peroxisomes, and by oxidation of certain compounds including ascorbic acid, thiols, and adrenaline in the presence of transition metal ions. The autoxidation of reduced transition metal can also generate the superoxide

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{-\bullet}$$

$$Cu^{+} + O_2 \rightarrow Cu^{2+} + O_2^{-\bullet}$$

Hydrogen peroxide is often produced in biological systems via the generation of superoxide: two superoxide molecules can react together to form hydrogen peroxide and oxygen

$$2O_2^{-\bullet} + 2H^{+} \rightarrow H_2O_2 + O_2$$

Although superoxide is a free radical, it is not a particularly damaging species and it does not appear to be capable of initiating lipid peroxidation. Its major significance is as a source of hydrogen peroxide and as a reductant of transition metal ion. Hydrogen peroxide is not a free radical but falls into the category of ROS. It is a source of hydroxyl radicals. In the presence of reactive transition metal ions, hydrogen peroxide can rather easily break down to produce the hydroxyl radical, the most reactive and damaging oxygen free radical that will attack most biological molecules and

initiate lipid peroxidation at diffusion-controlled rates

$$H_2O_2 + Fe^{2+} \rightarrow OH^{\bullet} + OH^{-} + Fe^{3+}$$

In summary, the ROS that initiate lipid peroxidation include oxygen itself, superoxide, hydrogen peroxide, transition metal ions, and the hydroxyl radical. These ROS are normally produced during metabolism and in the absence of adequate defense mechanisms ROS can attack DNA, proteins, and lipids in the body. However, ROS are not always harmful. For example, they are involved in the destruction of pathogens by phagocytes.

Biological Effects of Lipid Peroxidation

Although ROS is continuously produced during normal metabolism, the integrated antioxidant systems in the body prevent lipid peroxidation. In the absence of adequate defense mechanisms, lipid peroxidation can directly damage the structure of the membrane. The occurrence of lipid peroxidation in biological membranes causes severe impairment of membrane functioning, changes in membrane fluidity, inactivation of membrane-bound receptors and enzymes, and increased nonspecific permeability to ions such as Ca^{2+}. Disruption of cellular membrane structure may further cause antioxidant systems to be ineffective. In addition, decomposition of lipid hydroperoxides produces hydrocarbon gases (such as ethane and pentane), more radical species, and cytotoxic aldehydes. Thus, lipid peroxidation can indirectly damage other cell components by these products of its decomposition.

Lipid peroxidation has been implicated in a wide range of tissue injuries, diseases, and even in the aging process. The liver toxicity of carbon tetrachloride (CCl_4) is a classic example of the destructive effects of lipid peroxidation. A very small portion of administered carbon tetrachloride is metabolized into trichloromethyl free radical ($CCl_3^{\bullet}$) by the action of cytochrome P450 in the liver. This radical reacts rapidly with oxygen and gives rise to the trichloromethylperoxyl radical

$$CCl_3^{\bullet} + O_2 \rightarrow CCl_3O_2^{\bullet}$$

This trichloromethylperoxyl radical can efficiently abstract hydrogen atoms from lipids and initiate lipid peroxidation

$$R{-}H + CCl_3O_2^{\bullet} \rightarrow R^{\bullet} + CCl_3O_2H$$

Eventually these reactions result in the oxidative destruction of cellular membrane and serious tissue damage in the liver even though $<0.5\%$ of CCl_4 is ever metabolized. An essential involvement of lipid peroxidation in the events leading to death of hepatocytes has been proved in the acute intoxication as well as with other haloalkanes such as bromotrichloromethane ($CBrCl_3$), dibromoethane, and halothane. Lipid peroxidation is also involved in the hepatotoxicity of ethanol, allyl alcohol, and some drugs like adriamycin.

Atherosclerosis is an irregular thickening of the inner wall of the artery that reduces the size of artery lumen, particularly near junctions in the arterial tree. Atherosclerosis involves the buildup of deposits in arterial walls, characterized by high concentrations of lipids that derive from plasma lipoprotein. Lipids are also involved in the formation of thrombi that may lead to the blockage of blood vessels narrowed by atherosclerosis. It is currently believed that lipid peroxidation is involved in the pathogenesis of atherosclerosis through oxidative modification of low-density lipoprotein (LDL) and peroxidation of the apoB100. The oxidized LDL has reduced affinity for the LDL-receptor, and instead become ligands for the family of scavenger receptors. Therefore, the macrophages may engulf large amounts of lipid in an uncontrolled manner with the development of foam cells and the initiation of the atherosclerotic lesion. The oxidized LDL deposited in the arterial wall may continuously release highly cytotoxic lipid peroxidation products, such as certain aldehydes, irritating the endothelial cell layer and causing a range of other effects that may contribute toward the development of the lesion.

Lipid peroxidation has also been suggested to be involved in the pathogenesis of several lung diseases and injuries. One of the important events for several lung diseases is arachidonic acid (AA) release induced by lipid peroxidation and metabolism of released AA to active products. Hydroperoxides have been shown to induce lipid peroxidation in the isolated perfused lung, which could lead to a perturbed plasma membrane and the activation of phospholipase A_2 (PLA_2). As a result of the activation of PLA_2, an excessive amount AA is released. The released AA and its metabolites of eicosanoids, such as prostaglandins, prostacyclin, thromboxane, and leukotriene, can lead to vasoconstriction and brochoconstriction as well as the development of edema.

Recent researches have indicated that lipid peroxidation is involved in the pathogenesis of other human diseases such as hypoxic-ischemic reperfusion injury, cancers, Alzheimer's disease, rheumatoid arthritis, renal dysfunction, and diabetes mellitus.

However, it must be pointed out that in most cases increase in the bulk peroxidation of cell membrane lipids is not the cause of cell damage, but a consequence of cell damage. Rises in intracellular Ca^{2+}, protein damage, and DNA damage are more important events in causing cell injury than is the bulk peroxidation of membrane lipids. Lipid peroxidation is often a late event, accompanying rather than causing final cell death and often occurring after cell death, leading to putrefaction and added generation of products such as ethane.

Defenses against Lipid Peroxidation

Because lipid peroxidation can be very damaging, organisms have evolved antioxidant defense systems to protect against it and also repair the system to prevent the accumulation of oxidatively damaged molecules. The antioxidant defenses consist of two categories: those preventing the generation of free radicals and those intercepting the generated radicals. The preventive defenses include efficiency of electron transfer (i.e., no 'leakage' of electrons from the respiratory chain) and sequestration of transition metal ions. Iron, for example, is held tightly bound to special proteins such as transferrin and ferrin. Another type of preventive antioxidant defense is the removal of peroxides that react with transition metal ions to form reactive free radicals. Catalase and glutathione peroxidase are examples of this type of defense. Catalase is mainly located in peroxisomes and acts with hydrogen peroxide; glutathione peroxidase is found in the cytosol of most cells and is active toward both hydrogen peroxide and fatty acid hydroperoxides

$$2H_2O_2 \rightarrow \text{catalase} \rightarrow 2H_2O + O_2$$

$$\begin{aligned} ROOH + 2GSH &\rightarrow \text{GSH peroxidase} \\ &\rightarrow ROH + GSSG \end{aligned}$$

The intercepting defenses 'scavenge' the generated free radicals. As mentioned previously, superoxide dismutase and α-tocopherol are good examples of enzyme and nonenzyme scavengers, respectively. Some dietary minerals are essential for the function of antioxidant enzymes (e.g., the various isoforms of superoxide dismutase use copper and zinc or manganese as cofactors, whereas an isoform of glutathione peroxidase uses selenium).

The repair system removes damaged biomolecules before cell metabolism or viability has been altered due to their accumulation. Oxidatively damaged nucleic acids are repaired by specific enzymes, oxidized proteins are removed by proteolytic systems, and oxidized membrane lipids are processed by lipases, peroxidases, and acyl transferases.

Measurement of Lipid Peroxidation

The extent of lipid peroxidation can be determined by measuring (1) losses of fatty acids; (2) amounts of primary peroxidation products; (3) amounts of secondary products, such as carbonyls and hydrocarbon gases; and (4) reduction in antioxidant activity. Some of the commonly used methods are described below. Analysis of fatty acids by gas liquid chromatography (GLC) or high-performance liquid chromatography (HPLC) is used for measuring the loss of unsaturated fatty acids, a consequence of lipid peroxidation. Lipid hydroperoxides, the primary product of peroxidation product, can be directly measured by HPLC with chemiluminescence detectors. Iodine liberation and glutathione peroxidase methods are often used for measuring lipid peroxides. Lipid peroxides oxidize I^- to I_2 for titration with thiosulfate and thus consumption of thiosulfate indirectly indicates the quantity of lipid peroxides. Hydrogen peroxides and hydroperoxides oxidize reduced GSH to oxidized glutathione (GSSG) and addition of glutathione reductase and NADPH reduces GSSG back to GSH, requiring consumption of NADPH, which can be related to peroxide content. Spin traps (phenyl *t*-butyl nitrone) are frequently used for trapping intermediate radicals. Products of lipid peroxide decomposition, such as hydrocarbon gases and cytotoxic aldehydes, can be measured by GLC or HPLC. Lipid peroxidation products may cause damage to DNA and formation of 8-oxo-2′-deoxyguanosine (8oxodG) is a marker of oxidative damage to DNA. Contents of 8oxodG in DNA can be quantified by HPLC with EC detector and by an immunochemical method (enzyme-linked immunosorbent assay).

The commonly used assays for measurement of lipid peroxidation are the thiobarbituric acid (TBA) test and diene conjugation determination. In the TBA test, lipid samples are heated with TBA at low pH and malondialdehyde (MDA), a lipid peroxidation product, reacts with TBA to develop a pink color. Darkness of the color is related to the extent of lipid peroxidation. Because of its simplicity and economy, this method is very popular. During the process of lipid peroxidation, diene conjugations (a double bond–single bond–double bond structure) are formed (see enzyme catalysed peroxidation) which absorb ultraviolet (UV) light in the wavelength range of 230–235 nm. The absorption of UV light at this wavelength range can be related to contents of diene conjugates in lipid extracts of tissues and, thus, to extent of lipid peroxidation.

One approach to determination of 'whole-body' lipid peroxidation has been measurement of exhaled hydrocarbons by GLC, especially ethane. Hydrocarbon gases are, however, minor end-products of peroxidation and their formation depends on the decomposition of peroxide. Recent studies have demonstrated that isoprostane is a good biomarker of lipid peroxidation in the human body. Isoprostanes are specific products arising from the peroxidation of unsaturated fatty acid residues in lipids and detection of them and their metabolites in urine is a useful assay of 'whole-body' lipid peroxidation. Isoprostanes can be accurately and sensitively measured by mass spectrometric techniques.

Since radical-scavenging antioxidant molecules are consumed in the process of protecting against oxidants, changes in antioxidant activity may also reflect previous or ongoing oxidative stress. Therefore, measurement of antioxidant activity under controlled oxidative stress has been also used to estimate extent of lipid peroxidation.

Because all these methods measure only one stage of lipid peroxidation, and not the whole process, caution must be taken in interpreting the results from these measurements. In addition, each method has its own limitation. The simple TBA test and diene conjugation methods without previous calibration against more sophisticated assays may result in incorrect estimate of lipid peroxidation.

See also: Acetaminophen; Carbon Tetrachloride; Ethanol; Kidney; Liver; Paraquat; Respiratory Tract.

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Lithium

Shayne C Gad

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- Chemical Abstracts Service Registry Number: CAS 7439-93-2
- Chemical/Pharmaceutical/Other Class: Alkali earth metals
- Chemical Formula: Li^+

Uses

The most widely known use of lithium is the use of lithium carbonate in treating manic-depressive affective disorders; the mechanism by which it alleviates depression in some people is not known. Industrially, lithium and its compounds are used in metal alloys, lubricants, nuclear reactor, coolant, ceramics, alkaline storage batteries, and electronic tubes. It is also used as a catalyst and as a reducing agent.

Background Information

Lithium was discovered as a salt in 1817. It does not occur in nature as a free metal. Lithium was used as a salt substitute and as a major constituent of the soft drink 7-Up before 1950.

Exposure Routes and Pathways

Ingestion is the most common exposure pathway; lithium carbonate is administered orally. Occupational exposures to lithium rarely produce toxicity. Lithium occurs in breast milk of nursing women.

Toxicokinetics

Lithium is readily absorbed when administered orally and is widely distributed in the body, mainly intracellularly. It is carried into the red blood cells by the sodium transport carrier and enters the central nervous system. Lithium can penetrate the placental barrier. It is excreted in the urine – excretion depends on sodium and water balance and the glomerular filtration rate.

Mechanism of Toxicity

The exact mechanism of lithium toxicity is unknown. It may be related to lithium's displacement of potassium, producing an unusual equilibrium within the cells. Another hypothesis is that lithium is competitive with sodium for binding sites in various organs such as the kidney and the central nervous system.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} of lithium chloride following subcutaneous administration in mice was $\sim$17–19 mmol kg^{-1} ($\sim$700–800 mg kg^{-1}). The acute oral LD_{50} in rats is $\sim$500 mg kg^{-1}. Acute lithium exposure elicits excessive urination and polydipsia.

Human

Lithium hydride rapidly converts to lithium hydroxide in contact with water. Lithium hydroxide is corrosive to all tissues, outer skin, as well as lung cells.

Chronic Toxicity (or Exposure)

Animal

Long-term dietary lithium can lead to renal failure, hypertension, and proteinuria. Lithium is neither mutagenic nor carcinogenic, but is toxic to the central nervous system. Interestingly, long-term exposure to lithium chloride dramatically protected cultured neurons against glutamate-induced excitotoxicity via apoptosis mediated by *N*-methyl-D-aspartate receptors. High dietary potassium can prevent lithium toxicity following repeated exposures in hamsters and rats.

Human

Lithium can cause kidney injury. The first sign of intoxication in patients is usually a fine hand tremor. Occupational lithium toxicity is rare. The use of lithium carbonate for depression may result in damage to the neuromuscular system resulting in ataxia and tremors. Toxicity often occurs after weeks of chronic intake. The first signs of toxicity are nausea, vomiting, and abdominal pain. The action on the central nervous system can result in tremors, epileptic-type seizures, impediment of speech, and even short blackout periods. The heart can also be affected resulting in hypertension and arrhythmias. Nephrotoxicity has been recorded in some patients. Long-term lithium treatment of pregnant women has been associated with fetal cardiac abnormalities. Chronic lithium treatment also appears to disrupt thyroid function and may lead to hypothyroidism.

Clinical Management

There is no specific antidote for lithium toxicity. For acute overdose, administration of syrup of ipecac followed by gastric lavage is recommended. Electrolyte replacement should follow. An infusion of mannitol or urea and increasing the alkalinity of the urine will enhance lithium excretion. For abnormal motor activity, a tranquilizer such as diazepam is helpful. High salt intake protects against lithium toxicity in the kidneys. Hemodialysis may also be used. As noted above, diets high in potassium may also afford protection against chronic lithium toxicity.

Environmental Fate

Concentrations of lithium in surface waters are typically very low (<0.04 mg l^{-1}). Seepage into groundwater and surface water from storage sites (e.g., the US Department of Energy's Y-12 plant) may lead to concentrations much higher (0.15 mg l^{-1}).

Ecotoxicology

The 68 h LD_{50} (gastric gavage) in common carp was >400 mg kg^{-1}. The 96 h LC_{50} in fathead minnows was 42 mg l^{-1}. The 96 h LC_{50} in *Fundulus heteroclitus* was 8–39 mg l^{-1}. Lithium was reported to induce microcephaly in frogs and salamanders.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (TLV) – time-weighted average for lithium hydride is 0.025 mg m^{-3}. The TLV for lithium chloride or lithium carbonate is not established.

See also: Kidney; Metals.

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Liver

Janet E Kester

Introduction

The liver is the largest organ in the human body, weighing ~1.5 kg in human adults. Although not an elegant organ in its structure, having (and needing) no constant form, its highly complex activities are essential for life. The liver accommodates its form to its surroundings and expands into any part of the abdominal area not occupied by other viscera. Details of arrangement and lobation vary greatly from species to species and even between individuals of the same species. Ancient priests divined the future from the liver patterns of sacrificed animals; they found plenty of scope for personalized interpretations!

The liver performs four basic functions that are essential for maintenance of homeostasis and integration of metabolism:

1. storage and filtration of blood;
2. metabolism and storage of many xenobiotics, as well as nutrients and endogenous compounds such as bile acids, fatty acids, and steroids and other hormones;
3. secretory and excretory activities involved in bile formation and flow (exocrine functions); and
4. synthesis of a variety of important constituents of blood plasma, which are secreted directly into the blood (endocrine functions).

Liver injury by chemical substances has been recognized for more than 100 years.

Review of Liver Structure and Function

Correlation of the liver's structure with its many functions, and an appreciation of the systemic consequences of toxic injury to this vital organ, depends on an understanding of its strategic location in the circulation.

The Liver Functions as an Interface Between the Digestive Tract and the Systemic Circulation

The liver's interposition between the digestive tract and the systemic circulation is responsible for both its primary role in metabolism of xenobiotics and its susceptibility to their toxic actions. It directly receives all the material (including xenobiotics) absorbed from the intestine (except the lipid chylomicrons, which are transported by lymphatic vessels). The absorbed materials are taken up and further metabolized by the liver or transformed and released to the blood for utilization or storage elsewhere.

The liver has a dual blood supply. Its principal afferent (incoming) blood vessel is the portal vein, which drains the capillary beds of the intestine and spleen at a rate of $\sim 1100\,\mathrm{ml\,min^{-1}}$ (~75% of the total). A smaller volume of freshly oxygenated arterial blood (~350 ml, or 25%) is carried by the hepatic artery. These two vessels (and the bile duct) enter the liver together at the porta (Latin for 'gate') and branch together into a second capillary bed, the hepatic sinusoids. The liver is drained by the efferent (outgoing) hepatic veins into the inferior vena cava near the heart. This special arrangement of blood vessels – where blood collected from one set of capillaries passes through a larger vessel into a second set of capillaries before entering the venous circulation – is called a portal system. The hepatic blood vessels are accompanied throughout the parenchyma by bile ducts and lymphatic vessels.

The hepatic sinusoids – the second capillary bed in the hepatic portal system – differ from ordinary capillaries in several respects. They are larger and more variable in diameter, and their walls are lined with both endothelial cells and very large, actively phagocytic Kupffer cells. Unlike most other blood vessels in vertebrates, the sinusoids have actual discontinuities of as much as a micrometer in diameter between the endothelial cells, allowing the blood plasma, including plasma proteins (but not blood cells), to pass freely through into the space of Disse and directly contact the liver cells. The direct access of the plasma to the surface of the liver cell is a structural feature of great functional importance in the active exchange of materials between the liver and the bloodstream.

The Liver Serves as a Blood Filter and Reservoir

Blood entering the hepatic sinusoids carries many bacteria from the digestive tract. The phagocytic Kupffer cells interspersed among the typical endothelial cells lining this specialized capillary bed rapidly phagocytize more than 99% of bacteria and other foreign particles in the blood.

Because the liver is a soft, expansible structure, large volumes of blood can be stored in its vessels. The normal hepatic blood volume is ~450 ml, or almost 10% of the normal human total blood volume. As much as an additional liter can be stored

when blood volume is high. Likewise, the liver can supply extra blood when the circulatory volume is diminished.

The Liver Is Composed Primarily of Hepatocytes

Although the liver consists of several cell types present which are vital for its function – endothelial cells lining the blood vessels, bile ductular cells, connective tissue cells, nerve cells, and the phagocytic Kupffer cells – the hepatocytes constitute ~80% of the cytoplasmic mass and carry out the liver's characteristic metabolic and synthetic functions. These cells are essential for life and have an extraordinary capacity to regenerate and flexibly respond to varying metabolic demands. Indeed, they are unrivaled in their functional diversity, complexity, and flexibility.

Although the hepatocyte is a highly differentiated cell that rarely divides in adult vertebrates, it possesses a tremendous capacity for compensatory hyperplasia after injury or removal of liver tissue. It appears that hepatocytes engaged in the regenerative process undergo quantitative rather than qualitative changes in gene expression. Preferentially expressed are stress proteins, the multidrug-resistance gene, and several protooncogenes.

Protooncogenes in particular are thought to play an essential role in cell proliferation and differentiation because they are highly conserved in evolution, differentially expressed during development, and known to play a role in malignant transformation. In fact, analysis of protooncogene expression during liver regeneration provided one of the first demonstrations that the expression of these genes is regulated during normal growth.

The Basic Anatomical Unit of the Liver Is the Classical Lobule

The hepatocytes are arranged in single-cell-thick plates or sheets that appear to radiate out from terminal branches of the hepatic veins. These have traditionally been termed 'central veins' because of their location in the polyhedral units of liver parenchyma that constitute the classical 'liver lobules', typically hexagonal structures 2 or 3 mm in length and 1 or 2 mm in diameter. Neighboring plates are separated by sinusoids, which are closely applied to the sheets of liver cells and intercommunicate through fenestrations ('windows') in them to form a labyrinthine system covering a very large area of liver parenchyma. The human liver contains 50 000–100 000 individual lobules. The corners of the polygonal lobules are each occupied by portal space containing portal triads: branches of portal venule, hepatic arteriole, and bile ductule (see **Figure 1** for anatomical illustration).

It was previously thought that blood flowed directly from the portal space vessels between plates to hepatocytes to be collected in the central veins. Although it is now clear that the actual flow of blood in these areas is not as previously envisioned, the lobule unit remains conceptually convenient because it exhibits morphologically distinguishable zones, for example, differential deposition of glycogen and fat, referred to as centrilobular, mid-zonal, or periportal. Furthermore, toxic agents of pathological conditions may selectively show their harmful effects in these areas.

The Basic Functional Unit of the Liver Is the Acinus

We now know that blood enters the sinusoids of the parenchyma via fine terminal branches of the afferent vessels, which leave the portal spaces at intervals, coursing perpendicular to the central vein and along the sides of the hexagons forming classical lobules. Each fine terminal afferent vessel supplies blood to only sectors of adjacent lobules. The associated mass of parenchymal tissue that they preferentially supply

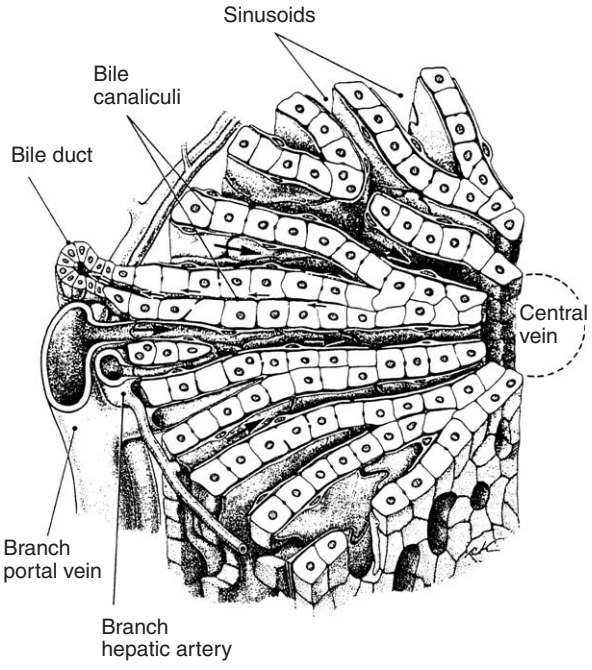

Figure 1 Diagram illustrating the basic anatomical unit of the liver, the liver lobule, showing (1) the radial disposition of the liver cell plates and sinusoids around the central vein, (2) the centripetal flow of blood from branches of the hepatic artery and portal vein, and (3) the centrifugal flow of bile (small arrows) to the small bile duct in the portal space. (Reproduced from Bloom W and Fawcett DW (eds.) (1968) *A Textbook of Histology*, 9th edn. Philadelphia: Saunders; redrawn and modified from Ham, *Textbook of Histology*. Philadelphia: Lippincott, with permission from Lippincott.)

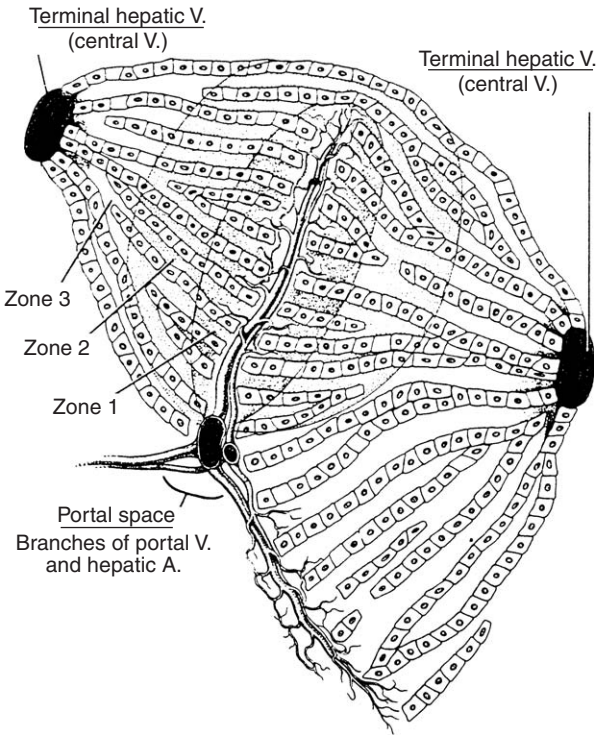

Figure 2 Diagram illustrating the basic functional unit of the liver, the acinus, consisting of the tissue centered around the terminal branches of the hepatic artery and portal vein. The cells in zone 1 nearest these vessels receive the highest concentrations of oxygen and nutrients, while those in zones 2 and 3 are exposed to progressively depleted blood. (Reproduced from Bloom W and Fawcett DW (eds.) (1968) *A Textbook of Histology*, 9th edn. Philadelphia: Saunders, with permission; redrawn from Rappaport A *et al.* (1954) *The Anatomical Record* 119: 11.)

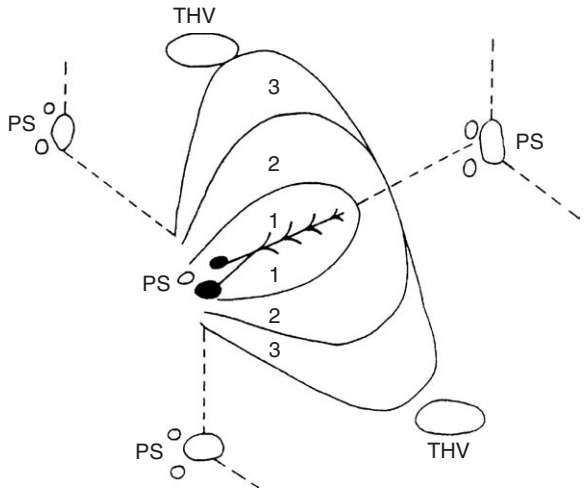

Figure 3 Schematic representation of a simple hepatic acinus. PS is the portal space, consisting of a branch of the portal vein, a hepatic arteriole, and a bile duct; THV is the terminal hepatic venule (central vein); 1, 2, and 3 represent the various zones surrounding the terminal afferent vessel. (Reproduced with permission from Klassen CD, Amdur MO, and Doull J (eds.) (1986) *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 3rd edn. New York: McGraw-Hill; © The McGraw-Hill Companies, Inc.)

Table 1 Predominant lobular/acinar localization of major liver functions

Periportal zone/Zone 1
Glucose release
Oxidative energy metabolism
Amino acid utilization
Protection against oxidants
Bile acid uptake and excretion
Bilirubin excretion
Centrilobular zone/Zone 3
Glucose uptake
Ammonia detoxification
Biotransformation reactions

is termed an acinus (Latin for 'berry') (**Figure 2**). The acinus lies between two or more terminal hepatic venules (central veins in the classical terminology), with which its vascular and biliary axis interdigitates. Acini are irregular in size and shape, and there is no physical separation between them.

As with the classical lobule, there are distinct circulatory zones within each acinus. They are typically divided into three, depending on their distance from the afferent vessels (**Figure 3**). Zone 1, being closest to the supply of fresh blood, is the first to receive oxygen and nutrients and the last to undergo necrosis. The more distal regions, zones 2 and 3, receive progressively depleted blood, and hence are possibly less resistant to hepatotoxicants and other vectors of hepatic injury. Furthermore, the fenestrations between sinusoidal endothelial cells gradually increase in number from the periportal to the pericentral end of the sinusoid, allowing the remaining nutrients greater access to the centrally located cells.

There is considerable evidence that position within the acinus also affects important aspects of hepatocyte function, including bile secretion and metabolic activity. Thus, periportal and pericentral cells differ both structurally and functionally, as summarized in **Table 1**. For example, periportal (zone 1) cells take up more bile acids from sinusoidal blood, and hence secrete more biliary constituents. Pericentral (zone 3) cells are specialized to perform higher levels of metabolic and degradative activities.

The Liver Is an Exocrine Gland, Secreting Bile

The liver functions as an exocrine gland, with each hepatocyte continually secreting a small amount of bile into tiny bile canaliculi located between adjacent pairs of parenchymal cells. These tiny vessels form a continuous network from lobule to lobule

throughout the organ. The canaliculi are smallest near the central vein, increasing in diameter with proximity to the portal triads. The canaliculi coalesce into biliary ductules, interlobular bile ducts, and larger hepatic ducts. The main hepatic duct joins the cystic duct from the gallbladder to form the common bile duct, which drains into the duodenum. The total secretion of bile by the human liver is $\sim$700–1200 ml day^{-1}.

Bile is Important Both as a Digestive Secretion and as a Medium of Excretion Bile is a complex mixture of bile salts, bile pigments, phospholipids, cholesterol, inorganic electrolytes, and endproducts of metabolism.

Bile Salts Enable the Digestion of Lipids Cholesterol is the precursor of both steroids and bile salts and is an integral component of cell membranes. It is eliminated from the body via conversion to bile salts and direct secretion into the bile. In fact, the word cholesterol (from the Greek 'chole' (bile) and 'stereos' (solid)) was used originally to describe the material of which gallstones are made. In the process of degradation, it is converted to the primary bile acids cholic acid and chenodeoxycholic acid in approximately equal amounts. The salts of these acids are excreted in bile. They perform two important functions in the digestive tract:

1. They act as detergents, emulsifying large fat droplets into small ones. This action creates a much larger surface area for the action of lipase in the small intestine, thereby increasing lipid absorption.
2. They form minute complexes called micelles with the emulsified lipids. The electrical charges of the strongly ionized bile salts render these micelles highly soluble, aiding in their transport to the absorptive surfaces of the intestinal brush border. The fats readily diffuse across the membrane, leaving the charged bile salts to retrieve more fats in a 'ferrying' activity.

Bile salts are extensively metabolized to secondary bile acids by intestinal microflora in the gut. Approximately 94% of the bile salts are reabsorbed at special mucosal receptor sites in the distal ileum and reused by the liver by the process of enterohepatic circulation. In enterohepatic circulation, compounds secreted in bile are reabsorbed in the gastrointestinal tract and returned to the liver. On reaching the liver in the portal blood, almost all of the bile salts are taken up across the sinusoidal membranes of hepatocytes (predominantly in periportal regions). The bile acids are then resecreted into the bile. On average, these salts make the entire circuit $\sim$18 times before being lost in the feces. Thus, enterohepatic circulation of toxicants increases their half-life in the body and hence their opportunity to exert toxic effects.

Bile Pigments are Products of Red Blood Cell Destruction Red blood cells circulate for $\sim$120 days before their membranes become very fragile and they rupture in vascular 'tight spots' like the spleen. The hemoglobin released when these cells rupture is rapidly taken up by tissue macrophages in many parts of the body, but especially by the Kupffer cells of the liver. The porphyrin portion of the hemoglobin molecule is oxidized in the macrophages to biliverdin, which is rapidly reduced to the yellow–green bile pigment bilirubin. The macrophages release bilirubin into the blood, where it binds tightly to albumin and is transported throughout the circulation to the liver. Inside hepatocytes, it associates with proteins that 'trap' it inside the liver cells. It is subsequently removed from these holding proteins and conjugated with glucuronide (80%), sulfate (10%), and other substances (10%). These conjugated forms are then actively transported into the bile canaliculi and largely eliminated with the feces.

This pigment is highly soluble in all cell membranes and also very toxic; hence, its excretion is one of the liver's most important functions. Interestingly, however, recent studies have demonstrated that bilirubin is an effective antioxidant of possible physiological importance. Along with urate and ascorbate (vitamin C), it is one of the three principal antioxidants in plasma. In membranes, its antioxidative efficacy rivals that of vitamin E. Thus, this toxic endproduct of a degradative pathway can also perform a beneficial function.

The Liver Plays a Key Role in the Integration of Metabolism

Rich in both phase I (principally the cytochromes P450, catalyzing hydrolysis, reduction, and oxidation reactions) and phase II (catalyzing conjugation of xenobiotic molecules with hydrophilic moieties) biotransforming enzymes, the liver is the metabolic center of the body. In fact, most of the field of biochemistry is concerned with its metabolic reactions. The liver essentially converts ingested food into a balanced cell culture medium via metabolic interconversion of amino acids, carbohydrates, and lipids and synthesizes many substances that are subsequently exported for use in other areas of

the body. It is also a major locus of biotransformation of xenobiotic compounds to both harmless (detoxification) and toxic (bioactivation) metabolites. The balance between bioactivation and detoxification reactions determines whether adverse effects occur. Given the complex metabolic machinery of the liver and the inevitable presence of multiple chemicals (both naturally occurring and xenobiotic), it is not surprising that metabolic and toxicologic interactions among chemicals are observed.

Carbohydrate Metabolism The liver serves as an energy reservoir, storing glucose as glycogen for release on demand. It thus plays a very important role in maintaining a normal blood glucose concentration. In the event of severe glucose deficiency, the liver can convert amino acids into glucose.

Fat Metabolism The liver also plays a central role in synthesis, oxidation, storage, and distribution of lipids. It not only aids in the absorption of fats through the action of the bile salts, but also (1) both synthesizes and oxidizes fatty acids, cholesterol, triacylglycerols, and phospholipids (the major components of cell membranes); (2) synthesizes most of the plasma lipoproteins; and (3) converts carbohydrates and proteins into fat.

About 80% of the cholesterol synthesized in the liver is converted into bile salts. The remainder of the cholesterol, triacylglycerols, other lipids, and hydrophobic substances (including xenobiotics) are transported to other tissues throughout the body by plasma lipoproteins. These lipoproteins, which are classified according to density, consist of apoproteins (also made by the liver) and various combinations of fat and fat-soluble compounds. The liver also stores vitamins, especially vitamin A but also vitamins D, E and K, as well as vitamin B12, in fat-storing Ito cells, located between endothelial cells and hepatocytes.

Protein Metabolism The most important functions of the liver in protein metabolism are (1) deamination of amino acids for use as energy or conversion into fats and carbohydrates, (2) synthesis and interconversion of amino acids and other metabolically important compounds, (3) formation of urea for excretion of ammonia, and (4) formation of plasma proteins.

Approximately 90% of the plasma proteins are formed by the hepatocytes. Among these important products are albumin (involved in the maintenance of osmotic pressure), clotting and anticlotting factors, and immunoglobulins. The remaining 10% are largely γ-globulins synthesized by plasma cells.

Toxic Liver Injury

The Liver Is Often a Target of Toxic Agents

Many toxic agents enter the body via the gastrointestinal tract and after absorption are carried directly to the liver. There, the drug metabolizing enzyme systems may detoxify them or, in some cases, create toxic intermediates which injure the liver and other tissues. Even chemicals that are successfully excreted in the bile can return to the liver via the cycle of enterohepatic circulation. Furthermore, the liver has a high concentration of binding sites and therefore a tendency to accumulate certain xenobiotics. The liver's position at the interface of the digestive tract and the blood, and its central role in the metabolism and excretion of foreign chemicals, therefore, renders it especially vulnerable to toxic injury.

Toxic Liver Injury Can Take Many Forms

Many chemicals are hepatotoxic, including thousands of synthetic drugs and chemicals as well as a plethora of natural compounds such as bacterial, fungal, plant, and animal toxins. Some examples of chemicals causing liver injury are shown in **Table 2**.

It is clear that a variety of pathologic processes are involved in toxic liver injury, depending on causative agent and duration of exposure. The incidence of

Table 2 Examples of liver toxicants causing specific types of injury

Necrosis and fatty liver
Carbon tetrachloride
Ethanol
Trichloroethylene
Acetaminophen
Cholestasis
Chlorpromazine
Diazepam
Estradiol
Sulfanilamide
Hepatitis
Isoniazid
Halothane
Indomethacin
Porphyria
Hexachlorobenzene
Cirrhosis
Ethanol
Carcinogenesis (in experimental animals)
Carbon tetrachloride
Dimethylbenzanthracene
Polychlorinated biphenyls and dioxins
Tri- and tetrachloroethylene
Vinyl chloride

hepatotoxic injury by a given chemical differs among species and individuals; a dose–response relationship may not always be evident. The toxicity of chemicals can be significantly modified by a number of host and other variables, particularly hepatic enzyme activity. Furthermore, different biochemical alterations in the liver can lead to the same toxic effect; no single mechanism seems to govern the appearance of degenerative changes in hepatocytes or alterations in their function. Virtually all forms of chemical-induced liver injury closely resemble other forms of liver disease not presently known to be produced by chemicals.

A variety of systems have been devised to impose some order on this profusion of toxic liver lesions. Our discussion resorts to the simple device of distinguishing between generally acute and generally chronic forms of toxic liver disease, followed by consideration of the mechanisms by which selected compounds exert these effects. A few other classification systems are mentioned here to provide the reader with a further conceptual framework for organization of current knowledge. In one such system, lesions are categorized by the duration of exposure to the causal agent. Some injuries typically occur after acute exposure, while others require chronic exposure. For example, an acute effect of ethanol is fatty change, metamorphosis, or accumulation (intracellular accumulation of triacylglycerols), while in the long term cirrhosis occurs.

Some injuries may be transient, while others are irreversible. For example, fatty change is often transient and not necessarily indicative of functional compromise; under certain conditions, hepatocytes with accumulated fat function normally. Malignant transformation, on the other hand, is irreversible and seriously disrupts hepatocellular function. Necrosis (cell death) may or may not be life-threatening, depending on its extent (see previous discussion of liver regeneration).

Yet another classification system refers to the nature of the host's response to the causative agent. Some agents, referred to as intrinsic hepatotoxicants, will cause hepatotoxicity in most individuals of most species. In the case of idiosyncratic hepatotoxicants, where a chemical's toxic effects are a function of unusual susceptibility of the exposed individual, it may not be clear whether the lesion is a manifestation of the hepatotoxic properties of the substance in question or a manifestation of the individual's untoward response to the agent. This response may mean hypersensitivity (allergic) reactions or exaggerated responses to minor alterations in liver function. For example, anabolic or contraceptive steroids cause diminished biliary excretion (cholestasis) in most humans, with a few showing jaundice. It is not clear whether the occasional jaundice is the result of an allergic response or an extreme reaction to diminished biliary excretion. Likewise, many drugs, for example, isoniazid and halothane, can precipitate a potentially fatal viral-like hepatitis in susceptible subjects.

Acute Toxic Effects

Acute exposures to hepatotoxicants may result in the following:

1. degenerative changes (lipid or water accumulation);
2. necrosis;
   a. zonal, focal, or massive,
   b. venoocclusive disease, and
   c. hepatitis
3. hepatobiliary dysfunction; and
4. acute porphyria

Degenerative Changes

Disturbances in cellular metabolism can cause swelling and accumulation of materials such as water or lipid. These changes are usually (but not always) reversible. Hydropic change (water accumulation) often precedes fatty metamorphosis. The fatty liver is grossly enlarged and pale yellow. Fat droplets consisting mostly of triacylglycerols and fatty acids are visible histologically using lipid stains. Fatty change may be brought about by several distinct mechanisms involving fatty acid and protein metabolism. Examples of causative agents are carbon tetrachloride, ethionine, and ethanol.

Necrosis

Cell swelling and lipid accumulation may precede cell necrosis. Necrosis can affect small groups of cells (focal), groups of cells located in discrete zones (zonal), or many cells (massive). Centrilobular necrosis refers to the death of cells surrounding the central vein of the classical lobule (zone 3 of acinar model). Typical causative agents are carbon tetrachloride, pyrrolizidine alkaloids, acetaminophen, bromobenzene, and isoniazid. An agent that causes periportal necrosis (cell death around portal spaces; zone 1 of acinus) is allyl alcohol. Mid-zonal (acinar zone 2) necrosis can be caused by beryllium and yellow phosphorus. Because of the dose-responsiveness and predictability of the lesions they cause, these agents are regarded as direct hepatotoxicants. Note, however, that many direct hepatotoxicants must

undergo biotransformation to reactive forms. A classic example of enzymatic bioactivation is the cytochrome P450-mediated conversion of carbon tetrachloride to a free radical that initiates lipid peroxidation.

Most of the unpredictable idiosyncratic forms of toxic liver injury are more diffuse, consisting of necrosis with significant inflammatory reaction. Nonspecific hepatitis, which can be caused by aspirin (acetylsalicylic acid), is characterized by a few scattered foci of necrosis. A clinical syndrome indistinguishable from viral hepatitis (hence the name viral-like hepatitis) has been associated with various drugs, for example, isoniazid and halothane. This appears histologically as generalized parenchymal damage with disruption of the normal liver cell arrangement, often accompanied by fever, rash, arthralgias, and eosinophilia. The cells may be swollen and hydropic, especially in the centrilobular regions. There may be a progression to a massive necrosis typical of viral hepatitis. Granulomatous hepatitis, characterized by well-demarcated aggregates of inflammatory cells, occurs with or without other types of hepatic injury in response to several drugs. Typical examples are sulfonamides and sulfonylurea derivatives.

Hepatobiliary Dysfunction

Interference with any step in the complex sequences of hepatic uptake and secretion could affect hepatobiliary function (see previous discussion). A number of agents cause cholestasis, generally defined as reduced bile flow. This condition can arise from extrahepatic obstruction of bile flow or intrahepatic alterations that reduce secretion and excretion of solutes and water. Because their elimination is impeded, bile substances (including bile acids and pigments, cholesterol, and various endogenous and exogenous conjugated products) accumulate within the liver and in the systemic blood. Cholestasis is generally difficult to reproduce in experimental animals using the drugs that cause it in humans. However, it is possible to produce cholestatic responses in animals following administration of certain compounds, including lithocholic acid, α-naphthylisothiocyanate, anabolic and contraceptive steroids, and chlorpromazine.

Acute Toxic Porphyria

The biochemistry of the porphyrins and the bile pigments is closely related because heme is synthesized from porphyrins and iron and the products of its degradation are bile pigments and iron. Porphyrins are cyclic compounds formed by the linkage of four pyrrole rings through methenyl bridges. Iron is complexed to the nitrogen atom of the pyrrole rings. The metalloporphyrin is in turn conjugated to proteins to form hemoglobin, cytochromes, and several other hemoproteins important in biological processes. The term 'porphyrin' is derived from the Greek word porphyra (purple) because mammalian porphyrins are deep red or purple in color (due to the iron atom). They are easily detected in blood, urine, and feces due to their color and fluorescent properties.

The porphyrias are a heterogeneous group of diseases, all of which involve disorders of heme biosynthesis, which result in accumulation and increased excretion of porphyrins or porphyrin precursors. The porphyrias can be divided into two kinds: the hereditary porphyrias, some of which can be exacerbated by exposure to certain chemicals, and the toxic porphyrias, which can be produced by exposure to certain chemicals alone. The pattern of excretion of porphyrins and porphyrin precursors is characteristic for each type. Clinical symptoms consist mainly of cutaneous photosensitivity and/or neurological disturbances. Hexachlorobenzene is a chemical inducer of porphyria.

Chronic Toxic Effects

Chronic exposure to certain chemicals can cause cirrhosis, a marked alteration of the entire liver structure with both degenerative and proliferative changes observed, and neoplasia.

Cirrhosis

Cirrhosis is characterized by diffuse destruction and partial regeneration of parenchymal tissue and the formation of collagen septa distributed throughout most of the liver. Separated by these fibrous sheaths, clusters of hepatocytes appear as nodules. The pathogenesis of cirrhosis is poorly understood. In most cases, the death of scattered single cells together with defective repair mechanisms appears to lead to scarring. In humans, the single most important cause of cirrhosis is chronic ingestion of alcohol. This condition is not easy to duplicate in laboratory animals with ethanol, leading to the suggestion that other factors unique to humans may play an important role in pathogenesis. A major factor might be diet since alcoholics usually suffer nutritional deficiency.

Neoplasia

Chemically induced liver tumors may arise from hepatocytes, bile duct cells, or sinusoidal lining cells (angiosarcoma). Susceptibility to liver tumors differs

significantly among species. A large number of chemicals, both anthropogenic and naturally occurring, are known to induce liver cancers in experimental rodents, acting by a variety of both DNA-damaging (genotoxic) and epigenetic (nongenotoxic) mechanisms. Although this classification is widely used and convenient, it is important to note that although nongenotoxic agents do not alter the primary sequence of DNA, they may directly or indirectly cause mutagenicity through oxidative damage. In addition, they may promote the clonal expansion of altered cells, by stimulating cell division and/or inhibiting apoptosis.

Genotoxic hepatic carcinogens include 2-acetylaminofluorene, aflatoxin, carbon tetrachloride, and vinyl chloride. The most potent known nongenotoxic rodent hepatocarcinogen is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin ('dioxin'), the best-studied of a large group of structurally similar halogenated aromatic hydrocarbon compounds (including the halogenated furans, biphenyls, triphenyls, azo- and azoxybenzenes, and naphthalenes) that exert their effects via interaction with the aryl hydrocarbon (Ah) receptor. Peroxisome proliferators are a large family of structurally diverse compounds that exert many of their effects, including rodent hepatocarcinogenicity, via transactivation of peroxisome proliferator-activated receptor α (PPARα). Examples of peroxisome proliferators include many drugs (e.g., fibrates, aspirin, and acetaminophen), phthalate plasticizers, pesticides, tri- and tetrachloroethylene, and natural compounds (hormones, eicosanoids, green and black tea, and omega-3 fatty acids).

The human hepatocarcinogenicity of many chemicals has not been well established. One of the difficulties in assessing the potential risks of human exposure to chemicals is the fact that the protocols typically used in chronic chemical toxicity assessment studies tend to overpredict human carcinogenicity. For example, several of the commonly used rodent species have a higher natural incidence of liver tumors than do humans, and the test procedures involve long-term administration of doses very much larger than those to which humans are likely to be exposed. Further, evolving tools of molecular biology continue to illuminate the mechanistic bases for observed differences in animal and human responses.

See also: Acetaminophen; Acetylaminofluorene; Acetylsalicylic Acid; Aflatoxin; Biotransformation; Blood; Bromobenzene; Carbon Tetrachloride; Chloroform; Dioxins; Distribution; Ethanol; Excretion; Immune System; Isoniazid; Lipid Peroxidation; Metallothionein; Peroxisome Proliferators; Tissue Repair.

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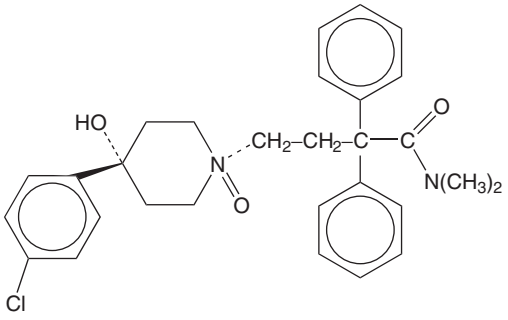
Loperamide

F Lee Cantrell

- Chemical Abstracts Service Registry Number: CAS 53179-11-6
- Synonyms: Imodium; Imodium AD; Imodium advanced, Imogen; Diarrid; Diar-aid; Neo-Diaral
- Chemical/Pharmaceutical/Other Class: Antidiarrheal
- Chemical Formula: $C_{29}H_{33}ClN_2O_2$
- Chemical Structure:



Uses

Loperamide is a nonprescription medication for the treatment of acute nonspecific diarrhea and chronic diarrhea associated with inflammatory bowel disease. It is also used to reduce the volume of discharge from ileostomies.

Exposure Routes and Pathways

Loperamide is available in capsule and liquid forms. Ingestion is the most common route of exposure.

Toxicokinetics

Loperamide is absorbed slowly from the gastrointestinal tract reaching peak levels within 4 h. It undergoes enterohepatic circulation. Protein binding is 97%. Loperamide is primarily excreted in the urine. The half-life can range from 7 to 15 h and is dose independent.

Mechanism of Toxicity

The mechanism of loperamide toxicity is related to opioid-like activity that causes depression of the central nervous system (CNS). The abuse potential for loperamide is low.

Acute and Short-Term Toxicity (or Exposure)

Animal

In dogs 1.25–5 $mg\,kg^{-1}\,day^{-1}$ has produced vomiting, CNS depression, severe salivation, and weight loss. Amounts $>5\,mg\,kg^{-1}\,day^{-1}$ have produced hemorrhagic enteritis and paresis.

Human

Children appear to be more susceptible than adults to the toxic effects of loperamide. These effects mimic opiate poisoning. Miosis, nausea, vomiting, and varying degrees of CNS depression exhibited by ataxia and drowsiness to prolonged coma can be seen. Loperamide is not recommended for use in children under 2 years of age. Death has occurred after misuse of loperamide in children 6.5 months of age and younger.

Chronic Toxicity (or Exposure)

Animal

Dogs dosed at $5\,mg\,kg^{-1}\,day^{-1}$ developed hemorrhagic enteritis. Doses of 1.5–5 $mg\,kg^{-1}\,day^{-1}$ produced vomiting, depression, severe salivation, and weight loss. Treatment of cerebellar symptoms with naloxone has been successful in the management of one puppy that was given 3 mg of loperamide.

Human

Loperamide has been used in the management of acute and chronic diarrhea, irritable bowel syndrome, fecal incontinence, ileostomy discharge, and Traveller's diarrhea. Adverse reactions tend to be uncommon and generally mild. Most side effects are gastrointestinal in nature (e.g., epigastric pain, nausea, vomiting, dry mouth, anorexia). Patients have reported development of hyperglycemia while taking loperamide. Volunteer studies have suggested that loperamide use may increase the likelihood for development of gallstones.

In Vitro Toxicity Data

Studies of human jejunal biopsy specimens from cystic fibrosis patients demonstrated that loperamide can restore sodium absorption to normal levels.

Clinical Management

Basic and advanced life-support measures should be utilized as needed. In patients presenting within 1 h of ingestion, activated charcoal may be considered. Supportive care should be provided as needed. Use of the narcotic antagonist naloxone may be beneficial in patients displaying opioid symptoms.

See also: Chloroquine; Codeine; Cromolyn; Diphenoxylate; Disulfiram; Liothyronine; Loxapine; Scombroid; Shellfish Poisoning, Paralytic.

Further Reading

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Lotronex

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 1-22852-69-1
- Synonyms: Alosetron hydrochloride; 2,3,4,5-Tetrahydro-5-methyl-2-[(5-methyl-1*H*-imidazol-4-yl)methyl]-1*H*-pyrido[4,3-*b*]indol-1-one, monohydrochloride
- Chemical/Pharmaceutical/Other Class: Serotonin 5HT3-receptor antagonist
- Chemical Formula: $C_{17}H_{18}N_4OHCl$

Uses

Lotronex is used for severe diarrhea-predominant irritable bowel syndrome (IBS).

Background Information

Animal models have shown alosetron to be active in anxiety, psychosis, cognitive impairment, emesis, and drug withdrawal, though its application in humans has been almost entirely restricted to IBS. Lotronex® (alosetron hydrochloride) was originally approved for IBS in the United States, and then removed from the market in 2000 due to serious gastrointestinal adverse events, some fatal. These events, including ischemic colitis and serious complications of constipation, have resulted in hospitalization, blood transfusion, surgery, and death. In 2002, the US Food and Drug Administration (FDA) approved the Supplemental New Drug Application (sNDA) for Lotronex® tablets under restricted conditions of use. The restrictions include a 'risk management program', the 'prescribing program' for Lotrenox™, which is a component of the 'risk management program', and a revised indication that reflects the intent to reserve Lotronex for patients in whom the medical benefits outweigh the risks, that is, women with severe diarrhea-predominant IBS. These changes are reflective of the serious gastrointestinal adverse events that were reported with the use of Lotronex. In addition, the initial dose of Lotronex was reduced to 1 mg QD (once daily) under the 'risk management program'. Lotronex tablets for oral administration contain 1.124 mg alosetron HCl (equivalent to 1 mg of alosetron).

Exposure Routes and Pathways

Oral, as tablet.

Toxicokinetics

Absorption: Alosetron is rapidly absorbed after oral administration with a mean absolute bioavailability of ~50–60% (approximate range 30% to >90%). After administration of radiolabeled alosetron, only 1% of the dose was recovered in the feces as unchanged drug. In patients with IBS, concentrations of alosetron are influenced by gender. The plasma concentrations of alosetron are 30–50% lower in men than in women given the same oral dose. Following oral administration of 1 mg alosetron dose to young men, a peak plasma concentration of $\sim 5\,ng\,ml^{-1}$ occurs at 1 h. In young women, the mean peak plasma concentration is $\sim 9\,ng\,ml^{-1}$, with a similar time to peak. Efficacy has not been established in men at any dose.

Food effects: Alosetron absorption is decreased by ~25% by coadministration with food, with a mean delay in time to peak concentration of 15 min.

Distribution: Alosetron demonstrates a volume of distribution of ~6–95 l. Plasma protein binding is 82% over a concentration range of 20–$4000\,ng\,ml^{-1}$.

Metabolism and elimination: Plasma concentrations of alosetron increase proportionality with increasing single oral doses up to 8 mg and more than proportionately at a single oral dose of 16 mg. Twice-daily oral dosing of alosetron does not result in accumulation. The terminal elimination half-life of alosetron is ~1.5 h (plasma clearance is $\sim 600\,ml\,min^{-1}$). Population pharmacokinetic analysis in IBS patients confirmed that alosetron clearance is minimally influenced by doses up to 8 mg.

Renal elimination of unchanged alosetron accounts for only 6% of the dose. Renal clearance is $\sim 94\,ml\,min^{-1}$.

Alosetron is extensively metabolized in humans, by multiple cytochrome P450 (CYP) enzymes, including CYP2C9 and CYP3A4. Metabolism is rapid and extensive with *N*-demethylation, hydroxylation, and oxidation. The biological activity of these metabolites is unknown. A mass balance study was performed utilizing an orally administered dose of unlabeled and ^{14}C-labeled alosetron. This study indicates that on a molar basis, alosetron metabolites reach addictive peak plasma concentrations ninefold greater than alosetron and that the additive metabolite AUCs (areas under the curve) are 13-fold greater than alosetron's AUC. Plasma radioactivity declined with a half-life twofold longer than that of alosetron, indicating the presence of circulating metabolites. Approximately 73% of the radiolabeled dose was recovered in urine

with another 24% of the dose recovered in feces. Only 7% of the dose was recovered as unchanged drug. At least 13 metabolites have been detected in urine. The predominant product in urine was a 6-hydroxy metabolite (15% of the dose). This metabolite was secondarily metabolized to a glucuronide that was also present in urine (14% of the dose). Smaller amounts of the 6-hydroxy metabolite and the 6-*O*-glucuronide also appear to be present in feces. A bi-oxidized dicarbonyl accounted for 14% of the dose and its monocarbonyl precursor accounted for another 4% in urine and 6% in feces. No other urinary metabolite accounted for more than 4% of the dose. Glucuronide or sulfate conjugates of unchanged alosetron were not detected in urine.

Mechanism of Toxicity

Alosetron is a potent and selective 5-HT3 receptor antagonist. 5-HT3 receptors are nonselective cation channels that are extensively distributed on enteric neurons in the human gastrointestinal tract, as well as other peripheral and central locations. 5-HT3 receptor antagonists such as alosetron inhibit activation of these channels that results in the modulation of the enteric nervous system. Activation of these channels and the resulting neuronal depolarization affect the regulation of visceral pain, colonic transit, and gastrointestinal secretions, processes that relate to the pathophysiology of IBS.

Acute and Short-Term Toxicity (or Exposure)

Human

Constipation is the most frequent adverse event, with a higher incidence of transient constipation in alosetron-treated patients, typically occurring in the first month of treatment. Significant side effects noted with the use of alosetron include severe constipation, fecal impaction, and ischemic colitis. Approximately 10% of patients on Lotronex in clinical trials withdrew because of constipation.

Chronic Toxicity (or Exposure)

Animal

In 2 year oral studies, alosetron was not carcinogenic in mice at doses up to $30\,mg\,kg^{-1}\,day^{-1}$ or in rats at doses up to $40\,mg\,kg^{-1}\,day^{-1}$. Reproduction studies have been performed in rats at doses up to $40\,mg\,kg^{-1}\,day^{-1}$ and rabbits at oral doses up to $30\,mg\,kg^{-1}\,day^{-1}$. These studies have revealed no evidence of impaired fertility or harm to the fetus due to alosetron.

Human

A small number of patients (2–3 per 1000) using Lotronex developed ischemic colitis in periods of use ranging from 3 to 6 months.

In Vitro Toxicity Data

Alosetron was not genotoxic in the Ames tests, the mouse lymphoma cell (L5178Y/TK $\pm$) forward gene mutation test, the human lymphocyte chromosome aberration test, and the rat hepatocyte unscheduled DNA synthesis test. Nonmutagenic in the rat micronucleus assay.

See also: Food and Drug Administration, US.

Relevant Websites

http://www.lotronex.com – GlaxoSmithKline, Prescribing Program for Lotronex®.

http://www.fda.gov – US Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER). Lotronex Information.

Love Canal

Michael A Kamrin

Introduction

Love Canal was an ordinary neighborhood in the City of Niagara Falls, New York until 25 years ago when it became the symbol of the dangers of hazardous wastes placed in and on the ground as a result of the boom in industrial activity during and after World War II. The national publicity devoted to this site focused public attention on the health threats to communities from buried hazardous wastes. As a result, Love Canal became the quintessential 'ticking time bomb' that citizens at contaminated sites around the country referred to when they asked the government for help with their problems.

The Love Canal incident brought to light a serious environmental problem that had not been taken care of by the environmental legislation passed in the 1970s that addressed air and water pollution as well as the future generation, transport and disposal of hazardous wastes. It was the catalyst for both federal and state legislation to address this gap. On the Federal level, it led to the enactment of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), also known as the Superfund Act, in 1980. This Act provided a mechanism for assessing and remediating the worst waste disposal sites across the United States and thus ensuring that there would be no more 'Love Canals'.

It was clear as soon as it was passed that CERCLA would address only the most serious sites and that there were literally thousands of other sites containing hazardous wastes that also needed to be dealt with. In the absence of federal resources for this task, a number of states passed their own legislation to identify, assess and remediate such sites. Activities undertaken under both the federal and state statutes revealed that hazardous wastes had been buried not only in industrially owned sites but also in municipal and private landfills. Indeed, some of these hazardous wastes were the result of citizen disposal practices. Thus, the dimensions of the problems that Love Canal uncovered were revealed to be larger than first thought and the resources needed to address them much greater than anticipated. Twenty-five years later, these problems persist and many sites still await remediation.

Love Canal

The neighborhood called Love Canal was named for a partially built canal that was abandoned late in the nineteenth century. The canal was used for a variety of purposes, mainly recreational, during the early twentieth century. However, by mid-century, it became the dumping grounds for wastes from a variety of sources, most importantly for chemical wastes produced by local industries, particularly Hooker Chemical Corporation which took title to the site in the 1940s. Intensive dumping of chemicals was carried out from ~1942 to1953 when the canal was covered over. It is estimated that Hooker disposed of ~40 million pounds of chemicals in the canal during this time.

As a result of the housing boom following World War II, the character of the Love Canal neighborhood changed from largely rural and agricultural to mainly residential. In light of this, the City of Niagara Falls was looking for sites for new school buildings at the time the Canal was covered over. After some negotiation, the City purchased the Love Canal site from Hooker Chemical for a nominal fee. A school was placed on a small part of the canal area and other parts of the site were used for the construction of roads. This, in turn, attracted more inhabitants and the number of homes adjacent to the Canal grew over time.

Soon after the Canal was first used as a disposal site, there were complaints about odors and chemical contamination. These complaints were ongoing and did not cease even after the disposal operations were discontinued. The postdisposal problems arose because buried chemicals were brought to the surface by natural percolation of liquids upwards in the soil as well as by disturbance of the soil during school and road construction. In addition to esthetic complaints, there were incidents of burns caused by contact with contaminated soil. This situation was exacerbated in 1976–77 because of much above normal rainfall. These rains raised the water table and brought more buried chemicals, as well as some buried drums, to the surface.

By early 1978, the problems at Love Canal had attracted the attention of the local media as well as the local member of congress. This led to sampling of the site and homes around the canal by the US Environmental Protection Agency (EPA) and the State of New York. These tests revealed the presence of benzene in indoor air and seepage of a number of chemicals off-site. These discoveries led to the conduct of preliminary health studies that indicated an increased rate of miscarriages in women living near the canal and identified several cases of congenital abnormalities in children from this same population.

Release of this information to the community resulted in citizen outrage and the formation of homeowner organizations to bring pressure for swift action to be taken. Soon afterwards, in early August, the New York State Commissioner of Health decided the data required the declaration of a health emergency and the recommendation that pregnant women and families with children under two years of age who were living adjacent to the Canal move from their homes.

In light of the organized public response to these recommendations, less than a week later the Governor of New York promised that the State would purchase all of the houses in the ring closest to the Canal; a total of almost 240 homes. A few months later remedial action was begun to reduce the threat of exposure from the canal to the remaining residents in the area. However, progress was slow and in 1979, the State Health Commissioner recommended that pregnant women and families with children under the age of 2, living in the ring of houses beyond those already vacated, temporarily relocate. In the face of continuing problems in controlling the chemicals in

the Canal, a few months later over 400 additional residents were temporarily relocated.

The concerns of the residents intensified in May, 1980 when the EPA announced that a study had found chromosome damage in 11 of 36 Love Canal residents tested. This study had serious scientific flaws but was widely publicized and became the focus of deep debate. The results of the chromosome study, as well as those from the studies of fetal outcomes, were evaluated by an expert panel which was established by the Governor of New York soon after the release of the chromosome damage reports. This expert group, known as the Thomas panel, came to the conclusion that no acute health effects related to exposure to the hazardous wastes had been established and that studies of chronic effects were inconclusive. Not surprisingly, the reactions to this report were mixed and reflected the positions staked out by the various actors in the Love Canal story, including a number of scientists.

Despite these findings, in October, 1980, President Carter signed a federal-state agreement for the purchase of the homes located in a ring just outside the homes that had previously been purchased by the State of New York. In December, 1980, the Superfund Act was signed into law and subsequently applied to Love Canal and thousands of other sites across the country. As part of the clean-up of the Love Canal site, the homes in the innermost ring closest to the landfill were razed and studies were conducted to decide on the habitability of the outer ring homes. The conclusion was that they were habitable and an authority was set up to rehabilitate and sell them. By 1996, almost all of these homes were sold and the neighborhood was renamed Black Creek Village.

Summary

From a societal perspective, probably the most important outcome of Love Canal was that it led to the enactment of national and state legislation, especially the federal Superfund Act, to deal with hazardous wastes that had been placed in the environment before stricter standards for waste disposal went into effect in the mid-1970s. The events at Love Canal were important because they set the tone for subsequent actions that were taken at contaminated sites around the country; in particular, these events revealed the potent role that citizen activism could play.

From a toxicological viewpoint, Love Canal illustrated the difficulties in assessing the relationship between chemical exposures and human health effects in an atmosphere of public activism and contentious policy debate. Instead of well-designed and peer-reviewed studies forming the scientific basis for assessing and managing the potential risks, preliminary and poorly designed studies served this purpose. In addition, data that might have been valuable in conducting better studies were lost in the heat of the events. Unfortunately, this pattern seems common as can be seen in looking at other notorious contamination incidents; for example, Times Beach, Missouri.

Love Canal also contributed strongly to the development of risk assessment methodologies since the Superfund Act required that determinations of clean-up levels be made based on a scientific assessment of data on the toxicity of contaminants found at the hazardous waste sites and the potential for exposure to those contaminants. In light of the limitations in available knowledge, new approaches had to be devised to answer the question of 'how clean is clean?' The methodology that was adopted married scientific data to value judgments about acceptable risk and margins of safety. This approach remains in use.

See also: Comprehensive Environmental Response, Compensation, and Liability Act, US; Silent Spring; Valley of the Drums.

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Lowest-Observed-Adverse-Effect Level *See* Levels of Effect in Toxicological Assessment

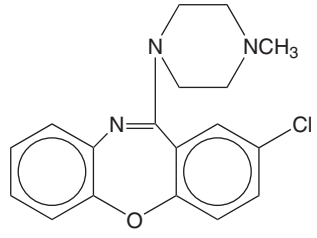
Lowest-Observed-Effect Level *See* Levels of Effect in Toxicological Assessment

Loxapine

F Lee Cantrell

This article is a revision of the previous print edition article by Douglas J Borys, volume 2, pp. 262–264, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 27833-64-3
- Synonyms: Loxapine hydrochloride; Loxapine succinate; 2-Chloro-11-(4-methylpiperazin-1-yl)dibenz[1,4]oxazepine; Oxilapine; Loxitane
- Chemical/Pharmaceutical/Other Class: Dibenzoxazepine antipsychotic
- Chemical Formula: $C_{18}H_{18}ClN_3O$
- Chemical Structure:



Uses

Loxapine is used to treat and control the psychotic symptoms of both acute and chronic schizophrenia. Other uses include treatment of dementia, anxiety neurosis, hostile/aggressive behavior, and psychotic depression.

Exposure Routes and Pathways

Loxapine is available in oral liquid, oral capsule, and injectable dosage forms. The principal exposure pathway is intentional ingestion by adults or accidental ingestion by children.

Toxicokinetics

Loxapine is readily but incompletely absorbed. Due to first-pass metabolism, oral bioavailability is 30% less than bioavailability after intramuscular injection. Peak blood levels occur 1 or 2 h after oral administration and 5 h after intramuscular injection. Loxapine is extensively metabolized in the liver through aromatic hydroxylation, *N*-demethylation, or *N*-oxidation. The metabolite amoxapine is active and marketed as an antidepressant. Loxapine is widely distributed throughout the body, including the central nervous system. The main metabolites are excreted both in the urine and feces, and 50% of a single oral dose is eliminated within 24 h. The mean half-life of oral loxapine is 4 h; the mean half-life of loxapine administered through intramuscular injection is 12 h.

Mechanism of Toxicity

The exact mechanism of action of loxapine is not known. It is thought to change the level of excitability in subcortical inhibitory areas of the brain by reducing the firing threshold of some polysynaptic neurons leading to seizure activity. It also appears to possess significant andrenergic and cholinergic blocking properties in overdose.

Acute and Short-Term Toxicity (or Exposure)

Animal

No specific information is available. Signs of toxicity are expected to include sedation, dullness, hypotension, respiratory depression, anorexia, colic, weakness, fever, icterus, restlessness, and seizures. Treatment consists of aggressive supportive care and gastric decontamination.

Human

Clinical signs of toxicity most frequently seen include sedation, coma, seizures, extrapyramidal effects, and rarely hypotension and cardiac arrhythmias. Coma and seizures may develop rapidly following an exposure to loxapine. Cardiac effects include prolonged QRS, Q–T intervals, and mild hypotension; however, the cardiac effects are less pronounced than those associated with tricyclic antidepressants. Anticholinergic effects, including dry mouth, blurred vision, and tachycardia, have been seen. Neuroleptic malignant syndrome has been reported after therapeutic use and acute intoxication. Hypokalemia has also been noted.

Chronic Toxicity (or Exposure)

Human

Adverse reactions following therapeutic use include sedation, dizziness, insomnia, agitation, tardive dyskinesia, dysphoria, dystonic reactions, tachycardia, syncope, anorexia, nausea, vomiting, constipation, diarrhea, and dyspepsia. The most frequently reported dystonic reactions include akathisia, stiff neck, stiff or protruding tongue, and tremor.

Clinical Management

Basic and advanced life-support measures should be utilized as needed. There is no antidote for loxapine exposure. In patients presenting within 1 h of ingestion, activated charcoal should be administered. Benzodiazepines are the drug of choice for seizures. Initial treatment of conduction disturbances should include electrolyte normalization and intravenous sodium bicarbonate. Antidysrhythmic class 1A agents should be avoided. Dystonic reactions respond well to intravenous benztropine or diphenhydramine. Oral therapy with diphenhydramine or benztropine should be continued for 2 days to prevent recurrence of the dystonic reaction. For patients with neuroleptic malignant syndrome, rapid external cooling, aggressive muscle relaxation with benzodiazepines or nondepolarizing neuromuscular blocking agents, and quality supportive care is the mainstay of therapy. Bromocriptine has been used in conjunction with other supportive measures. Hemodialysis and hemoperfusion have not been shown to be effective.

See also: Benzodiazepines; Diphenhydramine.

Further Reading

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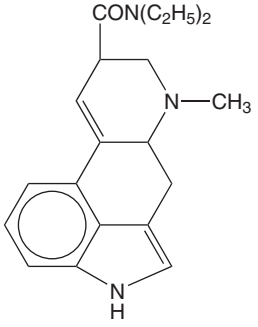
Mazzola CD, Miron S, and Jenkins AJ (2000) Loxapine intoxication: Case report and literature review. *Journal of Analytical Toxicology* 24: 638–641.

LSD (Lysergic Acid Diethylamide)

Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 50-37-3
- Synonyms: 9,10-Didehydro-*N*,*N*-diethyl-6-methylergoline-8b-carboxamide; Acid; Beast; Ben; Blotter; Blue caps; Blue drops; Brain buster; Brown caps; Cubes; Face melter; Ghost; Green caps; Hawk; Heavenly blue; Microdots; Paper acid; Pearly gates; Pink drops; Purple haze; Purple wedges; Royal Blue; Sunshine; Wedding bells; White lightning; Window Pane; Yellow caps; Yellow drops
- Chemical/Pharmaceutical/Other Class: Lysergamides
- Chemical Structure:



Uses

Lysergic acid diethylamide (LSD) is an illicit drug abused as a hallucinogen.

Exposure Routes and Pathways

The most common route of exposure is oral ingestion. Nasal insufflation or intravenous injection is also utilized.

Toxicokinetics

The absorption of LSD is described as rapid, with clinical effects within 15 min and peak concentrations within 60 min after ingestion. Protein binding is greater than 80% and the volume of distribution is $0.31\,kg^{-1}$. LSD is metabolized to inactive metabolites with less than 1% excreted unchanged. The drug penetrates into the central nervous system (CNS), concentrating in the visual brain areas and the reticular activating systems. The elimination half-life is $\sim$2–5 h.

Mechanism of Toxicity

The mechanism of action of LSD is incompletely understood. LSD's hallucinogenic effects are secondary to its ability to increase central serotonin activity. LSD also stimulates both D_1 and D_2 dopamine receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rats exposed to large doses of LSD have developed respiratory failure. Rabbits have also developed hyperthermia when large doses are administered.

Human

LSD produces distortion in perception. These changes in perception include sight, time, touch, odor, hearing, and sensation of body movement and image. These are usually identified by the intoxicated person as not real occurrences and may be considered pseudohallucinations. True hallucinations, which the individual believes are real, are less common. Other possible CNS effects include depersonalization, decreased ability to think and make judgments, and changes in mood and behavior. Patients are generally quiet and withdrawn, though aggression and bizarre behavior can occur. Acute panic attacks may occur, especially with unexpected use and less experienced users. Acute psychotic reactions can also occur. Seizures, hyperthermia, rhabdomyolysis, hypertension, hyperreflexia, tremors, anisocoria, hippus, and coma are associated with more severe LSD intoxication. Serotonin syndrome may occur.

Chronic Toxicity (or Exposure)

Animal

LSD has been studied as a model for schizophrenia in rats.

Human

Chronic toxic effects include flashbacks and psychosis. Flashbacks are the recurrence of the CNS changes associated with acute LSD use, which can occur up to 4 years after last use. Flashbacks occur in 15–77% of persons who use LSD and may be memory recall of the acute intoxication. Both mental and physiological stresses can precipitate flashbacks. LSD may cause chromosomal aberrations and increased risk of congenital abnormalities in the fetus when used during pregnancy.

In Vitro Toxicity Data

Leukocyte culture studies have demonstrated chromosomal breakage when exposed to LSD. Clastogenicity studies have also demonstrated suppression of mitosis. Other studies have shown LSD to have either no or only weakly mutagenic effects.

Clinical Management

The patient's airway, breathing, and circulation should be assessed and supportive care instituted as necessary. Many patients are anxious and respond to reassurance and a quiet, nonthreatening, nonstimulating environment. Benzodiazepines may be necessary for agitation or anxiety. Hyperthermia and seizures should be managed using standard therapy, a cool mist spray and fans for hyperthermia, and a benzodiazepine for seizures. Psychosis may require treatment with haloperidol.

See also: Benzodiazepines; Drugs of Abuse.

Further Reading

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Brown RT and Braden NJ (1987) Hallucinogens. *Pediatric Clinics of North America* 34: 341–347.

Kulig K (1990) LSD. *Emergency Medicine Clinics of North America* 8: 551–558.

Lubricating Oil *See* Oil, Lubricating

Lung *See* Respiratory Tract

Lye

Samantha E Gad

This article is a revision of the previous print edition article by Shayne C Gad, volume 2, pp. 265–266, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 1310-73-2 (Sodium hydroxide); CAS 1310-58-3 (Potassium hydroxide)
- Synonyms: Caustic potash; Caustic soda
- Chemical/Pharmaceutical/Other Class: Alkali

Uses

Lye is used in household drain cleaners, ammonia, automatic dishwasher detergents, Clinitest tablets, oven cleaners, and bleaches. It is also used in the manufacture of soaps and cleaners and in chemical synthesis.

Background Information

Lye generically refers to any strong alkali, usually sodium hydroxide or potassium hydroxide.

Exposure Routes and Pathways

Exposure to lye may occur via dermal, inhalation (as a mist or spray), or oral routes.

Mechanism of Toxicity

The mechanisms of toxicity for lye are those common to alkalis, saponification of fats and solubilization of proteins, corrosion, reduction, and protein denaturation. The severity of corrosion is determined by pH, viscosity, concentration, volume ingested, and contact time.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity of lye is essentially the same as of household bleaches. Lye has been shown to be a severe dermal and eye irritant.

Human

Lye is a strong eye, skin, and mucous membrane irritant and corrosive. Ingestion is followed by severe pain, vomiting, diarrhea, and collapse.

Clinical Management

Alkalis penetrate the skin slowly, making the extent of damage dependent on the duration of contact. Affected skin should be washed with running water until free of alkali as indicated by the disappearance of the 'soapy' feeling.

In cases of eye exposure, eyes should be washed with running water continuously for 15 min and then irrigated for 30–60 min with normal saline solution. Neutralizing agents should not be used.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists has a ceiling limit of $2.0\,mg\,m^{-3}$ and the US Occupational Safety and Health Administration has a permissible exposure limit (PEL), 8 h time-weighted average of $2.0\,mg\,m^{-3}$. In addition, the US National Institute for Occupational Safety and Health recommends a 15 min ceiling value of $2.0\,mg\,m^{-3}$ and an 'immediately dangerous to life or health' value of $10\,mg\,m^{-3}$. Other occupational permissible levels include Australia ($2.0\,mg\,m^{-3}$ peak limit), Federal Republic of Germany ($2.0\,mg\,m^{-13}$ short-term level, and $4.0\,mg\,m^{-3}$ for 5 min, eight times per shift), Sweden ($2.0\,mg\,m^{-3}$ ceiling limit), and the United Kingdom (10 min short-term exposure limit (STEL) of $2.0\,mg\,m^{-3}$.

See also: Alkalies; Corrosives.

Further Reading

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Relevant Websites

http://www.inchem.org – Alkalis. Poisons Information Monograph from the International Programme on Chemical Safety (IPCS INCHEM) System.

http://hpd.nlm.nih.gov – US National Library of Medicine, Household Products Database (search for sodium hydroxide and potassium hydroxide).

Lyme Disease

Michael A Kamrin

Introduction

Lyme disease is named after Lyme, Connecticut, where it was first identified in the late 1970s as the result of an investigation of a cluster of children with arthritis. The disease has now been found nationwide although it is mostly localized to the northeast, mid-Atlantic, and upper north-central regions of the United States. However, it has also been found in several northwestern counties of California. In 1999, over 16 000 cases were reported to the Centers for Disease Control and Prevention and over 90% of these occurred in northeastern, mid-Atlantic, and north-central states. The State of New York had the greatest number of cases from 1989 to 1998 while the incidence was highest in Connecticut.

In the early 1980s, the vector for the disease – black-legged ticks – and the causative agent – the bacterium *Borrelia burgdorferi* – were identified. These ticks, also known as deer ticks, are much smaller than ticks commonly found in pets and farm animals. Most often, they become infected after feeding on rodents, which serve as reservoirs for the *Borrelia* bacteria.

Lyme Disease

In general, the incubation period in humans after tick exposure is 7–14 days. The characteristic symptom seen in most infected individuals is a red, slowly expanding, 'bulls-eye' rash. This is accompanied by general malaise, fever, headache, muscle aches, and joint pain. If the infection is not treated, the exposed individual may develop arthritis, neurological symptoms; e.g., facial palsy, nerve, and/or brain inflammation; and, rarely, cardiac abnormalities.

Diagnosis is usually based on clinical signs and known, or putative, exposure. Serological testing may be performed to support the diagnosis and also to assess the severity of the disease. However, because antibodies may persist for months or years after successful treatment, serological endpoints cannot be used as markers of active disease.

Early disease is treated with antibiotics, such as doxycycline or amoxicillin, over a 3–4 week period. If the disease has progressed significantly, it may be necessary to administer intravenous antibiotics for four or more weeks. In some cases, there may be relapses and retreatment may be necessary. In a limited number of cases, Lyme disease may lead to chronic, disabling effects. Even more rarely, it may be fatal.

While Lyme disease is treatable, it can cause serious health effects and the best way to avoid these is prevention – minimizing the possibility of being infected by the deer tick. There are several ways to accomplish this. First is to recognize those areas that are likely to be tick infested and avoid them as much as possible. A second step is to use insect repellents, such as DEET, to discourage ticks from attaching to skin. Third is to understand that clothing can be an effective barrier if it is worn properly; e.g., pants tucked into socks or high rubber boots. A fourth and very important step is to check for ticks after being in areas where exposure is likely. The checks should be done soon after every possible exposure since it generally takes $\sim$36 h after a tick bite for infection to occur. Embedded ticks should be removed with tweezers and the area cleansed with an antiseptic.

In the mid-1990s, an effective vaccine against Lyme disease was developed. However, soon after it was brought to market there were claims that the vaccine caused serious side effects including a form of arthritis. While the merits of this claim were debated, sales of the vaccine decreased significantly and the manufacturer withdrew it from the market in 2002. Research into a new Lyme disease vaccine is continuing and a new vaccine may be in the market in the near future.

It has also been suggested that antibiotics be administered prophylactically to individuals who think they might have been exposed to the infectious agent. However, there is one serious drawback to this approach; it would likely expose a large number of individuals to antibiotics unnecessarily. This, in turn, could contribute to the development of antibiotic resistant organisms and thus compromise the health of others who depend on these same drugs. On balance, it appears that the risks of routine use of antibiotics outweigh the benefits.

Summary

Lyme disease is a serious problem in certain areas of the United States and can lead to serious long-term effects. However, preventive steps can significantly reduce the risks and the disease can be treated effectively, especially when detected early. No vaccine against Lyme disease is currently available but researchers are working at filling this gap.

See also: DEET (Diethyltoluamide); Public Health Service, US.

Further Reading

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Rahn DW and Evans J (eds.) (1998) *Lyme Disease*. Philadelphia, PA: American College of Physicians.

Relevant Website

http://www.cdc.gov – Centers for Disease Control and Prevention (2003) Lyme Disease Home Page. Centers for Disease Control, Atlanta, GA.

M

Mad Cow Disease *See* Bovine Spongiform Encephalopathy (Mad Cow Disease).

Magnesium

Russell Barbare

- Representative Chemicals: Magnesium sulfate (Epsom salts); Magnesium hydroxide (in suspension: milk of magnesia); Magnesium citrate
- Chemical Abstracts Service Registry Number: CAS 7439-95-4
- Chemical/Pharmaceutical/Other Class: Alkaline earth metal
- Chemical Formula: Mg^{2+}

Uses

The elemental form of magnesium is used in light metal alloys, some aspects of metallurgy, and in the production of precision instruments and flares. Many foods contain magnesium and vitamins are often supplemented with it. Magnesium sulfate may be used topically as a soak, internally as a laxative, or intravenously during pregnancy to control eclamptic seizures and uterine activity. Many antacids contain magnesium oxide or trisilicate as active ingredients.

Background Information

Magnesium is the most abundant divalent cation in cells, where it is essential for a wide range of cellular functions. Magnesium is the sixth most abundant metal on earth and dissolved magnesium constitutes 0.13% of seawater. It is found naturally only in the form of its salts. First obtained in metallic form in 1808, it is an essential nutrient necessary for human, animal, and plant health as it is an important component of red blood cells, a cofactor in over 300 cellular processes, and central to the chlorophyll molecule. The physiological role of magnesium was essentially ignored until recently. With the development of new technologies to measure the intracellular free concentration of magnesium ($[Mg^{2+}]_i$), the biologically important fraction, there has been a large increase of interest in the molecular, biochemical, physiological, and pharmacological functions of magnesium. Moreover, improved methods for assessing magnesium status in the clinic have contributed to the further understanding of magnesium regulation in health and disease. Magnesium deficiency is now considered to contribute to many diseases and the role for magnesium as a therapeutic agent is being tested in numerous large clinical trials. Specific clinical conditions in which magnesium deficiency has been implicated to play a pathophysiological role include hypertension, ischemic heart disease, arrhythmias, preeclampsia, asthma, and critical illness. There are two conditions where magnesium is now considered the therapeutic agent of choice, preeclampsia and torsades de pointes. Future research at the fundamental and clinical levels will lead to further increases in the understanding of how magnesium contributes to pathological processes and under what circumstances it should be used therapeutically.

Exposure Routes and Pathways

The primary route of exposure is ingestion. Secondary routes can include intravenous, ocular, or inhalation.

Toxicokinetics

Homeostasis of magnesium is tightly regulated and depends on the balance between intestinal absorption and renal excretion. Thirty-to-forty percent of ingested magnesium is absorbed from the gastrointestinal system, mostly by the small bowel. Most of the magnesium in the body is stored intracellularly or

in the skeleton; $<1\%$ is extracellular. In plasma, $\sim 65\%$ is in ionic form, with the rest being bound in proteins. The primary route of excretion is through the kidneys, but it is also excreted in sweat and breast milk. Various hereditary disorders of magnesium handling have been clinically characterized, and genetic studies in affected individuals have led to the identification of some molecular components of cellular magnesium transport.

Mechanism of Toxicity

Magnesium levels outside of the normal range alter cellular ion balances and activity, especially Ca^{2+} activity, which directly affects neural and muscular functions. One study found magnesium in relatively high amounts in about half of human colon cancers, but the relationship is unknown and animal studies have found that magnesium actually reduces sarcoma incidence in some nickel- and cadmium-induced tumors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute animal toxicity resembles acute human toxicity. A unique effect of magnesium when introduced in small amounts into the skin of animals has been called 'gas gangrene' or 'magnesiogenous pneumagranuloma'. Necrosis and tumor-like formation are caused by the production of hydrogen and magnesium hydroxide when metallic magnesium reacts with water of body fluids.

Human

Magnesium is a skin, eye, and pulmonary irritant. Inhalation of fumes can cause metal fume fever. Acute systemic toxicity, defined as serum concentrations $>2.8\,\mathrm{mEq\,l^{-1}}$, is almost always caused by both overingestion and reduced renal excretion together. Hypotension starts around $3\,\mathrm{mEq\,l^{-1}}$ and significant prolongation of cardiac intervals occurs between 4 and $6\,\mathrm{mEq\,l^{-1}}$. Higher serum levels lead to coma and paralysis and heart stoppage occurs around $14–15\,\mathrm{mEq\,l^{-1}}$.

Chronic Toxicity (or Exposure)

Animal

Chronic animal toxicity resembles human toxicity.

Human

There is no hormonal regulation of systemic magnesium levels, so toxic effects occur frequently with both hypermagnesemia and hypomagnesemia but systemic toxicity is rare in adults unless there is impaired renal function. Hypomagnesemia is most commonly associated with alcoholism or small bowel disease and is often accompanied by other electrolyte deficiencies, mostly hypokalemia (K deficit) and hypocalcemia (Ca shortage). The symptoms most commonly include tremor, neuromuscular irritability, and widening of the QRS complex. Human hypermagnesemia is generally caused by either increased ingestion or renal impairment. The symptoms of moderate increases include hypotension, sedation, and somnolence. The possible association between the risk of ovarian cancer and the levels of calcium and magnesium in drinking water from municipal supplies was investigated in a matched case–control study in Taiwan. The results of the study show that there may be a significant protective effect of magnesium intake from drinking water on the risk of ovarian cancer death. Another study has produced data supporting a protective role of higher intake of magnesium in reducing the risk of developing type 2 diabetes, especially in overweight women.

Clinical Management

Hypomagnesemia is treated initially with oral, intramuscular, or intravenous administration of magnesium salts. Immediate control of the symptoms of acute hypermagnesemia is obtained with doses of intravenous calcium repeated hourly but extreme toxicity may require cardiac support or mechanical ventilation. Calcium gluconate and calcium chloride can also be administered as antidotes. Serum levels are lowered by reducing intake and by normal methods of excretion, with diuretics given to patients with normal renal function. Other accompanying electrolyte imbalances should be treated concurrently, followed by treatment of the condition(s) that lead to the imbalances.

Environmental Fate

Elemental magnesium oxidizes and joins the natural environmental reserve.

Ecotoxicology

Magnesium and its compounds are not significantly ecotoxic.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average, is 10 mg m^{-3}.

See also: Calcium Channel Blockers; Metals; Vitamin A; Vitamin D; Vitamin E.

Further Reading

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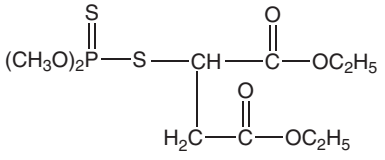
Relevant Website

http://ods.od.nih.gov – US National Institutes of Health (NIH) Magnesium (from NIH's Office of Dietary Supplements).

Malathion

Kevin N Baer

- Chemical Abstracts Service Registry Number: CAS 121-75-5
- Synonyms: *O,O*-Dimethyl-*S*-(1,2-dicarbethoxyethyl)phosphorodithioate; Chemathion; Karbophos; Cythion; Malaspray; Malathiozol
- Chemical Class: Organophosphorus insecticide
- Chemical Structure:



Uses

Malathion is an insecticide and acaricide for control of mosquitoes, household insects, and human head and body lice.

Exposure Routes and Pathways

Poisonings have occurred mainly from accidental or intentional ingestion, although dermal exposure has resulted in systemic symptoms.

Toxicokinetics

Malathion is absorbed through the skin, lungs, and gastrointestinal tract. However, skin absorption is fairly low. Most organophosphate insecticides require activation by oxidation of the P = S bond to the more toxic P = O compound by microsomal enzymes of the liver and other organs, including the brain. However, the carboxyethyl ester groups in malathion are rapidly hydrolyzed by malathion esterases. This action effectively detoxifies malathion and is the reason for the relatively low mammalian toxicity compared with many other organophosphates. The liver and kidney are primary sites of distribution and reflect the rapid detoxification and clearance of malathion. Malathion is rapidly excreted in the urine ($\geq$90%) after 24 h. The half-life following intravenous administration in human volunteers was approximately 3 h.

Mechanism of Toxicity

Malathion is converted to the toxic oxygen analog (replacement of covalent sulfur with oxygen) by microsomal enzymes. The oxygen analog then inhibits acetylcholinesterase as do other organophosphates. As a result, acetylcholine accumulates at cholinergic nerve endings with subsequent hyperstimulation of postsynaptic cells.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral and dermal LD_{50} values in rats and mice range from 1 to 12 g kg^{-1}. Domestic animals exhibit similar signs of cholinergic toxicity as seen in humans. Chickens may be somewhat more sensitive to acute toxicity from malathion exposure, but delayed neurotoxicity is not caused by this agent.

Human

Malathion exhibits very low toxicity compared with other organophosphates. The lethal dose in a 70-kg man is estimated to be $\geq$60 g. However, commercial preparations of malathion may contain organophosphate impurities that can lead to increased toxicity by interference with the detoxification systems. Signs and symptoms of severe malathion poisonings are similar to those of parathion and other organophosphates. They include an increase in secretions, gastrointestinal cramps, diarrhea, urination, slow pulse, uncontrollable muscle twitches followed by muscle weakness, paralysis, confusion, dizziness, ataxia, cyanosis, convulsions, and coma. However, life-threatening respiratory or cardiac involvement typical in parathion poisoning is usually not associated with malathion.

Chronic Toxicity (or Exposure)

Animal

As with most organophosphorus insecticides, acute toxicity is predominant. However tolerance to repeated exposures can occur. The no-observed-adverse-effect level (NOAEL) established from a rabbit developmental toxicity study was 50 mg kg^{-1} day^{-1} based on maternal toxicity (i.e., reduced body weight gain). Developmental toxicity studies were negative in rats and rabbits. A two-generation reproductive toxicity study in rats showed no increased sensitivity in pups compared to dams. Repeated exposure to malathion does not cause delayed neurotoxicity. The NOAEL of 2.4 mg kg^{-1} day^{-1} was established based on plasma cholinesterase inhibition in a long-term dosing study in rats.

Human

Generally, the onset and course of toxicity is rapid. However, a number of poisoning cases have shown prolonged symptoms including weakness of proximal limb muscles, cranial nerve palsies, and respiratory depression. As with other organophosphorus anticholinesterases, it is possible to accumulate acetylcholinesterase inhibition with repeated exposures, leading to signs of acute cholinergic toxicity.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be moved to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion, to be the most effective. Initial management of acute toxicity is the establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered, followed by 2–4 mg (in adults) or 0.015–0.05 mg kg^{-1} (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Exposure Standards and Guidelines

The acute population adjusted dose is 0.5 mg kg^{-1} day^{-1}. The chronic population adjusted dose is 0.024 mg kg^{-1} day^{-1}.

See also: Carboxylesterases; Cholinesterase Inhibition; Neurotoxicity; Organophosphates; Pesticides; Veterinary Toxicology.

Further Reading

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Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Malathion.
http://www.epa.gov – United States Environmental Protection Agency.

Male Reproductive System *See* Reproductive System, Male.

Mancozeb

Mona Thiruchelvam

- Chemical Abstracts Service Registry Number: CAS 8018-01-7
- Synonyms: Manganese–zinc ethylenebis(dithiocarbamate); Carbamic acid ethylenebis(dithio) manganese–zinc complex; Dithane; Manzeb; Manzate; Zimaneb
- Chemical/Pharmaceutical/Other Class: Ethylene(bis)dithiocarbamate
- Chemical Formula: $C_4H_6N_2S_4 \cdot Mn \cdot Zn$
- Chemical Structure:

$$[-SCSNHCH_2CH_2NHCSSMn-]_x(Zn)_y$$

Uses

Mancozeb is an ethylene(bis)dithiocarbamate fungicide. Mancozeb is classified as a contact fungicide with preventive activity. It is widely used to control fungal diseases in conifer and fir trees. It is also used to control blight in potatoes. It is also used to protect many other fruit, vegetable, nut, and field crops against a wide spectrum of fungal diseases. It is also used for seed treatment of cotton, potatoes, corn, safflower, and cereal grains.

Mancozeb is available as dusts, liquids, water-dispersible granules, wettable powders, and as ready-to-use formulations. It is commonly found in combination with maneb and zineb.

Exposure Routes and Pathways

Exposure routes and pathways to mancozeb are similar to the other commonly used ethylene(bis)-dithiocarbamates, maneb. Mancozeb has been shown to cross sensitize with zineb and maneb. Inhalation exposure can lead to upper respiratory tract irritation. Ingestion of mancozeb can lead to nausea, dizziness, headache and diarrhea. Severe overexposure can lead to convulsions and coma.

Toxicokinetics

The absorption and metabolism of mancozeb is similar to maneb. Mancozeb does not accumulate at high levels in most organs due to its rapid turnover rate. In experiments where rats were dosed with ^{14}C-mancozeb repeatedly for 7 days and sacrificed 1 day after the last dose, radioactivity was detected in various organs, with highest levels found in the liver, followed by the kidney and thyroid glands, with traces found in all other organs.

Mechanism of Toxicity

Mancozeb has been classified as a contact fungicide with preventive activity. It inhibits enzyme activity in fungi by forming a complex with metal-containing enzymes including those that are involved in the production of ATP.

Mancozeb has effects on various organ systems. Its primary mechanism of toxicity is via skin contact, leading to contact dermatitis and dermal sensitization. Mancozeb has also been shown to have teratogenic and reproductive effects. Mancozeb exposure also alters the reproductive and endocrine structures, leading to decreased fertility. Animals orally exposed to mancozeb showed thyroid hyperplasia, probably via its ability to inhibit the synthesis of thyroxin. Additionally, mancozeb exposure produces neurotoxicity via yet an unknown mechanism.

Similar to maneb, mancozeb also has chelating properties, allowing it to possibly interfere with a number of enzyme systems that contain metals, such as zinc, copper, and iron (e.g., dopamine β-hydroxylase).

Acute and Short-Term Toxicity (or Exposure)

The acute toxicity of mancozeb is rather low both in humans and experimental animals. Thus acute poisoning is highly unlikely unless large amounts are ingested. Mancozeb is slightly toxic via the dermal route. Contact with mancozeb leads to inflammation and/or irritation of the skin, eyes, and respiratory tract. Acute exposure to mancozeb may lead to effects such as hyperactivity, incoordination, loss of muscular tone, nausea, vomiting, diarrhea, loss of appetite, weight loss, drowsiness, slowed reflexes, and respiratory paralysis.

Animal

In general, mancozeb is not very toxic acutely unless high levels of exposure occur. The acute LD_{50} for mancozeb is $4500\,mg\,kg^{-1}$ in laboratory animals. The acute dermal LD_{50} is greater than $5000\,mg\,kg^{-1}$ in rodents. Dermal exposure to mancozeb leads to mild irritation to the skin. Exposure to the eye also leads to moderate irritation. Inhalation of mancozeb leads to irritation of the respiratory tract, with LC_{50} of greater than $5.14\,mg\,l^{-1}$.

A single exposure to mancozeb to relatively high doses at day 11 of gestation produced substantial malformations in the surviving animals. The malformations observed were cleft palate, hydrocephaly, and other serious defects. There was also an increase in the rate of resorption.

Human

Since the acute toxicity of mancozeb is relatively low as is with most dithiocarbamates, acute intoxication in humans is unlikely to occur unless large amounts are ingested. Mostly mancozeb is known for its irritant and allergic potential in occupational exposures. Skin irritation and sensitization has been studied in humans and have shown mild erythema and itching.

Chronic Toxicity (or Exposure)

Animal

There is limited information regarding the chronic toxicity of mancozeb. It has been indicated that mancozeb has low toxicity in most experimental animals. Its major metabolite, ethylenethiourea (ETU), has been shown to produce carcinogenic and teratogenic effects in laboratory animals at high dose levels.

Studies in dogs and mice indicate that mancozeb does not have carcinogenic effects; however, in rats there was an increase in thyroid tumors. The tumors as well as the inhibition of thyroid function due to these tumors are thought to be due to its metabolite, ETU.

Inhalation exposure of rats to mancozeb, exposed everyday for 4 months indicated an increase in irritation of the mucous membrane of the upper respiratory tract and concentration-related nonspecific changes to the liver and kidneys. Exposure was in the form of dispersed aerosols at concentrations ranging from 2 to $135\,mg\,m^{-3}$. At the lower concentrations, there were no observable effects. In animals exposed repeatedly to high doses of mancozeb (dust) equivalent to 150–250 times the acceptable exposure limit (AEL), reduced body weight, inflammation of the lungs, and abnormal thyroid function were observed.

Toxic effects in animals from repeated ingestion of high doses include reduced body weight and thyroid effects. Increased incidences of thyroid tumors and ocular lesions (retinopathy) were observed in rats administered 750 ppm (equivalent to $\sim 35\,mg\,kg^{-1}\,day^{-1}$) of mancozeb in their diet for 2 years. This compound is considered to show weak carcinogenic activity. Tests in some animals indicate that the compound may produce embryo and fetal toxicity, but only at maternally toxic doses. Multigeneration studies in animals demonstrate no reproductive toxicity. Although there have been isolated reports in the scientific literature of mutagenic activity of mancozeb, in general mancozeb is not genotoxic in animals or in cell cultures. Mancozeb has not been tested for heritable gene mutation. It has been shown to exert a dose-dependent adverse effect to gonads of male and female rats, with reproductive and endocrine structures being affected leading to decreased fertility. The exposure paradigm utilized here was twice a week for 4.5 months. Mancozeb also has been shown to produce teratogenic effects, with gross malformations observed in surviving rats of exposed dams.

ETU, a breakdown product and a minor metabolite of mancozeb, was shown to induce liver tumors in mice but not in rats or hamsters, and caused thyroid tumors in rats. ETU is not genotoxic. ETU has been categorized as a probable human carcinogen by the International Agency for Research on Cancer and as group B carcinogen by the National Toxicology Program. At sufficiently high doses, ETU also causes birth defects in laboratory animals.

Human

Exposure of mancozeb to humans can occur via absorption through the gastrointestinal tract, absorption through the skin or lungs. Human exposure to mancozeb, similar to maneb, has been calculated for

the population of the United States on the basis of estimated consumption of dietary residues of ETU in treated crops. Please refer to the maneb entry for more specifics on mancozeb human toxicity.

Most human exposure to mancozeb is via occupational exposure. Cases of diffuse erythema and eczematoid dermatitis have been observed among agricultural workers. Overexposure to mancozeb by skin contact may initially include skin irritation with discomfort or rash. The compound has been infrequently associated with skin sensitization in humans. Significant skin permeation and systemic toxicity after contact appears unlikely. Eye contact may initially include eye irritation with discomfort, tearing, or blurring of vision. Based on animal studies, long-term exposure to high levels of mancozeb may cause abnormal thyroid function. Individuals with preexisting diseases of the thyroid may have increased susceptibility to the toxicity of excessive exposures.

In Vitro Toxicity Data

In vitro systems have been developed to try and understand the mechanism of action of mancozeb, similar to other dithiocarbamates. The genotoxic, cytotoxic, and neurotoxic effects of mancozeb have been studied using a variety of primary cultures as well as cell-lines.

Clinical Management

Mancozeb can be absorbed into the body by inhalation, though the skin, and by ingestion.

If swallowed, large amounts of water should be ingested, only if person is conscious, to dilute the concentration of the compound and a physician should be called immediately. Vomiting can also be induced. Upon inhalation exposure, the exposed individual should be removed to fresh air, away from the contamination site. If skin contact occurs, all contaminated clothing should be removed and the area exposed should be washed with copious amounts of water and soap. If the product is present in the eyes, large amounts of water should be used to flush the eye for at least 15 min.

Environmental Fate

Mancozeb is generally not active in the soil. It rapidly degrades in the soil into numerous secondary products, principally ETU and eventually CO_2. Plants however can absorb ETU. Because it degrades so quickly, very little mancozeb gets adsorbed by the soil and its breakdown products are highly soluble and do not get adsorbed to soil particles.

Its persistence is very low in soil. One study recovered only 1.16% of mancozeb 7 days after application to silt loam soils, while the half-life was measured as only 3 days in fine sand. Lots of soil microorganisms readily break down mancozeb.

Ecotoxicology

Mancozeb is generally of low toxicity to most wildlife. It is practically nontoxic to birds and honey bees. It has a relatively high toxicity to fish. The 48 h LC_{50} for goldfish is $9\,mg\,kg^{-1}$, and for rainbow trout it is $2.2\,mg\,kg^{-1}$.

Mancozeb has been shown to reduce the population of soil organisms, and in soil nitrification has been reported at concentrations ranging from normal to 10 times the normal field application rates. These changes have tended to be temporary and reversed within 3 months.

Mancozeb is toxic to some plants such as marigold at normal field application rates. Some genetic effects were seen in onion cells exposed to mancozeb.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration: $5\,mg\,m^{-3}$ ceiling.
- American Conference of Governmental Industrial Hygienists: $5\,mg\,m^{-3}$ time-weighted average (TWA).
- National Institute for Occupational Safety and Health: $1\,mg\,m^{-3}$ recommended TWA; $3\,mg\,m^{-3}$ recommended short-term exposure limit.
- Threshold limit value: 5 mg (Mn) m^{-3}.

Miscellaneous

Mancozeb is a grayish-yellow powder with a musty odor, which is practically insoluble in water as well as most organic solvents. It is a polymer of maneb combined with zinc. While it is relatively stable and noncorrosive under normal, dry storage conditions, it is decomposed at high temperatures by moisture and by acid. Mancozeb may produce flammable products upon decomposition. It is also unstable in acidic conditions.

See also: Dithiocarbamates; Maneb; Pesticides.

Further Reading

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Maneb

Mona Thiruchelvam

- Chemical Abstracts Service Registry Number: CAS 12427-38-2
- Synonyms: Manganese ethylenebis(dithiocarbamate); Ethylene bis(dithiocarbamic acid)-manganese salt; Farmaneb; Manesan; Manex; Manzate; Nereb; Newspor
- Chemical/Pharmaceutical/Other Class: Ethylene(bis)dithiocarbamate
- Chemical Formula: $C_4H_6N_2S_4 \cdot Mn$
- Chemical Structure: $[–SCSNHCH_2CH_2NHCSS-Mn–]_x$

Uses

Maneb is an ethylene(bis)dithiocarbamate fungicide used in the control of early and late blights on potatoes, tomatoes and many other diseases on various fruits, vegetables, field crops, and ornamentals. Maneb has been shown to be effective on a wider spectrum of fruit, vegetable, and turf diseases caused by fungi compared to other fungicides. It is available as granular, wettable powder, flowable concentrate, and ready-to-use formulations.

Maneb is also used for the protection of wheat because of its growth inhibition properties and in the plastics and rubber industries as accelerators and catalysts.

Exposure Routes and Pathways

Exposure to maneb can occur via several routes, including dermal, oral, and inhalation. Skin contact with maneb can result in contact dermatitis and in some cases lead to sensitization. Besides dermal exposure, maneb can also be absorbed when inhaled or ingested.

Occupational exposure during manufacturing, mixing/loading, spraying, and harvesting to this compound can occur via dermal deposition and inhalation. Numerous studies have examined the effects of long-term occupational exposure to maneb at various steps in the manufacturing and application process of maneb. These studies have led to the implementation of preventive measures to reduce occupational exposure to maneb. Human exposure can also occur via consumption of treated crops. Residues of maneb and its metabolites have been found in and/or on treated crops. The residue levels change during storage, processing, and cooking due to environmental factors and during these processes the parent compound may be transformed.

Toxicokinetics

Maneb is absorbed via the skin, mucous membrane, respiratory, and gastrointestinal tracts. Its absorption through the skin and the gastrointestinal tract are poor due to its metal-complexed state. Maneb is metabolized to ethylene thiourea (ETU), ethylenediamine, ethylenebisisothiocyanate sulfide (EBIS), and carbon disulfide. ETU is further broken down to molecules that can be incorporated into compounds such as oxalic acid, glycine, urea, and lactose. Due to its rapid metabolism, maneb does not accumulate at high levels in most organs. Most of what is excreted in the urine and feces is in the form of the metabolite, ETU, with very little of the parent compound being eliminated unchanged.

Mechanism of Toxicity

Maneb has effects on various organ systems. Its primary mechanism of toxicity is via skin contact, leading to contact dermatitis, erythema, and even dermal sensitization. Maneb has also been shown to have teratogenic and reproductive effects. Exposure to pregnant animals has been shown to have adverse effects on the fetus. Maneb exposure has also been

shown to alter the reproductive and endocrine structures, leading to decreased fertility. Animals orally exposed to maneb showed thyroid hyperplasia, probably via its ability to inhibit the synthesis of thyroxin. Additionally, maneb exposure produces neurotoxicity via yet unknown mechanism. Humans exposed to maneb show signs of parkinsonism with tremors and slowed movement and gait, developing after years of unprotected handling of exceptionally large amounts of this compound.

Maneb possesses chelating properties, allowing it to possibly interfere with a number of enzyme systems that contain metals such as zinc, copper, and iron (e.g., dopamine β-hydroxylase). It is also capable of inhibiting sulfhydryl-containing enzymes and some other enzyme systems involved in glucose metabolism.

Acute and Short-Term Toxicity (or Exposure)

The acute toxicity of maneb is rather low, and thus acute intoxication is unlikely to occur.

Maneb is practically nontoxic by ingestion. Via the dermal route, it is slightly toxic. Contact with maneb leads to inflammation and/or irritation of the skin, eyes, and respiratory tract. Acute exposure to maneb may lead to effects such as hyperactivity, incoordination, loss of muscular tone, nausea, vomiting, diarrhea, loss of appetite, weight loss, drowsiness, slowed reflexes, and respiratory paralysis.

Animal

In general the acute oral and dermal toxicity of maneb for most mammals is relatively low. The acute oral LD_{50} for rats is $>5000\,mg\,kg^{-1}$. The acute dermal LD_{50} for rabbits is $>5000\,mg\,kg^{-1}$ and for rats is $>10\,000\,mg\,kg^{-1}$. It is a moderate skin and eye irritant.

Rats exposed to maneb produced dose-dependent signs of decreased movement, disturbances of coordination, lack of appetite, and general weakness. Teratogenic and embryogenic toxicity has been observed with single exposures to maneb. In rats given a single dose of $770\,mg\,kg^{-1}$ maneb on the 11th day of gestation, early fetal deaths occurred. Fetal abnormalities of the eye, ear, body, central nervous system, and musculoskeletal system were seen in rats given this single dose. In mice a single oral toxic dose of $1420\,mg\,kg^{-1}$ during gestation caused toxicity to the fetus. Relatively high acute doses of maneb are required to observe adverse consequences.

Human

Since the acute toxicity of maneb is relatively low as is with most dithiocarbamates, acute intoxication in humans is unlikely to occur. A case was reported where a 62-year-old man suffered acute kidney insufficiency following maneb application; however, the precise contribution of maneb exposure was unclear as the patient had other health complications.

Maneb is primarily known for its irritant and allergic potential in occupational exposures. Skin irritation and sensitization has been studied in humans: mild erythema and itching are common.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to maneb has been related to reproductive, embryotoxic teratogenic, and neurotoxic effects. Although the toxicity associated with maneb exposure is low, it has been shown that in combination with other toxicants such as metals, other fungicides and herbicides the effects of maneb may be more pronounced, leading to more severe deficits.

Rats fed maneb for 2 years at a dose of $12.5\,mg\,kg^{-1}$ showed no adverse effects; however, when fed with $67.5\,mg\,kg^{-1}$ maneb for only 97 days, rats showed reduced growth rate and increased thyroid weight. Dogs treated orally with $200\,mg\,kg^{-1}\,day^{-1}$ maneb for 3 or more months developed tremors, lack of energy, gastrointestinal disturbances, and incoordination. Additionally, spinal cord damage was observed. Rats exposed to $1500\,mg\,kg^{-1}\,day^{-1}$ for 10 days showed evidence of weight loss, weakness of hind legs, and increased mortality.

Inhalation exposure to maneb in rats produced irritation to the upper respiratory tract, and led to nonspecific changes to the liver and kidneys.

Chronic exposure to maneb also affects reproductive abilities. Rats fed maneb for 3 months before mating showed decreased fertility, and changes to reproductive and endocrine structures.

Teratogenic effects of maneb are observed at relatively high levels of exposure. Progeny of albino rats treated with either 700 or $1400\,mg\,kg^{-1}$ maneb twice a week for 4.5 months showed congenital deformities in the caudal vertebrae, palates, limbs, and tail. However, in the mouse the teratogenic effects of maneb exposure were much milder, with almost no deformities observed.

Little or no mutagenic potential has been detected in any assays with maneb.

Most dithiocarbamates have neurotoxic effects, including maneb. Rats exposed orally to maneb twice a week for 4 months at doses of 350 and $1750\,mg\,kg^{-1}$ produced high mortality and paresis in the hind limb progressing to complete paralysis. Exposure to maneb in combination with some

known dopaminergic neurotoxicants (e.g., MPTP and paraquat) has been shown to potentiate changes to the dopaminergic system even though exposure to maneb alone showed no significant alterations. In combination with these other toxicants, signs reminiscent of Parkinson's disease have been observed.

Human

Exposure of maneb to humans can occur via absorption through the gastrointestinal tract, and through the skin or lungs. Human exposure to maneb (and other ethylenebisdithiocarbamates) has been calculated for the population of the USA on the basis of estimated consumption of dietary residues of ETU in treated crops. Upper and lower limits of exposure have been assigned by the US EPA. Food residues have been detected and usually are analyzed as a collective level because analysis is accomplished by measuring carbon disulfide levels. Residues are regularly detected in fruit and vegetables, but mostly at levels below the maximum residue level. However, repeated exposure via ingestion can lead to a chronic exposure state, potentially leading to cumulative toxic effects.

Most human exposure to maneb is via occupational exposure. Cases of diffuse erythema and eczematoid dermatitis have been observed among agricultural workers. Studies on maneb production workers showed elevated levels of ETU in the urine and high blood levels of manganese. Very slight alterations to thyroid function were observed.

In Vitro Toxicity Data

In vitro systems have been developed to try and understand the mechanism of action of maneb. In particular, the mechanism of toxicity of maneb on the central nervous system using synaptosomal and mitochondrial preparations from brain tissue has been utilized. These studies have shown that maneb has adverse effects on the dopaminergic system, via mechanisms that relate to mitochondrial inhibition and altered neurotransmitter uptake. The genotoxic, cytotoxic, and neurotoxic effects of maneb have been studied using a variety of primary cultures as well as cell lines, including human lymphocytes. As noted above, maneb has little mutagenic potential.

Clinical Management

The extent of exposure will determine the initial treatment. On skin contact, contaminated clothing should be removed immediately followed by washing contaminated skin with soap and water to remove the chemical from the body. Similarly, if exposure to eyes occurs, large amounts of water or isotonic saline for at least 15 min should be used to flush the eye, occasionally lifting upper and lower lids.

If inhalation exposure occurs, the person should be removed from the exposure area to an area with fresh air. If needed, rescue breathing should be administered and medical attention sought immediately.

Upon ingestion, vomiting should be induced in the conscious patient. Activated charcoal should be administered to adsorb the remaining fungicide, followed by a sodium or magnesium cathartic.

Environmental Fate

Maneb has low persistence, with a reported field half-life of 12–36 days. It is readily transformed to ETU, which is much more persistent. Maneb strongly binds to most soils and is not highly soluble in water; therefore, it is not very mobile. It therefore does not represent a significant threat to groundwater. However, its breakdown product, ETU, may be more mobile. Maneb breaks down under both aerobic and anaerobic soil conditions. In one particular study, it was shown that maneb does not leach below the top 5 in. of soil.

Maneb degrades very quickly in water, with a half-life less than 1 h. Its main breakdown product is ETU. Significant amounts of ETU have been found in vegetables treated with maneb. Vegetables such as spinach, carrots, and potatoes that are treated with maneb after harvest produce a significant amount of ETU in the cooking process. Washing the vegetables or fruits before cooking or eating eliminated a majority of the residues.

Ecotoxicology

Maneb is practically nontoxic to birds. A 5 day dietary LC_{50} for maneb in bobwhite quails and mallard ducklings is greater than 10 000 ppm.

Maneb is however highly toxic to fish and other aquatic species. The 96 h LC_{50} for maneb is 1 mg l^{-1} in bluegill sunfish. The reported 48 h LC_{50} is 1.9 mg l^{-1} in rainbow trout and 1.8 mg l^{-1} in carp. Maneb-treated crop foliage may also be toxic to livestock.

Exposure Standards and Guidelines

- OSHA ceiling limit is 5 mg m^{-3}.
- ACGIH TWA is 1 mg m^{-3} (NIOSH recommended TWA).
- NIOSH recommended STEL is 3 mg m^{-3}.

- Mine Safety and Health Administration (MSHA) Standard air ceiling concentration is 5 mg (Mn) m^{-3}.
- Occupational Safety and Health Administration (OSHA) permissible exposure limit (general industry, construction, shipyards, federal contractors) ceiling concentration is 5 mg (Mn) m^{-3}.

Miscellaneous

Maneb is a yellow powder with a faint odor. It is a polymer of ethylenebisdithiocarbamate units linked with manganese. It is highly insoluble. Its water solubility is 6 mg l^{-1} and is practically insoluble in common inorganic solvents.

See also: Dithiocarbamates; Manganese.

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Manganese

Shayne C Gad

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- Chemical Abstracts Service Registry Number: CAS 7439-96-5
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Mn^{2+}

Uses

Manganese is used in ceramics, glass, dyes, dry-cell batteries, and special high-carbon steels. It is also added to fertilizers and animal food. Potassium permanganate is used as an oxidizing agent, and several antioxidant drugs now under development incorporate manganese in an organic matrix. Manganese is an essential trace element, and its concentrations are highest in tissues rich in mitochondria, where it forms stable complexes with ATP and inorganic phosphate. Manganese functions as a constituent of metalloenzymes and an activator of enzymes.

Exposure Routes and Pathways

Ingestion is the primary exposure pathway for the general population; sources of exposure include grains, nuts, fruits, and tea. Inhalation is a significant exposure pathway in industrial settings. Air and water pollution are minor sources in most areas. Manganese is a ubiquitous constituent in the environment, occurring in soil, air, water, and food. Thus, all humans are exposed to manganese, and manganese is a normal component of the human body. Food is usually the most important route of exposure for people, with typical daily intakes of 2.5–5 mg day^{-1}.

Toxicokinetics

Less than 5% of ingested manganese is absorbed from the gastrointestinal tract. Manganese is carried in blood serum by a β-globulin, which may be specific for this metal. Manganese is a cofactor for enzymes related to synthesis of cholesterol and also fatty acids. It is necessary for phosphorylation reactions. In some cases it can substitute for magnesium. Manganese is excreted in the bile, but systematic loads are slowly cleared.

Mechanism of Toxicity

Brain extracellular concentrations of amino acids and divalent metals (e.g., manganese) are primarily regulated by astrocytes. Adequate glutamate homeostasis is essential for the normal functioning of the central nervous system (CNS), for example, glutamate is important for nitrogen metabolism and, along with aspartate, is the primary mediator of the excitatory

pathways in the brain. Similarly, the maintenance of proper manganese levels is important for normal brain functioning. *In vivo* and *in vitro* studies have linked increased manganese concentrations with alterations in the content and metabolism of neurotransmitters, for example, dopamine, γ-aminobutyric acid, and glutamate. Rat primary astrocytes exposed to manganese display decreased glutamate uptake, thereby increasing the excitotoxic potential of glutamate. Furthermore, decreased uptake of glutamate has been associated with decreased gene expression of glutamate–aspartate transporter in manganese-exposed astroctyes. Other studies suggest that attenuation of astrocytic glutamate uptake by manganese may be a consequence of reactive oxygen species generation. These data suggest that excitotoxicity may occur due to manganese-induced altered glutamate metabolism, representing a proximate mechanism for manganese-induced neurotoxicity.

Acute and Short-Time Toxicity (or Exposure)

Human

Available human toxicity data are limited to the industrial setting, where adverse health effects have resulted from inhalation of manganese (primarily as manganese dioxide). Inhalation of particulate manganese compounds such as manganese dioxide (MnO_2) or manganese tetroxide (Mn_3O_4) can lead to an inflammatory response in the lung.

Acute inhalation exposure produces manganese pneumonitis; the incidence of respiratory disease among exposed workers is higher than that of the general population.

Chronic Toxicity (or Exposure)

Human

In workers with chronic inhalation exposure, iron deficiency and liver cirrhosis are commonly observed. Chronic inhalation exposure also affects the CNS, resulting in Parkinsonian-like symptoms. Mental aberrations are also observed. The psychiatric disturbance has been called 'manganese madness'. Symptoms include confusion, unusual behavior, and sometimes hallucinations. Apathy, difficulty with speech, and loss of balance are most common. Other symptoms include difficulty with fine motor movement, anxiety, and pain. Manganese intoxication can result in a syndrome of parkinsonism and dystonia. If these extrapyramidal findings are present, they are likely to be irreversible and may even progress after termination of the exposure to manganese. Clinical features are usually sufficient to distinguish these patients from those with Parkinson's disease. The neurological syndrome does not respond to levodopa. Imaging of the brain may reveal magnetic resonance imaging signal changes in the globus pallidus, striatum, and midbrain. Positron emission tomography reveals normal presynaptic and postsynaptic nigrostriatal dopaminergic function. The primary site of neurological damage has been shown by pathological studies to be the globus pallidus. The mechanism of toxicity is not clear. The US Environmental Protection Agency (EPA) lists manganese as category D, that is, it is not classifiable as to human carcinogenicity. While rare in occurrence, manganese deficiency in humans has been reported. It is characterized by skeletal abnormalities and seizure activity, probably due to decreased MnSOD and glutamine synthetase activities.

Clinical Management

Many symptoms of manganese toxicity disappear after the victim is removed from the source of exposure. L-Dopa (levodopa) can reverse some symptoms, but complete recovery is not expected. Calcium-EDTA (the calcium disodium salt of ethylenediaminetetraacetic acid) will help improve an acute manganese-induced psychosis.

Environmental Fate

Higher levels of environmental exposures to manganese are most likely to occur in or near a factory or a waste site that releases manganese dust into air. Manganese is also released into air by combustion of unleaded gasoline that contains manganese as an antiknock ingredient. Some manganese compounds are readily soluble, so significant exposures can also occur by ingestion of contaminated drinking water. However, manganese in surface water may oxidize or adsorb to sediment particles and settle out. Manganese in soil can migrate as particulate matter in air or water, or soluble compounds may be dissolved by water and leach from the soil. Elemental manganese and inorganic manganese compounds have negligible vapor pressures, but may exist in air as suspended particulate matter derived from industrial emissions or the erosion of soils. The half-life of airborne particles is usually on the order of days, depending on the size of the particle and atmospheric conditions.

The transport and partitioning of manganese in water is controlled by the solubility of the specific chemical form present, which in turn is determined

by pH, Eh (oxidation–reduction potential), and the characteristics of available anions. The metal may exist in water in any of four oxidation states (2+, 3+, 4+, or 7+). Divalent manganese (Mn^{2+}) predominates in most waters (pH 4–7), but may become oxidized at pH greater than 8 or 9. The principal anion associated with Mn^{2+} in water is usually carbonate (CO_3^{2-}), and the concentration of manganese is limited by the relatively low solubility (65 mg l^{-1}) of $MnCO_2$. In relatively oxidized water, the solubility of Mn^{2+} may be controlled by manganese oxide equilibria, with manganese being converted to the (3+) or (4+) valence states. In extremely reduced water, the fate of manganese tends to be controlled by the formation of the poorly soluble sulfide.

Manganese in water may be significantly bioconcentrated at lower trophic levels.

Manganese is a natural component of most foods. The highest manganese concentrations (up to 40 ppm) are found in nuts and grains, with lower levels (up to 4 ppm) found in milk products, meats, fish, and eggs. Concentrations of manganese in infant formulas range from 34 to 1000 ppb, compared to concentrations of 10 ppb in human milk and 30 ppb in cow's milk.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average (TWA), is 0.2 mg m^{-3} for elemental manganese and inorganic compounds. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA, is 5 mg m^{-3} for manganese as a fume and 0.2 mg m^{-3} for manganese as particulate matter. The US EPA recommends a concentration of manganese in drinking water not in excess of 0.05 ppm. The US Food and Drug Administration has set the same level for bottled water.

See also: Metals.

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Margin of Exposure (MOE)

Udayan M Apte and Harihara M Mehendale

Definition

Margin of exposure (MOE) is defined as the ratio of the no-observed-adverse-effect level (NOEAL) to the estimated exposure dose:

$$\text{MOE} = \frac{\text{NOEAL}}{\text{Estimated exposure dose}}$$

Introduction

The determination of MOE is a part of the risk characterization process of a compound. MOE is a way to express the risk of noncarcinogenic effects of a compound. It utilizes the NOEAL determined in animals and estimated exposure dose to human population. NOEAL is the highest dose level of a chemical that does not produce a significantly elevated increase in an adverse response. NOEAL is determined in test animals such as rats and is expressed in milligram per kilogram per day. The estimated exposure dose is determined by estimating amounts of the chemical in the sources of contamination (e.g., water supply) and is expressed in milligram per kilogram per day. MOE indicates how close the estimated exposure of the toxicant is to the dose, which produces no observable adverse effect in a test animal. Low values of MOE indicate that the human exposure of the chemical in the target population is close to the NOEAL in the animals. MOE values below 100 are considered unacceptable and generally demand further investigation. Higher

values of MOE indicate that the exposure of the chemical is much lower than the NOEAL in animals. It should be noted that the MOE calculation does not take into account the differences in animal-to-human susceptibility and/or the extrapolation of dose from animals to humans.

Example of MOE

Consider that the human exposure of a chemical X calculated via drinking water supply is 2 ppp, that is, $2\,mg\,l^{-1}\,day^{-1}$. Suppose a 70 kg man consumes 2 l of drinking water per day then the estimated exposure dose would be $2\,mg\,kg^{-1}\,day^{-1} \times 2\,l\,day^{-1}$ divided by 70 kg (body weight), which is equal to $0.057\,mg\,kg^{-1}\,day^{-1}$. Suppose that the NOEAL of chemical X is $150\,mg\,kg^{-1}\,day^{-1}$, then the MOE would be more than 2600. This indicates that the exposure of chemical X is much below its NOEAL and the risk to public health is very low.

See also: Risk Assessment, Human Health.

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Marijuana

Christopher P Holstege

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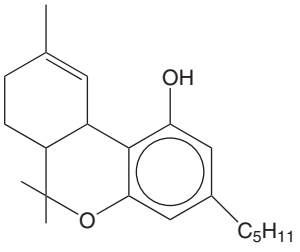
- Chemical Abstracts Service Registry Number: CAS 7663-50-5
- Synonyms: Tetrahydrocannabinol (THC); Bhang; Dronabinol; Cannabis; Ganja; Grass; Hashish; Hemp; Honey oil; Marihuana; Marinol; Mary Jane; Pot; Refeer; Weed
- Chemical/Pharmaceutical/Other Class: Psychoactive substance
- Chemical Structure:



Uses

Dronabinol is prescribed for its antiemetic and appetite stimulant properties. Marijuana is primarily a drug of abuse, although it is currently used by patients for the same purposes as dronabinol.

Exposure Routes and Pathways

Inhalation of marijuana smoke is the most common method of use followed by ingestion. Parenteral use is uncommon. Dronabinol is an oral capsule.

Toxicokinetics

After smoking, 18–50% of the available THC is absorbed, the onset of clinical effects occurs within 10 min, and effects continue for 2–4 h. Peak plasma levels occur within 5–12 min of smoking with peak clinical effects noted at 20–30 min later, after distribution into brain and other tissues. Following ingestion, only 5–20% of THC is bioavailable, the onset of effects begins within 30–60 min, and effects persist for 4–6 h. Gastrointestinal absorption is increased by fatty foods or a lipid vehicle. Peak plasma levels occur 2–3 h after THC ingestion. THC is 97–99% protein bound with a volume of distribution of $\sim 10\,l\,kg^{-1}$. THC undergoes substantial first-pass metabolism by the liver. THC is metabolized primarily to 11-hydroxy-delta-9-THC. The 11-hydroxy-delta-9-THC is pharmacologically active, but is further metabolized to inactive metabolites, primarily 11-nor-delta-9-THC carboxylic acid. Less than 1% of THC is excreted unchanged in the urine. The high lipid solubility results in an initial short plasma half-life, but this adipose storage produces a biologic half-life of 25–30 h. THC may be detectable in plasma for up to 15 days. With chronic high-dose use of marijuana, the presence of metabolites of THC in the urine can be detected for 6–8 weeks.

Mechanism of Toxicity

The mechanisms involved in THC's central nervous system (CNS) and cardiovascular effects have not been well delineated. Specific cannabinoid receptors in the cerebral cortex may be responsible for the pharmacologic effects of THC. THC also has immunosuppressive effects and results in depression

of both B- and T-cell activity and depression of tumor necrosis factor levels by macrophages. The antiemetic effect appears to involve the CNS vomiting control center.

Acute and Short-Term Toxicity (or Exposure)

Animal

The clinical effects of marijuana in animals are similar to those observed in humans. Clinical effects may be more pronounced after ingestion of marijuana than those seen with inhalation exposure.

Human

Toxicity primarily involves the CNS and cardiovascular system. Euphoria, increased apparent visual and auditory sensory perception, and altered perceptions of time and space are common with mild intoxication. Larger doses can impair memory, decrease attention and cognition, and result in lethargy. Impaired sensory interpretation and performance of complicated mental tasks increases the risk of trauma with activities such as operating a motor vehicle. Decreased balance, ataxia, and muscle incoordination can occur. Anxiety, panic attacks, paranoia, depression, confusion, and hallucinations can occur with high doses; these effects are more common in less experienced, younger users. Cardiovascular effects include increased heart rate and cardiac output and decreased exercise tolerance. Bronchodilation and, less frequently, bronchoconstriction may be seen. The pupils will constrict slightly and the conjunctiva will become red secondary to congestion of the blood vessels. A dry mouth is common. The intravenous administration of marijuana has been associated with severe multiple organ system failure, including renal failure, rhabdomyolysis, increased hepatic enzymes, shortness of breath, headaches, and hypotension.

Chronic Toxicity (or Exposure)

Animal

Nonhuman primates have displayed behavioral signs of withdrawal after chronic administration of THC. Chronic administration of THC via gavage over 2 years found no evidence of carcinogenic effect in rats and equivocal findings in mice at higher doses. Chronic use of THC has been shown to induce tumor regression in rodents.

Human

Chronic use can result in an amotivational state, paranoid behavior, worsening muscle incoordination, slurred speech, and delusions. Smoking marijuana is implicated in both chronic lung disease and the development of lung cancer. Fertility can be impaired in both males (decreased sperm count and activity) and females (decreased ovulation and abnormal menses). Prenatal marijuana use by the mother correlates with increased hyperactivity, impulsivity, and delinquency in the child. Tolerance to some CNS effects may develop with chronic use, and a withdrawal syndrome is possible after chronic high-dose use.

In Vitro Toxicity Data

The active moieties of marijuana have been studied for medicinal purposes in a variety of models. Some canabinoids have displayed effects on neuronal transmission and alterations of calcium homeostasis. Other cannabinoids have been shown to stimulate cell death (apoptosis), which may help explain observed antitumor effects in some animal models.

Clinical Management

Clinical management is primarily supportive. Reassurance is generally effective in treating alterations in thought process, although benzodiazepines may be necessary in uncommon, severe toxicity. If large amounts of marijuana are ingested, activated charcoal administration may be considered for recent exposures.

See also: Drugs of Abuse.

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Marine Organisms

William R Kem

A wide variety of natural toxins, from small heterocyclic molecules to large proteins, occurs in marine organisms. The phyletic diversity of plants in the ocean is far less than on land, while the number of marine animal phyla significantly exceeds that on land. Thus, it is not surprising that many of the known marine toxins are of animal origin. In this article, we will not only focus upon the toxins of unicellular organisms and marine animals, but also consider a few seaweed toxins.

What is a toxin? First, the word denotes a single chemical entity or compound which possesses a defined chemical composition and covalent structure. Generally, this word is reserved for molecules that occur naturally within an organism. Vertebrate (and human) toxins include the complement system and defensin peptides which serve as one of our chemical defenses against infectious bacteria. Toxic substances made with human hands (and minds) are generally referred to as poisons. A venom is a mixture of substances secreted together by an animal to either defend itself and/or capture prey. Animal venoms usually are mixtures of enzymes and toxins that, acting together, are more effective than when acting separately. For instance, phospholipases are commonly present in venoms because they facilitate the distribution of the toxins in the venom by digesting lipids in lipid membranes which act as barriers to the distribution of toxins throughout the body. Conversely, some membrane-disrupting toxins also enhance lipid digestion by phospholipases. Enzymes, hyaluronidase and collagenase, break down macromolecules responsible for holding cells together, also enhance the distribution of venoms in the body.

Many toxins act rapidly on their victims. This certainly makes sense if the toxin is being used to immobilize prey or to escape from predators. Rapidly acting toxins generally affect excitable cells such as nerves and muscles (including the heart myocardium) which allow movement. Their targets (receptors) include voltage-gated ion channels involved in the generation of nerve and muscle action potentials, which share many of the same characteristics. These ion channels are membrane-penetrating proteins which open in response to a change in the electrical potential across the membrane, allowing sodium or calcium ions to diffuse inwards, causing a rapid (millisecond timescale) depolarization of the membrane sufficient to serve as an electrical stimulus for the adjacent membrane and thereby causing the conduction of an electrical signal called an action potential. This depolarizing wave rapidly propagates down the length of the cell, ultimately causing contraction (muscle) or release of a neurotransmitter (nerve). Either process activated by an action potential involves the opening of calcium-selective ion channels, which allows calcium ions to rush into the cell and trigger either contractile proteins or release of packets of neurotransmitter at the nerve terminal. Many toxins, aquatic and terrestrial, attack the sodium or calcium channels involved in these processes, since their alteration usually causes paralysis and possibly death of the affected organism.

A neurotransmitter diffuses a very short distance to reach its receptor on a nearby cell which has formed a synapse with it; there the neurotransmitter activates what is called a ligand-gated ion channel which also usually generates a smaller electrical signal which can be excitatory (depolarizing, causing another action potential to be generated on the other side of the synapse) or inhibitory (suppressing action potential generation in the postsynaptic cell). There are many toxins which affect the release of neurotransmitters from their presynaptic sites or the subsequent effect of the neurotransmitter on its receptor. These effects also can cause a very rapid paralytic effect on a victim. In the following discussion of marine toxins we will at least briefly consider what is known about the sites and modes of action of a toxin.

Dinoflagellate Toxins

Single-celled organisms (formerly referred to as protozoans but more recently as prokaryotes) abound in aquatic environments including the seas and oceans. Much is known about their biology as they can often be cultured in the laboratory and their unicellular nature makes them excellent subjects for many cell biology studies. While most prokaryotes do not contain toxins, some marine dinoflagellates can secrete or release upon death very potent toxins capable of causing harm to a variety of animals including humans. The most cosmopolitan type of toxic dinoflagellate (genus *Gonyaulax*) contains a toxin called saxitoxin which blocks voltage-gated sodium channels in nerve and skeletal muscle and thereby inhibits excitability. Saxitoxin is concentrated by clams and mussels as well as other filter-feeding animals which feed upon *Gonyaulax*. Although these animals are relatively insensitive to this toxin (otherwise they could not feed upon this

dinoflagellate!), animals which feed upon bivalves containing sufficient amounts of this or closely related saxitoxin analogs can be paralyzed by sodium channel blockade caused by this toxin. In many ways saxitoxin acts like a local anesthetic (e.g., lidocaine) on the nerve impulse, blocking the sodium channels and causing paralysis. However, there are two obvious differences. First, saxitoxin much more selectively blocks the sodium channels and at over 1000-fold lower concentrations. Second, since saxitoxin is a much more polar molecule, it does not enter the brain readily from the systemic circulation, and thus acts primarily on the peripheral neuromuscular system causing relaxation of skeletal muscles. Depression of breathing by inhibiting the intercostals and diaphragm skeletal muscles can be fatal! Fortunately, our myocardial sodium channel is less sensitive to saxitoxin and thus cardiac depression is rare. Shellfish beds which are harvested for human consumption are monitored by federal agencies for dinoflagellate toxin levels to assure their safe consumption. When saxitoxin or related intoxication occurs, symptomatic treatment in a hospital setting is used to get the patient through the critical period of respiratory weakness.

Besides paralytic shellfish poisoning (PSP) there is also neurotoxic (NSP) and diarrhetic (DSP) shellfish poisoning due to other dinoflagellates in the marine environment. NSP is relatively rare, but in 1987 received considerable attention when there was an occurrence of this type of poisoning in Nova Scotia. NSP victims showed central nervous system cognitive deficits such as amnesia. The causative agent was later found to be domoic acid, which is known to be toxic to excitatory synapses in the brain which involve the neurotransmitter glutamic acid. This toxin is a chemical analog of glutamic acid, which is not readily removed from the nervous system and thus causes persistent stimulation of such synapses, which results in a massive calcium elevation which proves lethal to neurons expressing glutamate receptors. Again, this dinoflagellate toxin was only retained and concentrated by the bivalve.

DSP is not as life-threatening as PSP and NSP. The main toxin, called ciguatera toxin, is actually a group of very similar polyether molecules which, like PSP, also affects voltage-gated sodium channels. However, ciguatoxin stimulates the opening of a small fraction of sodium channels and this causes an increase in nerve excitability in contrast with saxitoxin's depressant action on excitability. Gastrointestinal cramps and diarrhea are the major effects. Ciguatoxin is made by a bacterium but because it is very lipophilic it is concentrated as it is passed up the food chain. Another chemically related marine bacterial toxin, maitotoxin, also causes ciguatera symptoms but acts by a different mechanism, enhancement of resting membrane calcium ion permeability. Thus predatory animals at the very top of the chain can accumulate the highest toxin concentrations. These include fish like barracuda. The highly lipophilic ciguatera toxins leave the victims very slowly, sometimes over months or a year, thus prolonging the misery.

There are several other marine dinoflagellates which secrete toxins into the sea water primarily when their high concentrations (blooms) cause a population crash, and the dead cells then release their toxins. In the United States, a very common organism causing massive fish mortalities is *Karenia* (formerly *Gymodinium*) *breve*. The so-called brevetoxins, like ciguatoxin, are large polyether molecules which tightly bind to voltage-gated sodium channels in excitatory cells and enhance their excitability. Because fish sodium channels are very sensitive to these toxins, they usually die before they are caught and consumed by humans. Thus this toxin is primarily injurious to marine ecosystems due to massive mortalities of fish and other animals. The only common human effect is bronchoconstriction of the airways resulting from inhalation of brevetoxins which can be airborne in coastal regions experiencing this red tide.

Although red tides occurred before human population density became high, the frequency and widespread occurrence of particular red tides is often attributed to eutrophic conditions along coasts caused by runoff of agricultural fertilizers and animal wastes. Unfortunately, the spores of these organisms are readily transported from one sea to another in the ballast waters of ships. It is thought that red tide dinoflagellates are now widely distributed around the oceans of our planet because of these human influences.

Increases in environmental pollution or nutrient levels, reduced oxygen levels, and other factors can change conditions significantly in marine environments, especially in protected coastal areas where tidal flushing currents may be slow. Sometimes when this occurs, different organisms that thrive under these altered conditions begin to emerge as do health concerns for both people and other species coming in contact with these species and the toxins they produce. One fairly recent example of this is a major outbreak of finfish kills and some human health problems (respiratory and eye irritation, skin rashes, gastrointestinal and neurological symptoms) reported along the middle Atlantic seaboard of the United States in the early 1990s. The cause of this appears to be exposure to dinoflagellate *Pfiesteria* sp. (including *Pfiesteria piscicida* and *Pfiesteria* shumwayae) and to

the yet unidentified substances that they produce. Such toxicity had not previously been detected in this region. This is just one illustration of how important it is to be aware of the impact of human activity on marine environments and the unintended changes our species may be bringing about.

Invertebrate Toxins

Sessile marine animals such as encrusting sponges, bryozoans, and tunicates are known to harbor a variety of toxins which may serve as chemical defenses against predators. These are filter feeding animals and thus many of the toxins and repellant substances obtained from these organisms may originally have been made by bacteria or other planktonic organisms which are concentrated by these animals. Certain sponges (the genus was originally *Haliclona*, but has been changed to *Amphimedon*) make pyridinium polymers called halitoxins which lyse blood and other cells which have been tested. Sponges containing high concentrations of this polymeric toxin are generally avoided by most predatory fish. The Carribbean Fire Sponge (*Tedania* sp.) possesses toxins which cause a delayed hypersensitivity as well as acute inflammatory reaction whose unpleasant nature the author has experienced. The active constituents of this and other inflammatory sponges have not yet been characterized.

Bryozoans look more like plants than animals and are common coastal animals growing on docks and boats in addition to the natural surfaces. A family of heterocyclic molecules aptly called bryostatins has been identified and is being tested as potential treatments for certain cancers. Similarly, tunicates, representing some of our most primitive chordate (backbone) ancestors, produce cyclic peptides which preferentially kill certain types of cancer cells. Vast numbers of sponge, bryozoan, and tunicate and other encrusting marine species are being extracted and tested for antineoplastic activity by a screening program sponsored by the National Cancer Institute and many lead compounds have already been identified.

The phylum Cnidaria consists of hydrozoans (including Portuguese Man O'War medusae and fire corals), scyphozoans (jellyfish), and anthozoans (soft corals, hard corals, and sea anemones). All of these animals are covered with stinging capsules (the cnidae) which are used to paralyze prey and defend against predators. The cnidae are located in cnidocytes, the epidermal cells which make the stinging capsules and eventually control their discharge. The wall of the stinging capsule has been shown to be impermeant to molecules larger than about 800. Since all of the known cnidocyst toxins are peptides or proteins exceeding this mass, they can be kept within the capsule without expenditure of energy. Jellyfish and hydrozoan toxins are relatively large, unstable proteins which form large pores in cell membranes, which cause their cells to swell up and burst due to the osmotic imbalance. The toxins of sea anemones are smaller and generally stable after isolation. The amino acid sequences of several sea anemone toxins are known. The toxins which affect excitable membranes are generally called neurotoxins, although they may be even more potent on heart sodium channels. These peptides of about 50–55 amino acid residues are known to prolong the repolarization phase of the action potential by delaying the process of sodium channel inactivation which is important for returning the nerve membrane to its resting state. This leads to an abnormally large release of neurotransmitters at nerve endings, and results in spastic paralysis of the victim. The other sea anemone toxins are larger peptides which form large ion channels pores in cell membranes, causing depolarization, loss of osmotic balance, and cell death (cytolysis). Particularly common are the 'actinoporins', which are $\sim$20 000 Da proteins, which, like the bacterial porins, possess large amounts of B-pleated sheet structures. A third, more recently discovered group of sea anemone peptide toxins block voltage-gated potassium channels at extremely low concentrations. One can imagine that when these three toxins act together on a nerve membrane that it will be depolarized much of the time! Soft corals, in contrast to the above-mentioned cnidarians, seem to rely upon small, repellant terpene molecules to deter predators.

Of the 25 animal phyla, almost half are worms. Thus, it is not at all surprising that some worms contain toxins. The nemertines are a phylum of over 800 known species which resemble flatworms but are active predators on crustaceans and other worms. This phylum is exceptionally toxic among the various worm phyla. The Heteronemertine side possesses peptide toxins which appear to be only defensive, as these animals have no means of injecting a venom. The peptides include neurotoxins, which enhance excitability of nerve membranes, and cytolysins, which permeabilize and destroy cell membranes. Members of the Hoplonemertine class inject a venom into their prey using a mineralized stylet located in their proboscis, which is also used to immobilize the prey. Their toxins are alkaloids similar to nicotine which in minute amounts paralyze crustaceans and annelid worms and primarily activate nicotinic acetylcholine receptors. Another well-known worm toxin is nereistoxin, a disulfide-containing alkaloid which also binds to nicotinic

receptors but is largely inhibitory to their normal functioning. This toxin was isolated after fisherman noticed that flies which ate the flesh of the dead worms were paralyzed. It later became an important agricultural insecticide because it is particularly effective on rice-stem boring insects.

Starfishes and sea urchins usually contain toxins serving as a chemical defense against predators and potential settling animals. Starfishes make saponins (diterpene glycosides) that are chemically similar to the saponins found in unripe tomatoes and in potato spuds. These enter the lipid bilayer part of the cell membrane and form complexes with cholesterol, a membrane-stabilizing lipid. This makes the membrane leaky to ions and water, causing cyolysis. Among the spines of sea urchins are found small flower-like appendages, pedicellariae, some of which are venomous. Their toxins are peptides and none have yet been characterized chemically. They can paralyze small animals which might otherwise attach (settle) to the surface of the urchin.

While most mollusks possess a protective shell, some also possess powerful venoms which can be used as a further defense against predators and also for paralysis of their prey. Undoubtedly, the best known group is one of marine snails known as 'cone' snails because their shells are often nearly perfectly conical. The genus *Conus* actually contains more than 300 species, and it is likely that all possess a venom harmful to some animal. Only $\sim$10% of the species are thought to be harmful to vertebrates and these are species that usually prey upon fish. Venoms of the others may also contain peptide toxins affecting vertebrates but are unlikely to be lethal. Most cones actually prey upon annelid worms or nonpoisonous snails (sometimes the cones battle as well, in a chemical warfare without backbones). Their venoms tend to be specialized for their molluscan or vermiferous prey rather than us vertebrates. Nevertheless, when scuba diving or snorkeling, it is best not to handle cones unless your skin is protected by gloves and wet suit. Since the venom is emitted from a tiny harpoon shot out with considerable force, it is also advised not to place the snail in a pocket! Octopuses are also venomous. Although the Australian blue-ringed octopus uses tetrodotoxin (TTX, see next section), most octopuses inject a salivary gland venom containing a protein (cephalotoxin) which paralyzes crabs in very small amounts. This toxin does not seem very potent when injected into vertebrates.

Vertebrate Toxins

Sea snakes (family Hydrophiidae) are close relatives of the cobra, coral, and other snakes belonging to the family Elapidae. While these snakes are usually not very aggressive, they are potentially dangerous, possessing venoms that on a unit weight basis are amongst the most potent of all snakes. Sea snakes are confined to the Pacific Ocean and contiguous tropical seas including the Red Sea. They use their venom to paralyze prey, primarily fish. Two peptide toxins and phospholipase A2 are generally present in these snake venoms. The most life-threatening toxin is the so-called α-neurotoxin, a peptide composed of $\sim$60 amino acid residues that is held together in a three-fingered loop structure by three disulfide bonds; the longer, middle loop binds to the nicotinic acetylcholine receptor on neuromuscular synapses and blocks the ability of the neurotransmitter acetylcholine to activate skeletal muscle. This sea snake toxin acts essentially like curare alkaloids and modern nondepolarizing muscle relaxants, but it binds more tightly to the receptor and thus the neuromuscular block takes more time to be reversed as the toxin disappears from the systemic circulation.

The second sea snake peptide toxin, cardiotoxin, is homologous (common ancestral gene) with the α-neurotoxin, but lacks the particular amino acid residues favorable for binding of the latter peptide to the nicotinic receptor. Cardiotoxin binds rather indiscriminantly to cell membranes including those of the heart and disrupts their normal structure such that they become more permeable to sodium, calcium, and other ions, which depolarizes the normal resting membrane sufficient to cause systolic arrest of the heart. It acts synergistically with phospholipase since it makes the membrane phospholipids more accessible to attack by the phospholipase A2 which is also a major enzymatic constituent of this venom. The most common means of treatment of sea snake envenomation involves intravenous injection of sea snake antivenin containing antibodies directed toward the various toxic constituents. When antivenin is unavailable cholinesterase inhibitors might be useful therapy when muscular paralysis is not complete. Artificial ventilation must be maintained until the victim is able to breathe spontaneously.

There are many poisonous fishes in the oceans of the world. Perhaps the most notorious is the puffer fish (family Tetrodontidae). Besides being able to inflate itself, thereby directing the spines on its skin toward a potential predator and becoming a large oval shape, this fish contains a heterocyclic toxin which, like saxitoxin, blocks some voltage-gated sodium channels at very low (nanomolar) concentrations. TTX was initially purified from a puffer fish prized as food in Japan, where chefs must pass a rigorous test demonstrating their ability to remove

the poisonous viscera and skin from the edible flesh. Puffers apparently use TTX only as a chemical defense against predators. TTX has been demonstrated to be produced by a bacterium which lives within the poisonous tissues of the fish. This may also explain why it also occurs in a wide variety of other animals including the California newt (an amphibian), the blue-ringed octopus, marine crabs, and worms. Fortunately, our myocardial (heart) sodium channels are relatively resistant to this toxin, as are the nerves of the puffer fish. Also, being ionized and very polar, the toxin does not readily penetrate across the blood brain barrier into the brain.

There are many fishes with poisonous spines, most notably the stone fishes and scorpion fishes occurring in Pacific and contiguous seas. The stone fish is an ugly fish that quietly sits upon the rocky substrate of shallow coastal waters waiting for its prey. Unlike other species it does not move when a human intruder appears, but rather holds its 'ground'. Thus, people who are wading in shallow waters sometimes step on these fishes with their upright dorsal fin spines which can puncture the skin readily and produce extremely painful stings that are usually not life threatening. Recent research has yielded several protein toxins which are currently being investigated. Scorpion fish have large pectoral and dorsal fins which have numerous poisonous spines also possessing protein toxins which depress neurotransmitter release from nerve terminals. Small scorpion fish are sometimes found in marine aquarium shops. Perhaps the most commonly encountered fishes with poisonous spines are sting rays. Unlike stone fish, sting rays usually swim away when disturbed. Waders in waters infested with these bottom dwelling fishes are advised to walk in a shuffling gait to provide the rays with enough advance notice of their presence and to wear boots when possible, to avoid being stuck by the 'whiplashing' tail spine. Some species of catfish also have stinging spines containing a venom which has not yet been characterized. Therapeutic treatments of individuals envenomated by poisonous fish spines are still largely symptomatic since antivenins are not usually available.

Treatment of Marine Envenomations and Intoxications

Relative to treatment of snake, spider, scorpion, and other terrestrial animal envenomations, the treatment of most envenomations due to marine animals is rather primitive. This is primarily due to our knowledge of these venoms being less complete. The incidence of jellyfish envenomation amongst swimmers is undoubtedly much higher than for stings of some of the above mentioned terrestrial serpents, but rarely are jellyfish stings life threatening unless the swimmer is stung over a large surface area by the Australian box jellyfish (*Chironex fleckeri*) or the hydrozoan Portuguese Man O'War (genus *Physalia*). However, marine 'toxinology' has made steady progress in the past two decades and one can expect antivenins for common marine envenomations to eventually become available. Antivenins are primarily useful for neutralizing proteinaceous venom constituents. If the effect of a venom is largely due to a single type of toxin, one can anticipate future treatments to be based on counteracting the effects of the toxin on its receptor target.

Toxins as Molecular Models for Development of New Drugs

Centuries ago the Swiss physician Paracelsus stated that all drugs are poisons and all poisons are drugs. While the first portion of this statement is generally considered valid, not all poisons are drugs. Nevertheless, there is a long tradition of developing materia medica from natural sources, generally plant extracts, which were used to treat a variety of disease conditions. An example would use of powdered leaves of the foxglove plant (and later purified digitalis alkaloids) to treat congestive heart failure. Toxins and other substances, because they often are potent modulators of particular ion channels or receptors, also can serve as 'lead' compounds for designing new drugs. Manipulation of the molecular structure frequently improves selectivity for a particular target (receptor) and thereby reduces the likelihood of adverse effects in therapeutic use. Toxic natural products isolated from several phyla of marine organisms have led to new drug candidates in recent years and there will likely be more in the not too distant future.

See also: Algae; Animals, Poisonous and Venomous; Saxitoxin; Shellfish Poisoning, Paralytic; Tetrodotoxin.

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Relevant Website

http://www.marine-medic.com.au – Marine-medic.com

Material Safety Data Sheets *See* Chemical Hazard Communication and Material Safety Data Sheets.

Maximum Allowable Concentration (MAC)

Shayne C Gad

Maximum allowable concentrations (MACs) are the maximum airborne concentrations that can be justified consistent with the objective of maintaining unimpaired health or comfort of workers or both. The criteria on which the standard is established are the avoidance of (1) undesirable changes in body structures or biochemistry, (2) undesirable functional reactions that may have no discernible effects on health, and (3) irritation or other adverse sensory effects.

MACs in the United States were established by The American National Standards Institute (ANSI); however, permissible exposure levels have superceded the use of MACs in the United States. Based on recommendations issued by the American Conference of Governmental Industrial Hygienists and ANSI, they serve the same function.

See also: American Conference of Governmental Industrial Hygienists; Exposure; Exposure Assessment; Occupational Toxicology.

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Maximum Tolerated Dose (MTD)

Shayne C Gad

The maximum tolerated dose (MTD) is commonly estimated to be the maximum dose that can be administered for the duration of a specific study that will not compromise the survival of the animals by causes other than carcinogenicity. If the MTD has been chosen appropriately, there should be no adverse effect on survival, only a modest decrement in body weight gain and minimal overt signs of toxicity. The MTD has been exceeded if there is increased mortality, severe body weight decrement, or marked signs of toxicity. It should be noted that another meaning for MTD has sometimes been 'minimum toxic dose'.

The information used for dose selection usually comes from subchronic toxicity studies, but other information about the pharmacological effects of a drug and its metabolism and pharmacokinetics may also be considered. The maximum recommended human dose (MRHD) of the drug might be an additional criterion, if this is known when the carcinogenicity studies are being designed.

For most pharmaceutical companies, the doses selected are as follows: The highest dose is selected to be the estimated MTD, the lowest dose is usually a small multiple of the MRHD (one to five times), and the mid-dose approximates the geometric mean of the other two doses.

The procedures for dose selection described previously are generally consistent with major regulatory

guidelines for carcinogenicity and other studies, for example, the *Redbook* from the US Food and Drug Administration. Earlier versions of the *Redbook* focused on direct food additives and color additives used in food. The *Redbook 2000* provides guidance for the safety assessment of food ingredients, including direct food additives, color additives used in food, Generally Recognized as Safe substances, food contact substances and constituents, or impurities of any of the above. There are, however, exceptions to the general approach described previously. For example, for nontoxic drugs, the difference between the high and the low doses may be many orders of magnitude if the high dose is set at the estimated MTD and the low dose is a small multiple of the clinical dose. Some guidelines require that the low dose be no less than 10% of the high dose. In this situation, it may be acceptable to set the high dose at 100 times the MRHD, even if the MTD is not achieved. Similarly, when a drug is administered in the diet, the highest concentration should not exceed 5% of the total diet, whether or not the MTD is achieved.

Metabolism and/or pharmacokinetic data, when available, should also be considered in the dose selection process. It is desirable that a drug not be administered at such a high dose that it is excreted in a different manner than at lower doses, such as the MRHD. Similarly, the high dose should not lead to the formation of metabolites other than those formed at lower (clinical) doses. If data show that a given dosage produces maximum plasma levels, administration of higher doses should be unnecessary. These considerations may be very useful when interpreting the results of the study or attempting to extrapolate the results to humans.

The dose range-finding study is necessary in most cases, but the suppression of body weight gain is a scientifically questionable benchmark when dealing with the establishment of safety factors. Physiologic, pharmacologic, or metabolic markers generally serve as better indicators of systemic response than body weight. A series of well-defined acute and subchronic studies designed to determine the 'chronicity factor' and to study onset of pathology can be more predictive for dose setting than body weight suppression.

Also, the MTD may well be at a level where the metabolic mechanisms for handling a compound at real-life exposure levels have been saturated or overwhelmed, bringing into play entirely artifactual metabolic and physiologic mechanisms. The regulatory response to questioning the appropriateness of the MTD as a high level has been to acknowledge that occasionally an excessively high dose is selected, but to counter by saying that using lower doses would seriously decrease the sensitivity of detection.

See also: Dose–Response Relationship; Food and Drug Administration, US; Investigative New Drug Application; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Pharmacokinetics/Toxicokinetics; Redbook.

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Mechanisms of Toxicity

Sanjay Chanda and Harihara M Mehendale

Toxicity is mostly caused by alterations in normal cellular physiology and biochemistry, eventually leading to cell death and tissue damage. Although many toxic responses are ultimately from cell death and loss of critical organ function, other responses may be the result of biochemical and pharmacological imbalances in normal physiological processes (genetic alterations) that do not result in cell death. Understanding of how different chemicals can affect these phenomena at biochemical and molecular level is essential to avert or prevent the toxicity. Despite a common outcome of toxicity, from all chemical-induced injury, the extent of tissue damage necessary to cause a life-threatening response varies depending on the tissue type and rate at which the injury is caused. Epithelial tissues (e.g., liver, kidney, lung, and intestine) and DNA have a great capacity to repair or regenerate in response to a loss of tissue mass or DNA architecture. Other tissues (e.g., neuronal tissues) either have a very poor capacity to regenerate

or do not regenerate at all. It is also true that organs have a capacity for function that exceeds the requirements for the normal homeostasis and is referred to as functional reserve capacity. Reserve capacity allows the body to survive severe toxic insults that lead to significant loss of organ functions. Humans functioning with one kidney, a part of the lung removed, only a portion of liver, or only half of the normal amount of hemoglobin are examples of the functional reserve capacity.

There are many ways in which a chemical can interfere with the normal biochemistry and physiology of the cells and a chemical may cause toxicity to multiple tissues by multiple mechanisms. The following general categories of the mechanisms are neither comprehensive nor mutually exclusive but represent the major mechanisms of toxicity of many drugs, chemicals, and environmental agents.

Covalent Binding to Macromolecules

Many toxic substances exert their toxic effects by covalently binding to proteins, thiols, and nucleic acids. The binding can be either very tight (e.g., covalent binding by shared electrons) or loose through other labile bonds. Covalent binding can lead to longer lasting toxic effects. Proteins constitute many enzymes and regulate many functions and structural components of membranes that are critical to cellular function. Binding of hydrogen cyanide to the ferric atom of cytochrome oxidase and thus preventing the electron transport and, as a result, blocking the transport of oxygen by hemoglobin in the blood is a classic example of toxicity caused through protein binding. Carbon monoxide, on the other hand, principally blocks delivery of oxygen to tissues by taking the place of oxygen on hemoglobin, the oxygen carrying protein of the red blood cells. Chemically induced porphyria caused by halogenated hydrocarbons (e.g., hexachlorobenzene) and metals (e.g., lead and mercury) in part is also caused by inhibition of specific enzymes (by protein binding) of the heme biosynthetic pathway. Many toxic trace metals (e.g., arsenic, cadmium, mercury, and lead) also bind to proteins with free sulfhydryl groups (also known as thiols of proteins, amino acids, etc.), resulting in toxicity.

Many chemicals form reactive, electrophilic intermediates and free radicals during their metabolism in the body. These can be formed via enzyme-mediated reactions (many of which are oxidations) or from autoxidation of small molecules like flavins and thiols. These electrophilic intermediates covalently react with nucleophilic sites in the cell, including glutathione (GSH) and thiol-containing proteins, causing cellular dysfunction and oxidative stress to the cell. Acetaminophen is one such drug that forms a metabolite, *N*-acetyl-*p*-aminobenzoquinoneimine, which first depletes GSH and then covalently binds to protein thiols to cause toxicity. Binding to protein thiols results in the loss of activity of thiol-containing enzymes. Calcium transporting ATPase is a thiol-containing enzyme that is affected by covalent binding with many electrophilic intermediates of chemical toxicants. Binding of this enzyme results in loss of adenosine triphosphate (ATP), important for cell survival, or excessive accumulation of extracellular calcium inside the cells and this results in cell death.

Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contain numerous nucleophilic sites that react readily with electrophilic chemicals. Binding to the nucleophilic sites of RNA leads to perturbed synthesis of proteins, which are very critical for the normal functioning of the body. Production of somatic mutations through DNA–adduct formation by certain chemicals may be responsible for chemical carcinogenesis. Adduct formation with RNA and DNA can also alter the expression of certain genes, thereby affecting the formation of certain gene products resulting in disruption of normal functions and life cycle of cells. N7, N2, C2, and O6 positions of guanine appear to be important in DNA–adduct formation and are known to cause mutagenicity and carcinogenicity.

Some chemicals may also enter normal cellular pathways of metabolism and cause perturbations in cellular metabolism. Fluoroacetate is a well-known example. Cell death occurs via interference with energy production. Fluoroacetate enters the citric acid cycle (Krebs cycle) with the formation of fluorocitrate. Isocitrate dehydrogenase, the normal enzyme in the sequential energy-producing pathway, is unable to handle the substrate, consequently blocking the energy production pathway. Another example is galactosamine, a naturally occurring amine derivative (present in high amounts in lobster shells) of sugar glucose. Galactosamine enters the normal galactose metabolism pathway of the liver cells because one of the enzymes accepts galactosamine in place of its normal substrate galactose. The impostor makes its way to form uridine diphosphogalactosamine (UDP-galactosamine), but the next enzyme (epimerase) detects the impostor and cannot act on this abnormal substrate. This results in the trapping of uridine into a useless intermediate causing cellular depletion of uridine, thereby causing depletion of UTP, inhibition of RNA and protein synthesis, and glycogen formation. This 'uridyl trapping' leads to cellular death, causing liver toxicity. The toxicity can be reversed by administering orotic acid, a precursor of uridine.

Interference with Calcium (Ca^{2+}) Homeostasis

Disruption of intracellular Ca^{2+} homeostasis can result from excessive Ca^{2+} influx or release of Ca^{2+} from intracellular stores or from inhibition of extrusion of Ca^{2+} by the plasma membrane. Cellular Ca^{2+} is closely regulated by living cells. Ca^{2+} concentration is ~5000- to 10 000-fold higher outside the cells. If higher amounts of Ca^{2+} enter the cells (e.g., due to perturbation of plasma membrane) the Ca^{2+} ATPase pumps in the plasma membrane pump the excessive Ca^{2+} out of the cell. Additional finer regulation of Ca^{2+} inside the cell is accomplished mainly by two mechanisms – mitochondria sequester larger fraction of Ca^{2+} and endoplasmic reticulum can sequester smaller amounts of Ca^{2+}. Interference with the normal processes responsible for regulation of intracellular Ca^{2+} plays a critical role in chemical-mediated cell injury and necrotic cell death. One or more of these regulatory mechanisms may be perturbed by toxic chemicals. Accumulation of Ca^{2+} in the cells has been correlated with necrotic cell injury and cell death from ischemia and a variety of toxic agents. Blebing or development membrane abnormalities with disruption of cytoskeletal structure (disruption of actin microfilaments by the activation of phospholipases and proteases) have been found *in vitro* after increased intracellular Ca^{2+}. Nitrophenols, quinones, peroxides, aldehydes, dioxins, halogenated alkenes, alkanes, and some metal ions cause toxicity by disrupting Ca^{2+} homeostasis.

Disruption of Ca^{2+} homeostasis has also been implicated in 'programmed cell death' or apoptosis. An increased Ca^{2+} level in the nucleus activates some endonucleases, which result in DNA fragmentation and chromatin condensation.

Lipid Peroxidation

Carbon tetrachloride (CCl_4) toxicity is a typical example of toxicity due to lipid peroxidation. The cleavage of a carbon–chlorine bond in CCl_4 by the cytochrome P450 mixed function oxidase system generates a trichloromethyl free radical ($^{\bullet}CCl_3$), which reacts rapidly with oxygen to form trichloromethyl peroxy radical ($^{\bullet}CCl_3O_2$). These free radicals can initiate a process of autocatalytic lipid peroxidation by attacking the bridges of unsaturated fatty acid side chains of microsomal lipids. Also, unsaturated fatty acids in other cellular membranes are affected. Once organic free radicals are generated in this manner, a self-propagating runaway series of reactions leads to rapid destruction of cellular membranes causing cell death.

Oxyradicals and oxyradical stress may also cause lipid peroxidation. Free radical forms of oxygen include superoxide anion (O_2^-), hydroxyradical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2). These radicals are formed by a stepwise one-electron reduction of O_2. One-electron reduction of H_2O_2 leads to the formation of water (H_2O). Superoxide anion (O_2^-) is dismutated by a cellular enzyme known as superoxide dismutase, resulting in the formation of H_2O_2. Cellular iron can metabolize O_2^- to $OH^{\bullet}$ and hydroxy anion (OH^-) radicals. Under certain circumstances a singlet O_2 (O_2) can also be formed from O_2. In the normal cellular metabolism small amounts of oxyradicals are generated. However, these are of no consequence since cellular defense mechanisms (e.g., superoxide dismutase, catalase, glutathione peroxidase, and vitamin E) mitigate these oxyradicals. Chemical toxicants may disrupt this balance to either produce excessive O_2 radicals and/or to compromise the cellular defense mechanisms.

Certain toxic chemicals may form organic radicals by being reduced by one electron, a reaction mediated by the flavin enzyme, cytochrome P450 reductase. These organic one-electron reduction products (semiradicals) can donate this electron to O_2 in the cells to form O_2^-. The organic toxic (parent) chemical is now free to be reduced again by the reductase and generate additional O_2^- radical. This reduction–oxidation cycle can continue as long as the chemical, cellular O_2, and reducing equivalents (NADPH) are available in the cell. Thus, redox cycling can generate a virtually unending supply of oxyradicals. The herbicide paraquat, anticancer agent bleomycin, and antibiotics nitrofurantoin and mitomycin are a few examples of chemicals that can undergo redox cycle.

Reperfusion injury is also thought to be associated with oxyradicals. After hypoxia during the cessation (or reduction) of blood supply to tissues (as in surgical procedures) the cells may shut down the normal defense mechanisms. When blood supply is restored after surgery, the tissue encounters a normal amount of O_2, leading to formation of oxygen free radicals. Regardless of how they are formed, free radical forms of O_2 can also initiate and propagate lipid peroxidation leading to tissue injury.

Interference with Endogenous Pathways

Many chemicals produce toxicity by interfering with different endogenous pathways (e.g., cellular energy production and excitable membrane functions). ATP is the main form of energy utilized by the living cells. ATP is thus necessary to maintain the normal functions of the cells and significant depletion will

lead to loss of cell function and cell death. Cyanide, hydrogen sulfide, and azides bind to cytochrome oxidase and thus block utilization of oxygen by different tissues and thus inhibit ATP production. Rotenone and antimycin A interfere with specific enzymes in the electron transport chain necessary to generate ATP, while sodium fluoroacetate blocks Krebs cycle.

Different ion channels maintain the stability of excitable membranes that are necessary for normal functioning of the body. Saxitoxin, tetrodotoxin, and DDT all cause toxicity by blocking the sodium channel in excitable membranes. On the other hand, organic solvents cause toxicity by changing the membrane fluidity of the neurones in the central nervous system.

Stimulation and Blockade of Cell Cycle Progression

Regardless of the mechanisms of cell and tissue injury, toxic or physical injury elicits an endogenous cell proliferative and tissue repair response in the affected tissues and organs in the body. This is a parallel but opposing response to tissue injury. Chemicals vary in their ability to induce the compensatory tissue repair response. This response also varies depending on the strains and species. The human body is also capable of this tissue repair response. Simultaneous with the initiation of tissue injury, the surviving cells respond by receiving/sending appropriate cellular and molecular signals that lead to cell cycle progression beginning with G_0 to G_1 and G_1 to S phase synthesis. The first line of defense of tissue, however, occurs by the release of normally occurring small population of G_2 cells to divide. This occurs within a few hours of exposure to toxic chemicals. When these cells divide, a host of molecular messages are expressed that facilitate surviving cells to divide. Thus, this tissue repair response usually takes the form of a biphasic cell proliferation response. At low to moderately toxic doses, tissue repair response shows a classic dose–response relationship. This allows the body to repair the injured tissue and restore the structure and function of the tissue, thereby permitting complete regression of injury, recovery, and survival. At high doses, two events occur which lead to unrestrained progression of injury initiated by the mechanisms that initiate injury. First, the tissue repair response is significantly diminished. Second, it is delayed considerably. Delay leads to unrestrained progression of injury, and diminished response is too little to cope with the progression of tissue injury and destruction. There is significant interest in understanding the biological events and the molecular regulation of these events. If tissue repair response is stimulated through specific methods of stimulation (e.g., partial hepatectomy, prior exposure to a low dose of a toxic chemical, nutritional supplementation with energy sources that facilitate cell division, and activation of molecular signals), complete protection can be demonstrated from even lethal doses of toxic chemicals, even though normally lethal massive injury may be inflicted by any of the mechanisms described earlier (Harihara M Mehendale).

Cell cycle progression is very important for the body; in newborns and young adults it helps in the normal development of the body, while in adults it is essential to replace cells that are dead or dying (either by normal aging or chemical-induced necrosis). Usually, most of the cells in an adult are in resting or G_0 phase of cell division. As a response to cell injury or impeding cell death, the cells go to G_1, S, G_2, and then M or mitotic phase of cell division to produce newly divided daughter cells. Cancerous growth is a result of abnormal and uncontrolled cell division. Chemical carcinogenicity (like cancers of unknown or viral etiology) leads to unregulated tumor growth due to uncontrolled cell division. Normally, each phase of cell division is finely regulated by growth factors, cytokines, and many other products of gene expression (e.g., cyclines). Cellular transduction mechanisms are also involved in the regulation of cell cycle progression. This finely regulated balance is perturbed by the cancer-causing chemicals. Many anticancer drugs cause toxicity by blocking the progression of cells through the cell cycle. They can either arrest the cells in different phases of cell division or can block specific enzymes by binding with the enzymes needed for cell cycle progression. Colchicine, a common anticancer drug, not only blocks the M phase of cell division but also inhibits the enzyme activity of thymidylate synthetase and thymidine kinase to arrest the cells in S phase of cell division. Taxol is another example of anticancer agent that works by blocking cell division. In contrast to colchicine, which blocks cell division by inhibition of S-phase synthesis and by preventing microtubular function, taxol works by interfering with microtubule aggregation.

Genetic Alterations

Chemicals that cause toxicity by genetic alterations of the somatic cells are called genotoxic carcinogens. Covalent interactions with DNA do not always lead to cell death. The vast majority of lesions in DNA are repaired; but in some cases the repair is incorrect or

incomplete, leading to a mutated DNA. From this time point on, all the daughter cells produced by the mutated cell(s) are also mutated. If this mutation occurs in a somatic cell then this may eventually lead to cancer but cannot be passed to future generations. It is believed that genotoxic chemicals induce cancer by altering the protooncogenes. Protooncogenes carry a cancerous phenotype. Many of the gene products are actually responsible for determining cells' response to growth factors and cytokines.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogenesis; Lipid Peroxidation; Modifying Factors of Toxicity; Molecular Toxicology–Recombinant DNA Technology; Tissue Repair; Toxicity Testing, Mutagenicity.

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Medical Surveillance

Christopher P Holstege

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The term *medical surveillance* is vague, but commonly utilized. There is no generally accepted definition of medical surveillance. Medical surveillance involves performing an observational study of either an individual or a population and includes collection, collation, analysis, and dissemination of data. The data acquired varies, and may be on exposures, disease, injury, death, and/or disability. There are numerous examples of medical surveillance with vastly different models. For example, the United States has formed the Army Medical Surveillance Activity (AMSA). Its main functions are to analyze, interpret, and disseminate information regarding the status, trends, and determinants of the health and fitness of America's Army and to identify and evaluate obstacles to medical readiness. AMSA is the central epidemiological resource for the Army.

In order to adequately develop a medical surveillance plan, one must perform a thorough assessment of the physical, biologic, and/or chemical hazards to which personnel may be exposed and which have the potential to cause adverse health effects. For certain chemical substances, medical surveillance is prescribed by law. The standards are set by the Occupational Safety and Health Administration (OSHA). Attention to sensitivity, specificity, and predictive value is particularly important in occupational screening programs. The primary purpose of medical surveillance is the elimination of exposures that cause disease. Medical surveillance's ultimate goal is prevention.

Prevention

Primary Prevention

Primary prevention pertains to actions that are taken before disease develops. The first line of defense against toxin-induced disease is the recognition that specific exposures are hazardous; this distinction is based on either prior human experience or experimental evidence. Recognized hazards may then be eliminated from the environment through substitution, through engineering controls, or through personal protective equipment.

Secondary Prevention

Secondary prevention occurs when a disease is detected early when the individual is asymptomatic. *Medical screening* is the periodic examination of an individual in order to detect preclinical disease. Examples of medical screening include use of urinary cytology in workers exposed to bladder carcinogens and mammography for asymptomatic cancer of the breast.

Tertiary Prevention

Tertiary prevention involves detecting clinical signs and symptoms of disease early before significant adverse consequences of that disease occur. Tertiary prevention is the delivery of optimal clinical care to minimize the consequences of symptomatic disease.

Biological Monitoring

Biological monitoring involves the examination of a sample from an individual (i.e., urine or blood) to look for evidence of exposure to chemical hazards.

Depending on the chemical of interest, biologic monitoring may evaluate the unchanged chemical in body fluids, a metabolite of the original chemical, an enzymatic alteration, a physiologic effect, or a secondary clinical finding. Examples of biological monitoring include obtaining a blood lead level and/or zinc protoporphyrin level in a worker with known lead exposure, obtaining a urinary phenol level in a worker with benzene exposure, and obtaining a red blood cell cholinesterase level in a worker with organophosphate pesticide exposure. The American Conference of Governmental Industrial Hygienists publishes guidelines and reference values (biologic exposure indices) for biologic monitoring.

Sentinel Events

Medical surveillance also includes monitoring for a single case of new disease caused by a chemical agent that triggers an alarm in an astute clinician. Such cases, called *sentinel events*, result in further study and analysis to determine a true cause and effect relationship. Historically, numerous chemical agent induced disease processes have been determined through medical surveillance and sentinel event determination. For example, the relationship between vinyl chloride exposure and hepatic angiosarcoma development was determined by astute medical surveillance.

Public Health Surveillance

Public health surveillance pursues a number of goals. The first is the estimation of the magnitude of disease occurrence and its trends over time. The second is the identification of new opportunities of prevention including entirely new diseases as well as old diseases in new circumstances. Cases of well-established occupational disease, with known preventable etiologies, are known as Sentinel Health Events, Occupational (SHEO). Each case represents a failure of prevention. Investigation of SHEOs and intervention may lead to the identification of root causes of the failure of prevention and hence to improved prevention. A third goal is identifying epidemic clusters, or epidemics of diseases, so that resources can be targeted toward their prevention.

Conclusion

The goal of medical surveillance is prevention. However, medical surveillance is an ambiguous term that includes surveillance of individuals (medical surveillance) as well as populations (public health surveillance). It includes interest in early disease (medical screening) as well as in detecting evidence of exposure (biological monitoring). Medical surveillance is most likely to lead to prevention. It is most important that medical surveillance work in conjunction with environmental monitoring.

Contact Details

- Occupational Safety and Health Administration (OSHA)
  Permissible exposure limits (PELs) and a guide to OSHA standards for screening and surveillance
  200 Constitution Ave. NW
  Washington, DC 20210, USA
  Tel.: + 1-202-693-2300
  URL: http://www.osha.gov

- American Conference of Government Industrial Hygienists
  Threshold limit values (TLVs) and biological exposure indices (BEIs)
  1330 Kemper Meadow Dr., Suite 600
  Cincinnati, OH 45240, USA
  Tel.: + 1-513-742-2020
  URL: http://www.acgih.org

- National Institute for Occupational Safety and Health (NIOSH)
  Recommended exposure limits (RELs)
  1600 Clifton Rd. NE
  Atlanta, GA 30333, USA
  Tel.: + 1-800-356-4674
  URL: http://www.cdc.gov/niosh

See also: American Conference of Governmental Industrial Hygienists; Biomonitoring.

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Meperidine

Michael Hiotis

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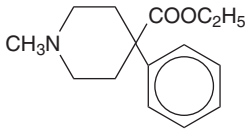
- Chemical Abstracts Service Registry Number: CAS 50-13-5
- Synonyms: Pethidine hydrochloride; Demerol; Sonipeccaine hydrochloride; Pethadol; Centralgion; Dolantin
- Chemical/Pharmaceutical/Other Class: Synthetic opioid analgesic; a phenylpiperidine derivative
- Chemical Formula: $C_{15}H_{21}NO_2$
- Chemical Structure:



Uses

Meperidine is used as analgesic for acute and severe pain, as a preoperative medication, as an obstetrical analgesic, and for support of anesthesia.

Exposure Routes and Pathways

Meperidine is commercially available in parenteral solutions for intravenous and intramuscular administration and in oral tablets and solution.

Toxicokinetics

Meperidine is well absorbed from all routes of administration. Following oral administration, meperidine undergoes extensive metabolism on first pass through the liver. Meperidine is less than one-half as effective when given orally as when given parenterally. Following oral administration peak analgesia occurs within 1 h with duration of 2–4 h. Peak analgesia occurs 30–50 min after parenteral administration with duration of 2–4 h. Meperidine is metabolized primarily in the liver. It is demethylated to form normeperidine, which is then hydrolyzed along with meperidine to normeperidinic acid and meperidinic acid. The acid metabolites are less active than the meperidine and are further metabolized through conjugation. Normeperidine is pharmacologically active. Meperidine distributes widely into the liver, kidneys, and muscle. The volume of distribution is $3.84\,l\,kg^{-1}$. Protein binding is 65–75%. Meperidine plasma half-life is 2.4–4.0 h. The half-life of normeperidine is 15–30 h. Meperidine is excreted in the urine, ~5% as unchanged drug. The elimination half-life is biphasic: $T_{1/2}$ (alpha) is 12 min and $T_{1/2}$ (beta) is 3.2 h in individuals with normal renal and hepatic functions.

Mechanism of Toxicity

Meperidine's chief pharmacological action is interacting with opioid receptors in the central nervous system (CNS). The highest concentration of stereospecific binding sites is in the limbic system, thalamus, striatum, hypothalamus, midbrain, and spinal cord. Meperidine's effects may result from mimicking the actions of enkephalins and endorphins and also from altering the release of neurotransmitters. Accumulation of the metabolite normeperidine can result in the toxic effects secondary to CNS stimulation such as seizures, agitation, irritability, nervousness, tremors, twitching, and myoclonus. Patients with decreased renal function are at a higher risk for developing seizures and other toxic effects of the metabolite normeperidine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs act similarly to humans when exposed to opiates. Symptoms may include drowsiness, ataxia, respiratory depression, miosis, coma, seizures, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone can be used at $0.02\,mg\,kg^{-1}$ if needed.

Human

When greater than therapeutic amounts are administered, CNS and respiratory depression, which may progress to cessation of respirations, may be seen. Pulmonary edema has been reported following therapeutic and toxic exposures to opiates. Peripheral vasodilation can cause hypotension and possibly circulatory collapse. In addition to the usual opiate toxicities, the course may be complicated by development of seizures. With daily doses of ≥ 3 g meperidine, convulsions may be seen due to metabolism of meperidine to normeperidine. Myoclonus will usually precede convulsions. Mydriasis may be present secondary to anoxia. Meperidine may also produce tachycardia through a vagolytic action, which increases ventricular response.

Semiquantitative and qualitative immunoassays can measure high concentrations of meperidine in the urine. Meperidine toxicity is reported with serum levels of 10–30 μg ml^{-1}; however, drug levels do not guide treatment. Normeperidine toxicity occurs with serum levels from 450 to 800 ng ml^{-1}.

Chronic Toxicity (or Exposure)

Animal

Chronic administration of meperidine in dogs at up to six times the maximum recommended therapeutic dose resulted in minor anorexia and weight loss.

Human

Addicts usually demonstrate symptoms of twitching, tremors, confusion, hallucinations, and convulsions at high doses. Meperidine use can produce physiologic dependence. An abstinence syndrome can begin within 3 h after use and peak at 8–12 h. The abstinence syndrome of meperidine consists of more severe muscular twitching and restlessness and fewer autonomic symptoms than other opiates.

In Vitro Toxicity Data

Studies of rat skeletal muscle mu 1 voltage dependent sodium channels have shown blockage of sodium channels; meperidine acts pharmacologically like a local anesthetic.

Clinical Management

Basic life-support measures should be instituted as necessary. Intensive support therapy may be required to correct respiratory failure and shock. Patients with mild to moderate toxicity may present with lethargy, miosis, decreased blood pressure, heart rate, and muscle flaccidity. With severe toxicity coma, respiratory depression, seizures, noncardiogenic pulmonary edema, apnea, and sudden death may occur. If taken orally, administration of activated charcoal is recommend as soon as possible to minimize absorption of meperidine. Emesis is contraindicated due to potential for seizures, and significant CNS and respiratory depression. The specific antagonist naloxone is used to counteract respiratory depression and coma. A dose of 0.4–2.0 mg is given intravenously and can be repeated at intervals of 2 or 3 min. The therapeutic effect of naloxone maybe of shorter duration than that of the opiate activity; therefore, a naloxone continuous infusion may then be of benefit. Nalmefene and naltroxone are other opioid antagonists. These antagonists are similar to naloxone but with longer half-life and may be considered as alternatives to naloxone. Naloxone does not antagonize the tremors or seizures caused by normeperidine. It will, however, antagonize the opiate effects. Seizures may be treated with intravenous benzodiazepines. Arterial blood gases, vital signs, and level of consciousness should be monitored continuously until cessation of symptoms. Patient should be monitored for at least 6–8 h after the last dose of naloxone is administered to prevent relapse of respiratory and CNS depression. Mepergan is a combination drug that contains meperidine and promethazine and when managing an overdose, the toxicity of both components should be considered.

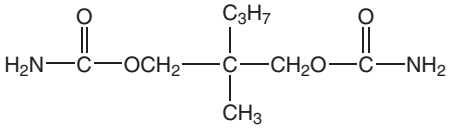
Further Reading

Goetting MG and Thirman MJ (1985) Neurotoxicity of meperidine. *Annals of Emergency Medicine* 14: 1007–1009.

Meprobamate

David Eldridge and Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 57-53-4
- Synonyms: Miltown; Equanil; Meprobam; Meprobamatum; Procalmadiol; 2,2-Di(carbamoyloxymethyl)pentane; Carbamic acid 2-methyl-2-propyltrimethylene ester; 2-Methyl-2-propyl-1,3-propanediol dicarbamate; 2-Methyl-2-propyltrimethylene carbamate
- Chemical/Pharmaceutical/Other Class: Carbamate derivative
- Chemical Formula: $C_9H_{18}N_2O_4$
- Chemical Structure:



Uses

Meprobamate is used as a minor tranquilizer and as an anxiolytic agent.

Exposure Routes and Pathways

Meprobamate is available as tablets, an oral liquid, and extended-release capsules (Meprospan-200). Meprobamate is also the active metabolite of carisoprodol (Soma) an oral drug that is used as a muscle relaxant.

Toxicokinetics

Meprobamate is well absorbed by the gastrointestinal tract and when taken orally, at therapeutic dosing, has a peak serum concentration within 2–3 h. When taken in overdose, absorption can be prolonged (reported up to 13 h) and clinical signs and symptoms may be delayed. Meprobamate is rapidly metabolized in the liver to inactive hydroxy and glucuronide metabolites.

After absorption, meprobamate can be found throughout the body and has a volume of distribution of $0.75\,l\,kg^{-1}$. Plasma protein binding is 15%. It is excreted by the kidneys either in its unchanged form (10%) or as inactive metabolites.

Mechanism of Toxicity

Meprobamate's chief toxicity is through central nervous system depression. Its precise mechanism of action is unknown. It appears to inhibit or affect neurotransmission in the thalamus, hypothalamus, limbic system, and spinal cord. In high doses, meprobamate can act as a general anesthetic with respiratory depression and cardiovascular collapse thought to be from a combination of vascular muscle and direct cardiac effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

The clinical effects of meprobamate in animals are similar to those observed in humans, although animals appear to be less sensitive to meprobamate than humans.

Human

No reliable toxic dose range of meprobamate is known. Death has resulted following the ingestion of 12 g, and survival has been reported at 40 g. Due to this variability, clinical symptoms should be the guide to intervention. Signs and symptoms of acute toxicity include central nervous system depression ranging from mild stupor to deep coma, slurred speech, weakness, vertigo, and ataxia. Profound hypotension and shock can occur. The most common pulmonary toxicity is respiratory depression, but pulmonary edema has been reported.

Chronic Toxicity (or Exposure)

Human

Prolonged use of high doses of meprobamate can cause slurred speech, ataxia, and vertigo. One of the most concerning problems with chronic use is the physical and psychological dependence that can both develop with regular use. Chronic use of high doses followed by abrupt cessation can produce a severe withdrawal syndrome. This syndrome can appear clinically similar to barbiturate withdrawal and can produce anxiety, tremors, insomnia, nausea, vomiting, delirium, hallucinations, and seizures. Meprobamate withdrawal is potentially life-threatening.

In Vitro Toxicity Data

Several mutagenicity studies in *Drosophila* models have been either inconclusive or negative. Studies of meprobamate activity from cultured rat hippocampal neurons have described meprobamate enhanced GABA-evoked responses in a concentration-dependent manner.

Clinical Management

Basic and advanced life-support measures are the most important component of clinical management. Serum levels of meprobamate are not routinely available and are therefore not useful in clinical management decisions. The administration of activated charcoal may be considered for substantial recent ingestions and a second dose may be used in patients who demonstrate continued drug absorption. The patient's level of consciousness and vital signs should be monitored closely. There is no antidote for meprobamate. If hypotension occurs, intravenous fluids should be infused. If hypotension is refractory to intravenous fluids, then vasopressors may be considered. Forced diuresis is ineffective and potentially harmful as it may lead to fluid overload. Hemodialysis has been reported to be efficacious at enhancing elimination of meprobamate, but should be considered only in severe cases when supportive care is insufficient to stabilize the patient. In chronic meprobamate users who suddenly stop therapy, withdrawal may be severe and life threatening. This withdrawal can be minimized by gradually weaning meprobamate over 2 weeks. If a

patient develops severe withdrawal symptoms, such as seizures, phenobarbital or benzodiazepines should be used to control symptoms.

See also: Barbiturates, Long-Acting; Barbiturates, Short-Acting; Benzodiazepines.

Further Reading

Allen MD, Greenblatt DJ, and Noel BJ (1977) Meprobamate overdosage: A continuing problem. *Clinical Toxicology* 11: 501–515.

Eeckhout E, Huyghens L, and Loef B (1988) Meprobamate poisoning, hypotension and the Swan–Ganz catheter. *Intensive Care Medicine* 14: 437–438.

Mercaptans

Lee R Shugart

- Representative Chemical: Methyl mercaptan
- Chemical Abstracts Service Registry Number: CAS 74-93-1
- Synonyms: Methanethiol; Mercaptomethane; Thiomethyl alcohol
- Chemical/Pharmaceutical/Other Class: Sulfur-containing organic chemical substances of the type R–SH (R = an alkyl group)
- Chemical Formula: CH_3SH

Uses

Used as an intermediate in the manufacture of jet fuels additives, pesticides, fungicides, plastics, and in the synthesis of methionine.

Background Information

Mercaptans are colorless and odorous gases linked to animal facilities, wastewater treatment plants, and paper and pulp manufacturing. It is released from decaying organic matter in marshes and is present in the natural gas of certain regions of the United States, in coal tar, and in some crude oils. If mercaptans are in the air, even at low concentrations, they are very noticeable.

Exposure Routes and Pathways

The main route of exposure is via inhalation.

Toxicokinetics

There are no toxicokinetic studies for methyl mercaptan via inhalation, oral, or dermal routes of exposure. *In vitro* studies in both humans and rats show that methyl mercaptan is oxidized (the carbon–sulfur bond is split) in blood by erythrocytes resulting in formic acid, sulfite, and sulfate ions.

Mechanism of Toxicity

Mercaptans act mainly as an irritant affecting the mucous membranes of the nose and respiratory tract.

Acute and Short-Term Toxicity (or Exposure)

Human

Mercaptans are considered to be slightly toxic if inhaled. Short-term exposure to slight amounts may result in coughing and irritation of the respiratory tract. Typically, these symptoms remit after a short period of time.

Chronic Toxicity (or Exposure)

Human

Exposure to high concentration (severe exposure) of mercaptans may produce central nervous system effects such as headache, staggering gait, muscular weakness, tremors, lung edema, convulsions, and paralysis of the respiratory center. Long-term health effects are not well documented.

Clinical Management

If inhaled and breathing is difficult, the person should be moved to fresh air and administered oxygen.

Environmental Fate

Relatively little is known about the fate of mercaptans once they are released to the environment.

Ecotoxicology

Animal toxicity: LC_{50} (mortality) for male and female rats is 675 ppm for 24 h. No mortality and no compound-related histopathological change were

noted in lungs of rats exposed continuously at 57 ppm for 3 months.

Exposure Standards and Guideline

The US Environmental Protection Agency requires that discharges, spills, or accidental release of 100 pounds or more of methyl mercaptan must be reported. Occupational Safety and Health Administration has set a permissible exposure limit of 20 mg of methyl mercaptan per cubic meter of air (20 mg m^{-3}) for an 8 h workday in a 40 h workweek. National Institute for Occupational Safety and Health recommends an occupational exposure limit of 1 mg m^{-3} for methyl mercaptan over 15 min and a threshold limit value for an 8 h time-weighted average of 0.5 ppm.

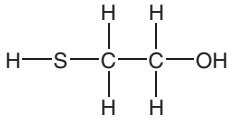
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Mercaptans.

Mercaptoethanol, 2-

Patricia J Beattie

- Chemical Abstracts Service Registry Number: CAS 60-24-2
- Synonyms: 2-ME; Mercaptoethanol; 1-Ethanol-2-thiol; 2-Hydroxy-1-ethanethiol; 2-Hydroxyethyl mercaptan; Monothioethyleneglycol; 2-Thioethanol; Thioglycol
- Chemical/Pharmaceutical/Other Class: Thiol; Mercaptan
- Chemical Formula: $HSCH_2CH_2OH$
- Chemical Structure:



Uses

2-Mercaptoethanol (2-ME) is used as an initiator for polymeric reactions; a reagent for diagnostic bioassays; a rust inhibitor for steel; a brightening agent in copper deposition; a tarnish remover for alloys and metals; an ingredient in hair permanent chemicals, and in hair and wool dyes; a stabilizer; and a mosquito control agent.

Exposure Routes and Pathways

Skin and eye contact and inhalation are the primary routes of exposure.

Toxicokinetics

2-ME is metabolized to 2-mercaptoacetate by alcohol and aldehyde dehydrogenase. Inhibition of alcohol dehydrogenase in experimental animals, blocking the formation of 2-mercaptoacetate, eliminated the adverse liver effects typically seen after exposure to 2-ME. No metabolic data with respect to biological half-life were found.

Mechanism of Toxicity

2-ME exposure in rats induced a fatty liver condition as shown by a significant rise in liver triacylglycerol and blood-free fatty acid levels, a slight reduction of liver phospholipids, and a progressive decrease of blood triacylglycerol (25%) and blood phospholipid levels (30%), as well as a reduction of hepatic ketone body levels. 2-Mercaptoacetate induced effects similar to those produced by 2-ME on the liver and blood, consistent with the data indicating that this is the metabolite responsible for the blood and liver toxicity seen following 2-ME exposure. 2-ME has also been reported to inhibit thymus deoxycytidine formation, inhibit mitotic activity in the rat intestine, inhibit thymidine incorporation in rat DNA, and cause moderate deoxyribonuclease inhibition.

Acute and Short-Term Toxicity (or Exposure)

Animal

In a number of studies, 2-ME has been reported to cause skin and eye irritation in mice and rabbits. Acute ingestion studies in mice indicate the LD_{50} is 348 mg kg^{-1}. The animals exhibited signs of central nervous system (CNS) depression with death due to respiratory failure. Microscopic examination found lymphocyte infiltration of the liver and kidneys, destruction and hemorrhaging of the lungs, and foci inflammation of the myocardium.

Human

In humans, 2-ME is extremely irritating to skin, eyes, and mucous membranes and may cause the development of contact dermatitis and pulmonary edema. Like other thiols, 2-ME may depress the CNS and cause respiratory paralysis and death. One case of an accidental spill of 2-ME was reported to the Centers for Disease Control and Prevention, with no ill effects observed in exposed individuals.

Chronic Toxicity (or Exposure)

Animal

A 6 month inhalation study was conducted in rats in which the animals were exposed daily to 10 mg m^{-3}. At 3 months, neuromuscular depression, lymphopenia, neutrophilia, and decreased oxygen consumption were observed. In the fifth month, increased organic sulfate elimination, variation in weight, arterial pressure, liver function, and protein metabolism were observed. Liver damage was reported upon histological examination. Studies on the teratogenic, embryotoxic, and cytogenic effects of 2-ME have been inconclusive.

Human

The literature contains no information on human long-term exposure to 2-ME.

Clinical Management

If contact with 2-ME occurs, the affected areas should be flushed immediately with large amounts of water. When eye contact has occurred, the affected person should be referred to a medical facility after eye washing has been completed. Victims who are overcome with fumes should be removed to fresh air. If breathing has stopped, artificial respiration should be administered. If ingested, medical attention should be obtained immediately.

Environmental Fate

In air, 2-ME degrades rapidly by reaction with photochemically produced hydroxyl radicals with an estimated half-life of 8.7 h. 2-ME added to soil at 10 000 ppm has been reported to biodegrade at a rate of 40 kg week^{-1}. 2-ME is miscible in water, but there is insufficient data to predict the biodegradation rate in water. The bioconcentration factor for 2-ME and its miscibility in water suggest that it will not bioconcentrate in aquatic organisms.

Exposure Standards and Guidelines

American Industrial Hygiene Association: workplace environmental exposure level: 8 h; time-weighted average: 0.2 ppm, skin.

Miscellaneous

It is manufactured by reacting ethylene chlorohydrin with sodium hydrogen sulfide or by reacting ethylene oxide with hydrogen sulfide. 2-ME is a white liquid with a strong unpleasant odor.

See also: Respiratory Tract; Sensory Organs; Skin.

Further Reading

Sax NI and Lewis RJ Sr (eds.) (1987) *Hawley's Condensed Chemical Dictionary*, 11th edn., p. 740. New York: Van Nostrand Reinhold Co.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Mercaptoethanol.

Mercuric Chloride

Vishal S Vaidya and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 7487-94-7
- Chemical/Pharmaceutical/Other Class: Mercurial compounds
- Chemical Formula: $HgCl_2$

Uses

Mercuric chloride is used in preservatives for wood and anatomical specimens, embalming solutions, disinfectants, photographic intensifiers, leather tanning, seed treatments, analytical reagents for organic syntheses, and the manufacture of other mercury-containing compounds. Pharmaceuticals containing mercuric chloride have also been used therapeutically as topical antiseptics and disinfectants.

Exposure Routes and Pathways

In its elemental form, mercury is a heavy silvery liquid at room temperature and has a very high vapor pressure. Mercury vapor is more soluble in plasma, whole blood, and hemoglobin than in distilled water, where it dissolves only slightly. The major natural sources of mercury are degassing of the earth's crust, emissions from volcanoes, and evaporation from natural bodies of water. (The worldwide mining of mercury is estimated to yield $\sim$10 000 tons per year. The activities lead to some losses of mercury and direct discharges to the atmosphere.) Other important sources are fossil fuel combustion, metal sulfide ore smelting, gold refining, cement production, refuse incineration, and industrial applications of metals.

Occupational exposure to inorganic mercury has been investigated in chloralkali plants, mercury mines, thermometer factories, refineries, and in dental clinics. High mercury levels have been reported for all these occupational exposure situations, although levels vary according to work environment conditions.

Toxicokinetics

Gastrointestinal absorption of mercuric chloride from food is less than 15% in mice and $\sim$7% in a study of human volunteers. In humans and other mammals, the kidneys are the primary targets where mercuric ions accumulate. Renal uptake and accumulation of mercury *in vivo* are rapid. As much as 50% of low dose of mercuric chloride ($0.5\,\mu mol\,kg^{-1}$) has been shown to be present in the kidney of rats within a few hours after exposure. Within the kidney it accumulates primarily in the cortex and outer stripe of outer medulla. Mercuric chloride does not readily cross the blood–brain barrier but will accumulate in the placenta. Urinary and fecal excretion of mercury is the principal means by which humans and other mammals eliminate the different forms of mercury from the body. Under most circumstances, a greater fraction of a dose of mercury is excreted in the feces than in the urine early after exposure to a nonnephrotoxic dose of mercuric chloride.

Mechanism of Toxicity

A reference from Middle Ages in Goldwater's book on mercury describes oral ingestion of mercury as causing severe abdominal cramps, bloody diarrhea, and suppression of urine. This is an accurate report of the effects following accidental or suicidal ingestion of mercuric chloride. Injection of mercuric chloride produces necrosis of the epithelium of the pars recta kidney. Cellular changes include fragmentation and disruption of the plasma membrane and its appendages, vesiculation and disruption of the endoplasmic reticulum and other cytoplasmic membranes, dissociation of polysomes and loss of ribosomes, mitochondrial swelling and loss of amorphous intramatrical deposits, and condensation of nuclear chromatin. Although exposure to high dose of mercuric chloride is directly toxic to renal tubular lining cells, chronic low-dose exposure may induce an immunologic glomerular disease. This form of chronic mercury injury to the kidney is clinically the most common form of mercury-induced nephropathy. Experimental studies have shown that the pathogenesis of chronic mercury nephropathy has two phases: an early phase characterized by antibasement membrane glomerular nephritis followed by a superimposed immune complex glomerulonephritis with transiently raised concentrations of circulating immune complexes.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute LD_{50} of mercuric chloride lies between 10 and $20\,mg\,kg^{-1}$ i.p. for rodents. The features of acute toxicity usually consist of shock, cardiovascular collapse, acute renal failure, and severe gastrointestinal damage. Exposure of animals to mercuric chloride primarily causes nephrotoxicity. Renal injury induced by mercuric chloride is generally expressed fully during the initial 24 h after exposure and can be induced in rats with single dose as low as $1.5\,\mu mol\,Hg\,kg^{-1}$. Rats tend to be more vulnerable to nephrotoxic effects of mercuric chloride than New Zealand White rabbits or several strains of mice. In rats, nephrotoxic doses of mercuric chloride produce selective alterations in the pars recta causing selective necrosis of the proximal tubules. *p*-Aminohippuric acid is produced in the pars recta, and its secretion is very sensitive to mercuric chloride.

A 16 day study was conducted by the National Toxicology Program in which rats and mice of each sex were administered six different concentrations (0–$20\,mg\,kg^{-1}$ for rats and 0–$80\,mg\,kg^{-1}$ for mice) of mercuric chloride in deionized water by gavage for 12 days. A significant mortality was recorded with administration of the highest dose of mercuric chloride in both rats and mice. Analysis of kidney, liver, and brain tissues for mercury residues revealed that the highest concentration was in the kidneys of rats and mice. Acute renal tubule nephropathy occurred in dosed rats and was slightly more severe in males than in females. Chemical-related lesions in mice included renal tubule necrosis, inflammation

and necrosis of the forestomach, and necrosis of the glandular stomach.

Human

Mercuric chloride is primarily a skin and mucous membrane irritant that is rapidly absorbed. Acute poisoning by ingestion or inhalation may cause severe nausea, vomiting, hematemesis, abdominal pain, diarrhea, melena, renal damage, and prostration. Ingestion of 1–2 g mercuric chloride may be fatal. Acute poisoning and death have also resulted from dermal applications of mercuric chloride solutions. In 1951, 18 cases of human poisoning were reported following oral ingestions of single dose of mercuric chloride, nine of which resulted in death. The lethal doses ranged from 29 mg kg^{-1} body weight to at least 50 mg kg^{-1}. The most common autopsy findings in these cases were gastrointestinal lesions (ranging from mild gastritis to severe necrotizing ulceration of the mucosa) and renal lesions that had resulted in renal failure.

Chronic Toxicity (or Exposure)

Animal

Groups of rats and mice ($n = 60$) of each sex received three different concentrations (0, 2.5, or 5 mg $HgCl_2$ kg^{-1} for rats and 0, 5, or 10 mg kg^{-1} for mice) in deionized water by gavage 5 days per week for 2 years. A 15 month interim evaluation suggested that the severity of nephropathy was increased in male rats and mice as compared to females. Chronic nephropathy appeared to develop at an accelerated rate and led to decreased survival in both dosed male rat groups at the end of 2 years. Secondary effects of renal dysfunction in dosed male rats resulted in increased incidences of fibrous osteodystrophy of the bone, mineralizations of various tissues, and parathyroid gland hyperplasia. Under the conditions of these 2 year gavage studies, focal papillary hyperplasia and squamous cell papillomas in the forestomach as well as thyroid follicular cell adenomas and carcinomas were observed in male rats. In the same study, evidence for increases in squamous cell papillomas in the forestomach of female rats was equivocal. An equivocal evidence for renal adenomas and adenocarcinomas was observed in male mice. This tumor type is rare in mice, and the increase in incidence was statistically significant when compared with historic controls. Two other nonpositive lifetime rodent studies were considered inadequate. Based on the absence of data in humans and limited evidence of carcinogenicity in rats and mice it has been termed as a Class 3 carcinogen. The relevance of the forestomach papillomas to assessment of cancer in humans is questionable because no evidence indicated that the papillomas progressed to malignancy. The relevance of the increase in thyroid tumors has also been questioned because these tumors are generally considered to be secondary to hyperplasia; this effect was not observed in the high-dose males. It should also be noted that the authors considered the doses used in the study to exceed the maximum tolerated dose for male rats.

Human

Most of the studies with potential mercury toxicity in humans deal with metallic mercury vapor or methylmercury. There are no adequate epidemiological studies with mercuric chloride exposure in humans.

In Vitro Toxicity Data

Results from genetic toxicity studies using a variety of assays indicate that mercuric chloride is not mutagenic in bacteria or yeast, but it may produce chromosomal damage and mitotic disruption (c-mitosis) in some plant and animal test systems.

Clinical Management

A patient airway should be established. Suction may be used if necessary. Signs of respiratory insufficiency should be watched out for and assisted ventilations provided if necessary. Oxygen should be administered by nonrebreather mask at 10–15 l min^{-1}. Pulmonary edema should be monitored and treated if necessary; shock should be monitored and treated if necessary. Seizures should be anticipated and treated if necessary. For eye contamination, eyes should be flushed immediately with available water. Each eye should be irrigated continuously with normal saline during transport. Emetics should not be used. For ingestion, the mouth should be rinsed and 5 ml kg^{-1} up to 200 ml of water for dilution should be administered if the patient can swallow, has a strong gag reflex, and does not drool. Activated charcoal should be administered.

Environmental Fate

Mercury adsorbed from mercuric chloride and 2-methoxy-ethylmercury chloride (Aretan) solutions by three contrasting soils showed a dependence on soil–solution ratio and initial mercury (Hg) concentration in soil solution. Changing the soil solution ratio from 1:10 to 1:100 but keeping the initial concentration constant resulted in an increase in initial concentration but, on the other hand, resulted in decrease in Hg adsorption. Upon manipulation of the pH of the surface soils, adsorption of mercuric

chloride at 100 mgHg l^{-1} concentration increased from ~70 to over 95 mgHg kg^{-1} when the pH was raised from 5.0 to 8.0. Precipitation of Hg may also have contributed to this trend. Removal of organic matter from soil resulted in large reductions of Hg adsorbed, as much as 95% from the mercuric chloride solutions. Mercuric compounds found in the atmosphere are likely to be transformed by chemical or physical processes. Theoretical calculations on the photodissociation of mercuric compounds have indicated that mercuric chloride and mercuric cyanide are stable, while mercuric hydroxide may dissociate in the gas phase. Exchange reactions between water and mercury compounds are likely to occur in the atmosphere. The result of these exchange reactions eventually results in the release of elemental mercury into the gaseous phase.

Ecotoxicology

The organic forms of mercury are generally more toxic to aquatic organisms than the inorganic forms. Aquatic plants are more affected by mercury in the water at concentrations approaching 1 mg l^{-1} for inorganic mercury (mercuric chloride) but at much lower concentrations of organic mercury. Aquatic invertebrates vary greatly in their susceptibility to mercury. Generally, larval stages are more sensitive than adults. The 96 h LC_{50} vary between 33 and 400 µg l^{-1} for freshwater fish and are higher for seawater fish. However, the organic mercury compounds are more toxic.

Environmental Bioconcentration

Bioconcentration factors of 10 000 and 40 000 have been obtained for mercuric chloride and methylmercury with oyster.

Atmospheric Concentrations

In the atmosphere, particulate bound mercury constitutes only ~2% of total mercury in the air and has normally been found to be less than 0.1 ng m^{-3} in regions unaffected by local sources. Some other mercury compounds, which may exist in the atmosphere, are mercuric chloride, mercuric bromide, mercuric hydroxide, mercuric sulfide, and mercuric cyanide. The rest is elemental mercury in the gaseous phase. In remote areas over the Atlantic and Pacific oceans, mercury bound to particulate matter concentrations are generally at or below the picogram per cubic meter level.

See also: Kidney; Mercury.

Further Reading

National Toxicological Program (1993) Toxicology and Carcinogenesis Studies of Mercuric Chloride (CAS No. 7487-94-7) in F344 Rats and B6C3F1 Mice (Gavage Studies), vol. 408, pp. 1–260.

Nordlind K (1990) Biological effects of mercuric chloride, nickel sulphate and nickel chloride. *Progress in Medicinal Chemistry* 27: 189–233.

Singer AJ, Mofenson HC, Caraccio TR, and Ilasi J (1994) Mercuric chloride poisoning due to ingestion of a stool fixative. *Journal of Toxicology: Clinical Toxicology* 32(5): 577–582.

Troen P, Kaufman SA, and Katz KH (1951) Mercuric bichloride poisoning. *New England Journal of Medicine* 244: 459–463.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Mercuric Chloride.

Mercury

Shayne C Gad

This article is a revision of the previous print edition article by Arthur B Furst and Shirley B Radding, volume 2, pp. 288–289, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7439-97-6
- Synonyms: Hydrargyrum; Liquid silver; Quicksilver
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Hg^{+}; Hg^{2+}

Uses

Mercury is a naturally occurring metal found throughout the environment as the result of normal breakdown of minerals in the earth's crust by weathering processes involving wind and water. With the exception of mercury ore deposits, the amount of mercury that naturally exists in any one place is usually very low. Natural phenomena such as

erosion and volcanic eruptions, and anthropogenic activities like metal smelting and industrial production and use may lead to substantial contamination of the environment with mercury.

Mercury was known in antiquity and utilized by alchemists. Its neurological effects were recognized early, and its use in the hat-making trade gave rise to the phrase 'mad as a hatter'. Mercury has been used commercially and medically for centuries. In the past it was a common constituent of many medications, for example, it was used in the treatment of syphilis. Use of mercury has been drastically reduced in recent years. Within the twentieth century, mercury used to be in every physician's or pharmacist's armamentarium, for example, calomel was commonly used in infant teething powders in the 1930s and 1940s.

The antibacterial and antifungal properties of organomercurials have resulted in their long-term use as topical disinfectants (thimerosal and merbromin) and preservatives in medical preparations (thimerosal) and grain products (both methyl and ethyl mercurials). Phenylmercury has been used in the past in paints, and dialkyl mercurials are still used in some industrial processes and in the calibration of certain analytical laboratory equipment. A major issue in recent years has been the presence of mercury in some vaccines, for example, in the vaccine preservative thimerosal – this has led to suspension of some vaccination programs and the development of preservative-free (i.e., mercury-free) vaccines as replacements.

Mercury can be used for the extraction of gold. In hospitals and homes, it is still used in thermometers and blood-pressure cuffs, can be found in batteries, switches, and fluorescent light bulbs. Large amounts of metallic mercury are employed as electrodes in the electrolytic production of chlorine and sodium hydroxide from saline. Today, exposure of the general population comes from three major sources: fish consumption, dental amalgams, and vaccines.

Despite the extensive knowledge of the dangers of mercury, it is still misused and mishandled. For example, mercury has been sold in recent years in the United States under the name Azogue in Hispanic botanicas for oral administration to treat constipation, colic, or stomachache, and there have also been cases of mercury poisoning due to the use of a beauty cream, Crema de Belleza–Manning (6–10% w/w mercury), produced in Mexico but 'commonly used among women of childbearing age'.

Exposure Routes and Pathways

Humans may be exposed to organic forms of mercury by either inhalation, oral, or dermal routes, and the effects of such exposure depend upon both the type of mercury to which exposed and the magnitude of the exposure. Most of the mercury found in the environment is inorganic mercury (metallic mercury and inorganic mercury compounds). This inorganic mercury can enter the air from deposits of ore that contain mercury, the burning of coal or garbage, and the emissions of factories that use mercury. Inorganic mercury may also enter water or soil from rocks that contain mercury, factories or water treatment facilities that release water contaminated with mercury, and the disposal of wastes. Inorganic or organic compounds of mercury may be released to the soil through the use of mercury-containing fungicides.

If mercury enters the water in any form, it is likely to settle to the bottom where it can remain for a long time. Mercury also remains in soil for a long time. Mercury usually stays on the surface of the sediments or soil and does not move through the soil to underground water. It is a liquid at room temperature, and evaporates into the air.

Inhalation and ingestion are the major routes of exposure, but it can also be absorbed through the skin. In its elemental form, mercury is the only metal that is in a liquid state at room temperature. It readily volatilizes at standard temperature and pressure, and its presence in open containers can result in biologically significant air concentrations in unventilated or poorly ventilated spaces. As an example, in a hospital laboratory study, mercury vapor levels of up to $0.71\,\mathrm{mg\,m^{-3}}$ were reported in the general air of the institutions' laboratories; this was not surprising given the approaches to mercury cleanup in nonchemical laboratory areas included 'sweeping up with Kimwipes', 'wipe up with filter paper', or 'try to retrieve it'.

Extensive studies have been conducted on the consumption of mercury (as methylmercury) from fish.

Toxicokinetics

Absorption varies significantly according to the form of exposure (elemental, inorganic, or organic). Inhaled mercury vapor crosses through the alveolar cells readily, is $\sim$75% absorbed, and is carried by the red blood cells. Catalase in these cells oxidizes elemental mercury almost at once to the divalent state. Alcohol inhibits the catalase activity; however, in the seconds it takes for a complete blood circulation cycle, a significant amount of free mercury can cross the blood–brain barrier.

Ingested or dermally applied elemental mercury is essentially not absorbed (an exception involved infants who absorbed mercury from disinfected

diapers). It is estimated that humans absorb $<10\%$ of ingested elemental mercury; whereas absorption of ingested methylmercury can be as high as 90%.

Mercury will cross the placental barrier. In mammalian tissue, organic mercury, especially alkyl mercury, is converted to inorganic forms but not vice versa. Inorganic forms of mercury (not organic forms) induce a metallothionein. Inorganic mercury concentrates mainly in the kidney. Organic mercury compounds, being lipid soluble, concentrate in adipose tissue and the brain. Elimination is primarily in the urine and the feces, with small amounts in breath, sweat, and saliva.

Mammalian tissue does not convert elemental mercury to methylmercury.

Mechanism of Toxicity

Mercury has a great affinity for sulfydryl moieties and, hence, binds and inactivates a variety of enzymes. Methylmercury also initiates lipid peroxidation, which can produce alterations in cell membranes. Mercury damages the microtubules in the brain by reacting with the protein tubilin.

Acute and Short-Term Toxicity (or Exposure)

Animal

Evidence of damage to brain, kidney, heart, and lungs have been reported in rabbits exposed acutely to metallic mercury vapor at certain concentrations. Both reversible and irreversible toxic effects may be caused by mercury and its compounds. The rabbit (inhalation) LC_{Lo} is $29\,mg\,m^{-3}$ over 30 h, and another rat (inhalation) TC_{Lo} is $4\,mg\,m^{-3}$ over 2 h a day for 11 days.

Human

Mercury is an accumulative poison. Its toxicity depends on its form. Symptoms may start rapidly after acute exposure to high air concentrations of mercury vapor, and can include fever, chills, and nausea. In severe cases (e.g., as a consequence of heating), pulmonary edema may cause death within a few days. Acute exposure to mercury vapor can also produce bronchitis and interstitial pneumonitis. The toxicity of mercuric chloride (i.e., corrosive sublimate) has been well established. Oral ingestion causes severe abdominal cramps, possible ulceration and bleeding of the gastrointestinal tract, and a bloody diarrhea. Loose teeth are noted and hepatitis has been recorded. Nephritis is common; if the renal tubes are extensively damaged, it could lead to a possible fatal uremia. Renal failure occurs rapidly and, when patients survive, they must be maintained by dialysis. Regeneration of some kidney cells is possible but the damage is usually permanent. Mercurous chloride is relatively insoluble and, thus, much less toxic than the soluble mercuric chloride.

Chronic Toxicity (or Exposure)

Animal

The carcinogenicity and mutagenicity of mercury have been claimed; however, further verification is needed. Although mercury is fetotoxic, the teratological aspect also needs further study. The rat inhalation TC_{Lo} is $1\,mg\,m^{-3}$ over 24 h a day for 5 weeks, $8\,mg\,m^{-3}$ over 6.5 h a day for 41 weeks, and $17\,mg\,m^{-3}$ over 2 h a day for 31 days.

Human

The neurotoxicity of mercury is devastating, especially for the central and peripheral nervous systems of children. Central nervous system defects and erethism as well as arrythmias, cardiomyopathies, and kidney damage have been associated with mercury exposure. The central nervous system symptoms include loss of memory, excitability, fever, and local tremors that can progress to the entire body. Necrotizing bronchitis and pneumonitis from inhalation of mercury vapor can lead to respiratory failure. Mercury is also considered a potent immunostimulant and suppressant, depending on exposure dose and individual susceptibility, producing a number of pathologic sequelae including lymphoproliferation, hypergammaglobulinemia, and total systemic hyper- and hypo-reactivities. Other clinical signs include inflammation of mouth and gums (gingivitis), tremors, loosening of teeth, jerky gait, personality change, depression, irritability, and nervousness. Other congenital abnormalities con occur with prenatal exposure to methylmercury.

The importance of workplace and home diligence in the handling and disposal of mercury is illustrated by a 9-year-old boy who developed encephalopathy and peripheral neuropathy as a result of having dismantled, 3 months before the visit to the doctor, a sphygmomanometer that had been provided by the hospital for monitoring of blood pressure at home. The family was unaware of the potential risks of mercury and when the boy informed his mother a few days after spilling the mercury in his bedroom, she attempted to dispose of the mercury by vacuuming it and then flushing it down the toilet. He was found to have a blood mercury level of $1000\,nmol\,l^{-1}$ (normal $<30\,nmol\,l^{-1}$) but slowly recovered after chelation.

Mammalian tissue does not convert elemental mercury to methylmercury; however, methylmercury can reside in the muscle and liver of fish, and has led to disasters such as the Minamata Bay and Niigata in Japan involving consumption of mercury-contaminated fish. Various ocean or river biota that had been eaten by the fish converted elemental mercury to the lipid-soluble mercury compound, methylmercury; clinical symptoms included encephalitis and disease or loss of the general senses (touch, smell, taste, hearing, and vision). Children are more sensitive to methylmercury poisoning than adults. In Iran, a local population ate bread that contained wheat seed that had been dusted with a fungicide consisting of methylmercury. The seed was intended for planting only. Symptoms of methylmercury poisoning included difficulty in walking, ataxia, paresthesia, sensory disturbance, and even deafness. A number of brain centers were damaged in the visual cortex and cerebellum.

Methylmercury crosses the placental barrier. Pregnant women who have not displayed any signs of mercury toxicity have given birth to infants with birth defects. Some infants were mentally retarded; some had palsy.

Other countries with mercury-related exposure issues include Iraq, Ghana, the Seychelles, and the Faroe Islands – the exposures and effects of which have been extensively studied.

Clinical Management

Normally British Antilewisite (2,3-dimercaptopropanol; BAL), administered intramuscularly, is used as an antidote for mercury poisoning. Oral D-penicillamine has been used for less severe cases. The I-acetyl derivative has been tested with good results. Experimentally, oral *m*-2,3-dimercaptosuccinic acid and the less toxic 2,3-dimercaptopropane-l-sulfonate are more effective than BAL.

Ecotoxicology

Mercury can be bioaccumulated in sea life and 'magnified' in the food chain.

Small fish and other organisms living in the water can take up methylmercury and inorganic forms of mercury. When larger fish eat small fish or other organisms that contain methylmercury, most of the methylmercury originally present in the small fish will be stored in the bodies of the large fish. As a result, large fish living in contaminated waters can collect a relatively large amount of methylmercury. Plants may have a greater concentration of inorganic mercury in them if they are grown in soil that contains higher than normal amounts of mercury.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average is $0.025\,mg\,m^{-3}$ for mercury vapor and inorganic mercury. The (US) National Institute for Occupational Safety and Health Immediately Dangerous to Life or Health value is $10\,mg\,m^{-3}$. The US Food and Drug Administration permits zero addition to the 20 μg of mercury contained in the average daily diet. Much of this comes from consumption of fish and seafood.

When mercury does spill, a thorough cleanup is necessary, and various commercial spill kits are available. Scrubbing with an aqueous solution of sodium thiosulfate has been reported to work well. A written set of work procedures and personal protective equipment should be considered in situations where mercury spills can occur.

See also: Disc Batteries; Environmental Processes; Kidney; Metals; Methylmercury; Neurotoxicity.

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Mercury.

Mescaline

Christopher P Holstege

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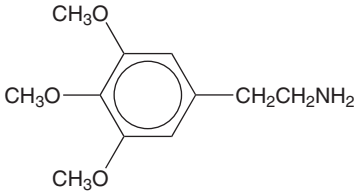
- Chemical Abstracts Service Registry Number: CAS 54-04-6
- Synonyms: 3,4,5-Trimethoxyphenethylamine; Peyote; Mescal; Mescal button
- Chemical/Pharmaceutical/Other Class: A phenylethylamine derivative alkaloid hallucinogen found in the North American small, blue-green, spineless cactus *Lophophora williamsii* and in several South American cacti of the *trichocereus* species
- Chemical Structure:



Uses

Mescaline does not have a therapeutic use. It is used as a Native American religious intoxicant. It is also a drug of abuse.

Exposure Routes and Pathways

The primary exposure pathway is oral ingestion of 'buttons'. These buttons are the round, fleshy tops of the cactus that are sliced and dried. Each button reportedly contains ~45 mg of mescaline.

Toxicokinetics

Each button of peyote reportedly contains ~45 mg of mescaline, with 6–12 buttons typically ingested to attain hallucinogenic effect. Mescaline is well absorbed from the gastrointestinal tract. Peak blood levels occur 2 h postingestion. Clinical effects occur within 1 h and last for typically 6–12 h. Mescaline does not bind to plasma proteins. The exact volume of distribution is unknown, but certainly above $1\,l\,kg^{-1}$. Mescaline is metabolized by the liver to inactive metabolites. Over 90% of a dose is recovered in the urine during the first 24 h, with 60% as mescaline and the remainder as mescaline metabolites.

Mechanism of Toxicity

The exact mechanism of mescaline has not been clearly defined. The central nervous system effects of mescaline appear to involve stimulation of both serotonin and dopamine receptors. In experimental studies, these effects can be blocked by either serotonin antagonists such as methysergide or dopamine antagonists such as haloperidol. Mescaline is structurally related to the amphetamines and cathine (khat). Sympathomimetic effects can occur and are thought to be centrally mediated. Mescaline does not appear to inhibit monoamine oxidase.

Acute and Short-Term Toxicity (or Exposure)

Human

Peyote buttons taste bitter. After ingestion, a transient initial phase of nausea, vomiting, and generalized abdominal discomfort typically occurs. This is followed by a sympathomimetic phase including increased blood pressure, tremor, mydriasis, diaphoresis, and tachycardia. Approximately 4–6 h after ingestion, a phase similar to lysergic acid diethylamide (LSD) intoxication occurs. This may include euphoria, depersonalization, disorientation, anxiety, ataxia, nystagmus, and vivid visual hallucinations. Changes in taste, smell, and hearing can also be present. Larger doses can produce bradycardia, hypotension, and respiratory depression. There is an increased risk for trauma in the mescaline intoxicated abuser due to the altered perception and increased emotional lability, panic attacks, and anxiety. Symptoms usually resolve over 6–12 h.

Chronic Toxicity (or Exposure)

Animal

Like LSD, mescaline has been used in animal models of schizophrenia. Both produce effects on 5HT2 receptors.

Human

Mescaline has been linked to a specific group of fetal abnormalities when used excessively. It is considered a potential teratogen. Studies of life-long peyote users did not find evidence of increased chromosomal

abnormalities. Mescaline may be associated with the phenomenon of 'flashbacks'.

In Vitro Toxicity Data

Recent studies have demonstrated mescaline extract toxicity in mouse and human leukocyte models of immune systems.

Clinical Management

Acute mescaline toxicity can be treated with supportive care. The patient's airway, breathing, and circulation should initially be assessed and therapy provided as required. Reassurance and provision of a quiet, nonthreatening environment may be effective in decreasing anxiety. Benzodiazepines should be utilized to alleviate sympathomimetic effects, anxiety, or panic attacks that do not respond to reassurance. If additional drug therapy is required for agitation or psychosis, haloperidol should be considered.

See also: Amphetamine; Drugs of Abuse; LSD (Lysergic Acid Diethylamide).

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Metabonomics

Vishal S Vaidya, Jeremy K Nicholson, and Harihara M Mehendale

What is Metabonomics?

Metabonomics, which can provide real biological endpoints, is defined as the quantitative measurement of the time-related multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification. The word metabonomics is derived from two Greek words 'meta' – meaning change – and 'ekynomous'– the word used to describe the behavior of a complex system. Its relationship to other 'omics' is shown in **Figure 1**.

Applying metabonomics involves generating metabolic databases for control animals and humans, diseased patients, and animals used in drug testing, and the simultaneous acquisition of multiple biochemical parameters on biological samples. Metabonomics is usually conducted with biofluids, many of which can be obtained noninvasively (urine) or relatively easily (blood), but other more exotic fluids such as cerebrospinal fluid, bile, seminal fluid, cell culture supernatants, tissue extracts, and similar preparations can also be used to determine the metabolites present, both in homeostasis and when the organism has been affected by factors such as environmental exposures.

Metabonomics is a promising approach because disease, drugs, or toxins cause changes in the concentrations and fluxes of endogenous metabolites involved in key cellular pathways. For example, the response of cells to toxic or other stressors generally results in an adjustment of their intra- or extracellular environment in order to maintain a constant internal environment (homeostasis). This metabolic change/adjustment is expressed as a fingerprint of biochemical perturbations, which is characteristic of the nature or site of a toxic insult or disease process. Urine, in particular, often shows changes in metabolic profile in response to toxic or disease-induced stress; because

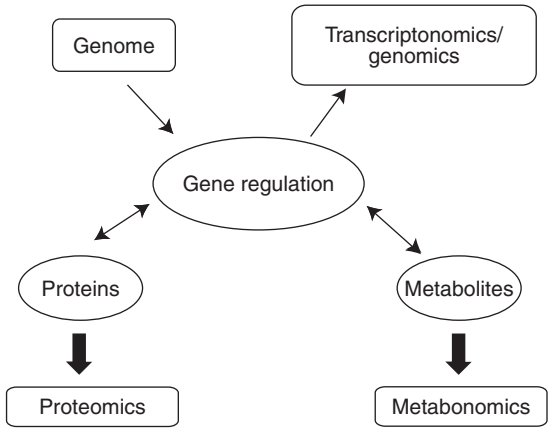


Figure 1 In the field of 'omics', the science for evaluating: protein expression changes is termed as 'proteomics'; gene expression changes is termed as 'transcriptonomics/genomics', and metabolite changes is termed as 'metabonomics'.

one way that the body's cellular systems attempt to maintain homeostasis after a toxic challenge is by modulating the composition of biofluids to eliminate substances from the body. Even when cellular homeostasis is maintained, subtle responses to toxicity or disease are expressed in altered biofluid composition. Metabonomics is also a powerful tool for investigating phenotypic abnormalities in mutant animals and human diseases and in modeling physiological variation in experimental animals and man.

Metabonomics is both systems biology and a dynamic approach, as metabonomic analysis can provide a description of the integrated physiological behavior of an entire living system across time. Metabolic profiling is a hugely complex undertaking, generating huge amounts of data to be analyzed and mined for gaining significant information about metabolic pathways and networks, novel biomarkers, and how metabolites interact not only with genes and proteins, but also with environmental, nutritional, and lifestyle factors. The integration of this array of variables holds the potential to tell researchers a great deal about human health and disease etiology.

Metabonomics versus Metabolomics

Metabolism is considered to be a phenotype. It integrates all the factors from nutrition to environment to genetics assessing one's current state of health. Metabolomics arose from metabolic control theory and was originally based on the metabolome, which is defined as the metabolic composition of a cell and is analogous to the genome or proteome. In metabonomics, static cellular and biofluid concentrations of endogenous metabolites are evaluated over a time-course.

In addition to providing molecular concentrations, metabonomics covers the study of molecular dynamic information, such as molecular reorientational correlational times and diffusion coefficients in intact tissues. Metabonomics can be regarded as a full systems biology approach; when a whole organism with separate organs and many cell types is studied, metabonomics can integrate the disparate effects that occur over both time and space. Metabolomics, which is the corresponding study in single cells, can be thought of as a subset of the systems covered by metabonomics.

Importance of Time-Course Estimations in Metabonomics

In a biological system, it is evident that the initiation of functionally connected gene expression events, cell signaling, protein synthesis changes, and metabolic responses to a stressor must be essentially sequential. Maturation and persistence of changes in gene expression, protein synthesis and posttranslational modification, and subsequent effects on metabolic processes also differ significantly. An event must therefore be evaluated in relation to time at each level of biomolecular organization if molecular responses are to be accurately associated with their macroscopic consequences in an organism. So, in metabolic studies, it is extremely important to measure time-dependent patterns of change in response to stimuli, because metabolic fluxes occur very rapidly, even in normal homeostasis, and consideration of the 'metabolite content' at only a fixed point in time can be misleading. The time-course studies are especially important in order to make a distinction between adaptive and toxic effects following exposure. This applies to the variables that change due to the initial adverse (mechanism-related) interactions, the homeostatic response to the cellular derangement (which could reflect entirely positive reactions of a healthy cell or tissue), and the changes due to cell death.

An important potential role of metabonomics is to direct the use and timing of proteomic and genomic analyses in order to maximize the probability of observing biological transitions that predict functional outcomes; this principle applies to human, animal, and microbial systems. In the case of single exposure to a toxic drug, there will be a response that takes time to complete, and the patterns that are observed in gene expression, proteins, and metabolites will therefore vary according to when the measurements are made. If the measurements are made long after dosing, it is possible that only profile changes will be due to biomarkers of recovery or cellular repair. In the case of a multidose study the second and subsequent doses of a compound might arrive before the effects of the first dose are cleared, complicating the profiles further. As the doses continue, there might be a rising curve of toxicity and there could be superimposed profile changes due to cell death and regeneration.

Quantitative Analysis of Metabonomics

To investigate the complex metabolic consequences of disease processes, toxic reactions, and genetic manipulation, nonselective but specific analytical approaches are required. Several spectroscopic methods in addition to nuclear magnetic resonance (NMR) can produce metabolic signatures of biomaterials, including mass spectrometry (MS), gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), liquid

chromatography/tandem mass spectrometry (LC/MS/MS), liquid chromatography/time-of-flight mass spectrometry (LC/TOFMS), high-performance liquid chromatography (HPLC), and optical spectroscopic techniques. Bioanalytically, NMR and MS are powerful means of generating multivariate metabolic data. NMR has the advantages of being nondestructive, applicable to intact biomaterials, and intrinsically more information rich with respect to the determination of molecular structures, especially in complex-mixture analyses. Furthermore, a technique known as magic angle spinning (MAS)-NMR can be used to carry out biochemical studies on intact tissues and cells, which, if carefully conducted, can preserve the samples for other studies and allow abnormal molecular compartmentation and interactions to be studied in intact tissues. MS is analytically more sensitive than NMR, but differential ionization suppression can make pattern quantification difficult, and extraction and derivatization might be necessary. The choice between NMR and MS approaches is ultimately matrix or problem dependent. Both technologies require further development, especially in terms of high-throughput and data processing methods, to optimize their use in complex metabolism studies.

NMR Spectroscopy of Biofluids

The successful application of ^{1}H NMR spectroscopy of biofluids to study a variety of metabolic diseases and toxic processes has now been well established and many novel metabolic markers of organ-specific toxicity have been discovered. ^{1}H NMR spectroscopy is well suited to the study of toxic events, as multicomponent analyses on biological materials can be made simultaneously, without bias imposed by expectations of the type of toxin-induced metabolic changes. This is particularly true for NMR spectra of urine in situations where damage has occurred to the kidney or liver. Quantitative changes in NMR spectroscopic metabolite patterns have also been shown to give information on the location and severity of toxic lesions, as well as insights into the underlying molecular mechanisms of toxicity.

The first studies of using pattern recognition to classify biofluid samples used a simple scoring system used to describe the levels of 18 endogenous metabolites in urine from rats that either were in a control group or had received a specific organ toxin that affected the liver, the testes, the renal cortex, or the renal medulla. This study showed that samples corresponding to different organ toxins mapped into distinctly different regions. Various refinements in the data analysis were investigated, including taking scored data at three time points after the toxin exposure for the nephrotoxins only as well as using a simple dual scoring system (the time and magnitude of the greatest change from control). The maps derived from the full time-course information provided the best discrimination between toxin classes. This study was further extended to incorporate actual metabolite NMR resonance intensities rather than simple scores. This was carried out for the nephrotoxins in the earlier group plus additional nephrotoxic compounds. A good separation of renal medullary from renal cortical toxins was achieved. In addition, it was possible to differentiate cortical toxins according to the region of the proximal tubule, which was affected, and also by the biochemical mechanism of the toxic effect.

Examples of toxicants investigated using this metabonomics approach are given below:

- *Kidney cortical toxins*: mercury chloride, *p*-aminophenol, uranyl nitrate, ifosfamide, cephaloridine.
- *Kidney medullary toxins*: propylene imine and 2-bromoethanamine hydrochloride.
- *Liver toxins*: acetaminophen, hydrazine, allyl alcohol, thioacetamide, α-naphthylisothiocyanate, and carbon tetrachloride.
- *Testicular toxin*: cadmium chloride using environmentally realistic levels.
- *Agents causing phospholipidosis*: amiodarone, chloroquine, DMP-777 (a neutrophil elastase inhibitor).
- Effect of dexamethasone on vascular lesions.
- Other studies include the toxicity of the aldose reductase inhibitor HOE-843 and lanthanum nitrate.
- Toxic stress in earthworms has also been investigated using metabonomics.

Extensions to the earlier chemometric approaches include a toxicological assessment approach based on neural network software to ascertain whether the methods provide a robust approach, which could lead to automatic toxin classification. The neural network approach to sample classification, based on ^{1}H NMR spectra of urine, was in general predictive of the sample class. It appears to be reasonably robust and once the network is trained, the prediction of new samples is rapid and automatic. However, the principal disadvantage is common to all neural network studies in that it is difficult to ascertain from the network which of the original sample descriptors are responsible for the classification. Although recently it has been suggested that probabilistic

neural networks appear to be a useful and effective method for sample classification. Recently, comprehensive studies have been published using pattern recognition to predict and classify drug toxicity effects, including lesions in the liver and kidney, and using supervised methods as an approach to an expert system.

It appears that using ^{1}H NMR spectroscopy to follow the biochemical responses of animals or cells to foreign compounds may confer significant analytical advantages. Currently, in order to evaluate the toxicity of a drug candidate by conventional toxicological procedures an array of biochemical methods is required. This is necessarily a complex and time-consuming process and if an inappropriate range of biochemical methods or metabolic parameters is used important metabolic disturbances may be overlooked. The role of NMR spectroscopy in analytical toxicology is thus essentially one of biochemical exploration; that is, determining the range of biochemical perturbations caused by exposure to a toxin and whether these are biologically significant.

MAS NMR of Tissues

As described above, NMR spectroscopy of biofluids when coupled with pattern recognition analysis can be an efficient new method of investigating toxicity profiles of xenobiotics. In addition, while NMR spectroscopy *in vivo* might be used to investigate abnormalities in whole animals, such studies are hampered by the heterogeneity of the sample, low magnetic fields of whole body scanners (low sensitivity and poor spectral dispersion), and short NMR relaxation times, all leading to broad lines and loss of resolution. Within the last few years, with the development of high-resolution MAS technology, it has become possible to obtain very high quality ^{1}H NMR spectra on small ($\sim$10 mg) samples of whole tissue.

MAS involves spinning the sample about an axis at 54.7° to the magnetic field direction. This process removes the line broadening caused by dipolar couplings, chemical shift anisotropies, and anisotropic magnetic susceptibility artifacts. Tissue metabolites already enjoy a degree of molecular mobility such that the line broadenings are greatly reduced from their true solid values and this means that only modest rotation rates ($<$10 kHz) are required. This approach has now been applied to cells and tissues. The technique opens up many diagnostic possibilities since information on a variety of metabolites in different cellular environments can be rapidly obtained and specialized NMR experiments, such as those to measure molecular diffusion coefficients, can be used to probe compartmentation. Confirmation of biochemical composition can be obtained using standard high-resolution NMR of both aqueous (protein-free) and methanolic extracts. This produces a comprehensive set of metabolic information that can be used in integrated metabonomics studies. MAS NMR data, like biofluid NMR spectra, can also be subjected to computer pattern recognition methods in order to classify toxicity type (target organ and biochemical mechanism) and to map time-related biochemical trajectories associated with drug-induced biochemical changes. The ability to compare biofluid and tissue NMR spectra may provide further insight into mechanisms of toxicity or target organ identification.

Chemometric Analysis of Metabolic NMR Data

One general procedure, which has found wide application, is to first simplify ^{1}H NMR spectra of biofluids by means of data compression, by producing a segmentation of each NMR spectrum (usually with $\sim$250 intensity values per spectrum), integrating peak intensity in each segment. Each of these acts as a metabolic descriptor with which to classify the NMR spectra according to biochemical features. These data are then constructed in the form of a spreadsheet, which is used as the input into a pattern recognition/multivariate statistics software suite. Appropriate data reduction routines, such as principal components analysis (PCA) or partial least squares discriminant analysis (PLS-DA), can be used to classify the NMR-generated toxicity data in terms of toxin type and dose. Multidimensional metabolic trajectories can be constructed in order to visualize the biochemical time-course of the toxic episodes. More complex expert systems based on chemometric models in the multidimensional metabolic space can be constructed and used for class prediction.

Expert System Development

A variety of different supervised pattern recognition methods have been evaluated for detecting abnormalities in metabolite profiles caused by toxins. These are of two main types, those that relate to overall variance in the data sets, such as those based on latent variables, for example, SIMCA and PLS-DA, and those that examine relationships in the data in different ways, such as neural networks and genetic algorithms. The basic procedure is to train types of expert systems to produce classifications of biofluid samples based on known toxicity type according to standard methods of evaluation, such as histopathology. These systems are then tested against standard toxicological assessment

procedures using toxins unknown to the model in order to evaluate the robustness of each expert system approach for toxicity screening.

Recently, LC/MS, LC/MS/MS, and LC/TOFMS have also become very popular techniques due to their sensitivity, speed, and specificity. However, the use of these techniques requires an expertise in the instrumentation to ensure high-quality reproducible data. Using the LC/TOFMS approach, drug metabolites have successfully been identified following drug candidate administration against a backdrop of potentially endogenous interferences. These measurements using any of the above-mentioned techniques, coupled with multivariate statistical chemometric methods for the purpose of latent information extraction and sample classification, offer a powerful new approach to the whole system of diagnostics and metabolic function to identify the metabolic phenotypes that result from a combination of genetic and environmental factors.

Applications of Metabonomics

Significant opportunities exist for the application of metabonomics to the field of environmental health sciences, particularly in the area of biomarkers of exposure and disease. When toxins interact with cells and tissues they disturb the ratios, concentrations, and fluxes of endogenous biochemicals in key intermediary cellular metabolic pathways. Under mild toxic stress, cells attempt to maintain homeostasis and metabolic control by varying the composition of the body fluids that either perfuse them or are secreted by them. In more severe toxicity states, cell death leads to loss of organ function and more marked biochemical changes occur in biofluids due to loss of whole body homeostasis and metabolite leakage from damaged cells. Consequently, following either scenario there are characteristic organ-specific and mechanism-specific alterations in biofluid composition. Clearly, the detection of toxic lesions via biochemical effects is most difficult close to the toxic threshold, yet these are often the most important effects to define.

^{1}H NMR spectroscopy has been used to study the composition of biofluids before and after administering a wide range of toxicants. Predictive statistical models have been constructed to deal with toxicological profiling on three levels. The first and most basic level is determining if the sample is normal, for example, whether it belongs to a control population. The second level involves fitting abnormal samples to known cases of tissue or mechanism-specific toxicity to predict the toxicity of novel pharmacological agents. The final level is to identify the spectral regions that are responsible for deviations from normal profiles and to determine toxicity biomarkers within those regions that may help elucidate mechanisms of toxicity.

Using pattern recognition methods, NMR spectra can be used to:

- Classify the sample as being normal or abnormal (example: patients suffering from coronary artery occlusion have been identified on the basis of ^{1}H NMR spectra of their blood serum).
- Establish normal physiological variance in a population of human urine samples.
- Classify target-organ toxicity and site and mechanism of action within the organ.
- Evaluate the time-course of the effect; for example, the onset, evolution, and regression of toxicity.
- Differentiate between tissue extract spectra obtained from normal tissues and to classify tumors by type such as pituitary tumor, fibrosarcoma, hepatoma.
- Classify several inborn errors of metabolism using urine spectra.
- Use in functional genomics: Metabonomics can be used to separate classes of experimental animals, such as mice and rats, according to their strain on the basis of the endogenous metabolite patterns in their biofluids. This is possible because differences in 'silent-gene' function between strains can influence the fluxes of metabolites through many key intermediary pathways resulting in distinct animal 'metabotypes'. There is also a strong indication for the use of metabonomics in the phenotype of mutant or transgenic animals and the investigation of the consequences of transgenesis.
- Identification of novel biomarkers of toxicity: Previously, the detection of novel biomarkers of toxic effect has mainly been serendipitous. However, it is now possible to use a combined NMR–expert systems approach to systematically explore the relationships between biofluid composition and toxicity and to generate novel combination biomarkers of toxicity. Pattern recognition maps can be examined for evidence of clustering of data according to site and type of toxic lesion.

Limitations of Metabonomics

As with all 'omics' platforms, metabonomics has certain limitations in terms of the recovery of biological information. In the case of toxicity assessment, it is possible to generate false-positive data in situations in which the compound of interest causes significant metabolic changes without associated toxicity, because of marked physiological

or pharmacological effects. For example, acetazolamide is a renal carbonic anhydrase inhibitor that massively reduces the excretion of intermediates in the citric-acid cycle. Misinterpretation can, however, be minimized by using supervised methods that include models of such effects.

Conversely, certain pathologies, such as liver fibrosis, are associated with negligible effects on biofluids, as metabolic derangement does not occur until there is significant tissue damage. In the case of low-potency compounds, there might be particular difficulties in separating toxicological from physiological effects. However, in previous dose–response studies, NMR-based metabonomic methods were at least as sensitive as conventional methods for detecting lesions at the 'threshold-dose' level, and even minor physiological changes were detected in normal animals.

There are obvious limitations in terms of choice of biofluid; for instance, urine might not be as appropriate as cerebrospinal fluid for studying neuropathology. There is also the potential for confusion with mixed-toxicity drugs that, for example, affect both liver and kidney, as the biomarkers of toxicity will be a complex combination that relates to both sites and possibly to the multiple mechanisms. However, this offset by the fact that mixed toxicities often have different timescales and such effects can therefore be deconvoluted by making repeated sequential measurements in individual animals.

Conclusion

Metabonomics is now recognized as an independent and widely used technique for evaluating the toxicity of drug-candidate compounds, and has been adopted by several pharmaceutical companies into their drug development protocols. It is possible to identify the target organ toxicity, derive the biochemical mechanism of toxicity, and determine the combination of biochemical markers for the onset, progression, and regression of the lesion. Furthermore, this technique can provide a metabolic fingerprint of an organism (metabotyping) – a key to functional genomics – and hence has applications in the design of drug clinical trials and evaluation of genetically modified animals as disease models.

A particular strength of spectroscopy-based metabonomic methods is that they are rapid, economic, and not labor intensive. In biological terms, the most important advantage of metabonomics is that individual animals and subjects can be followed noninvasively (urine collection) through a disease-related metabolic trajectory, yielding a holistic picture of integrated biological function over time. Finally, metabonomics can be a very effective tool for biomarker discovery following disease states or toxicant exposure.

A large challenge in the future will be to create a database of metabolic profiles with linkages to protein and gene expression databases. It is envisioned that a new and fundamental understanding of organism's responses to environmental insult will emerge from the integration of metabonomic data with those obtained from the study of global patterns of gene and protein expression. Such integration of data types will also pave the way to understanding the relationships between gene function and metabolic control in health and disease.

See also: Biomarkers, Human Health; Biomonitoring; Genomics, Toxicogenomics; Proteomics.

Further Reading

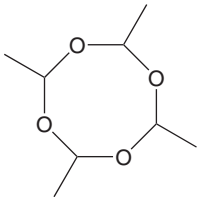
Nicholson JK, Connelly J, Lindon JC, and Holmes E (2002) Metabonomics: A platform for studying drug toxicity and gene function. *Nature Reviews. Drug Discovery* 1: 153–161.

Metaldehyde

Guangping Chen

- Chemical Name: *r*-2,*c*-4,*c*-6,*c*-8-Tetramethyl-1,3,5,7-tetroxocane or 2,4,6,8-Tetramethyl-1,3,5,7-tetraoxacyclooctane
- Chemical Abstracts Service Registry Number: CAS 108-62-3
- Synonyms: Acetaldehyde tetramer; Metacetaldehyde; Acetaldehyde polymers; Acetaldehyde homopolymer; Antimilace; Antimitace; Ariotox; Cekumeta; Deadline; Halizan; Limatox; Meta; Metason; Namekil; Ortho Metaldehyde 4% Bait; Slug Death; Slug Pellets, Slug-Tox; Slugit; Limax; Limovet; Polyacetaldehyde; Schneckokorn; Schnex-schneckentod; Terrasan-schneckentod Gekoernt

- Chemical/Pharmaceutical/Other Class: Molluscicide
- Chemical Formula: $C_8H_{16}O_4$
- Chemical Structure:



Uses

Metaldehyde is the most common molluscicide for controlling slugs and snails. It is used in a variety of vegetable and ornamental crops. Slug and snail baits generally contain 3% metaldehyde. It is also used as a fuel for lamps and stoves in Europe.

Exposure Routes and Pathways

Metaldehyde poisoning typically results from the ingestion of products containing the active ingredient. The use of molluscicides increases the risk of exposure for pets with access to treated areas.

Toxicokinetics

Metaldehyde is readily absorbed from the gastrointestinal tract. Metaldehyde's primary decomposition product in the body is acetaldehyde. Metabolites can cross the blood–brain barrier and enter the central nervous system.

Mechanism of Toxicity

Products containing metaldehyde are in the Environmental Protection Agency Toxicity Class II or III. The toxic dosage can range from 100 to 1000 mg kg^{-1} of body weight. Metaldehyde toxicity causes rapid onset of neurological signs/symptoms that can be fatal if untreated. Signs of poisoning begin within 1–4 h of exposure. Repeated seizures due to metaldehyde poisoning can cause very high body temperature, which can lead to complications similar to those observed from heatstroke.

Acute and Short-Term Toxicity (or Exposure)

Metaldehyde is moderately toxic by ingestion. Metaldehyde is also moderately toxic by inhalation. It can cause irritation to skin, eyes, and mucous membranes of the upper airways and gastrointestinal tract. The following symptoms appeared in humans hours following ingestion: abdominal pain, nausea, vomiting, diarrhea, fever, convulsions, coma, and persistent memory loss. High acute exposure can also lead to tachycardia, respiratory panting, acute asthmatic reaction, depression, drowsiness, high blood pressure, excessive urination and defecation, muscle tremors, sweating, excessive salivation, tearing, cyanosis, acidosis, stupor, and unconsciousness. Some signs and symptoms can persist for months following acute poisoning.

Chronic Toxicity (or Exposure)

Long-term exposure to metaldehyde may result in dermatitis and affect brain function in humans.

Clinical Management

An emetic (ipecac) should be administered to induce vomiting and prevent further absorption. Gastric lavage can be performed to remove metaldehyde from the gastrointestinal tract. If hyperthermia is noted, a cool-water bath can be given to lower body temperature. Sedatives, e.g., diazepam, can be given to control anxiety, seizures, and tremors. Intravenous fluids should be given to correct dehydration and acidosis.

Environmental Fate

Metaldehyde is of low persistence in the soil (days). It weakly adsorbs to soil. Metaldehyde is soluble in water. Metaldehyde undergoes rapid hydrolysis to acetaldehyde in an aquatic environment.

Ecotoxicology

Metaldehyde is toxic to birds, wildlife, pets, and poultry. Metaldehyde is reported to be virtually nontoxic to aquatic organisms. Bait agents with 6% active ingredient are nontoxic to bees. Many types of flowers lose their color following contact with metaldehyde.

See also: Acetaldehyde.

Further Reading

Booze TF and Oehme FW (1985) Metaldehyde toxicity: A review. *Veterinary and Human Toxicology* 27(1): 11–19.

Relevant Websites

http://ace.orst.edu – Extension Toxicology Network.
http://www.inchem.org – International Programme on Chemical Safety.
http://www.petplace.com – Webpage of Pet Place.com.

Metallothionein

Shayne C Gad

Metallothioneins (MTs) are a class of intracellular, low molecular weight (6000–7000 Da), cysteine-rich proteins. They are ubiquitous in eukaryotes, and have unique structural characteristics giving them potent metal-binding and redox capabilities. Although MTs were discovered many years ago, their functional significance remains obscure, that is, the primary role for MTs has not been identified and new functions are being discovered. MTs have been shown to be involved in many pathophysiological processes such as metal ion homeostasis and detoxification, protection against oxidative damage, cell proliferation and apoptosis, chemoresistance and radiotherapy resistance. MTs have been implicated as a transient response to any form of stress or injury providing cytoprotective action. Further, MT isoforms have been shown to be involved in the carcinogenic process, cancer development and progression; however, the use of MT as a potential marker of tumor differentiation or cell proliferation, or as a predictor of poor prognosism, remains unclear.

Four major MT isoforms, MT-1, MT-2, MT-3, and MT-4, have been identified in mammals. The most widely expressed isoforms in mammals, MT-1 and MT-2, are rapidly induced in the liver by a wide range of metals, drugs, and inflammatory mediators. In the gut and pancreas, MT responds mainly to Zn status. A brain isoform, MT-3, has a specific neuronal growth inhibitory activity, while MT-1 and MT-2 have more diverse functions related to their thiolate cluster structure. These include involvement in Zn homeostasis, protection against heavy metal (especially Cd) and oxidant damage, and metabolic regulation via Zn donation, sequestration, and/or redox control. A possible role for MT-4 is related to copper requirements in epithelial differentiating tissues.

MTs are key in protecting organs and tissues against the toxic effects of heavy metals. Although found throughout the body, metallothionein is most prevalent in the kidneys and liver. MTs bind with metals, rendering them biologically inactive as they enter the kidneys and liver. Once within these organs, the metal–MT complex breaks down, releasing the metal, which may cause toxic effects.

Outside the hepatic system, MTs facilitate absorption of metals such as cadmium into the kidneys and liver. Also, the induction of MTs via reactive metals protects hepatic enzymes against cellular damage. Without this intervention, zinc may stimulate glutathione concentration and reduce catalase activity. Cadmium reduces the amount of cytochrome P450 and its activity toward testosterone oxidation. The liver usually can produce sufficient amounts of the protein to bind to any free metals.

The kidneys are at greater risk for toxicity because they must manage free metals that enter without MTs binding as well as those freed through biotransformation. In the kidney, the cadmium–MT compound is filtered by the glomerulus and then reabsorbed by the proximal tubule. Lysomal degradation in the tubular cells may break the molecular binding and release free cadmium into the kidney, which prompts further MT production. If the production within the kidney is insufficient to absorb the concentration of a free metal, it may produce kidney damage or other pathologies. Similar effects are seen with aluminum as well, which may create tubular damage in free concentrations.

Wilson's disease is an autosomal recessive inherited disorder of copper metabolism resulting in accumulation of copper in various tissues. Rats raised to have a large accumulation of copper had ~ 80 times greater concentration of MTs in their liver compared to controls ($5016\,\mu g\,g^{-1}$ vs. $65\,\mu g\,g^{-1}$).

MTs appear to respond to other reactive substances such as trihalomethanes. A single dose of chloroform significantly increased metallothionein levels for up to 6–12 h following exposure. Although this indicates a response, it is not clear whether metallothionein protects against the harmful effects of this substance as well.

Toxicity due to insufficient MTs depends on factors such as age, gender, and health status. Young mice were found to have a fourfold greater accumulation of cadmium in their brains than adult mice, and significantly less metallothionein. Because cadmium interacts with calcium, women may lose cellular calcium. Diabetics experience glomeral damage and greater sensitivity.

In protecting the body against toxic metals, MTs interact with endogenous metabolic enzymes such as glutathione and mercapturic acid. This makes sense since cysteine is a substrate of glutathione. Supplements such as cysteine may elevate levels of MTs and zinc in the kidneys.

See also: Glutathione; Kidney; Liver; Metals.

Further Reading

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Tío L, Villarreal L, Atrian S, and Capdevila M (2004) Functional differentiation in the mammalian metallothionein gene family. metal binding features of mouse MT4 and comparison with its paralog MT1. *Journal of Biological Chemistry* 279: 24403–24413.

Metals

Shayne C Gad

This article is a revision of the previous print edition article by Arthur B Furst and Shirley B Radding, volume 2, pp. 291–292, © 1998, Elsevier Inc.

No general principles that govern the toxicity of all metals and their compounds exist; however, a few generalizations are possible. For the purposes of this discussion, the term metals will include both their ions and compounds.

Oxidation state and solubility are critical factors in toxic reactions. Metals can react with enzymes, cell membranes, and specific cell components. These reactions can inhibit or stimulate the actions of these substances and components.

Metals are generally circulated bound to some blood protein and can selectively bioaccumulate; thus, metals can affect either specific target organs or multiple anatomical sites. For example, lead can deposit in the bone, affect the central nervous system (CNS), and interfere with the metabolism of the heme in hemoglobin; cadmium appears to concentrate in the kidneys and the liver, mercury is a CNS toxin.

The metabolic product of the metal can determine the action in the organ in which the metal is deposited. Usually, metabolism of metals can lead to detoxification and often to excretion. Some metals, such as selenium metal and oxides, are converted to the volatile trimethyl derivative and are exhaled. On the other hand, mercury is converted to methyl mercury chloride, which is soluble in lipids and appears to be concentrated overtime in organs with high lipid content.

Some metals (cadmium, zinc, copper, and mercury) induce special protein complexes called metallothioneines. Iron forms a number of other protein complexes (ferritin, hemosidern, and transferrin).

For the general population, inhalation is a secondary exposure route for metals. Usually, metals are ingested with food or with drinking water.

Acute toxicity from metals can follow similar patterns; these can be essentially nonspecific like nausea and vomiting. A few metals and their compounds are carcinogenic to humans; the vast majority is not.

A few metals, such as lead and mercury, can cross the placental barrier. The very young population and the older population are most susceptible to metal toxicity.

Some metals are essential for good health (e.g., copper, chromium, selenium). Others are suspected not to be essential (e.g., beryllium, lead, tin). Still others are under investigation (e.g., arsenic).

See also: Aluminum; Antimony; Arsenic; Barium; Beryllium; Bismuth; Boron; Cadmium; Cesium; Chromium; Cobalt; Copper; Gallium; Iron; Lead; Lithium; Manganese; Mercury; Metallothionein; Molybdenum; Nickel and Nickel Compounds; Platinum; Potassium; Selenium; Silver; Sodium; Tellurium; Thallium; Tin; Titanium; Tungsten; Uranium; Vanadium; Zinc.

Further Reading

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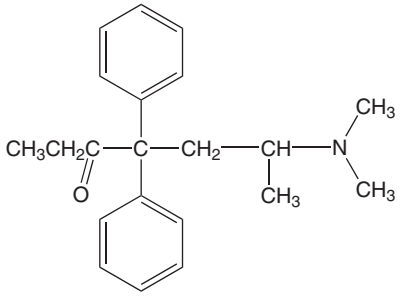
Xianglin S, Castranova V, Vallyathan V, and Petty WG (eds.) (2001) In: *Molecular Mechanisms of Metal Toxicity and Carcinogenesis*, Developments in molecular and cellular biochemistry, vol. 34. Dordrecht: Kluwer.

Methadone

Michael Hiotis

This article is a revision of the previous print edition article by Linda Hart, volume 2, pp. 292–293, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: CAS 297-88-1; CAS 76-99-3
- Synonyms: 4,4-Diphenyl-6, dimethylaminoheptan-3-one
- Chemical/Pharmaceutical/Other Class: Synthetic opioid
- Chemical Formula: $C_{21}H_{27}NO$
- Chemical Structure:



Uses

Methadone is a synthetic opioid used primarily for detoxification and maintenance in patients who are dependent on opiates, mainly heroin, and in the treatment of chronic severe pain.

Methadone is a Schedule II controlled substance under the Federal Controlled Substances Act.

Exposure Routes and Pathways

Oral and parenteral administrations are the most common routes of exposure.

Toxicokinetics

Methadone is rapidly absorbed after all routes of exposure. When administered orally, methadone is approximately one-half as potent as when given parenterally. Oral administration results in a delay of the onset, a lowering of the peak, and an increase in the duration of analgesic effect. It is metabolized primarily in the liver where it undergoes *N*-demethylation. Protein binding is 85%. Urinary excretion of methadone and its metabolites is dose dependent and comprises the major route of excretion only in doses exceeding 55 mg day^{-1}. It is excreted by glomerular filtration and undergoes renal reabsorption that decreases as urinary pH increases. Half-life ranges between 13 and 47 h with an average of 25 h.

Mechanism of Toxicity

Its mechanism of action is similar to morphine and it stimulates a number of specific opioid receptors in the brain, causing central nervous system (CNS) and respiratory depression.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methadone, like other opiates and their derivatives, cause miosis and CNS and respiratory depression, but has an excitatory effect on the CNS in cats and horses. Naloxone can be used at 0.02 mg kg^{-1}, if needed.

Human

Intentional or accidental overdose of methadone can lead to lethargy, miosis in mild intoxication, and stupor, coma, bradycardia, hypotension, hypothermia, respiratory depression, pulmonary edema, cardiovascular collapse, and death with higher doses. After abrupt discontinuation or administration of an antagonist such as naloxone, withdrawal syndrome can develop, consisting of lacrimation, rhinorrhea, sneezing, nausea, vomiting, fever, chills, tremor, tachycardia, and agitation. Accidental ingestions of methadone by children can lead to significant toxicity and even death.

Chronic Toxicity (or Exposure)

Animal

Pregnant rabbits and rats dosed up to 40 mg day^{-1} of methadone showed no fetal abnormalities. In B6C3F1 mice, doses of 1–30 mg kg^{-1} day^{-1} resulted in overall weight loss in the animals.

Human

Methadone has Food and Drug Administration indications for the management of pain in adults and for adult narcotic addiction. Adverse events are those expected from opiate exposures: gastrointestinal symptoms, CNS depression, and respiratory depression in larger doses, bradycardia, and constipation. Methadone appears to be fairly well tolerated by

most patients. Cases of edema have been described. Methadone appears to increase prolactin levels and can lead to gynecomastia.

In Vitro Toxicity Data

In *Drosophila* studies, methadone has not been shown to be mutagenic.

Clinical Management

Activated charcoal is the preferred method of decontamination. The use of syrup of ipecac is contraindicated because of the potential for serious CNS and respiratory depression. Naloxone, a specific opioid antagonist, should be used to reverse significant respiratory depression. Nalmefene and naltroxone are other opioid antagonists, with limited usefulness, similar to naloxone but with longer half-life and may be considered as an alternative to naloxone. The duration of naloxone effect is much shorter than that of methadone and relapse of opioid symptoms are common. For this reason naloxone infusion may be necessary and symptomatic patients should be monitored for 2 days.

See also: Heroin; Morphine.

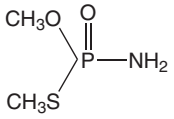
Further Reading

Wolff K (2002) Characterization of methadone overdose: Clinical considerations and the scientific evidence. *Therapeutic Drug Monitoring* 24(4): 457–470.

Methamidophos

Kevin N Baer

- Chemical Abstracts Service Registry Number: CAS 10265-92-6
- Synonyms: *O*,*S*-Dimethyl phosphoramidothioate; Tamaron; Tamanox; Monitor; Acephate-met
- Chemical/Pharmaceutical/Other Class: Organophosphorus insecticide
- Chemical Structure:



Uses

Methamidophos is used as an insecticide and acaricide. In the United States, cotton, potatoes and tomatoes are the principal crops for methamidophos use.

Exposure Routes and Pathways

Poisonings can occur from inhalation, skin absorption, or ingestion.

Toxicokinetics

Methamidophos can be readily absorbed through the skin, lung, and gastrointestinal tract. The bioactivation of methamidophos (replacement of covalent sulfur with oxygen) is accomplished primarily by liver microsomal enzymes. A variety of hydrolysis reactions to alkyl phosphates and various leaving groups can occur. Methamidophos is fairly well distributed throughout the tissues, with marked accumulation in the liver, kidney, and adipose tissue. Excretion in the urine is the major elimination pathway. Methamidophos is formed from biotransformation of another organophosphorus insecticide, acetphate.

Mechanism of Toxicity

Methamidophos is a potent, direct acetylcholinesterase inhibitor that acts by interfering with the metabolism of acetylcholine. As a result, acetylcholine accumulates at neuroreceptor transmission sites. Some evidence suggests that biotransformation of methamidophos may produce a more potent anticholinesterase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methamidophos has high mammalian toxicity. The oral LD_{50} value in rats is $\sim 20\,mg\,kg^{-1}$. Dermal LD_{50} values in rats and rabbits are about $100\,mg\,kg^{-1}$. The no-observed-adverse-effect level (NOAEL) from an acute neurotoxicity study based

on inhibition of plasma, erythrocyte, and brain cholinesterase in rats was 0.3 mg kg^{-1} day^{-1}.

Human

Classic signs of acute toxicity include pinpoint pupils, muscular fasciculations, slow pulse, excessive salivation and lacrimation, and gastrointestinal symptoms (nausea, abdominal cramps, diarrhea, and loss of sphincter control). In severe cases, convulsions, coma, and heart block are common. Death is generally attributed to respiratory insufficiency caused by the combination of respiratory center depression, paralysis, and increased bronchial secretions. In children, the classic signs described previously may be infrequent, with the major symptoms being central nervous system depression, stupor, flaccidity, dyspnea, seizures, and coma.

Chronic Toxicity (or Exposure)

Animal

Methamidophos can cause delayed neurotoxicity in hens. The NOAEL from a long-term dosing study in rats was 0.03 mg kg^{-1} day^{-1} based on brain cholinesterase inhibition.

Human

Cholinesterase inhibition may persist for 2–6 weeks. Signs of delayed neuropathy in humans have been reported following dermal and/or inhalation exposures. Progressive distal weakness, ataxia, flaccid paralysis, or quadriplegia may ensure. Additional chronic toxicities, such as memory impairment, language defects (slowed speech and slurring), and behavior disorders have been reported.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. Medical attention is necessary if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

For ingestion victims, vomiting should be induced, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with 2-PAM (PAM, Pralidoxime) can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered, followed by 2–4 mg (in adults) or 0.015–0.05 mg kg^{-1} (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Ecotoxicology

Methamidophos may cause harm to nontarget species with approved applications. Field studies indicate bird mortality can occur with methamidophos use. Methamidophos residues on food that birds may eat (e.g., leaves, insects, invertebrates) show high acute and persistent exposure. In addition, residue data on food that wild mammals may eat indicate that there would be sufficient persistent residues to cause adverse chronic effects. Methamidophos is highly toxic to bees and some beneficial insects. Freshwater and estuarine invertebrate aquatic species may be affected with normal use of methamidophos but acute risks to fish are minimal.

Exposure Standards and Guidelines

The acute reference dose is 0.001 mg kg^{-1} and the chronic reference dose is 0.0001 mg kg^{-1} day^{-1}.

See also: Cholinesterase Inhibition; Organophosphates; Pesticides.

Further Reading

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Relevant Website

http://www.epa.gov – United States Environmental Protection Agency.

Methane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 74-82-8
- Synonyms: Natural gas; Fire damp; Marsh gas; Methane (compressed, UN1971, DOT); Methane (refrigerated liquid, UN1972, DOT); Methyl hydride
- Chemical Formula: CH_4

Uses

In industry, methane is used to make methanol, halogenated methanes, ethylene, carbon tetrachloride, chloroform, acetylene, hydrogen cyanide, and methyl chloride. In the form of natural gas, methane is used as a fuel, as a source of carbon black, and as a starting material for the manufacture of synthetic proteins. It is also used in gas-fired utilities and in the home (home heating, gas dryers, and gas cooking).

Exposure Routes and Pathways

Because methane exists as a gas at normal temperatures and pressure, exposure generally occurs by inhalation. It is possible to spill liquid methane from a refrigerated tank, causing frostbite upon contact with the skin due to rapid evaporation and loss of heat.

Mechanism of Toxicity

Methane acts as an asphyxiant at concentrations that are high enough to displace oxygen (87–90%).

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity of methane is similar to that in humans; that is, no direct toxicity but can cause asphyxiation at concentrations high enough to displace oxygen required for normal respiration.

Human

Methane is not toxic to humans but acts as an asphyxiant at high enough concentrations. A threshold concentration of 1000 ppm is commonly assumed. The American Conference of Governmental Industrial Hygienists suggests that methane be treated as a simple asphyxiant.

Clinical Management

Persons who are exposed to high concentrations should vacate or be removed from the source of the gas and seek fresh air.

Environmental Fate

Methane is likely to be in the vapor phase in the atmosphere where it may react with hydroxyl radicals. Volatilization is the most important fate process in soil and water. It is not expected to hydrolyze, adsorb to soil or sediment, or bioaccumulate to any great extent.

Other Hazards

Methane is highly flammable and is therefore an explosion and fire hazard; the lower explosive limit is 5–15% by volume. Extreme care must be taken to keep areas of high concentration free from ignition sources, such as sparks from static electricity. Explosion-proof equipment should be used in these areas. Many people believe that methane is an important greenhouse gas, and that the apparent threefold increase in atmospheric concentrations over the last 200 years affects the stratospheric ozone layer and the oxidizing capacity of the atmosphere.

Miscellaneous

Methane is a colorless, odorless, highly flammable gas that is lighter than air. It occurs in natural gas at a concentration of 60–80%. It evolves naturally in marshes and highly reducing sediments as a result of microbiological decay of vegetation and organic matter. Ruminants also produce large concentrations as a by-product of their digestive process (anaerobic fermentation). It is also found in coal deposits and is produced as a by-product of some industrial processes, including some types of fermentation and sludge digestion. Methane is also found in tobacco smoke and in emissions from the incomplete combustion of coal, wood, and petroleum fuels.

See also: Respiratory Tract; Veterinary Toxicology.

Further Reading

Patty F (ed.) (1963) *Industrial Hygiene and Toxicology, vol. II: Toxicology*, 2nd edn., p. 1196. New York: Interscience Publishers.

Methanol

Greene Shepherd

This article is a revision of the previous print edition article by Bradford Strohm, volume 2, pp. 295–297, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 67-56-1
- Synonyms: Methyl alcohol; Carbinol; Wood spirits; Wood alcohol
- Chemical/Pharmaceutical/Other Class: Alcohols
- Chemical Formula: CH_4O

Uses

Methanol is used primarily as an intermediate in the production of formaldehyde, acetic acid, and methyl *t*-butyl ether. Methanol itself has been used as an oxygenated fuel additive, as well as an alternate transportation fuel in addition to its use as a solvent.

Background Information

Large epidemics of methanol poisoning have resulted from it being substituted for ethanol in Moonshine (illegally produced drinking alcohol). Recently, a new antidote, fomepizole, has been approved for use in the United States.

Exposure Routes and Pathways

Exposure to methanol can occur via inhalation, ingestion, and skin absorption.

Toxicokinetics

Irrespective of route of exposure, methanol distributes readily and uniformly to all organs and tissues as a function of their water content. Absorption via inhalation has been reported to be $\sim$60% of the inhaled dose. Methanol is absorbed through intact human skin. Upon absorption, methanol is excreted unchanged in urine, exhaled in breath, or metabolized in the liver and eventually excreted as carbon dioxide. Greater than 90% of the administered dose is metabolized with $\sim$2% excreted unchanged in expired air and 1% unchanged in urine. Depending on dose, half-lives for methanol elimination range from $\sim$1 day or more for doses of $1\,g\,kg^{-1}$ or greater to $\sim$3 h for doses of $<0.1\,g\,kg^{-1}$.

The oxidation of methanol to CO_2 proceeds through several enzymatic steps. Methanol is first converted to formaldehyde via either a catalase/peroxidase reaction (prevalent in rats) or an alcohol dehydrogenase oxidation (predominant in humans and monkeys). Methanol-derived formaldehyde is then conjugated with glutathione to form *S*-formylglutathione via a nicotinamide adenine nucleotide-dependent formaldehyde dehydrogenase. The *S*-formylglutathione conjugate is subsequently hydrolyzed by thiolase to form formic acid and glutathione. Formic acid, in the form of formate, then enters the body's carbon pool in the liver through complexation with tetrahydrofolate, where upon subsequent oxidation it is released as CO_2.

Mechanism of Toxicity

The toxic properties of methanol are the result of accumulation of the formate intermediate in the blood and tissues of exposed individuals. Formate accumulation produces metabolic acidosis leading to the characteristic ocular toxicity (blindness) observed in human methanol poisonings.

Humans and primates appear particularly sensitive to methanol toxicity when compared to rats. This is attributed to the slower rate of conversion in humans of the formate metabolite via tetrahydrofolate. This step in methanol metabolism occurs in rats at a rate $\sim$2.5 times that observed in humans.

Formate appears to directly affect the optic nerve. It is believed that formate acts as a metabolic poison inhibiting cytochrome oxidase. The optic nerve is composed of cells that normally have low reserves of cytochrome oxidase due to their low metabolic requirements and thus may be particularly sensitive to formate-induced metabolic inhibition.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute lethality of methanol is low based on animal testing via oral, dermal, and inhalation routes of exposure. The acute oral lethal dose (LD) observed in rats, rabbits, and monkeys range from $\sim 7\,mg\,kg^{-1}$ (monkey) to $14.4\,mg\,kg^{-1}$ (rabbits). Acute dermal LD in rabbits have been reported as $\sim 20\,mg\,kg^{-1}$ and inhalation lethal concentrations ranged from $\sim$31 000 ppm (18 h exposure, rats) to 72 000 ppm (54 h exposure, mice).

Sublethal doses have been shown to elicit central nervous system (CNS) effects, metabolic acidosis, ocular toxicity, and liver effects. Rats receiving oral doses of 1.0, 100, or 500 mg kg^{-1} day^{-1} methanol for 1 month showed liver changes characterized by enlarged hepatic cells and changes in some microsomal enzymes. Rabbits exposed via inhalation to 46.6 ppm methanol for 6 months exhibited changes in the photoreceptor cells of the retina which were observed upon electron microscopic examination. Monkeys exposed to 0, 10, 100, or 1000 ppm methanol, 22 h day^{-1} for up to 2 years exhibited slight changes in the liver and kidney at the 1000 ppm dose level. Pathologic changes in the nervous system of all animals at 1000 ppm were observed but were considered transient and probably reversible.

The developmental toxicity of methanol has been examined in rats and mice via inhalation and oral exposure. Rats exposed by inhalation to 10 000 and 20 000 ppm methanol 7 h day^{-1} during gestation have produced offspring with reduced body weights and a high incidence of malformations. Similarly, mice exposed to 4000 and 5000 ppm methanol 7 h day^{-1} during gestational days 6–15 experienced a high incidence of embryotoxicity and encephalopathy in surviving offspring. Single oral dosing of rats on gestational day 10 produced an increase in malformations at 1.3, 2.6, and 5.2 ppm methanol. These malformations are likely due to toxic mechanisms discussed above and folate depletion that can occur as the body metabolized formic acid.

Human

Historically, injuries and fatalities have been reported from acute methanol overexposure via ingestion, inhalation, as well as prolonged or repeated skin contact. Inhalation toxicity can occur in occupational settings or as a result of inhalant abuse (huffing). Clinical studies of individuals acutely poisoned by methanol ingestion have identified visual disturbances and possibly blindness as the most notable toxic effects in humans. Methanol is also a CNS depressant, although less potent than ethanol, and has also been shown to produce liver damage upon overexposure.

At high doses, methanol can cause reversible or permanent blindness, and in severe cases, death. Visual problems include eye pain, blurred vision, constriction of visual field, and possibly permanent blindness, which can develop in as little as 48 h. The lethal dose of methanol in untreated individuals is estimated to be in the range of 0.8–1.5 mg kg^{-1}, which translates into ~56–100 g, or 70–130 ml of 100% methanol, for the average individual (70 kg). Typically, the effects noted in methanol poisoning can be divided into three stages: (1) narcosis or CNS depression similar to that observed in ethanol intoxication; (2) a latent period, generally 10–15 h but can be prolonged if ethanol is coingested; and (3) visual disturbances, metabolic acidosis and possibly multiorgan failure leading to death.

Chronic Toxicity (or Exposure)

Animal

Rats exposed during pregnancy to doses up to 20 000 ppm in air for 7 h day^{-1} developed fetal skeletal, cardiac, and urologic abnormalities.

Human

Methanol is a normal part of the human diet (via fresh fruits and vegetables) and is produced in the body by metabolic breakdown of other products.

In Vitro Toxicity Data

Mutagenicity studies in Syrian hamster embryos and *Neurospora crassa* have been negative.

Clinical Management

Gastric decontamination by gastric lavage may be beneficial for patients that present less than an hour after a large ingestion of methanol. Administration of ethanol or preferably fomepizole, to competitively block the metabolism of methanol, is the first line of therapy. Fomepizole has significantly higher binding affinity for ADH than ethanol with lesser side effects and consequently is the preferred therapy. Blocking therapy is preventative in nature and most effective when started soon after an overdose. Leucovorin (folininic acid) may be beneficial for patients that present in stage three as it provides a folate source to help detoxify circulating formate/formic acid. Hemodialysis to remove methanol, toxic metabolites and to correct acid-base disorders has been found to be the most effective therapy in methanol poisonings.

Environmental Fate

Methanol can be released from natural sources (e.g., emissions from certain plants, as a by-product of degradation of organic material) as well as from human use of methanol as a solvent. Methanol has a low vapor pressure and will volatilize into air. With

volatilization into the air, methanol degrades via reaction with airborne hydroxyl radicals and has a half-life of ~18 days. Methanol can be removed from air via rainfall. If released into water, methanol decomposition is expected to occur via biodegradation. If released to soil, methanol is expected to degrade and be susceptible to leaching. Because of the low vapor pressure of methanol, rapid evaporation from dry surfaces occurs.

See also: Ethanol; Ethylene Glycol; Formaldehyde.

Further Reading

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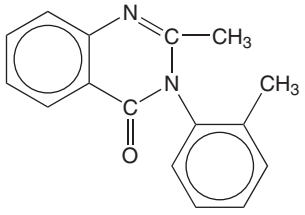
Kulig K, Duffy JP, and Linden CH (1984) Toxic effects of methanol, ethylene glycol, and isopropyl alcohol. *Emergency Medicine* 14–29.

Methaqualone

Christopher P Holstege

This article is a revision of the previous print edition article by Linda Hart, volume 2, pp. 297–298, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: CAS 72-44-6 (base); CAS 340-56-7 (hydrochloride)
- Synonyms: Ludes; Mequin; Parest; Quaalude; Sopes
- Chemical/Pharmaceutical/Other Class: Sedative–hypnotic
- Chemical Structure:



Uses

Historically, methaqualone was used as a hypnotic for the treatment of insomnia. However, it is less effective than the benzodiazepines for this indication. It also has anticonvulsant, antitussive, and weak antihistaminic properties. It no longer has clinical therapeutic value and is not manufactured in the United States for legitimate pharmaceutical purposes. It is manufactured by clandestine laboratories for drug abuse purposes.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures.

Toxicokinetics

Methaqualone is completely absorbed from the gastrointestinal tract within 2 h. The rate of absorption of the HCl salt is faster than that of the freebase form because of faster dissolution in the stomach. Methaqualone is nearly completely metabolized by the liver by hydroxylation. It is highly lipophilic with a volume of distribution of 2–6 l kg^{-1}. The elimination half-life is ~40 h for therapeutic doses and may be prolonged after overdose. Metabolites are excreted in the urine. These metabolites may be found in the urine up to 7 days postingestion.

Mechanism of Toxicity

Methaqualone is a central nervous system depressant similar to other sedative–hypnotics that cause enhanced gamma-aminobutyric acid activity.

Acute and Short-Term Toxicity (or Exposure)

Human

Signs and symptoms reported in overdose have included gastrointestinal distress, drowsiness, ataxia, slurred speech, paresthesias, agitation, convulsions, and coma. Unique among sedative–hypnotic drugs, hypertonicity, myoclonia, positive Babinsky responses, clonus, and hyperreflexia may develop following severe methaqualone poisoning. The cough reflex is decreased with this drug. Methaqualone inhibits platelet aggregation, prolongs prothrombin time, and partial thromboplastin time, and decreases factors V and VII, all of which may lead to conjunctival, retinal, and gastrointestinal hemorrhage.

Chronic Toxicity (or Exposure)

Animal

In chronic dosing studies in rats and dogs, 30 mg $kg^{-1} day^{-1}$ of methaqualone produced no toxic effects. Similar studies using doses of 130 mg kg^{-1} day^{-1} resulted in mortality of 60%.

Human

Tolerance and physical dependence may develop in persons who chronically use methaqualone. Abrupt discontinuation of chronic methaqualone abuse may result in a withdrawal syndrome consisting of anxiety, agitation, insomnia, tremors, headache, myalgias, nausea, vomiting, diaphoresis, and hyperpyrexia.

In Vitro Toxicity Data

Methaqualone use has been associated with reports of bleeding in humans. *In vitro* studies have demonstrated methaqualone induced blood platelet dysfunction in human platelets.

Clinical Management

Basic and advanced life-support measures should be implemented as necessary. Gastrointestinal decontamination procedures should be used as appropriate based on the patient's level of consciousness and history of ingestion. Activated charcoal can be used to adsorb methaqualone. The patient's level of consciousness and vital signs should be monitored closely. Obtunded patients with reduced gag reflex should be intubated to prevent pulmonary aspiration. Respiratory support, including oxygen and ventilation, should be provided as needed. If hypotension occurs it should be treated with standard measures including intravenous fluids, Trendelenburg positioning, and dopamine by intravenous infusion. Forced diuresis, hemoperfusion, and hemodialysis are of no value in methaqualone toxicity. Seizures should be treated with benzodiazepines. Coagulation studies and platelet counts should be obtained. If withdrawal signs and symptoms develop, treatment should consist of benzodiazepine therapy with a gradual dose reduction.

See also: Benzodiazepines; Drugs of Abuse.

Further Reading

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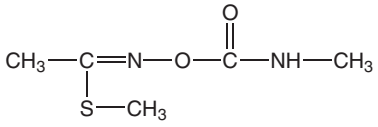
Methomyl

Carey N Pope

This article is a revision of the previous print edition article by Janice Reeves and Carey N Pope, volume 3, pp. 298–299, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 16752-77-5
- Synonyms: Nudrin; Lannate; Methyl-*N*-((methylcarbomoyl)oxy)thioacetimidate
- Chemical/Pharmaceutical/Other Class: Carbamate insecticide
- Chemical Structure:



Uses

Methomyl is a broad-spectrum insecticide registered for use on several agricultural crops and commercially grown ornamental plants.

Exposure Routes and Pathways

Oral exposures are most common. Inhalation and dermal exposures are also possible, particularly in the workplace.

Toxicokinetics

Methomyl is rapidly absorbed from the oral route. Dermal exposure is less hazardous. It is biotransformed to acetonitrile and carbon dioxide. Methomyl is well distributed to the tissues. Elimination is rapid: less than 10% remains 24 h after an oral exposure. Approximately 75% of an absorbed oral

dose of methomyl is eliminated via exhalation either as CO_2 or as acetonitrile. The remainder is excreted in urine as polar metabolites.

Mechanism of Toxicity

Methomyl exerts toxicity by inhibiting acetylcholinesterase. As with other carbamate insecticides, acetylcholinesterase inhibition is much less persistent than with organophosphate intoxication.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methomyl is extremely toxic to animals; the range of toxicity depends on the route and rate of exposure. Oral LD_{50} values in laboratory rats, mice, and guinea pigs range from about 10 to 24 mg kg^{-1}. The 4 h inhalation LC_{50} in rats was 0.3 mg l^{-1}. It is only slightly toxic by dermal application (LD_{50} in rabbits >5 g kg^{-1}).

Human

Methomyl is highly toxic by the oral route, moderately toxic by inhalation, and has low dermal toxicity. The primary symptom in acute methomyl poisoning is severe headaches, which may be accompanied by less severe symptoms such as nausea, vomiting, salivation, and abdominal pain. Other general symptoms are cramps, diarrhea, sweating, lassitude, weakness, runny nose, chest tightness, and blurring or dimness of vision. Sometimes the effects of acute exposure to carbamates can be long lasting. The probable lethal oral dose in humans is 5–50 mg kg^{-1}. The actual lethal dose of methomyl depends on the route and rate of exposure and the aggressiveness of the treatment used.

Chronic Toxicity (or Exposure)

Animal

In a 2 year feeding study in dogs, the no-observed-adverse-effect level was 5 mg kg^{-1} day^{-1}. Methomyl is not teratogenic or a reproductive toxicant.

Human

Because of its rapid biotransformation, methomyl does not tend to cause cumulative toxicity. However, repeated, frequent exposures could lead to cumulative inhibition of cholinesterase, resulting in flu-like symptoms including weakness, loss of appetite, and myalgia.

In Vitro Toxicity Data

Methomyl was negative in a variety of mutagenesis, DNA damage, and cytogenesis studies.

Clinical Management

Basic life-support measures must be maintained. The patient should be moved to fresh air, and exposed eyes and skin should be irrigated with large amounts of water. Atropine is used to counteract muscarinic side effects. Pralidoxime is contraindicated. Charcoal may be used to absorb methomyl.

Environmental Fate

Methomyl has moderate persistence in soil (half-life of 14 days). It is highly water soluble and can translocate to the groundwater. Methomyl readily undergoes microbial degradation in soil under anaerobic conditions. Dissipation in soil was influenced by differences in soil moisture content, which likely affect microbial activity, irrigation, and thus leaching. It is degraded in water with aeration, in sunlight, and under alkaline conditions (half-life in surface water of 6 days) but is more stable in neutral or acidic conditions.

Ecotoxicology

Methomyl is highly toxic to birds (oral LD_{50} in quail was 24–34 mg kg^{-1}, 28 mg kg^{-1} in chickens, and 10–45 mg kg^{-1} in other species). Methomyl is moderately to highly toxic to fish and aquatic invertebrates. In rainbow trout, the 96 h LC_{50} was 3.4 and 0.8 mg l^{-1} in bluegill. The 48 h LC_{50} for *Daphnia* was 0.0287 mg l^{-1}. Methomyl does not bioaccumulate. It is highly toxic in bees.

Exposure Standards and Guidelines

The chronic reference dose is 0.008 and the acceptable daily intake is 0.03 mg kg^{-1} day^{-1}.

See also: Carbamate Pesticides; Cholinesterase Inhibition; Pesticides.

Further Reading

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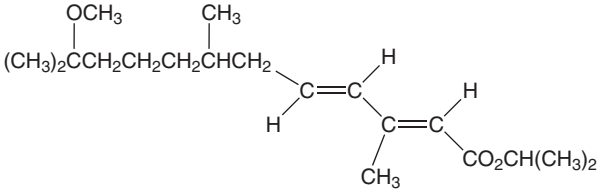
Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Methoprene

Eric M Silberhorn

- Chemical Abstracts Service Registry Number: CAS: 40596-69-8
- Synonyms: Altosid; Altosand; Apex; Manta; Minex; Diacon; Dianex; Kabat; Pharorid; Precor; Isopropyl(2*E*,4*E*,7*R*,*S*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate
- Chemical/Pharmaceutical/Other Class: Synthetic terpenoid
- Chemical Formula: $C_{19}H_{34}O_3$
- Chemical Structure:



Uses

Methoprene is a broad-spectrum synthetic juvenile hormone mimic, which acts as an insect growth regulator (insecticide). It prevents larval insect stages from undergoing metamorphosis to viable adults and thus acts as a larvicide. It is useful for control of a variety of insect pests including ants, mosquitoes, flies, fleas, beetles, lice, and moths, but is only effective against larvae, not adults or pupae. Many different products (e.g., pesticides, veterinary drugs) and formulations containing methoprene are commercially available. Methoprene products used for protecting pets such as cats and dogs include capsules administered orally and flea collars used externally. Production animals (e.g., cattle) typically receive methoprene in the diet as a food additive. Other formulations of methoprene include emulsifiable concentrates, pellets and tablets, granules, and aerosols. Some of these are applied to water for mosquito control whereas others are sprayed in areas where foods are stored to prevent insect infestations.

Background Information

Methoprene is the common name for a racemic mixture of two enantiomers (*R* and *S* in a ratio of 1:1). The activity of the compound as a juvenile hormone mimic is restricted to the *S* enantiomer.

Exposure Routes and Pathways

Dermal contact and eye contamination are the most common routes of exposure for humans.

Toxicokinetics

Methoprene may be absorbed from the gastrointestinal tract, through the intact skin, and by inhalation of spray mist. The metabolism of methoprene has been studied in rat, mouse, guinea pig, cow, and chicken given single doses. No studies of metabolism after repeated exposures have been conducted. Available studies indicate that methoprene is metabolized rapidly and extensively when given in low doses and excreted in urine, feces, and expired air. Metabolism is primarily via hepatic microsomal esterases, which initially produce methoprene acid. This compound undergoes both alpha- and beta-oxidation to form acetate, of which the majority is transformed via the Kreb's cycle into carbon dioxide and respired as such. Some carbon dioxide was also incorporated into natural products such as triglycerides, bile acids, and cholesterol. When administered at high dose levels, large quantities of methoprene are excreted unchanged in the feces, but are not found in the urine or blood, suggesting that intestinal absorption is slowed greatly but that metabolism is not saturated.

Mechanism of Toxicity

Juvenile hormones are one of three types of internal regulators of insect growth and metamorphosis. These hormones are synthesized and released in a regulated way into the hemolymph. Immature larvae require these hormones to progress through the regular larval stages. Methoprene disrupts these hormonal processes and prevents metamorphosis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methoprene has extremely low acute toxicity in laboratory animals. The oral LD_{50} value for rats is $>30\,000\,mg\,kg^{-1}$ and the value for dogs is between 5000 and $10\,000\,mg\,kg^{-1}$. The acute dermal LD_{50} for rabbits is $>2000\,mg\,kg^{-1}$. The acute (4 h) inhalation LC_{50} in the rat and guinea pig is $>210\,mg\,l^{-1}$. Methoprene is not irritating to the eye or skin when tested on rabbits and did not act as a skin sensitizer when tested on guinea pigs.

Human

No clinical effects or overt signs of toxicity are known to have occurred in humans due to methoprene exposure. Because of its mechanism of action, methoprene has high selectivity for insects and no acute toxicity is expected in humans even after ingestion of large doses.

Chronic Toxicity (or Exposure)

Animal

Several chronic and subchronic toxicity studies have been conducted with methoprene and show that the compound has little toxic potential. The main effect at high dose levels is an increase in the liver weight compared to body weight. Rats receiving up to 5000 ppm in the diet for 2 years did not exhibit any increases in tumor incidences; however, increased numbers of hepatic lesions were found at the highest dose level. The no-observed-adverse-effect level (NOAEL) was 1000 ppm, equivalent to 44 mg kg^{-1} body weight per day. Similarly, in mice fed up to 2500 ppm in the diet for 18 months, no toxicologically relevant effects were observed except for focal accumulations of macrophages with brownish foamy cytoplasm in livers and an increased frequency of amyloidosis of the intestine at 2500 ppm. The no-observed-effect level (NOEL) for systemic toxicity in this study was established at 1000 ppm, equivalent to 130 mg kg^{-1} body weight per day. In separate 90 day feeding studies, the NOEL in both rats and dogs was 500 ppm in the diet. Increased liver weights in rats and dogs, and renal tubular degeneration effects in some rats were observed at higher dose levels; however, the significance of these effects is questionable because they were not observed in longer feeding studies. In a 30 day dermal toxicity study on rabbits, the NOEL values for systemic effects and local effects were 300 and 100 mg kg^{-1}, respectively. In a 21 day inhalation toxicity study in rats, the NOEL for methoprene was 20 mg l^{-1}.

Methoprene is not teratogenic or strongly fetotoxic based on the results of several developmental and reproductive toxicity studies conducted in rabbits, rats, and mice. In a study in which mice were treated on days 7–14 of gestation, no toxicologically relevant effects were observed in dams or fetuses at any dose tested; the NOAEL was 570 mg kg^{-1} body weight per day, the highest dose tested. This study was extended and pups in some litters were observed for an additional 7 weeks. Effects on organ weights were observed at the highest dose; therefore, the NOAEL for toxicity to offspring was 190 mg kg^{-1} body weight per day. In a similar study with rabbits that were treated on days 7–18 of gestation, the NOAEL for maternal, embryo and fetotoxicity was 190 mg kg^{-1} body weight per day. There was an increase in fetal deaths and abortions at the highest dose tested, 1900 mg kg^{-1} body weight per day. The NOAEL in a three-generation reproduction study conducted in rats was 500 ppm in the feed (equivalent to 29 mg kg^{-1} body weight per day), based on reduced growth of pups and a slight increase in the number of pups of the F_3 litters that were born dead.

Human

No clinical effects or symptoms of toxicity are known to have occurred in humans due to methoprene exposure alone; however, some commercial formulations may potentially contain additional ingredients that cause skin or eye irritation, or allergic reactions after repeated exposures. Based on negative results in several genotoxicity studies and the results of the studies of carcinogenicity with methoprene, it is very unlikely that methoprene poses a carcinogenic risk to humans.

In Vitro Toxicity Data

Methoprene is not genotoxic based on negative results in the Ames *Salmonella* test, several other genotoxicity assays, and a dominant lethal study in rats.

Clinical Management

In cases of skin exposure, the exposed area should be thoroughly washed with soap and water. Eyes should be washed with copious amounts of room-temperature water in cases of eye contamination. If small amounts are ingested, no treatment is generally needed. Emesis is seldom necessary due to low toxicity; it is contraindicated when methoprene is in a hydrocarbon base. Activated charcoal is preferred. It can be administered as aqueous slurry or as a mixture of charcoal with saline cathartics or sorbitol. Symptomatic treatment is recommended.

Environmental Fate

Methoprene degrades rapidly in sunlight, both in water and on inert surfaces. It is metabolized rapidly in soil under both aerobic and anaerobic conditions (half-life = 10–14 days). The major microbial degradation product is carbon dioxide. Degradation in both freshwater and saltwater is also quite rapid with a half-life of 10–35 days at 20°C. Methoprene is not very soluble in water (< 2 ppm) and as a result is not

highly mobile in soil. Because of this and its rapid biodegradation, methoprene does not persist for long periods in soil and is unlikely to contaminate groundwater. Based on studies with bluegill sunfish, significant bioconcentration of methoprene is not expected in fish tissues as a result of aquatic exposures.

Ecotoxicology

Methoprene is practically nontoxic to birds with an acute oral LD_{50} of $>2000\,mg\,kg^{-1}$ in mallard ducks and an 8 day dietary $LC_{50} > 10\,000$ ppm in bobwhite quail. In avian reproduction studies, methoprene had no effects on reproductive parameters at dietary concentrations of 30 and 3 ppm in quail and mallards, respectively.

Methoprene toxicity has been studied extensively in aquatic life as a result of concern over possible effects on nontarget organisms. Toxicity to fish is slight to moderate with a 96 h LC_{50} of $1.5\,mg\,l^{-1}$ (ppm) for bluegill sunfish and $>50\,mg\,l^{-1}$ for rainbow trout. Toxicity to aquatic invertebrates is generally moderate to high, although there is considerable species-to-species variation in sensitivity. Acute LC_{50} values for most invertebrates are $>900\,\mu g\,l^{-1}$ (ppb). Effects on reproductive parameters have been observed at lower concentrations in studies with invertebrates. For example, exposures of *Daphnia pulex* to nominal concentrations of 10 and $100\,\mu g\,l^{-1}$ caused changes in the incidences of all-male and all-female broods compared to controls. Methoprene has also been shown to produce developmental toxicity (malformations) in exposed amphibians, but only at concentrations that produce other toxic effects and those that are greater than those typically resulting from field applications of mosquito control products.

Exposure Standards and Guidelines

The acceptable daily intake (ADI) for racemic methoprene is up to $0.09\,mg\,kg^{-1}$ body weight. For *S*-methoprene, the ADI is up to $0.05\,mg\,kg^{-1}$ body weight.

See also: Pesticides.

Further Reading

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Methoxychlor

Guangping Chen

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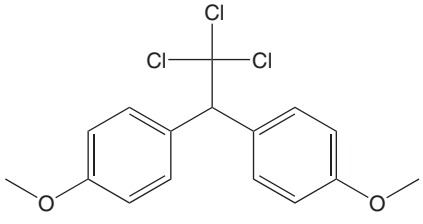
- Chemical Abstracts Service Registry Number: CAS 72-43-5
- Synonyms: 1,1,1-Trichloro-2,2-bis(*p*-methoxyphenyl)-ethane; 1,1′-(2,2,2-Trichloroethylidene)bis(4-methoxy-benzene); Dimethoxy-DT; DMDT; ENT 1716; Higalmetox; Methoxychlore; Marlate; Methoxy-DDT; OMS 466; Prentox; Marlate®; Metox®; Chemform
- Chemical/Pharmaceutical/Other Class: Organochlorine insecticide
- Chemical Formula: $C_{16}H_{15}Cl_3O_2$
- Chemical Structure:



Uses

Methoxychlor is a manufactured chemical. Pure methoxychlor is a pale-yellow powder. Methoxychlor is used as an insecticide against flies and a wide variety

of other insects. It is also used on agricultural crops and livestock, home garden, and on pets. Methoxychlor is used against the beetle vectors of Dutch elm disease.

Exposure Routes and Pathways

Ingestion and dermal contact are possible routes of exposure. Individuals may be exposed by ingestion of food or drinking water contaminated with methoxychlor.

Toxicokinetics

Chlorinated hydrocarbon insecticides, when dissolved in oil or other lipid, are readily absorbed by the skin and alimentary canal. Although methoxychlor is slowly metabolized to a small extent by pathways similar to those of DDT, the major pathway is by *O*-demethylation and subsequent conjugation. Methoxychlor has been detected in the blood of agricultural workers. All organochlorines are likely to be excreted in the milk of lactating women. Methoxychlor is excreted mainly in feces, and to a lesser extent in urine.

Mechanism of Toxicity

Methoxychlor undergoes hepatic microsomal monooxygenase(s)-mediated activation and the resultant reactive metabolites (possibly free radicals) bind covalently to microsomal components.

Animal Toxicity

Ocular toxicity has been reported from systemic exposure to methoxychlor. Chronic intoxication of dogs at dosages of $2000\,mg\,kg^{-1}\,day^{-1}$ in the diet led to convulsions in 6 weeks and death in 9 weeks; weight loss, high alkaline phosphatase and serum transaminase, and intestinal congestion were seen; and swine showed kidney injury and uterine and mammary enlargement. Rabbits given $200\,mg\,kg^{-1}\,day^{-1}$ orally died after four or five doses; autopsy findings included mild liver damage and nephrosis. Atrophy of the testes was observed in rats given 1% methoxychlor in the diet. In rats given 100 or $200\,mg\,kg^{-1}\,day^{-1}$, arrested spermatogenesis was noted after 70 consecutive days of treatment; corpora lutea failed to develop in female rats treated with similar dosages for 14 days before and after mating. Administration of $1000\,mg\,kg^{-1}$ in the diet to pregnant rats caused early vaginal openings in their offspring; both male and female offspring had reduced fertility when they attained maturity. No abortions were observed in pregnant cows given methoxychlor at $10\,mg\,kg^{-1}\,day^{-1}$. In a 27-month study in rats, 1000 ppm methoxychlor was administered in the diet; there were no differences in deaths or the incidence and distribution of benign or malignant tumors in treated animals versus controls. There is no evidence of carcinogenicity in animals. Results were negative in the *Escherichia coli* WP2, UVRA reverse mutation assay. Injection of a 0.1% solution of methoxychlor did not induce sex-linked recessive lethals in male *Drosophila melanogaster*.

Human Toxicity

In extreme overdoses, central nervous system depression may occur. In general, for chlorinated hydrocarbon insecticides, aspiration of insecticide-containing petroleum distillate may result in pneumonitis. In addition, nausea, vomiting, and diarrhea may follow ingestion; blood dyscrasias, anemia, and leukemia have been associated with organochlorine exposure, and extensive contact results in dermal irritation. The approximate fatal dose is $6\,g\,kg^{-1}$. Chronic exposure may cause kidney damage. There is no evidence of carcinogenicity in humans.

Clinical Management

In general, following acute exposure to chlorinated hydrocarbon insecticides, blood chlorinated hydrocarbon levels are not clinically useful; for most compounds it reflects cumulative exposure over a period of months rather than recent exposure. Emesis may be indicated and is most effective if initiated within 30 min postingestion. In addition, an activated charcoal/cathartic may be given. For seizures, diazepam should be administered as an intravenous bolus. Oils should not be given by mouth. Adrenergic amines should not be administered because they may further increase myocardial irritability and produce refractory ventricular arrhythmias. If clothing is contaminated, it should be removed.

Environmental Fate

Methoxychlor is very persistent in soil. Its half-life is $\sim$120 days. Methoxychlor degrades much more rapidly in aerobic conditions than in anaerobic conditions. Methoxychlor is tightly bound to soil and is insoluble in water. The risk to groundwater should be low. Movement of the pesticide is likely via adsorption to suspended soil particles.

Ecotoxicology

Methoxychlor is slightly toxic to bird species. Methoxychlor is highly toxic to fish and aquatic invertebrates. Methoxychlor accumulates in aquatic organisms because these organisms metabolize methoxychlor very slowly. The compound is relatively nontoxic to bees.

See also: Cholinesterase Inhibition; Cyclodienes; Pesticides.

Further Reading

Cummings AM (1997) Methoxychlor as a model for environmental estrogens. *Critical Reviews in Toxicology* 27(4): 367–379.

Gore AC (2001) Environmental toxicant effects on neuroendocrine function. *Endocrine* 14(2): 235–246.

Kanno J, Onyon L, Peddada S, *et al.* (2003) The OECD program to validate the rat uterotrophic bioassay. Phase 2: Coded single-dose studies. *Environmental Health Perspectives* 111(12): 1550–1558.

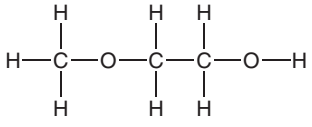
Relevant Websites

http://ace.orst.edu – Extension Toxicology Network.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Methoxychlor.
http://www.epa.gov – United States Environmental Protection Agency.
http://www.oehha.ca.gov – California Environmental Protection Agency.

Methoxyethanol

Michael J Brabec

- Chemical Name: Ethylene glycol monomethyl ether
- Chemical Abstracts Service Registry Number: CAS 109-86-4
- Synonyms: 2-Methoxyethanol; Methyl cellosolve; Methyl glycol; Amsco-Solv EE; Dowanol EM; Ektasolve EM; Glycol methyl ether; Monoethylene glycol methyl ether; Methyl oxitol; Monomethyl glycol ether
- Chemical/Pharmaceutical/Other Class: Glycol ether
- Chemical Formula: $C_3H_8O_2$
- Chemical Structure:



Uses

Methoxyethanol is an industrial solvent with high water miscibility. It is widely used in paints, lacquers, stains, inks, surface coatings, and food-contact plastics. Products have applications in printing, leather processing, photography and photolithography processes, such as in the semiconductor industry, and in textile finishing. It is used as an anti-icing ingredient in jet fuels and lubricants.

Exposure Routes and Pathways

As a solvent with a relatively high vapor pressure, exposures to both liquid and vapor states can simultaneously occur. About equal amounts can be absorbed via inhalation and dermal exposure to methoxyethanol vapors. Oral exposure would be unlikely except by deliberate or accidental ingestion of methoxyethanol-containing liquids. Unfortunately, since methoxyethanol has a mild ethereal odor, is not a surface irritant, and lacks an unpleasant flavor, it has weak warning properties.

Exposure to methoxyethanol can also occur after exposure to the acetic acid ester, methoxyethyl acetate, since the ester is readily hydrolyzed to release methoxyethanol by esterases in tissues lining the respiratory system, by blood cells, and in deep body organs.

Toxicokinetics

Methoxyethanol is rapidly absorbed through the skin and lungs into the blood. Its water solubility favors distribution to all body tissues except adipose tissue. Metabolism occurs via two pathways. Methoxyethanol is a substrate for alcohol dehydrogenase, and the resultant methoxyacetaldehyde is metabolized to methoxyacetic acid by aldehyde dehydrogenase. In rats, pretreatment with phenobarbitol decreased formation of methoxyacetaldehyde but accelerated formation of methoxyacetate in liver cytosolic fractions. A minor pathway involves demethylation by undefined enzymes to ethylene glycol and CO_2.

In Sprague–Dawley rats, males and females quickly eliminate methoxyacetate at similar rates in the

urine, but the male excretes the unmetabolized compound more slowly ($t_{1/2}$ of 50 min vs. 30 min). This may be because the activity of alcohol dehydrogenase in the liver is higher in female rats than in the male rats. Methoxyacetate and methoxyacetylglycine are the principal metabolites in rat urine, with minor amounts of sulfate and glucuronide derivatives also excreted. Human volunteers exposed to low levels (5 ppm) of methoxyethanol vapors for 4 h excreted ~90% of the absorbed dose as methoxyacetate in the urine. The half-life of methoxyethanol is estimated to be ~77 h in humans. The compound does not bioaccumulate.

Mechanism of Toxicity

High acute doses of methoxyethanol have a narcotic effect. Kidney and lung damage, accompanied by hemoglobinuria, follow exposures to high doses. Toxicity is attributed to metabolites, including the putative intermediate methoxyacetaldehyde and methoxyacetate. *In vitro* studies with radiolabeled methoxyethanol indicate that formation of methoxyacetyl-coenzyme A may lead to the formation of methoxyacetyl derivatives of Krebs cycle intermediates. Methoxyacetate produces the same testicular lesions in rodents as does the parent compound, although the immunosuppression elicited by methoxyethanol exposure may depend on the putative metabolite, methoxyacetaldehyde. In both the testicular lesion and the immune suppression, some data suggest that the pattern of cell death termed apoptosis may be stimulated. Methoxyacetate stimulates synthesis of progesterone by luteal cells in culture. This disturbance of luteal function may be related to the prolongation of gestation in rodents. Teratogenicity appears to be related to interference by methoxyethanol, or its metabolites, with one carbon metabolism in the synthesis of nucleotide precursors, and can be relieved by administration of other substrates, such as serine and glycine, which also provide substrates for nucleotide synthesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral LD_{50} values range from 1 to 3 g kg^{-1} in rodents. A dermal LD_{50} of 1.3 g kg^{-1} was determined in rabbits. An inhalation LC_{50} of 4600 ppm was determined in mice. In animals, systemic effects from sublethal, or short-term exposures, are kidney damage, thymic involution, depression of blood cell counts, and depression of bone marrow cellularity. Depression of the primary antibody plaque-forming cell response in rats suggests that methoxyethanol could inhibit the humoral immune system. Testicular atrophy and depression of sperm counts are noted in animal studies. Methoxyethanol, along with several other known testicular toxins, causes an increase in the urinary creatine/creatinine ratio as one of the earliest and most sensitive markers of testicular damage after acute exposure. Methoxyethanol is considered to be a mild skin and eye irritant. Methoxyethanol is not a contact sensitizer.

Human

Deliberate or accidental human exposures have defined the toxicity expected from acute and short exposures to methoxyethanol by all routes. High doses can kill by severe central nervous system (CNS) depression. A human death resulted from hemorrhagic gastritis and degeneration of liver, kidney, and pancreas following ingestion of ~225 ml of the neat compound. Two individuals who drank ~100 ml each of methoxyethanol survived, but were severely ill with symptoms of severe intoxication, metabolic acidosis, hyperventilation, tachycardia, nausea, weakness, and cyanosis.

Chronic Toxicity (or Exposure)

Animal

Reproductive toxicity in both sexes is the most significant effect of chronic exposures to low doses of methoxyethanol in humans and animals. Both chronic and subchronic studies indicate that rabbits are more sensitive to methoxyethanol than rats and mice, and primates, including human, are more sensitive to the teratogenic effects of methoxyethanol than any rodent species. A dose of 12 mg kg^{-1} day^{-1} during the second trimester of pregnancy in monkeys produced 29% dead or resorbed fetuses, while 36 mg kg^{-1} day^{-1} caused 100% fetal death. Doses of 25 mg kg^{-1} day^{-1} are the lowest reported to cause fetal abnormalities in rodents, with skeletal abnormalities of the extremities as one remarkable feature. Dose levels of 25 mg kg^{-1} day^{-1} during the sensitive period of gestation in rodents are also associated with reduced litter size, reduced litter weight, and prolongation of pregnancy. Examination of rodent fetuses at different periods during development indicate that repair of early damage occurs when the exposure ceases, which may contribute to the greater tolerance rodents display toward methoxyethanol.

Methoxyethanol is a male reproductive toxin. Males display testicular atrophy, tubular degeneration, and reduced sperm counts at doses as low as

25 mg kg^{-1} day^{-1} (rabbits) with symptoms increasing in severity as dose levels increase. Loss of pachytene spermatocytes in a stage-specific manner is the earliest noted lesion. Effects are reversible after low doses.

As the dose level of chronic exposures increases, methoxyethanol becomes immunosuppressive. Thymic involution, decrease of spleen weight, and suppression of various lymphocyte and antibody responses are noted at doses as low as 50 mg kg^{-1} in rats and mice, although comparison of several studies indicate that rats may be more susceptible than mice. Effects became more severe as the dose level of methoxyethanol increased in the studies.

Human

Evidence gathered from pregnant women exposed to methoxyethanol in the semiconductor industry would strongly suggest that methoxyethanol is a human teratogen as well as a reproductive toxin. Decreased fertility, increased incidence (roughly twofold) of spontaneous abortion, and prolongation of the menstrual cycle were found in women exposed to a variety of solvents that included methoxyethanol. In one report, six children delivered by five women heavily exposed to methoxyethanol in the workplace displayed characteristic dysmorphic features. Cleft palate, CNS malformations, mental retardation, and musculoskeletal malformations were noted in the offspring born to mothers with exposure to methoxyethanol. Thirty-five children delivered by a matched population of 23 nonexposed women in the same workforce did not present a similar pattern of lesions. Although it is difficult to ascertain the exposure history associated with the human subjects, effects were noted at exposure levels below 1 ppm, with more dramatic effects as exposures increased.

Reduced sperm counts, decreased testes size, decreased circulating testosterone and follicle stimulating hormone (FSH) levels, and an increase in luteinizing hormone (LH) were reported in men occupationally exposed to methoxyethanol at levels in the range 5.4–8.5 ppm. A tendency toward reduced fertility in the spouses of men exposed to methoxyethanol was also observed in this study. Increases in the incidence of oligospermia and azoospermia were found in painters exposed to methoxyethanol (mean level of exposure less than 1 ppm). However, no reduction of fertility was noted. The study was complicated by the simultaneous exposure of the workers to ethoxyethanol. No paternal effects on offspring have been associated with human methoxyethanol exposures.

Examinations of male and female workers exposed to methoxyethanol reveal hematopoietic effects manifested as anemia and alterations in numbers of white blood cells. Exposures were estimated as at, or below, 1 ppm.

In Vitro Toxicity Data

Methoxyacetate inhibits lactate production by testicular cells in culture.

Short-term tests for mutagenicity, such as the Ames assay, and various mammalian cell culture assays, indicate that methoxyaldehyde may be mutagenic at high doses but methoxyethanol does not produce any positive response. Thirty-two millimole concentrations of methoxyethanol (but not equimolar concentrations of methoxyacetate) inhibited gap junction communication in cultures of rat myometrial myocytes. The failure of the end metabolite, methoxyacetate, and the relatively high concentrations required to produce a positive result led the authors to conclude that inhibition of gap junction communication was not likely as a mechanism of female reproductive toxicity.

Clinical Management

Symptoms of patients acutely exposed to methoxyethanol depend on route of exposure. Inhalation exposure can lead to irritation of the respiratory tract and delayed presentation of pulmonary edema. Oral consumption will produce systemic toxicity, so prompt induction of vomiting following several glasses of water can reduce exposure. Contaminated clothing should be removed and methoxyethanol washed away with soapy water as quickly as possible after dermal exposure. Prompt flushing with copious amounts of water is recommended after eye contact. Observation of the patient for 24 h after exposure is important because presentation of pulmonary, liver, and kidney damage may not be evident for 12 h or longer after exposure. Animal studies indicate that attempts to alter the pharmacokinetic behavior of methoxyethanol by simultaneous, or subsequent, consumption of ethyl alcohol were of limited success in alleviating toxicity.

Environmental Fate

Since methoxymethanol is highly miscible with water and readily breaks down by several enzymatic routes, bioconcentration is unlikely and has not been shown to occur.

Ecotoxicology

The acute and chronic effects of methoxyethanol and its acetate ester on aquatic ecosystems have been examined. The effects of methoxyethanol are judged to

be negligible at the range of concentrations expected in the environment.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists exposure limit is 5 ppm, with a warning about dermal exposure routes. The Occupational Safety and Health Administration recommends a permissible exposure limit, 8 h time-weighted average, of 25 ppm. Exposure controls include the use of personal protective equipment such as respirators, chemical-resistant gloves, and chemical safety goggles. Contaminated clothing should be washed before reuse. Spills on skin, or eye splash should be thoroughly removed by flushing with copious amounts of water. Methoxyethanol is subject to SARA section 313 reporting requirements.

See also: Jet Fuels; Reproductive System, Female; Reproductive System, Male.

Further Reading

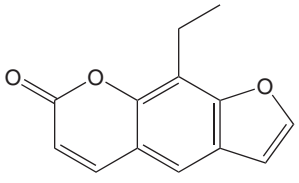
Johanson G (2000) Toxicity review of ethylene glycol monomethyl ether and its acetate ester. *Critical Reviews in Toxicology* 30(3): 307–345. Material Safety Data Sheets may be obtained from principal suppliers of methoxyethanol.

Vincoli JW (ed.) (1997) Ethylene glycol monomethyl ether. *Risk Management for Hazardous Chemicals*, vol. 1, pp. 1317–1320. Boca Raton, FL: CRC Press, Lewis Publishers.

Methoxypsoralen, 8-

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 298-81-7
- Synonyms: Xanthotoxin; 9-Methoxypsoralin; Ammoidin; Oxsoralen; Methoxsalen; 8-MOP
- Chemical/Pharmaceutical/Other Class: Coumarin
- Chemical Formula: $C_{12}H_8O_4$
- Chemical Structure:



Uses

8-Methoxypsoralen and other psoralens are naturally found in plants, including common fruit and vegetable crops. Synthetic forms of 8-methoxypsoralen and 5-methoxypsoralen (bergapten) are widely used as drugs in skin photochemotherapy, for example, with long-wave ultraviolet (UV) light in the treatment of psoriasis, vitiligo, and mycosis fungoides. The combination of UV light and psoralens is called PUVA therapy. They have also been used as tanning activators in many sunscreen preparations. Unfortunately, the use of psoralens in skin photochemotherapy has been shown to have major side effects. Concomitant therapy with 8-methoxypsoralen and other systemic or topical photosensitizing agents (e.g., anthralin, coal tar or coal tar derivatives, griseofulvin, phenothiazines, nalidixic acid, halogenated salicylanilides, sulfonamides, tetracyclines, thiazides, or certain organic staining dyes such as methylene blue, toluidine blue, rose bengal, and methyl orange) may produce additive photosensitizing effects. Particular caution is necessary if 8-methoxypsoralen is administered concomitantly with any topical or systemic photosensitizing agent.

Exposure Routes and Pathways

Dermal and oral exposures are the most likely to occur.

Toxicokinetics

8-Methoxypsoralen is extensively metabolized, and is demethylated to 8-hydroxypsoralen (8-HOP). 8-Methoxypsoralen and 8-HOP are conjugated with glucuronic acid and sulfate; other unidentified metabolites have also been detected. 8-Methoxypsoralen and 8-HOP and their conjugates are excreted in urine. Following oral administration of 8-methoxypsoralen, 80–90% of the drug is excreted in urine within 8 h as hydroxylated, glucuronide, and sulfate metabolites; less than 0.1% of a dose is excreted in urine as unchanged drug. About 95% of the drug is excreted in urine within 24 h as metabolites. When oral 8-methoxypsoralen is administered with food, the extent of absorption and the peak serum concentration appear to be increased. The mechanism of this interaction is not known but may involve the effect of food on dissolution or hepatic metabolism of 8-methoxypsoralen.

Mechanism of Toxicity

The toxic effects of psoralens almost never occur without exposure to UV light. These are photosensitizing

materials that exert their primary effect on the skin. 8-Methoxypsoralen, when activated by long-wavelength UV light in the range of 320–400 nm is strongly erythemogenic, melanogenic, and cytotoxic in the epidermis. The mechanisms of action of 8-methoxypsoralen in inducing repigmentation of vitiliginous skin have not been established. Repigmentation depends on the presence of functioning melanocytes and UV light. 8-Methoxypsoralen may activate the functional and dihydroxyphenylalanine-positive melanocytes present in vitiliginous skin. An increase in the activity of tyrosinase, the enzyme that catalyzes the conversion of tyrosine to dihydroxyphenylalanine (a precursor of melanin), has been shown in melanin-producing cells exposed *in vitro* to trioxsalen and UVA light. In addition, binding of photoactivated psoralens (in triplet states) to pyrimidine bases of nucleic acids, with subsequent inhibitions of DNA synthesis, cell division, and epidermal turnover, has been demonstrated. Following photoactivation, 8-methoxypsoralen forms covalent bonds with DNA to produce monofunctional (addition to a single strand of DNA) and bifunctional adducts (cross-linking to both strands of DNA). Reactions with other proteins also occur. Psoralens may also increase melanin formation by producing an inflammatory reaction in the skin. Other mechanisms of increased pigmentation may include an increase in the number of functional melanocytes (and possibly activation of dormant melanocytes); enhancement of melanin granule synthesis; stimulation of the movement of melanocytes up hair follicles resulting in melanocytic repopulation of the epidermis; and/or hypertrophy of melanocytes and increased arborization of their dendrites. Since psoriasis is a hyperproliferative disorder and other agents effective in the treatment of psoriasis are known to inhibit DNA synthesis, the therapeutic effect of 8-methoxypsoralen in the treatment of psoriasis probably involves binding to DNA and inhibition of DNA synthesis resulting in decreased cell proliferation; other vascular, leukocyte, or cell regulatory mechanisms may be involved. It has been suggested that at low drug load, 8-methoxypsoralen binds to DNA as an intercalator, whereas at higher ratios of 8-methoxypsoralen to DNA, it binds to the outside of DNA, probably in the minor groove and causes some compaction in DNA. Protective eyewear is used to prevent irreversible binding of 8-methoxypsoralen to proteins and DNA components of the lens. The central hypothesis for the reproductive toxicity of 8-methoxypsoralen is that it produces reproductive effects by disrupting the hypothalamus–pituitary axis, and the alternate hypothesis is that this compound targets gonadal function, resulting in alteration of pregnancy outcome.

Acute and Short-Term Toxicity (or Exposure)

Animal

The rat LD_{50} is $>51\,mg\,kg^{-1}$. Female rats had reduced birth weights, a reduced number of implantation sites, pups, and corpora lutea when given 8-methoxypsoralen.

Human

Since 8-methoxypsoralen is a strong photosensitizer capable of producing severe burns if used improperly, it should be used only under the supervision of a physician with special training and experience in photochemotherapy. 8-Methoxypsoralen lotion should be applied only by a physician under controlled conditions for light exposure and subsequent light shielding. The 8-methoxypsoralen lotion should be applied only to small, well-defined vitiliginous lesions, preferably those lesions that can be protected by clothing or a sunscreen from subsequent exposure to UVA light. Because of the potential for serious adverse effects (e.g., ocular damage, aging of the skin, and skin cancer (including malignant melanoma)) resulting from PUVA therapy, the patient should be fully informed by the physician of the risks associated with the treatment. To prevent serious adverse effects, the physician should carefully instruct the patient to adhere to the prescribed 8-methoxypsoralen dosage regimen and schedules for UVA exposure.

Side effects after oral 8-methoxypsoralen therapy are usually mild and include gastric discomfort, nausea, nervousness, insomnia, and depression. Mild, transient erythema occurring 24–48 h after PUVA therapy is an expected cutaneous reaction, and indicates that a therapeutic interaction between 8-methoxypsoralen and UVA has occurred. Areas of skin showing fiery erythema with edema should be shielded during subsequent UVA exposures until the erythema has resolved. Fiery erythema with edema that occurs within 24 h following UVA exposure may indicate a potentially severe burn, since the peak erythemal reaction usually occurs 48–72 h following PUVA therapy. Following 8-methoxypsoralen ingestion and controlled exposure to UVA or sunlight, patients must avoid additional, direct, or indirect exposure to sunlight for at least 8 h; following topical treatment with 8-methoxypsoralen, additional exposure to UV light should be avoided for at least 12–48 h. If exposure to sunlight cannot be avoided, the patient should wear protective clothing (e.g., hat, gloves) and/or apply sunscreens to all areas of the body that may be exposed to the sun (including lips).

Exposure of animals to large doses of PUVA without eye protection has produced cataracts and 8-methoxypsoralen enhances this effect; however, in patients receiving PUVA therapy who use appropriate eye protection, there is no evidence for an increased risk of cataract formation. Prior to the initiation of PUVA therapy and yearly thereafter, patients should have an ophthalmologic examination because of the cataractogenic potential of psoralens.

Because psoralens have caused photoallergic contact dermatitis and may precipitate sunlight allergy, 8-methoxypsoralen should be used with caution in patients with a family history of sunlight allergy. The drug should also be used with caution in patients with gastrointestinal diseases or chronic infection. Oral or topical 8-methoxypsoralen should be used with particular caution in patients receiving topical or systemic therapy with known photosensitizing agents. Oral or topical 8-methoxypsoralen is contraindicated in patients exhibiting idiosyncratic reactions to psoralens or with a history of a sensitivity reaction to the drugs; in patients with diseases associated with photosensitivity (e.g., lupus erythematosus, porphyria cutanea tarda, erythropoietic protoprophyria, variegate porphyria, xeroderma pigmentosum, albinism, hydroa vacciniforme, leukoderma of infectious origin, polymorphous light eruptions), except under special circumstances; in patients with melanoma or history of melanoma; and in patients with invasive squamous cell carcinoma. Oral 8-methoxypsoralen is also contraindicated in patients with aphakia (absence of lenses) because of the increased risk of retinal damage.

Damage to the nail beds can be induced by 8-methoxypsoralen and sunlight. Histological examination of the nail beds showed that the photosensitizing effect of the drug induced the generation of many multinucleated epithelial cells and fibroblasts in the dermis.

The 8-methoxypsoralen concentrations and UV irradiation conditions to which human lymphocytes are exposed therapeutically *in vivo* have been shown to be too low to induce observable numbers of sister chromatid exchanges. However, more point mutations, as indicated by the increased incidence of 6-thioguanine-resistant lymphocytes, were observed in patients treated with psoralen drugs and UV irradiation than in healthy controls. Because oral 8-methoxypsoralen and UVA radiation therapy is mutagenic, concern exists about the potential for teratogenic effects resulting from the use of this therapy at the time of conception and during pregnancy. The pregnancy outcomes among over 1000 patients who were documented and who received UVA radiation treatments have been examined in a prospective study. Although the power of this study to detect an increase in the risk of specific defects is limited, the results found no evidence to suggest that UVA radiation is a potent teratogen.

Chronic Toxicity (or Exposure)

Animal

8-Methoxypsoralen is a reproductive and developmental toxicant in rats; however, in rabbits, doses that caused minor maternal toxicity did not affect fetal growth, viability, or morphological development. The International Agency for Research on Cancer (IARC) has deemed 8-methoxypsoralen to be carcinogenic to animals. For example, rats exposed by gavage for 2 years had clear evidence of carcinogenic activity of 8-methoxypsoralen without UV radiation for male rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney and carcinomas of the Zymbal gland. Subcutaneous tissue fibromas and alveolar/bronchiolar adenomas of the lung in male rats may have been related to 8-methoxypsoralen administration. Dose-related nonneoplastic lesions in male rats included increased severity of nephropathy and mineralization of the kidney and forestomach lesions. There was no evidence of carcinogenic activity of 8-methoxypsoralen for female rats in the same study.

Human

8-Methoxypsoralen can cause hyperpigmentation and abnormal nail pigmentation. The IARC has classified 8-methoxypsoralen to be in group 1 (i.e., the agent is carcinogenic to humans). Studies have shown the patients with a history of methotrexate, ionizing radiation, or skin types I or II have an increased chance of developing cutaneous carcinomas when psoralen–UV light therapy is used.

In Vitro Toxicity Data

8-Methoxypsoralen is mutagenic in the Ames *Salmonella* assay, and in yeast, human lymphocyte, sister chromatid exchange, unscheduled DNA synthesis (UDS), and Chinese hamster ovary chromosome aberration studies. In a micronucleus test using V79 cells adapted to photogenotoxicity testing, 8-methoxypsoralen was found to cause micronuclei toxicity upon photochemical activation. Induction of UDS by 8-methoxypsoralen plus UVA was investigated in the epidermis of female hairless mice by means of an *in vivo–in vitro* assay using a liquid scintillation counting method. The results showed that PUVA causes a small induction of UDS, which might be due to slow DNA excision repair over a long period.

Clinical Management

Many of the acute symptoms of psoralen toxicity can be avoided by simply avoiding UV light. Severely exposed patients should be kept in a darkened room for 8–48 h depending on which psoralen is involved. Treatment of burns after PUVA therapy is symptomatic and supportive. UV absorbing wrap should be used around sunglasses for 24 h after 8-methoxypsoralen ingestions to decrease the potential for cataract formation. The body should be shielded from sunlight for at least 48 h after 8-methoxypsoralen exposure.

See also: Photoallergens; Toxicity Testing, Mutagenicity.

Further Reading

Arabzadeh A, Bathaie SZ, Farsam H, *et al.* (2002) Studies on mechanism of 8-methoxypsoralen–DNA interaction in the dark. *International Journal of Pharmacy* 237: 47–55.

Chignell CF, Haseman JK, Sik RH, Tennant RW, and Trempus CS (2003) Photocarcinogenesis in the Tg.AC mouse: Lomefloxacin and 8-methoxypsoralen. *Photochemistry Photobiology* 77: 77–80.

International Agency for Research on Cancer (IARC) (1987) *8-Methoxypsoralen (Methoxsalen Plus Ultraviolet Radiation)*. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, supplement 7, pp. 243–244.

Kersten B, Zhang J, Brendler-Schwaab SY, Kasper P, and Móuller L (1999) The application of the micronucleus test in Chinese hamster V79 cells to detect drug-induced photogenotoxicity. *Mutation Research* 445: 55–71.

Mori M, Kobayashi H, Katsumura Y, and Furihata C (2001) Induction of unscheduled DNA synthesis in hairless mouse epidermis by 8-methoxypsoralen plus ultraviolet A (PUVA). *Journal of Toxicolgical Sciences* 26: 1–8.

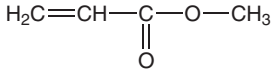
Relevant Website

http://ntp-server.niehs.nih.gov – (US) National Institute of Environmental Health Sciences, National Toxicology Program (NTP). 8-Methoxypsoralen (testing status from the NTP).

Methyl Acrylate

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 96-33-3
- Synonyms: Methyl 2-propenoate; 2-Propenoic acid methyl ester; Acrylic acid methyl ester
- Chemical/Pharmaceutical/Other Class: Ester
- Chemical Formula: $C_4H_6O_2$
- Chemical Structure:



Uses

Methyl acrylate is combined with other monomers to produce copolymers used in the production of surface coatings (e.g., latex finishes, floor and fabric finishes), acrylic fibers (used in carpets, clothing, blankets, and curtains), plastics (e.g., medical and dental prostheses), as well as adhesives, detergents, and sealants.

Exposure Routes and Pathways

Exposures to methyl acrylate monomer are most likely to occur in an occupational environment via skin contact and inhalation. However, these exposures are limited since methyl acrylate is a chemical intermediate that is manufactured and processed within closed systems accompanied by the use of industrial hygiene controls and personal protective equipment. The acrid odor of methyl acrylate, which can be detected at 0.002–0.014 ppm, also serves to limit exposure. Studies in workers involved in the production of the monomer have indicated that mean exposures to methyl acrylate are typically less than 1 ppm. The general population is not expected to receive a significant exposure to methyl acrylate monomer due to low concentrations of residual monomer in consumer products. The public may be exposed to methyl acrylate via ingestion because it is found naturally in some foods (e.g., pineapple puree).

Toxicokinetics

Data from animal experiments indicate that methyl acrylate is readily absorbed from the respiratory and gastrointestinal tracts. Absorption of methyl acrylate through the skin occurs less readily and may be limited by evaporation of methyl acrylate if the applied dose is unoccluded.

The primary route of methyl acrylate metabolism is its rapid hydrolysis by tissue and circulating carboxylesterases to acrylic acid and methanol, which

undergo further metabolism. Another route of methyl acrylate metabolism is conjugation with the sulfhydryl group of glutathione. Methyl acrylate is rapidly distributed throughout the body. Methyl acrylate and/or its metabolites can be detected in all organ systems, with the highest concentrations being present in the urine, expired air, and organ of entry (i.e., stomach, upper respiratory tract, and skin). More than 90% of orally administered methyl acrylate is excreted within 72 h via the lungs (>50%) as CO_2 and via the kidneys (40–50%) as products of methyl acrylate–glutathione.

Mechanism of Toxicity

Pretreatment of rats with a carboxylesterase inhibitor enhances the respiratory irritation and lethality produced by the inhalation of methyl acrylate. This observation suggests that the toxicity of methyl acrylate becomes manifest when local detoxification/defense mechanisms become overwhelmed.

Acute and Short-Term Toxicity (or Toxicity)

Animal

The acute oral LD_{50} in rat is 765 mg kg^{-1}; the rabbit dermal LD_{50} is 1250 mg kg^{-1}. The 4 h LC_{50} for methyl acrylate vapor in rat is 1600 ppm. Methyl acrylate may produce an allergic contact dermatitis and may cross-react with other acrylic esters. Methyl acrylate was not clastogenic in two (oral and inhalation) *in vivo* mouse micronucleus assays.

Human

Methyl acrylate can be highly irritating to the skin, eyes, and the respiratory tract. The severity of the reaction will depend on the concentration of the applied dose as well as the duration and frequency of contact. Methyl acrylate may cause an allergic contact dermatitis that may cross-react with other acrylate esters.

Chronic Toxicity (or Exposure)

Animal

Repeated inhalation exposures to irritating concentrations of methyl acrylate can damage the respiratory tract and eyes, while systemic toxicity is relatively minor, being limited to some organ weight changes without accompanying histopathology. Toxicity associated with repeated oral exposures is generally limited to decrements in body weight and the exacerbation of spontaneously occurring disease states.

In a 3 month drinking water study, rats were exposed to methyl acrylate at doses of 0, 1, 5, and 20 mg kg^{-1}. Toxicity was limited to the high dose and consisted of slight decreases in body weight gain and water consumption as well as an increase in relative kidney weight and in the severity of chronic progressive nephropathy that occurs spontaneously in Fischer 344 rats. The histopathology of other organs, including the sex organs, was normal. The study no-observed-adverse-effect level (NOAEL) was 5 mg-kg^{-1}. In a lifetime inhalation study, rats were exposed to methyl acrylate concentrations of 0, 15, 45, and 135 ppm for 6 h day^{-1}, 5 days week^{-1}. At these irritating concentrations, the primary effect was damage to the nasal and respiratory mucosa and the eyes at all three concentrations; systemic toxicity was observed primarily in the high dose group and was limited to a slight but reversible body weight gain and changes in organ weights without associated histopathology. The histopathology of the sex organs was normal. Tumor incidences were not increased.

In a developmental study, pregnant rats were exposed to methyl acrylate at concentrations of 0, 25, 50, or 100 ppm for 6 h day^{-1} on days 6–20 of gestation. The two highest doses produced marked decrements in maternal body weight gain and food consumption. Decrements in fetal body weight were observed only at 100 ppm; decreases in embryonic survival and increases in fetal malformations were not observed at any concentration. The NOAELs for maternal toxicity, developmental toxicity, and teratogenicity were 25, 50, and 100 ppm, respectively.

The normal sex organ histopathology noted in animals above combined with the occurrence of rat fetotoxicity only in the presence of maternal toxicity suggests that methyl acrylate does not pose a significant reproductive and developmental hazard to humans.

Human

No data on the chronic toxicity of methyl acrylate in humans are available.

In Vitro Toxicity Data

Data from several *in vitro* mutagenicity studies were negative, both with and without metabolic activation. However, methyl acrylate was clastogenic in Chinese hamster cells in the absence of metabolic activation.

Clinical Management

Clinical management involves removal from exposure and treatment of symptoms.

Environmental Fate

Methyl acrylate is a volatile (89 hPa at 20°C) liquid under normal environmental conditions. At equilibrium in the environment, methyl acrylate will partition primarily to air (92%) with lesser amounts to water (7.2%), soil (<1%), and sediment (<0.1%). In air, methyl acrylate will be removed by reaction with photochemically produced hydroxyl radicals (13.6 h half-life). When released to water, methyl acrylate will be removed by volatilization to air (Henry's law constant of $1.5\,Pa\,m^3\,mol^{-1}$) or biodegradation (60% removal in 28 days). Based on its estimated organic-carbon partition coefficient (K_{oc} of 6.4), methyl acrylate will exhibit high mobility in soil where it may leach to ground water or volatilize to air from surface soils. Similarly, it is not expected to bind significantly to sediments or suspended particulate matter. Based on its relatively low octanol–water partition coefficient ($\log K_{ow}$ of 0.74), methyl acrylate does not pose a significant bioaccumulation hazard.

Ecotoxicology

Methyl acrylate is acutely toxic to aquatic organisms. In a series of studies with analytically measured concentrations, methyl acrylate exhibited a 96 h LC_{50} of $3.4\,mg\,l^{-1}$ in freshwater fish (rainbow trout) and $1.1\,mg\,l^{-1}$ in marine fish (*Cyprinodon variegatus*), a 48 h EC_{50} (immobilization) of $2.6\,mg\,l^{-1}$ in an aquatic invertebrate (*Daphnia magna*), and a 96 h growth rate EC_{50} (biomass) of $1.99\,mg\,l^{-1}$ in algae (*Selenastrum capricornutum*).

Other Hazards

Methyl acrylate is highly flammable with lower and upper explosive limits of 2.1 and 14.5%, respectively.

Exposure Standards and Guidelines

International occupational exposure limits (OEL) for methyl acrylate generally range from 2 to 10 ppm as an 8 h time-weighted average (TWA); 2 ppm is the TWA OEL established by the American Conference of Governmental Industrial Hygienists. International short-term excursion limits generally range from 5 to 20 ppm. The US Occupational Safety and Health Administration lists a permissible exposure limit of 10 ppm for methyl acrylate (TWA). The National Institute of Occupational Safety and Health has a recommended exposure limit of 10 ppm methyl acrylate (TWA) and lists 250 ppm methyl acrylate as immediately dangerous to life or health. The International Agency for Research on Cancer indicates that methyl acrylate is not classifiable as to its carcinogenicity to humans (group C).

See also: Carboxylesterases; Glutathione; Polymers; Respiratory Tract.

Further Reading

Murphy SR and Davies JH (1993) Methyl acrylate health effects overview. In: Tyler TR, Murphy SR, and Hunt EK (eds.) *Health Effect Assessments of the Basic Acrylates*, pp. 33–52. Boca Raton, FL: CRC Press.

Methyl Bromide

Danny Villalobos and Marilyn Weber

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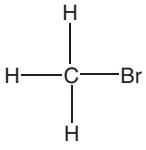
- Chemical Abstracts Service Registry Number: CAS 74-83-9
- Synonyms: Bromomethane; Monobromomethane; Halon 1001; Haltox; Zytox
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbon/fumigant
- Chemical Formula: CH_3Br
- Chemical Structure:



Uses

The uses of methyl bromide include preplant soil fumigant treatment for production of flowers, nursery crops, tomatoes, strawberries as well as other produce including carrots, legumes, and other assorted vegetables. The largest worldwide end use of methyl bromide is for soil fumigation; however, it is also used to fumigate durable and perishable commodities. Methyl bromide is also used to fumigate structures, dwellings, office buildings, warehouses, silos, mills, vaults, shipping, and freight cars for the control of fungi, insects, and rodents. As an outside fumigant, methyl bromide is typically used under approved gas-proof sheeting or tenting to control pests in soils and orchards. The largest usage of methyl bromide in developed countries occurs in the United States, Japan, Italy, France, Belgium, and

South Africa. It is also used in chemical synthesis. The use of methyl bromide in the United States as a fumigant is scheduled to cease in 2005.

Exposure Routes and Pathways

Exposures may occur via inhalation of the vapor or by dermal contact with the liquid. Ingestions are unlikely due to its gaseous form at room temperature. Childhood exposures may result in higher doses due to greater lung (surface area)/(body weight) ratios and increased minute volumes/weight ratios. Additionally, due to methyl bromide's density, children's shorter stature may also lead to relatively higher levels of exposure.

Toxicokinetics

Methyl bromide is rapidly absorbed by inhalation. Rats are more efficient than humans at absorbing methyl bromide following inhalation exposure, with absorption being directly proportional to air concentration up to ~about 300 ppm. Absorption is also rapid and extensive (97%) in rats after oral administration. Methyl bromide is distributed in fat, lung, liver, adrenals, and kidney, with less distribution into brain. Methyl bromide is rapidly and extensively metabolized to methanol, bromide, and finally to CO_2. After oral or inhalation exposure in rats, 85% was eliminated within 65–72 h. Most (30–50%) was recovered as expired CO_2 and 4–20% as expired parent compound. About 16–40% was recovered in the urine and a small percentage was eliminated in the feces. Extensive enterohepatic circulation is possible. Tissue half-lives of methyl bromide range from 0.5 to 8 h.

Mechanism of Toxicity

Methyl bromide methylates sulfhydryl groups of enzymes, causing cellular disruption and reduced glutathione levels. Cellular disruption, primarily in the central nervous system, results in progressive dysfunction. In sublethal poisoning, a latency period of 2–48 h can occur between exposure and onset of symptoms. Methanol, a metabolite of methyl bromide, may also contribute to the neurologic and visual effects, but this is only likely to be significant at high levels of exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Methyl bromide is moderately toxic by the oral and inhalation routes. The oral LD_{50} in rats was 104–214 mg kg^{-1}. Toxicity by the inhalation route is both time and concentration dependent. In mice, LC_{50} values were 1700 ppm (~6.6 mg m^{-3}) for a 30 min exposure to 405 ppm (~1.6 mg m^{-3}) for a 4 h exposure. In rats, the LC_{50} for a 30 min exposure was reported as 2833 ppm (~11 mg m^{-3}) while for an 8 h exposure the value was 302 ppm (1.2 mg m^{-3}).

Human

Primary effects of methyl bromide are on the nervous system, lungs, nasal mucosa, kidneys, eyes, and skin. Neurologic symptoms include blurred vision, mental confusion, paresthesias, tremors, and speech defects. Severe exposure may result in narcosis, seizures, coma followed by respiratory paralysis, and circulatory failure. Contact with the skin and eyes can lead to irritation and burns. After an acute single, small with prompt recovery, no delayed or long-term effects are likely to occur. In larger exposures inhalation can cause injury to the nervous system, lungs, and throat. High doses can also injure the kidneys and liver.

Chronic Toxicity (or Exposure)

Animal

Rats, rabbits, or female rhesus monkeys exposed to 0, 17, 33, 66, 100, or 220 ppm methyl bromide 7–8 h per day, 5 days per week for 6 months exhibited mortality in rats and monkeys at 100 ppm. Rabbits showed mortality at 33 ppm. Severe effects, including paralysis, were seen after exposure to 66 ppm in rabbits and monkeys. No signs of overt toxicity were noted at 17 ppm. Other studies have shown focal lesions in the brain and heart in rats after inhalation of 150 ppm methyl bromide (4 h per day, 5 days per week for 11 weeks). Rats exposed to 0, 200, 300, or 400 ppm methyl bromide (4 h per day, 5 days per week for 6 weeks) exhibited coronary lesions and exposures of 300 ppm or greater resulted in neurologic dysfunction including ataxia and paralysis. Testicular atrophy was noted at 400 ppm.

Rabbits appear more sensitive than rats to neurotoxicity of methyl bromide. Rats and rabbits were exposed to 0 or 65 ppm methyl bromide (7.5 h per day, 4 days per week for 4 weeks) but nerve conduction velocity and eyeblink reflex were impaired only in the rabbits.

Rats were exposed to 0, 3, 30, or 90 ppm methyl bromide (6 h per day, 5 days per week) and observed at 14, 53, and 105 weeks of exposure. Exposures of males and females to 90 ppm resulted in reduced body weight and significant lesions in the heart (cartilaginous metaplasia and thrombus in males; myocardial

degeneration and thrombus in females). Exposure at 30 or 90 ppm led to decreased kidney weights. Histological changes in the nose, heart, esophagus, and forestomach were noted. At the lowest concentration (3 ppm), slight degenerative changes in the nasal epithelium and olfactory basal cell hyperplasia were noted in both sexes at termination. In a 13 week study, mice were exposed to 0, 30, 60, or 120 ppm methyl bromide (6 h per day, 5 days per week). Serious effects, including 58% body weight loss, 17% mortality, and severe curling and crossing of the hindlimbs were observed with the high dose. Exposure of males to 40 ppm or higher led to decreased hemoglobin and increased red blood cell count.

No teratogenic effects were noted in rats or rabbits exposed to 20 ppm methyl bromide (7 h per day, 5 days per week for 3 weeks) during gestation days 1–19 (rats) or 1–24 (rabbits).

A two-generation reproductive and developmental toxicity study in rats was conducted using 0, 3, 30, or 90 ppm methyl bromide (6 h per day, 5 days per week during premating, gestation, and lactation through two generations). Significant decreases in body weight were observed in males exposed to 90 ppm. Neonatal body weights were decreased by exposure to 30 ppm. There was a decreased cerebral cortex width in the 90 ppm F1 group, reduced brain weight in 30 ppm F1 females, and reduced fertility in the 30 and 90 ppm F2b groups.

Methyl bromide induced sister chromatid exchanges and micronuclei in bone marrow cells in both male and female rats and mice following inhalation exposures.

There were no significant increases in tumors in rats exposed to concentrations up to 90 ppm methyl bromide for 29 months or mice exposed to concentrations up to 100 ppm for 2 years. Degeneration and hyperplasia of the nasal olfactory epithelium were noted in both species. No increased tumor incidence was seen in a 2 year dietary study in rats.

Human

Repeated exposures have been associated with peripheral neuropathies (especially sensory neuropathy), impaired gait, behavioral changes, and mild liver and kidney dysfunction. Visual impairment secondary to atrophy of the optic nerve has been reported. Chronic exposure may be more serious for children because of their potential longer latency period. Workers exposed to methyl bromide had a higher rate of neurological symptoms and performed less well on several behavioral tests. Several confounding factors (age, alcohol consumption, prescription medication, illegal drug use, education, ethnicity) may have influenced these findings, however.

In Vitro Toxicity Data

Methyl bromide was positive in *Salmonella typhimurium* strain TA100, with or without exogenous metabolic activation; negative results were obtained with TA98 in this assay.

Clinical Management

There is no antidote for methyl bromide and patients are treated symptomatically. Although there is no specific laboratory data to detect the presence of or diagnose an exposure to methyl bromide, serum bromide levels may be used to document that an exposure occurred. Bromide levels do not aid in the acute treatment of an exposure and do not accurately predict clinical course.

Environmental Fate

As a soil fumigant methyl bromide leaves no toxic residue in soils. The volatile gas rises into the atmosphere. Methyl bromide is an ozone-depleting substance. Although methyl bromide is very soluble in water, its high vapor pressure in various soil types indicates a low tendency to adsorb to soils and rapid evaporation. Methyl bromide has a half-life in air estimated from 0.3 to 1.6 years. Degradation is primarily due to photolysis. In soils, the half-life is 0.2–0.5 days. In water, a half-life of 3 h was calculated.

Ecotoxicology

Methyl bromide does not accumulate in aquatic species. Methyl bromide causes acute lethality in a number of aquatic species at concentrations of 0.7–20 $mg\,l^{-1}$. A 96 h LC_{50} in trout was 3.9 $mg\,l^{-1}$, with NOECs of 1.9 and 2.9 $mg\,l^{-1}$ for clinical signs and mortality, respectively. In *Daphnia*, a 48 h LC_{50} was 2.6 $mg\,l^{-1}$.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration ceiling limit is 20 ppm (skin).
- Reference exposure level is 5 $\mu g\,m^{-3}$. The reference concentration is also 5 $\mu g\,m^{-3}$.
- National Institute for Occupational Safety and Health IDLH (immediately dangerous to life or health) value is 250 ppm.
- American Industrial Hygiene Association ERPG-2 (the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms which could impair an individual's ability to take protective action) value is 50 ppm.

Miscellaneous

Methyl bromide is a colorless, odorless gas or liquid at low concentrations. At very high concentrations, it has a sweet, fruity odor. Additives such as chlorpicrin are often mixed with methyl bromide to warn of its presence.

See also: Bromine; Carbon Tetrabromide; Ethyl Bromide; Methanol.

Further Reading

Yang RS, Witt KL, Alden CJ, and Cockerham LG (1995) Toxicology of methyl bromide. *Reviews of Environmental Contamination and Toxicology* 142: 65–85.

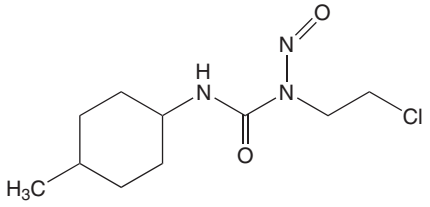
Relevant Websites

http://www.chem.unep.ch – United Nations Environment Programme.
http://www.epa.gov – United States Environmental Protection Agency.

Methyl CCNU

Jaya Chilakapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 13909-09-6
- Synonyms: 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea; Semustine; Methyl-CCNU
- Chemical/Pharmaceutical/Other Class: Alkylating agent
- Chemical Formula: $C_{10}H_{18}ClN_3O_2$
- Chemical Structure:



Uses

MeCCNU is an investigational drug used in chemotherapy to treat various types of cancers like Hodgkin's disease, malignant gliomas, gastrointestinal tract adenocarcinomas, breast carcinomas, and squamous-cell carcinomas, malignant melanoma and epidermoid carcinoma of the lung.

Exposure Routes and Pathways

The most common exposure pathway is ingestion.

Toxicokinetics

MeCCNU easily crosses the blood–brain barrier as it is lipid soluble. It has a short plasma half-life. This allows good therapeutic utility in various CNS neoplasms.

Mechanism of Toxicity

MeCCNU exerts its toxicity by cross-linking, that is, DNA alkylation, carbamoylates proteins and causes DNA strand breakage. It is cytotoxic in all stages of the cell cycle.

Acute and Short-Term Toxicity (or Exposure)

Animal

MeCCNU causes toxicity to kidney in male Fischer 344 rats. Even a single acute dose may lead to chronic and irreversible effects on the kidney. But lethal doses of MeCCNU (100–180 $mg\,kg^{-1}$) produced minimal proximal tubule injury. A 250 $mg\,kg^{-1}$ (1 $mmol\,l^{-1}$) dose of MeCCNU resulted in massive papillary necrosis within 7 days, with only limited necrosis to the proximal tubules. Sublethal doses resulted in a similar, chronic, progressive nephropathy, which was delayed in onset and was characterized by polyuria, enzymuria, a decrease in urine concentrating ability, and in renal slice organic ion accumulation. Studies suggest that hepatic metabolism contributes significantly to the alkylating activity of MeCCNU in the liver and the kidney, and indicate that a liver-derived metabolite may be responsible for the renal toxicity of MeCCNU.

Human

Protracted myelosuppression, a condition in which bone marrow activity is decreased, resulting in fewer red blood cells, white blood cells, and platelets, is the dose-limiting toxicity of MeCCNU. Renal and hepatic toxicities and pulmonary fibrosis are seen infrequently after exposure to MeCCNU. It exhibits dose-dependent nephrotoxicity. Proximal tubular cells are attacked, with renal failure occurring when

high doses are administered. It also produces acute nausea and vomiting.

Chronic Toxicity (or Exposure)

Animal

Data on methyl-CCNU were included in a report in which a large number of cancer chemotherapeutic agents were tested for carcinogenicity by intraperitoneal injection in Sprague–Dawley rats and Swiss-Webster mice. In male rats injected with methyl-CCNU thrice weekly for 6 months, total tumor incidence was reported to be increased 1.5–2-fold over that in controls at 18 months. A slight increase in tumor incidence was reported in mice. Intravenous administration of methyl-CCNU to rats induced lung tumors.

In another study, a single subcutaneous injection of MeCCNU (20–140 mg kg^{-1}) resulted in rapid decrease in renal function leading to a chronic progressive nephropathy in male Fischer 344 rats.

Human

Adjuvant treatment with methyl-CCNU has been evaluated in 3633 patients with gastrointestinal cancer treated in nine randomized trials. Among 2067 patients treated with methyl-CCNU, 14 cases of acute nonlymphocytic leukemia (ANLL) occurred, whereas one occurred among 1566 patients treated with other therapies. Cumulative (actuarial) risk was 4% at 6 years and was not affected by concomitant radiotherapy or immunotherapy. A subsequent report described a strong dose–response relationship, adjusted for survival time, giving a relative risk of almost 40-fold among patients who had received the highest dose.

Clinical Management

A patent airway should be established. Suction can be used if necessary. Signs of respiratory insufficiency should be watched for and ventilations should be assisted if needed. Oxygen should be administered by nonrebreather mask at 10–15 l min^{-1}. Pulmonary edema and shock should be monitored and treated if necessary. Seizures should be anticipated and treated if necessary. For eye contamination, eyes should be flushed immediately with water. Emetics should not be used.

See also: Alkyl Halides; Methylnitrosourea.

Further Reading

National Toxicology Program (2002) 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCnU). *Report on Carcinogens: Carcinogen Profiles* 10: 53–54.

Methyl Disulfide

Sara J Risch

- Chemical Abstracts Service Registry Number: CAS 624-92-0
- Synonym: Dimethyl disulfide
- Chemical/Pharmaceutical/Other Class: Alkyl sulfide
- Chemical Formula: $C_2H_6S_2$
- Chemical Structure: $CH_3–S–S–CH_3$

Uses

Methyl disulfide is used as a component of flavoring materials. It has an intense onion odor by itself. It is used in combination with other flavor compounds in food products including baked goods, frozen dairy products, meat products, soft candy, gelatin, puddings, and both alcoholic and nonalcoholic beverages. This compound has been found in nature.

Exposure Routes and Pathways

Dimethyl sulfide can be either a liquid or vapor by itself or in combination with other materials that can come into contact with the skin or be inhaled. It can be irritating to the skin and eyes. Based on single exposure animal tests, dimethyl sulfide is considered moderately toxic if swallowed, slightly toxic if absorbed through the skin, and slightly toxic if inhaled. The compound has a strong, objectionable odor that can cause nausea, dizziness, or headache.

Occupational exposure may occur through inhalation or dermal contact where the compound is produced, in the manufacture of flavoring materials where it is a component of that flavor, or when the final flavor is being added to a food product. Most exposure for the general population is through various food products.

Toxicokinetics

Methyl disulfide has been found as a normal component of mouse urine vapor, indicating that it

is not metabolized but is excreted. It has also been found in the expired air of both diabetics and nondiabetics as well as some samples of human milk.

Acute and Short-Term Toxicity (or Exposure)

Prolonged contact can result in the removal of oil from the skin and may dry the skin and cause irritation, redness, and a rash. High vapor concentration may be irritating to the eyes and respiratory tract. High vapor concentrations may also result in headache, dizziness, drowsiness, nausea, vomiting, and loss of muscle coordination. In severe exposure, loss of consciousness is possible.

Animal

Oral exposure showed methyl disulfide to be moderately toxic to rats (LD_{50} 190 mg kg^{-1}). Dermal testing with rabbits showed it to be no more than slightly toxic (LD_{50} >2000 mg kg^{-1}). Inhalation exposure for rats showed the material to be slightly toxic (4 h LC_{50} 805 ppm). Methyl disulfide is slightly irritating to the eyes and skin of rabbits.

A single application to the skin of rabbits produced no mortality but did result in eye irritation and an effect on the central nervous system and respiratory system. All of the effects disappeared within a day after exposure.

In rats, 5–40 µl was found to inhibit the thyroid function. Toxic levels were between 50 and 400 µl. In pigs, there was no effect on thyroid function at 100 nmol ml^{-1}.

Human

The principal hazard of methyl disulfide is as an irritant to the skin and eyes.

Chronic Toxicity (or Exposure)

Animal

Toxicity testing using rats showed that exposure to levels of 250 ppm by inhalation, up to 6 h day^{-1}, 5 days $week^{-1}$ for up to 4 weeks resulted in lethargy, respiratory difficulties, low weight gain. On autopsy, the organs were found to be congested. Exposure under the same conditions to levels of 100 ppm showed no toxic signs and the organs were normal.

Oral administration for 5 months found a no-effect level of 0.007 mg kg^{-1}. At 0.5 mg kg^{-1}, there were cardiovascular and kidney effects with dystrophic disease of the kidneys and cardiac muscles as well as impairment of the oxidation process.

Human

No specific exposure levels have been reported and there are no airborne exposure guidelines. The effects in humans are not known; however, excessive exposure may result in similar effects to what was observed in rats.

In Vitro Toxicity Data

Methyl disulfide was classified as a nonirritant in human *in vitro* studies. In a study of the inhibition of soy lipoxygenase, the IC_{50} was determined to be 1090 µmol l^{-1}.

Clinical Management

Eyes should be flushed with a large amount of water for at least 15 min. Skin should be washed with soap and water. If irritation continues, medical attention should be sought.

If inhalation exposure occurs, the person should be removed to fresh air immediately and monitored for respiratory distress. If breathing has stopped, artificial respiration should be initiated and oxygen administered if necessary.

For a person who is conscious, two glasses of water or milk should be given if the material is ingested. Vomiting should be induced and medical attention sought. If the person is unconscious or drowsy, liquids should not be given and medical attention should be sought immediately.

Environmental Fate

Methyl disulfide is not readily biodegradable and thus will be present in the environment. It is sometimes used as a marker of pollution.

Ecotoxicology

Methyl disulfide has been found to be moderately toxic to *Daphnia* with a 48 h LC_{50} of 4 mg l^{-1} and to trout with a 120 h LC_{50} of 1.75 mg l^{-1}. It is slightly toxic to algae with a 72 h LC_{50} of 11–35 mg l^{-1} and to guppies with a 96 h LC_{50} of 50 mg l^{-1}.

Exposure Standards and Guidelines

There are no established airborne exposure guidelines.

Miscellaneous

Methyl disulfide is a widely used flavoring ingredient. It is Food and Drug Administration approved as a

flavor material (21 CFR 172.515) and is FEMA GRAS number 3536. The applications for use as a flavor material include alcoholic and nonalcoholic beverages, baked goods, relishes, frozen dairy, gelatin and puddings, meat products, soft candy, and sweet sauces. It has been noted that the powerful and penetrating odor of this material may limit voluntary exposure.

See also: Food Additives.

Further Reading

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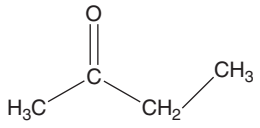
Transy MF, Kendall FM, Fantasia J, *et al.* (1981) Acute and subchronic toxicity studies of rats exposed to vapors or methyl mercaptan and other reduced sulfur compounds. *Journal of Toxicology and Environmental Health* 8: 71–88.

Methyl Ethyl Ketone

Samantha E Gad

- Chemical Abstracts Services Registry Number: CAS 78-93-3
- Synonyms: MEK; 2-Butanone; 2-Oxobutane; Ethyl methyl ketone
- Chemical/Pharmaceutical/Other Class: Ketone
- Chemical Formula: $CH_3COCH_2CH_3$
- Chemical Structure:



Uses

Methyl ethyl ketone (MEK) is used as a solvent for various coating systems, for example, vinyl, adhesives, nitrocellulose, and acrylic coatings. It is used in paint removers, lacquers, varnishes, spray paints, sealers, glues, magnetic tapes, printing inks, resins, rosins, cleaning solutions, and for polymerization. It is found in other consumer products, for example, household and hobby cements, and wood-filling products. MEK is used in dewaxing lubricating oils, the degreasing of metals, in the production of synthetic leathers, transparent paper and aluminum foil, and as a chemical intermediate and catalyst. It is an extraction solvent in the processing of foodstuffs and food ingredients. MEK can also be used to sterilize surgical and dental equipment.

In addition to its manufacture, environmental sources of MEK include exhaust from jet and internal combustion engines, and industrial activities such as gasification of coal. It is found in substantial amounts in tobacco smoke. MEK is produced biologically and has been identified as a product of microbial metabolism. It has also been found in plants, insect pheromones, and animal tissues, and MEK is probably a minor product of normal mammalian metabolism. It is stable under ordinary conditions but can form peroxides on prolonged storage; these may be explosive.

Exposure Routes and Pathways

Inhalation, ingestion, and dermal contact are all possible routes of exposure. MEK is a natural component in some foods. Indoor air pollution (and inhalation) can occur from volatilization of MEK from consumer and building products.

Toxicokinetics

MEK is rapidly absorbed by inhalation, skin contact and ingestion and transferred into the blood and other tissues. MEK is metabolized in the liver, mainly to 3-hydroxy-2-butanone and 2,3-butanediol that are eliminated in urine. Most MEK probably enters the general metabolism in the body and is converted to acetate that is eventually broken down to carbon dioxide and water that are then eliminated in exhaled air and urine. Small amounts of MEK itself are also eliminated in exhaled air and urine. MEK and its metabolites are mostly cleared from the body within 24 h, and MEK does not accumulate in the body.

Mechanism of Toxicity

There is very limited information on the mechanisms of toxicity of MEK. Relatively high inhaled concentrations of 1475–29 500 mg m^{-3} (500–10 000 ppm) caused pulmonary vasoconstriction and hypertension

in cats and dogs. There are several human case reports of neurological effects resulting from high exposure to MEK in combination with other solvents, and animal studies have confirmed synergism between MEK and ethyl *n*-butyl ketone, methyl *n*-butyl ketone, *n*-hexane, carbon tetrachloride, 2,5-hexanedione, and chloroform. The main target organs involved in toxicological interactions are the nervous system and liver, and the lung has also been mentioned.

Acute and Short-Term Toxicity (or Exposure)

Animal

MEK causes central nervous system depression in animals. The oral LD_{50} in rats is $6.86\,ml\,kg^{-1}$ ($2737\,mg\,kg^{-1}$) and $4050\,mg\,kg^{-1}$ in mice. Inhalation LC_{50} is $23\,500\,mg\,m^{-3}$ in rats and $32\,mg\,m^{-3}$ in mice. The intraperitoneal LD_{50} is $607\,mg\,kg^{-1}$ in rats, $616\,mg\,kg^{-1}$ in mice, and $2\,g\,kg^{-1}$ in guinea pigs. Animal studies indicate that MEK is a mild-to-moderate skin irritant, and a moderate to severe eye irritant. MEK was not mutagenic in mouse and hamster micronucleus cytogenetic assays. Pregnant rats were exposed by inhalation to up to 3000 ppm on days 6–15 of gestation; at 3000 ppm, there was a low but statistically significant increase in malformations. Sternebral and soft tissue anomalies were also increased. There was also a statistically significant increase in total skeletal anomalies at 1000 ppm. Maternal toxicity was not observed. In subsequent studies, pregnant rats and mice were exposed to up to 3000 ppm by inhalation during days 6–15 of gestation. There were no embryotoxic or teratogenic effects at any exposure level. There were fetotoxic effects (increased incidence of minor skeletal variations; delayed bone formation; reduced fetal weight) with very slight maternal toxicity at 3000 ppm.

Human

The major acute toxicity of MEK is mucosal irritation, and MEK can irritate the eyes, nose, and the respiratory system. No irritation was produced when 20% MEK in petrolatum was applied to volunteers for 48 h in a closed patch test; however, it can be irritating to the skin by defatting. It can cause dizziness, fatigue, memory alteration, dermatitis, headaches, nausea, and paresthesia of extremities, diminished vision acidosis, and vomiting. Acute inhalation can cause central nervous system depression. MEK vapor is irritating to mucous membranes and conjunctivae at 200 ppm after 15 min, but the odor is noticeable at 25 ppm. The results from animal evidence suggest that MEK can be aspirated during ingestion or vomiting, and could result in severe lung damage (edema), respiratory failure, cardiac arrest, and death.

Chronic Toxicity (or Exposure)

Animal

Exposure to 5000 ppm for 13 weeks produced an exposure-related effect on body and liver weights in rats, as well as a depression in brain weight in females. Guinea pigs and rats were exposed to 235 ppm for 12 weeks ($5\,day\,week^{-1}$, $7\,h\,day^{-1}$). There were no deaths and no signs of toxicity. Extensive neurological studies with high exposures have shown no effects. In one study, rats were initially exposed to 10 000 ppm that was reduced to 6000 ppm due to severe irritation of the upper respiratory tract. Temporary signs of muscle incoordination and gait disturbances were observed. Exposures continued for only 7 of the planned 15 weeks because some animals died of bronchopneumonia; there were no neurological symptoms. In the other study, rats were exposed to 1125 ppm continuously for up to 55 days with no signs of neurotoxicity.

Human

A mortality study of hundreds of workers who had worked at MEK dewaxing plants concluded that there was no evidence of a cancer hazard. The average follow-up was 14 years. This study is limited by the small size of the cohort and the relatively short follow-up period. The International Agency for Research on Cancer has not evaluated the carcinogenicity of MEK, the American Conference of Governmental Industrial Hygienists has not assigned a carcinogenicity designation for MEK, and the US National Toxicology Program has not listed MEK in its report on carcinogens. MEK is classified as a Group D chemical by the US Environmental Protection Agency, that is, it is not classifiable as to human carcinogenicity.

In Vitro Toxicity Data

MEK was not mutagenic in Ames (*Salmonella*) and *Escherichia coli* tests, but induced aneuploidy in *Saccharomyces cerevisiae*. MEK was not found to be genotoxic in the mouse lymphoma assay, in Chinese hamster ovary cell, unscheduled DNA synthesis assay, and micronucleus assays.

Clinical Management

Vomiting should not be induced.

Environmental Fate

MEK evaporates readily into the atmosphere and is subject to rapid photochemical decomposition. It reacts to form a haloform that is more toxic than the original compound in water containing free halogens or hypohalites. MEK does not accumulate in any environmental compartment, and it is rapidly metabolized by microbes and mammals. There is no evidence of bioaccumulation.

Ecotoxicology

MEK is not acutely toxic to fish or aquatic invertebrates. The LC_{50} values range from 1382 to 8890 mg l^{-1}. MEK is produced by fungi to concentrations that affect the germination of some plants.

Exposure Standards and Guidelines

The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h time-weighted average (TWA) is 200 ppm, and the (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day is 200 ppm. The NIOSH short-term exposure limit, for a 15 min exposure, is 300 ppm.

See also: Neurotoxicity.

Further Reading

Noraberg J and Arlien-Soborg P (2000) Neurotoxic interactions of industrially used ketones. *Neurotoxicology* 21: 409–418.

Yang RS (1986) The toxicology of methyl ethyl ketone. *Residue Reviews* 97: 121–143.

Relevant Websites

http://www.inchem.org – International Programme on Chemical Safety (IPCS). Methyl Ethyl Ketone (Environmental Health Criteria 143).

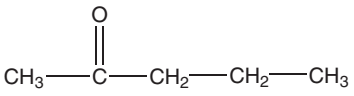
http://www.intox.org – International Programme on Chemical Safety. Methyl Ethyl Ketone.

Methyl Isobutyl Ketone

Samantha E Gad

This article is a revision of the previous print edition article by Shayne C Gad, volume 2, pp. 312–314, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 108-10-1
- Synonyms: MIK; Hexone; 4-Methyl-2-pentanone; Isopropyl acetone; MIBK; Isobutyl methylketone
- Chemical/Pharmaceutical/Other Class: Ketone
- Chemical Formula: $C_6H_{12}O$
- Chemical Structure:



Uses

Methyl isobutyl ketone (MIK) is used as a solvent for vinyl, epoxy, acrylic, natural resins, nitrocellulose, paints, varnishes, lacquers, protective coatings, rare metal extraction, and dyes. In addition, it is used as a denaturant for rubbing alcohol, as a synthetic flavoring adjuvant, and as a fruit flavoring agent. Other uses include in extracting uranium from fission products, dewaxing mineral oils, manufacturing antibiotics, dry-cleaning preparations, limited reported usage in cosmetics, and in the synthesis of methyl isobutyl carbinol.

Exposure Routes and Pathways

The general population can have inhalation and dermal contact during use of consumer products that contain MIK, together with oral exposures to MIK via its natural occurrence in oranges, grapes, and vinegar. Some segments of the general population may also be exposed to MIK via inhalation of contaminated air near industrial users, or areas near landfills and by ingestion of contaminated drinking water. In the workplace, inhalation of vapors and skin and eye contact are the most likely routes of exposure.

Toxicokinetics

MIK is absorbed by ingestion, inhalation, and dermal exposure. The metabolism of MIK involves oxidative hydroxylation, followed by reduction to the secondary alcohol. A single intraperitoneal administration of 450 mg kg^{-1} MIK to male guinea

pigs yielded two serum metabolites: 4-hydroxy-4-methyl-2-pentanone and 4-methyl-2-pentanol. The biological half-life for MIK elimination from the serum of guinea pigs was 66 min. The elimination times for MIK and 4-hydroxy-4-methyl-2-pentanone from these animals were 6 and 16 h, respectively.

The maximum percutaneous absorption rate in guinea pigs is 1.1 $\mu mol\,min^{-1}\,cm^{-2}$ at 10–45 min. The toxicokinetics of MIK were studied in human volunteers during inhalation exposure. The relative pulmonary uptake was ~60% and the total uptake increased linearly with increasing exposure concentration. The concentration of MIK in blood rose rapidly after the onset of exposure and no plateau level was reached during exposure. The concentration of unchanged MIK in the urine after exposure was proportional with the total uptake. Only 0.04% of the total MIK dose was eliminated unchanged via the kidneys within 3 h postexposure.

Mechanism of Toxicity

Organic solvents in general have the potential at acute high-level vapor pressure to cause narcosis and death, likely as a result of physical interaction of the solvent with cells of the central nervous system (CNS). MIK has been observed to enhance the neurotoxicity of *n*-hexane. This may be related to its ability to induce liver microsomal cytochrome P450, resulting in increased metabolic activation of *n*-hexane to more potent neurotoxic metabolites. MIK has also been observed to enhance the CNS effects of ethanol by reducing the action of alcohol dehydrogenase, thereby reducing the rate of ethanol metabolism and elimination.

Acute and Short-Term Toxicity (or Exposure)

Animal

MIK has a low acute toxicity; for example, the oral lethal doses for mice and rats were 2.85 and 4.6 $g\,kg^{-1}$, respectively. MIK causes eye and skin irritations and causes narcosis at high concentrations.

Human

MIK vapors may cause headaches and dizziness, are anesthetic, and may have other CNS effects. Small amounts of the liquid aspirated into the respiratory system during ingestion, or from vomiting, may cause bronchiopneumonia or pulmonary edema. An odor threshold for MIK has been reported at 15 ppm. The exposure to 200 ppm causes irritation of the eyes, nose, and throat.

Chronic Toxicity (or Exposure)

Animal

Effects from chronic exposure to MIK have included kidney damage and behavioral effects. Increased kidney/body weight ratios in rats occurred following exposure via inhalation to 100 ppm MIK for 2 weeks. Kidney and liver weights and the organ body weight ratios were also increased after exposure to 200 ppm for 2 weeks, and to 100 ppm for 90 days; however, dogs and monkeys did not demonstrate these effects after the 2 week exposures. Rats exposed to 100 ppm for 2 weeks experienced kidney damage in the form of hyaline droplet degeneration of the proximal renal tubules with occasional focal tubular necrosis; the tubular damage was considered to be transient and reversible. Chronic inhalation studies in rats have found TC_{Lo} levels of 410 $mg\,m^{-3}$ for 90 days and 1002 ppm for 6 $h\,day^{-1}$ for 14 weeks. In baboons, discriminatory behavior and memory were not affected by exposure to 20–40 ppm MIK; however, there was impairment on the accuracy of performance of tasks in a delayed match-to-sample discrimination test at an exposure of 50 ppm for 7 days. Inhalation exposure of rats to 86–127 $mg\,m^{-3}$ MIK for 4 $h\,day^{-1}$ for 4.5 months caused disturbances in the conditioned reflexes, and in the detoxifying function of the liver; a decrease of the eosinophil count in the blood was also observed.

MIK did not induce any treatment-related increases in embryotoxicity or fetal malformations in inhalation studies of pregnant rats or mice at concentrations of 300, 1000, or 3000 ppm. There was evidence of treatment-related maternal toxicity only at the highest concentration tested. MIK applied to the tail of rats daily at 300 or 600 $mg\,kg^{-1}$ for 4 months produced changes in the testes, including a reduction in the number of spermatocytes, spermatids, and spermatozoa. A carcinogenicity study of MIK is being conducted by the US National Toxicology Program.

Human

Repeated or prolonged inhalation may cause mucous irritation. Repeated or prolonged contact may cause defatting and drying of the skin. MIK is known to enhance the neurotoxicity of linear six-carbon solvents. Chronic exposure may cause axonal neuropathy, paresthesia, muscle weakness, and kidney damage.

In Vitro Toxicity Data

MIK was not mutagenic in the Ames test or in a mitotic gene-conversion assay in bacteria. MIK was also nonmutagenic in the mouse lymphoma,

unscheduled DNA synthesis, micronucleus, cell transformation, and chromosomal aberration test systems.

Clinical Management

Affected eyes should be flushed immediately with large amounts of water for at least 15 min. Affected skin should be flushed immediately with large amounts of water; soap should be used if available. Contaminated clothing, including shoes, should be removed after flushing has begun. In cases of inhalation, the victim should be removed from exposure, be kept at rest, and receive prompt medical attention. If breathing has stopped, artificial respiration should be administered. In cases of ingestion, vomiting should not be induced. Instead, 4–8 oz of milk or water (not to exceed 15 mg kg^{-1} in a child) should be given to dilute. The victim should get medical attention.

Environmental Fate

MIBK has a short half-life in the atmosphere and is also biodegraded in water. It is not expected to bioaccumulate. Based on an experimental vapor pressure of 19.9 mmHg at 25°C, MIBK is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase MIBK is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals with an estimated atmospheric half-life of ~27 h. Methyl isobutyl ketone is expected to have high mobility in soils based upon an estimated K_{oc} value of 123. Volatilization from dry soil surfaces is expected based upon the vapor pressure of this compound.

Ecotoxicology

Numerous ecotoxicology studies are available for MIK; for example, the oral LD_{50} for *Angelaius phoeniceus* (Redwinged blackbird) is 100 mg kg^{-1}, the LC_{50} for *Carassius auratus* (goldfish) is 460 mg l^{-1} for 24 h of exposure, and the LC_{50} for *Pimephales promelas* (fathead minnow) is 505 mg l^{-1} in a 96 h flowthrough bioassay. MIBK can contribute to the formation of photochemical smog when it reacts with other volatile organic carbon substances in air.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, 8 h time-weighted average (TWA), is 50 ppm, and the ACGIH short-term exposure limit (STEL) is 75 ppm. The US Occupational Safety and Health Administration permissible exposure limit, 8 h TWA, is 100 ppm (410 mg m^{-3}). The US National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day, is 50 ppm (205 mg m^{-3}), the NIOSH STEL, for a 15 min exposure, is 75 ppm (300 mg m^{-3}), and the NIOSH Immediately Dangerous to Life or Health level is 500 ppm. MIK is listed by the US Environmental Protection Agency as a Clean Air Act hazardous air pollutant generally known or suspected to cause serious health problems.

See also: Neurotoxicity.

Further Reading

Johnson W Jr. (2004) Safety assessment of MIBK (methyl isobutyl ketone). *International Journal of Toxicology* 23(Suppl. 1): 29–57.

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Phillips RD, Moran EJ, Dodd DE, *et al.* (1987) A 14-week vapor inhalation toxicity study of methyl isobutyl ketone. *Fundamental and Applied Toxicology* 9: 380–388.

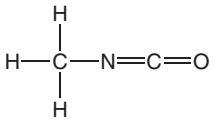
Relevant Website

http://cira.ornl.gov – US Oak Ridge National Laboratory, Center for Integrated Risk Assessment. Toxicity summary for Methyl Isobutyl Ketone (May 1994).

Methyl Isocyanate

Pallavi B Limaye and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 624-83-9
- Synonyms: Isocyanomethane; Isocyanatomethane; Methylcarbylamine, MIC
- Chemical Formula: C_2H_3NO
- Chemical Structure:



Uses

Methyl isocyanate (MIC) is used as an intermediate in organic synthesis, especially in the production of carbamate based pesticides. It is also used to produce polyurethane foams and plastics. Occasionally, it is present in cigarette smoke in very minute quantities.

Background Information

MIC is produced industrially by reacting methylamine with phosgene. At temperatures below 39°C (102°F), MIC is a highly flammable, colorless liquid. When exposed to air it readily evaporates. MIC in gaseous form is ~1.4 times heavier than air. Therefore, it tends to settle near the ground.

Exposure Routes and Pathways

Inhalation is the major route of exposure of MIC. MIC in the gaseous form is readily absorbed through the lungs. The odor threshold is ~2–5 ppm. However, odors of MIC may not provide adequate warning of hazardous concentrations since the immediately dangerous to life or health (IDLH) limit is only 3 ppm. Acute exposure to MIC vapors below the odor threshold can be irritating to the eye and respiratory epithelium. Acute exposure to higher vapor concentrations may cause severe pulmonary edema and injury to the alveolar walls of the lung leading to death due to respiratory failure. Significant exposures to MIC occur primarily in occupational settings or due to accidental release as occurred in Bhopal, India in 1984. The primary adverse effect was pulmonary edema with some alveolar wall destruction leading to respiratory failure that took several lives. Exposure in poorly ventilated, enclosed, or low-lying areas could result in asphyxiation. Children exposed to the same levels of MIC as adults may receive larger doses because they have relatively greater lung surface area:body weight ratios and higher minute volume:weight ratios. In addition, they may be exposed to higher levels than adults in the same location because of their short stature and the higher levels of MIC found nearer to the ground since it is heavier than the air.

Toxicokinetics

Studies conducted in Swiss Webster mice (males and pregnant females) indicate that the absorption of MIC is very rapid and it appears in the arterial and venous blood within few minutes after exposure to MIC vapors. Clearance of MIC is slower and may take ~3 days. The clearance is more rapid in urine than in bile. The highest concentrations of MIC in male mice 2 hours after exposure are found in the lung, sternum, gastrointestinal tract, spleen, and kidney. Twenty-four hours after exposure, highest MIC concentrations are found in the blood and lungs. In female mice, the highest concentrations at 2 h after exposure are found in the lungs, fetus, spleen, uterus, and kidney. After 24 h, the highest concentrations appear in the lung, spleen, and fetus.

Since MIC is highly reactive, it is not metabolized in the classical sense. Conjugation of MIC with glutathione (GSH) forming an adduct *S*-(*N*-methylcarbamoyl) glutathione, and corresponding cysteine adduct, *S*-(*N*-methylcarbamoyl) cysteine appears to represent an important pathway of biotransformation of MIC in the rats exposed to MIC intraperitoneally. The reaction of MIC with GSH and with cysteine is reversible, and can provide a source of free MIC in the tissues. It is speculated that these carbamate thioester conjugates of MIC may actually contribute to toxic effects of MIC. Similar studies in experimental animals exposed to MIC by the inhalation route have not been reported.

Mechanism of Toxicity

The exact mechanisms of MIC toxicity are not known, however, carbamylation of globin and other blood proteins have been speculated to contribute to MIC-induced toxicity. Acute exposure via inhalation of MIC vapors is known to cause irritation to the respiratory tract causing severe pulmonary edema and injury that can lead to death. It is also corrosive to the eyes causing severe corneal damage. Survivors of acute exposures may exhibit long-term respiratory and ocular effects. Direct skin contact of MIC in the liquid or gaseous form causes irritation of the skin.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute and subacute toxicity studies conducted in Charles Foster rats showed that MIC exposure significantly inhibits weight gain in a dose-dependent manner. These rats showed pathological lesions in the viscera, bronchial tree, lungs, liver, and kidneys. In another study, F344 rats exposed to 3 ppm MIC for 6 h day^{-1} for 4 days showed significant mortality within 28 days.

According to one report, in case of the Bhopal accident several thousand animals (cattle ~4000)

were reported dead due to MIC leakage. Information on fowl and other animals is not available.

Human

The respiratory system is the major target for MIC toxicity. In addition, MIC is also corrosive to skin and eyes. Upon ingestion, MIC shows corrosive action in the gastrointestinal tract. MIC exposure is rare in the general population except for the exposure occurring due to accidental release as occurred in Bhopal accident. The estimated immediate mortality resulting from this accidental exposure to MIC is believed to be between 2500 and 5000. Respiratory failure due to MIC inhalation was the principal cause of death. MIC caused bronchial necrosis and pulmonary edema. Within the first 24 h after the accident, around 90 000 patients were admitted in local hospitals and clinics with multiple symptoms of respiratory distress, breathlessness, choking, cough, chest pain, and hemoptysis. Acute ophthalmic effects were also reported with severe eye irritation and watering of the eyes.

Reproductive and gynecological effects were investigated by retrospective cohort studies. In an epidemiological survey conducted nine months after the accident, it was seen that 43% of 865 pregnancies amongst exposed women suffered fetal loss, as compared to 6–10% among the general Bhopal population. The spontaneous abortion rate was highest among those exposed during their first trimester. A study conducted by Shilotri *et al.* after 105–110 days of the accident showed a higher incidence of abnormal uterine bleeding and abnormal Pap smears amongst exposed women in the childbearing age.

Few immunological toxicity studies of MIC have been reported. A study of humoral and cell mediated immunity, in exposed subjects two months after exposure, found that cell-mediated immunity was suppressed, and that MIC-specific antibodies persisted for several months after the accident.

Chronic Toxicity (or Exposure)

Animal

No data were found.

Human

Around 50 000 survivors are estimated to be suffering from long-term health effects that are termed as 'Bhopal syndrome' due to a lack of information on the exact constituents of the gas cloud other than MIC. The Indian Council for Medical Research established a field office called Bhopal Gas Disaster Research Centre (BGDR) immediately after the accident. In addition, International Medical Commission on Bhopal (IMCB) was established in 1993 comprising 15 professionals from 12 different countries. BGDR and IMCB have reported that after 15 years of exposure, the affected population is still suffering from multisystemic toxicities. The major long-term health effects observed are shortness of breath, chest pain, muscle/bone pain, asthma, reproductive problems in the form of increased spontaneous abortions, and certain psychological problems. A randomized retrospective cohort study undertaken 10 years after exposure by Cullinan *et al.* indicates the presence of persistent small airways obstruction. The lung examination carried out in the survivors several months later exhibited presence of obliterative bronchiolitis and interstitial fibrosis. Thirty-nine percent of 783 patients examined showed ventilatory impairment.

A recent study published in the *Journal of the American Medical Association* in October of 2003 indicates that even the second generation of the exposed population is adversely affected. According to this study, a significant growth retardation was observed in boys who were either exposed to the gases as toddlers or born to exposed parents. Interestingly, no significant effects have been observed in girls.

In Vitro Toxicity Data

In vitro studies in Chinese hamster ovary cells indicate that MIC is capable of inducing chromosomal aberrations and sister chromatid exchanges in the 0.9–3.1 $\mu g\,ml^{-1}$ dose range, however it is not a mutagen.

Clinical Management

In acute exposure prompt medical attention is critical. Persons exposed only to MIC gas pose no risk of secondary contamination to rescuers. Persons whose skin or clothing is contaminated with liquid MIC can secondarily contaminate response personnel by direct contact or through off-gassing of vapor. There is no antidote for MIC poisoning. Treatment consists of removal of the victim from the contaminated area and support of respiratory and cardiovascular functions. In the event of ingestion of MIC, intragastric instillation of activated charcoal is useful; however, emesis is strictly avoided since this may cause additional corrosion of the gastrointestinal tract. Upon eye and skin exposures, washing the exposed area with ample amount of water is necessary. If the injury and pain are evident, the patient should be transferred to the Critical Care Unit to ensure continuous support therapy.

Environmental Fate

Terrestrial Fate

If MIC is released to soil, it is expected to rapidly hydrolyze if the soil is moist. Since it rapidly hydrolyzes, adsorption to and volatilization from moist soil are not expected to be significant processes, although no specific data regarding the fate of MIC in soil are available.

Aquatic Fate

If MIC is released to water, it is expected to rapidly hydrolyze with half-lives of 20 and 9 min at 15°C and 25°C, respectively. The products of hydrolysis may include *N*-carboxymethylamine, methylamine, carbon dioxide, and *N*,*N*′-dimethylurea. Since MIC rapidly hydrolyzes, bioconcentration, volatilization, and adsorption to sediment and suspended solids are not expected to be significant processes. However, some studies have shown that the alkylisocyanates like MIC are relatively resistant (compared to the arylisocyanates) to hydrolysis in water. Hence, despite the high water reactivity of MIC, this compound could possibly persist in the environment for many days after an initial release. No data were found concerning biodegradation of MIC.

Atmospheric Fate

If MIC is released to the atmosphere, it is expected to exist almost entirely in the vapor phase, based upon a reported vapor pressure of 348 mmHg at 20°C. It is also susceptible to photooxidation via vapor phase reaction with photochemically produced hydroxyl radicals, though this process is slow ($t_{1/2}$ 3 months).

Other Hazards

MIC reacts violently with water. MIC is incompatible with oxidizers, acids, alkalis, amines, iron, tin, and copper.

Exposure Standards and Guidelines:

Over an 8 h workshift, the Occupational Safety and Health Administration permissible exposure limit is 0.02 ppm. The IDLH established by the National Institute for Occupational Safety and Health is 3 ppm for MIC.

American Industrial Hygiene Association Emergency Response Planning Guidelines-2 (AIHA ERPG-2) defined as maximum airborne concentration below which nearly all persons could be exposed for up to 1 h without experiencing or developing irreversible or other serious health effects or symptoms that could impair their abilities to take protective action is 0.5 ppm. The current exposure standards and guidelines are summarized in **Table 1**.

Table 1 Summary of exposure criteria for MIC

Agency	*Criteria*		*Averaging time*
NIOSH	IDLH	3 ppm	NA
OSHA	PEL	0.02 ppm	8 h workshift per day
CalEPA	Chronic REL	0.001 mg m^{-3}	

EPA has not established a reference concentration or a reference dose for MIC. CalEPA stands for California Environmental Protection Agency.
Conversion: 1 ppm = 3.19 mg m^{-3}.

See also: Isocyanates; Bhopal.

Further Reading

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Relevant Website

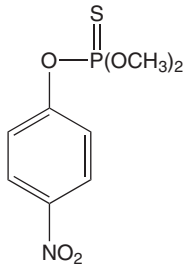
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Methyl Isocyanate.

Methyl Parathion

Kelly McCracken

This article is a revision of the previous print edition article by Carey N Pope, volume 2, pp. 317–318, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 298-00-0
- Synonyms: Penncap-M; Bladan M; Dalf; Folidol-M; Metacide; Nitrox
- Chemical/Pharmaceutical/Other Class: Synthetic organophosphorus insecticide in the phosphorothionate class
- Chemical Formula: $C_8H_{10}O_5NPS$
- Chemical Structure:



Uses

Methyl parathion is a contact insecticide for use on a variety of crop insects.

Background Information

In March 1997, there were reported misuses of methyl parathion leading to prosecution. Over 15 000 homes and businesses in Mississippi and Ohio were sprayed with methyl parathion by unlicensed operators. Methyl parathion is prohibited for use indoors. Authorities had to relocate over 1100 people to temporary accommodations with clean-up costs approaching $50 million dollars. With these reported misuses, local veterinarians reported deaths of household pets due to methyl parathion exposure. In July 1997, there was also an illegal application of methyl parathion by an illegal applicator to control cockroaches in the Chicago area.

Exposure Routes and Pathways

The dermal and inhalation routes are the most important means of occupational exposure. Accidental exposure through the oral route has also been reported. Methyl parathion is available as emulsifiable concentrates, wettable powders, and dusts of various concentrations.

Toxicokinetics

Methyl parathion is rapidly absorbed by all routes. Maximum tissue levels are achieved in 1 or 2 h following oral exposure. Methyl parathion is activated via the P450 mixed function oxidase system to the oxygen analog, methyl paraoxon. The oxon is metabolized in the liver to *p*-nitrophenol and dimethyl phosphate and these can be conjugated as glucuronides and glycosides. Glutathione-mediated demethylation also occurs. Methyl parathion is rapidly distributed to various tissues. The water-soluble metabolites are primarily excreted through the urine. A trace amount of the unmetabolized parent compound is also eliminated through the urine. Excretion of the major metabolite, *p*-nitrophenol, is essentially complete in 24 h following oral exposure. The excretion of dimethylphosphate is more protracted.

Mechanism of Toxicity

As with other organophosphorothioate agents, the toxicity of methyl parathion is due to inhibition of acetylcholinesterase by the active metabolite (i.e., methyl paraoxon), resulting in stimulation of the central nervous system, the parasympathetic nervous system, and the somatic motor nerves.

Acute and Short-Term Toxicity (or Exposure)

Animal

Clinical signs include hypersalivation, gastrointestinal hypermotility, abdominal cramping, vomiting, diarrhea, sweating, dyspnea, cyanosis, miosis, muscle fasciculations (in extreme cases, tetany followed by weakness and paralysis), and convulsions. The oral LD_{50} in adult rats and mice is $\sim 20\,mg\,kg^{-1}$. As with many other organophosphate insecticides, young animals appear to be more sensitive than adults to acute toxicity (lethality) from high doses. These age-related differences appear to be related in part to maturation of detoxification processes.

Human

Eye contact may cause pain, moderate eye irritation, and temporary corneal injury. Prolonged exposure may cause skin irritation. Ingestion of methyl parathion has caused typical symptoms of acute organophosphorus poisoning including headache, weakness, incoordination, fasciculations, tremor, nausea, cramps, diarrhea, and sweating. When inhaled the first adverse effects include bloody or

runny nose, coughing, chest discomfort, and difficulty breathing.

Chronic Toxicity (or Exposure)

Animal

Dietary administration of methyl parathion in dogs ($1.25\,mg\,kg^{-1}day^{-1}$) for 12 weeks caused red blood cell and plasma cholinesterase inhibition. A three-generation reproductive toxicity test in rats (0.5 and $1.5\,mg\,kg^{-1}\,day^{-1}$) led to reduced weanling survival, reduced birth weight at both dosages and increased stillbirths at the high dose only. In developmental studies, subacute exposures ($3\,mg\,kg^{-1}\,day^{-1}$ for 15 days) caused fetal toxicity but no teratogenic effects. In a 2 year study with mice, methyl parathion was not carcinogenic at dietary levels up to 50 ppm (9.2 mg per kg body weight per day).

Human

With repeated exposures, acetylcholinesterase inhibition can persist without indications of toxicity. In most cases, cholinesterase inhibition is without overt effects. Methyl parathion cannot cause delayed neurotoxicity.

In Vitro Toxicity Data

Methyl parathion is not mutagenic in standard *in vitro* assays.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and $0.15\,mg\,kg^{-1}$ in children) are initially administered, followed by 2–4 mg (in adults) or $0.015–0.05\,mg\,kg^{-1}$ (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Exposure Standards and Guidelines

Methyl parathion is a restricted use pesticide. The chronic reference dose for methyl parathion is $0.000\,02\,mg\,kg^{-1}\,day^{-1}$.

See also: A-Esterases; Carboxylesterases; Cholinesterase Inhibition; Neurotoxicity; Organophosphates; Pesticides.

Further Reading

Garcia SJ, Abu-Qare AW, Meeker-O'Connell WA, Borton AJ, and Abou-Donia MB (2003) Methyl parathion: A review of health effects. *Journal of Toxicology and Environmental Health, Part B: Critical Review* 6(2): 185–210.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Methyl Parathion.

http://www.epa.gov – US Environmental Protection Agency.

Methylamine

Dale J Marino

- Chemical Abstracts Service Registry Number: CAS 74-89-5
- Synonyms: Methanamine; Monomethylamine; Aminomethane; Carbinamine; MMA
- Chemical/Pharmaceutical/Other Class: Amine
- Chemical Formula: CH_5N
- Chemical Structure: CH_3NH_2

Uses

Methylamine is primarily used in the organic synthesis of pharmaceuticals, insecticides, herbicides, pesticides, fungicides, electrostatic automotive coatings,

as well as certain solvent and paint strippers, photographic developers, and water treatment chemicals. Methylamine has also found use in tanning and dyeing processes, in rocket propellants, and in the manufacture of rubber chemicals, surfactants, surface-active agents, and accelerators.

Background Information

Methylamine has been reported to occur in various fresh fruits and vegetables, coffee, tea, and cocoa, as well as cheeses, and fish.

Exposure Routes and Pathways

Exposure to methylamine primarily occurs in occupational settings. Because methylamine is a gas, such exposures typically occur via inhalation, although dermal contact (and to a lesser extent ocular contact) with liquefied methylamine or aqueous solutions of methylamine would also be possible. The general population is potentially exposed to low concentrations of methylamine by ingestion from food, and by inhalation from releases to air.

Toxicokinetics

The metabolism of methylamine is believed to occur in two stages. The amino group is initially dehydrogenated to an intermediate imine (methyl imine), which reacts spontaneously with water, forming the corresponding aldehyde (formaldehyde) and ammonia. The final metabolic products, reported to be formic acid and urea or methylurea, are excreted in the urine. To a lesser extent, methylamine is also metabolized to dimethylamine. Methylamine is a normal constituent of mammalian and human urine.

Mechanism of Toxicity

The toxic effects of methylamine are due primarily to its corrosive action on tissues.

Acute and Short-Term Toxicity (or Exposure)

Methylamine is corrosive to the eyes and skin, and is a severe respiratory tract irritant.

Animal

The acute rat oral LD_{50} of a 40% aqueous solution of methylamine is $100\,mg\,kg^{-1}$. Reported inhalation LC_{50} values in rats and mice are 448 ppm ($570\,mg\,m^{-3}$) (2.5 h) and $2400\,mg\,m^{-3}$ (2 h), respectively. In rabbits, a 40% aqueous solution of methylamine produced corneal damage and dermal necrosis, and a 5% aqueous solution caused conjunctival hemorrhages, superficial corneal opacities, and edema.

Human

Brief exposure to concentrations of 20–100 ppm produced transient irritation of the eyes, nose, and throat. A concentration of 2–60 ppm has been reported to cause bronchitis.

Exposure to elevated concentrations of various amines, and presumably methylamine, is known to cause transient visual disturbance called glaucopsia, which is often referred to as blue haze or halovision. Exposure to elevated levels of methylamine is expected to potentially cause severe ocular and respiratory tract irritation with eye burns and pulmonary edema possible. Dermal contact with aqueous methylamine is expected to cause severe skin irritation and skin burns, with cold burns/frost bite also possible following dermal contact with liquefied methylamine. Ingestion of aqueous methylamine is expected to cause burns of the mouth, throat, esophagus, and stomach with bleeding, vomiting, diarrhea, and possible perforation of the esophagus and stomach.

Chronic Toxicity (or Exposure)

Animal

Repeated inhalation exposure of rats to methylamine for $6\,h\,day^{-1}$, $5\,days\,week^{-1}$ for 2 weeks produced mild nasal irritation at 75 ppm; damage to the respiratory mucosa of the nasal turbinates at 250 ppm; and bodyweight loss, liver damage, and nasal degenerative effects at 750 ppm.

No adverse reproductive effects or fetal abnormalities were observed in CD-1 mice treated daily with 0.25, 1, 2.5, or 5 mmol methylamine per kg by intraperitoneal injection during gestation days 1–17.

Human

No symptoms of irritation were evident following repeated exposure to 10 ppm. Repeated exposures to higher concentrations are expected to produce irritation of the eyes, nose, and throat. Repeated exposures could potentially aggravate existing respiratory diseases.

In Vitro Toxicity Data

In vitro mutagenicity assays yielded negative (not mutagenic) results in the *Salmonella*/microsome

reverse mutation assay, and positive (mutagenic) results in the mouse lymphoma cell forward mutation assay.

Clinical Management

Exposed skin and eyes should be irrigated with copious amounts of water. After inhalation exposures, the victim should be moved to fresh air and monitored for respiratory distress. Humidified, supplemental oxygen (100%) with assisted ventilation should be administered as required. If coughing or breathing difficulties are noted, the patient should be evaluated for irritation, bronchitis, or pneumonitis, including chest X-rays and determination of blood gases. If pulmonary edema is present, positive-end expiratory pressure ventilation and steroids should be considered. For ingestion exposures, emesis or lavage should be avoided. Use of diluents is controversial. Delayed abdominal pain and tenderness or shock may indicate gastric or esophageal perforation.

Environmental Fate

Given its high vapor pressure of over 2 atm at 25°C, methylamine will remain in the vapor phase, if released to the atmosphere where it will react with photochemically produced hydroxyl radicals ($T_{1/2}$ of ~18 h). Dissolution into rain droplets is also an important removal process. Other atmospheric removal processes, for example, photolysis and hydrolysis are not significant.

The predominant form of methylamine under environmental conditions is the ionized (protonated) species, which is expected to bind to soil constituents, suspended sediments, and bed sediments to a greater degree than the neutral form. As such, migration from soil to groundwater is expected to be less than would be anticipated for the neutral form. Volatilization from moist soils or surface water is not expected to be an important fate process. Biodegradation is expected to be an important loss process in both soil and water. The potential for bioconcentration in aquatic biota is low.

Ecotoxicology

The reported nonlethal concentration in algae (*Scenedesmus*) is 4 mg l^{-1}. The reported no-observed-effect concentration in crustaceans (*Daphnia*) is 480 mg l^{-1}. The 24 h LC_{50} in fish (cheek chub) is 10–30 mg l^{-1}.

Other Hazards

High airborne concentrations of methylamine can form, given its vapor pressure, with the potential for severe eye, nose, and respiratory tract irritation; escape impairment; and possible death. The immediately dangerous to life or health concentration for methylamine is 100 ppm. Anhydrous methylamine is a flammable gas, and aqueous methylamine is a flammable liquid. Vapors can travel a considerable distance to an ignition source and flash back because methylamine vapor density is heavier than air.

Exposure Standards and Guidelines

Occupational exposure standards and guidelines for methylamine include the following:

- United States: Occupational Safety and Health Administration permissible exposure limit (Table Z-1) is 10 ppm (12 mg m^{-3}).
- United States: National Institute for Occupational Safety and Health recommended exposure limit is 10 ppm (12 mg m^{-3}).
- United States: American Conference of Governmental Industrial Hygienists threshold limit value is 5 ppm (6.4 mg m^{-3}), with a 15 min short-term exposure limit of 15 ppm (19 mg m^{-3}).
- Australia: 10 ppm.
- Germany: 10 ppm, with a short-term level of 20 ppm for 10 min (four times/shift).
- Sweden: 10 ppm, with a short-term value of 20 ppm for 15 min, (skin notation).
- United Kingdom: 10 ppm.

See also: Corrosives; Respiratory Tract.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Methylamine.

http://www.state.nj.us/health/eoh/rtkweb/1225.pdf – Methylamine (Right-to-Know Hazardous Substance Fact Sheet from the state of New Jersey).

Methylcholanthrene, 3-

Richard A Parent

This article is a revision of the previous print edition article by R A Parent, T R Kline, and D E Sharp, volume 2, pp. 305–306, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 56-49-5
- Synonyms: 3-MC; 3-MCH; 3-Methyl-I,j-cyclopentabenz(*a*)anthracene; 3-Methylcyclopentabenzophenathrene
- Chemical/Pharmaceutical/Other Class: Polynuclear aromatic hydrocarbon (PAH); Polycyclic aromatic hydrocarbon
- Chemical Formula: $C_{21}H_{16}$

Uses

3-Methylcholanthrene (3-MC) is used experimentally as a positive control in cancer research and in biochemical research to induce specific forms of cytochrome P450. Other than this, there is no particular use for this chemical except as a possible chemical intermediate.

Exposure Routes and Pathways

3-MC may be absorbed via inhalation, ingestion, or dermal contact. It is contained in coal tar pitch volatiles which may result in dermal or ocular exposure.

Toxicokinetics

Animal studies with structurally related polynuclear aromatic hydrocarbons (PAHs), such as benzo(*a*)pyrene, benz(*a*)anthracene, and 3-MC, confirmed that intestinal transport readily occurs primarily by passive diffusion after oral dosing. From the partitioning parameters, the rate-limiting step involves solvation of transfer species in the interfacial water at the phospholipid surface.

Metabolic products vary with the type of enzyme inductions. In fetal rat livers, several compounds, such as 1- or 2-hydroxy-, *cis*- and *trans*-dihydroxy-, 11,12-dihydroxy-11,12-dihydro-, and 1- and 2-keto-3-cholanthrene have been identified. Most frequently, it is the liver that produces a variety of electrophilic reactants that covalently bind to macromolecules. Metabolism or bioactivation may also be extramicrosomal or be carried out by fetoplacental tissue or gut bacteria.

PAHs are highly soluble in adipose tissue and lipids. *In vivo* binding of 3-MC to liver and lung DNA was studied in A/J mice and demonstrated DNA binding in the liver and lung.

Mechanism of Toxicity

Metabolic activation of PAHs consists of an oxidation of the rings of unsubstituted PAHs. These oxidations are carried out by mixed function oxidases of the liver which contain cytochromes P450 and P448 and require reduced nicotine adenine dinucleotide and oxygen. In this oxidation, an epoxide intermediate is formed which has been shown to have the requisite chemical reactivity to bind covalently with DNA and histones and to serve as the ultimate carcinogenic form of PAH. Administration of 3-MC to rats increased hepatic nuclear proteins and caused a turnover of protein of the endoplasmic reticulum. Studies of ^{14}C amino acid incorporation showed that 3-MC causes increased protein synthesis and reduced degradation of protein.

Acute and Short-Term Toxicity (or Exposure)

Animal

3-MC has a low order of acute toxicity but at high concentrations it can produce irritation of mucous membranes.

Human

The minimum lethal human exposure and the maximum tolerated human exposure to this agent have not been delineated. PAHs are eye irritants and produce photosensitivity, respiratory irritation, cough, mild hepatotoxicity, and nephrotoxicity. The minimal lethal dose of 3-MC in humans has not been established.

Chronic Toxicity (or Exposure)

Animal

In mice, skin application leads rather quickly to carcinoma formation. Subcutaneous injection produces sarcomas in rats or mice. Oral administration in sesame oil to female Sprague–Dawley rats results in rapid induction of breast cancer, while oral administration to mice during the last week of pregnancy produced a threefold increase in incidence of tumors. The most common types were lymphoma and lung tumors. 3-MC has reportedly produced skin tumors in mice, injection site tumors in rats, large

intestinal tumors in Sprague–Dawley rats treated intrarectally, lung adenomas in mice treated via intraperitoneal injection, and lung tumors in neonatal mice treated transplacentally.

3-MC is a powerful irritant and is an experimental carcinogen producing neoplastic responses by various dosing routes including oral, dermal, intravenous, parenteral, subcutaneous, intrarenal, intrapleural, intratracheal, and implant. 3-MC is hepatotoxic, nephrotoxic, and immunotoxic and has been reported to produce agranulocytosis, anemia, leukopenia, and pancytopenia in exposed animals.

Exposure of mice to 3-MC produces a marked depression in serum antibody response to shee erythrocytes. Subsequent studies confirmed that 3-MC does indeed suppress the immune system resulting in long term immunsuppression.

Human

Chronic exposure to 3-MC can result in irritation, chronic cough, bronchitis, and bronchogenic cancer. Leukoplakia and cancers of the lip and oral cavity can develop. Dermal contact has been associated with precancerous lesions called 'coal tar warts' which are enhanced by exposure to UV light. Erythema, dermal burns, acneiform lesions, photosensitization and cancer may develop upon chronic exposure.

Workers routinely exposed to PAHs have been reported to show increased incidences of skin, bladder, lung, and gastrointestinal cancers. Other studies also demonstrated increased incidences of lung and scrotal cancer. 3-MC is a PAH and is considered to be carcinogenic in humans.

PAHs as a class of compounds are generally classified by the International Agency for Research on Cancer as being possibly carcinogenic to humans while mixtures of these compounds such as coal tar pitch and coke production are classified as being carcinogenic to humans. Increased incidences of skin, bladder, lung, and possibly gastrointestinal tract cancers have been reported in polynuclear aromatic hydrocarbon (PNA)-exposed workers, particularly associated with coal carbonization, coal gasification, and coke oven work.

Increased numbers of chromosomal aberrations have been reported to be a sensitive marker for exposure to PAHs.

In Vitro Toxicity Data

3-MC is mutagenic in a number of *in vitro* and *in vivo* assays and is used regularly as a positive control in these assays, and it has been shown to covalently bind to DNA and other macromolecules. Using TA100 strain of Salmonella in the Ames test, S-9 liver microsomal fractions from mice, rats, hamsters, pig, and humans produced a doubling of the reversion rate for all liver fractions except the pig. 3-MC has also produced positive findings in the Ames test using S9 rat liver microsomal fractions stimulated with PCB Aroclor 1254 in strains TA100, TA1535, TA1537, TA1538, and in TA1538, TA98 with rat liver S9 fractions stimulated with phenobarbital. Positive mutagenic findings were also noted in Chinese hamster V-79 thioquanine assay, in unscheduled DNA assays done in human fibroblasts, and in rodent primary cells and hepatocytes, in Chinese hamster ovary HGPRT assay but not in several *Escherichia coli* WP2 UVRA assays with S9 activation. Positive findings were also reported in human lymphocyte assays showing increased sister chromatid exchanges. Numerous other reports of both positive and negative findings may be found in the literature.

Clinical Management

Acute toxicity relating to ingestion of PAHs is highly unlikely. Inhalation exposure to PAHs may involve other materials capable of causing acute respiratory and systemic effects. Treatment should be according to symptomatology. For dermal contact, it is important to remove contaminated clothing and wash the exposed area with soap and water. Burns should be treated in the usual manner.

Environmental Fate

3-MC, when released to soil should adsorb strongly to the soil and not leach. It will not biodegrade or hydrolyze significantly but may evaporate from dry soil. If released to water, it is expected to adsorb strongly to sediment and to bioconcentrate in aquatic organisms. Again it will not biodegrade or hydrolyze significantly. If released into the atmosphere, it may be subject to direct photolysis since it absorbs strongly in the UV spectrum of light. It may also react with peroxyl radicals already present in the atmosphere. Its estimated half-life in the atmosphere is 2.81 h. Considering an octanol/water log concentration ratio of 6.42, a bioconcentration factor of 45 000 has been estimated.

Other Hazards

Treated pregnant rats produce offspring with major birth defects including open neural tubes, abnormal flexure rotation and proencephalic defects, among others. In experimental animals, PAHs such as 3-MC and metabolites have been noted to produce

decrements in fertility as a result of decreased numbers of oocytes. PAHs are strongly lipophilic and are excreted in breast milk thereby resulting in a secondary exposure to nursing infants. They also cross the placenta adding an additional burden to the fetus and neonate.

Exposure Standards and Guidelines

The US Environmental Protection Agency's Carcinogen Assessment Group has placed 3-MC on a list of compounds for which strong evidence exists for either causing cancer in humans or in multiple animal species.

No occupational exposure limits have been established for 3-MC. There may be no safe level of exposure.

Miscellaneous

3-MC can be isolated by recrystallization from benzene/ether producing pale yellow slender prisms with a melting point of 280°C. It is highly soluble in organic solvents including the BTEX solvents, chlorinated aliphatics, and various ketone and ester solvents. It strongly absorbs UV light with a maximum absorption at 327 nm. Its vapor pressure is low (3.8×10^{-6} mmHg) limiting is volatility. One part per million in the environment is equivalent to $10.98\,\mathrm{mg\,m^{-3}}$.

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Relevant Websites

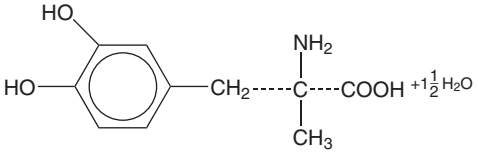
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Methylcholanthrene, 3-.

http://www.state.nj.us – New Jersey Department of Health and Senior Services, Hazardous Substance Fact Sheet, 3-Methylcholanthrene; August 1988.

Methyldopa

Elizabeth J Scharman

- Chemical Abstracts Service Registry Number: CAS 555-30-6 (anhydrous)
- Synonyms: 3-Hydroxy-α-methyl-L-tyrosine; α-Methyldopa; Methyldopate hydrochloride; Aldomet®
- Chemical/Pharmaceutical/Other Class: Hypotensive agent, centrally acting
- Chemical Formula: $C_{10}H_{13}NO_4$
- Chemical Structure:



Uses

Methyldopa is used in the management of moderate to severe hypertension and in the management of hypertension in pregnant women.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to methyldopa. Methyldopa is available in an oral dosage form (alone or in combination with a thiazide diuretic, hydrochlorothiazide) and a parenteral dosage form (methyldopate hydrochloride).

Toxicokinetics

Approximately 50% of an oral dose is absorbed, individual variations occur. The peak effect occurs within 4–6 h; pharmacologic effects do not correlate with plasma concentrations. Metabolism is mainly by conjugation in the gastrointestinal tract and the liver. In patients with renal insufficiency, the rate of conjugation is decreased. Oral administration results in more sulfate conjugation than when methyldopa is given via intravenous administration. The sulfate conjugate may be active therapeutically. Urinary metabolites include: α-methyldopa-mono-*O*-sulfate; 3-*O*-methyl-α-methyldopa; 3,4-dihydroxyphenylacetone; α-methyldopamine; 3-*O*-methyl-α-methyldopamine, and their conjugates. Seventy percent of an absorbed dose is excreted in urine as methyldopa and the mono-*O*-sulfate conjugate. The volume of distribution is $0.371\,\mathrm{kg^{-1}}$. Less than 15% is protein bound. Methyldopa crosses the placenta and is excreted into breast milk. Dialysis and peritoneal dialysis will remove methyldopa. Elimination is biphasic. The half-life during the first phase is 1.8 h. The half-life during the second phase is longer.

Mechanism of Toxicity

In the brain, methyldopa is enzymatically decarboxylated to α-methyldopamine, which undergoes subsequent enzymatic conversion, via hydroxylation, to α-methylnorepinephrine, an α-adrenergic agonist. Stimulation of central inhibitory α-adrenergic receptors causes a decrease in sympathetic outflow manifested as a decrease in blood pressure. A decrease in plasma renin activity may also play a role in methyldopa's hypotensive effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Specific information on the effects of methyldopa toxicity in domesticated animals is lacking.

Human

A minimum toxic dose is not well defined. Drug levels do not guide treatment. Hypotension, bradycardia, weakness, dizziness, sedation, and coma may occur. Gastrointestinal effects may include nausea, vomiting, and diarrhea. A withdrawal syndrome is not expected.

Chronic Toxicity (or Exposure)

Animal

Similar effects are seen in animals as those seen in humans (e.g., primarily central nervous system depression). Two year feeding studies of rats demonstrated no evidence of carcinogenicity despite doses up to 6300 ppm methyldopa in diet.

Human

Side effects may include drowsiness, headache, dry mouth, nasal congestion, and constipation. If orthostatic hypotension occurs, the dosage should be decreased. Sodium retention requiring concurrent therapy with a diuretic may occur. A positive Coombs' test has been reported in 10–20% of patients taking methyldopa; most commonly 6–12 months after starting therapy. However, not all patients with a positive test develop hemolytic anemia. A drug-induced fever may occur during the first 3 weeks after therapy is initiated. Methyldopa should be discontinued if fever, jaundice, or alterations in liver function tests occur.

In Vitro Toxicity Data

Ames *Salmonella* assays of mutagenicity have been negative.

Clinical Management

Activated charcoal will adsorb methyldopa and should be considered in patients with substantial recent ingestions. Standard supportive therapies, such as support of airway, breathing, and circulation, should be utilized as clinically necessary. Administration of vasopressors may be required for patients experiencing profound cardiovascular effects. Hemodialysis is of theoretical value if standard therapies fail.

Further Reading

Johnston GD and Smith AMJ (1990) Management of overdose due to antihypertensive agents. *Adverse Drug Reaction and Acute Poisoning Review* 9: 75–89.

Shnaps Y, Almog S, and Halkin H (1982) Methyldopa poisoning. *Journal of Toxicology Clinical Toxicology* 19: 501–503.

Methylene Chloride

Richard A Parent

This article is a revision of the previous print edition article by R A Parent, T R Kline, and D E Sharp, volume 2, pp. 308–310, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 75-09-2
- Synonyms: Dichloromethane; 1,1-Dichloromethane; DCM; Methane, dichloro
- Chemical/Pharmaceutical/Other Class: Chlorinated aliphatic hydrocarbon
- Chemical Formula: CH_2Cl_2

Uses

Methylene chloride has been used as a solvent in paint stripping formulations; as a propellant in aerosols; as a process solvent in the manufacture of drugs, pharmaceuticals, and film coatings; as a metal

cleaning and finishing solvent in electronics manufacturing; and as an agent in urethane foam blowing. Aerosol products in which methylene chloride may be found include paints, automotive products, and insect sprays. Methylene chloride has also been used as an extractant solvent for spice oleoresins, hops, and for the removal of caffeine from coffee.

Exposure Routes and Pathways

Methylene chloride is a widely used industrial chemical with reported atmospheric emissions of more than 126 million pounds annually in the United States. The principal route of exposure for the general population to methylene chloride is by inhalation. Occupational and consumer exposure to methylene chloride commonly occurs from spray painting and contact with consumer products such as paint strippers or aerosol cans, that contain methylene chloride. Exposures may occur as a result of breathing the vapors given off by the product or from direct dermal contact. Occupational exposure to methylene chloride by the inhalation route offers the most opportunity for exposure but it can also be absorbed through the skin.

Toxicokinetics

The principal route of human exposure to methylene chloride is inhalation, but dermal exposure has been noted albeit at a slower rate and oral ingestion has also been reported. During absorption through the lungs, the concentration of methylene chloride in alveolar air, in equilibrium with pulmonary venous blood content, approaches the concentration in inspired air until a steady state is achieved. After tissue and total body steady state is achieved through the lungs and other routes, uptake is balanced by metabolism and elimination. Elimination is mostly via the lungs in the exhaled air. Steady-state blood methylene chloride concentrations appear to be reached after 2–4 h of exposure. Evaluation of pulmonary uptake in humans indicated that 70–75% of inhaled methylene chloride vapor was absorbed. Uptake also increases with the percentage body fat since methylene chloride dissolves in fat to a greater extent than it dissolves in aqueous media. Once exposure ceased, methylene chloride was rapidly cleared from the blood. In animals, limited available data suggest that methylene chloride is easily absorbed from the gastrointestinal tract.

Methylene chloride is removed from the body primarily in expired air and urine. Methylene chloride excretion in the expired air was most evident in the first 30 min after exposure. In rats, methylene chloride was excreted in the expired air, urine, and feces following a single 6 h exposure to methylene chloride. Exhaled air accounted for 58–79% of the dose.

Mechanism of Toxicity

Available data suggest that there are two pathways by which methylene chloride is metabolized. One utilizes the mixed function oxidase enzymes and produces carbon monoxide, while the other pathway involves glutathione transferase and produces carbon dioxide. The mixed function oxidase pathway seems to be the preferred pathway for methylene chloride metabolism following inhalation exposures. In addition to carbon monoxide and carbon dioxide, methylene chloride is also metabolized to a lesser extent to formaldehyde and formic acid.

Human subjects exposed by inhalation to 500 ppm or greater for 1 or 2 h experienced elevated carboxyhemoglobin concentrations indicating that methylene chloride was metabolized to carbon monoxide by the mixed function oxidase pathway. Metabolism of methylene chloride in animals is similar to that in humans. Animal data on metabolism indicate that the process is similar for both inhalation and oral exposures.

When methylene chloride is absorbed through the lungs, it is thought that it will dissolve in the lipoprotein components of the blood and be distributed from the systemic circulation to the body organs.

Distribution studies in rats demonstrate that methylene chloride and its metabolites are present in the liver, kidney, brain, lungs, muscle, and adipose tissue after inhalation exposures. One hour after exposure, the highest concentration of radioactively labeled material was found in the adipose tissue followed by the liver. The concentrations in the kidney, adrenals, and brain were less than half of that in the liver. The affinity of methylene chloride for nucleophilic groups such as mercaptans present in proteins and DNA itself is thought to be a key factor in its mechanism of action.

Acute and Short-Term Toxicity (or Exposure)

Animal

Studies in animals confirm that methylene chloride may be lethal after inhalation exposure at high concentrations. Acute exposure to 16 000–19 000 ppm methylene chloride for 4–8 h caused death in rats and mice. Data suggest there is a narrow margin between concentrations causing anesthesia and those causing death. Repeated exposure in longer-term studies at levels from 1000 to 16 000 ppm has been reported to

cause increased deaths in rats, mice, guinea pigs, rabbits, dogs, and primates. Exposure to methylene chloride has reportedly resulted in fatty changes in the liver and elevated plasma enzymes. These effects were reversible after exposure ceased. A 28 day study in rats also showed elevated hepatic microsomal enzyme activities at 250 ppm in air. Nonspecific tubular degenerative and regenerative changes were observed after continuous exposure in rats at 25 and 100 ppm for 100 days, while others report splenic fibrosis and decreased cerebellar enzyme levels in rats and splenic atrophy in dogs. Methylene chloride has also been shown to cross the placental barrier but has not been shown to be teratogenic in rats and mice.

Human

Methylene chloride is a skin irritant on prolonged contact and eye irritant at high airborne concentrations and as a liquid. Case studies of methylene chloride poisoning during paint stripping operations have demonstrated that inhalation exposure can be fatal in humans. Quantitative estimates of exposure levels were not reported; however, methylene chloride was detected at autopsy in various tissues, including the liver ($14.4\,mg\,dl^{-1}$), blood ($50\,mg\,dl^{-1}$), serum ($29\,\mu g\,ml^{-1}$), and brain (24.8 mg per 100 g). The cause of death in these cases was uncertain; however, myocardial infarction was reported in one case.

Acute and prolonged exposures to methylene chloride have been reported to result in a number of signs and symptoms including headache, lightheadedness, nausea, vomiting, eye irritation, pulmonary irritation and cough, paresthesias, somnolence, altered sleep patterns, changing cardiac patterns, syncope, memory loss, intellectual impairment, cardiac sensitization, and gastrointestinal ulceration and bleeding, the latter resulting only from oral exposure. Less common symptoms include delerium, auditory and visual hallucinations, spontaneous abortions, hepatic effects, and renal effects including acute tubular necrosis. At very high levels of exposure, euphoria, central nervous system (CNS) depression with associated respiratory failure, seizures, and death have been reported. Single exposures to methylene chloride at 300 ppm caused decreased visual and auditory functions. These effects were reversible once exposure ceased. Similarly, psychomotor performance was impaired, but this occurred at higher exposure levels (800 ppm for 4 h). Alterations in visual evoked response have been observed in humans exposed to methylene chloride at 515–986 ppm for 1 or 2 h. Reversible CNS depression and carboxyhemoglobin formation are considered the major human toxicological effects of exposure to methylene chloride.

Chronic Toxicity (or Exposure)

Animal

Chronic oncogenicity studies in animal models have led to methylene chloride being classified as an animal carcinogen. In treated mice, hepatocellular carcinomas and broncho/alveolar neoplasm have been reported to be significantly elevated over controls, while salivary gland sarcomas have been noted in male rats and increased incidences of leukemia and mammary adenomas found in female rats. Other lesions found in oncogenicity studies include mesotheliomas at multiple sites, mononuclear cell leukemias and hemangiomas.

Human

Long-term low-level exposures have been known to result in neurophysiological and neurobehavioral disturbances.

Several epidemiological studies have detected no excess risk of death from malignant neoplasms in workers exposed to methylene chloride, while at least one study suggests an increased risk of pancreatic cancer.

Methylene chloride is considered to be a probable human carcinogen based on animal carcinogenicity studies and genotoxicity studies. Human studies are deemed inadequate in terms of establishing the human carcinogenic properties of methylene chloride.

In Vitro Toxicity Data

Methylene chloride has been shown to be genotoxic in some short-term assays including *Salmonella typhimurium* and *S. crevisiae*, but in other tests equivocal or negative results were reported. Methylene chloride has been reported to induce gene conversion, mitotic recombination, and gene mutations in *Saccharomyces cerevesia* D7 when cells were grown under conditions that lead to production of endogenous cytochrome P450 but did not induce DNA damage in a DNA repair assay in isolated rat hepatocytes. Other genotoxicity assays produced mixed findings. Attempted cell transformation using the BALB/3T3 mouse cell line was unsuccessful as was an attempt to cause chromosomal aberrations in the bone marrow cells of Sprague–Dawley rats.

Clinical Management

After inhalation exposures, the patient should be moved out of the exposure area and administered 100% oxygen. The carboxyhemoglobin level may be monitored as an indicator of state of intoxication.

Should seizures occur, intravenous benzodiazepam may be considered and if they continue, phenobarbital may be appropriate. For oral exposure, emesis is not recommended because of the potential for seizures and CNS depression. Gastric aspiration may be accomplished using a flexible nasogastric tube when ingestion is considerable.

Environmental Fate

When released into the environment as a vapor, methylene chloride does not usually undergo direct photolysis but does degrade by reaction with photochemically produced hydroxyl radicals. Its half-life in the atmosphere is estimated at 119 days. In soil and water, a major fate pathway involves volatilization to the atmosphere and subsequent degradation. Activated sludge studies have, however, demonstrated biodegradation of methylene chloride. Little bioconcentration in aquatic species can be expected.

Exposure Standards and Guidelines

- US Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for an 8 h period is 25 ppm time-weighted average (TWA).
- US OSHA short-term exposure limit (STEL) for 15 min sampling is 125 ppm.
- American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for 8 h TWA is 50 ppm.
- ACGIH TLV – TWA for 30 min exposure during workday is 250 ppm.
- US National Institute of Safety and Health recommends that methylene chloride be handled as an occupational carcinogen.
- US Environmental Protection Agency classifies methylene chloride as a B2 carcinogen, a probable human carcinogen based on inadequate human data but sufficient evidence for carcinogenicity in animals.
- ACGIH classifies methylene chloride as an A3 carcinogen, a confirmed animal carcinogen with unknown relevance to man.
- International Agency for Research on Cancer has classified methylene chloride as a group 2B carcinogen, possibly carcinogenic to humans based on sufficient evidence in experimental animals.
- Methylene chloride has been designated as a hazardous air pollutant under Section 112 of the Clean Air Act and is subject to effluent limitations since it has been designated as a toxic pollutant pursuant to Section 307(a) (1) of the Federal Water Pollution Control Act.
- The maximum contaminant level promulgated in the National Revised Primary Drinking Water Regulations for methylene chloride in community and nontransient noncommunity water systems is $0.005\,\mathrm{mg\,l^{-1}}$.
- EPA's Federal Drinking Water Standard for methylene chloride is $5\,\mu\mathrm{g\,l^{-1}}$.

Miscellaneous

Methylene chloride is a clear volatile colorless liquid having a chloroform-like odor that is detectable at concentrations from 540 to $2160\,\mathrm{mg\,m^{-3}}$ in air. At atmospheric pressure it boils at $\sim 40^{\circ}\mathrm{C}$ and is soluble in organophilic solvents and oils. Its vapor has a density of 2.93 in air and a vapor pressure of 435 mmHg at 25°C. One part per million in air is equal to $2.48\,\mathrm{mg\,m^{-3}}$. Combustion of methylene chloride may result in the production of highly toxic gases including hydrogen chloride, phosgene, and carbon monoxide. Smoking in a methylene-contaminated atmosphere can result in toxic inhalation of these potentially lethal pyrolysis products.

See also: Aerosols; Caffeine; Pollution, Air.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Methylene Chloride.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Methylene Chloride.

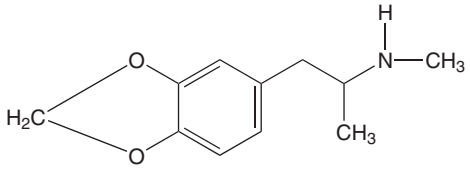
http://www.epa.gov – Methylene chloride (dichloromethane), US EPA Technology Transfer Network Air Toxic Website.

Methylenedioxymethamphetamine

Alexander B Baer and Christopher P Holstege

This article is a revision of the previous print edition article by Janet E Bauman, volume 2, pp. 311–312, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 69610-10-2
- Synonyms: 3,4-Methylenedioxymethamphetamine; Adam; Bean Doctor; E; Ecstasy; Essence; MDM; MDMA; M & Ms; Roll; The Substance; X; XTC
- Chemical/Pharmaceutical/Other Class: Synthetic phenylalkylamine derivative of amphetamine
- Chemical Structure:



Uses

Formerly a psychotherapeutic agent, methylenedioxymethamphetamine (MDMA) is now abused as a hallucinogenic amphetamine.

Exposure Routes and Pathways

MDMA is available illicitly in tablet, capsule, and powder forms. It is most commonly ingested, but insufflation and intravenous injection has also been reported.

Toxicokinetics

MDMA is rapidly absorbed with the onset of effects occurring in 20–60 min. Concentrations peak at 2–3 h and typically last 4–6 h. Prolonged effects lasting up to 48 h may be seen following large doses. MDMA is metabolized in the liver by cytochrome P450, chiefly CYP2D6, to form methylenedioxyamphetamine (MDA). The volume of distribution is considered large ($>5\,l\,kg^{-1}$). MDMA and its metabolites are excreted renally, with 75% as unchanged MDMA and 7% as MDA. Elimination is usually complete within 24 h.

Mechanism of Toxicity

MDMA induces norepinephrine release from presynaptic vesicles. MDMA also effects serotonin neurotransmission by causing release of serotonin (5-hydroxytryptamine (5-HT)) and inhibiting its uptake. In animal models, it has been demonstrated to cause long-term destruction of 5-HT axons. Studies demonstrate lowered concentrations of the 5-HT metabolite 5-hydroxyindoleacetic acid in the cerebrospinal fluid of regular MDMA users. This correlates with a similar decrease reported in primates with brain damage induced by MDMA.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, a toxic dose is estimated to be $10–30\,mg\,kg^{-1}$. Reported effects in small animals include hyperthermia, rapid respirations, rapid heartbeat, dilated pupils, lactic acidosis, hypertension, arrhythmias, vomiting, and diarrhea. Renal failure, seizures, and coma are possible.

Human

Symptoms noted in acute toxicity include anxiety, mydriasis, hypertension, tachycardia, tachypnea, hallucinations, bruxism, and diaphoresis. Hyperthermia, arrhythmias, hyperreflexia, seizures, metabolic acidosis, ischemia, rhabdomyolysis, and renal failure may be seen in severe toxicity. Hyponatremia, hyperkalemia, coagulopathies, pulmonary edema, and adult respiratory distress syndrome have also been reported. MDMA may cause liver injury. While the vast majority of these cases have spontaneous recovery, an increasing number of fulminate hepatic failure reports are now appearing in the literature. Hyponatremia is a recognized complication that is thought to have several contributing factors including sodium loss through excessive sweating, hemodilution with large free water volume intake, and inappropriate secretion of antidiuretic hormone leading to water retention. Sudden death is likely due to cardiac arrhythmias, seizures, and central nervous system depression. Blood levels do not correlate with toxicity but can confirm exposure.

Chronic Toxicity (or Exposure)

Animal

Several models of MDMA exposure in animals have described long-term adverse effects on emotion. These effects have responded to treatment with serotonergic agents such as fluoxetine.

Human

Positron Emission Tomography (PET) scans of former abusers of MDMA have revealed a decrease

in the brain serotoninergic neurons. These changes are of unknown consequences but may include depression, anxiety, and memory impairment. Chronic paranoid psychosis, depression, flashbacks, panic disorders, and some impairment of cognitive function have been related to long-term use.

In Vitro Toxicity Data

Several studies have demonstrated that MDMA can suppress neutrophil phagocytosis as well as suppress the production of tumor necrosis factor-alpha and interleukin.

Clinical Management

There is no antidote for MDMA poisoning. General supportive care is the mainstay of therapy. Activated charcoal may be used to adsorb the MDMA within an hour of ingestion. Benzodiazepines may be a useful adjunct for the immediate management of an acutely agitated or psychotic patient that poses an immediate threat to healthcare staff or self.

Hypertensive emergencies with end-organ ischemia may be treated with antihypertensive agents such as nitroprusside. Beta-blocking agents should be used with caution due to concern of causing unopposed alpha agonism leading to worsening end-organ ischemia. Phentolamine may be useful in cases of hypertensive emergencies or end-organ ischemia refractory to nitrate infusions.

Hypothermic blankets, ice water baths, chilled intravenous fluids, gastric and bladder lavage with cooled fluids may be needed to reduce body temperature. Dantrolene has also been utilized for the treatment of MDMA-related hyperthermia. Measuring creatinine phosphokinase and urine myoglobin levels can be helpful in recognizing those at risk of developing acute renal failure due to rhabdomyolysis. Ensuring adequate urine output with intravenous fluids is the mainstay of treatment for preventing acute tubular necrosis.

See also: Drugs of Abuse.

Further Reading

Dowling GP, McDonough ET, and Bost RO (1987) 'Eve' and 'Ecstasy'. A report of five deaths associated with the use of MDEA and MDMA. *Journal of the American Medical Association* 257: 1615–1617.

Henry JA, Jeffreys KJ, and Dawling S (1992) Toxicity and deaths from 3,4-methylenedioxymethamphetamine ('ecstasy'). *Lancet* 340: 384–387.

McCann UD, Slate SO, and Ricaurte GA (1996) Adverse reactions with 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy'). *Drug Safety* 15: 107–115.

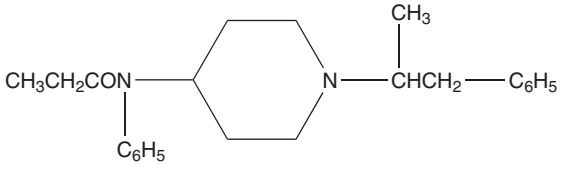
Milroy CM (1999) Ten years of 'ecstasy'. *Journal of the Royal Society of Medicine* 92: 68–72.

Shannon M (2000) Methylenedioxymethamphetamine (MDMA, "Ecstasy"). *Pediatric Emergency Care* 16: 377–380.

Methylfentanyl, α-

Abraham Dalu

- Chemical Abstracts Service Registry Number: CAS 79704-88-4
- Synonyms: *N*-[1-(1-Methyl-2-phenylethyl)-4-piperidinyl]-*N*-phenylpropanamide; China white
- Chemical/Pharmaceutical/Other Class: α-Methylfentanyl is a narcotic analgesic, a designer drug derived from fentanyl
- Chemical Formula: $C_{23}H_{30}N_2O$
- Chemical Structure:



Uses

α-Methylfentanyl is not medically used per se, although it is a derivative of fentanyl with higher analgesic effects. α-Methylfentanyl is a designer drug that has been synthesized for its analgesic and euphoric effects. Due to its high potency (1000–2000 times more potent than heroin) and fast-acting narcotic analgesia, it has high abuse potential and is sold on the street as synthetic heroin. α-Methylfentanyl also has a high abuse potential in racing horses for its analgesic and stimulant actions. Therefore, α-methylfentanyl is a controlled substance listed in the US *Code of Federal Regulations*, Title 21, Part 1308.11 (1987).

Exposure Routes and Pathways

Most common exposure pathways to α-methylfentanyl are via intramuscular or intravenous injection.

Toxicokinetics

Limited information indicates that α-methylfentanyl is rapidly absorbed and distributed to the central nervous system (CNS). Elimination of this drug is primarily via the kidneys.

Mechanism of Toxicity

α-Methylfentanyl is believed to exert its toxic effects by binding to opiate receptors (μ-agonist) at many sites in the CNS.

Acute and Short-Term Toxicity (or Exposure)

Animal

In experimental horses, α-methylfentanyl induces locomotive responses (as quantified by counting the number of footsteps taken per unit of time), indicating that it is a morphine-like narcotic agonist in horses. The maximum effect can be seen $\sim$10 min after treatment of horses with greater than 4 $\mu g\,kg^{-1}$ body weight.

Human

α-Methylfentanyl is fast acting. At high doses it causes euphoria, marked muscular rigidity, and respiratory depression. The literature indicates that α-methylfentanyl overdose deaths are primarily due to respiratory paralysis. In addition to its high potency and fast action, further danger is due to its poor ability to mix with the cutting agents used with illicit drugs.

Chronic Toxicity (or Exposure)

Information on chronic toxicity in animal and human is not available.

Clinical Management

Since α-methylfentanyl exerts its toxicity as a μ-agonist, its toxicity can be managed with narcotic μ-antagonists such as naloxone and respiratory support with resuscitative equipment.

See also: Fentanyl; Fentanyl Derivatives, Illicit.

Methylmercury

Shayne C Gad and Kevin N Bayer

- Chemical Abstracts Service Registry Number: CAS 22967-92-6
- Synonyms: Alkyl, alkoxyalkyl mercury compounds
- Chemical/Pharmaceutical/Other Class: Organometals
- Chemical Formula: The term 'methylmercury' generally refers to monomethylmercury, but several organic forms can exist. CH_3Hg^+ (methylmercury cation) is usually associated with simple anions, that is, Cl^-. $(CH_3)_2Hg$ is dimethylmercury

Uses

Organomercury compounds, such as aryl and alkoxy-aryl, have been used in medicine, agriculture, and laboratory research. Their use in fungicides has been greatly reduced or eliminated.

Exposure Routes and Pathways

The major route of general population exposure to methylmercury is through the consumption of contaminated fish and fish products. This occurs as a result of inorganic mercury from natural or man-made sources being methylated by microorganisms in aquatic sediments. Methylmercury is then biomagnified in the food chain with relatively high concentrations accumulating in the edible tissues of fish. A minor route of exposure is through inhalation of vaporized methylmercury and organomercurials from the atmosphere or industrial workplace.

Toxicokinetics

Methylmercury from dietary and inhalation exposures is almost completely absorbed ($\sim$90%) into the bloodstream. Methylmercury may be converted to inorganic mercury in both experimental animals and humans by intestinal flora and macrophage cells. Glutathione and sulfhydryl peptide complexes have been observed in the bile. Methylmercury is rapidly distributed to all tissues, with high concentrations accumulating in the brain, the target organ of toxicity. Methylmercury moves readily across the placenta, and higher concentrations are found in cord blood compared to maternal blood. The fecal pathway is responsible for $\sim$90% of the total elimination of mercury following methylmercury exposure. The

majority of methylmercury resulting from biliary secretion is demethylated by intestinal flora and eliminated in the feces as inorganic mercury. The remaining methylmercury can enter the enterohepatic circulation, while a small percentage of inorganic mercury is absorbed and distributed to the tissues. The half-life of methylmercury in fish-eating humans is estimated to be between 39 and 70 days.

Mechanism of Toxicity

All mercury compounds exhibit high affinity for sulfhydryl groups in proteins. As a result, a variety of enzymes and structural proteins containing free sulfhydryl groups can be modified and their functions affected. Inhibition of protein synthesis is an early biochemical event following exposure. The integrity of the blood–brain barrier can be disrupted by methylmercury, which results in the alteration of amino acid uptake and subsequent brain metabolism. Methylmercury can alter cell division during critical stages of central nervous system (CNS) development, at least in part through inhibition of microtubule function. However, there is uncertainty whether methylmercury or the mercuric ion following cleavage from methylmercury is the ultimate toxicant.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for methylmercury in rats is $58\,mg\,kg^{-1}$. Methylmercury causes mutations in rodents at $20–40\,mg\,l^{-1}$.

Human

Although methylmercury is generally recognized as a cumulative poison, acute effects, such as headache, gastrointestinal irritation (nausea, vomiting, abdominal pain, and diarrhea), and paresthesia of the extremities, have been reported. Severe neurologic toxicity, described below, may occur several weeks or months following exposure. Renal failure normally associated with inorganic mercury poisoning is seldom observed with methylmercury. At high concentrations, methylmercury is corrosive to the skin and eye.

Dimethylmercury is more toxic than the monomethyl form. In a well publicized case, it was associated with the fatality of a research chemist. The researcher was exposed by dermal absorption after spilling a small amount of the compound on her latex gloves. Dimethylmercury was found to penetrate disposable latex and polyvinyl chloride gloves in 15 s or less. Where possible, the use of inorganic mercury salts is recommended as a substitute in laboratory research. These compounds are less volatile and lipid soluble than dimethylmercury and scientists face a lesser risk of exposure to mercury.

Chronic Toxicity (or Exposure)

Animal

Methylmercury causes neurotoxicological effects in cats and dogs. Cats ingesting contaminated fish around Minamata Bay, Japan, died after paroxysmal fits (i.e., 'cat-dancing disease'). Mink are particularly sensitive to the toxicity of methylmercury. Methylmercury is also fetotoxic and teratogenic in laboratory mammals.

Human

Poisoning episodes in humans have occurred as a result of environmental contamination of fish due to industrial discharges (Minamata and Niigata, Japan) and through seed grains contaminated with a methylmercury fungicide (Iraq). In these episodes, most of the signs and symptoms of methylmercury poisoning were attributed to damage to the CNS; effects on nonnervous tissue were absent or negligible.

Characteristics of methylmercury poisoning in adults include a long latent period (several months) and a continuation of early nonspecific symptoms such as paresthesia, blurred vision, and malaise. A 5% increase in the incidence of paresthesia has been linked to a daily methylmercury intake of $3–7\,\mu g\,kg^{-1}$ body weight. Daily intakes of $0.4\,\mu g\,kg^{-1}$ body weight will not result in any detectable adverse effects. After time, additional signs may appear including concentric constriction of the visual field, deafness, speech difficulties, and ataxia (known as the Hunter Russell syndrome). Severely exposed patients may lapse into a coma and ultimately die, although there is no clear pattern of mercury-related deaths. Many effects in severe cases are irreversible due to destruction of neuronal cells. In less severe cases, some degree of recovery in each symptom may occur depending on the compensatory function of the CNS. At high doses, methylmercury also causes neuromuscular weakness from effects on the peripheral nervous system. The developing CNS is more sensitive to damage than the adult CNS. Some infants who have been exposed to high maternal blood levels of methylmercury were born with cerebral palsy. The main pattern of severe toxic effects includes microcephaly, hyperreflexia, and gross motor and mental impairment, sometimes associated with blindness and deafness. Milder degrees of the affliction show mainly as psychomotor impairment and persistence of pathological reflexes.

In Vitro Toxicity Data

In vitro exposure of primary cultures of rat cerebellar granule cells to methylmercury resulted in a time- and concentration-dependent cell death. Some *in vitro* models have shown that organic mercury may interfere with the bacteriocidal capacity of polymorphonuclear leukocytes.

Clinical Management

There is no known useful treatment for methylmercury poisoning. A variety of chelating agents, such as D-penicillamine, 1-acetyl-D,L-penicillamine, thiol resins, activated charcoal, BAL (British Antilewisite; 2,3-dimercaptopropanol), and meso-2,3-dimercaptosuccinic acid, have been used to treat methylmercury exposure but with limited to no success.

Thus, the best approach is prevention. Since young children appear to be the most sensitive to methylmercury toxicity, children under 7 years and pregnant or breast-feeding women should limit their consumption of fish that are known to have high levels of methylmercury in their edible tissues.

Environmental Fate

Inorganic mercury introduced as a pollutant into natural waters is scavenged by particulate matter and deposited into bottom sediments. Free Hg^{2+} is gradually released from this pool of slightly soluble inorganic mercury and is then transformed by microbial activity into methylmercury. Methylmercury diffuses into the water column and is taken up by fish and other organisms (either directly through water or through the food chain), and accumulated in their tissue. The degree to which mercury is transformed into methylmercury and transferred up the food chain through bioaccumulation depends on a variety of factors, including water chemistry and the complexity of the food web.

Exposure Standards and Guidelines

The US Environmental Protection Agency has set a criterion of 0.3 mg methylmercury per kg in fish tissue that should not be exceeded to protect the health of consumers of noncommercial freshwater/estuarine fish.

The joint expert committee for food additives and contaminants revised the Provisional Tolerable Weekly Intake for methylmercury, recommending that it be reduced to $1.6\,\mu g\,kg^{-1}$ body weight per week in order to sufficiently protect the developing fetus.

See also: Levothyroxine; Mercury; Metals; Neurotoxicity.

Further Reading

Castoldi AF, Coccini T, and Manzo L (2003) Neurotoxic and molecular effects of methylmercury in humans. *Reviews on Environmental Health* 18(1): 19–31.

Dourson ML, Wullenweber AE, and Poirier KA (2001) Uncertainties in the reference dose for methylmercury. *Neurotoxicology* 22(5): 677–689.

Tsubaki T and Irukayama K (1977) *Minamata Disease*. New York: Elsevier.

World Health Organization (WHO) (1989) *Environmental Health Criteria 86: Mercury-Environmental Aspects*. Geneva: WHO.

World Health Organization (WHO) (1990) *Environmental Health Criteria 101: Methylmercury*. Geneva: WHO.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Methylmercury.

http://www.epa.gov – Methylmercury Criteria Document (from the US Environmental Protection Agency).

Methylnitrosourea

Robin C Guy

- Representative Chemicals: Some chemotherapeutic agents
- Chemical Abstracts Service Registry Number: CAS 684-93-5
- Synonyms: *N*-nitroso-*N*-methylurea; 1-Methyl-1-nitrosourea; MNU; *N*-Nitroso-*N*-methylcarbamide; Nitrosomethylurea; *N*-Methyl-*N*-nitrosourea
- Chemical/Pharmaceutical/Other Class: Alkylating agent; DNA alkylating agent; Chemotherapeutic agent

Uses

N-Nitroso-*N*-methylurea was once widely used to synthesize diazomethane in the laboratory; however, this use was replaced by other reagents. It has been studied as a chemotherapeutic agent in cancer treatment, either alone or in combination with

cyclophosphamide. Small quantities are used in research to study its mutagenic effects on plants.

Exposure Routes and Pathways

The potential for human exposure is limited because *N*-nitroso-*N*-methylurea is not produced or used in large quantities in the United States.

Occupational exposure may occur through oral, inhalation, or dermal contact at facilities where this chemical is used in research. In air, it exists solely as vapor where it is degraded (estimated half-life of 10 days) by reaction with photochemically produced hydroxyl radicals. It hydrolyzes in water (half-life of 1.2 h at pH 7 at 20°C). A limited number of research laboratory workers may also be possibly exposed; several accidents have been reported in which laboratory personnel were exposed when the compound exploded at room temperature.

The potential for direct exposure exists when injecting cancer patients with *N*-nitroso-*N*-methylurea in conjunction with cyclophosphamide, as a chemotherapeutic agent. Health professionals such as pharmacists, physicians, and nurses are potentially exposed to the compound during the preparation and administration of the pharmaceuticals or during clean-up.

Toxicokinetics

Methylnitrosourea is rapidly absorbed from the gastrointestinal tract. To form *N*-nitroso compounds *in vivo*, there may be a reaction of nitrite with secondary amines or amides in food or water. Whole body autoradiography showed that 2 min after an intravenous dose of [^{14}C]*N*-methyl-*N*-nitrosourea to rat, ^{14}C was fairly evenly distributed in most tissues. According to the World Health Organization, the biological half-life of *N*-nitroso compounds appears to be less than 24 h.

Mechanism of Toxicity

Methylnitrosourea is a DNA alkylating agent. Methylnitrosourea causes dermal sensitization. It is also an inhibitor of protein and nucleic acid synthesis in tissues.

Acute and Short-Term Toxicity (or Exposure)

Animal

As methylnitrosourea is a DNA alkylating agent, the major toxic effects result from severe damage to hematopoietic, lymphoid, and other tissues that have rapid rates of cell turnover. ICR male mice were given intraperitoneal injections at 5, 15, or 25 mg kg^{-1} day^{-1} for 5 days, then were mated on days 1–7, 8–14, 15–21, or 64–80 after the last dose and their progeny were observed on day 18 of pregnancy; there was an increased postimplantation loss, in addition to cleft palate, and fused ribs.

N-Nitroso-*N*-methylurea is carcinogenic in all animal species tested: mice, rats, Syrian golden, Chinese, and European hamsters, guinea pigs, rabbits, gerbils, pigs, dogs, and monkeys. It induces benign and malignant tumors following its administration by different routes, including ingestion. It produces tumors at different sites, including the nervous tissue, stomach, esophagus, pancreas, respiratory tract, intestine, lymphoreticular tissues, skin, and kidney. It is carcinogenic following its administration prenatally and in single doses.

It was positive for gene mutations (HGPRT and TK genes) as well as for chromosomal aberrations, sister chromatid exchange, and DNA repair-deficient bacterial assays.

Human

Nausea and vomiting were seen after an intravenous injection of 4 mg kg^{-1} to patients.

Chronic Toxicity (or Exposure)

Human

Methylnitrosourea causes dermatitis; however, it appears to be due to sensitization rather than primary irritation. Although carcinogenicity data are not available for humans, *N*-Nitroso-*N*-methylurea is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals.

Clinical Management

Affected persons should be decontaminated with caution because methylnitrosourea is probably carcinogenic to humans. Symptoms should be treated as they appear.

Ecotoxicology

Fish, mollusks, and phytoplankton are all adversely affected by methylnitrosourea.

See also: Chromosome Aberrations; Sensitivity Analysis; Sister Chromatid Exchanges; Skin.

Further Reading

International Agency for Research on Cancer (IARC) (1978) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some *N*-Nitroso Compounds. Vol. 17. 365 pp. Lyon, France.

International Agency for Research on Cancer (IARC) (1987) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity. Supplement 7. 440 pp. Lyon, France.

WHO Environmental Health Criteria 5: Nitrates and Nitroso Compounds, 1978.

Relevant Websites

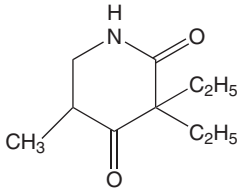
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Methylnitrosourea.

http://www.inchem.org – International Agency for Research on Cancer (IARC) – Summaries and Evaluations, *N*-nitroso-*N*-methylurea.

Methyprylon

S Rutherfoord Rose

- Chemical Abstracts Service Registry Number: CAS 125-64-4
- Synonyms: Methypryl; Methyprylone; 2,4-Dioxo-3,3-diethyl-5-methyl piperidine; 3,3-Diethyl-5-methyl-2,4-piperidinedine; 3,3-Diethyl-5-methyl-2,4-piperidinedione; Noludar; Noctan; Methyl-2,4-peperidine; 3,3-Diethyl-5-methyl-2,4-piperidinedione; Noludar; Noctan; Dimerin
- Chemical/Pharmaceutical/Other Class: Piperidinedione derivative
- Chemical Formula: $C_{10}H_{17}NO_2$
- Chemical Structure:



Uses

Methyprylon is a sedative–hypnotic agent that has been used in the treatment of anxiety, nervousness, and sleep disorders (insomnia). The drug was withdrawn from the US market in 1988. It was a commonly abused sedative–hypnotic agent.

Exposure Routes and Pathways

Methyprylon is only available in solid oral dosage forms. Poisoning occurs following ingestion.

Toxicokinetics

Following therapeutic doses, methyprylon is rapidly absorbed, with peak plasma levels occurring within 1 or 2 h. Greater than 95% of a dose undergoes oxidation and dehydrogenation in the liver; the remainder is excreted unchanged in the urine. At least two of four metabolites may have central nervous system (CNS) activity. The volume of distribution with therapeutic dosing is $\sim 1\,l\,kg^{-1}$. The extent of methyprylon binding to plasma proteins is unknown. Therapeutic plasma levels occur at $\sim 10\,mg\,l^{-1}$, with levels above $30\,mg\,l^{-1}$ considered toxic. The serum elimination half-life is ~ 3–5 h following therapeutic doses. The toxicokinetics of methyprylon are poorly understood. Both rates of absorption and elimination are prolonged following overdose. Pharmacokinetics appears to be dose dependent, and elimination becomes nonlinear at high doses.

Mechanism of Toxicity

Methyprylon produces depression of the CNS and decreases rapid eye movement sleep in a fashion similar to the barbiturates. Overdoses may also result in cardiovascular depression.

Acute and Short-Term Toxicity (or Exposure)

Human

Overdose results in dose-dependent CNS depression, ranging from mild lethargy to coma. Slurred speech, ataxia, nystagmus, headache, and gastrointestinal upset may occur. Paradoxical CNS stimulation (excitement) has been reported. Severe toxicity (ingestions exceeding 3 or 4 g) may produce hypotension, shock, or pulmonary edema. Death has occurred following ingestion of 6 g, and survival has occurred following ingestion of 30 g.

Chronic Toxicity (or Exposure)

Human

Chronic use may result in addiction, physical dependence, and withdrawal upon abrupt discontinuation of the drug.

Clinical Management

The basis of clinical management is supportive care. The airway should be secured and protected as needed. Symptomatic patients should have intravenous access and cardiac monitoring. Accidental ingestions exceeding 500–800 mg, and all intentional overdoses, should be treated with oral activated charcoal if patients present within 60 min of exposure. Seizures should be treated with benzodiazepines, or phenobarbital if refractory. Hypotension should be treated with intravenous fluids and vasopressors (dopamine or norepinephrine) if needed. Hemodialysis or hemoperfusion may enhance elimination of both the parent compound and metabolites, but the clinical value of extracorporeal drug removal is unknown. There is no known antidote.

Further Reading

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Metronidazole

David Eldridge and Christopher P Holstege

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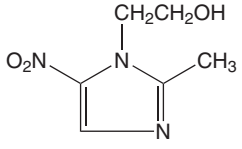
- Chemical Abstracts Service Registry Number: CAS 443-48-1
- Synonym: Flagyl
- Chemical/Pharmaceutical/Other Class: An antibiotic of the nitroimidazole class
- Chemical Structure:



Uses

Metronidazole is used as an antibiotic for certain bacterial and parasitic infections.

Exposure Routes and Pathways

Metronidazole is available as oral, topical, and parenteral preparations.

Toxicokinetics

Bioavailabilty of oral doses of metronidazole approaches 100% and peak serum concentrations occur within 1–2 h postingestion. The volume of distribution is $\sim 0.74\,l\,kg^{-1}$. Total protein binding is less than 20%. It readily crosses the placenta (contraindicated in the first trimester of pregnancy) and is excreted in breast milk at levels that approximate that of the serum concentration in the mother. Metronidazole is metabolized in the liver and undergoes biotransformation through hydroxylation, oxidation of side chains, and glucuronidation. Both unaltered metronidazole and its metabolites are excreted primarily by the kidney, although biliary excretion does occur. Metronidazole pigments may cause a dark brown discoloration of urine. The half-life of metronidazole is typically 8 h. The elimination half-life is unchanged except in patients with severe renal impairment or possibly in those with hepatic insufficiency.

Mechanism of Toxicity

Metronidazole possesses selective bactericidal and antiparasitic activity. Its mechanism of action is complex and not thoroughly understood but is thought to include interference with nucleic acid synthesis. Metronidazole is also capable of producing a disulfiram-type reaction with ethanol ingestion. This reaction is hypothesized to occur due to metronidazole inhibition of aldehyde dehydrogenase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Metronidazole is also used as an antiparasitic in domestic animals. Manifestations of overdose in animals include vomiting, nystagmus, decreased appetite, and ataxia.

Human

There is no documented acute lethal dose in humans. Commonly seen acute effects include nausea, vomiting, and ataxia. When taken concurrently with ethanol, a disulfiram-type reaction can occur with nausea, flushing, hypotension, headache, and shortness of breath.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies of rats and mice have demonstrated increased tumor development in treated animals compared to controls.

Human

With chronic toxicity, nausea, anorexia, headache, vomiting, and metallic taste in the mouth may occur. Chronic use has been associated with extremity numbness and parenthesis. Other neurological effects including insomnia, dizziness, and vertigo have been reported. White blood count suppression has been seen but is generally reversible.

Metronidazole use can lead to problematic drug interactions. It potentates the effects of oral anticoagulants and increases their physiologic effect. Acute psychosis and confusion has been reported in patients simultaneously receiving disulfiram and metronidazole. Its use can lead to elevated lithium and cyclosporine levels.

In Vitro Toxicity Data

Metronidazole produces inhibition of alcohol dehydrogenase *in vitro*.

Clinical Management

Clinical management of acute metronidazole ingestion is supportive. Administration of activated charcoal can be considered for patients with substantial ingestions that present within an hour of the exposure. There are no specific laboratory tests indicated for this isolated ingestion. With chronic metronidazole toxicity or toxicity resulting from interaction with other drugs, discontinuation of metronidazole is recommended. Supportive care is usually sufficient for the vast majority of patients.

See also: Disulfiram.

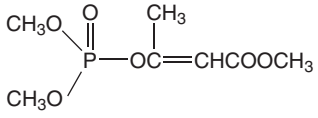
Further Reading

Kusumi RK, Plouffe JF, and Wyatt RH (1980) Central nervous system toxicity associated with metronidazole therapy. *Annals of Internal Medicine* 93: 59–60.

Mevinphos

Priya Raman*

- Chemical Abstracts Service Registry Number: CAS 7786-34-7
- Synonym: *O,O*-Dimethyl-1-carbomethoxy-1-propen-2-yl phosphate
- Chemical/Pharmaceutical/Other Class: Organophosphate pesticide
- Chemical Structure:



Uses

Mevinphos is used as a broad-spectrum insecticide for control of a variety of insects. It is also used as an acaricide that kills or controls mites and ticks. It acts quickly both as a contact as well as a systemic insecticide, being extremely effective at very low dosage rates.

Exposure Routes and Pathways

Oral and dermal routes are the most common routes of accidental and intentional exposure to mevinphos.

Toxicokinetics

Mevinphos is efficiently absorbed from the gut, through the skin, and across the pulmonary membrane. The compound is hydrolyzed in the body to alkyl phosphate. Mevinphos is rapidly degraded by the liver. Consequently, it is more toxic by peripheral exposure routes, such as dermal or intravenous, that bypass the liver.

*Clinical Management section prepared by Carey Pope.

Mechanism of Toxicity

The organophosphorus insecticide, mevinphos, exerts its acute toxicity by directly inhibiting the hydrolytic enzyme, acetylcholinesterase. This causes an increased accumulation of acetylcholine at the synaptic nerve terminals, thereby resulting in excessive stimulation of the cholinergic nerves. Recent studies have demonstrated that mevinphos intoxication may result from nitric oxide produced upon activation of the M2 subtype of muscarinic receptors by the progressive accumulation of acetylcholine.

Acute and Short-Term Toxicity (or Exposure)

Mevinphos is highly toxic through all routes of exposure, including ingestion, dermal absorption, and inhalation. Mevinphos poisoning affects the central nervous system (CNS), the cardiovascular system, the respiratory system, and the eyes.

Animal

Mevinphos has been reported to have an oral LD_{50} of 3–12 mg kg^{-1} in rats and 4–18 mg kg^{-1} in mice. It is highly toxic via the dermal route as well, with dermal LD_{50} value reported to be 4.2 mg kg^{-1} in rats and 40 mg kg^{-1} in mice. The 1 h LC_{50} for mevinphos was reported to be 0.125 mg l^{-1} in rats, indicating high inhalation toxicity. A 1 h exposure of rats to the above concentration of mevinphos resulted in acute pulmonary edema accompanied with changes in the structure or function of salivary glands.

Human

Effects of acute mevinphos exposure are similar to those due to exposure to other organophosphates. However, acute mevinphos toxicity may occur at much lower doses than with other organophosphates. Symptoms of acute exposure include numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, respiratory depression, and slow heartbeat. Very high doses may result in unconsciousness, incontinence and convulsions, or fatality. The greatest occupational hazard of mevinphos is its absorption through the skin, lungs, and mucous membranes. Symptoms of mevinphos poisoning may appear as early as 15 min to 2 h following exposure to mevinphos. However, the onset of symptoms may be delayed for as long as 2 days. Persons with respiratory ailments, recent exposure to cholinesterase inhibitors, impaired cholinesterase production, or with liver malfunction are at increased risk from exposure to mevinphos.

An impairment of judgment in the ability to reason is an early and important symptom of mevinphos poisoning from dermal exposure. When inhaled, the first effects are usually respiratory and may include bloody or runny nose, coughing, chest discomfort, difficulty breathing, or shortness of breath. Following exposure by any route, systemic effects may begin within a few minutes or be delayed for up to 12 h. These may include pallor, nausea, vomiting, diarrhea, abdominal cramps, blurred vision, constriction or dilation of the eye pupils, etc. Severe poisoning will affect the CNS, producing incoordination, slurred speech, loss of reflexes, weakness, fatigue, involuntary muscle contracts, and eventually paralysis of the body extremities and respiratory muscles. Death may be caused by respiratory failure or cardiac arrest.

Chronic Toxicity (or Exposure)

Animal

The oral and dermal LD_{50} of mevinphos in male rats is 6.1 and 4.7 mg kg^{-1}, respectively. Following exposure to 50 ppm mevinphos for 60 days, rats showed reduced growth, slight tremors, and brain cholinesterase that was 20% of normal levels. Other signs and symptoms in rats following exposure to mevinphos include nonspecific degeneration of the liver and kidneys and degeneration of the epithelial cells lining ducts and acini of salivary, lacrimal, and other exocrine glands. Dogs exposed to mevinphos at a dietary level of 0.1 mg kg^{-1} day^{-1} for 14 weeks showed a reduction in both erythrocyte and plasma cholinesterase activity; however, the brain enzyme remained normal. Administration of 20 mg kg^{-1} day^{-1} of mevinphos to rats in their diets for 13 weeks resulted in death of the animals. Dietary doses of 10 mg kg^{-1} day^{-1} for 14 weeks proved to be lethal for dogs. Rats given dietary doses of 10 or 20 mg kg^{-1} day^{-1} for 13 weeks exhibited degeneration of the liver, kidney, and cells lining the salivary, tear, and other glands.

Human

The lowest oral dose of mevinphos responsible for toxic effects (peripheral nervous system effects) in humans was 690 μg kg^{-1} when given intermittently over 28 days. Repeated or prolonged low-level exposure to mevinphos may cause effects similar to those observed with acute exposure. Mevinphos is a compound of high toxicity, not only orally but also dermally. It is a direct inhibitor of

acetylcholinesterase. Signs and symptoms involving overstimulation of the muscarinic receptors include bronchoconstriction, increased bronchial secretion, bradycardia, salivation, lacrimation, diaphoresis, vomiting, diarrhea, and pupillary constriction (miosis). The nicotinic effects following exposure to mevinphos include tachycardia, hypertension, muscle fasciculations, weakness, muscle cramps, and respiratory paralysis. Excessive stimulation of the CNS receptors (both muscarinic and nicotinic) is responsible for some of the higher order symptoms such as anxiety, restlessness, CNS depression, agitation, confusion, delirium, coma, and seizures. Mevinphos-induced cholinesterase inhibition can persist for 2–6 weeks. Monitoring of cholinesterase levels through regular blood testing is highly recommended for individuals exposed to mevinphos.

Clinical Management

For exposure to eyes, eyelids should be held open and the eyes flushed with copious amounts of water for 15 min. For exposure to skin, affected areas should be washed immediately with soap and water. The victim should receive medical attention if irritation develops and persists.

For exposure through inhalation, the victim should be removed to fresh air and, if not breathing, given artificial ventilation. The victim should receive medical attention as soon as possible.

First aid for ingestion victims would be to induce vomiting, keeping in mind the possibility of aspiration of solvents. Gastric decontamination should be performed within 30 min of ingestion to be most effective. Initial management of acute toxicity is establishment and maintenance of adequate airway and ventilation. Atropine sulfate in conjunction with pralidoxime chloride can be administered as an antidote. Atropine by intravenous injection is the primary antidote in severe cases. Test injections of atropine (1 mg in adults and 0.15 mg kg^{-1} in children) are initially administered, followed by 2–4 mg (in adults) or 0.015–0.05 mg kg^{-1} (in children) every 10–15 min until cholinergic signs (e.g., diarrhea, salivation, and bronchial secretions) decrease. High doses of atropine over several injections may be necessary for effective control of cholinergic signs. If lavage is performed, endotracheal and/or esophageal control is suggested. At first signs of pulmonary edema, the patient should be placed in an oxygen tent and treated symptomatically.

Environmental Fate

Mevinphos does not readily adsorb to soil particles, having a soil half-life of 3 days. It is readily hydrolyzed resulting in loss of its insecticidal activity within 2–4 weeks.

Ecotoxicology

Mevinphos is highly toxic to birds, fish, and bees. Consequently, areas frequented by wildlife, including lakes or ponds inhabited by fish, should not be contaminated by use of mevinphos.

Exposure Standards and Guidelines

Mevinphos air concentration of 40 mg m^{-3} is immediately dangerous to life or health. The Occupational Safety and Health Administration (OSHA) time-weighted average (TWA) (skin), American Conference of Governmental Industrial Hygienists (ACGIH) TWA (skin) and National Institute for Occupational Safety and Health (NIOSH) recommended TWA (skin) for mevinphos is 0.1 mg m^{-3}. The OSHA short-term exposure limit (STEL), ACGIH STEL, and NIOSH recommended STEL for mevinphos is 0.3 mg m^{-3}. Based on a 2 year rat feeding study and a 10-fold safety factor, the acceptable daily intake (ADI) for mevinphos is 0.0025 mg kg^{-1} day^{-1}.

Miscellaneous

Pure mevinphos is a colorless liquid with a very mild odor or is practically odorless. Mevinphos has a molecular weight of 224.15 and a specific gravity of 1.25. It is completely miscible with water, alcohols, ketones, chlorinated hydrocarbons, aromatic hydrocarbons, and most organic solvents. Mevinphos has a melting point of 21°C (*cis*-isomer), 6.9°C (*trans*-isomer), and a boiling point of 99–103°C at 0.03 mmHg. Some commonly used trade names of mevinphos are Phosfene, Phosdrin, Mevinox, Meniphos, Fosdrin, and Apavinphos.

See also: Cholinesterase Inhibition; Neurotoxicity; Organophosphates; Pesticides.

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Microarray Analysis

Kartik Shankar and Harihara M Mehendale

The genetic information stored in the DNA is first transcribed into message called RNA, which is further translated into proteins that carry on the various functions of living cells. Monitoring changes in messenger RNA (mRNA) in cells has been used as an indirect measure to characterize changes in proteins. Gene expression changes have been traditionally monitored using Northern blot analyses and more recently by reverse-transcriptase polymerase chain reaction (PCR) and Rnase protection assays. DNA microarrays provide an unprecedented and revolutionary platform to perform genome-wide expression analyses. There are basically two types of DNA microarrays: oligonucleotide-based arrays and cDNA arrays. Oligonucleotide arrays are made either by spotting prefabricated oligonucleotides or by using specific chemical synthesis steps using photolithographic mask, light, or other methods to generate the sequence order in the synthesis. This results in the formation of high-density short oligonucleotide probes that are synthesized at specific predefined positions. cDNA microarrays, on the other hand, can be used to study differences in gene expression between a control and experimental group. Partial cDNAs (500–2000 bp) that correspond to unique gene sequences are spotted onto surfaces of treated glass using a high-speed robotic printer. Spotted cDNAs represent either known genes or collections of partially sequenced cDNA derived from expressed sequence tags (ESTs) corresponding to mRNAs of genes of unknown function. It is possible to print greater than 25 000 cDNAs on a single microarray.

A standard microarray experiment using a cDNA microarray is summarized in **Figure 1**. RNA from either experimental or control (tissue or cells) sample is extracted. Fluorescent cDNA probes are generated from control and experimental RNA samples in a single round of reverse-transcription in the presence of fluorescently labeled dUTP, such that control and test products are labeled with different fluorescent labels (e.g., control Cy3-dUTP and test is Cy5-dUTP). The labeled cDNAs from both control and test groups are then mixed and hybridized to a glass microarray. The fluorescent signal is detected using a scanning confocal microscope equipped with lasers for fluorescence excitation. This method eliminates the need for a separate hybridization for control samples. The data acquired are generally represented as ratios of the fluorescent signals (Cy3/Cy5). The methodology for analyzing gene expression via

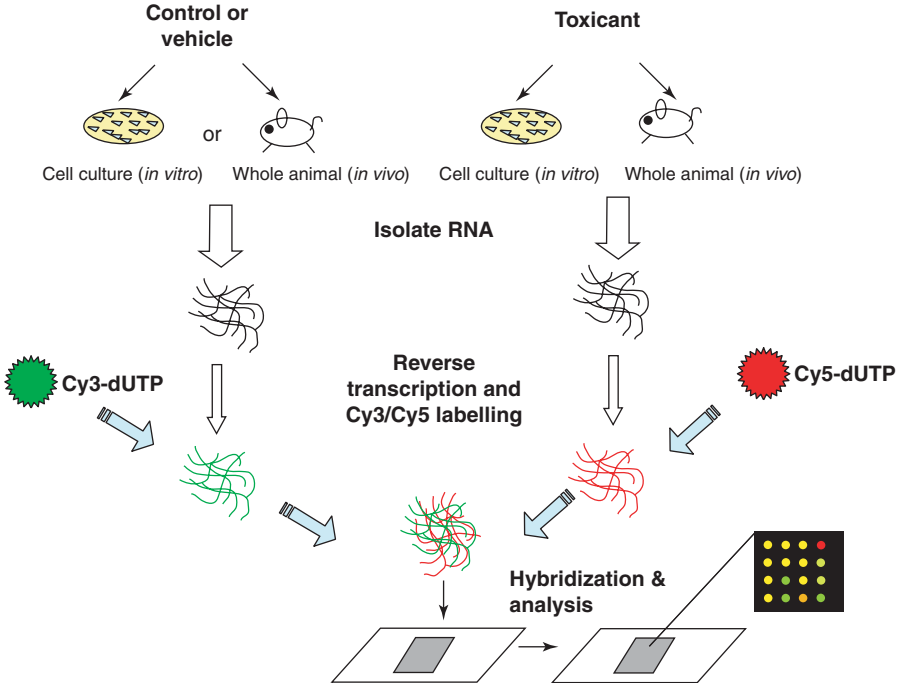

Figure 1 Schematic of a standard microarray experiment using a cDNA microarray.

cDNA arrays and oligonucleotide arrays is different. In case of oligonucleotide microarrays sample preparation involves generation of ds-cDNA from either total RNA or poly(A) + RNA followed by antisense RNA synthesis in an *in vitro* transcription reaction with biotinylated or fluortagged nucleotides. The RNA probe is then fragmented to facilitate hybridization and visualization done using a confocal scanner. Data generated via microarray analyses is difficult to analyze without the utilization of computational tools that handle large volumes of data. A large variety of bioinformatic software is now available both as free and commercial software for data acquisition and analysis.

See also: Bioinformatics; Genomics, Toxicogenomics.

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Micronucleus Assay

Robin C Guy

Background Information

The micronucleus assay is an *in vivo* or *in vitro* assay for the detection of chromosome damage. An *in vitro* micronucleus test with mammalian cell culture and a modified *Salmonella* reverse mutation (Ames) assay are frequently used for screening purposes early in the development and evaluation of a new chemical. Further, the micronucleus assay is one of the three assays that usually constitute the minimum test battery satisfying global regulatory requirements for evaluation of mutagenic potential. This set includes an *in vivo* rodent bone marrow micronucleus test, a bacterial reverse mutation assay, and an *in vitro* cytogenetic test with mammalian cell culture, or an *in vitro* gene mutation assay in mammalian cell cultures; supplementary studies might be conducted as a follow-up to the findings from this initial testing battery and/or to satisfy a regulatory requirement. *In vivo* assays have an advantage over *in vitro* assays, as metabolism and other physiological functions and interactions are able to occur as part of the study.

Reticulocytes, or polychromatic erythrocytes, are newly formed, immature red blood cells. These are larger than red blood cells and have retained some ribosomal RNA. These cells are detectable from normochromatic erythrocytes, mature erythrocytes that lack ribosomes, by their staining properties. Micronuclei are small nuclei, separate from and additional to the main nuclei of the cell. Micronuclei are formed due to breakage of chromatin or chromosomes, from spindle fiber or chromosome abnormalities, or from an entire chromosome that may have lagged behind in anaphase. After an insult that would increase the frequency of micronuclei, micronucleated polychromatic erythrocyte levels would increase at ~10–12 h and remain elevated for 20 h, with a possible twofold increase at 24 h. Micronucleated polychromatic erythrocyte levels may take longer to be at their peak, if there is a mitotic delay or slower uptake of the material or a metabolite due to metabolism. Therefore, it is prudent to have dose groups sacrificed over more than 1 day.

The *in vitro* assay primarily utilizes human lymphocytes, but other mammalian cells have been used. One technique incorporates a cytokinesis-block technique. Cytoplasmic division is inhibited, thereby resulting in binucleated and multinucleated cells.

In the *in vivo* assay, animals are dosed with the test materials by the appropriate route, and subsequently cells are collected for cytogenic analyses. The primary animal species for these tests are mice and rats. Chinese hamsters are also used for this assay; however, the US Food and Drug Administration (FDA) *Redbook* requires justification for any other species used besides the mouse and rat.

Rodents are typically administered a single dose of the test material. To obtain micronucleus information from a subgroup of a larger, repeated daily dose toxicity study lasting over 4 weeks, the ratio of micronucleated mature (normochromatic) erythrocytes in the peripheral blood to mature erythrocytes are determined. As long as there is no proof that the test material or a metabolite cannot act on the bone marrow, this assay may be used.

The US Environmental Protection Agency (EPA) and the US FDA *Redbook* state that there should be five analyzable animals per sex per group. The International Conference on Harmonisation (ICH) guidelines recommended by the FDA's Center for Drug Evaluation and Research state males are the most sensitive gender and that unless there are obvious metabolism differences between male and females, or that a gender-specific material is to be tested, males should be used. Gender differences have been shown in at least one experiment, so studies utilizing different genders are important.

The EPA and FDA state that there is no standard treatment schedule; therefore, one, two, or more doses every 24 h is acceptable as long as toxicity has been demonstrated, or that a limit dose has been achieved. In addition, if a large volume of material needs to be administered to the rodents, it may be administered as a divided dose, as long as the doses are not separated by more than a few hours. Doses may be selected from the results of a range finding study, or any preliminary tests done with the rodents after administration via the same route. The highest dose selected for the main assay would produce some bone marrow toxicity, including, in the bone marrow or peripheral blood, a decreased number of immature erythrocytes to total erythrocytes. In the case of a nontoxic test article, the limit dose is defined as $2000\,\mathrm{mg\,kg^{-1}}$.

Negative (solvent) and positive controls must be utilized for a valid study. A historical database must be maintained for these results. A positive control must produce a noticeable increase in micronucleated cells as compared to the solvent controls. Examples of positive control substances include:

- Ethyl methane sulfonate (CAS 62-50-0).
- Ethyl nitrosourea (CAS 759-73-9).
- Mitomycin C (CAS 50-07-7).
- Cyclophosphamide (CAS 50-18-0 (monohydrate form: CAS 6055-19-2)).
- Triethylenemelamine (CAS 51-18-3).

After sacrifice, bone marrow cells are extracted from femurs or tibias, prepared and placed on slides, and then stained for microscopic evaluation. When peripheral blood is used, the blood is collected at appropriate times after treatment and smear preparations are made and stained. If using peripheral blood, care should be taken to ensure that the species selected for study had a spleen that cannot remove micronucleated erythrocytes. The mouse was the species of choice for the measurement of micronucleated immature (polychromatic) erythrocytes in peripheral blood, but the rat has also been shown to have good results in this test.

EPA and the FDA's Center for Food Safety and Applied Nutrition toxicology study guidance and requirements (*Redbook* 2000) state that the proportion of immature erythrocytes among the total erythrocytes for each animal is determined by counting at least 200 erythrocytes for bone marrow and 1000 erythrocytes for peripheral blood. At least 2000 immature erythrocytes per animal should be scored for a micronucleated immature erythrocyte count. Statistical studies have been performed that show between 4000 and 5000 polychromatic erythrocytes are needed to detect a doubling of spontaneous frequencies that fall into the 1–3% range. Micronuclei may also be counted in the mature erythrocytes.

Many compounds that are positive in the micronucleus assay are mammalian carcinogens; however, there is no exact correlation between the micronucleus assay and carcinogenicity. Correlation may be dependent on chemical class.

The micronucleus assay is one type of study recommended by the FDA *Redbook* and ICH guidelines as part of a standard genetic toxicology battery. The other assays include the Ames (bacterial reverse mutation) and mouse lymphoma tests.

To ensure that the results of an assay are valid, specific criteria have been determined. The frequency of micronucleated cells need to be within the normal historical control range. The frequency of micronucleated cells in the positive controls need to be significantly increased over the vehicle control. In addition, there must be five surviving animals per group.

There are several criteria for determining a positive result, such as a dose-related increase in the number of micronucleated cells, or a statistical, repeatable increase in the number of micronucleated cells in a single dose group at a single sampling time. Biological relevance of the results should be considered even if there is statistical significance. Negative results indicate that the test substance does not produce chromosomal or spindle damage leading to the formation of micronuclei in the immature erythrocytes of the test species. An equivocal study may result from only one group with a statistically significant increase and should be elucidated by further testing, preferably using a modification of experimental conditions.

See also: Ames Test; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Good Laboratory Practices (GLP); International Conference on Harmonisation; Micronucleus Assay; Genetic Toxicology; Toxicity Testing, Mutagenicity; Redbook.

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Relevant Websites

http://www.epa.gov – US Environmental Protection Agency website. Health Effects Test Guidelines OPPTS 870. 5395 Mammalian Erythrocyte Micronucleus Test.

http://www.fda.gov – US Food and Drug Administration website. Specific Aspects of Regulatory Genotoxicity Test for Pharmaceuticals.

Microtox

Gary P Bond and John Martin

Published by Elsevier Inc.

Overview

Microtox is an *in vitro* test system which uses bioluminescent bacteria for the detection of toxicity. It is used as a screening system to provide an indication of the relative toxicity of a sample. Applications which have been published include the testing of air, samples containing biological toxins, industrial effluent, industrial process waters, municipal effluent, drinking water, ecotoxicological samples, hazardous waste, soil, sediments, storm water, and medical products for bioreactivity, among others. The Microtox test provides both acute toxicity and genotoxic analyses. (Microtox is a registered trademark of Strategic Diagnostics, Inc., Newark, DE.)

The Microtox test system utilizes a strain of naturally occurring luminescent bacteria – *Vibrio fischeri*. Exposure to a toxic substance causes a disruption of the respiratory process of the bacteria resulting in reduced light output. The effective concentration (EC_{50}) is determined as the concentration of a toxicant that causes a 50% reduction in light output over a prescribed period of time (typically 5, 15, or 30 min). The test is fast, fairly simple to conduct, uses small sample sizes, and is relatively inexpensive. Results correlate well with those from other toxicity bioassays such as fish and *Daphnia*. The test is used extensively in the measurement of toxicity of effluent and drinking waters, as well as an early screening tool for relative toxicity as part of a test battery.

Principle of Operation

Vibrio fischeri are nonpathogenic, marine, luminescent bacteria which are sensitive to a wide range of toxicants. The organisms are supplied for use in a standard freeze-dried (lyophilized) state, which serves to maintain the sensitivity and stability of the test organisms. Disruption of the respiratory process, by exposure to a toxicant affects the metabolic pathway that converts chemical energy via the electron transfer system of the bacteria to visible light. The process occurs in and close to the cell membrane so target sites are close to the cell surface. Each test utilizes approximately 1 million organisms and each organism is less than 1 μm in diameter, so a very high surface to volume ratio is presented. Sensitivity and statistical significance are therefore high; the response being an integrated effect of the toxicant on the entire population, which is very large.

The Microtox equipment includes a self-calibrating analyzer which incorporates a photomultiplier tube, a data collection and reduction system, and software. The temperature-controlled analyzer maintains the test organisms and samples at a standard temperature of 15°C. It also detects the light intensity at 490 nm, the wavelength emitted by the bacteria.

Advantages

The Microtox test has many advantages over other bioassays which makes it a useful tool in monitoring programs and screening studies. These include cost-effectiveness, simplicity, rapidity, precision, statistical significance, and the requirement for a small sample volume. The Microtox test is one of the least volume-intensive toxicity assays, using only 2.5 ml of sample per test. The rapidity of the test means that real-time data can be generated as part of a monitoring program, allowing fast corrective actions to be taken. Acute test protocols take ~45 min from start to finish when conducted by a trained operator (genotoxic protocols are conducted in 16, 20, and 24 h periods). Twenty-nine individual samples may be tested at one time for determination of relative toxicity or three complete serial dilution bioassays can be run simultaneously to provide effect concentrations.

Use as Part of a Test Battery and Correlation with Other Bioassays

No single test can ensure the detection of all toxic effects in a complex mixture. A test battery should have good sensitivity to a broad range of toxicants, while possessing unique characteristics making it a useful detector of certain types of toxicity. The Microtox test has been extensively evaluated for its correlation with other bioassays. Regression analyses of the inhibitory effects of chemicals on *Vibrio fischeri* with their acute toxicities to a variety of aquatic species show a high degree of collinearity over several orders of magnitude. Species tested include, *Daphnia magna* (water flea), *Pimephales promelas* (fathead minnow), *Leuciscus idus melanotus* (goldorfe), *Poecilia reticulata* (guppy), *Lepomis macrochirus* (bluegill sunfish), *Ictalurus punctatus* (channel catfish), *Carassius auratus* (goldfish), *Gambusia affinis* (mosquito fish), *Salmo trutta* (brown trout), *Cyprinodon variegatus* (sheepshead minnow), and *Oncorhynchus mykiss* (rainbow trout). Additional studies have also shown significant relationships between the Microtox EC_{50} and rat and mouse LD_{50} values.

Effluent test results support the use of Microtox as part of a test battery. Uniform response of sublethal effects to 50 effluents from pulp and paper mills was obtained with the Microtox test compared to the results with fish and algae, which varied greatly with age and genetic variation within the population. Microtox has also been used to detect other sublethal effects (e.g., chronic toxicity). For example, the bacterial response can be used to quantify the stress on the immunological defense systems of mussels exposed to toxins in polluted rivers or wastewaters. In this case, the bacteria are exposed to hematocytes extracted from the mussels for 30 min at standard temperature. The phagocytic activity of the hematocytes on the bacteria decreases bioluminescence by a quantifiable amount.

While it alone cannot substitute for acute or sublethal hazard assessment which covers all species, Microtox can be useful in a battery of screening tests or to supplement data obtained in other toxicity bioassays.

Regulatory Status

The Microtox test is recognized as an approved protocol or under consideration for acceptance by the regulatory agencies of many nations including the United States, Canada, and countries from the European Union, South America, and the Pacific Rim. The US Environmental Protection Agency has specifically referred to Microtox as an inexpensive tool to test effluent toxicity. Test protocols involving Microtox have been approved by the International Standards Organization, American Society for Testing and Materials, the Organization for Economic Cooperation and Development (OECD) and are included in Standard Methods for the Examination of Water and Wastewater ('Standard Methods').

See also: Biomonitoring; Effluent Biomonitoring; *In Vitro* Test; Toxicity Testing, Aquatic; Toxicity Testing, Mutagenicity.

Further Reading

Abbondanzi F, Cachada A, Campisi T, *et al.* (2003) Optimisation of a microbial bioassay for contaminated soil monitoring: Bacterial inoculum standardisation and comparison with Microtox((R)) assay. *Chemosphere* 53(8): 889–897.

Banks MK, Schwab P, Liu B, *et al.* (2003) The effect of plants on the degradation and toxicity of petroleum contaminants in soil: A field assessment. *Advances in Biochemical Engineering and Biotechnology* 78: 75–96. (review).

Nalecz-Jawecki G, Rudz B, and Sawicki J (1997) Evaluation of toxicity of medical devices using Spirotox and Microtox tests: I. Toxicity of selected toxicants in various diluents. *Journal of Biomedical Materials Research* 35(1): 101–105.

Relevant Websites

http://caat.jhsph.edu – John Hopkins University Center for Alternatives to Animal Testing Workshop that includes Microtox as a valuable ecotoxicology test.

http://www.sdix.com – Microtox is registered trademark of Strategic Diagnostics, Inc.

http://www.wrrc.hawaii.edu – Honolulu Board of Water Supply proposed use of Microtox for first tier monitoring in event of contamination as a result of terrorism.

Minamata

Stephen C Bondy

History

The Chisso Corporation of Japan used inorganic mercury as a catalyst in the production of acetaldehyde. A fraction of this mercury was discharged within waste water into Minamata Bay. This contamination occurred between 1932 and 1968, with an estimated total of 150 tons of mercury delivered to the bay. This led to a gradual but severe poisoning of a considerable fraction of the 50 000 people living in a town adjacent to the bay, where the primary industry was fishing. Concentrations of mercury in the fish (such as mullet, sea beam, rockfish, and mackerel) were found to be over 10 ppm mercury.

Around 900 people died as a result of mercury poisoning and over 3000 were severely affected. Hair concentrations as high as 705 ppm were present in some victims. It is likely that well over 15 000 people were adversely affected by ingestion of mercurials within fish. Symptoms were both neurological and systemic. In adults, the lowest levels affected individuals by causing parasthesia. Symptoms resulting from higher exposures led to other sensory disorders such as pain, paralysis, headaches, tremors, and also deficits in hearing, vision (concentric visual field constriction and blindness), and speech. In addition, motor deficits such as poor coordination were manifested. Postmortem pathological findings revealed cerebellar atrophy and enlarged cerebellar folia. The cerebellar granule cell population was most affected. Diffuse cortical and subcortical atrophy with gliosis and macrophage infiltration was also apparent. Hypothalamus and substantia nigra were relatively unaffected.

Fetal damage following intrauterine poisoning was especially pronounced. Affected infants were born with both physical malformations and profound mental retardation. Symptoms resembled cerebral palsy. Several infants were born completely blind. Pathology in these cases involved the cerebral hemispheres to a major extent and microcephaly was found in several instances. Cerebral cortices were not only underdeveloped but their convolutions were also narrowed. The corresponding maternal toxicity in these cases was minimal and rapidly reversible. The observable postnatal effects at the lowest levels of fetal exposure, was psychomotor retardation expressed as delayed behavioral development, often after a latent period of apparently normal maturation.

After an extended period of controversy, some corporate liability was admitted and limited compensation was paid to victims of this exposure. Discharge into the bay was stopped, and dredging was commenced to recover mercurials in the sediment at the bottom of the bay. A net was placed across the bay to prevent exodus of contaminated fish. This net was removed in 1997 and fishing was again allowed, since mercury levels in fish were found to be below 0.4 ppm and this was deemed safe.

Commentary

The Minamata Bay tragedy, whereby many inhabitants of the region died or were permanently injured as a result of methylmercury poisoning is a classic example of a harmful neurotoxic exposure. A series of unforeseen circumstances combined to make this a very severe outbreak with long lasting consequences. Although the harmfulness of methylmercury had long been known, the severity of the toxic exposures involved can be attributed to several factors:

1. The environmental fate of mercury was incompletely understood
   a. *Assumed:* The mercurial wastes that were discharged into the bay were not considered potentially harmful since they contained relatively low concentrations of much less toxic inorganic mercury, and it was believed that tidal action would be sufficient to flush the bay and prevent the accumulation of excess mercury.
   b. *Unanticipated:* The bacterial conversion of a large fraction of effluent mercury to an organic form led to an extensive bioaccumulation of methylmercury within the food chain. The consumption of microorganisms including phytoplankton, was followed by their consumption by zooplankton, which in turn were the prey of marine invertebrates and other plankton filtering species. The progression up the food chain then proceeded by ingestion of contaminated animals by small fish and ultimately larger species of fish which provided a nutritional source for humans. This led to extensive contamination of an important food source for inhabitants living in fishing villages around the bay.
2. The cause of the problem was initially misidentified
   a. *Assumed:* Signs of toxicity had a gradual onset and occurred over an extended period. This

occluded the association between the development of neurological disease and methylmercury for a considerable time. When an epidemic of severe neurological disorders, especially associated with newborn infants, became apparent, an epidemiological study revealed the derangement to be confined to low lying marshy areas around the bay. Since drier areas at higher altitudes and more removed from the bay were much less affected, an insect-borne disease of viral origin was suspected.
b. *Unanticipated:* Some clues that might have helped make fish suspect as disease vectors, were ignored. Fish in the preceding years had become rather sluggish and easier to catch than usual. Abnormal hyperreactive behavior in domestic cats which lived primarily on fish wastes, were humorously noted but the critical association was not made. Smaller mammals have higher metabolic rates so that these changes may have preceded signs of human toxicity.

3. Assumptions about recovery were incorrect being based on a different exposure scenario
   a. *Assumed:* The extent of recovery from methylmercury poisoning can be quite marked in cases of more acute poisoning that occurred in Iraq in 1971 following ingestion of grain treated with methylmercury as a fungicide. These doses were high and thus signs of intoxication arose shortly after exposure. The time frame of any recovery was relatively short in such circumstances.
   b. *Unanticipated:* The very extended low-level exposure to methylmercury following fish consumption led to a slow development of symptoms. The recovery from this exposure scenario was very gradual and often very limited. Any partial improvement observed took place over an extended time. Thousands of victims still remain handicapped by the exposure.
4. Appropriate warnings and other actions to protect public health were delayed
   a. *Assumed:* Safeguarding the welfare of the general population is a primary responsibility of government. It also a duty of industrial enterprises to protect its workers and other individuals living adjacent to manufacturing sites. Thus, when evidence for a causal relation between an industrial product and an adverse effect on the environment is noted, prompt action to mitigate the hazard, taken by both industry and the state, is necessary and to be expected.
   b. *Unanticipated:* There was a great reluctance on the part of both the Chisso Corporation and the Japanese governmental authorities to acknowledge the relation between mercurials in Minamata Bay and the prevalent and serious disease outbreak within the town. This delay, in part due to misplaced traditional concepts of loyalty, greatly expanded the scope of the outbreak. For example, the fishermen of Minamata were at one point allowed to eat but not to sell suspect fish. This regulation, apparently an attempt by government to absolve itself of responsibility, led to increased local fish consumption by inhabitants of Minamata and worsened the condition of the most high-risk families.
5. The magnitude of the outbreak was underestimated
   a. *Assumed:* Upon investigation by medical authorities, large number of exposed victims were found to exhibit marked neurological deficits. It was recognized that there might be a larger number of people who might experience subclinical deficits and that these would be difficult to locate.
   b. *Unanticipated:* A significant number of the exposed population initially exhibited either no or very minor symptoms, but experienced a progressive worsening of their health. It was not understood that a lesser exposure to methyl mercury could lead to delayed onset of toxicity. Thus, in some cases toxicity could first be manifested many years after cessation of exposure. This has now been confirmed in animal models where exposure of young animals to methylmercury, was found to lead to behavioral deficits that were only apparent as the animal aged. This is a classic example of a prolonged period of asymptomatic latency following toxic exposure.

See also: Methylmercury; Pollution, Water.

Further Reading

Eto K (2000) Minamata disease. *Neuropathology* 20(Suppl): S14–S19.

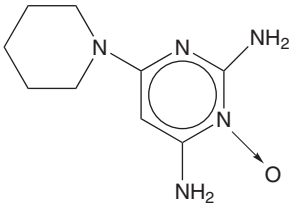
Harada M (1995) Minamata disease: Methylmercury poisoning in Japan caused by environmental pollution. *Critical Reviews in Toxicology* 25: 1–24.

Rice DC (1996) Evidence for delayed neurotoxicity produced by methylmercury. *Neurotoxicology* 17: 583–596.

Minoxidil

Elizabeth J Scharman

- Chemical Abstracts Service Registry Number: CAS 38304-91-5
- Synonyms: 2,6-Diamino-4-piperidinopyrimidine; 6-(1-Piperidinyl)-2,4-pyrimidinediamine-3-oxide; Rogaine®; Loniten®
- Chemical/Pharmaceutical/Other Class: Hypotensive agent; Vasodilating agent
- Chemical Formula: $C_9H_{15}N_5O$
- Chemical Structure:



Uses

Minoxidil is used for severe hypertension, and used when the patient is symptomatic or when end-organ damage is present. Minoxidil is also used topically to stimulate hair regrowth in patients with alopecia androgenetica (male pattern baldness).

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to minoxidil. Toxicity may also occur via dermal exposure. Minoxidil is available in an oral dosage form and in a 2% or 5% topical formulation.

Toxicokinetics

Minoxidil, given orally, is almost completely absorbed ($\geqslant$90%). Peak plasma concentrations are reached within 1 h and fall rapidly afterwards. Pharmacodynamic effects do not mirror the drug's pharmacokinetics. With administration of a single dose, hypotensive effects begin within 30 min, peak within 2–8 h, and may continue for 75 h. Following oral administration, ~90% of the drug is metabolized, primarily by glucuronidation. The metabolite, minoxidil-*o*-glucuronide, is active but is not as active as the parent drug. The metabolite, but not the parent drug, may accumulate when renal impairment is present. Minoxidil is widely distributed with a volume of distribution of 2–3 l kg^{-1}. Minoxidil is found in breast milk; it is not protein bound. Dialysis and peritoneal dialysis will remove minoxidil. The half-life is 4.2 h. Percutaneous absorption is low through intact skin (average $<$2% of the applied dose).

Mechanism of Toxicity

Minoxidil acts as a direct vasodilator of vascular smooth muscle which decreases peripheral vascular resistance and blood pressure. Veins are affected to a lesser extent than arterioles. The resulting hypotensive effect induces a reflex increase in heart rate, cardiac output, and stroke volume. Sodium and water retention also occur leading to edema; plasma renin activity is increased.

Acute and Short-Term Toxicity (or Exposure)

Human

Experience with acute toxicity is limited. A minimum toxic dose is not defined. Headache, dizziness, hypotension, tachycardia, sodium and water retention, and cardiac dysrhythmias may be seen following overdose. Severe hypotension may result in myocardial ischemia; other end-organs may also be affected.

Chronic Toxicity (or Exposure)

Animal

Chronic therapy in animals has caused cardiac hypertrophy, cardiac dilation, and epicarditis. The characteristic lesion in rats and dogs is focal necrosis of the papillary muscle and subendocardial areas of the left ventricle.

Human

Side effects associated with chronic therapy include tachycardia, sodium and water retention, and hypertrichosis. Sodium and water retention can cause complications secondary to the expanded fluid volume; for example, congestive heart failure. Pericardial effusion has been documented in 3% of patients. Topical therapy may cause contact dermatitis.

In Vitro Toxicity Data

Minoxidil has not been shown to be mutagenic in a variety of studies including the Ames *Salmonella*

assay, DNA damage alkaline elution assay, or mouse bone marrow micronucleus assay.

Clinical Management

Activated charcoal will adsorb minoxidil following recent oral ingestions. Serum levels are not readily available and do not guide treatment. Standard supportive therapies, such as vasopressors, should be utilized as clinically necessary; however, epinephrine and norepinephrine should be avoided. Hemodialysis is of theoretical value if standard therapies fail; however, clinical experience is unavailable to support use.

See also: Ames Test.

Further Reading

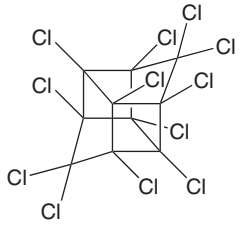
MacMillan AR, Warshawski FG, and Steinberg RA (1993) Minoxidil overdose. *Chest* 103: 1290–1291.

Poff SW and Rose SR (1992) Minoxidil overdose with ECG changes: Case report and review. *Journal of Emergency Medicine* 10: 53–57.

Mirex

Carey N Pope

- Chemical Abstracts Service Registry Number: CAS 2385-85-5
- Synonyms: GC-1283; Dechlorane
- Chemical/Pharmaceutical/Other Class: Chlorinated hydrocarbon insecticide
- Chemical Formula: $C_{10}Cl_2$
- Chemical Structure:



Uses

Mirex has not been manufactured or used in the United States since 1978. Mirex was used to control fire ants, and as a flame retardant from 1959 to 1972.

Exposure Routes and Pathways

Exposure to mirex occurs primarily from direct contact with contaminated soil or from consumption of contaminated food (e.g., fish). Mirex has been found in at least seven sites on US Environmental Protection Agency's National Priorities List of contaminated waste sites. Thus, since the use of mirex was eliminated in the United States in 1978, exposure would be most likely around contaminated waste sites. Mirex may still be used in other countries, however, leading to more exposure in the general population.

Toxicokinetics

Mirex is poorly absorbed from the oral tract. About half of an oral dose in rats was recovered unchanged in the feces. After an initial relatively rapid rate of excretion, the half-life was estimated at 100 days. Storage in tissues is high, often failing to reach a plateau with long-term exposures. To a very limited degree, some mirex is converted into 2,8-dihydromirex and 5,10-dihydromirex. Mirex is excreted unchanged in milk, thus posing a hazard for nursing infants. Approximately 90% of mirex residues are in fat.

Mechanism of Toxicity

Mirex is a potent microsomal enzyme inducer. Mirex may inhibit Na,K-ATPase and interfere with energy production and utilization. Mirex is also a nonphorbol ester tumor promoter.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in rats ranged from 600 to $2000\,mg\,kg^{-1}$. The acute oral LD_{50} in dogs was $>1\,g\,kg^{-1}$. A single dose of mirex in rats was reported to elicit hepatic centrilobular hypertrophy with a marked increase (twofold) in liver weight. High levels of mirex can affect the stomach, intestine, liver, kidneys, eyes, thyroid, and nervous and reproductive systems. Multiple doses over a number of days appear to elicit more extensive toxicity.

Human

There is little information on effects of acute exposure to mirex in humans.

Chronic Toxicity (or Exposure)

Animal

Mirex is more toxic with repeated dosing. Lethality was increased in adult male rats at dosages as low as $5\,mg\,kg^{-1}\,day^{-1}$ for 30 days and in adult females at dosages as low as $6.2\,mg\,kg^{-1}\,day^{-1}$ for 90 days. In mice, 100% mortality occurred following exposure at $1.3\,mg\,kg^{-1}\,day^{-1}$ for 60 days. Dietary mirex for 148 days in rats caused reduced motor activity, irritability, and tremors. Similar dosing led to decreased litter sizes and decreased mating in rats. A number of studies reported cataract formation in offspring from reproductive toxicity studies. Dietary exposure with mirex ($2\,mg\,kg^{-1}\,day^{-1}$ for 3 months) led to impaired reproductive performance in male mice.

Human

There are no reports of chronic toxicity in humans with mirex exposure. On the basis of findings in animal studies, mirex should be considered a potential carcinogen for humans.

Environmental Fate

Mirex degrades slowly in the environment. Residues can remain in soil and water for years. Mirex does not evaporate to any degree from surface water or soil. Mirex is practically insoluble in water, but adsorbs to soil and sediment. Mirex does not appreciably leach into groundwater. Bioaccumulation in fish and other aquatic organisms and animals that eat these organisms is possible.

Ecotoxicology

Mirex is highly toxic to a number of aquatic organisms with crustaceans including commercially important species of shrimps and crabs being particularly sensitive.

Further Reading

Faroon O, Kueberuwa S, Smith L, and DeRosa C (1995) ATSDR evaluation of health effects of chemicals. II. Mirex and chlordecone: Health effects, toxicokinetics, human exposure, and environmental fate. *Toxicology and Industrial Health* 11(6): 1–203.

Hayes WJ (1982) Chlorinated hydrocarbon insecticides. In *Pesticides Studied in Man*, pp. 172–283. Baltimore, MD: Williams and Wilkins.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Mirex.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Mirex.

http://www.inchem.org – International Programme on Chemical Safety.

Mistletoe

Christopher P Holstege

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- SYNONYMS: American mistletoe: *Phoradendron flavescens*, *Phoradendron juniperinum*, *Phoradendron leucarpum*, *Phoradendron macrophyllum*, *Phoradendron serotinum*, *Phoradendron tomentosum*; European mistletoe: *Viscum album*

Uses

Mistletoe has been advocated for the treatment of hypertension, cancer, muscular spasms, arthritis, and as an abortifacient. It is also widely used in Christmas decorations.

Background Information

The term mistletoe is used for a number of different parasitic plants from the Genus *Phoradendron* and *Viscum*. It is a semiparasitic shrub with ovate, opposite leaves. It is found growing on trees, especially oaks. The berries grow in grapelike clusters and are typically white, round, and translucent.

Exposure Routes and Pathways

All parts of the plant may cause toxicity when ingested, with berry ingestion being the most common

source of exposure. Exposure may also occur by herbal extracts derived from mistletoe.

Mechanism of Toxicity

Viscum contain lectins that are cytotoxic by inhibiting protein synthesis on the ribosomal level in a manner similar to the toxalbumins ricin and abrin. Viscotoxin and phoratoxin are cardiac toxins and vasoconstrictors. Both produced reflex bradycardia, negative inotropic effects, and, in high doses, vasoconstriction of skin and skeletal muscle vessels in animals.

Acute and Short-Term Toxicity (or Exposure)

Human

Reported human toxicity occurs from either ingestion of the plant itself or from ingestion of a herbal remedy derived from the mistletoe. Ingestion of the plant, most commonly the berries, may be associated with the development of gastrointestinal distress consisting of nausea, vomiting, abdominal cramps, and diarrhea. Mistletoe berry exposures most commonly occur in children during the Christmas season; development of symptoms is rare.

Rare cases of cardiovascular collapse have been reported. It is unclear if this effect is due to direct cardiotoxicity or due to hypovolemic shock caused by protracted vomiting and diarrhea. Rare cases of central nervous system effects have also been reported and include drowsiness, ataxia, and seizures.

Chronic Toxicity (or Exposure)

Human

Extracts of *Viscum album* have been used as a traditional remedy for diabetes.

In Vitro Toxicity Data

Extracts of mistletoe have demonstrated antitumor qualities in numerous tumor cell lines.

Clinical Management

The mainstay of treatment is supportive care. Fluid and electrolyte replacement may be needed in patients manifesting gastrointestinal signs and symptoms. Seizures can be managed with common anticonvulsant therapy. Pregnant patients should be monitored for premature uterine contractions.

See also: Plants, Poisonous.

Further Reading

Krenzelok EP, Jacobsen TD, and Aronis J (1997) American mistletoe exposures. *American Journal of Emergency Medicine* 15: 516–520.

Mithramycin

Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 18378-89-7
- Synonyms: Aurelic acid; Aureolic acid; Mithracin; Mithramycin A; Mitramycin; Plicamycin
- Chemical/Pharmaceutical/Other Class: Antineoplastic

Uses

Mithramycin is used as a DNA binding fluorescent dye, as an antineoplastic agent, and to reduce hypercalcemia, especially due to malignancies. Mithramycin is a potent inducer of fetal hemoglobin production in erythroid cells and is being investigated to alleviate the symptoms underlying beta-thalassemia and sickle cell anemia.

Exposure Routes and Pathways

Intravenous infusion is the only reported exposure pathway.

Toxicokinetics

Mithramycin peak levels are achieved immediately through the intravenous route. It is rapidly cleared from the blood within the first 2 h. Nearly 70% is excreted in the urine within the first 4 h and over 90% is recovered within the first 24 h. There is no evidence of protein binding.

Mechanism of Toxicity

Mithramycin inhibits RNA and protein synthesis by adhering to DNA. Mithramycin appears to effect bone resorption by stimulating osteoclast activity and results in hypocalcemia and hypophosphatemia.

Acute and Short-Term Toxicity (or Exposure)

Human

Mithramycin is toxic to bone marrow, liver, and kidneys.

Chronic Toxicity (or Exposure)

Animal

Mithromycin is occasionally used in veterinary practice as an antineoplastic. Expected toxicities are related to bone marrow suppression and bleeding.

Human

Mithromycin produces hemorrhagic diathesis in up to 10% of patients treated daily. These hemorrhagic diathesis may manifest early with epistaxis or gastrointestinal bleeding, with laboratory findings significant for thrombocytopenia, increased coagulation times, leukopenia, and/or anemia. Adverse gastrointestinal, cutaneous, and neurological manifestations may occur and include anorexia, nausea, vomiting, diarrhea, stomatitis, fever, malaise, headache, facial flushing, and skin rash. Mithramycin-induced hypocalcemia may result in fatigue, depression, muscle cramps, fasciculations, and tetany.

In Vitro Toxicity Data

Mithramycin was found to be mutagenic in the Chinese hamster ovary cell assay.

Clinical Management

In patients manifesting clinical toxicity, discontinuation of mithramycin should be considered and supportive care instituted. Administration of calcium and blood products may be necessary depending upon the degree of toxicity.

See also: Blood; Veterinary Toxicology.

Further Reading

Ashby MA and Lazarchick J (1986) Acquired dysfibrinogenemia secondary to mithramycin toxicity. *The American Journal of the Medical Sciences* 292(1): 53–55.

Mitomycin C

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 50-07-7
- Synonyms: Ametycine; MMC; Mutamycin; Mit-C; Methylmitomycin
- Chemical/Pharmaceutical/Other Class: Fungal metabolite
- Chemical Formula: $C_{15}H_{18}N_4O_5$

Uses

Mitomycin C is used as an antineoplastic agent and for slowing of fibroblast formation in open-angle glaucoma. Recently, mitomycin C has been used to induce tumor responses in patients with many types of cancer. For example, mitomycin C is used in the palliative treatment of various solid tumors such as non-small cell lung, cervical, colorectal, breast, bladder, pancreatic, and esophageal carcinomas. In addition to its systemic use in combination regimens for these tumors, mitomycin has been used as a single agent given by intrahepatic infusion for hepatic metastases from colorectal carcinoma and by intravesical instillation for carcinoma *in situ* of the bladder.

Exposure Routes and Pathways

Mytomycin C is administered intravenously as a therapeutic agent.

Toxicokinetics

Mitomycin C is absorbed inconsistently from the gastrointestinal tract. The volume of distribution is $16–56\,l\,m^{-2}$. The primary means of elimination is by hepatic metabolism, but it is also excreted in urine. $T_{1/2} = 8$ min.

Mechanism of Action

Mitomycin C inhibits DNA synthesis and cross-links DNA at the N6 position of adenine and at the O6

and N2 positions of guanine. In addition, single-strand breakage of DNA is caused by reduced mitomycin C (this can be prevented by free radical scavengers). Its action is most prominent during the late G1 and early S phases of the cell cycle. Mitomycin C can inhibit RNA and protein synthesis at high concentrations.

Mechanism of Toxicity

Mytomycin C is an aneuploidy-inducing agent. Oxygen and radiation therapy have shown to enhance the development of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The intravenous LD_{Lo} is $1500\,\mu g\,kg^{-1}$. In rats the intravenous TD_{Lo} is $2.6\,mg\,kg^{-1}$ and the oral LD_{50} is $68\,mg\,kg^{-1}$. Oral mouse LD_{50} is $88\,660\,\mu g\,kg^{-1}$; and oral LD_{Lo} in dogs and monkeys is $\sim 10\,mg\,kg^{-1}$. At the highest concentration of mitomycin C, in a rabbit experiment, the cornea was inflamed, with stromal necrosis and marked endothelial loss. Hemorrhagic iris necrosis was also observed.

Human

Hemolytic anemia, thrombocytopenia and renal dysfunction, leading to potentially fatal hemolytic uremic syndrome, can occur in patients given tamoxifen with or shortly after mitomycin C. Mitomycin C is contraindicated in patients with preexisting myelosupression and anemia.

Vinblastine and vindesine can increase the pulmonary toxicity of mitomycin C. Severe and life-threatening bronchospasms and two cases of fatal acute respiratory failure have been reported.

Chronic Toxicity (or Exposure)

Animal

Mitomycin C is a carcinogen (sarcomas and other cancers) in mice and rats after intraperitoneal, intravenous, or subcutaneous injections. It is teratogenic in mice (including skeletal defects). It is nephrotoxic. It is mutagenic in sister chromatid exchange assays.

Human

Intravenous administration of mitomycin C has resulted in dyspnea and lung fibrosis. Other effects include dermatitis, nausea, myelosuppression, fever, malaise, and glomerular damage. On rare occasions, interstitial pneumonitis, hemolytic uremic syndrome, and pulmonary fibrosis have occurred. It is a nephrotoxin and a male reproductive toxin. Mitomycin C causes alopecia and pulmonary damage and can cause severe tissue damage if it escapes from vasculature.

Serious and potentially life-threatening intravascular hemolysis and kidney failure may develop after long-term use of mitomycin and fluorouracil. The International Agency for Research on Cancer concluded that antimony trioxide was possibly carcinogenic to humans (group 2b) on the basis of the animal studies noted above.

In Vitro Toxicity Data

Mitomycin C has exhibited mutagenic properties *in vitro*. It induced chromosomal aberrations in drosophila oocytes and dominant and recessive mutations in wasp habrobracon.

Clinical Management

Early withdrawal of the drug and administration of corticosteroids appear to significantly improve the outcome. Topical application of dimethyl sulfoxide may be effective for management of mitomycin C extravasation.

Miscellaneous

Drug Interactions

Aclarubicin The bone marrow depressant effects of aclarubicin can be increased by previous treatment with mitomycin.

Doxorubicin (adriamycin) An increased incidence of late onset congestive heart failure has been seen in patients treated with mitomycin that had previously been given doxorubicin.

See also: Kidney; Reproductive System, Male; Sister Chromatid Exchanges; Toxicity Testing, Mutagenicity.

Further Reading

Bradner WT (2001) Mitomycin C: A clinical update. *Cancer Treatment Reviews* 27(1): 35–50.

Dorr RT and Frity WI (eds.) (1980) *Cancer Chemotherapy Handbook*. New York: Elsevier.

Mixtures, Toxicology and Risk Assessment

Glenn Rice, Linda K Teuschler, Jane Ellen Simmons, and Richard C Hertzberg

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Human health risk assessment of chemical mixtures is the process of evaluating the biological consequences of human exposures to groups of chemicals. This entry provides a broad overview of chemical mixtures risk assessment, introducing fundamental concepts and describing approaches for evaluating the risks mixtures pose. Here, a mixture is defined as a combination of two or more chemicals that influences a population's risk of chemical toxicity. We note that exposures to mixtures could occur at different times and through different routes; chemicals forming a mixture may originate from different sources. We encounter many different mixtures in our environment; for example, there may be mixtures of small quantities of dioxins in our food. Exposures to some of these mixtures can be deleterious to our health. Identifying these potentially harmful mixtures and quantifying the risks they pose are among the goals of mixture risk analysis. While we focus on the evaluation of human health risks, most of the concepts are applicable to ecological risk assessment. For a detailed treatment of mixtures risk assessment, read the US Environmental Protection Agency's (EPA's) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures or the Health Council of the Netherlands Exposure to Combinations of Substances: A System for Assessing Health Risk.

Basic Mixtures Concepts

For environmental health hazards, we typically rely on the US National Research Council's Risk Assessment Paradigm to guide the evaluation of whether or not a risk to human health is posed. This Paradigm is composed of a series of interconnected processes, including hazard identification, dose–response assessment, exposure assessment, and risk characterization. Mixtures are evaluated through the same processes used in single chemical risk assessment, but with ancillary considerations such as interactions between compounds, changes in the composition of the mixture over time, and the similarity between mixtures that have been tested toxicologically and the mixtures we encounter in our environment.

Specific terms are used to describe potential interactions between and among the chemical components of mixtures. Additivity implies that the effect caused by a mixture can be estimated directly from the sum of the scaled exposure levels (dose addition) or the sum of the risks (risk or response addition) of the individual components in the mixture. When evaluating the risk posed by a mixture, it is initially assumed that the components act in an additive manner. Mixtures producing risks greater than expected from additivity are described as synergistic, those producing risks less than expected from additivity are described as antagonistic. The designation of synergism and antagonism, therefore, depends on the definition of additivity.

Assessing exposures to mixtures can be complex. The initial composition and physical characteristics of a mixture can differ depending on how the mixture is produced; even the composition of mixtures produced by the same general process can differ dramatically (e.g., the components formed in chlorinated drinking water vary by temperature and source of the water). Once released into the environment the composition of a mixture typically changes. Some components may be selectively retained in one environmental medium (e.g., certain dioxin congeners released from the stacks of combustion sources appear to be selectively taken up and retained in plant tissues. See the work of Lorber). Cacela shows that other components such as polychlorinated biphenyls may be transformed by biological, chemical, or physical processes in the environment. These differences in the composition of mixtures result in differences in the exposures experienced by the population, potentially resulting in different biological consequences. Given the large number of mixtures and the variability of their components, we cannot test the toxicity of every mixture to examine the biological consequences.

The term 'similar mixtures' describes test mixtures that differ slightly in composition from an environmental/toxicological mixture of interest, but that are expected to share comparable environmental fates, and toxicokinetic and toxicodynamic processes. Similar mixtures may have the same components but in slightly different proportions, or have most components in nearly the same proportions with only a few different (more or fewer) components than an environmental mixture of interest. Risk assessors judge whether or not the toxicity of a test mixture is sufficiently similar to that of the environmental mixture of interest. If judged to be 'sufficiently similar', then the toxicity exhibited by the test mixture is used as a surrogate for the environmental mixture of interest (i.e., the biologic consequences of exposure to the two mixtures are judged indistinguishable).

Chemical Mixtures Toxicology

Chemical mixtures toxicity test strategies may analyze a whole mixture, a defined mixture, or individual mixture components. Samples of whole mixtures may be comprised of both known and unknown component chemicals, where the fraction of the mixture mass represented by known chemicals may often be smaller than that of the unknown chemicals. The whole-mixture approach offers a considerable advantage because it captures the toxicity of all of the components and all interactions between and among the known and unknown component chemicals. However, toxicological evaluation of the whole mixture is often fraught with technical difficulties. As concentration of environmental mixtures is typically required prior to conducting a toxicologic evaluation, careful consideration must be given to the environmental realism of the resulting sample. Have toxicologically important chemicals been lost or chemical artifacts been introduced into the sample during the concentration process? Are the relative proportions (the mixing ratios) of the resulting concentrate similar to those in environmental samples? Is the sample matrix compatible with the biological test system? Given that careful attention must be paid to sample quality, and sample preparation is often time-consuming and costly, toxicological evaluation of complex environmentally realistic mixtures may best be focused on those classes of mixtures, such as mixtures resulting from chlorination of drinking water, for which there is widespread human exposure, significant portions of the mixture mass are unknown and there is some, even if limited, epidemiologic information suggestive of adverse human health effects.

The toxicological testing of defined mixtures includes the preparation of chemical mixtures by adding individual components known to comprise whole mixtures. The component concentrations are designed to mimic the component ratios observed in whole mixtures (or a range of such ratios). The defined mixture approach has suffered historically from lack of experimental designs and corresponding statistical analysis techniques that compensate for the fact that traditional full-factorial experimental designs become technically infeasible as the number of both component chemicals and dose levels increase. Increasingly, less-than-full factorial designs, such as those described by Teuschler, are being developed and used. Toxicological evaluation of defined mixtures is most useful when: the research effort targets data collection either on mixtures of component chemicals that frequently occur together in the environment or on toxicologically significant combinations of chemicals; the experimental design includes environmentally-relevant mixing ratios; the experimental design includes data points at the low end of the dose–response curve; and, efficient experimental designs are employed and predictive modeling is incorporated into the study.

Toxicity tests conducted on individual components of whole mixtures also provide useful information to risk assessors. These tests may be conducted in isolation, in combination with other components of the mixture, or as an element of a defined-mixture study. Individual component approaches can be used to examine the assumptions underlying component-based risk assessment methods described in the next section. Toxicity tests on individual components are less expensive and faster than whole mixture and defined mixture approaches but lack information on interactions and unknown components.

Mixtures Risk Assessment Methods

When possible, risk assessors prefer to make assessments using epidemiologic or toxicologic data on the environmental mixture to which people are exposed. A second approach, described above, uses data from a test mixture judged to be sufficiently similar to the environmental mixture. A third option is to use data from a group of sufficiently similar mixtures; for example, a group of similar mixtures could be generated by the same commercial process or from similar emissions sources (e.g., diesel engines from emergency generators or from off-road vehicles). These mixtures may be readily available for testing, but may vary in composition. Based on toxicity test results from any of these three sources of whole mixtures, a safe level (e.g., a reference dose (RfD)) could be developed from an experimental no-observed-adverse-effect level or a benchmark dose, as could a cancer risk slope.

Component methods include those based on the assumption of response addition (e.g., addition of probabilistic cancer risks) or dose addition (e.g., relative potency factors (RPFs), hazard indexes (HI)). The advantages of component methods include an ability to utilize single chemical exposure and dose–response information to estimate a mixture risk and the flexibility to compare mixtures containing the same chemicals, but in different concentrations and proportions.

The RPF method is based on dose addition and assumes that the chemicals in a mixture share a common toxic mode of action; this means that when tested in the same bioassay, the dose response curves of each component should be similarly shaped. The components in this 'similarity group' are assumed to be true 'toxicologic clones' of each other and have

similar toxicokinetics, so that isoeffective doses differ by a fixed proportionality constant. The RPF method also requires the identification of an index chemical (IC). The IC is the mixture component that best represents the toxicity of the other members of the mixture and preferably has the highest quality dose–response information.

In the RPF method (eqn (1)), the user must identify the constraints of the application of a set of RPFs. For example, the health effect, dose range of component doses, route(s) of exposure, and duration(s) of exposure for which the RPFs can be applied must be specified (e.g., a set of RPFs may be constrained to oral exposures and not be used for exposures to the same mixture through the inhalation route). To apply the method, an RPF is estimated for each mixture component; the RPF estimates the toxicity of the component relative to that of the IC. RPFs commonly are estimated from a ratio of equally toxic doses of the individual dose–response functions for the component and the IC. For example, the quotient of the effective dose at which ten percent of a test population exhibits an effect (ED_{10}) of the IC and the component could serve as a value for the component's RPF; obviously, the RPF for the IC equals 1. The index chemical equivalent dose of an individual component is the product of the component dose and the RPF of the component. These equivalent doses are summed across all components. The risk posed by the mixture is estimated by comparing the summed index chemical equivalent doses of the mixture to the dose–response function of the IC:

$$R_{\mathrm{m}} = f_1\left(\sum_{i=1}^{n} \mathrm{RPF}_i \times D_i\right) \qquad (1)$$

where R_{m} is the risk posed by chemical mixture, f_1 (*) is the dose–response function of index chemical, D_i is the dose of the ith mixture component ($i = 1,\ldots,n$), and RPF_i is the toxicity proportionality constant relative to the index chemical for the ith mixture component ($i = 1,\ldots,n$). n is the number of mixture chemicals in the similarity group.

The hazard index, as described by Svensgaard, is another commonly used component method based on dose addition, where the component doses are scaled by their relative toxicity and then summed. HI serves to indicate concern rather than predict risk. When HI is less than 1, the mixture exposure is usually considered to pose no appreciable risk. Typically, the inverse of an acceptable, safe level (e.g., an RfD) is used to scale the component's toxicity. As described by the US EPA in 1989, the HI formula for oral exposures using RfDs appears in eqn (2):

$$\mathrm{HI} = \sum_{i=1}^{n} \frac{E_i}{\mathrm{RfD}_i} \qquad (2)$$

where HI is the hazard index, E_i is the oral exposure dose for the ith chemical, and RfD_i is the reference dose for the ith chemical. E_i and RfD_i have the same units and n is the number of chemicals in the mixture.

A more general component formula can be applied to mixtures of synergistic or antagonistic chemicals. US EPA (2000) describes a formula incorporating available toxicological interaction studies for pairs of component chemicals, based on the formula in eqn (2). For each chemical pair, a determination is made of the weight-of-evidence (WOE) that an observed toxicological interaction is relevant to human risk. The WOE classification is converted to a score that is used along with the component doses to estimate the mixture response. The formula for this interaction-based HI ($\mathrm{HI}_{\mathrm{INT}}$) is in eqn (3).

$$\mathrm{HI}_{\mathrm{INT}} = \sum_{j=1}^{n}\left(\mathrm{HQ}_j * \sum_{k\neq j}^{n} f_{jk} M_{jk}^{(B_{jk}g_{jk})}\right) \qquad (3)$$

where B_{jk} is the WOE interaction score for the influence of chemical k on chemical j, M_{jk} is the interaction magnitude of the influence of chemical k on chemical j, f, and g are functions of the component exposure levels, and HQ_j, the hazard quotient (equal to E_i/RfD in eqn (2)), represents the toxicity contributed by the jth component chemical if there were no interactions.

This formula adjusts each chemical's toxicity by the information on pairwise toxicological interactions involving that chemical. ATSDR has posted several interaction profiles with WOE determinations. If no interactions existed, then the second sum is always 1 and the formula reduces to the HI formula based on dose addition given in eqn (2); Hertzberg provides additional details about this equation.

While it appears complicated, this formula is the sum of chemical toxicities adjusted by pairwise interactions. Because there is no extrapolation parameter that can be gradually varied to move from the component and pairwise data to the mixture response, this interaction formula can be viewed as an extrapolation from dose addition to the mixture response. Comparing eqns (2) and (3), the pairwise interactions can be viewed as 'correction steps' roughly accounting for all the interactions (pairwise, three-way, etc.) in the mixture. (See the work of Hertzberg). As long as each pairwise interaction magnitude is fairly small, the estimated mixture

response will be a minor extrapolation away from dose addition. Few interaction studies attempt to quantify the interaction magnitude. Among those that do, the magnitude, expressed as a change in effective dose, is usually less than fivefold; the US EPA (2000) correspondingly set the default interaction magnitude at 5. This default magnitude limits the interaction-based HI to five-fold change in the additive HI. The strongest influence of the interactions will be when the evidence is excellent so the WOE scores are highest ($B = 1$), when all the pairs are at equitoxic levels (so every $g = 1$), and when all interactions are in the same direction. For example, if all pairwise interactions were synergistic, then the resulting calculation gives a maximum interaction-based index of $HI_{INT} = 5^{*}HI$.

Note that the interaction WOE score is based on judgment: the weaker the evidence, the closer the score is to zero, and the less impact the interaction has on the estimated mixture response. For most mixtures, no data exist on the whole mixture, so that accuracy checks are usually not possible. As a consequence, the quality of the component-to-mixture extrapolation is then judged by the quality and relevance of both the component data and pairwise interaction data.

See also: Aggregate Exposures; Dose–Response Relationship; Risk Assessment, Ecological; Risk Assessment, Human Health.

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Mode of Action

Lynne Haber

Mode of action refers to how a chemical exerts its toxic effects. Its meaning is similar to 'mechanism of toxicity' or 'mechanism of action'. Some scientists use the terms interchangeably, while others draw distinctions, with 'mechanism' indicating a more detailed understanding. For example, the International Programme on Chemical Safety (IPCS) framework for evaluating the mode of action for chemical carcinogenesis states "a supported *mode of action* would have evidence provided by robust mechanistic data to establish a biologically plausible explanation. *Mechanism of action*, in contrast, relates to sufficient understanding of the molecular basis to establish causality; it is at the other end of the continuum from little or no evidence of *mode of action* to scientific proof of *mechanism of action*."

The US Environmental Protection Agency (EPA) focuses more on the level of detail, rather than the degree of scientific support, in distinguishing mechanism and mode of action, stating that mechanism of action is used to imply a detailed understanding, often at the molecular level, of how a chemical exerts its toxic effect, while mode of action refers to a more general understanding of the process. The US EPA defines mode of action as "a sequence of key events starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation."

Although the term mode of action appears sometimes in the context of noncancer effects, it finds particular use in the context of cancer risk assessment, where mode of action forms the basis for: (1) determining whether tumors observed in animals are relevant to humans, and (2) determining the approach for quantitative cancer risk assessment. IPCS has developed a conceptual framework for evaluation mode of action for chemical carcinogenesis, based partly on a modification of the Bradford–Hill criteria for causality. A similar approach is used by the US EPA. Under this framework, each mode of action is analyzed separately, noting that multiple modes of action may contribute to the development of a given tumor type, and that a single chemical may cause tumors in different tissues by different modes of action. This framework includes:

1. Introduction – identifies the observed cancer endpoints, and which one(s) are being addressed in the analysis.
2. Postulated mode of action – brief description of the sequence of events on the path to cancer.
3. Identification of the 'key events', measurable events that are critical to the induction of tumors as hypothesized in the mode of action.
4. Dose–response relationship – evaluation of whether the dose–response for the key events parallels the dose–response for tumors.
5. Temporal association – evaluation of the sequence of events, and whether the key events precede the tumor response.
6. Strength, consistency, and specificity of association of tumor response with key events – discussion of the weight of evidence linking the key events, precursor lesions, and tumors.
7. Biological plausibility and coherence – consideration of whether the postulated mode of action is consistent with current understanding of carcinogenesis in general (biological plausibility) and the specific chemical (coherence).
8. Other modes of action – discussion of alternative modes of action that logically present themselves.
9. Assessment of postulated mode of action – overall conclusion, with the level of confidence in the postulated mode of action.
10. Uncertainties, inconsistencies, and data gaps.

There are a variety of possible carcinogenic modes of action. The following examples of modes of action are not intended to be exhaustive, but they do provide examples of the major modes of action relevant to carcinogenesis:

- Mutagenicity – interacting with DNA to cause DNA changes that can be inherited by daughter cells. The reaction with DNA may be direct or indirect.
- Mitogenesis – stimulation of cell division. Examples include disruption of hormone homeostasis and interfering with the cell signaling pathways.
- Inhibition of cell death. Programmed cell death (apoptosis) is used by organisms to eliminate damaged cells. Inhibition of this process can allow cells with damage to the cell cycle control system to proliferate.
- Cytotoxicity with reparative cell proliferation.
- Immune suppression. Decreased activity of the immune cells that monitor for damaged cells (NK, or natural killer cells) can allow the damaged cells to proliferate.

Once a chemical's mode(s) of action has been identified, the risk assessor can proceed with the evaluation of carcinogenic potential, including consideration of the relevance of the mode of action to humans. Further consideration of mode of action distinguishes between a genotoxic (or sometimes more rigorously described as DNA-reactive) mode of action, for which there may be some risk at every nonzero dose, and nongenotoxic (or nonmutagenic) modes of action, for which threshold or nonlinear dose–response curves may apply.

See also: Carcinogenesis; Risk Assessment, Human Health.

Further Reading

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Models *See* Animal Models.

Modifying Factors of Toxicity

Frank C Lu and Sam Kacew

The nature and extent of the toxic manifestations in an organism that is exposed to a toxic substance depend on a variety of factors. Exposure to a substance in certain instances produces a reaction in one individual but not in another. While there may be a difference in sensitivity between these individuals, it is also possible that the responsive person was previously exposed (i.e., this individual is actually subjected to a higher concentration of chemical). Two of the factors known to modify the toxic consequences of exposure to chemicals are the dose and duration. This section will focus on the modification of toxicity by other factors including the species and strain of the animal, its sex and age, and its nutritional and hormonal status. Various physical factors also play a part. In addition, the toxic effect of a chemical may be influenced by simultaneous and consecutive exposure to other chemicals. The toxic effects may be modified in a number of ways: alteration of the absorption, distribution, or excretion of a chemical; an increase or decrease of its biotransformation; and changes of the sensitivity of the receptor at the target organ.

It should be remembered that the outcome of adverse effects predicted in humans is based on data generated in animals since drugs released on the market for prescription in humans must be tested on animals. Hence, a clear understanding of the existence of these factors and of their mode of action is important in designing the protocols of toxicologic investigation. It is equally important in evaluating the significance of the toxicologic data and in assessing the safety/risk to humans under specified conditions of exposure. This is not always possible, however, because in certain circumstances the testing in animals is improper (i.e., a test for toxicity is carried out in an animal, but the human responds differently). The use of thalidomide in pregnant women as a sedative resulted in fetal malformations in humans, but this drug did not produce these effects in rats. Another example of a lack of specific conditions of human exposure is related to a long latency period. Pregnant mothers were administered diethylstilbesterol to prevent miscarriage, only to discover that 20 years later female offspring developed vaginal cancer. An industrial accident involving the release of methyl isocyanate, an intermediate in carbaryl synthesis, by Union Carbide in Bhopal, India, resulted in toxicity and death. The toxicological profile of methyl isocyanate was not known, and the chemical was released under uncontrolled conditions. Industrial accidents by definition are not specified conditions of exposure but must be borne in mind as a factor to consider. With increased knowledge of chemicals and the factors that affect these chemicals, the ability to assess and react to adverse conditions is enhanced.

Modifying Factors

Species

Differences of adverse effect from one species to another have long been recognized. Knowledge in this field has been used to develop, for example, pesticides, which are more toxic to pests than to humans and other mammals. Among various species of mammals, most effects of toxicants are somewhat similar. This fact forms the basis for predicting the toxicity to humans from results obtained in toxicologic studies conducted in other mammals, such as the rat, mouse, dog, rabbit, and monkey. There are, however, notable differences in toxicity even among mammals.

Some of these differences can be attributed to variations in detoxication mechanisms. For example, the loss of consciousness induced in several species of laboratory animals by hexobarbital (a barbiturate derivative that depresses the central nervous system (CNS)) shows marked differences; these are attributable to the activity of the detoxication enzyme that inactivates this chemical. In the mouse, the activity of the detoxifying enzyme is 16-fold greater than that in the dog, which is reflected by 12 min of hexobarbital-induced sleep in the mouse versus 315 min of sleep in the dog. There are other examples of species-related differences in the ability to detoxify chemicals that consequently result in differences in toxicity. Other examples include the industrial chemicals, ethylene glycol and aniline. Ethylene glycol is metabolized to oxalic acid, which is responsible for its toxicity, or to carbon dioxide. The rank order of ethylene glycol toxicity in animals is as follows: cat $\geqslant$ rat $\geqslant$ rabbit; this is the same for the extent of oxalic acid production. Aniline is metabolized in the cat and dog mainly to *o*-aminophenol, and these species are more prone to toxicity; however, in the rat and hamster aniline is metabolized mainly to I-aminophenol and thus these species are less susceptible to aniline toxicity.

A more serious example in which animal data are of little clinical relevance is the release of butadiene gas in the production of rubber products.

Butadiene is converted to butadiene monoepoxide, which is believed to be responsible for carcinogenesis in rodents but not in humans. In humans, butadiene monoepoxide is further converted to butenediol and conjugation with glutathione results in no toxicity. In rodents, however, there is direct conjugation of butadiene monoepoxide with glutathione, which presumably is not adequate, and thus cancer initiation occurs. In this example, it can be seen that rodent data are a poor indicator for prediction of risk in humans because the detoxification pathways differ.

Differences in bioactivation also account for dissimilarities in toxicity. A notable example is 2-naphthylamine, which can produce bladder tumors in the dog and human but not in the rat, rabbit, or guinea pig. Dogs and humans, but not the others, excrete the carcinogenic metabolite 2-naphthyl hydroxylamine. Acetylaminofluorene (AAF) is carcinogenic in many species of animals but not in the guinea pig. The *N*-hydroxy metabolite of AAF, however, is carcinogenic to all animals including the guinea pig, demonstrating that the difference between the guinea pig and the other animals is not in their response to the toxicant but in the bioactivation, that is, the guinea pig lacks the ability to form the toxic metabolite.

There are other factors, including absorption, distribution, and excretion of chemicals, to consider in trying to understand differences in toxicity between individuals. Paraoxon is the active metabolite of the organophosphate insecticide parathion. Hence, a difference in the rate of formation of this metabolite is attributable to a difference in toxicity. This has been reported in rats, in which females are more susceptible to parathion neurotoxicity than males and in which paraoxon formation occurs faster in the female resulting in greater toxicity in this sex. This phenomenon has been widely studied in humans with the drug isoniazid. For example, there are 'slow inactivators', who are deficient in acetyltransferase. Such individuals acetylate isoniazid only slowly and are thus likely to suffer from peripheral neuropathy resulting from an accumulation of isoniazid. On the other hand, people with more efficient acetyltransferase, termed 'fast metabolizers', require larger doses of isoniazid to obtain its therapeutic effect. Since large amounts of drug are needed, these individuals are more likely to suffer from hepatic damage caused by isoniazid because isoniazid also acts on the liver.

Strain

It is essential to determine the safety of new pharmaceutical agents for humans using the appropriate mammalian toxicity tests mandated by governmental regulatory agencies (e.g., US Food and Drug Administration and Health and Welfare Canada). This necessitates the use of a species that can be compared to humans for factors such as pharmacokinetics, metabolism, excretion, absorption, and distribution of the test material. The rat is one of the species that has proven to be extremely useful in pharmacologic and toxicologic research because there are many similarities between rat and human metabolic pathways; many anatomical and physiological characteristics are similar, allowing for comparisons in absorption, excretion, and pharmacokinetics. The rat is also of a convenient size, is relatively docile, has a short life span and gestation period, and is economical to maintain. There is a large database of its characteristics which is invaluable in the interpretation of the relevance of animal data for humans.

There are three main classes of rats used in research; these are inbred strains, outbred stocks, and mutants (including transgenic stocks). It is very important that research workers understand the characteristics of these three classes of stock because they may have a profound influence on the quality of their research. For example, outbred stocks such as Wistar rats may be segregating at many genetic loci, which are important in drug metabolism, so that different individuals within a colony will react differently. In many cases it does not make much sense to do detailed pharmacological studies against such a variable genetic background. The characteristics of the three main classes of stock are briefly summarized in the following sections.

Inbred Strains

Inbred strains are produced by at least 20 generations of brother $\times$ sister mating, with all individuals being derived from a single breeding pair in the 20th or subsequent generation (this eliminates parallel sublines). For most practical purposes, an inbred strain can be regarded as an immortal clone of genetically identical individuals. Inbred strains have a number of properties that make them the animal of first choice for most types of research. Because the strain is isogenic (i.e., all individuals are genetically virtually identical), the genotype of the whole colony can be determined at a particular genetic locus by typing a single individual. Many genetic markers are fixed in each strain so that the authenticity of the strain can be determined. This can now be done using DNA markers detected by the polymerase chain reaction. This contrasts sharply with outbred rats, in which currently there are not even any genetic markers that can be used to distinguish between Wistar and Sprague–Dawley stocks.

All inbred animals are homozygous at all genetic loci, so there are no 'hidden' recessive genes that could cause confusion in experiments involving breeding. As a result of this homozygosity, the strain stays genetically constant for many generations. This is valuable because it makes it possible to build up background data on genetic characteristics that should remain valid for a long period of time. Of course, the phenotype (but not the genotype) may alter if the diet, environment, or associated microorganisms change. However, over a period of several generations, an inbred strain will remain much more constant than an outbred stock.

The isogenicity and homozygosity together tend to lead to greater phenotypic uniformity of inbred animals. This is important because greater uniformity leads to more statistically powerful experiments that are able to detect a given biological effect with fewer animals. The degree of the contrast with outbred stocks depends on the character being studied. Clearly, for characters controlled by a single or small numbers of genetic loci, such as the major histocompatibility complex or the drug metabolizing enzymes, animals within an inbred strain will be uniform, whereas animals of an outbred stock will usually not. However, the greater uniformity of inbred animals may not be apparent for characters such as body and organ weights (which also depend on environmental and chance factors), unless very large numbers are studied.

Each inbred strain has its own unique pattern of behavior, growth patterns, reproductive performance, spontaneous disease (including tumors), and response to xenobiotics. Differences between strains are an indication that the observed character is under genetic control. Currently, there are over 200 inbred rat strains.

Outbred Stocks

So-called 'outbred stocks' are usually maintained as closed colonies of rats of undefined genotype and sometimes known by generic names, such as Wistar, Sprague–Dawley, or Long–Evans, which indicate their historical origin. The amount of genetic variation present in any given colony depends on its history. At one extreme, if the colony has been maintained as a closed colony for many years with small numbers of breeding animals each generation, it may be genetically highly uniform to the extent that it will closely approximate an inbred strain. If the colony has become inbred, it may have gone through a period of rapid genetic drift so that it will differ from other colonies with the same historical origin. At the other extreme, a colony that has recently been crossed to an unrelated stock should be genetically highly variable.

Mutants or Transgenics

Over 300 genetic loci associated with mutants and polymorphisms of various sorts have been described in the rat. A mutant can be created by insertion of DNA from an external source, be it a virus, recombinant DNA, etc. The inserted DNA is referred to as a transgene and the recipient host a transgenic animal, and the methodology for insertion of DNA is termed transgenic technique. The sequence of DNA in the transgene can be similar to sequences already present in the host or the sequence can be different. If the inserted DNA sequence is not novel, the new mutant created is termed a 'knockout' mutant or animal. Some of these, such as the polymorphisms associated with drug metabolizing enzymes and mutants such as acholuric jaundice (widely known as the 'Gunn' rat) and the Rowett, a thymic nude, are important in pharmacological and toxicological research. Recently, 'mutants' such as the 'Big Blue' rats have been produced using transgenic techniques. Transgenic and knockout rats produced by gene targeting techniques are likely to be of increasing importance in toxicological research. Mutants and transgenes can be placed on any genetic background by suitable breeding techniques. Thus, the jaundice gene from the Gunn rat is available on the inbred ACI, LEW, R/A, and RHA genetic background, as well as on a number of outbred genetic backgrounds. For this reason, it would be incorrect to discuss drug metabolism in 'the Gunn rat' (or any other mutant or transgenic) without specifying its genetic background because drug metabolism will depend on many genes in addition to the specific locus that is abnormal in the Gunn rat.

Choice of Strain in Research and Screening

There seem to be no serious disadvantages (apart from cost) and many advantages in the use of inbred strains rather than outbred stocks in academic research. These animals offer the nearest equivalent to pure reagents that is possible when using animals in research, particularly if they are also of a high health status. In disciplines other than toxicology, there has been a relentless trend toward the increased use of inbred strains. It is not entirely clear why their use is not more widespread in toxicological research. Any disadvantage in terms of initial cost should be amply compensated for by improved research quality and the need for fewer animals. In toxicological screening

the relative merits of inbred strains versus outbred stocks has been debated for more than 50 years without reaching a consensus. An inbred rat strain F344 is used in the National Toxicology Program Carcinogenesis Bioassay, but most commercial screening is done using outbred stocks.

Sex

Male and female animals of the same strain and species usually react to toxicants similarly. It must be borne in mind, however, that there are marked differences in the hormonal makeup between sexes, and this can result in notable differences in responses. Chloroform produces damage to liver and kidney in humans and mice. In mice, however, chloroform produces nephrotoxicity only in males. Furthermore, administration of testosterone (male hormone) to the female mouse followed by chloroform results in kidney damage. Clearly, there are androgen (male) receptors in the kidney that sensitize males to chloroform-induced nephrotoxicity. In rats, exposure to the hydrocarbon decalin results in a renal nephropathy and tumor formation in the male but not female, and this is associated with an α_2-globulin protein accumulation. Treatment of females with testosterone followed by decalin also produces renal toxicity and protein accumulation. These examples demonstrate that kidney function differs between the sexes and, consequently, toxic manifestations will vary between males and females.

There are metabolic differences between the sexes. Many barbiturates induce more prolonged sleep in female rats than in males. The shorter duration of action of hexobarbital in male rats is related to the higher activity of the liver microsomal enzymes stimulated by testosterone to hydroxylate this chemical. This higher activity can be reduced by castration or pretreatment with estrogen (female hormone).

Female rats are also more susceptible than males to such organophosphorus insecticides as azinphosmethyl and parathion. Castration or estrogen treatment of the male reverses this difference. The male rat is far more susceptible to carcinoma than the female as shown in the following examples: Males are more susceptible to the induction of pancreatic tumors by azaserine, colonic carcinoma by dimethylhydrazine, intestinal tumors by dimethylnitrosamine, renal tumors by decalin, and liver cirrhosis by AAF. In the case of hydroquinone, which is present in photographic material, acute exposure produced renal toxicity in the female; but in a chronic 2 year study, the male and not the female was found to have tubular degeneration and adenoma.

Imbalances of nonsex hormones can also alter the susceptibility of animals to toxicants. Hyperthyroidism, hyperinsulinism, adrenalectomy, and stimulation of the pituitary–adrenal axis have all been shown to be capable of modifying the effects of certain toxicants. One of the functions of thyroid hormone involves the maintenance of normal heart activity; in hyperthyroidism, however, there is tachycardia and hypertension. In normal circumstances, ingestion of caffeine, which is a cardiac stimulant, does not affect heart function but large doses of caffeine produce cardiac arrhythmias. It is thus evident that a hyperthyroid patient drinking excess coffee would be more prone to cardiac dysfunction than a normal individual. Hyperinsulinism is manifested by a hypoglycemic coma through a depletion of carbohydrate stores and lack of CNS energy supply. The insecticide DDT in toxic doses is known to produce CNS excitability, tremors, and convulsions and is associated with carbohydrate store depletion. Thus, it can be seen that in conditions of hyperinsulinemia, exposure to DDT or the heavy metal cadmium, which acts in a similar fashion, results in a greater sensitivity of the CNS to toxicity.

Age

The pharmacokinetic principles applied in pediatric drug therapy are, in general, similar to those utilized for adults. Data obtained in adult studies, however, are not always applicable to rational therapy in infants or young children. The infant must be regarded as a distinct organism (not a small adult), and lack of appreciation of this fact can result in serious harm and potentially in death.

A number of important characteristics exist that distinguish drug therapy in infants from adult medication protocols. For example, after intramuscular administration, drug absorption is partially dependent on blood flow in the muscle bed. Abnormal drug absorption following intramuscular injection can occur in premature infants, in whom muscle mass is small and blood flow to the musculature is poor. Examples of adverse effects attributed to altered drug absorption are the reactions of infants to cardiac glycosides and anticonvulsants.

In the infant, absorption from the gastrointestinal tract of an orally administered drug differs from that in adults. Certain toxicants are absorbed to a greater extent by the young than by the adult. For example, young children absorb four or five times more lead than adults and 20 times more cadmium. In both adults and infants, the rate and extent of drug absorption depend on the degree of ionization, which, in turn, is influenced by pH. Within the first

24 h of life, gastric acidity increases rapidly, and this is followed by an elevation in alkalinity over the next 4–6 weeks. These conditions result in drugs existing in the infant gastrointestinal tract in states of ionization other than might be observed in adults. The higher incidence of methemoglobinemia in young infants has been explained on the basis that their lower gastric acidity allows upward migration of intestinal microbial flora and the reduction of nitrates to a greater extent. Furthermore, infants have a higher proportion of fetal hemoglobin, which is more readily oxidized to methemoglobin. Other factors that modify gastrointestinal drug absorption in the young infant include an irregular neonatal peristalsis, a greater gastrointestinal tract surface to body ratio, and enhanced β-glucuronidase activity in the intestinal tract. The significance of the β-glucuronidase is that it converts drug-bound glucuronide to the free form and thus increases drug bioavailability.

Differences exist in the organ distribution of drugs between newborns and adults. The greater susceptibility of the young to morphine is attributable to a less efficient blood–brain barrier. In the newborn, a higher percentage of body weight is represented by water, so extracellular water space is proportionally larger. To initiate a receptor response, the distribution of drugs must occur predominantly in the extracellular space, so the amount of drug reaching the receptor sites is higher in neonates. Furthermore, the ability of newborn infants to bind drugs in plasma is significantly less than that in adults. This again suggests that neonates could be expected to be more susceptible to the effects of drugs. Differences also exist with respect to drug-metabolizing enzymes. It has been clearly demonstrated that the drug inactivation rate is generally slower in newborns. The available information indicates that the greater susceptibility of the young animals to many toxicants can be attributed to deficiencies of various detoxication enzyme systems. Both phase I and phase II reactions may be responsible. For example, hexobarbital at a dose of $10\,mg\,kg^{-1}$ induced a sleeping time of longer than 360 min in 1-day-old mice compared to 27 min in the 21-day-old mice. The proportion of hexobarbital metabolized by oxidant in 3 h in these animals was 0% and 21–33%, respectively. On the other hand, chloramphenicol (an antibiotic) is excreted mainly as a glucuronide conjugate. When a dose of $50\,mg\,kg^{-1}$ was given to 1- or 2-day-old infants, the blood levels were $15\,\mu g\,ml^{-1}$ or higher over a period of 48 h. In contrast, children aged 1–11 years maintained such blood levels for only 12 h.

Not all chemicals, however, are more toxic to the young. Certain substances, notably CNS stimulants, are much less toxic to neonates. The acute toxicity of DDT was reported more than 20 times smaller in newborn rats than in adults, in sharp contrast to the effect of age on malathion.

Furthermore, the ability of the neonate to eliminate drugs via the kidney, the major excretion pathway, is significantly limited by the state of development of these organs. Penicillin and tetracycline (two antibiotics) are excreted more slowly and hence are more toxic in the young. Consideration of these factors indicates that the susceptibility and responsiveness of newborns to drug therapy are different from those of adults.

Old animals and humans are also more susceptible to certain chemicals. This problem has not been studied as extensively as in the young. The available evidence indicates that the aged patients are generally more sensitive to many drugs. A prime example is the use of antibiotics to treat infections in geriatric patients. Since the detoxification of antibiotics is dependent on renal clearance, which is generally slower in the aged, drug accumulation and toxicity are higher in the older patient. The possible mechanisms include reduced detoxication and an impaired renal excretion. In addition, the distribution of chemicals in the body may also be altered because of increased body fat and decreased body water. A number of drugs have been found to be likely to induce more severe signs of toxicity. These include most CNS depressants, certain antibiotics, cardiac glycosides, and hypotensive agents.

Pregnancy

During the course of a pregnancy a mother is likely to take a number of drugs for therapeutic reasons. In addition, with many more women in the work force, there is an increased potential of exposure to a variety of chemicals under occupational conditions. Furthermore, a large number of women indulge in a variety of recreational chemicals including cigarettes and alcohol. The consequences attributed to exposure to a pharmaceutical product can be advantageous to the mother; however, in many instances the effects are deleterious to the fetus. Exposure to occupational and/or environmental chemicals is more likely to result in adverse effects than to be beneficial. Thus, it may be stated that the fetus is at some jeopardy as a result of exposure to foreign chemicals.

Nutrients essential for fetal growth and development require an active transport system to be moved from the maternal circulation to the fetal circulation against a concentration gradient. By contrast, drugs and other chemicals cross the placenta by simple diffusion. The amount of a chemical that is transferred to the fetus is dependent on lipid solubility,

the degree of ionization, and the molecular weight. Lipophilic chemicals tend to diffuse across the placenta readily, while highly ionized compounds penetrate the placental membrane slowly. The molecular weight of a chemical affects placental transfer, with the larger molecules crossing the placental barrier less readily. Protein binding of a chemical or its metabolites will affect the rate and the amount transferred to the fetus. Exposure of fetal target tissues to chemical entities may also be influenced by metabolism in the placenta or the fetal liver.

An important component to consider is the stage of fetal development at the time of chemical exposure. During the first week of development after fertilization, the embryo undergoes the process of cleavage and gastrulation. Exposure to chemicals or drugs such as antimetabolites, ergot alkaloids, or diethylstilbestrol at this stage can result in termination of pregnancy. Organogenesis is the next developmental stage covering weeks 2–8 of gestation. Exposure to drugs or other chemicals including thalidomide, alcohol, and phenytoin during this phase can result in serious structural abnormalities. Drugs and chemicals such as cigarette smoke, heavy metals, or carbon monoxide may affect development during the remaining gestational period ranging from 9 weeks to 9 months. Predominant effects are alteration in the differentiation of the reproductive system and CNS. Consequently, altered brain function and growth retardation are some of the principal adverse effects due to exposure at this stage.

Lactation

The nursing mother can serve as a source of exposure for the neonate to drugs and environmental chemicals. Most drugs are detectable in breast milk regardless of whether they are over-the-counter medication or something prescribed by a physician. In addition, exposure of the nursing mother to environmental pollutants can result in chemical contamination of breast milk. The presence of a drug or chemical in maternal milk may be construed as a potential hazard to the infant even though only 1% or 2% of total intake is likely to be found here. Hence, the primary consideration in maternal drug therapy or exposing a lactating mother to industrial chemicals is the risk to the nursing infant rather than the mere presence of a xenobiotic in the milk.

Several factors play a role in determining the quantity of a drug or chemical that will be transferred to breast milk. The amount of drug or chemical that is actually available for transfer to milk is dependent on certain maternal factors including amount of drug or chemical absorbed, frequency and route of exposure, xenobiotic biotransformation, and protein binding and excretion.

Drug utilization can to a large extent be controlled, so the prudent use of drugs during lactation is imperative because of the potential transfer of these agents or their metabolites into the milk. Certain drugs should be totally avoided during lactation. Certain foods or nutritional supplements have also been shown to cause adverse effects in the infant as a result of lactational exposure.

It is much more difficult to control exposure to environmental chemicals. For example, a mother has no knowledge of what pesticides may have been used on the fruits or vegetables purchased for consumption. Nor is there an easy way to protect oneself from ambient industrial pollution. Some examples of environmental agents known to produce adverse effects in the nursing infant include lead and tetrachloroethylene (dry cleaning solvent).

Nutritional Status

The principal biotransformation of toxicants is catalyzed by the microsomal mixed function oxidase system (MFO). A deficiency of essential fatty acids generally depresses MFO activities. This is also true with protein deficiency. The decreased MFO has different effects on the toxicity of chemicals. For example, hexobarbital and aminopyrine are detoxified by these enzymes and are thus more toxic to rats and mice with these nutrient deficiencies. On the other hand, the toxicity of aflatoxin is lower in such animals because of their depressed bioactivation of this toxicant. MFO activities are decreased in animals fed high levels of sugar.

A number of carcinogenesis studies have demonstrated that restriction of food intake decreases tumor yield. Deficiency of protein generally lowers tumorigenicity of carcinogens, such as aflatoxin and dimethylnitrosamine. It is well known that enzymes, derived from protein, are required to produce reactive, toxic metabolites of aflatoxin or dimethylnitrosamine. Hence, with protein deficiency the toxic metabolite cannot be generated. The importance of diet on carcinogenesis is further demonstrated by the fact that rats and mice fed diets rich in fats have higher tumor incidences compared to those that are given a restricted diet.

Chemical Interaction

The toxicity of a chemical in an organism may be increased or decreased by a simultaneous or consecutive exposure to another chemical. If the combined effect is equal to the sum of the effect of each

substance given alone, the interaction is considered to be additive, for example, combinations of most organophosphorus pesticides on cholinesterase activity. If the combined effect is greater than the sum, the interaction is considered to be synergistic, for example, carbon tetrachloride and ethanol on the liver and asbestos exposure and cigarette smoking on the lung. In the latter example, there can be a fivefold increase in lung cancer incidence among asbestos workers, an 11-fold increase among cigarette smokers, and a 55-fold increase among asbestos workers who are cigarette smokers. The term potentiation is used to describe the situation in which the toxicity of a substance on an organ is markedly increased by another substance that alone has no toxic effect on that organ. For example, isopropanol (a solvent) has no effect on the liver, but it can increase considerably the hepatotoxicity of carbon tetrachloride (another solvent).

The exposure of an organism to a chemical may reduce the toxicity of another. Chemical antagonism denotes the situation wherein a reaction between the two chemicals produces a less toxic product, for example, chelation of heavy metals by dimercaprol. Functional antagonism exists when two chemicals produce opposite effects on the same physiologic parameters, such as the counteraction between CNS stimulants and depressants. Competitive antagonism exists when the agonist and antagonist act on the same receptor, such as the blockade of the effects of nicotine on ganglia by ganglionic blocking agents. Noncompetitive antagonism exists when the toxic effect of a chemical is blocked by another not acting on the same receptor. For example, atropine reduces the toxicity of acetylcholinesterase (AChE) inhibitors not by blocking the receptors on the AChE, but by blocking the receptors for the ACh accumulated.

Chemical interactions are achieved through a variety of mechanisms. For instance, nitrites and certain amines can react in the stomach to form nitrosamines, the majority of which are potent carcinogens, and thus greatly increase the toxicity. On the other hand, the action of many antidotes is based on their reactivity with the toxicants; for example, thiosulfate is used in cases of cyanide poisoning. Furthermore, a chemical may displace another from its binding sites on plasma protein and thereby increase its effective concentration. A chemical may modify the renal excretion of weak acids and weak bases by altering the pH of urine. Competition for the same renal transport system by one chemical can hinder the excretion of another. A notable example is the administration of the drug probenecid along with the antibiotic penicillin to reduce the renal excretion of the antibiotic, thereby prolonging its duration of action.

One important type of interaction involves the binding of chemicals with their specific receptors. An antagonist blocks the action of an agonist, such as a neurotransmitter or a hormone, by preventing the binding of the agonist to the receptor.

Another important type of interaction results from alterations of the biotransformation of a chemical by another. Some chemicals are inducers of xenobiotic-metabolizing enzymes. They augment the activities of these enzymes, perhaps mainly by *de novo* synthesis, a fact that is consistent with the finding that repeated administrations are necessary. The common inducers include phenobarbital, 3-methylcholanthrene (3-MC), polychloro biphenyls, DDT, and benzo(*a*)pyrene. The inducers may lower the toxicity of other chemicals by accelerating their detoxication. For example, pretreatment with phenobarbital shortens the sleeping time induced by hexobarbital and the paralysis induced by zoxazolamine. In addition, 3-MC pretreatment greatly reduces the liver injury produced by bromobenzene, probably by increasing the activity of the epoxide hydrase. On the other hand, pretreatment with phenobarbital augments the toxicity of acetaminophen and bromobenzene, apparently by increasing the toxic metabolites formed. Repeated administration of a chemical may induce its metabolizing enzymes, as has been shown with the industrial chemical vinyl chloride.

Piperonyl butoxide, isoniazid, and SKF 525A and related chemicals are inhibitors of various xenobiotic-metabolizing enzymes. For instance, piperonyl butoxide increases the toxicity of pyrethrum (an insecticide) by inhibiting MFO activity in insects that detoxifies this agent. Isoniazid, when taken along with phenytoin, lengthens the plasma half-life of the antiepileptic drug and increases its toxicity. Iproniazid inhibits monoamine oxidase and increases the cardiovascular effects of tyramine, which is found in cheese and which is normally readily metabolized by the oxidase.

See also: Absorption; Analytical Toxicology; Biotransformation; Distribution; Exposure; Mechanisms of Toxicity; Mixtures, Toxicology and Risk Assessment; Pharmacokinetics/Toxicokinetics; Resistance to Toxicants.

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Mold

Martha Boss

Mold is part of the taxonomic fungi kingdom. These kingdoms (Monera, Protista, Fungi, Plantae, Animals) represent the known life-forms on the planet Earth.

Fungi reproduce through alteration of generations, from asexual to sexual to asexual, and so on. Their vegetative body may be unicellular (yeasts) or composed of microscopic threads called hyphae. The general term for the latter is molds, and in certain environmental conditions, yeasts being dimorphic can grow using hyphae. Fungi are eukaryotic, nonvascular, and reproduce by means of spores. Both sexual (meiotic) and asexual (mitotic) spores may be produced.

Fungi are heterotrophic (must feed on preformed organic material) rather then being autotrophic (make their own food by photosynthesis). Unlike animals (also heterotrophic), which ingest then digest, fungi digest then ingest. Fungi secrete exoenzymes to digest their food.

Fungi are not dependent on light and can grow in dark habitats in any direction. As they grow, the fungi can imbed their absorptive filaments (mycelia) into their growth substrate surface and, thus, obtain nutrients and water.

In our outdoor environment, fungi interacts with the other life-forms and inanimate objects of our planet. Molds are present in various geographic regions of our world and in the microhabitats in these regions.

Fungi Classification

Fungi are classified into phyla based on the morphology of their spores produced for sexual and asexual reproduction. This classification system has been heavily dependent on how the spores looked under a microscope. Fungi may be reclassified as sexual spores are identified or the genetic structure of the fungi is determined.

Currently, four major phyla are recognized. Chytridiomycota, which has sexual and asexual spores. The gametes have posterior flagella and, thus, are motile in a liquid environment. Zygomycota, which has sexual spores called zygospores that are thick-walled and asexual spores contained in a sac structure called a sporangium. Ascomycota, which has spores borne internally in a sac called an ascus. The asexual spores may be borne at the tips or sides of hyphae. These asexual spores (if dust like in size) may be termed conidia and are borne on a conidiophore. Basidiomycota, which has spores borne externally on a club-shaped structure called a basidium. The majority of Basidiomycota do not produce asexual spores.

Fungal Cellular Structures

The cell walls of fungi are structurally similar to plants; however, the chemical composition is different. Fungi cell walls are composed mostly of chitin plant walls whereas plant cell walls are composed mostly of cellulose. Only one subgroup of Chytridiomycota, the Hyphochytrids, has cellulose as well, a trait unique among living fungi. The cytoplasmic ultrastructure of fungi is broadly similar to plant cells; however, the types of organelles present are significantly different.

Most fungal cell walls contain glucose polymers as structural components. These glucans can be chemically bound to chitin. Glucans have been implicated in toxicological effects. A-(1-3)-D-Glucan is a known and potent T-Cell adjuvant, simulates macrophages and neutrophils, plays a role in organic dust toxic syndrome, and may be involved in hypersensitivity pneumonitis.

Microbial Volatile Organic Compounds

Molds while growing produce various chemicals as a result of their primary metabolic processes. These processes are needed to ensure the continuation of the mold's life cycle. The gaseous metabolic products are collectively referred to as microbial volatile organic compounds (mVOCs). Some mVOCs are primary solvents and are chemically identical to those originating from solvent-based building materials and cleaning supplies (e.g., alcohols, aldehydes, ketones, hexane, methylene chloride, benzene, and acetone).

Health effects from mVOCs have not been comprehensively studied. Some mVOCs are implicated in trigeminal nerve irritation and odor-related health complaints.

Mycotoxins

Molds also produce complex products of secondary metabolism. These secondary metabolites include chemicals used to ensure that the molds maintain their niche within their current habitat. These chemicals may suppress the growth of bacteria or other molds (antibiotic effect), or may be toxic to other eukaryotic cells (mycotoxic effect),. Mycotoxins may function as inhibitors of DNA, RNA, and protein synthesis. The production of secondary metabolites

takes energy away from the growing fungi, and is a process used only as needed.

Mycotoxin formation is more likely when a mixture of microorganisms is present. This is often the case when molds grow indoors. Conversely, molds isolated and grown in pure cultures (cultures containing only one species/strain of organisms) stop making mycotoxins after a few generations.

The growth substrate, temperature, humidity, the species and strains of fungi, and the presence of competitive organisms determine the potential and rate of mycotoxin formation. The mycotoxins vary in toxic potency, mechanism, target species, and target organs.

Over 200 mycotoxins have been identified as being produced by some 300 different genera of mold. Some of the mycotoxins and the mold genera that produce them, include:

- Aflatoxin and sterigmatocysti form *Aspergillus* (various species).
- Anthoquinones (e.g., rugulosin), from *Penicillium islandicum*.
- Substituted coumarins (aflatoxins), from *Aspergillus flavus* and *A. parasiticus*.
- Epipolythiodioxoperazines (gliotoxin), from at least six species of *Aspergillus*, *Penicillium*, and *Stachybotrys*.
- Ergot alkaloids, from *Claviceps purpurea* and species of *Aspergillus, Rhizopus*, and *Penicillium*.
- Substituted furans (e.g., citreoviridin), from *Penicillium citreoviride*.
- Griseofulvins, from *Aspergillus*, *Memnoniella,* and *Penicillium*.
- Ochratoxins, from several species of *Aspergillus*, *Memnoniella*, and *Penicillium*.
- Quinones (citrinins), from several species of *Aspergillus* and *Penicillium*.
- Trichothecenes from *Fusarium*, *Stachybotrys*, and *Trichoderma*, among others. (*Note*: Trichothecenes include sesquiterpenes with a trichothecane skeleton, olefinic groups at C-9 and C-10, and epoxies at C-12 and C-13. Macrocyclic trichothecenes have a carbon chain between C-4 and C-15 in an ester or ether linkage (e.g., T-2 toxin, DON, satratoxins G and H; verrucarins B and J, trichoverrins A and B)).

Mycotoxins may include: butanol, estrogenic compounds (e.g., zearalenone), heptanone, lactones, lactams (patulin), stachybotrylactones, stachybotrylactams, 2-pentylfuran, 2-hexanone, 2-methyl-1-propanol, 3-methylfuran, 2-methylisoborneol, 3-methyl-2-butanol, and macrocyclic trichothecenes (Satratoxins F, G. and H, Roridine, Verrucarinj, and Trichoverrols).

Different strains of the same mold may make differing amounts and types of mycotoxins. Certain strains may not always produce mycotoxins, making it impossible to predict mycotoxin levels based solely on spore concentrations in air.

Mycotoxin Exposure Routes

Some mycotoxins cling to the mold spores' surfaces and may be found in dust as adsorbed or absorbed chemically on the dust particulates' matrix. Thus, the exposure pathways for mycotoxins include inhalation of mVOC vapors and inhalation of associated dusts or mold spores. Ingestion and dermal exposure through initial skin contact is also possible.

Aspergillus flavus and *A. parasiticus* produce the mycotoxin aflatoxin B1. Aflatoxin B1 is a carcinogenic chemical that can cause liver cancer. Both ingestion and inhalation are proven exposure routes. Aflatoxin B1 has been found on contaminated grains, peanuts, and other foodstuffs. *A. flavus* and *A. parasiticus* are not commonly considered an indoor contaminant, unless the grains, peanuts, cereal-based animal food, or other foodstuffs are stored in an indoor environment.

Studies that have investigated inhalation of mold and mold products found that inhalation produces more potent effects than ingestion. These effects are as potent as intravenous administration. Mycotoxins upon inhalation may produce immunosuppression, carcinogenesis, cytotoxicity, neurotoxicity (including acute or chronic central nervous system damage), mucous membrane irritation, skin rash, nausea, acute or chronic liver damage, and endocrine effects. These effects may be independent of infection or stimulation of antibodies (in contrast to the *Mycobacterium* mycotoxins).

Ingestion of moldy foods may also cause health effects such as liver damage, nervous system damage, and immunological effects.

Studies have included the effects from various exposure routes, including intravenous, intradermal, intramuscular, and intraperitoneal routes, as well as more natural dermal, ingestion, or inhalation routes.

Mycotoxin Toxicity

The mycotoxins differ in their absorption, toxicokinetics, toxicodynamics, target organs, metabolism, detoxification, and elimination due to differences in chemical structure. Mycotoxins also differ in potency, ranging from a lethal dose 50% (LD_{50}) in fractions of milligrams per kilogram to hundreds of milligrams per kilogram.

Some mycotoxins have only been tested for cytotoxicity, which is a relatively crude measure of effect

involving testing the toxin against isolated cells or tissues in culture.

The US Food and Drug Administration has set regulatory limits for aflatoxins produced by *Aspergillus flavus*. Health Canada has set limits for zearalenone (from *Fusarium* and some other molds). However, the majority of mycotoxins do not have regulatory limits.

The US Army Medical Research Institute for Infectious Disease has investigated a number of toxins for their potential to be used as weapons. Other toxins have been investigated because of their large economic impact on agricultural animals and crops.

With the exception of mycotoxins examined for military use, the bulk of research with animals has focused on the ingestion route given the potential for contaminated feed and fodder. The World Health Organization has investigated and sought control of mycotoxin exposure to humans.

More research is needed on other mycotoxins, including penicillic acid, roquefortine, cyclopiazoic acid, verrucosidin, rubratoxins A and B, PR toxin, luteoskyrin, cyclochlorotine, rugulosin, erythroskyrine, secalonic acid D, viridicatumtoxin, kojic acid, xanthomegnin, viomellein, chaetoglobosin C, echinulin, flavoglaucin, versicolorin A, austamide, maltoyzine, aspergillic acid, paspaline, aflatrem, fumagillin nigragillin chlamydosporol, and isotrichodermin.

Exposure Routes and Pathways

Effect often varies, depending on the degree of access of the exposure route to blood or lymph pathways. These pathways provide a means of distribution to target tissues for the specific poisons.

Dose and Effect

The doses necessary to establish effect levels for most types of mold exposures have not been established. Risk assessments are difficult due to the complex effects of mixtures of toxins and other physiologically active molecules produced by molds.

Animal experiments are unlikely to detect the incidence of toxic effects because of the low power of such studies. Low power is defined herein as investigations involving a small number of animals and animal homogeneity. This resultant small degree of test population variability requires that high doses of challenge agents be administered to observe an effect (e.g., 10 animals per dose group).

Allergic Responses

Adverse respiratory effects have been documented due to allergic responses to mold vegetative growth and spore proteins. Asthma can be triggered by a specific allergy, including an allergy to fungi.

Mucus within the respiratory tract traps fungi. If the fungi particulate is inhaled into the bronchi, the cilia of the bronchi attempt to move the mucus and entrapped mold particulate to the mouth and nose.

Chronic respiratory conditions are diagnosed by the examination of this mucus. However, for conditions requiring surgical intervention, the mucus is routinely suctioned out and discarded. Consequently, this mucus has not been available for examination and the subsequent examination of excised tissue may underestimate the fungi present.

Some research has concluded that in certain patients a cascading effect occurs. Eosinophils cluster around the fungi in the respiratory tract and produce toxins to subdue the fungi. In sinusitis, these toxins also destroy the outer lining of the sinus tissue, clearing the way for a bacterial infection that causes inflammation and sinusitis.

See also: Aflatoxin; Immune System; Mycotoxins; Penicillin; Respiratory Tract.

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Molecular Toxicology–Recombinant DNA Technology

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Molecular technology and recombinant DNA technology have revolutionized the field of toxicology. These techniques are powerful tools for better understanding and assessment of the mechanisms of action of substances that may adversely affect human and environmental health.

The application of recombinant DNA technology has accelerated scientific discovery in all areas of biological research to an unprecedented rate. This technology has enabled researchers to examine the molecular mechanisms and structures responsible for such complex processes as cell growth, metabolism, differentiation, and development. More significantly, it provides a means to manipulate molecules critical to these processes and an opportunity to examine the effects of these manipulations in living systems and elucidate the physiological roles of the protein under investigation. There is also a general consensus among researchers that the application of recombinant DNA techniques to problems in toxicology will have a profound impact on the future direction of scientific research.

Molecular toxicology has enabled toxicologists to understand events at the molecular level and examine alterations in fundamental biological processes that lead to the manifestation of toxic responses. As a result, toxicologists are examining the mechanisms of action of toxic substances in order to identify molecular changes predictive of exposure to harmful substances. This information can be used to identify susceptible groups within a population or establish safe levels of exposure using a mechanistic approach as an alternative to association of risk based on extrapolation of 'high-dose' studies in rodent models to low-level human exposures. This molecular or 'reductionist' approach is not intended to circumvent *in vivo* studies but to introduce mechanistic data into risk assessment in order to define the possible implications of exposure to potentially harmful substances. The inclusion of recombinant DNA technology in toxicological research has facilitated our understanding of the mechanisms of action of several toxic substances. This information has subsequently been used by toxicologists in risk assessment and in the development of assays that identify and assess the potential adverse effects posed by uncharacterized toxic substances.

Perhaps the single most important advance in the last 40 years of medical science was the development of the polymerase chain reaction (PCR) protocol. PCR exploits features of DNA replication, which provide researchers endless possibilities in the manipulation of DNA. The most powerful advantage of this technology is its ability to produce enormous numbers of copies of a specific DNA sequence from a minute sample. The technique involves two primers that are designed to be complementary to the boundaries of the desired DNA segment and DNA polymerase that produces copies of the DNA sequence between the primers (**Figure 1**). The cycling of this replicative reaction results in the exponential production of a DNA fragment whose boundaries are determined by the primers. PCR can also be used to isolate, characterize, or quantify mRNA through the use of reverse-transcriptase PCR (RT-PCR), which uses viral reverse transcriptase enzymes to produce complimentary DNA (cDNA) sequences from isolated mRNA. PCR has applications not only in research

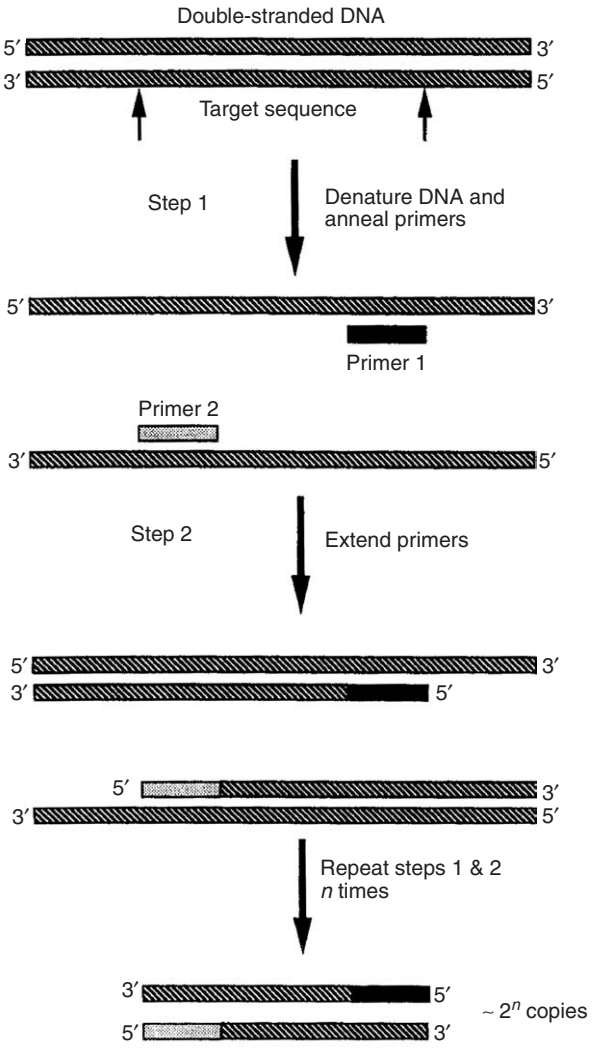

Figure 1 A schematic representation of the polymerase chain reaction (PCR). Repetitive cycles of DNA denaturation followed by annealing of the primers and primer extension results in the exponential amplification of the DNA fragment whose boundaries are determined by the primers.

but also in forensic toxicology, evolutionary studies, and in the diagnosis of infectious diseases, genetic abnormalities, and cancer. This technique is so sensitive that forensic scientists can obtain sufficient amounts of DNA extracted from saliva left on postage stamps or discarded cigarettes to implicate individuals in criminal activities. More recent advances in PCR technology have produced 'real-time' PCR, in which the PCR reaction can be monitored in real-time with the use of fluorescent dyes or labeled DNA probes. This technique is even more sensitive than standard PCR, and has proved to be significantly more effective for quantification of gene expression.

Recombinant DNA technology has provided a dramatic expansion in our knowledge of the structure, function, multiplicity, and regulation of xenobiotic metabolizing enzyme (XME) superfamilies. Its use has resulted in the sequencing of the entire human genome, and the cloning of cDNA sequences that encode or act as the blueprint for the synthesis of proteins that are important in toxicology. The increased availability of cDNA and deduced protein sequences has provided a rational foundation for the development of standardized nomenclatures based on amino acid sequence similarities. These systems are a welcome alternative to the multiple, laboratory-specific classifications that have led to considerable confusion particularly in the P450 superfamily, in which over 200 unique cDNAs have been identified. In addition to the characterization of the human enzymes, XMEs from a variety of other species have been cloned using the same techniques. Comparisons of the XME sequences between species can enhance our understanding of the critical amino acids for enzyme functionality, and has led to rapid advances in the field of evolutionary toxicology.

A major emphasis in drug metabolism research has been focused on determining the role of individual XMEs in the *in vivo* biotransformation of drugs and chemicals in order to understand their role in eliciting adverse drug reactions. The identification of specific XMEs that are responsible for adverse drug effects has been difficult since multienzyme superfamilies made up of enzymes with diverse structure and overlapping substrate specificities are involved in metabolism. A further complication has been the relatively low abundance of individual forms of XMEs. This limitation has been overcome by using heterologous expression systems to produce large quantities of specific XMEs. These systems use cloned XME cDNAs and take advantage of the protein production machinery in bacteria, yeast, insect, or mammalian cells in culture to overexpress large quantities of a desired protein. Depending on the system that is utilized, proteins can be generated by simply introducing the appropriate vector containing the desired cDNA into the cells of choice. Expression of these proteins can be greatly enhanced through the use of a vector containing a strong enhancer element, such as the SV40 enhancer. The enzymatic activity of recombinant proteins can be directly measured from whole cells or isolated subcellular fractions. Heterologous expression systems have also been used to produce sufficient quantities of desired protein for subsequent purification to homogeneity and biophysical investigations. Many heterologous expression systems are commercially available and provide all the necessary materials to express the desired product in a variety of organisms.

Bacterial, yeast, and mammalian cells have been utilized in novel ways to develop or improve assays

for the identification of drug metabolizing enzymes involved in the bioactivation and detoxification of xenobiotics. In addition to the use of immortalized cell lines, more effective techniques of isolating and culturing primary cell lines have enabled the use of these cell types as well. Protocols have been developed which allow for the expression of XMEs in these cell culture systems, as well as manipulation of XME structure, function, and expression levels (**Figure 2**). However, these studies are limited by the use of a single cell type, which may not properly model the *in vivo* toxicity. For example, xenobiotics which are bioactivated in one organ system or tissue, but exert their toxic effects in another, require the use of two different cell lines for proper understanding of the overall mechanism. Another complication in the use of these expression systems can be altered physiology brought on by *in vitro* culturing. Maintenance of cells under these artificial growth conditions can lead to changes in gene and protein expression. This is particularly important when considering toxicants that may effect the regulation of cellular proliferation, which is typically altered in immortalized cell lines. In addition, results produced using human cell lines do not reflect the overall genetic variability of our species, but rather only the particular XME genotype of the individual(s) from which the cell line was produced.

Cell lines have also been produced which stably express a specific XME isozyme. For example, cDNAs encoding for detoxification enzymes, such as *N*-acetyltransferase and glutathione *S*-transferase, have been introduced into Ames tester strains and mammalian cells, which are naturally devoid of these enzymatic activities. This has enabled researchers to examine the mutational specificity of a chemical following metabolism or bioactivation by specific detoxification enzymes. Furthermore, a series of bioengineered lymphoblastoid cell lines expressing various human P450s and phase II enzymes have been prepared and are commercially available. Results from these *in vitro* test systems combined with data gained from experiments investigating differential regulation, tissue-specific expression, and interindividual variation in human XMEs should provide a sound mechanistic basis for assessing human risk following procarcinogen and promutagen exposures.

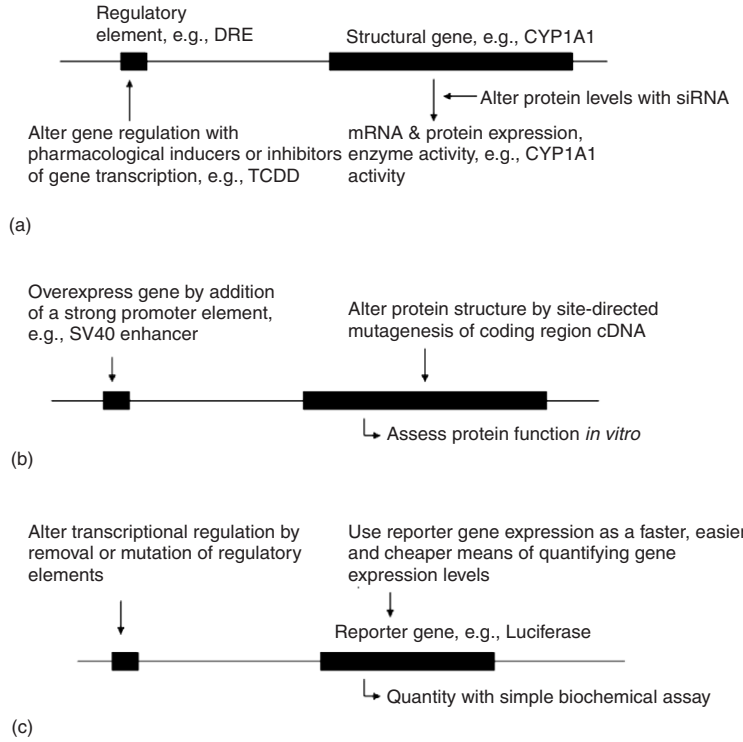

Figure 2 Methods of altering XME gene expression *in vitro*. (a) Modulation of endogenous gene activity. Gene transcription can be induced by the application of pharmacological inducers, such as TCDD for CYP1A1, or inhibitors. siRNA can be used to 'knock down' protein expression. (b) Expression of functional cDNAs. Vectors containing XME cDNAs can be introduced to a cell culture expression system. Strong positive regulators such as the SV40 enhancer can be used to drive overexpression of the XME gene product. XME structure and function can be altered by site-directed mutagenesis of the cDNA. (c) Expression of regulatory region and reporter gene. The 5′ regulatory region or 'promoter' can be fused to a reporter gene such as luciferase, which allows for the rapid analysis of gene expression using a simple biochemical assay. Specific regulatory elements within the promoter can then be removed or mutated, allowing for identification of the critical elements for control of XME expression.

The recent development of short-inhibitory RNA (siRNA) has added even more power to the use of cell-culture-based expression systems. siRNA takes advantage of an unexpected mechanism by which a short, double-stranded RNA of known sequence will initiate the degradation of complimentary RNA. This allows researchers to 'knock-down' the expression of proteins by introducing these siRNAs via the same protocols commonly used to introduce exogenous cDNA expression or reporter constructs. Although some difficulties in design and effectiveness of siRNAs still exists, this technique, together with traditional expression vectors, allows for the inhibition or overexpression of most genes whose mRNA sequences have been characterized. Importantly, these techniques allow toxicologists the opportunity to modulate the function of endogenous XMEs within an expression system.

In addition to the controlled expression of genes made possible by available expression systems and siRNA, specific control of gene expression in the whole animal has been made possible by recombinant DNA technology. These transgenic and 'knock-out' animals have been invaluable in the dissection of a number of important toxicological pathways, and are discussed further elsewhere in the encyclopedia.

Recombinant DNA technology has also led to techniques in which individual bases in a cDNA sequence can be changed with great precision in the laboratory. This allows for site-directed mutagenesis, which in addition to altering the sequence of a cDNA, can alter the amino acid sequence of the protein produced in expression systems. This is of great importance in the study of the molecular mechanisms of XMEs, as individual amino acids within the active sites of these enzymes can be changed sequentially to determine their relative importance in the functionality of the enzyme. Heterologous expression technology has also been an important technique in assessing the functional consequences of XME polymorphisms. Expression of cDNAs with variant DNA sequences using heterologous systems allows one to identify protein products with altered catalytic activity without having to perform lengthy, labor-intensive purification protocols from tissue samples from multiple phenotypic populations. In addition, these studies may assist in the identification of critical residues within XMEs that are required for optimal catalytic activity. This information may lead to improved drug design and efficacy by introducing modifications that minimize metabolic transformations to reactive metabolites that are responsible for eliciting adverse reactions, or facilitate synthesis of prodrugs that exhibit better pharmacokinetic properties.

The advancement of recombinant DNA technology has also allowed for the study of proteins in a 'modular' sense. Individual domains of proteins can be removed entirely by manipulation of the cDNA sequence to determine their function. Furthermore, domains from entirely different proteins can be fused together to create fusion proteins. One example of the usefulness of this technique is the creation of GST-fusion proteins, in which a small domain from the glutathione-*s*-transferase (GST) gene is fused to a gene of interest before introduction into an expression system. The small GST domain usually does not impact the functionality of a protein, but it allows for the fast and simple purification of the fusion protein using an affinity column. A second example of the usefulness of fusion proteins is the yeast two-hybrid system. In this system, a gene of interest is fused to the DNA binding domain of a yeast transcription factor, such as Gal-4, and a library of cDNA sequences from the same species or cell type are fused to the transactivation domain of the same yeast transcription factor. These fusion cDNAs are then introduced into an expression system in yeast, and proteins that physically interact with the protein of interest bring the DNA binding domain and the transactivation domain of Gal-4 into close proximity, activating expression of a reporter gene. The cDNA sequences can then be isolated, cloned, and the identity of the interacting proteins discovered, even if they were not previously characterized. This technique is a powerful tool for identification of proteins that interact directly with XMEs or transcription factors that regulate XME expression.

In addition to investigating the structural features of drug metabolizing enzymes, molecular biology has also enhanced our understanding of how the expression of these enzymes is regulated. The XME whose transcriptional regulation is the most well characterized is CYP1A1. Its expression has been linked to the bioactivation of a number of carcinogens and studies have found that it is inducible by a variety of structurally diverse compounds, including 3-methylcholanthrene, β-naphthoflavone, and halogenated aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The induction of CYP1A1 expression is mediated by a specific cytosolic protein referred to as the aryl hydrocarbon receptor (AhR). The AhR is a ligand-inducible transcription factor that contains separable and distinct domains for ligand binding, DNA binding, and transcriptional activation for gene expression. The AhR is a member of PAS (PER, ARNT, SIM) family of proteins which also includes its dimerization partner, the Ah receptor nuclear translocator (ARNT), hypoxia inducible factor-alpha (HIF1-Alpha), and period

(PER1). The members of this protein family tend to act as environmental sensors. For instance, while the AhR mediates responses to xenobiotics, HIF1-Alpha mediates responses to low oxygen levels, and PER1 and MOP3 are involved in the circadian rhythmicity, which, in part, is a response to light.

Ligand binding to the AhR causes the release of cytoplasmic cofactors, which enables the receptor to move into the nucleus and heterodimerize with ARNT. The resulting complex exhibits high affinity for DNA and seeks out specific DNA sequences referred to as dioxin or xenobiotic response elements (DREs or XREs) located in the 5′ regulatory region of the CYP1A1 gene. Binding of the complex to DREs results in the recruitment of factors that facilitate the increased rate of CYP1A1 gene transcription. Analysis of the CYP1A1 5′ regulatory region has identified six DREs, all of which contain the core sequence, 5′-GCGTG-3′, and have a role in the dramatic increase in CYP1A1 activity following treatment with TCDD. In addition to CYP1A1, other TCDD-inducible genes such as the rat glutathione S-transferase Ya subunit, CYP1A2, and quinone oxidoreductase also possess DREs in their 5′ regulatory regions. Although the mechanism by which TCDD and related compounds cause toxicity has not been fully elucidated, it is generally believed that the AhR plays a role. In support of this, mice lacking the AhR are virtually immune to the effects of TCDD, and CYP1A1 null mice are resistant to some, but not all, of the toxic effects of this toxicant. Molecular toxicology has also provided great advances in our understanding of the role of XME and XME-regulating genes in normal physiology. For instance, mice lacking the AhR exhibit a number of cardiovascular and reproductive defects that suggest that this protein plays a role in normal physiology, in addition to its control of XME induction.

Receptors are not the only mechanisms used by toxic substances to affect gene regulation. Reactive oxygen intermediates (ROIs), such as superoxide and hydroxyl radicals, are continuously produced in cells as side products of electron-transfer reactions. Oxidative stress occurs when abnormally high levels of ROIs are produced within a cell following exposure to physical, chemical, and biological agents including UV radiation, alkylating agents, hydrogen peroxide, metals, cytokines, and other natural ligands for cell surface receptors. In addition to causing cellular damage, ROIs also induce the expression of a number of genes by the induction and activation of transcription factors such as c-*fos*, c-*jun*, and NF-kappa-B. c-*Fos* and c-*jun* are members of the AP-1 transcription factor family, which forms *fos-jun* or *jun-jun* dimers prior to binding to specific DNA sequences located in the 5′ regulatory region of target genes. These specific DNA sequences or response elements have been found to regulate the expression of numerous genes, including enzymes with radical scavenging or DNA repair activities, which serve to protect the cell from oxidative damage and repair damage that has already occurred.

Methods for investigating the control of XME gene expression by the AhR, AP-1, and other transcription factors have also been greatly enhanced through the use of recombinant DNA technology. The 5′ regulatory region, or promoter sequences, of XME genes can be linked to reporter genes that are then controlled in an expression system via the same mechanisms by which XME expression is controlled. This allows for the study of critical response elements that are involved in the control of XME expression. Examples of reporter genes include firefly luciferase, bacterial chloramphenicol acetyltransferase, bacterial β-galactosidase, green fluorescent protein, and heat-resistant mammalian alkaline phosphatase. Reporter genes provide sensitive, rapid, and easily measured enzymatic activities that are usually absent in the host cell. The XME promoter can then be altered by the same site-directed mutagenesis methods that are used to alter amino acid sequence in the coding region of the XME for functional analysis. Results of these studies can pinpoint the regulatory elements involved in controlling gene expression. For instance, removal of the DRE sequences in the CYP1A1 promoter eliminates the ability of TCDD to induce expression of this gene.

Reporter gene technology has also been exploited to develop bioassays that assist toxicologists in the detection and assessment of potentially toxic substances. These bioassays consist of reporter genes whose expression is controlled by the 5′ promoter of a target gene. This allows for identification of substances that activate gene expression with a simple biochemical assay, and without direct mRNA quantification. Several different reporter gene bioassays are currently being used to assess the potential toxicity of individual chemicals as well as complex mixtures. These bioassays can detect a number of different substances including halogenated aromatic hydrocarbons, sex steroid mimetics, peroxisome proliferators, metals, and inducers of oxidative stress.

Pharmacogenetics, the study of genetically determined variations in drug response, has been profoundly transformed by molecular biology. Genetic polymorphisms have been traditionally defined as the occurrence in a population of more than one allele of a particular gene with the prevalence of the less common form being at least 1%. Prior to recombinant DNA technology, genetic polymorphisms were

identified by familial and population studies following the observation of an atypical drug reaction in a population. Classification of individuals as poor or extensive metabolizers was determined by measuring drug clearance of a substance that was metabolized by a specific XME. On this basis it became evident that there were genotypic variants in a wide variety of human XMEs. Recombinant DNA technology has advanced the characterization and study of these polymorphisms tremendously. Literally hundreds of polymorphisms in cytochrome P450s and other drug metabolism enzymes have now been identified (**Table 1**). It is believed that adverse drug reactions account for up to 5% of all hospital admissions in the United States, and many of these reactions may be due to XME polymorphisms. Also, polymorphisms in proteins which are not involved in xenobiotic metabolism, but which have substantial effects on the therapeutic effectiveness or tolerance of certain drugs have also been identified. For example, polymorphisms in glucose-6-dehydrogenase, which reduce the activity or expression of this enzyme affect more than 400 million people worldwide, and can cause adverse reactions to primaquine and sulfonamides.

In addition to altering the metabolism of, and causing adverse reactions to pharmacological agents, XME polymorphisms also have the potential to alter the susceptibility of individuals to environmental toxicants. Many xenobiotics are metabolized by XMEs to form toxic metabolites which then led to toxicity, and functional alterations of these XMEs could lead to altered production of these toxic metabolites. For example, a number of XME polymorphisms are linked to increased risk for smoking-related cancers. However, the link between XME polymorphisms and cancer risk is poorly understood. Epidemiological studies of large populations have shown only a moderate increase in risk for the development of these cancers in individuals with XME polymorphisms. These results are confounded by the involvement of multiple polymorphisms, the genetic variability of the human population in general, and in difficulties in assessing exposure levels. Transgenic mice may add to our understanding of the role of XME polymorphisms in potentiation or protection from xenobiotic toxicity. For example, mice lacking the AhR are immune to benzo(*a*)pyrene carcinogenicity, presumably due to an inability to induce CYP1A1 expression.

While these results are intriguing, there are no known human polymorphisms that completely disrupt the function of the AhR or CYP1A1. Interestingly, however, persons with 'high-inducibility' of P4501A1 have been shown to be at a higher risk for the development of certain smoking-related cancers.

By combining allele-specific DNA sequence information with PCR, noninvasive assays have been developed that can be rapidly performed to identify individuals susceptible to adverse drug reactions. The three strategies that have been successfully employed include the amplification of a DNA sequence that contains a restriction fragment length polymorphism (RFLP) which identifies a variant phenotype, the use of allele-specific PCR primers designed to hybridize to a sequence only if a known variation is present, and the use of allele-specific DNA probes during real-time PCR (**Figure 3**).

RFLPs involve the digestion of DNA with purified bacterial enzymes, known as restriction endonucleases, which recognize and cleave specific DNA sequences. The digested DNA fragments are subsequently separated based on size using gel electrophoresis and the resultant DNA fragment lengths are determined by comparison to a known standard. These fragments can then be further amplified and sequenced for exact characterization of the polymorphism. RFLPs have been used in the identification of DNA sequence changes that are responsible for genetic diseases as well as in forensic science in order to establish an association between an individual and the human tissues such as blood, saliva, or semen collected at a crime scene. Genetic polymorphisms that involve changes in DNA sequence at restriction enzyme recognition sites can be directly detected following restriction enzyme digestion of both normal and variant DNA. However, polymorphisms that alter

Table 1 Some common XME polymorphisms and their clinical importance

Enzyme	*Substrates*	*Functional defect*	*Adverse clinical effects*
CYP1A2	Olanzapine	Reduced enzyme induction	Enhanced side-effects
Cyp2C8	Taxol	Reduced metabolism	Altered pharmacokinetics, toxicity
CYP2C9	Phenytoin warfarin	Reduced metabolism	Lower therapeutic doses, toxicity
CYP2C19	Diazepam	Reduced metabolism	Prolonged sedation
CYP2D6	Perhexilene	Reduced metabolism	Hepatotoxicity and neuropathy
CYP3A4	Nefedipine	Reduced metabolism	Altered pharmacokinetics
NAT2	Sulfonamides	Reduced activity	Hypersensitivity
Aldehyde dehydrogenase 2	Ethanol	Inactive enzyme	Decreased tolerance for alcohol

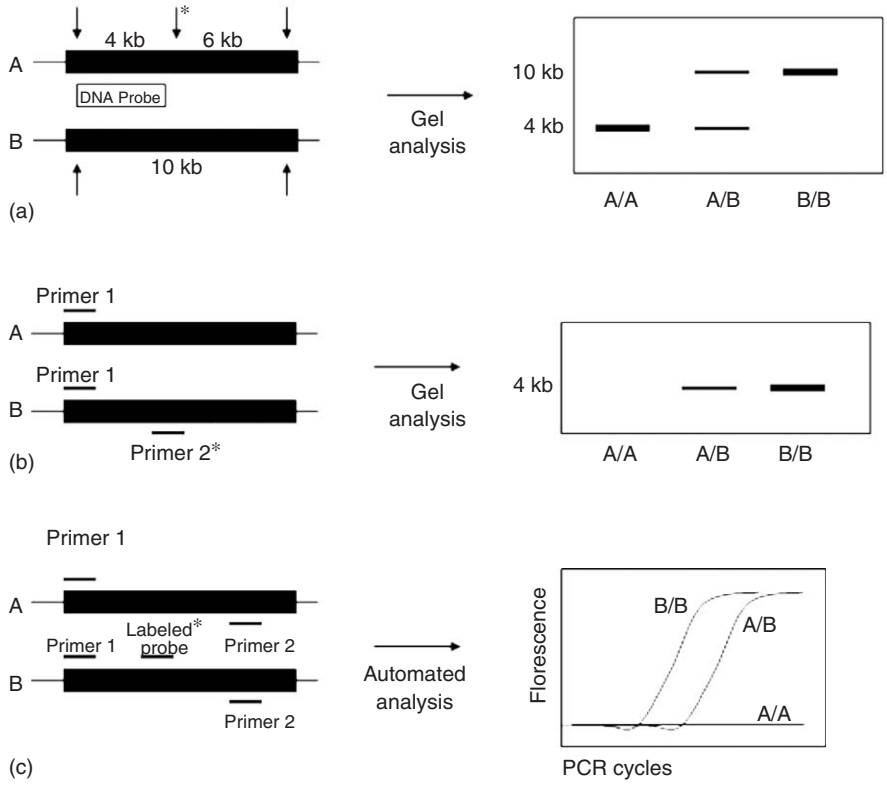

Figure 3 Methods for identification of XME polymorphisms. (a) RFLP analysis. Genomic DNA is digested with restriction endonucleases, hybridized to a radioactive DNA, and run on a gel. The asterisk indicates a restriction endonuclease site which is found only in variant A. Gel analysis demonstrates three different banding patterns, corresponding to the three possible genotypes. (b) XME polymorphism identification using allele-specific primers. Standard PCR is performed using a primer that only recognizes one allele. The resulting products are run on a gel, and visualized as bands following staining with an intercalating dye. No bands are seen in A/A individuals, a strong band in B/B individuals, and an intermediate band in A/B individuals. (c) XME polymorphism identification using real-time PCR. A florescent-labeled DNA probe which only recognizes variant B is used to monitor the progress of the PCR reaction. A/A individuals produce no florescence, because the probe will not bind variant A. B/B individuals will produce a rapid increase in florescence as the product is produced, and A/B individuals will produce a slower increase in florescence, due to the 50% reduction in available B-alleles at the start of the PCR reaction.

restriction endonuclease recognition sites and cause abnormalities in protein function are rare. It should also be emphasized that the majority of the DNA sequence variations located in or around a gene do not necessarily explain the observed phenotypic variants since many of the differences detected by RFLP analysis are silent polymorphisms that do not enchanges at the protein level and, therefore, have no functional consequences. For example, in the CYP2D6 polymorphism, digestion of genomic DNA with 20 different restriction enzymes identified the presence of 14 different RFLPs. However, only two of these RFLPs correlated with specific DNA sequence variations that were associated with the poor metabolizer phenotype.

In contrast to RFLP analysis and standard PCR techniques, real-time PCR analysis can easily and rapidly detect a single nucleotide polymorphism (SNP) in a gene of interest without the need to run the products on a gel. This technique uses a florescent dye-labeled probe that specifically recognizes and binds to polymorphic sequences during the PCR reaction, and is detected in real-time using a sensitive camera. Real-time PCR is extremely fast and sensitive, and has been used successfully to genotype a number of important polymorphic genes.

The development of these techniques for rapid analysis of XME polymorphisms may eventually lead to routine, detailed genotyping in a clinical setting. Indeed, the use of PCR-based genotyping to determine the proper pharmacological intervention in patients who may be treated with drugs which are metabolized by polymorphic XMEs has been widely suggested. This may be particularly useful prior to the use of drugs metabolized by CYP2C9, CYP2D6, and CYP3A4, which are responsible for metabolism of 60–70% of all therapeutic drugs, and are highly polymorphic. However, the use of these technologies

to determine individual XME genotypes within a clinical setting must be balanced with ethical concerns regarding patient confidentiality.

The ability of toxic substances to induce gene expression has been successfully used to develop assays that assist in the identification of substances that may cause adverse effects. In the past, toxicologists relied on physiological responses, such as decreased body weight or lethality, to assess the effects of a toxic substance. As more information regarding the mechanism of action of compounds was acquired, biochemical responses such as enzyme activities were used to determine the potential adverse health effects resulting from exposure to toxic substances. Molecular toxicology provides techniques such as RT-PCR and real-time PCR, which have enabled toxicologists to directly measure subtle changes in the expression of specific target genes. Recombinant DNA technology has also provided strategies for toxicologists to identify target genes that are directly involved in eliciting the observed toxic responses. Differential hybridization to microarrays containing thousands of genes has been successfully used to identify structurally unrelated genes that are regulated by a common mechanism such as exposure to a specific chemical agent. These powerful techniques are discussed in a separate chapter.

The development of recombinant DNA technology and molecular biology techniques has accelerated the rate of discovery in all major areas of biological research. The incorporation of this technology into the field of toxicology will enhance both the basic and applied aspects of the discipline. These technological advances have already enabled toxicologists to elucidate the mechanisms of action of toxic substances at the molecular level and this information has been successfully used to engineer and improve the sensitivity and specificity of a number of assays commonly used to assess the potential toxicity of a substance. Furthermore, the use of this technology has also contributed to our understanding of the normal physiological roles of proteins and enzymes that are disrupted by toxic substances. In conclusion, the use of recombinant DNA technology will extend the comprehensive nature of toxicology and will assist toxicologists in identifying and predicting the potential risks an unknown substance may pose to human health and environmental quality.

See also: Analytical Toxicology; Carcinogen–DNA Adduct Formation and DNA Repair; Developmental Toxicology; Genetic Toxicology; Genomics, Toxicogenomics; GF; Mechanisms of Toxicity; Microarray Analysis; Risk Assessment, Human Health; Toxicity Testing, Mutagenicity; Transgenic Animals.

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Molinate

Danny Villalobos

- Chemical Names: *S*-Ethyl azepane-1-carbothioate; *S*-Ethyl perhydroazepine-1-carbothioate; *S*-Ethyl perhydroazepine-1-thiocarboxilate; *S*-Ethyl hexahydro-1*H*-azepine-1-carbothioate (9CI)
- Chemical Abstracts Service Registry Number: CAS 2212-67-1
- Synonyms: Hydram; Ordram; Yalan
- Chemical/Pharmaceutical/Other Class: Thiocarbamate herbicide
- Chemical Formula: $C_9H_{17}NOS$

Uses

Molinate is a selective thiocarbamate herbicide used to control broad-leaf and grassy plants primarily in rice production. Molinate is available in granular and emulsifiable liquid formulations. Recently, US manufacturers of molinate have requested voluntary cancellation of all uses of molinate by 2009.

Exposure Routes and Pathways

Potential routes of exposure to molinate include inhalation (for mixers, applicators, field workers, and residents of rice-growing regions), dermal (for mixers, applicators, field workers, and anyone exposed to drift of spray droplets or residues on plants), and dietary (from drinking water sources contaminated with molinate and from residues on rice and rice products).

Toxicokinetics

Molinate is well absorbed by the oral route. It is widely distributed with highest concentrations remaining in the circulation. There is no evidence for accumulation of molinate. It is nearly completely excreted within 48 h, primarily via the urine. The primary biotransformation pathway for molinate in rats is *S*-oxidation to sulfoxide, followed by hydrolysis (hexamethyleneimine) or conjugation (mercapturate). In humans, ring hydroxylation is the primary route followed by glucuronidation.

Mechanism of Toxicity

Reproductive effects of molinate may depend on inhibition of neutral cholesterol ester hydrolase, leading to disruption of mobilization of cholesterol from high-density lipoprotein, a process selective for rodents. However, the selectivity of this mechanism or others in rodents relative to reproductive toxicity remains unclear. Molinate can inhibit acetylcholinesterase as well as aldehyde dehydrogenase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Molinate is moderately toxic by ingestion, with reported oral LD_{50} values of 369–720 mg kg^{-1} in rats and 530–795 mg kg^{-1} in mice. Dermal LD_{50} values are 4000–4800 mg kg^{-1} in rats. It is mildly irritating to rabbit skin and moderately irritating to rabbit eyes, and is not a skin sensitizer. A 4 h inhalation LC_{50} of 1.36 mg l^{-1} indicates moderate toxicity by this route as well. Some formulations show a lower degree of acute toxicity. Molinate is more acutely toxic to developing organisms.

Human

Persons poisoned by contaminated well water demonstrated rapid onset signs and symptoms including abdominal and gastrointestinal disorders, fever, weakness, and conjunctivitis. These signs and symptoms disappeared rapidly and there was no evidence of persistent sequelae.

Chronic Toxicity (or Exposure)

Animal

Molinate has been shown to adversely affect reproduction/fertility in the rat. Administration of molinate to young male rats at a dose of 3.6 mg kg^{-1} day^{-1} for 2 months caused changes in spermatozoa but did not decrease sperm fertility. When these rats were mated to normal females, many of the embryos were resorbed and postnatal mortality was increased. Sertoli cells appeared to be directly affected as neither gonadotropin nor androgen levels were affected. Female reproductive function also appeared sensitive to molinate. Molinate also led to developmental defects in rats including reduced/retarded uterine growth, incomplete ossification, and dilated brain ventricles, but a potential role for maternal toxicity was unclear. Molinate is a cholinesterase inhibitor and elicits signs of neurotoxicity, with excess salivation being a relatively sensitive response. Molinate did not cause segmental demyelination as do some other thiocarbamates (e.g., disulfuram). Molinate has been reported to potentiate the delayed neurotoxicity of some

organophosphorus toxicants. Interestingly, while not an organophosphorus molecule, there are data suggesting that molinate can cause delayed neurotoxicity in the hen.

Human

In a study of male workers between 1980 and 1982 at a molinate production facility, measurements were made on reproductive parameters including sperm concentration, motility, morphology, and serum follicle-stimulating hormone (FSH), leuteinizing hormone (LH), and testosterone levels. The study provided little evidence of an effect of molinate on sperm or serum hormone levels. Subsequent analysis by US Environmental Protection Agency concluded there was a slight decrease in number of children especially for the high exposure classification, both between production cycles and during peak production exposure. Molinate showed no evidence of skin sensitization in agricultural workers.

In Vitro Toxicity Data

Molinate was positive in the mouse lymphoma (with metabolic activation) and mouse micronucleus assays. It was negative in other mutagenesis tests including Ames assays.

Clinical Management

Symptoms of exposure to molinate include skin sensitization, nausea, diarrhea, abdominal pain, fever, weakness, and conjunctivitis.

Molinate has the potential to interact with the endocrine system. The primary target organ affected by molinate is the thyroid.

Environmental Fate

Chlorination during water purification treatment converts molinate to molinate sulfoxide. Complete degradation to noncarbamate compounds requires other oxidants, such as chloramination, potassium permanganate, or ozone. Molinate is of low persistence in the soil environment, with a half-life of 5–21 days. It is poorly bound to soils, relatively soluble in water, and thus may be mobile. Soil microorganisms are responsible for most degradation of molinate. Molinate may rapidly volatilize if not plowed into the soil, and may undergo degradation by sunlight. Molinate may be degraded by hydrolysis. Molinate is rapidly taken up by plants and transported to leaves. In the leaves, molinate inhibits leaf growth and development.

Ecotoxicology

Molinate appears to be practically nontoxic to birds. It poses an acute risk to fish, amphibians, and aquatic invertebrates, limited to organisms in small streams and tributaries that are subject to high exposure from rice drainage. Chronic risk to freshwater fish is possible. Molinate also poses a chronic risk to freshwater invertebrates that live in agricultural drains and small rivers. Molinate poses low acute risk to estuarine fish and invertebrates. It poses a risk to nontarget aquatic plants.

Exposure Standards and Guidelines

The reference dose for molinate is $0.002\,\mathrm{mg\,kg^{-1}\,day^{-1}}$.

Miscellaneous

Based on currently available animal data, molinate is shown to be a reproductive toxicant. The registrant (Syngenta) and the California Rice Commission are developing mechanistic data that they believe will support their conclusion that this effect is rodent specific.

See also: Carbamate Pesticides; Cholinesterase Inhibition.

Further Reading

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Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

http://www.epa.gov – US Environmental Protection Agency.

Molybdenum

Robert Kapp

- Related Compounds: Molybdenum dioxide, MoO_2 (CAS 18868-43-4); Molybdenum trioxide, MoO_3 (CAS 1313-27-5); Molybdenum pentachloride, $MoCl_5$ (CAS 10241-05-1)
- Chemical Abstracts Service Registry Number: CAS 7439-98-7
- EINECS No.: 231-107-2
- Synonyms: Amperit (105.054); Amperit (106.2); MChVL Metco (63); Molybdenum, metallic; TsM_1
- Chemical/Pharmaceutical/Other Class: Transition metal.

Uses

Molybdenum occurs naturally in various ores, the most important being molybdenite (MoS_2), which is converted to molybdenum trioxide (MoO_3) for use in ferro- and manganese alloys, chemicals, catalysts, ceramics, and pigments. Molybdenum is a valuable alloying agent because it contributes to the hardening and resilience of quenched and tempered steels. It also increases the strength of steel at high temperatures. Molybdenum is used in electrodes for heated glass furnaces, in nuclear energy applications, in missile and aircraft parts, in the production of tungsten, in glass to metal seals, and in colloidal form as a lubricant additive. It has additional applications in petroleum refining as a catalyst. Generally, molybdenum is prepared from the powder made from hydrogen reduction of purified molybdic trioxide or ammonium molybdenate. Molybdenite is also recovered as a by-product of copper and tungsten mining operations.

Exposure Routes and Pathways

The primary pathway for molybdenum exposure is ingestion by water or food. Molybdenum is found in leafy vegetables, legumes, meat, and many grains. Molybdenum does not appear to be absorbed dermally. Dusts and fumes may be inhaled. In ambient air in urban areas, molybdenum ranged from 0.01 to 0.03 $\mu g\,m^{-3}$, and in nonurban areas it varied between 0.001 and 0.0032 $\mu g\,m^{-3}$.

Toxicokinetics

Water-soluble molybdenum compounds are readily taken up through the lungs and the gastrointestinal tract; but insoluble compounds are not. Following absorption, molybdenum is distributed throughout the body with the highest levels generally found in the liver, kidneys, spleen, and bone. Limited data suggest that 25–50% of an oral dose is excreted in the urine, with small amounts also eliminated in the bile. The biological half-life may vary from several hours in laboratory animals to as much as several weeks in humans. The vast majority of molybdenum found in the liver is concentrated in the outer membrane of the mitochondria where it is readily available as a cofactor in enzyme reactions. Molybdenum is excreted rapidly primarily as in the urine as molybdate.

Mechanism of Toxicity

The physical and chemical state of molybdenum, route of exposure, and compounding factors such as dietary copper and sulfur levels may all affect toxicity. The mechanism of molybdenum toxicity is not yet understood, but is assumed that a primary factor is the formation of a copper–tetrathiomolybdate complex in the reduction medium of the gastrointestinal tract, reducing the biological utility of copper. Molybdenum is considered an essential trace element. It functions as an electron transport agent in the molybdenum–flavoprotein enzyme, xanthine oxidase, and is also a cofactor for aldehyde oxidase, NADH-dehydrogenase, xanthine dehydrogenase, and sulfite oxidase. The molybdate ion (MoO_4^{2-}) inhibits glutaminase and sulfoxidase. The absence of molybdenum creates an interruption of sulfur-containing amino acid metabolism.

Molybdenum deficiency is manifest by alterations in the uric acid and sulfite metabolism, often noted by the development of mouth and gum abnormalities, hypouricemia, hyperoxypurinemia, and eventually coma.

Acute and Short-Term Toxicity (or Exposure)

Animal

Severe gastrointestinal irritation, diarrhea, coma and death from cardiac failure can be symptoms of acute exposure to molybdenum (molybdenosis). The rat oral LD_{50} values are 188 $mg\,kg^{-1}$ (125 $mg\,Mo\,kg^{-1}$) for molybdenum trioxide, and 680 $mg\,kg^{-1}$ (370 $mg\,Mo\,kg^{-1}$) for ammonium molybdate. Oral LD_{100} values of 2200 $mg\,kg^{-1}$ (1200 $mg\,Mo\,kg^{-1}$), 1870 $mg\,kg^{-1}$ (1020 $mg\,Mo\,kg^{-1}$), and 2400 $mg\,kg^{-1}$ (1310 $mg\,Mo\,kg^{-1}$) have also been reported for

guinea pigs, rabbits and cats, respectively, dosed with ammonium molybdate. Inhalation exposures to molybdenum compounds have resulted in respiratory tract irritation, pulmonary hemorrhages, perivascular edema, and liver and kidney damage. Other effects reported in animals include muscle incoordination, loss of hair, loss of weight, changes in electrocardiograms, increased arterial blood pressure, increased serum lactate dehydrogenase, increased cardiac adrenaline and noradrenaline levels, and inflammation of the uterine horns with necrotic foci and endometrial atrophy. Some molybdenum compounds, such as molybdenum trioxide and sodium molybdate (Na_2MoO_4), are strong eye and skin irritants; however, others such as calcium and zinc molybdate are not primary irritants. Molybdenum was one of seven metals reported to cause abnormalities in chick embryos following injection of 4–1000 g sodium molybdate into the air sac of the egg on day 2 of incubation. Neck defects, hemorrhages and reduced body size were the most common abnormalities; however, there was no clear dose–response relationship. Diffuse pneumoconiosis with interstitital pneumonia was observed after 9 months upon histological examination in rabbits that had been given a suspension of powdered molybdenum intratracheally in doses of 70–80 mg kg^{-1}.

Human

In general, molybdenum and its compounds are considered to be of low toxicity to humans; however, molybdenum dust and fumes can cause irritation of the eyes, nose, throat, and respiratory tract. The trioxide and ammonium molybdate are more toxic than the ore molybdenite, the metal or the dioxide. It is not irritating to the skin, and is not a sensitizer. Mild cases of molybdenosis may be clinically identifiable only by biochemical changes (e.g., increases in uric acid levels due to the role of molybdenum in the enzyme xanthine oxidase). Excessive intake of molybdenum causes a physiological copper deficiency, and conversely, in cases of inadequate dietary intake of copper, molybdenum toxicity may occur at lower exposure levels.

Chronic Toxicity (or Exposure)

Animal

Water-insoluble molybdenite (MoS_2) is practically nontoxic; rats dosed with up to 500 mg molybdenite daily for 44 days exhibited no adverse effects. In contrast, animals dosed subchronically with water-soluble molybdenum compounds exhibited gastrointestinal disturbances, growth retardation, anemia, hypothyroidism, bone and joint deformities, liver and kidney abnormalities, and death. Fifty percent mortality was reported in rats maintained for 40 days on molybdenum-enhanced diets containing 125 mg Mo kg^{-1} (as molybdenum trioxide, MoO_3), 100 mg Mo kg^{-1} (as calcium molybdate, $CaMoO_4$), or 333 mg Mo kg^{-1} (as ammonium molybdate, $(NH_4)_2MoO_4$). A dietary level of 0.1% sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) for several weeks was lethal to rabbits. Growth retardation was observed in rats maintained on diets containing 0.04–0.12% molybdenum. Evidence that the toxic effects of molybdenum might be caused by a secondarily acquired copper deficiency was shown in a study where a significant reduction in growth occurred in rats after 11 weeks on a diet containing 20 ppm molybdenum and 5 ppm copper; whereas, growth was not affected by molybdenum dietary levels as high as 80 ppm when the dietary level of copper was increased to 20 ppm. Hypothyroidism, as evidenced by decreased levels of plasma thyroxin, was found in rabbits maintained on a diet containing 0.3% Mo (as sodium molybdate) for several weeks or longer.

Anemia, as well as anorexia, weight loss, alopecia, and bone deformities occurred in young rabbits maintained for 4–17 weeks on a diet containing 0.1% molybdenum (as sodium molybdate). Anemia was also observed in rats maintained on a diet containing 0.04% Mo (as sodium molybdate) for 5 weeks, in rabbits on a dietary level of 0.2% sodium molybdate for 5 weeks, and in chicks on a dietary level of 0.4% sodium molybdate for 4 weeks. Signs of anemia and marked erythroid hyperplasia of the bone marrow were observed in rabbits maintained for 11 days on a diet containing 0.4% sodium molybdate. Bone and connective tissue disorders observed in animals receiving dietary levels of molybdenum 0.04% for 4 weeks or longer included mandibular exostoses, joint deformities, detachment of tendons, epiphyseal line fractures, and epiphyseal plate widening.

The liver can be affected to varying degrees by excessive intake of molybdenum. Significantly elevated levels of serum bilirubin were observed in dogs receiving 20 mg kg^{-1} of ammonium molybdate in their diet for 5.5 months. Fatty changes in the liver occurred in rabbits dosed with 50 mg kg^{-1} day^{-1} of ammonium molybdate for 6 months, and in guinea pigs dosed with 25 mg kg^{-1} day^{-1} of molybdenum dioxide for 14 days. Histological changes in the liver and altered glycolytic enzyme activity were observed in rats dosed with 289 mg Mo kg^{-1} day^{-1} (as ammonium molybdate) in drinking water for 28 days. Severe liver damage, consisting of perilobular necrosis, nuclear clumping and an increase in Kupfer cells, occurred in rats receiving 489 mg Mo kg^{-1} day^{-1}

(as ammonium molybdate) in their diet for 20 days. A 72% reduction in glycogen levels occurred in rats receiving the same dietary level for 30 days. An increase in kidney weight and indications of mild renal failure (decreased glomerular filtration as measured by a reduction in creatinine clearance) occurred in rats dosed for 8 weeks by gastric intubation with 80 mg Mo kg^{-1} day (as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$). Histological changes in kidneys were also observed in rats dosed with 289 mg Mo kg^{-1} day^{-1} (as ammonium molybdate) in drinking water for 28 days. Severe kidney damage, including glomerular shrinkage and epithelial alterations in the distal and proximal renal tubules, occurred in rats receiving 1000 mg kg^{-1} day^{-1} of ammonium molybdate (489 mg Mo kg^{-1} day^{-1}) in their diet for 20 days.

In sheep and cattle, a condition known as 'teart disease' occurs when these animals graze on plants containing abnormally high amounts of molybdenum. Dietary levels of ~10 ppm molybdenum and higher can cause teart disease. Symptoms that may occur within 24 h include weakness and diarrhea. Longer exposure can lead to decoloration of hair, skeletal deformities, sterility due to damage to testicular interstitial cells, poor conception and deficient lactation.

Rats exposed to molybdenum dust (19.7 mg Mo m^{-3}, 4 h daily for 4 months) exhibited inflammation of the uterine horns with necrotic foci and endometrial atrophy. Severe demyelinization of the central nervous system occurred in newborn lambs born to dams maintained on high-molybdenum diets during pregnancy. Seventy-five percent of male rats maintained on a diet containing 80 or 140 ppm Mo (as sodium molybdate dihydrate) from weaning until mating became sterile, and histological examination revealed seminiferous tubule degeneration. Female fertility, gestation, and litter size were not affected by these dietary levels of molybdenum; however, weaning weight of offspring was reduced, indicating deficiencies in lactation. Sterility due to damage to testicular interstitial cells, poor conception, and deficient lactation have also been reported in cattle ingesting large amounts of molybdenum. A three-generation study conducted on mice found that 10 ppm molybdenum in drinking water (1.9 mg Mo kg^{-1} day^{-1}) resulted in a significant increase in the number of dead offspring in the F_1 and F_3 generations compared to the controls; however, the total number of litters per generation and the average litter size per generation were not affected by the molybdenum treatment.

There are no published carcinogenicity studies on molybdenum, and it is not listed as a carcinogen by the (US) Environmental Protection Agency (EPA), the International Agency for Research on Cancer, the (US) National Institute of Environmental Health Sciences' National Toxicology Program (NTP), the (US) Occupational Safety and Health Administration (OSHA), and the American Conference of Governmental Industrial Hygienists (ACGIH). Animal data indicate that Mo may have an inhibitory effect on esophageal and mammary carcinogenesis. However, intraperitoneal injections of MoO_3 in mice produced a significant increase in the number of lung adenomas per mouse and an insignificant increase in the number of mice bearing tumors.

Human

There is no information available on the subchronic oral and inhalation toxicity of molybdenum in humans. In studies conducted in a region of Armenia where levels of molybdenum in the soil are high (77 mg Mo kg^{-1}), many of the adults examined were found to have elevated concentrations of uric acid in the blood and urine, increased blood xanthine oxidase activity, and gout-like symptoms such as arthralgia, articular deformities, erythema, and edema. The daily molybdenum intake was estimated to be 10–15 mg. An outbreak of genu valgum (knock-knees) in India was attributed to an increase in Mo levels in sorghum, the main staple food of the region (the estimated daily Mo intake was 1.5 mg).

An investigation of the incidence of gynecological diseases in female workers at an integrated copper–molybdenum mill in the former Soviet Union did not reveal any evidence of molybdenum toxicosis. Studies of workers chronically exposed to Mo indicate a high incidence of weakness, fatigue, headache, irritability, lack of appetite, epigastric pain, joint and muscle pain, weight loss, red and moist skin, tremor of the hands, sweating, and dizziness. Joint pains, backaches, headaches, and nonspecific hair and skin changes were also the most frequent complaints of male workers at a US molybdenum-roasting plant. The exposed workers, who had been employed for 0.5–20 years, had high levels of molybdenum in the plasma and urine and significantly higher levels of serum ceruloplasmin and uric acid when compared with values for a control group. Elevated blood uric acid levels, as well as symptoms of arthralgia occurred in most Russian workers at a copper–molybdenum plant. Russian studies have also suggested that exposure to molybdenum can result in increased serum bilirubin levels and decreased blood A/G globulin ratios due to a rise in α-immunoglobulins, with the latter interpreted as evidence of liver dysfunction. Pulmonary effects of chronic exposorted in a study in which some workers exposed to Mo and MoO_3 (1–19 mg m^{-3}) for 3–7 years were symptomatic and had X-ray findings indicative of

pneumoconiosis. Adverse reproductive or developmental effects have not been observed in molybdenum workers. Molybdenum is placed in the US EPA's group D, that is, it is not classifiable as to carcinogenicity in humans.

Clinical Management

Upon ocular exposure, the eyes should be generously washed with tap water. Molybdenum ingestion is treated using gastric lavage and saline catharsis.

Environmental Fate

Molybdenum occurs as iron molybdates in nature. Exposure occurs via weathering and release into rivers, from mining, and by the combustion of oil and coal. Terrestrial plants can contain enough Mo to be toxic to animals but the plants can still grow normally. Adding lime to soil increases Mo availability.

Ecotoxicology

A series of experiments were conducted to determine the physiological impact of acute sublethal molybdenum exposure to juvenile kokanee salmon (*Oncorhynchus nerka* Kennerlyi). Molybdenum was relatively nontoxic to juvenile kokanee as the 96 h LC_{50} was $>2000\,mg\,Mo\,l^{-1}$. Exposure to either 25 or $250\,mg\,Mo\,l^{-1}$ for 7 days was found to stimulate a significant dose-dependent increase in ventilation. Acute sublethal molybdenum exposure was found to have little or no impact on kokanee oxygen consumption at rest or immediately following about of forced activity, or on physiological indicators of stress such as plasma lactate, sodium, and cortisol. Despite these findings, prior exposure to 25 or $250\,mg\,Mo\,l^{-1}$ resulted in postexercise loss of equilibrium and exercise-induced delayed mortality. The findings suggest that despite the nontoxic nature of molybdenum, acute sublethal exposure to this metal has physiological consequences to fish exposed for only a brief period.

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average (TWA), is $10\,mg\,m^{-3}$ (as Mo metal and as insoluble compound, inhalable fraction), and $3\,mg\,m^{-3}$ (as Mo metal and as insoluble compound, respirable fraction). The US OSHA permissible exposure limit, 8 h TWA, is $15\,mg\,m^{-3}$ (as Mo metal and as insoluble compound, inhalable fraction), limit. The US NIOSH immediately dangerous to life or health value is $5000\,mg\,m^{-3}$ (as Mo). The US EPA has set a federal drinking water guideline of $40\,\mu g\,l^{-1}$ and an oral reference dose of $0.005\,mg\,kg^{-1}\,day^{-1}$.

See also: Metals.

Further Reading

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Monoamine Oxidase Inhibitors

Rebeca Gracia

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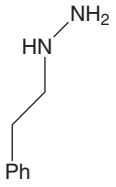
- Representative Chemicals: Phenelzine (Nardil, Stinerval, Monofen, Fenelzin, Kalgan, Nardelzine); Phenethylhydrazine hydrogen sulfate
- Chemical Abstracts Service Registry Number: CAS 51-71-8
- Synonyms:
  Monoamine oxidase inhibitors (MAOI)
  - Nialamide (Niamide): *N*′-(2-Benzylcarbamoylethyl)isonicotinohydrazide
  - Tranylcypromine (Parnate): (±)-*trans*-2-Phenylcyclopropylamine sulfate (2:1)
  - Iproniazid (Marsilid): 2′-Isopropylisonicotinohydrazide
  - Isocarboxazid (Marplan): 5-Methyl-3-isoxazolecarboxylic acid-2-benzylhydrazide or 2′-benzyl-5-methylisoxazole-3-carbohydrazide

- Moclobemide (Avrorix): 4-Chloro-*N*-(2-morpholinoethyl)benzamide
- Selegiline, l-Deprenyl (Eldepryl): (*R*)-(−)-*N*-2-dimethyl-*N*-2- propynylphenethylamine
- Others: Pargyline, clorgyline, pheniprazine, toloxatone, benmoxin, echinopsidine iodide, etryptamine, iproclozide, mebanazine, metfendrazine, phenoxypropazine, pivhydrazine

- Chemical/Pharmaceutical/Other Class: Antidepressants
- Chemical Structure: Phenelzine is the prototype monoamine oxidase inhibitor



Uses

Monoamine oxidase inhibitors are used to treat depression, atypical depression, bulimia, posttraumatic stress reactions, obsessive–compulsive disorder, panic attacks, narcolepsy, phobias, hypochondria, anxiety, and many other psychiatric disorders as well as night tremors, parkinsonism, postural hypotension, headache, and aphthous stomatitis.

Exposure Routes and Pathways

Monoamine oxidase inhibitors are available orally. Accidental or intentional ingestion are the most common routes of exposure.

Toxicokinetics

Monoamine oxidase inhibitors are rapidly and completely absorbed orally reaching peak blood levels within 2 h. Monoamine oxidase inhibitors are acetylated in the liver to many active and inactive metabolites. The volume of distribution is estimated to range from 1 to 4 l kg^{-1}. The inactive metabolites are excreted by the kidneys. The elimination half-lives of monoamine oxidase inhibitor parent compounds range from 15 min to 3.5 h. The biologic half-life often significantly exceeds the elimination half-life.

Mechanism of Toxicity

Monoamine oxidase is the enzyme principally responsible for degradation of amine neurotransmitters (norepinephrine, epinephrine, serotonin, and dopamine). In general, monoamine oxidase inhibitors irreversibly bind to monoamine oxidase leading to neurotransmitter accumulation. Moclobemide, the exception, binds reversibly. They do not have any effect on monoamine oxidase production. The enzyme then regenerates over many weeks. Monoamine oxidase inhibitors may also stimulate the release of norepinephrine from some nerve endings while having a sympatholytic effect at postganglionic terminals. In high doses, monoamine oxidase inhibitors also inhibit other enzymes, which may cause many toxic effects. Iproniazid, isocarboxazid, nialamide, phenelzine, and tranylcypromine are nonselective monoamine oxidase inhibitors. Clorgyline, moclobemide, and toloxatone are type A monoamine oxidase inhibitors whereas selegiline is a type B monoamine oxidase inhibitor. The type B receptors are less commonly found in the alimentary tract; therefore, selegiline does not result in as many drug–food interactions as the other monoamine oxidase inhibitors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Monoamine oxidase inhibitors are not used therapeutically in animals. Toxicity would be expected to resemble that observed in humans.

Human

Any ingestion of 2 or 3 mg kg^{-1} is potentially toxic. At therapeutic levels, the most commonly reported adverse effects are dizziness, headache, nervousness, sleep disorders, drowsiness, ataxia, constipation, dry mouth, weight gain, postural hypotension, and edema. Monoamine oxidase inhibitors combined with sympathomimetic drugs or tyramine-containing foods, in either therapeutic amounts or in overdose, may result in a hypertensive crisis characterized by severe headache, tachycardia, diaphoresis, and hyperpyrexia. In very severe cases, subarachnoid hemorrhage and death have resulted. The clinical effects of monoamine oxidase inhibitor overdose have been categorized into four phases. Phase 1 is an asymptomatic period of 12–24 h. Sympathomimetic stimulation characterizes Phase 2. Symptoms in this phase include headache, agitation, mydriasis, tachycardia, drowsiness, hyperreflexia, flushing, and nausea. Symptoms may worsen to coma, muscle rigidity, hyperpyrexia, hypotension, seizures, and cardiac arrest. Phase 3 is cardiovascular or central nervous system (CNS) collapse. The fourth and last phase is marked by secondary complications that may include renal failure, pulmonary edema, and asystole.

Chronic Toxicity (or Exposure)

Animal

Procarbazine has been associated with increased development of tumors in several animal models compared to controls.

Human

Expected symptoms include drowsiness/CNS depression, and other CNS effects (e.g., psychosis, myoclonus, seizures, extrapyramidal effects).

Clinical Management

Basic and advanced life-support measures should be aggressively implemented. Gastric decontamination with activated charcoal should be performed in patients with recent ingestions. Hypertension should be managed with intravenous sodium nitroprusside or with phentolamine. Agitation, muscle rigidity, and seizures may be controlled with intravenous diazepam or other benzodiazepines. The hypotensive patient should be placed in Trendelenburg's position and given intravenous fluids and pressors such as norepinephrine or dopamine as needed. External cooling should be used in hyperpyrexic patients. Dantrolene has also been used in patients with hyperpyrexia. Hemodialysis and hemoperfusion have not been shown to lead to any improvement in clinical status.

Further Reading

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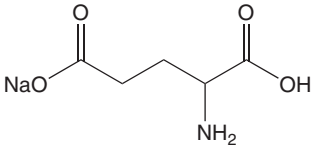
Monomethylhydrazine *See* Mushrooms, Monomethylhydrazine.

Monosodium Glutamate

Arezoo Campbell

This article is a revision of the previous print edition article by Linda Larsen, volume 2, pp. 345–346, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 142-47-2
- Synonyms: L-Glutamate; Sodium glutamate; Glutamic acid monosodium salt; L-Glutamic acid monosodium salt; MSG; Chinese seasoning
- Chemical/Pharmaceutical/Other Class: Acidic amino acid salt
- Chemical Formula: $C_5H_8NO_4Na$
- Chemical Structure:



Uses

Monosodium glutamate (MSG) is used as a food additive, mainly in Oriental cuisine, to enhance and impart a 'meaty' flavor.

Background Information

MSG is the sodium salt of glutamic acid, one of the most abundant nonessential amino acids found in nature. Virtually all foods including meat, vegetables, poultry, and fish contain glutamate. It can be synthesized *in vivo*. By weight, ~10–40% of proteins are composed of the amino acid.

In 1908, Professor Kikunae Ikeda of Tokyo Imperial University extracted crystals of glutamic acid from broth prepared from a type of seaweed. He recognized that the compound imparted a taste distinct from sweet, salty, bitter, and sour. He termed this new taste 'umami'(savoury) and decided to use the newly isolated glutamic acid as a seasoning which enhanced the original flavor of food. The use of MSG in Chinese cuisine has been connected to a complex of symptoms termed 'Chinese restaurant syndrome' (CRS). The most common symptoms are burning sensations in the mouth, facial pressure, chest pain, flushing, headache, tingling, numbness, and generalized weakness. It is thought that ~1–2% of the population is sensitive to MSG. However, two extensive scientific reviews, one in 1987 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and another in 1995 by the Federation of

American Societies for Experimental Biology (FASEB), both concluded that concentrations of MSG in food are not hazardous to human health. The latter source does indicate, however, that there is a subset of individuals who are sensitive and may suffer transient adverse symptoms due to consumption of large amounts of MSG.

Exposure Routes and Pathways

Ingestion of food containing monosodium glutamate is the main route of exposure.

Toxicokinetics

Absorption of dietary glutamate occurs by an active amino acid transport system. The mucosal cells of the gastrointestinal tract then metabolize and utilize it as an energy source. Thus, the amount of glutamate that actually enters the portal blood supply is very low. However, if very large concentrations >5 g of MSG are ingested, the plasma levels can rise significantly. Ingestion of carbohydrates significantly attenuates this effect. The blood–brain barrier is very effective in blocking the passive transport of glutamate into the central nervous system even when the levels of glutamate are elevated in the plasma. The fetus is also protected from any adverse effects because the placenta is impermeable to glutamate. Studies of human infants show that they are capable of metabolizing glutamate similarly to adults.

Mechanism of Toxicity

Although several mechanisms have been proposed to be responsible for causing CRS, none has been extensively studied. One hypothesis has been that the effects are due to an immediate hypersensitivity reaction. Since no IgE-mediated reaction has been documented, there is no direct evidence that this is the case. Another hypothesis is that vitamin B_6 deficiency plays a role in the response because the symptoms were prevented by supplementing individuals with the vitamin. Since glutamate can be converted to acetylcholine by the tricarboxylic acid cycle, it has also been proposed that the effects are due to an increase in acetylcholine levels. It has been noted that after MSG ingestion, there is a decrease in cholinesterase levels. Due to inadequate investigations, it is not currently known if any or all of these mechanisms are responsible for CRS. The neurotoxicity of MSG, demonstrated after exposure to very large doses only in rodent species and rabbits, is attributed to excitotoxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Large concentrations of MSG administered by gavage or intravenous injection causes focal lesions in the hypothalamus of rodents and rabbits. These occur only hours after exposure. The mouse appears to be the most sensitive species affected. Neonatal animals are more sensitive to MSG neurotoxicity. Neuronal damage occurs in neonatal mice when plasma levels reach between 100 and 300 $\mu mol\,dl^{-1}$. In adults, the plasma levels have to reach $>630\,\mu mol\,dl^{-1}$ before similar effects are noted. None of the primate studies were able to demonstrate hypothalamic lesions after exposure to MSG.

Human

The plasma levels of glutamate necessary to cause hypothalamic lesions in mice are never reached voluntarily in humans since the highest palatable dose is well below these concentrations. However, because of the animal studies, MSG is not recommended as an ingredient in baby formulas.

Double-blind controlled trials have failed to demonstrate an unequivocal link between exposure to MSG and CRS because many of the individuals who had a history of suffering from symptoms after ingestion of MSG also reacted positively to the placebo. Furthermore, upon second challenge, the symptoms were different than what they had experienced previously. Therefore, there are cases of individuals who are sensitive to MSG. However, the response is difficult to evaluate clinically. It has been suggested that MSG can trigger an asthma attack in sensitive individuals suffering from severe unstable asthma. However, the studies investigating this link have been inconclusive.

Chronic Toxicity (or Exposure)

There is no evidence of chronic toxicity in either animals or humans.

Clinical Management

The symptoms caused by MSG exposure are uncomfortable but not serious. Those who experience adverse effects should avoid foods containing MSG.

Exposure Standards and Guidelines

An acceptable daily intake has not been set for MSG because the levels typically found in foods do not pose a health threat. However, the FASEB review indicates that a subset of MSG-sensitive individuals will experience CRS after ingestion of >3 g bolus of MSG in the absence of food.

See also: Food Additives.

Further Reading

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Relevant Websites

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http://www.foodstandards.gov.au – Food Standards, Australia, New Zealand.

Monte Carlo Analysis

M A Jayjock, Paul Price, and Cristine F Chaisson

A Brief History and Introduction of Uncertainty Analysis in Risk Assessment

For much of its early and brief history, the field of human exposure/risk assessment has focused on characterizing the highest levels of exposure to substance that will occur to an individual or a population over time as the result of the interaction with a specific source. Examples of substances and their sources include residues in drinking water, chemicals present indoors in a factory or residence, contaminants at a waste disposal site, or a pesticide-containing product used by a consumer or worker. The approach that has been used is to characterize the upper bound of exposure using simple models of the doses received from exposure sources. These source-to-dose models are then evaluated with a series of conservative model inputs. This approach has great value for screening out exposures that are of little concern, and it has formed the basis for US Environmental Protection Agency (EPA) and other exposure and risk assessment guidance, for example, EPA's Risk Assessment Guidance for Superfund and the Office of Pesticide Programs' Residential Standard Operating Procedures (SOPs).

Some risk assessors and risk managers followed these initial efforts by beginning to seek analyses that are more sophisticated. Instead of just an upper bound worst-case overestimate, they wanted more information on the actual range of variation of the exposure, and some measure of the uncertainty associated with the estimate.

It has been stated that anytime we are absolutely certain of a fact, we are almost surely wrong. Indeed, one cannot measure any physical quantity without error, and any activity that aspires to gain and transmit knowledge, including exposure and risk assessment, requires a frank and explicit understanding and communication of inherent uncertainty associated with that practice.

Analyzing Uncertainty: Elements and a Simple Example

The following discussion centers on uncertainty related to the estimation of human exposure. Many of the general principles will be applicable to both exposure and toxicology, and the connection and context of this treatment of uncertainty associated with toxicological determinations will be made later in this discussion.

Uncertainty concerning the determinants of human exposure can be considered as coming from two sources or types

1. The natural variability of these predictors in any particular scenario of interest.
2. Our lack of knowledge about the basic nature of these variables (i.e., our fundamental ignorance of the reality and relationships within that reality that cause the exposure to occur).

We can describe type 1 uncertainty with sampling statistics. These, in turn, describe a tolerance of knowledge around the measurement or estimate.

The second source of uncertainty (type 2 from our lack of basic knowledge) is typically much more troublesome, and tends to dominate because it is often much larger than that posed by type 1. Thus, it is incumbent on the risk assessor to understand and describe this unavoidable subjectivity in as much detail as possible to facilitate the understanding of those who rely on the work, allowing them to comprehend and appreciate its boundaries and limitations.

Historically, the first and more conventional method to examine uncertainty in human exposure

assessment is to look at predictions of dose based on reasonable worst-case scenarios and the impact or sensitivity of the uncertainty for individual variables.

An example for this discussion is a simple indoor air model

$$C = \frac{G}{Q}$$

where C is the equilibrium airborne concentration of a toxicant ($mg\,m^{-3}$), G is the steady (unvarying) generation rate ($mg\,h^{-1}$), and Q is the steady (unvarying) ventilation rate ($m^3\,h^{-1}$).

A conventional way of addressing uncertainty in risk assessments is to estimate and assign 'reasonable' worst-case conditions for our evaluations or models. Thus, in this case one would typically pick the worst case (highest G and lowest Q) to estimate a worst case for C. Next, this could then be combined with 'best case' estimates (lowest G and highest Q) to provide a range for C. Finally, the impact or sensitivity of G or Q on either best or worst-case scenarios could be determined by calculating the results of varying these predictors from maximum to minimum individually in each.

Unfortunately, when a single or 'bright-line' prediction for exposure potential is required, often only the worst-case estimate is reported and used. This single worst-case value is the compounding of all the worst-case uncertainty in all of the predictors. Our example has only two variables, but in some cases with many predictors the estimate of exposure becomes compounded to a much higher order. Historically, the mention or note of the 'average case' or 'best case' is often omitted. Doing so essentially hides valuable information about the uncertainty since those viewing the results have no knowledge and thus no sense of the relative width of the error band around the prediction.

Using the example assume that one has data on the source rate (G) which indicates that between residence values are normally distributed with a mean of $50\,mg\,h^{-1}$ and a standard deviation of $5\,mg\,h^{-1}$ for the particular source of interest. (This is an example of uncertainty type 1 above – a known and measured quantity with natural variability.)

We might take a worst-case estimate of G to be the mean + 3 standard deviations. This is $50 + 15$ or $65\,mg\,h^{-1}$, which is a value greater than 99.8% of the values in this predicted set of values. Best case would be the mean -3 standard deviations or $35\,mg\,h^{-1}$. Thus

- Reasonable worst case, $G = 65\,mg\,h^{-1}$
- Average case, $G = 50\,mg\,h^{-1}$
- Reasonable best case, $G = 35\,mg\,h^{-1}$

However, for the ventilation rate (Q) in this case there is much less certain information or knowledge available. (This is an example of uncertainty type 2 – uncertainty from ignorance or a basic lack of knowledge.) Assume that this particular source will be used in large and small industrial settings almost invariably without benefit of local exhaust. Experience indicates that the general ventilation rate will not likely be less than 0.2 mixing air changes per hour and will most likely not be higher than 30 air changes per hour. (Note Q = (air change per hour)(-room volume).) However, the average or 'most likely' level of ventilation is essentially unknown. Venturing a guess that it is halfway in between 0.2 and 30 without data or confident knowledge would be unwise. So we have

- Worst Case air change per hour = 0.2
- Best Case air change per hour = 30

Using the traditional 'reasonable worst case' approach one could simply take a worst case estimate of G (the mean + 3 standard deviations $= 50 + 3(5) = 65$) and use the worst case estimate of ventilation as 0.2 air changes per hour. Assuming a relatively small room of $3\,m \times 3\,m \times 2\,m$:

$$C = \frac{65\ mg\ h^{-1}}{(0.2\ h^{-1})(18\ m^3)} = 18\ mg\ m^{-3}$$

Best case is

$$C = \frac{35\ mg\ h^{-1}}{(30\ h^{-1})(18\ m^3)} = 0.065\ mg\ m^{-3}$$

There is no average case since there is not enough confidence in any estimate of an average ventilation rate to use it.

The uncertainty range (prediction of exposure) in this analysis varies 277-fold from best to worst case.

Monte Carlo simulation modeling represents the next stage or advancement of uncertainty analysis. This computer-aided stochastic (i.e., random, involving chance) probability analysis technique allows one to more transparently and completely present information about the predictions of exposure and the uncertainty associated with these predictions. In this method the predictor variables, in this case G and Q are described as distributions rather than point estimates of best, worst or average.

Figure 1 shows the attributed distribution for the source rate (G) in the example where G = normal or Gaussian distribution with mean = 50 and standard deviation = 5.0.

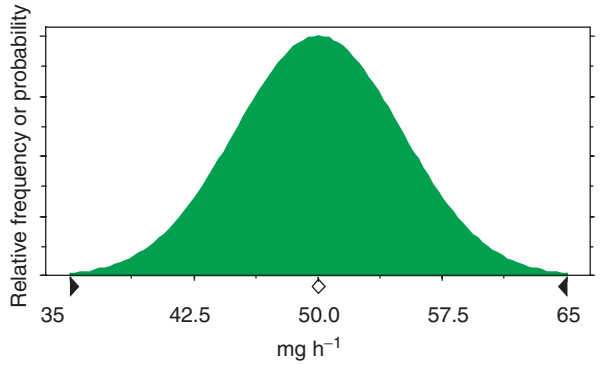

Figure 1 Measured/fitted probability distribution of source generation rate (*G*).

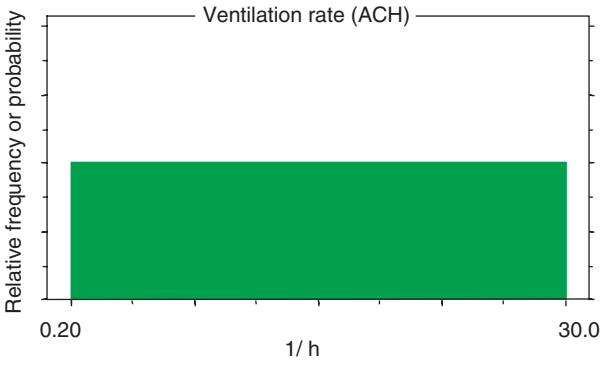

Figure 2 Ascribed probability distribution of ventilation rate (*Q*).

Figure 2 shows the attributed distribution for the ventilation rate expressed in air changes per hour (ACH) as a uniform (i.e., totally random) distribution from 0.2 to 30 air changes per hour. In a room 3 m × 3 m × 2 m (18 m^3) this is a uniform distribution of ventilation rate expressed in $m^3 h^{-1}$ of $Q = 3.6$ to 540 $m^3 h^{-1}$. That is, a distribution in which there is an equal probability of any values within this range occurring and a zero probability of any value occurring outside this range.

The distribution chosen for the air changes per hour is ascribed based on professional judgment; as such, it is a direct result of our lack of knowledge about it. It is important to note that this distribution is not reality, but instead is our best subjective description of our knowledge of reality. There is most likely some finite probability of air change rates being below 0.2 or above 30, and there is certainly some central tendency to this universe of values; however, this distribution represents the quantification of our best knowledge and professional judgment of this situation. Additional data could be used to refine this estimated distribution to be closer to the truth.

Thus, this analysis allows for a distribution (more accurately termed a probability density function or PDF) of values to be used for these two input variables (*G* and *Q*), and these PDFs reflect the quality of our understanding and data. Using a personal computer and readily available Monte Carlo modeling software, a large number (normally 10 000 or more) of independent 'samples' consisting of sets of values for each of the input variables are obtained, and the corresponding distribution of predicted airborne concentration is calculated. This is done by repeated computer runs through the concentration estimation algorithm using PDF selected values for the input parameters. These values are constrained by the known or inferred ranges, means and probability distributions of the individual input parameters. The resulting output is displayed as a forecast graph that shows the entire range of possible outcomes and the likelihood of achieving any of them. This includes a mean concentration and the probability for any concentration above and below the mean. It also provides the upper and lower limits as a measure of dispersion. **Figure 3** shows the output distribution for this example.

This distribution has the following properties

- Median = 0.19 mg m^{-3}
- Mean = 0.46 mg m^{-3}
- 5%tile = 0.09 mg m^{-3}
- 95%tile = 1.7 mg m^{-3}

It is interesting to note that our 'worst case' estimate of 18 mg m^{-3} was not reached in the 10 000 run simulation; the highest prediction in this run (i.e., the 100th percentile) was 14.5 mg m^{-3}. Similarly, the lowest value (i.e., the 0%tile) was 0.07 mg m^{-3}, which is relatively close to the 0.065 mg m^{-3} value as the absolute best case.

An added benefit of Monte Carlo analysis is that a common by-product of this computerized examination is a sensitivity analysis that shows how much each predictor variable contributed to the uncertainty or variability of the predictions. This, in turn, tells both the risk assessor and risk manager which portion of the variability is from natural fluctuation versus how much is caused by lack of knowledge. Given this information, decisions can be made as to where the most cost-effective allocation of resources may occur to refine the estimate of exposure and risk. In the example, the sensitivity analysis shown in **Figure 4** presents the apportionment of variance for the model.

In this analysis estimates of *G* contributed 2.4% of the variance of the predictions while estimates of *Q* contributed 97.6% of the variance of the predictions.

Clearly and not surprisingly the lack of knowledge about the ventilation (*Q*) in this scenario added most of the uncertainty of this analysis. Most important, the estimate of 95th percentile of concentration is

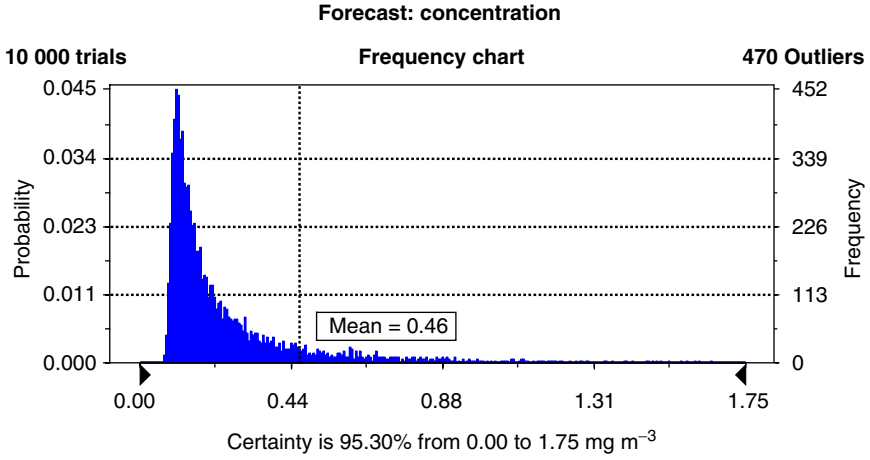

Figure 3 Forecast concentration distribution (C) in $mg\,m^{-3}$.

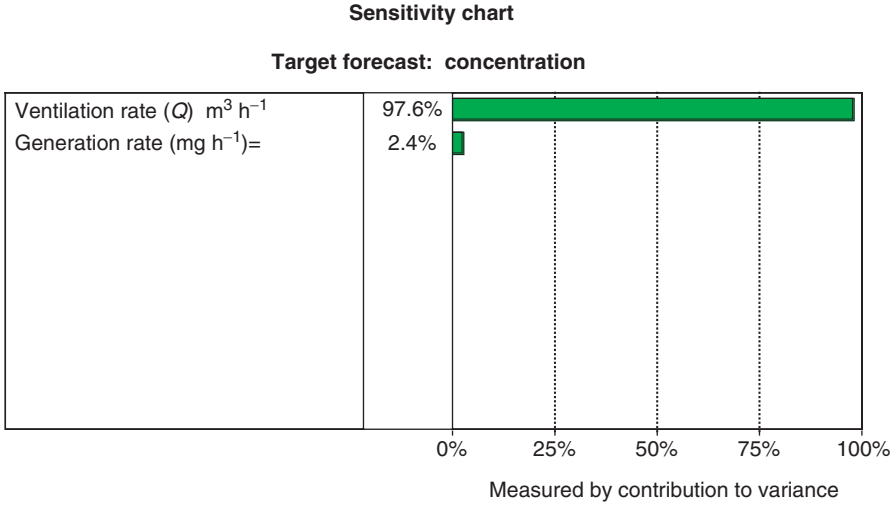

Figure 4 Sensitivity: relative contribution to model variance by each predictor variable.

significantly higher than it would have been with a more accurate description of the ventilation rates in these scenarios.

Relevance to Toxicology and Risk Assessment

The general equation for risk assessment is the product of exposure and toxic potential. The above discussion, focused on human health exposure assessment, is clearly applicable to the analysis of uncertainty in the determinants and predictions of toxic effect per unit exposure. Indeed the complete assessment of risk and its uncertainty comes from the integrated evaluation of uncertainty associated with both toxicity and exposure. It is simply an extension of the technique demonstrated above with the addition of algorithms for toxic effect yielding a forecasted distribution of predicted risk.

Direction of the Field and Related Topics

The next step in the fields of exposure and risk assessment is simulation modeling. Simulation models of exposure and risk have been developed in recent years by the US EPA, industry trade associations, private firms, nonprofit organizations, and academia to answer a need, the obligation to understand aggregate and cumulative exposure.

These simulation models use probabilistic approaches, but are much more complex than the simply Monte Carlo models described above that have been used in exposure assessments. Monte Carlo analysis has been applied to simple spreadsheet calculations of dose using 'add-in' software programs such as @Risk™ or Crystal Ball™. These analyses seek to understand the uncertainty and variation in the predictions of these simple dose models. In contrast, these new models are stand-alone computer

programs that are written in computer languages such as C++, Visual Basic, or SAS. This modeling is sufficiently complex that it can only be investigated using probabilistic techniques.

While the specific models vary in their details and in the sources of exposure that they consider, simulation models have a set of common characteristics. The primary characteristic is a focus on modeling people, not sources of pollution. The models seek to simulate people, their patterns of daily activities (e.g., what they eat, what they do, what consumer products they buy, the kinds of residences they live in, where they live geographically, and how long they live in a residence before moving to a new residence) and their physiological characteristics (e.g., how much they weigh, their breathing frequencies and breathing volumes, and the sizes of various organs of relevance to the assessment). These simulations seek to characterize both how one individual varies from another, and how each individual varies over time.

Each of the models defines individuals' exposures as a series of separate events. These events are defined in a way that allows the calculation of the dose received during the event. An event may consist of eating a specific food (an apple, a slice of pizza, or a fish from a contaminated river) or a specific act (mowing a lawn, applying a pesticide, or refueling a car).

The characteristics of these exposure events are allowed to change from one event to the next. The changes can reflect the day-to-day variation in the types and amounts of foods consumed, the levels of chemical residues in the foods, and in the activities and the characteristics of the person. These changes can reflect factors such as seasonal changes in diet or pesticide residues on foods, seasonal activities such as fishing or gardening applications, changes in activities on weekends versus weekdays, and physiological changes as a person grows from childhood to adult. The result is a set of time-varying doses that occur at specific times in an individual's life. Together the doses create an exposure history for a simulated individual.

These exposure histories can be used in a number of ways. The doses from all of the events in a day can be summed to give an estimate of the daily dose. The daily doses can be averaged to produce long-term estimates of dose (7 day, seasonal, annual, and, in some cases, lifetime). The models can also be used to look at doses when the simulated individual was a child or during certain seasons of the person's life.

By repeating this process for different types of individuals, the models create a picture of the exposure histories of a representative population of individuals. The variation of exposures in such a population can be used to estimate the variation in the US population or other population of interest.

The models provide a powerful tool for evaluating aggregate exposures (the simultaneous exposure to multiple sources of a single chemical) and cumulative exposures (simultaneous exposures to multiple chemicals). They provide the framework for the accurate portrayal of human exposure in the real world, including its uncertainty, while pointing the way to critical research needs in the realm of exposure assessment and toxicology. Indeed, the models have the potential to fully integrate exposure and toxicity models. By defining the person exposed and the temporal patterns of dose, the simulation models can serve as the starting point for pharmacokinetic and pharmacodynamic models of the occurrence of adverse effects. Such models may replace the current system of toxicological safety factors and policy-driven assumptions with actual predictions of risk to human from chemical exposure.

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Morning Glory

Christine Stork and Jeanna Marraffa

- SYNONYMS: *Ipomoea violacea*; *Ipomoea tricolor*; Pearly gates; Wedding bells; Heavenly blue; Blue star

Uses

Morning glory seeds are abused for their hallucinogenic effect.

Background Information

Morning glory is a climbing vine with blue, white, or red trumpet-shaped flowers that open in the morning and close in the afternoon. The leaves are green and heart shaped. A papery thin pod holds small black seeds.

Exposure Routes and Pathways

Exposure is via ingestion. The seeds must be pulverized (chewed) to induce toxicity because the intact seed coat prevents absorption.

Mechanism of Toxicity

Morning glory seeds contain the toxin lysergic acid hydroxyethylamide, which has one-tenth the potency of lysergic acid diethylamide (LSD). The toxic effect, at an equivalent dose, is similar to that of LSD. To avoid abuse of morning glory seeds, commercial seed producers treat the seeds with essential oils, which are irritants, which induce nausea, vomiting, and abdominal pain which precede the psychedelic effects.

LSD acts on several sites in the central nervous system. It is a nonselective serotonin, or 5-hydroxytryptamine (5-HT) agonist on both presynaptic and postsynaptic receptor sites. The 5-HT_{2A} receptor agonism is implicated in the modulation of hallucinations. In addition to the role of serotonin in causing hallucinations, other neurotransmitters, including glutamate and D_1 and D_2 dopamine receptors, are implicated; yet, their role remains elusive.

Acute and Short-Term Toxicity (or Exposure)

Human

Symptoms include nausea, disorientation, delusions, hallucinations, psychosis, panic reactions, paranoia, violent behavior, prolonged changes in perception and sensation, and suicidal ideations. Morbidity and mortality generally result from complications of hyperthermia including rhabdomyolysis, renal failure, and disseminated intravascular coagulopathy. Hyperthermia occurs secondary to autonomic instability and an increase in serotonergic activity. Hyperthermia ensues when there is excess muscle activity in the face of agitation. Sedation with benzodiazepines is usually sufficient to treat the excess agitation and muscle activity; however, occasionally hyperthermia may require more aggressive therapy with active cooling measures and muscle relaxants. Excessive physical restraint should be avoided to prevent further hyperthermia and excess muscle activity. A few morning glory seeds are unlikely to cause significant problems. Several packages of seeds must be eaten to produce toxic effects in adults; however, nausea and vomiting generally precede its psychedelic effects. The contents of the seeds are not absorbed unless chewed. Three hundred seeds have a potency equivalent to 200–300 g of LSD, an amount sufficient to produce an altered state of consciousness. Twenty to fifty seeds may result in increased sociability, restlessness, and alertness followed by a period of relaxation. About 100–150 seeds result in hallucinations, perceptual changes, and improved mood lasting up to 4 h. About 200–500 seeds will cause euphoria, hallucinations, and philosophical thought. Adverse side effects are likely at this dose and include nausea, vomiting, abdominal pain, fatigue peripheral temperature, and sensation changes.

Clinical Management

Standard decontamination methods are rarely needed due to the rapid absorption of the agent and the likelihood that their use will exacerbate the patient's emotionalism. The patient's environment should be managed to prevent self-harm and to promote calmness. Significant agitation, dysphoria, or autonomic instability can be effectively treated with benzodiazepines. Phenothiazines have been utilized but are associated with an increased risk of hallucinogen persisting perception disorder (flashbacks) as well as increased risk of hyperthermia and agitation secondary to its anticholinergic properties. Symptoms usually resolve in 8 h. Close psychiatric follow-up may be needed if symptoms persist.

See also: LSD (Lysergic Acid Diethylamide); Plants, Poisonous.

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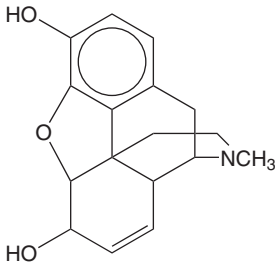
Morphine

Michael Hiotis

- Chemical Abstracts Service Registry Number: CAS 57-27-2
- Synonyms: 7,8-Didehydro-4, 5-epoxy-17- methyl-morphinan-3,6-diol morphine acetate; Morphine CHM; Morphine sulfate
- Chemical/Pharmaceutical/Other Class: Opiate analgesic; an alkaloid and phenanthrene derivative of opium
- Chemical Structure:



Uses

Morphine is used as an analgesic for acute and severe pain, as a sedative, as an antitussive, and for treatment of dyspnea in left ventricular failure and acute pulmonary edema. It is DEA Class II and has high drug abuse potential.

Exposure Routes and Pathways

Morphine is available for oral, rectal, and parenteral administration. Oral formulations include immediate and controlled release tablets, as well as oral solution regular strength and concentrate. Parenterally, it can be administered subcutaneously, intramuscularly, intravenously, or by continuous infusion. It is a drug of abuse and can be nasally insufflated.

Toxicokinetics

Morphine is rapidly absorbed from the gastrointestinal tract after oral administration and its bioavailability is ~40%, since it undergoes extensive first-pass metabolism in the liver and gut. Oral morphine is about one-sixth as potent as morphine administered parenterally. After parenteral injection, morphine is readily absorbed into the blood and peak effects occur within 30 min to 1 h. Morphine is metabolized in the liver by N-demethylation. The majority of a dose of morphine is conjugated with glucuronic acid to its major metabolite morphine-3-glucuronide (M3G) which is inactive, and the active metabolite morphine-6-glucuronide (M6G). Other active metabolites include normorphine, codeine, and morphine ethereal sulfate. Enterohepatic circulation of conjugated and intestinally deconjugated morphine has been reported. Morphine is distributed throughout the body, but mainly in the kidneys, liver, lungs, and spleen. The volume of distribution is 3 or $4\,l\,kg^{-1}$. Mean plasma elimination half-life is 1.7 h for morphine and 2.4–6.7 h for M3G. Up to 10% of a dose may eventually be excreted, as conjugates, through the bile into the feces. The remainder is excreted in the urine, mainly as conjugates.

Mechanism of Toxicity

Morphine is the prototype for the class of natural and synthetic opioid analgesics and its toxicity stems mainly from its extensive effect on the central nervous system (CNS), principally that of a descending depression. Opioids interact with stereospecific and saturable binding sites mostly located in the CNS. Interaction with the opioid receptors mimics the actions of endogenous enkephalins and endorphins. Morphine is a pure opiate agonist and exerts its activity primarily on the mu receptor. Activity also appears to involve an alteration in the release of neurotransmitters, such as the inhibition of acetylcholine, norepinepherine, and dopamine. These actions result in the therapeutic effects of analgesia, sedation, euphoria, and decreased gastrointestinal motility; however, in toxic amounts they can lead to

respiratory depression, coma, and cardiovascular collapse.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs and monkeys act similarly to humans – symptoms may include drowsiness, ataxia, vomiting, respiratory depression, miosis (mydriasis in monkeys), coma, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone may be used at $0.02\,mg\,kg^{-1}$ if needed. Morphine is used in veterinary practice for the treatment of pain and for cough suppression.

Human

Symptoms of toxicity may occur in varying degrees in nontolerant adults who receive greater than a therapeutic amount of morphine. The primary insult is respiratory depression from direct depression of the CNS. This state may then progress to apnea or respiratory arrest. Pulmonary edema is a common complication. Therapeutically, morphine results in analgesia; however, when toxic doses are used, CNS depression results in coma. Miosis is frequent, but in an acidotic or asphyxiated state, pupils may be dilated. From a cardiovascular perspective, morphine causes a decrease in systemic vascular resistance, which may result in a fall in systemic arterial pressure, thus leading to severe hypotension. A decrease in sympathetic tone can yield bradycardia. Hypothermia may also ensue with peripheral vasodilation. Laboratory analysis of morphine is useful only as confirmation of its presence; it does not dictate treatment. Semiquantitative and qualitative EMIT homogenous enzyme immunoassays are available for measurement of opiates in urine. Lastly, toxicity can result from therapeutic mistakes such as dispensing the wrong formulation of morphine, for example if solution concentrate is dispensed instead of the regular strength.

Chronic Toxicity (or Exposure)

Animal

As in humans, animals can become dependent on morphine after chronic use. Dependence has been produced with as few as two injections per day. Chronic dosing studies during pregnancy in rats have not produced teratogenic effects. However, growth retardation was observed in the intermediate dosing group ($35\,mg\,kg^{-1}\,day^{-1}$).

Human

Opiates have a high potential for abuse. Chronic users may develop tolerance, thus necessitating larger doses for the desired effect. Toxic effects in chronic abuse can result in decreased immunity. Abrupt cessation can cause withdrawal, yielding restlessness, agitation, yawning, vomiting, and diarrhea. It has been reported that patients with significant renal impairment may develop toxicity from accumulation of the active metabolite M6G.

In Vitro Toxicity Data

Drosophila studies of morphine have been either negative or inconclusive.

Clinical Management

Basic life-support measures should be instituted, as necessary, and initial management should include establishment of secure airway and support of ventilation and perfusion. Patients with mild to moderate toxicity may present with lethargy, miosis, hypotension, bradycardia, and muscle flaccidity and may require only supportive care. With more severe toxicity, respiratory depression, coma, noncardiogenic pulmonary edema, apnea, cardiovascular collapse, and death may occur. If taken orally, administration of activated charcoal is recommended, as soon as possible, to minimize absorption of morphine. Gastric lavage may be considered if time of ingestion is less than 1 h but should not delay administration of activated charcoal. Whole bowel irrigation may be considered for ingestions of controlled-release products. Emesis is contraindicated due to potential for significant CNS and respiratory depression. The specific antagonist naloxone is used to counteract respiratory depression and coma. A dose of 0.4–2.0 mg is given intravenously and can be repeated at intervals of 2 or 3 min. Naloxone can precipitate withdrawal syndrome in patients with physical dependence and caution is advised. The therapeutic effect of naloxone maybe of shorter duration than that of morphine activity; therefore, a naloxone continuous infusion may then be of benefit. Nalmefene and naltroxone are other opioid antagonists that are similar to naloxone but with longer duration of action and may be considered as an alternative to naloxone. These agents also have partial opiate agonist activity. Arterial blood gases, vital signs, and level of consciousness should be monitored continuously until cessation of symptoms.

See also: Poisoning Emergencies in Humans.

Further Reading

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Mothball *See* Naphthalene.

Mouse Lymphoma Assay

Robin C Guy

Background

The mouse lymphoma (MOLY) assay is an *in vitro* mammalian cell gene mutation test that can be used to detect gene mutations induced by chemical substances. The cell line used is the L5178Y MOLY cell. In these cell lines the most commonly used genetic end points measure mutation at the thymidine kinase (TK) locus on the mouse chromosome 11b.

In the early 1950s, while attempting to induce tumors in female DBA/2 mice by painting them with 3-methylcholanthrene, Dr Lloyd W. Law at the National Cancer Institute isolated the L5178Y cell line. Later, in 1958, Dr G. Fischer at Yale University was successful in getting the L5178Y cells to grow *in vitro*, using a semi-defined medium (Fischer's medium). In the early 1970s, D. Clive *et al*. developed the MOLY forward mutation assay.

TK is an enzyme that allows cells to take up thymidine from the surrounding medium for incorporation into the DNA. Specifically for this assay, the $TK^{+/-}$ phenotype is used. If a thymidine analog were added to suspension or soft agar, it would eventually be incorporated into the DNA, thereby resulting in the death of the cell. Cells deficient in thymidine kinase ($TK^{-/-}$) due to the mutation ($TK^{+/-} \rightarrow TK^{-/-}$) are resistant to the cytotoxic effects of the pyrimidine analogue trifluorothymidine (TFT). TK-proficient cells are sensitive to TFT, which causes the inhibition of cellular metabolism and halts further cell division. Thus mutant cells are able to proliferate in the presence of TFT, whereas normal cells, which contain thymidine kinase, are not.

When TFT is used as the selective agent and the ($TK^{+/-} \rightarrow TK^{-/-}$) mutation occurs, a possibility of two colony sizes of mutants may be observed. Large colonies of $TK^{-/-}$ mutants would have cytogenically normal 11b chromosomes while smaller colonies of $TK^{-/-}$ mutants would have cytogenically damaged 11b chromosomes. Therefore, increased numbers of the smaller mutant colonies are generally considered to be the result of a clastogen. This assay is unique in that it can detect chromosomal damage because the TK is functionally heterozygous. Therefore, if a deleted essential function is supplied by the homologous portion of the homologous chromosome, the cells will survive, but the colony may be slower growing and hence, smaller. Other mammalian mutagenesis assays do not pick up the chromosomal damage.

The preferred selective agent, 5-trifluorothymidine (TFT), may be used in this study as a thymidine analog. 5-Bromo 2′-deoxyuridine (BUdR) has also been successful in selecting mutants; however, BUdR is mutagenic in mammalian cells and it requires several cell divisions before triggering cell death, thereby giving a hazy background lawn, and is presently not used very often. Cells exposed to TFT do not need to go through the prolonged cell division, and therefore, treating with TFT creates a clearer background lawn.

Many compounds that are positive in the MOLY assay are mammalian carcinogens; however, there is not an exact correlation between the MOLY assay and carcinogenicity. Correlation may be dependent on chemical class. There is a possibility that pseudodiploid transformed cells may affect the response and thymidine analogs are not recommended for testing with this assay. Care should be taken to avoid conditions that would lead to results not reflecting authentic mutagenicity. Positive results that do not reflect authentic mutagenicity may arise from changes in pH, osmolality (including very high

concentrations of test article), extended exposure to S9 (a rat liver homogenate prepared from the livers of rodents treated with enzyme-inducing agents such as Aroclor 1254), or high levels of cytotoxicity.

The MOLY assay is recommended by ICH guidelines as part of a standard genetic toxicology battery. The other assays include the Ames (bacterial reverse mutation) and micronucleus tests.

Cultures of established cell lines or cell strains should be used. These should be determined to be mycoplasm free and should be karyotyped. To reduce the spontaneous frequency of the $TK^{-/-}$ mutants, the cells should be cleansed, that is, exposed to conditions that inhibit this phenotype, then returned to normal growth media for a few days before the start of the study. The cells used are selected on the basis of growth ability in culture and stability of the spontaneous mutation frequency.

Tests conducted *in vitro* generally require the use of an exogenous source of metabolic activation. This metabolic activation system simulates the metabolic characteristics of a mammal under *in vivo* conditions. Therefore, a typical assay should determine the chemical's mutagenic potential in the absence and presence of a metabolic activation system (S9). For both of the metabolic situations, a negative (solvent) and the appropriate positive control should be tested concurrently.

The MOLY assay consists of a preliminary dose range-finding phase and the final mutagenicity phase. For the dose range-finding phase, cells in suspension or monolayer culture are exposed to the test substance, both with and without metabolic activation, for about 4 h or any other suitable period of time. Nine to 10 concentrations should be used. They are then subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection. The vehicle used may include sterile water, dimethylsulfoxide, ethanol, or acetone. Cytotoxicity is usually determined after 24–48 h by measuring the relative cloning efficiency (survival) or the reduction in relative total growth of the cultures after the treatment period as compared to the negative controls.

Concentrations for the mutagenicity phase are then selected by determining at least four, but up to 10 concentrations that cover a range of 0–50% to 80–90% reduction in growth. Test and control cultures are prepared with freshly cleansed cells. The cells, with and without S9, should then be exposed to the test article, as appropriate. The cells are treated for 4 h, then are washed and placed in growth medium. The cells are counted and diluted twice in the next 2 days. After the second dilution, cultures are cloned; a specific number of cells are added to a flask containing cloning medium. Some of those cells are then exposed to TFT, and some of the cells are allowed to grow for the determination of cloning efficiency of the cells. Cells are incubated for a sufficient period of time to allow for phenotypic expression of induced mutations (10–12 more days). After this incubation time, colonies are counted. Mutant frequency is determined by seeding known numbers of cells in medium containing the selective agent to detect mutant cells, and in medium without selective agent (nonselective) to determine the cloning efficiency (viability). The mutant frequency is the number of mutant cells observed divided by the number of viable cells.

A typical MOLY study utilizes 10 ml of test article solution. A newer screening study only requires 2 ml of solution, thereby reducing the amount of test article needed for exposure to the cells.

Negative (solvent) and positive controls must be utilized for a valid study. A historical database must be maintained for these results. Examples of positive control substances that detect both large and small colonies include:

- Absence of exogenous metabolic activation:
  - Methylmethanesulfonate (CAS 66-27-3)
- Presence of exogenous metabolic activation:
  - cyclophosphamide (monohydrate) (CAS 50-18-0 (6055-19-2));
  - benzo(*a*)pyrene (CAS 50-32-8); and
  - 3-methylcholanthrene (CAS 56-49-5).

To ensure that the results of an assay are valid, specific criteria have been determined. The mutation frequency of the positive control group should be twice that of the solvent control group. The solvent controls should have a spontaneous mutation frequency between 20 and 100 per 10^6 surviving cells. In addition, the plating efficiency of the solvent controls must be $>0\%$.

Once the data are available for analyses, evaluation of the results follows. A positive result is one from which the test article, at two or more concentrations that produce $>10\%$ growth, produces a concentration-related increase in mutant frequency that is twofold greater than the background level. An equivocal result is one from which the test article, at a concentration that produces $>10\%$ growth, produces a mutant frequency that is twofold greater than the background level. In this case, the study should be repeated and if the results were repeatable, the study is positive. A negative result is one from concentrations that produced $>10\%$ growth; there were no concentration-related increase in mutant

frequency and no twofold greater increase in the background level.

See also: Ames Test; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Genetic Toxicology; Good Laboratory Practices (GLP); International Conference on Harmonisation; Micronucleus Assay; Redbook; Toxicity Testing, Mutagenicity.

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Relevant Websites

http://www.epa.gov – US Environmental Protection Agency.
http://www.fda.gov – US Food and Drug Administration Website.

Mouthwash

Nancy Linde

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- SYNONYMS: Cepacol; Gel-kam (Rx); Hexetidine; Listerine; Listermint; Plax; Prevention Mouth Rinse; Scope; Signal; Therasol; Viadent

Use

Mouthwash is used to improve oral hygiene through the reduction and/or prevention of dental plaque and gingivitis.

Background Information

Over-the-counter (OTC) mouthwashes may contain ethanol in concentrations of 14–27% (v/v), water, flavor, sweetener, preservative, color, and an astringent. Ethanol is a universal diluent that is mildly polar, able to easily cross cell membranes, and considered the toxic constituent in mouthwashes. Active ingredients are generally considered safe and may include combinations of eucalyptol (0.02–0.1%), menthol (0.04–2.0%), methyl salicylate (0.06–0.4%), and thymol (0.06%), as well as cetylpyridinium chloride, stannous fluoride, hydrogen peroxide, and povidone iodine.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to mouthwash. Ocular exposure is also a possible route of exposure.

Toxicokinetics

Ethanol absorption through the stomach wall is minimal. Since rapid absorption occurs in the small intestine, factors that delay or enhance gastric emptying will influence the rate of absorption of ethanol into the blood. The enzymatic oxidation of ethanol occurs primarily in the liver, first to acetaldehyde by the enzyme alcohol dehydrogenase and then conversion to acetic acid by the enzyme aldehyde dehydrogenase. Acetic acid is available for the formation of acetyl coenzyme A, which enters the Krebs cycle and is eventually metabolized to carbon dioxide and water. Ethanol is uniformly distributed throughout all tissues and body fluids. The volume of distribution approximates 0.47–0.61 kg^{-1}. Approximately 2–10% is eliminated by the kidneys and lungs. Ethanol follows Michaelis–Menton kinetics. Therefore, half-life determination is not meaningful. An average adult decreases blood ethanol levels by 15–20 mg dl^{-1} h^{-1}.

Mechanism of Toxicity

The toxic component of commercial mouthwash products is ethanol. Ethanol is a central nervous system depressant that selectively depresses the reticular

activating system, resulting in disruption of the motor and thought processes. Preferential suppression of inhibitory neurons most likely causes the excitation seen at low ethanol concentrations.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal toxicity corresponds to ethanol toxicity in humans.

Human

Since common commercially available mouthwashes contain only moderate concentrations of ethanol, casual ingestions will produce no toxicity. Significant ingestions, which result in blood alcohol levels of $\geqslant 100\,\mathrm{mg\,dl^{-1}}$ may result in ataxia, slurred speech, decreased motor skills, diplopia, and decreased attention. Unpredictable hypoglycemia may occur in children. Extreme ingestions, resulting in blood alcohol concentrations of $\geqslant 300\,\mathrm{mg\,dl^{-1}}$, may result in vision impairment, stupor, or respiratory failure. Mouthwashes may be irritating to the eyes on contact.

Chronic Toxicity (or Exposure)

Animal

Animal toxicity corresponds to ethanol toxicity in humans.

Human

The complications of ethanol abuse are many. Ethanol-containing mouthwash products may be used by a chronic abuser as an alcohol substitute.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be used as deemed appropriate to the patient's level of consciousness and the history of the ingestion. Casual ingestions of mouthwash do not necessitate treatment.

Ocular exposures require immediate flushing of the affected eye(s) with a steady stream of tepid water for a minimum of 15 min. If ocular irritation persists, an ophthalmology consultation is required.

Treatment of chronic alcohol toxicity involves replacement of nutritional deficiencies such as thiamine, pyridoxine, and vitamins K and C. Correction of dehydration, electrolyte imbalance, and acid–base imbalances is of paramount importance. Chronic abuse may be associated with dependence liability.

Environmental Fate

Normal uses of mouthwash do not present environmental concerns. The major ingredient, ethanol is water soluble and readily biodegrades.

Ecotoxicology

Normal uses of mouthwash do not present ecotoxicological concerns. The major ingredient, ethanol, is water soluble and does not bioaccumulate.

Exposure Standards and Guidelines

There are no exposure standards for mouthwash. Formulations, doses, and labeling are regulated by the Center for Drug Evaluation and Research of the US Food and Drug Administration (FDA). The US FDA also has requirements for manufacturing products according to Good Manufacturing Processes, and requires that all manufacturers, distributors, and mouthwash products be formally registered.

See also: Ethanol; Eye Irritancy Testing; Food and Drug Administration, US.

Further Reading

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Mouthwash.

Multiple Chemical Sensitivities

Kathleen Rodgers

Introduction

The constellation of symptoms that has come to be known as multiple chemical sensitivities (MCS) or idiopathic environmental intolerance (IEI) is increasingly recognized, although the definition of the phenomenon is elusive and its pathogenesis as a distinct entity is unconfirmed. Reports of patients with MCS are increasing, but information on its natural history is lacking. Individuals diagnosed with MCS do not exhibit a specific symptom pattern; a wide range of individual symptoms have been described with the most prominent symptoms being cardiorespiratory (nasal congestions, sore throat, and breathing difficulties), constitutional (fatigue, headache, joint aches, back pain, muscle aches, and weakness), and neuropsychologic (memory loss, forgetfulness, and mood or personality changes). These symptoms are similar to those seen in other syndromes such as chronic fatigue syndrome. In MCS, however, the symptoms have been attributed to chemical exposure. The diagnostic label of MCS is primarily used by a group of physicians who are called clinical ecologists. A description or diagnostic categorization of MCS, which would more readily allow animal modeling and clinical testing, has been difficult due to the involvement of multiple organ systems, lack of consistent symptomatology, and/or the absence of objective and measurable end points (either physical or laboratory findings). No pathophysiologic mechanism for these symptoms has been established although several speculative theories have been proposed: (1) MCS is a purely biologic or psychobiologic response to exposure to low levels of chemicals, (2) MCS is a misdiagnosis of physical or psychologic illness, (3) MCS is a illness belief system shaped by the patient's culture and is possibly iatrogenic, or (4) MCS is a somatoform disorder with genetic factors.

Four hypotheses with regard to the first theory have been invoked to explain the symptoms of MCS: (1) altered immune regulation, (2) neurogenic inflammation, (3) neurologic sensitization of the limbic system by odor, and (4) variations in an individual's biochemical makeup. Experimental models to test these hypotheses have not been established, thereby slowing research into mechanisms.

Definition

The first definition of MCS was created by Theron Randolph in 1962. Randolph described MCS as a condition that (1) is acquired; (2) includes physical and mental symptoms that can be triggered by chemical exposure; (3) has a specific adaptation syndrome (i.e., adaptation to chemicals is followed by chronic illness, withdrawal symptoms upon removal, and shock upon re-exposure); (4) is characterized by a spreading phenomena (i.e., an intolerance to an increasing number of environmental chemicals); and (5) may be resolved by avoidance of chemicals.

Another definition was put forth by ME Cullen and his clinical definition is most commonly used. Although no definition is widely accepted, the Cullen definition allows physicians to distinguish MCS from other collections of similar, commonly experienced symptoms. There are four important characteristics of this definition:

(1) MCS is acquired in relation to some documentable environmental exposure that may initially have produced a demonstrable toxic effect. This aspect excludes patients with long-standing health problems who later attribute certain symptoms to chemical exposure. (2) Symptoms involve more than one organ system, and recur and abate in response to predictable environmental stimuli. (3) Symptoms are elicited by exposures to chemicals that are demonstrable but very low. The exposures eliciting symptoms may be several standard deviations below the average exposures known to cause toxic or irritant health effects in humans and typically involve chemicals of widely varied structural classes and different mechanisms of toxicologic action. (4) The manifestations of MCS are subjective. No widely available test of organ system function can explain symptoms, and there is no objective evidence of organ system damage or dysfunction.

This definition is derived from Cullen (1987) and quoted exactly due to the need to precisely define the contribution of environmental exposure and to distinguish MCS from objectively defined illnesses such as asthma.

One problem with these definitions is that the relationship between the symptoms and the exposure is solely dependent on the patient's report. Further, patients with MCS are generally polysymptomatic and report sensitivities to multiple unrelated substances. In addition, cross-sectional studies indicate that persons diagnosed with MCS have impaired social and occupational adjustment and exhibit other characteristics

of disability. A recent definition by Ashford and Miller is an operational definition; that is, if symptoms disappear on removal from the agent and recur with specific challenges, then one can infer a causal association. In this definition, the rechallenge must be done under strictly controlled environmental conditions.

Theories of Etiology of MCS

Biologic and Psychobiologic Mechanisms

MCS, as defined by clinical ecologists, results from chemical exposure; however, the mechanisms that have been proposed include altered immune function, neurogenic inflammation, neurologic sensitization, and conditioned reflexes. To date, no mechanism has been established.

Various immunologic mechanisms have been postulated based on case reports. Alterations in various measures of antibody and cell-mediated immunity have been measured in patients with MCS, but no consistent pattern of abnormalities has been observed. Several factors, however, confound these studies: lack of standardization of protocols, wide variability (day to day and person to person) with most tests, lack of control for variables known to modulate the immune system (e.g., stress and smoking), and lack of concordance in reports of immune function response. In addition, despite some similarities in symptomatology, MCS is distinctly different from traditional allergy. Patients with allergy generally have well-defined, clinical reactions to allergens and symptoms of rhinitis, asthma, urticaria, or gastrointestinal symptoms occurring shortly after exposure. In addition, if a substance acts as an allergen, a specific cell- or antibody-mediated response develops so the body will only recognize the precise antigen or one with the same structure. It is difficult to explain how structurally different chemicals could result in such diverse symptomatology and organ involvement due to an adverse effect on the immune system.

Another postulated mechanism is an altered function of the central nervous and respiratory systems through an amplification of a nonspecific inflammatory response to low-level irritants (neurogenic inflammation hypothesis). This suggests that MCS may be initiated by the interaction of chemical irritants with sensory nerves or C-fiber neurons, a nonspecific response pathway. It is proposed that inhaled chemicals stimulate irritation receptors which activate sensory nerves to release mediators producing vasodilation, edema, and other manifestations of inflammation, leading to neurogenic inflammation. There is some evidence in animals for this theory, though similar studies in humans do not generally support this theory. In animals, nasal irritation activates systemic reflexes, producing increased blood pressure and bradycardia.

Another hypothesis is that environmental chemicals gain access to the central nervous system via the olfactory and limbic pathways. The absence of a blood–brain barrier in the olfactory system could permit direct access of environmental chemicals through the nasal mucosa to the olfactory bulb. The olfactory and limbic systems are anatomically linked and participate directly and indirectly in the regulation of cognitive, endocrine, and immune functions. In this hypothesis, chemical exposure could induce lasting changes in limbic and neuronal activity and alter a broad spectrum of behavioral and physiological functions.

Animal studies show the olfactory and limbic pathways are particularly susceptible to kindling, the ability of a stimulus previously unable to induce a seizure to later induce one. Animal studies also show that acute administration of a high dose or intermittent repeated low-dose exposures to chemicals cause limbic 'kindling', and that this response is amplified depending on the time between stimuli. Kindling without a seizure has been shown to cause affective behavior changes in animals. Kindling could amplify reactivity and lower the threshold response to low levels of chemicals.

Similar to the hypothesis of kindling is neural sensitization with progressive host amplification of polysymptomatic responses elicited by chemical exposures following an initiating event. In this theory, repeated exposures to the same or cross-sensitizing stimuli elicit enhanced responses. Within this framework, there may be genetic components that are associated with greater susceptibility to sensitization. These include female gender, alcohol-preferring parents, and preferences for carbohydrates. It is suggested that sensitization acts as an adaptive, sentinel function that allows adaptation to threatening environments. The hypothesis further suggests that individual response specificity rather than toxicant properties may determine the central, automic, and/or peripheral nervous system dysfunctions that are manifest in the disorder.

Additionally, cacosmia, a subjective sense of altered olfactory function and feeling ill on exposure to chemical odors, which is experienced by many MCS patients, might be associated with neurocognitive dysfunction. However, MCS patients do not demonstrate a consistent or specific pattern of neurocognitive deficits and disturbances of memory and attention, which may be a result of depression and anxiety. Some have suggested that cascosmia as

well as MCS may be a manifestation of a stress response. The 'precipitating event' leads to stress and may lead to heightened sensitivity to chemical odors or irritants. Animal studies suggest that exposure to a stressor produces a long-lasting sensitization to some drugs. In surveys of students, individuals reporting extreme cascosmia also had higher incidences of anxiety and depression.

Biochemical mechanisms have also been suggested to explain the symptoms associated with MCS. One hypothesis states that individuals who have genetically or nutritionally defective enzyme detoxification systems might be more susceptible to exposure to low levels of chemicals. Another hypothesis states that chemicals may cause blood vessel constriction, inflammation, or leaking in multiple organ systems which would produce various combinations of symptoms.

Arguing against biologic alterations by chemicals as a cause of MCS symptoms is the observation the patients attribute their symptoms to levels of chemicals much lower than those to which others are occupationally exposed with no adverse effects. Therefore, the relationship of MCS symptoms to chemical exposure does not meet accepted toxicologic principles. In addition, the current evidence and models do not meet the criteria set forth in toxicology to establish a causal relationship between chemicals and MCS, such as strength of association, consistency, specificity, temporality, biologic gradient, and plausibility.

Misdiagnosis

As stated, some physicians believe MCS symptoms are not due to chemical exposure but may be the result of misdiagnosed physical or psychologic illness. Many investigators have concluded that MCS patients are not significantly different from psychiatric patients who do not project their disorder onto the environment. Studies show that patients with MCS meet criteria for depression, anxiety, somatization, and obsessive–compulsive and personality disorders. Psychiatric evaluations have shown that MCS may be a new variant of a 'somatoform disorder'. In this disorder, the patients have a psychological tendency to somatocize or misconstrue normal physical sensation. In addition, studies show that psychologic mechanisms are important in the manifestation, if not the etiology, of MCS. Since it is theoretically possible that MCS produces the psychiatric symptoms, through chemical exposure, lifestyle limitations, or nonbelief of family members, studies have been conducted to attempt to evaluate the presence of psychiatric illness in patients that predated the onset of MCS symptoms. While these studies are ongoing and there is difficulty in discerning the temporal pattern of disease, most of them indicate that the prevalence of somatization symptom pattern among MCS patients prior to onset of symptoms attributed to chemical exposure was significantly greater than that in matched controls. In a group of patients followed over 9 years, 83% met DSM-IV criteria for lifetime mood disorder, 56% for lifetime anxiety disorder, and 56% for lifetime somatoform disorder. These studies also show that there was no apparent difference in the prevalence of preexisting anxiety or depression. However, there are a small number of persons diagnosed with MCS who do not have histories of psychiatric disorders who should be more closely examined for possible mechanisms.

MCS as a Belief System

Others suggest that MCS is a belief system that is supported and reinforced by the clinical ecology subculture. Physicians within this subculture claim special expertise in the diagnosis and treatment of MCS, perpetuating the belief that this disorder is widespread and is responsible for substantial suffering and disability. Within this model, the cause of the illness is believed to be outside the control of the patients and leads to the role of a victim with adversarial interactions with those who do not share the belief system, such as conventional health care providers. MCS shares many features, such as pain, fatigue, and headache, with several syndromes, such as chronic fatigue syndrome, fibromyalgia, and neurasthenia, with few or no objective findings of pathology which encompass patients with functional disability. A factor that may contribute to this culturally shaped illness belief system is the increasing concern of the public regarding health effects of chemical exposure. It is unlikely that the majority of MCS patients are simulating their symptoms or that the symptoms result from suggestion. However, it is likely that the attribution of the symptoms to chemical exposure is due to suggestion in some cases. It is also likely that a patient's beliefs regarding illness modify the expression of symptoms even when resulting from a direct toxic effect of a chemical.

Conclusions

To understand the phenomenon of MCS will require interaction among many disciplines to allow for the examination of the influence of the mind on the body and the influence of physical disease on the psyche. Illness should not be regarded as less 'real' because psychogenic mechanisms may play a major role in

causation, and this should not prevent treatment of the symptoms. In addition, patients diagnosed with MCS are heterogenous and there may be more than one causal mechanism in each person. Since none of the hypotheses described have substantial scientific support, dogmatic adherence to any one theory is unwise, particularly by the physician.

See also: Immune System; Neurotoxicity; Occupational Toxicology; Psychological Indices of Toxicity.

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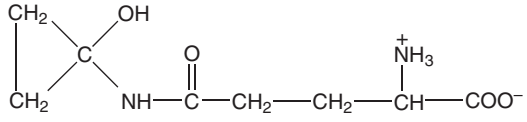
Muscarine *See* Mushrooms, Muscarine.

Mushrooms, Coprine

Anthony S Manoguerra

- Chemical Names: *Coprinus atramentarius; Coprinus insignis; Coprinus variegatus* (also known as *C. quadrifidus*); possibly other *Coprinus* species
- Synonym: Inky-caps
- Chemical Structure:



Uses

There are no uses for mushrooms in this group. They are usually consumed when mistaken for edible mushrooms.

Exposure Routes and Pathways

Ingestion is the route of exposure.

Toxicokinetics

Symptoms occur within 20 min to 2 h after the ingestion of this mushroom and ethanol. The effect of coprine may persist for up to 5 days after ingestion of the mushroom.

Mechanism of Toxicity

Poisoning with mushrooms in this group occurs when ethanol is consumed shortly before or within 5 days after eating the mushrooms. Coprine (*N*(5)-(1-hydroxy cyclopropyl)-L-glutamine) is the active constituent in these mushrooms and has been shown to inhibit liver aldehyde dehydrogenase. The active metabolite, cyclopropanone hydrate, has also been shown to possess similar activity. This inhibition of ethanol metabolism at the point of aldehyde dehydrogenase results in accumulation of acetaldehyde. In the absence of concurrent ethanol consumption, these mushrooms are edible.

Acute and Short-Term Toxicity (or Exposure)

Animal

The consumption of mushrooms and ethanol concurrently is a typically human occurrence; therefore, poisoning in animals is unlikely.

Human

When these mushrooms and ethanol are consumed within the appropriate time frame, symptoms typically develop within 20 min to 2 h. The reaction resembles a disulfiram–ethanol reaction. Symptoms commonly include nausea, vomiting, facial flushing, throbbing headache, weakness, and paresthesias. Less frequently, chest pain, hypotension, and shortness of breath have been seen. No laboratory methods are available for determining the presence of coprine in biologic fluids.

Clinical Management

Because the syndrome is self-limiting and recovery is complete, symptomatic care is often all that is required. Emesis is not indicated because often many hours have elapsed since the ingestion of the mushroom. Also, vomiting is a prominent feature of this type of poisoning and induced emesis may worsen fluid and electrolyte losses. There is no evidence that activated charcoal and/or cathartics provide any benefit in this syndrome. Experimentally, 4-methylpyrazole inhibits the production of acetaldehyde by blocking alcohol dehydrogenase. The clinical usefulness in this type of treatment in mushroom poisoning has not been demonstrated.

See also: Disulfiram; Ethanol; Plants, Poisonous.

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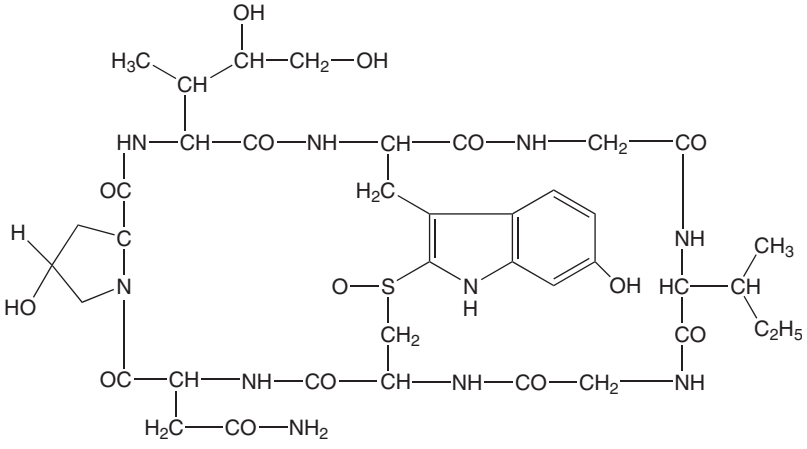
Mushrooms, Cyclopeptide

Anthony S Manoguerra

- Chemical Names: Several genera of mushrooms contain toxic cyclopeptides; these mushrooms include but are not limited to *Amanita bisporigera*; *Amanita hygroscopica*; *Amanita ocreata*; *Amanita phalloides*; *Amanita subalbiacea*; *Amanita tenuifolia*; *Amanita verna*; *Amanita virosa*; *Galerina autumnalis*; *Galerina fasciculata*; *Galerina marginata*; *Galerina sulcipes*; *Galerina venenata*; *Lepiota castanea*; *Lepiota helveola*; *Lepiota subincarnata*; *Lepiota josserandii*; *Conocybe filiaris*
- Synonyms: Destroying angel (*Amanita virosa*); Death cap (*Amanita phalloides*)
- Chemical Structure: α-Amanitin



Uses

There are no uses for mushrooms in this group. They are usually consumed when mistaken for edible mushrooms.

Exposure Routes and Pathways

Ingestion is the route of exposure.

Toxicokinetics

Amatoxins are absorbed rapidly from the gastrointestinal tract. These toxins may be detected in the urine as early as 90–120 min after ingestion of the mushrooms. Radiolabeled amatoxins given to a dog model showed a volume of distribution equal to the volume of extracellular water (160–290 ml kg^{-1}). Amatoxins

disappear rapidly from the blood because they are taken into cells rapidly. No biotransformation of amatoxins occurs. Amatoxins are found in the urine shortly after ingestion of the mushrooms and continue to be detectable for up to 96 h after ingestion. They have also been detected in diarrhea fluid and bile.

Mechanism of Toxicity

Cyclopeptide mushrooms contain both amatoxins and phallotoxins. Studies in animals have shown that, although the phallotoxins are highly toxic when given parenterally, they are not absorbed from the gastrointestinal tract and do not produce toxicity when given orally. The toxicity of cyclopeptide mushrooms is believed to be due to the amatoxins. At least six amatoxins have been identified that differ according to amino acid substitutions on the peptide ring. The α- and β-amanitins are felt to be the predominant cyclopeptides producing systemic toxicity. The phallotoxins may contribute to gastrointestinal symptoms but this is unclear. Amatoxins interfere with RNA and DNA transcription by interfering with RNA polymerase II. Cells with the highest rate of turnover are affected most severely.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ingestion of these mushrooms in dogs have taken place both accidentally and experimentally. The toxic effects mimic those seen in humans.

Human

There is typically a delay of 6–24 h between the ingestion of the mushrooms and the onset of symptoms. The first symptoms are nausea, severe vomiting, and diarrhea, which may result in severe dehydration and electrolyte imbalances. Gastrointestinal symptoms may persist for several days. Over the course of the next 24–36 h, evidence of hepatic injury becomes evident both clinically and by laboratory measurements. Patients may progress to hepatic failure with coma, hemorrhage, and renal failure or begin to recover depending on the degree of injury. In fatal poisonings, death usually occurs after 5 or 6 days. Mortality ranges up to 50% in untreated patients and appears to be $<5\%$ in patients who receive modern intensive supportive care. The severity of poisoning also seems to correlate with the amount of toxin ingested on a body weight basis. The mortality in children appears to be greater probably because of the larger amount of toxin ingested in relation to their body size.

Clinical Management

Immediate and vigorous fluid and electrolyte replacement must be carried out. Oral activated charcoal may be given if the ingestion occurred within the previous 24 h and severe vomiting has not yet begun. No specific antidotal therapy exists for the treatment of this ingestion, although many substances have been tried. The mainstay of therapy is meticulous supportive care.

Some authors have advocated the use of multiple dose activated charcoal or gastroduodenal drainage, but evidence for the effectiveness of these procedures is lacking. Treatment of hepatic failure follows the routine supportive procedures standard for this process. Liver transplantation has been used successfully in patients who appeared to have developed irreversible hepatic failure. Experimental studies in animals have suggested the use of high-dose penicillin G and silibinin. These substances may inhibit the uptake of amatoxins into the liver and are often given in combination in Europe. Silibinin is not available in the United States. The efficacy of these treatments in humans is unknown. Thioctic acid has also been advocated; its efficacy is unclear. It does not appear to provide any protection in experimental animal studies. It also is not available in the United States. High-dose cimetidine, high-dose vitamin C, and *N*-acetylcysteine have been studied in animals and do not provide protection against toxicity. Based on the fact that large amounts of amatoxins are found in the urine during the first 24 h after ingestion, it has been suggested that forced diuresis may increase the excretion of the toxin. Proof of efficacy is lacking. Hemodialysis or hemoperfusion have not been shown to enhance survival and hemodialysis is only indicated as a supportive measure in patients who develop renal failure.

Further Reading

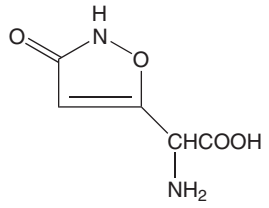
Bartoloni ST, Omer FB, and Giannini A (1985) Amanita poisoning: A clinical-histopathological study of 64 cases of intoxication. *Hepato-gastroenterology* 32: 229–231.

Hanrahan JP and Gordon MA (1984) Mushroom poisoning. *Journal of the American Medical Association* 251: 1057–1061.

Mushrooms, Ibotenic Acid

Anthony S Manoguerra

- Chemical Names: *Amanita muscaria*; *Amanita pantherina*; *Amanita corthurnata*
- Synonyms: Fly-agaric; Panther
- Chemical Structure:



Uses

There are no uses for this mushroom. It is typically consumed for its psychopharmacologic effects.

Exposure Routes and Pathways

Ingestion is the route of exposure.

Toxicokinetics

The symptoms of intoxication seen after ingestion of this mushroom appear ~1 h after ingestion. Ibotenic acid is converted to muscimol by decarboxylation. Ibotenic acid and muscimol can both be detected unchanged in the urine. Other metabolites found in the urine include pantherin, tricholomic acid, and solitaric acid. Intoxication with this mushroom peaks at ~5 h after ingestion and lasts for up to 10 h with a hangover effect the next day.

Mechanism of Toxicity

Ibotenic acid is structurally similar to glutamic acid, whereas muscimol closely resembles gamma-aminobutyric acid (GABA). Muscimol has an affinity for GABA receptors in the central nervous system, functioning as a false neurotransmitter, and appears to mimic the effects of GABA.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal ingestions of these mushrooms are rare and, therefore, experience is limited. Of nine puppies that supposedly had ingested *Amanita pantherina*, three died and the others developed seizures and the inability to walk. All survivors fully recovered by 17 h after ingestion.

Human

Following the ingestion of a single mushroom, symptoms of intoxication typically appear within an hour. Nausea and vomiting are common. Users describe a typical experience where they can "view themselves from outside their own bodies along with a sense of being freed from gravity." Small objects appear large. The appearance of the user during this time may resemble someone with ethanol intoxication. On occasion, users appear to have the desire to carry out extreme physical activity followed by a deep, 'death-like' sleep from which arousal is difficult. Upon wakening, users describe vivid visions during this dream period. Severe poisoning is rare, but seizures have been reported to occur in children. Deaths are said to have occurred from these mushrooms but the documentation of these occurrences is poor and the deaths may have been related to medical problems that were exacerbated by the intoxication.

Clinical Management

Most patients who ingest these mushrooms require no treatment other than observation. In recent, accidental ingestions, activated charcoal may be administered, although the efficacy of this treatment is unknown. In severe cases, when seizures occur, benzodiazepine therapy may be required. Long-term anticonvulsant therapy should not be required because the effects of the mushroom are short-lived. All other treatment is supportive and symptomatic in nature. Atropine should not be administered because these mushrooms, despite their name, contain only trace to no amounts of muscarine or other cholinergic substances.

Further Reading

Benjamin DR (1992) Mushroom poisoning in infants and children: The *Amanita pantherina*/muscaria group. *Clinical Toxicology* 30: 13–22.

Carter CA, Wojciechowski NH, and Skoutakis VA (1983) Management of mushroom poisoning. *Clinical Toxicology Consultant* 5: 103–118.

Mushrooms, Monomethylhydrazine

Anthony S Manoguerra

- Chemical Names: *Gyromitra esculenta; Gyromitra fastigiata; Gyromitra infula; Gyromitra ambigua; Gyromitra brunnea; Gyromitra californica; Gyromitra korfii; Gyromitra sphaerospora; Gyromitra giga*. All other species of Gyromitra should be considered toxic unless proven edible. Gyromitra sp. mushrooms are nongilled mushrooms in the class Ascomycetes
- Synonyms: False morel; Beefsteak mushroom; Elephant ears mushroom
- Chemical Structure: Monomethylhydrazine $CH_3–NH–NH_2$

Uses

There are no uses for mushrooms in this group. They are usually consumed when mistaken for edible mushrooms.

Exposure Routes and Pathways

Ingestion is the most likely route of exposure; however, it has been stated (without good documentation) that poisoning can occur from inhaling monomethylhydrazine vapors that come off in the steam during boiling of the mushrooms.

Toxicokinetics

The absorption rate is not known. Symptoms typically occur after a latent period of 6–24 h after ingestion of the mushroom. Gyromitrin is converted to methylformylhydrazine (MFH) and then to monomethylhydrazine (MMH). Some MFH is also converted to nitrosamide, which causes liver tumors in experimental animals.

Mechanism of Toxicity

Gyromitra sp. mushrooms contain ~0.12–0.16% of the toxin *Gyromitrin* (*n*-methyl-*N*-formylhydrazone). This compound is very unstable and undergoes hydrolysis at low cooking temperatures to the toxic compound MMH. In some species of *Gyromitra*, MMH may be found in its free form and, therefore, cooking may not be required for toxicity to occur. Because MMH is highly volatile, it has been suggested that boiling of the mushroom and discarding the water may yield an edible mushroom, although cases exist in the European literature in which poisoning occurred even after the mushroom was boiled and the water discarded. MMH is thought to act similar to other naturally occurring and synthetic hydrazines by acting as a pyridoxine antagonist. Because many enzyme systems require pyridoxine as a cofactor, hydrazines are capable of affecting numerous metabolic pathways. For example, hydrazines interfere with pyridoxine utilization by both glutamic acid decarboxylase and γ-aminobutyric acid (GABA) transaminase leading to decreased concentrations of the inhibitory neurotransmitter, GABA, in the brain and producing the resultant seizures. Hepatotoxicity is thought to result from a direct toxic action of a metabolite similar to that which occurs with isoniazid. MMH has also been shown to inhibit glycolysis; blood glucose levels fall markedly in experimental animals after exposure to MMH. MMH may also cause methemoglobinemia and hemolysis of red blood cells.

Acute and Short-Term Toxicity (or Exposure)

Animal

The same pattern of toxicity that occurs in humans has been induced experimentally in small animals. The treatment is similar.

Human

Symptoms typically appear following a 6–8 h latent period; however, symptoms have been reported to occur as early as 2 h after ingestion or as long as 24 h after ingestion of the mushrooms. The initial symptoms are vomiting, fatigue, dizziness, and headache. In some cases, but not consistently, a watery diarrhea may also occur. In mild cases, recovery then occurs over a period of 2–5 days. In severe cases, the poisoning may progress to coma, seizures, and hepatic injury leading to hepatic coma and death. Hemolysis with a resultant anuria has also been reported.

Chronic Toxicity (or Exposure)

Animal

MMH and its precursor metabolite nitrosamide are low-grade carcinogens in experimental animals. How this relates to chronic, subacute consumption of this substance in mushrooms is unknown.

Clinical Management

As most patients do not present for care until symptoms develop, emesis or gastric lavage are unlikely to provide much benefit. Activated charcoal administration may be considered to reduce absorption of any remaining material in the gastrointestinal tract, but proof of efficacy is lacking. Based on the resemblance to isoniazid and other hydrazine toxicity, pyridoxine has been suggested, although experience is limited. The recommended dose is $25\,mg\,kg^{-1}$ given as an intravenous infusion over 15–30 min. It can be repeated to treat neurologic symptoms (seizures and coma) as needed, up to a maximum of $15–20\,g\,day^{-1}$. Seizures may also be controlled with benzodiazepines or barbiturates. Severe hemolysis may require blood transfusions and anuria may require short-term hemodialysis until renal function recovers. Hepatic injury leading to hepatic coma is treated with supportive care.

See also: Hydrazine; Plants, Poisonous; Pyridoxine.

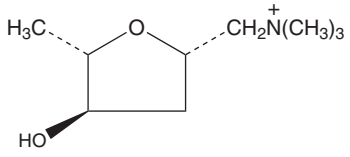
Further Reading

Braun R, Greeff U, and Netter KJ (1979) Liver injury by the false morel poison gyromitrin. *Toxicology* 12: 155–163.
Giusti G and Carnevale A (1974) A case of fatal poisoning by *Gyromitra esculenta*. *Archives of Toxicology* 33: 49–54.

Mushrooms, Muscarine

Anthony S Manoguerra

- Chemical Names: Muscarine and muscarine-like compounds are present in varying quantities in many different species of mushrooms but reach clinically significant quantities only in certain species of *Clitocybe*, *Inocybe*, and *Omphalotus* mushrooms. These species include but are not limited to *Clitocybe dealbata*, *Clitocybe rivulosa*, *Clitocybe dilatata*, *Inocybe agglutinata*, *Inocybe cincinnata*, *Inocybe entheles*, *Inocybe fastigata*, *Inocybe geophylla*, *Inocybe godeyi*, *Inocybe griseolilacina*, *Inocybe lacera*, *Inocybe lilacina*, *Inocybe mixtilis*, *Inocybe napipes*, *Inocybe obscuroides*, *Inocybe pallidipes*, *Inocybe patouillardi*, *Inocybe pudica*, *Inocybe purica*, *Inocybe rimosus*, *Inocybe sororia*, *Inocybe subdestricta*, *Inocybe umbrina*, *Omphalotus illudens*
- Synonym: 'Jack-O-Lantern' mushroom (*Omphalotus illudens*)
- Chemical Structure:



Uses

There are no uses for mushrooms in this group. They are usually consumed when mistaken for edible mushrooms.

Exposure Routes and Pathways

Ingestion is the route of exposure.

Toxicokinetics

Symptoms occur typically within 15–120 min following ingestion of the mushroom.

Mechanism of Toxicity

Mushrooms in these genera may contain varying amounts of muscarine isomers and muscarine-like compounds. L(+)Muscarine is the most potent of the muscarine isomers found. The varying amounts of these isomers in different mushrooms account for the diversity of reports of the severity of symptoms produced by muscarine-containing mushrooms.

Muscarine binds to the so-called 'muscarinic' receptors in the parasympathetic nervous system. These are primarily postganglionic cholinergic receptors in smooth muscle and glands. Muscarine does not act on so-called 'nicotinic' receptors, which are found in ganglionic synapses and at the neuromuscular junction. Muscarine is a tertiary amine structure and, therefore, does not diffuse into the central nervous system to an appreciable extent. Symptoms are, therefore, limited to the peripheral nervous system.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxic reactions similar to those found in humans can be induced in experimental animals and are, therefore,

expected to occur following ingestion of the mushrooms. Treatment is similar to that for humans with the use of atropine.

Human

Stimulation of nicotinic receptors produces small pupils, blurred vision, excessive perspiration, salivation and lacrimation, bradycardia, increased intestinal peristalsis, increased pulmonary secretions, and decreased blood pressure. Acute asthma exacerbations may occur in patients with reactive airway disease. The onset is typically within 15–30 min after ingesting the mushrooms but may be delayed by up to 120 min. Nausea and vomiting are often the first symptoms to occur. The rare deaths reported appear to occur from cardiovascular collapse and respiratory failure.

Clinical Management

Atropine is a competitive antagonist of muscarine at the cholinergic receptors and is, therefore, 'antidotal' in these types of poisonings. Doses sufficient to reverse the effects of excessive pulmonary secretions and bradycardia should be administered. Activated charcoal may be useful in recent ingestions, but because most patients have vomiting and diarrhea, its usefulness is limited and unproved.

See also: Atropine; Neurotoxicity.

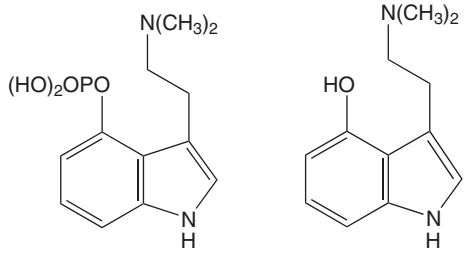
Further Reading

Benjamin DR (1995) *Mushrooms – Poisons and Panaceas*. New York: WH Freeman & Co.

Mushrooms, Psilocybin

Anthony S Manoguerra

- Chemical Names: Psilocybin and psilocin have been found in the following mushroom species (as well as in others): *Psilocybe aztecorum; Psilocybe baeocystis; Psilocybe bonetti; Psilocybe caerulescens; Psilocybe caerulipes; Psilocybe candidipes; Psilocybe cambodginiensis; Psilocybe coprinifacies; Psilocybe cubensis; Psilocybe cyanescens; Psilocybe fimetaria; Psilocybe mexicana; Psilocybe pelliculosa; Psilocybe quebecensis; Psilocybe semilanceata; Psilocybe sempervira; Psilocybe serbica; Psilocybe strictipes; Psilocybe stuntzii; Psilocybe subaeruginosa; Psilocybe zapatecorum; Conocybe cyanopus; Conocybe smithii; Gymnopilus aeruginosa; Gymnopilus validipes; Panaeolus africanus; Panaeolus ater; Panaeolus cambodginiensis; Panaeolus fimicola; Panaeolus foenisecii; Panaeolus subalteatis; Panaeolus tropicalis*
- Synonyms: Hallucinogenic mushrooms; Magic mushrooms; 'Blue legs'; 'Liberty caps'
- Chemical Structures: Psilocybin and Psilocin



Psilocybin Psilocin

Uses

There are no uses for mushrooms in this group. These mushrooms are intentionally ingested for their hallucinogenic activity.

Exposure Routes and Pathways

Ingestion is the route of exposure.

Toxicokinetics

The onset of symptoms typically begins 20–60 min following ingestion of the mushroom, although it has been reported to be delayed up to 3 h. The typical hallucinogenic experience lasts ~3 or 4 h.

Mechanism of Toxicity

These mushrooms contain the psychoactive compound psilocybin and, in some cases, also the lesser active substance psilocin. Psilocybin is highly stable and is not destroyed by cooking or drying. Psilocin is rapidly destroyed by oxidation. Psilocybin can be extracted from the mushroom by boiling the mushroom in water. The exact mechanism of action of psilocybin has not been determined but as an indoleamine it is thought to act similarly to LSD, as an agonist at 5-hydroxytryptamine receptors in the central nervous system.

Acute and Short-Term Toxicity (or Exposure)

Animal

Horses and dogs have been reported to have eaten these mushrooms with predictable effects on their behavior. Treatment has included sedation or placement of the animal in a darkened, quiet environment. In severe cases seizures and hyperthermia may occur.

Human

These hallucinogenic mushrooms are small and, therefore, consumption of more than a single mushroom is required for the effects to occur. For example, two or three mushrooms of *P. cubensis* will produce the desired hallucinogenic experience, while 20–40 *P. cyanescens* are required for an equivalent experience. In addition, the concentrations of psilocybin vary significantly between species of mushrooms. Following the ingestion of an effective number of mushrooms, dizziness, giddiness, muscle twitching, restlessness, and anxiety begin within 30 min. Approximately 20% of users will also experience some nausea and vomiting. In 30–60 min, the user will begin to experience visual hallucinations and perceptual distortions. The user may experience a 'good' or 'bad' 'trip' depending on mood, environment, and prior hallucinogenic experience. Mild tachycardia and hypertension are common during this time period. The effects of psilocybin are short lived and recovery is typically complete in 4–6 h. The experience usually ends with drowsiness progressing to sleep. Rare cases of flashbacks occurring 2 weeks to several months after the initial experience have been reported. Severe or life-threatening toxicity is not expected except in children or in adults who have ingested large amounts. These cases are reported to have had seizures and hyperthermia.

Clinical Management

No specific treatment is indicated for the ingestion of these mushrooms. Patients having a bad trip may require isolation in a dark, quiet environment, and calm reassurance until the effects of the drug have worn off. In severe cases, sedation may be indicated. In severely symptomatic cases, seizures can be controlled with benzodiazepines and hyperthermia treated with external cooling.

See also: Drugs of Abuse; LSD (Lysergic Acid Diethylamide); Plants, Poisonous.

Further Reading

Peden NR, Bissett AF, and Macaulay KE (1981) Clinical toxicology of 'magic mushroom' ingestion. *Postgraduate Medical Journal* 57: 543–545.

Southcott RV (1974) Notes on some poisonings and other clinical effects following ingestion of Australian fungi. *South Australian Clinics* 6: 441–478.

Mustard Gas

Harry Salem*

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- Chemical Name: bis(2-Chloroethyl) sulfide
- Chemical Abstracts Service Registry Number: CAS 505-60-2
- Synonyms: Sulfur mustard; 1,1-Thiobis(2-chloroethane); 1-Chloro-2-(β-chloroethylthio)ethane; 2,2′-Dichlorodiethyl sulfide; Distilled mustard; S mustard; S-lost; Schwefel-lost; Yellow cross liquid; Yperite; Kampstoff lost; HD; HT; H
- Chemical/Pharmaceutical/Other Class: Thioether
- Chemical Formula: $C_4H_8Cl_2S$
- Chemical Structure:

$$Cl–CH_2–CH_2–S–CH_2–CH_2–Cl$$

*The views of the author do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Uses

Mustard gas is a chemical warfare agent belonging to the blister agent/vesicant class. It is a cytotoxic alkylating compound similar to the other type of vesicants or blister agents such as nitrogen mustard, Lewisite, and phosgene oxide. Mustard causes blistering of the skin and mucous membranes.

Exposure Routes and Pathways

Actually, mustard gas is not a gas, but a dense, yellow to brown oily liquid with a low vapor pressure and relatively high melting point. Mustard can also be released in the air as a vapor and thus exposure of

skin, eyes, and respiratory tract can be to vapor or liquid. If sulfur mustard is released into water supplies, exposure can occur from drinking contaminated water or getting it on the skin. Although it currently has no medical use, it was available for use as a treatment for psoriasis. Mustard may smell like garlic, onions, mustard, or have no odor.

Toxicokinetics

Mustard gas is lipophilic and will accumulate in brain and fatty tissue. Mustard gas is soluble in water to less than 0.1% and hydrolyzes in water with a half-life of 3–5 min. It is freely soluble in organic solvents. It has been detected in the blood after dermal or inhalation exposure. Although mustard dissolves slowly in aqueous solution, it must first dissolve in sweat or extracellular fluid to be absorbed. Following dissolution, mustard molecules rapidly rearrange to form extremely reactive cyclic ethylene sulfonium ions that immediately bind to intracellular and extracellular enzymes, proteins, and other cellular components. Mustard binds irreversibly to tissues within several minutes after contact. If decontamination is not done immediately after exposure, injury cannot be prevented. However, later decontamination might prevent a more severe lesion.

The biological half-life of the chemical has not been published, but products are excreted in the urine for several days after acute exposure. Traces of the agent are exhaled and excreted in the feces. Several hours can pass before symptoms become manifest but this is attributed to the mechanism of action and not to direct effects of residual levels of the agent. Mustard gas is a greater threat in hot and humid climates.

Mechanism of Toxicity

Although the exact mechanism by which mustard produces tissue injury is not known, the mechanism of action has been suggested to be its ability to directly alkylate DNA. This DNA alkylation and cross-linking in rapidly dividing cells such as basal keratinocytes, mucosal epithelium, and bone marrow precursor cells leads to cellular death and inflammatory reactions. Systemic effects with extensive exposures include bone marrow inhibition, with a drop in the white blood cell count, and gastrointestinal tract damage.

Human Toxicity

Mustard gas is a powerful irritant and vesicant. Dermal effects range from itching to erythema, blistering, corrosion, and necrosis. Dermal blistering is delayed in onset and slow to heal. Ocular irritation, conjunctivitis, and blindness can occur. Respiratory symptoms include a harsh and painful cough, bronchitis, sneezing, rhinorrhea, and sore throat. Mustard gas is not acutely lethal; 2–3% of soldiers exposed to mustard gas during World War I died of its direct effects. Death is generally due to respiratory collapse, shock, and secondary infections.

The estimated human incapacitating and lethal dosages by different routes of exposure are listed below:

	ICt_{50} ($mg\,min\,m^{-3}$)	LCt_{50} ($mg\,min\,m^{-3}$)	LD_{50} ($mg\,kg^{-1}$)
Eyes	200	–	–
Inhalation	–	1 500	–
Skin	2000	10 000	9
Oral	–	–	0.7

The time-weighted average exposure limit for the workplace is estimated at $0.003\,mg\,m^{-3}$, while for the general population it is estimated to be $0.0001\,mg\,m^{-3}$. The inhalation minimal risk level (MRL) for acute exposure for 14 days or less is $0.0007\,mg\,m^{-3}$ and for 15–364 days it is $0.00002\,mg\,m^{-3}$. The oral MRL is $0.0005\,mg\,kg^{-1}\,day^{-1}$ for 14 days or less, and $0.00007\,mg\,kg^{-1}\,day^{-1}$ for 15–364 days.

Survivors may be susceptible to bronchitis and pneumonia, and may be at an increased risk to develop tumors of the respiratory tract. Mustard gas produces tumors in animal studies.

The US National Advisory Committee has developed acute exposure guidelines (AEGLs) to protect people from harmful effects of short-term exposures (8 h or less) to sulfur mustard. The AEGL-1 for a 10 min exposure is $0.40\,mg\,m^{-3}$ and it is $0.008\,mg\,m^{-3}$ for an 8 h exposure. Exposure to higher concentration may result in eye irritation. The AEGL-2 for 10 min is $0.60\,mg\,m^{-3}$ and for 8 h it is $0.013\,mg\,m^{-3}$. Exposure to higher concentration may result in swelling of the eyes, sensitivity to light, and eye irritation. The AEGL-3 is $3.9\,mg\,m^{-3}$ for 10 min and for 8 h it is $0.27\,mg\,m^{-3}$. Exposure to higher concentrations may result in death.

Sulfur mustards are vesicants and alkylating agents; however, the biochemical mechanisms of action are not clearly understood. They are highly reactive and combine rapidly with proteins, DNA, or other molecules. Therefore, within minutes following exposure, intact mustard or its reactive metabolites are not found in tissue or biological fluids. Sulfur mustards also have cholinergic activity, stimulating both muscarinic and nicotinic receptors. The onset of clinical symptoms and their time of onset depend on the severity of exposure. The death rate

from exposure to sulfur mustard is low (2–3% during World War I). Death usually occurs between the fifth and tenth day due to pulmonary insufficiency complicated by infection due to immune system compromise.

The eye is the most sensitive tissue to sulfur mustard effects. Sulfur mustard vapor or liquid may cause intense conjunctival and scleral pain, swelling, lacrimation, blepharospasm, and photophobia; however, these effects do not appear for an hour or more. Miosis due to cholinergic effects may occur. High concentrations of vapor or liquid can cause corneal edema, perforation, blindness, and later scarring.

Direct skin exposure to sulfur mustards causes erythema and blistering. Generally, a pruritic rash will develop within 4–8 h followed by blistering 2–18 h later. Contact with the vapor may result in first- and second-degree burns, while contact with the liquid typically produces second- and third-degree chemical burns. An area of burn covering 25% or more of the body surface area may be fatal.

Dose-dependent inflammatory reactions in the upper and lower airway begin to develop several hours after exposure and progress over several days. Burning nasal pain, epistaxis, sinus pain, laryngitis, loss of taste and smell, cough, wheezing, and dyspnea may occur. Necrosis of respiratory epithelium can cause pseudomembrane formation and local airway obstruction.

Ingestion may cause chemical burns of the gastrointestinal tract and cholinergic stimulation. Nausea and vomiting may occur following ingestion or inhalation. Early nausea and vomiting is usually transient and not severe. Nausea, vomiting, and diarrhea occurring several days after exposure indicate damage to the gastrointestinal tract and is thus a poor prognostic sign.

High doses of sulfur mustards can cause hyperexcitability, convulsions, and insomnia. Systemic absorption of sulfur mustard may induce bone marrow suppression and an increased risk for fatal complicating infections, hemorrhage, and anemia.

Relapsing keratitis or keratopathy may develop years after apparent healing of severe eye lesions. Persistent eye conditions, loss of taste and smell, and chronic respiratory illness including asthmatic bronchitis, recurrent respiratory infections, and lung fibrosis may persist following exposure to sulfur mustards. Prolonged or repeated acute exposure to sulfur mustards may cause cutaneous sensitization and chronic respiratory disease. Repeated exposures result in cumulative effects because mustards are not naturally detoxified by the body.

The International Agency for Research on Cancer has classified sulfur mustard as carcinogenic to humans (Group I). Epidemiological evidence indicates that repeated exposures to sulfur mustard may lead to cancers of the upper airways. There is limited evidence that repeated exposures to sulfur mustards may cause defective spermatogenesis years after exposure. Sulfur mustard has been implicated as a potential developmental toxicant because of its similarity to nitrogen mustard; however, data are inconclusive.

Chronic Toxicity (or Exposure)

Production workers exposed to mustard gas had increased rates of bronchitis and pneumonia. Increased incidence of tumors of the respiratory tract, lung cancer, bladder cancer, and leukemia were also found. Mustard gas is classified as a human carcinogen.

Clinical Management

It is important that the agent be washed from the skin with soap and water as soon after exposure as possible. The eyes should be thoroughly flushed. The onset of symptoms typically is delayed and an absence of immediate effect does not rule out toxicity. Although ingestion is unlikely, due to the sources of mustard gas, an emetic should not be administered because of the extreme caustic nature of the chemical. If the patient is not comatose, dilution of stomach contents with milk or water, prior to gastric lavage, may be attempted. Application of a solution of sodium thiosulfate to the skin and inhalation of a nebulizing mist of sodium thiosulfate may speed inactivation of mustard gas. Animal studies have shown that administration of corticosteroids (e.g., dexamethasone) and antihistamines (e.g., promethazine) may prove beneficial.

Animal Toxicity

The irritant, vesicant, and respiratory effects of mustard gas are the same in animals and humans, although the hair coat and lack of extensive sweat glands may somewhat protect the animal from the dermal effects of the agent. The grooming habits of animals may result in some exposure by the oral route.

The estimated animal toxicity is listed below:

Species	*Percutaneous LD_{50} ($mg\,kg^{-1}$)*	*Inhalation LCt ($mg\,min\,m^{-3}$)*
Rats	18	800
Rabbits	100	900
Dogs	–	600
Goats	–	900

Other H-Series Blister Agents

Sulfur mustard is a component of the H-series blister agents including undistilled sulfur mustard (H; sulfur mustard with 20–30% impurities, also known as Levinstein mustard), distilled sulfur mustard (HD or HS; ~96% pure), a mustard–lewisite mixture (HL), and HD/agent T mixture (HT; a mixture of HD and nonvolatile agent T), and an HD/agent Q mixture (HQ; a mixture of HD and nonvolatile Agent Q (Agent Q is also known as sesquimustard).

Sulfur Mustards

- 2-Chloroethyl chloromethylsulfide: CAS 2625-76-5
- Bis(2-chloroethylthio)methane: CAS 63869-13-6

Sesquimustard

- 1,2-Bis(2-chloroethylthio)ethane: CAS 3563-36-8
- 1,3-Bis(2-chloroethylthio)-*n*-propane: CAS 63905-10-2
- 1,4-Bis(2-chloroethylthio)-*n*-butane: CAS 142868-93-7
- 1,5-Bis(2-chloroethylthio)-*n*-pentane: CAS 142868-94-8
- Bis(2-chloroethylthiomethyl)ether: CAS 63918-90-1

See also: Bio Warfare and Terrorism: Toxins and Other Mid-spectrum Agents; Blister Agents/Vesicants; Chemical Warfare Delivery Systems; Nerve Agents; Nitrogen Mustard.

Relevant Websites

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

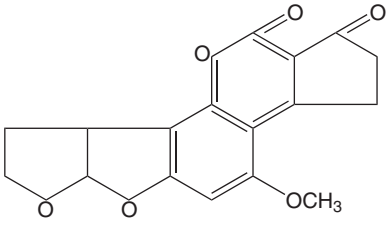
Mutagenicity Tests *See* Sister Chromatid Exchanges; Ames Test.

Mutagenicity Toxicity Testing *See* Toxicity Testing, Mutagenicity.

Mycotoxins

Samantha E Gad and Shayne C Gad

- Representative Compounds: Aflatoxins; Citrinin; Ergot alkaloids; Fumonisins; Ochratoxin A; Patulin; Trichothecenes; Zearalenone; Stachybotrys toxin
- Chemical Formula: $C_{17}H_{12}O_6$ (Aflatoxin B_1)
- Chemical Structure: Aflatoxin B_1



Uses

While most of the mycotoxins do not have beneficial uses, some mycotoxin derivatives have been used as antibiotics, growth promoters, and other kinds of drugs. In addition, some mycotoxins have been implicated as chemical warfare agents.

Background Information

Mycotoxins are metabolites of fungi that are generally produced during the growth or storage of plant products (e.g., cereal grains, nuts, corn, sorghum, rice, dried beans, and apples). However, recently mycotoxins have been found in the indoor environment where they are produced by molds growing in damp environments. Mycotoxins represent a very diverse group of chemicals that differ from each other structurally and in their ability to cause adverse effects. They may be classified in a variety of ways; for example, by the type of toxicity they cause, by the organism that produces them, or by their chemical structures. While from 300 to 400 compounds have been identified as mycotoxins, only a limited number have been studied in detail. Most of these are not of significant human health concern; rather they have

more impact on food animals. There has been recent concern about possible adverse effects in humans from toxins produced by molds in the indoor environment but this has not been fully researched. Based on extensive data, the mycotoxin that has the greatest public health impact is one of the aflatoxins, aflatoxin B_1, and this will be the focus of the remainder of this entry.

Exposure Routes and Pathways

The main route of human exposure is ingestion of foods contaminated with mycotoxins. Skin exposure to and inhalation of moldy materials may also be significant sources of exposure in some populations.

Toxicokinetics

Aflatoxin B_1 is absorbed into the blood, excreted mainly through bile, and can cross the placenta. In urine, aflatoxin B_1 is excreted as aflatoxin M_1. The liver is the primary site of metabolism, with P450 CYP 3A4 and 1A2 being primarily involved in the activation to highly reactive species. Half lives in humans are short (8 h or less).

Mechanism of Toxicity

Aflatoxin B_1 is transformed in the liver into a highly reactive epoxide that forms covalent bonds with DNA, RNA, and proteins. Toxicity and carcinogenicity are attributed to interaction with nucleic acid and proteins resulting in the inhibition of nucleic acid synthesis.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of Aflatoxin B_1 is $9\,mg\,kg^{-1}$ in the mouse, $4.8\,mg\,kg^{-1}$ in the rat, and $10\,mg\,kg^{-1}$ in the hamster. Acute exposures to Aflatoxin B_1 have been shown to cause liver damage in rodents. Dermal effects are apparent at $2–12\,mg\,kg^{-1}$.

Human

Aflatoxin B_1 can cause a rare condition known as acute aflatoxicosis, which may result in death in some cases. At lower exposures, this mycotoxin has been linked to liver damage.

Chronic Toxicity (or Exposure)

Animal

In 1960–63, the death of turkeys in England (referred to as turkey X disease) was associated with the consumption of peanut meal feeds containing aflatoxins. Death usually occurs from hepatoxicity. Aflatoxin B_1 is carcinogenic to a wide variety of animal species; rats are particularly sensitive to this effect. It is also mutagenic and teratogenic in rodents.

Human

Long-term exposure to low levels of Aflatoxin B_1 can cause liver damage and this mycotoxin is considered to be the most potent natural carcinogen known. It is associated with liver cancer, especially in individuals who have been exposed to hepatitis B. Aflatoxin B_1 and hepatitis B appear to be synergistic in the induction of liver cancer. This mycotoxin has also been linked to cancers in other organs, particularly the lungs. Aflatoxin B_1 is classified as carcinogenic to humans by International Agency for Research on Cancer.

Clinical Management

Reducing the opportunity for exposure is the first line of defense. After high-dose exposure watch for signs of pulmonary insufficiency and provide ventilation if needed. Monitor for shock and treat if necessary. For eye contamination, flush eyes immediately with water and then irrigate with saline. For ingestion exposure, rinse mouth and use water for dilution if the patient can swallow. Do not use emetics.

Ecotoxicology

The LD_{50} (oral) of Aflatoxin B_1 in a day-old duckling was $18.2\,\mu g\,50\,g^{-1}$ body weight. Chronic low dose ($0–1.5\,\mu g\,kg^{-1}\,day^{-1}$) dietary exposure of rainbow trout to Aflatoxin B_1 resulted in liver cancer.

See also: Kidney; Mold; Veterinary Toxicology.

Further Reading

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N

Nails (of the Fingers and Toes)

Pertti J Hakkinen

The nails of the fingers and toes have received little attention by toxicologists, and by exposure and risk assessors. However, chemicals coming in contact with the outer surface of the nails can be taken up into the nails. Further, nails have been shown to be useful in some biomonitoring studies and in the development of some pharmaceuticals. The structure of nails includes strongly linked keratinocytes surrounded by phospholipid layers. The factors that affect drug and chemical uptake and permeation through the nail plate include solute molecular size, hydrophilicity/hydrophobicity charge, and the nature of the vehicle. Further, research has found ways of enhancing drug transport into and through the nail plate, and diseased or hydrated nails can have altered penetration characteristics.

The possible roles of nails (fingers and toes) in exposure to chemicals include direct contact with the cuticle and nail, inhalation from volatilization of a chemical applied to the nails, and oral intake via nail biting and finger sucking. Consumer products of relevance include nail lacquers ('polishes') and nail lacquer removers applied via various means (applicator, cotton ball, etc.). Also, handwashing, dishwashing, shampoo, hard surface cleaning, etc., products would involve nail contact, as would contact with residential water and soil, and paints and paint removers, and petrol. Nail lacquers can include toluene, 1,1,1-trichloroethane, and phthalates, while nail lacquer remover can include ethyl acetate.

Nails have been used in the biomonitoring of various elements, for example, arsenic, fluoride, mercury, nickel, and thallium. The advantages of analyzing nail samples include the easy and noninvasive collection of the samples, the small sample size required for analysis, and their easy storage at room temperature. The great toenail, which reflects body exposure in the previous 12 months, has been stated to be the nail best utilized for biomonitoring because it is less exposed to external contamination.

Nail clippings have also been used in the monitoring of creatinine during the diagnosis and treatment of renal failure, and as noted above, can be used in the monitoring of fluoride exposure. The absorption of drugs into nails following topical application to the nail plate has been shown to be useful for treating nail disorders, such as onychomycosis (fungal infections of the nail), and is known as ungual drug delivery. Finally, the fingernail clippings of victims have been utilized in looking for the DNA of aggressors in cases where the victims struggled to defend themselves.

See also: Biomarkers, Human Health; Biomonitoring; Exposure; Exposure Assessment.

Further Reading

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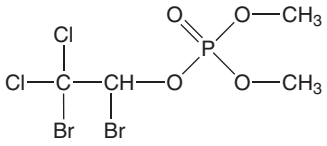
Naled

Danny Villalobos

- Chemical Name: (*RS*)-1,2-Dibromo-2,2-dichloroethyl dimethyl phosphate
- Chemical Abstracts Service Registry Number: CAS 300-76-5
- Synonyms: Dibrom; Bromchlophos; BRP; Bromex; Fly Killer-D; Lucanel
- Chemical/Pharmaceutical/Other Class: Organophosphorus insecticide and acaricide
- Chemical Formula: $C_4H_7Br_2Cl_2O_4P$
- Chemical Structure:



Uses

Naled is a fast acting, nonsystemic contact and stomach organophosphorus insecticide used to control aphids, mites, mosquitoes, and flies on crops and in greenhouses, mushroom houses, animal and poultry houses, kennels, food processing plants, and aquaria. Naled is also used in outdoor mosquito control. Liquid formulations can be applied to greenhouse heating pipes to kill insects by vapor action. It has been used by veterinarians to kill parasitic worms (other than tapeworms) in dogs. Naled is available in dust, emulsion concentrate, liquid, and ultra-low volume (ULV) formulations.

Exposure Routes and Pathways

Inhalation, dermal, and gastrointestinal exposures are possible.

Toxicokinetics

Naled is readily absorbed by inhalation, through intact skin, and through the gastrointestinal tract. Naled is rapidly hydrolyzed to give a number of metabolites which include dichlorvos, dichlorobromoacetaldehyde, dimethyl phosphate, and complex amino acid conjugates. When 25 mg kg^{-1} of ^{32}P naled was given to a cow by oral intubation, 9% was recovered in urine and 34% in feces up to 1 week after dosing. Rats given 1/10 of the LD_{50} daily for 9 weeks showed moderate inhibition of blood and brain cholinesterase.

- *Inhalation:* No toxic effects were observed in rats and guinea pigs exposed to vapor at a concentration of 19 μg l^{-1} for 6 h per day, 5 days per week for 5 weeks.
- *Cumulation of compound:* Naled is not cumulative in body tissues.
- *Cumulation of effect:* Repeated exposure to naled may have a cumulative effect on cholinesterase levels.

Mechanism of Toxicity

Naled is an inhibitor of acetylcholinesterase, and can lead to typical signs and symptoms of cholinergic crisis through elevation of tissue acetylcholine levels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Naled is moderately to highly toxic via the oral route, with oral LD_{50} values of 91–430 mg kg^{-1} in rats and mice. Dermal LD_{50} values were 1100 mg kg^{-1} in rabbits and 800 mg kg^{-1} in rats. Naled can cause dermatitis and sensitization, and can be corrosive to skin and eyes. Effects due to naled exposure are similar to those caused by other organophosphorus pesticides.

Human

Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, and slow heartbeat. Very high doses may result in unconsciousness, incontinence, and convulsions or fatality.

The most commonly reported symptoms from acute naled exposures have been limited to contact dermatitis, erythematous and maculopapular rashes, which may be followed by edematous vesiculating blisters, which become dry, itchy, and flake off.

The acute toxicity data, inhalation data, and experience in the early reports indicated that naled is not a highly toxic organophosphate.

Effects due to naled exposure will be similar to those caused by other organophosphate pesticides, including inhibition of cholinesterase and neurological and neuromuscular effects.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure may lead to nervous system toxicity due to cumulative acetylcholinesterase inhibition. There are little data on the carcinogenic or teratogenic effects of naled. Dietary administration of 1, 5, and 25 mg naled kg^{-1} diet to three generations of albino rats had no effect on mating and fertility indices, incidence of pregnancy, parturition or gestation times, lactation indices, offspring and their survival. Rats treated with 28 mg kg^{-1} day^{-1} naled for 9 weeks showed cholinesterase inhibition but no overt signs of neurotoxicity.

Human

Little information is available on effects of naled in humans. Repeated exposure to naled may have a cumulative effect on cholinesterase levels. Chronic exposure to organophosphates may cause the neurological and neuromuscular effects associated with cholinesterase inhibition.

In Vitro Toxicity Data

Naled did not influence DNA repair in *Proteus mirabilis*e, but did increase the frequency of mutations in the Ames assay.

Clinical Management

Therapy for acute naled poisoning: (1) Respiration should be supported. The airways should be kept clear, and artificial respiration with oxygen should be used if cyanosis is indicated. Death from pesticide poisoning is usually due to respiratory failure. (2) Decontamination should be done as indicated. Contaminated clothing should be removed. Skin, hair, and fingernails should be washed with soap and water, and eyes should be rinsed. Dermal exposures should be treated symptomatically as indicated. If ingested, gastric lavage may be indicated as may the administration of activated charcoal, 1–2 g kg^{-1} body weight. First-aid and medical personnel should be protected. (3) Five milliliters of heparinized blood should be drawn for cholinesterase determination. First urine and first/early vomitus samples should be saved for possible laboratory analysis. (4) Insecticide label should be consulted under 'active ingredients' for specific chemicals involved. (5) When mixtures of organophosphates and chlorinated hydrocarbons are involved, specific treatment should be given for organophosphates first and indicated support therapy and decontamination.

Adults: After cyanosis is overcome, atropine sulfate should be used, 2–4 mg i.v. Doses should be repeated at 5–10 min intervals until signs of atropinization appear. This should be maintained for 24 h or longer if necessary. 2-PAM (pralidoxime chloride) should be given. Adult dose: 1 g, slowly, intravenously. Contraindicated are morphine, aminophylline, theophylline, phenothiazine tranquilizers, and barbiturates.

Children: Atropine sulfate in proportion to body weight: ~ 0.05 mg kg^{-1}. 2-PAM, 0.25 g, should be given slowly, intravenously.

Environmental Fate

Naled is practically nonpersistent in soil, with half-life of less than 1 day. It degrades in sunlight to dichlorvos (DDVP). Naled does not bind strongly to soils, but is not highly soluble in water. It is moderately volatile. Soil microorganisms break down most of the naled in the soil. Naled is rapidly broken down in water, with a half-life of ~ 2 days. Plants reductively eliminate bromine from naled to form DDVP, which may evaporate or be further modified.

Chemical hydrolysis and biodegradation are the major processes involved in the transformation of naled. Volatilization from soils and/or from water is the major mode of transport for degraded naled and its bioactive degradate DDVP, as opposed to leaching to ground water.

A major route of contamination of surface waters by naled is spray drift and direct application for mosquito abatement. There are no data on the fate and transport of degradates containing only the organophosphate group, which form by cleavage of the P–O bond in naled and/or DDVP.

Ecotoxicology

Based on acute toxicity data, naled is moderately to highly toxic to birds. Acute oral LD values in birds ranged from 37 to 65 mg kg^{-1}. On a subacute dietary basis, naled is slightly toxic to birds. Naled is highly toxic to honey bees.

Naled is moderately to highly toxic in freshwater fish, with 96 h LC_{50} values ranging from 87 ppb to 3 ppm. Growth in fathead minnow was impaired at concentrations of greater than 6.9 ppb. Naled was very highly toxic to Daphnia: length was affected at concentrations > 0.045 ppb.

Exposure Standards and Guidelines

US Environmental Protection Agency (EPA) toxicity class I.

- Threshold limit value (TLV): 3 mg m^{-3} A4 (skin) (American Conference of Governmental Industrial Hygienists, ACGIH 1996).
- Acceptable daily intake (ADI): Not available
- Maximum contaminant level (MCL): Not available
- Reference dose (RfD): 0.002 mg kg^{-1} day^{-1}
- Permissible exposure limit (PEL): 3 mg m^{-3} (8 h)
- Health advisory (HA): Not available

See also: Cholinesterase Inhibition; Dichlorvos; Organophosphates.

Further Reading

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Relevant Websites

http://www.epa.gov – US Environmental Protection Agency.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Naled.
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Nanotechnology

David B Warheit

Nanotechnology is an emerging multidisciplinary science that deals with the creation and use of molecules a few billionths of meter in size. Assessing the potential hazards of this technology's nanoparticles, and the nanomaterials manufactured using the nanoparticles is an emerging area in toxicology and health risk assessment. The development of toxicity data sets and exposure assessments for various nanoparticles and nanomaterials is ongoing as new particles and materials are developed. A related issue in toxicology and risk assessment is the extent to which nanoparticle toxicity can be extrapolated from existing toxicology databases for particles and fibers. Other information needs being addressed include the environmental and biological fate, transport, persistence, and transformation of manufactured nanoparticles, and the recyclability and overall sustainability of manufactured nanomaterials.

Ultrafine or nanoparticles are generally defined as particles in the size range <100 nm. A nanometer (nm) is roughly the width of 10 hydrogen atoms. The terms 'ultrafine' and 'nano' can be used interchangeably, with the latter being the current nomenclature. Having been called 'the next big thing' in science and the foundation for the 'next industrial revolution', governments and companies around the world are providing billions of dollars into nanotechnology research. As other emerging technologies have over the years, nanotechnology has attracted the attention of the public and others. This has led to concerns about potential effects of materials made using nanotechnology on human health and environmental impact.

In theory, nanoparticles can be produced from nearly any chemical. Most of the current nanoparticles are made from transition metals, silicon, carbon, and metal oxides. Manufactured nanoparticles display physicochemical characteristics and coatings that provide highly desirable electrical, thermal, mechanical, and imaging properties for various technology applications.

The field of nanotechnology is currently undergoing a large amount of research and development. Nanotechnology can be used to manufacture materials, devices or systems less than 1000th the width of a human hair. Materials made using nanotechnology are already used in hundreds of products, including sunscreens and cosmetics to make them clear (e.g., the sunscreens have ultraviolet-absorbing nanoparticles so small they cannot reflect light, making them transparent), in textiles using nanofibers to make them stain-resistant; and in power machinery to add durability. Further examples are tennis rackets and airplane bodies made using nanomaterials with carefully arranged atoms to make the materials especially strong.

In addition, nanotechnology is expected to become very important in various biomedical applications such as in drug delivery, molecular imaging, biomarkers, and biosensors. Target-specific drug therapy and methods for early diagnosis and therapy of

diseases are among the priority research areas for application of nanotechnology. Further, nanotechnology could play a very important role in environmental science, for example, via the development and usage of nanospheres to trap polychlorinated biphenyls and toxic metals, and via nanopore materials that can filter out bacteria, viruses, and toxins from water.

Given their extremely small size, a key area of toxicology for nanotechnology thus far has been the evaluation of pulmonary toxicity. Pulmonary toxicology studies in rats demonstrate that nanoparticles administered to the lung produce an enhanced pulmonary inflammatory response when compared to larger particles of identical chemistry at equivalent mass concentrations. Particle surface area and particle number appear to play important roles in the mechanisms of nanoparticle toxicity. Contributing to the effects of inflammation-promoting effects of nanoparticles is their very high size-specific deposition when inhaled as singlet particles rather than as aggregated particles. Some evidence suggests that inhaled nanoparticles, after deposition in the lung, largely escape alveolar macrophage surveillance and transmigrate through epithelial cells to the pulmonary interstitium, generally considered a vulnerable anatomical compartment of the respiratory system.

It is important to note that most of the early published lung toxicity studies with nanoparticles have been conducted in laboratory animals at very high particle concentrations, which significantly exceed workplace or ambient exposures. These hazard-based toxicity studies are designed to assess pulmonary effects caused by particles at high concentrations and can result in the induction of lung tumors in rats following 2 year exposures. Specifically, chronic inhalation studies with nano- and fine-sized titanium dioxide (TiO_2) particles (average primary particle sizes $\sim$20 and $\sim$270 nm, respectively) have shown that ultrafine particles are greater than 10 times more potent than fine particles in producing pulmonary fibrosis and consequent lung tumors in rats.

Additional studies have been conducted using intratracheal instillation exposures to aggregates of ultrafine and fine carbon black, as well as to TiO_2 particles in rats. The results have demonstrated a significantly enhanced lung inflammatory potency of the ultrafine particles when compared to fine-sized particulates of similar composition. However, when the instilled doses were expressed in terms of particle surface area, the responses of the ultrafine and fine TiO_2 particles fell on the same dose–response curve. This is because a given mass of ultrafine particles has a much greater surface area (and particle number) than the same mass of fine, yet respirable (3 μm) particles and therefore is more likely to cause particle overload in the lung. Thus, from a risk assessment and regulatory viewpoint, it will be important to delineate the pulmonary toxicity effects of ultrafine particles in rats at overload versus nonoverload conditions.

It may be surprising to note that the total lung toxicity database for systematic comparisons of the effects of ultrafine/nanoparticles versus fine-sized particles in rats consists of studies on only three particle types: namely titanium dioxide, carbon black, and diesel exhaust particles. Moreover, as stated above, the rat model, for which most if not all of the nano versus fine-size comparisons have been reported, is known to be an extremely sensitive species for developing adverse lung responses to particles, particularly at overload concentrations. As a consequence, long-term (2 year), high-dose, inhalation studies in rats with poorly soluble, low-toxicity dusts can ultimately produce pulmonary fibrosis and lung tumors via an 'overload' mechanism. The tumor-related effects are unique to rats and have not been reported in other particle-exposed, rodent species such as mice or hamsters, under similar chronic conditions. For the mechanistic connection, it has been postulated that the particle-overload effects in rats result in the development of 'exaggerated' lung responses, characterized by increased and persistent levels of pulmonary inflammation, cellular proliferation, and inflammatory-derived mutagenesis in the rat, and this ultimately results in the development of lung tumors following high dose, long-term exposures to a variety of particulate-types.

In contrast to the response in rats, evidence from numerous studies demonstrate that particle-exposed mice and hamsters do not develop sustained inflammation, mesenchymal cell alterations, and consequent lung tumors following high-dose, long-term exposures to low-toxicity dusts. Therefore, species differences in lung responses to inhaled particles are important considerations for assessing the health risks to nanoparticles.

To complicate further our perceptions of nanoparticle toxicity, some recent evidence suggests that, on a mass basis, not all nanoparticle-types are more toxic than fine-sized particles of similar chemical composition. As mentioned previously, the limited numbers of studies that have been reported suggest that ultrafine TiO_2 particles produced greater pulmonary inflammation when compared with fine-sized TiO_2 particles. However, in contrast to the conclusions of the earlier findings, the results of recent preliminary studies comparing the effects of nano- versus

fine-sized particles, have indicated that pulmonary exposures in rats to uncoated TiO_2 nanorods (200 nm lengths $\times$ 30 nm diameters) and TiO_2 nanodots (particle size <30 nm) did not produce enhanced lung inflammation in rats when compared to fine-sized TiO_2 particle exposures (particle size ~ 270 nm).

Other lung bioassay studies have compared the toxicity effects in rats of uncoated nanoscale quartz particles (50 nm) versus fine-sized quartz particles (particle size ~ 1.6 μm). In pulmonary instillation studies, at equivalent mass doses, the nanoquartz particles produced less intensive and sustained pulmonary inflammatory and cytotoxic responses when compared to the effects produced by the Min-U-Sil quartz particles. This result is intriguing since crystalline quartz silica particles are classified as a Category 1 human carcinogen by the International Agency for Research on Cancer. In summary, the preliminary findings from these two studies suggest that particle size is only one factor in determining pulmonary toxicity.

In addition to the issues of particle size and species differences as discussed above, several additional variables are likely to play important roles in modifying the pulmonary toxicity of nanoparticles. For example, the surface coatings on particles may play an important role in influencing pulmonary effects. In this respect, a pulmonary bioassay toxicity methodology was used to assess the pulmonary toxicity of a number of commercial formulations of fine-sized TiO_2 particles in rats, each formulation with different surface coatings/treatments. The results demonstrated that one of the formulations containing enhanced amounts of amorphous silica and alumina surface coatings on the TiO_2 particle produced greater pulmonary inflammation and cytotoxic effects when compared to the other formulations containing different surface treatments.

The degree to which engineered nanoparticles aggregate in the ambient aerosol and subsequently disaggregate following inhalation will strongly influence particle deposition patterns and interactions with lung cells. If the ultrafine particles disaggregate upon interaction with alveolar lung fluids, then they could behave as discrete individual nanoparticles and may stimulate enhanced inflammatory cell recruitment and/or the particles could preferentially translocate to more vulnerable compartments of the lung.

An additional factor which may modify the lung toxicity and corresponding risk following exposures to engineered nanoparticulates is the electrostatic attraction/aggregation or agglomeration potential of some nanoscale materials, such as single wall carbon nanotubes (SWCNT). The dimensions of individual SWCNTs have been reported as 1 nm (diameter dimension) by >1 μm (length dimension). SWCNTs, however, rarely exist as discrete individual particles, and due to their strong electrostatic characteristics, form agglomerates of 'nanoropes' or 'nanomats', consisting of agglomerates of 10–200 individual SWCNTs.

Two recently reported pulmonary bioassay studies with SWCNTs have been reported in mice and in rats. In one study, groups of rats were exposed by intratracheal instillation with multiple doses of SWCNTs, quartz particles (positive control), or carbonyl iron particles (negative control). Exposures to high-dose ($5\ mg\ kg^{-1}$) SWCNTs resulted in mortality in about 15% of the instilled rats within 24 h postinstillation exposure. This mortality was not due to inherent toxicity of the nanotubes, but resulted from mechanical blockage of the large airways by the instilled agglomerated SWCNT nanoropes. Exposures to quartz particles produced significant increases versus controls in lung inflammation responses, cytotoxicity, and lung parenchymal cell proliferation indices, while exposures to SWCNTs produced transient lung inflammation. Histopathological observations revealed that exposures to quartz particles produced inflammation, foamy alveolar macrophage accumulation, and tissue thickening (fibrosis). In contrast, pulmonary exposures to SWCNTs produced a non dose-dependent series of multifocal granulomas. Contained within the granulomas were agglomerated carbon nanotubes surrounded by monocytic cell-types. Similar findings in SWCNT-exposed mice have been observed.

It is noteworthy that, unlike the results with quartz particles, the finding of unusual pulmonary lesions (i.e., multifocal granulomas) in rats was not consistent with indices of lung cellular injury and sustained lung inflammation. In addition, the results of two recent independent exposure assessment studies have reported very low respirable aerosol SWCNT concentration exposures at the workplace. Thus, the physiological relevance of these pulmonary bioassay findings remains to be determined and can only be reconciled by conducting an inhalation toxicity study in rats with aerosols of SWCNTs. Moreover, it must be noted that single wall carbon nanotubes, due to their unique electrostatic and agglomerative characteristics, are not likely to be representative of other nanoscale particulates.

At this point in time, no generalized conclusions can be drawn regarding the human health effects of inhaled engineered nanoparticulates. This is due, in large part, to the following:

- A paucity of data exists on the pulmonary toxicity of nanoparticles.

- On a mass basis, nano or ultrafine particles (i.e., <100 nm) are considered to produce greater pulmonary toxicity when compared to fine-sized particles (i.e., size range from 100 nm to >3 μm) of identical composition. This conclusion has been derived based only on comparisons of two or three particle types.
- Some recently reported findings indicated that, even on a mass basis, some nanoparticle-types are not more inflammogenic and cytotoxic than fine-sized particulates of similar or identical chemical composition.
- It seems likely that in addition to particle size, other factors such as surface coatings, aggregation/disaggregation potential, origin and method of particle synthesis/composition (e.g., gas phase (fumed) versus liquid phase (colloidal/precipitated)), and surface charge will have a significant impact on modifying potential toxicity of inhaled engineered nanoparticles.
- The impact of surface coatings on biological effects will predominate, particularly on smaller nanosized particles (i.e., particles <25 nm), wherein surfaces will comprise 25–50% of the particle composition.
- It is expected that much additional safety and mechanistic toxicology data on nanoparticulates will be generated by about the year 2010.
- As a consequence, no general conclusions regarding nanoparticle toxicity can be made. Thus, it is important that assessments of safety and health risks of newly developed engineered nanoparticles should be made following relevant testing on a case-by-case basis for each nanoparticle type.

See also: Respiratory Tract.

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http://www.nano.org.uk – The Institute of Nanotechnology (IoN) was one of the world's first nanotechnology information providers, and leads the European network of networks, Nanoforum (www.nanoforum.org) as well as working closely with governments, universities, researchers and companies worldwide on micro and nanotechnology.

http://www.nano.gov – The National Nanotechnology Initiative (NNI) is a US program established to coordinate the efforts of eighteen federal agencies in nanoscale science, engineering, and technology.

Naphthalene

Heriberto Robles

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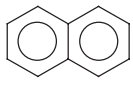
- Chemical Abstracts Service Registry Number: CAS 91-20-3
- Synonyms: Naftalen; Naphthene; NCI-C52904; Albocarbon; Dezodorator; Camphor tar; Mothballs; Moth flakes; Tar camphor; Naftaleno
- Chemical/Pharmaceutical/Other Class: Polynuclear aromatic hydrocarbon
- Chemical Formula: $C_{10}H_8$
- Chemical Structure:



Uses

Naphthalene is commonly used in the manufacture of dyes, resins, and mothballs and may also be found in association with coal tar production and in wood preservatives. It is also used as a chemical intermediate in the synthesis of pharmaceuticals, insect repellents, and pesticides.

Background Information

Naphthalene is a component of crude oil and is found in petroleum-derived fuels and consumer products. The most common use of naphthalene in consumer products is in the production of mothballs. The two active ingredients in mothballs are naphthalene and paradichlorobenzene.

Exposure Routes and Pathways

The primary exposure route for naphthalene is via inhalation, although it may also be absorbed into blood from the gastrointestinal tract and the skin. However, percutaneous absorption is too limited to produce acute systemic reactions except in newborns.

Toxicokinetics

Naphthalene ingestion can result in acute as well as delayed toxicity. The primary target organs of toxicity are the blood and eyes. Individuals deficient in glucose-6-phosphatase dehydrogenase are especially sensitive to the hemolytic effects of naphthalene. Normal individuals may also develop hemolysis when exposed to high doses.

Mechanism of Toxicity

Systemic absorption of naphthalene vapor may result in cataracts. The biochemical basis for naphthalene cataract has been investigated. Naphthalene is metabolized in the liver to 1,2-dihydro-1,2-dihydoxynaphthalene. Lenticular catechol reductase biotransforms 1,2-dihydro-1,2-dihydroxylnaphthalene to 1,2-dihydroxynaphthalene, which in turn is autooxidized in air at neutral pH to 1,2-naphthaoquinone and hydrogen peroxide. Ascorbic acid reverses the latter reaction and forms dehydroascorbic acid, which diffuses out of the lens very slowly. Dehydroascorbic acid has been shown to accumulate in the lens of rabbits fed naphthalene and lens incubated *in vitro* with 1,2-dihydro-1,2-dihydroxynaphthalene. The sequence of reactions involves reduction of ascorbic acid by 1,2-naphthoquinone in the aqueous humor to dehydroascorbic acid, which rapidly penetrates the lens and is reduced by glutathione. Oxidized glutathione and 1,2-naphthoquinone may compete for enzyme glutathione reductase, which normally maintains high reticular levels of reduced glutathione. A reduction in the concentration of these coupled with the removal of oxygen from the aqueous humor due to the autooxidation of 1,2-dihydroxynaphthalene may make the lens sensitive to naphthalene toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute ingestion of naphthalene by rabbits produced effects similar to those observed in humans. Rabbits fed acute doses of naphthalene presented browning of the lenses and eye humors and developed cataracts. In contrast to humans, where the major target organ of toxicity is the blood and eyes, for rats and mice the major target organ of toxicity is the lung.

The maximum air concentration of naphthalene that can be generated (78 ppm) has not been shown to be lethal to rats. Animal studies report that the oral LD_{50} for naphthalene ranges from 354 mg kg^{-1} in mice to 2400 mg kg^{-1} in rats. In extreme circumstances, death may also occur as a result of dermal exposure.

Human

Skin or eye surface contact may result in naphthalene cataracts, ocular irritation, skin irritation, and in the case of a sensitized person, severe dermatitis. Topical lesions will clear spontaneously, as soon as the exposure is terminated.

Inhalation of vapor may result in headache, confusion, excitation, nausea, and sometimes vomiting and extensive sweating. Dysuria, hematuria, and acute hemolytic reaction can also be seen.

Ingestion may cause abdominal cramps with nausea, vomiting, diarrhea, headache, profuse perspiration, listlessness, confusion, and coma with or without convulsions in a case of severe poisoning. It also may cause irritation of the urinary bladder, dysuria, the passage of brown or black urine with or without albumin and casts, and acute intravascular hemolysis.

Adverse neurologic effects have also been reported in humans who ingested naphthalene. Signs and symptoms reported include vertigo, lethargy, muscle twitching, decreased response to painful stimulus, and coma. It has been speculated that the neurological symptoms may have resulted from cerebral edema produced by acute hemolysis and not from a direct toxicological effect of naphthalene.

There are no reported cases of death due to inhalation of naphthalene. Naphthalene-induced deaths are usually related to mothball ingestion during suicide attempts. Based on suicide data, the lethal dose in humans is estimated to range from 319 to 574 mg kg^{-1}. The most prominent effect of high-dose naphthalene exposure in humans is hemolytic anemia. There is a report of an infant who died of acute hemolytic anemia after being exposed to

mothball-treated diapers. Another infant reportedly experienced skin rashes, systemic poisoning, and then death apparently due to naphthalene exposure from mothballs used with clothes or blankets that had been stored in or near the infant's room.

Reported effects of naphthalene overexposure in humans include hemolytic crisis, characterized by increased bilirubin levels, and the appearance of Heinz bodies and fragmented red blood cells. Other effects that have been associated with ingestion of high doses include gastrointestinal distress, vomiting, nausea, jaundice, proteinurea, hemoglobinuria, hemoglobinuria, methemoglobinemia, kernicterus, and coma.

A 69-year-old woman exposed to naphthalene and *p*-dichlorobenzene developed aplastic anemia 2 months after exposure. A 36-year-old pharmacist was given 5 g of unpurified naphthalene in an emulsion of castor oil in divided doses over 13 h. On awakening 8 or 9 h later, he had severe pain in the bladder and found that he was nearly blind, although he had had no eye problems. After 1 year, an examination showed that his vision was reduced to the ability to count fingers at 1.5 m and his visual fields were constricted 30–50°. The condition was unimproved by glasses.

Chronic Toxicity (or Exposure)

Animal

Naphthalene does not appear to be teratogenic. Mice exposed to $300\,mg\,kg^{-1}\,day^{-1}$ produced normal offspring, although a decrease in litter size was reported.

Oral administration of $1\,g\,kg^{-1}\,day^{-1}$ in rabbits leads to lenticular changes, initially observed as swelling in the peripheral portion of the lens. Within 2 weeks, the whole lens is affected with mature cataract. The biochemical basis for cataract has been shown to be related to a liver metabolite of naphthalene, 1,2-dihydro-1,2-dihydoxynaphthalene.

Selective lung damage and necrosis occurred in Clara cells of mice that were administered naphthalene. It produced selective depression of pulmonary monooxygenase activities without accompanying changes in hepatic monooxygenease. A dose-dependent alteration of Clara cells (bronchiolar epithelial cells) was noted.

Mice exposed to naphthalene vapor at concentrations as high as 30 ppm in air for 6 h a day, 5 day a week for 104 weeks developed nose and lung lesions. These lesions were described as nose inflammation accompanied by metaplasia and hyperplasia of the olfactory and respiratory epitheliums.

Carcinogenicity studies conducted in rats and mice prior to 1992 reported either negative or non-conclusive results. Continued efforts to determine the potential carcinogenicity of naphthalene has resulted in improved design studies. Some of these studies have reported evidence of carcinogenic activity for naphthalene. For example, rats of both sexes exposed to naphthalene by inhalation presented a dose-dependent increased incidence of respiratory epithelial adenoma and olfactory epithelial neuroblastoma.

Human

Reports of adverse effects following chronic naphthalene exposure include the development of cataracts and retinal hemorrhage in a 44-year-old man occupationally exposed to powdered naphthalene. Unilateral chorioretinitis was reported for a coworker and cataracts developed in 8 of 21 workers exposed to naphthalene fumes or dust for ~ 5 years in an industrial setting. Chronic exposure to powdered naphthalene in the workplace has been associated with an increased incidence of cataracts. However, few of these effects have been confirmed in animal studies.

Carcinogenicity studies conducted in the late 1990s and early 2000s have found some evidence that naphthalene may be carcinogenic to rats. These findings have prompted the International Agency for Research on Cancer to classify naphthalene as a chemical that is possibly carcinogenic to humans (group 2B classification).

In Vitro Toxicity Data

Several *in vitro* genotoxicity studies have been conducted for naphthalene. Naphthalene has not been found to be genotoxic in the *Salmonella* reverse mutation assay. However, naphthalene has been reported to have genotoxic effects in nonmammalian assay studies. In various *in vitro* studies, naphthalene has been shown to have the potential to induce chromosomal damage in mammalian cells.

Clinical Management

There is no specific antidote for naphthalene toxicity. Treatment is symptomatic and supportive. Gastric decontamination should be considered with emesis or lavage, followed by activated charcoal. Hemolysis may require urinary alkalinization and transfusion. Methemoglobinemia may be treated with methylene blue. Emesis is more useful for mothballs because of size. Lavage may be useful for ingestion of flakes. Information on activated charcoal is scant, but adsorption is thought to occur. Mothballs dissolve slowly; gastric decontamination should be performed

even in patients presenting late after ingestion. Emesis may be indicated in recent substantial ingestion unless the patient is or could rapidly become obtunded, comatose, or convulsant. It is most effective if initiated within 30 min of ingestion. The recommended dose of ipecac syrup is 30 ml for an adult and 15 ml for a child.

Gastric lavage may be indicated if performed soon after ingestion or in patients who are comatose or at risk of convulsing. The airway should be protected by placement in Trendelenburg and left lateral decubitus position or by cuffed endotracheal intubation.

Environmental Fate

Naphthalene may be released to the environment as a wood and fossil fuel combustion product or from unintentional, accidental release of petroleum fuels. It is relatively volatile at room temperature and tends to evaporate readily. In air, naphthalene will react with hydroxyl and nitrate radicals. This compound has a relatively short half-life in soil and water due to its volatility and rapid degradation. The half-life of naphthalene in soil ranges from 2 to 18 days.

Exposure Standards and Guidelines

- US Environmental Protection Agency (EPA) drinking water standard for naphthalene is $20\,\mu g\,l^{-1}$.
- State regulated drinking water standards for naphthalene range from $6.8\,\mu g\,l^{-1}$ in Florida to $300\,\mu g\,l^{-1}$ in Minnesota.
- The US EPA 10-day Health Advisory (HA) for a 10 kg child is $\sim 0.5\,mg\,l^{-1}$.
- The US EPA long-term HA for a 10 kg child is $\sim 0.4\,mg\,l^{-1}$.
- The US EPA long-term HA for a 70 kg adult is $\sim 1\,mg\,l^{-1}$.
- The US EPA lifetime HA for a 70 kg adult is $\sim 0.02\,mg\,l^{-1}$.

See also: Blood; Carcinogenesis; Polycyclic Aromatic Hydrocarbons (PAHs); Sensory Organs.

Further Reading

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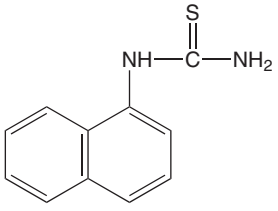
Naphthyl Thiourea, α-

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 86-88-4
- Preferred Name: ANTU
- Synonyms: α-Naphthyl urea; 1-(1-Naphthyl)-2-thiourea; α-Naphthyl thiocarbamide
- Chemical Formula: $C_{11}H_{10}N_2S$
- Chemical Structure:



Uses

ANTU is a single-dose rodenticide used as bait and tracking powder and is specifically used against Norway rats. It is ineffective against all species of field rodents. It is used in baits in concentrations of 1–3%. Because of its specificity to Norway rats and its tendency to cause resistance, this poison rapidly lost popularity and is no longer manufactured. It is not produced commercially in the United States.

Background Information

ANTU is a gray crystalline odorless powder with a bitter taste. It has a molecular weight of 202.7. It has a melting point of 198°C and does not ignite readily. On contact with strong oxidizers it may cause fire

and explosions. Fire may produce irritating or poisonous gas. Hazardous decomposition products include sulfur dioxide, oxides of nitrogen, and carbon monoxide.

Exposure Routes and Pathways

Inhalation, ingestion, and dermal contact are possible routes of exposure.

Toxicokinetics

Limited data on the toxicokinetics of ANTU are available. However, absorption does occur following oral administration. ANTU toxicity in the rat is thought to depend on metabolic activation via the hepatic and lung microsomal enzymes to form a hydrosulfide and α-naphthyl urea.

Mechanism of Toxicity

ANTU toxicity in the rat is thought to depend on metabolic activation via the hepatic and lung microsomal enzymes to form a hydrosulfide and α-naphthyl urea. The metabolites are covalently bound to lung macromolecules. However, it is not known if these metabolites are produced in humans. ANTU presumably acts on some enzyme system involving the sulfhydryl group. Analogous pulmonary edema is produced by sulfhydryl inhibitors, such as alloxan, iodoacetamide, and oxophenarsine. Production of oxygen free radicals via the cyclooxygenase pathway has been implicated in mediating ANTU-induced lung damage. Following exposure to ANTU, there are a number of biochemical events, such as alteration in carbohydrate metabolism, adrenal stimulation, and interaction of the chemical with sulfhydryl groups, but none of these appear to bear any relationship to the observed signs of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity of ANTU is strikingly higher for wild Norway rats than for other species. Mice and dogs rank next in susceptibility. Young animals are less resistant. Rats that survive sublethal doses develop a high degree of tolerance owing partly to refusal to eat freely. Symptoms in rats appear within 12–25 min with a sharp fall of body temperature, huge pleural and intraalveolar edema, anuria, dyspnea, and death. Blood sugar rises to nearly three times the normal level within 2.5 h with a severe fall of liver glycogen and failure to deposit liver glycogen. Observations in experimental animals indicate that the principal organ affected is the lung; pulmonary edema and pleural effusion develop due to the action of ANTU on pulmonary capillaries causing marked edema of the subepithelial spaces of the alveolar walls; pericardial effusion is less marked.

Dogs are quite susceptible to toxicity but may be protected by prompt vomiting. Pulmonary effusion in dogs showed an increase in albumin globulin ratio. Hemorrhagic glomerular nephritis has been seen after acute exposure in rats. Hyperglycemia has been reported in experimental animals. LD_{50} (dog) is $16\,mg\,kg^{-1}$ ip and $380\,\mu g\,kg^{-1}$ po.

Human

The estimated mean lethal dose in humans is 25 g/70 kg. ANTU is classified as being moderately toxic. No human fatalities have been reported. It is probably not toxic to humans except in large amounts. Although it appears that humans are resistant to ANTU intoxication, probably because insufficient quantities are ingested, poisonings have occurred, with tracheobronchial hypersecretion of a white, nonmucous froth containing little protein, pulmonary edema, and respiratory difficulty. Ingestion may cause vomiting, shortness of breath, and bluish discoloration of the skin. Inhalation of ANTU powder may result in dyspnea, rales, cyanosis, and pulmonary edema or effusion. A case of contact eczema due to handling a rat poison containing ANTU as a base has been reported.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure in rats results in stunted growth, thinning and coarsening of hair, deformities of the legs and feet, hyperplasia of the thyroid and splenic pulp, hyaline changes in the hepatic cells, decreased thickness of the adrenal cortex, and calcified tubular casts. Continued administration to cats produces fatal intrahepatic obstructive jaundice without pulmonary lesion. Available data were inadequate to evaluate the carcinogenicity of ANTU in experimental animals.

Human

Chronic exposure to ANTU led to the investigation of two cases of bladder cancer in two rodent operators. Therefore, the use of ANTU was restricted to professional operators. Available data were inadequate to evaluate carcinogenicity in humans. Chronic sublethal exposure may result in antithyroid activity and hyperglycemia.

Clinical Management

For ingestion, emesis is indicated unless the patient becomes comatose or shows convulsions. Emesis is most effective if initiated with 30 min of ingestion. Syrup of ipecac can be used for inducing emesis. Charcoal slurry, aqueous or mixed with saline cathartic, or sorbitol may be used. Treatment would be by liberal gastric lavage, the substance being only slightly soluble. Ventilation and oxygenation with close arterial blood gas monitoring should be maintained in case of pulmonary edema. In case of an inhalation exposure, the patient should be moved to fresh air and monitored for respiratory distress. The person should also be evaluated for respiratory tract irritation, bronchitis, or pneumonitis. Humidified supplemental oxygen (100%) with assisted ventilation may be administered as required. Exposed eyes should be irrigated with copious amounts of tepid water. For dermal exposure, the affected skin should be washed with soap and water. No antidotes are established.

Since ANTU is a sulfhydryl blocking agent, cysteine has been tried in rats and was effective in some cases. There is no human experience with cysteine and its use is not recommended.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value time-weighted average (TWA) and the Occupational Safety and Health Administration permissible exposure limit – TWA are both $0.3\,mg\,m^{-3}$.

See also: Pesticides.

Further Reading

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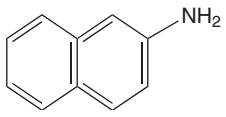
Naphthylamine, 2-

Glenn Talaska

- Chemical Abstracts Service Registry Number: CAS 91-59-8
- Synonyms: 2-Aminonaphthalene; *β*-Naphthylamine; 2NA; BNA; Fast Scarlet Base B
- Chemical/Pharmaceutical/Other Class: Aromatic amine
- Chemical Formula: $C_{10}H_7NH_2$
- Chemical Structure:



Uses

2-Naphthylamine (2NA) was used as an intermediate in the dye industry and as an antioxidant in the rubber industry (e.g., the last company to manufacture it in the United States supposedly ceased production in 1972). However, it probably still presented an exposure hazard for at least some time afterward as a contaminant of dye stocks such as Broenner's acid and replacement antioxidants that retain the 2NA nucleus; for example, Nonox S. In addition, antioxidants in the rubber industry such as *N*-phenyl-2-naphthylamine and *N*,*N*′-di-2-naphthyl-*p*-phenylenediamine have been shown to be metabolized to 2NA following absorption. 2NA and other aromatic amines such as 4-aminobiphenyl are also produced during the burning of tobacco, especially low temperature burning, and when cooking fats and oils are heated. Trace amounts of 2NA have been found in dye-containing products such as children's finger paints.

Exposure Routes and Pathways

2NA is well absorbed through the skin, as well as via the gastrointestinal and respiratory tracts. With this and other aromatic amines, the skin appears to be a significant, if not the major occupational exposure pathway. Workers tolerate skin contamination since the acute effects of exposure are minimal. Inhalation

is the major route of exposure for tobacco smokers. There have been reports that passive burning of tobacco (environmental tobacco smoke) produces larger amounts of 2NA and other aromatic amines on a per cigarette basis than is seen with active smoking. Thus, there is some concern that passive smoke exposure may contribute to the burden of urinary bladder cancer in the nonsmoking population.

Occupational exposure to compounds containing a 2NA nucleus can result in 2NA exposure if metabolic enzymes can degrade the material. For example, workers inhaling ~ 30 mg *N*-phenyl-2-naphthylamine in 1 day excreted 3–4 μg 2NA in their urine over the next 24 h. This is the 2NA exposure equivalent of smoking ~ 5 packs of cigarettes.

Toxicokinetics

Aromatic amines are well absorbed from the skin, the gut, and the respiratory tract. Aromatic amines like 2NA are metabolized rapidly and several systems compete for these agents as substrate. For example, the majority of 2NA is excreted in the urine as the glucuronide that is deconjugated prior to analysis. Ring oxidation and *N*-acetylation are considered detoxification reactions and this is evidenced by the finding that persons with the slow *N*-acetyltranferase 2 phenotype and exposed to many aromatic amines are at elevated risk of urinary bladder cancer in comparison to their fast acetylating cohorts. *N*-Oxidation by cytochrome P450 enzymes is considered activating for bladder carcinogenesis.

Mechanism of Toxicity

The acute toxicity of 2NA is low and due to the formation of methemoglobin. However, this is greatly overshadowed by the urinary bladder carcinogenicity of this compound. The proposed mechanism for this affect includes *N*-oxidation and excretion of the amine into the blood in an unconjugated form. Then the *N*-hydroxy-2-naphthylamine is co-oxidized to the corresponding nitroso form while hemoglobin is oxidized to methemoglobin. 2-Nitrosonaphthalene is then capable of covalent binding with sulfhydral groups on hemoglobin, forming stable hemoglobin adducts.

The mechanism of chronic toxicity is related, but not identical. 2NA and other aromatic amines including benzidine and 4-aminobiphenyl are potent human urinary bladder carcinogens. Apparently the *N*-hydroxy-2-naphthylamine is *N*-glucuronide and the product is transported from the liver to the urinary bladder, where the glucuronide can be hydrolyzed liberating *N*-hydroxy aromatic amine. This material is capable of binding with DNA in the urothelium of the exposed persons. While this pathway has not been shown specifically using 2NA as a substrate in humans, it is consistent with the animal data.

The low acute toxicity of 2NA masks its extreme carcinogenicity as exposed persons experience no or very slight ill effects and assume that the material is not toxic. The latency period for 2NA urinary bladder cancer is estimated from 16 to 30 years after initial exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs and cats have been shown to be sensitive to methemoglobinemia following exposure to 2NA. A dose of 200 mg kg^{-1} was found to produce this effect 'reliably'.

Human

Methemoglobin formation was considered the most serious health outcome following exposure to 2NA until the increased rate of urinary bladder tumors were confirmed in the workers. Methemoglobinemia is symptom-less until acutely toxic. In one report workers exposed to 2NA were required to pass through the medical department following their shift so that any cyanosis could be ascertained.

Chronic Toxicity (or Exposure)

Human

Humans exposed to 2NA during the production of this compound are at dramatically increased risk of urinary bladder cancer. According to one report all 15 workers involved with distilling the product fell victim to the disease. In other studies the relative risk of urinary bladder cancer ranged from 30 to 60 times higher than expected; it not being uncommon that 50% of the exposed workforce was prevalent cases. As noted above the estimates of the so-called 'latency' (time between first exposure and disease) period ranged for 16–30 + years for 2NA-exposed workers.

Clinical Management

Fortunately, urinary bladder cancer is amendable to effective treatment if detected early. A full spectrum of biomarkers and early diagnostic screens are available to alert the health professional when exposure to 2NA has occurred and when changes consistent with early neoplasia are occurring.

The US Occupational Safety and Health Administration (OSHA) standard for β-naphthylamine, 29 CFR 1910.1009, contains regulations covering periodic medical surveillance, examinations, and medical records for current employees who may have been exposed to 2NA. However, it should be noted that these regulations do not apply to former employees and that medical surveillance or treatment of former employees is not regulated or required by OSHA.

Exposure Standards and Guidelines

As indicated above, 2NA is one of the carcinogens covered under a specific OSHA regulation, 29 CFR 1910.1009. The American Conference of Governmental Industrial Hygienists indicates that exposure by all routes to 2NA should be controlled to levels as low as possible.

Miscellaneous

The molecular weight of β-naphthylamine is 143.2. It has a negligible vapor pressure until heated; at 200°C the vapor pressure is 1 mmHg. The concentration of 2NA in the air can be determined using National Institute for Occupational Safety and Health method 5518 and it oxidizes in air. Biological monitoring has been done for this material using urinary analysis of metabolites by high-performance liquid chromatography, hemoglobin adducts using gas chromatography–mass spectrometry, and DNA adducts in lymphocytes and exfoliated urothelial cells using ^{32}P-postlabeling.

See also: Dyes; Tobacco Smoke.

Further Reading

Felknor SA, Delclos GL, Lerner SP, *et al.* (2003) Bladder cancer screening program for a petrochemical cohort with potential exposure to beta-napthylamine. *Journal of Occupational and Environmental Medicine/American College of Occupational and Environmental Medicine* 45: 289–294.

IARC (1983) *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Rubber Manufacture*. Lyon, France: International Agency for Research on Cancer.

Talaska G and Al-Zoughool M (2003) Aromatic amines and biomarkers of human exposure. *Journal of Environmental Science and Health* 21: 133–164.

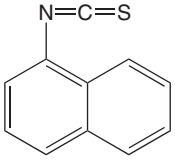
Relevant Website

http://www.osha.gov – OSHA website for analytical method #93, 4-aminobiphenyl, 1-naphthylamine, and 2-naphthylamine.

Naphthylisothiocyanate

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 551-06-4
- Synonyms: α-Naphthylisothiocyanate; ANIT; 1-Isothiocyanato-naphthalene
- Chemical Formula: $C_{11}H_7NS$
- Chemical Structure:



Uses

1-Naphthylisothiocyanate is used as an ingredient in insecticides, and as a laboratory agent for inhibiting microsomal based metabolism. It is also found in cyanamide, which is used in many industrial applications.

Exposure Routes and Pathways

Inhalation, ingestion, and dermal contact are all possible routes of exposure.

Mechanism of Toxicity

1-Naphthylisothiocyanate causes separation of extracellular tight junctions that seal bile canaliculi, impairing bile formation. 1-Naphthylisothiocyanate inhibits microsomal drug-metabolizing activity. It has also been suggested that ANIT depletes hepatocytes of glutathione through a reversible process.

Acute and Short-Term Toxicity (or Exposure)

Animal

A single dose can induce intrahepatic cholestatis (reduction in bile flow) in rats, producing hyperbilirubinemia. In China, several herbal formulations have been shown to reduce the liver damage caused by naphthylisothiocyanate in rats. The oral LD_{50} in rats is 200 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

1-Naphthylisothiocyanate is a potent hepatotoxin and mutagen in animals.

Human

1-Naphthylisocyanate can cause liver and kidney damage as well as dermatitis, ocular irritation, and corrosion.

Clinical Management

After ocular exposure, eyes should be immediately flushed with water for at least 15 min. Following skin exposure, the skin should be flushed with water for at least 15 min. If ingested, vomiting should not be induced. If conscious, the individual should ingest two to four cupfuls of milk or water. After inhalation exposure, the individual should be removed to fresh air immediately and provided breathing support if necessary. Mouth-to-mouth resuscitation should not be used.

See also: Kidney; Liver; Pesticides.

Further Reading

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, 5th edn. New York: Wiley.

Hill DA and Roth RA (1998) Alpha-naphthylisothiocyanate causes neutrophils to release factors that are cytotoxic to hepatocytes. *Toxicology and Applied Pharmacology* 148(1): 169–175.

Roth RA and Dahm LJ (1997) Neutrophil- and glutathione-mediated hepatotoxicity of alpha-naphthylisothiocyanate. *Drug Metabolism Reviews* 29(1–2): 153–165.

National Environmental Policy Act, US

Samantha E Gad and Shayne C Gad

- Agency: US Council on Environmental Quality
- Year Passed: 1969
- Groups Regulated: US government agencies

Synopsis of Law

The National Environmental Policy Act (NEPA) was signed into law in 1970, and established a national policy to protect the environment, created a Council on Environmental Quality (CEQ), and required that environmental impact statements be prepared for major federal actions having a significant effect on the environment. The CEQ's efforts laid the groundwork for almost all current US environmental legislation, except for Superfund and asbestos control legislation. The CEQ also developed guidelines for the environmental impact statement process. The NEPA process resulted in a major change in the way governments deal with environmental issues, and this model has been replicated in whole or in part in 23 states.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Ethanol; Resource Conservation and Recovery Act, US; Toxic Substances Control Act, US

Relevant Websites

http://www4.law.cornell.edu – National Environmental Policy (from the US Code).

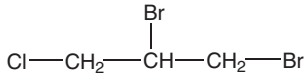
http://ceq.eh.doe.gov – NEPANet.

http://www.epa.gov – US Environmental Protection Agency (EPA) website on 'NEPA: Past, Present, and Future'.

Nematocides

Samantha E Gad

- Representative Compound: Nemagon (1,2-dibromo-3-chloropropane)
- Chemical Abstracts Service Registry Number: CAS 96-12-8 (Nemagon)
- Chemical/Pharmaceutical/Other Class: Nema-tak; Halogenated hydrocarbons
- Chemical Formula: $C_3H_5Br_2Cl$
- Chemical Structure:



Uses

Nematocides are pesticides that kill parasitic worms such as roundworms or threadworms. Early nematocides were used as soil fumigants. Since the 1960s, a completely new group of 'nonfumigant' nematocides has been developed. All are organophosphorus or carbamate pesticides with marked (acute oral and dermal) toxicity to humans.

Exposure Routes and Pathways

Dermal contact, inhalation, and ingestion are possible exposure pathways.

Toxicokinetics

In rats, 98% of nemagon is absorbed into the stomach. Within 3 days, 90% of the compound is excreted. Within the first 24 h period, 49% is excreted through urine, 14% through feces, and 16.5% through expired air.

Mechanism of Toxicity

Defatting creates cell necrosis. Nematocides also reduce cell P450 content. Sulphyril, but not glutathione, mediates toxic effects. Biotransformation (hydrolysis and oxidation) is via the mercapturic acid route, producing α-chlorohydrin and α-bromohydrin, two antifertility agents. Further oxidation of these substances may produce oxalic acid, which causes liver and kidney damage.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of nemagon is 100 mg kg^{-1} in rabbits and 260 mg kg^{-1} in mice.

Human

Acute exposure to high concentrations produces dyspnea, gasping, and coughing.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure results in eye damage, kidney degeneration, and central nervous system (CNS) effects. It is a carcinogen to nasal passages, pharynx, and respiratory tract. A dose–response relationship exists between exposure and damage to the reproductive system. Age or stage of sexual development also mediated damage.

Human

Chronic exposure affects the liver, kidneys, and heart. Other symptoms include CNS depression and pulmonary congestion. Nemagon is a reproductive toxin resulting in reduced sperm count. Adverse effects are presumed to be reversible. It is a possible carcinogen. Most human exposure to nematocides is as trace residues in meat.

Clinical Management

The victim should be moved from exposure site and given respiratory therapy. Treatment should be symptomatic.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 1 ppb per 8 h.

See also: Federal Insecticide, Fungicide, and Rodenticide Act, US; Pesticides.

Further Reading

Krieger R (2001) *Handbook of Pesticide Toxicology*. San Diego, CA: Academic Press.

Neon

Lynda M Ewers

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-01-9
- Chemical/Pharmaceutical/Other Class: Neon is an element in the family referred to as the inert, rare, or noble gases. Members of this family, in increasing order by atomic number, are helium, neon, argon, krypton, xenon, and radon. All of these gases are colorless, odorless, and nonflammable. The noble gases are thought to originate primarily under conditions of high pressure and temperature such as existed at the origin of the universe and continues to exist in some stars. Neon

is highly inert and does not form chemical compounds under normal environmental conditions.

Uses

Neon is primarily used in luminous tubes (vacuum electric discharge tubes), airplane beacons, helium–neon lasers, high-voltage indicators, cryogenic refrigerant, and laboratory experiments.

Background Information

In 1898, a Scottish scientist and a British scientist, Sir William Ramsay and Morris M Travers, respectively, discovered neon as a condensation product in liquefied air, a process similar to that used to collect neon today. Neon's use in lighting evolved from the discoveries that gases under low pressure will conduct electricity. When some of the flowing electrons collide with residual gas in an evacuated glass tube, the resulting ions emit light as they return to their nonexcited state. The color of the light produced depends on the residual gas; neon gas produces a red color and argon, another inert gas often used in tubes (which are frequently and incorrectly called "neon" lights), produces a blue color. These two basic colors are often modified into many different hues by the addition of such elements as mercury and cadmium. The neon found on the earth is considered to be primordial in origin. Most of the neon is sequestered in the earth's rocks or dissolved in water, with small amounts escaping into the atmosphere during geologic weathering. The escaped gas is slowly lost into space faster than it is replenished. Consequently, neon constitutes only a small part (0.0018%) of the earth's atmosphere, although this element is estimated to be the fourth most abundant in the universe.

Exposure Routes and Pathways

The most important route of exposure is inhalation, occurrs when neon escapes from natural sources (rocks and water) or neon-containing products (see Uses). Skin exposure and ingestion can also occur; however, only the inhalation route is considered to be important from a toxicological standpoint, because of the way inhalation of excessive concentrations of this inert gas can still potentially produce harmful effects (see Mechanism of Toxicity).

Mechanism of Toxicity

Neon is a simple asphyxiant. It displaces the oxygen necessary to support life. When normal levels of oxygen are not present in the body, then all tissues, organs, and organ systems eventually malfunction. Tissues with particularly high oxygen and energy requirements, including the brain and heart, are particularly susceptible to harmful effects resulting from reduced levels of oxygen in the body.

Acute and Short-Term Toxicity (or Exposure)

The primary adverse health effect attributed to neon exposures is simple asphyxiation due to the displacement of oxygen necessary for life. No animal or *in vitro* studies were found in scientific literature, but it is known that humans and other animals requiring oxygen can die by asphyxiation, if exposed to high concentrations of neon.

Chronic Toxicity (or Exposure)

The typically small quantities of the gas in the environment and amounts used in manufacturing consumer products result in very low levels of neon in workplace and ambient environments, and thus cause negligible health risks to workers and the general public who may experience chronic exposures.

Clinical Management

Oxygen should be provided to the affected individual.

Ecotoxicology

No known reports on ecotoxicology of neon could be found. Neon is very inert and does not deplete ozone.

Other Hazards

Neon is not explosive or flammable. Hazards related to neon include use of cryogenic neon-gas tanks, emission of eye-damaging light from lasers, or escape of mercury, cadmium, or lead from luminous tubes. The manufacture of neon-type advertising signs are of special concern because such signs are often produced by small business artisans who may have limited knowledge or resources to deal with hazardous material, such as mercury and lead, which have been documented to contaminate these workplaces.

Exposure Standards or Guidelines

No standards or guidelines are available for neon.

See also: Cadmium; Lead; Mercury.

Further Reading

Ewers L, Page E, and Mortimer V (2003) Hazards associated with the manufacture and repair of neon lights. *Applied Occupational and Environmental Hygiene* 18(1): 1–9.

Neonicotinoids

Josef Seifert

History of Neonicotinoid Development

Neonicotinoids are a new class of synthetic insecticides that became commercially available in the 1990s. Currently there are only a few neonicotinoid insecticides on the market but those are being increasingly used with a good prognosis for their further development. These new-generation pesticides have potential as replacements for some of the more toxic organophosphorus and methylcarbamate insecticides.

Nicotine (**Figure 1**) isolated from tobacco plants (*Nicotiana tabacum*) has been used as a systemic insecticide against sucking insects for centuries. The nicotinic acetylcholine receptor-Na^+/K^+ ionophore of the insect central nervous system is the target site, and the consequences of the altered cholinergic neurotransmission provides the mechanism of insecticidal action. Since nicotine is equally or more toxic to mammals than to insects, the major objective in developing new insecticides modeled on nicotine has been to change this unfavorable feature and synthesize compounds with greater selectively and toxicity to insects. Preservation of the nicotinic acetylcholine receptor as a target for these novel insecticides is important in order to address the development of insect resistance to other insecticides. Nicotine and its analogs, such as nornicotine or anabasine (**Figure 1**), are grouped together as nicotinoids. Neonicotinoids are synthetic, newly developed insecticides with the nicotinic acetylcholine receptors as their target but, in contrast to nicotinoids, have a high degree of selectivity toward insects.

Nithiazine (**Figure 2**) was the first neonicotinoid developed by Shell (Modesto, USA) in the 1970s. Nithiazine is a 2-nitromethylene tetrahydro-1, 3-thiazine selected from a series of nitroalkyl heterocyclic compounds, the molecular models being distinct from nicotine but acting on the nicotinic acetylcholine receptors like nicotine. Nithiazine is selectively toxic to insects but its field application is limited because of its low photostability.

Nithiazine was the lead compound in syntheses of the first commercially successful neonicotinoids that surpassed the parent compound in both insecticidal properties and environmental stability. A 6-chloro-3-methylpyridine moiety and a pharmacophore of varying structures (**Figure 2**) are the two components of a neonicotinoid molecule. Insecticides of the first generation of neonicotinoids are best represented by imidacloprid (Nihon Bayer Agrochem, Japan) (**Figure 2**) and are also called chloronicotinyls or chloropyridyls.

The most recent efforts in the development of neonicotinoids focused on the search for heterocycles and pharmacophores that would further improve insecticidal properties of the current compounds. This search must be a compromise between the requirements for the optimal electron distribution in the pharmacophore needed for insecticide binding to the receptor subsites and the need for hydrophobicity of a neonicotinoid for efficient penetration through the protective lipoid shield that surrounds the insect central neural system. The synthesis of thiamethoxam (compound CGA 293 343; Novartis, Switzerland) from a heterocycle 2-chloro-5-methylthiazine and a pharmacophore 4-nitroimino-N^5-methyl-1,3,5-oxadiazinane was the first success in the development of the second generation of neonicotinoids also called thianicotinyls. Examples of the second generation of neonicotinoids that have been or are being introduced on the market are shown in **Figure 3**. Most recently, the third generation of neonicotinoids, furanicotinyl compounds (e.g., dinotefuran, **Figure 3**), has been developed.

Mechanism of Neonicotinoid Action

The nicotinic acetylcholine receptors of the neural excitatory cholinergic system are the targets for both nicotine and neonicotinoids in mammals and insects.

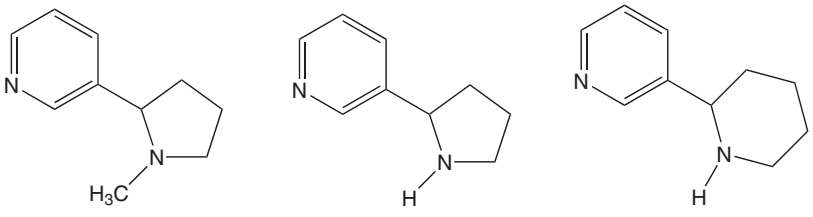


Figure 1 Nicotinoids.

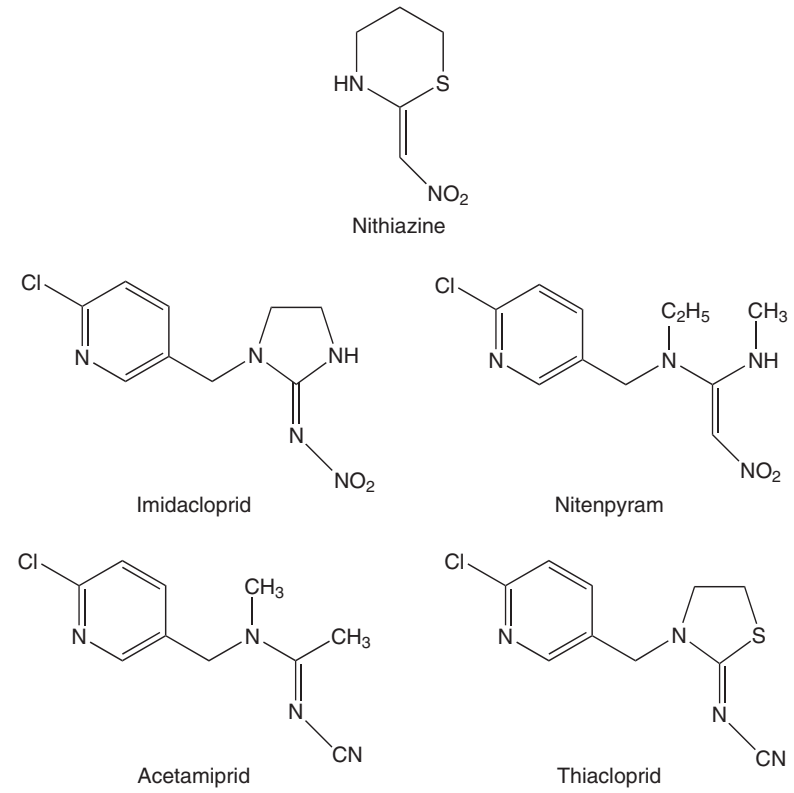


Figure 2 Nithiazine and the first generation of neonicotinoids.

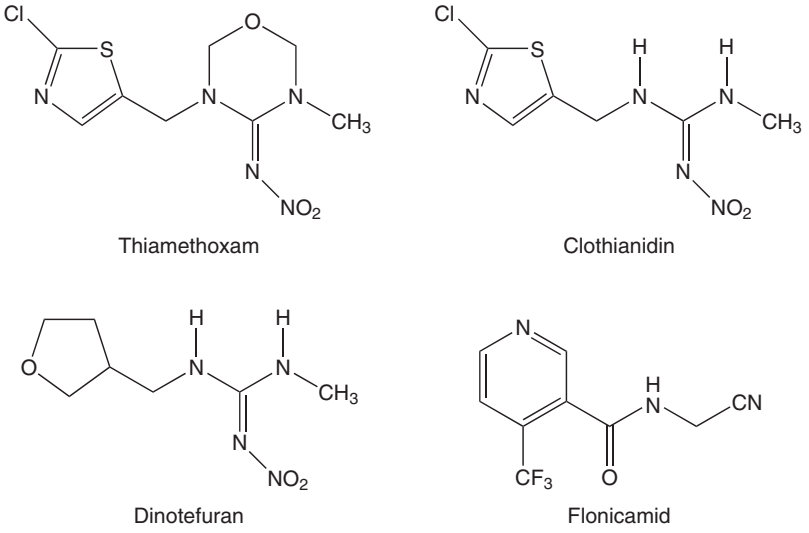


Figure 3 The second and third generations of neonicotinoids.

Nicotinic acetylcholine receptors regulate the flow of Na^+ and K^+ through the channels in the neural postsynaptic membranes. Opening and closing of the channels by acetylcholine maintains the dynamic ratio of the intracellular to extracellular concentrations of Na^+ and K^+ which is needed for the initiation of the electric signal in the postsynaptic neuron. Nicotine and neonicotinoids are agonists, both of which act at the nicotinic acetylcholine receptor – Na^+/K^+ ionophore. The structural differences between the insect and mammalian receptors define the selectivity of neonicotinoid toxicity to insects and nicotine toxicity to vertebrates. The proposed concept of the neonicotinoid electronegative pharmacophore

model (**Figure 4**) considers the presence of a positively charged site unique for the insect receptor that interacts specifically with the negatively charged tip of neonicotinoid pharmacophores. On the other hand, protonation of the nicotinoid nitrogen at physiological pH is the determining factor for their strong binding to the vertebrate receptors. Ionization of nicotinoids also negatively affects their penetration into the insect central nervous system, in contrast to the nonionized and more hydrophobic neonicotinoids.

Neonicotinoid Toxicity to Nontarget Species

Acute Toxicity

Unlike nicotine, neonicotinoids are only moderately toxic to mammals mainly because of their lower affinity for the mammalian neural and muscle nicotinic acetylcholine receptors. In laboratory animals, high neonicotinoid doses that are near the LD_{50}s cause tremor, gait incoordination and hypothermia appearing 2–6 h after oral administration. The signs generally cease within 24 h following treatment. LD_{50}s of the currently used neonicotinoids are in the range of 170–2000 mg kg^{-1} for oral administration and $\geqslant$2000 mg kg^{-1} with dermal administration, dependent on animal species and the type of neonicotinoid. Death from oral neonicotinoid overdose occurs within 3–7 h.

Subchronic and Chronic Toxicity

Tests of neonicotinoids for neurotoxicity, reproductive toxicity, teratogenesis and mutagenesis in a variety of laboratory animals, generally conducted for the purpose of insecticide registration, were negative. Neither of the neonicotinoids induced growth of malignant tumors in laboratory animals. Based on the current knowledge, neonicotinoids can be considered safe for both humans and farm animals or pets.

Environmental Toxicity

Some toxic consequences of neonicotinoids for nontarget beneficial aquatic and terrestrial arthropods such as bees can be expected since these creatures have nicotinic acetylcholine receptors as functional components of the cholinergic system similar to those of insect pests. Surprisingly, neonicotinoid toxicity to numerous nontarget insect species and wildlife marker vertebrates, for example, rainbow trout, is lower than expected. In general, the environmental safety of neonicotinoids surpasses that of other insecticides.

Neonicotinoid Stability

Physical–Chemical Factors

Neonicotinoids, products with medium to high water solubility, are relatively stable in water, buffers or physiological media in pH range 5–7. Their stability decreases with an increasing pH (e.g., $t_{1/2}$ for thiamethoxam at pH 5–7 is $\geqslant$1 year while only a few days at pH 9). Photostability of neonicotinoids with a nitromethylene group ($=CH-NO_2$) is low since this group absorbs strongly sunlight in the range of 290–400 nm. For instance, degradation of

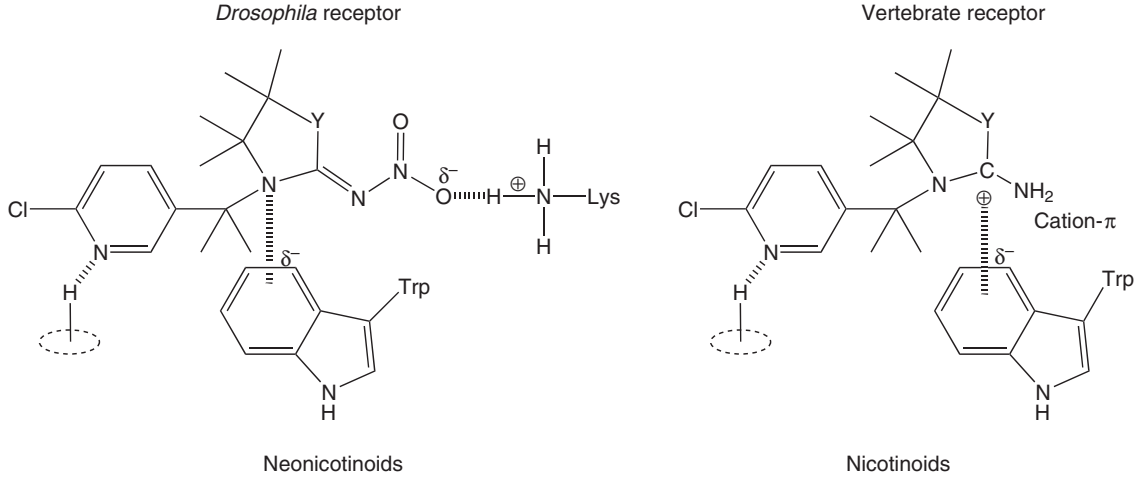


Figure 4 Molecular models of binding subsites in the *Drosophila* or vertebrate nicotinic acetylcholine-regulated receptors. (Reprinted with permission from Tomizawa *et al.* (2003) The neonicotinoid electronegative pharmacophore plays the crucial role in the high affinity and selectivity for the *Drosophila* nicotinic receptor: An anomaly for the nicotinoid cation-pi interaction model. *Biochemistry* 42: 7819–7827; © American Chemical Society.)

nithiazine in direct sunlight is complete within several minutes. The photodegradation products are inactive as insecticides. Replacement of the nitromethylene group with groups that absorb less, or do not absorb sunlight such as the nitroimine ($=N-NO_2$) in imidacloprid or cyanoimine ($=N-CN$) in acetamiprid, significantly improved photostability.

Metabolism

The metabolism of neonicotinoids in vertebrates, insects, and plants has many common features. It may result in cleavage and the separation of the heterocyclic and pharmacophore moieties, or modifications of a pharmacophore in an intact parent molecule. Oxidations, reductions, and elimination reactions are the major mechanisms that result mostly in a reduced or diminished insecticidal potency of the metabolites. Dehydration of the 4-hydroxyimidazolidinyl resulting in a formation of the imidazolinyl (olefin) or reduction of the *N*-nitroimine ($=N-NO_2$) to *N*-nitrosoimine ($=N-NO$) group (**Figure 5**) are examples of a limited number of metabolic conversions leading to increased insecticidal potency. *N*-desmethylation of *N*′-methyl in nitempyram pharmacophore and formation of a nonsubstituted imine represent detoxification reactions for insects but activation for mammals. Similarly, loss of an *N*-nitro-group from *N*-nitroimine in imidacloprid or *N*-cyano-group from *N*-cyanoimine in thiacloprid is a detoxification process in insects but an activation step in mammals. Oxidative metabolism catalyzed by cytochrome P450s can be prevented by using piperonyl butoxide-type synergists. Generally, excretion of the metabolites, some as conjugates, is fast with only low accumulation of the parent compound in organisms treated with neonicotinoids. Fast recovery from poisoning by neonicotinoids is in accordance with their high rates of absorption, distribution, and elimination.

The metabolism of imidacloprid (**Figure 5**) provides a typical example of the metabolic behavior of chloronicotinoids. In rats, imidacloprid is readily absorbed from the gastrointestinal tract and distributed into the organs within 1 h. Liver and kidney are the target organs. No residues of imidacloprid were

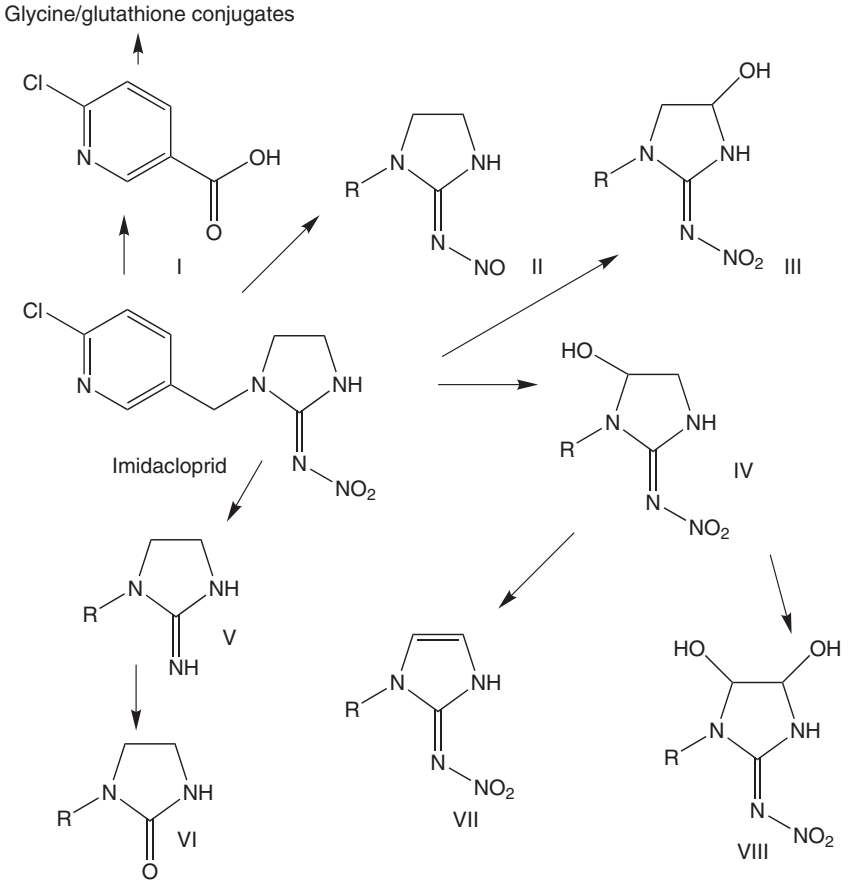


Figure 5 Imidacloprid metabolites in rats, insects and plants. I – 6-chloronicotinic acid (mammalian route of elimination); II – nitrosoimine; III – 4-hydroxy; IV – 5-hydroxy; V – guanidine; VI – urea; VII – olefin; VIII – 4,5-dihydroxy derivatives.

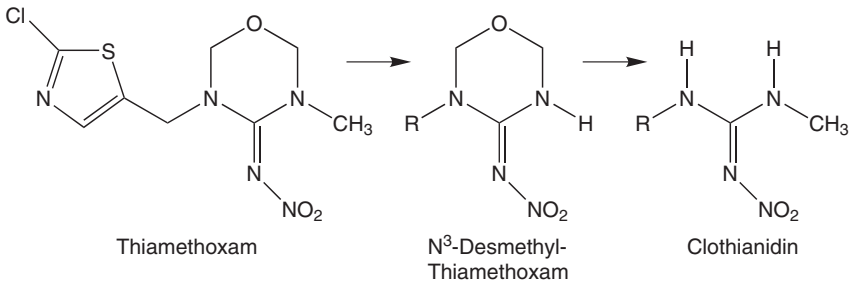


Figure 6 A proposed activation of thiamethoxam in insects and plants.

found in the central nervous system or in fat tissues or bones. Imidacloprid is metabolized in two distinct pathways in rats. In one pathway, it is oxidatively cleaved into 6-chloro-3-methylpyridine and 2-nitroiminoimidazolidine. The latter product is excreted directly in urine while 6-chloro-3-methylpyridine is eliminated as a glycine or glutathione conjugate. More than 90% of the administered compound is excreted within 24 h with the majority of the metabolites (~80%) in urine; the rest is eliminated in feces. In the second metabolic pathway, the imidazolidine ring of imidacloprid undergoes hydroxylation at the 4- and/or 5-position possibly followed by dehydration to imidazolinyl (olefin). The latter metabolite has higher affinity for the nicotinic acetylcholine receptors and is a more potent insecticide. Reductions of a nitroimino group to nitroso- and hydrazono-, or breaking the N–N bond and forming a guanidine-like derivative followed by a possible oxidation to urea-like derivative are the optional routes in insects, vertebrates, and plants.

The toxicokinetics of the thianicotinyl thiamethoxam is similar to that of imidacloprid. When applied orally to rats, goats, or chickens, thiamethoxam is rapidly and almost quantitatively absorbed. Its excretion, predominantly in urine, is fast. Accumulation in tissues is negligible. Thiamethoxam itself does not bind strongly to the neonicotinoid binding site of the nicotinic acetylcholine receptor but it is reported to be converted to clothianidin, a neonicotinoid with high affinity for the insect receptors, in insects and plants (**Figure 6**). It is possible that this activation proceeds via formation of an *N*-desmethyl thiamethoxam intermediate, another compound that acts at the neonicotinoid-binding site.

Uses

Nithiazine

The low photostability of nithiazine limits its commercial use to fly traps.

Imidacloprid and Other Neonicotinoids

Neonicotinoids are effective against homopterans, coleopterans, and lepidopterans. They act systemically because of their water solubility, being especially active against sucking insects. Their water solubility makes them useful for application in seed treatments. Low mammalian toxicity allows their use for flea control in dogs and cats. Their environmental stability at neutral or mild acidic media is valuable in soil applications, for example, against termites.

See also: Acetylcholine; Cholinesterase Inhibition; Nicotine.

Further Reading

Tomizawa M and Casida JE (2003) Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annual Review of Entomology* 48: 339–364.

Yamamoto I and Casida JE (eds.) (1999) *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor.* New York: Springer.

Neoplasia *See* Carcinogenesis.

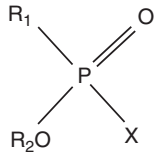
Nephrotoxicity *See* Kidney.

Nerve Agents

Harry Salem and Frederick R Sidell*

Published by Elsevier Inc.

- Preferred Name: Nerve agents. Also known as nerve gases
- Synonyms: Tabun (GA; CAS 77-81-6); Sarin (GB; CAS 107-44-8); Soman (GD; CAS 96-64-0); Cyclosarin (GF; CAS 329-99-7); G agents; Organophosphates (OP): VX (CAS 20820-80-8); V agents; Chemical warfare agents; Irreversible cholinesterase inhibitors; Anticholinesterase compounds
- Description: Nerve gases are clear liquids; therefore, the term 'gas' is a misnomer. The preferred term is 'nerve agents'. Because of the chiral phosphorus in their structure, the nerve agents contain various steroisomers. Soman, sarin, tabun, and VX contain equal amounts of (+) and (−) enantiomers. The G agents are volatile and thus present both vapor and liquid hazard. In decreasing order of volatility are sarin, soman, tabun, and VX. VX presents a negligible vapor hazard, but its volatility increases with increasing temperature. At temperatures above 40°C it also presents a vapor hazard
- Chemical Structures:



	X	R_1	R_2
GA (Tabun)	–CN	$-N(CH_3)_2$	$-C_2H_5$
GB (Sarin)	–F	$-CH_3$	$-CH(CH_3)_2$
GD (Soman)	–F	$-CH_3$	$-CH(CH_3)C(CH_3)_3$
GF (Cyclosarin)	–F	$-CH_3$	$-\text{cyclo-}C_6H_{11}$
VX	$-SCH_2CH_2N(CH(CH_3)_2)_2$	$-CH_3$	$-C_2H_5$

Uses

Nerve agents are used in chemical warfare.

Exposure Routes and Pathways

Casualties are caused primarily by inhalation; however, they can occur following percutaneous and ocular exposure, as well as by ingestion and injection.

Toxicokinetics

Nerve agents are absorbed both through the skin and via respiration. Because VX is an oily, nonvolatile liquid it is well absorbed through the skin (persistent nerve agent), although it can also be absorbed by inhalation. Thus, VX is more of a percutaneous threat than by inhalation, whereas the G agents (nonpersistent), which are also liquids, pose more of an inhalation hazard because of their vapor pressure. Sarin (GB) is the most volatile, but evaporates less readily than water, while cyclosarin (GF) is the least volatile of the G agents.

Nerve agents are hydrolyzed by the enzyme organophosphate (OP) hydrolase. The hydrolysis of GB, soman (GD), tabun (GA), and diisopropyl fluorophosphate occurs at approximately the same rate. The isomers of the asymmetric OPs may differ in overall toxicity, rate of aging, rate of cholinesterase inhibition, and rate of detoxification. The rates of detoxification differ for different animal species and routes of administration. The onset of effects from nerve agents depends on the route, duration, and amount of exposure. The effects can occur within seconds to several minutes after exposure. There is no latent period following inhalation exposure of high concentrations where loss of consciousness seizures has occurred within 1 min. At low concentrations; however, miosis, rhinorrhea, and other effects may not begin for several minutes. Maximal effects usually occur within minutes after contamination ceases.

Mechanism of Toxicity

The nerve agents inhibit the enzymes butrylcholinesterase in the plasma, acetylcholinesterase on the red blood cell, and acetylcholinesterase at cholinergic

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products

receptor sites in tissues. These three enzymes are not identical. Even the two acetylcholinesterases have slightly different properties, although they have a high affinity for acetylcholine. The blood enzymes reflect tissue enzyme activity. Following acute nerve agent exposure, the red blood cell enzyme activity most closely reflects tissue enzyme activity. However, during recovery, the plasma enzyme activity more closely parallels tissue enzyme activity.

Following nerve agent exposure, inhibition of the tissue enzyme blocks its ability to hydrolyze the neurotransmitter acetylcholine at the cholinergic receptor sites. Thus, acetylcholine accumulates and continues to stimulate the affected organ. The clinical effects of nerve agent exposure are caused by excess acetylcholine.

The binding of nerve agent to the enzyme is considered irreversible unless removed by therapy. The accumulation of acetylcholine in the peripheral and central nervous systems (CNS) leads to depression of the respiratory center in the brain, followed by peripheral neuromuscular blockade causing respiratory depression and death.

The pharmacologic and toxicologic effects of the nerve agents are dependent on their stability, rates of absorption by the various routes of exposure, distribution, ability to cross the blood–brain barrier, rate of reaction and selectivity with the enzyme at specific foci, and behavior at the active site on the enzyme.

Red blood cell enzyme activity returns at the rate of red blood cell turnover, which is $\sim 1\%$ per day. Tissue and plasma activities return with synthesis of new enzymes. The rates of return of these enzymes are not identical. However, the nerve agent can be removed from the enzymes. This removal is called reactivation, which can be accomplished therapeutically by the use of oximes prior to aging. Aging is the biochemical process by which the agent–enzyme complex becomes refractory to oxime reactivation. The toxicity of nerve agents may include direct action on nicotine acetylcholine receptors (skeletal muscle and ganglia) as well as on muscarinic acetylcholine receptors and the central nervous system.

Recent investigations have focused on OP nerve agent poisoning secondary to acetylcholine effects. These include the effects of nerve agents on γ-aminobutyric acid neurons and cyclic nucleotides. In addition, changes in brain neurotransmitters, such as dopamine, serotonin, noradrenaline, as well as acetylcholine, following inhibition of brain cholinesterase activity, have been reported. These changes may be due in part to a compensatory mechanism in response to overstimulation of the cholinergic system or could result from direct action of nerve agent on the enzymes responsible for noncholinergic neurotransmission.

Human Toxicity

The human toxicity estimates for the nerve agents are summarized in **Table 1**.

Rhinorrhea may precede miosis as the first indication of exposure to even small amounts of nerve agent vapor. After exposure to high concentrations/doses by any route, rhinorrhea occurs as part of the generalized increase in secretions. Direct ocular contact to nerve agents may cause miosis, conjunctival injection, pain in or around the eyes, and dim or blurred vision.

Acute exposure of 3 mg min m^{-3} of GB vapor will produce miosis in most of the exposed population. Other routes of exposure may not cause any eye effects or cause a delayed onset of them, but will cause vomiting, sweating, and weakness.

The onset of miosis is within seconds to minutes following aerosol or vapor exposure but may not be maximal for up to 1 h, especially at low concentrations. The duration of miosis varies and is dependent on the extent of exposure. The ability of the pupil to dilate maximally in darkness may not return for up to 6 weeks. There is no correlation between miosis and blood cholinesterase levels.

Respiratory distress also occurs within seconds to minutes following vapor exposure. The symptoms include tightness of the chest, shortness of breath, and gasping and irregular breathing leading to apnea. Bronchoconstriction and bronchial secretions contribute to this. With larger concentrations, cyanosis and audible pulmonary changes occur, which can only be relieved by therapeutic intervention. Death due to nerve agent intoxication is

Table 1 Human toxicity estimates for nerve agents

Agent	*Inhalation LCt_{50} (mg min m^{-3})*	*Intravenous LD_{50} (mg kg^{-1})*	*Percutaneous LCt_{50} (mg min m^{-3})*	*Percutaneous LD_{50} (mg kg^{-1})*	*Oral LD_{50} (mg kg^{-1})*
GA	135	0.08	20 000	14	0.36–0.71
GB	70	0.014	12 000	24.3	0.07–0.29
GD	70			5	0.07–0.29
GF					0.14
VX	30	0.008		0.143	0.04–0.14

attributable to respiratory failure resulting from bronchoconstriction, bronchosecretion, paralysis of skeletal muscles, including those responsible for respiration, and failure of the central drive for respiration. Nerve agent intoxication causes skeletal muscles to fasciculate, twitch, and fatigue prior to paralysis.

The cardiovascular effects of nerve agent exposure are variable. Bradycardia may occur via vagal stimulation, but other factors such as fright, hypoxia, and adrenergic stimulation, secondary to ganglionic stimulation may produce tachycardia or hypertension. Following inhalation exposure to large amounts of nerve agent, the CNS effects will cause loss of consciousness, seizure activity, and apnea within 1 min.

Following skin contact with large amounts of liquid, the dermal effects may be delayed up to 30 min. Long-term exposure to an OP, diisopropyl phosphorofluoridate, used in the treatment of myasthenia gravis, caused side effects including nightmares, confusion, and hallucinations.

US Public Law 91-145 (50 USC 1521) and an International Treaty mandate that stored Chemical Warfare Agents (CWA) be destroyed by the US Department of Defense (DoD). Public Law 91-121 and 91-441 (50 USC 1512) mandate that the US Department of Health and Human Services (DHHS) review DoD plans for disposing of the stored munitions and make recommendations to protect public health. Thus, DHHS and Centers for Disease Control and Prevention (CDC) revised the airborne exposure criteria for GA, GB, and VX and proposed revisions for sulfur mustard. Worker population limits (WPLs), short-term exposure limits (STELs), general population limits (GPLs), and immediately dangerous to life or health (IDLH) values are used to protect workers and the general population during routine chemical demilitarization activities. The recommended airborne exposure limits for GA, GB, and VX (in $mg\,m^{-3}$) in demilitarization are as follows:

Agent	*GPL (24 h)*	*WPL (8 h)*	*STEL (15 min)*	*IDLH (30 min)*
GA	1×10^{-6}	3×10^{-5}	1×10^{-4}	0.1
GB	1×10^{-6}	3×10^{-5}	1×10^{-4}	0.1
VX	6×10^{-7}	1×10^{-6}	1×10^{-5}	0.003
HD (proposed)	0.000 02 (12 h)			0.7 (30 min)

Note: Five-minute ceiling level is 0.003.

Over the last two decades, two groups have addressed acute exposure guideline levels for hazardous substances. One group, sponsored by the American Industrial Hygiene Association, develops these advisory numbers for a 1 h exposure as emergency response planning guidelines (ERPG-1, ERPG-2, and ERPG-3). ERPG-1 is the level associated with slight irritation, ERPG-2 with developmental or subchronic toxicity, while ERPG-3 is associated with acute lethality. The other committee, sponsored by the US Environmental Protection Agency, developed 3 acute exposure guideline levels (AEGL) for 10 and 30 min, and for 1, 4, and 8 h. These are threshold exposure limits for the general public and are applicable to emergency exposure periods that would occur infrequently in a person's life. The AEGL-1 is the airborne concentration above which the general population, including sensitive individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. The effects are not disabling and are transient and reversible upon cessation of exposure. AEGL-2 is the airborne concentration at which the same population experiences irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape. AEGL-3 is the airborne concentration predicted to cause life-threatening health effects or death. For GB and VX, these are as follows:

AEGL-1 (nondisabling)

	10 min	*30 min*	*1 h*	*4 h*	*8 h*
GB					
ppm	0.001 2	0.000 68	0.000 48	0.000 24	0.000 17
$mg\,m^{-3}$	0.006 9	0.004 0	0.002 8	0.001 4	0.001 0
VX					
ppm	0.000 052	0.000 030	0.000 016	0.000 009 1	0.000 006 5
$mg\,m^{-3}$	0.000 57	0.000 33	0.000 17	0.000 10	0.000 071

AEGL-2 (disabling)

	10 min	*30 min*	*1 h*	*4 h*	*8 h*
GB					
ppm	0.015	0.008 5	0.006 0	0.000 29	0.002 2
$mg\,m^{-3}$	0.087	0.050	0.035	0.017	0.013
VX					
ppm	0.000 65	0.000 38	0.000 27	0.000 14	0.000 095
$mg\,m^{-3}$	0.007 2	0.004 2	0.002 9	0.001 5	0.001 0

AEGL-3 (lethal)

	10 min	*30 min*	*1 h*	*4 h*	*8 h*
GB					
ppm	0.064	0.032	0.022	0.012	0.0087
$mg\,m^{-3}$	0.38	0.19	0.13	0.070	0.051
VX					
ppm	0.002 7	0.001 4	0.000 91	0.000 48	0.000 35
$mg\,m^{-3}$	0.029	0.015	0.010	0.005 2	0.003 8

The DoD in 1996 and 1997 originally set an army safety limit intended for monitoring potential public exposure to US chemical agents. This limit, termed GPL dosage was the smallest dose of an agent causing noticeable effects such as miosis. This was calculated by applying numerous safety factors to establish a level at which the general population could be exposed 24 h day^{-1} for a lifetime without experiencing any adverse health effects. DoD and the (US) Central Intelligence Agency selected the GPL as their threshold since it was considered a scientifically based standard. These GPLs were used for modeling nerve agents, and were revised in 2000 and 2001 as follows:

Agent	*Modeling year*	*Time basis (h)*	*Dosage (mg min m^{-3})*
GB	1996–97	72	0.013 0
GB	2000–01	24	0.043 2
GF	1996–97	72	0.013 0
GF	2000–01	24	0.014 4
HD	1996–97	72	0.432
HD	2000–01	24	0.288

Following the events of September 11, 2001, in the United States, concern with acts of terror have been heightened and resulted in President George W. Bush's declaration of war on terrorism. The President's Commission concluded that water supplies to US communities are potentially vulnerable to terrorist attack, especially if contaminants were inserted at critical points in the system. Contamination of water supplies goes back to antiquity and continues until today. In the United States and in areas around the world where US troops are deployed, concern for ensuing safety of water supplies can be traced back to at least the 1980s when Triservice Standards for some chemical warfare agents were established. These are presented in the following table:

Triservice Standards for military drinking water ($\mu g\,l^{-1}$)

Agent	*2 l day^{-1}*[a]	*5 l day^{-1}*	*15 l day^{-1}*
GA	175.0	70.0	22.5
GB	34.5	13.8	4.6
GD	15.0	6.0	2.0
VX	18.7	7.5	2.5
HD	350.0	140.0	47.0
BZ	17.5	7.0	2.2

[a]Civilian consuming 2 l of water per day.

These are guidelines established by the National Research Council and based on Triservice Standards developed by the US Army in collaboration with Lawrence Livermore Laboratory. These levels are those that should not cause acute adverse health effects or degrade military performance following ingestion for 7 days of 5 or 15 l of water per day. The 5 l day^{-1} consumption is considered the average for a soldier under normal working conditions, while under stress and exertion, consumption may rise to 15 l day^{-1}. The average daily drinking water for civilians is considered to be 2 l.

Animal Toxicity

Small doses of nerve agents can produce tolerance.

The cause of death is attributed to anoxia resulting from a combination of central respiratory paralysis, severe bronchoconstriction, and weakness or paralysis of the accessory muscles for respiration.

Signs of nerve agent toxicity vary in rapidity of onset and severity. These are dependent on the specific agent, route of exposure, and dose or concentration. At the higher doses or concentrations, convulsions, apnea, and neuropathies are indications of CNS toxicity. Following nerve agent exposure, animals exhibit hypothermia resulting from the cholinergic activation of the hypothalamic thermoregulatory center. In addition, plasma levels of pituitary, gonadal, thyroid, and adrenal hormones are increased during OP intoxication. The nerve agents are anticholinesterases and as such inhibit the cholinesterase enzymes in the tissues resulting in the accumulation of acetylcholine at its various sites of action in both the autonomic nervous system and the CNS. These include the endings of the parasympathetic nerves to the smooth muscles of the iris, ciliary body, bronchial tree, gastrointestinal tract, bladder, blood vessels, the secretory glands of the respiratory tract, the cardiac muscles, and the endings of sympathetic nerves to the sweat glands. Accumulation of acetylcholine at these sites results in characteristic muscarinic signs and symptoms, while the accumulation at the endings of the motor nerves to voluntary muscles and in the autonomic ganglia results in nicotinic signs and symptoms. The accumulation of acetylcholine in the brain and spinal cord results in the characteristic CNS signs and symptoms.

Nerve agents inhibit the activity of acetylcholinesterase by attaching to its active sites so that it cannot hydrolyze the neurotransmitter acetylcholine into choline, acetic acid, and regenerated enzyme. Thus, acetylcholine cannot attach to the enzyme, is not hydrolyzed, and continues to produce action potentials until the mechanism is fatigued. The biological effects of the nerve agents result from the excess of acetylcholine.

Animal toxicity values for nerve agents are listed as follows:

Agent	LCt_{50} (mg min m^{-3}) inhalation	LD_{50} (mg kg^{-1})			
		SC[a]	IV[b]	IP[c]	PC[d] (mg kg^{-1})
Rat					
GA	450	300	66	490	12.6
GB	220	103	39	218	2.5
GD	230	71	44.5	98	14.3
GF	180		5.3	400	1.8
VX	17	12	7.9		0.1
Rabbit					
GA	960	375	63		2.5
GB	120	30	15	275	4.4
GD	160	16	11		1.54
GF		63	15	550	0.3
VX	25	14	8.4	66	0.025
Dog					
GA	135		84		30
GB	60		19		10.8
GD		12	5		4.9
GF					
VX	15		63		0.054
Monkey					
GA	187		50		9.3
GB	74		22.3		
GD		13			
GF	130				
VX	50		8.4		0.065

[a]Subcutaneous.
[b]Intravenous.
[c]Intraperitoneal.
[d]Percutaneous (depilated).

Although there is a lack of information on the general toxicological effects of low-level, and sublethal repeated exposures, there are studies on the behavioral effects of such exposures to nerve agents in animals free of observed signs of intoxication. These were conducted in an effort to determine whether behavioral studies can provide markers of early neurotoxicity that are more sensitive than neurochemical and neuropathological changes.

Although blood cholinesterase activity correlations with nerve agent effects are equivocal, they are indicative of exposure but do not reflect changes in the CNS. In rodents repeated subcutaneous injections of GD over a 5 day period at doses less than one-half the LD_{50} caused a significant decline in cholinesterase activity in all regions of the brain examined. The regional sensitivities were in agreement with the studies employing acute high-level soman exposures. In all cases, the neostriatum was the area of the brain least sensitive to nerve agents. These results are consistent with those of GD, GB, and GA at doses of 30%, 40%, and up to 85% of the LD_{50}. No evidence of tolerance to the direct inhibitory effects of GD during 5 days of repeated injections was observed. However, a tolerance to GD-induced hypothermia was reported. GD at 35 mg kg^{-1} injected subcutaneously up to 36 days at regular intervals reduced body temperature after the third injection, and then a steady tolerance developed to the drop in body temperature even though brain cholinesterase levels were inhibited. Brain cholinesterase levels did not parallel the recovery of serum cholinesterase following cessation of GD injection. Red blood cell cholinesterase recovery more closely reflected brain cholinesterase recovery than did serum cholinesterase. Daily doses of 25–54 mg kg^{-1} of GD for 5 days with survival times of 7–35 days were consistent with previous studies in that the area of the brain most sensitive to nerve agents was the piriform cortex and the least sensitive areas were the hypothalamus and neostriatum. This was demonstrated in both neurochemical and neuropathological studies. GD-induced brain damage was similar in severity and locus whether administration was single or in repeated doses. However, the progression of brain degeneration following repeated dosing was more protracted. In rodents and nonhuman primates, the performance dose response was very steep, indicating that small changes in dose caused a large change in performance. Pretreatment plus use of antidote drugs was ineffective in preventing soman-induced performance decrements.

Clinical Management

Following exposure the victim should be removed from the area to avoid further contamination and decontaminated (water/hypochlorite) by adequately protected (protective clothing and gas mask) and trained attendants. Contaminated clothing should be removed carefully so as to avoid further contamination. Respiration should be maintained and drug and supportive therapy instituted. If exposure is anticipated, pretreatment with carbamates (pyridostigmine bromide) may protect the cholinesterase enzymes before GD and possibly GA exposures, but not for GB and VX exposures. The three types of therapeutic drugs to be administered following nerve agent exposure are (1) a cholinergic blocker, anticholinergic or cholinolytic drug such as atropine; (2) a reactivator drug to reactivate the inhibited enzyme, such as the oxime pralidoxime chloride; and (3) an anticonvulsant drug such as diazepam or benzodiazepine. Oxygen may be indicated in respiratory failure.

Miosis, pain, dim vision, and nausea may be relieved by topical atropine in the eye. Atropine, a cholinergic blocker or anticholinergic drug, is effective in blocking the effects of excess acetylcholine at peripheral muscarinic sites. The usual dose is 2 mg for minor exposures and 6 mg for severe exposures.

The dose should be repeated at 3–5 min until there is improvement. Pralidoxime chloride (protopam chloride) is an oxime used to break the agent–enzyme bond and restore the normal activity of the enzyme. This is most apparent in organs with nicotinic receptors. Abnormal activity decreases and normal strength returns to skeletal muscles, but no decrease in secretions is seen following the oxime treatment. The usual dose is 1000 mg (intravenous or intramuscular). The injection contains 600 mg, which is not a high enough dose for a severe exposure. In a severe exposure, three of these (1800 mg) would be given. This is not an item generally used by civilians. The administration of oxime may be repeated two or three times at hourly intervals either by intravenous or by intramuscular injections. Diazepam, an anticonvulsant drug, is used to decrease convulsive activity and reduce brain damage that may occur from prolonged seizure activity. It is suggested that all three of these drugs be administered at the onset of severe effects from nerve agent exposure, whether or not seizures occur. The usual dose of diazepam is 10 mg.

Pyridostigmine bromide is available as a pretreatment for GD and possible GA exposures, but not for GB and VX. It is available in 30 mg tablets, which should be administered every 8 h. When used prior to exposure, it should be followed by atropine and pralidoxime chloride after exposure. LD_{50} values in animals were increased several-fold and survival rates were also increased in experiments with GD and these therapies.

Supportive therapy may include ventilation via an endotracheal airway if possible and suctioning of excess secretions from the airways.

See also: G-Series Nerve Agents; Organophosphates; Sarin; Soman; Tabun; V-Series Nerve Agents: Other than VX; VX.

Relevant Websites

http://www.bt.cdc.gov – (US) Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – (US) National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Neurotoxicity

Peter S Spencer and Pamela J Lein

Introduction

Neurotoxicity refers to the direct or indirect effect of chemicals that disrupt the nervous system of humans or animals. Numerous chemicals can produce neurotoxic disease in humans, and many more are used as experimental tools to disturb or damage the nervous system of animals. Some act directly on neural cells, others interfere with metabolic processes on which the nervous system is especially dependent. Some only disrupt neural function; others induce maldevelopment or damage to the adult nervous system. Perturbations may appear and disappear rapidly, evolve slowly over days or weeks and regress over months or years, or have permanence if they are acquired during development. Neurotoxicity is usually self-limiting after exposure ceases and rarely progressive in the absence of continued exposure. Links between chemical exposure and long-latency neurodegenerative diseases are suspected but rarely proved.

Occurrence

Chemicals with the potential to disrupt the mammalian nervous system may occur naturally (neurotoxins) or arise by synthesis (neurotoxicants). While chemicals with neurotoxic potential are conveniently termed 'neurotoxins' or 'neurotoxicants', this is not an intrinsic property but rather the description of an effect that may occur if the tissue concentration exceeds a certain threshold. Biological chemicals with neurotoxic properties often have high target specificity and toxic potency, discrete biological actions, and are among the best understood mechanistically. Examples of chemicals with direct or indirect neurotoxic potential are found in bacteria, algae, fungi, plants, coelenterates, insects, arachnids, mollusks, amphibians, reptiles, fish, and certain mammals (**Table 1**).

Other less potent naturally occurring substances exhibit neurotoxic effects when encountered in large concentration for sufficient periods of time. Examples include metals (arsenic, lead, mercury) and certain compounds containing these elements (methylmercury) (**Table 2**). Some elements (manganese, selenium) and compounds (vitamin B_6) in this group, while neurotoxic in sustained heavy doses, are required in smaller amounts to support normal physiological function, including that of the nervous system.

Natural substances (thiaminase) that interfere with required substances (thiamine) are also associated with neurological disease in animals and humans.

Synthetic chemicals with neurotoxic potential (**Table 2**) are most commonly encountered in the form of prescription (ethambutol, isoniazid, vincristine) and over-the-counter pharmaceutical agents (bismuth preparations), domestic products used in antidandruff shampoos (pyridinethione), fragrance raw materials (2,6-dinitro-3-methoxy-4-*t*-butyltoluene), pyrolysis products in broiled, baked, or fried food (acrylamide), beverages (ethanol), workplace chemicals (*n*-hexane), pest-control agents (aldrin), environmental pollutants (mercury), and substances (methamphetamine) used to induce euphoria. Others are associated with special applications, such as chemical warfare in military and civilian settings (sarin).

Table 1 Naturally occurring substances with mammalian neurotoxic potential

Life form	*Substance with neurotoxic potential*
Bacterium	Diphtheria toxin
Alga	Anatoxin-a
Fungus	3-Nitropropionic acid
Plant	β-*N*-Oxalylamino-L-alanine
Coelenterate	Palytoxin
Insect	Apamin
Arachnid	Scorpion toxins
Mollusc	Conotoxins
Fish	Ciguatoxin
Amphibia	Batrachotoxin
Reptile	Dendrotoxin
Bird	Batrachotoxin
Mammal	Vitamin A

Direct-acting substances with neurotoxic potential are supplemented by other agents that initiate neurological change as a consequence of effects on another organ system on which the brain depends for normal function. Substances that target the liver, kidneys, or lungs fall into this category, as do agents that interfere with the continuous supply of oxygen (cyanide, azide) and glucose (6-chloro-6-deoxyglucose) required by the nervous system for normal function. Chronic liver failure and manganese intoxication are both associated with high signal abnormalities in the basal ganglia on T1-weighted

Table 2 Heavy metals and synthetic substances with neurotoxic potential

Substance	*Primary neurotoxic effects*
Acrylamide	Peripheral neuropathy (axonal degeneration); cerebellar ataxia
Arsenic	Acute encephalopathy (brain swelling and hemorrhage); peripheral neuropathy (axonal degeneration)
Barbiturates	Acute encephalopathy (sedation and coma), chronic encephalopathy, developmental neurotoxicity; facilitated GABA neurotransmission
Carbamate pesticides	Acute encephalopathy (cholinergic syndrome); neuromuscular transmission dysfunction; acetylcholinesterase inhibition
Carbon disulfide	Acute psychosis; chronic peripheral neuropathy (axonal degeneration); parkinsonism
Carbon monoxide	Encephalopathy/delayed parkinsonism; neuronal and tissue necrosis secondary to hypoxia
Carbon tetrachloride	Acute encephalopathy, visual dysfunction
Doxorubicin	Progressive ataxia (rodents); sensory neuronal degeneration
Ethanol	Fetal alcohol syndrome; acute encephalopathy (agitation, sedation, ataxia, coma); chronic encephalopathy (cognitive impairment, dementia); myopathy; peripheral neuropathy (vitamin B_1 deficiency?)
n-Hexane	Peripheral neuropathy (axonal degeneration)
Lead, inorganic	Peripheral neuropathy (axonal loss and demyelination); acute encephalopathy (seizures); cognitive dysfunction
Manganese, inorganic	Emotional disturbance, psychoses; parkinsonism/dystonia; neuronal degeneration in striatum and globus pallidus
Mercury, inorganic	Cerebellar syndrome (tremor, ataxia); psychobiological reaction (anxiety, personality changes, memory loss)
Methanol	Optic neuropathy (axonal degeneration, primary demyelination); extrapyramidal syndrome (necrosis of putamen); retinopathy (edema)
Methyl mercury	Developmental toxicity and teratogenesis; visual dysfunction (tunnel vision); cerebellar syndrome (ataxia); peripheral neuropathy; chronic encephalopathy (cognitive dysfunction)
Organophosphorus compounds (pesticides and warfare agents)	Cholinergic syndrome (certain compounds); peripheral neuropathy (certain compounds only); acetylcholinesterase inhibition
Phenytoin	Fetal phenytoin syndrome; cerebellar syndrome (ataxia, nystagmus); chronic encephalopathy (cognitive dysfunction); extrapyramidal syndrome (chorea, dyskinesia); peripheral neuropathy
Toluene	Acute encephalopathy (sedation, coma); chronic encephalopathy (cognitive dysfunction)
Tricyclic antidepressants	Seizure disorder (myoclonus); psychobiological reaction (serotonin syndrome, anticholinergic syndrome); tremor; extrapyramidal syndrome (dyskinesia)
Trimethyltin	Acute encephalopathy (neuronal degeneration of limbic system) – rodents; chronic encephalopathy (cognitive dysfunction, neuronal loss in hippocampus)

magnetic resonance images, suggesting that the metal accumulates because it cannot be cleared normally by the liver.

Neurotoxic Effects

The nervous system has a vast repertoire of functional reactions to chemical perturbation, and these responses give rise in the aggregate to a plethora of neurological and psychiatric phenomena, many of which recapitulate the clinical manifestations of disease of nontoxic origins. Large single doses of certain substances such as organic solvents (ethanol, toluene) induce functional changes in the organism that appear and disappear rapidly. Other agents, such as the anticholinesterase nerve agents, induce functional changes that reverse when the inhibited target protein is reactivated or replaced. Sometimes, as in the case of methanol, the latent period (hours) between exposure and effect is associated with the production and action of a toxic metabolite (formate). Single exposures to large amounts of other agents (arsenic, mercury, thallium) may be followed by a latent period of days or weeks before structural and functional changes become clinically evident. While certain substances (acrylamide) can induce neurological damage after single large exposures, smaller doses over a long period of time are also effective. The pattern of neurological deficit may be distinct in the two dosing scenarios. Neurotoxic disorders typically progress during the period of exposure and immediately following exposure, when the pathological events already in progress may take time to unfold before stabilization or recovery can begin. Prospects for functional recovery depend on the presence or absence of tissue damage, the extent of damage, and whether the central nervous system (CNS) is involved (poor prognosis).

An unanswered question is whether in some instances disease may progress or recur after chemical exposure has ceased. Certainly, catastrophic and fatal neurodegeneration may follow acute carbon monoxide poisoning, but this is an isolated example. Relapses occur in ciguatoxicity presumably because the offending agent is released from fat stores under certain physiological conditions. Progressive visual defects may occur from release of chloroquine stored in the choroid layer of the eye. There is also concern that certain substances (carbon disulfide) might predispose or accelerate the onset of age-related diseases of the nervous system, such as Parkinson's disease. Finally, research is underway to determine whether DNA-damaging agents (cycasin) may predispose neurons to tardive degeneration because of their low capacity for DNA repair.

Principles of Nervous System Vulnerability

There are many factors that determine the response of the nervous system to chemical exposure. Species, gender, genotype, age, and nutritional status are key determinants, as are chemical access, structure, and biotransformation. Metabolic activity of the brain may serve to activate a neurotoxic substance, as in the conversion of methylphenyltetrahydropyridine (MPTP) to the mitochondrial toxin *N*-methyl-4-phenylpyridinium ion (MPP+), which targets substantia nigral neurons and precipitates parkinsonism in humans and other mammals. Hepatic biotransformation is broadly concerned with the conversion of lipophilic chemicals to less toxic hydrophilic metabolites (phase 1) and their conjugation (phase 2) prior to excretion. Occasionally, phase 1 metabolism may increase the neurotoxic potential of an exogenous substance, as in the case of the stepwise conversion of *n*-hexane to 2,5-hexanedione, an agent that targets neuroproteins and causes nerve fiber degeneration in the CNS and peripheral nervous system (PNS). Coexposure to substances such as methyl ethyl ketone or toluene may impact phase 1 enzyme function and thereby markedly modify the quantitative neurotoxic response to *n*-hexane.

Most mammalian species reproduce the qualitative response of the human nervous system to chemical perturbation, but the sensitivity may differ markedly among species. For example, relative to humans, primates and cats, rodents are relatively refractory to organophosphate-induced axonal degeneration. Gender as a determinant of biotransformation may be less important in humans than in rats, where marked differences in the metabolism and consequent functional responses to individual chemicals such as parathion may be noted. Differences in genotype may determine the presence of neurotoxic responses, as in the rare mitochondrial polymorphism that results in hypersensitivity to aminoglycoside-induced deafness. Age in humans is generally associated with increased susceptibility to many chemical substances largely because of reductions in biotransformation, renal clearance, and biliary excretion, as well as factors such as reduced body weight, inanition, and polypharmacy that promotes drug and chemical interactions. In addition, neurons display age-related increases of DNA damage, regional nerve cell loss (e.g., substantia nigra), and axonal pathology (e.g., spinal roots). Nutritional status may predispose to human neurotoxicity as seen in minimally nourished Africans who develop spastic paraparesis from a combination of sulfur amino acid deficiency and exposure to cyanogenic substances, both of

which arise from dietary dependence on the root crop cassava (*Manihot esculenta*). Agents that interfere with vitamin production or utilization, including thiamine (pyrithiamine), riboflavin (quinacrine), niacin (3-acetylpyridine, 6-aminonicotinamide) or cyanocobalamin (nitrous oxide) produce various types of severe neurological deficit, as does excessive exposure to vitamin A. Other types of neurodegeneration are seen with substances that chelate physiologically important metals ions (pyridinethione, 8-hydroxyquinolines, ethambutol), such as zinc. Excessive dietary intake of sulfur and selenium produce neuronal lesions in ruminants and pigs, respectively.

The structure of chemicals and their differential access to the nervous system are of critical importance in determining the presence and nature of the neurotoxic response. While access to nervous tissue dictates the possibility of a direct neurotoxic effect, neurotoxicity ultimately depends on the ability of the substance to bind to neural tissue targets and interfere with functional or structural integrity. Structure–activity relationships are therefore of cardinal importance. For example, 1,2-diacetylbenzene but not 1,3-diacetylbenzene induces leg weakness because only the former binds to and crosslinks neuroproteins. Triethyltin targets the myelin sheath, trimethyltin damages neurons, but tributyltin lacks neurotoxic properties – another illustration of the critical importance of chemical structure in determining the presence and nature of the neurotoxic response.

The large majority of the nervous system is protected from direct exposure to chemicals in the bloodstream and the cerebrospinal fluid (CSF) by blood–brain/nerve/CSF barriers. These barriers separate the microenvironment of the brain parenchyma from changes in circulating ion and metabolite concentrations. Regulation of blood–brain/nerve interchange is a key function of capillaries coursing through nervous tissue. Structural specializations of capillary walls in the form of tight junctions between adjacent endothelial cells constitute a diffusion barrier. This allows the endothelium to regulate the selective transport and metabolism of substances from blood to brain and vice versa. While gases (oxygen, carbon monoxide, nitrous oxide) cross capillary walls with ease, many substances are excluded or their access to nervous tissue impeded by the presence of the capillary barrier. Lipophilicity and size are key elements in regulating the passage of macromolecules across the blood–brain barrier. Key nutrients and macromolecules required by the brain cross via facilitated diffusion or specific carriers. The epithelial junctions that constitute the blood-CSF barrier at the choroid plexus are somewhat more permeable and allow greater passage of drugs and toxicants into the interstitial fluid that bathes brain tissue. Many metals are required for normal CNS function and are thus transported across the blood–brain and blood-CSF barriers. However, excess metal may accumulate in their endothelial cells and give rise to toxic damage of the cellular barrier. Several metals are known to accumulate in both barriers, including substances with neurotoxic potential such as lead, mercury, arsenic, and manganese. Lead accumulates in the choroid plexus and alters key functions, including transthyretin, the binding protein for thyroid hormone. High concentrations of lead damage the blood–brain barrier, cause vascular leakage, and may result in brain swelling accompanied by herniation, ventricular compression, and petechial hemorrhages.

Some regions of the brain (e.g., hypothalamus) and PNS (spinal and autonomic ganglia) lack capillary barriers and are thus directly exposed to chemicals circulating in the bloodstream. Damage to the hypothalamus can have far reaching effects on somatic metabolism, reproductive function, and growth. The adult obesity of rats treated postnatally with monosodium glutamate exemplifies the effect and raises important questions for human health in regard to past exposure to glutamate-rich foods during postnatal development. In the PNS, the selective loss of sensory neurons in rats treated with doxorubicin arises because spinal ganglia lack a protective capillary barrier.

Certain other substances (tetanus toxin) reach nerve cells directly via distal axonal entry. Tetanus toxin is transported to the spinal anterior horn cell, subsequently translocates and binds to presynaptic inhibitory (glycinergic) nerve terminals impinging on the motor nerve cell, and thereby suppresses the inhibition of motor neuron activity leading to hyperexcitation. Violent and sustained muscle contraction (tetany) results in response to external stimulation. Another example of peripheral entry to the CNS is the transport and delivery of metals (manganese, aluminum) from the nose along olfactory neurons to the brain of laboratory animals.

The mammalian nervous system has functional design features that predispose it to chemical perturbation. Consequently, neurological dysfunction is among the most common of the toxic responses of humans to chemical substances. Some neurotoxic agents perturb energy generation by interfering with glycolysis (arsenic) while others (3-nitropropionic acid, cyanide) disrupt sites in the electron transport chain. Some agents (metronidazole, misonidazole) damage brain regions such as the brainstem nuclei that seem to have a high requirement for glucose.

Architectural design is another important determinant of vulnerability both at the cellular and organ level. Unlike most organs (liver, kidney, testes), the nervous system is regionally organized for functions such as memory, vision, audition, and olfaction. Whereas small lesions of the liver and kidney have little functional impact, a chemical that damages the hippocampus (domoic acid), visual cortex (methylmercury), or the peripheral vestibular and auditory system (streptomycin) may have devastating effects on the function of the organism. Moreover, whereas tissue repair follows damage to many organs, the CNS recovers from injury poorly. Some recovery may be afforded by reorganization of synaptic connections on surviving nerve cells, but regrowth and reconnection of damaged axons is impeded by postinjury proliferation of astrocytes.

Cellular architecture is yet another important factor in determining vulnerability to chemical attack. Unlike most types of mammalian cells, mature neurons and myelinating cells in the CNS (oligodendrocytes) and PNS (Schwann cells) possess elongated cellular processes that expose vast surface areas of membrane to chemicals present in extracellular fluid (**Figure 1**). Additionally, each of these cells has a segregated anabolic region (cell body) that is responsible for supplying the metabolic needs of proportionately huge volumes of cytoplasm (axons, dendrites, myelin). The unusual architecture of these cells demands the presence of a distribution system that efficiently transports materials from sites of production to sites of utilization. Within neurons, chemicals that disrupt axonal transport are known to induce axonal degeneration with consequent effects on sensory and motor function. The special vulnerability of the longest and largest-diameter axons leads clinically to sensory dysfunction and motor weakness in a stocking-and-glove distribution.

Neural cells are highly interconnected and dependent upon each other's presence and physiological activity for normal function. Thus, chemicals that disrupt Schwann cells (diphtheria toxin) or myelin (hexachlorophene) secondarily disturb nerve conduction along the axonal processes of neurons with which they are physically associated. Other agents interfere directly with electrical transmission (pyrethroids) or the orderly conveyance of signals via synapses (certain organophosphates) that connect nerve cells to each other. Similarly, agents (nitrofurantoin) that cause peripheral axons to degenerate thereby severe neuronal connections with muscle cells and cause muscle weakness.

Given the nervous system has built-in redundancy, the loss of neurons or axons must exceed a certain threshold for clinical effects to become apparent. That redundancy of nerve cells may be substantially reduced with the advance of age, such that regional loss of CNS striatal dopaminergic neurons is thought to be a factor in dictating susceptibility to agents (carbon disulfide) that can trigger parkinsonism. Similarly, age-related spinal nerve root demyelination and distal axonal degeneration of long and

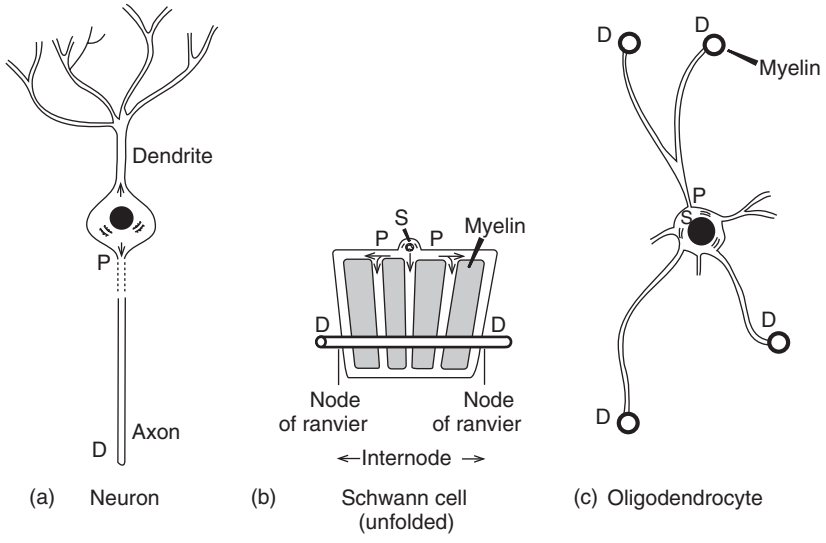


Figure 1 Diagram of (a) neurons, (b) Schwann cell with myelin sheath 'unrolled', and (c) oligodendrocyte, to illustrate that each cell has a restricted cell soma (S) and elongated processes that can be divided into proximal (P) and distal (D) portions. The elongated processes of these cells provide a huge area for chemical attack. (From Spencer PS and Schaumburg H (eds.) (2000) *Experimental and Clinical Neurotoxicology*, 2nd edn. New York: Oxford University Press; © Oxford University Press, Inc. Used by permission of Oxford University Press, Inc.)

large-diameter axons may predispose elderly individuals to substances that cause peripheral neuropathy (*vide infra*).

Vulnerability of Neurons and Their Processes

Nerve cells are vulnerable to chemical attack at many loci, including protein synthesis, mitochondrial and nucleic acid function. Somal DNA is predisposed to damage by reactive oxygen species because of the high oxygen consumption and metabolic activity of neurons. The cycad genotoxin methylazoxymethanol (MAM), an alkylating agent that promotes DNA adduct formation, interferes with neuronal development and has been implicated in a long-latency progressive neurodegenerative disease. Repair of DNA damage in neurons is poor relative to that in astrocytes, such that long-term neuronal effects might occur as a consequence of DNA damage. Mitochondrial DNA chain growth is blocked by certain anti-HIV drugs (2′,3′-dideoxyinosine, 2′,3′-dideoxycytidine) that cause painful axonal neuropathy. Interference with neuronal protein synthesis occurs with agents (ricin) that disrupt polypeptide elongation and trigger axonal degeneration. Chemicals that alter the mitochondrial electron transport chain (cyanide) have a propensity to induce basal ganglia damage. Substances (bromethelin, pentachlorophenol) that uncouple electron transport and oxidative phosphorylation elevate temperature and induce tremor and hyperexcitability. Several neuronal enzymes are key sites of chemical attack, including synaptic acetylcholinesterase (carbamates) and neuropathy target esterase (organophosphates), with resulting neuroexcitation and axonal degeneration, respectively. Lead interferes with oxidative phosphorylation by potently activating protein kinase C.

The excitable membrane of neurons is the target of a rich array of neurotoxins that interfere with ion channels required for the proper functioning of neurons, axons, muscle, and glial cells. These agents are often complex structures generated by invertebrate species and plants presumably for purposes of chemical defense. Sodium ion channels are common targets. Agents that bind to the outer surface of the Na^+ channel and prevent ion influx are found in dinoflagellates (saxitoxin); vertebrates, including fish, octopus and salamander (tetrodotoxin); and cone snails (geographutoxin). Lipid-soluble anesthetics (lidocaine, procaine) bind to a hydrophobic site in the channel and interfere with the gating mechanism. Activation of Na^+ channels by opening or impeding normal closure is seen with certain plant chemicals (grayanotoxin; pyrethrin), dinoflagellate chemicals (ciguatoxin) stored in fish, amphibian skin toxins (batrachotoxin), scorpion toxins, and synthetic pesticides (pyrethroids). Interference with Na^+ channel function is usually heralded by abnormal sensation (paresthesias) in the tongue, around the mouth, and in the extremities. While usually transient, ciguatoxin is a fat-soluble agent that may cause repeated neurotoxic events after single exposures presumably because of sequestration and periodic release within the affected subject. K^+-channel toxins with selective blocking actions have been identified in the venom of certain scorpions, bees, and snakes. Thallium and bromine ions are transported by K^+ and Cl^- channels, respectively, with significant neurological and psychiatric consequences for the affected subject. Calcium channel blockers are produced by certain plants, insects, spiders, snails, and snakes. Divalent lead ions interfere with intracellular processes regulated by Ca^{2+} ions and accumulate in the same intra-mitochondrial compartment as calcium.

Neurotransmitter Systems

Numerous substances target mechanisms involved in neurotransmitter (NT) synthesis, transport, synaptic release, re-uptake, the interaction between NT and postsynaptic receptor, or the removal of NT from the synaptic gap. Neurotoxicity occurs when the agent reduces or increases NT release, alters NT concentration or resident time, or acts as an agonist or antagonist at a postsynaptic receptor.

Acetylcholine synapses at neuromuscular junctions are targets for a number of biologic and synthetic substances (**Figure 2**). Neurotransmission is disrupted by agents that interfere with choline transport (hemicholinium, choline), with acetylcholine synthesis (triethylcholine), or synaptic vesicle uptake (vesamicol). Other agents (β-bungarotoxin, crotoxin) target mechanisms involved in presynaptic NT release. Botulism arises from the action of a zinc-endopeptidase (the light chain of the botulinum toxin dipeptide) in blocking synaptic transmission by cleaving synaptic-vesicle fusion proteins required for NT exocytosis. Botulinum-induced blockade of normal depolarization-induced NT release at the neuromuscular junction leads to flaccid muscle weakness. Conversely, α-latrotoxin in venom of the black widow spider causes massive NT release at the vertebrate neuromuscular junction resulting in a painful disorder featured by dysarthria, tremor, clonic muscle contraction, and paralysis. Numerous chemicals interfere with acetylcholinesterase, the enzyme that terminates NT action at the neuromuscular junction and other synapses within the CNS and PNS. Certain

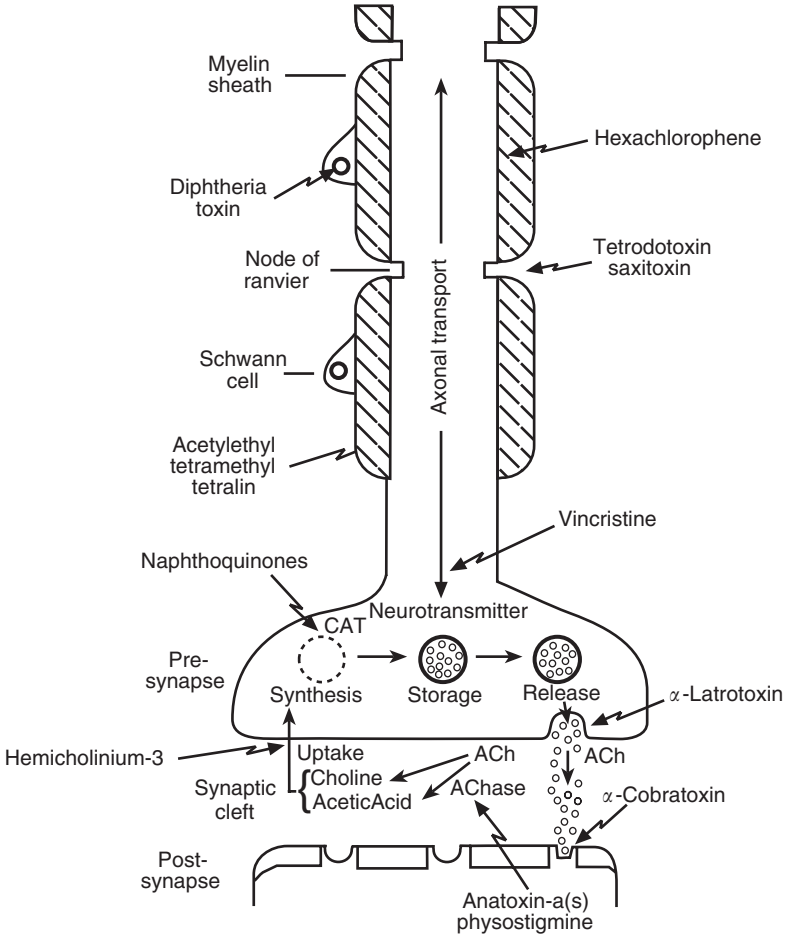


Figure 2 Targets of neurotoxic agents acting on PNS cholinergic nerve fibers (upper portion), terminals (lower midportion) and neuromuscular synapses (lowest portion). Ach, acetylcholine; AChase, acetylcholinesterase; CAT, choline acetyltransferase. (From Spencer PS and Schaumburg H (eds.) (2000) *Experimental and Clinical Neurotoxicology*, 2nd edn. New York: Oxford University Press; © Oxford University Press, Inc. Used by permission of Oxford University Press, Inc.)

anticholinesterases (edrophonium) bind directly to the active center of the enzyme and act rather briefly; others (physostigmine) carbamylate the enzyme and have long-lasting actions. The large class of organophosphates, which include high-potency agents used in chemical warfare and less hazardous materials employed as agricultural pesticides, interact with the anionic and/or esteratic sites in the active center of acetylcholinesterase to form complexes; the stability of the phosphorylated enzyme is further enhanced by the loss of one of the alkyl groups, a phenomenon known as (chemical) aging. Biological anticholinesterases include the product of a cyanobacterium (anatoxin-a(s)) and certain snake toxins (fasciculins).

Acetylcholine receptors provide another target for chemicals with neurotoxic potential; most of these act as antagonists. D-Tubocurarine is the classical nicotinic receptor antagonist, and curare-like substances are found in elapid and hydrophid snakes (α-neurotoxins) such as cobra (α-cobratoxin) and krait (α-bungarotoxin), mollusks (α-conotoxin), corals (lophotoxin), and certain plants such as delphinium (methyllcaconitine). Anatoxin-a is a potent agonist at neuromuscular, autonomic, and brain nicotinic receptors. Muscarinic receptor antagonists include atropine, scopolamine, and the synthetic warfare agent quinuclidinyl benzilate, which induces dryness of the mouth, blurred vision, confusion, delirium, and coma.

Glutamate receptors, which mediate most excitatory synaptic traffic in the CNS, are another important target of chemical substances linked to human disease. Fast synaptic transmission is mediated by DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors, a target of the grass pea AMPA agonist *β-N*-oxalylamino-L-alanine that causes pyramidal tract degeneration and paraparesis (lathyrism) in heavy consumers. Seaweed (kainic acid) and dinoflagellate (domoic acid) toxins act as agonists at the kainate subclass of glutamate receptors, the latter causing significant CNS neuronal

excitotoxicity and CNS degeneration in humans. Glutamate receptors that respond to *N*-methyl-D-aspartate (NMDA) have numerous antagonists, including the anesthetic agent ketamine and phencyclidine (angel dust). Termination of excitatory neurotransmission by removal of synaptic glutamate is perturbed by dithiocarbamate pesticides. Ionotropic glutamate receptors also play an important role in mediating neuronal death secondary to energy deficits induced by hypoxia.

Gamma-aminobutyric acid (GABA), which is synthesized by glutamic acid decarboxlase (GAD), is the major inhibitory NT in the mammalian brain. GAD inhibition (2-amino-4-pentenoic acid) induces convulsions. Numerous compounds bind to and act as antagonists (picrotoxin) or agonists (muscimol) at $GABA_A$ receptors, which employ Cl^- channels to mediate fast inhibitory postsynaptic potentials. Enhanced GABAergic transmission occurs under the action of benzodiazepine and barbiturate drugs, while organochlorine insecticides (lindane, aldrin) exert convulsant effects mediated through picrotoxin-binding sites.

Glycine receptors, which mediate inhibitory neurotransmission in the brainstem and spinal cord, have a number of plant-derived antagonists (strychnine, hydrastine) that elicit hyperreflexia and tetanic muscle contraction among other signs. Tetanus toxin from *Clostridium tetanii* triggers generalized muscle rigidity by binding to glycinergic nerve terminals (and inhibiting glycine NT release) after crossing from anterior horn cells that retrogradely transport the agent from a peripheral wound site.

Several other NT systems and pathways are impacted by chemicals that exhibit neurotoxicity. Dopamine mediates communication in a number of important pathways, including the nigrostriatal tract (a key component of basal ganglia mechanisms for control of the quality of motor movement) that degenerates in Parkinson's disease and MPP+-induced toxicity. Extrapyramidal movement disorders occur as a side effect of a number of therapeutic drugs acting on dopaminergic pathways. Chemicals that perturb adrenergic function may interfere with NT synthesis (α-methyltyrosine), serve as false NTs (methyl-dopa), block vesicular uptake (reserpine), inhibit the cleavage enzyme catechol-*O*-methyltransferase (pyrogallol), or promote NT release (amphetamine). Substituted amphetamines (3,4-methylenedioxymethamphetamine) damage axons derived from the dorsal raphe serotonergic projection system of rodents and primates. Natural substances in plants interfere with substance P neurotransmission (capsaicin), cannabinoid receptors (cannabis), purinoceptors, and adenosine receptors (caffeine). Caffeine intoxication is characterized by anxiety, sleep disturbance, and mood changes; while caffeine withdrawal in sensitive subjects leads to vascular headache, drowsiness, and fatigue.

Axonal Transport

Disruption of the transport of materials along axons is another method by which chemicals perturb neural function and induce changes, including focal axonal pathology and axonal degeneration (axonopathy). Some agents interfere with the anterograde transport of materials from sites of synthesis (cell body) to sites of utilization (axon and terminal) (β,β′-iminodipropionitrile); some with retrograde transport (acrylamide), others with the return of materials from nerve terminals (zinc pyridinethione). Certain plant-derived chemicals (colchicine, vinca alkaloids) bind to tubulin, inhibit microtubule-based function, and thereby block bidirectional axonal transport. Retrograde transport may also serve to ferry foreign substances (ricin, tetanospasmin, certain metals) to targets in neuronal somata.

Neuroglia and Myelin

Neuroglia include (1) ependymal cells lining the ventricles of the brain and the central canal of the spinal cord, (2) cells of the PNS (Schwann cell) and CNS (oligodendrocyte) that wrap around axons to form compacted plasma membranes (myelin) that provide electrical insulation to speed nerve conduction, (3) cells (astrocytes) that interface between nerve cells and capillaries in the CNS, regulate interstitial water content, K^+ concentration, remove and metabolize certain NT molecules, and proliferate following injury.

Ependymal cells are susceptible to agents (amosconate) present in the CSF. Astrocyte foot processes investing cerebral capillaries undergo marked swelling in water intoxication, lead encephalopathy and in hypercapnia, and after the experimental administration of 6-aminonicotinamide, isoniazid, misonidazole, or ouabain. Astrocytes increase their glycogen content in a variety of insults (methionine sulfoxamine), form intranuclear inclusion bodies in lead intoxication, and greatly increase the relative size of the nuclear compartment in hepatic encephalopathy, which is thought to be triggered by hyperammonemia.

Myelinating cells are susceptible to agents that disrupt the synthesis of myelin components, the best example of which is diphtheria toxin, which has access to peripheral nerves where it inhibits Schwann cell protein synthesis and causes primary demyelination. Oligodendrocyte demyelination can be induced experimentally by diphtheria toxin and by other

protein synthesis inhibitors such as ethidium bromide and actinomycin D. The latter induces widespread status spongiosus of white matter, with edema fluid in the periaxonal space and between myelin membranes split open at the intraperiod line. Cuprizone (biscyclohexanone oxalylhydrazone) induces oligodendrocyte degeneration with intramyelinic edema. Several other agents (cycloleucine, hexachlorophene, triethyltin) trigger reversible changes in CNS and/or PNS myelin without apparent damage or loss of the myelin-forming cells.

System Vulnerability

Developing Nervous System

The intrinsic neural factors that determine or influence response to chemicals differ in development, at maturity, and in late life. The dominant host factor influencing response to exogenous chemicals is developmental state. As a result, neurotoxic effects observed following developmental exposure differ both quantitatively and qualitatively from those seen following adult exposure. Quantitatively, the developing nervous system is generally more sensitive to neurotoxic agents as exemplified by observations that NMDA causes a greater neurotoxic response in the immature rat brain than in the adult animal. Similarly, studies of both humans and rodents indicate that intrauterine exposure to lead or to polychlorinated biphenyls (PCBs) seem to be more damaging than exposure later in life. The nature of the neurotoxic effect may also differ as a function of developmental state. For example, infants of mothers treated with the anticonvulsant drug sodium valproate may display congenital malformations, including neural tube defects, whereas neurotoxicity in the adult is manifest as tremor, a confusional state and, in rare cases, parkinsonism.

The influence of developmental state on the response to neurotoxic agents largely reflects qualitative differences between the developing and adult nervous system. Neurodevelopment is a complex process that is precisely regulated in time and space, with the basic framework of the nervous system laid down in a step-by-step process in which each step is dependent upon the proper completion of the previous one. In both humans and rodents, normal neurodevelopment begins very early in the fetus and is not complete until puberty. Active organogenesis, which occurs during the period from implantation through mid-gestation, requires the concomitant and coordinated ontogeny of cell proliferation, migration, and differentiation. During late gestation and the early neonatal period, the processes of synaptogenesis, apoptosis, and myelination are predominant. These processes continue throughout later stages of neurodevelopment, and together with elimination of extraneous synapses via axon and dendrite retraction, function to refine patterns of neuronal connectivity. There are data to suggest that errors in timing, spatial resolution, or magnitude of any of these developmental events can have clinical consequences. For example, magnetic resonance imaging studies of schizophrenia indicate excessive pruning of axons and dendrites in the cortex during late adolescence, coincident with the onset of symptoms. Importantly, substances have been identified that interfere with each of these processes in animal models and, in some instances, humans, and the outcome of such interactions ranges from death to gross structural abnormalities to subtle defects in structure or function. For example, early gestational exposure to substances such as fumonisins can produce neural tube defects; exposure to ionizing radiation produces altered brain morphology and mental retardation; exposure to high concentrations of ethyl alcohol causes mental retardation, while moderate levels of alcohol exposure can delay motor development; intrauterine exposure to cocaine causes excessive outgrowth of dendrites, and exposure of infants and children to relatively low levels of lead is linked to reduced scores on tests of cognitive development and to increased aggressive tendencies.

The single most important determinant of the pattern of damage induced by neurotoxic agents is the timing of exposure relative to ongoing ontogenetic events. There are at least four scenarios to explain how timing of exposure influences neurotoxic outcome. First, because later stages of neurodevelopment depend on successful completion of early stages, even relatively minor disturbances early in neurodevelopment may cause significantly more damage than perturbations occurring at later stages. For example, inhibition of cellular proliferation by agents such as MAM, lead, mercury or ethanol, can subsequently impact migration, differentiation, and ultimately neuronal connectivity. Second, individual neurodevelopmental events may be differentially vulnerable to a specific substance. When proliferation is actively occurring within a given region of the brain, that region is vulnerable to anti-mitotic agents, such as MAM, but when cell proliferation ceases, the brain region is more resilient to MAM. Similarly, vitamin A and other retinoic acid derivatives can cause marked and irreversible abnormalities, including anencephaly and spina bifida, when exposure occurs during gestation days 5–10 in rats. In contrast, administration of retinoic acid on gestational day 12 fails to perturb brain development. Third,

since different brain regions develop on different time lines during prenatal and postnatal life, a chemical may produce impairment in different functional domains depending on the time of exposure. Thus, in a rat model of fetal alcohol syndrome changing the time at which neonatal rats are exposed to ethanol triggered neuronal cell loss via apoptosis from different brain regions, thereby giving rise to different profiles of functional deficits. Finally, the expression and/or function of many proteins targeted by neurotoxicants can vary with development. The α subunit of the glycine receptor exists in several isoforms that are transcriptionally regulated during development. The adult isoforms of the α subunit have a higher affinity for strychnine than the neonatal isoforms; thus, the developing nervous system is less vulnerable to strychnine intoxication than the adult. Another critical example includes NTs and enzymes that metabolize NTs, such as acetylcholinesterase. In the adult nervous system, these proteins function in neurotransmission; however, during development, NTs and acetylcholinesterase act as morphogenic factors that modulate patterns of neuronal connectivity. Therefore, substances that target NT systems, such as certain pesticides (*vide supra*), may have quite different effects on the developing fetus or child compared to the adult, and this has been demonstrated in animal models. As with the adult nervous system, the dose and duration of exposure also influence the response of the developing nervous system to exogenous chemicals. Physiological differences between developing and adult organisms underlie potentially significant differences in distribution, metabolism, and excretion of neurotoxic agents.

The potential for chemical exposure to the fetus begins before conception in that prior parental exposure to toxicants can have a major impact on the developing fetus. Parental exposures threaten fetal health by either altering maternal or paternal reproductive organs directly or via release of stored neurotoxic agents from maternal tissues during pregnancy. Yusho disease is a tragic example of preconception exposure influencing fetal neurodevelopment. Women in the Japanese town of Yusho who consumed cooking oil contaminated with PCBs, up to a year prior to conception, gave birth to infants exhibiting a constellation of symptoms including dysmorphism, skin lesions, hepatic dysfunction, and cognitive abnormalities. PCBs stored in maternal tissues were mobilized during pregnancy. Similarly, lead can be mobilized from storage depots in bone during pregnancy. Once in the maternal blood supply, chemicals may diffuse across the placenta and enter the fetal circulation. Some agents (organoarsenicals) accumulate in the placenta, which shields the offspring from exposure. However, the placenta does not block small molecular weight compounds (carbon monoxide), lipophilic compounds (PCBs, ethanol, methylmercury), or compounds using active transport mechanisms (lead). Once in the fetal circulation, these agents can readily enter the developing nervous system since the blood–brain barrier is not completely developed until after birth (6 months in humans). The lack of a fully formed blood–brain barrier explains why many systemically distributed compounds, such as lead salts, which generally do not elicit brain damage in adults, cause severe encephalopathy in newborn animals and humans. Prior to keratinization of the human fetal epidermis, beginning at 20 weeks of gestation, exogenous chemicals may also diffuse from the amniotic fluid into the developing fetus. After birth, exposure to potential toxicants may occur via breastfeeding and consumption of other contaminated foodstuffs, oral contact via hand-to-mouth activity, dermal contact, or inhalation. Compared with adults, children in all postnatal developmental stages have higher rates of respiration and energy consumption per kilogram of body weight, which increases their exposure rates. In addition, the skin is highly permeable during the newborn period and several epidemics of developmental neurotoxicity have been described involving percutaneous absorption of chemicals. These include hypothyroidism from iodine in povidone iodine (Betadine) scrub solutions and myelin disorders consequent to bathing infants in hexachlorophene. The expression of phase I and II metabolic enzymes is also developmentally regulated, resulting in altered abilities of developing organisms to detoxify and excrete chemicals relative to adults. This difference may confer increased resistance when a substance must be metabolized to an active metabolite. But, more frequently, the metabolic differences manifest as a decreased capacity of children to excrete toxins as compared to adults, and thus they are more vulnerable to neurotoxic agents. The lack of functional paroxonase, the enzyme that detoxifies many organophosphates, contributes to the increased vulnerability of the developing nervous system to the neurotoxic effects of these agents.

Very many chemicals are recognized teratogens in animals; a significantly smaller subset of these is known or suspected to be developmental neurotoxicants in humans. Some of the more significant of the latter group include ethanol, which causes a constellation of effects ranging from fetal alcohol syndrome to alcohol-related neurodevelopmental disorder; maternal smoking of tobacco (fetal tobacco syndrome); excess vitamins A and D; heavy metals, particularly

inorganic and organic mercury, lead and cadmium; anticonvulsants, such as phenytoin, valproate, phenobarbital, carbamazepine and primidone; drugs of abuse, including cocaine, cannabis and mescaline; persistent aromatic hydrocarbons, especially PCBs; and both organochlorine and organophosphate pesticides.

A significant challenge in the field of developmental neurotoxicity is to identify agents with developmental neurotoxic potential in humans. Detecting effects in the human population is difficult because they may be subtle (small shifts in IQ scores, slight changes in behavior) or because neurotoxicity does not become manifest until a significant period of time after the developmental exposure. Delayed neurotoxicity may arise via two different mechanisms. One of these involves the occurrence of a toxic insult early in neurodevelopment, but with manifestation of the pathological change much later in neurodevelopment when function of the affected cells is normally activated. An agent that causes this type of delayed neurotoxicity is the food additive, monosodium glutamate (MSG). MSG causes excitotoxicity via activation of glutamate receptors, and the developing brain is more sensitive than the adult brain to the toxic effects of glutamate agonists. Developmental exposure to MSG causes excessive apoptosis of neurons in the developing hypothalamus. However, the fetal loss of these hypothalamic neurons becomes evident (as hypogonadism and infertility) only in adolescence when the neuroendocrine function of these neurons is normally activated. A second mechanism of delayed neurotoxicity involves a developmental insult in which both anatomical and/or functional effects may be masked or attenuated initially because of compensatory mechanisms or plasticity. However, these developmental perturbations predispose the individual to neural deficits following subsequent insults such as chemical exposure, disease, or aging because of decreased reserve capacity. This phenomenon has been demonstrated in both animal models and humans following developmental exposures to methylmercury.

A current goal in developmental neurotoxicity is to develop screening methods to identify agents with the potential to cause developmental neurotoxic effects in humans. However, designing a screening method that is humane, scientifically valid and mechanistically driven represents a significant scientific and technical challenge in large part because there is a paucity of information regarding the molecular mechanism(s) by which agents perturb neurodevelopmental events. However, with the application of recent advances in the cell and molecular biology of normal neurodevelopment, these gaps in the database are being addressed. For example, the balance of activity between excitatory glutamate receptors and inhibitory GABA receptors modulates neuronal apoptosis in the developing brain. Developmental exposure of rats to ethanol significantly increases the percentage of apoptotic neurons via simultaneous inhibition of excitatory glutamate receptors and activation of inhibitory GABA receptors. Ethanol disrupts neuronal migration, axon outgrowth, and synaptogenesis in cultured neurons via interference with the L1 adhesion molecule. Significantly, prenatal exposure to ethanol mimics the brain defects observed in humans with congenital mutations in the L1 adhesion molecule. What is not yet clear is whether ethanol acts similarly in the developing human brain and under what conditions of dose and timing of exposure either of these mechanisms predominates. Also unanswered is whether these mechanisms are specific to ethanol or represent generalized mechanisms by which broad categories of agents cause developmental neurotoxicity.

Adult Nervous System

Chemicals generally perturb neurological function of the adult by interfering differentially with the structure and function of specific neural pathways, circuits, and systems. Vulnerable circuits within the brain include those that modulate and affect efferent output. Most commonly affected, however, are the peripheral neurons in pathways that relay information to and from the brain.

The special senses of vision, audition, balance, gestation, and olfaction depend on neural pathways that originate in peripheral receptors and terminate in the brainstem or cerebral cortex. Afferent pathways for taste, smell, hearing, and balance employ sensory neurons in ganglia that lack a blood-nerve barrier. However, chemicals that perturb the special senses seem most commonly to interfere with the structure or function of the peripheral sensory receptors. Olfaction and gustation are subserved by cilia-bearing sensory neurons that are continuously generated from stem cells, a process of cellular replacement that is disrupted by antiproliferative drugs such as vincristine and doxorubicin. For vision, the function of retinal cells is perturbed by a large number of substances, some of which produce reversible change (cardiac glycosides and trimethadone), while others (aminophenoxyalkanes) elicit morphological damage. For retinal ganglion cells, the nerve fibers that form the optic pathway are sites of vulnerability to toxic attack. Substances that impair energy metabolism (thallium, cyanide) tend to damage proximal regions of axons projecting into the optic nerve from the papillomacular bundle, while distal axonal

degeneration, with damage to the optic tracts, is seen with drugs such as clioquinol and ethambutol. Vestibular and auditory function may be affected concurrently by agents that target receptor cells in the inner ear of rodents (2-butenenitrile, 2-pentenenitrile) and humans (streptomycin). Other potentially ototoxic substances include cisplatin, furosemide and imipramine. Noise may exacerbate the neurotoxic effects of some ototoxic agents. Disturbance of human oculomotor function may take the form of nystagmus (carbon monoxide) or opsoclonus (chlordecone), two types of abnormal eye movement. Certain neurotoxic substances produce lesions of vestibular and cochlear nuclei in rodents (6-chloro-6-deoxyglucose) and primates (1-amino-6-chloropropane).

Sensorimotor function is altered by a number of chemicals that act at different sites in the neuraxis. Most affect the axons or somata of lower motor neurons in the anterior horn of the spinal cord or primary sensory neurons in dorsal root ganglia. Some substances target the nerve cell body of sensory neurons that detect touch and vibration (methylmercury), position sense (pyridoxine) or pain (capsaicin), or of neurons that regulate cardiac muscle (doxorubicin) or voluntary muscle (domoic acid, *β*-*N*-methylamino-L-alanine) function. Others target motor nerve terminals (botulinum toxin, α-latrotoxin) or the enzyme that targets acetylcholine (anticholinesterases), both of which produce acute alterations of neurotransmission associated with reduced or enhanced synaptic transmission. Temporary disruption of electrical impulse conduction is another neurotoxic effect. Agents (pyrethroids, ciguatoxin, tetrodotoxin) that perturb electrical activity in the excitable membrane (axolemmal) of the nerve cell produce rapidly reversible sensory abnormalities (paresthesias) in the distribution of trigeminal and elongate peripheral nerves. Those substances (hexachlorophene, ethidium bromide) that attack the myelin sheath or myelinating cell precipitate focal demyelination and remyelination, with consequent disruption and restoration of nerve conduction and associated sensory-motor phenomena over a period of several weeks. Focal demyelination and remyelination may also result from exposure to chemicals that block neurofilament transport and cause focal axonal swellings proximally (1,2-diacetylbenzene, 3,4-dimethyl-2,5-hexanedione) or distally (2,5-hexanedione, carbon disulfide, acrylamide). Chemicals that produce distal (acrylamide), but not proximal (*β*,*β*′-iminodiproprionitrile), giant axonal neurofilamentous swellings trigger retrograde distal axonal degeneration (distal axonopathy). Long and large-diameter myelinated axons in the PNS and CNS are vulnerable to distal axonopathy caused by a number of compounds (organophosphates, isoniazid), the resulting clinical picture being one of symmetrical sensory loss and muscle weakness in the distal extremities (stocking-and-glove polyneuropathy). Shorter and thinner nerve fibers, including postganglionic nerves of the autonomic nervous system, may become involved in distal axonopathies. This common type of neurodegeneration usually occurs after repeated exposure, evolves during and shortly after the period of intoxication, and then reverses as regenerating axons reestablish functional contact with denervated sensory terminals and muscle. Significant atrophy may result from prolonged skeletal muscle denervation, and recovery of sensory motor function may be slow, progressive and incomplete. When central motor pathways are heavily affected by distal axonopathy (leptophos, clioquinol), affected subjects may display permanent residual spasticity.

The motor pathway from brain to muscle is modulated by two other CNS structures that are vulnerable to substances with neurotoxic potential, namely the basal ganglia and cerebellum. Damage to the cerebellum may result in loss of coordination of limb and eye movement and in an ataxic gait (methylmercury, 3-acetylpyridine). The cerebellum and basal ganglia are both sensitive to hypoxia and related states induced by agents that impair energy metabolism (cyanide) and promote glutamate-mediated excitotoxicity. Other energy-disrupting agents elicit neuronal damage in the putamen (3-nitropropionic acid, methanol), pallidum (carbon monoxide), substantia nigra (MPTP), or other parts of the basal ganglia (manganese). These types of neurotoxicity may find clinical expression as parkinsonism, tremor, dystonia, and other extrapyramidal dysfunction. Movement disorders of various types may result from the side effects of several therapeutic agents (amphetamines, anticonvulsants, anticholinergics, neuroleptics, dopamine agonists, lithium, tricyclic antidepressants).

Autonomic regulation of the pupil, lacrimal and salivary glands, airway, heart, gut, bladder, genitalia, and blood vessels involves sympathetic, parasympathetic, and enteric neurons. Unlike their somatic counterparts in the spinal cord, efferent neurons of the autonomic nervous system are housed in peripheral ganglia with permeant blood vessels. Autonomic function is disrupted by agents that target synapses that utilize acetylcholine since this is the principal NT for all preganglionic autonomic fibers, all postganglionic parasympathetic fibers, and some postganglionic sympathetic fibers. Drugs that selectively block nicotinic receptors (curare) curtail

ganglionic output, while muscarinic agents (atropine) block transmission to effector cells. Anticholinesterase agents (fasciculins, organophosphates) stimulate sympathetic and parasympathetic activity, sometimes with the dramatic clinical consequences of a cholinergic crisis (nerve agents). Direct contact of anticholinesterase nerve agents (sarin, VX) with the eye, nasal passages, and bronchi leads to pupillary constriction (miosis), blurred vision, rhinorrhea, bronchoconstriction, and increased secretions. Systemic exposure results in increased salivation, bradycardia, enhanced lacrimation, urination, and defecation. Attendant muscle fasciculation, weakness, and seizures arise from peripheral somatic and CNS actions of anticholinesterase nerve agents.

The autonomic nervous system and endocrine function are regulated by the hypothalamus and associated limbic structures of the brain. Hypothalamic functions, including the regulation of temperature, heart rate, blood pressure, blood osmolarity, circadian control, and water and food intake, may be impacted by a range of chemicals. Parts of the hypothalamus lack a blood–brain barrier: infant mice treated with excitotoxic agents (glutamate, aspartate, cysteic acid) display extensive damage of the arcuate nucleus and develop a syndrome of obesity, skeletal stunting, reproductive failure, and gonadal hypoplasia. The hypothalamus receives major input from the hippocampus, which functions in the storage of declarative memory, uses cellular circuitry that involves glutamertergic synapses vulnerable to excitotoxins (domoic acid) and certain other agents (trimethyltin, trimethyl lead, soman), the latter possibly as a result of seizure-associated hypoxia.

See also: Carbamate Pesticides; Cholinesterase Inhibition; Metals; Nerve Agents; Organophosphates; Pesticides.

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New Drug Application *See* Investigative New Drug Application.

Niacin

Diana Ku

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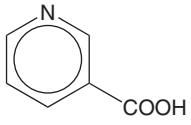
- Chemical Abstracts Service Registry Number: CAS 59-67-6
- Synonyms: Vitamin B_3, Nicotinic acid; Nicotinamide; Pellagra-preventative factor; 3-Carboxypyridine
- Chemical/Pharmaceutical/Other Class: Water-soluble vitamin
- Chemical Formula: $C_6H_5NO_2$
- Chemical Structure:



Uses

Niacin is a nutritional supplement used during periods of deficiency known as pellagra and for the treatment of dyslipidemia. Niacin needs may increase during chronic illness such as diabetes mellitus, malignancy, metabolic diseases, hyperthyroidism, infections, chronic fever, alcoholism, and during pregnancy and lactation.

Exposure Routes and Pathways

Routes of exposure are oral and intravenous. It can also be given intramuscularly or subcutaneously but intravenous administration is recommended when possible. Dietary sources of niacin are green vegetables, eggs, milk, and other dairy products, legumes, yeast, whole grains, lean meats, liver, and fish.

Toxicokinetics

Niacin is readily absorbed from the gastrointestinal tract. The peak serum concentration for an immediate release oral dosage form is usually seen within 45 min of niacin ingestion; 4–5 h for an extended release tablet. Niacin is hepatically metabolized and widely distributed into body tissues. Niacin is renally excreted. Excess amounts of niacin, beyond daily needs, are excreted largely unchanged in the urine. The plasma half-life is $\sim$45 min.

Mechanism of Toxicity

Niacin-induced vasodilation is believed to be mediated by prostaglandins. The mechanism of hepatotoxicity associated with niacin use is unknown.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity is not expected.

Human

Toxicity is unlikely even after acute ingestions of 50–100 times the recommended daily allowance. Side effects include nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and dryness of the skin. Niacin flush is a sensitivity reaction that causes warmth and finishing of the face and neck lasting $\sim$2 or 3 h. This event usually occurs with doses of $>$1 g. The symptoms are self-limiting and tend to occur less frequently with increased tolerance or premedication with aspirin or ibuprofen.

Chronic Toxicity (or Exposure)

Animal

It would be unlikely for animals to be given chronic niacin overdoses.

Human

Chronic megadoses of niacin may be associated with hyperglycemia, hyperuricemia, cardiac arrhythmias, hepatotoxicity, cystoid maculopathy, myopathy, peptic ulcers, and hyperkeratotic pigmented skin lesions. These problems may occur with doses exceeding 3 g day^{-1}.

In Vitro Toxicity Data

In one study of 87 infants born to women who were given therapeutic doses of niacin at any time during pregnancy, there were two infants born with congenital anomalies.

Clinical Management

Acute ingestions seldom require treatment. Reassurance that the niacin flush will gradually resolve over the next couple of hours should be given. In cases of chronic excessive use, the patient should be instructed to discontinue the supplement. Any toxic symptoms should be treated symptomatically.

See Also: Dietary Supplements.

Further Reading

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Nickel and Nickel Compounds

Shayne C Gad

This article is a revision of the previous print edition article by Betty J Locey and Arthur Furst, volume 2, pp. 415–416,

- Representative Chemicals: Metallic nickel (Ni); Nickel subsulfide (Ni_3S_2); Nickel sulfate ($NiSO_4$); Nickel carbonyl ($Ni(CO)_4$); Nickel oxide (NiO). Nickel can exist in a variety of other forms, but this text focuses on the primary environmentally relevant forms.
- Chemical Abstracts Service Registry Number: CAS 7440-02-0
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: $Ni^{+2,0}$

Uses

Nickel and nickel compounds are widely used in plated coatings, nickel–cadmium batteries, certain pigments, ceramic glazes, and as industrial and laboratory catalysts. Nickel subsulfide is used in refining certain ores and in smelting operations. Nickel is commonly used in alloys such as stainless steel, alloy steel, and nonferrous metal mixtures. Coins, costume jewelry, plumbing equipment, and electrodes are often made from nickel-containing alloys.

Background Information

Discovered in 1751, nickel is rare in earth's crust, but is believed to be more common in molten core.

Nickel is ubiquitous; it is found in air, water, soil, food, certain work environments, and in certain products. Low concentrations are found in soils and in plant and animal tissues. Nickel may be released into the ambient air with emissions from certain industrial processes and with smoke from the combustion of coal and petroleum products. Cigarette smoke may contain up to 3 μg of nickel per cigarette.

Exposure Routes and Pathways

Exposure to nickel and its compounds may occur through ingestion, inhalation, or dermal contact. The specific characteristics of the compound determined the likelihood of exposure through a particular route and the amount absorbed in to the body through that route.

Nickel is commonly ingested with food, and is found at low levels in drinking water. Elemental nickel, nickel oxide, and nickel subsulfide, may occur as a particulate or adsorbed onto other particles in ambient air. Nickel carbonyl is a highly reactive gas, with a half-life in air of ~100 s, and so exposure would occur only in the immediate vicinity of a release. Skin exposure may occur during contact with nickel-containing products (e.g., jewelry).

Toxicokinetics

The toxicokinetics of nickel compounds depends on their solubility in water and biological fluids. Nickel sulfate and nickel chloride are highly soluble in water, while nickel oxide is insoluble. Nickel subsulfide is poorly soluble, but is more soluble in biological fluids, presumably due to the effects of the proteins and other cellular components. Nickel and its inorganic compounds are not well absorbed through the skin or the gastrointestinal tract. Absorption is higher when soluble forms of nickel are administered in drinking water or to fasting subjects than when administered in food. The absorption of inhaled nickel particles depends, in part, on the size of the particles and the solubility of the compound, with soluble forms being rapidly absorbed and distributed, and insoluble forms being retained in the respiratory tract for much longer periods. Nickel carbonyl is rapidly absorbed from the lungs. Generally, absorption for nickel carbonyl > soluble compounds > insoluble compounds. Once absorbed, nickel is transported with the plasma, in a form bound to serum albumin, amino acids, polypeptides, and other small organic molecules. Nickel is found at elevated levels in the kidneys, liver, and brain. Nickel has been found in adipose tissue. Nickel may act at the point of contact (e.g., in the skin or in the lung) or systemically.

The half-life of nickel in nickel platers (exposed primarily to nickel sulfate) was found to be 20–34 h in plasma and 17–39 h in urine. In refinery workers (exposed to a mixture of soluble and insoluble forms of nickel), the half-life in the nasal mucosa was found to be several years.

The major route of excretion for nickel is in urine. Animal studies indicate that 60% of the nickel introduced into the body via injection is excreted in the urine and to a lesser extent through the bile into the feces. Some nickel is excreted in perspiration. Ingested nickel is primarily eliminated in the feces, with only ~10% excreted in the urine. Nickel crosses the placenta.

Mechanism of Toxicity

Skin sensitization is believed to occur as a result of nickel binding to proteins (particularly on the cell surface) and hapten formation. Essentially, the body perceives the nickel-protein complex as foreign and mounts an immune reaction to it. For example, sweat may react with the nickel in plated jewelry that comes in direct contact with skin; dissolved metal may penetrate and react with proteins in the skin and lead to immune sensitization. Nickel may substitute for certain other metals (especially zinc) in metal-dependent enzymes, leading to altered protein function. High nickel content in serum and tissue may interfere with both copper and zinc metabolism. It also readily crosses the cell membrane via calcium channels and competes with calcium for specific receptors.

Nickel carbonyl can cross-link amino acids to DNA and lead to formation of reactive oxygen species. Nickel carbonyl can also suppress natural killer cell activity and production of some interferons.

Responses in many of these assays were weak and occurred at toxic doses, and were affected by tissue culture conditions modifying uptake by the cell. The mechanism of nickel carcinogenesis is controversial, and is likely to vary with the form of nickel. The nickel ion (Ni^{2+}) alone does not form premutagenic DNA lesions, suggesting that nickel causes indirect DNA damage, perhaps due to oxidative stress or blocking DNA repair mechanisms.

Nickel is an essential trace nutrient in plants and certain animal species (e.g., rat and chick); however, it has not been shown to be essential in humans.

Acute and Short-Term Toxicity (or Exposure)

Human

The skin and respiratory tract are primary target organs. Nickel carbonyl is very reactive, and highly acutely toxic. Nickel carbonyl is very irritating to the respiratory tract, and exposure may lead to pulmonary edema, pneumonia, and death. Adverse reactions on exposure to other forms of nickel may occur at the site of contact (skin, respiratory tract, and gastrointestinal tract) or systemically (heart, blood, and kidneys). Ingestion of high doses of nickel and certain nickel compounds has been shown to cause stomach pain, increases in the number of red blood cells, and kidney damage.

Chronic Toxicity (or Exposure)

Animal

Different nickel compounds have been shown to have varying toxicity in animals. Both soluble and insoluble forms of nickel have been shown to damage the lung. Chronic inhalation studies (certain forms) have shown pulmonary inflammation, damage to certain regions of the respiratory tract mucosa and epithelium, and damage to the nasal olfactory epithelium. Nickel has been shown to be carcinogenic in animals via injection and implantation. Nickel subsulfide and nickel carbonyl have been shown to be carcinogenic via inhalation. Inhaled nickel oxide was carcinogenic in rats, but not in mice.

Nickel has been shown to adversely affect the blood (e.g., severe erythrocytosis) in experimental rats. Oral exposure to soluble nickel has been shown to cause increased prenatal or neonatal mortality.

Human

Nickel and nickel compounds are skin sensitizers, leading to irritation, eczema and allergic contact dermatitis. Oral exposure may elicit allergic dermatitis in sensitized individuals. Allergy-related asthma and skin reactions ('nickel itch' and contact dermatitis) have been associated with exposure. Skin sensitivity may even develop from contact with jewelry or coins made of nickel-containing alloys. Approximately 2.5–5% of the general population may be sensitized to nickel. A higher percentage of women than men is sensitized, probably because of direct contact with nickel-plated jewelry. Skin sensitization reactions can progress to erythema, some eruption, and in more extreme cases to pustules and ulcers. Severe skin reactions are most likely to occur in occupational settings where higher exposure is likely.

Exposure to certain nickel compounds is associated with development of cancer. Nickel particulate (e.g., elemental and subsulfide) has been associated with nasal and lung cancer after workplace exposures. Chromosomal aberrations have been noted in lymphocytes in occupationally exposed individuals. The American Conference of Governmental Industrial Hygienists (ACGIH) classifies inhalable nickel particulate – insoluble compounds as confirmed human carcinogens (A1). US Environmental Protection Agency (EPA) classifies nickel refinery dust and nickel subsulfide as known human carcinogens (A) and nickel carbonyl as a probable human carcinogen (B). The International Agency for Research on Cancer (IARC) classifies nickel and nickel compounds as having sufficient evidence of cancer in humans (group 1); however, IARC notes that the evaluation applies to the group in general and not necessarily to all compounds in the group.

In Vitro Toxicity Data

Nickel compounds are generally negative in bacterial gene mutation assays, but positive responses have often been found in *in vitro* mammalian cell assays.

Clinical Management

For inhalation exposure (typically to nickel carbonyl), the victim should be moved from the source of the exposure to fresh air. Contaminated clothing should be removed and contaminated skin washed. Blood, urine, and fecal nickel levels may be used as indicators of the level of recent exposure.

Chelating agents may be used to reduce the body burden after exposure. Diethyldithiocarbamate is the preferred chelating agent. D-Penicillamine and calcium ethylenediaminetetraacetate may also be effective in enhancing excretion of nickel.

Oral toxicity of elemental nickel is low. Treatment of illness caused by ingestion of nickel salts is usually limited to fluid replacement in cases of severe vomiting and diarrhea. Once sensitization has occurred, contact with nickel should be strictly avoided since reactions may occur after exposure to very low levels. This is particularly important in the workplace where high-level exposures are more likely to occur.

Environmental Fate

Nickel and its compounds are naturally present in the earth's crust, and releases to the atmosphere occur from natural discharges such as windblown dust and volcanic eruptions, as well as from anthropogenic activities. It is estimated that 8.5 million kilograms of nickel are emitted into the atmosphere from natural sources such as wind-blown dust, volcanoes, and vegetation each year. Five times that quantity is estimated to come from anthropogenic sources. Nickel releases are mainly in the form of aerosols that cover a broad spectrum of sizes. Particulates from power plants tend to be associated with smaller particles than those from smelters. Atmospheric aerosols are removed by gravitational settling and dry and wet deposition. Submicrometer particles may have atmospheric half-lives as long as 30 days. Monitoring data confirm that nickel can be transported far from its source. The form of nickel emitted to the atmosphere will vary according to the type of source. Species associated with combustion, incineration, and metals smelting and refining are often complex nickel oxides, nickel sulfate, metallic nickel, and in more specialized industries, nickel silicate, nickel subsulfide, and nickel chloride.

Nickel may be transported into streams and waterways from the natural weathering of soil as well as from anthropogenic discharges and runoff. This nickel can accumulate in sediment, with the adsorption of the metal to the soil depending on pH, redox potential, ionic strength of the water, concentration of complexing ions, and the metal concentration and type.

Ecotoxicology

The speciation and physicochemical state of nickel is important in considering its behavior in the environment and availability to biota. For example, the nickel incorporated in some mineral lattices may be inert and have no ecological significance. Most analytical methods for nickel do not distinguish the form of nickel; the total amount of nickel is reported, but the nature of the nickel compounds and whether they are adsorbed to other material is not known. This information, which is critical in determining nickel's lability and availability, is likely to be site specific.

In rainbow trout, 96 h LC_{50} values were from $\sim$8–36 mg Ni l^{-1} with highly soluble compounds (e.g., chloride, sulfate, and acetate salts). LC_{50} values in fathead minnows ranged from 3 to 90 mg l^{-1}. In studies with *Daphnia*, 48 h LC_{50} values ranged from 0.5 to 7 mg Ni l^{-1}.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit (PEL) time-weighted average (TWA) for nickel metal and other nickel compounds as nickel is 1 mg m^{-3}; the PEL TWA for nickel carbonyl is 0.007 mg m^{-3}. The ACGIH threshold limit values (TLVs) for nickel metal, insoluble compounds, soluble compounds, nickel carbonyl, and nickel subsulfide, all expressed as nickel, are 1.5, 0.2, 0.1, 0.12, and 0.1 mg m^{-3}, respectively. Except for nickel carbonyl, all of the TLVs are expressed as inhalable particulate. The US EPA reference dose for soluble nickel salts is 0.02 mg kg^{-1} day^{-1}, but this value is undergoing reevaluation, due to the availability of several relevant new studies.

See also: Hypersensitivity, Delayed Type; International Agency for Research on Cancer; Kidney; Metals; Respiratory Tract; Skin.

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Relevant Websites

http://www.nickelinstitute.org – Nickel Institute.
http://www.epa.gov – US Environmental Protection Agency.

Nickel Chloride

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7718-54-9 (Previously CAS 37211-05-5)
- Synonyms: Nickel dichloride; Nickelous chloride
- Chemical/Pharmaceutical/Other Class: Metal salt
- Chemical Formula: $NiCl_2$

Uses

Nickel chloride is used for nickel plating cast zinc, as an agent in electrolytic refining of nickel, as a chemical intermediate for nickel catalysts and complex nickel salts, as an absorber of ammonia gas in industrial gas masks, as a catalyst in diarylamine and silicon tetrachloride production, as an agent in electrodeless plating of nickel, as an agent in tin–nickel alloy plating, and as a fungicide for control of rust and rustlike disease. However, workers exposed to different forms of nickel have an elevated risk of lung cancer. Besides, Ni and its compounds have been reported to be potent carcinogens and toxic agents in humans and experimental animals. Therefore, Ni compounds are considered to be an industrial/occupational health hazard.

Exposure Routes and Pathways

Humans are frequently exposed to metals due to their ubiquity, wide use in industries, and persistence in the environment. Many nickel compounds are released into the atmosphere during mining, smelting, and refining operations. Although nickel is poorly absorbed from the gastrointestinal tract, exposure via food and drinking water provide most of the intake of nickel and nickel compounds. Humans and animals absorb ~1–10% of dietary nickel, and similar values were reported for drinking water exposure. Nickel metal is poorly absorbed dermally but some nickel compounds such as nickel chloride or nickel sulfate can penetrate occluded skin resulting in up to 77% absorption within 24 h. Nickel is excreted in the urine and feces, but because it is poorly absorbed, most ingested nickel is excreted in the feces. About 80–90% of nickel chloride is excreted and only a small amount is retained. The average daily intake of nickel in food is ~0.002 mg Ni kg^{-1} day^{-1} and the tolerable intake (TI) of nickel chloride is 0.0013 mg Ni kg^{-1} day^{-1}.

Toxicokinetics

Adverse effects can result from ingestion, skin contact, inhalation, or parenteral routes of exposure; nickel may be absorbed from the gastrointestinal and respiratory tracts as well as percutaneously; however, it is poorly absorbed orally. Nickel is bound to albumin and α_2-microglobulin in the circulation. Nickel chloride is a water soluble salt, and respiratory absorption with secondary gastrointestinal absorption of nickel (insoluble and soluble salts) is the major route of entry during occupational exposure. A significant quantity of inhaled material is swallowed following mucocillary clearance from the respiratory tract. Percutaneous absorption is negligible, but is important in the pathogenesis of contact hypersensitivity. Absorption is related to the solubility of the compound, and nickel given orally to rats as the chloride in the drinking water was eliminated mainly in the feces. Intubation of rats with nickel chloride led to 3–6% absorption of the labeled nickel, regardless of the administered dose. These studies suggest that

very little nickel in water or beverages is bioavailable, and that large doses are required to overcome the intestinal absorption-limiting mechanism.

Mechanism of Toxicity

As parent metal alters sodium balance and lipid metabolism; it induces metallothionein synthesis. Nickel chloride affects the T-cell system and suppresses the activity of natural killer cells. If given orally or by inhalation, nickel chloride has been reported to decrease iodine uptake by the thyroid gland.

Acute and Short-Term Toxicity (or Exposure)

Animal

The mouse oral LD_{50} for nickel chloride is $48\,mg\,kg^{-1}$ and the intraperitoneal LD_{50} is $11\,mg\,kg^{-1}$. Nickel chloride is a dermal sensitizer, and when injected interperitoneally into mice and rats caused a rapid decrease in body temperature. The alveolar macrophage was a cellular target for nickel toxicity following parenteral exposure to nickel chloride. Subcutaneous administration of nickel chloride to rats caused nickel uptake into and activation of alveolar macrophages, followed by reduced phagocytic capacity. Nickel chloride induces DNA damage in mouse leukocytes. The significant DNA damage observed after treatment with nickel chloride agrees with the results obtained for other metals like lead, chromium, and cadmium in mice and mercury in rats. The ability of nickel chloride to induce chromosome aberrations in male mice was tested by the micronucleus test and the dominant lethality test. Nickel chloride failed to produce micronuclei in polychromatic erythrocytes whereas cyclophosphamide, used as positive control, raised their incidence markedly. In contrast to the results obtained with cyclophosphamide, nickel chloride did not increase the rate of postimplantation death, but did decrease significantly the rate of pregnancy as well as the amount of preimplantation loss. Taking into account these results and the data in the literature, it was concluded that nickel probably has no clastogenic properties in mammals. Administration of nickel chloride to pregnant rats on days 7–11 of gestation resulted in significant embryotoxic effects such as increased resorption rate, decreased fetal weight, delays in skeletal ossification, and a high incidence of malformation.

Human

Inhalation of dust causes irritation of nose, throat, and eyes. Nickel poisoning has been reported in electroplaters who accidentally ingested water contaminated with nickel chloride, and there was rapid development of symptoms (e.g., nausea, vomiting, abdominal discomfort, diarrhea, giddiness, lassitude, headache, cough, dyspnea) that typically lasted a few hours, but persisted for 1–2 days in some cases.

Chronic Toxicity (or Exposure)

Animal

There is sufficient evidence in experimental animals for the carcinogenicity of metallic nickel, nickel monoxides, nickel hydroxides, and crystalline nickel sulfides. There is limited evidence in experimental animals for the carcinogenicity of nickel alloys, nickelocene, nickel carbonyl, nickel salts, nickel arsenides, nickel antimonide, nickel selenides, and nickel telluride. There is inadequate evidence in experimental animals for the carcinogenicity of nickel trioxide, amorphous nickel sulfide, and nickel titanate. In a two-stage carcinogenesis assay, orally administered nickel chloride enhanced the renal carcinogenicity of *N*-ethyl-*N*-hydroxyethyl nitrosamine in rats, but not the hepatocarcinogenicity of *N*-nitrosodiethylamine, the gastric carcinogenicity of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, the pancreatic carcinogenicity of *N*-nitrosobis(2-oxopropyl)amine, or the skin carcinogenicity of 7,12-dimethylbenzanthracene.

Human

Chronic exposure to aerosols of nickel chloride, emitted as mists from electroplating baths, may lead to chronic rhinitis, nasal sinusitis, anosmia, and perforation of the nasal septum. Asthma and chronic restrictive lung disease, nasal polyps and nasal septum perforation can also occur if nickel chloride is inhaled. Orally, nickel chloride can lead to cardiomyopathies. The International Agency for Research on Cancer deems nickel compounds to be carcinogenic to humans (group 1), that is, there is sufficient evidence in humans for the carcinogenicity of nickel sulfate, and of the combinations of nickel sulfides and oxides encountered in the nickel refining industry. There is inadequate evidence in humans for the carcinogenicity of metallic nickel and nickel alloys.

In Vitro Toxicity Data

Nickel chloride induced substantial induction of DNA repair synthesis in cultured Syrian hamster embryo and Chinese hamster ovary cells. Nickel chloride was not mutagenic in an *Escherichia coli* assay. The ability of nickel chloride to induce chromosomal

aberrations in Chinese hamster ovary and C3H1OT1/2-mouse cell lines was investigated, and nickel chloride induced chromosomal aberrations primarily in heterochromatin in both cell lines.

Clinical Management

Fluid replacement is indicated in the case of ingestion causing serious vomiting and diarrhea. For inhalation exposures, the patient should be moved to fresh air, and be provided with symptomatic and supportive treatment. Diethyldithiocarbamate is the chelating agent of choice. Disulfiram has been used to clear cases of nickel dermatitis.

Environmental Fate

Nickel chloride is water soluble and would be expected to release divalent nickel into the water.

Ecotoxicology

Numerous LC_{50} values are available for nickel chloride, for example, the LC_{50} for *Daphnia magna* (cladoceran) was $510\,\mu g\,l^{-1}$ for a 48 h test and $0.13\,mg\,l^{-1}$ for a 3 week test. Reproductive impairment of *Daphnia magna* was observed at 30–$95\,\mu g\,l^{-1}$ in a 64 h study.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average is $0.1\,mg\,m^{-3}$ as inhalable fraction. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA, is $1.0\,mg\,m^{-3}$. The (US) National Institute for Occupational Safety and Health (NIOSH) considers nickel metal and other compounds (as Ni) to be a potential occupational carcinogen. NIOSH usually recommends that occupational exposures to carcinogens be limited to the lowest feasible concentration.

Nickel chloride is listed as a US hazardous air pollutant, generally known or suspected to cause serious health problems. The Clean Air Act, as amended in 1990, directs the US Environmental Protection Agency (EPA) to set standards requiring major sources to sharply reduce routine emissions of toxic pollutants. EPA is required to establish and phase in specific performance based standards for all air emission sources that emit one or more of the listed pollutants. Nickel is also a toxic pollutant designated pursuant to Section 307(a) (1) of the Clean Water Act and is subject to effluent limitations.

See also: Metallothionein; Nickel and Nickel Compounds.

Further Reading

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Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.

International Agency for Research on Cancer (IARC) (1990) *Chromium, Nickel and Welding, Nickel and Nickel Compounds*, vol. 49, pp. 447–525. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Lyon, France: IARC Scientific Publications.

Maibach HI and Menne T (1989) *Nickel and the Skin: Immunology and Toxicology*. Boca Raton, FL: CRC Press.

Relevant Website

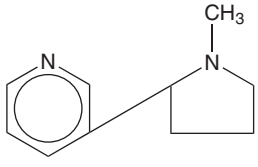
http://www.intox.org – (UK) National Poisons Information Service Centre of the United Kingdom. Nickel Chloride (an UKPID Monograph).

Nicotine

Brian Hughes

This article is a revision of the previous print edition article by Bonnie S Dean, volume 2, pp. 417–418, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 54-11-5
- Synonym: Methylpyridylpyrrolidine
- Chemical/Pharmaceutical/Other Class: Ganglionic cholinergic-receptor agonist
- Chemical Structure:



Uses

Nicotine is a highly toxic alkaloid found principally in tobacco products (cigarettes, 15–20 mg; cigars,

15–40 mg; snuff, 4.6–32 mg) and in smoking cessation products such as nicotine gum (2–4 mg per piece), lozenges (2–4 mg per piece), transdermal patches (7–52.5 mg per patch), nasal sprays (0.5 mg per spray), and inhalers (2–4 mg per use). It is also used as an insecticide.

Exposure Routes and Pathways

Exposure to nicotine occurs mainly through smoking tobacco products or inhalation of side-stream smoke. Dermal contact occurs most often through the use of therapeutic preparations (transdermal patch) designed for use in smoking cessation programs. Workers harvesting tobacco are also exposed dermally. Oral exposure occurs through the use of nicotine gum and accidental ingestion of tobacco products. Nicotine can also be absorbed through the nasal mucosa via the use of snuff.

Toxicokinetics

Absorption

Nicotine is a weak base with a pK_a value of 7.9. It is readily absorbed via the lung, oral mucosa, gastrointestinal (GI) tract, and skin. The dose of nicotine received via tobacco smoke varies a great deal and depends on the smoking behavior of the individual. Absorption of nicotine via deep lung inhalation of tobacco smoke is ~90% with a high amount of individual variability. Transdermal patches deliver 82% of the impregnated nicotine into systemic circulation. Absorption through the GI tract is limited in the stomach but extensive in the intestines due to a higher pH. However, extensive first pass metabolism by the liver limits the amount reaching the systemic circulation to 25–30% of the dose.

Distribution

Nicotine has an apparent volume of distribution in adults of ~1.7–3.0 l kg^{-1}. Plasma concentrations of nicotine appear to decline in a biphasic manner. Protein binding is ~4.9%. Under steady-state conditions, cotinine concentrations in the serum can be up to 10 times that of the parent compound. The half-life of nicotine in the initial phase is reportedly 2 or 3 min, and the half-life in the terminal phase is reportedly ~30–120 min. Nicotine is widely distributed in the body to the brain, lungs, adrenals, heart, GI tissue, spleen, thymus, kidney, skeletal muscle, saliva, and breast milk. Nicotine can also pass the placental barrier and enter the fetal tissue. Penetration through biological membranes occurs via passive diffusion rather than active transport.

Metabolism

Nicotine undergoes a large first-pass effect during which the liver metabolizes 80–90%. Small amounts are metabolized in the lungs and kidneys. The major metabolic pathway of nicotine is the C-oxidation to cotinine through a nicotine-Δ-1′-(5′)-iminium ion intermediate catalyzed by CYP2A6. Metabolism also occurs via *N*-oxidation, and glucuronidation of nicotine, cotinine, and *trans*-3-hydroxycotinine. Nicotine-1′-*N*-oxide is reduced to nicotine by bacterial flora in the large intestine via an *N*-oxide reductase system and subsequently undergoes enterohepatic circulation and repeat metabolism in the liver.

Excretion

Nicotine and its metabolites are quickly excreted in the urine. The half-life of nicotine in plasma is 1.6–2.8 h and that for cotinine is 8–29.3 h. Approximately 10–20% of the absorbed nicotine dose is excreted unchanged. Urinary excretion of nicotine is pH dependent, increasing in acid urine.

Mechanism of Toxicity

Nicotine exerts its effects by binding to nicotinic cholinergic receptors found in ganglia, the neuromuscular junction and the central nervous system. The prominent effects relate to an initial transient stimulation of the adrenal medulla, central nervous system (CNS), and cardiovascular system due to the release of catecholamines, gastrointestinal tract due to parasympathetic stimulation, salivary and bronchial glands, and the medullary vomiting center. Following these initial effects, nicotine causes blockade of the autonomic ganglia and the neuromuscular junction transmission, inhibition of catecholamine release from the adrenal medulla, and CNS depression.

Acute and Short-Term Toxicity (or Exposure)

Animal

Nicotine causes initial hyperexcitability, hyperpnea, salivation, vomiting, and diarrhea and then depression, incoordination, and paralysis in both small and large animals. The reported oral LD_{50} values for the male laboratory rat range from 50 to 188 mg kg^{-1}, and an LD_{50} of 288 mg kg^{-1} in female rats. The oral LD_{50} values for mice range from 3.34 to 24 mg kg^{-1}. Nicotine tends to be equally toxic through several exposure routes. The dermal LD_{50} for rabbits and mice are 50 and 140 mg kg^{-1}, respectively.

Human

Nicotine is a highly toxic alkaloid. It is water soluble, colorless, and bitter tasting. Few deaths have been reported from its use with the onset of symptoms much more rapid following the ingestion of liquid nicotine-containing products rather than with nicotine-containing organic material. Clinical manifestations include nausea, vomiting, abdominal pain, and increased salivation. Severe toxicity might result in headache, confusion, agitation, and restlessness, followed by lethargy, seizures, and coma. Severe toxicity with hypertension, tachycardia, and vasoconstriction has occurred from buccal absorption after biting a transdermal nicotine patch. The lethal oral dose of nicotine for adults has been established to be ~40–60 mg. Survival has been reported after ingestion of 1–4 g. Pediatric ingestion of 1–1.5 cigarettes resulted in episodes of weakness, limb jerking, and unresponsiveness. Children were asymptomatic if less than one cigarette was ingested.

Chronic Toxicity (or Exposure)

Animal

There are no apparent species differences in nicotine toxicity between animals and man. The toxic sequelae following chronic nicotine exposure to animals is similar to that in man including GI problems, cardiovascular effects, changes in neurochemistry, and reproductive effects such as decreased birth weight and length of gestation.

Human

Chronic exposure through tobacco use can produce nicotine dependence disorders. Tolerance may occur in some individuals. Effects that reinforce nicotine use include improved cognitive function and mood. Withdrawal symptoms following cessation of cigarette smoking may include anxiety, impaired concentration and memory, depression, hostility, sleep disturbances, and increased appetite. Correlation between smoking and alcoholism has been observed. Nicotine may also in part be responsible for cardiovascular diseases, lung cancer, and chronic pulmonary lung disease. Women who smoke during pregnancy also have greater risk of having children with low birth weight, and attention deficit disorders in latter in life.

In Vitro Toxicity Data

Nicotine was not found to be mutagenic in the Ames assay using several strains of *Salmonella typimurium* with or without S9 activation. Tests for aneuploidy/chromosome aberrations using *Neurospora crassa* were also negative. Nicotine did induce a slight increase in sister chromatid exchange rate in Chinese hamster ovary cells. Nicotine may be converted into other carcinogenic/mutagenic compounds such as nitrosonornicotine.

Clinical Management

Nicotine's rapid absorption, swift onset of symptoms, and quick metabolism and excretion necessitates the need for early supportive measures to be instituted in cases of severe acute intoxication. In mild toxicity, clinical effects may last 1–2 hours while in severe toxicity effects may persist for 8–24 h. Monitoring the patient's vital signs (pulse, blood pressure, respiration), and neurologic function, will enable one to determine if interventions are indicated. Such interventions may include continuous monitoring of the heart rate and rhythm with an electrocardiogram (if available) in the presence of an irregular and/or a rapid pulse, the administration of intravenous 0.9% NaCl, dopamine, or norepinephrine for hypotension and providing mechanically assisted respiration which may also include the use of supplemental oxygen (if accessible) for signs of respiratory depression. If seizure activity is noted, the use of diazepam or barbiturates should be considered as therapy for seizure control. Atropine may be used to control excess bronchial secretions, salivation, and diarrhea. For oral exposure to nicotine, GI decontamination procedures may be considered if performed soon after ingestion and only if the patient's level of consciousness allows this method to be used. If so, slurry of activated charcoal may be administered orally or via gastric lavage, since this will serve to decrease nicotine absorption in the intestinal tract. Emesis is usually spontaneous and the alkalinity of an antacid increases the absorption of nicotine. Therefore, neither ipecac for emesis nor antacid by mouth should be administered, as such treatment is contraindicated.

Environmental Fate and Toxicity

Environmental releases of nicotine occurred mainly from its use as an insecticide. At ambient temperatures, nicotine will exist as a vapor. The half-life in the atmosphere is ~4 h because it reacts with photochemically produced hydroxyl radicals. Nicotine's mobility in soil is somewhat dictated by the pH. Absorption on soil particles occurs in neutral and acidic soils when, as a weak base, nicotine is protonated. Nicotine has a high mobility in alkaline soils. Likewise, in water nicotine is not expected to bind to

sediment or suspended particles unless the pH is neutral or acidic. Information on the degradation of nicotine in soil is limited. The breakdown products include oxynicotine, 3-pyridylmethyl ketone, 2-3-dipyridyl, and *N*-methyl myosmine. The ability of nicotine to bioconcentrate in aquatic organisms is low (bioconcentration factor = 5).

Ecotoxicology

Nicotine was evaluated for acute aquatic toxicity in rainbow trout and dapnia. The mean 96 h LC_{50} for rainbow trout and the 48 h EC_{50} for dapnia are 4 and 0.24 mg l^{-1}, respectively. Nicotine sulfate was also evaluated in multiple aquatic species for lethality. The species and the corresponding toxicity are as follows: fathead minnow (96 h $LC_{50} = 19.7$ mg l^{-1}), rainbow trout (96 h $LC_{50} = 7.31$ mg l^{-1}), bluegill (96 h $LC_{50} = 4.31$ mg l^{-1}), goldfish (96 h $LC_{50} = 13.1$ mg l^{-1}), daphnia magna (48 h $EC_{50} = 3.25$ mg l^{-1}), midge (48 h $LC_{50} > 27$ mg l^{-1}), crayfish (96 h $LC_{50} > 38.2$ mg l^{-1}), and snail (96 h $LC_{50} > 38.2$ mg l^{-1}).

Other Hazards

There is a moderate explosion hazard when nicotine is exposed to heat or flame. Decomposition products include nitrogen oxides, carbon monoxide, and other highly toxic fumes. Nicotine is stable under normal conditions. Contact with oxidizing materials should be avoided. Heat or flames should be avoided.

Exposure Standards and Guidelines

The Occupational Safety and Health Association has set a permissible exposure limit of 0.5 mg m^{-3} (skin) as an 8 h time-weighted average. The American Conference of Governmental Industrial Hygienists guidelines are also set at this limit of 0.5 mg m^{-3}. The National Institute for Occupational Safety and Health recommended exposure limit is 0.5 mg m^{-3}. The level that is immediately dangerous to life or health is 5 mg m^{-3}.

Nicotine is a hazardous substance under the Comprehensive Environmental Response, Compensation, and Liability Act. Releases of 100 lb or more are reportable to the National Response Center. SARA Title III has established a threshold planning quantity requiring a facility that receives or produces 100 lb of nicotine to notify state emergency response and local emergency planning commissions. SARA Title III also requires state and local reporting of nicotine releases greater than or equal to 100 lb. The Federal Insecticide, Fungicide, and Rodenticide Act has established a tolerance of 2 ppm of nicotine on cucumber, lettuce, and tomatoes.

See also: Developmental Toxicology; Neurotoxicity; Tobacco; Tobacco Smoke.

Further Reading

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Gorrod J and Wahren J (eds.) (1993) *Nicotine and Related Alkaloids: Absorption, Distribution, Metabolism, Excretion*. New York: Chapman and Hall.

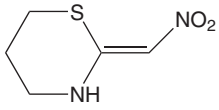
Piasechki M and Newhouse P (eds.) (2000) *Nicotine in Psychiatry: Psycopathology and Emerging Therapeutics*. Washington, DC: American Psychiatric Press.

Yildiz D (2004) Nicotine, its metabolism and an overview of its biological effects. *Toxicon* 43(6): 619–632.

Nithiazine

Josef Seifert

- Chemical Abstracts Service Registry Number: CAS 58842-20-9
- Synonyms: (*E*,*Z*)-2-Nitromethylene-1,3-thiazinane (IUPAC); Tetrahydro-2-(nitromethylene)-2*H*-1,3-thiazine (CAS); 2*H*-1,3-Thiazine; Tetrahydro-2-(nitromethylene) (label)
- Chemical/Pharmaceutical/Other Class: Neonicotinoid insecticide
- Chemical Formula: $C_5H_8N_2O_2S$
- Chemical Structure:



Uses

Nithiazine is used as an active ingredient in fly strips (QuickStrike® Fly Abatement Strip). QuickStrike® strips are most effective against houseflies (*Musca*

domestica). The strips can be placed in or around the house, animal housing, recycling facilities, or other facilities where abundant houseflies are a nuisance.

Background Information

Nithiazine has historical importance as a compound on which neonicotinoids, a new class of insecticides, have been modeled. Its synthesis was accomplished by Shell (Modesto, CA) in the 1970s based on selection from a series of nitroalkyl heterocyclic compounds.

Exposure Routes and Pathways

Exposure due to inhalation is minimal because nithiazine has a low vapor pressure (4×10^{-7} mmHg). Accidental dermal contact and ingestion are two potential exposure routes.

Mechanism of Toxicity

Nithiazine is an agonist acting at the neural nicotinic acetylcholine receptor – Na^+/K^+ ionophore in mammals and insects. The nicotinic acetylcholine receptors regulate the flow of Na^+ and K^+ through the channels in the neural postsynaptic membranes. Opening and closing of the channels by acetylcholine maintains the dynamic ratio of the intracellular to extracellular concentrations of Na^+ and K^+ needed for the initiation of the signal in the postsynaptic neuron. The structural differences between insect and mammalian receptors determine the high selectivity of nithiazine toxicity to insects.

Acute and Short-Term Toxicity (or Exposure)

Animal

The data on nithiazine toxicity are scarce since nithiazine is being registered only for terrestrial nonfood use. High oral dosages ($>100\,mg\,kg^{-1}$ body weight) of nithiazine to male rats caused a decrease in body temperature, decreased locomotor activity (rearing), lower arousal, increased salivation and increased fecal boluses. These signs were more extensive in female rats. Additional functional observations in female rats were tremors, abnormal cage behavior, increased urination, ataxic gait, reduced reaction to visual stimuli and tail pinch, and reduced visual placing response. The results of gross necropsy and neurohistology were normal.

Human

Very little is known regarding acute toxicity of nithiazine in humans.

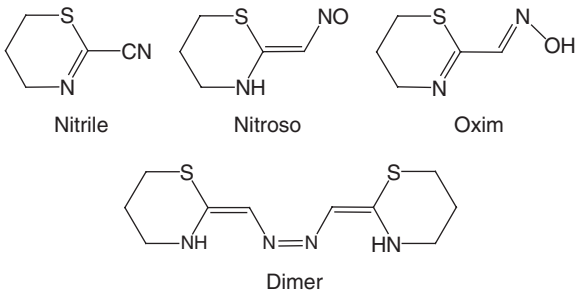


Figure 1 The major products of nithiazine photoreduction and dimerization.

Chronic Toxicity (or Exposure)

Little is known regarding chronic toxicity of nithiazine in humans or animals. It is reasonable to conclude, however, based on the data obtained in animal studies and *in vitro* tests, and on nithiazine affinity for insect nicotinic acetylcholine receptors, that nithiazine toxicity to humans and domestic animals or pets is low.

In Vitro Toxicity Data

Tests for genetic toxicity (Ames assay using *Salmonella typhimurium*, mouse bone marrow micronucleus assay, chromosome aberrations in human lymphocytes and/or Chinese hamster ovary cells, *in vitro* unscheduled DNA synthesis assay in primary rat hepatocytes) were all negative.

Environmental Fate

Nithiazine is extremely unstable in sunlight due to the nitromethylene chromophore. This group absorbs strongly in water at 365 nm with an extinction coefficient $\sim 40\,000\,M^{-1}\,cm^{-1}$. Direct irradiation results in a loss of the insecticidal activity and formation of a mixture of more than 40 degradation products. The major products of nithiazine photoreduction and dimerization from irradiation at 360 nm in water are nitrile, nitroso, and oxim derivatives, and dimers (**Figure 1**). Consequently, nithiazine half-time on foliage is only ~ 30 min. Low photostability is the primary reason that nithiazine is not used as an insecticide for field and other environmental applications.

See also: Acetylcholine; Neonicotinoids.

Further Reading

Kollmeyer WD, Flattum RF, Foster JP, *et al.* (1999) Discovery of the nitromethylene heterocycle insecticides. In: Yamamoto I and Casida JE (eds.) *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*, ch. 3, pp. 71–89. Berlin: Springer.

Nitric Oxide

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 10102-43-9
- Synonyms: Mononitrogen monoxide; Nitrogen monoxide
- Chemical/Pharmaceutical/Other Class: Gas
- Chemical Formula: NO
- Chemical Structure: N = O

Uses

Nitric oxide is used in the manufacturing of nitric acid. It is also used as a stabilizer for propylene and methyl ether, and to bleach rayon. Nitric oxide is a natural product of fuel combustion and a component of smog.

Exposure Routes and Pathways

Since it is a gas, inhalation is the most likely route of exposure. However, nitric oxide is also produced endogenously from arginine by humans.

Toxicokinetics

When exposed to air, nitric oxide may be converted to nitrogen dioxide or nitrogen tetraoxide, both of which are highly toxic. Conversion is slower at concentrations below 1 ppm. Other contaminants, such as ozone in the air, expedite the conversion process.

Mechanism of Toxicity

A cytotoxic free radical, nitric oxide, impairs mitochondrial ATP synthesis by inhibiting the citrate cycle and other cellular mechanisms of electron transport.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation can lead to the formation of methemoglobin and resultant toxic effects; for example, respiratory distress. The inhalation LC_{50} in rats is 1068 mg m^{-3}. In mice, $LC_{Lo} = 320$ ppm.

Human

Contact with skin and mucous membranes, such as those in the eyes and lungs, may be highly irritating. Inhalation leads to methemoglobin formation, which interferes with normal oxygen utilization. This can lead to fatigue, uneasiness, and respiratory distress.

Chronic Toxicity (or Exposure)

Animal

Nitric oxide is a mutagen in somatic cells. It has been shown to cause lung damage after long exposure periods.

Human

Effects similar to those seen on acute exposure are also seen chronically. In addition, if the compound is converted to nitrogen dioxide or nitrogen tetraoxide, toxicities associated with these compounds can also be observed.

Clinical Management

Oxygen therapy should be provided for cyanosis of dyspnea. Prednisone or predinsolone should be given at 5 mg, every 6 h, to reduce inflammation in the lungs.

Environmental Fate

Nitric oxide is converted spontaneously in the air to nitrogen dioxide; hence, some of the latter gas is present whenever nitric oxide is found in air (at concentrations below 50 ppm).

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 25 ppm.

See also: Photochemical Oxidants; Pollution, Air.

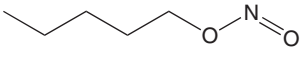
Further Reading

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, vol. 3. New York: Wiley.
Wang T, El Kebir D, and Blaire G (2003) Inhaled nitric oxide in 2003: A review of its mechanisms of action. *Canadian Journal of Anaesthesia* 50(8): 839–846.
Weinberger B, *et al.* (2001) The toxicology of inhaled nitric oxide. *Toxicological Sciences* 59(1): 5–16.

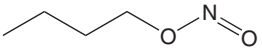
Nitrite Inhalants

Keiko Okamoto

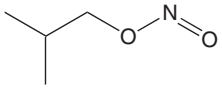
- Representative Chemicals: Amyl nitrite; Butyl nitrite; Isobutyl nitrite
- Chemical Abstracts Service Registry Numbers: Amyl nitrite (CAS 463-04-07); Butyl nitrite (CAS 544-16-1); Isobutyl nitrite (CAS 542-56-3)
- Synonyms:
  - Amyl nitrite: Isoamyl nitrite; *n*-Amyl nitrite; Nitramyl; Nitrous acid; Nitropentane; Nitrous acid; Pentyl alcohol nitrite; Pentyl ester; Pentyl nitrite
  - Butyl nitrite: 1-Butyl-nitrite; 2-Methylpropyl ester; Butyl ester; Isobutyl ester; Isobutyl nitrite; *n*-Butyl nitrite; *s*-Butyl nitrite; *t*-Butyl nitrite; Cyclohexyl nitrite; Aimies; Ames; Amys; Bang; Bolt; Bullet; Climax; Discoroma; Flash; Hardware; HiBall; Jack aroma; Jungle Juice; Lightning Bolt; Liquid Gold; Locker Room; Mama Poppers; Natural Brutes; Odor of Man; OZ; Poppers; Quick Silver; Ram; Rush; Satan's Secret; Snappers; Sweat; Thrust
- Chemical/Pharmaceutical/Other Class: Volatile organic nitrites; Aliphatic nitrites
- Chemical Formulas:
  - Amyl nitrite: $C_5H_{11}NO_2$
  - Butyl nitrite: $C_4H_9NO_2$
  - Isobutyl nitrite: $C_4H_9NO_2$
- Chemical Structures:



Amyl nitrite



Butyl nitrite



Isobutyl nitrite

Uses

Amyl nitrite has been used as a vasodilator drug, a diagnostic agent, and a cyanide treatment adjunct; it is an abused inhalant. Butyl nitrite is an abused inhalant. Isobutyl nitrite is an ingredient of various incenses or room odorizers, and it is also used as a jet propellant and in the preparation of fuels. It is an abused inhalant.

Exposure Routes and Pathways

Nitrites are usually inhaled but have been ingested, either accidentally or with suicidal intent.

Toxicokinetics

Effects following inhalation occur in 10 s, peak at 30–60 s, and last ~3–5 min. Nitrites are hydrolyzed to the nitrite ion and alcohol within seconds. Approximately 60% of the nitrite ion is biotransformed; ammonia is a metabolite. Almost 40% of the nitrite ion is excreted unchanged via the kidneys. Elimination follows first-order kinetics.

Mechanism of Toxicity

Nitrites produce relaxation of vascular smooth muscles, causing cardiovascular effects through coronary and peripheral vasodilation. They are oxidizing agents and, in excess, induce formation of methemoglobin, abnormal hemoglobin that is unable to bind and transport oxygen or carbon dioxide. Nitrites can also precipitate an intravascular hemolysis with formation of Heinz bodies. The resulting anemia can compound the hypoxic effects of methemoglobinemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hearing loss, weight loss, lacrimation, changes in visual acuity, nausea, vomiting, decreased motor performance, tachypnea, seizures, immunosuppression, decrease in immune cells, methemoglobinemia, and alteration in hepatic angiogenic gene expression have been induced in animal studies.

Human

Users claim aphrodisiac effects from heightened stimulation, enhanced penile erection, and relaxation of the anal sphincter and rectum. Because of the rush of blood and oxygen to the brain, facial flushing and a temporary euphoria occur several seconds after inhalation. Perception of time is slowed.

Typical effects of inhalation abuse are dizziness, palpitations, blurred vision, headache, nausea, and stinging of the nasal passages, eyes, and throat. There is a fall in blood pressure with a reflexive increase in pulse rate. Pulmonary irritation, tachypnea, and shortness of breath are described. Cardiovascular

collapse, coma, anion gap acidosis, and seizures can occur in severely poisoned patients. Methemoglobinemia may occur, characterized by cyanosis and respiratory depression. Ingestion of nitrites seems to produce a more rapid and malignant methemoglobinemia than inhalation. Blood methemoglobin levels should be monitored in symptomatic patients. Methemoglobin levels of 20–30% produce mild symptoms, levels of 30–45% produce moderate effects, and levels of 50–70% are associated with severe toxicity. Levels $\geqslant$70% are often lethal if untreated. Plasma nitrite levels are not clinically useful.

Chronic Toxicity (or Exposure)

Animal

A significant evidence of increase in tumor growth and carcinogenic activity of isobutyl nitrite were observed in male and female rats in 2 year inhalation studies.

Human

Repetitive abuse can cause crusting skin lesions and telangiectasis (angioma or hyperemic spots). Tracheobronchial irritation with dyspnea and hemoptysis has been reported. Withdrawal from industrial exposure has resulted in respiratory failure, left ventricular hypertrophy, and myocardial infarctions. Damage to the lungs, liver, kidneys, bone marrow, and brain is possible. Nitrite inhalants are thought to be carcinogenic and immunosuppressive. Tolerance occurs.

In Vitro Toxicity Data

Isobutyl nitrite was found to be mutagenic in *in vitro* studies.

Clinical Management

Airway management, respiratory support, and high-flow oxygen are indicated for the cyanotic patient. The cardiovascular, neurological, and metabolic effects should respond to the usual therapeutic agents. Methemoglobinemia is treated with 1 or 2 mg kg^{-1} of methylene blue, given intravenously over a 5 min period and repeated if needed. Methylene blue is recommended for symptomatic patients and for patients with methemoglobin levels $\geqslant$30%. Patients with preexisting anemia or cardiovascular disease may need treatment even if their methemoglobin levels are as low as 15%. Exchange transfusion has been employed in severely symptomatic patients who were unresponsive to methylene blue treatment. Hyperbaric oxygen can be supportive while preparing for exchange transfusion. Gastric decontamination is indicated for oral exposure. It will be necessary to monitor complete blood count and arterial blood gases.

See also: Amyl Nitrite; Butyl Nitrite.

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Relevant Website

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Nitrites

Betty J Locey

This article is a revision of the previous print edition article by Robin Guy, volume 2, pp. 420–421, © 1998, Elsevier Inc.

- Representative Chemical: Sodium nitrite
- Chemical Abstract Service Registry Numbers: CAS 14797-65-0 (Nitrite); CAS 7632-00-0 (Sodium nitrite)
- Chemical Formulas: NO_2^- (Nitrite); $NaNO_2$ (Sodium nitrite)

Uses

Nitrites have been used as vasodilators, as circulatory (blood pressure) depressants, as antidotes for cyanide and hydrogen sulfide poisoning (amyl and sodium nitrites), and to relieve smooth muscle

spasm. Topical silver nitrate is used in burn therapy. Sodium nitrite is commonly used to cure meats. It retards the onset of rancidity and the development of unpalatable odors and flavors during storage and delays the development of botulinal toxin. In addition, nitrites produce a desirable flavor and pink color in the cured meat. Sodium nitrite has also been used as an anticorrosive agent in cooling fluids. The greatest use of nitrates is as a fertilizer.

Exposure Routes and Pathways

Exposure may occur through oral, dermal, ocular, and inhalation routes. People may be exposed to nitrites through foods such as cured meats and selected vegetables (e.g., broccoli, spinach, cauliflower, collard greens, and root vegetables). Accidental poisonings have occurred when people have mistaken sodium nitrate for table salt.

People may also be exposed through contaminated drinking water. Individual wells drawing from shallow groundwater in areas where nitrogen-based fertilizers are used are at higher risk of contamination. Of particular concern are potential infant exposures both through water and through water used to prepare formula.

Nitrites and nitrates have a number of medicinal uses. Abuse of volatile nitrites (amyl, butyl, and isobutyl nitrites intended for medical use) as recreational drugs (e.g., psychedelics) has been reported. On the street, they may be called 'rush', 'poppers', and 'snappers'.

Toxicokinetics

Nitrites are absorbed orally, dermally, and through the lungs. Nitrites (NO_2^-) and nitrates (NO_3^-) are interconverted in the body. Ingested nitrates are rapidly absorbed through the upper intestine (proximal small bowel) and then rapidly distributed throughout the body. Nitrates in the blood then enter the lower, large intestine, where they are converted to reactive nitrites by bacteria in the gut (fecal organisms). Nitrites are then reabsorbed from the lower intestine back into the blood where they react with the ferrous ion (Fe^{2+}) in deoxyhemoglobin in red blood cells converting it to methemoglobin (containing ferric iron; Fe^{3+}). Some nitrate in the blood may be excreted in saliva through an active blood nitrate transport system and reintroduced into the beginning of the gastrointestinal tract. Nitrates are also metabolized in the liver. Nitrates and metabolites are excreted in the urine. The half-life of nitrate is generally less than 1 h; however, metabolites have half-lives from 1 to 8 h.

Transplacental passage of nitrite occurred in pregnant rats given doses of 2.5–50 mg kg^{-1} orally. In mice given 400, 800, or 1200 mg sodium nitrite orally in drinking water, 99.1–99.5% of the dose was eliminated. The remaining nitrite was transformed into nitrate and recovered from the liver and muscle.

Mechanism of Toxicity

Nitrites cause relaxation of smooth muscle and the conversion of hemoglobin to methemoglobin. Nitrites in the blood react with the ferrous ion (Fe^{2+}) in deoxyhemoglobin in red blood cells, converting it to methemoglobin containing ferric iron (Fe^{3+}). Methemoglobin with ferric iron (Fe^{3+}) cannot bind and transport oxygen to tissues and cells throughout the body. Oxygen is necessary for cells to generate energy and function. Systems in the body with the highest oxygen demand are most vulnerable. These include the central nervous system, particularly the areas that control breathing, and the heart.

In general, the effects associated with exposure to nitrite (NO^{2-}) and nitrite-containing compounds are the same whether ingested, inhaled, or produced *in vivo* (e.g., produced from nitrate). Ingested nitrate (NO^{3-}) is generally metabolized and excreted without producing adverse effects unless conditions are favorable for conversion to nitrite (e.g., higher pH, presence of certain intestinal flora).

Acute and Short-Term Toxicity (or Exposure)

Animal

Nitrite is irritating to eyes, skin, and respiratory tract. Exposure to sufficiently high concentrations can cause death. Many animal species lack the nitrate-reducing bacteria and therefore do not provide a good model for methemoglobin formation.

Significant levels of methemoglobinemia were produced when mice were exposed to butyl nitrites via inhalation. Pretreatment of the mice with methylene blue prevented the methemoglobin formation associated with the butyl nitrite exposure. A single intravenous dose of 30 mg kg^{-1} of sodium nitrite caused methemoglobinemia in dogs. The minimum lethal dose of sodium nitrite is estimated to be 150–170 mg kg^{-1} in cattle and ~70–75 mg kg^{-1} in pigs.

Human

Nitrite is a severe respiratory, eye, and skin irritant. Acute exposure to high levels of nitrites can be fatal. Exposure may cause visual field defects, hypotension, tachycardia, respiratory depression, and cyanosis due to formation of methemoglobinemia. Symptoms may

also include headache, confusion, dizziness, convulsions, unconsciousness, nausea, vomiting, and diarrhea.

Infants are much more sensitive to nitrite and nitrate toxicity. Infants have a higher stomach pH (generally greater than 4) than adults (generally a pH of ~ 2), generally have higher levels of nitrate reducing bacteria in their gut, have lower enzymatic capacity to reduce methemoglobin to hemoglobin, and the presence of hemoglobin F, which is more susceptible to oxidation by nitrites.

In addition, fetal hemoglobin (hemoglobin F) is oxidized by nitrite to methemoglobin at a rate twice as that of adult hemoglobin (hemoglobin A). Furthermore, enzymatic capacity of erythrocytes of newborn infants to reduce methemoglobin to hemoglobin appears less than that of adults. Methemoglobin anemia can cause the child's health to degrade in several days and can ultimately cause death. Symptoms include shortness of breath and a blue cast to the skin. In infants the condition has been referred to as the blue baby syndrome.

Chronic Toxicity (or Exposure)

Animal

In one study, rats received sodium nitrite at 100 mg kg^{-1} in drinking water daily during their entire life span over three generations and no evidence of chronic toxicity, carcinogenicity, or teratogenicity was observed.

Human

Long-term exposure to nitrites and nitrates at high enough levels may cause an increase in the formation of urine by the kidney (diuresis), increased starchy deposits, and bleeding of the spleen.

Nitrites are generally not classified as human carcinogens. Under certain conditions nitrites may combine with amines in the body to form nitrosamines. There are a number of different nitrosamines; many are regulated as human carcinogens. Certain chemicals, such as vitamin C (ascorbic acid), can limit the transformation of nitrites to nitrosamines. US Department of Agriculture (USDA) requires the addition of ascorbic acid or erythorbic acid to bacon cure to reduce the risk of nitrosamine formation.

In Vitro Toxicity Data

Nitrites have tested negative in DNA repair bacterial assays. Positive results have been reported in mammalian cytogenetics and sister chromatid exchange studies.

Clinical Management

If exposed via inhalation, the victim should be moved to fresh air and monitored for respiratory distress. Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. Exposed areas of skin should be washed thoroughly with soap and tepid water. Medical attention should be sought.

Emesis and/or activated charcoal/cathartic may be indicated if the patient is conscious. For seizures, diazepam may be administered as an intravenous bolus. For hypotension, intravenous fluids may be indicated.

Methemoglobinemia may be noted and associated with a cyanosis. In general, plasma levels of nitrites and related compounds are not clinically useful. Methemoglobin concentration should be determined in all cyanotic patients or patients experiencing respiratory distress. Arterial blood gases should be monitored in symptomatic or cyanotic patients. Methemoglobinemia is often treated by intravenous administration of methylene blue (dose generally 1–2 mg kg^{-1}) over a 5–10 min period. Cyanosis should begin to improve within 15 min of treatment with methylene blue. A second dose of methylene blue may be indicated if improvement is not observed. Methylene blue is not indicated if the patient is G-6-PD deficient as hemolytic anemia can develop. If the condition is life-threatening, treatment may include transfusion and hyperbaric oxygen therapy.

Environmental Fate

Nitrite (NO_2^-) and nitrate (NO_3^-) are naturally occurring inorganic ions that can combine with various organic and inorganic species and compounds. Microbial action in soil or water decomposes wastes containing organic nitrogen first into ammonia, which is then oxidized to nitrite and nitrate (part of the 'nitrogen cycle'). Nitrite is easily oxidized to nitrate under environmental conditions and nitrates are more commonly found in surface water and groundwater. The levels of nitrate found in water are generally increased if impacted by organic wastes, fertilizers, and/or ammonia. In soil, nitrate-containing compounds are generally water soluble and readily leach from soil to groundwater.

Ecotoxicology

Nitrites are toxic to aquatic organisms. *Nitrosomonas* sp. bacteria oxidize ammonia to nitrite. Ammonia is produced by decomposing organic mater and excreted fish. Nitrites are generally less toxic to fish than ammonia; however, chronic exposure to low levels increase stress on the fish population leading to stress-related disease states such as fin rot and bacterial ulcers. At higher concentrations, nitrites can cause damage to fish skin and gills and increase

the likelihood of bacteria infections and the success of parasitic organisms. In addition, higher levels in the blood stream may lead to conversion of hemoglobin to methemoglobin, reducing the fish's ability to transport oxygen potentially leading to asphyxiation.

Other Hazards

Nitrites are generally incompatible with flammable materials, strong oxidizing agents, reducing agents, organics, and finely powdered metals. They tend to be hygroscopic (draw moisture from the atmosphere). Sodium nitrite is not combustible; however, it may enhance the combustion of other compounds. Reactions may lead to fire and/or explosions. Fires may produce irritating and toxic fumes.

Exposure Standards and Guidelines

The US federal primary drinking water standard, maximum contaminant level (MCL), and maximum contaminant level goal (MCLG) for nitrite (measured as nitrogen) are both set at $1\,mg\,l^{-1}$. The MCL was established to be protective of infants (below 6 months of age). The MCL and MCLG for nitrates are $10\,mg\,l^{-1}$.

The US Environmental Protection Agency Integrated Risk Information System provides a chronic oral reference dose (RfD) of 0.1 mg/kg-day (file last update September 1997). The RfD is based on a critical effect of methemoglobinemia in infants chronically exposed to nitrites in drinking water.

The US Food and Drug Administration (US FDA) is the federal agency responsible for monitoring proper use of nitrite by meat processors. USDA established guidelines to reduce/eliminate nitrosamine formation in nitrite and nitrate cured meats in 1973 (see *Federal Register*, Vol. 38, No. 221, Friday, November 16, 1973, p. 31 679). Levels of sodium nitrite that can be used in curing meat are defined in under the Meat Inspection Regulations (Title 9, Chapter 111, Subchapter A, Code of Federal Regulations, 1974). The final calculated concentration of sodium nitrite in cured meat products (processed with nitrites, nitrates, or combination) cannot exceed 200 ppm.

See also: Blood; Food Additives; Food and Drug Administration, US; Food Safety and Toxicology; Gastrointestinal System; Nitrite Inhalants; Nitrosamines; Respiratory Tract.

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USEPA (1990) *National Pesticide Survey: Summary Results of Pesticides in Drinking Water Wells*. Washington, DC: US Environmental Protection Agency, Office of Pesticides and Toxic Substances.

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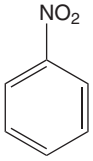
http://books.nap.edu – National Academy Press website.

http://www.epa.gov – USEPA. Integrated Risk Information System. Nitrite. CAS 14797-65-0. See also: USEPA. Office of Groundwater and Drinking Water. Consumer Factsheet on Nitrates/Nitrites.

Nitrobenzene

Robin C Guy

- Chemical Abstracts Service Registry Number: CAS 98-95-3
- Synonyms: Nitrobenzol; Essence of mirbane; Essence of myrbane: Mirbane oil
- Chemical/Pharmaceutical/Other Class: A chemical intermediate and solvent
- Chemical Formula: $C_6H_5NO_2$
- Chemical Structure:



Uses

Nitrobenzene is an industrial chemical. Most of the nitrobenzene produced in the United States is used to manufacture aniline. Nitrobenzene is also used to produce lubricating oils such as those used in motors and machinery. A small amount of nitrobenzene is used, sometimes as a solvent, in the manufacture of dyes, polishes, paints, drugs, pesticides, and synthetic rubber (Office of Pollution Prevention and Toxics, OPPT).

Exposure Routes and Pathways

Dermal contact, inhalation, and ingestion are possible exposure pathways.

Toxicokinetics

Nitrobenzene activation in rats to methemoglobin-forming metabolites appears to be mediated to a significant degree by intestinal microflora. In animal studies, the major part of nitrobenzene ($\sim$80% of the dose) is metabolized and eliminated within 3 days (WHO). The remainder is eliminated slowly. The slow compartment is likely due to erythrocyte recycling of nitrobenzene redox forms and glutathione conjugates. Covalent binding, presumably to sulfhydryl groups of hemoglobin, was demonstrated. In rodents and rabbits, *p*-nitrophenol and *p*-aminophenol are major urinary metabolites. In humans, part of the absorbed dose is excreted into the urine; 10–20% of the dose is excreted as *p*-nitrophenol (which thus may be used for biological monitoring). The half-life of elimination for *p*-nitrophenol is estimated to be ~ 5 h (initial phase) and > 20 h (late phase). The urinary metabolite *p*-aminophenol is significant only at higher doses.

Mechanism of Toxicity

Intestinal microflora present in animals may be responsible for reduction of nitrobenzene *in vivo* and subsequent methemoglobin formation.

Acute and Short-Term Toxicity (or Exposure)

Animal

In subchronic oral and dermal studies in mice and rats, central nervous system lesions in the cerebellum and brain stem were apparent and included petechial hemorrhages (WHO). These may be direct toxic effects or mediated by vascular effects of hypoxia or hepatic toxicity. Depending on the dose, these neurotoxic effects were grossly apparent as ataxia, head-tilt and arching, loss of righting reflex, tremors, coma, and convulsions. Other target organs included kidney (increased weight, glomerular and tubular epithelial swelling, and pigmentation of tubular epithelial cells), nasal epithelium (glandularization of the respiratory epithelium, pigment deposition in and degeneration of olfactory epithelium), thyroid (follicular cell hyperplasia), thymus (involution) and pancreas (mononuclear cell infiltration), while lung pathology (emphysema, atelectasis and bronchiolization of alveolar cell walls) was reported in rabbits.

Nitrobenzene causes toxicity in multiple organs by all routes of exposure. Methemoglobinemia results from oral, dermal, subcutaneous, and inhalation nitrobenzene exposure in mice and rats, with consequent hemolytic anemia, splenic congestion and liver, bone marrow and spleen hematopoiesis. In rodents, methemoglobinemia, hematological effects, impaired male fertility with significant testicular toxicity, and, in the inhalation studies, effects on the respiratory system were found at the lowest doses tested. Methemoglobinemia, bilateral epididymal hypospermia and bilateral testicular atrophy were observed at the lowest exposure level studied, 5 mg m^{-3} (1 ppm), in rats. The oral LD_{50} in rats was 640 mg kg^{-1}.

In mice, there was a dose-related increase in the incidence of bronchialization of alveolar walls and

alveolar/bronchial hyperplasia at the lowest dose tested of 26 mg m^{-3} (5 ppm).

Human

WHO reports that acute poisonings by nitrobenzene in consumer products have occurred frequently in the past. Significant human exposure is possible, due to the moderate vapor pressure of nitrobenzene and extensive skin absorption. Furthermore, the relatively pleasant almond smell of nitrobenzene may not discourage people from consuming contaminated food or water.

A small amount of nitrobenzene may cause mild irritation if it contacts the skin or eyes directly. Repeated exposures to a high concentration of nitrobenzene can result in methemoglobinemia, a condition in which the blood's ability to carry oxygen is reduced. During this condition, the skin may turn a bluish color and nausea, vomiting, and shortness of breath may occur. Effects such as headache, irritability, dizziness, weakness, and drowsiness may also occur. There is also some evidence that breathing high concentrations of nitrobenzene may damage the liver and spleen.

Nitrobenzene is toxic by all routes of exposure. Symptoms may be delayed for up to 1–4 h. Methemoglobinemia associated with headache, nausea, lethargy, depressed respiration, and cyanosis may occur. A bitter almond odor may be present, which suggests cyanide poisoning, but cyanide produces symptoms much more rapidly than nitrobenzene. Tachycardia, hypotension, respiratory depression/failure, and cardiac arrhythmias may be observed. Repeated exposure may be followed by liver impairment up to yellow atrophy, hemolytic icterus, and anemia of varying degrees, with the presence of Heinz bodies in the red blood cells. Pregnant women may be especially at risk due to transplacental passage. Individuals with glucose-6-phosphate dehydrogenase deficiency may also be special-risk groups (Environmental Protection Agency, EPA). Additionally, because alcohol ingestion or chronic alcoholism can lower the lethal toxic dose of nitrobenzene, individuals consuming alcoholic beverages may be at risk.

Chronic Toxicity (or Exposure)

Animal

Carcinogenic response was observed after exposure to nitrobenzene in rats and mice: mammary adenocarcinomas were observed in female B6C3F$_1$ mice, liver carcinomas in male Fischer-344 rats and thyroid follicular cell adenocarcinomas in male Fischer-344 rats. Benign tumors were observed in five organs.

Human

As repeat exposure to nitrobenzene in air over a lifetime causes cancer in animals, nitrobenzene may likewise cause cancer in humans (OPPT). The International Agency for Research on Cancer (IARC) has determined that nitrobenzene is possibly carcinogenic to humans.

In Vitro Toxicity Data

Nitrobenzene was nongenotoxic in *Salmonella typhimurium* and mammalian cells *in vitro* and in mammalian cells *in vivo*. Studies reported included DNA damage and repair assays, gene mutation assays, chromosomal effects assays, and cell transformation assays.

Clinical Management

Nitrobenzene is toxic by all routes including skin absorption. Systemic effects may be delayed a few hours. Poisoning closely resembles aniline. Initial care should include adequate gastrointestinal (gastric lavage as indicated and activated charcoal) and dermal decontamination. The patient should be given oxygen and monitored for cyanosis. Cardiac rhythm should be monitored in symptomatic patients.

Plasma nitrobenzene levels are not clinically useful. The metabolites in urine, *p*-nitro- and *p*-aminophenol, primarily in long-term exposure to nitrobenzene can be used as evidence of exposure. Methemoglobin levels should be determined in all cyanotic patients; cyanosis that does not respond to oxygen therapy may appear when the plasma methemoglobin level is 15%. Symptomatic methemoglobinemia should be treated with methylene blue. For seizures, diazepam should be administered via an intravenous bolus.

Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min and exposed areas should be washed extremely thoroughly with soap and water.

Ecotoxicology

Very low levels of nitrobenzene may be found in air. It may be present in water from industrial releases but in water, nitrobenzene is broken down by sunlight. Nitrobenzene is a liquid that does not bind well to soil; therefore, in the soil, it can move into the groundwater, be taken up by plants, evaporate to the air, and be broken down by bacteria. It does not appear to concentrate in fish or other aquatic animals. Most releases of nitrobenzene to the US environment are to underground injection sites. In 1992, only a small percent (6%) of environmental releases of

nitrobenzene was to air (OPPT). It can also evaporate slowly from water and soil exposed to air.

WHO summarized air quality data from various sources and reported that some measured levels in air in US cities in the early 1980s ranged between <0.05 and $2.1\,\mu g\,m^{-3}$ (<0.01 and 0.41 ppb). Data reported by the US EPA in 1985 indicated that less than 25% of air samples in the United States were positive, with a median concentration of $\sim 0.05\,\mu g\,m^{-3}$ (0.01 ppb); in urban areas, mean levels were generally less than $1\,\mu g\,m^{-3}$ (0.2 ppb), with slightly higher levels in industrial areas (mean $2.0\,\mu g\,m^{-3}$ (0.40 ppb)). Of 49 air samples measured in Japan in 1991, 42 had a detectable level, measured as 0.0022–$0.16\,\mu g\,m^{-3}$. Levels over urban areas and waste disposal sites were significantly lower (or undetectable) in winter than in summer.

WHO also summarized water quality data from various sources and reported that data on nitrobenzene levels in surface water appear to be more extensive than data on levels in air. While levels are variable depending on location and season, generally low levels (~ 0.1–$1\,\mu g\,l^{-1}$) have been measured. One of the highest levels reported was $67\,\mu g\,l^{-1}$, in the river Danube, Yugoslavia, in 1990. However, nitrobenzene was not detected in any surface water samples collected near a large number of hazardous waste sites in the United States (reported in 1988). Based on limited data, it appears that there may be greater potential for contamination of groundwater than of surface water; several sites measured in the United States in the late 1980s had levels of 210–250 and $1400\,\mu g\,l^{-1}$ (with much higher levels at a coal gasification site). Nitrobenzene has been reported in studies conducted in the 1970s and 1980s on drinking water in the United States and the United Kingdom, albeit in only a small proportion of samples, but was not detected in 30 Canadian samples (1982 report).

Exposure Standards and Guidelines

The estimated mean lethal adult dose is ~ 1–5 g. Children may be much more susceptible.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Comprehensive Environmental Response, Compensation, and Liability Act, US; Consumer Product Safety Commission; National Institute for Occupational Safety and Health; Occupational Safety and Health Administration; Pollution Prevention Act, US; Toxic Substances Control Act, US.

Relevant Websites

http://www.who.int – World Health Organization (WHO), Environmental Health Criteria, No. 230: Nitrobenzene.
http://www.epa.gov – US Environmental Protection Agency. Chemical Fact Sheet on Nitrobenzene.
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Nitrobenzene.

Nitrocellulose

Dennis J Naas

- Chemical Abstracts Service Registry Number: CAS 9004-70-0
- Synonyms: Cellulose nitrate; Nitrocotton; Soluble gun cotton; Pyroxylin; Various trade names (C 2018, E 1440, H 1/2, BK2-W, BK2-Z, CA 80-15, Celex, Celloidion, Collodion Cotton, Collodion Wool, Xyloidin); Iodion cotton; Pyroxylin; Colloxylin; Paraladion
- Chemical/Pharmaceutical/Other Class: Nitric acid esters
- Chemical Formula: $C_6H_7N_3O_{11}$

Uses

Nitrocellulose is a product that has found many uses in everyday life. Its physical form can vary widely from white fibers to thin sheets to thick liquid. Nitrocellulose is used to make everything from smokeless gun powder to waterproof fuses in pyrotechnics, inks, adhesives, varnishes, resins, lacquer coatings, embedding sections in microscopy, photography, electrotechniques, galvanoplasty, and even certain plastics, such as what is used in ping-pong balls. It can be a white, yellow, or transparent plastic, which can be anywhere from brittle to flexible. It can have properties ranging from a strong, resistant plastic to an unstable class B (highly flammable, explosive when confined) explosive material, all determined by the nitrogen content. Other current uses include the making of membranes that are used to immobilize

DNA, RNA, or protein, which can then be probed with a labeled sequence or antibody (Western blot assays), microscopy embedding, electrotechniques, skin protectants, microfilters, and others. Nitrocellulose continues to be used in photography, the manufacture of lacquers, patent and natural leathers, artificial pearls, process engraving, and cements.

Background Information

In the 1830s and 1840s, European chemists discovered that cotton dipped in nitric acid produced an explosive material. This early form of nitrocellulose was too unstable to be used safely in explosives production. Scientists later converted nitrocellulose into a stable base for improved gunpowder known as smokeless powder. Further experiments revealed that a combination of nitrated natural fibers with ether and alcohol produced a nonexplosive solution that hardened into a film. This discovery led to a wide array of end uses for nitrocellulose including plastics, lacquers, and photographic film.

Exposure Routes and Pathways

Accidental oral exposure is most likely. Skin exposure and inhalation of airborne fibers are also possible in occupational settings but these are unlikely routes for the general public.

Mechanism of Toxicity

No information is available on specific effects of nitrocellulose exposure.

Acute and Short-Term Toxicity (or Exposure)

Human

Although the primary danger of nitrocellulose is physical harm from fire or explosion, there are a number of clinical case reports on ingestion of Collodion, which contains nitrocellulose along with ether (70%) and ethanol (24%). Symptoms are similar to ethanol intoxication (exhilaration, talkativeness, impaired motor coordination, slowed reaction time, ataxia, flushing, drowsiness, etc.) except that onset is more rapid and the stomach becomes promptly distended because of the volatility of Collodion. One or two ounces of Collodion may be fatal if swallowed.

Collodion is classified as moderately toxic. Probable oral lethal doses for humans are 0.5–5.0 g kg^{-1} or between 1 ounce and 1 pint (1 lb) for a 70 kg person.

Chronic Toxicity (or Exposure)

Human

Earlier proportional mortality studies of workers in a plastics-producing plant indicated excess mortality from certain digestive and genitourinary cancers. To more definitively examine mortality, a retrospective cohort study was conducted for 2490 male wage earners who worked at least 1 year during 1949–66. Possible associations warranting continued surveillance were found between rectal cancer and cellulose nitrate production.

Clinical Management

Recommended treatment for acute colloidon ingestions is similar to that recommended for ethanol or ether overdose, including gastric lavage.

Environmental Fate

Degradation of nitrocellulose involves a complex chemical dissociation into a wide variety of products. Extremely high concentrations of nitrate and nitrite (NO_3^-/NO_2^-) are present in leachate from nitrocellulose landfills. Low permeability of the sludge and especially soil/sludge mixture will attenuate effects over long period of time.

Ecotoxicology

No acutely toxic effects of nitrocellulose were observed among fish, invertebrate, or algal species except the green alga *Selenastrum capricornutum*. Sediments containing nitrocellulose indicated no adverse effects among chironomid populations exposed to 540 mg kg^{-1} in sediment over two generations. Four species of invertebrates and four species of fish were unaffected by nitrocellulose concentrations as high as 1000 mg l^{-1}. Four species of algae were exposed up to 1000 mg l^{-1}. Three were unaffected and *Selenastrum capricornutum* showed a 96 h EC_{50} of 731 mg l^{-1}.

Other Hazards

Nitrocellulose is very flammable and may explode or ignite without warning when dry.

Nitrocellulose is classified under Organization for Economic Cooperation and Development (OECD) standards as: R1 – Explosive when dry; R3 – Extreme risk of explosion by shock, friction, fire, or other sources of ignition; R11 – Highly flammable; S16 – Keep away from sources of ignition; S33

– Take precautionary measures against static discharges; S35 – This material and its container must be disposed of in a safe way; S37 – Wear suitable gloves; S39 – Wear eye/face protection.

NFPA (National Fire Protection Association) Hazard Classification: Health: 1. 1 = Materials that, on exposure, would cause irritation, but only minor residual injury, including those requiring the use of an approved air-purifying respirator. These materials are only slightly hazardous to health; only breathing protection is needed. Flammability: 4. 4 = This degree includes flammable gases, pyrophoric liquids, and Class IA flammable liquids. The preferred method of fire attack is to stop the flow of material or to protect exposures while allowing the fire to burn itself out. Reactivity: 0. 0 = This degree includes materials that are normally stable, even under fire exposure conditions, and which do not react with water. Normal firefighting procedures may be used.

Exposure Standards and Guidelines

According to the US Food and Drug Administration requirements, colloidon is an indirect food additive for use only as a component of adhesives that could be used in packaging.

See also: Phthalate Ester Plasticizers.

Further Reading

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Relevant Website

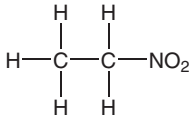
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Nitrocellulose.

Nitroethane

Christopher P Holstege

This article is a revision of the previous print edition article by Daniel Steinmetz, volume 2, pp. 423–424, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 79-24-3
- Chemical/Pharmaceutical/Other Class: Aliphatic nitro compounds
- Chemical Formula: $C_2H_5NO_2$
- Chemical Structure:



Uses

Nitroethane is used as a propellant; as a solvent for nitrocellulose; resins, waxes, and dyestuffs; and in chemical synthesis.

Exposure Routes and Pathways

Exposure to nitroethane may occur by inhalation, dermal contact, and ingestion.

Toxicokinetics

In studies involving animals, 50–70% of nitroethane vapors were absorbed through the upper respiratory tracts. Nitroethane is metabolized rapidly, with increased nitrite and nitrate levels detected in the blood. Animal studies suggest that absorbed nitroethane is eliminated within 48 h.

Mechanism of Toxicity

The mechanisms of toxicity for nitroethane are unknown. Methemoglobinemia associated with nitroethane is due to its metabolism to nitrite compounds.

Acute and Short-Term Toxicity (or Exposure)

Animal

Nitroethane produces similar effects in animals to those that appear in humans. Chronic nitroethane vapor administration has produced methemoglobinemia, pulmonary edema, narcosis, and liver and kidney damage in various animal models.

Human

Nitroethane is an irritant to the eyes and the respiratory tract. Vapors may exacerbate preexisting respiratory conditions, such as emphysema and asthma. Neither the odor nor irritation provide dependable warning properties. Overexposure to inhalation of the vapors may cause narcosis, headaches, and dizziness. The liquid is a mild irritant to the skin and can cause defatting and dermatitis. A number of cases of toxicity were reported in children who ingested nitroethane. These children developed prolonged methemoglobinemia following ingestions of small quantities of nitroethane-containing artificial nail removal products. Methylene blue therapy reduced the methemoglobin level in all of these children; however, methemoglobin levels increased again several hours later in some of the children and they required additional methylene blue.

Chronic Toxicity (or Exposure)

Animal

Chronic inhalation studies in rats and mice at doses of 0, 100, 350, and 1000 ppm for 6 h a day, 5 days a week demonstrated increased methemoglobin levels. At the highest dose tested, rats showed evidence of hepatic damage (vacuolization) and mice developed multinucleated spermatids.

In Vitro Toxicity Data

Assessment of mutagenicity using the Ames *Salmonella* and micronucleus assays has been negative or inconclusive.

Clinical Management

If dermal or eye contact with the liquid occurs, the affected areas should be flushed thoroughly with water for at least 15 min and the patient observed for resulting skin or eye irritation. In case of inhalation, the victim should be moved to fresh air and the patient should be monitored for respiratory irritation and pulmonary edema. If ingestion occurs, basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures are unlikely to provide clinical benefit. The use of methylene blue should be considered in the treatment of nitroethane-induced methemoglobinemia. Repeat doses of methylene blue may be necessary for patients with profound methemoglobinemia.

Environmental Fate

Nitroethane has a wide variety of industrial uses. Because of the huge production and widespread use of this substance, releases into the environment can and have occurred. Release at ambient temperature and pressure will result in nitroethane existing solely as a vapor. Nitroethane is broken down in the atmosphere by hydroxyl radicals and has a half-life of 107 days.

See also: Dyes; Nitrocellulose.

Further Reading

Hornfeldt CS and Rabe WH III (1994) Nitroethane poisoning from an artificial fingernail remover. *Journal of Toxicology. Clinical Toxicology* 32: 321–324.

Osterhoudt KC, Wiley CC, Dudley R, Sheen S, and Henretig FM (1995) Rebound severe methemoglobinemia from ingestion of a nitroethane artificial-fingernail remover. *Journal of Pediatrics* 126: 819–821.

Nitrogen Mustard

Harry Salem and Frederick R Sidell*

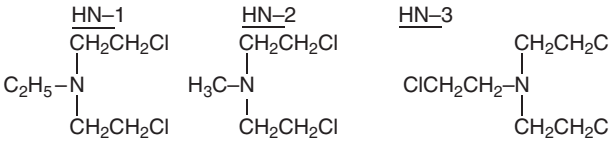
Published by Elsevier Inc.

- Chemical abstracts Service registry Numbers:
  CAS 538-07-8 (HN-1)
  CAS 51-75-2 (HN-2)
  CAS 555-77-1 (HN-3)

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

- Synonyms:
  HN-1: Bis(2-chloroethyl)ethylamine; 2-Chloro-*N*-(2-chloroethyl)-*N*-ethylethanamine; 2,2[1]-Dichlorotriethylamine; Ethylbis (2-chloroethyl)amine; Ethyl-S
  HN-2: MBA; Mechlorethamine; Mustine; 2,2[1]-Dichloro-*N*-methyldiethylamine; Dichloren; Caryolysin; Chlormethine; Bis(2-chloroethyl)methylamine; Leukeran
  HN-3: Tris(2-chloroethyl)amine; 2-Chloro-*N*,*N*-bis(2-chloroethyl)ethanamine; Trichlorotriethylamine; Nitrogen mustard-3; Mechlorethamine; Mustargen

- Chemical/Pharmaceutical/Other Class: Vesicant; Alkylating agent
- Chemical Formulas: $C_6H_{13}CI_2N$ (HN-1); $C_5H_{11}CI_2N$ (HN-2); $C_6H_{12}CI_3N$ (HN-3)
- Chemical Structures:



Uses

Nitrogen mustards are among the blister agents/vesicants used in chemical warfare. HN-1 was originally designed to remove warts and later identified as a potential chemical warfare agent. HN-2 was designed as a military agent, but later used in cancer chemotherapy. HN-3 was developed as a military agent.

Exposure Routes and Pathways

Ocular, percutaneous, inhalation, ingestion, and injection are all possible routes of exposure. Effects may be local, systemic, or both. All of the nitrogen mustards are oily liquids that are colorless to pale yellow and evaporate slowly. They are more dangerous than sulfur mustard but, like sulfur mustard, they are derivatives of ammonia. The most toxic and most volatile of the three nitrogen mustards is HN-2, but HN-3 is used more because it is stable.

Toxicokinetics

Like sulfur mustard, the nitrogen mustards combine predominantly with the thiol group of molecules and are excreted as conjugated cysteinyl derivatives. Both nitrogen and sulfur mustards have structural similarities and have common chemical reactions. A key reaction is the intramolecular cyclization in a polar solvent such as water to form a cyclic onium cation and a free anion. Nitrogen mustards form the immonium cation, while the sulfur mustard forms the sulfonium cation. The cyclized form is responsible for the varied effects of mustards, which are similar. In nitrogen mustard the sulfur is replaced by nitrogen.

Animal Toxicity

The animal toxicity reported for HN-3 is described in **Table 1**. Evidence shows it causes leukemia and cancers of the lungs, liver, uterus, and large intestine in animals. Nitrogen mustards also produce developmental effects in animals.

Human Toxicity

Nitrogen mustards produce damage to the eyes, respiratory tract, skin, and suppress the immune system. Systemically they produce cytotoxicity, with the hematopoietic and lymphoid tissues being especially sensitive.

The estimated inhalation median lethal dosage (LCt_{50}) for HN-3 in humans is 1500 mg min m^{-3}. The estimated percutaneous vapor LCt_{50} is 10 000 mg min m^{-3}, and the estimated percutaneous liquid LD_{50} is 700 mg per 70 kg. The percutaneous median incapacitating dosage (ICt_{50}) in humans has been estimated at 2500 mg min m^{-3}; the dose to produce eye injury has been estimated at 200 mg min m^{-3}. The airborne exposure limit (AEL) for HN-1 is 0.003 mg m^{-3} as a time-weighted average, while none exist for HN-2 and HN-3.

Irritation of eyes following a single exposure to nitrogen mustards occurs at doses that do not affect the skin or respiratory tract and appears sooner than with sulfur mustard. Mild-to-moderate exposures cause slight smarting and lacrimation within 20 min,

Table 1 Animal toxicity values for nitrogen mustards

Species	*Toxicities*
Inhalation Time (min)	*LCt_{50} (mg min m^{-3})*
Mouse (10)	165 (vapor)
	345 (aerosol)
Rat (10–100)	670 (vapor)
Rat (0.25–2.0)	800 (aerosol)
Cat (10)	400–1000
Dog (10)	400–1000
Percutaneous	*LD_{50} (mg kg^{-1})*
Mouse	7.0
Rat	4.9
Rabbit	19.0
Dog	10.0
Intravenous	*LD_{50} (mg kg^{-1})*
Mouse	1
	2
Rat	0.7
Rabbit	2.5
Dog	1.0
Subcutaneous	*LD_{50} (mg kg^{-1})*
Mouse	2.0
Rat	2.0
Rabbit	2.0
Dog	
Intragastric	*LD_{50} (mg kg^{-1})*
Mouse	
Rat	2.5
Rabbit	
Dog	

becoming persistent ~2.5 h later, and reaching a maximum at 8–10 h. The effects include erythema and edema of the palpebral and bulbar conjunctiva with superficial, steamy haziness of the cornea, irritation, lacrimation, deep eye pain, miosis, and photophobia.

Following severe exposure these symptoms progress for 24 h or longer, and are followed by spotty hemorrhagic discoloration of the iris and roughened lusterless surface of the corneal epithelium, which demonstrate punctuate fluorescein staining. The corneal epithelium may exfoliate.

Clouding and edema of the cornea and necrosis may cause rupture of the globe.

There may be no skin lesions following mild vapor exposures. However, severe vapor or liquid exposure to nitrogen mustard will produce effects similar to those of sulfur mustard (but the onset is sooner than with sulfur mustard). These effects include erythema, irritation, and itching, with blisters developing in the erythematous areas.

Exposure of the respiratory tract to nitrogen mustard produces the same effects as sulfur mustard. These include the delay in onset, irritation of the nose and throat, hoarseness progressing to aphonia, and persistent cough, fever, dyspnea, and moist rales. After 24 h, chemical pneumonitis may appear.

Following oral administration or systemic absorption of nitrogen mustards, the intestinal tract may be damaged. In animals, severe diarrhea occurred, which may be hemorrhagic. The lesions were most marked in the small intestine and consisted of degenerative changes and necrosis in the mucosa. In humans, ingestion of 2–6 mg causes nausea and vomiting.

Following absorption of nitrogen mustard from intact skin or respiratory or gastrointestinal tract, the most specific effects are on the hematopoietic and lymphoid tissues. In bone marrow, the degenerative changes can be detected within 12 h and may progress to severe aplasia. The thymus, spleen, and lymph nodes involute rapidly with necrosis and phagocytosis of their lymphocytes. This is evident from the transient leukocytosis in the blood, which is followed by severe lymphopenia, granulocytopenia, and thrombocytopenia, for 5–10 days following exposure. The white blood cell count may fall to 500 cells mm^{-3} or lower. The various nitrogen mustards differ in their ability to produce these changes.

The chronic physiological effects may include, for severe exposure, scarring of the cornea, and the iris frequently becomes discolored and atrophied. Repeated skin burns may lead to hypersensitivity of the skin, which is an effect similar to that of sulfur mustard. That is, reexposure will cause erythema, with or without edema, and pronounced itching and burning occurring within 1 h. Lower concentrations will produce these effects in sensitized persons. Vesication heals more rapidly. Frequent manifestations of reexposure in sensitized individuals include a morbilliform rash and eczematoid dermatitis surrounding old lesions. The International Agency for Research on Cancer has classified nitrogen mustard as probably carcinogenic to humans (group 2A). Evidence shows it causes leukemia in humans.

Clinical Management

The victim must be removed from the source of contamination quickly by adequately protected attendants and then decontaminated using a solution of sodium hypochlorite, liquid household bleach, or fuller's earth. Oxygen and/or artificial respiration should be administered if dyspnea is present or breathing has stopped.

Erythema should be treated with calamine or other soothing lotions or creams to reduce burning and itching. Large blisters should be unroofed and covered with a sterile dry dressing if the patient is ambulatory or left uncovered if the patient is not ambulatory. Denuded areas should be irrigated with saline or soapy water and covered with a topical antibiotic (e.g., silver sulfadiazine or mefanide acetate). Multiple or large areas of vesication require hospitalization and whirlpool irrigation. Systemic analgesics are indicated especially prior to manipulation of the patient or irrigation of the burn areas. Systemic antipruritics (e.g., trimeprazine) may also be used.

Treatment of ocular injury includes thorough irrigation, application of homatropine (anticholinergic) ophthalmic ointment, and topical antibiotics several times daily. Vaseline or similar products should be applied regularly to the edges of the eyelids to prevent them from sticking together. Topical analgesics may be useful in severe blepharospasm for examination of the eye but should be used sparingly. Sunglasses may reduce discomfort from photophobia, and the victim must be reassured that complete healing and restoration of vision will result.

Steam inhalation and cough suppressants may relieve upper airway symptoms, sore throat, nonproductive cough, and hoarseness. Appropriate antibiotic therapy should only be instituted following confirmation of infection by positive sputum tests (Gram stain and culture). Intubation should be accomplished prior to the development of laryngeal spasm or edema so that adequate ventilation is established and suction of necrotic and inflammatory debris can be facilitated. Oxygen may be required as well. Early use of positive expiratory pressure (PEEP) or

continuous positive airway pressure (CPAP) may be useful. Bronchoscopy may be required if pseudomembrane has developed to permit suction of the necrotic debris by direct vision. Bronchodilators or steroids may also be used to relieve bronchospasm.

Death may occur between the fifth and tenth day postexposure because of pulmonary insufficiency complicated by a compromised immune response from mustard-induced bone marrow damage.

Atropine (0.4–0.6 mg intramuscular or intravenous) or other anticholinergic or antiemetic drugs may be used to control nausea and vomiting.

Sterilization of the gastrointestinal tract by nonabsorbable antibodies may reduce the possibility of infection from enteric organisms. Bone marrow transplants or blood transfusions may be indicated. The recent introduction of granulocyte colony stimulating factor may offer hope in the management of bone marrow depression.

A victim of nitrogen mustard exposure also requires the general supportive care given to a severely ill patient as well as the specific care given to a burn patient. This includes the liberal use of systemic analgesics and antipruritics and the maintenance of fluids and electrolyte balance. Parenteral food supplements and vitamins may also be beneficial.

See also: Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents; Blister Agents/Vesicants; Chemical Warfare Delivery Systems; Mustard Gas; Nerve Agents.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Nitrogen Oxides

Lee R Shugart

- Chemical Abstracts Service Registry Number: CAS 10102-44-0
- Synonyms: Nitrogen dioxide (Nitrogen peroxide)
- Chemical/Pharmaceutical/Other Class: Inorganic, gas
- Chemical Formula: NO_2

Uses

Used in the nitration of organic compounds and explosives, in the manufacture of oxidized cellulose compounds, and as an oxidizing agent in rocket propulsion. It is an intermediate in the production of nitric and sulfuric acids.

Background Information

Nitrogen dioxide belongs to a family of highly reactive gases called nitrogen oxides (NO_x). These gases form when fuel is burned at high temperatures and come principally from motor vehicle exhaust and stationary sources such as electric utilities and industrial boilers. A suffocating, brownish gas, nitrogen dioxide is a strong oxidizing agent that reacts in the air to form corrosive nitric acid, as well as toxic organic nitrates. It also plays a major role in the atmospheric reactions that produce ground-level ozone (or smog).

Exposure Routes and Pathways

Inhalation, skin contact, eye contact, and/or ingestion are the main routes of exposure.

Mechanism of Toxicity

Nitrogen dioxide acts mainly as an irritant affecting the mucosa of the eyes, nose, throat, and respiratory tract.

Acute and Short-Term Toxicity (or Exposure)

Human

The acute toxicity of nitrogen dioxide by inhalation is high. Toxic effects may occur after exposure to concentrations of 10 ppm for 10 min and include coughing, chest pain, frothy sputum, and difficulty in breathing. Nitrogen dioxide at concentrations of 10–20 ppm is mildly irritating to eyes; higher concentrations are corrosive to the skin, eyes, and mucous membranes. Short-term exposure following ingestion includes nausea, vomiting, and stomach pain.

Chronic Toxicity (or Exposure)

Animal

Animal testing indicates that nitrogen dioxide does not have carcinogenic or reproductive effects and it does not produce heritable genetic damage.

Human

Chronic toxicities following exposure include shortness of breath and pulmonary edema, which may progress to respiratory infections, reduction in the blood's oxygen carrying capacity, lung disorders, eye damage, and digestive disorders.

In Vitro Toxicity Data

Nitrogen dioxide produces no genetic damage in bacterial and mammalian cells in cultures.

Clinical Management

If inhaled and breathing is difficult, the person should be moved to fresh air and administered oxygen. For skin contact, the exposed area should be washed with soap and water. For eye contact, the eyes should be flushed with water.

Environmental Fate

Highly reactive with air and decomposes in water.

Ecotoxicology

Nitrogen oxides significantly contribute to a number of environmental effects such as acid rain and eutrophication in coastal waters like the Chesapeake Bay as well as ozone formation, all of which can have adverse effects on both terrestrial and aquatic ecosystems. Fish toxicity: LC_{50} (hematological) for Red drum (*Sciaenops ocellatus*) is $3\,mg\,l^{-1}$ for 24 h. Invertebrate toxicity: LC_{50} (mortality) for Redtail prawn (*Penaecus penicillatus*) is $3.03\,mg\,l^{-1}$ for 144 h. Animal toxicity: LC_{50} (mortality) for rat by inhalation is 88 ppm for 4 h.

Exposure Standards and Guidelines

The US Environmental Protection Agency's health-based national air quality standard for nitrogen dioxide is 0.053 ppm (measured as an annual arithmetic mean concentration). National Institute for Occupational Safety and Health and Occupational Safety and Health Administration recommended exposure limit is 1 ppm ($1.8\,mg\,m^{-3}$).

See also: Chemicals of Environmental Concern; Nitrocellulose; Ozone.

Further Reading

Anyanwu E (1999) Complex interconvertibility of nitrogen oxides (nox): Impact on occupational and environmental health. *Reviews on Environmental Health* 14(3): 169–185.

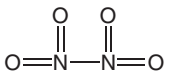
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Nitrogen Oxides.

Nitrogen Tetraoxide

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 10544-72-6
- Synonyms: Liquid nitrogen dioxide under pressure; Nitrogen peroxide; Dinitrogen tetroxide; Dinitrogen tetraoxide; Tetra oxide
- Chemical/Pharmaceutical/Other Class: Nitrogen oxides
- Chemical Formula: N_2O_4
- Chemical Structure:



Uses

Nitrogen tetraoxide is formed by pressurizing liquid nitrogen dioxide. It is a gas at normal temperature and pressure, and is used in the manufacture of explosives and rocket fuels. Nitrogen tetraoxide is used as a catalyst in oxidation reactions and in many other industrial applications. It is also a component of nitric and sulfuric acid.

Exposure Routes and Pathways

Principal routes of exposure are generally inhalation; for example, inhalation of industrial gases, fumes resulting from the welding process, vapors arising from the contact of nitric acid with organic materials, from the exhaust of metal cleaning processes, vapors associated with electroplating, engraving, and photogravure operations, dynamite blasting, diesel engine

exhaust, and polluted air resulting from internal combustion engine exhaust. Dermal exposure is rare.

Toxicokinetics

Toxic effects may result from inhalation exposures and from skin absorption.

Mechanisms of Toxicity

Nitrogen tetraoxide is absorbed through the respiratory system and reacts with blood, reducing fluid levels, inducing massive pulmonary edema, and a severe reduction in hemoglobin levels.

Acute and Short-Term Toxicity (or Exposure)

Animal

The inhalation LC_{50} is 315 ppm in rabbits. Nitrogen tetraoxide as a liquid can cause severe burns from even brief contact with the skin or eyes.

Human

The liquid is highly corrosive to the skin and may cause chemical burns. The vapor is extremely irritating to the eyes, and is capable of causing pain and severe conjunctivitis. A review by the American Conference of Governmental Industrial Hygienists (ACGIH) suggests that a 60 min exposure to 100 ppm leads to pulmonary edema and death; 50 ppm to pulmonary edema with possible subacute or chronic lesions in the lungs; and 25 ppm to respiratory irritation and chest pain. About 50 ppm is moderately irritating to the eyes and nose; 25 ppm is irritating to some people. The effects of exposure are insidious, leaving an exposed person asymptomatic for days, even at a fatal dosage. Only high concentrations show immediate toxic effects. The latent period may be from 5 to 72 h, and initial symptoms include coughing and nausea. Vapor can cause pain, severe conjunctivitis, and other effects to the eye. The liquid is highly corrosive to the skin. Nitrogen tetraoxide is a class A poison (US Code of Federal Regulations (CFR) 173, Section 173.326).

Chronic Toxicity (or Exposure)

Human

Long-term exposure of small levels can cause bronchitis, interstitial edema, epithelial proliferation, and possible emphysema and fibrosis. It is classified by the ACGIH as Category A4 (not classifiable as causing cancer in humans).

Clinical Management

Exposure to nitrogen tetraoxide in the missile industry can lead to symptoms identical to those from nitrogen dioxide. Medical assistance should be sought immediately after any inhalation exposure, however small. If eyes or skin are exposed these should be well rinsed with water.

Environmental Fate

Nitrogen tetroxide is released to the atmosphere where it can undergo reactions, including leading to air pollution.

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average, is 3 ppm ($6\,mg\,m^{-3}$), and the short-term exposure limit (STEL) is 5 ppm ($10\,mg\,m^{-3}$). The US National Institute for Occupational Safety and Health (NIOSH) STEL, for a 15 min exposure, is 1 ppm ($1.8\,mg\,m^{-3}$), and the NIOSH Immediately Dangerous to Life or Health value is 20 ppm.

See also: Nitrogen Mustard; Nitrous Oxide.

Further Reading

Liekauf GD and Prows DR (2001) Inorganic compounds of carbon, nitrogen, and oxygen. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 3, pp. 607–730. New York: Wiley.

Relevant Websites

http://www.intox.org – International Programme on Chemical Safety (IPCS). Nitrogen Oxides, 2nd edition (Environmental Health Criteria 188).

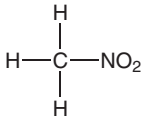
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Nitrogen Tetraoxide.

Nitromethane

Richard D Phillips

This article is a revision of the previous print edition article by Daniel Steinmetz, volume 2, pp. 427–428, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 75-52-5
- Synonym: Nitrocarbol
- Chemical Formula: CH_3NO_2
- Chemical Structure:



Uses

Nitromethane has been used as a chemical stabilizer for a variety of halogenated hydrocarbon solvents and aerosol propellants. In addition, it is a solvent, a chemical intermediate, a fuel for professional racing and hobby cars, an explosive when mixed with ammonium nitrate, and a rocket propellant.

Exposure Routes and Pathways

Nitromethane is a colorless, oily liquid with a moderately strong, somewhat disagreeable odor. Production of nitromethane and its use as a solvent, fuel additive stabilizer for halogenated alkanes and intermediates, may result in its release into the environment, principally the atmosphere. Human exposure to nitromethane may additionally occur via dermal contact and accidental ingestion.

Toxicokinetics

Toxicokinetic data on nitromethane are limited. Nitromethane may be metabolized to formaldehyde based on *in vitro* studies with liver microsomes, but only in trace amounts. In addition, nitromethane may form a cytochrome P450 NO complex. Nitromethane appeared to compete with carbon monoxide for a common binding site. However, nitromethane appears to undergo limited metabolic denitrification.

Mechanism of Toxicity

Nitromethane affects the central nervous system (CNS) via narcosis as a solvent. It is also a mild pulmonary irritant. In addition, nitromethane produces histidinaemia in rats by decreasing hepatic histidase activity, leading to increased tissue levels of histidine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs were more sensitive than other species following an oral dose with 125 mg kg^{-1} causing a toxic effect. Doses ranging from 250 to 1500 mg kg^{-1} caused death in all dogs tested. Pathologic lesions were seen in the liver and kidney.

Death occurred in one monkey exposed at 1000 ppm vapor for 48 h. A concentration of 5000 ppm for 3 h resulted in death to guinea pigs. Nitromethane concentrations of 500 ppm were tolerated for 140 h (6 h day^{-1} exposure) by guinea pigs, rabbits, and monkeys. The most common signs of toxicity were CNS depression and slight irritation in the respiratory tract. Histopathologic changes were mainly in the liver and kidneys with liver showing the most prominent injury.

The US National Toxicology Program (NTP) published the results from a 13 week subchronic inhalation study of nitromethane in both sexes of F344/N rats and B6C3F1 mice. Exposures were at 0, 94, 187, 373, 748, and 1500 ppm of nitromethane. At 1500 ppm, a significant decrease in body weight gain was observed for male rats. A similar trend (though not statistically significant) was observed in female mice exposed to 1500 ppm. Body weights were not depressed in the other rat groups or in the mice.

Neurological effects were observed in all male and female rats in the 1500 ppm groups and partially in the 748 ppm group. There were no exposure-related clinical signs of toxicity in mice. In addition, nitromethane caused exposure-related microcytic responsive anemia in male and female rats.

Exposure to nitromethane was also associated with minimal to mild hyperplasia of the bone marrow in both rats and mice.

In a 6 month inhalation study, New Zealand White rabbits and Sprague–Dawley rats were exposed by inhalation to 0, 98, or 745 ppm (0, 245, or 1860 mg m^{-3}) nitromethane for 7 h day^{-1}, 5 days per week for 6 months. Decreased body weight gain was observed in rats after 8 weeks of exposure to 745 ppm. The most notable response in rabbits was an effect on the thyroid: increased thyroid weight and decreased serum thyroxine levels. There were no exposure-related gross or microscopic lesions in either rats or rabbits exposed to 98 or 745 ppm.

Human

Signs and symptoms of toxicity include dermatitis due to solvent action of nitromethane. By analogy, with effects seen in laboratory animals, nitromethane may cause mild pulmonary irritation, weakness, and ataxia muscular incoordination at fairly high levels. More severe effects such as convulsions, liver and kidney injury are possible under conditions of severe overexposure. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value time-weighted average (TLV-TWA) of 20 ppm ($50\,mg\,m^{-3}$) for an occupational exposure to nitromethane to reduce the potential for adverse thyroid effects and pulmonary hemorrhage reported for inhalation exposed rabbits and rats.

Chronic Toxicity (or Exposure)

Animal

Nitromethane was administered $7\,h\,day^{-1}$, 5 days per week for 2 years via inhalation to male and female Long–Evans rats at 100 and 200 ppm. There were no pharmacologic effects for the exposure at either dose rate. The body weights of the female rats for both exposure groups were slightly less than the control. There was no effect on the hematology, no clinically significant effects of serum chemistry, no effects on organ weight, and no significant differences in the nonneoplastic or neoplastic pathology related to exposure.

The NTP-sponsored a 2 year chronic inhalation study on male and female rats (F344/N) and male and female mice (B6C3F1) with exposures at 6 h, 5 days per week for 103 weeks. The rats were exposed to 0, 94, 188, or 375 ppm; the mice were exposed to 0, 188, 375, or 750 ppm. Clinical findings consistent with mammary gland neoplasms were noted in female rats, but not in males, at some of the concentrations tested.

For mice, clinical findings at the highest doses included swelling around the eyes and exophthalmos in exposed males and females. This is consistent with harderian gland adenoma or carcinoma (combined) in exposed mice with increasing exposure. Female mice also had increased liver and lung tumors and nonneoplastic nasal damage. Males had lung and nonneoplastic nasal damage

The NTP study concluded that there was (1) clear evidence of carcinogenic activity from nitromethane in female F344/N rats, based on increased incidences of mammary gland fibroadenomas and carcinomas; (2) clear evidence of carcinogenic activity in male B6C3F1 mice, based on increased incidences of Harderian gland adenomas and carcinomas; (3) clear evidence of carcinogenic activity in female B6C3F1 mice, based on increased incidences of liver neoplasms and harderian gland adenomas and carcinomas; and (4) male F344/N rats showed no evidence of carcinogenic activity from nitromethane.

Human

There is no epidemiological evidence or case reports specific to exposure to nitromethane in the published scientific literature.

In Vitro Toxicity Data

Nitromethane has given consistently negative results in bacterial mutagenicity assays, and *in vitro* mammalian tests for sister chromatid exchanges and chromosomal aberrations. It was not mutagenic in *Drosophila*. It did not induce micronuclei *in vitro* in Syrian hamster embryo cells or *in vivo* in mice. However, nitromethane did show a positive response at high concentration in a cell transformation assay in Syrian hamster embryo cells. The results of short-term tests on nitromethane do not indicate that the compound has genotoxic activity.

Clinical Management

Appropriate procedures should be instituted for anyone overcome by nitromethane (e.g., removal from exposure to fresh air, washing skin areas, and irrigation of eyes with water). Treatment for pulmonary irritation or dermatitis may be needed if symptoms are present.

Exposure Standards and Guidelines

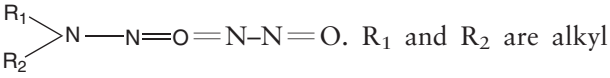
ACGIH TLV-TWA of 20 ppm ($50\,mg\,m^{-3}$).

Relevant Website

http://monographs.iarc.fr – International Agency for Research on Cancer (IARC, 2000) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 77 – Some Industrial Chemicals, pp. 487–502.

Nitrosamines

Heriberto Robles

- Preferred Name: *N*-Nitrosamines
- Chemical/Pharmaceutical/Other Class: Dialkylnitrosamines; Nitrosamines
- Chemical Structure: The name nitrosamines applies to a family of compounds that have an alkyl and an aryl group attached to the chemical group,  . R_1 and R_2 are alkyl and aryl groups

Uses

Nitrosamines have no known industrial use. However, they can be found in processed foods as unintentional by-products of food preparation and processing.

Background Information

Nitrosamines are formed by a reaction between nitrates or nitrites and certain amines. Nitrosamines and their precursors can be found in diverse consumer products such as processed meats, alcoholic beverages, cosmetics, and cigarette smoke. Nitrosamines are considered to be strong carcinogens that may produce cancer in diverse organs and tissues including lung, brain, liver, kidney, bladder, stomach, esophagus, and nasal sinus.

In 1957, a new malignant disease was reported in Norway's fur farms. Farmed mink fed a diet containing fish (herring) meal developed an unknown liver disease. Later, in the early 1970s, additional outbreaks of liver disease and cancer were reported in Norway's farm animals. Feeding experiments and extensive toxicological research using herring meal on cows and sheep resulted in liver damage and death of some animals. Upon examination, nitrite-treated herring meal was found to contain up to 100 ppm of dimethylnitrosamine. At the time of the incident, sodium nitrite was being used in Norway as a preservative for fish meal. It is now known that sodium nitrate reacted with amines normally present in fish to produce dimethylnitrosamine, a potent nitrosamine carcinogen.

Exposure Routes and Pathways

The most common route of exposure is by oral ingestion of nitrosamines in food. It has been estimated that the general population consumes approximately 0.1 μg of nitrosamines per day in their diet. Nitrosamines can be found in foods preserved with nitrates as well as in untreated foods such as mushrooms, alcoholic beverages, smoked fish, bacon, ham, and some cheeses. Nitrosamines have also been found in tobacco smoke and urban air. Nitrosamines can also be formed in the mouth or stomach if the food contains nitrosamine precursors. Under acidic pH in the mouth or stomach, nitrite or nitrates added to food or naturally occurring may combine with amines to form nitrosamines.

While the major route of exposure for the general population is through the consumption of nitrosamines in food, the total dose consumed by cigarette smokers is considerably larger. It has been estimated that cigarette smokers may inhale up to 17 μg of nitrosamines per day.

Toxicokinetics

The physical properties of nitrosamines vary widely depending on the nature of the substituent groups R_1 and R_2. Similarly, the nature of the substituent group has an effect on the toxicological properties of nitrosamines. For example, the LD_{50} of nitrosamine compounds is directly proportional to the carbon chain length of the substituent. Long-chain substituents have a higher LD_{50}. Also, the nature of the substituent group has an effect on the carcinogenicity properties of nitrosamines. Dimethyl and diethyl compounds cause predominantly liver tumors, while dibutyl compounds tend to cause bladder tumors.

Nitrosamines have a short half-life that has been measured in the order of minutes.

Mechanism of Toxicity

Nitrosamines are not carcinogenic at the point of application. They require bioactivation. One possible mechanism of biotransformation is by enzymatic transformation to a carbonium ion. Activation is known to proceed first by hydroxylation of an α-carbon. The resulting hydroxyalkyl moiety is eliminated as an aldehyde, and an unstable primary nitrosamine is formed. The unstable nitrosamine ultimately tautomerizes to a carbonium ion. The highly reactive carbonium ion readily alkylates with the nearby cellular macromolecules. Cancer and mutagenicity develop when reactive nitrosamine metabolites alkylate to genetic macromolecules.

Acute and Short-Term Toxicity (or Exposure)

Animal

Nitrosamines are strong hepatotoxic agents. Large, acute doses produce liver necrosis and hemorrhages in the liver and other tissues.

Chronic Toxicity (or Exposure)

Animal

Nitrosamines and *N*-nitroso compounds are strong carcinogens that produce cancer of the liver and kidneys. In experiments conducted to date, 75–80% of nitrosamines tested have been found to be carcinogenic to mammals. Dimethylnitrosamine, a member of the nitrosamine family, is highly carcinogenic to the liver and kidneys of almost all the mammalian species tested.

Rats exposed to tobacco-derived nitrosamines developed tumors at the nose, mouth, esophagus, lung, and pancreas. Tobacco-derived nitrosamines caused upper respiratory tract cancers in exposed hamsters.

It appears that all animals are susceptible to the carcinogenic action of nitrosamines. For example, dimethylnitrosamine given by gavage, in drinking water or in the feed, produced liver tumors in rats, mice, guinea pigs, hamsters, rabbits, dogs, and monkeys.

Human

Chronic, continuous exposure to low doses of nitrosamines in the diet is considered to be of toxicological importance to humans. There is a significant body of epidemiological data that links exposure to nitrosamines and human cancer.

Tobacco-derived nitrosamines are considered to be one of the major cancer-causing agents found in tobacco smoke and tobacco products. This is of importance as up to 90% of human lung cancers can be linked to cigarette smoking. The most potent carcinogen found in tobacco is the nicotine-derived nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

In Vitro Toxicity Data

In vitro studies have demonstrated the mutagenic activity of nitrosamines. For example, mutagenicity assays using dimethylnitrosamines have been positive for *Salmonella typhimurium*, *Escherichia coli*, and *Neurospora crassa*. Dimethylnitrosamine also produced mitotic recombination in *Salmonella cerevisiae*, recessive lethal mutations in *Drosophila melanogaster*, and chromosomal aberrations in mammalian cells.

Clinical Management

Nitrosamine exposure is not an acute hazard. Health hazards associated with nitrosamine exposure are limited to cancer, and liver and kidney damage associated with chronic exposure. No specific treatment exists for nitrosamine intoxication. Supportive and symptomatic treatment should be provided.

Since nitrosamines and their precursors are present in the food, exposure to nitrosamines cannot be avoided. However, recent studies have shown that ingestion of adequate quantities of vitamin E and selenium may reduce the risk of cancer. It is known that carcinogenic nitrosamines are formed from the reaction of some amines with nitrites and nitrates present in the diet. Vitamin E and selenium have been found to minimize or prevent the reaction of nitrites/nitrates with amines and hence prevent or reduce the formation of carcinogenic nitrosamines.

Vitamin C (ascorbic acid) is known to inhibit nitrosamine formation. For this reason, manufacturers of cured meat are now required to add vitamin C to their meat products.

Exposure Standards and Guidelines

The US Environmental Protection Agency (EPA) has included some nitrosamines under its B2 – probably human carcinogens – classification.

The US EPA has estimated oral cancer slope factors for some of the most common nitrosamines. Cancer slope factors published in the EPA's Integrated Risk Information System range from $4.9 \times 10^{-3}\,\mathrm{mg^{-1}\,kg^{-1}\,day^{-1}}$ for *N*-nitrosodiphenylamine (CAS 86-30-6) to $150\,\mathrm{mg^{-1}\,kg^{-1}\,day^{-1}}$ for *N*-nitrosodiethylamine (CAS 55-18-5). The same agency has established inhalation unit risk factors that range from $6.4 \times 10^{-4}\,\mu\mathrm{g^{-1}\,m^{-3}}$ for *N*-nitrosopyrrolidine (CAS 930-55-2) to $4.3 \times 10^{-2}\,\mu\mathrm{g^{-1}\,m^{-3}}$ for *N*-nitrosodiethylamine.

See also: Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogenesis; Epidemiology; Nitrites; Tobacco Smoke.

Further Reading

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Nitrous Oxide

Shayne C Gad

This article is a revision of the previous print edition article by Jayne E Ash and Shayne C Gad, volume 2, pp. 430–431, © 1998, Elsevier Inc.

- Chemical Service Registry Number: CAS 100-24-97-2
- Synonyms: Dinitrogen monoxide; Laughing gas; Hyponitrous acid anhydride; Factitious air; Nitrogen monoxide; Entonox; Nitronox
- Chemical/Pharmaceutical/Other Class: An oxide of nitrogen
- Chemical Formula: N_2O
- Chemical Structure: $-N=N^+=O$

Uses

Nitrous oxide is used therapeutically as an anesthetic or analgesic. It is also used in the formulation of rocket fuel and as a propellant for whipped cream. It occurs endogenously. Nitrous oxide is a common inhalant drug of abuse.

Exposure Routes and Pathways

Inhalation is the route of exposure.

Toxicokinetics

Nitrous oxide is rapidly absorbed from inspired air. Some patients lose consciousness when breathing 30% nitrous oxide in oxygen and most will become unconscious with 80%. It is almost entirely eliminated through the lungs, with small amounts through the skin and in urine.

Mechanism of Toxicity

High concentrations of nitrous oxide have a narcotic and/or asphixiant effect. By inactivating vitamin B_{12}, a critical cofactor in hematopoiesis and lipid membrane formation, nitrous oxide can cause anemia and neuropathy via selective inhibition of methionine synthase, a key enzyme in methionine and folate metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

An asphixiant and narcotic at higher concentrations. The inhalation LC_{50} is $160\,m^{-3}$ in rats.

Human

Nitrous oxide causes drowsiness and headache. Anesthesia with nitrous oxide as the sole anesthetic in normal humans for periods of 2–4 h has induced tachypnea, tachycardia, increased systemic blood pressure, atrioventricular junctional rhythm, acute cardiovascular failure, mydriasis, diaphoresis, and occasional clonus and opisthotonus.

Chronic Toxicity (or Exposure)

Animal

Teratogenicity has been observed in studies of rats, rabbits, cat, and hamsters exposed to nitrous oxide. A carcinogen bioassay of nitrous oxide in mice exposed for $4\,h\,day^{-1}$, $5\,days\,week^{-1}$ for 78 weeks found no neoplastic or non-neoplastic lesions judged to be related to nitrous oxide.

Human

Occupational exposure has been associated with impairment of psychological functions, but these effects do not occur with trace concentrations. A recent review of the available data concluded that exposure to trace amounts of nitrous oxide is not associated with impaired fertility or an increased risk of developing cancer; however, recent studies seem to suggest a correlation between nitrous oxide anesthesia and hyperhomocysteinemia, an independent risk factor for coronary artery disease. Long-term exposure to high concentrations of nitrous oxide may cause megaloblastic bone-marrow depression and neurological symptoms. Bone-marrow depression was observed in humans exposed for 4 days to high concentrations of nitrous oxide in the treatment of tetanus. Nitrous oxide is a common inhalant drug of abuse, and severe myeloneuropathy has been observed as a complication. Nitrous oxide is listed as A4 (not classifiable as a human carcinogen) by the American Conference of Governmental Industrial Hygienists (ACGIH).

Clinical Management

Exposure should be terminated immediately. Oxygen therapy should be provided if respiratory difficulties are present.

Environmental Fate

Emission of nitrous oxide from medical use has been estimated to contribute less than 0.05% to total annual greenhouse gas emission.

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average (TWA), is 50 ppm, and the US National Institute for Occupational Safety and Health recommended exposure level is 25 ppm as a TWA during the period of anesthetic administration.

See also: Nitric Oxide.

Further Reading

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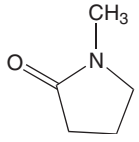
N-Methylpyrrolidone

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 872-50-4
- Synonyms: *N*-Methyl-2pyrrolidone; 1-Methyl-2-pyrrolidone; NMP
- Chemical/Pharmaceutical/Other Class: Cyclic amide
- Chemical Structure:



Uses

N-Methylpyrrolidone (NMP) is used as an extraction solvent in the petrochemical industry, as a paint stripper in occupational (e.g., graffiti removal) and consumer (e.g., furniture) settings, as a solvent in the microelectronics industry, and as a chemical reaction medium. NMP enhances the penetration of topically applied pharmaceuticals and is used as a formulating agent in pigments, dyes, inks, and pesticides. It has been used increasingly as a replacement for chlorinated solvents that may pose a greater health risk.

Exposure Routes and Pathways

NMP is a liquid under normal environmental conditions. Due to its low vapor pressure, human exposures are primarily limited to dermal contact. Significant inhalation exposures to NMP are possible during applications that generate NMP aerosols (e.g., graffiti removal) or vapors (e.g., unventilated cleaning baths of heated NMP). Due to its complete miscibility in water, NMP vapor concentrations in the atmosphere are limited by the relative humidity, ranging from 0 ppm (100% relative humidity) to 315 ppm (0% relative humidity).

Toxicokinetics

NMP is freely soluble in both polar and nonpolar solvents and should readily cross biological membranes. This expectation is consistent with experimental studies indicating NMP is well absorbed following inhalation (40–60%), oral (about 100%), and dermal ($\leqslant$100% depending on conditions) exposures. Dermal absorption has been extensively studied as it typically poses the greatest potential for human exposure. Due to its irritant properties, neat NMP is unlikely to remain in voluntary contact with the skin for more than several hours. During this time, the flux of NMP through human skin is about $2\,\mathrm{mg\,cm^{-2}\,h^{-1}}$. The presence of cosolvents can

affect NMP fluxes. Water inhibits dermal absorption while other organic solvents (e.g., D-limonene) can increase it. For example, the dermal flux of 10% NMP in water is about $0.01\,mg\,cm^{-2}\,h^{-1}$. Prolonged NMP exposures can increase the permeability of the skin to NMP and other compounds; the dermal permeability to water increases sevenfold following a 4–6 h exposure to neat NMP.

Following absorption, NMP is uniformly distributed throughout all major organs in the rat with a volume of distribution (about $0.71\,kg^{-1}$) that approximates total body water. In both the rat and man, NMP is eliminated primarily by metabolism to other compounds via a saturable process; only about 2% of the absorbed NMP is excreted unchanged. The major metabolite is 5-hydroxy-NMP (50–70%) with lesser amounts of *N*-methylsuccinimde, 2-hydroxy-*N*-methylsuccinimide, and possibly other unidentified metabolites. The half-life of NMP in plasma is $\sim$4 h. Studies with radiolabeled NMP indicate the most of the radiolabel is excreted in the urine ($\leqslant$95%), with lesser amounts in the feces ($\leqslant$5%) and expired air ($\leqslant$2%). Ongoing studies are investigating the use of 5-hydroxy-NMP as a urinary biomarker for human exposures to NMP.

Mechanism of Toxicity

Developmental toxicity is the most sensitive endpoint associated with NMP exposures in experimental animals. While the mechanism responsible for this effect is unknown, available data suggest that NMP may be the proximate toxin.

Acute and Short-Term Toxicity (or Exposure)

Animal

NMP has a low acute toxicity. The oral LD_{50} values for NMP in multiple species range between 3500 and $7900\,mg\,kg^{-1}$; the dermal LD_{50} values in rats and rabbits range between 4000 and $10\,000\,mg\,kg^{-1}$. Respiratory tract irritation but no deaths were observed in rats exposed nose-only to a NMP vapor/aerosol mixture of $5100\,mg\,m^{-3}$ for 4 h. NMP is irritating to the skin and eyes. When instilled into the eye, NMP also causes corneal opacity, iritis, and conjunctivitis; however, these effects were reversible within 21 days. Sensitization studies have been negative. In 28 day feeding studies, body weight decrements, clinical chemistry changes, centrilobular liver hypertrophy, and testicular degeneration were observed in rats at $\geqslant 1230\,mg\,kg^{-1}$; in similarly exposed mice, toxicity was limited to epithelial swelling of the kidney distal tubules at $\geqslant 2130\,mg\,kg^{-1}$. The 28 day no-observed-adverse-effect levels (NOAELs) in rats and mice were about 450 and about $800\,mg\,kg^{-1}$, respectively. In 90 day feeding studies in rats, body weight decrements and changes in 3 of 36 neurobehavioral parameters occurred at $\geqslant 430\,mg\,kg^{-1}$ while increased liver weights (with centrilobular hypertrophy) and increased kidney weights (without associated histopathology) were observed at $\geqslant 1340\,mg\,kg^{-1}$. In 90 day feeding studies in mice, toxicity was limited to centrilobular liver hypertrophy at $\geqslant 620\,mg\,kg^{-1}$. The 90 day NOAELs in rats and mice were about 180 and about $280\,mg\,kg^{-1}$, respectively. NMP is not clastogenic *in vivo*.

Human

Few data on the acute toxicity of NMP in humans are available. Volunteers exposed to $\leqslant$12 ppm NMP for 8 h did not experience any eye or respiratory tract irritation, symptoms such as headache, dizziness, or nausea, or changes in pulmonary function measured by spirometry. At 12 ppm NMP, two of six subjects reported an acetone-like odor. NMP did not produce signs of sensitization in a repeated-insult patch test with NMP, although a minor and transient irritation was observed.

Chronic Toxicity (or Exposure)

Animal

Whole body exposure of rats to $\leqslant$100 ppm NMP vapor for $6\,h\,day^{-1}$, $5\,days\,week^{-1}$ for a lifetime resulted in only a slight decrement in male body weight at 100 ppm. In another study, rats receiving a lifetime dietary exposure to NMP exhibited decrements in body weight and an increase in the severity of chronic progressive nephropathy (males only) at the highest doses tested, $678\,mg\,kg^{-1}$ (males) and $939\,mg\,kg^{-1}$ (females); the lifetime NOAELs were $207\,mg\,kg^{-1}$ (males) and $283\,mg\,kg^{-1}$ (females). In both studies, sex organ histopathology was normal, and the incidence of cancer was not increased. Mice receiving a lifetime dietary exposure to NMP exhibited an increase in hepatocellular adenomas and carcinomas at the highest doses tested, $1089\,mg\,kg^{-1}$ (males) and $1399\,mg\,kg^{-1}$ (females); centrilobular liver hypertrophy was also noted in males at the high dose. Sex organ histopathology was normal. The lifetime NOAELs in mice were $173\,mg\,kg^{-1}$ (males) and $221\,mg\,kg^{-1}$ (females). The increased tumor incidence seen in mice may occur via a nongenotoxic mechanism given the negative results seen with NMP in both *in vitro* and *in vivo* genotoxicity tests. The human relevance of these positive results in mice is unknown.

In two multigeneration rat reproduction studies conducted under current guidelines, dietary exposures to NMP at the highest dose tested ($350\,mg\,kg^{-1}$) produced some signs of systemic toxicity in parental animals but did not affect reproductive performance or fertility; however, this dose resulted in decrements in pup survival and body weights. Sex organ histopathology and sperm parameters were normal. The NOAEL for reproduction was $>350\,mg\,kg^{-1}$; the NOAEL for developmental toxicity was $160\,mg\,kg^{-1}$. The normal sex organ histopathology noted in chronic and multigeneration reproduction studies combined with the normal reproductive performance in the latter studies suggest that NMP does not pose a significant reproductive hazard to humans.

Developmental studies have been performed via the dermal, oral, and inhalation routes of exposure in both rats and rabbits. Based on results of these studies, it appears NMP can sometimes cause developmental effects in the absence of maternal toxicity. Developmental toxicity is typically manifested by fetotoxic effects (e.g., decrements in fetal body weight), although malformations have been observed above fetotoxic doses. NMP also appears to be a more potent developmental toxin via the inhalation route (lowest-observed-adverse-effect level or LOAEL equivalent to about $120\,mg\,kg^{-1}\,day^{-1}$) than by either the oral (LOAEL about $250\,mg\,kg^{-1}\,day^{-1}$) or dermal (LOAEL about $750\,mg\,kg^{-1}\,day^{-1}$) routes; NOAELs associated with these exposure routes are about twofold lower than the LOAELs.

Human

No data on the chronic toxicity of NMP in humans are available.

In Vitro Toxicity Data

Data on the *in vitro* mutagenicity and clastogenicity of NMP are negative.

Clinical Management

Exposed skin and eyes should be irrigated immediately with water to minimize irritation and systemic absorption. Medical attention should be sought if symptoms or health concerns persist.

Environmental Fate

When released to the environment, NMP is expected to partition at equilibrium almost exclusively to water where it is readily biodegraded. Due to its low Henry's law constant ($1.6 \times 10^{-3}\,Pa\,m^3\,mol^{-1}$), significant volatilization of NMP from water is not expected. NMP that does reach the atmosphere will be removed by reaction with photochemically produced hydroxyl radicals (5.2 h half-life) and rain washout. NMP is not expected to adsorb significantly to soil and sediment matrices based on its calculated absorption coefficient (K_{oc}) of 9.6; the half-lives of NMP in various soil matrices are 4 days (clay), 8 days (loam), and 12 days (sand). Based on its low bioconcentration factor (0.16) and log octanol–water partition coefficient (-0.73), NMP should not pose a significant bioaccumulation hazard.

Ecotoxicology

The 96 h LC_{50} values for a variety of fish species range between 680 and $4000\,mg\,l^{-1}$. For a variety of aquatic invertebrates, the 48 h LC_{50} values are $>1000\,mg\,l^{-1}$; in algae the EC_{50} values are $>500\,mg\,l^{-1}$.

Exposure Standards and Guidelines

International occupational exposure limits (OELs) for NMP generally range between 5 and 50 ppm as an 8 h time-weighted average (TWA). The American Conference of Governmental Industrial Hygienists has not established an 8 h TWA OEL for NMP. Several countries have established a short-term excursion limit of 75 ppm. The California Office of Environmental Health Hazard Assessment has identified NMP as a reproductive toxin and established maximum allowable daily limits for exposure of $3200\,\mu g\,day^{-1}$ (via inhalation) and $17\,000\,\mu g\,day^{-1}$ (via dermal contact).

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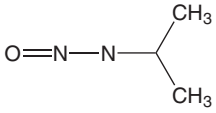
N-Nitrosodimethylamine

Sidhartha D Ray and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 62-75-9
- Synonyms: Nitrosamine; Dimethylnitrosamine; *N*-Methyl-*N*-nitroso-ethanamine
- Chemical Formula: $C_2H_6N_2O$
- Chemical Structure:



Uses

Dimethylnitrosamine (DMN) is used commonly as an industrial solvent in the production of dimethylhydrazine; formerly used in the production of hypergolic rocket fuels, thiocarbonyl fluoride polymers, and as soluble cutting oil. It is presently used as an antioxidant, as an additive for lubricants, gasoline, and as a softner of copolymers. Nitrosamines have also been found in multiple cosmetic products, hand and body lotions, shampoos, and have been patented for use in pesticides and nematocides. Alarming levels have been found in soil, postulated to be from the use of triazine herbicide, which can react with ubiquitously used nitrogenous fertilizers. DMN is a research chemical, air and water pollutant, and tobacco smoke condensate. Presence of this compound has also been found in nonfat dry milk, gastric juices, rubber products, rubber manufacturing, metal industries, and certain chemical manufacturing.

Exposure Routes and Pathways

The common exposure routes are inhalation (contaminated air), skin contact, ingestion through food and water, and *in vivo* formation from amines and nitrates; it is classified as a potential human carcinogen. Maximal exposure occurs through food (e.g., nitrite cured meat, fish, or malt beverages), house hold goods (e.g., rubber), tobacco and tobacco smoke, cosmetics, drugs, pesticides, and indoor air (e.g., frying of nitrite cured meat with release of volatile nitrosamines).

No indication can be given about the rate in which a harmful concentration in the air is reached on evaporation of this substance at 20°C. DMN is used extensively in cancer research facilities. Human exposure occurs when unchanged DMN is excreted by the laboratory animals. DMN has been used as an industrial solvent and as a chemical intermediate in the production of 1,1-dimethylhydrazine. Other uses or proposed uses include: as a nematocide, as a lubricant additive, as an antioxidant, as a softener for copolymers, and in electrical condensers to increase the dielectric constant. Inhalation risks are very high in those settings.

Toxicokinetics

DMN is rapidly absorbed from all the routes of exposure. This chemical is dangerous because, in most instances, it is produced inside the body. *Biotransformation*: DMN (and many other related) is intrinsically not reactive, and requires metabolic activation by the P450 system for its biological effects. Upon metabolism, very reactive electrophilic carbonium ions (e.g., methyldiazonium) are formed, which in turn attack cellular macromolecules, e.g., protein, DNA, and RNA. *Distribution and elimination*: No clear-cut human data are available. An intravenous bolus dose of $1.35\,\mu mol\,kg^{-1}$ ($100\,\mu g\,kg^{-1}$) to Fisher 344 rats revealed a predominantly hepatic clearance of 2.5 min to 3 h. Orally administered DMN (2.02 or $4.05\,\mu mol\,kg^{-1}$) cleared between 5 and 120 min. Intravenously injected DMN concentrations declined in a biexponential manner with a terminal half-life of 10 min. The apparent total systemic blood clearance distribution was $39\,ml\,min^{-1}\,kg^{-1}$. The apparent steady-state volume of distribution was $297\,ml\,kg^{-1}$. The rate of metabolism of DMN after larger oral doses ($10\,mg\,kg^{-1}$) is $5\,mg\,kg^{-1}\,h^{-1}$ and follows zero-order kinetics. Terminal biological half-life is 11 min, and elimination predominantly by liver. The substance decomposes on heating producing nitrogen oxides. It reacts with strong oxidants and strong bases.

Mechanism of Toxicity

NDMA is a potent alkylating agent. It can cause DNA single-strand breaks, double-strand breaks, and fragmentation in the form of a ladder in cells and tissues. It is well recognized that metabolic activation of NDMA and other *N*-nitrosodialkylamines is required for the generation of electrophilic species that can elicit genotoxic or other damage in cells. This metabolic activation process is believed to be an important factor in determining the tissue and species specificities of some of these carcinogens. NDMA is activated to an alkylating electrophile by *N*-demethylation that is generally accepted as the rate-limiting step in the activation of DMN, which is catalyzed by hepatic cytochromes P450. Different cytochrome P450 species are involved in the metabolism of NDMA in microsomes. They show substrate

specificity and alkyl group selectivity in the metabolism of *N*-nitrosodialkylamines. Cytochrome P450IIE1 displays low K_m and high turnover numbers in catalyzing the demethylation and denitrosation of NDMA and other nitrosamines. It exists in untreated rats, rabbits, and other animals and is inducible by a variety of inducers such as acetone, ethanol, pyrazole, and isoniazid as well as by physiological conditions such as fasting and diabetes. Rat cytochrome P450j is involved in bioactivation of *N*,*N*-dimethylnitrosamine. The K_m value for NDMA demethylase determined in acetone induced rat liver microsomes was found to be 20–30 μM *N*-Nitrosodimethylamine1. Other P450 species contribute to the metabolism of NDMA when this substrate is present at high concentrations, especially cytochrome P450IIB1 suggesting high K_m values. Since animals and humans are rarely exposed to such high concentrations of NDMA, it is believed that P450IIE1 species are the predominant forms responsible for the metabolism of carcinogenic levels of these compounds. Microsomal activation of DMN is decreased by protein and protein-choline deficient diets and is increased by pretreatment with microsomal enzyme inducers. With human liver microsomes of differing cytochrome P450 contents, similar correlation is obtained. Oxidative demethylation of DMN by mouse liver microsomes and the activation of DMN to a mutagen follow similar kinetics. Micronutrients may have an effect on the levels of cytochrome P450 enzymes. Ascorbate deficiency can result in a depression of the levels of cytochrome P450 and cytochrome b5. But excessive intake of vitamin C does not significantly enrich microsomes in cytochrome P450 and b5 content. Different inducers are known to affect the metabolism of the two alkyl groups of an *N*-nitrosodialkylamine differently. Acetone/ethanol-inducible P450IIE1 is more efficient in catalyzing the α-oxidation of the methyl and ethyl groups of NDMA and other nitrosamines than other constitutive forms. The phenobarbital-induced P450(s), on the other hand, is less active in catalyzing the oxidation of these groups in NDMA and NEMA but more active in catalyzing the α-oxidation of the butyl group of NBMA. However, in comparing metabolic activities different results are obtained depending on the substrate concentration.

Acute and Short-Term Toxicity (or Exposure)

Animal

DMN is carcinogenic to all the 10 species tested in single bolus dose or multiple low doses. Primary target organs include liver, kidney, thymus, spleen, lung, skin, and trachea. Swiss mice fed a diet containing 0.005% DMN for 1 week developed tumors of the kidney and lung. Hamsters fed a diet containing 0.0025% for 11 weeks developed liver tumors. ICR mice injected 25–50 mg kg^{-1} (one dose) developed severe hepatotoxicity within 12 h. The LD_{50} in the rat for DMN is 26 mg kg^{-1} and the LC_{50} is 78 ppm over 4 h. A single dose of about 25 mg kg^{-1} DMN administered orally to the rat, or by intravenous, intraperitoneal, or subcutaneous injection produces serious destruction of liver tissue accompanied by hemorrhages into the liver and lungs. Often there occurs a serious accumulation of fluid in the abdominal area and blood in the lumen of the intestines. Death usually occurs in 2–4 days or the animal recovers completely. Rabbits, mice, guinea pigs, and dogs all develop similar liver damage.

Human

Usually does not involve irritation of skin or mucous membranes. Acute poisoning may invoke headaches, malaise, fever, or general weakness. Gastrointestinal effects include abdominal cramping, nausea, and vomiting, eventually leading to diarrhea. Hepatomegaly and jaundice may follow if the exposure is severe or prolonged.

Chronic Toxicity (or Exposure)

Human

Chronic exposure may cause liver disease with jaundice and swelling with a precipitous drop in the platelet count. It is a suspected human carcinogen. Nitrosamines can form in the gastric juice of the human stomach. This is commonly referred to as endogenous nitrosation. Bacteria in the mouth chemically reduce nitrate, which is prevalent in many vegetables, to nitrite, which in turn can form nitrosating agents. Many foods contain amines that can react with nitrosating agents in the acidic stomach to form nitrosamines. While it has been demonstrated that ascorbic acid can reduce nitrosation in the stomach, more research will be required for a fuller understanding of endogenous nitrosation and its ramifications for health and disease. It is carcinogenic to all the other species tested upon prolonged low level exposure. The lowest lethal oral dose in humans has been reported at 10 mg kg^{-1} per 80 week intermittent exposure.

Effects of Long-Term or Repeated Exposure

N-Nitrosodimethylamine (NDMA) produced liver tumors in rats when administered in drinking water

or in the diet. DMN is also known to produce many hemangiomatous tumors and some parenchymal cell tumors in the livers of rats after oral administration. NDMA acts as a transplacental carcinogen when administered to pregnant rats, mice, and Syrian golden hamsters by several routes. Increases in lung, liver, and kidney tumors were observed in both rats and mice exposed by inhalation. Mink are very sensitive to the tumerigenic effects of DMN.

Continuous low doses (adequate for animal survival) of DMN exposure for 7–8 months to the rat resulted in liver cancer. Higher concentrations for shorter periods, or as a single dose, resulted in kidney tumors. Continuous low-level exposures generally cause cancer (or tumor) of the liver and higher concentrations for short periods (or as a single dose) result in kidney tumors. Although, in the rat, cancers of the liver and the kidney are the most frequent outcomes, DMN can be immunotoxic (and immunocarcinogenic) and gastrointestinal-toxic as well. The fact that a single exposure to DMN can result in cancer has tremendous implications with respect to man. While there is no direct evidence that exposure to DMN leads to cancer in humans, indirect evidence, obtained from laboratory experiments that measured the relative metabolic rates of DMN by rat and human liver slices, indicate that man is probably about as sensitive to the carcinogenic action of DMN as is the rat.

NDMA is mutagenic for *Escherichia coli*, *Salmonella typhimurium*, and *Neurospora crassa*. It can produce mitotic recombination in *Sacharoyus cerevesiae* species, recessive lethal mutations in *Drosophilla melanogaster*, and chromosomal aberrations in mammalian cells. Mutagenic responses in bacterial cells are dependent upon the addition of a mammalian drug metabolism system (specific form of cytochrome P450).

Clinical Management

Quick removal of the affected workers from the site of exposure is required and respiration should be established. Absorption should be prevented by repeated flushing with water if exposure to mucous membranes is suspected. Parts of the body that were exposed should be decontaminated with soap and water. It is necessary to evaluate hepatic and renal function tests as thoroughly as possible, while paying special attention to liver size. Chest X-ray and cancer screening are necessary. Human exposure to nitrosamines results from contact with mixtures containing these compounds (e.g., cutting oils, tobacco products). Because of potential confounding by the other substances in these mixtures, data from human exposure is of limited use in the evaluation of carcinogenicity of individual nitrosamines.

Environmental Fate

Very little experimental data are available to predict the environmental fate of *N*-nitrosomethylethylamine in soil, atmosphere, or elsewhere in the environment. Nitrosamines photolyze rapidly in aqueous solution; therefore, photolysis is expected to occur on surfaces exposed to sunlight. Insufficient data are available to predict the relative importance of biodegradation or other transformation processes in soil. Based upon estimated K_{oc} values of 4–73 (2–3, SRC), *N*-nitrosomethylethylamine will be highly mobile in soil and can be expected to leach. Insufficient data are available to predict the relative importance of biodegradation in natural water. Volatilization from water is slow; the estimated volatilization half-life from a shallow, rapidly moving model river is 81 days (2, SRC). Since *N*-nitrosomethylethylamine is relatively soluble in water (30%), adsorption to sediment and bioconcentration in aquatic organisms are not expected to be important fate processes. Based upon an extrapolated vapor pressure of 1.1 mmHg at 20°C, *N*-nitrosomethylethylamine will exist primarily in the vapor phase in the ambient atmosphere (2, SRC). It will degrade rapidly in the vapor phase via direct photolysis in sunlight; the photolysis half-life at a solar zenith angle of 40°N is approximately 5.8 min (3, SRC). By comparison, reaction with photochemically produced hydroxyl radicals is a minor degradation process with an estimated half-life of 1.6 days (4, SRC). NDMA is released to the environment in mainstream and sidestream tobacco smoke. It may be formed in the nighttime atmosphere by reaction of atmospheric amines with nitrous acid. If released to the atmosphere, *N*-nitrosomethylethylamine will degrade rapidly through direct photolysis in sunlight (estimated half-life of 5.8 min at a solar zenith angle of 40°N). If released to soil or water, it will degrade rapidly at the water surface or on soil surfaces exposed to sunlight. Insufficient data are available to predict the relative importance of biodegradation within soil or water. NDMA is highly soluble in water and is therefore expected to leach in soil. The general population is exposed to *N*-nitrosomethylethylamine through inhalation of tobacco smoke and through consumption of various foods.

It is more difficult to determine point sources for DMN emissions than for other toxic agents because DMN is not extensively used by industry and most current occupational exposures will probably occur as a result of the chemical formation of the compound from its precursors rather than from the known utilization of the chemical. The chemical reaction, in the condensed phase, between nitrous acid

and dimethylamine (DMA) or trimethylamine (TMA) to form DMN is well known. Recently, it has been shown that DMA and TMA can react with oxides of nitrogen in the vapor phase to give DMN as a reaction product. This means that even though DMN is not used at a particular location, it may be formed from its precursors and therefore be found in the occupational environment. The fact that oxides of nitrogen can chemically react with amines to produce airborne nitrosamines is of special interest in light of the fact that there appears to be a statistical correlation between high concentrations of NO_2 and high incidence of cancer in some urban areas. NO_2, in itself, is probably not a carcinogen.

Exposure Standards and Guidelines

Current exposure level is $\sim 0.1\,\mu g\,day^{-1}$ due to successful efforts over the last two decades to reduce nitrosamine formation in foods and beverages. In contrast, the National Academy of Sciences report estimated an exposure of $17\,\mu g\,day^{-1}$ from cigarette smoking, although the use of filters has somewhat lowered smokers' exposure. Recent reports indicate that industrial exposure, such as found in a rubber or chemical manufacturing plant, can be relatively high. Nonoccupational exposures to DMN are predominantly via food products. Industrial effluents, automobile exhaust, and environmental auto reduction processes are additional natural burden.

The substance can be absorbed into the body by inhalation and by ingestion. The threshold limit value is A3, skin (American Conference of Governmental Industrial Hygienists, in 2000). MAK: Class 2 (in 2000).

Therapeutic Uses

There are no therapeutic uses. NDMA was a potent carcinogen to all the animal species tested and is a suspected human carcinogen. The lowest lethal oral dose in humans has been reported at $10\,mg\,kg^{-1}$ per 80 week intermittent exposure.

See also: Gasoline; Pesticides; Shampoo; Tobacco Smoke.

Further Reading

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Relevant Websites

http://www.who.int – *N*-Nitrosodimethylamine (Concise International Chemical Assessment Document 38). International Programme on Chemical Safety, World Health Organization, Geneva, 2002.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for *N*-Nitrosodimethylamine.

Nonionizing Radiation *See* Radiation Toxicology, Ionizing and Nonionizing.

Non-Lethal Weapons, Chemical

Patricia M Nance

Non-lethal weapons (NLW) or less-than-lethal weapons have been defined by the US Department of Defense (DoD) to be "explicitly designed and primarily employed so as to incapacitate personnel or materiel, while minimizing fatalities, permanent injury to personnel, and undesired damage to property and the environment." NLWs are being developed and evaluated because the US military and other military and law enforcement organizations globally have increasingly been involved in operations that call for different types of weapons and tactics. For example, nontraditional military operations such as those conducted in the 1990s in Bangladesh, Haiti, Somalia, and Bosnia demanded greater flexibility because they commonly involve close and continual interaction between US forces and noncombatant civilians.

The DoD issued a formal Directive (3000.3) on NLWs in 1996. It states that NLWs should enhance the capability of US forces to accomplish objectives including "to discourage, delay, or prevent hostile

actions; to limit escalation; to take military action in situations where use of lethal force is not the preferred option; to better protect our forces; and to temporarily disable equipment, facilities, and personnel." The Directive also states that NLWs are not required to have a zero probability of producing fatalities or permanent injuries; rather they should significantly reduce fatalities and injuries when compared with use of lethal force. The concept of risk–benefit analysis is highlighted in the Directive, which calls for these weapons to "achieve an appropriate balance between the competing goals of having a low probability of causing death, permanent injury, and collateral materiel damage, and a high probability of having the desired anti-personnel or anti-materiel effects."

Riot control agents (RCAs), also known as 'crowd control agents', are a broad category of chemical NLWs that are in use by military and law enforcement agencies around the world. RCAs are intended to temporarily disable a targeted individual by way of irritating the skin and mucous membranes. These agents are generally regarded as safe, with low toxicity, when used as intended, but under increased exposure levels or prolonged durations of exposure they can have toxic effects. There is the potential of dermal, ocular, or pulmonary injury when exposed to high levels in enclosed areas. There is, however, a large margin between the dose intended to harass and the dose that would likely cause a serious adverse effect.

The chemicals in RCAs can be classified physiologically as lacrimators, vomiting agents, or respiratory irritants. The first group will cause eye irritation and lacrimation, the second group will also causing vomiting, and the final group produces uncontrollable sneezing, coughing, and sometimes vomiting. Typical characteristics of RCAs include rapid onset, short period of activity after exposure ceases, and relatively high margins of safety. The lacrimatory effects of many of the chemicals can range from mild to severe, including stinging of the eyes and tearing at low concentrations that can cause temporary disablement. At low levels these compounds will cause reversible effects with no serious injury. At higher levels or prolonged durations of exposure there is serious potential of injuries. At the ocular level such injuries as corneal edema, corneal ulceration and scarring, corneal opacification, and corneal vascularization may occur.

Oleoresin capsicum (OC), pelargonic acid vallinylamide (PAVA), and capsaicin are derived from the pepper plant. The ingredients in hot peppers that are responsible for 'the heat' are called capsaicinoids. Capsaicinoids are a family of chemicals and they come with various heat qualities. The mixture used contains the active ingredient capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) as well as other compounds. PAVA is a pepper derivative that is extremely hot. PAVA (capsaicin II) is the hottest of the capsaicin family. It generates immediate effects after exposure and usually begins to lessen with 15–30 min after removal from the exposure. Occasionally, ocular and mucous membrane effects can last up to 24 h. PAVA and OC are highly effective irritants, generally considered safe, although this is not necessarily accurate. Both sprays will quickly produce lacrimation and closure of the eyes as well as respiratory responses such as bronchoconstriction, severe coughing and sneezing, shortness of breath, and nasal irritation. Other potential effects are a burning sensation on the skin and loss of motor control. Pulmonary system effects of capsaicin and capsaicinoids are dominant, including bronchospasms, respiratory arrest, and pulmonary edema. OC can also cause hypertensive crises and hypothermia. At suprathreshold levels serious respiratory and cardiovascular effects as well as permanent damage to the sensory nervous system may occur. There have been a considerable number of deaths linked to OC although no actual causal relationship has been determined. Most of these deaths occurred within 1 h of exposure.

The compound *o*-chlorobenzylidene malononitrile (CS) is a potent, safe RCA. CS and other chemicals in this class of compounds can lead to toxic reactions in experimental animals and humans if there is a high air concentration. CS is highly irritating to the mucous membranes that cover or line the eyes, nose, throat, and stomach. It also causes intense eye irritation, excessive tearing, and the nose and mouth may feel a stinging or burning sensation as well as rhinorrhea. If the respiratory tract is irritated, as is frequent postexposure, the individual may also have excessive coughing and sneezing, increased tracheobronchial secretions, and tightness in the chest. CS can cause death by way of serious lung injury leading to respiratory and circulatory failure, when exposure lasts for long durations. Diarrhea and vomiting will occur if the gastrointestinal tract is irritated. Burning sensation on the skin followed by inflammation and erythema are results of skin exposure. If exposure occurs in hot, humid conditions the effects will be more severe. CS produces some or all of its effects within 30 s of exposure, some so severe that the individual will seek escape from exposure. CS is also less toxic than CR or CN (see below).

Dibenz[*b*,*f*]1:4-oxazepine (CR) is a potent sensory irritant with low toxicity. The effects of CR on the eyes and skin are more transitory than with other agents. CR is not associated with contact sensitization.

Experiments done on various species using various routes have shown CR to have a low acute toxicity, much less than CN or CS. Overdose in animals will cause rapid breathing, incoordination, spasms, and convulsions. The effects generally subside gradually over a period of 15–60 min at which point the animal will either appear normal or have respiratory distress leading to death.

Chloroacetophenone (CN) is a white crystalline solid with an apple-blossom odor. It is also known as tear gas or Mace®. CN acts directly on mucous membranes to produce intense ocular and respiratory irritation as well as burning and pain of the eye, nose, throat, and lungs. Effects can include blepharospasms (i.e., eye blinking), conjunctivitis, sneezing, coughing, secretions, nasal congestion, and a sense of suffocation. The onset of some symptoms is immediate and persists for up to 20 min after the individual leaves the contaminated atmosphere. The primary cause of death related to CN is a result of inhalation effects on the pulmonary system.

CS and CN are by far the most important irritants described above. CN was the primary pulmonary irritant after World War I until CS was developed in 1928. CS has replaced CN as the principal military and law enforcement RCA, while CN as Mace® is available over the counter for personal protection in some places. Capsaicin as pepper spray has somewhat replaced CN as a personal protective agent. Other chemicals in this class that are worthy of mention are chloropicrin (PS) and bromobenzenecyanide (CA). PS and CA were developed before World War I, but have largely have been replaced because they were too lethal for their intended effects but not lethal enough to compete with the more effective blistering and nerve agents. PS still is seen occasionally as a soil sterilant or grain disinfectant. The creation of CNB (CN, carbon tetrachloride, and benzene), chloroacetophenone in chloroform (CNC), and CNS (CN, chloroform, and PS) were attempts to make CN more effective. However, CS proved more effective and less toxic than any of the CN series and largely has replaced them. CR, as a more recent tear gas (first synthesized in 1962), is not yet used widely.

Diphenylaminochloroarsine (adamsite or DM) is one of several military vomiting agents used. This compound is more toxic than many other RCAs and is potentially dangerous.

See also: Arsenical Vomiting Agents; Riot Control Agents.

Further Reading

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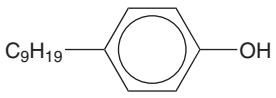
Nonsteroidal Antiinflammatory Agents *See* Acetaminophen; Acetylsalicylic Acid; Ibuprofen.

Nonylphenol

Alan L Blankenship and Katie Coady

- Chemical Abstracts Service Registry Numbers: CAS 25154-52-3 (mixed isomer); CAS 104-40-5 (4-nonylphenol); CAS 136-83-4 (2-nonylphenol)
- Synonyms: Nonylphenol; *p*-Nonylphenol-branched; 4-Nonylphenol; 2,6-Dimethyl-4-heptylphenol
- Chemical/Pharmaceutical/Other Class: Nonylphenol (NP) is a member of the alkylphenol class of chemicals. Alkylphenols are produced from cyclic intermediates during refining of petroleum and coal-tar crudes. Specifically, NP is manufactured by alkylating phenol with mixed isomeric nonenes in the presence of an acid catalyst. Thus, the product is a mixture of alkylphenols that vary in the length of carbon chain(s) and can vary in branching and substitution patterns. The product mixture contains predominantly *para*-substituted (4-nonylphenol) and occasionally *ortho*-substituted (2-nonylphenol), with various isomeric, branched-chain nonyl (nine carbon) groups. Theoretically, there can be 211 isomers present in a nonylphenol mixture due to different branching and substitution patterns

- Chemical Formula: $C_{15}H_{24}O$
- Chemical Structure:



Uses

Nonylphenol (NP) has not been widely used in commercial products, except in limited applications such as mixed with diisobutyl phthalate to color fuel oil for taxation purposes. However, it has been widely used as an intermediate in the production of nonionic surfactants of the nonylphenol ethoxylate (NPE) type. These nonionic surfactants are used as oil-soluble detergents and emulsifiers, lubricants, oil additives, and antioxidants for rubber manufacture. The occurrence of NP in the environment is mostly the result of metabolic degradation of NPEs and related alkylphenol ethoxylates (APEs). APEs are surfactants that are used in domestic and industrial detergents such as de-resinating agents, wetting agents, and degreasers. APEs are also components of biocides, plastics, and paints.

Background Information

NPs were first introduced in the United Kingdom in 1944 and they have subsequently been used in industry for over 50 years. Production of NP in the United States was ~147 million pounds in 1980 and grew to over 230 million pounds in 2000.

Exposure Routes and Pathways

Occupational exposure to NP may occur through inhalation and dermal contact with this compound at workplaces during its production and formulation into commercial products. Monitoring data indicate that the general population may be exposed to NP via dermal contact with products (e.g., nonionic surfactants) containing NP and ingestion of water containing NP. The primary route of environmental exposure to NP is through municipal wastewater treatment plants, as well as discharges of various industrial effluents from industries such as chemical plants, textile mills, and pulp and paper mills.

Toxicokinetics

By oral administration, nonylphenol is quickly absorbed in the gastrointestinal tract, distributed via the bloodstream, and finally excreted via urine and feces. In a human study in which one volunteer received 5 mg (66 $\mu g\,kg^{-1}$) ^{13}C-NP orally and a second volunteer received 1 mg (14 $\mu g\,kg^{-1}$) of ^{13}C-NP intravenously, NP was found to be ~20% bioavailable after oral application (relative to intravenous administration). The greatest concentration measured in blood after oral administration occurred after 60 min, followed by a distribution and elimination phase. By 10 h, NP could no longer be detected at a detection limit of 20 $pg\,g^{-1}$ in blood. From this very limited data, an elimination half-life of 163 min was calculated for humans.

Following dietary exposure to Sprague–Dawley rats, NP was rapidly absorbed and eliminated in the blood serum (absorption and elimination half-lives of 0.8 and 3.5 h, respectively). The predominant metabolite was a glucuronide, although NP aglycone was also observed. Other metabolic reactions of NP include sulfuration. In another investigation, ~70% of the orally administered NP dose in rats was recovered in feces and 20% in urine within 4 days.

Percutaneous penetration and absorption of ^{14}C-NP was found to be $<5\%$ and $<1\%$, respectively, in the skin of humans, pigs, and rats. NP was mainly present in the corneal layer of the skin.

Mechanism of Toxicity

There has been considerable interest in characterizing the biological effects of NP and related alkylphenols, ever since some were reported to exhibit estrogenic activity. Structure–activity studies with alkylphenols have shown clearly that the branching pattern, length of carbon chain, and substitution patterns on the phenolic ring have dramatic effects on the biological activity.

NP is able to interact and bind to the estrogen receptor, and is therefore capable of modulating estrogen receptor-mediated gene expression, such as that responsible for vitellogenin in fish. However, the estrogenic potency of NP is ~1000 times less than that of 17β-estradiol.

Acute and Short-Term Toxicity (or Exposure)

Animal

Published LD_{50} values for laboratory mammals include 1600 $mg\,kg^{-1}$ bw (rat, oral) and 2140 $mg\,kg^{-1}$ (rabbit, dermal), and 2031 $mg\,kg^{-1}$ (rabbit, oral). A number of acute aquatic toxicity tests have been conducted with 96 h LC_{50} values of 0.135 $mg\,l^{-1}$ (fathead minnow, flow through), 3.0 $mg\,l^{-1}$ (bay mussel, unspecified conditions), and 0.56–0.92 $mg\,l^{-1}$ (rainbow trout, unspecified conditions).

Some of the most sensitive biomarkers of effect to estrogenic chemicals are uterotrophic assays. For example, after oral and subcutaneous administration of 4-NP at 0, 25, 50, 100, and 200 mg kg^{-1} day^{-1} to female Long Evans rats (21 days old) for 3 days, an increase in uterine weight was observed at concentrations greater than or equal to 50 mg kg^{-1} day^{-1}. Some studies have also reported effects of NP in male rats. For example, male Sprague–Dawley rats (12 weeks old) exposed orally for 10 weeks experienced atrophy of seminiferous tubules ($\geqslant$100 mg kg^{-1} day^{-1}), decreased epididymis weight ($\geqslant$250 mg kg^{-1} day^{-1}), and decreased testis weight and decreased number of sperm ($\geqslant$400 mg kg^{-1} day^{-1}).

Human

Acute NP exposure, such as may occur in occupational settings, can produce severe irritation to the eye, skin, and respiratory system. Symptoms of such acute toxicity include a burning sensation, cough, labored breathing, sore throat, unconsciousness, abdominal pain, diarrhea, nausea, skin irritation, and burns. Other than these acute effects, there is no conclusive evidence that typical exposure to NP causes adverse health effects in humans.

Chronic Toxicity (or Exposure)

Animal

There are considerable data on the chronic toxicity of NP in laboratory animals. The focus of these investigations has typically been evaluation of the potential reproductive and developmental effects of NP, due to its ability to modulate estrogen receptor-mediated responses. Many endpoints are not consistently observed across studies. Some of this variability may be due to differences in the conditions, design, and other test-specific variables of the toxicity tests. For example, since phytoestrogens are abundant in most laboratory animal feeds (such as found in soy and alfalfa) and are known to modulate estrogen receptor-mediated responses, phytoestrogens may be confounding factors as a result of the feed selection.

In one study, Sprague–Dawley rats were exposed over three generations to 4-NP via diet at concentrations of 0, 200, 650, and 2000 ppm in order to assess potential reproductive effects. The F0 generation was exposed to 4-NP as adults and were bred once to produce the F1 generation, who were bred once to produce the F2 generation, who, in turn, were bred once to produce the F3 generation. Parameters evaluated over the course of the study included body weights, feed consumption, clinical observations, estrous cyclicity, reproductive performance, anogenital distance, pup survival, sexual development, sperm analysis, gross pathology, organ weights, and limited/selected histopathology. Feed consumption, clinical observations, and mortality were not adversely affected by NP administration. Nor were there any treatment-related changes observed in the litter data from all three mating trials. Effects that were observed included 7–12% reductions in terminal body weights, 14–18% increase in estrous cycle length, acceleration of vaginal opening by 1.5–7.3 days at 650 ppm and by 2.9–6.0 days at 2000 ppm in all three generations, a 8–13% decrease in epididymal sperm density in the F2 males at 650 ppm (sperm endpoints were unchanged in the F0 and F1 generations), increased relative kidney weight in adult males from the F0, F1, and F2 generations and in the F1 2000 ppm adult females, an increase in the incidence of renal tubular degeneration/dilatation in the males from all generations and in the 2000 ppm females from the F1, F2, and F3 generations, and in the 200 and 650 ppm females in the F3 generation, and a decrease in ovarian weights in the F2 generation and at 2000 ppm in the F1, F2, and F3 generations. The results of this study show that NP is a male and female reproductive toxicant at concentrations equal to or greater than 650 ppm based on decreased epididymal sperm density in males, as well as increased estrous cycle length and decreased ovarian weights observed in females.

Human

There are insufficient data to characterize chronic toxicity or exposure in humans.

In Vitro Toxicity Data

NP is an estrogenic compound that alters estrogen receptor-mediated gene expression, cell proliferation, and progesterone receptor responses in human estrogen sensitive MCF-7 breast tumor cells and other cellular models. For example, NP (10 μmol l^{-1}) induces mRNA expression of pS2 (a trefoil peptide expressed in breast cancer cells), MUC1 (a member of the mucin family), and estrogen receptor. Nonylphenols have been shown to be weakly estrogenic as indicated by elevated vitellogenin production in cultured rainbow trout hepatocytes ($ED_{50} = 16.15$ μmol l^{-1}).

Environmental Fate

NP partitions effectively into sediments following its release into aquatic environments ($K_{oc} = 31\,000$). In the mid-1990s, the average concentration of NP in US river sediments was determined to be $\sim$162 μg kg^{-1} (2960 μg kg^{-1}). In 1989, NP was detected in 17 of 30

US river samples at concentrations ranging from 0.11 to 0.64 $\mu g\,l^{-1}$. Concentrations of NP were greater (up to 600 $g\,l^{-1}$) in effluents from municipal wastewater treatment plants and industrial plants in the mid-1970s. While NP bioaccumulates in low-level aquatic organisms to a limited extent, NP is not expected to bioaccumulate appreciably in higher organisms ($\log K_{ow} = 4.48$). In freshwater fish for example, lipid-normalized bioconcentration factors ranged from 39 to 209 times the water concentration. Bioaccumulation was apparently greater in saltwater organisms where bioconcentration factors ranging from 78.7 to 2170 were measured.

Aerobic conditions favor the biotransformation and degradation of APE metabolites such as NP. Volatilization can be a major fate process for NP in moist soil due to a Henry's law constant of $1.1 \times 10^{-6}\,atm\,m^3\,mol^{-1}$. However, volatilization is likely not a major fate process from dry soil due to adsorption to soil particles ($K_{oc} = 31\,000$). NP is also susceptible to photochemical degradation (half-life 10–15 h in noon summer sun conditions).

Ecotoxicology

Due to concerns over potential exposure of aquatic organisms to NP, a number of acute and chronic toxicity tests have been conducted for both freshwater and saltwater species of invertebrates, fish, and aquatic plants. NP is considered an endocrine disruptor chemical and induces production of vitellogenin in male rainbow trout, a process that normally occurs only in female fish in response to estrogenic hormones during the reproductive cycle. NP also induces precocious development of ovaries and an intersex condition in some fish species.

Acute toxicity values (LC_{50}) for freshwater organisms ranged from 55.7 $\mu g\,l^{-1}$ for the amphipod (*Hyalella azteca*) to 774 $\mu g\,l^{-1}$ for the snail (*Physella virgata*). No relationships have been demonstrated between water quality characteristics (such as hardness and pH) and toxicity. The freshwater final acute value (FAV) for NP is 55.7 $\mu g\,l^{-1}$ which is equal to the LC_{50} for the most sensitive tested species, *H. azteca*. Acute toxicity values (LC_{50}) for saltwater organisms ranged from 17 $\mu g\,l^{-1}$ for the winter flounder (*Pleuronectes americanus*) to 209.8 $\mu g\,l^{-1}$ for the sheepshead minnow (*Cyprinodon variegatus*). The saltwater FAV for NP is 13.35 $\mu g\,l^{-1}$.

Chronic toxicity levels of NP for freshwater organisms ranged from 7.86 $\mu g\,l^{-1}$ for the rainbow trout (*Oncorhynchus mykiss*; based on growth), and 10.18 $\mu g\,l^{-1}$ for the fathead minnow (*Pimephales promelas*; based on survival) to 157.9 $\mu g\,l^{-1}$ for a freshwater cladoceran (based on reproduction). The chronic toxicity of NP for saltwater organisms was tested in only one species. A saltwater chronic value of 5.11 $\mu g\,l^{-1}$ was determined for the mysid (*Americamysis bahia*; based on reduced growth). Data were available to calculate a final acute–chronic ratio for NP of 9.41 based on a freshwater cladoceran, a saltwater mysid, and rainbow trout.

Two species of aquatic plants were exposed to NP and were found to be as sensitive as animals to NP exposure, showing effects that ranged from 27 to 410 $\mu g\,l^{-1}$.

Exposure Standards and Guidelines

Draft EPA water quality criteria for freshwater: 5.9 $\mu g\,l^{-1}$ (4 day average); 27.9 $\mu g\,l^{-1}$ (1 h average). Draft EPA water quality criteria for saltwater: 1.4 $\mu g\,l^{-1}$ (4 day average); 6.7 $\mu g\,l^{-1}$ (1 day average).

See also: Phenol.

Further Reading

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No-Observed-Adverse-Effect Level *See* Levels of Effect in Toxicological Assessment.

No-Observed-Effect Level *See* Levels of Effect in Toxicological Assessment.

Norbormide

Lynn Weber

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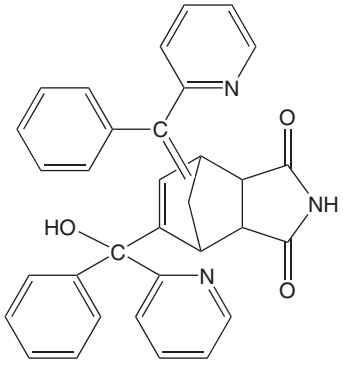
- Synonyms: 6-(α-Hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-norbor-5-ene-2,3-dicarboximide; McN-1,025; S-6,999; Shoxin; Raticate; ENT 51,76
- Chemical/Pharmaceutical/Other Class: Heterocyclic dicarboximide
- Chemical Structure:



Uses

Norbormide was first introduced in the market in 1964 as a selective rodenticide. This compound is highly specific for rats. Norbormide is on the World Health Organization 'obsolete' pesticide list.

Exposure Routes and Pathways

Norbormide can enter the body through oral, dermal, and inhalation exposures.

Mechanism of Toxicity

Norbormide causes an extreme and irreversible vasoconstriction in small arteries in rats following both systemic and local administrations. However, large rat arteries (e.g., aorta), nonvascular rat smooth muscles (e.g., duodenum and trachea) and all smooth muscles from nonrat species are not constricted by norbormide even at high concentrations/doses. The peripheral vasoconstriction in small rat arteries subsequently reduces coronary blood flow rate, leading to cardiac arrhythmias, which can lead to death. The norbormide-induced vasoconstriction is mediated by activation of phospholipase C/protein kinase C and calcium influx via L-type voltage dependent calcium channels. In contrast, norbormide-resistant arteries and smooth muscles exhibit inhibition of L-type voltage dependent calcium channels and instead exhibit a mild relaxation response. Sex- and species-related differences in sensitivity to norbormide may be attributed partially to the differences in metabolism of this compound. A lethal dose of norbormide in rats ($1\,g\,kg^{-1}$) can also elevate the blood glucose level twofold with a decrease in both liver and muscle glycogens. Exposed animals became comatose within 30 min to 2 h after treatment. The hyperglycemic effect of this compound in rats is considered to be a secondary effect.

Acute and Short-Term Toxicity (or Exposure)

Animal

Laboratory rats showed difficulties in locomotion and ataxia but no hindlimb paralysis after treatment with norbormide. Death occurs within 15 min to 4 h after struggling, dysnpea, hypothermia, and convulsions. Oral LD_{50} values for Norway rats ranged from 5.3 to $15\,mg\,kg^{-1}$. Rodents other than rats have considerably higher oral LD_{50} values (e.g., hamster, $140\,mg\,kg^{-1}$; guinea pigs, $620\,mg\,kg^{-1}$; mice, $2250\,mg\,kg^{-1}$). However, no effect was detected with $1000\,mg\,kg^{-1}$ doses of norbormide in dogs, cats, monkey, sheep, pigs, or chickens. Interestingly, L-type voltage dependent calcium channels in guinea pig heart were relatively selectively inhibited by norbormide in the sino-atrial and atrio-ventricular nodes, suggesting a possible therapeutic benefit for norbormide in treating supraventricular arrhythmias in heart failure.

Human

Human toxicity due to norbormide exposure is highly unlikely because of its relatively selective toxicity in rats. Human volunteers given 20–300 mg showed only a minimal hypotensive effect, which returned to control levels within 2 h. A dose of 300 mg corresponds to 60 g of the 0.5% bait and 30 g of the 1% bait. Although a hypotensive effect of norbormide was cited as a potential toxic sign, the maximum reduction in body temperature was found to be 0.7°C following 20–80 mg of norbormide in human volunteers. The hyperglycemic effect could not be demonstrated in humans.

Chronic Toxicity (or Exposure)

Little information is available on chronic effects of norbormide in humans or animals. Due to the selective toxicity in rodents, in particular Norway rats, little persistent toxicity would be expected in other species.

Clinical Management

As mentioned earlier, human toxicity due to overdose of norbormide is not expected because of its selectivity for rats. The only sign identified was a mild reduction of systolic blood pressure for a short period of time; therefore, only symptomatic and supportive care has been recommended in cases of norbormide ingestion. However, emesis may be induced in case of recent substantial ingestion of norbormide. Exposed eyes should be washed with tepid water for 15 min. In case of dermal exposure, the contaminated area should be washed with a sufficient amount of soap and water.

See also: Pesticides.

Further Reading

Bova S, Cima L, Golovina V, Luciani S, and Cargnelli G (2001) Norbormide: A calcium entry blocker with selective vasoconstrictor activity in rat peripheral arteries. *Cardiovascular Drug Reviews* 19(3): 226–233.

Pelfrene AF (2001) Rodenticides. In Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1793–1836. San Diego, CA: Academic Press.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency. Chemical Profile on Norbormide.

Norepinephrine *See* Catecholamines.

Notorious Poisoners and Poisoning Cases

Joanna Willis, Thomas Holdsworth, and Katherine Cathrow

Introduction

In Greek mythology, Hercules was said to have dipped his arrows in snake venom to render them more deadly. This may explain the origins of the word 'toxic', thought to be derived from the ancient Greek word 'toxon', meaning arrow. Therefore, although most cases of poisoning seen today are either accidental or as an act of deliberate self-harm, the origins of toxicology bring to mind a more sinister role of poison, that is, its use in dispatching of enemies.

Poison has been called 'the coward's weapon' as it is administered by stealth without any chance of defense on the victim's part. Therefore, murderers who have made poison their weapon of choice are often seen as more sinister and cold-blooded than those who face their victims with a gun or a knife, and at the other end of the scale, chemical warfare is seen to betray the ancient principles of chivalry and bravery in battle.

What follows are a few famous cases through history where poison has been used intentionally to destroy life, from individual murders to chemical warfare. However, it must be pointed out that these cases only skim the surface of what is an extensive history of murder by poison.

Socrates (Greece, 420 BC)

Socrates was born around 470 BC in Athens and was taught his family's trade of sculpture, as well as receiving an education in geometry and astronomy. At first he was an enthusiastic student in the sciences. However, he soon came to regard his teachers as merely imparting received knowledge that they could not themselves prove, and he set out to seek true knowledge of 'causes' and 'good'.

Socrates became involved in Athenian life because of his ideas, and was friendly with many of those in power. As was required of all citizens, he also served as a soldier and we know that he was decorated for bravery. He came to be widely known and respected for his wisdom, although he famously said that his wisdom relied on the fact that he fully recognized his own ignorance.

Socrates made it his mission to seek out people whose reputation in society he felt was undeserved. He confronted these people and questioned them on their positions and views before leading them

through further questioning into inevitable contradictions. He called this questioning 'elenchus'. Despite the fact that Socrates was polite and considerate in these exchanges, they were often conducted in public places. The Athenian youth came to view these public humiliations as a form of entertainment, and others used the same method in a less polite and more personal manner.

In 399 BC Socrates was accused of impiety, neglect of the gods whom the city worships, practice of religious novelties, and corruption of the youth. It is probable that the charges resulted from resentment of influential figures towards Socrates methods of questioning, and the way in which younger members of society were using it to upset the establishment. Socrates had also openly ridiculed the method of election in parliament by lot. 'In no other craft', he claimed, "would the craftsmen be chosen in this way." In addition, references he made to his personal spirit or 'daimonion', raised public suspicion that he rejected the state religion.

Socrates was found guilty of these charges and sentenced to death. Though his friends were willing to organize his escape, Socrates accepted his fate despite feeling the sentence to be unjustified. The rule of law decreed death by drinking the state poison. The Greeks were fond of the poison Hemlock, and it was often used for suicidal purposes. The state poison was a species of Hemlock known as cicuta. However, the administered dose was often not fatal and had to be repeated. Plato famously describes how Socrates was put to death by drinking the state poison in prison in 402 BC.

The Borgia Family (Italy, Fifteenth and Sixteenth Century)

The Borgias, a noble Spanish family from Valencia, established themselves in Italy during the fifteenth and sixteenth centuries. Alfonso de Borgia pursued an ecclesiastical career and became Pope Calixtus III in 1455. Rodrigo Borgia followed in his footsteps and became a cardinal of the Roman Catholic Church and then Pope Alexander VI in 1492. Rodrigo and his mistress Vannozza Catanei had five children, two of whom, Cesare and Lucrezia, became notorious (along with their father) for their supposed use of a secret poison, known as 'La Cantarella', to dispatch of several of their rivals. Although the exact composition of 'La Cantarella' is not known, it is thought to have been a mixture of copper, arsenic, and phosphorus, prepared in the decaying carcass of a hog, itself poisoned with arsenic.

Cesare Borgia (1475–1507) rose to the level of cardinal as his father had done before him, but his skills lay in politics, and when his father became pope in 1492, Cesare became his personal advisor. However, when Cesare's younger brother Juan overtook Cesare in status by being made Duke of Gandía, Cesare reputedly became jealous, and when Juan was mysteriously murdered in 1497, many suspected Cesare of killing his brother. This was never proved and Cesare went on to marry and receive the title of Duke of Valentinois. As captain general of the papal army, and with his father's support, Cesare attempted to establish a secular kingdom in central Italy. He was determined to establish himself as an Italian Prince before his father's death deprived him of the papal support he relied on. Cesare was ruthlessly single-minded in this quest and he is reported to have assassinated numerous political figures who were standing in his way.

Cesare's sister Lucrezia Borgia (1480–1519) helped to raise the political profile of the Borgia family by marrying into prominent families. However, her first two marriages ended unhappily. Her marriage to Giovanni Sforza, Lord of Pesaro, Milan, was annulled by her father, the Pope, after Giovanni fled Rome in response to the Pope making an enemy of Milan. Lucrezia's second husband, Alfonso, Duke of Bisceglie (son of Alfonso II of Naples) was murdered by Lucrezia's brother Cesare. It is not known whether Cesare murdered Alfonso to sever Rome's ties with Naples or whether it was the result of a personal vendetta. In any case, it was seen as highly suspicious that Lucrezia allowed the murder to go ahead. Lucrezia's reputation was further soiled by the appearance of her son in 1501, rumored to be the child of either Alexander or Cesare Borgia. Despite this reputation, Alfonso d'Este, son of the Duke of Ferrara, married Lucrezia in 1501.

Lucrezia Borgia has long been accused of sharing in her father's and brother's crimes by poisoning rivals with 'La Cantarella' powder from a ring on her finger, and her reputation as a ruthless murderer has been immortalized in Victor Hugo's drama and Donizetti's opera. However, it would appear on hindsight that she was simply an instrument for the political ambitions of her family.

In 1503, Pope Alexander VI (father to Lucrezia and Cesare) died. Ironically, it is thought that his death was the result of poisoned wine, although it is not known whether he was accidentally served wine meant for one of his rivals, or whether one of his rivals had in fact intended to poison him. Following the Pope's death, an enemy of the Borgias, Giuliano della Rovere, was elected as Pope. Cesare Borgia was arrested and imprisoned under his rule and although he escaped in 1506, he died in battle in 1507. Lucrezia Borgia retired to the Ferrara courts after her

father's death and lived out her remaining days there as a patron of the arts. She died at the age of 39.

The Borgia family began to decline towards the end of the sixteenth century and by the mid-eighteenth century the name had disappeared completely.

The Murder of Sir Thomas Overbury (England, 1615)

Thomas Overbury was a poet and essayist who moved to London to seek his fortune. With his friend Robert Carr (page to Lord Dunbar) he managed to secure an appointment at the court of King James I, where they both received expeditious promotion. Carr acquired the title of Lord Rochester and Overbury was knighted.

Rochester soon became infatuated with the Countess of Essex, who divorced her husband for him. However, Overbury did not approve of the Countess and tried to oppose the match. Rochester married the Countess against Overbury's advice and he and his wife turned against Overbury.

Soon afterwards, Overbury, on the advice of Rochester, refused an invitation from the King to become Ambassador to Russia. The King was greatly offended by this refusal and sent Overbury to the Tower of London.

The Countess of Essex, still consumed by hate for Thomas Overbury, plotted to kill him. She procured the help of an apothecary named Franklin. Franklin supplied poisons, including rosalger (a compound of arsenic), sublimate of mercury and white arsenic, to Weston, the under-keeper of the Tower. Weston, under heavy bribery from the Countess, mixed small quantities of poison into Overbury's food over the course of 4 months. Overbury became very unwell and eventually died. At first his death was thought to be the result of syphilis, but after some time, suspicion was aroused and the case was brought to trial.

Franklin pleaded guilty and was hanged. Weston was also hanged although he maintained his plea of innocence throughout the trial. Rochester and the Countess were also found guilty but their death sentences were retracted and they were eventually pardoned by the King.

The Affair of the Poisons (France, 1679)

In seventeenth century France, it was fashionable among Parisians to seek advice and aphrodisiacs from fortune-tellers. These fortune-tellers, however, also sold poisons (often called 'inheritance powders' as they enabled those who made use of them to claim their inheritance ahead of time by dispatching of their parents or spouse).

An inquiry lead by Nicolas de La Reynie in 1679 exposed a number of these 'fortune-tellers' and as a result, a special tribunal, known as the Chambre Ardente ('burning court'), was set up for the trial of those accused of witchcraft and poisoning.

Perhaps the most famous case to be tried in the Chambre Ardente was that of the midwife and fortune-teller La Voisin (born Catherine Deshayes Monvoisin). She was exposed by La Reynie as a supplier of poisons, put to trial and sentenced to death by burning; a sentence carried out in 1680. La Voisin was supposed to have sold mixtures of arsenic, aconite, belladonna, and opium in many forms including cosmetics. Several members of French society were implicated following the exposure of La Voisin, including Madame de Montespan, the mistress of King Louis XIV. She was accused by La Voisin's daughter of seeking poison and black magic from La Voisin in her attempts to win the King's affections and then later in (failed) attempts to dispatch of her rivals in court and ultimately of the King himself. Although these charges against Madame de Montespan were never proved, a permanent stain was left on her name and she eventually left the King's court in 1691 to join a convent.

Other prominent French figures connected with La Voisin include Olympe Mancini (niece of Cardinal Mazarin and mother of Prince Eugene of Savoy), her sister Marie Anne Mancini, and Marshal Luxembourg (duke and peer of France and one of the military heroes of the time). The Duke of Buckingham was also rumored to have been one of La Voisin's clients.

Following the success of the Chambre Ardente in imprisoning and executing poisoners such as La Voisin, the poisoning epidemic that had taken hold of France for so long came to an end.

Toffana (Italy, 1690)

Perhaps the most notorious poisoner of the seventeenth century was an Italian woman named Madame Giulia Toffana. She invented an arsenical solution in 1690, called 'Aqua Toffana', which she sold in phials bearing the representation of a saint, usually Saint Nicholas of Bari (Bari was a town whose water was supposed to have had healing properties). The phials were sold to women under the pretence that Aqua Toffana was good for a woman's complexion (as arsenic is), but Toffana also sold her solution to women who wanted to rid themselves of their husbands. It was apparently colorless, tasteless, and miscible with wine, and therefore very easy to administer. Toffana is said to have been responsible for as many as 600 murders and for this she was executed in Naples in 1709. However, Toffana had

her followers and one of them, Hieronyma Spara, developed her own version of the poisonous solution, named Aquetta di Perugia, in Rome in 1695. She too was responsible for a number of deaths and, like Toffana, was executed for her crimes.

Thomas Wainewright (England, 1830)

At 30, Thomas Wainewright was a popular and successful gentleman in the literary and artistic circles of London society; he was a friend of William Blake and Charles Dickens, a published writer and an exhibited artist. At 40 he was working on a chain gang in a Tasmanian penal colony, shackled to thieves and murderers.

Born in 1794 and orphaned at a young age, Thomas Griffiths Wainewright was brought up by his grandfather, the editor of *The Monthly Review* (London's first literary magazine) into London's high society which was, at the time of the Romantic Revolution, a world of dandies and dilettantes, painters and poets. With an artistic temperament and considerable wit and charm, Wainewright seemed perfectly suited to the lifestyle of dinner parties and art galleries. He showed talent as a painter himself, exhibiting on several occasions at The Royal Academy, and wrote regularly for various magazines and journals. However, his glamorous lifestyle was expensive, and by his late 20 s, he found himself in financial difficulties.

In 1822, by which time he was married, Wainewright turned his artistic talents to forgery, counterfeiting signatures on documents to allow him immediate access to some of his inheritance, which was held in a trust fund. By 1824, he had got his hands on the full legacy of £5250. However, this money did not last long, and he was soon borrowing money from loan sharks and friends, and running up large debts.

In 1828 Wainewright, his wife Eliza, and their son moved into Linden House, the impressive country home in which he had grown up, and which was now owned by his uncle. Within a year, his uncle had died in mysterious circumstances, leaving his house and estate to Wainewright. The Wainewrights then invited Eliza's mother and two sisters to come and stay with them at Linden House. In the following months, Wainewright, Eliza, and Helen Abercrombie, the youngest sister, set up an elaborate insurance fraud. They insured Helen's life with five different companies and on false pretences, lying about Helen's age and the Wainewrights' financial situation.

Helen's mother seems, understandably, to have disapproved of this, but in 1830 she too died suddenly in mysterious circumstances. The fraud was completed in the same year, Helen's life being insured for a total of £16 000. It is hard to imagine why Helen would co-operate with a scheme that it seems could only have been successfully completed by her own death, but she was present at many of the negotiations and aware of at least some of the deception involved. It seems likely that she was being manipulated and deceived by Wainewright, and possibly by her sister, although the extent of Eliza's involvement is unclear. One actuary reported that, when asked why she wanted to insure her life, Helen said that "she had been told it was proper to do it." Almost as soon as the insurance policies were in place, Helen, up until then a healthy 21 year old, was taken ill. She died within a few days, in the grip of painful convulsions which one servant of the household reported were identical to those experienced by both Wainewright's uncle and Mrs Abercrombie. Helen's death certificate recorded the cause of death as "cerebral haemorrhage." However, the insurance companies refused to pay out on the basis of "misrepresentation", having identified the fraud. Wainewright promptly initiated legal action against them, but his reputation had taken a serious blow and there were widespread suspicions about the deaths. He decided it would be prudent to lie low, and moved to France, where he stayed for the next 5 years.

No charge of poisoning was ever brought against Wainewright, as there was insufficient evidence. It cannot be said with absolute certainty that he was responsible for his relatives' deaths, although the circumstantial evidence seems reasonably compelling. At this time forensic detection of poisons was difficult, and if Wainewright was indeed a poisoner, he was canny and skilful. The most widely accepted story of Helen's death is that she was poisoned first with antimony, causing her to suffer from symptoms such as nausea and vomiting. However, at the same time the Wainewrights served a relatively indigestible meal, providing an alternative explanation for her sickness. Then, after a couple of days and a visit from the doctor, she was fed jelly laced with strychnine. The bitter taste of the strychnine would have been masked by the sweet jelly, and she may have been told that the powder was an all-purpose remedy, such as a 'black draught' laxative, which was widely used at the time. In her weakened state, she would have died almost immediately. The convulsions described by the servant as being common to all three deaths are characteristic of poisoning with strychnine, which would have been easily available from an apothecary. There are also reports that Wainewright had several books on poisons in his library.

After five difficult and largely impoverished years in France, Wainewright returned to London, but was

promptly arrested for the forgery he had committed 10 years previously. He was held in Newgate Prison for some time, and then transported to Hobart Town in Tasmania, where he lived until his death in 1847. Initially he worked on a chain-gang building roads, but eventually he was allowed to work as a hospital orderly and even paint again. He painted portraits of many of the local dignitaries and their families, which are generally thought to be his most accomplished works as an artist.

William Palmer (England, 1855)

Lauded as the 'Prince of Poisoners' by the press of the time, William Palmer is believed to have murdered using strychnine. He was Britain's first recorded 'Serial Killer' and his effigy stood in the Chamber of Horrors at Madam Tussaud's Waxworks for 127 years.

Palmer was born in Staffordshire, England on August 6, 1824, the sixth of seven children. At the age of 10 he was send to Rugeley Free Grammar School, which he attended as a day scholar. Some accounts state that he was a boisterous child and a bully; others claim that he was the most well behaved of all the children. It would seem that his true personality fails to have been documented. When Palmer was 12, his father died. Without their father's strict governance, William and his siblings were now free to run wild.

At 17, Palmer left school and began an apprenticeship which his mother had arranged for him at a wholesale chemists in Liverpool. This job ended abruptly when his employers discovered that Palmer had been stealing money from them. He was in fact stealing money to buy gifts to impress his girlfriend. His mother intervened and paid back the money he had stolen to prevent his employers reporting him to the police. A second apprenticeship was arranged with a physician in a medical practice, but this too was doomed to fail as Palmer, still obsessed with the same girlfriend, stole money from his new employer. His mother's attempts to have William forgiven were futile and he was sent to Stafford Infirmary to serve an apprenticeship as a 'walking pupil'. His love of women and alcohol, however, continued to grow.

It was during his time at Staffordshire Infirmary that Palmer is thought to have become interested in poisons and he may have been behind a suspicious death of a patient whilst a student at the infirmary. It is claimed that Palmer laced brandy with poison and challenged his patient to a 'drinking contest' after which the patient died. Palmer continued his training at St Bartholomew's Hospital in London. However, things did not go well, and he persisted in drinking and womanizing. It was only after his mother intervened yet again and employed a private tutor that Palmer managed to settle, study and eventually qualify as a doctor in 1846.

Upon returning to Rugeley in 1847 Palmer set up a surgery from a rented house. Business went well and he was soon able to afford an assistant. However, he became heavily involved in horse racing and gambling, and at one point owned 15 racehorses. Eventually, he could no longer sustain his gambling addiction and fell into debt. His financial situation became so desperate that at one point he resorted to drugging a competing horse. This earned him the title of 'nobbler', and he was discredited by the racing authorities. Despite this, his gambling addiction grew and Palmer came to rely on moneylenders to finance his habit. In order to secure loans to buy more horses, he began to forge his mother's signature. By the autumn of 1855 he owed £15 000 and had outstanding bills for a further £11 500.

Palmer met his wife, Annie Thornton in 1845. She had completed her studies at finishing school, and Palmer was attracted to her beauty, charm, and wealth. They married in 1847, despite the audible displeasure of her mother, a rich widow. Palmer and his mother-in-law did not have a good relationship. She was a difficult woman by all accounts, drinking heavily and becoming violent. It is even supposed that she drove her own husband to suicide. Following a particularly heavy drinking binge, she became so ill that she needed to be nursed at her daughter and son-in-law's house. Within 2 weeks she was dead. At the time it was presumed that she had died from the effects of prolonged alcohol abuse, but later, as suspicions surrounding Palmer's activities grew, many began to suspect that Palmer was responsible for his mother-in-law's death.

Four of Annie and William Palmer's five children died within weeks of their birth. The death certificate in each case cited convulsions as the cause of death. However, the Palmers' housekeeper claimed that Palmer had murdered the children by dipping his finger in poison, then honey and then into their mouths. She claimed he had commented that he could not afford so many mouths to feed. Whether this was the case, or whether there was a medical explanation for the deaths of Palmer's children has never been established.

Palmer had a close friend, fellow gambler John Parsons Cook. Cook had inherited £12 000 and retired from his work as a solicitor, choosing to spend his time and money on horse racing. Having never enjoyed good health, his new-found wealth led him into a more 'riotous' lifestyle and he is reported to have caught syphilis, for which he was treated by Palmer.

In mid-November 1855, Palmer and Cook attended the Shrewsbury Races, where Cook won ~£3000. Palmer was not so lucky and he had begun to receive threatening letters from his money-lenders. Shortly after his friend's win, both were dining at a local inn, when Palmer was seen mixing up some kind of concoction in a room away from his friend. He returned to his friend and a tray of brandy was brought in. Upon drinking his brandy, Cook complained that it burnt his throat and thought it may be drugged. He retired to bed feeling unwell. Over the next few days, his condition deteriorated. Palmer attended to him, and even traveled to London with Cook's betting books to collect his winnings for him (money, Palmer later claimed, that was owed to him). By the 20th of November, Cook was very unwell, and in the early hours of the 21st, he suffered violent convulsions and subsequently died.

Palmer was arrested on suspicion of Cook's murder and taken to Stafford jail where he went on hunger strike before being threatened with force-feeding by the jail governor. On May 4, 1856, Palmer was transferred to London for his trial in Westminster.

Reports claiming that Palmer had bought strychnine around the time of Cook's death, and claims by maids in attendance of Cook that food sent by Palmer had made them sick resulted in Palmer being the first man in British history to be tried for murder by strychnine poisoning. However, strychnine was never found in the body of John Parsons Cook and although this was blamed on an inadequate postmortem, many, including Cook's own doctor who was present at the time of death, believed that Cook had died of tetanus.

During Palmer's trial, other suspicious deaths were investigated. Palmer had insured his wife's life for £13 000 in spring 1854 and by the autumn of the same year she was dead. Her death certificate stated that she died from English cholera. Her symptoms were recorded to include retching and vomiting, but no convulsions. Annie Palmer's body was exhumed for examination of her stomach contents. There were no traces of strychnine, but a small amount of antimony was found. Antimony can be used as a poison but at the time Annie Palmer died it was also often used to treat symptoms such as the ones she was suffering from.

Similarly, Palmer had insured his brother Walter's life. It is widely accepted that Palmer was defrauding the insurance companies as he actually employed someone to keep Walter sober while medical clearance was obtained for the insurance to be validated. Walter died soon after and the money went to Palmer. As with Annie, Walter's body was ordered to be exhumed when Palmer was awaiting trial for the murder of Cook. However, Walter's body was in such a state of decomposition that the coroner was unable to perform a satisfactory postmortem, and the case was dropped. Whether the death was due to alcohol or poisoning by Palmer is still unclear.

Other suspicious deaths were also investigated: an uncle of Palmer died shortly after a night of drinking brandy with him, and a friend of Palmer's died after being treated by him.

After a 12 day trial, the jury of 12 men took less than 2 h to reach a unanimous verdict of guilty. Palmer was sentenced to death for the murder of John Parsons Cook, and was publicly hanged in Stafford on of June 14, 1856, at the age of 31, still protesting his innocence.

It is supposed that Palmer poisoned at least 11 victims, and as many other suspicious deaths also carry his hallmarks. However, much debate still exists as to whether Palmer actually was a murderer, or whether he was simply labeled a killer by the misfortune of circumstantial evidence.

Madeleine Smith (Scotland, 1857)

In 1857, Madeleine Smith was tried for the murder, by arsenic poisoning, of her lover, Emile L'Angelier.

Madeleine was the 22-year-old daughter of a wealthy and well-respected family in Glasgow. Emile was a poor immigrant from Jersey, employed as a clerk at a Glasgow seed-merchant. They met in the spring of 1855 and became friends over the next few weeks. When Madeleine moved with her family to their summer house at Rowaleyn a few weeks later, Madeleine and Emile continued their evolving friendship through numerous letters. Madeleine's father soon found out about the friendship and, because of Emile's social standing, was not happy about it. However, Madeleine and Emile's relationship carried on in secrecy and they soon declared their love for each other. They had occasional clandestine meetings and Emile's letters reached Madeleine through her maid, Christina. During the summer of 1855 Emile and Madeleine became secretly engaged and planned their wedding for September of 1856.

Madeleine and Emile's relationship continued like this for over a year. However, in July of 1856 Madeleine was introduced to William Minnoch, a wealthy businessman Madeleine's father knew and intended his daughter to marry. Over the next few months Madeleine's letters to Emile began to lose their previous enthusiasm and Madeleine decided to postpone the wedding. She knew of her impending proposal from Minnoch and did not know what to do about it. Minnoch formally proposed to Madeleine in January of 1857 and Madeleine accepted.

Madeleine then made several attempts to end her relationship with Emile and asked him to return all of her letters so that no one would find out about the relationship and jeopardize her engagement. Emile refused to return Madeleine's letters and, instead, threatened to send them to her father. To prevent him from carrying out his threat and to attempt to pacify him, Madeleine agreed to continue seeing Emile.

On February 21 Madeleine went to a local apothecary and bought sixpenny worth of arsenic. She told the clerk that the poison was needed to kill rats and signed the Poison Book, as was required by law. It is not known whether Madeleine and Emile met that night, but the next morning Emile suffered from stomach cramps, nausea, and vomiting, which kept him at home for a week. Madeleine bought sixpenny worth of arsenic twice more in the next few weeks, again claiming that it was to kill rats. Madeleine and Emile met on the night of the 22nd of March; at half past two the next morning Emile arrived at his lodging house doubled-up in pain and he vomited many times over the next few hours. At 10 O'clock on the morning of March the 23rd, Emile died.

Over the course of the next week, Madeleine's letters to Emile were found and on postmortem examination, Emile's body was found to contain large amounts of arsenic. Madeleine was arrested for the murder of her lover. The trial began on June 30, 1857 and by this time there was considerable public interest in the case. Madeleine pleaded not guilty but was not allowed to speak for her defense in the trial, in accordance with the law. However, she recorded a statement before the trial began claiming that she had last seen Emile three weeks before his death and that the arsenic she had bought was for cosmetic purposes. In the course of the trial many people were questioned about Madeleine and Emile's relationship, about the events that occurred on the night before Emile's death, and about the fate of the arsenic Madeleine purchased. The prosecution concentrated on the fact that Madeleine's arsenic purchases coincided perfectly with Emile's periods of ill health. They suggested that Madeleine had become so afraid that Emile would jeopardize her engagement to William Minnoch that she poisoned cocoa with arsenic and gave it to Emile during one of their secret meetings. The defense concentrated on a number of people's accounts of Emile as an unstable man who was capable of suicide, and suggested that he was so angry about Madeleine's rejection that he tried to frame her for his murder. According to some of the defense witnesses, Emile had taken arsenic in small doses as a tonic.

On July 9, 1857 the jury returned a verdict of not proven on the charge of murder (a verdict unique to Scotland, which allows the defendant to go free but carries a stigma, as it not only states that the prosecution failed to prove its case, but also indicates that the defense failed to convince the jury of the defendant's innocence). Madeleine Smith walked free from the court and fled to Rowaleyn. Her engagement to William Minnoch was called off and the Smith family tried to forget about the unfortunate incident. However, public interest in the case refused to die down.

Not long after the trial, Madeleine moved to London and married a draftsman. They had two children but separated after 28 years of marriage. Madeleine emigrated to America a few years later and eventually married again and lived in New York. She died on April 12, 1928.

What actually happened to Emile L'Angelier in the early hours of March 23, 1857 will never be known, although speculation continues.

The Umbrella Assassination (London, 1977)

Georgi Markov was a Bulgarian writer who lived in his home country until 1969, when at the age of 40 he defected to the West. Living in London, he worked as a broadcast journalist for the BBC, Radio Free Europe, and the German Deutsche Welle.

He had a large audience in Bulgaria, and his outspoken views against the ruling communist party were seen as the inspiration for Bulgarian dissident movements. The leader of the Bulgarian communist party, Zhivkov Todor, decided in June 1977 that he wanted Markov silenced, and informed a politburo meeting of his wishes. The job of assassinating Markov was given to the interior minister Dimiter Stoyanov, who requested KGB assistance. The KGB chairman Yuri Andropov agreed provided there would be no trail left to the Soviet Union.

There were three attempts on Markovs' life. During a dinner party given by friends at Radio Free Europe, someone slipped a poison into his drink. However, this and another attempt on his life in Sardinia failed. The successful attempt took place in London on September 7, 1977.

Markov worked a double shift at the BBC, and after working the early morning shift, he went home to rest. On returning to work he parked his car South of Waterloo Bridge and made his way to the bus stop to catch the bus to the BBC headquarters. As he neared the people queuing for the bus, he felt a stabbing pain in his right thigh and turned to see a man facing away from him stoop and pick up an umbrella. The man apologized in a foreign accent and departed hurriedly in a taxi. Markov later described the man as thick set and ~40 years old. In pain, Markov

boarded the bus for work, where he told colleagues what had happened. He noticed a spot of blood on his jeans, and showed a friend a pimple-like red swelling on his thigh. When he returned home he became very ill, with a high fever.

The next day Markov was admitted to St James's hospital in Balham. Examination of his right thigh showed a central puncture wound of ~2 mm diameter, and a circular area of inflammation. A diagnosis of septicemia was made, due to the very high white cell count. Mr Markov died 3 days after he had been injured.

During the postmortem, a single metal sphere the size of a pinhead was excised from the wound. It was 1.52 mm in diameter and composed of 90% platinum and 10% iridium. It had two holes bored through it, with diameters of 0.35 mm, leaving 0.28 mm^3 available for toxin retention.

Dr. David Gall at the government chemical defence establishment Porton Down, hypothesized that ricin could be the only possible poison used, owing to the exceptionally small dose and the symptoms Markov had experienced.

After the fall of the Soviet Union it was revealed that ricin was used in an umbrella mechanism for injecting poison spheres into victims, a technique developed in the secret KGB laboratory 'the Chamber'. Two former KGB officers Oleg Kalugin and Oleg Gordievsky publicly admitted to Soviet involvement in Markov's death and it was reported that the Bulgarians had used a low-level Italian criminal to carry out the murder. The man was located in Denmark but questioning remained inconclusive; he then fled to Hungary and the Czech Republic and his current whereabouts are unknown.

Harold Shipman (England, 2000)

Harold Frederick Shipman was born to a working class family in Nottingham on the 14 of January 1946. Intelligent and successful at school, he endeavored to study medicine after the death of his mother from lung cancer when he was 17. In 1965, he realized this ambition and began a medical degree at Leeds University. Graduating in 1970, Shipman qualified as a General Practitioner (GP) in 1974, and went to work at the Abraham Ormerod Medical Practice in Todmorden, West Yorkshire.

It was during his time at this practice that colleagues discovered his addiction to the opioid pethidine. Shipman was disciplined by the General Medical Council (GMC), but was not struck off the medical register. It is now understood that Shipman murdered his first victim during his time there in 1975. As a result of his newly discovered history of opioid abuse, Shipman was dismissed from the Todmorden practice. However, he reappeared as a GP in Hyde, Greater Manchester in 1977, this time working at the Donneybrook House Practice.

Solid indications that he had been conducting murderous activities became apparent during his time at Donneybrook House, when the death rate amongst his patients was three times higher than that of his colleagues in the same practice. It is believed that Shipman murdered a further 71 patients whilst at this practice.

In 1993 Shipman set up his own practice on Market Street in Hyde, after falling out with the partners at Donneybrook House. He was to go on to murder another 143 patients whilst in this practice. It was not until 1998 that Shipman's crimes first came to light when the daughter of one of his victims grew suspicious following her mother's death.

Kathleen Grundy, an 81-year-old previous mayoress, was found dead in her home. Shipman had visited her house on the morning of her death. His visit had been prompted by a consultation the previous day, when he had requested her help in a survey of aging, as he proposed that she was extremely fit and well for her age. Mrs Grundy readily agreed, and Shipman arranged to take the required 'blood sample' for inclusion in the survey. Under the pretences of taking such a sample, he in fact injected her with a lethal dose of diamorphine, which led to swift respiratory depression and death. He was to state on her death certificate that she died simply from 'old age' – a strange conclusion to draw considering his conversation with her the previous day!

Mrs Grundy's last will and testament were scrutinized. A recently redrafted will replaced the original her family were familiar with, and to their surprise left all estate and monies to the sum of £386,000 to Dr Shipman, in recognition of his 'attentive care'. The new document was poorly drafted and the signature of Mrs Grundy did not correspond to her usual hand.

Mrs Grundy's daughter, Angela Woodruff, voiced her suspicions to the police that Dr Shipman may have forged her mother's will, and possibly undertaken more sinister actions. Owing to the severity of the allegations, the body of Kathleen Grundy was exhumed for postmortem 1 week after her burial in Hyde Chapel cemetery. Forensic teams took prints from the body looking for matches on the will to indicate her handling of the document: none were found. Tissue samples were taken from thigh muscle and liver for drug levels. Using mass spectrometry, it was ascertained that diamorphine was indeed present at highly toxic levels in the samples. The levels were consistent with those found in fatal overdose cases.

Rumours about Shipman spread, and on September 19, 1997, the *Manchester Evening News* published a story detailing these. In response, many concerned families came forward, and as a result, further bodies of elderly females who had lived alone, and died in 'suspicious circumstances' were also exhumed and tested for diamorphine. Similar results were found.

Shipman was arrested on suspicion of murder on September 7, 1998, just over a year since the investigation had begun. At no point did Shipman confess to having any knowledge regarding the murders. He claimed that many of these women had in fact been substance abusers. He cited mainly codeine as the probable drug of abuse and said that he could prove his claims by retracing medical records where he had made entries indicating his suspicions. Forensic document analysts and computer experts were later to show that Shipman had in fact tampered with records on the actual day of the deaths (some hours after he had administered lethal doses of opioid to them). The truth was uncovered when the in-built clock in his practice computer verified the exact time of new entries to medical records, regardless of their apparent chronological ordering to the onlooker. Shipman's 'perfect' plan was crushed. His fabricated suggestions that patients had suffered from life-threatening illnesses or drug abuse were thrown out in court on the basis of the computer evidence.

However, an important question remained: how did Shipman obtain so much diamorphine in order to murder so many? After his reprimand from the GMC in the 1970s following his pethidine addiction, he was banned from holding controlled drugs in his surgery. However, calling upon the help of local pharmacists, police discovered in controlled drug registers that Shipman had indeed prescribed morphine and diamorphine for many patients – both those with, and those without terminal illnesses.

When local registered nurses working in home care settings of the terminally ill were interviewed, it became apparent that Shipman had frequently failed to deliver the full prescription of controlled drugs to patient's homes when he had collected them from pharmacies on their behalf. Shipman was effectively stealing up to 60% of the diamorphine prescribed for his patients.

Shipman's claims that those patients who were found with opiates in their dead body tissues were habitual drug abusers still needed to be quashed. The prosecution brought in an expert forensic hair analyst. As hair grows at a rate of 1 cm per month, the expert could identify evidence of opioid use by quantifying levels in strands of hair. Using mass spectrometry, he discovered that the amount present in the victims' hair was consistent with opioid use on only one or two occasions. In a narcotic abuser, it is normal to find 2 ng of the substance per milligram of hair – this level was 200 times more than that found in the victims' hair samples.

Expert forensic psychiatrists believe that Shipman was indeed a psychopath, feeding a need to maintain a perfect public and personal perception of himself. His addiction to the power of a perfect murder grew in strength and momentum over time, and cost the lives of many of those who entrusted their care to him. John Pollard, a coroner who knew and worked with Shipman has theorized that "The only valid possible explanation for it is that he simply enjoyed viewing the process of dying and enjoyed the feeling of control over life and death."

On January 31, 2000, Dr Harold Shipman was given 15 life sentences and 4 years to serve in prison for 15 murders and the forging of a will. A public enquiry later concluded that Shipman had killed at least 215 of his patients over a period of 23 years. The youngest victim was a 41-year-old man, the oldest a 93-year-old woman.

Shipman never publicly accepted responsibility for the death of his victims and showed no remorse for his crimes. He was found hanging from bedsheets in his prison cell in the early hours of January 13, 2004, one day before his 58th birthday. He left behind his wife, Primrose, three sons and a legacy of misunderstanding, doubt and public loss of trust in the medical profession. The full extent of his crimes will never be known.

Chemical Warfare

Records of poisonous chemicals being used to maim and kill date back to ancient Greek times, when pitch and sulfur were combined to make 'Greek Fire', which was launched at enemies in battle. Poisons were also used in medieval battles although their use was seen to betray the tenets of chivalrous conduct. The First Hague Convention at the end of the nineteenth century resulted in prohibition of the use of chemical weapons in war. This seemed to have little effect however, as the first time chemical weapons were used on a large scale was in World War I.

From the beginning of the War in 1914, both sides made use of various tear gases, although the German chemist Fritz Haber was working on more effective chemical means of penetrating Allied defenses. In 1915, the German military's certainty of victory began to waver, and they began planning chlorine gas attacks. The first of these, and perhaps the most famous, took place in Ypres, Belgium on April 22, 1915, against French and Algerian troops. The Germans set up more than 5000 cylinders of chlorine and when the valves were opened, a dense green

cloud of chlorine gas at 1000 ppm settled over Allied lines, killing thousands of soldiers. Ironically, although a number of these attacks were carried out over the next year, German forces were unable to take advantage of the breach in Allied lines as they themselves had limited protective supplies.

By 1916, poison gas in the form of chlorine and phosgene (both respiratory agents) had become a standard weapon used by both German and Allied forces, and primitive gas masks were standard issue among troops. Research into agents for chemical warfare was heavily funded on both sides, and the British established a large facility at Porton Down on Salisbury Plain for this purpose. However, in practice, gas attacks rarely went according to plan and although the British used phosgene very successfully at the battle of the Somme in June 1916, many British soldiers were killed by their own gas.

The race was on to develop the most effective poisons and the best methods of delivery as well as the most impenetrable gas masks. By 1918, gas mask technology had rendered chlorine and phosgene ineffective at normal concentrations, but the Germans had started using 'mustard gas', a liquid blistering agent (vesicant) that was rarely lethal but could incapacitate men who came in contact with it. Again, within months, mustard gas was being heavily used by both sides. Ill-prepared forces, such as the Russian troops on the Eastern Front, always suffered the most.

Chemical warfare continued until the armistice was declared in November 1918. By this time gas was estimated to have killed more than 100 000 men and injured more than a million (a small proportion, nonetheless, of the total number of casualties and deaths in the First World War).

By the end of World War I, a number of other agents had been developed, including the arsenical Lewisite (another blistering agent developed by the Americans), and agents that blocked the absorption of oxygen in the blood, such as hydrogen cyanide. Despite the Treaty of Versailles in 1919 and the Geneva Protocol in 1925, both of which forbade the use of chemical agents in warfare, research and development continued in secret. Fritz Haber continued his research into poisonous gases under the guise of 'pest control'. He developed an insecticide called 'Zyklon B' in the form of a crystalline material that released hydrogen cyanide fumes. Hydrogen cyanide had never achieved widespread use in World War I as it dissipated too easily in the open air. However, in enclosed spaces, it was very effective (see discussion on Holocaust gas chambers, below). Because of his Jewish background, Haber resigned when the Nazis came to power in Germany in 1933. However, he was soon replaced by Gerhard Schrader, who had accidentally come across a highly lethal organophosphate compound in December 1936 while carrying out genuine research into insecticides. Named 'tabun', it became the first in a long line of nerve agents. Tabun was invisible, odorless, and could kill in very small quantities by being absorbed through the skin (thereby rendering gas masks ineffective). With Nazi funding, Schrader soon developed an even more lethal nerve agent, which he named 'Sarin'.

Chemical warfare was beginning to resurface all over the world in the few years before World War II. The Italians dropped mustard gas from planes during their campaign in Abyssinia (now Ethiopia) in 1937 and the Japanese reportedly used mustard gas against the Chinese. The fear of chemical warfare in the imminent war resulted in exhaustive civil defense programs in Britain with the distribution of 30 million gas masks (useless of course in the face of a nerve agent attack).

When World War II broke out in 1939, stockpiling of poison gases recommenced. However, contrary to the predictions made before the war, chemical weapons were hardly used. Battles moved more rapidly than they had done in World War I, rendering many agents useless, and new explosives were capable of far more destruction than poison gases. The Germans produced nerve agents and, by 1944 they possessed enough tabun to kill vast numbers of people. However, they never used it, possibly because they believed (incorrectly) that the Allies knew about nerve agents and had their own supplies.

After World War II, large quantities of chemical weapons that had been stockpiled but never used, were very publicly disposed of. However, the existence of nerve gas was no longer a secret as Russian and British intelligence had uncovered its production in Germany at the end of the war. The Cold War saw massive development of nerve agents all over the world and while the British renounced the use of offensive chemical weapons in 1965, the Americans continued to develop new agents. By 1967 vast quantities of the nerve agent 'VX' had been produced. VX was less volatile and more toxic than previous nerve agents.

The Americans, who had not ratified the original Geneva Protocol in 1925, eventually ratified it in 1975. They then began an international campaign to limit the use of chemical weapons.

By 1980, although chemical weapons were still the subject of much debate and suspicion (including accusations of the use of 'Yellow Rain' in South East Asia and the use of 'knock down agents' by USSR forces in Afghanistan) they had not been used on a large scale since World War I. However, all that was to change during the Iran–Iraq war in the 1980s when the Iraqis resorted to chemical warfare to

compensate for their small numbers. Mustard gas, Lewisite and nerve agents (including VX) were all produced and mustard gas and sarin were used in attacks on Iranian forces. This was the first time in history that the use of chemical weapons had actually contributed to the defeat of a side (the Iranians). Then, following the success of these attacks, Sadam Hussein used mustard gas and the nerve agents sarin, tabun, and VX against Iraqi Kurds as part of a campaign aimed at depopulating rural Kurdistan. The most deadly of these attacks (of which there were more than 100) took place in Halabja in March 1988 and took the lives of thousands of civilians.

During the Gulf War in 1991, there were widespread fears that Saddam Hussein would use chemical and biological weapons on Coalition forces. However, these fears were not realized and no weapons of mass destruction were deployed. UN Inspection teams proceeded to destroy many of Iraq's stockpiles following Iraq's defeat, although whether stores remain is still a matter of considerable debate today.

The collapse of the USSR in the late 1980s and early 1990s was a step forward in controlling chemical weapons. However, by this time, destruction of chemical weapons was becoming increasingly difficult due to environmental issues. Incineration is now the only acceptable method of disposal and as yet only a limited number of suitable incinerators exist as the process is dangerous and expensive.

Although there have been no large-scale attacks using chemical weapons since the 1980s, a number of smaller attacks have occurred. Perhaps the most famous of these recent attacks took place on March 20, 1995, when containers of a liquefied form of sarin were placed on five different subway cars on three different lines in the Tokyo subway system by members of a Japanese religious sect named the 'Aum Shinrikyo' (Supreme Truth). Five thousand people were injured and 12 died. The leader of the sect, Shoko Asahara (born Chizuo Matsumoto) was arrested along with other members. Shoko Asahara confessed to the subway attack (as well as a number of other terrorist attacks, many of which had been thought to be accidental) and has since been sentenced to death.

In the aftermath of the September 11, 2001 attacks in the United States, many of the captured Islamic terrorists revealed how they had been trained in methods of dispersing hydrogen cyanide into the ventilation systems of buildings.

Clearly the threat of chemical warfare is still very real, and research continues into defensive technology such as nerve gas vaccines and chemical sensors. The Chemical Weapons Convention came into effect in 1997 and it has taken the Geneva Protocol one step further, banning the manufacture and storage of lethal and nonlethal chemical weapons as well as their use.

Holocaust Gas Chambers, World War II

Perhaps the most atrocious killings of the two World Wars took place not on a battlefield, but in the Nazi concentration camps. By 1941 prison camps were filling up with POWs and Jews but the Nazis had no efficient method of disposing them. Then, in September 1941, as an experiment, they tried using Zyklon B in Block 11 at Auschwitz I. Six hundred Soviet POWs and 250 Poles were killed and the Nazis had found an efficient and very effective method of destroying their prisoners.

Zyklon B was the trade name of a pesticide developed in the 1920s by the German (and ironically, Jewish) chemist Fritz Haber. It was originally used in concentration camps to delouse prisoners and control typhus. It consisted of small pellets or discs of wood pulp or diatomaceous earth impregnated with hydrocyanic acid (HCN). When the pellets were removed from their airtight containers they evolved hydrogen cyanide as a gas. The pellets normally contained an irritant warning chemical, but when the Nazis began to experiment with it in gas chambers they ordered Zyklon B to be produced without the warning chemical. After the War two directors of the company that supplied this modified Zyklon B were sentenced to death by a British Military court.

Zyklon B achieved its most widespread use in the gas chambers at Auschwitz II, Birkenau. There were four chambers at Birkenau, each capable of killing up to 2000 people at once. The system worked very efficiently; a death train would arrive at the camp in the morning and by the afternoon, the prisoners would have been poisoned and then either buried in mass graves or burned.

Other camps, such as Treblinka and Chelmno used carbon monoxide in their gas chambers.

The use of the word 'zyklon' (German for cyclone) continues to prompt angry reactions from Jewish groups. In 2002, both Bosch Siemens and Umbro were forced to withdraw from attempts to use or trademark the term for their products.

Conclusions

Murder by poison has changed a great deal over the centuries. In ancient times, poisons were often thought of as spiritual or witchcraft, and were used with little knowledge of how they worked; there was no such thing as toxicology, let alone forensic toxicology. For this reason, detection of these murders was

often very difficult. However, poisoning is no longer a mystery to us. Toxicology has given us insight into the mechanisms by which poisons act, allowing us to detect their use and prevent and treat their effects.

However, with knowledge comes the power to misuse it. This is demonstrated by the (thankfully rare) cases of medical professionals, such as Harold Shipman, who abuse their position of trust and murder patients, and it is also seen on a far larger scale when countries at war resort to the use of chemical weapons. Most of the chemical warfare agents used recently, such as sarin, have been developed with a full understanding of the potentially devastating effects they have on the human body.

It is clear that murder by poison, from individual cases to chemical warfare, will continue in the future, and advancements in toxicology will inevitably aid as well as hinder its use.

In fact, dioxin was the poisoning agent in a high profile political incident in 2004. It was ultimately identified as the cause of the disfiguring acne-like skin condition suffered by Ukrainian opposition leader Viktor Yushchenko a few months before the first presidential election. The suspicion is that the dioxin was placed into soup ingested by Mr. Yuschenko. Despite his condition, Mr. Yuschenko continued to campaign and was the ultimate victor when a second election was held after the first outcome was invalidated. The acne-like skin condition is the most recognizable hallmark of dioxin poisoning in humans. It is expected that at least most of his skin condition is reversible; however, his situation is unique as a known case of high-exposure-dioxin poisoning, with severe effects, in a human. Futher, it is not known what other effects to his body related to the poisoning might surface in future months and years. The actual intake of dioxin in this poisoning is unknown.

See also: Arsenic; Hemlock, Poison; Nerve Agents; Strychnine.

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Nutmeg

Christopher P Holstege

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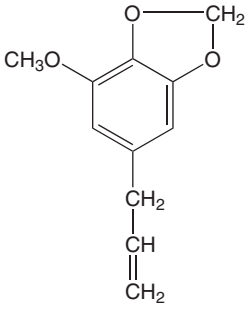
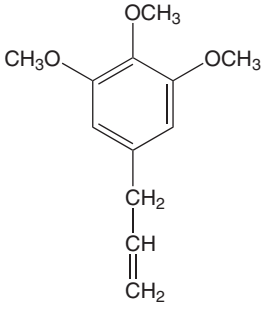
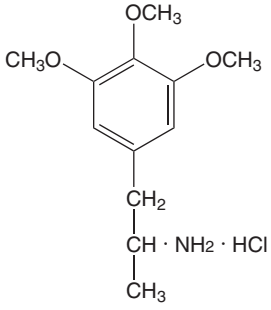
- Chemical Abstracts Service Registry Number: CAS 8008-45-5
- Synonyms: *Myristica fragrans*; Brown slime; Mace; Madashaunda (narcotic fruit); Pala banda; Spice of madness
- Chemical Structures:



Myristicin



Elemicin



Amphetamine corresponding to elemicin

Uses

Nutmeg is used as a spice, as a hallucinogen, and as an herbal remedy for ailments such as rheumatism, diarrhea, anxiety, and excessive flatulence.

Background Information

Nutmeg is the seed of *Myristica fragrans*, an aromatic evergreen tree cultivated in Indonesia and Grenada.

Exposure Routes and Pathways

Nutmeg is ingested whole, in ground or grated form, or as a slurry of water and powder (brown slime). Nutmeg powder is occasionally sniffed.

Toxicokinetics

The volatile oils in nutmeg consist of allylbenzene derivatives and terpines. Myristicin, elemicin, and safrole comprise 80% of the allylbenzenes. Myristicin and elemicin may be biotransformed into MMDA (3-methoxy-4,5-dimethylene-dioxamphetamine) and TMA (3,4,5-trimethoxyamphetamine), respectively, both consisting of a difference of only an amine group added to the side chain. Symptoms occur within 3–8 h, followed by 6–24 h of alternating periods of stupor and delirium. Recovery normally occurs within 24 h but may take several days.

Mechanism of Toxicity

A probable metabolite of myristicin is MMDA and of elemicin is TMA. Both of these metabolites are psychoactive compounds related to amphetamine. Other components of the volatile oil, such as eugenol, isoeugenol, safrol, and linalool, are structurally similar to some serotonin agonists and may contribute to the psychological effects. The terpine hydrocarbons are unlikely contributors to the psychomimetic effects but may increase absorption of the allylbenzenes by irritation of the stomach. Nutmeg has weak monoamine oxidase-inhibiting abilities. Nutmeg inhibits the synthesis and activity of prostaglandin B in the colon, giving an antidiarrheal effect. It also has antiinflammatory properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD of nutmeg oil is 2600 mg kg^{-1} in rats, 4620 mg kg^{-1} in mice, and 6000 mg kg^{-1} in hamsters. Fatty degeneration of liver has been noted in dogs and cats.

Human

Nutmeg is abused for its narcotic and hallucinogenic properties. One to three seeds or 5–30 g of the ground nut are used to attain psychogenic effects. One tablespoon of ground nutmeg or one grated nutmeg yields ~7 g. A fatality was reported in an 8 year old who ate two nutmegs. A 55 year old was also suspected to have died from acute nutmeg poisoning and was found to have a blood level of 4.0 μg ml^{-1}. Nutmeg may produce symptoms similar to those of an anticholinergic poisoning. The reported initial neurological effects include giddiness, tingling, euphoria, and hallucinations that may include distortion of time and space, detachment from reality, sensation of separation from one's limbs, and fear of impending death. This is followed by alternating delirium and extreme drowsiness or stupor. However, common unpleasant side effects occur and include headache, nausea, vomiting, abdominal pain, dizziness, chest pain, flushing, tremor, and tachycardia. The blood pressure may slightly increase, but a marked decrease with cyanosis and shock has been reported. Palpitations, agitation, anxiety, dry mouth, chest tightening, and blurred vision were reported in a pregnant woman in her third trimester who ingested one tablespoon of nutmeg. The fetal heartbeat was increased for 12 h. Levels for myristicin and elemicin are not generally available. Myristicin has been isolated from nutmeg using high-performance liquid chromatography. Other laboratory values have been reported to be normal.

Chronic Toxicity (or Exposure)

Human

Chronic nutmeg abuse has been reported to induce psychosis that is reversible after cessation of nutmeg ingestion.

Clinical Management

Treatment should focus on keeping the patient calm while hallucinations are occurring, maintaining blood pressure, and controlling nausea and vomiting. Gastric emptying can be considered if the ingestion was recent. Activated charcoal and a cathartic may be given. Benzodiazepines have been used to decrease anxiety and agitation. Intravenous fluid administration along with antiemetics may be indicated to treat dehydration, nausea, and vomiting.

See also: Amphetamine; Benzodiazepines.

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Occupational Exposure Limits

Andrew Maier

Occupational exposure limits (OELs) provide health and safety professionals an important tool for protecting worker health. OELs provide health and safety guidance to chemical users, inform workers of potential adverse effects of chemical exposure, and provide a scientific basis for evaluating whether existing environmental exposure controls are adequate.

Many organizations around the world develop OELs using approaches that fit the unique needs of the constituencies involved and the mission of the organization. For example, some organizations set health-based guidelines that reflect best scientific judgment regardless of other considerations, while many regulatory organizations evaluate policy and management issues such as implementation costs and technical feasibility as part of the OEL determination. Nevertheless, the general scientific approach used by most organizations is similar and includes a detailed critical review of the epidemiology and toxicology information to identify potential hazards, selection of sensitive adverse effects, dose-response estimation to determine appropriate thresholds, and an evaluation of tenant uncertainties to ensure the desired margin of safety.

There are several general categories of OELs for airborne chemical exposure, which differ primarily on the duration of exposure considered relevant for preventing the effect of concern. The common OEL duration categories include:

- *Time-weighted average (TWA)*: These limits are generally developed to protect from health effects caused by longer-term or chronic exposures (e.g., chronic target organ damage) and are compared against air concentrations measured over full-shift exposure durations (e.g., 8 or 10 h, depending on the organization). Note that methods to adjust OELs for other durations based on toxicokinetic considerations have been developed for cases involving exposures that occur during nonstandard work schedules.
- *Short-term exposure limit (STEL)*: These limits are generally developed for substances that induce effects of concern following fairly brief periods of exposure. For example, many STELs are based on thresholds for the induction of irritant responses or central nervous system depression, or for preventing chronic or irreversible damage due to brief periods of exposure. Many organizations establish STELs as a 15 min TWA air concentration that should not be exceeded during a work shift. Many compounds do not have sufficient data to serve as the basis for developing a STEL. However, some organizations recommend general excursion limits that are a multiple of the full-shift TWA limit (e.g., three times the TWA), as a measure of protection from peak exposures even when no STEL has been established.
- *Ceiling limit*: These limits are generally developed to protect from effects caused very quickly if a threshold concentration is exceeded. For example, ceiling limits are established for many highly potent irritants. The ceiling limit generally refers to the maximum concentrations in air that should not be exceeded at anytime during the work period.

Most published OELs are derived on the basis of preventing adverse effects arising from occupational exposures due to contaminant concentrations in the air. However, dermal exposures may also contribute to the overall body burden. Most OEL-setting organizations have developed qualitative notations to identify those substances for which dermal exposure may contribute significantly to the total body burden. For substances with a skin notation, caution should be used in interpreting the level of protection afforded by the OEL if skin exposure may occur.

Most OEL-setting organizations also establish qualitative notations to indicate the ability of a compound to induce dermal or respiratory sensitization. This approach is used since dose-response thresholds for the induction of these sensitization responses are generally not well understood, and sensitized individuals may respond to very low exposure

concentrations and may not be protected by an OEL based on other toxicity endpoints.

Organizations differ in the approaches used to develop OELs for carcinogens. Some organizations develop OELs using a threshold-based assumption for all compounds, while others do not set health-based limits for carcinogens that are thought to act via linear (e.g., genotoxic) mechanisms. Most organizations do provide some classification approach to identify carcinogens.

Other Types of OELs

Several other types of occupational exposure limits are derived in addition to the OELs derived to protect against airborne chemical exposures in traditional workplace settings.

Dermal contact is the primary route of exposure for many substances that have low vapor pressure and are not aerosolized. For these substances and exposure situations, it can be valuable to develop dermal exposure limits. Few dermal exposure limits have been published by the primary OEL-setting organizations. Nevertheless, the field is maturing with increasing publication of proposed reference values for dermal exposure in the literature.

Most OELs are developed to protect workers from the development of any adverse effects. However, in some cases, guidance on thresholds for effects of greater severity is an important tool – in particular for emergency response applications. There are a number of different organizations that establish acute emergency exposure values. In occupational settings, immediately dangerous to life or health (IDLH) values are often used for setting protective equipment requirements and in emergency planning.

Assessing exposures to physical agents is also important for protecting worker health. Many organizations establish workplace exposure guidelines for physical agents and stresses, including noise, heat or cold, nonionizing and ionizing electromagnetic radiation, and ergonomic stressors such as vibration and repetitive trauma.

Key OEL-Setting Organizations

There are many other organizations that establish workplace exposure guidance. The list in the Relevant Websites section at the end of the article represents those organizations that establish OELs that are frequently cited for international application, but this list is not comprehensive. In addition, many companies develop OELs for their specific products. These company limits are not often published, but they can be requested from product suppliers or be found on company product literature (such as Material Safety Data Sheets).

See also: American Conference of Governmental Industrial Hygienists; American Industrial Hygiene Association; Exposure Criteria; National Institute for Occupational Safety and Health; Occupational Safety and Health Administration; Occupational Toxicology.

Further Reading

American Conference of Governmental Industrial Hygienists (2001) *Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices*, 7th edn. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

DiNardi SR (ed.) (2003) *The Occupational Environment: Its Evaluation and Control*. Fairfax, VA: American Industrial Hygiene Association Press.

Harris RL (ed.) (2000) *Patty's Industrial Hygiene*, 5th edn. New York: Wiley.

Relevant Websites

http://www.acgih.org – American Conference of Governmental Industrial Hygienists (ACGIH). The Threshold Limit Value (TLV) Chemical Substance Committee of ACGIH develops TLVs®, which are health-based OEL guidelines.

http://www.aiha.org – American Industrial Hygiene Association (AIHA). The Workplace Environmental Exposure Level Committee establishes WEEL Guidelines which are health-based OELs.

http://www.cdc.gov – (US) National Institute for Occupational Safety and Health (NIOSH) develops health-based recommended exposure limits (RELs).

http://www.osha.gov – (US) Occupational Health and Safety Administration (OSHA). OSHA has regulatory authority in the United States for workplace health and safety. The OELs promulgated by OSHA are Permissible Exposure Limits (PELs).

http://europe.osha.eu.int – (EU) Scientific Committee on Occupational Exposure Limits (SCOEL). The EU committee establishes health-based OELs for use by EU member countries. Occupational Exposure Limits: Summary of Information from EU Member States and Other Sources.

http://www.hse.gov.uk – (UK) Health and Safety Executive. The Health and Safety Commission's Advisory Committee on Toxic Substances (ACTS) recommends new OELs or revision of a current OEL value, which can be adopted as enforceable limits by the regulatory authority.

www.hvbg.de – (Germany) The DFG Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work area – the MAK Commission sets health-based OELs for threshold substances (carcinogens that act via genotoxic mechanisms are addressed through a separate process).

Occupational Safety and Health Act, US

Michael A Kamrin

The Occupational Safety and Health Act (OSHAct) is administered and enforced by the Occupational Safety and Health Administration (OSHA). Both OSHA and OSHAct were created in December 1970, the same month the US EPA (Environmental Protection Agency) was created. Unlike the US EPA, OSHA is essentially an enforcement organization and most of its employees are inspectors who perform thousands of workplace inspections per year; it is a division of the Department of Labor. The OSHAct assures, as far as possible, that all working men or women have risk-free working environments; and imposes on employers the obligation to provide employees with workplaces that are free from recognized health and safety hazards and to maintain compliance with specific OSHA standards.

Many states and territories also have their own occupational safety and health plans that have been approved by OSHA and many of these are more stringent than the federal OSHA requirements.

The OSHA Hazard Communication Standard, better known as the "Right-to-Know" law, requires that the hazards of all chemicals produced in or imported into the United States are evaluated and that employers provide their employees with all appropriate hazard information. This involves providing employees with hazard communication/training programs and access to material safety data sheets (MSDSs) and written records. OSHA considers the MSDS the primary vehicle for transmitting detailed hazard information to downstream employers and employees.

Chemical manufacturers and importers must make a "hazard determination" of the chemicals with which they are involved. This involves an assessment of the physicochemical properties of a material (e.g., flammability, explosivity, corrosivity, and reactivity) as well as potential acute and chronic toxicity. However, manufacturers and exporters are not required to conduct additional testing. Typically, the hazard determination is made on the basis of existing company data or information from the published scientific literature.

Worker exposure to chemicals in the workplace is regulated through the promulgation of permissible exposure limits (PELs) that are maximum allowable exposure limits or maximum time-weighted average limits over an 8 h working day. These are complemented by short-term exposure limits. In March 1989, OSHA reduced the PELs for many substances and set new ones for substances previously not regulated; OSHA is still in the process of developing permanent health-based workplace standards. Many of the standards are based on recommendations made in criteria documents prepared by the National Institute for Occupational Safety and Health (NIOSH), although OSHA has its own standards office. Another listing of exposure limits contains the threshold limit values (TLVs) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH). The standards recommended by OSHA, NIOSH, and ACGIH often differ from each other and may be quite controversial. As a result, standards may become mired in hearings and in the courts.

See also: American Conference of Governmental Industrial Hygienists; Carcinogen Classification Schemes; Medical Surveillance; National Institute for Occupational Safety and Health; Occupational Safety and Health Administration; Occupational Toxicology.

Further Reading

DiBerardis LJ (ed.) (1998) *Handbook of Occupational Safety and Health*, 2nd edn. Hoboken, NJ: Wiley-Interscience.

Occupational Toxicology

Elizabeth V Wattenberg

Introduction

The aim of occupational toxicology is to help create a safe workplace. Historically, occupational studies have provided some of the strongest evidence that exposure to xenobiotics (chemicals or other agents that are foreign to the body) in the environment can cause disease in humans. As early as 370 BC, Hippocrates described symptoms of lead poisoning in a metal worker. In 1775, Sir Percival Pott recognized that soot played a role in the high rate of scrotal

cancer among chimney sweeps. In 1977, case studies on pesticide workers indicated that exposure to dibromochloropropane could cause infertility and sterility in men. Such observations have sparked interest in investigating how xenobiotics injure or disrupt biological systems. In turn, results from toxicological studies are used to develop methods to assess workplace exposures and to establish occupational exposure limits.

Occupational toxicology draws from the same framework as other disciplines in toxicology (**Figure 1**). This framework outlines the major physiological steps that can influence the dose–response for a xenobiotic. A dose can be defined as the amount of a xenobiotic a worker is exposed to in an occupational setting, and a response is some overt physiological effect, such as organ damage. The growing field of molecular toxicology aims to refine the characterization of the dose–response, such that the dose reflects the amount of the xenobiotic or active metabolite that reaches a critical target in the body, and the response is an early, subtle change in cells or components of cells that precedes clinical disease.

Developing occupational exposure guidelines involves risk assessment – the evaluation of toxicological and exposure data to determine the health risks presented by using xenobiotics in the workplace. The toxicological characterization of xenobiotics contributes to the risk assessment process by providing information for the dose–response and hazard identification steps (**Figure 2**). Hazard identification describes the types of physiological effects a xenobiotic can cause – for example, reproductive toxicity, cancer, respiratory problems, or allergic reactions. Toxicology may also play an increasingly important role in exposure assessment as advances in molecular toxicology promise to improve the measurement of the dose of a xenobiotic that is absorbed by the body or that hits a critical target tissue. Risk characterization synthesizes the information gathered in the dose–response, hazard identification, and exposure assessment steps for use in risk management decisions. Risk management strategies can range from recommending the use of protective equipment to setting occupational exposure limits, or possibly to eliminating a xenobiotic from the workplace. Making informed decisions regarding worker health requires a clear understanding of health risk information.

Use of Toxicological Data

Although epidemiological data provide the strongest evidence that specific chemicals affect human health, occupational exposure limits and other preventive measures typically are not based on these types of data alone. The number of chemicals to which workers are exposed far exceeds the number of solid epidemiological studies. Therefore, many occupational health standards and protective health measures are based on data from toxicology studies. Consequently, one of the major challenges that faces an occupational toxicologist is how to interpret the results obtained from animal studies and molecular studies for application to humans.

Occupational toxicology relies on information from both traditional toxicology studies and investigations into the molecular mechanisms of xenobiotics. Toxicology studies involve exposing groups of animals to various doses of a xenobiotic and observing the response. These are the types of toxicology studies that have traditionally been used to develop exposure guidelines. Very simply, toxicologists use these studies

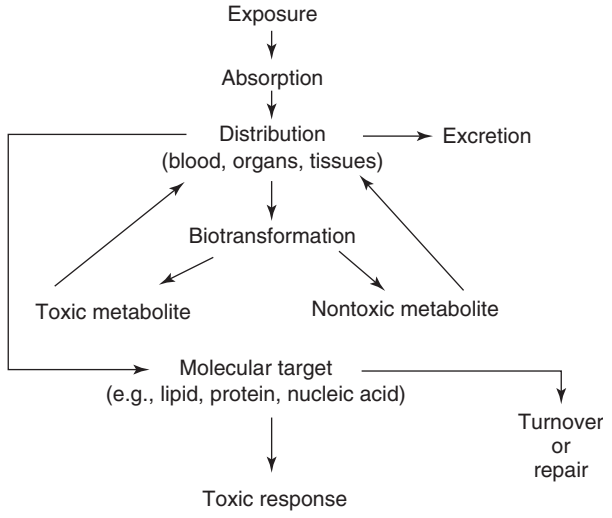

Figure 1 Toxicology framework.

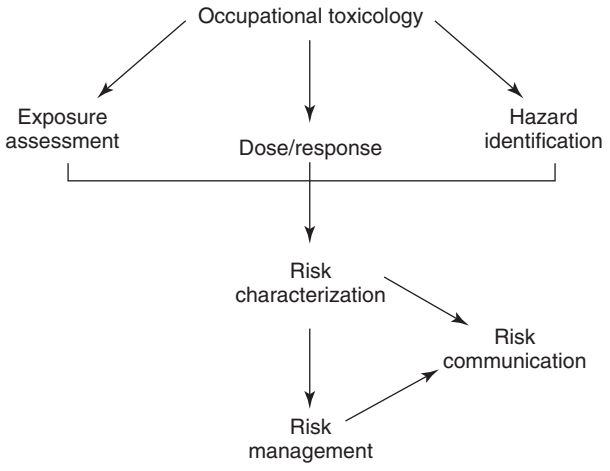

Figure 2 Occupational toxicology and risk assessment.

to determine what dose presents little or no risk of causing a harmful effect in humans. Classic toxicology studies also provide information on the types of effects the xenobiotic can cause, such as cancer, neurotoxicity, or organ damage.

Molecular toxicology investigates the biochemical steps that lead to the physiological response observed in the classic studies. Identifying the critical molecules and cellular processes involved in toxicity can help determine whether the effects seen in animals are likely to occur in humans. For example, studies suggest that the protein α-2μ-globulin plays a key role in the induction of kidney tumors by D-limonene. This protein is synthesized in large quantities by adult male rats, but not by humans, suggesting that although D-limonene causes kidney tumors in male rats, it may not cause the same effect in humans. Characterizing the molecular target of a xenobiotic can also provide clues for the prevention of toxicity or perhaps aid in developing therapies or antidotes for toxic effects. For example, neurotoxic organophosphate insecticides inactivate the enzyme acetylcholinesterase. Pralidoxime acts as an antidote for this type of pesticide poisoning by reactivating this enzyme. Finally, molecular toxicology can help identify biological markers, such as xenobiotics bound to DNA or proteins, altered or unusual macromolecules, or changes in gene expression, which can be used to indicate exposure or preclinical toxic effects.

Exposure

When choosing a study for applications in occupational toxicology, it is important that the exposure protocol be relevant to the exposure scenario in the workplace. The route, duration, and frequency of exposure can have a significant effect on the toxicity of a xenobiotic agent.

The route of exposure determines both the initial physiological barrier faced by the xenobiotic and its initial metabolic fate. Two of the primary defense mechanisms against xenobiotics are barriers, such as skin and membranes, and the biotransformation or breakdown of toxic compounds to nontoxic products. Xenobiotics must elude these defense mechanisms in order to reach the target tissue and cause damage.

Physiological barriers, such as the cells that line the gastrointestinal tract and respiratory tract, and make up the skin, determine the amount and the rate of absorption of specific xenobiotics into the body. Some xenobiotics do act directly at the site of exposure. For example, epoxy resins can cause allergic contact dermatitis, and UV light can cause skin cancer. Other xenobiotics damage tissues that are distant from the site of exposure. For example, many solvents that are inhaled, such as trichloroethylene and perchloroethylene, cause liver damage. These types of xenobiotics reach their targets by being absorbed into the circulatory system.

The toxicological effects observed due to exposure from one route cannot necessarily be used to predict the toxicological effects that would result from exposure via another route. For example, inorganic lead is almost completely absorbed through the lower respiratory tract but does not easily penetrate the skin. Ease of absorption through a particular physiological barrier depends on the physical and chemical characteristics of xenobiotics. In contrast to inorganic lead, organic lead, such as tetraethyl lead, is readily absorbed through the skin. Therefore, the results from a dermal toxicology study on inorganic lead cannot directly predict the dose–response for dermal exposure to organic lead. Likewise, reducing dermal exposure to organic lead would require stricter protective measures than that for inorganic lead.

In addition to presenting the initial physiological barrier to a xenobiotic, the route of exposure also determines the initial metabolic fate of the agent. The liver, which contains the highest concentration of enzymes involved in biotransformation, is the primary site for detoxification. In addition, some xenobiotics can be broken down by the acid pH of the stomach or enzymes present in the gastrointestinal tract. Chemicals that enter the body orally will face both of these defense systems before they are absorbed into the general circulatory system. By contrast, xenobiotics that are absorbed through the respiratory tract or through the skin are transported to the general circulatory system without first passing through the liver. Therefore, they may reach a target tissue before exposure to the detoxifying enzymes in the liver. Injection can be a particularly dangerous route of exposure because the agents bypass all of the barrier properties of the skin and directly enter the bloodstream.

The duration and frequency of exposure can also influence the physiological effect of xenobiotics. The health effects following a short-term, high-dose (acute) exposure to a xenobiotic can differ dramatically from the effects of a long-term, low-dose (chronic) exposure. For example, acute inhalation exposure to vinyl chloride causes respiratory tract irritation, lethargy, and headache. Chronic exposure to vinyl chloride can cause hepatic angiosarcoma. Whereas short-term, low-dose exposure to a specific xenobiotic may not be toxic, prolonged or frequent exposure to the same xenobiotic may deplete detoxifying or repair systems and therefore result in the

accumulation of damaged tissue. Toxicologists interpret short-term animal studies with care because many xenobiotics have latent effects, such that the toxic response is not detected until long after the time of exposure. This also holds true for the interpretation of epidemiological data. For example, latency is well established for carcinogens. Tumors may not appear in animals for weeks or months, and cancer may not appear in humans until decades after exposure. Other xenobiotics, such as neurotoxins, may also have latent effects.

Exposure scenarios differ depending on the type of occupation and the physical workplace. Inhalation is a common industrial route of exposure. Some xenobiotics can be absorbed through the eyes. Many types of work involve dermal exposure to xenobiotics. Medical personnel are at risk of being exposed to a variety of agents through injection. To ensure worker safety, all of the possible pathways of exposure should be examined.

Factors That Affect Toxicity

Both environmental factors and an individual's characteristics can affect the toxicity of a given xenobiotic. For example, exposure to other xenobiotics, diet, age, sex, and genetics can alter the toxicity of a given xenobiotic, in part, by modulating its biotransformation. Biotransformation can detoxify xenobiotics, but this process can also bioactivate xenobiotics – that is, transform a relatively benign parent compound into a more toxic intermediate that can go on to interact with a target and cause damage. Ultimately, the effect of environmental agents on endogenous macromolecules, such as hormones and the enzymes and cofactors involved in biotransformation, determines the fate of a xenobiotic.

In general, biotransformation reactions convert lipophilic (fat-soluble) xenobiotics into compounds that are hydrophilic (water-soluble). As a result, lipophilic xenobiotics can be excreted from the body instead of accumulating or damaging a target tissue. The biotransformation process is broadly divided into phase I and phase II reactions. Phase I reactions prime xenobiotics for phase II reactions by adding or exposing a functional group (e.g., –OH, –SH, $–NH_2$, or –COOH). Phase II reactions take advantage of the functional group produced by the phase I reactions and add a water-soluble molecule to the xenobiotic, making it even more hydrophilic and therefore more readily excreted. Xenobiotics that already have a functional group can also undergo phase II reactions. Biotransformation requires a variety of enzymes and a set of endogenous cofactors. Biotransformation takes place primarily in the liver, which contains a very high level of the enzymes involved in phase I and phase II reactions. Although biotransformation also takes place in other organs, such as the lung, stomach, intestine, skin, and kidneys, the liver generally has a wider variety of metabolic enzymes than the other tissues and therefore can modify a broader range of compounds.

Phase I reactions are primarily catalyzed by a family of enzymes called cytochrome P450s. Like the immune system, which has evolved to combat a broad range of foreign antigens, the cytochrome P450 system has evolved to modify remarkably diverse classes of xenobiotics. Specific isoenzymes of cytochrome P450 catalyze reactions involving particular classes of xenobiotics. For example, CYP1A1 catalyzes the hydroxylation of benzo(*a*)pyrene, and CYP2E1 catalyzes the oxidation of alcohols.

Phase II reactions generally require a transferase type of enzyme and an endogenous cofactor to add a water-soluble molecule to a xenobiotic. Phase II reactions are also called conjugation reactions, and the modified products of these reactions are called conjugates. Like the cytochrome P450s, specific transferase enzymes and their associated cofactors tend to catalyze conjugation reactions with particular structural classes of compounds. For example, the enzyme UDP-glucuronosyl transferase, along with the cofactor UDP glucuronic acid, catalyzes the formation of glucuronide conjugates of aliphatic or aromatic alcohols, carboxylic acid, sulfhydryl compounds, and amines. Glutathione *S*-transferases use the cofactor glutathione to catalyze the formation of conjugates with a number of reactive intermediates, including epoxides.

Some xenobiotics can undergo competing biotransformation reactions. The extent to which a given xenobiotic will be detoxified or bioactivated depends, in part, on the physiological levels of specific enzymes and cofactors required for each biotransformation pathway. For example, nutritional status, sex, age, genetics, and the presence of or previous exposure to other xenobiotics can influence the toxicological fate of xenobiotics by affecting enzyme levels and cofactor pools. If toxicologists understand how these factors modulate toxicity, they can more accurately determine if the results of animal studies can be extrapolated to humans. In addition, this information may be used to identify individuals who might be particularly sensitive to certain types of exposures.

One xenobiotic can affect the toxicity of another by either increasing enzyme activity or depleting essential cofactors. Many of the enzymes involved in biotransformation reactions are inducible, such that

certain compounds can cause an increase in the levels of specific isoenzymes. An increase in the level of an isoenzyme usually results in an increase in the rate of the specific reaction catalyzed by that isoenzyme. Some xenobiotics induce the expression of enzymes that catalyze their own biotransformation. For example, polycyclic aromatic hydrocarbons induce the expression of an isoenzyme of cytochrome P450 that catalyzes the hydroxylation of benzo(*a*)pyrene, and ethanol induces the expression of an isoenzyme that catalyzes the oxidation of ethanol. Xenobiotics can also induce enzymes that catalyze the biotransformation of other compounds. For example, pretreatment of rats with 3-methylcolanthrene increases the biotransformation of aniline. Common inducers of cytochrome P450s include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), halogenated pesticides, such as DDT, and polychlorinated biphenyls. Natural substances in the diet can also induce some of the enzymes involved in biotransformation, as can steroid hormones. Some of the enzymes involved in phase II reactions are also inducible. For example, 3-methylcholanthrene increases the expression of glutathione-*S*-transferases. Specific isoenzymes of glucuronosyltransferases are also inducible. Finally, an inadequate supply of cofactors can limit phase II reactions. Exposure to xenobiotics can deplete cofactor pools. For example, exposure to high doses of acetaminophen can deplete the supply of phase II cofactors sulfate and glutathione. Nutritional status may also affect cofactor pools.

Because many xenobiotics can modulate biotransformation pathways, it is difficult to predict the health effects or dose–response that would result from exposure to multiple xenobiotics. Mixtures of xenobiotics can cause an additive response, where the mixture acts like one xenobiotic of a dose equal to the sum of the doses of the individual xenobiotics. Xenobiotics can also act synergistically, that is, cause a response that is greater than additive. The actions of different compounds may also be antagonistic such that the response is less than additive. Finally, the components of a mixture may act independently, resulting in no detectable change in response. Few xenobiotics have been tested extensively enough to determine how they might interact with other compounds. Instead, most toxicological studies investigate exposures to a single compound. Where studies on mixtures do exist, they often investigate the interaction of only two compounds. The biological response to two compounds could be altered by the presence of a third. The problem grows more complex as the number of xenobiotics increases. Finally, the type of interaction may depend on the doses of the specific components of a mixture. Clearly, there is no simple formula for accurately predicting the health risk from exposure to mixtures.

Nevertheless, workers are often exposed to more than one xenobiotic. The American Conference of Governmental Industrial Hygienists (ACGIH) has developed exposure limits for airborne substances called threshold limit values (TLVs). To estimate the TLV for a mixture, the ACGIH recommends using a model based on the assumption that when xenobiotics "act upon the same organ system … [i]n the absence of information to the contrary, the effects of the different hazards should be considered as additive" (ACGIH, 2003). This model generates a hazard index (HI) that indicates whether a mixture has exceeded an exposure guideline:

$$\mathrm{HI} = C_1/T_1 + C_2/T_2 + \cdots + C_x/T_x$$

where C_x is the atmospheric concentration of the xth (e.g., first, second) xenobiotic and T_x is the TLV for the xth xenobiotic.

An $\mathrm{HI} \geqslant 1$ indicates that the mixture exceeds the TLV. A separate HI is calculated for each set of xenobiotics in a mixture that causes the same type of toxicological effect. For example, an HI would be calculated for all liver toxicants in the mixture, a separate HI would be calculated for xenobiotics that cause kidney damage, etc. Further refinement of the additivity model or development of an alternative model will require more extensive research.

The ACGIH does recognize that some xenobiotics may act synergistically. For example, exposure to asbestos and cigarette smoking are synergistic for the induction of lung cancer. The ACGIH also recognizes that compounds absorbed through different routes of exposure can act synergistically. For example, drinking alcohol can affect the toxicity of a solvent, like trichloroethylene, that is inhaled. With regard to possible synergistic interactions, the ACGIH maintains that "[s]uch cases at present must be determined individually" (ACGIH, 2003).

The level and activity of specific enzymes involved in biotransformation can differ depending on the species, strain, age, and sex of the test animal. For example, cats cannot carry out glucuronidation reactions, newborn rats have relatively low cytochrome P450 activity, and male rats are more sensitive to carbon tetrachloride toxicity than female rats. These differences are important to consider when interpreting the results from toxicological studies. The observation that age, sex, and genetics can significantly influence biotransformation reactions in animals raises the question of whether these characteristics also affect the biotransformation capacity of humans.

The field of toxicogenetics focuses on the genetic basis for the differences in xenobiotic metabolism. This field has grown out of an interest in identifying individuals who may be particularly sensitive to certain types of drugs or environmental exposures. For example, sensitivity to certain compounds may differ as much as 200-fold among individuals. Individual differences in xenobiotic metabolism result from polymorphisms among the population. That is, some members of the population express different forms of enzymes involved in biotransformation. While these polymorphisms have largely been characterized in terms of drug metabolism, these differences also have implications for exposures to other types of xenobiotics.

One common polymorphism in the United States is for *N*-acetyltransferase, an enzyme involved in phase II reactions. *N*-acetyltransferase catalyzes the acetylation of aromatic amines and hydrazines, and other classes of xenobiotics. People characterized as 'slow acetylators' have relatively low *N*-acetytransferase activity. Consequently, slow acetylators are more sensitive to the toxic effects of certain types of drugs, including sulfa drugs. In addition, a study of workers exposed to benzidine in the dye industry suggested a link between the 'slow' acetylator phenotype and the development of bladder cancer.

Polymorphisms also exist for specific isoenzymes of cytochrome P450. For example, there is a polymorphism within the human population for a cytochrome P450 isoenzyme that catalyzes the 4-hyroxylation of the drug debrisoquine. 'Extensive metabolizers' hydroxylate this drug 10–200 times faster than 'poor metabolizers'. Poor metabolizers express much less of the isoenzyme involved in this reaction than extensive metabolizers. This polymorphism also appears to affect the metabolism of environmental agents. For example, there appears to be an association between the poor-metabolizer phenotype and Parkinson's disease. By contrast, the extensive-metabolizer phenotype may be correlated with an increased risk of developing cancer.

The use of genetic information to identify sensitive individuals raises difficult policy questions. For example, individuals with certain polymorphisms might be sensitive to a xenobiotic at concentrations below the TLV. Although this information could be used to recommend additional protective measures for sensitive workers, there is also concern that the identification of susceptible individuals could result in job discrimination.

Male and female animals can have dramatically different responses to certain xenobiotics. For example, TCDD induces liver tumors in female rats but not in male rats. By contrast, chloroform is a more potent kidney toxin in male mice than in female mice. Female rats are more sensitive to the organophosphate insecticide parathion than male rats. Castration increases the sensitivity of the male rats to parathion. In addition to their role in xenobiotic biotransformation, cytochrome P450s are also involved in the biotransformation and synthesis of endogenous compounds, including fatty acids, prostaglandins, and steroid hormones. Therefore, it should not be surprising that hormones can regulate the activity of specific cytochrome P450 isoenzymes. The question of to what extent men and women differ in their responses to xenobiotic agents is still under investigation. The composition of the work force has changed dramatically in the past few decades, with an increasing number of women holding positions that traditionally had been held by men. Therefore, it is important to consider possible sex-specific differences in toxic responses if results from occupational studies of male workers or other epidemiological data on men are used to set occupational standards that are applied to both men and women.

Biomonitoring: Molecular Targets

One aim of occupational toxicology is to improve exposure assessment. The field of molecular toxicology provides information on the types of molecules and macromolecules that can serve as indicators of exposure. Whereas classic toxicology studies usually examine gross effects, such as changes in body weight, organ damage, or tumor development, molecular toxicology investigates the biochemical mechanisms of action of xenobiotics. In other words, molecular toxicology investigates the molecular or cellular events that eventually lead to the gross effects observed in classic toxicology studies. The results from molecular toxicology studies can be used to develop biological markers, also called biomarkers. Biomarkers are generally defined as 'cellular, biochemical, or molecular alterations' that can be measured in 'biological media such as human tissues, cells, or fluids'. Biomarkers can be applied in occupational settings to measure exposure to xenobiotic agents and to detect an early response that could lead to toxic injury or disease.

Estimating exposure usually requires measuring the amount of the xenobiotic in the air, water, dust, or other media. These types of measurements may be technically difficult or expensive to do. In addition, concentrations of xenobiotics can vary depending on time and location. Therefore, these measurements may not give an accurate estimate of past exposures. Furthermore, monitoring the levels of xenobiotics in the workplace does not necessarily indicate how

much of the xenobiotic has been absorbed into the body or how much reaches the target tissue. One potential use of biomarkers in occupational settings is to refine exposure assessment. Common types of biomarkers include parent compounds or metabolites that can be measured in urine or exhaled breath and metals that can be measured in hair. Development of other types of biomarkers relies on the identification of a molecular target. For example, lead decreases ferrochelatase activity, an enzyme important in heme biosynthesis. As a result, some red blood cells contain zinc–protoporphyrin instead of hemoglobin. Therefore, zinc–protoporphyrin levels in erythrocytes have been used as a biomarker of lead exposure. Likewise, since organophosphate and carbamate pesticides inhibit acetylcholinesterase, measurement of inhibition of choline esterase activity in the blood has been used as a biomarker of exposure to these pesticides. Some xenobiotic agents bind directly to DNA or to proteins. For example, benzo(*a*)pyrene forms DNA adducts and ethylene oxide forms protein adducts. Researchers continue to investigate whether measurement of DNA adducts or protein adducts in easily accessible samples, such as blood, can accurately reflect the interaction of xenobiotics with critical toxicological target tissues such as the lung. Microarray technology can be used to measure global effects on gene expression and protein levels and modifications. Such profiles may be used to assess exposure.

Biomarkers can also be used to detect an early biological response to a xenobiotic agent that precedes serious damage or disease. Examples of precursory responses include mutations in critical genes, changes in hormonal status, and altered gene expression. For example, substantial evidence indicates that carcinogenesis involves the conversion of protooncogenes (normal genes, many of which code for proteins critical for regulating cellular growth control) into oncogenes (genes that can transform normal cells into cancerous cells). Some xenobiotics can cause mutations that convert protooncogenes into oncogenes. These mutations can result in the overproduction of a protein or in the expression of an altered protein, also called an oncogene product. Microarray technology may also reveal early, preclinical responses to chemical exposure and perhaps indicate if workers are at increased risk of developing cancer or other diseases.

A number of parameters must be established in order to validate the use of biomarkers. If a biomarker is used to assess exposure, it is important to determine how long the biomarker persists following exposure. Likewise, the time interval between exposure and biomarker appearance should be determined. In addition, biomarkers often cannot be measured in the target tissue, such as the lung, because the tissue is not easily accessible for sampling. Instead, the biomarker is measured in a surrogate tissue, such as red blood cells. If this is the case, it should be established that the persistence and levels of the biomarker in the surrogate tissue reflect those of the target tissue.

Regulation

The US Congress passed the Occupational Safety and Health Act in 1970. This act created the Occupational Safety and Health Administration (OSHA) in the federal Department of Labor to establish and enforce safety standards for the workplace. OSHA standards are called permissible exposure limits (PELs). Many PELs have been adopted from ACGIH TLVs. TLVs are generally defined as air concentrations of chemicals that most workers can be exposed to for an 8 h workday, 40 h week^{-1} for a working lifetime without suffering adverse effects. TLVs are not guaranteed as safe exposure levels for the entire population. Employers may also institute voluntary exposure limits either because an OSHA standard has not been promulgated for a xenobiotic of concern or because they want to apply an exposure limit that is more protective than either the PEL or the TLV.

Occupational exposure limits are not always based on toxicology alone. The incorporation of toxicological information into the development of occupational policy can depend on economics, technology, and the sociopolitical climate. The history of occupational policies that apply specifically to women illustrates the complex meshing of toxicology and social factors in the development of occupational policy. Although some sex-specific measures were progressive and eventually led to greater occupational safety for both men and women, it has also been argued that other sex-specific measures were instituted to restrict the role of women in the workplace, and that the intent of the policies depended, in part, on whether women were considered a dispensable part of the work force.

For example, in the late nineteenth century, factory inspectors in England recognized lead poisoning as one of the most widespread industrial diseases. In 1882, the Chief Inspector of Factories, Alexander Redgrave, submitted a report that lead to the Factories (Prevention of Lead Poisoning) Act, 1883. In contrast to the Consolidating Act of 1878, which excluded children and young people from working in the white lead industry, Redgrave specifically advised against banning women from this work.

He apparently recognized the economic role of women in industrial society and realized that if women lost their jobs, they would have a difficult time finding other employment. Instead of banning women from the lead industry, he recommended further protective measures that would improve working conditions. Approximately a century later in the United States, new discoveries on the toxicology of lead and a changing work force led to the implementation of just the sort of policy that Redgrave sought to avoid.

The 1970s saw an influx of women entering the US work force. Growing numbers of women began occupying industrial positions that had been traditionally held by men, including positions in companies that manufactured lead batteries. At the same time, there was growing concern that exposure to levels of lead once considered safe could be harmful. For example, toxicological and epidemiological studies suggested that lead was not only a neurotoxin but also a reproductive toxin. As a result of both the influx of women into 'nontraditional' jobs and the toxicological data on lead, some companies instituted so-called fetal protection policies. In contrast to Redgrave's recommendations a century earlier, these policies excluded fertile women from the workplace, regardless of age or intent to have children, rather than institute additional measures to lower exposure to lead. Although toxicological and epidemiological studies also indicated that lead was a reproductive toxin in men, the fetal protection policies did not apply to fertile male employees. Employees from a number of companies sued, and in 1989 the Supreme Court banned fetal protection policies on the grounds that they constitute discrimination. Although OSHA did not lower the PEL for lead, the lead standard was amended in 1991 to include the warning that "Chronic overexposure to lead impairs the reproductive systems of both men and women."

Policymakers develop workplace standards or institute protective measures by considering health risk data along with economics, the available technology, and the sociopolitical climate. The role of occupational toxicology in the development of sound and equitable safety measures is to provide the most accurate interpretation of toxicological and epidemiological data possible.

See also: American Conference of Governmental Industrial Hygienists; Biomarkers, Human Health; Biotransformation; Dose–Response Relationship; Exposure; Hazard Identification; Medical Surveillance; Occupational Safety and Health Administration; Psychological Indices of Toxicity; Risk Assessment, Ecological; Risk Assessment, Human Health.

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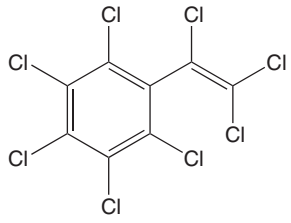
Octachlorostyrene

Alan L Blankenship

- Chemical Abstracts Service Registry Number: CAS 29082-74-4
- Synonyms: Pentachloro(trichloroethenyl)-benzene
- Chemical/Pharmaceutical/Other Class: Octachlorostyrene is a persistent, bioaccumulative, halogenated aromatic compound. Related compounds include other chlorinated styrenes that differ in the number of chlorines (i.e., ranging from 1 to 8)
- Chemical Formula: C_8Cl_8
- Chemical Structure:



Uses

Octachlorostyrene was never commercially produced. Rather, it was historically produced as an inadvertent by-product of high-temperature industrial processes involving chlorine such as the electrolytic production of chlorine gas or magnesium, the refining and degassing of aluminum smelt, and the chlorination and distillation processes involved in niobium and tantalum production. However, recent advances have been made in process technology and pollution prevention practices in some of these industries, such as largely eliminating the electrolytic manufacture of chlorine and aluminum degassing with hexachloroethane, both of which have likely resulted in reductions in known sources of octachlorostyrene.

Exposure Routes and Pathways

Occupational exposure to octachlorostyrene may occur from inhalation of dust and dermal contact in environments where octachlorostyrene is formed as a by-product. The general population may be exposed to octachlorostyrene via consumption of fish and shellfish that contain octachlorostyrene.

Toxicokinetics

In rats, following oral administration, ^{14}C-octachlorostyrene was absorbed and distributed with a rank order of tissue concentrations (greatest to least) of: fat > adrenal glands > skin > lung. After intravenous administration, ~8% of the dose was excreted in feces over the course of 7 days with only a negligible amount in urine. Greater than 90% of the radioactivity in feces was found to be unchanged octachlorostyrene with the remainder being heptachlorostyrene and pentachlorophenyldichloroacetic acid. Approximately 1% of the administered dose was detected as $^{14}CO_2$ in expired air.

Recent data comparing ratios of 4-hydroxy heptachlorostyrene (a metabolite of octachlorostyrene) to octachlorostyrene suggest that there are likely species differences in the ability to metabolize octachlorostyrene. For example, data from an earlier study suggest that polar bears can metabolize octachlorostyrene to 4-hydroxy heptachlorostyrene at a much faster rate than ringed seals.

Mechanism of Toxicity

The mechanism(s) of toxicity and human toxicological properties of octachlorostyrene have not been well characterized. In laboratory animals, histological changes in liver, kidney, and thyroid tissues were observed, but potential impairment in function was not well quantified. In rats, a 50 mg kg^{-1} dose (via intraperitoneal administration) resulted in increased microsomal protein and cytochrome P450 content and also induction of the activities of several enzymes including cytochrome P450 reductase, acetanilide 4-hydroxylase, ethylmorphine *N*-demethylase, and 4-nitroanisole *O*-demethylase. However, there was no induction of benzo(*a*)pyrene hydroxylase activity. Metabolites of octachlorostyrene, such as 4-hydroxy heptachlorostyrene, have been shown to bind to transthyretin in polar bears with a similar degree of affinity as to thyroxine (T_4). This suggests that there is a potential for disruption of T_4 and retinol transport by metabolites of octachlorostyrene in some species.

Acute, Subacute, and Chronic Toxicity (or Exposure)

Animal

In an acute toxicity study with male rats dosed by gavage with single doses of octachlorostyrene at 1300, 1690, 2190, 2850, and 3710 mg kg^{-1}, test animals were sacrificed 14 days later. At all but the lowest dose, there was an increase in liver weight, hepatic microsomal aniline hydroxylase and aminopyrine demethylase activities, serum cholesterol, and uric acid levels.

In a subacute study, both male and female rats were fed diets containing octachlorostyrene at 0.5, 5.0, 50, and 500 mg kg^{-1} for 28 days. Histological changes were observed in the liver and thyroid of rats exposed to doses equal to or greater than 5 mg kg^{-1}. Hepatic microsomal enzyme induction and liver hypertrophy were observed in the two highest dose groups. At 500 mg kg^{-1}, there was an increase in serum cholesterol, total protein, potassium, and sorbitol dehydrogenase.

In a chronic study, weanling Sprague–Dawley rats (20 animals of each sex per exposure group) were fed (*ad libitum*) diets containing 0, 0.005, 0.05, 0.5, 5.0, or 50 mg kg^{-1} octachlorostyrene in diet (fed *ad libitum*) for 12 months. While there was some mortality, it did not appear to be related to treatment. Similarly, tumor incidence was infrequent and appeared unrelated to treatment. However, 5.0 and 50 mg kg^{-1} exposures resulted in kidney effects (e.g., dose-related dilation of proximal tubules and cytoplasmic eosinophilia along with granular casts and proteinaceous losses), and induction of aniline hydroxylase and aminopyrine demethylase activities in hepatic microsomes of both sexes. At the highest exposure level only (50 mg kg^{-1}), there was a

statistically significant increase in relative liver to body weight. The chronic dietary no-observed-adverse-exposure level (NOAEL) from this study was determined to be 0.5 mg kg^{-1}. Corrected for body weight and ingestion rate, the actual dose for the NOAEL is 0.031 and 0.044 mg kg^{-1} day^{-1}, respectively, for males and females.

Human

The effects of octachlorostyrene exposure on humans are not well known. Most of the available human data are from monitoring studies in which tissue residue concentrations have been determined for occupational and nonoccupational populations, including consumers of seafood. As discussed earlier, potential human exposure pathways for octachlorostyrene are through ingestion (especially of contaminated fish), inhalation, and absorption through the skin. Occupational exposure has been shown to result in a greater than 70-fold increase in mean concentrations of octachlorostyrene in the blood in foundry workers (mean of controls, 0.7 ng g^{-1}, lipid; mean of exposed, 54.6 ng g^{-1}, lipid) who use hexachloroethane as a degassing agent for aluminum. Octachlorostyrene has also been detected in the blood of humans ingesting contaminated fish at levels generally ranging up to a few parts per billion (nanograms per gram), and in the breast milk of nonoccupationally exposed women at levels generally less than 1 ng g^{-1}. However, the toxicological relevance of these exposure levels in humans is not known.

Environmental Fate

In aquatic systems, octachlorostyrene is expected to adsorb to suspended solids and sediments based on its K_{oc} value ranging from 200 000 to 10 000 000. Octachlorostyrene has been detected in water at concentrations as high as 7.2 ng l^{-1} but levels typically are well below 1 ng l^{-1}. While there is the potential for volatilization from aquatic systems based on an estimated Henry's law constant of 2.3×10^{-4} atm m^3 mol^{-1}, volatilization is likely attenuated by adsorption to particles. Bioaccumulation by aquatic organisms is likely based on a bioconcentration factor that is estimated to range from 8100 to 33 000. Field estimates of bioaccumulation factors range up to 1 400 000 (from water to rainbow trout in Lake Ontario). Mean concentrations in Lake Ontario sediments and rainbow trout were 13.6 ng g^{-1} (dry weight) and 2.6 ng g^{-1} (wet weight), respectively. The highest concentrations found in fish as part of the National Study of Chemical Residues in Fish (conducted by the US Environmental Protection Agency (EPA)) were from Bayou D'Inde, Louisiana (138 ng g^{-1}), Freeport, Texas (65.3 ng g^{-1}), River Rouge, Michigan (50.7 ng g^{-1}), and Olcott, New York (49.6 ng g^{-1}). Temporal studies, while limited, have indicated a substantial decline in concentrations of octachlorostyrene since the 1970s.

In terrestrial systems, octachlorostyrene is expected to bind to soil particles. In the atmosphere, octachlorostyrene (in the vapor phase) is degraded by reactions with photochemically produced hydroxyl radicals. Octachlorostyrene weakly absorbs ultraviolet light between 295 and 310 nm with slow photolysis. Major transformation products of photolysis include heptachlorostyrene and two isomers of hexachlorostyrene, while minor transformation products of photolysis include pentachlorostyrene and tetrachlorostyrene.

Ecotoxicology

Among its potential adverse effects, octachlorostyrene has the potential to interfere with metabolism in fish and to inhibit photosynthesis in algae. EPA has determined that 'aquatic toxicity values indicate that octachlorostyrene is toxic at relatively low concentrations and thus is highly toxic to aquatic organisms'. Metabolites of octachlorostyrene, such as 4-hydroxy heptachlorostyrene, may have the potential to disrupt T_4 and retinol transport by binding to transthyretin in some species. Bioaccumulation into higher trophic level species has been documented to occur with several species including herring gulls, double-crested cormorants, black-crowned night herons, beluga whales, polar bears, and ringed seals. However, the toxicological significance of the concentrations found in wildlife is not clear at this time.

See also: Styrene.

Further Reading

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Octane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 11-65-9
- Synonyms: *n*-Octane (UN1262, DOT); Oktan (Polish); Oktanen (Dutch); Ottani (Italian)
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon
- Chemical Formula: C_8H_{18}

Uses

It is used as a solvent and raw material for organic synthesis reactions and is a very important chemical in the petroleum industry. It is also widely used in the rubber and paper processing industries. Isooctane, along with other *n*- and isoparaffins, are used in the blending of fuels to achieve desired antiknock properties. A total of 17 isomers of octane are known to exist; isooctane (2,2,4-trimethylpentane) is a principal ingredient of gasoline.

Exposure Routes and Pathways

Because octane can exist as a liquid or vapor at normal temperature and pressure, exposure could occur by either dermal contact or inhalation (1 ppm air = 4.67 mg m^{-3}); oral exposure would most likely be either incidental or accidental. Isooctane, an octane isomer, can comprise up to 1% of the total hydrocarbons emitted from the exhaust of diesel and gasoline engines.

Toxicokinetics

After absorption, octane is most likely converted to a hydroxy derivative (e.g., alcohol) via the cytochrome P450 oxidase system.

Mechanism of Toxicity

The molecular mechanism of toxicity is not known, although based on its solvent properties a direct physical alteration or disruption of cellular membrane structures and organelles is suspect.

Acute and Short-Term Toxicity (or Exposure)

Animal

Octane has been shown to have narcotic effects in both mice and rats after acute exposure at high concentrations. One study estimated a 4 h LC_{50} in rats of 118 000 mg m^{-3} (25 260 ppm). The lowest concentration to cause an effect on righting reflexes in mice was 35 mg l^{-1} (7490 ppm), while complete loss was seen at 50 mg l^{-1} (10 700 ppm). For 2,5-dimethylhexane (an octane isomer), the narcotic concentration in mice was 70–80 mg l^{-1} (14 980–17 120 ppm), and the effects were less severe than those seen for octane. In rats, oral administration of isooctane caused moderate toxicity, and pulmonary lesions were observed following aspiration of octane into the lungs. None of the branched octane isomers are known to have neurotoxic properties; some types of soil-dwelling bacteria can exist using branched chain octanes as the sole carbon sources.

Human

Octane is moderately toxic if taken orally and more toxic than the lower molecular weight analogs by this route. It is similar in potency to heptane (especially with regard to narcotic effects) but is apparently without the associated neurotoxic signs of heptane and hexane. If it is aspirated into the lungs, it may cause rapid death due to cardiac arrest, respiratory paralysis, and asphyxia. At high air concentrations (generally between 5000 and 13 700 ppm for 30 min) it will have an acute narcotic effect but no adverse effects are apparent in humans at concentrations below 500 ppm.

Clinical Management

Persons who are exposed to high air concentrations should vacate or be removed from the source of the gas and seek fresh air. Upon oral ingestion, persons should not be induced to vomit as pulmonary aspiration may occur, resulting in severe narcosis and/or death.

Ecotoxicology

Young Coho salmon showed no significant mortality in water containing <100 mg l^{-1} octane. No significant mortality of the eggs of the Pacific oyster is seen at concentrations <3500 mg l^{-1}. An EC_{50} of 120 μg l^{-1} was calculated based on effects on the feeding behavior of a test population of blue mussels.

Other Hazards

Extreme care must be taken to keep areas of expected high concentration free from ignition sources; for example, sparks from static electricity. Only explosion-proof equipment should be used in these areas. The lower and upper explosive limits for octane are 1% and 4.7%, respectively.

Exposure Standards and Guidelines

The permissible exposure limit (time-weighted average, TWA) for octane is 500 ppm (2335 mg m^{-3}) while the American Conference of Governmental Industrial Hygienists recommends a TWA threshold limit value of 300 ppm (1400 mg m^{-3}). National Institute for Occupational Safety and Health has recommended a maximum human exposure level of 75 ppm for octane and 350 mg m^{-3} for a mixture of C5–C8 hydrocarbons.

Miscellaneous

Octane is a colorless, highly flammable liquid that is lighter than water. It has an odor that can be detected at 400 ppm. It occurs in natural gas but is principally derived from crude oil.

See also: Gasoline; Petroleum Distillates; Petroleum Hydrocarbons.

Ocular Toxicology *See* Eye Irritancy Testing; Sensory Organs.

OECD *See* Organisation for Economic Cooperation and Development.

Oil, Crude

Michael J Sullivan

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- Chemical Name: Petroleum
- Representative Chemicals: Aliphatic, aromatic, paraffinic hydrocarbons; Naphthenic hydrocarbons; Asphaltic hydrocarbons; Trace metals
- Chemical Abstracts Service Registry Number: CAS 8002-05-9
- Synonyms: Petroleum; Naphtha; Petrol; Rock oil
- Chemical/Pharmaceutical/Other Class: Petroleum hydrocarbons

Uses

The separation of the components of crude oil into useable products is known as refining. Each of the crude oil fractions finds its way into consumer products. A typical list of fractions is: gasoline, kerosene and fuel oil, gas oil, wax distillate, and bottoms or asphaltics. Refineries must be designed to handle the type of crude oil they are going to process. For example, if a crude oil is highly paraffinic in nature, it will yield a lower amount of gasoline fuel by distillation. Highly paraffinic oils may be processed into lubricating stock. The chemical fraction consisting of chemicals with the largest carbon numbers, the asphaltic fraction, is used as roof or road tar.

Background Information

Crude oil is a complex mixture of chemicals. The relative composition of these chemicals will be different in crude oil from different sources. However, the overall composition (i.e., the chemicals present) remains fairly consistent between sources. The chemical classes present in crude oil include paraffinic hydrocarbons, long-chain straight or branched carbon-based chemicals and naphthenic hydrocarbons, multiple-ringed carbon-based chemicals. Also present will be low percentages of sulfur, nitrogen, and oxygen compounds, and trace quantities of many other elements.

Regulatory agencies have classified crude oil into categories that are useful to help understand how the oil will behave if released into the environment. These categories are summarized in **Table 1**.

Table 1 Categories of crude oil

Category	*Name*	*Description*
Class A	Light, volatile oils	Highly fluid Strong odor Spread rapidly High volatiles Penetrates soil Flammable
Class B	Nonsticky oils	Adheres to surface Waxy feel Can be washed away Mild volatiles Nonpenetrating
Class C	Heavy, sticky oils	Tarry/sticky Adheres to surfaces Cannot be washed away Nonpenetrating Low volatiles
Class D	Nonfluid oils	Black/brown solid Nonpenetrating Cannot be washed away Melts upon heating Nonvolatile

Exposure Routes and Pathways

At any one time worldwide, up to 1 000 000 workers are employed in crude oil exploration, production, and refining. Workers employed in these fields can be exposed to crude oil. Activities associated with exposure include drilling, pumping, and transportation of crude oil as well as the cleaning and maintenance of the equipment used in these activities.

Exposure to crude oil can occur through both direct contact with the material and contact with environmental media contaminated with crude oil. The primary route of exposure in the environment to crude oil is direct dermal contact with liquid oil. In the workplace, the primary route of exposure is also dermal contact. Inhalation exposure to crude oil could occur through the production of oil mists or through inhalation of the volatile fraction of the crude oil. Exposure to crude oil through contact with environmental media also constitutes significant routes of exposure. This can occur at spill sites, in former oil fields developed into other uses, or areas of natural oil seeps. Dermal contact or incidental ingestion of soil contaminated with crude oil, as well as the inhalation of dust from crude oil-contaminated soil are common at crude oil contaminated sites. Both human and animal exposures can occur when crude oil is released into the aquatic environment.

Toxicokinetics

Exposure to crude oil is a concern for the organ of contact. For dermal exposure the concern is for the skin. For inhalation exposure, the concern is for the respiratory system. Therefore, absorption and distribution kinetics are not well studied because of these site of contact concerns. However, it would be expected that individual chemicals present in crude oil can be absorbed and would have biological fate appropriate for that chemical or chemical class (e.g., polynuclear aromatic hydrocarbons).

Mechanism of Toxicity

The concern for both dermal and inhalation exposures is the site of contact and effects on that tissue. The mechanism of crude oil toxicity is mediated through its irritant effects which after sufficient exposure duration and concentration result in tissue hyperplasia. Chronic hyperplasia leads to subsequent loss of tissue integrity and damage and in some animal models of cancer. It has been suggested that at exposures below levels that cause chronic irritation, other long-term effects would not be expected.

Acute and Short-Term Toxicity (or Exposure)

Crude oil contains many chemicals considered toxic and the effects of these individual chemicals should be evaluated if exposure is possible. These chemicals are aromatic solvents including benzene, aliphatic chemicals including hexane, and naphthenic chemicals including the polynuclear aromatic hydrocarbons.

Animal

Both eye and dermal irritation have been noted in animal testing. Systemic effects have not been noted and oral toxicity is low. Dermal irritation has been reported in test animals at 24 h doses of 100 mg.

Human

The acute effect of crude oil on humans is narcosis. The effect is reversible even after exposure to high concentrations. Inhalation of vapors can produce pneumonitis.

Chronic Toxicity (or Exposure)

Crude oil has low chronic toxicity. Exposures insufficient to cause tissue irritation may also not be sufficient to cause other, more-serious chronic effects.

Animal

Dermal application of crude oil to the shaved backs of animals has produced limited adverse effects (see section Carcinogenicity). A dose of 25 mg, three times per week for 105 weeks produced dermal irritation at the site of application. No systemic effects were noted. Some limited evidence of developmental toxicity of crude oil has been reported but only at doses that were high enough to cause significant maternal toxicity.

Samples of crude oil have been tested for carcinogenicity in animals. Crude oil samples from single sources or composites of several sources were tested by dermal application of mice. Crude oil from single sources produced both benign and malignant tumors. Some composites have produced a low incidence of skin carcinomas whereas other composites have not. When crude oil fractions were applied to the skin of mice, skin tumors were also produced. In the rabbits tested, a single crude oil source produced skin papillomas in rabbits in one experiment but no effects were seen in rabbits using other sources. In all studies where crude oil was reported to be associated with skin cancer, there was significant damage to the skin and effects including drying, cracking, irritation, and hyperkeratosis. In summary, there is limited evidence for carcinogenicity in experimental animals.

Human

Adverse effects of crude oil on the skin have been reported in petroleum workers. These effects include dryness, pigmentation, hyperkeratosis, warts, and eczema.

Epidemiology studies of workers exposed to crude oil have been performed in petroleum producing, pipeline, and production operations. In a retrospective cohort mortality study, deaths from all types of cancers were low with decreases in lung and testicular cancer related deaths. Thyroid cancer-related deaths were increased. In a case–control study, an elevated risk for lung cancer was observed among older men. These workers were also exposed to welding fumes and paints, and smoking was not controlled. In another case–control study, an excess risk for testicular cancer was observed among petroleum and natural gas extraction workers; however, this isolated finding is inconsistent with other studies. Another case–control study exposure to crude oil was related to rectal and lung cancers. However, the authors noted that the study numbers were small and the finding may have been confounded by lifestyle factors.

In the summary, there is inadequate evidence for carcinogenicity in humans. Overall, crude oil is not classifiable as to its carcinogenicity to humans. Therefore, it is placed in the International Agency for Research on Cancer (IARC) classification Group 3.

In Vitro Toxicity Data

Crude oil did not increase the number of sister chromatid exchanges in cultured human lymphocytes. However, in studies of mice treated *in vivo* crude oil did cause an increase in the number of sister chromatid exchanges at the highest dose tested. No effects were observed in bone-marrow cells or sperm. Sister chromatid exchanges were caused by the aromatic fraction of crude oil in cultured mammalian cells. Crude oil extracts did not induce mutation in bacteria. However, the neutral fractions of crude oil which contain aromatic or polycyclic aromatic compounds generally had mutagenic activity in bacteria.

Environmental Fate

When released to the environment, crude oil undergoes the process of 'aging'. This occurs by both abiotic processes (volatilization and oxidation) and biotic processes including biodegradation. Often the abiotic aging will occur before the biotic one. The chemical-specific properties will determine how an individual chemical or chemical class fares during this aging. For example, small volatile compounds would be expected to be lost first from both land and water releases. Large paraffinic compounds would be expected to be somewhat resistant to aging. The asphaltic compounds would be the residual material and have limited exposure opportunities due to their properties. Terrestrial releases of crude oil do not lead to bioaccumulation in terrestrial organisms.

In aquatic environments, the heavier and less volatile/soluble compounds in crude oil will adsorb to suspended solids and subsequently settle in the sediments. Some heavy fractions with high density may sink into the sediment. This happens after the initial removal of the smaller and more volatile chemicals by either dissolution or volatilization. This is followed by biodegradation of those crude oil constituents that can serve as a food source for bacteria. Biodegradation is a significant mechanism for removal of hydrocarbons released into the environment. However, this generally occurs on the order of months and years. It is not believed that there is significant bioaccumulation of petroleum hydrocarbons in aquatic organisms.

Exposure Standards and Guidelines

No exposure standards for crude oil are available. Occupational exposures to oil mists are a concern. Both the Occupational Safety and Health

Administration (OSHA) permissible exposure limit (PEL) and the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) are $5\,\mu g\,m^3$ for oil mists.

Long-term animal studies of dermal exposure to crude oil can be used to set a no observed adverse effect level (NOAEL) that can be used to predict safe human exposure levels for both dermal and systemic effects. A reference dose of $0.04\,mg\,kg^{-1}\,day^{-1}$ has been suggested for exposures to crude oil. The individual aliphatic and aromatic fractions of crude oil have also been evaluated for toxicity and sufficient information exists to set references doses for these fractions. An understanding of the exposure to the individual fractions is necessary to use this process. The use of the reference dose for either crude oil as a whole or the individual fractions is preferable to evaluating only the toxic constituents in crude oil. This latter strategy is commonly employed in risk assessment however; it ignores the hydrocarbon matrix within which these toxic chemicals are found. This hydrocarbon matrix affects the exposure to these toxic constituents which is not accounted for in typical exposure assessments.

See also: Petroleum Distillates; Petroleum Hydrocarbons.

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Oil, Lubricating

Michael J Sullivan

- Representative Chemicals: Aliphatic, aromatic, paraffinic hydrocarbons; Naphthenic oil
- Synonyms: Lub oil; Crankcase oil; Motor oil
- Chemical/Pharmaceutical/Other Class: Petroleum hydrocarbons

Uses

Lubricating oil is used to lubricate the parts of motors. The purpose is to reduce friction between parts, remove heat (i.e., act as a coolant), and act as a sealing liquid. Oils can also be used in machining and applications where friction protection and heat removal are needed.

It is also worth noting that both paraffinic and naphthenic oils are used as food additives and in cosmetics. Two chemical classes (paraffin waxes, CAS 8002-74-2, and petrolatum, CAS 8009-03-8), are considered Generally Recognized as Safe food ingredients by the Food and Drug Administration.

Background Information

Lubricating oil is a generic name for a wide range of products that are characterized by hundreds of base chemicals and additives. The most common lubricating oils are crude oil distillate fractions although both synthetic and plant-based lubricating oils are used. This article focuses on petroleum-based lubricating oils.

Lubricating oils are composed of 80–90% petroleum hydrocarbon distillate with 10–20% additives to impart specific properties to the oil. The petroleum hydrocarbon distillate generally consists of paraffinic or naphthenic compounds, whose properties are listed in **Table 1**.

Unused lubricating oil changes under the use conditions of heat and friction and, if appropriate,

Table 1 Properties of paraffinic and naphthenic oils

Paraffinic oil	*Property*	*Naphthenic oil*
Long carbon chains	Chemical structure	Multiple carbon rings
High	Resistance to oxidation[a]	Medium
High	Pour point[b]	Low
High	Viscosity[c]	Low
Low	Volatility[d]	High
Low	Specific gravity[e]	High

[a] Measure of stability/chemical breakdown.
[b] Lowest temperature at which oil will pour.
[c] Resistance to flow/shear.
[d] Property of transition to vapor state.
[e] Density related to water.

exposure to exhaust gases of internal combustion engines. Used lubricating oil or used crankcase oil generally have higher concentrations of polynuclear aromatic hydrocarbons than unused oils. Used oils are not specifically addressed in this article but would be considered to be more toxic because of increased presence of toxic constituents.

Environmental releases of unused lubricating oil have been reported. The largest releases have been associated with lubricating oil manufacture and storage. These oils, stored in large above-ground tanks, have been the subject of large releases similar to other petroleum hydrocarbon materials (e.g., fuels). Soil and groundwater beneath these tanks can become contaminated with concentrations ranging up to saturation limits.

Exposure Routes and Pathways

Exposure to lubricating oil can occur through both direct contact with the material and contact with environmental media containing the oil. The primary route of exposure in the environment is direct, through dermal contact with the liquid oil. In the workplace, the primary route of exposure is the inhalation of oil mists generated during machine lubricant use. Exposure to oil mists in the occupational environment is of sufficient concern that an exposure standard has been set. Secondary exposure to lubricating oil occurs through contact with environmental media. These consist of exposure to contaminated soil via dermal contact, incidental ingestion, or inhalation of dust particulates. When lubricating oil is released to water, exposure can occur through ingestion and dermal contact with contaminated water.

Toxicokinetics

Exposure to lubricating oil is a concern for the organ of contact. For dermal exposure the concern is for the skin. For inhalation exposure, the concern is for the respiratory system. Therefore, absorption and distribution kinetics are not well studied because of these sites of contact concerns. However, it would be expected that individual chemicals present in lubricating oil can be absorbed and would have biological fate appropriate for that chemical or chemical class (e.g., polynuclear aromatic hydrocarbons).

Mechanism of Toxicity

The concern for both dermal and inhalation exposures is the site of contact and effects on that tissue. The mechanism of lubricating oil toxicity is mediated through its irritant effects, which after sufficient exposure duration and concentration result in tissue hyperplasia. Chronic hyperplasia leads to subsequent loss of tissue integrity and damage and in some animal models cancer.

Acute and Short-Term Toxicity (or Exposure)

Lubricating oils have low acute toxicity. Their sublethal acute effects are generally limited to irritation of those tissues in contact with the oil.

Animal

Low animal toxicity has been demonstrated in various studies. For example, a published oral LD_{50} for a mouse is $22\,g\,kg^{-1}$. Dermal irritation has been reported in rabbits at a total dose of 100 mg for 24 h.

Human

Low human acute toxicity would be expected due to the use of oils in food and cosmetic products. However, exposure to oils can cause irritation of the eyes, skin, and respiratory tract.

Chronic Toxicity (or Exposure)

Lubricating oils have low chronic toxicity although chronic exposures to levels that cause irritation can lead to other effects.

Animal

A 90 day study of various oils was performed in rats exposed with doses from 2 to $2000\,mg\,kg^{-1}$ in their feed. No treatment-related effects were reported in rats exposed to lubricating oil (paraffinic and naphthenic) with carbon ranges above C30. Rats fed oils with lower molecular weight fractions, carbon ranges C15–C30, showed histological changes in the liver and lymph nodes, which were noted at doses $20\,mg\,kg^{-1}$ and higher. Females were more sensitive than males.

Human

Low human chronic toxicity would be expected due to the use of oils in food and cosmetic products.

Carcinogenicity

Various lubricating oils have been tested for their carcinogenicity in animals using dermal application. Many studies have reported an increase in the number of tumors and this is associated with chronic skin irritation and hyperplasia. At doses where these skin effects were not noted, there were no

increases in tumor incidence rates. It has been suggested that unused lubricating oils with low polynuclear aromatic hydrocarbon content are not carcinogenic. This may be due to 'matrix' effects of the hydrocarbon base material. It has also been suggested that at exposures below those associated with dermal irritation and hyperplasia, these lubricating oils are not carcinogenic.

A single listing in Registry of Toxic Effects of Chemical Substances (RTECS) for lubricating oil suggest tumorgenic potential in humans exposed via inhalation to $5\,mg\,m^{-3}$ for 5 years. However, this reference was not reviewed and is not consistent with other reported chronic human exposures.

In Vitro Toxicity Data

A number of *in vitro* assays for mutagenicity have been performed on a variety of lubricating oils and their fractions. With the exception of oils with high polynuclear aromatic hydrocarbon content, the results of the studies on lubricating oil are negative.

Clinical Management

The clinical signs of overexposure to lubricating oil either by dermal contact or inhalation of mists would be irritation and inflammation of the contact tissues. This irritation is reversible and effective management is cessation of exposure by change of activities and effective use of dermal and/or pulmonary personal protective equipment.

Environmental Fate

Since lubricating oils contain a wide range of chemicals, the environmental fate of a release of lubricating oil is dependent on the chemical make-up of that oil. Generally, the chemicals in lubricating oil have low water solubility and high binding constants to organic carbon. Their low water solubility limits the potential for impacting deep groundwater yet these oils cause surface sheens and visible contamination when released to surface waters. When released to soil, the oils would not be expected to migrate far from the point of release although once bound to soil, these chemicals can spread as contaminated soil is spread.

Exposure Standards and Guidelines

Occupational exposures to oil mists are a concern. Both the Occupational Safety and Health Administration permissible exposure limit and the American Conference of Governmental Industrial Hygienists threshold limit value are $5\,\mu g\,m^{-3}$ for oil mists.

The toxicity studies of lubricating oil in animals suggest that no-observed-adverse-effect levels (NOAELs) can be set and used to predict safe human exposure levels. Lubricating oils with carbon ranges above C30 have shown NOAELs $\sim 2000\,mg\,kg^{-1}$ for a 90 day study. Oils with smaller carbon numbers have reported NOAELs $\sim 20\,mg\,kg^{-1}$. These values would result in oral reference doses in the range of $0.2–20\,mg\,kg^{-1}\,day^{-1}$ using appropriate safety factors.

Miscellaneous

The evaluation of petroleum hydrocarbon toxicity and it application to human health risk assessment is complicated. Strategies range from considering only the known toxic constituents (e.g., benzene or naphthalene) and ignoring the paraffinic and naphthenic fractions (i.e., not evaluating the hydrocarbon base material) to only evaluating the hydrocarbon base material. Given the uncertainty inherent in both of these strategies, an alternative would be to evaluate both the hydrocarbon base material and the toxic constituents. This strategy however suffers from the potential to double count the toxicity of those toxic constituents that are in both evaluation processes.

See also: Fuel Oils; Oil, Crude.

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Oleander

Fermin Barrueto Jr.

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- SYNONYMS: *Nerium oleander*; *Nerium indicum*; *Nerium odorum*; Common oleander; Rose-bay; Yellow oleander, *Thevetia peruviana*, Rose laurel

Uses

Oleander is used as an ornamental shrub along road sides and in gardens. The plant is also used in rodenticides, insecticides, and homeopathic remedies. In some countries, particularly Sri Lanka, this shrub has become a notorious method of suicide.

Background Information

Oleander is in the Apocynaceae (dogbane) plant family. The large ornamental evergreen shrub may grow 20–25 ft in height. Leaves are long and narrow, with pointed tips. During the summer months large clusters of white, pink, or red flowers appear at the ends of the branches.

Exposure Routes and Pathways

Common exposure pathways include ingestion of plant parts and inhalation from the burning of oleander bushes. Homeopathic extracts, insecticides, and rodenticide extracts are other available sources.

Mechanism of Toxicity

Common oleander contains at least five cardiac glycosides and the toxicity is identical to digitoxin poisoning where there are two main mechanisms of toxicity. First, these cardioactive steroids (like digoxin) increase the vagal tone, which leads to bradycardia but can lead to further AV nodal dysfunction and heart block. Second, these cardiac glycosides inhibit the Na^+,K^+-ATPase enzyme system. This causes a disturbance in the sodium gradient causing increased intracellular sodium and extracellular potassium. The excess intracellular sodium leaves the cell in exchange for Ca^{2+} through an antiport system. Sodium exits the cell and there is a subsequent increase in intracellular calcium. This calcium binds the ryanodyne receptor on the sarcoplasmic reticulum leading to a larger efflux of calcium to bind the myosin–actin filaments responsible for muscle contraction. Due to the increased intracellular sodium, there is an increase in the resting membrane potential leading to myocardial excitability. This is why the most common dysrhythmia seen in digoxin toxicity is premature ventricular contractions, though any dysrhythmia can be seen except a supraventricular tachycardia.

Chronic Toxicity (or Exposure)

Animal

Animals have the same potential for toxicity as humans. Cases have been documented in cows of oleander ingestion, which have shown similar effects to those that appear in humans.

Human

The range of toxicity is dependent on how it is ingested, what part of the plant is ingested, and the presence of any comorbidity. Significant oleander poisoning closely resembles digitoxin poisoning and can be treated as such. Gastrointestinal and cardiac symptoms predominate. Within the first several hours, nausea, vomiting, and abdominal pain are characteristically present. Cardiotoxic effects, such as conduction abnormalities, ventricular dysrhythmias, and asystole, can be present. Poisoned patients can present with bradycardia; first-, second-, and third-degree heart block; normotensive or hypotension; and hyperkalemia.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. With significant recent ingestions, decontamination with activated charcoal may be considered. Continuous cardiac monitoring and serial potassium levels should be performed. Atropine is useful in managing bradycardia and varying degrees of heart block. Low-dose phenytoin improves atrioventricular conduction and can terminate heart block, though with digoxin-specific Fab this has fallen out of favor. Ventricular dysrhythmia can be managed with phenytoin and/or lidocaine but is best managed with digoxin-specific Fab. Intravenous glucose and insulin and sodium bicarbonate can be used in life-threatening hyperkalemia though the most effective treatment is administration of digoxin-specific Fab. For patients who have persistent severe cardiovascular disease, an electrical pacemaker should be considered after administration

of digoxin-specific Fab. Digoxin-specific Fab, which is used to treat severe digitalis glycoside poisoning, has been demonstrated to be effective in the management of oleander poisoning and decreases morbidity and mortality.

See also: Digitalis Glycosides; Plants, Poisonous.

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Olfaction *See* Sensory Organs.

Opium

Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 8008-60-4
- Synonyms: Crude opium; Gum opium; Powdered opium; Raw opium; Standardized opium powder
- Chemical/Pharmaceutical/Other Class: Opiate agonist

Uses

Opium is used for analgesia, sedative–hypnotic narcosis, antiperistalsis, and in the treatment of neonatal withdrawal. It is also a drug of abuse.

Background Information

Opium is the air-dried milky exudate obtained by incising the unripe capsules of *Papaver somniferum*.

Exposure Routes and Pathways

Opium can be solubilized and given parenterally. Tinctures and suspensions are available for oral administration and suppositories for rectal administration. In addition, it can be insufflated as a powder.

Toxicokinetics

Opium contains several alkaloids, including no less than 10% anhydrous morphine and small amounts of codeine and papverine. After oral administration, morphine is absorbed from the gastrointestinal tract. The drug is rapidly metabolized after oral administration and plasma concentrations of unconjugated morphine are lower than those achieved after parenteral administration. Activity following parenteral administration of concentrated opium alkaloids is similar to parenterally administered morphine. Peak analgesia occurs within 60 min and can be maintained for up to 7 h. Rectal adsorption is erratic. Morphine is distributed throughout the body. Approximately 35% is protein bound. The volume of distribution is 3–4 $l kg^{-1}$. Opium preparations are metabolized in the liver. The major pathway for the metabolism of morphine is conjugation with glucuronic acid to form both active and inactive products. Morphine-6-glucuronide is a major metabolite of morphine that is twice as potent as morphine. In patients with renal failure, an accumulation of morphine-6-glucuronide may cause toxicity in the absence of significant morphine levels. A small percentage of morphine is excreted unchanged. Nearly

all morphine is metabolized and eliminated by the kidneys, with the major metabolite being morphine-3-glucuronide.

Mechanism of Toxicity

Morphine, the major active principle of powdered opium, is responsible for the action of opium, although other alkaloids contribute to it. Morphine's toxicity is a result of its extensive effect on the central nervous system (CNS), mainly that of a descending depression. Opiates interact with stereospecific and saturable binding sites primarily located in the CNS. Interaction with these receptors mimics the actions of endogenous enkephalins and endorphins. Their action also appears to involve an alteration in the release of neurotransmitters, such as the inhibition of acetylcholine, norepinephrine, and dopamine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dogs act similarly to humans – symptoms may include drowsiness, ataxia, vomiting, respiratory depression, miosis, coma, and hypotension. Opiates and their derivatives have an excitatory effect on the CNS of cats and horses. Naloxone may be used at $0.02\,mg\,kg^{-1}$ if needed.

Human

Symptoms of toxicity may occur in varying degrees in nontolerant individuals who receive greater than a therapeutic dosage. The primary insult is respiratory depression from direct depression of the CNS. This state may then progress to apnea or respiratory arrest. Pulmonary edema is a common complication. Therapeutically, opium results in analgesia. In toxic doses, CNS depression ensues and can progress to coma. Miosis is frequent, but in an acidotic or asphyxiated state the pupils may be dilated. Opium can cause hypotension and bradycardia. Hypothermia may also develop if there is peripheral vasodilation. Laboratory analysis does not dictate treatment but can confirm the presence of opiates.

Chronic Toxicity (or Exposure)

Human

Opiates have a high potential for abuse. Chronic users may develop tolerance, thus necessitating larger doses for the desired effect. Abrupt cessation can cause withdrawal, yielding restlessness, vomiting, and diarrhea.

Clinical Management

In patients presenting with opium toxicity, the airway should be patent and adequate ventilation assured. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk for subsequent CO_2 narcosis, worsening acidosis, and/or aspiration. If necessary, endotracheal tube intubation should be performed. The initial treatment of hypotension consists of intravenous fluids. Close monitoring of the patient's pulmonary exam should be performed to assure that pulmonary edema does not develop as fluids are infused. The patient should be placed on continuous cardiac monitoring with pulse oximetry. Frequent neurological checks should be made. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered to patients who have ingested opium and present early. Syrup of ipecac is contraindicated after overdose with the opium due to the potential for rapid clinical deterioration. Gastric lavage should be avoided.

Naloxone may be of benefit in reversing the neurological and respiratory depressant effects of opium. A dose of 0.4–2.0 mg is given intravenously slowly, titrated to resumption of adequate respirations, and can be repeated as needed. The therapeutic effect of naloxone may be of shorter duration than that of opium activity; therefore, it is imperative that opium intoxicated patients who demonstrated improvement after naloxone be closely monitored for resedation. Vital sign measurements and neurological checks should be monitored frequently until resolution.

See also: Codeine; Drugs of Abuse; Morphine.

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Organochlorine Insecticides

Benny L Blaylock

Organochlorine insecticides are chlorinated hydrocarbon compounds that fall into three basic structure classifications: aryl (aromatic), carbocyclic, and heterocyclic.

They may be differentiated from other chlorinated hydrocarbon compounds (e.g., solvents) by molecular weight. Organochlorine insecticides, by virtue of their cyclic structure, have molecular weights ranging from 291 to 545, whereas chlorinated hydrocarbon solvents and fumigants have molecular weights that generally are less than 236.

Organochlorine insecticides may be divided into three broad groups: dichlorodiphenylethanes, such as DDT and methoxychlor; cyclodienes, such as chlordane and dieldrin; and hexachlorocyclohexanes, such as lindane. Mirex and chlordecone, however, are organochlorine insecticides whose caged structures do not fit well into the previous groups.

The first organochlorine synthesized was DDT. Although it was first synthesized by Zeidler in 1874, it was not produced or used for many years. Mueller rediscovered DDT in 1939 and won the Nobel Prize for his efforts in 1948. The first major uses for DDT were vector control of typhus and malaria and control of lice and other pests during World War II.

Other organochlorine compounds were synthesized and came into general use during the late 1940s, thus introducing the synthetic insecticide era. From the 1940s through the 1960s, organochlorine insecticides were used extensively to control insect pests in both agricultural and domestic settings and for vector control of malaria, typhus, and other diseases affecting human health. Head and body lice are still treated effectively today with lindane (although the pyrethroids are now more commonly used).

Low volatility, lipid solubility, and environmental persistence are characteristic of organochlorine insecticides. Initially, these properties helped make organochlorines very useful and effective. However, starting in the 1960s and 1970s, environmental problems began to emerge as a result of environmental persistence of these insecticides. Bioaccumulation in the food chain and the resulting toxicity led to decreased use of these insecticides and eventually to their general ban in the United States and Europe. By 1973, DDT use had ceased in the United States. By 1988, chlordane and heptachlor were no longer produced for use in the United States. Biologically, the organochlorine insecticides are generally nervous system stimulants, although there are distinct differences in the activities of the individual chemicals. The mode of action for organochlorine insecticides in general is alteration of enzymatic and electrophysiological properties of nerve cell membranes. Ion flow is altered by inhibiting Na^+, K^+, and Ca^{2+} adenosine triphosphatases that pump ions across neuronal membranes. The Na^+ channel activation is normal but its closing is prolonged. Additionally, the cyclodiene group inhibits the uptake of Cl^- ions by γ-aminobutyric acid. These activities inhibit the repolarization of neurons after excitation. The nerve remains partially depolarized and extremely sensitive to complete depolarization by very small stimuli.

All organochlorine insecticides may be absorbed through the gastrointestinal tract, respiratory tract, and skin, although there is variation among classes. Organochlorine insecticides are, in general, very lipophilic and tend to accumulate in the mammalian system in adipose tissue and/or organs with high fat content. Biotransformation of organochlorine insecticides is slow. Metabolism is by liver microsomal P450 enzymes to hydroxyl derivatives by dechlorination, conversion to stable epoxides, and/or *O*-dealkylation and hydroxylation, depending on the class. Excretion of the parent compound is usually in bile or through the intestinal wall. In either case, final elimination is usually in the feces. Urinary excretion after glutathione conjugation is also an important route of excretion. Organochlorine insecticides have been found in both cows' milk and human milk. In the liver, most organochlorine insecticides induce cellular hypertrophy, granule margination and the production of lipospheres containing fat droplets. Focal necrosis is observed with high doses. Nodules of hypertrophied hepatocytes appear in the centrilobular area with a loss of lobular architecture.

Although generally negative in mutagenicity tests, organochlorine insecticides are associated with liver tumors in rodents. Whether this is a direct carcinogenic effect or due to promotion of spontaneous tumorigenic events is not currently known. Though conclusive proof of carcinogenicity in humans is lacking but tumor potential, based on animal data, it cannot be totally discounted in humans.

Endocrine disruption has recently become a significant concern for organochlorine insecticides in both human and environmental health. Many organochlorine chemicals, including cyclodiene insecticides, mirex, and toxaphene as well as PCBs and PCDDs have been shown to have endocrine disrupting properties.

Symptomatology includes paresthesia, ataxia, nausea, vomiting, fatigue, and, in more acute cases, tremor, convulsions, coma, respiratory arrest, and death.

Clinical management is generally symptomatic. Convulsions are usually controlled using diazepam, pentobarbital, or phenobarbital. In some instances, treatment with activated charcoal is effective in increasing the excretion of the pesticide. Cholestyramine has been proven effective in chlordecone poisoning. In severe cases, mechanical maintenance of cardiac function and respiration is necessary.

See also: Chlordane; DDT (Dichlorodiphenyltrichloroethane); Dieldrin; Lindane; Methoxychlor.

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Relevant Website

http://npic.orst.edu – National Pesticide Information Center, Oregon State University and the US Environmental Protection Agency.

Organophosphate Poisoning, Delayed Neurotoxicity

Rudy J Richardson

The term 'delayed neurotoxicity' may be used to describe any type of toxicity to the nervous system involving a delay between the precipitating chemical exposure and the appearance of neurological signs or symptoms. However, this designation usually refers to organophosphorus (OP) compound-induced delayed neurotoxicity (or delayed neuropathy) (OPIDN), also known as OP compound-induced delayed polyneuropathy (OPIDP).

The particular syndrome of OPIDN is produced by certain organic compounds of pentacovalent phosphorus. The less common and relatively unstable organic compounds of trivalent phosphorus, such as triphenyl phosphite, can produce a different spatial–temporal pattern of neurodegeneration, which is distinct from OPIDN.

The underlying pathology in OPIDN involves bilaterally symmetrical degeneration of sensory and motor axons in distal regions of peripheral nerves and spinal cord tracts. Generally, the longest, largest diameter fibers tend to be preferentially affected. The most prominent lesions are often found in the dorsal columns of the cervical spinal cord, especially in the fasciculus gracilis. Injury to this tract results in specific sensory deficits, including loss of recognition of limb position (proprioception) and vibration sensitivity. Pathogenesis studies indicate that the primary lesion in OPIDN is in the axon rather than the myelin sheath or the cell body of the neuron, and that demyelination occurs secondarily to axonal degeneration. The process has been likened to a 'chemical transection' of the axon, with subsequent Wallerian-type degeneration, as opposed to a 'dying back' of the axon following an insult to the cell body as once hypothesized.

Signs and symptoms of axonopathy appear after a delay of at least 8 days following absorption of an effective dose of an OPIDN-producing (neuropathic) OP compound and will consist of abnormal sensations (paresthesias) in the extremities, including numbness and tingling. There may also be pain, particularly in the calves of the legs. Distal reflexes may be absent or attenuated. The feet and lower legs are usually affected predominantly and before involvement of the hands and arms, but severe cases will involve the upper and lower limbs in a 'glove and stocking' distribution. Incoordination of movement (ataxia) develops at about the same time as the sensory disturbances and may progress to partial flaccid paralysis (paresis) after ~10–21 days. Recovery from severe disease is usually poor, and there is no specific treatment. Over a period of months to years, flaccidity may be replaced by spasticity, reflecting regeneration of peripheral nerve injury with residual damage to descending upper motor neuron pathways in the spinal cord.

Because of the ubiquity of OP compounds and the serious and often irreversible nature of OPIDN, much effort has been expended to develop ways to identify the OP compounds that pose a genuine risk of causing this condition. Consequently, although the pathogenic mechanism remains unknown, human OPIDN is now an extremely rare disease, with a worldwide incidence of only about two cases per year, usually from intentional ingestion of massive

doses of OP compounds in attempted suicides. Sporadic episodes of OPIDN affecting domestic animals and livestock also occur, largely from misapplication of OP compounds used directly on the animals for control of insect or arachnid pests. Most of the ~30 000 human cases that occurred between 1930 and 1960 arose from contamination of cooking oil or beverages with tri-*o*-cresyl phosphate (TOCP; also known as tri-*o*-tolyl phosphate, TOTP). Over half of the cases of OPIDN have been attributed to consumption of an alcoholic extract of Jamaica Ginger ('Ginger Jake') that had been adulterated with solvents containing TOCP. Ginger Jake was used as a source of alcohol during Prohibition in the United States. The resulting paralysis became known as 'Jake Leg' or 'Jake Walk'. Awareness of OPIDN coupled with the advent of improved methods for assessing the relative potential of OP compounds to produce the disease has led to the virtual elimination of human cases. Nevertheless, neuropathic OP compounds and OPIDN continue to be active fields of study. This apparent paradox arises from the importance of OP chemistry in diverse applications, the threat of neuropathic OP compounds as agents of terrorism or warfare, and the promise of neuropathic OP compounds as tools in neurological research.

Experimental studies have identified the adult chicken as the species of choice for testing OP compounds for their potential to cause OPIDN. Hens of greater than 8 months of age are now used in routine testing. Other species in addition to humans and chickens that are known to be susceptible to single doses of neuropathic OP compounds include certain nonhuman primates, water buffalo, cattle, swine, sheep, dogs, and cats. Rats and mice have been considered resistant to the clinical manifestations of OPIDN. However, recent studies have shown that histopathological lesions, particularly in the spinal cord, can be produced in these species by compounds known to cause OPIDN in the adult hen. The apparent resistance of rodents to OPIDN may be due, at least in part, to the fact that relatively young (less than 3 months of age) animals have been used in most studies. Generally, the young of a given species are much more resistant to OPIDN than adults are. For example, chicks younger than ~50 days of age will not develop OPIDN after a single dose of a neuropathic OP compound. Moreover, chicks are resistant to repeated doses if they are younger than ~14 days of age. Species and age differences in susceptibility to OPIDN have been attributed to long axons in large animals and robust repair of neural injury in young animals.

The complete mechanism of OPIDN has not been elucidated. However, there is good evidence that the disease is initiated by a concerted two-step reaction involving inhibition and aging of a critical amount of a protein called neuropathy target esterase (neurotoxic esterase, NTE) in target neural tissues. The net result of the aging step is the rapid formation of a

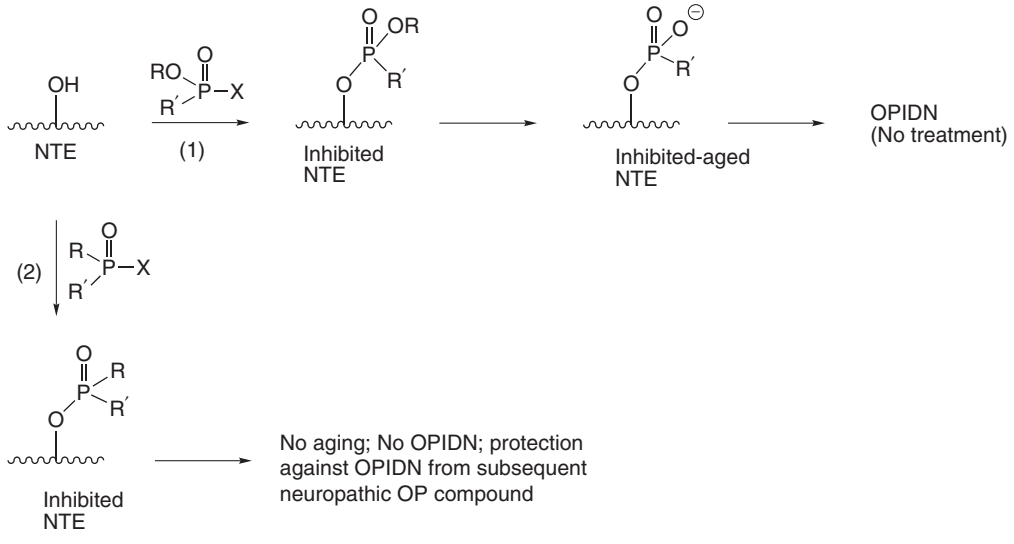

Figure 1 Inhibition and aging of NTE are required for initiation of OPIDN. NTE is represented by a wavy line containing the active site serine hydroxyl group. Pathway (1) shows inhibition by a phosphonate, which undergoes rapid aging to yield a negatively charged phosphonyl adduct. OPIDN follows within 8–21 days and is not treatable. Pathway (2) shows inhibition by a phosphinate, which cannot undergo aging. The neutral phosphinylated adduct does not trigger OPIDN; however, it confers protection against subsequently administered neuropathic (ageable) NTE inhibitors. For each type of inhibitor, R and R′ may be substituted or unsubstituted alkyl or aryl groups. X is the primary leaving group that is displaced by the serine hydroxyl of NTE and may be, for example, substituted or unsubstituted alkoxy, aryloxy, or fluorine.

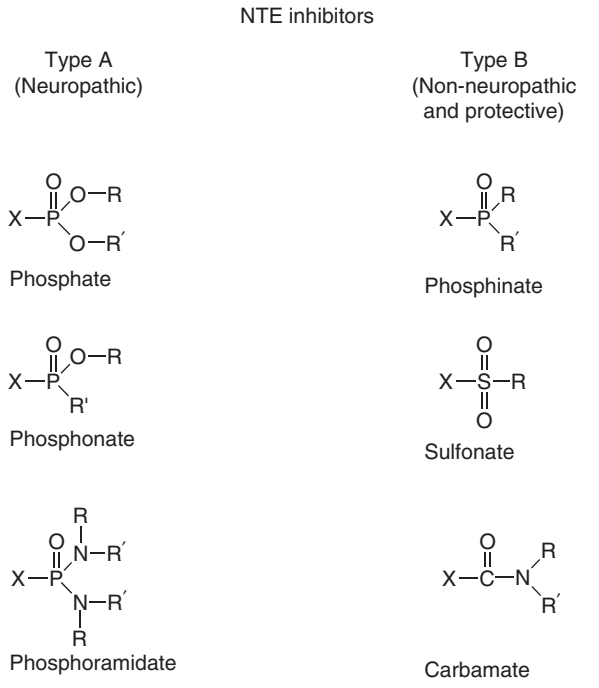

Figure 2 NTE inhibitors. For each type of inhibitor, R and R′ may be substituted or unsubstituted alkyl or aryl groups. X is the primary leaving group that is displaced by the serine hydroxyl of NTE and may be, for example, substituted or unsubstituted alkoxy or aryloxy. Fluorine can be a leaving group for the OP NTE inhibitors and is the most common leaving group for sulfonate NTE inhibitors. Type A inhibitors are neuropathic and include certain phosphates, phosphonates, and phosphoramidates. Mixtures of subtypes are possible. Phosphonates are intrinsically asymmetric and enantiomers may have different inhibitory and/or aging properties. Some phosphoramidates may have one or more hydrogen atoms as R-groups. Type B inhibitors are not neuropathic, but pretreatment protects against OPIDN from subsequent exposure to Type A inhibitors. Type B inhibitors include certain phosphinates, sulfonates, and carbamates.

negatively charged species in the active site of the enzyme (**Figure 1**). Such a reaction can take place with OP inhibitors of NTE such as phosphates, phosphonates, or phosphoramidates, which have an ester or amide group in addition to the leaving group (**Figure 2**). Phosphates and phosphonates undergo aging by net loss of an R-group. Phosphoramidates having only a single R-group attached to the phosphoramidate nitrogen appear to age by loss of the phosphoramidate proton rather than by loss of an R-group. Compounds that do not inhibit NTE do not cause OPIDN, even if they belong to a structural class capable of undergoing the aging reaction. For example, although paraoxon belongs to the phosphate class of OP compounds, it does not produce OPIDN because it is a poor inhibitor of NTE.

NTE inhibition and aging transpire within minutes to hours following absorption of an effective dose of a neuropathic OP compound. Thus, events that remain to be elucidated contribute to the delay of 8–21 days between exposure and the initial signs of ataxia and paresis. However, if inhibition but no aging occurs by dosing with an NTE inhibitor that is incapable of generating a negative charge at the active site, no OPIDN ensues. Furthermore, an animal whose NTE is inhibited with a nonaging compound is protected against a subsequent dose of an OP compound that would be neuropathic in a naive animal (**Figure 1**). Nonaging inhibitors of NTE include representatives from the phosphinate class of OP compounds, certain carbamates, and sulfonyl fluorides, such as phenylmethanesulfonyl fluoride (PMSF) (**Figure 2**).

The threshold of NTE inhibition in target neural tissue that correlates with the development of OPIDN after a single dose of a neuropathic OP compound is ~70%. For many compounds, inhibition measured in brain is paralleled in spinal cord and peripheral nerve, and brain values are often used in screening tests in hens to assess relative neuropathic potency. Repeated dosing also appears to require that a high point of inhibition be reached before OPIDN will develop. The threshold appears to be the same as for acute dosing for some compounds, but for some others, the critical level of inhibition may be as low as 50%. With repeated dosing, there still appears to be a delay of ~8–21 days between the time inhibition exceeds the threshold value and the appearance of signs of OPIDN.

NTE has also been found in circulating lymphocytes and platelets, where its inhibition has found some use as a biomarker of exposure to neuropathic OP compounds. There is a reasonably good correlation between inhibition of NTE in leukocytes and brain when the measurements are carried out within 24 h of an acute exposure. However, a good correlation might not be found later (even by 48 h) or under conditions of repeated exposures. Nevertheless, leukocytes provide an accessible source of NTE for detection of inhibition by neuropathic OP compounds. Currently, there is considerable interest in using protein mass spectrometry to detect OP adducts on NTE as sensitive and specific biomarkers of exposure to neuropathic OP compounds.

It is important to realize that OPIDN depends on a particular type of chemical modification of NTE rather than mere inhibition of its enzymatic activity. Inhibition of NTE is a necessary, but not sufficient, condition for OPIDN. Aging of the inhibited enzyme results in a complete change in the toxicological outcome. Whereas inhibition without aging results in no clinically apparent injury, suprathreshold inhibition with aging triggers an inexorable neurodegenerative process leading to evident disease. The

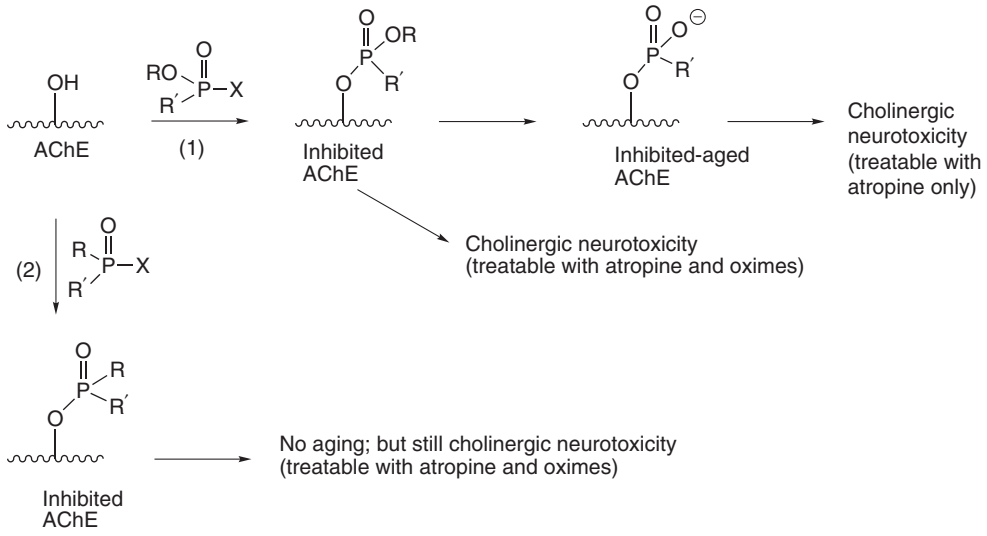

Figure 3 Inhibition of AChE is sufficient for cholinergic neurotoxicity. AChE is represented by a wavy line containing the active site serine hydroxyl group. Pathway (1) shows inhibition by a phosphonate leading directly to cholinergic toxicity, treatable by both atropine (acetylcholine antagonist) and oximes (AChE reactivators). If aging occurs, the type of toxicity does not change, but oxime reactivators are no longer effective. Pathway (2) shows inhibition by a phosphinate, which cannot undergo aging. Cholinergic toxicity still occurs and is treatable by both atropine and oximes. R and R′ may be substituted or unsubstituted alkyl or aryl groups. X is the primary leaving group that is displaced by the serine hydroxyl of AChE and may be, for example, substituted or unsubstituted alkoxy, aryloxy, or fluorine. Neither inhibition nor aging of inhibited AChE can produce OPIDN – this requires inhibition and aging of NTE (see **Figure 1**).

situation with NTE is completely different from that with acetylcholinesterase (AChE). Inhibition of a sufficient amount of AChE will produce cholinergic toxicity, regardless of whether or not aging of inhibited AChE occurs (**Figure 3**). Aging of inhibited AChE does not alter the type of toxic response, but it does change the options available for therapy against cholinergic toxicity. For example, oximes such as pralidoxime methiodide (2-PAM) are used to reactivate inhibited AChE, but these agents are ineffective if aging of the enzyme has occurred. Moreover, oximes do not appear to affect the clinical course of OPIDN following administration of a neuropathic OP compound, except to allow survival of an otherwise lethal dose of a compound that also has cholinergic toxicity.

In a homologous series of OP compounds, increasing potency for AChE inhibition and cholinergic toxicity correlates with decreasing potency for NTE inhibition and OPIDN. The relative inhibitory potency (RIP) of an OP compound or its active metabolite for NTE versus AChE *in vitro* can be used as a convenient index of the probable neuropathic potential of the compound. A commonly used measure of inhibitory potency is the IC_{50}, the concentration required to inhibit 50% of the enzyme activity under a standardized set of reaction conditions and time of incubation of the inhibitor with the enzyme preparation. A better measure of inhibitory potency is the bimolecular rate constant of inhibition, k_i. When pseudo-first-order kinetics are observed, it is valid to use the relationship, $IC_{50} = 0.693/k_i t$, where t is the time of preincubation of the inhibitor with the enzyme. Comparisons of AChE/NTE k_i ratios or NTE/AChE I_{50} ratios *in vitro* (RIPs) with toxicity data *in vivo* have shown that values less than 1 indicate that the dose required to produce OPIDN is less than the median lethal dose (LD_{50}). In contrast, RIP values greater than 1 correspond to doses greater than the LD_{50} being required to produce OPIDN. The higher the RIP, the safer the compound with respect to its capacity to produce OPIDN. Thus, insecticidal OP compounds will generally be much more potent inhibitors of AChE than NTE and will not produce OPIDN except at doses that would require treatment for cholinergic toxicity. On the other hand, compounds can be made that are better inhibitors of NTE than AChE. If such compounds can also undergo aging, not only will they produce OPIDN; they will do so at doses that elicit little or no cholinergic toxicity.

Marginal or subclinical OPIDN can be potentiated to full-blown disease by subsequent treatment with nonaging inhibitors of NTE. The phenomenon is called 'promotion' by some authors, which is an appropriate term if the initial insult is undetectable. Potentiation was initially a surprising finding, especially in view of the fact that reversing the order of dosing of the nonaging and aging NTE inhibitors affords protection against OPIDN. However, it now

appears that the outcome of many types of neural injuries can be exacerbated by dosing with nonaging NTE inhibitors as well as with inhibitors of other serine esterases or proteases. The apparent indifference to the method of producing the initial lesion suggests a general mode of action for potentiation, such as interference with regeneration and repair. With respect to understanding the potentiation of OPIDN and its practical significance, more data are needed to answer the following questions: What is the extent to which the NTE inhibition threshold for initiation may be lowered by potentiators? What are the potencies of potentiators at a given dose level of initiator? What are the potencies of initiators at a given dose level of potentiator? What are the structure–activity relationships of potentiators? What is/are the mechanism(s) of action of the effect?

Although the physiological function of NTE is currently unknown, knocking out the gene is embryonic-lethal in mice, indicating an essential role in development. NTE is an integral membrane protein concentrated in the endoplasmic reticulum. It contains a domain with homology to cyclic nucleotide-binding regions in other proteins, implying a regulatory or signaling function. Mutation of a homologous protein called SWS in *Drosophila* results in a spongiform neurodegenerative disease, suggesting that NTE might be linked to neurological or neurodevelopmental disorders. Thus far, the goal of using cell expression systems to produce full-length NTE for study has proved to be elusive. However, it has been possible to generate the NTE catalytic domain, called NEST. The enzymological properties of this truncated NTE are similar to those of the full-length protein, and it is being used to examine mechanisms of inhibition and aging by neuropathic OP compounds. A recent intriguing finding is that NEST mediates an ionic conductance across liposome membranes, which is selectively disrupted by aging inhibitors of the enzyme. Certainly, much work remains to be done to elucidate the normal and pathogenic roles of NTE, but the accomplishments thus far are proving to be useful in a wide range of fields, from toxicological risk assessment to developmental neurobiology.

Acknowledgment

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See also: Acetylcholine; A-Esterases; Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates.

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Organophosphate Poisoning, Intermediate Syndrome

Ramesh C Gupta

Organophosphate (OP) insecticide-induced intermediate syndrome (IMS) was reported for the first time in human patients in Sri Lanka in 1987. Thereafter, this syndrome has been diagnosed in OP-poisoned patients in South Africa (1989), Turkey (1990), Belgium (1992), United States (1992), Venezuela (1998), France (2000), and elsewhere. IMS is usually observed in patients who have ingested a massive dose of an OP insecticide either accidentally or in a suicidal attempt. A similar syndrome has also been observed in dogs and cats poisoned maliciously or accidentally with massive doses of certain OPs.

IMS is clearly a separate clinical entity from acute cholinergic crisis and delayed neuropathy. The acute cholinergic crisis usually emerges within a few minutes to a few hours and is due to acetylcholinesterase (AChE) inhibition resulting in acetylcholine accumulation at the synapses in the nervous system and at the neuromuscular junctions. Patients acutely poisoned with OPs exhibit muscle fasciculations,

convulsions, seizures, salivation, lacrimation, tracheobronchial secretion, and diarrhea due to overstimulation of muscarinic and nicotinic receptors within the peripheral and central nervous systems. Delayed neuropathy, commonly referred to as organophosphate-induced delayed neurotoxicity (OPIDN), a neurological manifestation of some OPs, usually occurs ~2 or 3 weeks after exposure. OPIDN occurs due to the inactivation of neurotoxic or neuropathy target esterase (NTE) and is characterized by predominately sensory, motor, distal, and symmetrical polyneuropathy.

IMS in OP-poisoned patients appears 24–96 h after an apparently well-treated acute cholinergic crisis phase. By definition, OP-poisoned patients should completely recover from the cholinergic crisis and then develop a syndrome. Clinically, IMS is characterized by acute paralysis and weakness in the territories of several cranial motor nerves, neck flexors, facial, extraocular, palatal, nuchal, proximal limb, and respiratory muscles 24–96 h after poisoning. Generalized weakness, depressed deep tendon reflexes, ptosis (drooping of the upper eyelids due to paralysis of the third cranial nerve), and diplopia (double vision of an object) are also evident. These symptoms may last for several days or weeks depending on the OP involved. Despite severe AChE inhibition, muscle fasciculations and muscarinic receptor-associated hypersecretory activities are absent.

OP compounds that are known to cause IMS are listed in **Table 1**. These compounds in general are highly lipid soluble, and in some cases, the metabolites of these OP compounds have a long-lasting half-life. Other contributing factors for IMS include the chemical structure of OP compounds, impairment of systemic functions (cardiovascular, hepatic, renal), and the time elapsed between ingestion of an OP and treatment.

Based on electromyographic (EMG) findings from OP-poisoned patients and experimental studies on laboratory animals, scientists have found that the defect in IMS is at the neuromuscular endplate and postsynaptic level, but the effects of neural and central components in producing muscular weakness have not been ruled out. EMG findings in the early stages revealed marked decrements at low rates of repetitive nerve stimulation and increments at a high rate, suggesting diverse types of impaired neuromuscular transmission. IMS seems to be due to persistent AChE inhibition at the endplate, presumably leading to combined pre- and postsynaptic impairment of neuromuscular transmission.

Table 1 Organophosphates known to cause intermediate syndrome in humans

Bromophos	Fenthion	Monocrotophos
Chlorpyrifos	Malathion	Omethoate
Diazinon	Merphos	Parathion
Dicrotophos	Methamidophos	Phosmet
Dimethoate	Methylparathion	Trichlorfon

Note: Exposure to a single or combination of these OP compounds can produce IMS.

Perhaps there are gradations of IMS and genetic or environmental factors which influence its onset. Some OPs may have a higher affinity for nicotinic acetylcholine receptors or selectively distribute to muscle, producing a neuromuscular dysfunction that is longer lasting than at muscarinic sites. There may also be differences in the onset of IMS, which would depend on OP distribution or metabolism to the active metabolite. Perhaps the lesions produced at the neuromuscular junction are more permanent than the muscarinic lesions. Currently, very little is known about the type of damage at the motor endplate or about risk factors associated with IMS. Thus, more detailed laboratory and clinical tests are necessary to determine the exogenous and endogenous factors contributing to its development.

Some investigators suggest that the IMS may result from inadequate oxime therapy, while others suggest that prolonged oxime therapy by continuous infusion has no role in the routine management of OP intoxication. The undisputed fact remains, that in patients with IMS, a long-lasting inhibition of AChE occurs due to the persistence of the OP or its active metabolite in the body. This appears to be a trigger for nicotinic receptor overstimulation and toxicity at the neuromuscular junction, leading to weakness and paralysis of respiratory and other muscles. Also in IMS patients, both clinical signs and AChE are unresponsive to atropine or oxime therapy. Signs and symptoms of IMS usually persist for 1–3 weeks, followed by a complete recovery. IMS makes the management of OP poisoning more complicated since the clinician has to observe the patients for an additional 3 or 4 days for a possible respiratory arrest. In such cases, urgent respiratory support is absolutely necessary.

Confirmatory tests for IMS include persistent inhibition of AChE, decreasing response on electromyography, and necrotizing myopathy on muscle biopsy. In most instances, recovery from IMS is complete, though a few deaths have been reported due to severe respiratory insufficiency. Only a few IMS patients have developed delayed neuropathy. The risk of death in IMS is as dangerous as it is in the cholinergic crisis phase. Due to development of severe respiratory distress, tracheostomy is recommended for ventilatory support. Patients may remain on a respirator for 7 days or more. In conclusion, prolonged inhibition of

cholinesterases and dysfunction of the neuromuscular junction are the pathophysiological hallmarks of IMS. Because of the risk of appearance of an IMS, a prolonged clinical medical supervision seems necessary after recovery from the cholinergic crisis. The administration of atropine sulfate and pralidoxime should be continued for a long period, even if efficiency of these drugs on the development of the IMS is limited. Evidently, a drug protocol that can clearly benefit the patients of IMS remains to be established.

See also: Cholinesterase Inhibition; Neurotoxicity; Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphates; Pesticides.

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Organophosphates

Marion Ehrich

The term ‘organophosphates’ (organophosphorus compounds and organophosphorus esters) generally refers to chemicals that are organic derivatives of phosphoric acid. These compounds contain both phosphorus and carbon atoms, which may be linked directly or through another atom such as oxygen. Each phosphorus atom has three such linkages, and the terms ‘phosphate’, ‘phosphonate’, and ‘phosphinate’ refer to the number of linkages made through oxygen atoms (three, two, and one, respectively). Phosphorus linkages to carbon can also be made through nitrogen atoms (‘phosphoroamidates’ if one such linkage; ‘phosphorodiamidates’ if two), or through sulfur atoms (‘phosphorothiolates’ if one such linkage; ‘phosphorodithiolates’ if two). Some organophosphates have a phosphorus–fluoride linkage (‘phosphorofluoridates’). As derivatives of phosphoric acid, four atoms are directly attached to the phosphorus atoms – three by single bonds, as described previously, and one represented as a double bond. The doubly bonded linkage to the phosphorus atom of organophosphates is with an oxygen atom or with a sulfur atom. The nature of the atom linked with a double bond to the phosphorus atom also affects the chemical nomenclature of the compound, with ‘thio’ used in the chemical name to identify the S-containing phosphorothionate compounds. It is generally recognized that these phosphorothionate compounds are not active toxicants but rather protoxicants. Oxidation, which results in exchange of the doubly bonded sulfur for a doubly bonded oxygen, is necessary for conversion of the protoxicant phosphorothionate compounds to active neurotoxicants.

Organophosphates first gained notoriety during World War II, when they were synthesized by German chemists for use as highly toxic nerve gases. The potential for their use as chemical warfare agents continues today. However, other less volatile, less toxic organophosphates have since been synthesized, and these are used as insecticides, defoliants, herbicides, therapeutic agents in human and veterinary medicine (e.g., as ophthalmic agents and antiparasitics, respectively), flame retardants, fuel additives, lubricants, and plasticizers. They are in especially widespread use as insecticides, with formulations prepared for use in homes and gardens, on pets and livestock, and on crops and fields. Their popularity as insecticides is based on their effectiveness and on their biodegradability.

Examples of organophosphates include the insecticides malathion, parathion, diazinon, fenthion, azinphos methyl, terbufos, dichlorvos, and chlorpyrifos; the nerve gases soman, sarin, tabun, and VX; the ophthalmic agent echothiophate; the anthelmintic trichlorfon; tricresyl phosphate-containing

industrial chemicals; and the herbicides/defoliants DEF and merphos.

Most organophosphates are lipid soluble and all can be hydrolyzed, a reaction that results in detoxification. Some of them are gases or volatile liquids. These chemical properties contribute to their usefulness and to their toxicity by affecting their absorption and metabolism. Lipid-soluble substances more readily pass membrane barriers than compounds that are less lipid soluble, resulting in more complete absorption regardless of the route by which humans or animals are exposed. This lipid solubility aids the passage of organophosphate insecticides through the chitin exoskeleton of insects. Due to their lipid solubility, however, most organophosphates can pass through the skin of humans and animals as well, although the extent of absorption and the time in which it occurs varies from compound to compound. Volatility means that exposure to some organophosphates can be by inhalation, with absorption via the lungs. Organophosphates are esters, which means that they can be split into their component acid and an alcohol by the addition of water across the ester bond. This degradatory reaction (hydrolysis) occurs when organophosphates are in the presence of water and, in particular, in the presence of enzymes (esterases) that catalyze this reaction. That organophosphates are subject to hydrolysis increases the probability that they will biodegrade in the environment and that they will not accumulate in the environment or in the bodies of exposed subjects.

Organophosphate insecticides inhibit neural acetylcholinesterase, an enzyme responsible for the degradation of the neurotransmitter acetylcholine. This is the means by which they are effective as insecticides and the means by which they are toxic to humans and animals. Acetylcholine is a neurotransmitter found in the brain, spinal cord, and peripheral nervous system. In the peripheral nervous system, it is the neurotransmitter at effector organs of the parasympathetic nervous system, at ganglia of the autonomic nervous system (both sympathetic and parasympathetic ganglia), and at junctions between nerves and skeletal muscles. The presence of excess acetylcholine, due to inhibition of acetylcholinesterase, at muscarinic receptors (which are at the effector organs of the parasympathetic nervous system) results in clinical signs that include blurred vision due to pupil constriction, tearing, breathing difficulty due to excessive respiratory secretions, vomiting and diarrhea due to increased activity of the gastrointestinal tract, increased frequency of urination, and slowing of the heart rate. The presence of excess acetylcholine, due to inhibition of acetylcholinesterase, at nicotinic receptors found in autonomic ganglia can result in exaggeration of the effects seen by stimulation of parasympathetic muscarinic receptors and, in addition, can cause hypertension and tachycardia due to concurrent stimulation of the sympathetic nervous system. The presence of excess acetylcholine at nicotinic receptors of neuromuscular junctions of skeletal muscles can result in muscle twitching, tremors, and cramps. This may be followed by muscle weakness and flaccid paralysis. Excess acetylcholine in the brain and spinal cord (central nervous system) may cause anxiety, restlessness, emotional instability, confusion, ataxia, weakness, convulsions, and/or coma. Death is usually due to respiratory failure, which may be the result of a combination of effects in the peripheral and central nervous systems. Some organophosphates are very toxic; others are not. Toxicity resulting from organophosphate exposure is dependent on the chemical structure, lipid solubility, formulation and formulation vehicle, dosage, and the absorption, distribution, metabolism, and excretion of the substance to which subjects are exposed.

The acetylcholinesterase enzyme contains two sites for binding of acetylcholine, its natural substrate. Organophosphates combine with one of these sites, called the esteratic site, preventing the attachment of acetylcholine. Treatment of acetylcholinesterase inhibition is directed toward protecting the acetylcholine receptor from excess neurotransmitter and toward removal of the organophosphate from the inhibited enzyme. Atropine competes with acetylcholine for the muscarinic receptors at which it acts in the parasympathetic nervous system; this drug is used to reduce symptoms associated with overstimulation of those receptors with the acetylcholine that accumulates as acetylcholinesterase is inhibited. Symptomatic treatment of organophosphate toxicity may also include diazepam, a tranquilizer and anticonvulsant. Oxime drugs (e.g., pralidoxime or 2-PAM) attach to the organophosphate itself, removing it from acetylcholinesterase. Oximes must be given in a relatively short time frame after exposures occur, however. Although initially reversible, with time the attachment between the organophosphate and the enzyme can become irreversible, a condition generally referred to as 'aging'. Once aging has occurred, treatment of toxicity with oximes is ineffective. Time is needed for synthesis of new acetylcholinesterase molecules before enzyme activities return to preexposure levels.

Prevention of organophosphate toxicity is aimed at protecting the acetylcholine receptor and/or acetylcholinesterase itself. Atropine can be used to prevent as well as to treat organophosphate poisonings. In addition, use of 'reversible' inhibitors of acetylcholinesterase has been used to prevent organophosphate

toxicity. The rationale behind use of such compounds (carbamates such as physostigmine) is that they occupy the site at which organophosphates could bind to acetylcholinesterase and, consequently, provide time for the organophosphate to be metabolized and excreted before sites become free for occupancy on acetylcholinesterase. Carbamates may actually be given after exposure to organophosphates, with the assumption made that absorption will take some time, so the carbamates can be used to occupy sites on acetylcholinesterase until the danger of further absorption of organophosphates is past. After sufficient time, the carbamate is withdrawn and the carbamylated enzyme is given time to spontaneously reactivate. Experimental therapies for prevention and/or treatment of organophosphate toxicosis with potential for use in extenuating circumstances (e.g., nerve gas exposure) include administration of enzymes responsible for organophosphate hydrolysis.

Esterases other than neural acetylcholinesterase may also be inhibited by organophosphates, although this is dependent on the compound and the species of animals exposed. These esterases include acetylcholinesterase of mammalian erythrocytes; pseudocholinesterases found primarily in nonneural sites, such as the liver and plasma; neurotoxic esterase (also known as neuropathy target esterase) found primarily in the nervous system; and carboxylesterases (also known as aliesterases), which are relatively nonspecific enzymes found in many cells, including those of the nervous system and liver. That organophosphates can inhibit esterases other than neural acetylcholinesterase provides an opportunity to monitor exposure using red blood cell acetylcholinesterase and/or serum pseudocholinesterase as markers. Organophosphate-induced signs of toxicity generally only occur after significant inhibition of neural acetylcholinesterase or significant inhibition and aging of neurotoxic esterase; inhibition of pseudocholinesterases or carboxylesterases causes no apparent clinical signs. The organophosphates that inhibit neurotoxic esterase do not include commonly used insecticides; toxicity that follows inhibition of this enzyme differs considerably from that caused by inhibition of acetylcholinesterase. Weeks after exposure, humans and certain species of animals develop progressive degenerative changes that can be seen on microscopic examination of peripheral nerves and/or the spinal cord. This organophosphate-induced delayed neuropathy can result in incoordination, ataxia, and paralysis. Specific treatments for this disorder have not been developed.

The capability of organophosphates to inhibit pseudocholinesterases and carboxylesterases without causing clinical signs provides a mechanism by which serial exposures to nontoxic dosages can result in toxicity. These enzymes provide sites additional to those on acetylcholinesterase at which organophosphates can attach, but once inhibited, they may not be available when humans or animals are exposed to organophosphates for a second time. Thus, more organophosphate is available to attach to acetylcholinesterase, resulting in toxicity at dosages that would not be toxic without prior exposure. Instead of the potentiation of toxicity seen with subsequent dosing as described previously, however, multiple administrations of low dosages of organophosphates over a sufficient period of time may result in tolerance as receptors for acetylcholine become desensitized or downregulated. Metabolism of a compound may also increase with repeated exposure.

Due to their widespread use, especially as insecticides, exposure to organophosphates may be intentional, accidental, or environmental. Once exposure occurs, regardless whether the route is dermal, oral, or by inhalation, absorption is likely because organophosphates have considerable lipid solubility. This property also increases the potential that they will be generally distributed throughout the body, and will easily pass into the nervous system, where they exert their toxic effects. Many organophosphates, especially the insecticides, enter the body as S-containing phosphorothionate protoxicants. These protoxicants are readily activated to esterase inhibitors by mixed function oxidase enzymes of the liver. Organophosphates are metabolized by a variety of esterases, including those that they may inhibit (B-esterases such as pseudocholinesterases and carboxylesterases) and those that they do not inhibit (arylesterases or A-esterases, also known as organophosphorus acid anhydrases, phosphohydrolases, or phosphorotriesterase hydrolases); metabolites may be excreted in urine, feces, and milk. Organophosphates are readily hydrolyzed in aqueous solutions with high pH; therefore, soapy water is useful for decontamination of skin and clothing.

Although the primary mechanism of toxicity associated with exposure to organophosphates has to do with inhibition of acetylcholinesterase, signs can still occur after the cholinergic crisis has resolved. Such signs include the delayed neuropathy that can occur weeks after inhibition of neurotoxic esterase, an intermediate paralytic syndrome that may occur several days after severe acetylcholinesterase inhibition, muscle damage that may begin during the cholinergic crisis and which may develop into a myopathy that can reverse within weeks of exposure, and cardiotoxicities that may be part of or occur after the acute syndrome. Residual neurobehavioral effects may remain following recovery

from significant acetylcholinesterase inhibition. In addition, there have been reports of effects that may be a consequence of repeated low-dose exposures at doses that are insufficient to cause clinical evidence of acetylcholinesterase inhibition in humans. The effects that have been reported to appear include anxiety, confusion, impairment of judgment, visual disturbances, behavioral changes, memory deficits, and incoordination. Certain organophosphates have also been reported to have immunotoxic or teratogenic effects.

Species differences in susceptibility to organophosphate toxicities are notable. For example, significant neurotoxic effects that remain after recovery of acetylcholinesterase activity have not been reported for studies performed in animals, but a number of reports suggest that a variety of long-lasting behavioral and functional changes could occur in some humans. Species differences in clinical manifestations of organophosphate-induced delayed neuropathy are also notable. Although locomotor difficulties occur in humans, hens, cats, sheep, cattle, and a variety of other species, they are not obvious in the rodent species commonly used for toxicity testing (rats and mice). Other species differences may be related to the pharmacokinetics (absorption, distribution, and clearance) of organophosphates. For example, insects, due to their small size and to their chitin exoskeleton (which does not provide an impediment to organophosphate insecticide absorption), are much more likely to succumb to organophosphate toxicity than are other animal species. Absorption also contributes to species differences among mammals. Cats are more likely to absorb organophosphates after exposure by the dermal route, and the propensity of this species to groom also increases the likelihood of oral exposure even when the original exposure was by the dermal route. Metabolic differences among species are a significant factor associated with species differences. Avians, for example, are more susceptible to organophosphate toxicity because they have less capability to hydrolyze these chemicals by enzymes that are not inhibited by the organophosphates.

Pesticide assessment guidelines under the Federal Insecticide, Fungicide, and Rodenticide Act stipulate that organophosphates proposed for use as insecticides be tested both for their capability to cause acute toxicities due to inhibition of acetylcholinesterase and for their potential to cause inhibition of neurotoxic esterase and subsequent delayed neuropathy. Testing could be performed in laboratory rodents because they, like all species, are susceptible to acetylcholinesterase inhibition, but rodents do not develop notable ataxia, and neuropathological manifestations are very restricted if these species are exposed to organophosphates that cause delayed neuropathy. Testing for the toxicity of organophosphates includes, therefore, adult hens as the animal model for organophosphate-induced delayed neuropathy. Inhibition of neurotoxic esterase, ataxia, and neuropathy are detectable in hens, and the relationship between dosages causing acetylcholinesterase inhibition and delayed neuropathy can be determined.

See also: Anticholinergics; Behavioral Toxicology; Carbamate Pesticides; Carboxylesterases; Cholinesterase Inhibition; Federal Insecticide, Fungicide, and Rodenticide Act, US; Nerve Agents; Neurotoxicity; Organophosphate Poisoning, Delayed Neurotoxicity; Organophosphate Poisoning, Intermediate Syndrome; Psychological Indices of Toxicity.

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Organotins*

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The organotins comprise a large class of organometallic compounds containing one or more tin atoms bound covalently to one or more carbon-containing moieties (R groups), which can be either alkyl or aryl groups. Because tin may assume either a $+2$ or $+4$ valence state, up to four R groups may replace inorganic anions, yielding mono-, di-, tri-, and tetra-substituted organotin compounds. As of 1982, 259 organotin compounds were described in the *CRC Handbook of Chemistry and Physics*, a number that far exceeds the number of organically substituted forms of any other metal, including mercury, lead, arsenic, and germanium. This large number of organotin compounds attests to their broad utility as industrial catalysts and stabilizers, as preservatives in construction material, and as agricultural and marine pesticides. More than 1000 patents have been granted for organotin stabilizer formulations alone, and annual world production has reached nearly 50 000 metric tons. Major uses of organotins include stabilization of polyvinyl chloride (PVC) plastics during heated polymerization and catalysis of the polymerization of urethane foams; a variety of monomethyl, dimethyl, dibutyl, and dioctyl tin derivatives are used for these purposes. Pesticidal usage relies primarily on trialkyl and triaryl tins, which provide effective rodent repellents, fungicides, insecticides, and molluscicides; bis(tri-*n*-butyl tin) oxide (TBTO) is used as a preservative for wood, paper, leather, and textiles.

Exposure to organotins may occur occupationally, during manufacturing processes, or during application of biocidal agents containing the compounds. The general public may also receive exposure from this latter route (e.g., during spray application of paints and wood preservatives). In addition, significant leaching of tin stabilizers from plastic packaging into foodstuffs has been demonstrated; thus, the US Food and Drug Administration closely regulates the use of organotins in the manufacture of these materials. Plastic tubing used for medical procedures may also contain organotin stabilizers, as well as PVC piping used in domestic water systems, leachates of which may gain entry directly into the body. Finally, inorganic tin may be biomethylated by microflora in marine and estuarine sediments, leading to human exposure via consumption of seafood as well.

In contrast to the low toxicity of inorganic tin salts, the toxic properties of organotins have been recognized since their discovery in the latter part of the nineteenth century. Investigation of the mammalian toxicology of organotins was spurred by several case reports of severe reactions to inhalation of trimethyltin vapors, and by the 'Stalinon' disaster in France in 1954. In this latter episode, preparations of diethyltin diiodide, designed for the treatment of skin disorders, were contaminated with triethyltin iodide. More than 100 people died and a similar number were disabled after taking this medication.

Subsequent studies of the toxicity of organotins have provided several generalizations based on the chemical characteristics of the compounds. First, organotins primarily affect the skin, immune and nervous systems; the liver and kidneys are less sensitive. Recent evidence indicates potent endocrine-disrupting effects in both invertebrates and mammals. Second, the toxicity of organotins tends to increase with the number of organic ligands. For example, trialkyl forms appear to be the most toxic of the alkyl tins, and tetraalkyl tins are metabolically dealkylated to trialkyl forms. Third, toxicity tends to decrease with increasing size and complexity of the R group because of reduced penetration into target organs with increasing chain length, and because tetraalkyl compounds with large alkyl groups are less readily dealkylated than those with small alkyl groups. For example, dioctyltin and triphenyltin are poorly absorbed from the mammalian gut, whereas trimethyltin and triethyltin are absorbed with high efficiency. Dioctyltin dichloride (DOTC) appears to be an exception, in that it causes a severe yet reversible immunotoxicity in rats, but not in mice, guinea pigs, quail, or chickens. Further, these effects are not observed when the carbon chain extends beyond eight, suggesting that the observed immunotoxicity may be specific to DOTC in rats. Fourth, the anionic component of the organotin contributes to the toxicity of the compound by affecting its volatility and solubility in water; those compounds more volatile and soluble will naturally gain access to target organs in preference to those less so. Fifth, among the highly toxic trialkyltins, the primary target of toxicity appears to be the nervous system for trimethyltin and triethyltin, whereas the immune, reproductive, renal, and hepatic systems are more sensitive to tripropyltin

*This manuscript has been reviewed by the National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

and tributyltin. The only aryltin with documented toxicity is triphenyltin, whose actions resemble those of tributyltin.

Mechanism of Toxicity

Organotin compounds exert a number of cellular, biochemical, and molecular effects, and toxicity is primarily influenced by bioavailability of the toxicant, route of exposure, and the level and time course of exposure. Both di- and trialkyltin compounds are potent inhibitors of mitochondrial oxidative phosphorylation. Tetra-substituted organotins have no direct selective action on mitochondria, but are converted *in vivo* to toxic tri-substituted species. Perturbation of calcium homeostasis has also been shown to be involved in the cytotoxic action of tributyltin. The effects of elevated calcium concentrations include thymocyte killing and stimulation of apoptosis. Trimethyltin, triphenyltin, and dibutyltin are less potent in altering calcium homeostasis in thymocytes; however, increased free intracellular Ca^{2+} in the neuron synapses appear to be involved in the neurotoxicity of both trimethyltin and triethyltin. Tri-substituted organotins also interact with various intracellular enzymes that may result in toxicity, mainly cytochrome P450-dependent monooxygenases, which serve an important role in detoxifying xenobiotics. Tributyltin and triphenyltin exert selective effects upon different components of this enzyme system, and significant species differences occur. Lastly, the reproductive effects of tributyltin appear to involve another P450-dependent system: inhibition of aromatase, an enzyme responsible for the conversion of testosterone to estradiol, has been implicated as the primary pathway by which the aromatase inhibitors tributyltin and triphenyltin cause endocrine disruption. Moreover, this mechanism seems to be conserved across a wide phylogenetic range, as tributyltin and triphenyltin have been shown to inhibit cytochrome enzymes in gastropods, fish, rats, and human tissue.

Effects on Humans and Experimental Mammals

All of the common organotins with low molecular weight are potent irritants to the skin and mucous membranes, and even brief contact can cause severe chemical burns and dermatitis. Dermal effects represent the most immediate hazard for workers handling these compounds, though these effects are reversible over time. Despite high acute toxicity, no organotins appear to be carcinogenic. Indeed, some evidence exists for a potential therapeutic role for organotin compounds in the diagnosis and treatment of tumors.

Next to skin, the immune system appears to be the organ system most sensitive to di- and tri-substituted organotins in mammals. Toxic effects have been reported in thymus, lymph, and spleen, with the thymus being the most sensitive target. For example, TBTO in the diet of rats has been shown to cause atrophy of the thymus and lymphoid organs, to deplete stores of iron in the spleen, to reduce hormonal activity of the pituitary–thyroid axis, and, of greater biological consequence, to decrease resistance to bacterial and parasitic infections. Many of these effects occur at lower doses of tributyltin and dioctyltin in neonatal rats than in adults. In adult rats, thymic atrophy also follows from dietary exposure to diphenyltin and triphenyltin, and to dialkyltins whose toxicity decreases with increasing chain length. Mice appear to be far less sensitive than rats and other species to the thymolytic effects of ingested organotins. This resistance is likely due to differences in the uptake and elimination of the compounds, because it is not observed after systemic injections of dialkyltins. The reversibility of the immunotoxic effects of organotins has not been well studied; existing data indicate that thymic atrophy after oral exposure to dibutyltin and dioctyltin recovers after termination of exposure, though more slowly in rats dosed perinatally than in adult rats.

The neurotoxicity of trialkyltins has been well studied, using primarily trimethyltin (TMT) and triethyltin (TET). These highly toxic compounds are not used commercially, but have proven to be useful tools for the study of the nervous system and its response to organotins. TMT is readily absorbed by any route and readily penetrates the central nervous system (CNS), where it destroys neurons, and for unknown reasons targets large pyramidal neurons. Damage is most prominent in the hippocampus but can also be detected in many regions of the CNS and spinal cord. Because the affected cells die, many functional changes caused by TMT (including emotional disorders, cognitive dysfunction, and hearing loss) are persistent. TET is also neurotoxic; however, its primary target cells are the neuroglia, which generate the myelin sheath surrounding the axons of large neurons in the CNS. Thus, TET toxicity is characterized by cerebral edema and demyelination instead of neuronal cell loss. Because these effects are slowly reversible, recovery after TET intoxication is more likely than after TMT intoxication. In addition to their CNS effects, both TMT and TET have been shown to produce peripheral neuron degeneration and central chromatolysis of specific neuron groups.

Hepatic effects of alkyltins involve injury to the bile duct, hepatocellular necrosis, and changes in the activity of some enzyme systems. The effects of dibutyltin on biliary function appear to occur only in species with common hepatic and pancreatic bile ducts (e.g., rats, mice, and hamsters). Species with separate pancreatic and hepatic bile ducts, including rabbits, guinea pigs, hens, and cats are not similarly affected, suggesting that humans, who also have separate systems, would not be affected in this manner. Damage to hepatocytes appears to involve a combination of secondary effects, including bile duct injury and direct toxicity to the cells. Changes have also been reported in the activity of enzymes involved in heme synthesis and metabolism of xenobiotics, including both cytochromes and mixed-function oxidases.

Organotins also exert hematological effects, including microcytic anemia and inhibition of platelet aggregation. The anemia appears to involve both interference with the synthesis of hemoglobin, perhaps via inhibiting iron uptake, and direct hemolytic effects. The inhibition of platelet aggregation has been attributed to tin-induced loss of serotonin from the cells.

While not yet studied thoroughly, reproductive effects of organotins have also been reported. Tributyltin has been shown to cause preimplantation embryonic loss in female rats, and to reduce testis weight, spermatid and sperm counts in male rats. However, most of the studies of reproductive and endocrine effects of organotins have involved invertebrates studied in an ecological context.

Ecological Effects

Unlike TMT and TET, tributyltin (TBT) and triphenyltin (TPT) are used commercially and are among the most harmful pollutants in aquatic ecosystems. TBT gained widespread application as a biocide in antifouling paints on ships and in wood protection. TPT, although sometimes employed as a cotoxicant with TBT in antifouling preparations, is mainly applied as an agricultural fungicide, entering the aquatic environment through runoff and atmospheric deposition. Although regulation of TBT and TPT has resulted in decreased contamination, complete removal from the environment can occur only through biodegradation, photolysis by sunlight, sedimentation, flux, and biological uptake. Little is known about the exact mechanisms contributing to TBT and TPT degradation in the environment, but only a limited number of microbes are capable of degrading TBT. As such, the potential consequences of biological uptake are of global concern. The endocrine disrupting effects of TBT are most evident in marine organisms; shellfish develop ambiguous genitalia and display increased androgen levels and decreased estrogen levels. To date, over 100 gastropod species have been adversely affected by TBT, in addition to both marine and freshwater fish. TPT residues have also been reported in freshwater and marine gastropods and fish; however, the toxic action of TPT has not been fully delineated. In addition to the direct toxic effects of organotins, exposure to TBT has been shown to exacerbate effects of infectious diseases, with TBT-exposed oysters succumbing to parasitic infection at levels markedly below those causing mortality in an unexposed group. Thus, organotins, like other anthropogenic stressors, may contribute to disease outbreaks by creating populations of immunosuppressed hosts.

See also: Pesticides; Tin.

Acknowledgments

We thank Ginger Moser and Robert Luebke for constructive reviews of this chapter.

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Ototoxicity

Michael J Sullivan

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Introduction

Ototoxicity is defined as affects on the organs of the inner ear. Oto means ear. The organs of the inner ear supply two key functions to the body: hearing and balance. Chemicals that cause ototoxicity can affect the cochlea (hearing) or the vestibular system (balance). Ototoxicity of the cochlea or the Organ of Corti may also be termed cochleotoxicity. Ototoxicity of the vestibular system may also be termed vestibulotoxicity. However, generally the term ototoxicity is used in this field of study. Both of these functions may also be impaired by effects on the vestibulocochlear nerve, the nerve sending balance/hearing information from the inner ear to the brain. Effects on either hearing or balance can be caused by effects on the brains itself. Effects on nerves or the brain would be classified as neurotoxic.

Figure 1 shows both a schematic of the inner ear (both cochlear and vestibular organs) and a histology cross section. Several turns of the cochlea can be seen rising from the middle ear in turns of decreasing diameter. Within the cochlea is the Organ of Corti with the functional cellular units of hearing, the hair cells. Damage to and permanent loss of hair cells by either chemicals or noise results in permanent hearing loss.

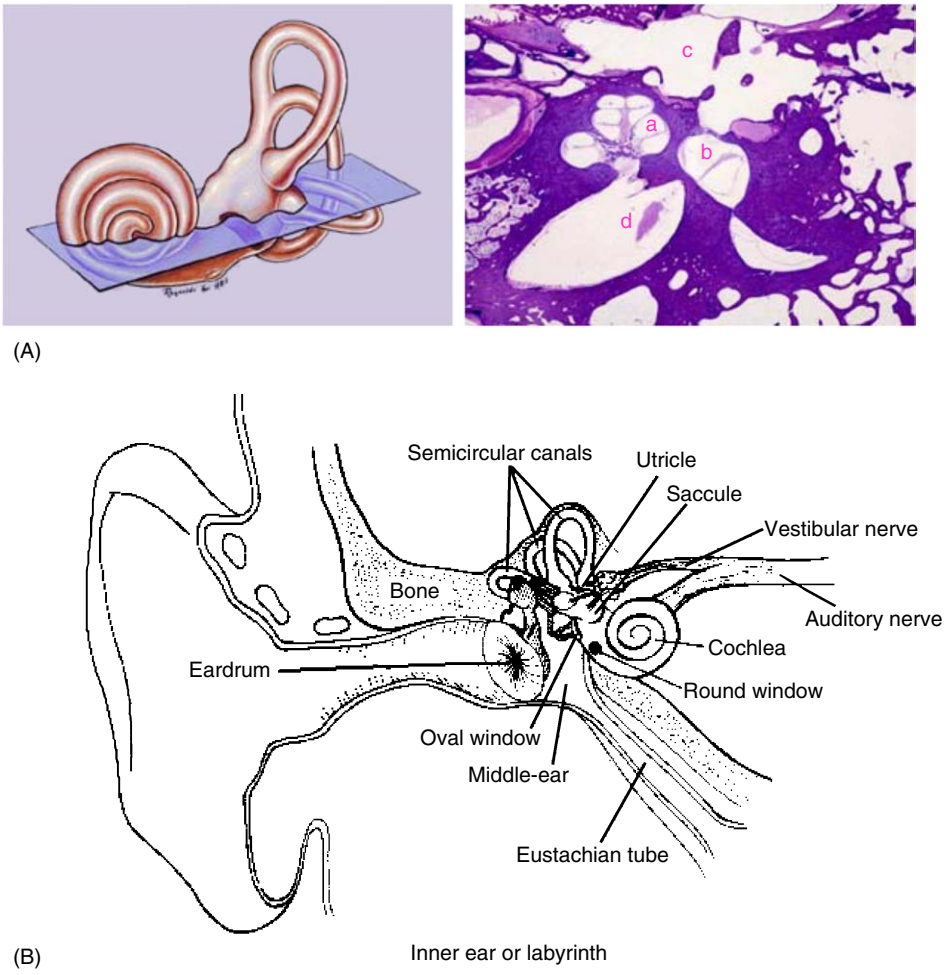

Figure 1 (A) Drawing of the inner ear to illustrate the plane of the microscopic section shown in (B). (B) Horizontal slice of the inner ear prepared for microscopic study: a, cochlea; b, vestibule (balance organs); c, middle ear space; d, portion of the hearing nerve in the internal auditory canal.

Symptoms of Ototoxicity

Ototoxicity may be manifest in many ways depending on the agent involved. Ototoxic effects may be severe or mild, permanent or temporary, or affect hearing or balance or both. An understanding of how the particular ototoxic agent causes the effects would be needed before an effective testing regime could be developed.

Determining effects on hearing may be complicated. Early signs of hearing loss may be the symptom of tinnitus or ringing sound in the ears. This can be caused by both direct effects on the cochlea or the vestibulocochlear nerve. Hearing loss is often manifested by the loss of changes in sensitivity to specific frequencies. Some ototoxic agents affect high frequencies (e.g., aminoglycoside antibiotics) and others affect middle-frequencies (e.g., toluene). Noise, although a physical agent is also ototoxic and can affect those frequencies that are characteristic of the sound pattern.

Testing for Ototoxic Effects

Estimates of how many people suffer from ototoxicity are not available. This is because in humans the early signs of ototoxicity will often not be reported. The slight ringing in the ears may be thought to be temporary or not noticeable. The initial loss of sensitivity to certain sound frequencies may be so slight that it would not be reported to a physician. The symptoms of high frequency hearing loss or loss of balance can also be incorrectly attributed to aging. For these reasons, ototoxicity can often remain undiagnosed.

In some cases, the ototoxicity may be manifest as a sudden onset of tinnitus or significant hearing loss. These symptoms should immediately be reported to a physician because both medicinal and industrial chemicals can be the cause. It would be advisable to complete a hearing test to determine a hearing baseline for that individual. A regular hearing monitoring program could be used to determine if hearing is worsening. If the patient is under a treatment regime that includes ototoxic agents, the use of these agents may need to be terminated before permanent hearing loss results.

Ototoxicity of the vestibular system will also be overlooked in patients. The loss of balance may manifest itself either sporadically (e.g., only a certain movement axis affected) or the symptoms my be mild and worsening slowly. There are tests available to determine if the vestibular system has been affected. These include both physical tests (e.g., balance tests) and electrosensory tests.

Testing regimes in animals are available to evaluate the potential ototoxic effects of chemicals. These tests range from the gross testing of animal reaction/reflexes to sudden noise (even different frequencies) to the more sensitive electrosensory tests. In treated animals, the cochlea may be harvested at the time of necropsy and the Organ of Corti examined for evidence of damage.

Damage to Tissues Caused by Ototoxic Agents

As mentioned previously, the effects of ototoxic agents can be on the cochlea or vestibular organs directly or on the vestibulocochlear nerve. Each of these types of damage will manifest itself in changes in hearing or balance. It is often these functional changes that are noticed and measured. However, when the effect of the ototoxic agent is directly on the cochlea or vestibular organs, there are pathological changes that can be found. Just like the harvesting and processing of other tissues, for example, liver, the organs of the inner ear can be harvested and examined microscopically.

In the cochlea, it is the Organ of Corti that is examined. Like some other organs, the Organ of Corti has a three-dimensional structure that is related to function. This organ, contained within the cochlea (named because of the spiraling shell shape) spirals from the oval window to the apex in turns of decreasing diameter. It is the distance from the oval window that determines the specific hearing function, that is, the frequency of sound detected. High-frequency sound with short wavelengths is detected by portions of the Organ of Corti close to the oval window. Low-frequency sound with long wavelengths is detected by portions of the Organ of Corti most distant from the oval window. The pathology of where the damage has occurred in the cochlea should match the functional hearing loss observed.

The functional unit in both organs of the inner ear is the hair cell. They are composed of long cylindrical cells that have hair-like features at one end. When physical energy in either the cochlea (caused by sound) or in the vestibular organ (caused by movement) causes the hair to bend, an electrical signal is sent to the brain that is interpreted as either hearing or movement. These hair cells are the target for some ototoxic agent. When hair cells die they are not replaced and function is lost. Maps of the cochlear hair cells, termed cytocochleograms, can be constructed to show where hair cells damage has occurred.

Agents that Cause Ototoxicity

Many classes of agents can cause ototoxicity. These classes include:

- physical,
- antibiotics,
- diuretics,
- industrial solvents/environmental chemicals, and
- chemotherapy for cancer.

Table 1 lists many of the agents that cause ototoxicity.

When new drugs or chemicals are being introduced, the battery of testing generally does not include testing for either form of ototoxicity. In some cases if there is a structural similarity between an existing ototoxic agent and the drug/chemical being tested, ototoxic testing may be requested. Generally, drugs and chemicals are only found to be ototoxic after a sufficient amount of use or exposure has accumulated in the population and these effects begin to be reported. This, of course, can take years before the extent of ototoxicity is known.

It is also possible that there can be exposure to multiple ototoxic agents simultaneously. For example, multiple antibiotics can be prescribed and the ototoxic effects could be additive. A patient may be on diuretics and receiving chemotherapy or antibiotic therapy. In these cases is has been observed that the effects of these two agents is synergistic, that is, the effects of the combined treatment is greater than the expected effect of each agent individually. Exposure of workers to industrial chemicals can also occur in a noisy occupational environment. Studies of the interaction of ototoxic solvents and noise have shown additive effects related to both functional changes and hair cell loss.

Table 1 List of ototoxic agents

Physical
Noise
Antibiotics
Streptomycin
Gentamicin
Tobramycin
Netilmycin
Neomycin
Erythromycin
Kanamycin
Vancomycin
Diuretics
Acetazolamide
Bumetanide
Furosemide
Ethacrynic acid
Industrial solvents and environmental chemicals
Butyl nitrite
Carbon disulfide
Lead
Mercury
Tin
Trichloroethylene
Carbon monoxide
Hexane
Manganese
Styrene
Toluene
Xylene
Chemotherapy for cancer
Cisplatin
Vincristine
Other
Aspirin

Reversibility of Ototoxicity

The reversibility of ototoxic effects is dependent on the dose, the duration, and the ototoxic agent. For aspirin, which can cause tinnitus, the effects can be transient and end soon after exposure ends. For the aminoglycoside antibiotics gentamicin, kanamycin, netilmycin, and tobramycin, the severity of hearing loss varies. With these agents hearing loss begins at the higher frequencies and with sufficient dosing cochlear hair loss is significant and hearing loss at selected frequencies permanent. Even when dosing is done carefully to balance the beneficial antibiotic effects and the harmful ototoxic effects it is estimated the chances of hearing recovery are low and thought to be $\sim$10%. The onset of hearing loss with diuretics is usually immediate and if symptoms are noticed and exposure stopped, the effects are reversible. The industrial solvent toluene has been found to cause hair cell loss in the middle frequency range. With only mild hair cell loss any functional loss of hearing would not be noticed. However, higher exposures leading to significant and permanent loss of hair cells (which are not replaced) could lead to permanent middle-range hearing loss.

See also: Acetylsalicylic Acid; Butyl Nitrite; Cancer Chemotherapeutic Agents; Carbon Disulfide; Cisplatin; Lead; Mercury; Tin; Trichloroethylene.

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Otto Fuel II

Richard D Phillips

- Chemical Abstracts Service Registry Number: CAS 106602-80-6
- Chemical Formulas: A mixture of propylene glycol dinitrate, $C_3H_6N_2O_6$; 2-Nitro-diphenylamine, $C_{12}H_{10}N_2O_2$; and Dibutyl sebacate, $C_{18}H_{34}O_4$

Uses

Otto Fuel II is a distinct-smelling, reddish-orange, oily liquid that the US Navy uses as a fuel for torpedo and other weapons systems. It is a mixture of three synthetic substances: mostly propylene glycol dinitrate (~75%), dibutyl sebacate (~23%), and 2-nitrodiphenylamine (~2%).

Propylene glycol dinitrate is the explosive part of Otto Fuel II. It is a colorless liquid with an unpleasant odor. Other names for propylene glycol dinitrate are PGDN, 1,2-propylene glycol dinitrate, and 1,2-propanediol dinitrate.

Dibutyl sebacate is a clear liquid. It is most often used for making plastics, many of which are used for packaging food. It is also used to enhance flavor in foods such as ice cream, candy, baked goods, and nonalcoholic drinks. Some consumer products, such as shaving creams, also contain dibutyl sebacate. Other names for dibutyl sebacate are decanedioic acid, dibutyl ester, sebacic acid, dibutyl ester, and dibutyl decanedioate.

2-Nitrodiphenylamine is a solid. Otto Fuel II contains 2-nitrodiphenylamine to control the explosion of propylene glycol dinitrate. It is also used as a solvent dye. Other names for 2-nitrodiphenylamine are 2-nitrobenzamine, 2-nitro-*N*-phenyl, 2-nitro-*N*-phenylaniline, and Sudan Yellow 1339.

Exposure Routes and Pathways

Exposure to Otto Fuel II may occur by the inhalation, oral, or dermal routes. Inhalation exposures to Otto Fuel II would consist primarily of inhalation exposure to propylene glycol dinitrate. Oral exposure to Otto Fuel II is possible through consumption of contaminated water. It is likely that significant ingestion of dibutyl sebacate may occur as a result of its civilian use in food packaging materials and as a flavor enhancer in ice cream, candy, baked goods, and nonalcoholic beverages. Dermal exposure to Otto Fuel II and its components is likely through contact with the fuel. Limited information was located regarding the degradation of the components in the environment, but the available data indicate that degradation would occur fairly rapidly (i.e., within days).

Humans are most likely to be exposed to Otto Fuel II or its components in areas where it is used as a torpedo fuel or where it is manufactured.

Toxicokinetics

There is evidence that the only volatile compound of Otto Fuel II, propylene glycol dinitrate, is absorbed following exposure. Also, there is evidence for dermal or oral absorption from toxicity studies but no definitive toxicokinetic work has been done.

The predominant metabolite of propylene glycol dinitrate was nitrate with propylene glycol 2-mononitrate. Information regarding the metabolism of the other two components is not available.

Animals given propylene glycol dinitrate subcutaneously rapidly excreted unmetabolized propylene glycol dinitrate and its metabolites in urine. Inorganic nitrate was the major metabolite excreted in urine, accounting for ~56% of the nitrate in the injected dose.

Mechanism of Toxicity

The mechanism of toxicity for Otto Fuel II is related to its major component, propylene glycol dinitrate. Propylene glycol dinitrate is an organic nitrate and shares many of the cardiovascular properties of therapeutic nitrates such as nitroglycerin for its vasodilating capacity. One of the earliest consequences of overexposure to propylene glycol dinitrate (or to Otto Fuel II) is a vasodilation of the cerebral vessels,

which is believed to be the major factor in the development of the typical 'trinitrotoluene' headache. Should the overexposure be more severe, the relaxation of the vascular smooth muscle can result in a fall in blood pressure followed by a compensatory vasoconstriction. However, a decrease in the magnitude of the vasodilating effect has been observed after repeated exposure to organic nitrates. Although the exact mechanism of initiation and maintenance of tolerance to organic nitrates is not known, several possibilities have been suggested, including depletion of sulfhydryl groups at the receptor sites, reduced availability or activity of the active intermediate *S*-nitrosothiol, and altered pharmacokinetics leading to decreased nitrate concentration in vascular tissues. Massive overexposure to propylene glycol dinitrate can produce toxic levels of methemoglobin. This property is shared by many organic and inorganic nitrates and also by aromatic amines, 2-nitrodiphenylamine among them.

Acute and Short-Term Toxicity (or Exposure)

Animal

An oral LD_{50} value of 2000 mg kg^{-1} in rats has been reported for Otto Fuel II. The oral LD_{50}s for the components of Otto Fuel II indicate relatively low toxicity. Oral LD_{50} values ranging from 250 to $\sim$2000 mg kg^{-1} have been reported for propylene glycol dinitrate in rats.

Exposure to propylene glycol dinitrate at concentrations up to 200 ppm for 4 h was tolerated in rats without signs of toxicity. Rats, guinea pigs, and dogs exposed continuously for 90 days at 35 ppm showed no treatment-related deaths.

Elevated methemoglobin levels were observed in a number of acute-, intermediate-, and chronic-duration exposure studies in laboratory animals exposed to propylene glycol dinitrate.

Continuous (24 h day^{-1}) exposure of rats, guinea pigs, dogs, and monkeys to propylene glycol dinitrate for 90 days resulted in elevated methemoglobin levels during exposure and histopathologic evidence of hemolysis in all four species. At concentrations as low as 10 ppm, hemosiderin deposits (indicating phagocytosis of oxidized hemoglobin released from hemolyzed red cells) were observed in kidneys and livers from dogs and in kidneys from some rats. At 16 ppm, hemosiderin deposits were observed in the liver of dogs and monkeys; at 35 ppm, in addition to the liver and kidneys, heavy hemosiderin deposits were observed in the spleens of these animals. At 35 ppm, all four species exhibited elevated methemoglobin levels.

Degenerative changes in the liver and kidneys were also observed in rats, guinea pigs, dogs, and monkeys exposed to propylene glycol dinitrate for 90 days.

Although no information was located regarding reproductive performance of Otto Fuel II or its individual components, limited data are available regarding effects of propylene glycol dinitrate on the gross and microscopic structure of reproductive organs and/or revealed no treatment-related effects.

Human

Exposure to Otto Fuel II is likely to cause eye and respiratory irritation in humans exposed to significant levels. In addition, headaches of presumed vascular origin are possible based on experience by torpedo maintenance workers. In experimental exposure to volunteers to propylene glycol dinitrate vapor, headaches were reported by some subjects at concentrations of 0.2 ppm for up to 8 h.

Studies designed to assess the neurological effects of Otto Fuel II and its component, propylene glycol dinitrate, in humans have indicated that an alteration of central nervous system activity may result from occupational exposures. In one study, workers were given tests of balance and oculomotor performance before and after a torpedo maintenance procedure. The maintenance procedures were $\sim$30–60 min in duration, and propylene glycol dinitrate concentrations measured in the work area ranged from 0 to 0.22 ppm. Subjects exposed to 0.2 ppm propylene glycol dinitrate for 1–8 h were observed to have altered visual evoked responses. With repeated 7.5–8 h exposures to 0.2 ppm, the change in the visual evoked response was observed to increase in magnitude indicating a cumulative effect. Exposure to 0.5 ppm for 8 h resulted in nausea, dizziness, and more markedly altered visual evoked responses. At the highest concentration tested, 1.5 ppm, subjects experienced coordination deficits and altered visual evoked responses and coordination.

Chronic Toxicity (or Exposure)

Animal

No chronic studies on Otto Fuel II are available. There is insufficient information to conclude whether or not Otto Fuel II or its components are carcinogenic.

In Vitro Toxicity Data

The US Navy concluded that Otto Fuel II assayed at toxic levels did not cause a significant increase in the

frequency of sister chromatid exchange in mouse lymphoma cells in the presence or absence of rat liver microsomes (S9). However, in the mouse lymphoma cell forward mutation assay, increased mutation frequencies and the number of mutant colonies were observed, indicating that at severely cytotoxic levels, the US Navy concluded that Otto Fuel II was mutagenic in the mammalian cell line. Otto Fuel II was not mutagenic in *Saccharomyces cerevisiae* D4 and several histidine-requiring mutant strains of *Salmonella typhimurium*.

Clinical Management

Based on currently available information, the constituent of Otto Fuel II that presents the main health concern is propylene glycol dinitrate. Exposure to propylene glycol dinitrate occurs primarily by inhalation or through dermal absorption. In an acute exposure situation, general recommendations include removing the exposed person from the source of exposure. Dermal absorption may be reduced by removing contaminated clothing, blotting any excess liquid material on the skin with an absorbent material, and washing the skin with copious amounts of water and mild soap. Contaminated eyes should be flushed with water or normal saline. If ingestion QJ;of Otto Fuel II or propylene glycol dinitrate has occurred, absorption from the gastrointestinal tract may be limited by administering water or milk for dilution and activated charcoal to adsorb the material.

Environmental Fate

The limited data located on the environmental fate of Otto Fuel II components indicate that propylene glycol dinitrate is removed from water primarily by volatilization. Neither 2-nitrodiphenylamine nor dibutyl sebacate is volatile or soluble enough for the partitioning to air or water to be important fate processes. The data on biodegradation of propylene glycol dinitrate and 2-nitrodiphenylamine are mixed. Some experiments indicate these compounds are readily degraded and others indicate limited biodegradation. A bioconcentration factor has been calculated only for 2-nitrodiphenylamine. It indicates that this chemical does not bioconcentrate in aquatic organisms or biomagnify in the food chain.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Otto Fuel II and Its Components.

Oxalates

Eric M Silberhorn

- Representative Chemicals: Oxalic acid; Calcium oxalate; Sodium oxalate
- Chemical Abstracts Service Registry Number: Oxalic acid (CAS 144-62-7)
- Synonyms: Ethanedioic acid; Ethane-1,2-dioic acid
- Chemical Formula: $C_2H_2O_4$

Uses

Oxalic acid is used in the manufacture of dyes, inks, bleaches, paint removers, varnishes, wood and metal cleaners, dextrin, cream of tartar, celluloid, tartaric acid, purified methyl alcohol, glycerol, and stable hydrogen cyanide. It is used in the following industries: photographic, ceramic, metallurgic, rubber, leather, engraving, pharmaceutical, paper, and lithographic.

Background Information

Oxalates, typically in the form of calcium oxalate, are present in a variety of common poisonous plants including caladium (*Caladium* spp.), dumbcane (*Dieffenbachia* spp.), elephant's ear (*Colocasia* spp.), Jack-in-the-pulpit (*Arisaema triphyllum*), *Philodendrum*, pothos (*Scindapsus aureus*), rhubarb (*Rheum rhaponticum*), sorrel (*Rumex crispus*), and skunk cabbage (*Symplocarpus foetidus*). It occurs in plants in a partly insoluble form as acid oxalate and free oxalic acid, and in a partly insoluble form as calcium oxalate. When present, oxalates usually occur in all parts of the plant, although in rhubarb they are found primarily in the leaves and much lower in the stalks, which are therefore edible. Cooking does not make rhubarb leaves edible.

Oxalic acid and its salts (i.e., calcium, sodium) are a product of normal human metabolism. Calcium oxalate is the major constituent of kidney stones. Oxalates may also be produced by several common molds. For example, *Penicillium* and *Aspergillus*

molds can convert sugar into calcium oxalate at very high yields.

Exposure Routes and Pathways

Exposure to oxalates may occur through consumption of certain plants and foods in which they are naturally present (see above). Exposure may also occur through contact with, or inhalation of, commercial products (e.g., bleaches, cleaners) or as a result of accidental ingestion or contact with some commercial antifreeze products that contain ethylene glycol, which is metabolized *in vivo* to oxalates.

Toxicokinetics

Oxalic acid is poorly absorbed with a bioavailability of 2–5%. It is excreted unchanged in the urine. Normal urinary oxalic acid excretion ranges from 8 to 40 mg day^{-1}.

The soluble oxalates found in rhubarb and most other poisonous plants are readily absorbed from the gastrointestinal tract and lead to systemic formation of calcium oxalate.

Oxalic acid, and ultimately calcium oxalate, may also be formed *in vivo* as a result of the normal metabolism and biotransformation of ethylene glycol and several other compounds (e.g., ascorbic acid, glycerol, xylitol, glycolaldehyde, glycolic acid, glycoxylic acid).

Mechanism of Toxicity

Oxalic acid may have a direct corrosive effect on the eyes, skin, and digestive tract after contact. However, once absorbed (or produced as a result of the metabolism of other compounds), oxalic acid and other soluble oxalates react with calcium in the plasma to form insoluble calcium oxalate. Systemic formation of calcium oxalate may produce hypocalcemia directly. Precipitation of calcium oxalate in the renal system (proximal tubules of the kidney) may lead to local necrosis of the tubular epithelium, producing kidney dysfunction and electrolyte imbalance. Precipitation of calcium oxalate may also occur in the blood vessels, heart, lungs, and liver leading to local effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxic dose of oxalic acid to the dog and cat is ~1 and 0.2 g, respectively.

Human

The oral lethal dose of oxalic acid for adults is 15–30 g, although the ingestion of as little as 5 g has caused death.

Oxalic acid exposure typically produces immediate irritation and local effects on the skin, eyes, and mucosal membranes of the gastrointestinal tract (if ingested) or respiratory tract (if inhaled). Slightly delayed effects may occur on the respiratory system and kidneys.

Early symptoms of rhubarb and other plant poisonings may include mucosal irritation of the gastrointestinal tract, including signs such as sore throat, nausea, vomiting, anorexia, diarrhea, and abdominal pain; however, these symptoms are not always present in rhubarb poisoning. Kidney dysfunction and electrolyte imbalance occur subsequently and may be severe enough to cause death. In severe cases, anuria, oliguria, proteinuria, hematuria, and oxaluria are present. Hypocalcemia may produce paresthesias, tetany, hyper-reflexia, muscle twitches, and muscle cramps.

Chronic Toxicity (or Exposure)

Animal

A reproduction and fertility assessment of oxalic acid in Swiss CD-1 mice has been conducted with administration through drinking water (0.05%, 0.10%, and 0.20%; equivalent to ~89, 162, and 275 mg kg^{-1} day^{-1}). In a preliminary dose-range-finding study, water consumption was reduced in the middle and high dose groups by ~25%, but produced no adverse clinical signs in adults. In definitive studies, effects on reproductive parameters (e.g., live pups/litter, pup weight, abnormal sperm forms) were found at the 0.2% exposure level in both F_0 and F_1 mice, leading the investigators to conclude that oxalic acid is a reproductive toxicant at concentrations that reduce parental water consumption, but that cause few other somatic effects.

Human

Chronic occupational exposure to oxalic acid fumes has been associated with headache, vomiting, pain of the lower back, anemia, and fatigue. Chronic exposure may also lead to chronic inflammation of the upper respiratory tract and hypocalcemia. Prolonged contact of oxalic acid with the hands or feet may produce dermatitis, localized pain, cyanosis, and possibly gangrenous changes as a result of localized vascular damage.

In Vitro Toxicity Data

Sodium oxalate acts as a uremic toxin, inhibiting endothelial cell replication and migration *in vitro* at concentrations greater than $30\,\mu mol\,l^{-1}$. The inhibitory effect was fully reversible upon removal of oxalate, but only if exposure was limited to 5 days or less. In *Salmonella* mutagenicity testing using the standard National Toxicology Program (NTP) protocol, oxalic acid produced negative results.

Clinical Management

For exposure to oxalic acid, the exposed area should be decontaminated and tissue injury treated as for other strong acids. If on skin or in eyes, there should be thorough rinsing with water for at least 15 min. If ingested, emesis should not be initiated. Rather immediately there should be dilution with milk or water and calcium gluconate or lactate administered ($150\,mg\,kg^{-1}$ orally). For dilute oxalic acid ingestions, activated charcoal should be administered (adult: 60–100 g; child: 30–60 g). This may not be advisable in concentrated ingestions due to possible necessity for endoscopy. An electrocardiogram and serum calcium monitoring are required. Renal failure may require hemodialysis.

For ingestion of oxalates from plants, treat similarly as for oxalic acid.

Environmental Fate

Oxalic acid will readily degrade in aquatic ecosystems and is expected to also degrade in soil. Under typical environmental conditions (pH 5–9), oxalic acid will exist as the oxalate ion in soil and water (pK_{a1} and pK_{a2} values are 1.25 and 4.28, respectively).

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit, time-weighted average (PEL–TWA) and short-term exposure limit (STEL) for oxalic acid in air are 1 and $5\,mg\,m^{-3}$, respectively. The American Conference of Governmental Industrial Hygienists PEL–TWA and STEL for oxalic acid in air are also 1 and $5\,mg\,m^{-3}$, respectively. The National Institute for Occupational Safety and Health IDHL (immediate danger to life or health) is $500\,mg\,m^{-3}$.

See also: Ethylene Glycol; Kidney; Plants, Poisonous.

Further Reading

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Konta T, Yamaoka M, Tanida H, *et al.* (1998) Acute renal failure due to oxalate ingestion. *Internal Medicine* 37: 762–765.

Oxidative Stress

Kartik Shankar and Harihara M Mehendale

Cellular Events Leading to Oxidative Stress

Oxygen is critical to sustenance of life in all anaerobes and higher organisms. However, catalytic reactions involving oxygen can also result in the formation of several free radicals. Formation of the superoxide ($O_2^{\bullet}$) and the hydroxyl ($^{\bullet}OH$) radicals normally occurs during various biochemical processes, which are duly kept in check by cellular mechanisms that are designed to quench free radicals. However, certain toxicants cause a perturbation in this balance by either causing an increase in the formation of oxidative free radicals (the bipyridyl herbicide, diquat) or inhibition of the cellular antioxidant defense mechanisms (buthionine sulfoxime). Others like the well-known model hepatotoxin carbon tetrachloride, after being bioactivated to a reactive trichloromethyl free radical initiate a 'run-away' process of peroxidation of cellular membrane lipids.

Several enzyme systems are also responsible for producing oxygen free radicals. Several examples of reactions catalyzed by the P450 and flavin monooxygenases lead to the generation of reactive oxygen species (ROS). Microsomal P450s are capable of undergoing futile cycling in the absence of substrate to produce ROS. The cytochrome P450, CYP2E1 is notorious in this regard and has been described as a 'leaky' enzyme. CYP2E1, and from recent reports CYP4A enzymes are a major source of hydrogen peroxide and NADPH-dependent lipid peroxidation. Other enzyme systems also produce oxidative reactive intermediates: the cyclooxygeneases, nitric oxide synthases, and prostaglandin synthases.

Cellular Defenses against Oxidative Stress

Since several processes can potentially result in a prooxidant state detrimental to cellular homeostasis, cells have developed a wide range of antioxidant defense mechanisms to mitigate oxyradicals. Cellular glutathione is one of the most ubiquitous of these antioxidant mechanisms. Glutathione is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) that is present in varying concentrations (0.5–10 mM) in different cell types. Most of the cellular glutathione exists in the reduced form (GSH, ~95%) while less than 5% is present as oxidized gluthathione disulfide (GSSG). Four enzymes are critical in maintaining appropriate levels of GSH in the cell. Gammaglutamyl cysteine is the enzyme that transfers a cysteine moiety in the final and rate-controlling step of GSH biosynthesis. Formation of glutathione conjugates of electrophilic compounds is mediated by a glutathione-*S*-transferases, a superfamily of enzymes that are distributed in both the cytosol and microsomal compartments of cells. The reduced glutathione pool in the cell can be oxidized via glutathione peroxidase, an enzyme that releases two molecules of hydrogen peroxide in the process. Conversely, GSSG can be 'regenerated' to the reduced form by glutathione reductase using NADPH as the proton donor.

Superoxide dismutases are enzymes that catalyze the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. Superoxide dismutases either have Cu^{2+} and Zn^{2+} (cytosolic isoform) or Mn^{2+} (mitochondrial isoform). Reduction of hydrogen peroxides in cells is accomplished via catalases to water and oxygen. Cellular organelles called peroxisomes contain large quantities of catalase. In addition, NADPH:quinone oxidoreductases (DT-diaphorase) also act as antioxidant enzymes by catalyzing two-electron reduction of quinones.

Oxidative (Redox) Status as a Signaling Event

Since an extreme pro-oxidant status of a cell can lead to a progressive loss of cellular function and eventual death, cells have developed mechanisms to detect changes in the redox status of cells. These 'switches' are used to regulate and turn on the antioxidant machinery of the cell. These signals are largely mediated by redox-sensitive transcription factors. Although a large number of very extensively studied transcription factors are now know to respond to redox status, nuclear factor-kappaB (NF-κB) deserves special mention. NF-κB usually resides in the cytoplasm bound to an inhibitor protein, IκB. Changes in redox status of a cell (increase in hydrogen peroxide) will activate enzymes like MAPK kinases, which will phosphorlyate and hence cleave the inhibitory protein from NF-κB. The free NF-κB now translocates into the nucleus and binds to particular DNA sequences on specific genes called consensus sequences. Binding of NF-κB to response elements on genes alters the transcription of those genes. Several genes including other transcription factors, which may exert a similar transcriptional control over gene expression, are also induced or repressed; hence creating a cascade of signaling events. Several genes regulated by NF-κB are involved in upregulating antioxidant defense mechanisms, including catalase. NF-κB also exerts significant effects on other signaling events that decide cell proliferation and survival. It must be noted that NF-κB is only one of numerous transcription factors regulated by redox status. AP-1 and nrf2 are other notable transcription factors.

Endogenous and Therapeutic Antioxidants

Antioxidant compounds have a useful place in preventing or reducing peroxidative damage to cellular macromolecules either due to an offending xenobiotic or altered pathological state (diabetes). Several vitamins, both lipid and water soluble, possess antioxidant properties. Ascorbic acid (vitamin C) is a water-soluble compound capable of one-electron reduction of several free radicals. Vitamin E and other related tocopherols, on the other hand, are lipid soluble and effectively prevent peroxidation of polyunsaturated lipids present in membranes. Carotenoids such as β-carotene (vitamin A) inactivate singlet oxygen molecules. Other synthetic compounds such as promethazine, diethyldithiocarbamate, ubiquinol, butylated hydroxyanisole, *N*-acetylcysteine (NAC), among others have shown to protect against oxidative and reactive intermediate injury in experimental studies. Indeed, NAC is the standard treatment for liver injury in humans following toxic ingestion of acetaminophen. The mechanism of NAC action is thought to be a precursor for increasing GSH stores and prevents reactive intermediate induced damage. Recent studies also suggest that improving status via GSH may also improve prognosis via enhancing liver tissue repair.

See also: Ascorbic Acid; Cytochrome P-450; Diquat; Glutathione; Mechanisms of Toxicity.

Further Reading

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Robertson G, Leclercq I, and Farrell GC (2001) Nonalcoholic steatosis and steatohepatitis. II. Cytochrome P-450 enzymes and oxidative stress. *American Journal of Physiology. Gastrointestinal and Liver Physiology* 281: G1135–G1139.

Oxygen

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7782-44-7
- Synonym: LOX
- Chemical/Pharmaceutical/Other Class: Elemental gas
- Chemical Formula: O_2
- Chemical Structure: O═O

Uses

Oxygen produces flame for welding and lighting. It is also used in the production of fuels. Therapeutically, it is used to relieve hypoxia and as a component of the gas mixture for respiratory support.

Background Information

Oxygen is essential for life in appropriate ranges of pressure and concentration. Normal air consists of 20.04% oxygen.

Exposure Routes and Pathways

Exposure route is inhalation. Industrial exposures to high oxygen pressure are uncommon. Caison workers and tunnel makers may be exposed to pressures high enough to cause lung damage. Some potential risks for intoxication with oxygen also exist for drivers and persons living or working in closed compartments, where the air is reconditioned by the addition of pure oxygen (e.g., submarines and spacecraft), should the regulation system malfunction.

Toxicokinetics

Oxygen is absorbed almost entirely through the lungs, but may be taken up through mucous membranes of the gastrointestinal tract, the middle ear, and the accessory sinuses. It diffuses through the lining of the lung alveoli into the blood capillaries, is dissolved in the blood plasma, diffuses into the red blood cells, and is bound to the hemoglobin that they contain. Toxicity occurs at elevated pressures (e.g., deep sea diving). The latent period is 2 h at 3 atm and 30 min at 4 atm. It rapidly equilibrates with external atmosphere.

Mechanism of Toxicity

The partial reduction of molecular oxygen in biological systems produces the cytotoxic intermediates superoxide, hydrogen peroxide, and hydroxyl radical. The superoxide radical plays a significant role in a number of pathophysiologic states including oxygen toxicity, radiation damage, phagocyte-mediated inflammation, and postischemic injury. Oxygen radical scavengers such as superoxide dismutase and catalase protect the body against normal levels of oxygen-free radicals.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animal studies have shown the importance of considering potential chemical interactions in the production of oxygen-related toxicity. For example, oxygen tolerance can occur in mice with pre-exposure to one concentration of oxygen mitigating later exposure to 100% oxygen by modifying cellular and enzymatic composition of the lung. Further, damage of the alveolar zone in mice by the antioxidant butylated hydroxytoluene (BHT) can be greatly enhanced by subsequent exposure to oxygen concentration which, otherwise, would have little if any demonstrable effect. The synergistic interaction between BHT and oxygen results in a resulting interstitial pulmonary fibrosis. Based on these types of data, it can be seen that acute or chronic lung disease may be caused not only by one agent, but very likely in many instances by the interaction of several agents.

Human

Convulsions have occurred in man after oxygen has been breathed for 45 min at 4 atm; after 1–3 h at 1 atm, neuromuscular coordination and power of attentions were adversely affected. To study the early changes in the lower respiratory tract in persons exposed to hyperoxia usually considered safe, normal subjects were evaluated by bronchoalveolar lavage before and immediately after ~17 h of breathing more than 95% oxygen. A significant alveolar-capillary 'leak' was observed, as detected by the presence of increased plasma albumin and transferrin in lavage fluid. Some of the effects of exposure to 17 h of more than 95% oxygen are reversible; however, hyperoxia for this length of time lowers the structural or functional barriers that normally prevent alveolar-capillary 'leak' and induces processes that can culminate in fibrosis of the alveolar wall.

Chronic Toxicity (or Exposure)

Animal

Dogs inhaling 100% oxygen at atmospheric pressure had adverse effects beginning after 36 h, with death in 60 h. Inhaling 90% oxygen required double the exposure time for similar results. Animal studies have found eye effects similar to those noted below from prolonged exposure to a high concentration of oxygen.

Human

Concentrations of over 60% cause respiratory/pulmonary irritation, reduce vital capacity, and cause substernal distress. Oxygen poisoning causes nervousness, muscular twitch, hilarity, convulsions, or unconsciousness. Severe retinal damage in adults is rare during hyperoxia; however, one case was an individual with myasthenia gravis who developed irreversible retinal atrophy after breathing 80% oxygen for 150 days. The retinal vasculature was markedly constricted with no blood flowing through both eyes.

In Vitro Toxicity Data

Hyperoxia was toxic to cultured human pulmonary endothelial cells, with impairment of replicative function (expressed as growth impairment index), monitored by cell number determination and tritiated thymidine incorporation.

Clinical Management

In cases of pulmonary irritation, the oxygen concentration should be reduced to 60% or less. With oxygen poisoning, the oxygen concentration should be reduced to 200 mm kg^{-1}.

Exposure Standards and Guidelines

The human TC_{Lo} is 100 ppm per 14 h exposure.

See also: Ozone; Respiratory Tract.

Further Reading

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Witschi HP and Hakkinen PJ (1984) The role of toxicological interactions in lung injury. *Environmental Health Perspectives* 55: 139–148.

Oxygenates *See* Fuel Oxygenates.

Ozone

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 10028-15-6
- Synonym: Triatomic oxygen
- Chemical/Pharmaceutical/Other Class: Elemental gas
- Chemical Formula: O_3

Uses

Ozone is used for water treatment for taste and odor control, in the deodorization of air and sewage gases, as a mold and bacteria inhibitor in cold storage, in the oxidation of furnace carbon black for ink black manufacture, for bleaching flour, oils, paper pulp, starch, sugar, textiles, and waxes, for aging liquor and wood, and for processing some perfumes, vanillin, and camphor. It is also used in the treatment of industrial wastes, in the rapid drying of varnishes and printing inks, and in the deodorizing of feathers.

Exposure Routes and Pathways

Inhalation and exposure to mucous membranes are possible exposure routes. Ozone is formed locally in air from lightning and equipment such as photocopiers and residential electronic air cleaners, and in the outer layers of the atmosphere by the action of solar ultraviolet radiation on the oxygen in the air. It is a significant component of photochemical smog, and is produced when nitrogen oxides and hydrocarbons from motor vehicle emissions and other sources react with oxygen and sunlight. Ozone also reacts with limonene from consumer products and other sources, and this leads to formation of chemicals (e.g., formaldehyde, formic acid, and autooxidation products of limonene) capable of producing sensory irritation, bronchoconstriction, and pulmonary irritation.

Toxicokinetics

Ozone rapidly oxidizes thiol-containing compounds and unsaturated fatty acids.

Mechanism of Toxicity

The biochemical mechanism of pulmonary injury is due to the formation of reactive free-radical intermediates from oxidization of thiol-containing compounds and unsaturated fatty acids. The primary site of injury is the lung, and the injury is characterized by pulmonary congestion, edema, and hemorrhage.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LC_{50} in rats is 4.8 ppm. No increase in chromosomal aberration levels was seen in hamsters that inhaled ozone, although small increases in chromatid aberration levels were seen. Pregnant rats exposed to up to 1.97 ppm during parts or all of organogenesis had no defects in their offspring.

Human

Short-duration exposures can lead to dryness of the throat and mucous membranes of the nose and eyes. In studies with exercising subjects, acute ozone exposure produces a variety of reversible symptoms, including cough, shortness of breath, and pain on deep inspiration. Alterations in lung function and an influx of inflammatory cells into the lungs have also been observed. Mild to moderate exposure produces upper respiratory tract symptoms and eye irritation (e.g., lacrimation, burning of the eyes and throat, nonproductive cough, headache, substernal soreness, bronchial irritation, and an acrid taste and smell). More severe exposures, such as that seen in an industrial setting, may produce significant respiratory distress with dyspnea, cyanosis, and pulmonary edema. Chest X-rays show increased bronchovascular markings and bilateral lung densities. Symptoms resolve over 1–2 weeks although fatigue, headache, and exertional dyspnea may persist for several months. Ozone may exacerbate the small airway impairment of smoking adults.

Chronic Toxicity (or Exposure)

Animal

Ozone produces cell injury and connective tissue alterations in the lungs. Rats exposed for 6 h day^{-1} to a combination of 0.8 ppm of ozone and 14.4 ppm of nitrogen dioxide began to demonstrate respiratory insufficiency and severe weight loss $\sim$7–10 weeks after the initiation of exposure. About half of the rats died between days 55 and 78 of exposure; no overt ill effects were observed in animals exposed to filtered air, to ozone alone, or to nitrogen dioxide. The biochemical findings (e.g., increased lung content of DNA, protein, and collagen) were consistent with extensive breakdown and remodeling of the lung parenchyma and its associated vasculature. Histopathologic evaluation showed severe fibrosis, alveolar collapse, honeycombing, macrophage and mast cell accumulation, vascular smooth muscle hypertrophy, and other indications of severe progressive interstitial pulmonary fibrosis and end-stage lung disease. This animal model of progressive pulmonary fibrosis was judged to resemble the final stages of human idiopathic pulmonary fibrosis.

In 2 year and lifetime inhalation studies, there was no evidence of carcinogenic activity of ozone in male or female rats exposed to 0.12, 0.5, or 1.0 ppm. There was equivocal evidence of carcinogenic activity of ozone in male mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. There was some evidence of

carcinogenic activity of ozone in female mice based on increased incidences of alveolar/bronchiolar adenoma or carcinoma. However, oxygen and ozone both have been found to enhance or to inhibit the development of tumors in mouse lung under various exposure conditions. As a general rule, preexposure to the oxidant, before administration of a carcinogen, or exposure to high levels for a comparatively short time immediately following carcinogen administration favors development of tumors. On the other hand, prolonged exposure begun after a certain time following carcinogen exposure inhibits tumor development. The paradoxical effects of the two oxidants depend on experimental design; results can be tentatively explained in terms of oxidant-induced cell proliferation or by oxidant-mediated cytotoxicity.

Human

Ozone can aggravate asthma and increase susceptibility to respiratory diseases such as pneumonia and bronchitis. The TC_{Lo} is 50 ppm. Pulmonary symptoms at low levels (60–200 ppm) include substernal pain, cough, dry throat, wheezing, and dyspnea. The American Conference of Governmental Industrial Hygienists (ACGIH) lists ozone as A4 (not classifiable as a human carcinogen).

In Vitro Toxicity Data

In a study of *in vitro* transformation, ozone (6 ppm for 10 min) acted in an additive fashion with ultraviolet light (4 J m^2) to produce enhanced levels of transformation in hamster embryo cells and mouse C3H/10T-1/2 cells. Mouse C3H10T1/2 cells were exposed to 5 or 1 ppm ozone for 5 min. Some of the cell cultures were exposed to gamma rays immediately before or after ozone treatment. Following 6 weeks in culture, transformation was scored using morphological criteria. Ozone (at 5 ppm) and radiation acted as independent carcinogens, and when the cells were first exposed to radiation, transformation was enhanced in a synergistic manner.

Clinical Management

The victim should be removed from exposure and monitored for respiratory distress.

Ecotoxicology

Ground-level ozone interferes with the ability of plants to produce and store food. This makes them more susceptible to disease, insects, other pollutants, and harsh weather. Ozone damages the leaves of trees and other plants. Ozone and the chemicals that react to form it can be carried long distances from their origins, thus causing air pollution over wide regions.

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average (TWA), is 0.05 ppm for heavy work, 0.08 ppm for moderate work, and 0.1 ppm for light work; 0.20 ppm is the level for heavy, moderate, or light workloads of $\leqslant$2 h. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA, is 0.1 ppm. The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure ceiling value is 0.1 ppm, and the NIOSH immediately dangerous to life or health value is 5 ppm. In 1997, the US Environmental Protection Agency (EPA) announced new stricter national ambient air quality standards for ground-level ozone, the primary constituent of smog. After a lengthy scientific review process, including extensive external scientific review, EPA determined that these changes were necessary to protect public health and the environment. The new standard is intended to be more protective of the health of children and adults who play and work outdoors in the summer. In establishing the 8 h standard, EPA set the standard at 0.08 ppm as an average over an 8 h period. Areas in the United States would have until 2010 to meet the new standard.

See also: Pollution, Air; Pollution, Air Indoor; Pollution, Water.

Further Reading

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Relevant Website

http://www.oma.org – OMA Ground Level Ozone Position paper (from the Ontario Medical Association).

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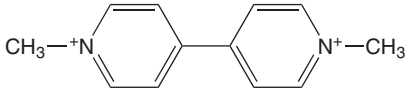
Paraoxon Detoxification *See* A-Esterases.

Paraquat

Kevin N Baer

- Chemical Abstracts Service Registry Number: CAS 1910-42-5 (dichloride)
- Synonyms: Paraquat dichloride; Methyl viologen; 1,1′-Dimethyl-4,4′-bipyridinium ion; Gramoxone
- Chemical/Pharmaceutical/Other Class: Quaternary nitrogen (bis-pyridinium) compound
- Chemical Structure:



Uses

Paraquat is used as a broad-spectrum herbicide on weeds and grasses in agricultural and nonagricultural areas. It is used as a desiccant on tomatoes, cotton, beans, soybeans, potatoes, sunflowers, and sugar cane to aid in harvesting and to induce resin soaking on pine trees.

Exposure Routes and Pathways

Accidental or intentional ingestion is the most common route of exposure. Poisonings from inhalation and dermal exposure have also occurred.

Toxicokinetics

Paraquat has low but rapid gastrointestinal absorption (5–10%) and low skin absorption. Peak plasma concentrations occur in less than 2 h following ingestion. Generally, paraquat is not metabolized to any large extent. In animal studies, metabolites have been detected in urine, possibly resulting from the action of intestinal microflora. Paraquat is actively transported to alveolar cells, where it is reduced to form highly reactive free radicals. The volume of distribution is large and has been estimated at $2.75\,l\,kg^{-1}$. Paraquat tends to attain higher and more prolonged levels in the lung. Clearance is rapid by the kidneys with 80–90% of the dose excreted in the urine after 6 h. The terminal half-life increases from 12 to 120 h or longer as renal failure begins.

Mechanism of Toxicity

Paraquat produces lung damage by all routes of exposure. Progressive and generalized proliferation of fibrous connective tissue is observed in the pulmonary alveoli where paraquat is selectively concentrated. The mechanisms of action result from a metabolically catalyzed single electron oxidation/reduction reaction resulting in NADPH depletion and the generation of oxygen free radicals. For example, superoxide radicals are formed and attack unsaturated lipids of cell membranes. This in turn produces lipid free radicals, resulting in membrane damage and loss of the functional integrity of the cell. In some animal models, paraquat leads to damage of dopaminergic cells in the subtantia nigra similar to that seen in Parkinson's disease.

Acute and Short-Term Toxicity (or Exposure)

Animal

Paraquat is highly toxic by inhalation. Particle sizes used in agricultural practices (400–800 μm) limits lower airway deposition, thereby lessening inhalation hazard. Paraquat is moderately toxic by the oral route and only slightly toxic by the dermal route. Paraquat causes moderate to severe eye irritation and minimal dermal irritation. The oral and dermal LD_{50}

values reported in rats and mice range from about 20 to 150 mg kg^{-1}.

Human

Paraquat may result in severe toxicity to all organ systems and death within 24 h after ingestion, inhalation, and dermal exposure. The initial symptoms after ingestion are burning in the mouth and throat with vomiting and diarrhea and subsequent fluid and electrolyte loss. Depending on the dose (>60 ml), esophageal perforation, renal failure, cardiac arrhythmias, convulsions, and coma can occur. Early death is usually due to hepatic and renal toxicities. The lethal dose in humans is estimated to be ~40 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

Subchronic exposure to paraquat led to pulmonary damage. A rabbit dermal toxicity study noted scabbing and inflammation when tested at the two highest doses (2.6 and 6.0 mg kg^{-1} group). Inhalation of small particles (<2μm in diameter) resulted in pulmonary changes and lesions in the larynx. Chronic exposure in dogs led to chronic pneumonitis. Paraquat did not appear to be carcinogenic in two long-term studies in rats and in mice. In developmental toxicity studies, paraquat caused delayed or retarded ossification in the forelimb and hindlimb digits and posterior portion of the skullat maternally toxic dosages only. Paraquat does not appear to influence reproduction.

Human

Survivors of the initial poisoning or from poisonings from as little as 10–15 ml of the concentrate often develop a progressive pulmonary fibrosis associated with dyspnea and pulmonary edema several days or weeks after exposure. As a result, death is due to asphyxia.

In Vitro Toxicity Data

Paraquat did not demonstrate mutagenic capacity.

Clinical Management

No specific treatment is known. All cases of paraquat exposure should be managed as a potentially fatal exposure. Basic life-support measures should be instituted; however, the administration of supplemental oxygen is not advised. Treatment must be instituted early, within 10 h after ingestion. Treatment involves removal of paraquat from the alimentary tract by gastric lavage and cathartics, prevention of further absorption by Fuller's earth (30% w/v), and removal of absorbed paraquat by hemodialysis or hemoperfusion. Use of various drugs, such as D-propranolol, prednisone, and vitamins E and C, has provided little benefit.

Signs of toxicity in animals are similar to those in humans. Paraquat has been shown to be mutagenic, carcinogenic, and teratogenic in experimental animals.

Environmental Fate

Paraquat is relatively immobile in soil. Paraquat resists hydrolysis, photodegradation in water, and microbial degradation under both aerobic and anaerobic conditions. Dissipation is primarily by adsorption to organic material and clay particles. As paraquat persists in clay soils, it may be found in surface water from erosion. Since it binds so strongly to clay particles, paraquat is not generally a groundwater concern.

Ecotoxicology

Paraquat is practically nontoxic to honey bees, only slightly toxic to fish, and moderately toxic to terrestrial animals. Hazard for birds and mammals is generally short lived after paraquat application.

Exposure Standards and Guidelines

The reference dose for paraquat is 0.0045 mg kg^{-1} day^{-1}. The acceptable daily intake for paraquat is 0.004 mg kg^{-1} day^{-1} while the threshold limit value is 0.1 mg m^{-3}.

See also: Lipid Peroxidation; Pesticides; Pollution, Water; Respiratory Tract.

Further Reading

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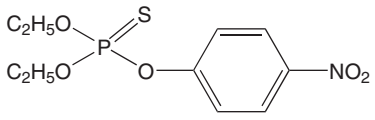
Lock EA and Wilks MF (2001) Paraquat. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1559–1604. San Diego: Academic Press.

Parathion

Jason R Richardson

This article is a revision of the previous print edition article by Tamal Kumar Chakraborti, volume 2, pp. 476–477, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 56-38-2
- Synonyms: Bayer E-605; Corthion; *O*,*O*-Diethyl-*O*-(*p*-nitrophenyl) phosphorothioate; Diethyl 4-nitrophenyl phosphorothionate; DNTP; DDPP; Ethyl parathion; AC 3422; Alkron; Alleron; Aphamite; Corothion; E-605; ENT 15108; Etilon; Fosferno 50; Niran; Nitrostigine; Orthophos; Panthion; Paramar; Paraphos; Parathene; Parawet; Pethion; Phoskil; Rhodiatox; Soprathion; Stathion; Sulphos; Thiophos
- Chemical/Pharmaceutical/Other Class: Organophosphorus insecticide
- Chemical Structure:



Uses

Parathion is an effective insecticide for a range of insect pests. It has nonsystemic, contact, stomach, and fumigant actions. Because of its high acute toxicity, parathion is no longer approved for use on fruit or vegetable crops and is severely restricted in grain crop and other uses. Parathion has no approved residential use.

Exposure Routes and Pathways

Fatal poisonings have occurred after oral, dermal, and inhalation exposure to parathion. The vapor pressure of the pure compound is generally not sufficient to lead to respiratory exposure alone. However, fine dusts or aerosol preparations may result in severe poisoning through the respiratory tract.

Toxicokinetics

Parathion is efficiently absorbed through any route of exposure. Signs of toxicity due to parathion generally appear within several hours following dermal exposure. The rate of dermal absorption in rabbits was found to be $0.059\,\mu g\,min^{-1}\,cm^{-2}$. There is considerable individual variation in dermal absorption rates in animals and humans. About 0.1–2.8% of the applied compound was absorbed through skin in human volunteers. The kinetics of absorption studied in isolated perfused porcine skin flaps indicates a linear, three-compartment model of absorption.

Parathion is preferentially distributed in the liver, the kidneys, and ordinary adipose tissue. It is also concentrated to a fairly high degree in gastric and intestinal walls, thyroid, spleen, and lungs. It can cross the blood–brain barrier because of its nonpolar nature and it accumulates to a lesser extent in the central nervous system. Parathion is metabolized in the liver and other extrahepatic sites by the mixed function oxidase enzyme system to paraoxon, which is considerably more toxic than the parent compound. The conversion of parathion to paraoxon requires the presence of NADPH and oxygen. Parathion is also metabolized to *O*-ethyl phosphoric acid, phosphoric acid, and inorganic sulfate. Paraoxon is an extremely potent inhibitor of brain cholinesterase, with an IC_{50} of $18\,nmol\,l^{-1}$ in rat brain homogenates. Paraoxon is efficiently detoxified by binding to carboxylesterases and, to a much lesser extent by A-esterases.

Metabolites of parathion are exclusively eliminated through urine. However, some of the unmetabolized parent compound may also be excreted through sebum. The elimination half-life of parathion was found to be 2.1 days. It was reported that, following oral administration of parathion (1 or $2\,mg\,day^{-1}$) in humans, 60% of parathion was excreted within 4 h and 86% within 8 h in the form of *p*-nitrophenol. The rate of excretion of diethyl phosphate was found to be slower than that of *p*-nitrophenol. Following dermal exposure of 5 g of a 2% dust for 2 h, the *p*-nitrophenol concentration reached a peak level by 5 or 6 h after initial exposure. In case of dermal exposure, the rate of excretion of the metabolites of parathion increases with temperature.

Mechanism of Toxicity

The mechanism of toxicity for parathion is similar to that of chlorpyrifos. Following activation to the potent anticholinesterase paraoxon, acetylcholinesterase is inhibited within synapses and acetylcholine levels accumulate. This leads to overstimulation of cholinergic receptors of neurons, muscle cells, and end-organs culminating in cholinergic toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for parathion is $2–30\,mg\,kg^{-1}$ in rats, $5–25\,mg\,kg^{-1}$ in mice, $8–32\,mg\,kg^{-1}$ in guinea pigs,

10 mg kg^{-1} in rabbits, 0.93 mg kg^{-1} in cats, and 3–5 mg kg^{-1} in dogs. The dermal LD_{50} is 6.8–50 mg kg^{-1} for rats, 19 mg kg^{-1} for mice, 45 mg kg^{-1} for guinea pigs, and 15 mg kg^{-1} for rabbits. The no-observed-adverse-effect level (NOAEL) based upon plasma and red-blood cell cholinesterase inhibition is 0.025 mg kg^{-1} day^{-1} in rats.

Human

The toxic effects of parathion in humans are similar to those of chlorpyrifos, although parathion is toxic at much lower levels. The lowest dose that results in toxic effects in humans has been estimated at 240 μg kg^{-1}. Parathion has the notorious distinction of being the synthetic pesticide that has killed more humans (both through accidental and intentional exposures) than any other.

Chronic Toxicity (or Exposure)

Animal

Parathion is neither mutagenic nor teratogenic. Parathion has been classified as a possible carcinogen. Parathion has been shown to produce adverse reproductive effects, although these are most likely secondary to the primary neurotoxic effects. Parathion has not been shown to cause delayed neuropathy. The lowest-observed-adverse-effect limit for parathion in rats has been determined to be 0.01 mg kg^{-1} day^{-1} in a 1 year feeding study in dogs, with no NOAEL established.

Human

Repeated or prolonged exposure to parathion can cause the same effects seen with acute exposures. In people working directly in the manufacture or application of parathion, impaired memory and concentration, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, and sleepwalking and drowsiness or insomnia have all been reported. Parathion cannot cause delayed neurotoxicity but has been reported to be associated with the intermediate syndrome.

In Vitro Toxicity Data

Paraoxon, the active metabolite of parathion, is a potent inhibitor of acetylcholinesterase, butyrylcholinesterase, and carboxylesterase, with IC_{50} values in the low- to mid-nanomolar range. Neither parathion nor paraoxon has been shown to be mutagenic when tested *in vitro*.

Clinical Management

Oral Exposure

Induction of emesis is contraindicated in the case of parathion poisoning due to the early onset of respiratory depression and seizures. Gastric lavage may be indicated if performed immediately after parathion ingestion. Activated charcoal/cathartic therapy may be adopted to retard the absorption from the gastrointestinal tract. Atropine should be administered intravenously until atropinization is achieved. In adults, 2–5 mg kg^{-1} should be administered every 10–15 min and in children 0.05 mg kg^{-1} must be given at the same frequency. Atropinization may require several hours to days depending on the severity of poisoning. 2-PAM (Pralidoxime) may be combined with atropine in case of severe poisoning (adult, 1 or 2 g intravenously at 0.5 g min^{-1}; children, 25–50 mg kg^{-1} over 5–30 min). Seizures may be treated with conventional anticonvulsants (e.g., diazepam, phenobarbital, and phenytoin).

Inhalation Exposure

The affected person should be moved immediately to fresh air and should be administered with 100% humidified supplemental oxygen with assisted ventilation.

Eye Exposure

Exposed eyes should be irrigated with copious amount of tepid water for 15 min. If irritation, photophobia, pain, or swelling persists, the patient should be admitted to a health care facility.

Dermal Exposure

The contaminated clothing should be removed and the contaminated area of the skin should be washed repeatedly with soapy water.

Environmental Fate

Parathion binds tightly to soil particles and has little or no potential for groundwater contamination. Residues of parathion may persist for days or weeks. Parathion readily undergoes photodegradation and sunlight can convert parathion into paraoxon. The breakdown of parathion in soil or water increases with alkalinity. Parathion residues on crops typically decay with a half-life of 1 day.

Ecotoxicology

Parathion is extremely toxic to birds, with LD_{50} values of 6, 3, and 2.1 mg kg^{-1} in bobwhite quail,

pigeons, and ducks, respectively. Parathion is moderately toxic to aquatic invertebrates and fish. The 96 h LC_{50}s for trout, catfish, and bluegill are 1.6, 2.7, and 0.02 mg l^{-1}. Parathion is extremely toxic to honeybees.

Exposure Standards and Guidelines

US Environmental Protection Agency has established an acute reference dose of 0.000 25 mg kg^{-1} day^{-1} and a chronic reference dose of 0.0000 33 mg kg^{-1} day^{-1} for parathion.

See also: A-Esterases; Carboxylesterases; Chlorpyrifos; Organophosphate Poisoning, Intermediate Syndrome; Organophosphates.

Further Reading

Eyer F, Meischner V, Kiderlen D, *et al.* (2003) Human parathion poisoning. A toxicokinetic analysis. *Toxicological Reviews* 22(3): 143–163.

Garcia SJ, Abu-Qare AW, Meeker-O'Connell WA, Borton AJ, and Abou-Donia MB (2003) Methyl parathion: A review of health effects. *Journal of Toxicology and Enivornmental Health, Part B: Critical Reviews* 6(2): 185–210.

Savolainen K (2001) Understanding the toxic actions of organophosphates. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1013–1041. San Diego, CA: Academic Press.

Relevant Website

http://www.inchem.org – International Programme on Chemical Safety.

Paregoric

Fermin Barrueto Jr.

- Chemical Abstracts Service Registry Number: CAS 8029-99-0
- Synonyms: Opium; Opium tincture; Hydrochloride of opium alkaloids, Camphorated tincture of opium
- Chemical/Pharmaceutical/Other Class: Paregoric is an opium preparation. It is composed of several alkaloids, including anhydrous morphine (9.5% or more) and smaller amounts of codeine and papaverine

Uses

Paregoric affects the gastrointestinal tract by inhibiting motility and propulsion and increasing smooth muscle tone. Thus, it has been used to treat diarrhea, opioid addiction, and neonatal abstinence syndrome.

Exposure Routes and Pathways

Paregoric is usually taken orally.

Toxicokinetics

Paregoric is well absorbed from the gastrointestinal tract. Peak serum levels are detectable ~1 h after ingestion. Opium preparations are metabolized in the liver by demethylation. Morphine undergoes conjugation with glucoronic acid at the 3-hydroxyl group. Secondary conjugation occurs at the 6-hydroxyl group to form 3,6-diglucuronide. Paregoric is 34–37% protein bound. The volume of distribution is 3 or 4 l kg^{-1}. From 8.5% to 12% is excreted unchanged in the urine, 7–10% excreted in feces as glucuronide conjugate, and 7–10% in the bile. The half-life is 1.9–2.6 h.

Mechanism of Toxicity

Opium and its derivatives cause depression of the central nervous system (CNS) and respiratory depression through binding of the opioid receptors (μ, δ, κ subtypes).

Acute and Short-Term Toxicity (or Exposure)

Animal

Cats and horses experience excitability and increased CNS effects. Dogs exhibit drowsiness, ataxia, seizures, coma, respiratory depression, hypotension, and vomiting. Treatment consists of decontaminating the gastrointestinal tract, maintaining the airway, administering naloxone, and controlling seizures with diazepam and/or phenobarbital. Hypotension is effectively treated with intravenous fluids and rarely needs pressor support. If ineffective, norepinephrine is the drug of choice. Dopamine and dobutamine can also be used. Monitoring needs to be

provided for at least 8 h after cessation of symptoms since relapse can occur.

Human

Paregoric produces CNS depression ranging from drowsiness to coma. These symptoms can be cyclic due to decreased gastric emptying. Respiratory depression occurs and progresses to Cheyne–Stokes respirations, cyanosis, and respiratory arrest. Pulmonary edema can also occur. Cardiac affects are characterized by bradycardia and hypotension. Other symptoms include hypothermia, flaccid skeletal muscles, cold and clammy skin.

Chronic Toxicity (or Exposure)

Human

Chronic use can produce psychological and physical dependence. Discontinuation of paregoric causes withdrawal symptoms.

Clinical Management

Life-support measures should be provided. Gastric decontamination with activated charcoal can be utilized in recent ingestions. Respiratory and CNS depression can be effectively reversed with naloxone, a pure opioid receptor antagonist. Naloxone administration will precipitate the opioid withdrawal syndrome and should be administered judiciously. Initial dose of 0.1 mg of naloxone can be administered intravenously and titrated until the respiratory depression is reversed. Until naloxone is administered, proper airway support is critical. If the patient has a second episode of respiratory depression after administration of naloxone, a drip utilizing two-thirds the dose needed to reverse the patient should be administered over 1 h.

See also: Codeine; Drugs of Abuse; Morphine; Opium.

Further Reading

Schoen AM (1984) Tincture of opium. *New England Journal of Medicine* 310(17): 1124.

Theis JG, Selby P, Ikizler Y, and Koren G (1997) Current management of the neonatal abstinence syndrome: A critical analysis of the evidence. *Biology of the Neonate* 71(6): 345–356.

Wagner CL, Katikaneni LD, Cox TH, and Ryan RM (1998) The impact of prenatal drug exposure on the neonate. *Obstetrics and Gynecology Clinics of North America* 25(1): 169–194.

PBT (Persistent, Bioaccumulative, and Toxic) Chemicals

Thomas M Murray

- Representative Chemicals: Aldrin (CAS 309-00-2); Dieldrin (CAS 60-57-1); Alkyl-lead (CAS 7439-92-1); Benzo(*a*)pyrene (CAS 50-32-8); Chlordane (CAS 57-74-9); Dichlorodiphenyltrichloroethane (DDT) (CAS 50-29-3); Hexachlorobenzene (CAS 118-74-1); Mercury and compounds (CAS 7439-97-6); Mirex (CAS 2385-85-5); Octachlorostyrene (CAS 29082-74-4); Polychlorinated biphenyls (PCBs) (CAS 11097-69-1 for Arochlor 1254); 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (CAS 1746-01-6) and chemically similar compounds collectively known as dioxins and furans; Camphechlor (toxaphene) (CAS 8001-35-2)

Persistent, bioaccumulative, and toxic (PBT) chemicals are a group of chemicals that meet the following criteria:

1. evidence of an environmental persistence value of more than 2 months in water and more than 6 months in soil or sediment or evidence of sufficient persistence to justify its consideration;
2. evidence of a bioconcentration factor (BCF) value or bioaccumulation factor (BAF) value of more than 5000 in aquatic species or, in lieu of such data, a log K_{OW} of more than 5 or evidence of high bioaccumulation in other species, evidence of high toxicity or ecotoxicity, or monitoring data in biota indicating a sufficient bioaccumulation potential to justify its consideration;
3. evidence of adverse effects to human health or the environment or toxicity or ecotoxicity data indicating the potential for damage to human health or to the environment; and
4. a long-range transport value of a half-life in air of more than 2 days, or other data/predictions of traveling long distances through air, water, or migratory species.

Uses

The uses of these PBT chemicals vary by chemical. The reader is referred to other sections of this encyclopedia for specific use information.

Exposure Routes and Pathways

PBT substances (and ones that could be classified as PBTs) enter the environment in various ways. Some, like dioxins, dioxin-like compounds, and numerous polycyclic aromatic hydrocarbons, are unintentional by-products of incomplete combustion or other high-temperature industrial processes involving organic materials. Some result from the environmental degradation of a released substance, such as hexachlorocyclohexane, which degrades to a γ-isomer with much higher PBT properties than the parent compound. Some substances, such as active ingredients of pesticide products, were designed to have a combination of molecular stability, biological uptake/retention, and a specific toxic endpoint – and only later were recognized to have broader environmental impacts. Finally, some substances were designed or discovered to have economically desirable properties like flame suppression, absorption of ultraviolet radiation, water and oil repellency, and even fragrance, and were later discovered to have unintended properties of toxicity, bioaccumulation, and persistence.

The food chain is the predominant source of human and wildlife exposure to most PBT chemicals, although drinking water (and dust and dirt for children) are also significant exposure pathways for lead. Within the food chain, the aquatic and marine food chains are significant sources of PBT exposure to humans and other terrestrial species. PBTs enter waterways in various amounts, both directly or by virtue of air deposition and runoff. Aquatic and marine organisms ingest PBT chemicals from the water column and sediments as they feed. Once ingested, the chemical properties of PBTs make them difficult for many organisms (depending on their physiological makeup) to excrete, thereby leading to bioaccumulation of PBTs in the organisms. As one organism feeds on another, this results in PBTs moving up the food chain. Many PBTs also biomagnify as they bioaccumulate, which means they increase in concentration within organisms as they move up the food chain. Mammals and birds high on the food chain can have levels of these PBTs that are at least 100 000–1 000 000 times greater than the concentrations found in ambient waters.

Bioconcentration in the marine food chain can lead to animals such as seals, beluga whales, seabirds, and polar bears having concentrations of toxaphene $\sim$10 million times higher than levels in the surrounding water. For polychlorinated biphenyls (PCBs), the amplification is $\sim$1000 million times.

Bioaccumulation also occurs directly within the terrestrial food chain for some PBT chemicals, without the involvement of aquatic organisms. For example, dioxins and furans are generated in combustion processes, are discharged to air, and then settle out on plant surfaces. These plants become food for farm animals and these PBTs become concentrated in animal tissue (many of the PBTs concentrate in fatty tissue). People are exposed via the terrestrial food chain when they consume meat, poultry, pork, and dairy products.

Mechanism of Toxicity

The toxic effects of PBT chemicals include neurotoxicity, reproductive toxicity, developmental toxicity, and cancer in humans and other species.

Animal

Birds and mammals high on the food chain are also at risk from exposure to PBTs, with similar concerns for their young exposed to PBTs in their eggs or through maternal milk. In both the North Temperate and Arctic zones, some marine mammal and bird populations are experiencing disease, reproductive problems, and population declines, probably in part or in whole due to contamination from PBT pollutants. Free-ranging Orca whales along the Pacific Northwest coast, whose numbers are falling appreciably, have PCB levels four to five times higher than highly-PCB-polluted St. Lawrence Beluga whales, who themselves have serious health problems. Populations of mink and otter continue to be depressed in certain regions where significant PCB concentrations have been reported. Canadian Arctic whales are providing the first statistical inference that PBT (specifically, PCB) levels in Arctic species may relate to subtle health effects.

Human

The developing human fetus and nursing infant are at particular risk for developmental problems. Studies show that mothers, who previously accumulated PBTs in their bodies, whether from the food chain or some other exposure pathway, transmit PBT contaminants through the fetal blood cord and breast milk to the fetus and nursing infant respectively. Most PBT chemical releases have occurred in the North Temperate zone, between the Arctic Circle and the Tropic of Cancer, where the majority of industrialized nations are located. In this region, the general population has detectable levels of 2,3,7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) – and chemically similar compounds collectively known as dioxins, furans, and coplanar PCBs – in their bodies as a result of eating meat, fish, eggs, and dairy products.

US Environmental Protection Agency's (EPA's) 2000 Draft Dioxin Reassessment estimated the cancer risk to the US population from this background exposure to be in the 1:10 000 to 1:1000 range, which is approaching levels associated with adverse noncancer effects.

For mercury, results from the 1999 US Natio nal Health and Nutrition Examination Surveys (NHANES), which measured mercury levels in hair and blood of US women, show that ~8% of US women of childbearing age have concentrations of mercury at blood levels higher than those associated with EPA's reference dose. About 75% of the 2618 US consumption advisories listed on US EPA's 2001 National Listing of Fish and Wildlife Advisories were issued at least partly due to mercury, and lake acres and river miles under mercury advisory continued to increase in 2001, a trend since 1993. Most US advisories involve mercury, PCBs, chlordane, dioxin, and DDT, with PCBs being the second highest cause of fish advisories.

Environmental Fate

Air deposition of PBTs can occur in three ways – as gases and particles trapped in rain, fog, or snow, as dry particles dropping onto surfaces, or as semivolatile organic chemicals (SVOCs) cycling between the gas phase in air and the particle phase in water. SVOCs and some trace metals like mercury can cycle between the atmosphere and the Earth's surface many times in the course of being transported long distances. This cycling slows or ceases in the colder Polar Regions and high-altitude regions, a phenomenon known as global distillation.

Atmospheric deposition of PBTs contributes significantly to the contamination of aquatic, marine, and terrestrial ecosystems and their food chains. Large water bodies, such as the Great Lakes, seas, and oceans, appear vulnerable to significant air-water exchange of SVOCs, and air deposition accounts for a significant percentage of toxics contained in water bodies such as the Great Lakes. Moreover, the circulation of PBTs in the atmosphere at regional and global scales can make it difficult to identify sources of contamination deposited via the atmosphere, since they may be far away. This point is perhaps best illustrated by the existence of PCBs in the Arctic snow pack and food chain, hundreds or thousands of miles from any possible source.

See also: Aldrin; Dieldrin; Dioxins; Polychlorinated Biphenyls (PCBs).

Further Reading

Arctic Monitoring and Assessment Programme (AMAP) (1997) *Arctic Pollution Issues: A State of the Arctic Environment Report*. ISBN 82-7655-060-6 (Oslo, Norway).

Arctic Monitoring and Assessment Programme (AMAP) (1998) *AMAP Assessment Report: Pollution Issues*. ISBN 82-7655-061-4 (Oslo, Norway).

Canada Department of Indian Affairs and Northern Development (1997) *Highlights of the Canadian Arctic Contaminants Assessment Report: A Community Reference Manual*.

Relevant Websites

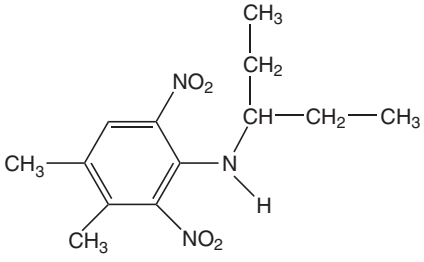
http://www.cdc.gov – Centers for Disease Control and Prevention, US Department of Health and Human Services, National Health and Nutrition Examination Surveys (NHANES), March 2, 2001: Mercury findings. Other NHANES results are available on the same website.

http://www.epa.gov – US Environmental Protection Agency (EPA), 2000 National Listing of Fish and Wildlife Advisories. Additional details and information on PBTs are also available on the same website.

Pendimethalin

K S Rao

- Chemical Abstracts Service Registry Number: CAS 40487-42-1
- Synonyms: (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine); Prowl; Squadron
- Chemical/Pharmaceutical/Other Class: Agricultural; Herbicide
- Chemical Formula: $C_{13}H_{19}N_3O_4$
- Chemical Structure:



Uses

Pendimethalin is used as a selective herbicide to control broadleaf weeds and grassy weed species in cereals, onions, garlic, corn, sorghum, rice, soy beans, peanuts, brassicas, carrots, celery, peas, potatoes, cotton, pome fruits, stone fruits, citrus, lettuce, tobacco, and tomatoes. It is also used on noncrop areas and on residential lawns and ornamentals.

Exposure Routes and Pathways

Primary exposure is through the dermal route to mixers, loaders, and applicators, during and after normal use. However, significant exposure to residues of pendimethalin in food crops can occur in consumers.

Toxicokinetics

Pendimethalin is absorbed rapidly and effectively by the oral route but less effectively by the dermal route. Following absorption, ~70% of pendimethalin is excreted in the feces and 20% in the urine in 24 h. The maximum tissue concentration of pendimethalin was seen at 6 h in liver, kidney, muscle, and fat. The major portion that is excreted in the feces is the parent compound. Pendimethalin is metabolized in rats mainly through oxidation of the 4-methyl group attached to the benzene ring as well as oxidation of the alkyl side chain of the N-substituted dinitroaniline compound.

Mechanism of Toxicity

The toxicity of pendimethalin is related to its effects on the thyroid. Treatment with pendimethalin (500 ppm for 90 days) produces a decreased total T_4, T_3, total free T_4 and increased percent T_3, increased follicular cell height and decreased area occupied by colloid. In addition, it produces an increased absolute (15%) and relative (23%) thyroid weight and ultrastructural thyroid changes. The ultrastructural thyroid changes are consistent with mild to moderate testicular stimulating hormone (TSH) stimulation except for the accumulation of dense bodies in the cytoplasm, which may be reaction products of pendimethalin. At higher doses (5000 ppm), increased liver weight, bile flow and cumulative biliary excretion of [^{125}I]-T_4 with a slight increase in T_4-glucuronytransferase activity was detected by generation of [^{125}I]-T glucuronide from [^{125}I]-T.

Acute and Short-Term Toxicity (or Exposure)

Animal

In studies using laboratory animals, pendimethalin generally has been shown to be of low acute toxicity with an oral LD_{50} of 1050 mg kg^{-1} in rats. It is slightly toxic to practically nontoxic by skin exposure, with reported dermal LD_{50} values of greater than 2000 mg kg^{-1}. It is not a skin sensitizer, but it causes mild eye irritation. The inhalation 4 h LC_{50} for pendimethalin in rats is 320 mg l^{-1}, indicating practically no toxicity via this route. Inhalation of dust or fumes of pendimethalin is irritating to the linings of the mouth, nose, throat, and lungs.

Human

A search in the Office of Pesticide Programs' Incident Data System identified 12 pendimethalin reports with three of these involving five humans (the remainder concern fish, wildlife, or domestic animals). The symptoms included signs of systemic illness: vomiting, diarrhea, chills, and shakiness. Three people were hospitalized when they were exposed to a mixture of pesticides including pendimethalin and nitrogen. The database does not indicate the associated use patterns or activities in which the poisoned individuals were involved. The California Pesticide Illness Surveillance Program for 1982–1992 contained six reports involving pendimethalin. In three of these reports, the effects were systemic (vomiting, diarrhea, etc.), two involved skin effects, and one involved eye effects. Pendimethalin was ranked 41st on a list of the top 200 active ingredients for which the National Pesticide Telecommunications Network (NPTN) received calls during 1982–1991. There were 682 calls, with 91 of them concerning human poisoning due to pendimethalin.

Chronic Toxicity (or Exposure)

Animal

In subchronic studies in rats at a low dose level of 100 ppm (5.0 mg kg^{-1} day^{-1}) there was decreased total T_4, rT_3, total free T_4 and increased percent T_3, increased follicular cell height and decreased area occupied by colloid. At 5000 ppm (245 mg kg^{-1} day^{-1}) exposed animals exhibited decreased body weight and food consumption compared to controls, increased thyroid weight, decreased total T_4, total T_3, rT_3, total free T_4 and [^{125}I]-T_4 to transthyretin bonding, increased percent free T_4, percent free T_3 and [^{125}I]-T_4 to albumin binding, increased follicular cell height and decreased area occupied by colloid and ultrastructural thyroid changes. Chronic exposure to pendimethalin has resulted in increased liver weights in test animals.

Pendimethalin induces a statistically significant increase in thyroid follicular cell adenomas in male and

female rats. Pendimethalin does not induce mutations, birth defects or reproductive effects.

Human

The Environmental Protection Agency has concluded that pendimethalin should be classified as a group C (possible human) carcinogen.

Clinical Management

In case of contact, the eyes and skin should be flushed immediately with water for at least 15 min to reduce exposure. Following accidental oral exposure, immediate dilution with 4–8 ounces (i.e., 118–237 ml) of milk or water is recommended. Following a known significant exposure, the patient should be subjected to a complete thyroid profile, to make sure thyroid function is intact. If thyroid function is affected, a specialist in endocrinology should be consulted.

Environmental Fate

Pendimethalin is moderately persistent, with a field half-life of 40 days. It does not undergo rapid microbial degradation. Pendimethalin is strongly adsorbed by most soils. Increasing soil organic matter and clay is associated with increased soil binding capacity. Under agricultural use conditions, pendimethalin is absorbed by plant roots and shoots and inhibits cell division.

Ecotoxicology

Pendimethalin is highly toxic to fish and aquatic invertebrates. The 96 h LC_{50} value for pendimethalin in rainbow trout is 138 $\mu g\,l^{-1}$. The bioconcentration factor for this compound in whole fish is 5100, indicating a moderate potential to accumulate in aquatic organisms.

Pendimethalin is slightly toxic to birds, with an acute oral LD_{50} of 1421 $mg\,kg^{-1}$ in mallard ducks.

See also: Pesticides; Thyroid Extract.

Relevant Websites

http://europa.eu.int – European Commission (2003) Review Report for the Active Substance Pendimethalin. Brussels: Health and Consumer Protection Directorate General.

http://www.epa.gov – US Environmental Protection Agency (1997) Reregistration Eligibility Decision (RED) Pendimethalin. Washington, DC: Office of Prevention, Pesticides and Toxic Substances.

Penicillin

Brenda Swanson-Biearman

- SYNONYMS: Aminopenicillins; Amoxicillin; Ampicillin; Carbenicillin; Bacampacillin. Extended spectrum: Carbenicillin; Mezlocillin, Piperacillins, Ticarcillins. Natural penicillins: Penicillin G; Penicillin V. Pencillinase-resistant: Cloxacillin; Dicloxacillin; Methicillin; Nafcillin; Oxacillin
- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Antibiotic

Uses

Penicillin is used to treat infections caused by Gram-positive cocci, Gram-negative aerobic cocci, and anaerobic bacteria. The penicillins may be bactericidal or bacteriostatic in action, dependent upon the concentration of drug attained at the sire of infection and susceptibility of the organism.

Exposure Routes and Pathways

Penicillin is available in tablet and suspension form for oral use and in injectable form for intravenous and intramuscular use. Ingestion of tablets and the suspension forms are the most common poisoning exposures.

Toxicokinetics

Following oral administration, absorption of penicillin occurs mainly in the duodenum and upper jejunum, with a small percentage absorbed in the stomach and insignificant amounts in the large intestine. The extent of absorption is variable and depends on several factors including the penicillin derivative, the dosage form administered, gastric and intestinal pH, and the presence of food in the gastrointestinal tract. Peak concentrations are generally seen within 1–2 h. Protein binding and the volume of distribution vary with each derivative. Following absorption from either the gastrointestinal tract or from

injection sites, penicillins are widely distributed into most body tissues. Most penicillins and their microbiologically active metabolites are excreted primarily unchanged in the urine by renal tubular secretion. Nonrenal elimination includes hepatic inactivation and biliary excretion. Renal clearance of penicillin is delayed in the neonate due to an immature tubular secretion mechanism. Children older than 3 months generally excrete drugs similarly to adults. Excretion is also delayed in geriatric patients due to diminished tubular secretion ability. Patients with renal impairment may also have altered tubular secretion ability and, therefore, have higher and more prolonged serum concentrations of penicillins. The rate of absorption from parenteral administration depends upon the dose, concentration, and solubility of the particular salt being administered. The elimination half-life depends on the derivative, but ranges from 0.5 to 2.5 h in the parent compound.

Mechanism of Toxicity

The primary toxic manifestations of penicillin overdose are due to inability of renal excretion due to age, kidney disease, or anaphylaxis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxic amounts of penicillin in animals are not established. Allergic reactions in animals have not been commonly reported in animals. In very large doses, penicillin VK has resulted in neurologic effects in mice and rats.

Human

Hypersensitivity reactions may follow exposure to any amount of penicillins and could result in anaphylaxis. Acute ingestion of large amounts of penicillins ($>250\,mg\,kg^{-1}$) in children less than 6 years of age may result in nausea, vomiting, diarrhea, and abdominal pain. Acute oliguric renal failure, hematuria, and crystalluria have been reported rarely. Intravenous use of penicillin in doses exceeding 10 million units may cause seizures and coma. Cardiac conduction defects have occurred after rapid infusion of potassium penicillin C and procaine penicillin C. Electrolyte abnormalities have been associated with large doses of potassium and sodium salts. Toxicity associated with chronic ingestions is expected to be similar.

Chronic Toxicity (or Exposure)

Animal

No evidence of carcinogenicity was documented in rats and mice receiving 500 or $1000\,mg\,kg^{-1}$ penicillin VK 5 days a week for 2 years.

Human

Most penicillins have significant renal clearance. Patients who have reduced renal function may accumulate large amounts of penicillin over time. Patients with very high serum levels are more likely to develop more serious toxicity (e.g., neurologic effects) than those with lower levels.

In Vitro Toxicity Data

Penicillin V was inconclusive in the *Bacillus subtilis* assay and negative in *Escherichia coli* and male mouse sperm assays.

Clinical Management

Gastric decontamination with activated charcoal may be warranted with large recent ingestions. Urinalysis, renal function tests, and evaluation of electrolytes may be indicated in large exposures. In all cases, regardless of the route of exposure, the mainstay of therapy is supportive care and discontinuation of the drug. For very large overdoses that result in renal impairment, dialysis may be considered for correction of acidosis and electrolytes, rather than for removal of penicillins. Ocular exposures necessitate thorough eye irrigation with water for 15 min. Anaphylaxis is treated with supportive care and the administration of epinephrine and diphenhydramine as symptoms dictate.

See also: Gastrointestinal System.

Further Reading

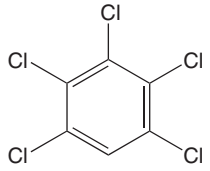
Swanson-Biearman B, Dean BS, and Lopez G (1988) The effects of penicillin and cephalosporin ingestions in children less than six years of age. *Veterinary & Human Toxicology* 30: 66–67.

Thethi AK and Van Dellen RG (2004) Dilemmas and controversies in penicillin allergy. *Immunology and Allergy Clinics of North America* 24(3): 445–461.

Pentachlorobenzene

Jing Liu

- Chemical Abstracts Service Registry Number: CAS 608935
- Synonyms: 1,2,3,4,5-Pentachlorobenzene; Benzene, pentachloro-; Quintochlorobenzene (QCB)
- Chemical/Pharmaceutical/Other Class: Chlorinated benzene
- Chemical Formula: C_6HCl_5
- Chemical Structure:



Uses

Pentachlorobenzene is a chemical intermediate in the production of the fungicide pentachloronitrobenzene. It can be found as a technical impurity in pentachloronitrobenzene formulations. It can also be used as a fire retardant.

Exposure Routes and Pathways

Inhalation and ingestion through contaminated food and water are the primary routes of human exposures.

Toxicokinetics

Pentachlorobenzene can be absorbed readily through gastrointestinal and respiratory tracts in humans and experimental animals. Chlorobenzenes accumulate primarily in fatty tissues and have been shown to cross the placenta. Pentachlorobenzene is metabolized via cytochrome P450-dependent processes to its major metabolites pentachlorophenol and 2,3,4,6-tetrachlorophenol. Food restriction was reported to increase its metabolism. Metabolites are excreted in the urine as mercapturic acids, glucuronic acid, or sulfate conjugates. Some proportion of the chemical is eliminated unchanged in the feces.

Mechanism of Toxicity

There is little information available that describes the exact mechanism of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values for pentachlorobenzene in rats were 940, 1080, and 1125 mg kg^{-1} for weanling females, adult females, and adult males, respectively. The oral LD_{50} values in mice were 1175 and 1370 mg kg^{-1} for males and females, respectively. With high dosages, animals exhibited signs of overt toxicity including tremors and narcosis. Acute toxicity of pentachlorobenzene has been reported to involve the liver, kidney, and thyroid. In one report, changes in plasma alanine aminotransferase (ALT) and liver histopathological profiles, presence of protein droplets in the tubular epithelial cells, and reduction of plasma thyroxine levels were observed in rats treated with relatively low intraperitoneal pentachlorobenzene exposures (1, 2, or 4 mmol kg^{-1}). In rats and mice exposed to pentachlorobenzene in the diet (33–2000 ppm) for 13 weeks, liver weights were increased and there was centrilobular hepatocellular hypertrophy and possible accumulation of porphyrins in the hepatocytes at higher concentrations. Male rats exhibited renal lesions that were characteristic of hyaline droplet nephropathy. Exacerbation of spontaneous nephropathy characterized by renal tubular cell regeneration and homogeneous intratubular protein casts was seen in both male and female rats. Urinary protein levels were increased at 1000 and 2000 ppm dietary pentachlorobenzene.

Human

The limited information regarding effects of chlorobenzenes on human health is restricted to case reports and to mono- and di-chloro congeners. Clinical signs and symptoms of excessive exposure include central nervous system effects, irritation of the eyes and upper respiratory tract, hardening of the skin, and hematological disorders. No report is available specifically regarding pentachlorobenzene in humans.

Chronic Toxicity (or Exposure)

As a family of chemicals, the long-term toxicity of chlorobenzenes increases with ring chloridation. The liver and kidney are the major target organs. Thyroid and hematologic toxicity including

hypothyroxinemia and decreased hematocrit, hemoglobin, and erythrocytes were also reported in animals exposed to higher doses. There is no available evidence that chlorobenzenes are teratogenic in rats or rabbits. Carcinogenicity of pentachlorobenzene has not been fully determined.

In Vitro Toxicity Data

The limited data from both *in vitro* and *in vivo* assays for chlorobenzene isomers other than 1,4-dichlorobenzene indicates that chlorobenzenes are not mutagenic.

Clinical Management

First aid should be provided based on the route of exposure. Clinical treatment is symptomatic.

Environmental Fate

Pentachlorobenzene is persistent and immobile in soil and sediment. Volatilization, adsorption, photooxidation, and aerobic biodegradation primarily control the fate of pentachlorobenzene in the environment. Bioaccumulation in the food chain may occur.

Ecotoxicology

Pentachlorobenzene is toxic to aquatic organisms. The 96 h LC_{50} value for the guppy (*Poecilia reticulate*) is $135\,\mu g\,l^{-1}$. For the water flea (*Daphnia magna*), a 48 h EC_{50} of $122\,\mu g\,l^{-1}$ was reported based on acute immobilization. No toxicity data are available on birds.

Exposure Standards and Guidelines

No threshold limit value is currently available. The reference dose is $0.0008\,mg\,kg^{-1}\,day^{-1}$ based on liver and kidney toxicities.

See also: Pentachloronitrobenzene; Pesticides.

Further Reading

den Besten C, Peters MM, and van Bladeren PJ (1989) The metabolism of pentachlorobenzene by rat liver microsomes: The nature of the reactive intermediates formed. *Biochemical Biophysical Research Communication* 163(3): 1275–1281.

den Besten C, Vet JJ, Besselink HT, *et al.* (1991) The liver, kidney, and thyroid toxicity of chlorinated benzenes. *Toxicology and Applied Pharmacology* 111(1): 69–81.

Linder R, Scotti T, Goldstein J, McElroy K, and Walsh D (1980) Acute and subchronic toxicity of pentachlorobenzene. *Journal of Environmental Pathology and Toxicology* 4(5–6): 183–196.

Umegaki K, Ikegami S, and Ichikawa T (1993) Effects of restricted feeding on the absorption, metabolism, and accumulation of pentachlorobenzene in rats. *Journal of Nutritional Science and Vitaminology* 39(1): 11–22.

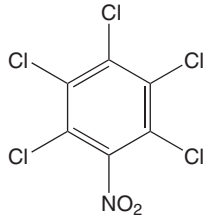
Relevant Websites

http://www.cdc.gov – National Institute of Occupational Safety and Health.
http://www.epa.gov – US Environmental Protection Agency.
http://www.hc-sc.gc.ca – Health Canada.
http://www.inchem.org – International Programme on Chemical Safety.
http://www.ntp-server.niehs.nih.gov – National Institute for Environmental Health Sciences.
http://www.speclab.com – Spectrum Laboratories.

Pentachloronitrobenzene

Jing Liu

- Chemical Abstracts Service Registry Number: CAS 82-68-8
- Synonyms: Quintozene (BSI and ISO preferred name); Avicol; Brassicol; Botrilex; Folosan; Fungiclor; Terraclor; Tilcarex; Tritisan; Tri-PCNB; Turfcide
- Chemical/Pharmaceutical/Other Class: Chlorinated nitrobenzenoid fungicide
- Chemical Formula: $C_6Cl_5NO_2$
- Chemical Structure:



Uses

Quintozene is used as a fungicide on seeds and soil. Most products containing quintozene have been banned in the United States.

Exposure Routes and Pathways

Dermal, oral, and inhalation routes of exposure are all possible. For the general population, exposure is mainly via residues in food.

Toxicokinetics

While very little information is available on absorption of pentachloronitrobenzene via inhalation or dermal contact, absorption from the gastrointestinal tract exhibited large differences among species. For example, quintozene is poorly absorbed from the gastrointestinal tract in rats. In contrast, it is well absorbed from the gastrointestinal tract in monkeys. In animals, quintozene is rapidly metabolized with main metabolites being pentachloroaniline (PCA) and mercapturic acids. A number of minor metabolites including pentachlorothioanisole (PCTA) and pentachlorophenol have also been identified. In soil and on plants, quintozene is metabolized to PCA and PCTA. Unabsorbed quintozene following oral exposure is excreted in the feces and the metabolites of quintozene are primarily excreted in the urine. There is virtually no bioaccumulation of quintozene in tissues. In dogs and rats fed at levels up to 1080 and 500 mg kg^{-1} of quintozene, respectively, for 24 and 33 weeks, no residues were detected in kidney, brain, fat, skeletal muscle, or liver.

Mechanism of Toxicity

There is no information on the exact mechanism of toxicity of quintozene. The aromatic nitro structure of quintozene and the aromatic amine structure of its main metabolite PCA, however, are the common parent structures of known methemoglobinemic agents. Hexachlorobenzene is a major contaminant in the technical material, which may be accountable for some aspects of quintozene's toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The reported oral LD_{50} values of quintozene in male and female rats were 1710 and 1650 mg kg^{-1}, respectively. When given as an aqueous suspension, the oral LD_{50} was greater than 30 g kg^{-1}. Dogs can tolerate quintozene orally up to 2500 mg kg^{-1} without lethality. No signs of toxicity or skin irritation were observed in rabbits exposed dermally once to 4 g kg^{-1} quintozene as a 30% solution. Oral administration of quintozene to cats caused methemoglobinemia and Heinz body formation in erythrocytes.

Human

Among 50 human volunteers, quintozene did not cause skin irritation after a single 48 h dermal exposure. Four of the 50 subjects, immediately after a second exposure 2 weeks later, showed erythema, edema, formation of small vesicles, and itching. Another nine people developed a delayed reaction. A case of keratoconjunctivitis was reported following ocular exposure.

Chronic Toxicity (or Exposure)

Animal

Growth and survival of both sexes of rats were affected by dietary quintozene (5000 mg kg^{-1} for 3 months), with male rats exhibiting higher sensitivity. Liver hypertrophy and fine vacuolization of liver cell cytoplasm were observed in these rats. In dogs consuming 500, 1000, or 5000 mg kg^{-1} quintozene in the diet, liver changes including fibrosis, narrowing of hepatic cell cords, increased periportal areas, and leukocyte infiltration occurred in a dose-related manner. The highest dose of quintozene also caused reduced hematopoiesis and atrophy of the bone marrow. No effects on reproduction and no teratogenic effect were observed in rats that received quintozene orally. There is little information on quintozene's carcinogenic potential. It may cause liver tumors in mice.

Human

There is little information available on chronic effects of quintozene in humans.

In Vitro Toxicity Data

There is no indication for mutagenic activity. Quintozene elicited negative results in the Ames assay and reverse mutation assay.

Clinical Management

Treatment is symptomatic.

Environmental Fate

Pentachloronitrobenzene has an estimated half-life of 1.8 days in water. It is more stable in acidic and neutral conditions. Volatilization, adsorption, and sedimentation as detritus are the major processes responsible for the rapid decrease in quintozene in water. Biodegradation and photolysis are not relevant pathways. In soils, volatilization and biodegradation are important pathways, with anaerobic conditions being more favorable for the degradation.

Ecotoxicology

Quintozene appears to be moderately bioaccumulated in aquatic animals and plants. The toxicity of quintozene for aquatic organisms depends on the species tested. The LC_{50} values in rainbow trout and bluegill sunfish were reported to be 0.55 and 0.1 mg l^{-1}, respectively. On the other hand, a 48 h LC_{50} value of 10 mg l^{-1} for carp and a 3 h LC_{50} value of 40 mg l^{-1} for *Daphnia* have been reported. Quintozene is practically nontoxic to birds and no information is available for bees. Quintozene has a significant effect on earthworm reproduction and survival.

Exposure Standards and Guidelines

The oral reference dose is 0.003 mg kg^{-1} day^{-1}, the threshold limit value is 0.5 mg m^{-3} (8 h), and the acceptable daily intake is 0.007 mg kg^{-1} day^{-1}.

See also: Hexachlorobenzene; Pentachlorobenzene; Pesticides.

Further Reading

Borzelleca JF, Larson PS, Crawford EM, *et al.* (1971) Toxicologic and metabolic studies on pentachloronitrobenzene. *Toxicology and Applied Pharmacology* 18(3): 522–534.

Choudhury H, Colemanm J, Minkm FL, De Rosam CT, and Staram JF (1987) Health and environmental effects profile for pentachloronitrobenzene. *Toxicology and Industrial Health* 3(1): 5–69.

Edwards R, Ferry DG, and Temple WA (1991) Fungicides and related compounds: Quintozene. In: Hayes WJ Jr. and Laws ER Jr. (eds.) *Handbook of Pesticide Toxicology*, pp. 1420–1422. San Diego, CA: Academic Press.

Schumann AM and Borzelleca JF (1978) An assessment of the methemoglobin and Heinz-body-inducing capacity of pentachloronitrobenzene in the cat. *Toxicology and Applied Pharmacology* 44(3): 523–529.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network. Oregon State University.

http://www.inchem.org – International Programme on Chemical Safety (IPCS).

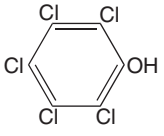
http://www.epa.gov – US Environmental Protection Agency.

http://www.speclab.com – Laboratory Inc.

Pentachlorophenol

Kevin N Baer

- Chemical Abstracts Service Registry Number: CAS 87-86-5
- Synonyms: PCP; Penchlorol; Penta; Pentacon; Penwar; Dowicide EC-7
- Chemical/Pharmaceutical/Other Class: Halogenated phenol
- Chemical Structure:



Uses

Pentachlorophenol was used extensively as an insecticide, herbicide, fungicide, and wood preservative. Since 1984, use of pentachlorophenol in the United States has been restricted to certified pesticide applicators. It is still used industrially as a wood preservative for utility poles, railroad ties, and wharf pilings.

Exposure Routes and Pathway

Dermal contact is the most frequent route of exposure and the majority of poisonings are occupational in origin.

Toxicokinetics

Pentachlorophenol is readily and almost completely absorbed through the skin and gastrointestinal tract. The majority of the compound is excreted primarily unchanged in the urine, although glucuronide conjugates have been detected. In humans, there is conflicting evidence as to whether pentachlorophenol is metabolized in the liver to any significant extent. One metabolite, tetrachlorohydroquinone, was produced in human liver homogenates and was detected in the urine of exposed workers, while in other studies no metabolites were found. Pentachlorophenol is well distributed throughout the tissues, with high concentrations found in the urine, liver, and kidneys. Greater than 90% in blood is bound to serum proteins. The kidney is the primary route of elimination with ~80% excreted in the urine and a smaller amount in the feces. In humans, discrepancies exist concerning the elimination half-life with values ranging from 10 h to 20 days. Although the exact reasons are not

known for the long half-lives, high protein binding with tubular reabsorption and possible enterohepatic circulation may be contributing factors.

Mechanism of Toxicity

Pentachlorophenol increases metabolic rate and elevates body temperature by uncoupling oxidative phosphorylation in tissues. The circulatory system and heart are particularly affected. Pentachlorophenol can be contaminated by dibenzodioxins and dibenzofurans.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute oral LD_{50} value for pentachlorophenol in rats, mice, and rabbits is $\sim$30–200 mg kg^{-1}. Dermal LD_{50} values in rats are somewhat higher ($\sim$100–300 mg kg^{-1}).

Human

Characteristic symptoms of poisoning are extremely high body temperature and profuse sweating. In fatal cases, rapid pulse, coma, heart failure, and death can occur within 3–30 h after initial symptoms appear. Symptoms in nonfatal poisonings include weakness, gastrointestinal upset, headache, dizziness, and seizures. Pentachlorophenol is a potent skin, eye, and upper respiratory tract irritant.

Chronic Toxicity (or Exposure)

Animal

Pentachlorophenol has been demonstrated to be fetotoxic and teratogenic with exposure during early gestation.

Human

Chloracne has been reported in chronic occupational exposure to pentachlorophenol. However, commercial preparations are commonly contaminated with dioxins and furans, and chloracne may be linked to these compounds. In addition, hemolytic and aplastic anemia and weight loss have been reported in humans. Pentachlorophenol is classified as a probable human carcinogen (group 2B).

Clinical Management

Rapid decontamination is important, especially with skin exposure. The primary treatment is supportive and symptomatic and consists of promoting heat loss, reducing anxiety, and replacing fluids and electrolytes lost during sweating. Following oral exposure, emesis, activated charcoal, and cathartics are recommended. Administration of salicylates to reduce the high body temperature is contrain dicated. Single-exchange transfusions have been successfully performed in infants poisoned by pentachlorophenol.

Environmental Fate

Pentachlorophenol is moderately persistent in the soil (half-life of 45 days). It degrades more rapidly under anaerobic conditions, higher temperatures, and in the presence of organic matter. Under alkaline conditions, it is less adsorbed and more mobile. It has been found in groundwater in a number of western states. In water, pentachlorophenol is primarily bound to sediments and suspended particles. The half-life in water ranges from hours to days. It is relatively nonvolatile and does not evaporate to a significant degree. Pentachlorophenol can be taken up by plants and is highly toxic in plants.

Exposure Standards and Guidelines

The time-weighted average (8 h) for pentachlorophenol is 0.5 mg m^{-3}. The reference dose is 0.03 mg kg^{-1} day^{-1}.

See also: Chlorophenols; Pesticides.

Further Reading

Proudfoot AT (2003) Pentachlorophenol poisoning. *Toxicological Reviews* 22(1): 3–11.

Relevant Website

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Pentane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 109-66-0
- Synonyms: Pentane (ACGIH, NIOSH, OSHA, DOT); Pentan (Polish); Pentanen (Dutch); Pentani (Italian); UN1265 (DOT)
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon (C5)
- Chemical Formula: C_5H_{12}

Uses

Pentane is present in volatile petroleum fractions and is used: (1) as a fuel; (2) in the production of ammonia, olefin, and hydrogen; (3) in the manufacture of artificial ice; (4) in low-temperature thermometers; (5) as a blowing agent for plastics and foams; and (6) in solvent extraction processes. Neopentane is important in the manufacture of rubber.

Background Information

Pentane is a colorless, flammable liquid (the first liquid member of the alkanes) that is lighter than water. It has a pleasant odor that can be detected at 900 ppm, and a moderate odor intensity is observed at 5000 ppm. It occurs as two other isomers, including isopentane [$(CH_3)_2CHCH_2CH_3$] and neopentane [$C(CH_3)_4$]. Isopentane (2-methylbutane) apparently has physical and physiological characteristics similar to straight-chain pentane. Neopentane (2,2-dimethylpropane) is similar to butane in physical and physiological characteristics. In air, one part per million of C5 pentane is equivalent to 3 mg m^{-3}.

Exposure Routes and Pathways

As pentane may exist as a vapor or liquid at normal temperature and pressure, exposure would be expected to occur either by inhalation or dermal contact. Oral ingestion would be expected to be incidental or accidental. Typical background concentrations that have been detected in major cities within the United States range from 0.05 to 0.35 ppm.

Mechanism of Toxicity

As seen with other short-chain alkanes, upon inhalation, pentane is moderately toxic and may cause irritation of the respiratory tract and narcosis. The narcotic action of pentane (observed in 1 h at 90 000–120 000 ppm) is, however, much less pronounced than effects seen following exposure to the C1–C4 alkanes. Although the actual biochemical mechanism of toxicity has not been discerned, the narcotic effects seen are most likely related to its physical solvent properties. The effect is similar to the 'high' experienced upon exposure to other aliphatic hydrocarbon solvents.

Acute and Short-Term Toxicity (or Exposure)

Animal

Mice that are acutely exposed to a concentration of 200 000–300 000 mg m^{-3} show 'incoordination and inhibition of the righting reflex' and a pronounced anesthetic effect is seen after 10 min at 7% or 1.3 min at 9% (death will ensue after 37 min of 12.8%). Pentane exposure in dogs will sensitize the heart to epinephrine. In rats, air concentrations of 10.4, 50.9, and 94.7 mg m^{-3} resulted in brain damage in the offspring. As with other aliphatic hydrocarbons, studies have shown that pentane can be utilized by certain microorganisms as a nutrient.

Human

According to a 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, NIOSH), the majority of persons exposed to pentane in the workplace are operators of molding and casting machines, lathes and turning machines, separating/filtering/clarifying machines, hand molders and shapers, computer operators, chemists, and biological/chemical technicians. It is generally known that human exposure for 10 min at 5000 ppm does not cause any adverse effects or irritation of the mucous membranes. Lethal effects have been observed, however, in estimated air concentrations of 130 000 ppm; the lowest concentration known to cause a toxic effect is 90 000 ppm. Some studies have implicated pentane as a neurotoxicant, but these study results are confounded by the presence of other compounds in the mixture.

Chronic Toxicity (or Exposure)

Human

Prolonged skin contact may cause drying, cracking, and dermatitis.

Clinical Management

Persons exposed to high concentrations should vacate or be removed from the source of the liquid or

vapor and seek fresh air. Extreme care must be taken to keep areas of high concentration free from ignition sources, such as sparks from static electricity and use of explosion-proof apparatus.

Environmental Fate

When released into soil, pentane may biodegrade to a moderate extent, is expected to quickly evaporate, and is not expected to leach into groundwater. Pentane has a half-life of less than 1 day in water with an estimated bioconcentration factor of less than 100 and a log octanol–water partition coefficient of greater than 3.0. Thus, it is not expected to significantly bioaccumulate. When released into the air, pentane is expected to be readily degraded by reaction with photochemically produced hydroxyl radicals.

Ecotoxicology

Young Coho salmon exposed to pentane in the laboratory (salt water) show no effect on lethality below $100\,mg\,l^{-1}$. A freshwater EC_{50} of $9.74\,mg\,l^{-1}$ has been reported for *Daphnia magna* (or the water concentration required to immobilize approximately one-half of the test organisms).

Other Hazards

Pentane is highly flammable and readily forms explosive mixtures with air.

Exposure Standards and Guidelines

NIOSH recommends a workplace standard of $350\,mg\,m^{-3}$ (120 ppm) for the C5–C8 alkanes, and a short-term exposure limit (STEL) of $1800\,mg\,m^{-3}$ (610 ppm). The American Conference of Governmental Industrial Hygienists suggests a workplace environmental standard of $1770\,mg\,m^{-3}$ (600 ppm) and a STEL of $750\,mg\,m^{-3}$ (2210 ppm). Chemical exposure kits are available for individual monitoring (e.g., wearing chemical detection badges) of pentane in the workplace. Pentane is also highly flammable and is therefore an explosion and/or fire hazard. The upper and lower explosive limits are 1.5% and 7.8% by volume, respectively. No threshold limit values are available for other isomers of pentane, so time-weighted average and ceiling values established for pentane are therefore recommended.

See also: Petroleum Hydrocarbons.

Further Reading

Stadler JC, O'Neill AJ, Elliott GS, and Kennedy GL Jr. (2001) Repeated exposure inhalation study of pentane in rats. *Drug and Chemical Toxicology* 24(2): 75–86.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Pentane.

Pentazocine

Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 359-83-1
- Synonyms: Fortral; Talacen; Talwin NX; Talwin
- Chemical/Pharmaceutical/Other Class: Synthetic opiate agonist and antagonist

Uses

Pentazocine is used as an analgesic. It may also be diverted as a drug of abuse.

Exposure Routes and Pathways

Exposures occur with ingestion or injection of solubilized tablets.

Toxicokinetics

Pentazocine is well absorbed from the gastrointestinal tract. The onset of action following oral administration occurs within 15–30 min, with peak blood levels occurring at 0.5–4.0 h. Following parenteral administration, the onset of effects are observed within 3 min with peak serum levels at 30 min. Protein binding is ~60%. The volume of distribution is $4–8\,l\,kg^{-1}$. Pentazocine is largely metabolized in the liver to *cis*-hydroxypentazocine and *trans*-carboxypentazocine. These metabolites are excreted in the urine with 5–8% of pentazocine excreted

unchanged in the urine. The elimination half-life ranges from 1.5 to 10 h.

Mechanism of Toxicity

Pentazocine is an opioid agonist and a partial opioid antagonist. It produces an analgesic effect by stimulating kappa and sigma receptors. It increases serotonin activity through sigma receptor agonism and produces partial opioid antagonism by inhibiting mu receptors. Pentazocine also potentially inhibits dopamine receptors and increases norepinephrine turnover.

Acute and Short-Term Toxicity (or Exposure)

Animal

In cases of acute exposure, dogs have experienced mild to moderate salivation, slight transient ataxia, fine tremors, and (infrequently) tonic convulsions. Horses, following intravenous administration, have experienced slight to moderate ataxia, localized muscular twitching, slight perspiration, unsteady gait, excitability, and nervousness.

Human

Acute toxicity induced by pentazocine is primarily associated with central nervous system (CNS) effects that include dizziness, anxiety, hallucinations, mood alterations, and seizures. Respiratory depression, increased P_aCO_2 levels, pulmonary edema, and apnea may occur. Tachycardia, increased systolic and diastolic blood pressure, pinpoint pupils, nausea, vomiting, and abdominal pain have also been reported. In a recently published case series, 40% of acute pentazocine overdose patients did not have the classic opioid toxidrome of CNS and respiratory depression with miosis.

Chronic Toxicity (or Exposure)

Human

Psychological and physical dependence may occur. Tolerance may develop, resulting in the need for higher and more frequent dosing. Some oral pentazocine preparations also contain naloxone (an opioid antagonist) to reduce parenteral abuse. Naloxone does not affect the efficacy of pentazocine administered by the oral route; naloxone does inhibit pentazocine's opioid effect if tablets are solubilized and injected. Pentazocine may be abused as a heroin alternative or in combination with other drugs. The most publicized combination was 'T's and Blues' (Talwin mixed with blue-colored pyribenzamine tablets). Since pentazocine can produce dependency, abrupt cessation may precipitate the opioid withdrawal syndrome. Chronic intramuscular pentazocine injections have been associated with fibrous myopathy and localized neuropathy.

In Vitro Toxicity Data

Pentazocine is used as a Sigma1 ligand in receptor binding studies. These ligands are of interest because of their potential role in the development of substance abuse syndromes as well as because of the changes in receptor quantity and binding affinity seen in humans and in animal brains during aging.

Clinical Management

Cardiac and respiratory stabilization are the first priorities following pentazocine poisoning. The patient's airway should be patent and adequate ventilation assured. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk for subsequent CO_2 narcosis with worsening acidosis or aspiration. If necessary, endotracheal tube intubation should be performed. Close monitoring of the patient's pulmonary exam should be performed to assure that pulmonary edema does not develop. The health care providers should place the patient on continuous cardiac monitoring with pulse oximetry and make frequent neurological checks.

Gastrointestinal decontamination should be considered for patients who have ingested pentazocine only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered. Syrup of ipecac is contraindicated after overdose with pentazocine due to the potential for rapid clinical deterioration. Gastric lavage is not indicated. Naloxone can be infused in an attempt to reverse respiratory and CNS depression. Naloxone administration may precipitate opioid withdrawal and should be administered slowly. Recent case series have demonstrated that naloxone may not result in clinical improvement in the majority of patients who have overdosed on pentazocine.

See also: Drugs of Abuse; Heroin.

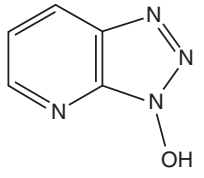
Further Reading

Challoner KR, McCarron MM, and Newton EJ (1990) Pentazocine (Talwin®) intoxication: Report of 57 cases. *Journal of Emergency Medicine* 8: 67–74.

Peptide Coupling Agents

Sang-Tae Kim

- Representative Chemicals: 1-Hydroxy-7-azabenzotriazole; *O*-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; 7-Azabenzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate
- Chemical Abstracts Service Registry Numbers: CAS 39968-33-7; (1-Hydroxy-7-azabenzotriazole); CAS 148893-10-1 (*O*-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate); CAS 156311-83-0 (7-Azabenzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate)
- Synonyms: HOAt: 1-Hydroxy-1*H*-v-triazolo[4,5-b]pyridine; 7-Aza-1-hydroxybenzotriazole; [1,2,3]Triazolo[4,5-*b*]pyridin-3-ol; 3-Hydroxy-3*H*-1,2,3-triazolo[4,5-*b*]pyridine HATU: 1*H*-1,2,3-Triazolo[4,5-*b*]pyridinium, 1-[bis(dimethylamino)methylene]-, hexafluorophosphate(1-), 3-oxide; HATU may crystallize as guanidinimum *N*-oxides (*N*-form), rather than the isomeric uranium structures (*O*-form) depending on storage conditions: *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridine-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate. PyAOP: Tri-1-pyrrolidinyl(3H-1,2,3-triazolo[4,5-b]pyridin-3-yloxy)-, hexafluorophosphate
- Chemical Structure:



HOAt

Uses

HOAt, as additives, and HOAt-based peptide coupling reagents, as activators, are used in peptide bond formation reactions both in solution and solid phase synthesis.

There are many different types of peptide coupling reagents (e.g., carbodiimides, aminium/uranium salts, and phosphonium salts). The choice of method(s) and reagent(s) depend on a variety of factors, including the specific sequence of peptide to be synthesized, the preferred method of deprotection, the preferred solvents, and the type of active intermediate desired.

Exposure Routes and Pathways

Exposure to HOAt- and HOAt-based peptide coupling reagents is most likely to occur in occupational settings. Inhalation and dermal exposure are the primary routes of exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

HOAt was nontoxic to rats when dosed at $2000\,mg\,kg^{-1}$ via the oral route. HOAt was not a skin irritant in rabbits. In rabbits, HOAt was found to be a slight eye irritant. Studies in guinea pigs revealed that HOAt did not induce contact allergy. HOAt did not induce micronuclei in bone marrow polychromatic erythrocytes in male mice when evaluated in the *in vivo* micronucleus assay.

HATU showed no mortality, but limited toxicity, to rats when dosed at $2000\,mg\,kg^{-1}$ via the oral route. HATU was not a skin irritant in rabbits. In rabbits, HATU was found to be a slight eye irritant. Studies in guinea pigs revealed that HATU induced contact allergy.

PyAOP was nontoxic to rats when dosed at $2000\,mg\,kg^{-1}$ via the oral route. PyAOP was not a skin irritant in rabbits. In rabbits, PyAOP was found to be a very severe eye irritant. Studies in guinea pigs revealed that PyAOP induced contact allergy.

Human

The principal hazard of concentrated HOAt-based peptide coupling reagents is the potential to cause contact allergy. Signs and symptoms of exposure to HATU may include skin rashes, 'red face', itching, local swelling, headaches, coughing, eye irritation and swelling, respiratory congestion, shortness of breath, and flue-like symptoms. Symptoms may progress to include severe dyspnea, cephalgia, rhinorrhea, flatulence, obstruction of bronchial airways, and permanent fine whistle of bronchial airways. The likelihood and severity of adverse health effects due to exposure to HATU depends on (1) the concentration in the air, (2) how long the individual is exposed, and (3) the individual's susceptibility to the effects of HATU.

Prolonged eye contact to the solid form of HOAt may cause eye irritation. Information on the toxic effects of HOAt by other routes of exposure in humans is not available.

In Vitro Toxicity Data

HOAt was mutagenic to *Salmonella typhimurium* and *Escherichia coli* strains.

HOAt was weakly mutagenic in mouse lymphoma L5178Y cells, in the absence of S9 mix. In the presence of S9 mix, the evidence was inconclusive. HATU and PyAOP were not mutagenic to *S. typhimurium* and *E. coli* strains.

Clinical Management

The victim should be removed from the exposure environment. Exposed skin and eye should be copiously flushed with water and thoroughly decontaminated to prevent further absorption. Contaminated clothing and shoes should be removed and isolated at the site.

Ecotoxicology

HOAt showed no significant inhibition in a study designed to evaluate the effects on the respiration rate of sewage sludge microorganisms contained in activated sludge. HOAt and HATU were not readily biodegradable when evaluated in 28 day modified Sturm tests.

See also: Skin.

Further Reading

Han S-Y and Kim Y-A (2004) Recent development of peptide coupling reagents in organic synthesis. *Tetrahedron* 60: 2447–2467.

Perchlorate

David R Mattie

Published by Elsevier Inc.

- Representative Chemicals: Ammonium perchlorate, Sodium perchlorate, Potassium perchlorate
- Chemical Abstracts Service Registry Numbers: CAS 14797-73-0 (Perchlorate); CAS 7790-98-9 (Ammonium perchlorate); CAS 7601-89-0 (Sodium perchlorate); CAS 7778-74-7 (Potassium perchlorate)
- Chemical/Pharmaceutical/Other Class: Ternary salts of alkali metals containing oxygen
- Chemical Formulas: ClO_4^- (Perchlorate); NH_4ClO_4 (Ammonium perchlorate); $NaClO_4$ (Sodium perchlorate); $KClO_4$ (Potassium perchlorate)
- Chemical Structures:

Uses

The primary source of perchlorate is the ammonium salt. Ammonium perchlorate is the oxidizer ingredient in solid propellant mixtures for rockets, missiles, and munitions. Other uses of perchlorate salts include medicine, matches, metal cation chemistry, and pyrotechnics (illuminating and signaling flares, colored and white smoke generators, tracers, incendiary delays, fuses, photo-flash compounds, and fireworks). Perchlorate is also found in lubricating oils, finished leather, fabric fixer, dyes, electroplating, aluminum refining, manufacture of rubber, paint and enamel production, as an additive in cattle feed, in magnesium batteries, and as a component of automobile air bag inflators.

Exposure Routes and Pathways

Perchlorate has been found in soil, surface and groundwater, at locations where perchlorate salts were manufactured, stored, or used. The salts are highly water soluble, fully ionize in water, and result in a perchlorate ion that is identical whether it comes from the ammonium, sodium, or potassium salt. The primary route of exposure for perchlorate is through ingestion of water from contaminated public drinking water supplies across the United States. Occupational exposure of workers during the commercial production or use of ammonium perchlorate is higher than potential exposures from drinking water sources. Exposure to perchlorate is primarily through inhalation of ammonium perchlorate dust with systemic absorption through mucous membranes in the respiratory and gastrointestinal tracts. Some ingestion through the oral route is possible, as is dermal contact, although significant absorption of perchlorate through intact skin is unlikely.

Toxicokinetics

Studies of absorption, distribution, metabolism, and elimination to measure perchlorate kinetics revealed

that there was no metabolism of perchlorate in either adult rats or humans. Perchlorate is rapidly excreted, with urinary half-lives on the order of 4 h in the rat and 6 h in humans. Kinetic studies were also conducted for fetal and lactational time points in rats. Kinetic studies were designed to aid quantitative interspecies extrapolation as well as form the basis for physiologically based pharmacokinetic (PBPK) models for adult rats and humans, as well as pregnant and lactating rats.

Mechanism of Toxicity

The perchlorate ion, because of its similarity to iodide in ionic size and charge, competes with iodide for uptake into the thyroid gland. At therapeutic dosage levels (100–1000 mg day^{-1}), this competitive inhibition results in reduced production of the thyroid hormones T_3 and T_4 and a consequent increase in thyroid stimulating hormone (TSH) via a negative feedback loop involving the thyroid, pituitary, and hypothalamus. The competitive inhibition of iodide uptake is the only direct perchlorate effect on the thyroid, leading to a reversible chemical-induced iodine deficiency. Inhibition of iodide uptake in the thyroid of adult male rats dosed intravenously was detected at a dose as low as 0.01 mg kg^{-1} perchlorate.

Acute and Short-Term Toxicity (or Exposure)

Animal

A 90 day subchronic bioassay determined that the thyroid was the only target organ in male and female rats exposed to perchlorate in drinking water (0, 0.01, 0.05, 0.2, 1.0, and 10 mg kg^{-1} day^{-1}) for 90 days. The no-observed-adverse-effect level (NOAEL) based on thyroid changes was 1 mg kg^{-1} day^{-1} but hormone changes, decreased T_4 and increased TSH, were still seen at lowest doses.

Developmental neurotoxicity studies exposed pregnant rats to perchlorate in drinking water (0, 0.1, 1.0, 3.0, and 10 mg kg^{-1} day^{-1}) during pregnancy through day 10 of lactation. No pup behavioral effects were seen except equivocal motor activity at one time point. An additional motor activity study with the same doses found no statistically significant effects in motor activity. However, Bayesian statistical analysis on the results of the two different motor activity studies combined resulted in a NOAEL at 1.0 mg kg^{-1} day^{-1}. Hormone changes, decreased T_4, increased TSH, were again seen at lower doses. Brain histology and morphometry observations in developmental studies are equivocal.

Genotoxicity assays showed that perchlorate is not genotoxic or mutagenic. Perchlorate is not a teratogen as no birth defects were found at doses as high as 100 mg kg^{-1} day^{-1} in the rabbit or as high as 30 mg kg^{-1} day^{-1} in the rat. Immunotoxicity studies were motivated by case reports of aplastic anemia and leukopenia in humans when perchlorate was used as an antithyroid drug. Studies using female mice did not demonstrate any adverse effects to the immune system. Evaluation of thyroid responses identified no alterations in T_3 and TSH, while T_4 was decreased after exposure to 1.0, 3.0, or 30 mg kg^{-1} day^{-1}. Thyroid changes detected histologically were not seen in all animals until the 30 mg kg^{-1} day^{-1} dose.

Human

Two 14 day studies were conducted in which 10 mg day^{-1} was provided in water to 10 male subjects and 3 mg day^{-1} was provided in drinking water to 8 male subjects. In both studies, each subject served as their own control by having measurements taken before and after perchlorate consumption. Iodide-123 was measured in the thyroid to obtain inhibition data, and iodide and perchlorate were determined in blood and urine. Perchlorate, at both 3 and 10 mg day^{-1}, caused inhibition of iodide uptake into the thyroid (38% and 10%, respectively). There were no changes seen in TSH or thyroid hormone levels in the blood. The extrapolated no-observed-effect level for iodide inhibition was 2 mg day^{-1} based on these two exposures.

Another 14 day study employed 10 subjects (5 male/5 female) for each dose (0.5, 0.1, 0.02, and 0.007 mg kg^{-1} day^{-1}) who also served as their own control. The parameters measured were iodide-123 uptake in the thyroid for inhibition data and iodide and perchlorate in blood and urine for kinetic data. There were no changes seen in TSH or thyroid hormone levels in the blood. The result of the iodide inhibition measurements was a NOAEL of 0.007 mg kg^{-1} day^{-1}, resulting in 4.8% iodide inhibition (equivalent to 0.5 mg day^{-1} perchlorate exposure). Data from these studies were used to develop the human PBPK model for perchlorate.

Chronic Toxicity (or Exposure)

Animal

A two-generation reproductive toxicity study was used to evaluate fertility in adult rats and viability/toxicity in their offspring. Reproductive parameters were tested over two generations of drinking water exposure to perchlorate. The reproductive NOAEL is

greater than the highest dose tested, 30 mg kg^{-1} day^{-1}. Thyroid histology changes were seen starting at 3 mg kg^{-1} day^{-1}. There were three rare benign thyroid tumors seen in two first generation (F1) pups at the 30 mg kg^{-1} day^{-1} dose.

Human

Employees were examined at an ammonium perchlorate production facility in Nevada and their findings compared to those of a large control population from the same chemical complex. The average working-lifetime cumulative dose in the higher exposure group was estimated to be 38 mg kg^{-1}. Based on both cumulative and single-shift perchlorate exposures there were no adverse effects on thyroid, kidney, liver, or bone marrow function. A cross-sectional health study of two similar worker populations, a group of ammonium perchlorate workers in three exposure groups and a comparison group of other workers from the same industrial complex, was conducted at a perchlorate manufacturing plant in Utah. More than 40% of the workers had been working with perchlorate for more than 5 years. There were no effects on blood and clinical chemical parameters at any level of exposure up to 34 mg day^{-1}.

School-age children were examined in three cities in Northern Chile where levels of perchlorate in the water supply were undetectable, 5–7, and 100–120 ppb. No changes were found in hormone levels, prevalence of goiter between cities, congenital hypothyroidism, and clinical differences between children from the three different cities.

Perchlorate has been found in one Southern Nevada county at levels averaging 14 μg l^{-1} and as high as 24 μg l^{-1}. The congenital hypothyroidism data from the neonatal screening program for 1996 and 1997 were examined, and no increase in congenital hypothyroidism was observed in the county. The monthly mean T_4 levels of neonates from Las Vegas (an area with perchlorate-contaminated drinking water at 9–15 ppb (μg l^{-1}) for eight of those months and nondetectable (i.e., <4 ppb) for 7 months) were compared with those of neonates from Reno (an area with no detectable perchlorate in its drinking water) for the 15 month period of April 1998 through June 1999. There were no differences in neonatal T_4 levels between Las Vegas and Reno. An analysis of the neonatal TSH levels of newborns from Las Vegas and Reno was conducted for those born between December 1998 and October 1999 with a birth weight of 2500–4500 g and sampled within the first month of life. The mean blood TSH levels were not different in Las Vegas versus Reno. Another Las Vegas versus Reno comparison examined prevalence rates among Medicaid-eligible residents for simple goiter, nodular goiter, thyrotoxicosis, congenital hypothyroidism, acquired hypothyroidism, thyroid cancer, or other thyroid diseases. Again there were no differences between Las Vegas and Reno.

Environmental Fate

Perchlorate is exceedingly mobile in aqueous systems and can persist for many decades under typical ground and surface water conditions. Plants and vegetables grown in perchlorate-containing soil or water may incorporate the perchlorate, posing a potential exposure if such vegetation is consumed.

Other Hazards

Safety concerns with ammonium perchlorate are greater than toxicity concerns because of its explosive potential.

Exposures Standards and Guideline

There is no occupational standard for perchlorate but the Occupational Safety and Health Administration regulates perchlorate as a nuisance dust, with an 8h time-weighted average permissible exposure limit of 15 mg m^{-3}. Studies have demonstrated that occupational exposures to perchlorate have not been hazardous to the health of workers at manufacturing plants.

There are no environmental standards for perchlorate; however, the Office of Research and Development of the US Environmental Protection Agency (ORD/EPA) released the 1999 Interim Guidance on June 18, 1999 because of significant concerns and uncertainties that needed to be addressed in order to finalize a human health oral risk benchmark for perchlorate. That guidance recommended that agency risk assessors and risk managers continue to use the standing provisional reference dose range of 0.0001–0.0005 mg kg^{-1} day^{-1} (4–18 ppb) for perchlorate-related assessment activities. In the absence of a finalized oral health risk benchmark for perchlorate, but in light of ongoing assessment activities by EPA, states, and other interested parties, the ORD/EPA reaffirmed this guidance on January 22, 2003 with an added suggestion to carefully consider the low end of the provisional 4–18 ppb range.

See also: Ammonium Perchlorate.

Further Reading

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Soldin O, Braverman L, and Lamm S (2001) Perchlorate clinical pharmacology and human health: A review. *Therapeutic Drug Monitoring* 23: 316–331.

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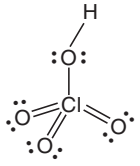
Perchloric Acid

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 7601-90-3
- Synonyms: Dioxonium perchlorate; Hydronium perchlorate
- Chemical/Pharmaceutical/Other Class: Halogenated acid
- Chemical Formula: $ClHO_4$
- Chemical Structure:



Uses

Perchloric acid is used to separate potassium from sodium, and in many laboratory tests and industrial processes. Uses for the salts of perchloric acid include explosives and plating metals. Perchloric acid may explode, and it decomposes on heating producing toxic and corrosive fumes. The substance is a strong oxidant and reacts violently with combustible and reducing materials, organic materials and strong bases, causing a fire and explosion hazard. It attacks many metals forming flammable/explosive gas. The acid is unstable if the concentration is over 72%; it may explode by shock or concussion when dry or drying. Mixtures with combustible material such as paper may ignite spontaneously at room temperature. Water should never be poured into perchloric acid; when dissolving or diluting always add perchloric acid slowly to the water. A mixture of perchloric acid and acetic anhydride exploded in a Los Angeles factory in 1947, killing 15, injuring 400, and causing $2 million in damage.

Exposure Routes and Pathways

Contact with skin and mucous membranes are the exposure pathways. Inhalation of mist formed from solutions is possible.

Toxicokinetics

Perchloric acid can be absorbed into the body by inhalation and by ingestion.

Mechanism of Toxicity

Perchloric acid's corrosive properties and ability to cause tissue oxidation are mechanisms of toxicity. Perchlorate (ClO_4^-) disrupts endocrine homeostasis by competitively inhibiting the transport of iodide (I^-) into the thyroid through the sodium iodide symporter. Potential human health risks exist from chronic exposure to perchlorate via drinking water. Such risks may include hypothyroidism, goiter, and mental retardation (if exposure occurs during critical periods in neurodevelopment).

Acute and Short-Term Toxicity (or Exposure)

Animal

Perchloric acid is a severe irritant and is corrosive to eyes, skin, and mucous membranes. The oral LD_{50} is $1100\,mg\,kg^{-1}$ in rats and $400\,mg\,kg^{-1}$ in dogs. The subcutaneous LD_{50} in mice is $250\,mg\,kg^{-1}$.

Human

Perchloric acid is corrosive to eyes, skin, and mucous membranes. Ingestion may produce mild to moderate oral and esophageal burns with more severe burns occurring in the stomach. Symptoms of lung edema might not become manifest until a few hours have passed, and are aggravated by physical effort.

Chronic Toxicity (or Exposure)

Human

Chronic exposure can cause bronchial irritation, gastrointestinal disturbances, rash, possible sensitization, dental erosion, and jaw necroses. Inhalation can cause upper respiratory tract effects and possible dyspenea and hemoptysis.

Clinical Management

Contaminated clothing should be removed and exposed body surfaces should be washed thoroughly. Respiratory assistance should be given if necessary. If ingested, a conscious patient should be given large amounts of water and 1 oz milk of magnesia if available. Vomiting should not be induced. Eyes should be flushed with large amounts of water if eye contact occurs. Treatment is the same as for exposure to any strong inorganic acid.

Ecotoxicology

Perchloric acid may be toxic to aquatic life. Degradation products are toxic.

See also: Acids; Ammonium Perchlorate; Corrosives.

Relevant Websites

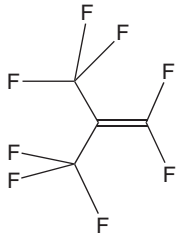
http://www.auburn.edu – Perchloric Acid (adapted from the *CRC Handbook of Laboratory Safety*).

http://www.inchem.org – International Programme on Chemical Safety (IPCS). Perchloric Acid (IPCS International Chemical Safety Card 1006).

Perfluoroisobutylene

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 3812-21-8
- Synonyms: Octafluoro-*s*-butene; Octafluoroisobutylene; PFIB; Isobutene; Octafluoroisobutene; Perfluoroisobutene
- Chemical/Pharmaceutical/Other Class: Perfluorocarbon
- Chemical Formula: C_4F_8
- Chemical Structure:



Uses

Perfluoroisobutylene or perfluoroisobutene (PFIB) is the monomer used in synthesis of Teflon®. It has possible use as chemical warfare agent, that is, it is a schedule 2A substance under the Chemical Weapons Convention (CWC).

Exposure Routes and Pathways

PFIB is produced by the pyrolysis, and as a by-product during the manufacture of 'Teflon' fluorocarbon resins, for example, polytetrafluorethylene (PTFE). Inhalation is the major exposure pathway.

Toxicokinetics

PFIB is readily absorbed. The retention in rats of inhaled PFIB in the upper airways and lungs was found to be ~25% of the amount inspired at the concentrations tested.

Mechanism of Toxicity

PFIB is a strong electrophile which reacts with nucleophiles. The toxicity of PFIB may be correlated with its susceptibility to nucleophilic attack and the generation of reactive intermediates.

Acute and Short-Term Toxicity (or Exposure)

Animal

Similar to phosgene but reported to be 10 times as acutely lethal. When PFIB is inhaled it produces a fulminating and sometimes fatal pulmonary edema similar to that of phosgene after a latent period of several hours. The rat LC_{50} is 500 ppb for a 6 h exposure. Lung injuries caused by the inhalation of PFIB have been examined in a study where rats were exposed to 50, 83, 90, 110, or 200 mg m^{-3} of PFIB for 10 min. At exposure to 90 mg m^{-3} or more, lung injuries began to be detected histologically within

hours after exposure, with the latency periods being inversely proportional to PFIB concentrations, so that at the high concentration of 200 mg m^{-3} no latency period was detectable. Significant accumulations of edema fluid were not apparent until 9 h after PFIB exposure. Significant amounts of fibrin were detected in alveolar spaces at 18–24 h after exposure, but no fibrin was evident at 48–72 h. Significant increases in alveolar macrophages (AMs) were observed at 10 h after exposure, with peak increases between 24 and 48 h postexposure. Even at 1 h after exposure, the alveolar epithelial cells and endothelial cells showed abnormal vacuolation and blebbing. Progressively, the alveolar surface was denuded, leading to edema and extravasation. The AM appeared relatively insensitive to the toxic effects of PFIB.

Human

The toxicity observed is called Polymer fume fever. The first symptom of poisoning is a cough and difficult breathing immediately after inhaling the fumes. The symptomology becomes progressively worse. Pathological changes in the lungs occur in the first 4–6 h postexposure and increase in severity in the first 2 days. Improvement occurs on the fifth to sixth days.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to 0.1 ppm PFIB for 6 h day^{-1}, 5 day week^{-1} for 2 weeks showed no compound-related pathological changes and only mild respiratory impairment and restlessness during their exposure. A repeat study using the same experimental conditions (0.1 ppm) found no effects in rats.

Human

No information could be found on chronic toxicity of PFIB in humans.

Clinical Management

Exposure should be terminated and supportive management provided.

Ecotoxicology

PFIB decomposes rapidly when dissolved in water, forming various reactive intermediates and fluorophosgene, which then decomposes into carbon dioxide, a radical anion and hydrogen fluoride.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists, (US) Occupational Safety and Health Administration, and the (US) National Institute for Occupational Safety and Health have not set exposure standards for PFIB. Although there is no hygienic standard for the safe handling and use of 'Teflon' fluorocarbon resins, specifically PTFE and fluorinated-ethylene–propylene polymers manufactured by the DuPont Company, a publication from the American Industrial Hygiene Association many years ago noted that a maximum atmospheric concentration of 15 mg m^{-3} may be tolerated over an 8 h period on a nuisance basis without significant hazard, since the oral and inhalation toxicities of the undecomposed polymers are practically nil. Further, it noted that: (1) decomposition products appear only at temperatures above 200°C, (2) no practical way has yet been devised to express safe concentrations of the various possible mixtures of the decomposition products, which include PFIB, (3) above 250°C, toxicologically significant amounts of these products are evolved and polymer fume fever may result from exposure to them or from smoking Teflon-contaminated cigarettes, and (4) the decomposition products become flammable above 690°C.

See also: Combustion Toxicology; Phosgene.

Further Reading

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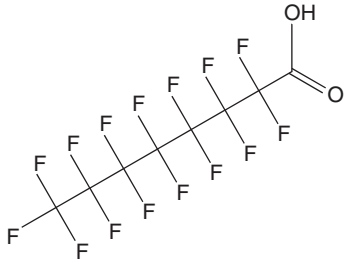
Van Helden HPM, van de Meent D, Oostdijk JP, *et al.* (2004) Protection of rats against perfluoroisobutene (PFIB)-induced pulmonary edema by curosurf and *N*-acetylcysteine. *Inhalation Toxicology* 16: 549–564.

Perfluorinated Chemicals *See* Perfluorooctanoic Acid (PFOA).

Perfluorooctanoic Acid (PFOA)

Cathy Villaroman and Ruth Custance

- Chemical Abstracts Service Registry Numbers:
  - Free acid ($X = OM^+$; $M = H$) (CAS 335-67-1)
  - Ammonium salt ($X = OM^+$; $M = NH_4$) (CAS 3825-26-1)
  - Sodium salt ($X = OM^+$; $M = Na$) (CAS 335-95-5)
  - Potassium salt ($X = OM^+$; $M = K$) (CAS 2395-00-8)
  - Silver salt ($X = OM^+$; $M = Ag$) (CAS 335-93-3)
  - Acid fluoride ($X = F$) (CAS 335-66-0)
  - Methyl ester ($X = CH_3$) (CAS 376-27-2)
  - Ethyl ester ($X = CH_2–CH_3$) (CAS 3108-24-5)
- Synonyms: 1-Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-PFOA; Pentadecafluorooctanoic acid; Pentadecafluoro-1-octanoic acid; Perfluorocaprylic acid; Perfluoroheptanecarboxylic acid; Perfluorooctanoic acid; Perfluoro-*n*-octanoic acid; Hexanoyl fluoride, 3,3,4,4,5,5,6,6,6-nonafluoro-2-oxo-; Pentadecafluoro-*n*-octanoic acid; Perfluoroctanoic acid; PFOA; Fluorad FC-26; 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluorooctanoic acid
- Chemical Formula: $C_8HF_{15}O_2$
- Chemical Structure:



Uses

Perfluorooctanoic acid, also known as PFOA, is used as a processing aid in the manufacture of fluoropolymers, which are used in a wide variety of consumer and industrial applications, including nonstick surfaces on cookware. However, finished products are not expected to contain PFOA. It may also be formed during the degradation of related chemicals, such as small polymers called telomers, which are used in a range of commercial products including fire fighting foams, as well as soil-, stain-, and grease-resistant coatings on carpets, textiles, paper, and leather.

Exposure Routes and Pathways

PFOA has been detected in workers and it has also been measured in the general population. The highest levels reported to date in the general population are similar to some of the lowest levels in workers exposed to PFOA occupationally. Neither the environmental concentrations of PFOA nor the pathways of exposure to the general population are known. The limited geographic locations of fluorochemical plants making or using the chemical suggest that there may be additional sources of PFOA in the environment and exposures beyond those attributable to direct releases from industrial facilities. But whether human exposures are due to PFOA in air, water, on dusts or sediments, in dietary sources, or through some combination of routes is currently unknown.

Toxicokinetics

Animal studies have shown that the ammonium salt of PFOA (ammonium perfluorooctanoate; APFO) is well absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. PFOA distributes primarily to the liver and plasma and may be detected in the blood stream after exposure. It does not partition to the lipid fraction or adipose tissue. PFOA is not metabolized and there is evidence of enterohepatic circulation of the compound.

Gender differences were observed in the elimination of PFOA in rats. The urine is the major route of excretion of PFOA in females, while the urine and feces are both main routes of excretion in males. Moreover, estimates of the serum half-life range from 1.9 to 24 h in female rats, while estimates of the serum half-life range from 4.4 to 9 days in male rats. Elimination of PFOA appears to be biphasic in female rats; a fast phase occurs with a half-life of ~2–4 h while a slow phase occurs with a half-life of ~24 h. The rapid excretion of PFOA by female rats is reportedly due to active renal tubular secretion (organic acid transport system), which is thought to be hormonally controlled. Hormonal changes during pregnancy do not appear to change the rate of elimination in rats.

Additionally, substantial differences have been observed in the half-life of PFOA in rats, monkeys, and humans. The gender and species differences are not completely understood; therefore, the extent of potential risks to humans is uncertain.

Mechanism of Toxicity

PFOA is thought to induce peroxisome proliferation and interfere with mitochondrial metabolic pathways. Direct measurements revealed that PFOA uncouple mitochondrial respiration by increasing

proton conductance. At sufficiently high concentrations, PFOA had the capacity to interfere with mitochondrial respiration by causing a slight increase in the intrinsic proton leak of the mitochondrial inner membrane, which resembled a surfactant-like change in membrane fluidity. The protonated nitrogen atom with a favorable pK_a is reportedly essential for the uncoupling action of perfluorooctane sulfonamides in mitochondria, which may be critical to the mechanism by which these compounds interfere with mitochondrial metabolism to induce peroxisome proliferation *in vivo*.

Acute and Short-Term Toxicity (or Exposure)

Animal

Most animal toxicity studies have been conducted with APFO, the most widely used salt of PFOA. Several animal toxicity studies have been conducted in rodents and monkeys and have shown that APFO exposure can result in a variety of toxic effects in animals including liver toxicity, developmental toxicity, and immunotoxicity.

Recent studies show organ weight changes among laboratory animals exposed to PFOA *in utero* and into early adulthood. The prenatal developmental toxicity studies in rats resulted in death and reduced body weight after exposure to oral doses of $100\,mg\,kg^{-1}\,day^{-1}$ or by inhalation to $25\,mg\,m^{-3}$ of APFO. There was no evidence of developmental toxicity after oral exposure to doses as high as $150\,mg\,kg^{-1}\,day^{-1}$, while inhalation exposure to $25\,mg\,m^{-3}$ resulted in reduced fetal body weights. In a rabbit oral developmental toxicity study, a significant increase in skeletal variations was observed after exposure to $50\,mg\,kg^{-1}\,day^{-1}$ APFO. No evidence of maternal toxicity at $50\,mg\,kg^{-1}\,day^{-1}$ was observed at the highest dose tested.

Human

APFO is a skin, eye, nose, and throat irritant. Evidence suggests that skin permeation can occur in amounts capable of producing the effects of systemic toxicity. Eye contact may cause eye irritation with discomfort, tearing, or blurring of vision. Inhalation may cause irritation of the upper respiratory passages, with coughing and discomfort. APFO ingestion could cause weight loss, gastrointestinal irritation and enlarged liver, or abnormal blood forming system function with anemia. Individuals with preexisting diseases of the liver or bone marrow may have increased susceptibility to the toxicity of excessive exposures.

A nonstatistically significant increase in estradiol levels in workers with the highest PFOA serum levels (>30 ppm) was reported; however, none of the other hormone levels analyzed indicated any adverse effects. At PFOA manufacturing plants where the serum PFOA levels were lower, cross-sectional and longitudinal studies found positive significant associations between PFOA and cholesterol and triglyceride levels. In addition, a positive, significant association was reported between PFOA and T3 hormone and a negative association with high-density lipoprotein in the cross-sectional study. These results must be interpreted carefully, as the studies conducted to date have many limitations.

Chronic Toxicity (or Exposure)

Animal

In a two-generation reproductive study, rats exposed to PFOA showed significant increases in absolute and relative liver and kidney weights, but reproductive indices were not affected in this F0 generation. In F1 animals exposed to PFOA *in utero* through early adulthood, a significant reduction in mean body weight was observed at the lowest doses tested. In F1 female rats, there was a significant increase in post-weaning mortality, a significant decrease in mean body weight, and a significant delay in sexual maturation at $30\,mg\,kg^{-1}\,day^{-1}$. Significant decreases in body weights and body weight gains, and significant changes in absolute liver and spleen weights and in the ratios of liver, kidney, and spleen weights-to-brain weights were observed in all treated F1 male groups. The lowest-observed-adverse-effect level (LOAEL) for the F1 females was $30\,mg\,kg^{-1}\,day^{-1}$, and the no-observed-adverse-effect level (NOAEL) was $10\,mg\,kg^{-1}\,day^{-1}$; the LOAEL for F1 males was $1\,mg\,kg^{-1}\,day^{-1}$ and an NOAEL was not determined. These gender differences in sensitivity are presumably related to gender differences in PFOA elimination. At the higher doses in the rat reproductive study (1 ppm), mortality was observed in 12% male and 10% female offspring. However, no excess mortality was found in F1 generation adult rats, which suggests that PFOA is less toxic in adults. The F2 generation pups were sacrificed at weaning; therefore, no treatment-related effects were observed.

Repeated exposures to APFO, the most widely used salt of PFOA, produced liver, kidney, pancreas and testes changes, anemia, and cyanosis. Tests in male rats demonstrated weak tumorigenic activity based on an increased incidence of benign testicular, pancreatic, and liver tumors. Tests in animals demonstrate no developmental toxicity.

Rodent bioassays have shown that chronic APFO (most widely used salt of PFOA) exposure is associated with a variety of tumor types. The mechanisms of APFO tumorigenesis are not clearly understood. The US Environmental Protection Agency (EPA) is currently evaluating the scientific evidence and has not reached any conclusions on the potential significance to humans of the rodent cancer data.

Human

Several epidemiological studies on the effects of PFOA in humans have been conducted on workers. These studies did not examine developmental outcomes. A retrospective cohort mortality study demonstrated a statistically significant association between prostate cancer mortality and employment duration in the chemical facility of a plant that manufactures PFOA. However, this result was not observed in an update to the study in which more specific exposure measures were used.

In Vitro Toxicity Data

In vitro mutagenicity assays with *Salmonella typhimurium* and *Saccharomyces cerevisiae* were conducted to evaluate the potential toxicity of APFO. All *in vitro* assays were negative for APFO mutagenicity.

Clinical Management

APFO is a skin, eye, nose, and throat irritant. If exposure to fumes from overheating or combustion occurs, the victim should be moved to fresh air and monitored for irritation. If eye or dermal contact occurs, affected areas should be flushed thoroughly with water for at least 15 min. The victim should be observed for resulting skin irritation. A physician should be consulted if symptoms persist. If ingestion occurs, two glasses of water should be immediately given and vomiting should be induced.

Environmental Fate

PFOA and related compounds are known to be highly persistent and widely distributed in the environment, and that they bioaccumulate. They do not further degrade and can remain in the body or environment for an extended period of time after exposure. PFOA also does not hydrolyze or photolyze under environmental conditions.

Although fluorinated telomers are not made using PFOA, some data indicate that certain telomers may break down or degrade to form PFOA in the environment, and may be metabolized to form PFOA if they manage to enter living organisms. Fluorinated telomers are small fluorine-containing polymers, synthetic chemicals produced by a specific process that utilizes the ability of certain chemicals to link together into chains of a defined length.

Ecotoxicology

Ammonium perfluorooctanoate can be very toxic to aquatic organisms and may cause long-term effects in the aquatic environment. It may produce a milky appearance if released into surface waters. The 96 h lethal concentration (LC_{50}) of APFO is 766 mg l^{-1} for fathead minnows and 569 mg l^{-1} for bluegill sunfish.

Other Hazards

Inhalation of fluoropolymer fumes from overheating or burning the resin may cause 'polymer fume fever'. High temperatures, such as in sintering operations, may release APFO vapors, which may condense as a solid or as a liquid solution in the oven, exhaust duct or stack, or on other cool surfaces.

Exposure Standards and Guidelines

The US EPA has entered into enforceable consent agreements with some parties under the Toxic Substance Control Act to control PFOA in the environment.

The occupational exposure standards and guidelines for APFO include the following:

American Conference of Governmental Industrial Hygienists threshold limit value of 0.01 mg m^{-3}.

The US Occupational Safety and Health Administration permissible exposure limit: not established.

See also: Blood; Carcinogenesis; Developmental Toxicology; Liver.

Further Reading

US EPA (2003) Environmental News: EPA Intensifies Scientific Investigation of a Chemical Processing Aid. Office of Pollution Prevention & Toxics (OPPT). April 14.

US EPA (2003) Fact Sheet. PFOA Q's & A's. Office of Pollution Prevention & Toxics (OPPT). April 14.

US EPA (2003) Perfluorooctanoic Acid (PFOA), Fluorinated Telomers. 18626 Federal Register, Vol. 68, No. 73, Wednesday, April 16.

US EPA (2003) Preliminary Risk Assessment of the Developmental Toxicity Associated with Exposure to Perfluorooctanoic Acid and Its Salts. Office of Pollution Prevention & Toxics (OPPT), Risk Assessment Division. April 10.

Relevant Websites

http://www.coating4ind.com – Material Safety Data Sheet. Manufacturer's Name: Coatings For Industry, Inc. Revised 9/00; reviewed 1/04.

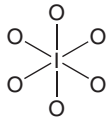
http://www.ewg.org – PFCs, A Family of Chemicals that Contaminate the Planet. Environmental Working Group.

Perfumes *See* Fragrances and Perfumes.

Periodic Acid

Samantha E Gad and Shayne C Gad

- Chemical Abstracts Service Registry Numbers: CAS 10450-60-9; CAS 13444-71-8
- Synonym: Paraperiodic acid
- Chemical/Pharmaceutical/Other Class: Inorganic acid; Periodate
- Chemical Formula: H_5IO_6
- Chemical Structure:



Uses

Periodic acid is used in organic synthesis. It is also used to fortify the wet strength of papers such as photographic paper, and in methods (e.g., oxidation in periodic acid followed by staining with Schiff's reagent) for staining tissues and cells for histopathology.

Exposure Routes and Pathways

Dermal contact, ingestion, and inhalation are possible routes of exposure.

Mechanism of Toxicity

Periodic acid is corrosive. It is also an oxidizing agent.

Acute and Short-Term Toxicity (or Exposure)

Human

The estimated lethal dose in humans is $1\,ml\,kg^{-1}$. Periodic acid is a strong corrosive to the skin, eyes, and mucous membranes. Symptoms of overexposure include respiratory distress, headache, nausea, and vomiting.

Clinical Management

The affected areas should be washed with copious amounts of water. If periodic acid has been ingested and the patient is conscious, the mouth should be washed with water and plenty of water given to drink.

Ecotoxicology

The US Environmental Protection Agency categorizes periodic acid as a corrosive hazardous waste. It is likely to reduce to iodides in the environment, and is possibly harmful to aquatic species.

See also: Corrosives.

Relevant Website

http://www.jtbaker.com – Periodic Acid (Material Safety Data Sheet from Mallinckrodt Baker, Inc.).

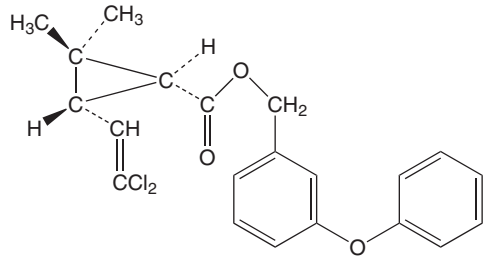
Permethrin

Paul R Harp

- Chemical Abstracts Service Registry Number: CAS 52645-53-1
- Synonyms: 3-Phenoxybenzyl-(1*R*,1*S*)-*cis*,*trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate; Adion; Ambush; Assithrin; Cliper; Coopex; Corsair; Dragnet; Dragon; Eksmin; Kafil; Pounce; FMC 33297; OMS 1821; NRDC 143;

SHA 109701. The *cis*-isomer is cispermethrin (NRDC 148) and the *trans*-isomer is biopermethrin (NRDC 147)

- Chemical/Pharmaceutical/Other Class: Type I pyrethroid insecticide
- Chemical Structure:



Uses

Permethrin is a broad-spectrum insecticide used in a variety of agricultural and commercial/residential applications. It is also used to control termites, ectoparasites on animals, and head lice and scabies (in combination with sulfur) in humans. Permethrin is available as a dust, smoke, wettable powder, emulsifiable concentrate, concentrate for ultra low volume application and as a lotion (for treatment of head lice).

Exposure Routes and Pathways

Human exposure to permethrin most commonly occurs through dermal contact.

Toxicokinetics

Pyrethroids are poorly absorbed through the skin and are only moderately absorbed in the gastrointestinal tract. One study estimated dermal absorption of permethrin to be $\leqslant 2$ mg per 12 h. Metabolism of permethrin occurs through hydrolysis, ester cleavage, and conjugation of the metabolites with glucuronic acid, glycine, or sulfuric acid. Isomeric configuration influences the rate of release from adipose tissues as well as the primary route of excretion (urinary versus fecal).

Mechanism of Toxicity

Several mechanisms of action have been identified for the pyrethroids with the primary mechanism related to a selective high affinity for membrane sodium channels. Closing of the channel, which ends the action potential, is slowed resulting in a prolonged 'tail' current and repetitive firing of presynaptic and accompanying postsynaptic cells following a single action potential. High enough doses can cause complete depolarization and blockade of nerve conduction. Permethrin also inhibits Ca^{2+},Mg^{2+}-ATPase.

Acute and Short-Term Toxicity (or Exposure)

Animal

In mammals, permethrin produces type I motor symptoms generally characterized by hyperexcitation, enhanced startle response, tremors, and prostration.

Human

Reports of human exposure have indicated only skin irritation.

Chronic Toxicity (or Exposure)

Chronic effects following permethrin exposure in humans have not been reported. US Environmental Protection Agency classifies permethrin as a possible human carcinogen based on findings of lung adenomas and combined adenomas/carcinomas and liver adenomas in mice.

Clinical Management

Exposed skin should be washed promptly with soap and water. Dermal application of vitamin E oil preparations may be used for both prophylaxis and treatment of paresthesia. For contact with eyes, eyes should be flushed immediately and for an extended period with generous amounts of clean water or saline. Gastric lavage is indicated if patient has ingested a large amount of pyrethroids and can be treated soon after exposure. For ingestion of smaller amounts or if treatment has been delayed, activated charcoal and catharsis are indicated. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenytoin or phenobarbital may be helpful for recurrent seizures. No specific antidotes for pyrethroid-induced neurotoxic effects have been approved for use in humans. Spontaneous recovery usually occurs with mild or moderate intoxication.

Environmental Fate

Permethrin is of low to moderate persistence in the soil (half-life of 30–38 days). Permethrin is readily degraded in most soils except those rich in organic matter, with microbial degradation predominant. Permethrin is tightly adsorbed to soil with little leaching and low mobility. Permethrin degrades rapidly

in water: the half-life in estuarine water was less than 3 days. Permethrin has little phytotoxic potential.

Ecotoxicology

Fish and crustaceans are extremely sensitive to pyrethroid compounds in laboratory settings. However, various factors (e.g., sediment binding) may reduce pyrethroid toxicity to these nontarget organisms in a natural environment.

Exposure Standards and Guidelines

The acceptable daily intake and reference dose for permethrin are both $0.05\,mg\,kg^{-1}\,day^{-1}$.

See also: Neurotoxicity; Pesticides; Pyrethrins/Pyrethroids.

Further Reading

Ray DE (2001) Pyrethroid insecticides: Mechanisms of toxicity, systemic poisoning syndromes, paresthesia, and therapy. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1289–1303. San Diego, CA: Academic Press.

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Permethrin.

Permissible Exposure Limit *See* Occupational Exposure Limits.

Peroxisome Proliferators

Abraham Dalu and Harihara M Mehendale

Introduction

One of the most rapidly developing areas of organelle biology, which has a major involvement in biochemical pharmacology, is the research into peroxisomal function. The process of xenobiotic-induced proliferation of the cytoplasmic organelle, the peroxisome, in mammalian liver cells has received considerable attention because of the proposed relationship between the induction of hydrogen peroxide (H_2O_2)-producing peroxisomal enzymes and the development of hepatocellular carcinomas in mice and rats. In rodents, the number and the size of peroxisomes are dramatically increased in the liver and, to a lesser extent, the heart and kidney in response to treatment with a variety of different amphipathic acids, including hypolipidemic drugs and plasticizers, which are collectively referred as 'peroxisome proliferators' (PPs). They encompass a diverse group of compounds with dissimilar structures capable of producing pleiotropic responses in experimental animals. The pleiotropic responses are predominantly observed in hepatocytes and are characterized by enlargement of the liver, increases in a relative number of peroxisomes in a cell, marked induction of fatty acid β-oxidation, carnitine acetyltransferase, lauric acid ω-hydroxylation activities, and hypolipidemia. Long-term exposure to PPs is associated with the induction of hepatocellular carcinoma. Currently, there are over 100 known PPs in use including herbicides, industrial solvents, plasticizers, and hypolipidemic agents. Peroxisome proliferation can occur in many organs and tissues. The effects of PPs have been also reported in the kidneys, the heart, the prostate, the pancreas, and the thyroid. Immune reaction may also be observed. Although a significant advance has been made in this area over the past few years, there is much yet to be investigated to elucidate the molecular and cellular mechanisms underlying PPs associated with hepatocellular carcinoma.

Peroxisomes

Peroxisomes, cytoplasmic organells of $\sim 0.5\,\mu m$ in diameter, are ubiquitous in eukaryotes. Peroxisomes consist of a single membrane that separates them from the cytosol, and participate in several important metabolic functions, including simple respiration characterized by H_2O_2 production and H_2O_2 degradation, β-oxidative chain shortening of long chain and very long-chain fatty acids, metabolism of glyoxalate, degradation of uric acid; and synthesis of ether phospholipids, cholesterol, and bile acids. Peroxisomes were discovered by Christian de Duve in 1965. The term 'peroxisome' was introduced to

emphasize the biochemical property of H_2O_2 production by the peroxisomes as a result of respiration mediated by peroxisomal oxidases such as d-amino acid oxidase and fatty acyl-CoA. Unlike lysosomes, peroxisomes are not formed in the Golgi apparatus, but self-replicate by dividing peroxisomes, normally enlarge, then divide. Peroxisomes are 0.2–1 μm and are most abundant in the liver.

The biogenesis of the peroxisome requires the formation of a lipid bilayer, the import of membrane proteins into that bilayer, and the transport of soluble proteins across the membrane into the peroxisomal matrix. Among the different organisms in which this process has been studied, 24 genes have been identified that encode proteins involved in peroxisome biogenesis. These genes are called *PEX* genes (*PEX1*, *PEX2*, *PEX3*, etc.), and their protein products are termed peroxins. Peroxisomes are ubiquitous single-membrane organelle present in all organelles both in animal and plant cells which exhibit numerous oxidases involved in several catalytic and anabolic pathways such as β-oxidation of very long fatty acids. Peroxisomes are also involved in bile acid synthesis, cholesterol synthesis, plasmalogen synthesis, amino acid metabolism, and purine metabolism. The importance of the peroxisome and these processes is underscored by the existence of numerous genetic disorders associated with defects in the peroxisome, which can be divided into two categories.

One of the main functions of peroxisomes is to detoxify the cell by splitting hydrogen peroxide. They contain the enzyme catalase. Catalase converts H_2O_2 (hydrogen peroxide, a toxic by-product of cellular metabolism) to H_2O and O_2, with $4H_2O_2 \rightarrow 4H_2O + 2O_2$. Peroxisomes also degrade fatty acids and toxic compounds and catalyze the first two steps required in the synthesis of ether-linked phospholipids (which are later used to build membranes) and sterols. In addition, it plays a role in isoprenoid biosynthesis and amino acid metabolism.

Although the mitochondria are the primary site of oxidation for dietary and storage fats, the peroxisomal oxidation pathway is responsible for the oxidation of very long-chain fatty acids, β-methyl branched fatty acids, and bile acid precursors. The peroxisomal pathway also plays a role in the oxidation of dicarboxylic acids. In addition, it plays a role in isoprenoid biosynthesis and amino acid metabolism. Peroxisomes are also involved in bile acid biosynthesis, a part of plasmalogen synthesis and glyoxylate transamination. Furthermore, the literature indicates that peroxisomes participate in cholesterol biosynthesis, hydrogen peroxide-based cellular respiration, purine, fatty acid, long-chain dicarboxylic acid, prostaglandin, and xenobiotic metabolism. Currently, there are ~ 50 known enzymes associated with mammalian peroxisomes including catalase, oxidases (H_2O_2 generators), acetyltransferases (carnitine acetyl-CoA and carnitine octanoyl-CoA), dehydrogenases (NAD and NADP), and others (enoyl-CoA hydratase, thiolase, fatty acetyl-CoA synthetase). Thus, any chemical capable of disrupting these enzymes perturbs the normal functioning of peroxisomes, leading to long-term adverse health effects. Several techniques are available to identify these organelles in hepatocytes and other cells. One such technique is the recently developed immunohistochemical protocol using antibodies raised against peroxisomal enzymes, or the 'protein A-gold' method.

Peroxisome Proliferators

PP in mammalian cells, first described over 30 years ago, represents a fascinating field of research. Agents known as PPs exert peroxisome proliferation through binding to the steroid hormone receptors known as PP-activated receptors (PPARs). They are an important group of chemicals that include certain hypolipidemic drugs, plasticizers, and pollutants. The term 'PP' was introduced by Reddy and coworkers in 1975 to designate a drug or xenobiotic which induces the proliferation of peroxisomes in the liver cells. PPs are structurally diverse compounds, which induce peroxisome proliferation. Many of these agents are known rodent liver tumor promoters and debate exists as to whether humans are at increased cancer risk following exposure to PPs. They have been shown to regulate hepatic lipid metabolism via activation of the PP-activated receptor alpha (PPARα). Recent studies have revealed that PPs also exert considerable influence on certain extrahepatic tissues, including adipose tissue and lymphoid organs, in an indirect fashion. Inhibition of the proliferation of thymocytes and splenocytes and alteration of fatty acid uptake into and release from adipose tissue might be consequences of the hypolipidemic effect of PPs involving both PPARα-dependent and -independent pathways. Exposure to PPs reduces the cholesterol content of circulating low-density lipoprotein (LDL), which is the major supply of this steroid to most peripheral tissues. In addition, PPs increase serum levels of high-density lipoprotein (HDL), which extracts cholesterol from peripheral tissues and returns it to the liver, thereby further decreasing the cholesterol content of peripheral tissues.

Earlier studies with the hypolipidemic agent clofibrate revealed that it induced a marked

hepatomegaly in male rats. Fine structural changes seen primarily as a massive increase in the number of dense particles or 'microbodies' were first described by Paget. Catalase and three H_2O_2-producing oxidases (urate oxidase, D-amino acid oxidase, and 1-α-hydroxy acid oxidase) had been found to cosediment with liver cell fractions containing particles identical to microbodies, and the term 'peroxisome' was coined to describe this organelle as a site of compartmentalized peroxide metabolism.

Currently, over 100 compounds have been identified as PPs. The literature indicates that induction of peroxisome proliferation is not limited to exogenous chemicals. A number of endogenous substances, such as the steroid hormones, thyroid hormones, morphogenes, and fatty acids, are also involved in peroxisome proliferation. Peroxisome proliferation in hepatic parenchymal cells of rats and mice following the administration of clofibrate has been reported by numerous investigators. Compounds that are structurally unrelated to clofibrate, such as acetaminophen and Wy-14,643, can also cause peroxisome proliferation (**Table 1**). The industrial solvent trichloroethylene, the industrial plasticizers di(2-ethyl hexyl) phthalate (DEHP) and di(2-ethyl hexyl) adipate (DEHA), have also been found to be hepatic peroxisome proliferators.

Newer compounds of interest include the perfluoroalkanoic acids, the steroid hormones, and anticarcinogen dehydroepiandrosterone; some structurally related leukotriene D_4 antagonists; certain chlorinated hydrocarbons, primarily those metabolized to tri- or di-chloro acetic acid; as well as structurally unrelated herbicides such as tridiphane, lactofen, and several of the chlorophenoxy acids (2,4-dichlorophenoxy acetic acid and 4-chloro-2-methylphenoxy acetic acid) (**Table 1**). PPs comprise chemicals of wide structural dissimilarity (**Figure 1**) and share a common property of inducing characteristic effects in the liver of treated rats and mice. Within a few days of exposure they produce a striking dose-dependent hepatomegaly accompanied by characteristic proliferation of the peroxisomal and microsomal compartments as assessed morphologically and biochemically.

Peroxisomes are also responsive to dietary and hormonal changes such as high-fat diets, particularly those with long-chain fatty acids, high-cholesterol diets, and vitamin E deficiency. Thyroid hormones produce moderate increases in the content of hepatic peroxisomes and peroxisomal enzymes. While these diets and physiological influences rarely induce changes in peroxisomes to the same extent as many xenobiotics, they are useful in exploring the mechanisms of regulation of peroxisomal proliferation.

Table 1 Selected representative PPs

Fibric acid hypolipidemic agents
Beclobric acid
Ciprofibrate
Clofibrate
Gemfibrozil
Simfibrate
Other xenobiotics
Bleached kraft mill effluents
Citral
Dimethrin
Garlic, ether extracts
Trichloroethylene
Nonfibric acid hypolipidemic agents
Gemcadiol
Niadenate
Tiadenol
Tibric acid
Wy-14,643
Herbicides
2,4-D (2,4-dichlorophenoxyacetic acid)
MCPA (2-methyl-4-chlorophenoxyacetic acid)
2,4,5-T (2,4,5-trichlorophenoxyacetic acid)
Lactofen
Tridiphane
Other drugs
Acetylsalicyclic acid
Benzobromarone
Bifonazole
Flurbiprofen
Valprioic acid
Other chlorophenoxy acids
2-Phenyopropionic acid
4-Chlorophenoxypropionic acid
4-Chlorophenoxybutyric acid
Fatty acid analogs
2,2,4,4,6,8,8-Heptamethylnonane
Perfluorobutyric acid
Perfluorodecanoic acid
Sorbic acid
Tetradecylthioacetic acid

Biomedical Responses to Acute Toxicity of PPs

Peroxisome proliferation is consistently associated with hepatomegaly, which arises from a combination of cellular hypertrophy and hyperplasia. Studies on fine structure of hepatocytes revealed that the increase in hepatocyte size was associated with the predominant increase in peroxisomes and modest increase in smooth endoplasmic reticulum (SER). Rats exposed to peroxisome proliferators exhibit 7- to 10-fold increases in peroxisomal relative volume and surface area as evidenced by morphometric analysis of liver sections. The increase in peroxisomal relative volume is due to the increases in both volume and number of peroxisomes. In contrast, the increase in SER surface area and volume rarely exceeds two-fold. The magnitude of increase in cellular DNA and

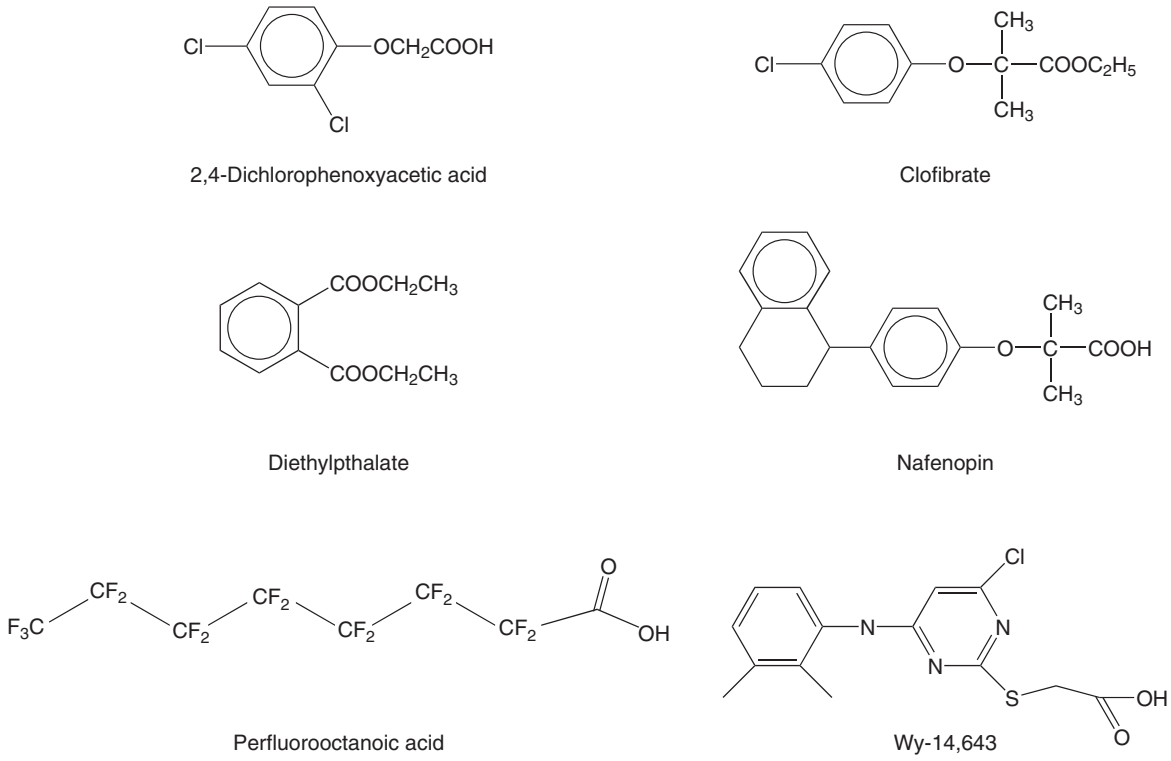


Figure 1 Structures of representative peroxisome proliferators.

peroxisomes is dose and compound dependent and correlates with the extent of hepatomegaly. Hyperplasia, however, does not correlate with the extent of peroxisomal proliferation. For example, clofibrate and DEHP produce little hyperplasia, while nefenopin and Wy-14,643 produce relatively extensive hyperplasia. Hypolipidemia is one of the characteristic responses to peroxisome proliferators. This can be evidenced by a remarkable decrease in triglyceride and cholesterol levels following exposure to nonhypolipidemic agents. Because of these properties, hypolipidemic drugs are primarily used to lower serum cholesterol and triglyceride. Earlier studies showed that clofibrate treatment increases liver carnitine acetyltransferase activity, but no correlation was established with peroxisome proliferation. It has also been reported by Moody and Reddy that an increase in carnitine acetyltransferase is a conforming response to a wide variety of peroxisome proliferators, with increase in specific activity ranging from 10- to 25-fold, the most predominant increase being in the peroxisomes. Increases in the medium- (5- to 10-fold) and long-chained (two- to fivefold) carnitine acetyltransferases also occur. Another obvious peroxisome response to peroxisome proliferators is increased β-oxidation of fatty acids in glyoxysome, a specific form of peroxisome, in germinated seeds which later was also reported to be present in rat liver peroxisome. Studies have shown that clofibrate treatment increases β-oxidation of fatty acids by $\sim$10-fold. Based on such studies, it has been concluded that β-oxidation of fatty acids is a generalized response to peroxisome proliferators.

Peroxisome proliferators are also involved in two other metabolic pathways of importance to lipid metabolism. Peroxisomes contain the most of dihydroxyacetone phosphate acetyltransferase and alkyldihydroxyacetone phosphate synthetase activities. Therefore, they are responsible for initiating most ether glycerolipid biosynthesis. These enzymes are also moderately induced by peroxisome proliferators. Induction of cytochrome P450s by peroxisome proliferators will be addressed separately.

Mechanism of Induction of Peroxisome Proliferation

Two widely accepted possible mechanisms for the induction of peroxisome proliferation are (1) activation of specific genes by the chemical or its metabolites, either directly or mediated by a specific receptor, and (2) substrate overload, either as a result of lipolysis occurring outside the liver and causing an influx of fatty acids into the liver or as a consequence of the peroxisome proliferators or their metabolites perturbing lipid metabolism.

The most likely mechanism of induction of peroxisome enzymes is interaction of peroxisome

proliferators with a cytoplasmic receptor of the hepatocytes. The peroxisome proliferator–receptor complex interacts with the chromatin to elicit selective increases in protein, translation of mRNA, and peroxisome-specific mRNAs. The second mechanism of peroxisome proliferation may be related to substrate overload in the hepatocytes of animals treated with various peroxisome proliferators. In rats, feeding of high-fat diet results in a very slight increase in peroxisome number. The administration of clofibrate or other peroxisome proliferators may lead to an influx of fatty acids into the liver as a result of lipolysis occurring outside the liver, or these compounds and their metabolites enhance the breakdown of triglycerides in the liver cell thereby causing an intrahepatic excess of fatty acids. The fatty acid overload may then trigger an increase in peroxisomal β-oxidation pathway.

In addition to the two previously mentioned mechanisms, some investigators have proposed another mechanism for induction of peroxisome proliferation. Peroxisomes contain a fatty acid β-oxidation system which preferentially oxidizes long-chain fatty acids (C_8–C_{20}). For example, the physicochemical properties of clofibric acid, the hydrolytic product of clofibrate, are very closely similar to those of naturally occurring C_{16}–C_{18} fatty acids. It is important, therefore, to examine whether the hypolipidemic drugs and/or their metabolites serve as substrates for the peroxisomal β-oxidation, thereby causing peroxisomal enzyme induction and possibly leading to increased production of H_2O_2 and other active oxygen species which ultimately lead to peroxisome proliferation.

Induction of Cytochrome P450s by PPs

Induction of the enzymes involved in xenobiotic biotransformation (phases I and II) is one of the characteristic responses to peroxisome proliferators. Attention has focused on these compounds initially because they were identified as epigenetic hepatocarcinogens in rodents. In addition, peroxisome proliferators received further attention as inducers of members of cytochrome P450 gene superfamily known to readily metabolize fatty acids. These metabolites have marked physiological activity, particularly those of arachidonic acid, which are vasoactive, regulate hormone release, and control renal ion flux. Therefore, regulation of cytochrome P450-dependent fatty acid hydroxylases by peroxisome proliferators is of particular interest in the field of physiology and pathophysiology. The cytochrome P450 gene superfamily consists of $\sim$250 different known genes which have substrate specificity in metabolizing a range of xenobiotics, including drugs, pesticides, food flavors and additives, and environmental chemicals. Furthermore, peroxisome proliferator-induced cytochrome P450s can also metabolize endogenous compounds other than fatty acids such as steroids, vitamins, and eicosanoids. It should be noted that only few of the cytochrome P450s induced by peroxisome proliferators have been isolated and studied. Cytochrome P450 4A1 in rat liver and P450 4A7 in rabbit lung are two such isozymes isolated and extensively characterized. Because a vast majority of endogenous and exogenous chemical substances are metabolized by cytochrome P450, their metabolism and effects can be modulated by exposure to peroxisome proliferators. The basic mechanism of action of peroxisome proliferators is shown in **Figure 2**.

Mechanisms of PP-Induced Cytochrome P450s and Other Enzymes

In general, the cytochrome P450 gene superfamily exhibits a range of induction mechanisms, including transcriptional gene activation, mRNA processing and mRNA stabilization, translational regulation, and protein stabilization. Current understanding indicates that xenobiotic-dependent transcriptional gene activation is the most common induction mechanism, and direct experimental evidence using nuclear run-on experiments has demonstrated that cytochrome P450 4A1 undergoes transcriptional gene activation by clofibrate. However, it is not clear whether or not the same induction mechanism is involved in the other cytochrome P450 4A gene superfamily. The question is then, do peroxisome proliferators directly activate the cytochrome P450 4A1 gene or do they require the intermediary of a protein factor/receptor to interact with the 5′ flanking regulatory element of the gene? Recent experimental findings suggest that peroxisome proliferator-induced cytochrome P450 and other enzymes are mediated through a receptor, better known as PPAR. The existence of multiple PPARs (PPAR-α, -β, and -γ) has been recently reported. These receptors are members of a superfamily that comprises at least 30 mammalian genes encoding receptors for the classical steroid hormones, thyroid hormones, vitamin D_3, and retinoic acid. These receptors have been implicated in the activation of some enzymes and have also been implicated in the activation of CYP4A6. Investigators have shown that the peroxisome proliferators' complex interacts with chromatin to result in selective increases in the transcription of peroxisomal fatty acid β-oxidation gene enzymes. The induction of P450 4A enzymes by PPs and fatty acids is now

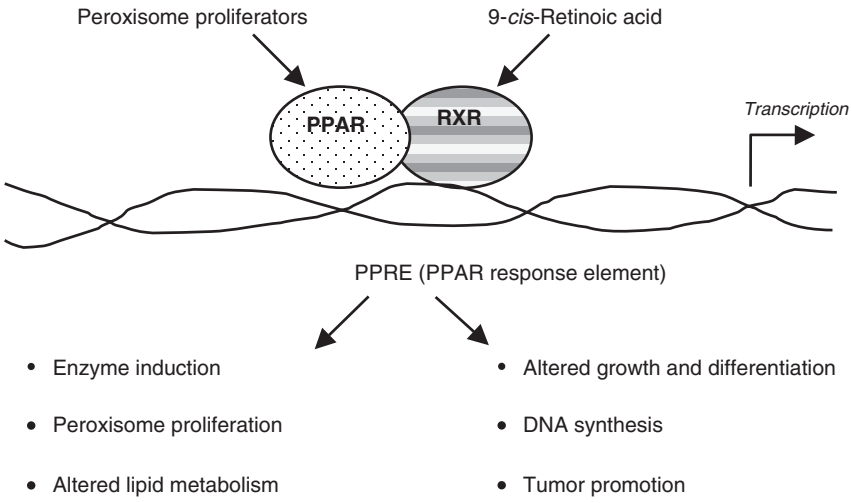


Figure 2 Basic mechanism of action of peroxisome proliferator-activated receptors. (Reproduced from Vanden Heuvel JP (1999) *Toxicological Sciences* 47: 1–8, with permission from The Society of Toxicology.)

known to be mediated by the PPARα that binds to response elements in target genes as a heterodimer with the RXR. The consensus sequence recognized by PPAR/RXR heterodimers contains an imperfect direct repeat of two nuclear receptor binding motifs separated by a single nucleotide. This repeat is preceded by a conserved A/T-rich sequence that is required for function. There is also evidence to indicate that the PPAR is inducible by the PP fenofibrate in rat liver as assessed by immunochemical methods and PPAR mRNA analysis by Northern blotting.

Peroxisome Proliferator-Activated Receptors

The past several years have seen an increasing interest in the PPARs, the subfamily of which consists of three isoforms by separate genes: PPAR alpha (α), PPAR delta (δ) (also referred to as PPAR beta (β)), and PPAR gamma (γ). PPARα was first cloned from mouse liver in 1990 by Issemann and Green; subsequently, Dreyer and associates cloned two other members of this subfamily (β (δ) and γ), which belong to the steroid receptor super-family. PPARs are now considered to be essential transcription factors regulating key cellular functions including lipid metabolism, xenobiotic metabolism, inflammation, cell differentiation, and cancer. These receptors are expressed in both embryonic and the adult organism. Each of the three PPAR subtypes is expressed in a distinct, tissue-specific pattern (**Table 2**) and differ considerably in their ligand-binding domains and specificities, attesting to the fact that they perform different functions in different cell types. PPARα is highly expressed in liver, skeletal muscle, kidney, heart and vascular wall, and brown adipose – tissues that are metabolically very active. PPARγ is expressed mainly in white and brown adipose tissue, large intestine, and spleen. In contrast to PPARα and PPARγ, which are abundantly expressed in just a few tissues, PPARδ is expressed in virtually all tissues at comparable levels and heterodimerized with retinoic acid X receptor (RXRα), another transcription factor activated by 9-*cis* retinoic acid. Like other members of the nuclear receptor superfamily, PPARα contains ~70 amino acid DNA-ligand-binding domain (LBD) of ~250 amino acids (**Figure 3a**). In addition to its ligand-binding capabilities, the LBD contains dimerization and transcriptional activation function 2 (AF-2), which is embedded in the extreme C-terminal portion of the receptor. The N-terminal domain of PPARα is less well characterized but appears to encode an additional transcriptional activation function.

Table 2 Tissue distribution of PPARs

PPAR isoform	*Liver*	*Intestine*	*Spleen*	*Fat*
α	++++	++++	+	–
β (δ)	++	+++	++	–
γ	–	++	+++	+++

PPARs contain a central cystein-rich zinc finger motif DNA-binding domain that recognizes DNA sequence elements, designated peroxisome proliferator response elements (PPREs), containing direct repeats of the hexanucleotide sequence AGGTCA separated by one nucleotide present in the 5′-flanking region of target genes. As shown in **Figures 2** and **3b**, PPARs bind to DNA as obligate

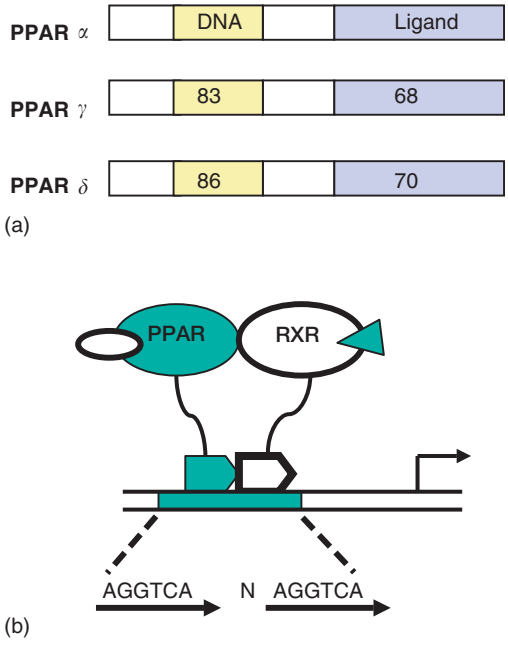


Figure 3 The PPAR family and its DNA properties. (a) The murine PPAR subfamily. The DNA and ligand-binding domains are indicated. Numbers represent percentage amino acid identity. (b) The PPARs bind to DR-1-type DNA response elements as heterodimers with RXR. The PPAR/RXR heterodimer can be activated by ligands for either PPAR or RXR. (Reproduced from Kliewer SA, Xu HE, Lambert MH, and Willson TM (2001) Peroxisome proliferator-activated receptors: From genes to physiology. *Recent Progress in Hormone Research* 56: 239–265, with permission from The Endocrine Society.)

heterodimers with the 9-*cis* retinoic acid receptors (RXRs). The PPAR/RXR heterodimers bind to two half sites of the consensus sequence AGGTCA. PPARs have been identified in the transcriptional regulatory regions of numerous genes involved in carbohydrate and lipid metabolism. There is emerging evidence that optimal binding sites differ slightly for each PPAR subtype. These subtle differences in binding site preference, together with differences with tissue expression patterns, undoubtedly contribute to the different biologies of the three PPAR subtypes.

PPARs (α and γ) are key regulators of lipid homeostasis and are activated by a structurally diverse group of compounds including fatty acids, eicosanoids, and hypolipidemic drugs such as fibrates and thiazolidinediones (see **Table 3** for representatives of the endogenous and exogenous ligands for PPARs). While thiazolidinediones and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 have been shown to bind to PPARγ, although it is unclear whether other activators mediate their effects through direct interactions with the PPARs or via indirect mechanisms. The most conclusive evidence that PPARs are key elements in peroxisome proliferator-mediated events comes from

Table 3 PPAR ligands

PPARα	*PPARγ*
Exogenous PPAR ligands	
WY14,643	Indomethacin
Clorfibrate	Ibuprofen
Gemfibrozil	Piroxicam
Nafenopin	Pioglitazon
Bezafibrate	Ciglitazon
	Englitazon
	BRL-49653
Endogenous PARP ligands	
Palmitic acid	Arachidonic acid
Stearic acid	Eicosapentaenoic acid
Palmitoleic acid	PGJ2
Oleic acid	15 deoxy PGJ12
Linoleic acid	
Arachidonic acid	
Eicosapentaenoic acid	

studies with transgenic mice performed by Frank Gonzalez and colleagues at NCI. When PPARα expression was disrupted in transgenic mice, the response to peroxisome proliferators was greatly altered. In mice with abrogated PPARα expression the prototypical peroxisome proliferation, hepatomegaly, and induction of fatty acid metabolizing enzymes were not observed. In addition, PPARα-null mice were refractory to the carcinogenic effects of peroxisome proliferators. Therefore, PPAR is required for both the effects on gene expression as well as the tumor-promoting activity of peroxisome proliferators in rodents. PPARs also play an important role during rodent hepatocarcinogenesis, inflammation, atherosclerosis development, lipid metabolism, diabetes, and cancer.

Species Differences in Response to Peroxisome Proliferation

Studies with a number of peroxisome proliferators have shown a high degree of specificity in the responsiveness of species to peroxisome proliferators. The increase in growth and peroxisome proliferation seen in rat and mouse liver is not seen in guinea pigs, dogs, nonhuman primates, and more importantly, humans. In humans these findings have come from epidemiological studies with hypolipidemic drugs and from *in vitro* experiments with human hepatocytes. Fibrate acid derivatives such as clofibric acid and fenofibrate were among the first xenobiotics to be associated with increased numbers of peroxisomes and augmented enzymatic activity associated with this organelle. The peroxisome proliferator response is most notable in the liver and kidney, and also shows a strong species difference with rats

and mice being sensitive while humans are relatively refractory. Peroxisome proliferation, cytochrome P450 induction, and induction of peroxisomal enzymes by hypolipidemic agents and other chemicals such as DEHP is well-established in rats and mice. Hamster liver is also responsive to these compounds, although to a lesser extent. Examination of human liver biopsy material, obtained from patients receiving clofibrate, gemfibrozil, or fenofibrate, has demonstrated marginal or no increase in peroxisomal volume densities or numbers. These studies suggest that there is a marked species difference in sensitivity to chemicals that cause hepatomegaly and peroxisome proliferation. In general, rats and mice are most responsive; hamsters, chickens, and pigeons exhibit an intermediate responsiveness; and dogs, rabbits, marmosets, and rhesus monkeys are least responsive to peroxisome proliferators. Human peroxisomes are also insensitive to these compounds. In contrast, hypolipidemic effect is quite evident in all of the primates and most other species studied. One of the reasons for interspecies differences in response to peroxisome proliferators may be attributed to the existence of multiple PPARs, each having different ligand-binding specificity and being differentially regulated and expressed in a tissue- and species-specific manner. In mice, chronic exposure to PPs results in PPARα-mediated liver hypertrophy, hyperplasia, and carcinogenesis accompanied by a proliferation of peroxisomes. In contrast, humans exhibit a reduced sensitivity to PP pathogenesis. This could reflect >10-fold lower PPARα levels in humans relative to mice as well as differences in gene network regulated by PPARs. In addition, species differences in response to peroxisome proliferators are reproducible in cell cultures since hepatocytes from rats and mice respond *in vitro* to peroxisome proliferators, whereas monkey and human hepatocytes are far less sensitive. In sharp contrast, guinea pigs do not respond to peroxisome proliferators in both *in vivo* and *in vitro* systems. Therefore, this cell culture system may provide a good method to study the effects of peroxisome proliferators in humans.

Gender Differences in Peroxisome Proliferation

There appears to be a general misconception that female rats are not responsive to peroxisome proliferators such as clofibrate. This misconception was based on earlier studies in which male F-344 rats, but not females, maintained on 0.25% clofibrate diet for up to 4 weeks exhibited hepatic peroxisome proliferation. However, subsequent studies adequately demonstrated that clofibrate, when given at 0.5%, 1%, and 2% in a diet, resulted in a marked peroxisome proliferation in liver parenchymal cells of both male and female rats. Therefore, gender differences can be obviated by increasing the dose of the inducing agent. On the other hand, it would be interesting to investigate the nonresponsiveness of female rats to the low dose of clofibrate in terms of risk assessment. No gender differences in the induction of peroxisome were seen in adult, fetal, and neonatal rats treated with nafenopin or other potent PPs. In terms of gender-related differences in cytochrome P450 induction, recent studies have shown that P450 4A1 and 4A3 mRNAs are induced to a much greater extent in male compared to female rats following clofibrate treatment. Cytochrome P450 4A2 mRNA is altogether absent from the female rat liver. Male-specific expression of P450 4A2 mRNA was also observed in kidneys. These observations suggest that the lower responsiveness of female rats to clofibrate-induced PP may reflect the lower inducibility of the P450 4A fatty acid hydroxylase enzyme in female rats. There is no gender-related difference in the induction of P450 4A12 mRNA in mice treated with the potent PP methylclofenapate. Thus, gender-related differences in chemical-induced peroxisome proliferation are dependent by and large on species, strains, and doses of a given agent.

Recent studies indicate that there are gender-related differences in the induction of hepatic catalase activity by clofibrate. Catalase, which is located in peroxisomes, catalyzes the reduction of hydrogen peroxide to water either directly or using small molecules, such as ethanol, formate, or methanol, as electron donors. Basal and clofibrate-induced hepatic catalase activity in male Sprague–Dawley rats was reported to be higher as opposed to the corresponding levels in females. Induction of hepatic catalase activity by clofibrate was decreased by 50% in castrated males compared to intact male rats. Clofibrate-fed castrated male rats challenged with estradiol benzoate showed greatly diminished induction of catalase activity. Uninduced ovariectomized female rats had hepatic catalase activity levels comparable to those of induced intact females. A marginal increase in hepatic catalase activity was observed in induced ovariectomized females compared to ovariectomized control females. Furthermore, a substantial increase in hepatic catalase activity was seen in induced ovariectomized females challenged with testosterone propionate. These observations clearly demonstrate gender-related differences in the induction of hepatic catalase activity by clofibrate in rats depending on the exposure level. A basis for these different responses could be attributed to factors

such as gender-dependent metabolic pathways. Induction of acetyl-CoA oxidase activity by peroxisome proliferators has recently been shown to be receptor mediated. If induction of hepatic catalase activity by peroxisome proliferators is shown to also be receptor mediated, then hormonal status could potentially interact with signal transduction pathways, resulting in differences in induction of catalase and other biochemical endpoints between the genders. On the other hand, findings on the gender differences in the induction of catalase activity in other species and with other peroxisome proliferators are less compelling. Unlike clofibrate, other peroxisome proliferators show no consistent pattern of gender differences in induction of hepatic catalase activity in either mice or rats. Therefore, the observed differences may relate to effective dose of the inducer rather than responsiveness to peroxisome induction.

The mechanism of trichloroethylene-induced liver peroxisome proliferation and gender-related differences in response was investigated using a wild-type Sv/129 and PPARα-null mice. Trichloroethylene treatment ($0.75\,g\,kg^{-1}$ for 2 weeks by gavage) resulted in liver peroxisome proliferation in wild-type mice, but not in PPARα-null mice, suggesting that trichloroethylene-induced peroxisome proliferation is primarily mediated by PPARα. No remarkable sex difference was observed in induction of peroxisome proliferation, as measured morphologically, but a markedly higher induction of several enzymes and PPARα protein and mRNA was found in males. On the other hand, trichloroethylene induced liver cytochrome P450 2E1, the principal enzyme responsible for metabolizing trichloroethylene to chloral hydrate, only in males, which resulted in similar expression levels in both sexes after the treatment. Trichloroethylene influenced neither the level of catalase, an enzyme involved in the reduction of oxidative stress, nor aldehyde dehydrogenase, the main enzyme catalyzing the conversion to trichloroacetic acid. These results suggest that trichloroethylene treatment causes a male-specific PPARα-dependent increase in cellular oxidative stress.

Peroxisome Proliferators and Hepatocarcinogenesis

In recent years a growing concern has developed with regard to long-term exposure to hypolipidemic agents, certain herbicides and industrial plasticizers (DEHP and DEHA) and the possible effect on human health. These concerns have basically centered on the tumorigenic property of peroxisome proliferators. There is a large body of evidence to indicate an association between peroxisome proliferation and hepatocarcinogenesis in rats and mice. Hepatocellular carcinogenesis is a property of all peroxisome proliferators, with few exceptions after discounting any direct genotoxic action of these compounds. The increased production of H_2O_2, which may overwhelm protective enzymes within the hepatocyte and produce indirect genotoxic injury, and the propensity of peroxisome proliferators to induce hepatocyte replication have both been argued to contribute to the carcinogenic action of these compounds. Furthermore, there is no evidence for the covalent binding of peroxisome proliferators to DNA in experimental animals. The lack of covalent DNA binding and mutagenic activity suggests that peroxisome proliferators do not react directly with DNA to produce injury and that electrophilic species generated by peroxisome proliferators interact with non-DNA target. Therefore, since peroxisome proliferators do not directly interact with and impair DNA, their mechanism of action is considered to be nongenotoxic, and is classified as a novel class of epigenetic chemical carcinogen. The understanding of the carcinogenic process induced by peroxisome proliferators is a continuing challenge. It is generally believed that a major contributing factor to cancer formation by nongenotoxic carcinogens, including peroxisome proliferators, is altered gene expression. That is, these agents effect the expression of genes that regulate cellular growth and/or differentiation.

Furthermore, epigenetic carcinogens operate by mechanisms such as chronic tissue injury, immunosuppression, solid-state effects, hormonal imbalance, cocarcinogenesis, or promotional activity. In the presence of tumor-initiating agents, peroxisome proliferators accelerate tumor formation. The distinctive phenotypic markers (GGT-positive foci) of the early stages of hepatocarcinogenesis are not observed, suggesting that pathways specific to peroxisome proliferators underlie the transformation of rodent hepatocytes. Peroxisome proliferators such as Wy-14,643 and clofibrate promote tumors after cell initiation. Recent developments also indicate that commonly used phthalate ester plasticizers DEHA and DEHP are capable of inducing hepatocellular carcinoma in rats and mice. These observations are of a serious concern since ~400 million pounds of DEHP plasticizers are used every year in the United States and many more million pounds elsewhere in the world.

The molecular and cellular mechanism of peroxisome proliferator-mediated hepatocarcinogenecity is not well understood. However, several hypotheses have been proposed for the hepatocarcinogenecity of these compounds. Accumulated experimental

evidence does not favor any single triggering event to explain the hepatocarcinogenic process by peroxisome proliferators. Some of the hypotheses are calcium mobilization, a cascade of oncogene activation, sustained cell growth, increased turnover of specific hepatocyte population, the effect of the activated PPAR on the differentiation state, and the long-term consequences of the metabolic imbalance resulting from increased peroxisomal enzyme activities; oxidative stress may also be involved in the carcinogenic process. Oxidative stress in various forms can lead to activation of NFκB. Studies have shown that treatment of rats with ciprofibrate increased hepatic NFκB activity. Thus, it is possible that the oxidative stress induced by peroxisome proliferators is responsible for activation of NFκB. Overall, these data suggest that NFκB may play an important role in liver homeostasis. And, possibly regulation of hepatocyte proliferation following treatment with peroxisome proliferators.

Another hypothesis suggests the cell proliferation observed after peroxisome proliferators treatment is due to the expression and release of TNF-α from the Kupffer cells. Kupffer cells also contain NFκB and can become activated in response to oxidative stress. The hypothesis is that the oxidative stress produced in hepatocytes by peroxisome proliferator treatment may activate NFκB in Kupffer cells and lead to cytokine synthesis and release. It is this cytokine that stimulates neighboring hepatocytes to undergo mitogenesis. Ames and associates have proposed that chemical carcinogens or promoters that are not mutagenic in *Salmonella* mutagenicity tests interact with cellular membranes and may cause DNA damage through stimulation of arachidonic acid cascade or the induction of an oxidative burst and lipid peroxidation. It is likely that the carcinogenicity of halogenated compounds is owing to their ability to form radicals which cause lipid peroxidation. Because lipid peroxidation is a chain reaction, it causes the production of a considerable number of reactive oxygen species, such as the hydroxyl radicals ($OH^{\bullet}$), H_2O_2, and the superoxide radical (O_2^-), which can damage DNA.

Chronic exposure to peroxisome proliferators results in accumulation of autofluorescent lipofuscin pigments. The accumulation of lipofuscin pigments is indicative of increased lipid peroxidation and is generally related to increased production of biologically damaging free radicals such as $OH^{\bullet}$. Furthermore, peroxisome proliferators can alter the peroxisomal enzyme profile such that the output of the oxygen species produced can be enhanced in that the increase in the H_2O_2 destructive enzyme catalase is proportionally small compared to the peroxisomal volume and H_2O_2 generating fatty acid β-oxidation is increased by peroxisomal proliferators. Peroxisome proliferators also increase the activity of uricase, which results in decreased levels of uric acid, a powerful antioxidant which is a scavenger for oxygen radicals. While it is known that excessive H_2O_2 is formed in the liver as a result of sustained proliferation, it is not well known whether H_2O_2 or other reactive oxygen species are directly involved in hepatocarcinogenesis.

Relevance to Public Health

The effect of peroxisome proliferators on human health is a fundamental toxicological concern primarily due to the pervasive presence of these chemicals from clinical (hypolipidemic drugs), occupational and environmental sources (industrial plasticizers). Therefore, from the public health perspective the concern is the ultimate outcome from chronic occupational exposure and long-term therapeutic effects of peroxisome proliferators. The available evidence indicates that potent peroxisome proliferators are carcinogenic in rats and mice. However, neither the mechanism of proliferation nor the events leading to the development of hepatocellular carcinomas are sufficiently well understood. Since peroxisomal proliferation was not observed in nonhuman primates and certain other species in a preliminary screening study conducted several years ago with clofibrate, the peroxisome proliferator-induced carcinogenic effects have been readily dismissed by some as being of no importance to humans. The question, 'are rodents a good model for human risk?' cannot be answered directly in this instance due to large species differences in response to peroxisome proliferators. Species such as the mouse, rat, and hamster are responsive to peroxisome proliferation, whereas guinea pig and monkey are not. Importantly, the morphological effects of these chemicals in liver have not been seen in humans or in human hepatocytes in culture. Rat liver peroxisomal proliferation was characterized as a unique atypical phenomenon restricted to the biology of peroxisomes in these species. However, in light of recent evidence that peroxisomal proliferation can be induced in a wide range of species, including subhuman primates, it seems appropriate to consider the biological implications of peroxisomal proliferators and assess their risk to humans.

Hypolipidemic drugs are being developed with the assumption that reduction of elevated serum lipid is necessary in order to control mortality and morbidity associated with cardiovascular disease. Although the causal relationship between the level of certain serum lipids and the development of the atherosclerotic

lesion and its ischemic complications is supported by experimental and human studies, there is little proof that either prevention or amelioration of coronary heart and peripheral atherosclerotic disease is achieved by lipid-lowering therapy. Studies have shown that newer lipid-lowering agents reduce the short-term risk of nonfatal and fatal myocardial infarcts and other debilitating complications of hypolipidemias. However, this has to be balanced with any long-term delayed carcinogenic risk which usually develops in 10–20 years. Thus, patients should be informed of the risks/benefits related to hypolipidemic long-term therapy. In contrast to the hypolipidemic agents, there is limited evidence for carcinogenicity of industrial plasticizers such as DEHP and DEHA in experimental animals. However, because the two compounds are widely used in the formulation of plastics, they may present a wider danger to the general public. Additional studies are needed to establish the carcinogenicity of these industrial plasticizers.

Accumulated experimental evidence suggests that carcinogenic and proliferative effects of peroxisome proliferators may not be related. Thus, the assumption that lack of or a minimal peroxisome proliferative response observed in the liver of some animals or humans to therapeutic dose levels of hypolipidemic drugs poses no danger to humans could be misleading, if carcinogenesis by these drugs is not mediated by proliferated peroxisomes. On the other hand, if carcinogenesis is directly related to their ability to induce both hepatomegaly and peroxisome proliferation, carcinogenic risk to humans could be predicted with some assurance by quantitative morphometric analysis of the alterations in peroxisome volume, numerical densities, and by changes in the levels of H_2O_2-generating peroxisomal oxidases including the β-oxidation system.

In summary, substantial progress has been made over the past few years in understanding the cytoplasmic organelle peroxisome and factors that alter its normal functions. Peroxisome proliferator-induced increase in the liver peroxisomes is associated with an approximately two-fold increase in catalase activity and several-fold increases in the activity of the peroxisomal fatty acid β-oxidation system. It is also evident from the available literature that hepatic peroxisomal proliferation appears to be a carcinogenic event in rodents, and this may depend on the potency of the inducer. However, there is no single mechanism that is attributed to the peroxisome proliferation or carcinogenesis induced by these agents. The hypothesis that peroxisome proliferator-induced carcinogenesis is mediated by disturbances in subcellular organelle homeostasis requires continued investigational attention because of the importance of these hypolipidemic drugs and industrial plasticizers to our society. In conclusion, advances in molecular biology that led to the discovery of PPARs in 1990 significantly enhanced our understanding that peroxisome proliferators exert their effects through activation of PPARs. For example, humans express hepatic PPARα and this receptor functions nearly identical to its rodent counterpart. However, human cells do not respond identical to the effects of peroxisome proliferators. Whether or not humans are at risk to the tumor-promoting effects of peroxisome proliferators will not be realized until the sequence of events initiated by ligand activation of PPAR and ultimately resulting in altered parameters of growth and differentiation in sensitive species such as rat or mouse is delineated.

See also: Chlorophenoxy Herbicides; Liver.

Further Reading

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Personal Care Products *See* Cosmetics and Personal Care Products.

Pesticide Residues: Joint FAO/WHO Meeting *See* Joint FAO/WHO Expert Meetings (JECFA and JMPR).

Pesticides

Carey N Pope

The word pesticide literally means an agent used to kill an undesirable organism. In the amended US Federal Insecticide, Fungicide and Rodenticide Act, the definition of an 'economic poison' or pesticide was expanded to include

(1) Any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insect, rodent, nematode, fungus, weed, other forms of terrestrial or aquatic plant or animal life or viruses, bacteria, or other microorganisms, except viruses, bacteria, or other microorganisms on or in living man or other animals, which the Administrator declares to be a pest) and (2) any substance or mixture of substances intended for use a plant regulator, defoliant, or desiccant.

The major classes of pesticides in use today include herbicides, fungicides, rodenticides, insecticides, nematocides, acaricides, and molluscides. Prior to about 1940, pesticides were primarily inorganic chemicals (e.g., arsenic) and a few natural agents from plant origin (e.g., nicotine and pyrethrum). With the discovery of the insecticidal activity of DDT, however, a burgeoning increase in the development and utilization of synthetic organic chemicals occurred. From about 1940 to 1980, an exponential increase in the production and use of these synthetic pesticides was evident worldwide. The major chemical classes of pesticides in use today include inorganic and organic metals, chlorinated hydrocarbons, organophosphorus compounds, carbamates, pyrethroids, substituted phenols, substituted ureas, coumarins, organic acids, organic amides, triazines, and neonicotinoids. Currently, $\sim$1 billion pounds of $\sim$600 different pesticides (active ingredients) are produced each year in the United States alone, with total worldwide production estimated at $\sim$5 billion pounds.

Insects were the first major focus of pest control, whether to prevent the destruction of food or fiber crops or to limit the spread of insect vectors of disease. There is little doubt that the use of insecticides had a profound impact on the further development of civilization. The control of anopheline mosquitoes and malarial infection, as well as vectors for typhus, plague, and yellow fever, by DDT undoubtedly saved millions of lives. Over the past several decades, however, the use of herbicides has dramatically increased and such efforts have markedly altered the methods of modern agriculture. As a result, herbicides now represent the most extensively used class of pesticides in the United States. Some food and fiber crops reportedly increased yields by 300–600% after the introduction and widespread use of synthetic herbicides.

While the public health and economic benefits of synthetic pesticide use over the past 50 years are indisputable, these benefits have not been without costs. Widespread environmental contamination by DDT and other organochlorine pesticides, reaching global proportions, with concomitant deleterious effects on some members of the food web heralded the end of an era for their extensive use. DDT was banned from use in the United States in 1972 and most other organochlorines were subsequently banned, being replaced by the less environmentally persistent organophosphates and carbamates. While these agents had considerably lower abilities to accumulate in environmental and biological media, they tended to be much more acutely toxic and thus more hazardous to utilize. The pyrethroids are generally regarded as safer than the anticholinesterase organophosphates and carbamates, but still constitute a smaller proportion of total insecticidal use. In general, herbicides exhibit markedly lower acute mammalian toxicity than other classes of pesticides. The relative toxicities of these agents are generally scaled, however, on the basis of acute reactions. More recent findings suggest that many pesticides may have actions at lower levels of exposure that are more subtle in nature but with long-lasting consequences. For example, a number of studies suggest that the organophosphorus insecticide chlorpyrifos may alter neurodevelopmental processes in the mammalian brain and that those effects may not be elicited through the common mechanism of toxicity for this class of pesticides, that is, through acetylcholinesterase inhibition. While herbicides as a class typically elicit selective toxicity in plants with markedly less toxicity in mammalian species, the common herbicide and groundwater contaminant atrazine can disrupt luteinizing hormone and prolactin secretion

through direct action on the hypothalamic–pituitary axis, possibly altering reproductive success. Endocrine disruption by direct interaction with hormone receptors or alteration of hormone metabolism is a real concern for a number of pesticides in different chemical classes, with possible adverse health consequences for both wildlife and humans. Knowledge of the long-term health consequences of prolonged, low-level exposure to various pesticide classes is still limited. A major challenge for toxicologists in the future is the continued acquisition of data pertaining to the long-term effects of low-level pesticide exposures. In contrast, recent events worldwide heighten concern that easily accessible and common pesticides might be used in chemical terrorism, with either direct human health consequences or long-term environmental contamination.

See also: Carbamate Pesticides; Chlorophenoxy Herbicides; Federal Insecticide, Fungicide, and Rodenticide Act, US; Nematocides; Occupational Toxicology; Organochlorine Insecticides; Organophosphates; Pollution, Soil; Pollution, Water; Psychological Indices of Toxicity; Pyrethrins/Pyrethroids; Veterinary Toxicology.

Further Reading

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Krieger R (ed.) (2001) Handbook of Pesticide Toxicology, 2nd edn. San Diego, CA: Academic Press.

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Relevant Website

http://www.epa.gov – US Environmental Protection Agency.

Petroleum Distillates

Stephen R Clough

- Representative Chemicals: Organic solvents
- Chemical Abstracts Service Registry Number: CAS 8052-41-3
- Synonyms: Petroleum distillates; Petroleum naphtha; Naphtha
- Chemical/Pharmaceutical/Other Class: Aromatic hydrocarbons

Uses

Petroleum distillates are used as general extractants and universal solvents for paints, varnishes, fats, furniture polishes, and waxes. They are also used as vehicles for medication and pesticide applications, as counter-irritants, and as degreasers, detergents, and fuel. Most can be purchased 'over the counter', particularly mineral spirits, which is a widely used solvent or diluent for nonlatex paints.

Background Information

The term 'petroleum distillates' generally refers to petroleum naphtha or petroleum ether, which contain the lower boiling fractions (boiling point range, 86–140°F) of petroleum, principally pentanes and hexanes, with minor amounts of paraffins ranging up to 13 carbons. Petroleum naphtha is also known by the following synonyms: Amsco H-J, Amsco H-SB, Benzin B-70, HI-Flash naphtha, hydrotreated naphtha, naphtha coal tar, naphtha, petroleum naphtha, solvent naphtha, petroleum benzin, petroleum-derived naphtha, petroleum oil, Super VMP, UN1255, UN1256, UN1270, UN2553.

A solvent obtained from higher boiling distillates (boiling point range, 203–320°F), generically known as ligroin or 'varnish-makers' and painters' naphtha' (VM&P naphtha), may also occasionally be referred to as 'petroleum distillates'. Other synonyms for this solvent include benzin, benzine (light petroleum distillate), benzoline, canadol, ligroin, painters naphtha, petroleum ether, petroleum spirit, refined solvent naphtha, Skellysolve F, Skellysolve G, UN1271, varnish makers' naphtha, VM and P naphtha, VM&P naphtha.

Mineral spirits, also known as petroleum spirits, is another commonly used solvent that distills at an even higher temperature than naphtha (boiling point range, 302–392°F). It is also known as Stoddard

solvent, refined petroleum spirits, white spirits, Amsco 140, Soltrol, Soltrol 50, Soltrol 100, or Soltrol 180.

Thus, the term petroleum distillates may be used generically and interchangeably for two or three different types of petroleum distillation fractions. It can be confusing in that many commercial products will list the term as an ingredient even though it does not contain petroleum naphtha (e.g., the label of a solvent may say 'contains petroleum distillates' simply because some of the components were derived from crude oil).

Exposure Routes and Pathways

The primary exposure pathway for these solvents is inhalation of volatile components or absorption through skin. Inhalation of fumes can be hazardous, especially in environments with poor ventilation. Residential exposures to vapors may be significant if volatile components are allowed to accumulate in enclosed areas (e.g., using paint containing petroleum distillates in a poorly ventilated, enclosed space such as closets).

Toxicokinetics

Petroleum distillates are well absorbed through the gastrointestinal track if ingested and through the skin if contact occurs. Vapors are well absorbed through the lung. Because distilled fractions are mixtures, the distribution, metabolism, and excretion would be different for the different types of naphthas or spirits.

Mechanism of Toxicity

Although the toxicity of Stoddard solvent is not attributable to any one type of constituent, the aromatic components are considered to be more toxic than the paraffin or naphtha/naphthene components. Solvents tend to cause a nonspecific narcosis. This is generally believed to be due to the disrupting (or solublizing) effect that the solvent has on cellular membranes, particularly those of the nervous system.

Acute and Short-Term Toxicity (or Exposure)

Acute toxicity is generally defined as a single (or short-term) exposure to a fairly high concentration of the chemical in question. Acute effects are generally reversible upon removal of the animal or person from the chemical source.

It can be assumed, based on the adverse effects following exposure to these mixtures, that the central nervous system (CNS) is a primary target organ. The most common symptom of overexposure is dizziness and headache. Effects are generally similar to those seen with the methane series.

Animal

Acute toxicity information on mineral spirits (as Stoddard solvent) is sparse. Short-term animal studies have shown depression of the CNS and irritation of the eyes, nose, and throat. Draize skin irritation tests on rabbits resulted in a final score of 'moderate'.

Toxicity tests on laboratory animals using different types of petroleum distillate formulations have shown oral LD_{50} values ranging from 4.5 to 25 ml kg^{-1}. Inhalation LC_{50} values have ranged from 1600 to 73 000 ppm. The majority of laboratory rats subjected to chemical aspiration experiments (up to 0.2 ml) do not survive.

The acute inhalation LC_{50} for the rat is greater than 5500 mg m^{-3} (4 h whole body exposure). The acute oral LD_{50} in the rat is greater than 5000 mg kg^{-1} and the acute dermal LD_{50} in the rabbit is greater than 3000 mg kg^{-1}. All three of these acute values indicate that the overall toxicity to laboratory animals is relatively low.

Human

Irritation of the skin and/or respiratory tract is a common symptom following acute exposures to petroleum ethers. Reactions of human skin include edema (swelling), erythema (reddening), and disruption of the horny layer. Acute inhalation of high concentrations of petroleum ether may cause cerebral edema. Accidental ingestion may cause aspiration pneumonia and pneumatoceles in children.

Chronic Toxicity (or Exposure)

Chronic toxicity is generally defined as the repeated exposure to relatively low concentrations of a chemical over a long period of time. Chronic effects are generally less reversible than acute affects and may have serious long-term consequences (such as emphysema or cancer).

Animal

In experiments with VM&P naphtha, temporary hematological effects have been observed. As in humans, CNS depression is commonly seen following exposure; at high concentrations, convulsions are sometimes seen.

Using Stoddard solvent, long-term (chronic inhalation) rat studies have shown no outward signs of distress and only slight effects on the lung (irritation) and the liver and kidney (190–330 ppm for 13 weeks). Chronic inhalation tests in other animals (dogs, monkeys, guinea pigs, rabbits) show similar findings: no significant outward signs of toxicity (80–200 ppm in air) with lung irritation (e.g., congestion, emphysema) as the primary adverse effect.

No adverse teratogenic effects were seen in rats exposed to air concentrations that were high enough to induce maternal toxicity (950 ppm).

Human

The naphtha mixtures that are distilled at a lower boiling temperature have a higher volatility and, generally speaking, a higher degree of toxicity than the higher boiling fractions. In some occupational settings, chronic exposure to petroleum distillate has resulted in damage to the CNS, sometimes irreversible. Adverse effects on blood-forming components have also been reported, although the frequency of this effect has decreased considerably since the removal of benzene from these mixtures.

Petroleum ether (ligroin) consists primarily of *n*-pentane and *n*-hexane. Therefore, the primary effects seen are on the CNS, including peripheral nerve damage and depression.

Effects related to *n*-hexane intoxication, including paresthesia, loss of appetite, muscle weakness, and impaired motor function have been seen in workers chronically exposed to petroleum ether in inadequately ventilated buildings.

VM&P naphtha, also known as 'light naphtha' and 'spotting naphtha', is used extensively in the thinning of lacquers, varnishes, and rapidly evaporating paint thinner. It is a mildly irritating to the nose and eyes. Workers exposed to this mixture have been known to experience symptoms typical of intoxication with aliphatic compounds, including lightheadedness, labored breathing, tremors, hyperactivity, and nausea. Petroleum-derived distillates have not been shown to be carcinogenic in humans.

In Vitro Toxicity Data

Stoddard solvent has not been shown to be mutagenic in rat or mouse bioassays.

Clinical Management

If overexposure occurs, medical attention should be sought immediately. Persons exposed to high vapor concentrations should vacate or be removed from the source of the vapor and put in fresh air. If there are breathing problems, respiratory support should be provided (artificial respiration or oxygen, as appropriate). If skin has been exposed, the exposed area should be washed promptly with soap and large amounts of tepid water. Contaminated clothing should be removed. If eyes have been exposed, they must be irrigated immediately with tepid water. If swallowed, vomiting should not be induced. Emergency treatments that could result in introduction of solvents in the lung should be avoided. Aspiration pneumonia can also occur in children who have ingested solvents and then accidentally inhaled the solvent during vomiting. Symptoms should be treated and medical attention should be sought.

Rescuers should take care in areas with high vapor concentration. Care should be taken to control any potential ignition sources, such as sparks from static electricity.

Environmental Fate

Petroleum distillates that are spilled onto the ground may migrate to, and contaminate, groundwater supplies. Because they are volatile chemicals, however, most environmental releases will ultimately end up migrating to the atmosphere.

Ecotoxicology

No environmental guidelines or criteria for petroleum distillates in water, sediment, or air were identified. Some state regulatory agencies, such as Massachusetts Department of Environmental Protection, do have health-based environmental criteria (principally for soil) for various 'fractions' of aliphatic or aromatic hydrocarbons.

Other Hazards

Petroleum distillates are highly flammable so care must be taken when using these solvents near ignition sources or devices that can induce sparks. Contact with strong oxidizers should be avoided. Persons using paint(s) containing petroleum distillates should exercise caution when painting in poorly ventilated, enclosed areas.

Exposure Standards and Guidelines

For Stoddard solvent, the Occupational Safety and Health Administration has established a time-weighted average (TWA) standard of 500 ppm (2900 mg m^{-3} of air) for an 8 h workday, 40 h workweek to prevent nervous system and skin damage.

The National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit TWA for VM&P naphtha is 350 mg m^{-3} TWA for a 10 h workday and a ceiling level of 1800 mg m^{-3} (15 min sampling period). NIOSH recommends preventing contact with eyes and skin. The immediately dangerous to life or health value is 10 000 ppm.

The American Conference of Governmental Industrial Hygienists threshold limit value, TWA, is 1370 mg m^{-3} (300 ppm) for VM&P naphtha. The lower explosive limit is 1.2%.

See also: Heptane; Hexane; Neurotoxicity; Octane; Pentane; Petroleum Ether; Petroleum Hydrocarbons; Stoddard Solvent.

Relevant Websites

http://www.intox.org – Canadian Center for Occupational Health and Safety. Cheminfo. Chemical Profiles Created by CCOHS.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Petroleum Distillates.

Petroleum Ether

Patricia J Beattie

- Chemical Abstracts Service Registry Number: CAS 8032-32-4
- Synonyms: Ligroin; Refined solvent naphtha; Varnish makers' and painters' naphtha (VM&P naphtha); Skellysolve
- Chemical/Pharmaceutical/Other Class: Petroleum ether is a petroleum distillate made up primarily of aliphatic and alicyclic hydrocarbons in the C_5–C_{10} range
- Chemical Formula: Petroleum ether is a complex mixture. The following is a typical composition; however, this will vary depending on petroleum feedstock and refining process:
  - 50–80% paraffins (C_5–C_{10}) (% by volume)
  - 20–40% monocycloparaffins
  - 2–10% aromatics
  - The boiling point range for petroleum ether is 38–150°C; the flash point range is −18–13°C. The most common production process used today results in petroleum ether with $\leqslant$0.002% benzene and $\leqslant$5.0% *n*-hexane

Uses

Petroleum solvents are typically grouped into three classes based on volatility and aromatic content. They are (1) special boiling range solvents, (2) white spirits, and (3) high boiling aromatics. Petroleum ether is in the special boiling range solvent class. It is used in the rubber industry and as a degreasing agent. Petroleum ether is a common constituent in adhesives, inks, paints, varnishes, and lacquers.

Exposure Routes and Pathways

Exposure occurs most commonly by either inhalation or through skin contact.

Toxicokinetics

Petroleum ether is absorbed by the lungs following inhalation exposure. It is metabolized by the liver with a biological half-life of 46–48 h.

Mechanism of Toxicity

The acute toxicity from overexposure to petroleum ether is manifested primarily in central nervous system (CNS) effects. The mechanism of toxicity is unknown; however, the general anoxia observed is most likely due to oxygen deprivation. The mechanism of toxicity from long-term overexposure to petroleum ether is dependent on the chemical makeup of the distillate. For example, if peripheral neuropathy is observed, it is most likely due to a high concentration of *n*-hexane in the petroleum ether. *n*-Hexane is known to cause axonal damage in peripheral nerves.

Acute and Short-Term Toxicity (or Exposure)

Animal

In general, the toxicity reported from exposure to petroleum ether is more pronounced with samples containing higher concentrations of aromatic compounds. The irritation potential of petroleum ether to skin and eyes as tested in rabbits in the Draize protocol ranged from minimally to moderately irritating. Inhalation toxicity of VM&P naphtha was studied in mice, rats, cats, and dogs at concentrations ranging from 280 to 15 000 ppm. Acute exposure to high concentrations resulted in loss of motor coordination and CNS depression. Eye irritation was reported in rats at 3400 ppm after 4 h and dogs at 3400 ppm after 2 h. Respiration rate was decreased

in mice after exposure for 1 min to $\geqslant$2600 ppm. The 4 h LC_{50} in rats was reported to be 3400 ppm.

Human

Skin contact with petroleum solvents can cause allergic contact dermatitis. Preexisting skin disease may increase the potential for adverse effects. Overexposure via inhalation of petroleum ether affects primarily the CNS. Short-term, high overexposure is associated with an excitatory phase followed by a depressive phase. Exposures of 100–400 ppm for 7 h have resulted in headaches, fatigue, and incoordination with dose-associated effects on equilibrium, reaction time, visuomotor coordination, and memory. Inhalation exposures of 445–1250 ppm resulted in blurred vision, a cold sensation in extremities, fatiguability, headache, fatty demylination of muscle fibers, and demylination and mild axonal degeneration. Exposure to 880 ppm produced eye and throat irritation with temporary olfactory fatigue.

Chronic Toxicity

Animal

Beagle dogs were exposed to 1200 ppm of petroleum ether for 6 h day^{-1}, 5 days $week^{-1}$ for 13 weeks. No significant toxicity was reported. In long-term mouse skin painting studies using petroleum distillate fractions similar to petroleum ether, local necrosis, ulceration, marked regenerative epidermal hyperplasia, and, in some cases, squamous cell carcinomas have been reported.

Human

Several cross-sectional epidemiology studies have investigated the CNS effects observed in industrial painters, house painters, car painters, shipyard painters, and floor layers. Subjective symptoms such as headache, fatigue, poor coordination, emotional instability, impaired memory and other intellectual functions, and impaired psychomotor performance have been reported. Because most of these workers were exposed to a multitude of chemicals, in addition to petroleum ether, it is difficult to evaluate the cause of the reported effects.

In Vitro Toxicity Data

The majority of data suggest that petroleum ether is not mutagenic, based on *in vitro* tests using cultured mammalian cells, yeast, or bacterial test systems. Genotoxic potential is correlated with polynuclear aromatic hydrocarbon concentration.

Clinical Management

Overexposure to vapors of petroleum ether is treated by removing the patient to fresh air. If skin or eye contact occurs, the affected areas should be flushed with water for at least 15 min to remove residual solvent. Good personal hygiene and regular washing of skin and clothes minimizes the potential for developing allergic contact dermatitis. If ingestion of petroleum ether occurs, vomiting should not be induced. This could result in aspiration of solvent into the lungs, leading to chemical pneumonitis, and pulmonary edema, which can be fatal. If ingestion is suspected and the patient is coughing, there is a good possibility that aspiration has occurred. The patient should be monitored closely; hospitalization may be indicated.

Environmental Fate

Petroleum ether degrades rapidly in soil and water. In air, it reacts with photochemically produced hydroxyl radicals with an estimated half-life of 4–8 days. Based on water solubility and estimated bioconcentration factors, petroleum ether is not expected to bioconcentrate in aquatic organisms.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration: Permissible exposure limit, 8 h time-weighted average (TWA) is 100 ppm (400 mg m^{-3}).
- American Conference of Governmental Industrial Hygienists: Threshold limit value, 8 h TWA is 300 ppm (1370 mg m^{-3}). A3 – Confirmed animal carcinogen with unknown relevance to humans.
- National Institute for Occupational Safety and Health: Recommended exposure limit, 10 h TWA is 100 ppm (400 mg m^{-3}).
- Immediately dangerous to life or health limit is 1000 ppm.

See also: Neurotoxicity.

Further Reading

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Petroleum Hydrocarbons

Shayne C Gad

Petroleum is a naturally occurring, oily, flammable liquid composed principally of hydrocarbons; it is occasionally found in springs or pools but is usually obtained from beneath the Earth's surface by drilling wells. Formerly called rock oil, unrefined petroleum is now usually termed crude oil. Crude oil is a highly complex mixture of paraffin, cycloparaffinic (naphthenic), and aromatic hydrocarbons, containing a low percentage of sulfur and trace amounts of nitrogen and oxygen compounds.

The most important petroleum fractions, obtained by cracking or distillation, are various hydrocarbon gases (butane, ethane, and propane), naphtha of several grades, gasoline, kerosene, fuel oils, gas oil, lubricating oils, paraffin wax, and asphalt. From the hydrocarbon gases, ethylene, butylene, and propylene are obtained; these are important industrial intermediates, being the source of alcohols, ethylene glycols, and monomers for a wide range of plastics, elastomers, and pharmaceuticals. Benzene, phenol, toluene, and xylene can be made from petroleum. Hundreds of other products, including biosynthetically produced proteins, are petroleum derived.

Petroleum is separated by distillation into fractions designated as (1) straight-run gasoline, boiling at up to ~390°F (200°C); (2) middle distillate, boiling at ~365–653°F (185–345°C), from which are obtained kerosene, heating oils, and diesel, jet, rocket, and gas turbine fuels; (3) wide-cut gas oil, which boils at ~653–1000°F (345–540°C), and from which are obtained waxes, lubrication oils, and feedstock for catalytic cracking to gasoline; and (4) residual oil, which may be asphaltic.

The physical properties and chemical composition of petroleum vary markedly, depending on its source. As it comes from the earth, it ranges from an occasional nearly colorless liquid consisting chiefly of gasoline to a heavy black tarry material high in asphalt content. Most crudes are black; many are amber, red, or brown by transmitted light and show a greenish fluorescence by reflected light and have a specific gravity in the range ~0.82–0.95.

Hydrocarbons constitute 50–98% of petroleum, and the remainder is composed chiefly of organic compounds containing oxygen, nitrogen, or sulfur and trace amounts of organometallic compounds. The hydrocarbon types found in petroleum are paraffins (alkanes), cycloparaffins (naphthenes or cycloaldanes), and aromatics. Olefins (alkenes) and other unsaturated hydrocarbons are usually absent.

The number of carbon atoms in hydrocarbons of a given boiling range depends on the hydrocarbon type. In general, gasoline will include hydrocarbons having 4–12 carbon atoms; kerosene, 10–14; middle distillate, 12–20; and wide-cut gas oil, 20–36. Five main classes of compounds are present in the gasoline fraction: straight-chain paraffins, branched-chain paraffins, alkylcyclopentanes, alkylcyclohexanes, and alkylbenzenes. Several physiochemical properties (viscosity, surface tension, and volatility) significantly influence toxicity.

Asphalt is a dark-brown to black solid or semisolid consisting of carbon, hydrogen, oxygen, sulfur, and sometimes nitrogen. It is made up of three components: (1) asphaltene, a hard, friable, infusible powder, (2) resin, a semisolid to solid ductile and adhesive material, and (3) oil, which is structurally similar to the lubricating oil fraction from which it is derived.

Acute exposure to unleaded gasoline and a variety of light hydrocarbons present in gasoline produces a nephropathy in male rats characterized by (1) an excessive accumulation of protein (hyaline droplets) in epithelial cells of proximal tubule, (2) accumulation of casts at the corticomedullary junction, and (3) evidence of mild tubular regeneration. This nephropathy only occurs in male rats; female rats and mice do not show any renal pathology. A number of chemicals present in unleaded petrol when tested alone have been shown to produce nephropathy and, in particular, 2,2,4-trimethylpentane and decalin have been used as model compounds. Certain other industrial chemicals (1,4-dichlorobenzene and isophorone), natural products (D-limonene), and pharmaceuticals (levamisole) also produce this male-rat-specific nephropathy. Chronic exposure of male rats to unleaded petrol, 1,4-dichlorobenzene, isophorone, or D-limonene ultimately leads to the induction of a low incidence of renal adenomas and carcinomas.

Studies on the mechanism of pathogenesis have shown that the protein which accumulates in the proximal tubular cells is α_{2u}-globulin, a low-molecular weight protein of 18 700 Da that is synthesized in the liver of adult rats and is freely filtered at the glomerulus. Female rats excrete less than 1% of the α_{2u}-globulin that male rats excrete. The chemical itself or a metabolite has been shown to bind reversibly to α_{2u}-globulin and this chemical-protein complex is then thought to be taken up by the proximal tubular cells (primarily in the S2 segment) by

endocytosis. These complexes appear to be quite resistant to, or impair, lysosomal degradation, which leads to their accumulation of polyangular droplets. Lysosomal overload is thought to lead to individual cellular necrosis, which is followed by repair and regeneration. It has been suggested that a sustained increase in renal cell proliferation can promote initiated cells to form preneoplastic foci and lead to renal neoplasia. The development of the renal toxicity and increased cell proliferation is dependent on the presence of α_{2u}-globulin The NCI Black–Reiter strain of male rat cannot synthesize α_{2u}-globulin and, by inference, would not be expected to be at risk. However, it is not known whether these hydrocarbons or their metabolites can bind to other low-molecular-weight proteins and, if so, whether the same biochemical events as those observed with α_{2u}-globulin could occur.

Products with viscosity in the range of 30–35 or lower present an extreme aspiration risk and include agents such as mineral seal oil, which is found in furniture polishes. It is important to realize that even small amounts of a low-viscosity material, once aspirated, can involve a significant portion of the lung and produce a chemical pneumonitis. Oral ingestion of hydrocarbons often is associated with symptoms of mucous membrane irritation, vomiting, and central nervous system depression. Cyanosis, tachycardia, and tachypnea may appear as a result of aspiration, with subsequent development of chemical pneumonitis. Other clinical findings include albuminuria, hematuria, hepatic enzyme derangement, and cardiac arrhythmias. Doses as low as 10 ml orally have been reported to be potentially fatal, whereas some patients have survived the ingestion of 60 ml of petroleum distillates. A history of coughing or choking in association with vomiting strongly suggests aspiration and hydrocarbon pneumonia. Hydrocarbon pneumonia is an acute hemorrhagic necrotizing disease that can develop within 24 h after the ingestion. Pneumonia may require several weeks for complete resolution.

Activated charcoal and/or emesis may be indicated in some hydrocarbon ingestions in which absorption may produce systemic effects. Agents such as asphalt, tar, heavy lubricants, vaseline, and mineral oil are considered relatively nontoxic and do not require removal. Chlorinated hydrocarbon solvents or any hydrocarbon or petroleum distillate with a potentially dangerous additive (camphor, pesticide, and heavy metals) in some cases may be treated with activated charcoal or emesis. Petroleum naphtha derivatives, gasoline, kerosene, and mineral seal oil (or signal oil) as found in furniture polish and oil polishes produce severe and often prolonged chemical pneumonitis. These compounds are poorly absorbed from the stomach but are very damaging to the lung if inhaled. They should not be removed by emesis unless very large amounts are ingested ($\geqslant$12–18 ml kg^{-1}). Gastric lavage is not indicated for hydrocarbon ingestion because of the risk of aspiration if the patient vomits around the lavage tube. X-rays taken early in the course of ingestion may not demonstrate chemical pneumonia; even if it is demonstrated, the clinical severity does not correlate well with the degree of X-ray findings. However, X-rays should be repeated on follow-up to detect the development of pneumonitis or demonstrate pneumatoceles. Patients who arrive coughing probably already have aspirated and should be monitored closely for the development of pneumonitis. The decision for hospitalization should be based on clinical criteria (e.g., cyanosis and respiratory distress) rather than on X-ray findings alone. Steroid therapy may be harmful. Antibiotics, oxygen, and positive end expiratory pressure should be instituted as indicated.

The usual cutaneous response to oil-based materials is an oil folliculitis that arises as a result of chemical irritation and mechanical plugging of the follicular canals. Onset of the problem usually occurs soon after the first exposure and is marked by acute reactions starting on the dorsal surfaces of the hands and fingers, the extensor surfaces of the forearms and thighs, and the abdomen (i.e., those surfaces that are in contact with oil or oil-soaked clothing). Comedones and perifollicular papules and pustules ('oil boils') develop. Secondary infections may occur, but the bacteria in the oil are rarely primary skin pathogens and are rarely the single cause of the folliculitis. Melanosis may appear later. Clinical manifestations clear rapidly with the termination of exposure and do not resolve if the exposure is continued. Exposure is controlled through proper machine design to prevent spattering, clean clothing, protective garments, and careful attention to hand washing and other aspects of personal hygiene.

Since emulsion and synthetic fluids are potent defatting agents, the skin reaction to them may include maceration, dryness and 'chapping', reddening, and vesiculation. Bacterial growths in the fluid do not appear to be directly injurious to workers, but rancid fluids and products of bacterial action can lead to skin disorders. As in the case of insoluble oils, both treatment and prevention are based on the control of exposure. Corticosteroid creams may be used as an adjunct in the treatment. The value of 'barrier' creams and other protective gels is not universally accepted but they do offer modest usefulness in certain situations and have been shown to reduce ultrastructural and cytoarchitectural changes in human epidermis after applications of acetone and kerosene.

Individual additives in cutting fluids can be a cause of either primary irritative or hypersensitive dermatitis. Detergents, soaps, and wetting agents defat the skin, and alkaline materials damage the keratin of the upper, protective skin layers. Ulcerative and erythematous lesions on the genitals and buttocks have been reported for workers wearing coveralls that had been dry-cleaned with Stoddard solvent, a mixture of petroleum distillates. Formalin in germicides is a sensitizer. Additives containing sulfur and chlorine are direct irritants, although so-called chloracne is not associated with cutting fluids. Nickel or chromates derived from metals being cut can be a source of allergic dermatitis. Harsh abrasive soaps and solvents, such as gasoline and kerosene, may contribute to chemical and traumatic dermatitis since these cleaning materials are common in machine shops. While grime and grease can certainly be removed from the skin with these substances, it is safer to utilize less injurious cleansers available commercially.

Certain petroleum oils have carcinogenic constituents; this is especially the case with shale oils, which are currently extracted and used outside of the United States. Since American potential supplies of oil shale tars constitute 94% of the known world resources, these substances may present toxic problems in the United States in the future. There are no good data that would establish the prevalence of skin cancers among machinists in this country, but scrotal and other skin cancers have been reported among British cotton mule spinners prior to 1953 and more recently among tool setters and machine operators in the British Midlands. Knowledge of occupational malignancies of the skin has a long and important history that dates back to 1775 when Pott identified scrotal cancer in English chimney sweeps. A particular set of carcinogenicity bioassays, the mouse skin painting studies, were developed specifically to assess the carcinogenic potential of petroleum products.

Exposure to mist sprays or insoluble oils used as coolants, cutting fluids, and lubricants in machine operations are usually not harmful to the respiratory tract, although worker discomfort occurs at oil mist levels above $5\,\mathrm{mg\,m^{-3}}$. Mineral oil droplets $< 5\,\mu\mathrm{m}$ in diameter may be inhaled and result in fibrotic nodules, paraffinomas, or in lipoid pneumonitis. There was no evidence that machinists exposed to cutting-oil mists had any unusual mortality from respiratory tract cancer.

See also: Kerosene; Oil, Crude; Oil, Lubricating; Polycyclic Aromatic Hydrocarbons (PAHs).

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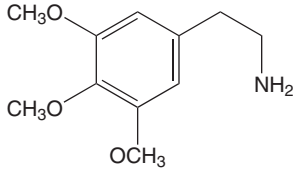
Peyote

Amanda Lofton

This article is a revision of the previous print edition article by Janet E Bauman, volume 2, pp. 500–501, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 11006-96-5
- Synonyms: 3,4,5-Trimethoxyphenethylamine; Anhalonium; Bad seed; Big chief; Button; Cactus; Indian dope; *Lophophora williamsii*; Mesc; Mescal; Mescal button; Mescaline; Moon tops; Peyotl; Turnip cactus
- Chemical/Pharmaceutical/Other Class: Mescaline, the major active alkaloid found in peyote, is a narcotic hallucinogen
- Chemical Formula: $C_{11}H_{17}NO_3$
- Chemical Structure:



Uses

Peyote is used as a Native American religious intoxicant, a hallucinogenic agent, and a folk remedy.

Background Information

Peyote is the common name given to *Lophophora williamsii*, the North American dumpling cactus. The

peyote cactus is a flowering plant of the family Cactaceae, a group of fleshy spiny plants found primarily in dry climates. Spines are present only in very young seedlings. The cactus areole, the area on the stem that usually produces flowers and spines, is well pronounced in peyote and is identified by a tuft of hairs or trichomes. Flowers arise from within the center of the plant. Leaves are greatly reduced and only microscopic in size. The plant also produces a pink bitter-tasting berry that contains black seeds.

Peyote has been used in tribal ceremonies by indigenous cultures in North America since 1000 BC. In the year 1560, Spanish priest Bernardino de Sahagún wrote about the use of peyote and hallucinogenic mushrooms by the Aztecs. The first proper botanical description of peyote was made by Hernandez, the naturalist of Philip II of Spain, in 1638. Dried peyote buttons were processed and distributed by Parke Davis and Company in 1887. By 1930, over a dozen states in the United States had outlawed the possession of peyote and in 1967 peyote was banned nationwide by the federal government.

Exposure Routes and Pathways

Peyote is ingested in various forms, including dried buttons, tincture of peyote (70% alcohol), and pan-peyote (chloroform extract of ground peyote). Synthetic mescaline has been administered orally, intravenously, subcutaneously, and intramusculary.

Toxicokinetics

Mescaline is rapidly absorbed, with peak blood levels noted within 2 h of ingestion. Gastrointestinal effects appear ~30–60 min after exposure and sensory effects peak between 4 and 6 h postingestion. Symptoms usually resolve within 12–14 h. Mescaline is not bound to plasma proteins, but to liver proteins. The volume of distribution is large, with the agent widely distributed among a number of organs. Brain and blood levels are nearly equal; concentrations in the kidneys, liver, and spleen are 3 to 5 times greater. Mescaline is metabolized in the liver to a variety of inactive metabolites. Approximately 60% of a dose is excreted unchanged in urine. The elimination half-life of the compound is ~55 min.

Mechanism of Toxicity

Mescaline causes hallucinogenic effects by stimulating serotonin and dopamine receptors in the central nervous system. The sympathomimetic effects of mescaline are probably also centrally mediated. Changes in catecholamine metabolism and adrenal medullary function may be responsible for the agent's peripheral effects. In animals, mescaline decreases the synthesis of the cofactor nicotinamide adenine dinucleotide in the brain. Mescaline may also produce cerebral vasospasm.

Acute and Short-Term Toxicity (or Exposure)

Animal

Doses of 20 mg kg^{-1} in animal studies led to bradycardia, hypotension, and peripheral vasodilation. The lethal dose of mescaline in animals ranged from 150 to more than 500 mg kg^{-1}, depending on the species and the route of administration. The terminal events in animals given mescaline overdoses were seizures followed by respiratory arrest.

Human

A dose of 5–8 mg kg^{-1} by any route causes the desired psychedelic effects. One dried peyote button contains ~45 mg of mescaline. Nausea, chills, and vomiting, which are often accompanied by anxiety and terror, occur first in most users. Diaphoresis, tachycardia, and hypertension are common. Photophobia secondary to mydriasis, nystagmus, tremors, ataxia, and hyperreflexia may also present. These sympathomimetic effects are followed by vivid visual hallucinations and exaggerated sensitivity to sound and other sensory perceptions. Users describe increased clarity and intensity of thought. Death from mescaline has not been reported. Hallucinations may lead to psychotic or suicidal behavior resulting in trauma and death. The qualitative presence of mescaline in urine can confirm the diagnosis; blood levels do not correlate with toxicity.

Chronic Toxicity (or Exposure)

Human

Flashbacks, or reoccurrence of hallucinogenic effects, have been reported. Persistent psychosis, anxiety, and depression have been described following mescaline use. Tolerance, but not physical dependence, to mescaline's effects has been reported in humans. Additionally, chronic users may demonstrate cross-tolerance to the effects of LSD or psilocybin.

Clinical Management

Treatment consists mainly of supportive care. A nonthreatening environment should be maintained and calm reassurance provided to the patient. Because mescaline is rapidly absorbed and vomiting is common, gastric decontamination is usually not necessary. However, activated charcoal will adsorb

mescaline and can be used prior to the occurrence of symptoms. Benzodiazepines are recommended to sedate agitated patients. Haloperidol can be administered to patients who fail to respond to benzodiazepines, but should not be used in children. Phenothiazines may increase the risk of flashbacks in later years. Patients with massive mescaline ingestion may require ventilatory support.

See also: LSD (Lysergic Acid Diethylamide); Mescaline; Plants, Poisonous.

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Pharmacokinetic Models

Natalie Eddington

Pharmacokinetics/toxicokinetics is the area of toxicology that is concerned with the role of absorption, distribution, metabolism, and excretion of toxicants in the body. These events, some of which may be interdependent, often have a very significant impact on the toxicity of a chemical in a specific species. Quantitative characterization of the time profile of absorption, distribution, metabolism, and excretion of xenobiotic compounds is included in the area of pharmacokinetics. In this sense, pharmacokinetics is used synonymously with toxicokinetics.

One of the methods of examining the kinetics of absorption, distribution, metabolism, and excretion of a xenobiotic, particularly of a toxicant, is the use of physiologically based pharmacokinetic (PBPK) models. PBPK models are mathematical models that permit predictions about body burdens, clearance profiles following cessation of the exposure, and provide other information that may aid in assessing the hazards of chemicals to humans. In these models, body is described as a series of relevant compartments in contact with the venous and arterial supplies of blood wherein anatomy and physiology decides the structure of model. The physiological information, such as blood flow rates to each compartment, the partition coefficients between the blood and organ tissues, and the different volumes of the various compartments, can then be used to build differential mass-balance equations that describe the rate of change of concentrations of the chemical of interest in the compartments.

PBPK models are especially useful for characterizing tissue-level doses when external exposures (exposures contacting the biological barriers or membranes of the organism (e.g., skin)) are repeated or intermittent in nature. Such models utilize physiological parameters and biochemical transformation data to determine the temporal relationships of the distribution and disposition of an administered dose.

PBPK models require three different types of information: (1) partition coefficients that describe the relative solubility or affinity of the compound for blood versus other tissues; (2) physiological constants, such as tissue and organ volumes and the relevant blood flows; and (3) rate constants for the key elimination pathways.

Often, PBPK models for toxicokinetics application require special considerations (e.g., volatile toxicants may incur tissue–air partition coefficients and alveolar elimination rates). Partition coefficients are generally obtained by measurement in the laboratory, tissue volume/blood flow data are mostly available from the scientific literature (with allometric scaling from species to species), and biotransformation data are usually obtained from *in vivo* and *in vitro* kinetic studies. Biochemical constants for metabolic pathways are captured using the maximum rate of reaction, or V_{max}, and the binding affinity of the particular substrate for the metabolizing enzyme.

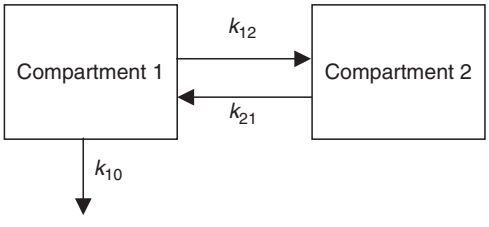
Two limiting cases relating to permeability of the cell membrane should be considered in developing a pharmacokinetic model for a given chemical. In the flow-limited model, transport of the chemical across the cell membrane is assumed to be so rapid that the rate of blood flow is taken to be the limiting process that determines the rate of uptake. In the membrane-limited model, cell permeability is assumed to be low, and thus the rate-limiting step, compared with the blood perfusion rate. The pharmacokinetics of volatile hydrocarbons and halogenated hydrocarbons, such as methylene chloride, are adequately described by a flow-limited model. Larger molecular weight compounds and many drugs, such as digoxin and

methotrexate, are most appropriately described by a membrane-limited model.

In constructing a pharmacokinetic model, it is not necessarily critical to place each tissue or organ into a separate compartment; rather, organs with similar blood flows, diffusion characteristics, and permeability properties can often be combined into single compartments. For example, the adrenals, kidneys, thyroid, brain, heart, and heptoportal system are sometimes pooled into one compartment because their perfusion-to-volume ratios are relatively high, facilitating their classification as a vessel-rich group. The liver, lungs, and gastrointestinal tract are usually represented separately. For toxicokinetics applications, however, the decision of inclusion/exclusion or lumping of compartments is greatly influenced by the properties of toxicant and nature of its toxicity (e.g., a localized toxicity to a specific organ tissue may require it to be included as an individual compartment).

PBPK models are particularly useful for interspecies extrapolations of dose–response data. In using a PBPK model of uptake, distribution, and elimination, an exponential power (e.g., 0.75) of the body weight is used to scale the cardiac output and ventilation rate between the laboratory species (typically rat) and humans. A PBPK model will therefore contain adequate logic to account for routes of administration, storage tissues and residence time therein, elimination rates, and sufficient mathematical detail to mimic the integration of these processes. It is important that the model parameters (e.g., elimination rates) be validated as much as possible by separate kinetic studies in the relevant species. The ultimate test of the model is how the model predictions are for parameters such as blood levels, rate of metabolism, and tissue concentrations relative to real-life animal data for the chemical.

The simplest model is a two-compartment model in which there is a plasma compartment and tissue compartment which have reversible flows of compound or metabolites or both between them. The compartment model for a drug which follows bi-exponential pharmacokinetics is shown below. In addition, the general forms of the equations that describe the rate of change of drug in the two compartments are presented below for both the central (compartment 1, eqn (1)) and the peripheral (compartment 2, eqn (2)) model, respectviely:



$$dX_1 = -(k_{12} + k_{10}) \cdot X_1 + k_{12} \cdot X_1 + k_{21} \cdot X_2 \quad (1)$$

$$dX_2 = k_{12} \cdot X_1 - k_{21} \cdot X_2 \quad (2)$$

X_1 and X_2 represent the doses of drug in the central compartment (1) and the peripheral compartments, respectively, k_{12} represents the intercompartment rate constant from the central to the peripheral compartment, k_{21} represents the intercompartment rate constant from the peripheral back to the central compartment, and k_{10} is the elimination rate constant.

Simultaneous integration of these two equations gives the explicit solution as a multiexponential equation, the exponents being expressed as a function of the distribution (α) and elimination rate constants (β), and factoring in the volumes of the compartment (V_c). The following equation (eqn(3)) represents the concentration versus time for a drug which follows a two-compartment model:

$$C_p = \frac{X_0(\alpha - k_{21})}{V_c(\alpha - \beta)} e^{-\alpha t} + \frac{X_0(k_{21} - \beta)}{V_c(\alpha - \beta)} e^{-\beta t} \quad (3)$$

PBPK have been developed and parameters defined for a number of chemicals, including methylene chloride, perchloroethylene, and pharmaceuticals (including anticancer drugs). The US Environmental Protection Agency commonly uses the Integrated Exposure Uptake Bio-kinetic Model for Lead to estimate the blood lead levels in children (up to 6 years) associated with multipathway environmental exposures. Acceptability of the concentration of lead in various environmental media is linked to a blood lead level in children believed to be protective of health. For methylene chloride, the mass-balance equations that form the model adequately account for the removal of the compound by the liver as well as the significant excretion through the lungs and incomplete retention of an inhaled dose, and they are useful for comparing organ-specific doses between different routes of exposure (e.g., oral and inhalation). Because of these features, the PBPK model prevents overestimation of dose that would be obtained, for example, if total absorption of an inhaled dose were assumed and if removal by the liver were ignored. Thus, such a model provides an effective tool for exposure assessment by quantifying the internal doses that are ultimately the most appropriate dose metrics to use in route-to-route comparisons.

See also: Absorption; Distribution; Excretion; Pharmacokinetics/Toxicokinetics.

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Pharmacokinetics/Toxicokinetics

Robert Tardif and Jules Brodeur

It is generally accepted that the intensity of toxic effects exerted by foreign chemicals (xenobiotics) is related to the concentration of the toxic moiety at the site of action in a target tissue or organ, whereas in many cases, the duration of the toxic effects depends on the time during which the toxic moiety remains at the site of action. However, there are numerous examples showing that the administration of two xenobiotics, at the same dose and with similar toxic potential, does not yield the same concentration of the toxic moiety for the same duration of time at a given site of action. The most plausible reason for such behavior is that the respective disposition of the two chemicals in the body can differ. Disposition may be regarded as the result of the absorption, distribution, and elimination processes acting on xenobiotics. In other words, disposition is what governs the fate of chemicals in the various compartments of the body and as such plays a key role in determining the concentration and toxicity of these chemicals at the site of action.

Pharmacokinetics/toxicokinetics may be defined as the study of the dynamic movements of xenobiotics during their passage through the body and as such encompass the concept of disposition described previously (**Figure 1**). In simpler words, it tells us what the body does to foreign chemicals. To that end, pharmacokinetic/toxicokinetic analysis uses mathematical terms, or equations, to describe the time course of the absorption and disposition of xenobiotics in the body and proposes simplified representations (models) of the relationship between time and movements of xenobiotics. Once the information on the concentration of a chemical in biologically relevant parts of the body is provided by pharmacokinetic/toxicokinetic studies, it then usually becomes possible to better understand, interpret, and even predict the nature and the extent of the biological effects of xenobiotics.

Etymologically, the term pharmacokinetics relates to the study of the movements of medicines or therapeutic agents within the body. From an historical point of view, principles and methods dealing with the study of the movements of chemicals within the body have evolved from data pertaining precisely

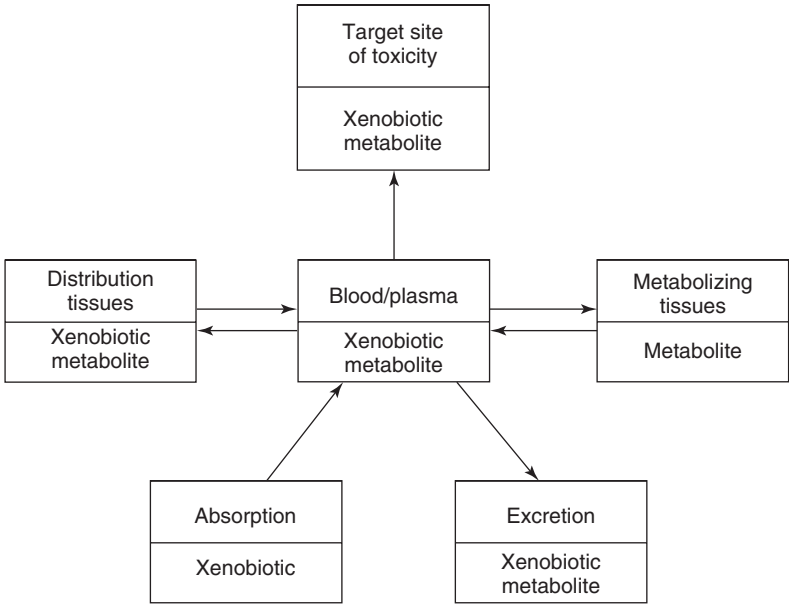

Figure 1 Schematic representation of the main biological processes involved in the disposition of xenobiotics in the body.

to medication. For example, pharmacokinetic data are used for optimal adjustment of the dosage regimen to obtain the best therapeutic effect without eliciting adverse side effects. The term toxicokinetics has a broader meaning in that encompasses the application of pharmacokinetic principles and methods to the prediction of occurrence and time course of toxic events related to foreign chemicals (medication, food additives, workplace products, environmental contaminants, etc.) encountered at levels of exposure likely to induce toxicity.

To facilitate the description of the biological fate of xenobiotics, toxicologists represent the body as compartments that correspond to the various tissues, organs, or fluids of the body. Such functional representation is known as pharmacokinetic modeling. Two types of models are currently available to toxicologists for that purpose: compartmental models and physiologically based pharmacokinetic (PBPK) models. Compartmental models have been extensively studied and used in pharmacokinetic modeling, but recently PBPK modeling has received increasing attention.

The purpose of this article is to introduce the reader to simple basic concepts and principles of pharmacokinetic/toxicokinetic analysis using both types of models – compartmental and physiologically based.

Pharmacokinetic Models

Compartmental Models

Compartmental models, also known as data-based models, are essentially used to fit curves to experimental data on blood, plasma, or urine concentrations of a chemical or its metabolite(s). In this approach, the body is represented as a single or a series of compartments that do not necessarily correspond to any physiological or anatomical reality. As mentioned previously, toxicologists are mainly concerned with avoiding toxicity in target organs/tissues that are presented with time-dependent concentrations of a chemical. It is not feasible, at least in humans, to determine the time course of the concentration of a xenobiotic at a target site (e.g., brain, liver, and kidneys). To overcome this problem, it is assumed, when using the compartmental modeling approach, that the biological effects which depend on the concentration at target site are also related to the concentration of a chemical in blood or plasma. This is the reason why almost all pharmacokinetic analyses are based on blood concentration.

Compartmental modeling consists of finding the proper mathematical equation of the curve that provides the best fit to the kinetic behavior of a xenobiotic (e.g., blood levels). In the simplest case, the body is represented as a single compartment (e.g., one-compartment model). When necessary, however, additional and usually limited numbers of compartments can be added to achieve a better description of the kinetic behavior of a particular xenobiotic (e.g., two- and three-compartment models).

When using such models, it is assumed that the disposition of a chemical is governed by first-order processes. This means that the rate of disappearance of a xenobiotic from the body, as a result of excretion and/or biotransformation, is proportional to the amount of the xenobiotic in the body at that time. In other words, the quantity of a xenobiotic that leaves the body is large when the amount of xenobiotic in the body is large (e.g., immediately after exposure), whereas this quantity is small when the amount in the body is small (e.g., several hours after exposure). Most xenobiotics exhibit this type of behavior, provided that the several biological mechanisms responsible for disposition are not saturated, i.e., not overwhelmed by large concentrations of xenobiotics (see section Dose-Dependent Kinetics).

One-Compartment Open Model In this simple model, the body is treated as a homogenous unit with an entry and an exit (i.e., open model) (**Figure 2a**). It is assumed that changes occurring in blood concentrations reflect similar changes in tissue levels as the xenobiotic rapidly equilibrates between blood and all the various tissues of the body.

Figure 2b illustrates the time dependency of the concentration of a xenobiotic in blood, following rapid intravenous administration. It is seen that the blood concentration decreases rapidly at the beginning and then falls more slowly thereafter. This is typical of first-order elimination as described previously. This curve can be described by using the following exponential term:

$$C_p = C_p^0 \times e^{-k \times t}$$

where C_p represents the blood (plasma) concentration of a xenobiotic at time t, C_p^0 is the blood initial concentration (i.e., extrapolated at time 0), and k is the first-order elimination rate constant. A more practical form of this equation is obtained by substituting the base 10 logarithm (**Figure 2c**):

$$\log_{10} C_p = \log_{10} C_p^0 - \frac{k \times t}{2.303}$$

This simple mathematical description is very useful for determining various kinetic parameters

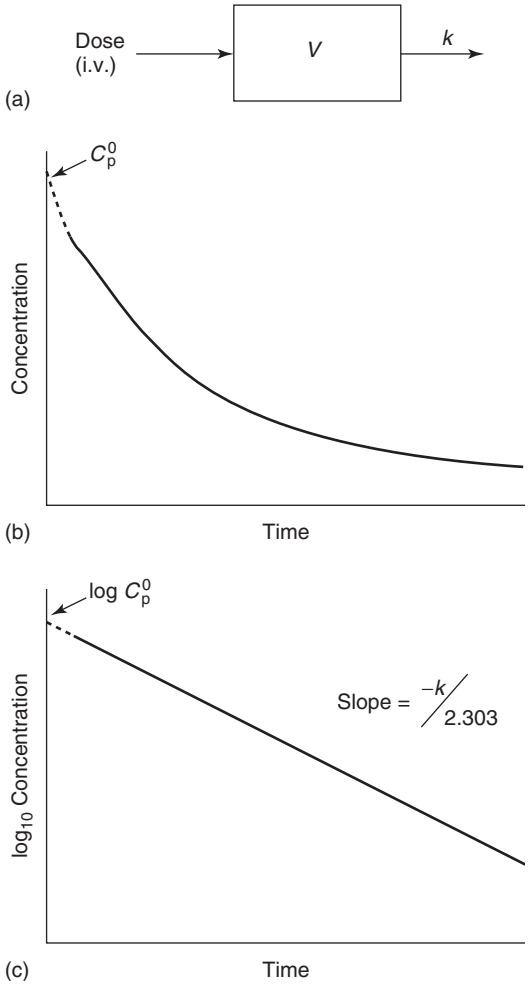

Figure 2 (a) Schematic representation of a one-compartment open model: i.v., intravenous administration; *V*, volume of the compartment; *k*, elimination rate constant (see text). (b) and (c) represent the time course of blood concentration of a xenobiotic following intravenous administration (b, linear scale; c, logarithmic scale); C_p^0, blood or plasma concentration at time zero.

that characterize the kinetic behavior of a given xenobiotic. These kinetics parameters are the volume of distribution, the elimination rate constant, the half-life of elimination, the clearance, the area-under-the-blood-concentration curve, and the bioavailability.

Volume of distribution (V_D) The volume of distribution is defined as the apparent volume (e.g., liter and milliliter) into which a chemical appears to have been dissolved, once it has penetrated in the body, to give an initial blood concentration equal to C_p:

$$V_D = \text{DOSE}/C_p$$

Usually, V_D does not correspond to any real biological volume and as such has no direct physiological meaning. In some cases, V_D may show values even larger than the volume of a standard human body. Indeed, since the value of V_D is inversely proportional to the blood concentration, chemicals showing an especially high affinity for fatty tissues (e.g., insecticides of the DDT family and several industrial solvents) and therefore having a strong tendency to leave the blood pool may exhibit large V_D values. On the other hand, a chemical that is firmly bound to blood components, such as the red cells and the proteins (e.g., warfarin, a blood anticoagulant), will remain in the blood pool and exhibit V_D values close to blood volume only.

Elimination rate constant (k) The elimination rate constant (i.e., usually a first-order rate constant) is a very useful value that represents the fraction of an agent that is eliminated from the body during a given period of time. For instance, when the value of the elimination rate constant of a xenobiotic is 0.25 per hour, this means that ~25% of the amount remaining in the body is excreted each hour. The rate constant is calculated from the slope ($-k/2.303$) of the curve relating blood concentration with time as shown in **Figure 2c**. Its value is affected by all processes (e.g., distribution, biotransformation, and excretion) that contribute to clear the substance from the blood.

Half-life of elimination ($t_{1/2}$) This is the time period (e.g., minutes, hours, and days) during which the blood concentration of a xenobiotic falls to one-half of its original value as a result of all processes of distribution, biotransformation, and excretion. The determination of $t_{1/2}$ is based on the calculation of the elimination rate constant described above (k):

$$t_{1/2} = 0.693/k$$

Xenobiotics that show small $t_{1/2}$ values (i.e., short half-lives) are those that are cleared rapidly from the body, whereas those with high values (i.e., long half-lives) are cleared more slowly and in some cases may accumulate in the body. Insecticides of the DDT family and heavy metals like lead, cadmium, and mercury all display long half-lives, whereas aspirin is a drug that exhibits a short half-life.

Clearance (CL) Clearance represents the volume of blood (e.g., milliliter and liter) that is completely cleared of a xenobiotic during a given period of time, usually 1 min or 1 h (e.g., ml min^{-1}, l h^{-1}). As such, the clearance is a quantitative measure of the rate of removal of a compound from the body. All routes of elimination (e.g., hepatic biotransformation, urinary, biliary, and pulmonary excretion) contribute to the clearance of a chemical from the body, and each one

exhibits a specific clearance value. Specific clearance values provide an indication of the ability of a particular organ to dispose of a substance. When the value for clearance is high, it suggests that the compound is removed rapidly from the body, whereas a low clearance value indicates slower removal. The value of the clearance is the product of the elimination rate constant (k) and the apparent volume of distribution (V_D) as described by the following equation:

$$CL = k \times V_D$$

Therefore, CL may be regarded as the apparent volume of blood from which the compound is removed during a given period of time.

Bioavailability (F) Bioavailability is a term used to describe the percentage (or the fraction F) of an administered dose of a xenobiotic that reaches the systemic circulation. Bioavailability is practically 100% ($F = 1$) following an intravenous administration. Bioavailability could be lower ($F \leqslant 1$) and in some cases almost negligible for other routes (e.g., oral, dermal, and pulmonary), depending on how efficiently a xenobiotic crosses various biological membranes (e.g., lungs, skin, and stomach) or whether or not tissues or organs (e.g., lungs, skin, and liver) through which xenobiotics pass before reaching the systemic circulation are capable of metabolizing the substance; the latter phenomenon is known as a first-pass effect. Bioavailability may vary considerably between compounds or even between batches of a given compound. For example, drugs commonly used as therapeutic agents must undergo bioavailability testing to ensure reliable dosing throughout treatment. The blood concentration of the administered drug is used as an index of bioavailability.

Area under the curve (AUC) The area-under-the-blood-concentration-time curve reflects the amount of a xenobiotic that has effectively reached the systemic circulation and as such is influenced both by the degree of bioavailability and by the rate at which a chemical is removed from the body. AUC is a good indicator of the internal exposure dose in the body since it takes into consideration not only the blood concentration of a xenobiotic but also the time a xenobiotic is present in the blood compartment and thus in the body.

In summary, the kinetic parameters described previously are used to describe the behavior of xenobiotics in the body following exposure via several routes: the extent of distribution within the body, the amount available for action and elimination, the contribution of specific organs in elimination, and the rate of elimination. Such information can be used to establish therapeutic drug regimens or to predict the extent and duration of contamination of exposed organisms.

Two-Compartment Open Model In certain circumstances, following the completion of the absorption phase, the curve that describes the time course of the blood concentration of a xenobiotic does not exhibit a single straight line but rather two segments (**Figure 3b**). Such biexponential decline can best be described by a two-compartment model (**Figure 3a**): a central compartment that usually refers to the blood pool and a peripheral compartment that represents various fluids and tissues of the body for which a xenobiotic may have a particular affinity. This system can be described mathematically by a differential equation comprising two exponential terms, one for each segment of the curve. Taken individually, each one of these terms is essentially similar to the one used to describe the curve corresponding to the one-compartment model:

$$\log_{10} C_p = \log_{10} A - \frac{\alpha \times t}{2.303} + \log_{10} B - \frac{\beta \times t}{2.303}$$

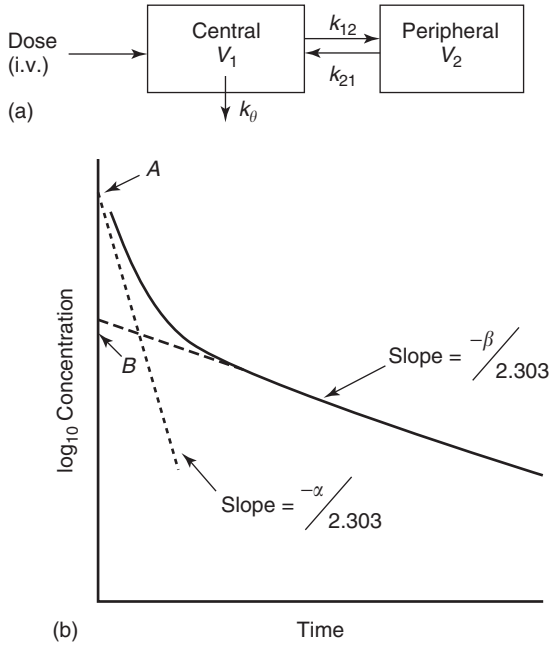

Figure 3 (a) Schematic representation of a two-compartment open model: i.v., intravenous administration, V_1 and V_2, respective volumes of compartments 1 and 2; k_{21} and k_{12}, transfer rate constants between compartments 1 and 2; k_θ, elimination rate constant. (b) Time course of blood concentration following intravenous administration: A and B, proportionality constants; α and β, elimination rate constants corresponding to each segment of the curve.

where A and B are proportionality constants for each compartment ($A + B = C_p$), and α and β are composite rate constants that can be regarded as the elimination rate constant of each segment of the curve (i.e., each compartment). The first segment is known as the α-phase, during which a chemical leaves the blood circulation to be distributed among the various organs and tissues, whereas the second segment corresponds to the β-phase, which mainly characterizes the processes leading to the elimination of a chemical. Accordingly, the $t_{1/2}$ of a xenobiotic displaying such kinetic behavior is calculated from the β-phase using an equation similar to the one previously described for a one-compartment model:

$$t_{1/2} = 0.693/\beta$$

How values A, B, α, and β are calculated falls beyond the scope of this text. Suffice it to say that these parameters represent values that contribute additively to the equation describing the two-compartment open model.

To facilitate the understanding of the pharmacokinetic concepts, the examples given previously are for the simplest and the most effective route of administration, that is, intravenous administration. When exposure is to toxic compounds (e.g., occupational or environmental exposure), however, other routes are frequently involved. These routes include respiratory, cutaneous, mucous, or oral uptake. In such cases, pharmacokinetic analyses are more complex since they should take into account the various processes responsible for the uptake of a xenobiotic. Usually, this consists of introducing into equations an additional term that contains a rate constant describing the uptake, operating in a direction opposite to, yet not conceptually different from the elimination rate constant.

Physiologically Based Models

Whereas compartmental models are abstract mathematical representations of an animal or a human body, in the form of a certain number of boxes, PBPK models describe the behavior of xenobiotics on the basis of the actual anatomy, physiology, and biochemistry of human beings and animals. Being realistically modeled on how the body functions, PBPK models take into consideration the complex relationships that exist between critical biological and physicochemical determinants such as blood flow, ventilation rates, metabolic rate constants, tissue solubilities, and binding to proteins (e.g., albumin and glycoproteins) or other macromolecules (e.g., DNA and hemoglobin).

Contrary to compartmental models, PBPK models allow one to describe the time course of xenobiotic concentration in any organ or tissue represented in the model. Since these models include anatomical, physiological, and biochemical determinants, they can account for any quantitative alterations of such determinants – for example, ventilation rates, organ pathology, or metabolic enzyme activity. Not only can they describe and model what is actually occurring under a given set of exposure conditions but also they can build on such a description and expand to any other condition likely to happen within the range of variation of the anatomical, physiological, and biochemical parameters.

A PBPK model comprises a series of anatomically well-defined compartments that represent organs or tissues in which a xenobiotic distributes or exerts its toxic effects (**Figure 4**). These anatomical compartments are interconnected by the blood circulation (i.e., arterial blood to and venous blood from the tissues). The physiological and anatomical determinants for different species, including humans (e.g., alveolar ventilation rate, blood flow rates, and tissue volumes), are usually abundantly documented in the literature. Physicochemical parameters – namely partition coefficients that describe the relative solubility

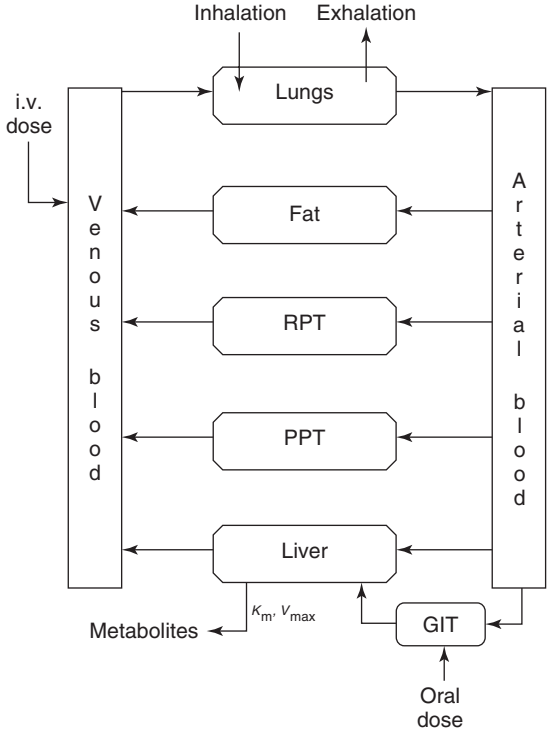

Figure 4 Schematic representation of a PBPK model with different routes of entry. RPT, richly perfused tissues (e.g., brain, kidneys, and spleen); PPT, poorly perfused tissues (e.g., muscles, skin, and bone; GIT, gastrointestinal tract. K_m and V_{max} are constants that characterize metabolizing tissues like the liver.

of a xenobiotic between air present in the lungs and blood, on the one hand, and between blood and tissues, on the other hand – may be obtained in some cases from the literature or otherwise determined experimentally in the laboratory. Usually, biochemical parameters, namely metabolic rate constants that describe the metabolic capacity of a tissue toward a given xenobiotic, are determined experimentally in the laboratory.

To each compartment corresponds a mass-balance differential rate equation that describes the rate of change in the amount (Amt) of a xenobiotic in this h tissue compartment, as the xenobiotic enters in, distributes within, and exits the tissue:

$$\frac{\mathrm{d}\ \mathrm{Amt}_i}{\mathrm{d}t} = Q_i(C_\mathrm{a} - C_\mathrm{vi})$$

where Q_i represents the volume of blood circulating throughout the tissue i per unit of time, C_a is the concentration of the xenobiotic in arterial blood entering the tissue, and C_vi is the concentration of the xenobiotic in venous blood leaving the tissue. For metabolizing tissues (e.g., liver), an additional term that takes into account the capacity of such tissues to operate the metabolic transformation of the xenobiotic is added to the basic differential equation described previously. Since the capacity of the liver and other metabolizing tissues is limited when large amounts of a xenobiotic are presented to the tissues, the basic equation contains terms (K_m and V_max) that account for such limitations:

$$\frac{\mathrm{d}\ \mathrm{Amt}_i}{\mathrm{d}t} = Q_i(C_\mathrm{a} - C_\mathrm{vi}) - \frac{V_\mathrm{max} \times C_\mathrm{vi}}{K_\mathrm{m} + C_\mathrm{vi}}$$

where the new terms K_m and V_max describe, respectively, the affinity of a xenobiotic for metabolizing enzymes and the maximum velocity of the enzymatic reactions.

The previously described equations are characteristic of blood flow rate-limited models; it is assumed that xenobiotics cross the cell membrane by simple diffusion and that equilibrium takes place instantaneously between blood and tissue compartments. This assumption is valid for a great number of chemicals. For certain xenobiotics, however, the kinetics of tissue uptake are not consistent with blood flow rate-limited processes since their distribution in a given tissue is limited by the resistance of the cell membrane to the passage of a xenobiotic. In these cases, the basic equation should account for such phenomena to describe adequately the time course of the xenobiotic disposition in the tissue.

Of course, various exposure routes (e.g., inhalation, intravenous, oral, and dermal) can be accounted for in PBPK models by incorporating the proper equation describing these uptake processes.

Once formulated, a PBPK model can be used to simulate the kinetic behavior of a xenobiotic (e.g., amount metabolized, blood or tissue concentrations, and percentage of dose excreted) in animals or humans. An important step in the development of PBPK model is its validation. Validation is usually based on the visual or statistical comparison of model predictions with experimental observations in humans or animals.

Once validated, PBPK models can be used by toxicologists for many purposes. For example, PBPK models can (1) provide an estimate of the time course distribution of xenobiotics and their metabolites in various parts of the body, including target organs/tissues; (2) allow various types of metabolic extrapolations between various species, from high doses of exposure to low doses, or from one route of exposure to another; (3) allow the examination of pharmacokinetic differences between species; (4) facilitate the setting and adjustment of exposure standards since it becomes possible to better estimate the concentration of a xenobiotic and its metabolite(s) in various body fluids or tissues, resulting from various exposure scenarios; and (5) predict changes in the disposition kinetics of xenobiotics resulting from physiological and pathological alterations in body function.

For all these reasons, PBPK models are and will continue to be increasingly used in toxicology. This is especially true in risk assessment studies since better definition of the internal tissue dose, may contribute to reduce the uncertainty associated with extrapolation to human beings of responses observed in animal toxicity studies in which animals usually receive high doses of xenobiotics by routes often different from the one(s) anticipated in human exposures.

Repetitive Exposures

Frequently, individuals are exposed repetitively to xenobiotics, be they medication, food additives, or environmental contaminants.

In general, chemicals exhibiting a short half-life (i.e., smaller than the period of time between each new exposure) are almost completely eliminated between exposures. Inversely, chemicals with a long half-life (i.e., longer than the period of time between exposures) tend to accumulate in the body leading eventually to increased risk of toxicity. In the latter case, if exposure continues at a relatively constant level, the accumulated chemical will reach a plateau, also called a steady-state level, when the amount of a xenobiotic that enters the body equals the amount eliminated during a given period of time.

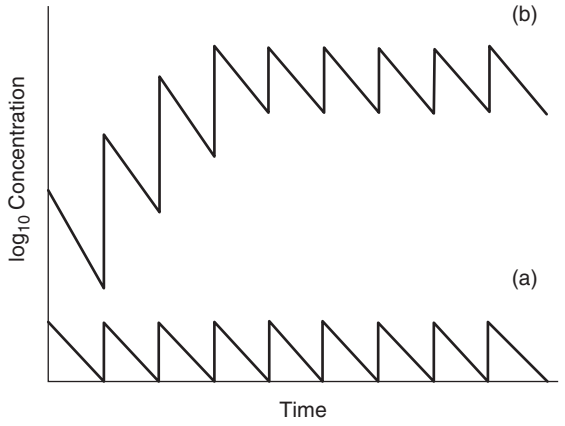

Figure 5 Time-course blood concentration of a xenobiotic following repeated intravenous administration: (a) xenobiotic half-life shorter than the period of time between exposures; (b) xenobiotic half-life longer than the period of time between exposures.

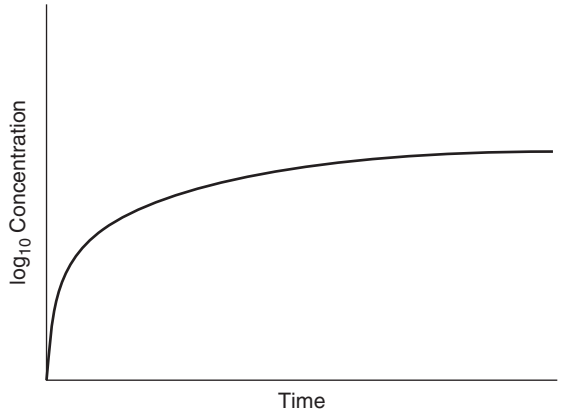

Figure 6 Time-course blood concentration of xenobiotic following continuous intravenous infusion or pulmonary inhalation until steady state.

Figure 5a and **b** illustrates the kinetics of a xenobiotic in blood resulting from repeated intravenous exposures. The time that is necessary to reach the steady state depends on the half-life of the xenobiotic and corresponds to about five times the half-life value, whereas the blood concentration is a function of the absorbed dose.

In contrast to the sawtooth pattern of blood concentrations during repeated, noncontinuous exposure to a xenobiotic (**Figure 5a** and **b**), the pattern resulting from continuous exposure is characterized by a single stable line (**Figure 6**). However, for both situations, the time to reach a plateau concentration and the amount present in blood obey the same rules of kinetics.

Thus, for a compound administered intravenously and described by a one-compartment model, the average steady-state blood concentration (C_{ss}) is

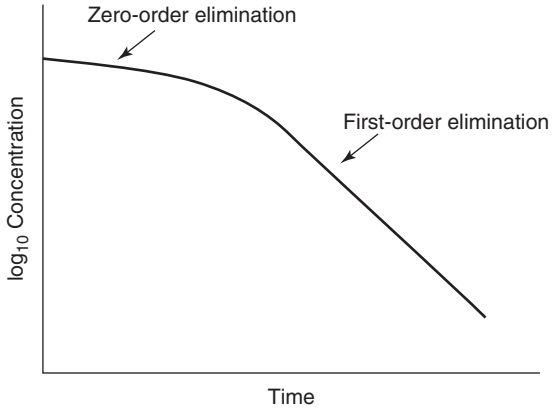

Figure 7 Time-course blood concentration of a xenobiotic exhibiting nonlinear (zero-order) kinetics following intravenous administration; when the biological processes responsible for the disposition of the xenobiotic are no longer saturated, first-order kinetics resume.

determined by the following equation:

$$C_{ss} = \frac{\text{Dosing rate}_{\text{i.v.}}}{V_D \times k \times \tau}$$

where V_D is the apparent volume of distribution, k is the elimination rate constant, and τ is the time interval between administered doses. As can be seen, the blood concentration is related proportionally to the administered dose but bears an inverse relationship to all other parameters.

Dose-Dependent Kinetics

As seen earlier, exposure conditions amenable to pharmacokinetic/toxicokinetic analysis are such that the rate of the biological processes (e.g., diffusion across membranes, biotransformation, excretion by glomerular filtration, etc.) is proportional to the concentration or amount of a xenobiotic in a given compartment such as blood. The rate is then said to be governed by first-order kinetics (see **Figures 2** and **3**).

There are biological processes, however, that involve saturable carrier or enzymatic systems, with a finite capacity for transport or catalysis. For instance, processes like active uptake at absorption sites, renal tubular secretion, or hepatic biotransformation of xenobiotics may become saturated at high exposure levels, yielding rates of disposition that are constant and independent of the concentration in blood. This is characteristic of zero-order kinetics. Biotransformations of ethanol in the liver and active tubular renal secretion of penicillin in urine are examples of biological processes that obey zero-order kinetics. **Figure 7** illustrates the blood concentration

of a chemical eliminated by zero-order kinetics. Since at high concentrations, the amount of a chemical that is biotransformated or excreted is limited by saturable processes, blood concentration falls less rapidly than when first-order kinetics prevails. This may result in more or less accumulation of that chemical in several tissues, including those that are especially sensitive to its toxic action.

Conclusion

Pharmacokinetic/toxicokinetic analysis is a very important tool that can help toxicologists understand how the body handles foreign chemicals. With a good knowledge of the time course relationship between exposure to chemicals and their concentration in various tissues and organs, toxicologists are in a position to better interpret and predict the nature and extent of toxicity.

More specifically, data pertaining to toxicokinetics are, and will increasingly continue to be, essential to properly:

- Predict the body burden of toxic chemicals in a critical organ or tissue.
- Understand the dose–response relationship of toxic chemicals.
- Assist in the selection of animal species that can act as a surrogate of human toxicity.
- Make rational extrapolations from high doses, as used in animal toxicity studies, to low doses, as encountered in the human environment.
- Set exposure limits to toxic chemicals for all kinds of living organisms, including humans.
- Identify potentially at-risk subgroups of exposed living organisms.

See also: Absorption; Distribution; Excretion; Pharmacokinetic Models.

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Renwick AG (2001) Toxicokinetics: Pharmacokinetics in toxicology. In: Hayes W (ed.) *Principles and Methods in Toxicology*, 4th edn., pp. 137–191. Philadephia: Taylor and Francis.

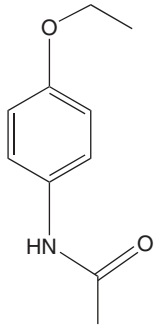
Rozman K and Klaassen CD (2001) Absorption, distribution, and excretion of toxicants. In: Klaassen CD (ed.) *Casarett and Doull's Toxicology. The Basic Science of Poisons*, 6th edn., pp. 107–132. New York: McGraw-Hill.

Pharmacology and Safety *See* Safety Pharmacology.

Phenacetin

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 62-44-2
- Synonyms: *p*-Acetophenetidide; 1-Acetamido-4-ethoxybenzene; Acetophenetin; 4-Ethoxy acetanilide; *p*-Acetylphenetidin
- Chemical/Pharmaceutical/Other Class: An NSAID (nonsteroidal antiinflammatory drug) with analgesic, antipyretic, and antirheumatic properties
- Chemical Formula: $C_4H_{13}O_2N$
- Chemical Structure:



Background Information

Phenacetin was introduced into the pharmaceutical market in 1887, and was withdrawn in 1983 in the United States due to unacceptable levels of interstitial nephritis in patients and potential risks of tumorgenicity. Like in the United States, most Western countries did not ban phenacetin from marketing until 1983.

Exposure Routes and Pathways

Possible exposure routes are oral and inhalation.

Toxicokinetics

Phenacetin is metabolized to acetaminophen and sulfhemaglobin-forming metabolite and other toxic metabolites. In the absence of adequate glutathione or a glutathione substitute, acetaminophen is further metabolized to cytotoxic and hepatotoxic molecules.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral rat LD_{50}: 3600 mg kg^{-1}; mouse 866 mg kg^{-1}; rabbit $>$500 mg kg^{-1}.

Human

Phenacetin is harmful if swallowed or inhaled, and may cause kidney, liver, and blood disorders. It may cause methemoglobinemia and hemolytic anemia due to acute toxicities, but more commonly as a result of chronic overdosage. A therapeutic plasma level was less than 20 μg ml^{-1}, with 50–150 μg ml^{-1} being toxic plasma levels in humans.

Chronic Toxicity (or Exposure)

Animal

Phenacetin is nephrotoxic, with positive results in mutagenicity and tumorgenicity studies.

An International Agency for Research on Cancer (IARC) Working Group reported that there is limited evidence of carcinogenicity of analgesic mixtures containing phenacetin in experimental animals.

Human

Phenacetin is group 2A (reasonably anticipated to be a human carcinogen) based on sufficient evidence of carcinogenicity in experimental animals according to the IARC Working Group noted above. The same group found that analgesic mixtures containing phenacetin are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans. The Human Health Assessment Group in (US) Environmental Protection Agency's Office of Health and Environmental Assessment has evaluated phenacetin for carcinogenicity. According to their analysis, the weight of evidence for phenacetin is group B2 (considered probably carcinogenic to humans), which is based on inadequate evidence in humans and sufficient evidence in animals.

Phenacetin is linked to hypertension, cardiovascular disease, and cancer, but was removed from the market primarily due to induction of chronic renal disease.

In Vitro Toxicity Data

Phenacetin was mutagenic to *Salmonella typhimurium* bacteria when tested in the presence of a metabolic system derived from hamster but not mouse or rat liver. The urine from phenacetin-treated Chinese hamsters, but not that from rats, was mutagenic to bacteria. It is activated to direct-acting mutagens by deacetylation, occurring more frequently in hamsters than rats. Phenacetin induced chromosomal aberrations in Chinese hamster cells *in vitro*, but not DNA strand breaks in rat hepatocytes. It did not induce sex-linked recessive lethal mutations in *Drosophila*.

Clinical Management

Methylene blue therapy (unlike with methemoglobinemia) does not help with the hematological effects.

Environmental Fate

Phenacetin is expected to leach into groundwater when released into the soil. When released into the water, this material is expected to have a half-life of more than 30 days. This material has an estimated bioconcentration factor of less than 100, and is not expected to significantly bioaccumulate. This material is expected to be readily degraded by reaction with photochemically produced hydroxyl radicals when released into the air. This material is expected to have a half-life of less than 1 day when released into the air.

Exposure Standards and Guidelines

The US Occupational Safety and Health Administration regulates phenacetin under the Hazard Communication Standard, and as a chemical hazard in laboratories. A reportable quantity (RQ) of 100 lb has been proposed for phenacetin under the EPA's

Comprehensive Environmental Response, Compensation, and Liability Act.

See also: Carcinogen Classification Schemes.

Further Reading

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Schnuelle P and van der Woude FJ (2003) Analgesics and renal disease in the postphenacetin era. *American Journal of Kidney Diseases* 42: 385–387.

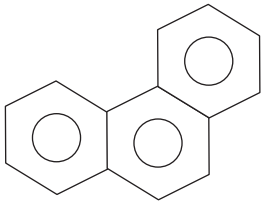
Relevant Website

http://ehp.niehs.nih.gov – US National Institute of Environmental Heath Sciences (NIEHS) National Toxicology Program (NTP) (2002) Phenacetin and Mixtures Containing Phenacetin (from the 10th Report on Carcinogens of the National Toxicology Program, 2002).

Phenanthrene

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 85-01-8
- Synonyms: Coal tar pitch volatiles; Phenanthrin
- Chemical/Pharmaceutical/Other Class: Polycyclic aromatic hydrocarbon
- Chemical Formula: $C_{14}H_{10}$
- Chemical Structure:



Uses

Phenanthrene is a polycyclic aromatic hydrocarbon (PAH) that can be derived from coal tar. Phenanthrene is used in the production of dyes, pharmaceuticals, and explosives, and in biochemical research. A derivative, cyclopentenophenanthrene, has been used as a starting material for synthesizing bile acids, cholesterol, and other steroids.

Exposure Routes and Pathways

Phenanthrene occurs in fossil fuels and is present in products of incomplete combustion. Some of the known sources of phenanthrene in the atmosphere are vehicular emissions, coal and oil burning, wood combustion, coke plants, aluminum plants, iron and steel works, foundries, municipal incinerators, oil shale plants, and tobacco smoke. It is widely distributed in the aquatic environment and has been identified in surface water, tap water, wastewater, and dried lake sediments. It has also been identified in seafood collected from contaminated waters and in smoked and charcoal-broiled foods. Phenanthrene has been identified in foods.

Human exposure occurs primarily through inhalation of tobacco smoke and other polluted air, and via ingestion of food or water contaminated by combustion effluents.

Toxicokinetics

Since it is the smallest aromatic hydrocarbon to have a 'bay-region' and a 'K-region', phenanthrene is often used as a model substrate for studies on metabolism of carcinogenic PAHs. Phenanthrene is absorbed following oral and dermal exposure. Data from structurally related PAHs suggest that phenanthrene would be absorbed from the lungs. Metabolites of phenanthrene identified in *in vivo* and *in vitro* studies indicate that metabolism proceeds by epoxidation at the 1-2, 3-4, and 9-10 carbons, with dihydrodiols as the primary metabolites.

Mechanism of Toxicity

Phenanthrene absorbs ultraviolet light and causes production of singlet oxygen, which in turn leads to free radical production. Although a large body of literature exists on the toxicity and carcinogenicity of other PAHs, primarily benzo[*a*]pyrene, toxicity data for phenanthrene are limited.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{Lo} in mice is 71 mg kg^{-1}. In mice, the oral LD_{50} is 700 mg kg^{-1}, the intraperitoneal LD_{50} is 700 mg kg^{-1}, and the intravenous LD_{50} is 56 mg kg^{-1}. Single doses of 100 mg kg^{-1} day^{-1} of phenanthrene administered by gavage for 4 days suppressed carboxylestrase activity in the intestinal mucosa of rats, but did not produce other signs of gastrointestinal toxicity. Phenanthrene had no effect on hepatic or extrahepatic carboxylesterase activities. Single intraperitoneal injections of phenanthrene produced slight hepatotoxicity in rats.

Human

Phenanthrene can cause phototoxicity and photosensitization of the skin. No other human data were available that addressed the acute toxicity profile of phenanthrene.

Chronic Toxicity (or Exposure)

Animal

Phenanthrene may cause skin allergy, and is considered phototoxic. It has induced sister chromatid exchanges in Chinese hamster cells. The available data are inadequate to permit an evaluation of the carcinogenicity of phenanthrene to experimental animals; however, a number of other PAHs have caused tumors in laboratory animals via oral, inhalation, and dermal exposures. A single oral dose of phenanthrene did not induce mammary tumors in rats, and a single subcutaneous injection did not result in treatment-related increases in tumor incidence in mice. Neonate mice administered intraperitoneal or subcutaneous injections of phenanthrene also did not develop tumors. No skin tumors were reported in two skin painting assays with mice. Phenanthrene was also tested in several mouse skin initiation-promotion assays. It was active as an initiator in one study, inactive as an initiator in four others, and inactive as a promoter in one study.

Human

Phenanthrene is classified as category D for human carcinogenicity by the US Environmental Protection Agency, that is, it is not classifiable as to human carcinogenicity.

In Vitro Toxicity Data

Phenanthrene induced mutations in a human cell in culture and in the Ames *Salmonella*. Phenanthrene was shown to inhibit colony formation of HeLa cells.

Environmental Fate

Release of phenanthrene most likely results from the incomplete combustion of a variety of organic compounds including wood and fossil fuels. Release to the soil will likely result in biodegradation. Volatilization from soil is not expected to be significant. Phenanthrene is expected to bind strongly to soil and not leach extensively to groundwater. When released to water, adsorption of phenanthrene to suspended sediments is expected to remove most of the compound from solution. Photolysis is expected to occur near the water surface and biodegradation in the water column is expected. Bioconcentration is not expected to be significant. Phenanthrene released to the atmosphere is expected to rapidly adsorb to particulate matter. It will react with hydroxyl radicals with an estimated half-life of less than 2 days. Uptake, accumulation, and translocation of phenanthrene and pyrene by 12 plant species grown in various treated soils was investigated, and the plant uptake and accumulation of both compounds was correlated with their soil concentrations and plant compositions.

Ecotoxicology

Phenanthrene has been shown to be toxic to marine diatoms, gastropods, mussels, crustaceans, and fish. The toxic effects of several aromatic hydrocarbons (benzene, toluene, naphthalene, 1-methylnaphthalene, anthracene, 9-methylanthracene, and phenanthrene) on the productivity growth rate of various marine planktonic algae (*Dunaliela biocula*, *Phaeodactylum tricornutum*, and *Isochysis galbaya*) increased with an increasing number of aromatic rings. The methylated compounds were most toxic. The TL_m (median lethal dose) for exposure of *Neanthes arenaceodentata*, a member of the polychaete family, to phenanthrene is 0.6 ppm for a 96 h exposure in seawater at 22°C in a static bioassay.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average (TWA) is 0.2 mg m^{-3} for coal tar pitch volatiles, as is the (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA. The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h workday, is 0.1 mg m^{-3} for coal tar pitch volatiles. Further, NIOSH considers coal tar

products carcinogenic and conditions should be made to keep exposures as low as possible. Current NIOSH research indicates that asphalt products are carcinogenic to laboratory animals and, therefore may be more toxic to humans than previously believed. Phenanthrene is a toxic pollutant designated pursuant to Section 307(a) (1) of the US Clean Water Act, and is subject to effluent limitations.

See also: Coal Tar; Polycyclic Aromatic Hydrocarbons (PAHs); Skin.

Further Reading

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International Agency for Research on Cancer (IARC) (1983) *IARC Monographs* 32: 419–430.

Relevant Website

http://risk.lsd.ornl.gov – US Oak Ridge National Laboratory, Risk Assessment Information System. Toxicity Profile for Phenanthrene.

Phenazopyridine

Christopher P Holstege

- Chemical Abstracts Service Registry Numbers: CAS 94-78-0; CAS 136-40-3 (hydrochloride salt)
- Synonyms: 3-Phenylazopyridine-2,6-diyldiamine; Pyridium
- Chemical/Pharmaceutical/Other Class: Azo dye

Uses

Phenazopyridine is utilized as an analgesic for the urinary tract, thereby providing relief of urinary urgency and/or frequency.

Exposure Routes and Pathways

Ingestion is the exposure pathway.

Toxicokinetics

Phenazopyridine is absorbed through the gastrointestinal tract. It is metabolized rapidly, producing aniline, 2,3,6-triaminopyridine, *p*-aminophenol, and *N*-acetyl-*p*-aminophenol. Renal clearance is the major route of elimination. Approximately 90% of a therapeutic dose is excreted in the urine within 24 h, with 41% as phenazopyridine, 24% as *p*-aminophenol, 18% as *N*-acetyl-*p*-aminophenol, and 6.9% as aniline. The color of the urine changes to orange or red.

Mechanism of Toxicity

Phenazopyridine may induce methemoglobinemia. Erythrocytes possess four hemoglobin chains, each of which contains a heme moiety. Methemoglobin occurs when phenazopyridine induces oxidation of the heme moiety, changing the normal oxygen-carrying ferrous (Fe^{2+}) state to the ferric (Fe^{3+}) state. Ferric heme is incapable of binding oxygen. Ferric heme also shifts the hemoglobin dissociation curve to the left, thereby impairing the release of oxygen from the remaining ferrous heme groups on the same hemoglobin tetramer. Oxygen delivery to tissues is therefore impaired. Red blood cells with methemoglobin also become rigid and are unable to traverse the spleen, with resultant destruction and anemia. Renal failure may result either due to the phenazopyridine itself, as a result of hemolytic anemia, or secondary to rhabdomyolysis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Phenazopyridine has been associated with methemoglobinemia, hemolysis, hepatic necrosis, muscle damage, and renal tubular necrosis in animals.

Human

Phenazopyridine-induced methemoglobinemia may manifest as dyspnea, tachycardia, cyanosis, dizziness, and syncope. Hemolysis may occur and result in anemia. Progressive oliguric renal failure may occur and is typically associated with methemoglobinemia and hemolysis, or with massive acute overdosage. Yellow discoloration of the skin and sclerae may occur due to deposition of this azo dye in the skin, primarily in patients with renal dysfunction. Rare cases of phenazopyridine-induced hypersensitivity hepatitis, rhabdomyolysis, and aseptic meningitis have been reported.

Chronic Toxicity (or Exposure)

Animal

Mice chronically fed phenazopyridine up to 1200 mg kg^{-1} 5 days a week for 80 weeks, survived. Animals receiving higher doses had greater rates of adenomas, adenocarcinomas, and carcinomas than the controls, and greater rates than those animals receiving lower doses.

Human

Patients have occasionally been treated with higher than therapeutic doses of phenazopyridine. Toxic effects (e.g., methemoglobinemia) are more commonly seen in patients with some degree of glucose-6-phosphate dehydrogenase deficiency. A man who took 600 mg phenazopyridine daily for 2 years developed pure phenazopyridine vesical calculi.

In Vitro Toxicity Data

Several carcinogenicity and mutagenicity studies have been performed on phenazopyridine. Mouse lymphoma studies have been mixed but rat hepatocyte studies have been positive.

Clinical Management

Symptomatic and supportive care is the mainstay of therapy. Adequate urine output should be assured. In acute overdose, charcoal may be considered. Methylene blue therapy may be considered for patients with methemoglobinemia. Dialysis has been used for phenazopyridine-induced renal dysfunction, but no studies have demonstrated an increased elimination of phenazopyridine with dialysis. In patients with hemolysis and marked anemia, transfusion may be necessary.

See also: Aniline; Carcinogenesis.

Further Reading

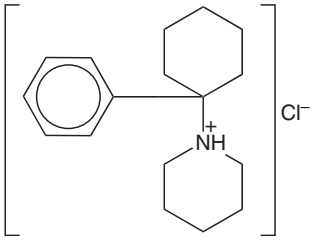
Mercieca JE, Clarke MF, and Phillips ME (1982) Acute haemolytic anaemia due to phenazopyridine hydrochloride in G-6-PD deficient subject. *Lancet* 2: 564.

Truman TL, Dallessio JJ, and Weibley RE (1994) Life-threatening pyridium plus(R) intoxication: A case report. *Pediatric Emergency Care* 10: 225–228.

Phencyclidine

Christopher P Holstege

- Chemical Abstracts Services Registry Numbers: CAS 77-10-1; CAS 956-90-1 (hydrochloride)
- Synonyms: 1-(1-Phenylcyclohexyl)piperidine; Angel dust; Busy bee; Crystal joint; Cyclones; DOA; Embalming fluid; Goon; Hog; Kay jay; Love boat; Lovely; Mint dew; Mist; Murder-1; Peace pill; PCP; Rocket fuel; Scuffle; Selma; Sernyl; Sernylan; Snorts; Soma; Star dust; Super grass; Super weed; Super kool; Surfer; Tranquilizer; Whacky weed; Zombie dust. Phencyclidine analogs with similar pharmacologic effects include phenylcyclohexylpyrrolidine (PHP), phenycyclopentylpiperidine (PCPP), thienycyclohexylpiperidine (TCP), and cyclohexamine (PCE)
- Chemical/Pharmaceutical/Other Class: Synthetic arylcyclohexylamine closely related to ketamine, a medically approved dissociative anesthetic
- Chemical Structure: Phencyclidine hydrochloride



Uses

Medical use in humans was discontinued in 1965 and veterinary use was discontinued in 1978. Today, phencyclidine is manufactured in illegal laboratories and utilized as a drug of abuse.

Exposure Routes and Pathways

Phencyclidine is sold illicitly as powder, tablets, liquid, rock crystal, or mixed with leaves (marijuana, oregano, mint, and parsley). Phencyclidine is ingested, smoked, snorted, and injected intravenously.

Toxicokinetics

When smoked or snorted, phencyclidine has an onset of action from 2 to 5 min; when ingested, effects are apparent within 30–60 min. Peak effects may be achieved 15–30 min after onset with effects persisting for as long as 24–48 h. Phencyclidine undergoes hepatic degradation by oxidative hydroxylation to two metabolites that have little psychotropic activity. The volume of distribution of phencyclidine is large, averaging $6\,l\,kg^{-1}$. Plasma protein binding is ~65%. Because of high lipid solubility, levels found in tissue far exceed those found in plasma. Phencyclidine follows first-order elimination kinetics. It undergoes enterohepatic recycling with subsequent excretion by the kidneys. The half-life of small doses is 1 h, increasing to 17.6 h (range, 7–50 h) in overdose.

Mechanism of Toxicity

The precise mechanisms by which phencyclidine causes its clinical effects have not been fully delineated. Phencyclidine blocks the *N*-methyl-*D*-aspartate (NMDA) receptors and thereby calcium influx into cells. Phencyclidine inhibits the biogenic amine reuptake complex and thereby inhibits norepinephrine and dopamine reuptake. Phencyclidine also increases adrenergic activity by indirectly releasing norepinephrine from presynaptic neurons. Phencyclidine in high doses stimulates sigma receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dose-dependent effects include depressed reflexes, tachycardia, twitching, dilated pupils, and hyperthermia. There are marked species differences in behavior. Mice primarily experience excitation. Dogs generally display depression at low doses and stimulation (e.g., seizures) at high doses.

Human

Phencyclidine use results in excitation with marked paranoid or aggressive behavior, which is often self-destructive. Effects include distortion of body image, diminished pain perception, illusions, and delusions, including a perception of superhuman strength and invulnerability. Miosis and nystagmus (horizontal, vertical, and rotary) may be seen in association with ataxia, bizarre behavior, and hallucinations. Tachycardia, hypertension, hyperreflexia, seizures, respiratory depression, and coma are reported with high doses. Dystonias and dyskinesias have been reported. Hypoglycemia can be seen. Rhabdomyolysis, acute renal failure, disseminated intravascular coagulation, liver necrosis, and traumatic injury are reported complications. The anesthetic dose of phencyclidine is $0.25\,mg\,kg^{-1}$ intravenously. Doses of 1–5 mg are purported to cause euphoria and numbness, 5–10 mg cause excitation and hallucinations, and 20 mg or more cause coma and serious toxicity or death. Plasma concentrations of phencyclidine vary widely after overdose. Phencyclidine crosses the placenta resulting in hyperirritability, tremors and hypertonia, depressed reflexes, and nystagmus in neonates.

Chronic Toxicity (or Exposure)

Animal

Rats demonstrate signs and symptoms of withdrawal after 7 days of receiving $45\,mg\,kg^{-1}\,day^{-1}$ phencyclidine.

Human

Cognitive decline, depression, anxiety, violent behavior, and weight loss are reported following chronic use of phencyclidine. Prolonged psychosis has been reported, which can mimic acute schizophrenia, and can persist for 4–6 weeks. Tolerance to the psychoactive effects can lead abusers to take increased doses. Psychological dependence has been noted, but no distinct withdrawal symptoms have been reported.

In Vitro Toxicity Data

Phencyclidine is extensively used in research because of its properties as a noncompetitive antagonist of NMDA glutamate receptors. *In vivo* and *in vitro* data have demonstrated that phencyclidine can produce apoptosis in the frontal cortex of rats.

Clinical Management

Adequate supportive care should be assured in the phencyclidine-intoxicated patient. There is no antidote for phencyclidine overdose. The patient should be isolated from all sensory stimuli as much as possible and protected from self-inflicted injury. Benzodiazepines should be administered liberally and titrated until the phencyclidine-intoxicated patient calms. Adequate hydration should be assured to maintain the urine output at $1–2\,cc\,kg^{-1}\,h^{-1}$. Although urine acidification theoretically enhances phencyclidine elimination, it is not recommended because of the high frequency of rhabdomyolysis and myoglobinuric renal failure seen with significant intoxication. Seizures should be treated with

benzodiazepines. Hypertensive crisis can be managed with nitroprusside. The patient's blood sugar, electrolytes, serum creatinine phosphokinase, urine myoglobin, and renal and hepatic function tests should be monitored.

See also: Benzodiazepines.

Further Reading

Aronow R, Miceli JN, and Done AK (1980) A therapeutic approach to the acutely overdosed PCP patient. *Journal of Psychedelic Drugs* 12: 259–266.

Patel R, Das M, and Palazzolo M (1980) Myoglobinuric acute renal failure in phencyclidine overdose: Report of observation in eight cases. *Annals of Emergency Medicine* 9: 549–553.

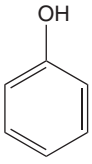
Phenelzine *See* Monoamine Oxidase Inhibitors.

Phenobarbital *See* Barbiturates, Long-Acting.

Phenol

Kathryn J Kehoe

- Chemical Abstracts Service Registry Number: CAS 108-95-2
- Synonyms: Carbolic acid; Hydroxybenzene; Phenic acid; Benzenol; Phenol alcohol
- Chemical/Pharmaceutical/Other Class: Phenol
- Chemical Formula: C_6H_5OH
- Chemical Structure:



Uses

Phenol is a product of the refining of oil and coal tar and is used industrially in the manufacture of pharmaceuticals, plastics, rubber, and plywood. It has antiseptic, germicidal, and anesthetic properties and may be used in disinfectants and preservatives. It is used in small amounts in many over-the-counter products with antiseptic properties. It is also a common reagent in nucleic acid/molecular biology research and is used to denature and remove protein from preparations of DNA and RNA.

Exposure Routes and Pathways

Phenol is readily absorbed from all surfaces of the body. Acute exposures by all routes (inhalation, skin contact, and ingestion) can be fatal. Inhalation exposure appears to be the most sensitive route of exposure, although limited, due to phenol's low volatility. Cigarette smoke contains phenol. Due to phenol's anesthetic properties initial exposure may not be painful; however in skin exposure, deep dermal damage progressing to gangrene is common.

Toxicokinetics

Phenol will undergo biotransformation to oxidation and conjugated products. Phenol and phenolic compounds can be oxidized by peroxidase-dependent prostaglandin H synthase to phenoxyl radicals. It is a reactive intermediate in the P450 oxidation of benzene to hydroquinone. Phenol that is not oxidized will undergo conjugation to etheral, sulfate, or glycuronate species. This is especially true of phenol introduced to the gastrointestinal tract. Conjugates appear less toxic than the parent compound and are subsequently excreted through the kidneys. A smaller amount may be eliminated through the lungs, as detected by an aromatic odor to the breath.

Mechanism of Toxicity

Phenol is a general protoplasmic poison. It can be oxidized to a reactive electrophile that combines with protein and DNA. The binding to hepatic or renal proteins leads to centrilobular and medullar damage, respectively.

Acute and Short-Term Toxicity (or Exposure)

Animal

Phenol shows high toxicity by all routes of exposure. The LD_{50} values in rats are as follows: 384 mg kg^{-1}

(oral), 669 mg kg^{-1} (skin), 250 mg kg^{-1} (interperitoneal), and 316 mg kg^{-1} (inhalation). The LD_{50} values for rabbits after dermal exposure is from 35 to 200 mg kg^{-1} body weight.

Human

Significant exposure to phenol by absorption through skin, by inhalation, or by ingestion can lead to death within minutes. Ingestion of even 1 g of phenol has been reported as lethal. It is extremely destructive to tissues of the mucus membranes and to the upper respiratory tract, skin, and eyes. Rapid death of nerve endings and tissue necrosis produces anesthesia and paralysis. Gastrointestinal, cardiovascular, and pulmonary symptoms will appear. Phenol effects on the gastrointestinal tract will result in pain, nausea, vomiting, and diarrhea. There may be cardiovascular collapse and subsequent shock. Pulmonary exposure can produce spasm, inflammation, and general edema. The central nervous system may have a transitory stimulation, followed by depression. Exposure to small amounts of phenol may result in a respiratory alkalosis similar to salicylate poisoning. This will be followed by an acidosis.

Chronic Toxicity (or Exposure)

Animal

Developmental studies performed in rats have shown decreased fetal weights and viability. Animal studies investigating long-term inhalation exposure to phenol have shown effects on the liver, kidney, respiratory, cardiovascular, and central nervous systems.

Human

Chronic exposure has been shown to damage the liver, kidney, and other major systems and has been correlated with an increased risk of ischemic heart disease. Literature reports of the human LD_{Lo} by the oral route range from 0.14 to 14 g kg^{-1}. Phenol is a known mutagen; however, conclusive carcinogenic data are not available. In laboratory experiments, it does show teratogenic and reproductive effects.

In Vitro Toxicity Data

Phenol had a direct toxic effect on human colonic epithelial cells *in vitro*. In this cell model glucuronidation was the preferred conjugation pathway involved in detoxification.

Clinical Management

Individuals exposed to phenol by inhalation should be removed to fresh air and given artificial respiration/cardiopulmonary resuscitation if necessary. Prompt transport to a medical facility is recommended with observation for up to 48 h. Treatment should be symptomatic, keeping in mind that effects such as pulmonary edema may be delayed. After ingestion, absorption should be delayed by giving milk, olive oil, castor oil, or polyethylene glycol 300, followed by repeated gastric lavage. Mineral oil or alcohol should not be administered because these can increase gastric absorption. Other therapy should be utilized as necessary noting edema and shock acidosis as predicted outcomes. After skin exposure, the affected area should be washed with soap and copious amounts of water for at least 10 min. Water alone may be harmful. Vegetable oil or polyethylene glycol should be applied with cotton swabs or dressings to assist in the removal of phenol from exposed skin.

Environmental Fate

Small, single releases of phenol into the air will be removed in less than a day. The oxidation of phenol will be accelerated by light and catalyzed by other atmospheric impurities. Phenol will persist up to 5 days in soil and even longer (9 days) in water.

Ecotoxicology

Phenol is biodegradable by both aerobic and anaerobic pathways. Little will accumulate in plants or animals and complete aerobic bacterial degradation will produce carbon dioxide. Still phenol is considered a potent insecticide, herbicide, and fungicide. The LC_{50} for aquatic organisms ranges from 12 to 68 mg l^{-1}.

Exposure Standards and Guidelines

Phenol is considered dangerous to life or health at 100 ppm. The permissible exposure limit – TWA (skin) is 5 ppm while the short-term exposure limit is 10 ppm. The odor threshold is 0.4–3.0 ppm.

See also: Skin.

Further Reading

Agency for Toxic Substances and Disease Registry (ATSDR) (1998) *Toxicological Profile for Phenol*. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

Relevant Website

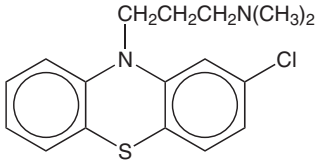
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Phenol.

Phenothiazines

Julie Weber

This article is a revision of the previous print edition article by Douglas J Borys, volume 2, pp. 515–516, © 1998, Elsevier Inc.

- Representative Chemicals: Chlorpromazine; Triflupromazine; Promazine; Promethazine; Acepromazine; Ethopropazine; Thioridazine; Mesoridazine; Piperacetazine; Fluphenazine; Perphenazine; Prochlorperazine; Trifluoperazine; Acetophenazine; Molindone; Pimozide; Haloperidol; Droperidol; Thiothixene; Chlorprothixene; Clozapine; Olanzapine; Risperidone; Quetiapine; Loxapine
- Chemical Abstracts Services Registry Number: Prototype compound, Chlorpromazine (CAS 50-53-3)
- Synonyms: Chlorpromazine, 2-Chloro-10-(3-dimethylaminopropyl)phenothizaine, Thorazine; Triflupromazine, 2-Trifluoromethyl-10-[3′-(1-methyl-4-piperazinyl)propyl]phenothiazine, Vesprin; Promazine, 10-(3-Dimethylaminopropyl)phenothiazine, Sparine; Promethazine, 10-(2-Dimethylaminopropyl)phenothiazine, Phenergan; Acepromazine, 1-[10-[3-(Dimethylamino)propyl]-10*H*-phenothiazin-2-yl]ethanone, Atravet; Ethopropazine, 10-(2-Diethylaminopropyl)phenothiazine, Parsidol; Thioridazine, 10-[2-(1-Methyl-2-piperidinyl)ethyl]-2(methylthio)-10*H*-phenothiazine, Mellaril; Mesoridazine, 10-[2-(1-Methyl-2-piperidinyl) ethyl]-2-(methylsulfinyl)-10*H*-phenothizaine, Serentil; Piperacetazine, 1-[10-[3-[4-(2-Hydroxyethyl)-1-piperidinyl]propyl]-10*H*-phenothizin-2-yl] ethanone, Quide; Fluphenazine, 4-[3-[2-(Trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl]-1-piperazineethanol, Prolixin; Perphenazine, 4-[3-(2-Chloro-10*H*-phenothiazine-10-yl)propyl]-1-piperazineethanol, Trilafon; Prochlorperazine, 2-Chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-10*H*-phenothiazine, Compazine; Trifluoperazine, 10-[3-(4-Methyl-1-piperazinyl)-propyl]-2-(trifluoromethyl)-10*H*-phenothiazine, Stelazine; Acetophenazine, 1-[10-[3-[4-(2-Hydroxyethyl)-1-piperazinyl]propyl]-10*H*-phenothiazin-2-yl]ethanone, Tindal; Molindone, 3-Ethyl-1,5,6,7-tetrahydro-2methyl-5-(4-morpholinylmethyl)-4*H*-indol-4-one, Moban; Pimozide, 1-[1-[4,4-Bis(4-fluorophenyl)butyl]-4-piperidinyl]-1,3-dihydro-2*H*-benzimidazol-2-one, Orap; Haloperidol, 4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone, Haldol; Droperidol, 1-[1-[4-(4-Fluorophenyl)-4-oxybutyl}1,2,3,6-tetrahydro-4-pyridinyl]-1,3-dihydro-2*H*-benzimidazol-2-one, Inapsine; Thiothixene, *N*,*N*-Dimethyl-9-[3-(4-methyl-1-piperazinyl) propylidene]thioxanthene-2-sulfonamide, Navane; Chlorprothixene, 3-(2-Chloro-9*H*-thioxanthen-9-ylidene)-*N*,*N*-dimethyl-1-propanamine, Taractan; Clozapine, 8-Chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine, Clozaril; Olanzapine, 2-Methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine, Zyprexa; Risperidone, 3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one, Risperdal; Quetiapine, 2-(2-(4-Dibenzo(*b*,*f*)(1,4)thiazepin-11-yl-1-piperazinyl)ethoxy)ethanol, Seroquel; Loxapine, 2-Chloro-11-(4-methyl-1-piperazinyl)-dibenz[*b*,*f*] [1,4]oxilapine, Loxitane
- Chemical/Pharmaceutical/Other Class: Neuroleptic agent, antipsychotic, major tranquilizer
- Chemical Formula: $C_{17}H_{19}ClN_2S$
- Chemical Structure: Chlorpromazine is the prototype phenothiazine



Uses

Phenothiazines are used to treat psychosis including schizophrenia; violent, agitated, disturbed behavior; and manic phase of bipolar disorder. Other uses include treatment of pain, headache, hiccups, acute severe anxiety, idiopathic dystonia, withdrawal, taste disorders, leishmaniasis, alleviation of nausea and vomiting, and acute intermittent porphyria. Phenothiazines permit smoother induction of anesthesia, potentiate anesthetic agents, and allow treatment of behavioral symptoms secondary to Alzheimer's disease and senile dementia. Some phenothiazines exert an antipruritic effect and are useful for the treatment of neurodermatitis and pruriginous eczema, and may relieve psychogenic itching.

Exposure Routes and Pathways

Phenothiazines are available in oral, parenteral, and rectal dosage forms. The principal exposure pathway is intentional ingestion in adults or accidental ingestion in small children.

Toxicokinetics

Phenothiazines are readily but incompletely absorbed due to first-pass metabolism. Oral bioavailability ranges from 10% to 69%. Peak serum levels are reached at 2–4 h after oral dosing and 0.5–1 h after immediate-release intramuscular injections. Phenothiazines are extensively metabolized in the liver through glucuronic acid conjugation, *N*-dealkylation, and sulfoxidation. Phenothiazines are widely distributed throughout the body, including the central nervous system (CNS). CNS levels may be up to 10 times greater than plasma levels. Phenothiazines are highly protein bound: 75–99% with a volume of distribution from 10 to 40 l kg^{-1}, with a mean of 20 l kg^{-1}. The main metabolites are excreted both in the urine and feces. Less than 1% is excreted in the urine unchanged. Elimination half-life ranges from 6 to 119 h, with an average of 18 h.

Mechanism of Toxicity

Phenothiazines primarily block postsynaptic neurotransmission by binding to dopamine (D_1 and D_2), muscarinic, histamine H_1, and serotonergic 5-HT_2 receptors. Phenothiazines also possess peripheral α-adrenergic receptor blockade and quinidine-like cardiac effects. Phenothiazines may also lower the seizure threshold.

Acute and Short-Term Toxicity (or Exposure)

Animal

Signs of toxicity reported in animals have included sedation, dullness, hypotension, respiratory depression, pulmonary edema, photosensitivity; photochemical reactions can cause acute keratitis and corneal ulceration in calves, weakness, anorexia, fever, icterus, colic, restlessness, seizures, anemia, and hemoglobinuria. Treatment consists of gastric decontamination and aggressive supportive care.

Human

Clinical signs of toxicity most frequently include sedation, coma, hypotension, extrapyramidal effects, and cardiac arrhythmias. Anticholinergic effects including blurred vision, decreased gastrointestinal motility, delirium, hallucinations, hyperthermia, and tachycardia have been seen. Cardiac effects include mild hypotension, prolonged Q–T interval, and ventricular dysrhythmias. Quinidine-like effects have rarely resulted in sudden cardiac death. The most commonly reported extrapyramidal symptoms include dystonia, akathisia, and parkinsonism. Respiratory depression, loss of gag reflex, and pulmonary edema may occur. Respiratory distress syndrome has been reported. Phenothiazines may interfere with the temperature regulating function of the hypothalamus; hyperthermia is seen more often in overdose, but hypothermia has been reported with haloperidol and thioridazine. Neuroleptic malignant syndrome has been reported after therapeutic use and acute intoxication.

Chronic Toxicity (or Exposure)

Animal

Phenothiazine is used as an antihelminthic in some animal species. Larger doses administered to sick animals have resulted in the development of neurologic effects. Horses seem more sensitive to phenothiazines than other animals and have been noted to develop hemolysis with phenothiazine exposure.

Human

Chronic dose-related exposure might cause tardive dyskinesia (lip smacking, tongue protrusion, grimacing, and chewing). Seizures are rarely seen, but are more common with loxapine and clozapine. The most commonly reported adverse reactions following therapeutic use include dry mouth, sedation, orthostatic hypotension, blurred vision, photosensitivity, anorexia, nausea, vomiting, constipation, diarrhea, and dyspepsia. Various hematologic changes have been reported. Clozapine has been linked to fatal agranulocytosis.

In Vitro Toxicity Data

Mutagenicity studies in Syrian hamster embryos have been positive but Ames Salmonella tests have been negative.

Clinical Management

Aggressive supportive care including airway management should be instituted when necessary. All patients with phenothiazine ingestion should have continuous cardiovascular monitoring and an electrocardiogram (EKG) performed. Emesis with syrup of ipecac is contraindicated due to the possible rapid onset of acute dystonic reaction and sedation. Lavage may be considered in massive, recent exposures, but is not routinely recommended. Phenothiazines readily bind to activated charcoal and it may be beneficial if given early after ingestion. Hypotension usually responds to intravenous fluids and placement of the patient in the Trendelenburg position. The vasopressor of choice is norepinephrine. Arrhythmias

should be treated with lidocaine and, if necessary, cardioversion and/or defibrillation. Quinidine, disopyramide, and procainamide are contraindicated. Benzodiazepines (diazepam or lorazepam) should be used to treat seizures, and if necessary use phenobarbital. Dystonic reactions respond well to intravenous benztropine or diphenhydramine. Oral therapy of diphenhydramine or benztropine should be continued for 1–2 days to prevent recurrence of the dystonic reaction. For patients suffering from neuroleptic malignant syndrome, dantrolene sodium and bromocriptine have been used in conjunction with cooling and other supportive measures. Hemodialysis and hemoperfusion have not been shown to be effective due to the high protein binding and large volumes of distribution. Fluids and electrolytes should be monitored closely. Baseline complete blood count (CBC), arterial blood gas (ABG) (if significantly CNS or respiratory depressed), and glucose should be obtained. Patient's temperature must be checked regularly. Creatine kinase (CK) must be monitored to detect elevation that may produce acute renal insufficiency or failure. Blood urea nitrogen (BUN), creatinine, and urinalysis should be monitored while looking for any symptoms of myoglobinuria, rhabdomyolysis, and renal insufficiency. Patients who have received adequate decontamination and remained asymptomatic with no vital-sign changes may be medically cleared after 4–6 h of observation.

Miscellaneous

Phenothiazines and metabolites have resulted in false positive results for tricyclic antidepressants using various screening methods. Unabsorbed phenothiazine may be radiopaque on abdominal X-ray. Use caution, as the absence of radiographic findings does not rule out ingestion.

See also: Benzodiazepines; Loxapine; Neurotoxicity; Quinidine.

Further Reading

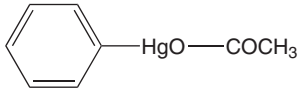
Buckley NA, Whyte IM, and Dawson AH (1995) Cardiotoxicity is more common in thioridazine overdose than with other neuroleptics. *Journal of Toxicology. Clinical Toxicology* 33: 199–204.

Henderson RA, Lane S, and Henry JA (1991) Life-threatening ventricular arrhythmia (torsade de pointes) after haloperidol overdose. *Human and Experimental Toxicology* 10: 59–62.

Phenylmercuric Acetate

Lynn Weber

- Chemical Abstracts Service Registry Number: CAS 62-38-4
- Synonym: Acetoxyphenyl mercury
- Chemical/Pharmaceutical/Other Class: Organomercurial
- Chemical Structure:



Uses

Phenylmercuric acetate is used as a seed dressing for the prevention of seed-borne diseases of vegetables, soybeans, cotton, peanuts, beets, and ornamental plants. Its use as a pesticide has been banned in the United States. Its use in paints was phased out in the United States in 1990–91. It was sometimes used as a food and cosmetic preservative as well as an antifungal agent. In paper, plastic, and fabric industries, this compound was also used as a preservative.

Exposure Routes and Pathways

Oral and dermal routes are the most common routes of exposure to phenylmercuric acetate.

Toxicokinetics

Phenylmercuric acetate is slowly absorbed through the skin; absorption is more efficient by the gastrointestinal tract. Relatively similar rates of absorption of phenylmercuric acetate and mercuric acetate were found in rat kidney slices. When absorption was studied in liver slices, however, the rate of absorption was found to be much higher (twice) for the organic form. Organic mercury has a greater affinity for the brain compared to inorganic mercury (probably because of its relative ease in crossing the blood–brain barrier).

Laboratory studies demonstrated that mercury from phenylmercuric acetate tends to distribute more

in the liver and kidneys compared to inorganic mercury. A chronic study with phenylmercuric acetate and mercuric acetate showed greater (10–20 times) distribution of the phenyl derivative into these tissues. Organomercury compounds usually undergo cleavage of the carbon–mercury bond in the body, releasing ionic inorganic mercury.

Phenylmercuric acetate is mainly excreted through urine. The excretion of phenylmercuric acetate in humans was reported to exhibit two phases. The first phase showed a transient increase in urinary mercury concentration followed by a second slower phase.

Mechanism of Toxicity

Toxic effects of phenylmercuric acetate are correlated with its rapid metabolic breakdown into the mercuric ion. Generally, mercury interferes with cellular enzymatic mechanisms by combining with sulfhydryl (–SH) groups of different enzymes and thereby produces nonspecific cell injury or death.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of phenylmercuric acetate in rats was $60\,mg\,kg^{-1}$. In mice, the oral LD_{50} was $70\,mg\,kg^{-1}$. A single oral exposure to $2\,mg\,kg^{-1}$ phenylmercuric acetate was found to be genotoxic in murine bone marrow and male germline cells. Succinate dehydrogenase and alkaline and acid phosphatase activities in the renal epithelium were reported to be altered following intragastric administration of phenylmercuric acetate in rats.

Human

Phenylmercuric acetate can be lethal with oral doses as low as 100 mg. The principal manifestations of mercury salt poisoning are gastrointestinal, hepatic, and renal damage.

Ingestion of phenylmercuric acetate may cause metallic taste, thirst, severe abdominal pain, vomiting, and bloody diarrhea, which may persist for several weeks. Acute renal failure characterized by decreased urine output was reported 1 day to 2 weeks after ingestion.

Chronic Toxicity (or Exposure)

Animal

Dietary mercury ($2\,mg\,kg^{-1}\,day^{-1}$ for 2 years) in the form of phenylmercuric acetate did not affect rat growth, mortality, or organ weights. However, a dietary level of 160 ppm ($8\,mg\,kg^{-1}\,day^{-1}$) of phenylmercuric acetate was found to retard the growth of rats and shorten their survival time. Histochemical changes in the rat kidney have been observed following a dietary level of 0.5 ppm of phenylmercuric acetate.

Human

Ingestion of phenylmercuric acetate over a prolonged period may cause skin disorders (urticaria and stomatitis), salivation, diarrhea, anemia, leukopenia, and hepatic and renal damage. Prolonged dermal exposure to phenylmercuric acetate may cause mercurialism.

In Vitro Toxicity Data

Matrix metalloproteinases may be targeted by phenylmercuric acetate. Incidence of sister chromatid exchanges in human lymphocytes was increased at $1–30\,\mu m\,mol^{-1}$ concentrations.

Clinical Management

In case of acute poisoning, emergency measures should be taken by immediately removing the ingested poison using gastric lavage with tap water or using emesis or catharsis. Dimercaprol may be administered as an antidote for mercury poisoning with subsequent hemodialysis to accelerate the removal of the mercury–dimercaprol complex from the body. Penicillamine may also be administered as an antidote.

Environmental Fate

If released into air, phenylmercuric acetate is expected to be bound to particulates. If released into soil, its mobility may be high based on a K_{oc} of 60 of the undissociated form, but is likely to be much lower because it will dissociate and the cation will sorb to organic matter or clay. Water releases would result in quick dissociation of the salt and sorption of the cation to particulates or humics, with little bioconcentration in aquatic species. Photolysis of phenylmercuric acetate and subsequent loss through volatilization of inorganic mercury is expected in superficial soils and water.

Ecotoxicology

Pheasants and Japanese quail exhibit decreased egg production, decreased fertility, and increased embryonic mortality after oral phenylmercuric acetate exposure. Feeding, growth, oxygen consumption, swimming performance, and reproduction are

impaired in mosquitofish (*Gambusia affinis*) and rainbow trout (*Oncorhynchus mykiss*). Five-day oral LC_{50} values have been reported for Japanese quail (1028 ppm), ring-necked pheasant (2350 ppm), and mallard duck (1175 ppm). The 48 h aqueous LC_{50} value for rainbow trout has also been reported (1780 ppm).

See also: Mercury; Metals.

Further Reading

Clarkson TW (2001) Inorganic and organometal pesticides. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1357–1428. San Diego, CA: Academic Press.

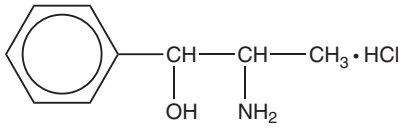
Relevant Website

http://www.inchem.org – International Programme on Chemical Safety.

Phenylpropanolamine

Brenda Swanson-Biearman

- Chemical Abstracts Service Registry Number: CAS 14838-15-4
- Synonyms: PPA; D,L-Norephedrine
- Chemical/Pharmaceutical/Other Class: A synthetic sympathomimetic drug structurally related to ephedrine and amphetamine
- Chemical Formula: $C_9H_{13}NO \cdot HCl$
- Chemical Structure:



Uses

Phenylpropanolamine (PPA) is used as a nasal decongestant and as an anorectic. As of November 2000, the US Food and Drug Administration (FDA) Nonprescription Drugs Advisory Committee (NDAC) determined that there is a significant association between PPA and hemorrhagic stroke and recommended that PPA not be considered safe for over-the-counter use. Products containing PPA such as Alka-Seltzer Plus®, Acutrim®, Contac®, Comtrex®, Dimetapp®, Triaminic®, Robitussin CF®, Dexatrim™ were reformulated. Although the risk of hemorrhagic stroke is very low, the FDA raised significant concerns because of the seriousness of a stroke and the inability to predict who is at risk. All drug companies have voluntarily discontinued marketing products containing PPA.

Exposure Routes and Pathways

PPA is available in liquid, tablet, and caplet dosage forms. Ingestion is the most common route of accidental and intentional exposure.

Toxicokinetics

Oral doses of PPA are rapidly and completely absorbed from the gastrointestinal tract, with maximal therapeutic effect in 1–3 h. In overdose, the peak toxic reaction is usually seen within 2 or 3 h following ingestion. In sustained release preparations, these effects may occur later and be prolonged. PPA is converted primarily to norephedrine. Small amounts of the drug are slowly metabolized in the liver to an active hydroxylated metabolite. PPA crosses the blood–brain barrier, resulting in central nervous system (CNS) effects. The brain-to-serum ratios are extremely close at 0.025 and 0.05 mmol kg^{-1}. The volume of distribution of PPA is 4.4þ1.2 l kg^{-1}. PPA is eliminated unchanged (80–90%) in the urine within 24 h, along with the metabolite norephedrine. PPA is a weak base and is eliminated more rapidly in acidic urine. Where the urine pH is normal (5.5–7.0), the plasma half-life is 3–7 h. In alkaline urine, the elimination half-life increased from a mean of 4.03 to 5.39 h.

Mechanism of Toxicity

The primary action of PPA is indirect alpha-adrenergic agonism, releasing norepinephrine at postganglionic sympathetic nerve terminals. PPA also possesses direct alpha-adrenergic agonist properties and, to a lesser degree, beta-adrenergic agonist activity. Hypertension results from alpha-adrenergic mediated vasoconstriction of peripheral blood vessels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Following the ingestion of PPA, dogs and cats may exhibit hyperactivity, mydriasis, depression, vomiting, hyperthermia, disorientation, and bradycardia. Therapy is directed at prevention of absorption and control of tachyarrhythmias with lidocaine (dogs only) or procainamide (dogs only). Diazepam may be used for control of symptoms of CNS stimulation.

Human

Hypertension is the most common and most serious toxic effect of PPA. Hypertensive crisis, cerebral arteritis, cerebral hemorrhage, psychoses, seizures, and myocardial ischemia may result. Tachycardia is most often seen with PPA where it is combined with antihistamines in multi-symptom products. Bradycardia (as a reflex response to hypertension) is more common when the PPA is ingested exclusively. Concurrent substances that are prevalent in combination products and may contribute to the toxicological presentation of PPA exposures include analgesics, antihistamines, and antitussives. Consideration should be given to the alcohol component of liquid preparations. Caffeine may be added to PPA in illicit stimulant and weight-loss preparations. PPA has a low therapeutic index and adverse effects can occur at doses two or three times the normal daily dose. The recommended adult daily dose is 75–150 mg. An amount over $10\,mg\,kg^{-1}$ is toxic in children. Other neurological symptoms include anxiety, confusion, headache, hallucinations, and altered mental status.

Chronic Toxicity (or Exposure)

Animal

PPA has been used in veterinary practice as an agent to help with urinary continence, primarily in dogs. Dogs commonly develop signs and symptoms of CNS stimulation.

Human

The use of PPA has been associated with increases in blood pressure and an increased risk of hemorrhagic stroke. In November 2000, the US FDA issued a public health advisory on the use of PPA and asked drug companies to voluntarily withdraw PPA products from the market.

In Vitro Toxicity Data

PPA has been demonstrated to competitively and reversibly inhibit monoamine oxidase activity in both human brain and rat liver.

Clinical Management

Basic and advanced life-support measures should be instituted as indicated. Gastric decontamination may be performed depending on the patient's symptomatology and the history of the ingestion. Activated charcoal may be used to adsorb PPA. Most overdoses require observation only for a period of 4–8 h; sustained-release preparations may require a longer period of observation. Careful monitoring of the cardiac and hemodynamic status should be performed. Antidysrhythmics and antihypertensive agents may be necessary in severe exposures. Management of poisoning with concurrent drugs ingested should be appropriate to the agent(s) involved. Laboratory analysis of creatine phosphokinase and urinalysis should be performed in those with severe symptoms.

See also: Diazepam; Lidocaine; Procainamide.

Further Reading

Cantu C, Arauz A, Murillo-Bonilla LM, Lopez M, and Barinagarrementeria F (2003) Stroke associated with sympathomimetics contained in over-the-counter cough and cold drugs. *Stroke* 34: 1667–1672.

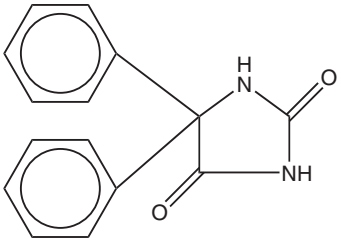
Edwards M, Russo L, and Harwood-Nuss A (1987) Cerebral infarction with a single oral dose of phenylpropanolamine. *American Journal of Emergency Medicine* 5: 163–164.

Lake CR, Gallant S, and Masson E (1990) Adverse drug effects attributed to phenylpropanolamine: A review of 142 case reports. *American Journal of Medicine* 89: 195–208.

Phenytoin

S Rutherfoord Rose

- Chemical Abstracts Service Registry Numbers: CAS 57-41-0; CAS 630-93-3
- Synonyms: Diphenylhydantoin (DPH); 5,5-Diphenylhydantoin, 5,5-Diphenylimidazolidine-2, 4-dione; Dilantin Infatabs®; Fenitoina; Phenantoinum; Phenytoin sodium (92% phenytoin); Diphenylhydantoin sodium; Diphenin; Phenytoinum natricum; Soluble phenytoin; Dilantin®; Epanutin®; Diphenylan®; Fosphenytoin is a water-soluble prodrug of phenytoin suitable for rapid intravenous administration
- Chemical/Pharmaceutical/Other Class: Hydantoin (a synthetic chemical that is structurally similar to barbituric acid)
- Chemical Formula: $C_{15}H_{12}N_2O_2$
- Chemical Structure:



Uses

Phenytoin is used as an anticonvulsant and rarely as an antidysrhythmic.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Phenytoin can also be administered intravenously.

Toxicokinetics

Oral absorption occurs in the small intestine and is dose dependent. Peak blood concentrations occur 2–4 h after single 100 mg doses but may be delayed by 4–12 h after a loading dose (600 mg). Peak levels may not occur for 2–7 days after an oral overdose. The oral bioavailability of phenytoin averages 90% (range, 70–100%). Intramuscular absorption is erratic and unpredictable.

The major pathway of biotransformation is via hepatic hydroxylation to *p*-hydroxyphenytoin, which is subsequently conjugated to glucuronide. Minor metabolites include *m*-hydroxyphenytoin and 3,4-dihydro-dihydroxyphenytoin. All metabolites are inactive. Phenytoin biotransformation is capacity limited, with linear (first-order) kinetics observed at low (therapeutic) doses, and zero-order (Michaelis–Menton) elimination observed at toxic and even high therapeutic doses. Phenytoin and its metabolites undergo enterohepatic recirculation prior to elimination.

The volume of distribution averages 0.5–0.8 l kg^{-1} and binding to plasma proteins is normally ~90%. Protein binding is altered in neonates, the elderly, and under many conditions including aremia, nephrotic syndrome, hypoalbuminemia, hyperbilirubinemia, and hepatic disease. Alterations in protein binding will result in variations in the amount of unbound (free) drug that is the active component. Thus, free phenytoin levels (therapeutic = 1–2 μg ml^{-1}) rather than total levels (therapeutic = 10–20 μg ml^{-1}) may correlate better with clinical efficacy and toxicity in the presence of these conditions. Phenytoin crosses the placenta and is excreted in breast milk.

Small amounts of phenytoin are excreted unchanged in the urine (2–4%) and feces (5%). Most is eliminated renally as inactive conjugated metabolites. The elimination half-life at linear doses averages 20–30 h (12–20 h in children) but may be as long as 60 h, and as high as 200 h after overdose, due to saturation of hydroxylation pathways. The maximum rate of metabolism is estimated at 6 mg kg^{-1} day^{-1}.

Mechanism of Toxicity

Phenytoin possesses anticonvulsant activity without significant central nervous system (CNS) depression. At various concentrations, phenytoin has been shown to inhibit inward Na^+ currents, outward K^+ currents, and Ca^{2+}-mediated action potentials. The ability to inhibit sodium channels is responsible for the antidysrhythmic action (class II-B) of phenytoin. Phenytoin can induce enzymes of the hepatic cytochrome P450 system.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity in animals has been similar to that observed in humans (e.g., effects are primarily neurologic).

Human

Clinical effects after overdose are generally dose related and primarily involve the peripheral and central nervous systems; nystagmus (>20 μg ml^{-1}),

ataxia ($>30\,\mu g\,ml^{-1}$), and lethargy ($>40\,\mu g\,ml^{-1}$) are most characteristic. Nausea, tremor, dysarthria, and confusion are also relatively common. Coma or significant cardiac dysrhythmias are unusual. Hypotension or dysrhythmias may be encountered with very rapid intravenous infusion. Paradoxical CNS excitation has been reported, but the potential of phenytoin to actually cause seizures at very high serum concentrations is unclear. This phenomenon has typically occurred in patients with a preexisting seizure disorder on chronic phenytoin therapy.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies have demonstrated increased development of tumors in mice compared with untreated controls.

Human

Chronic toxic effects are dose related and typically involve cerebellar and vestibular functions (nystagmus and ataxia). Nausea, dizziness, diplopia, behavioral changes, gingival hyperplasia, hirsutism, hyperglycemia, osteomalacia, pancytopenia, and skin eruptions are reported complications of chronic therapy. Hypersensitivity (idiosyncratic) reactions, including hepatic necrosis and Stevens–Johnson syndrome, can occur and are potentially fatal.

Phenytoin use during pregnancy has been associated with intrauterine growth retardation, mental retardation, craniofacial abnormalities, and digital hypoplasia (e.g., fetal hydantoin syndrome).

In Vitro Toxicity Data

Studies of sister chromatid exchange have been positive, Ames *Salmonella* tests have been negative, mouse lymphoma tests have been negative, and Chinese hamster ovary assays have been negative for mutagenicity.

Clinical Management

The basis of treatment is supportive care. Hypotension is usually associated with rapid infusion of injectable phenytoin and should respond to slowing the infusion rate and intravenous fluid therapy. Seizures should be treated with intravenous doses of diazepam or lorazepam and discontinuation of phenytoin. Assessments of toxicity should be based on serum drug levels rather than the amount of drug ingested. Serum phenytoin concentrations should be determined in all symptomatic patients or patients with ingestions exceeding $20\,mg\,kg^{-1}$. Serial levels are needed to determine peak (highest measured) concentration. Serum levels of electrolytes, glucose, hepatic enzymes, blood urea nitrogen, and bilirubin should be determined in hospitalized patients. Activated charcoal is useful to prevent gastrointestinal absorption and to enhance the elimination of the absorbed drug (i.e., gastrointestinal dialysis). Multiple oral doses of charcoal are indicated to facilitate the lowering of toxic blood levels that possibly would require days to decline in conditions of zero-order metabolism. However, care must be taken to ensure that patients have gastrointestinal motility before using multiple doses of activated charcoal. Other measures to enhance phenytoin elimination are not warranted. Continuous cardiac monitoring is not necessary in the absence of preexisting cardiac disease or massive overdose with hemodynamic compromise. Patients should be monitored until serum levels are (near) normal and they are neurologically competent. Death resulting from oral ingestion is rare.

See also: Charcoal; Diazepam.

Further Reading

Evers ML, Izhar A, and Aqil A (1997) Cardiac monitoring after phenytoin overdose. *Heart & Lung* 26: 325–328.

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Mellick LB, Morgan JA, and Mellick GA (1989) Presentations of acute phenytoin overdose. *American Journal of Emergency Medicine* 7: 61–67.

Morkunas AR and Miller MB (1997) Anticonvulsant hypersensitivity syndrome. *Critical Care Clinics* 13(4): 727–739.

Phorbol Esters

Samantha E Gad and Shayne C Gad

Tumor promotion is described as 'the process by which an agent brings about the selective expansion of initiated cells which increase the probability of malignant transformation'. Such promotion can also be considered nongenotoxic or epigenetic carcinogenesis. The concept of tumor promotion came from studies which found that a single application of coal tar or a polycyclic hydrocarbon to the skin of rabbits or mice in subcarcinogenic amounts would initiate the process of skin carcinogenesis if followed by a promotional event.

The characteristic of a promoter in the mouse skin model can be described as follows:

1. That it should not be carcinogenic *per se*.
2. That it should not increase tumor yield if administered before the initiating carcinogen.
3. That when applied after an initiating, subcarcinogenic dose of the carcinogen, it should accelerate the rate of development of tumors and thus increase the total, time-related tumor incidence.
4. That the yield of tumors produced should be related to the dose of the initiator, not to dose of the promoter, providing the promoter is used in excess of the minimum amount required to promote all initiated cells.
5. That, unlike initiation, which can take place rapidly during a single exposure to the initiator and which is a permanent event, promotion requires long exposure to the promoter before the changes induced become irreversible.

Phorbol esters were first detected in oil prepared from seeds of *Croton tiglium*, and are the most widely studied skin tumor promoters; however, many other chemical compounds have been shown to possess skin tumor-promoting properties, for example, phenobarbitol, DDT, and the peroxisomal proliferators. Within a few hours after application of a single effective dose of phorbol 12-myristate 13-acetate (also known as TPA and 12-*O*-tetradecanoyl-phorbol-13-acetate, CAS 16561-29-8) to mouse skin, localized edema and erythema characteristic of inflammation and irritation are evident, and within 24 h there is leukocytic infiltration of the dermis. Within 1 or 2 days after a single promoter treatment, stimulation of mitotic activity in the basal cell layer of the epidermis is evident and continues for several days. This results in an increased number of nucleated cell layers, and is followed by a phase of increased keratinization of the upper layers of the epidermis. Without additional promoter treatments, these responses to the promoter gradually subside and the epidermis regains its normal appearance within ~2 or 3 weeks of treatment. Repeated promoter treatment, however, prevents this decrease in response, and the skin appears to be in a chronic state of irritation and regenerative hyperplasia. Phorbol esters have been shown to transform cultured fibroblasts and embryonic cells that have been previously exposed to polycyclic aromatic hydrocarbons *in vitro*.

The best known receptors for phorbol esters and their derivatives are the isozymes of protein kinase C (PKC), which bind phorbol esters and the physiological second messenger diacylglycerol (DAG) by cysteine-rich domains, the C1 domains. The exact functions of the different PKC isozymes is not known at present; however, they have been shown to be involved in synaptic transmissions, the activation of ion fluxes, secretion, cell cycle control, differentiation, proliferation, tumorigenesis, metastasis, and apoptosis.

Phorbol esters also target numerous C1-containing receptors unrelated to PKC. Identifying and understanding the complete set of key mediators for the physiological DAG responses and phorbol ester-induced tumorigenesis will help in the understanding of signal integration, and can also help in the development of new strategies for therapeutic cancer intervention. For example, individual PKC isozymes appear to have opposite effects on skin carcinogenesis despite being all activated by phorbol esters in the mouse skin chemical carcinogenesis model, and a better understanding of the different epidermal expression patterns and substrate proteins are needed to explain their opposing effects on skin carcinogenesis.

See also: Carcinogenesis; DDT (Dichlorodiphenyltrichloroethane); Peroxisome Proliferators; Polycyclic Aromatic Hydrocarbons (PAHs); Skin; Toxicity Testing, Dermal.

Further Reading

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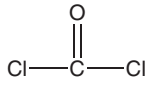
Saraiva L, Fresco P, Pinto E, and Goncalves J (2004) Characterization of phorbol esters activity on individual mammalian protein kinase C isoforms, using the yeast phenotypic assay. *European Journal of Pharmacology* 491: 101–110.

Phosgene

Samantha E Gad

- Chemical Abstracts Service Registry Number: CAS 75-44-5
- Synonyms: Carbonyl chloride; Chloroformyl chloride; Carbon oxychloride
- Chemical/Pharmaceutical/Other Class: Haloform
- Chemical Formula: $COCl_2$
- Chemical Structure:



Uses

Phosgene is widely used as a chemical intermediate. It is used in metallurgy and in the production of pesticides, herbicides, and many other compounds. It is a by-product of chloroform biotransformation and can be generated from some chlorinated hydrocarbon solvents under intense heats. Phosgene has been used as a chemical warfare agent.

Exposure Routes and Pathways

Inhalation and exposure to skin and mucous membranes are possible exposure routes. Potential for toxicity depends on concentration, route of exposure, and length of time exposed.

Toxicokinetics

Phosgene is absorbed by the lungs and excreted via the liver and kidneys.

Mechanism of Toxicity

Rapid-onset ocular, nasal, and airway irritations from high levels of phosgene are caused by hydrochloric acid released during hydrolysis. The carbonyl group (C = O) participates in acylation reactions with amino ($-NH_2$), hydroxyl (–OH), and sulfhydryl (–SH) groups. These reactions may account for some toxic effects of phosgene. At alveolar-capillary membranes, these reactions can cause fluid leakage into the interstitial lung. Leakage of fluid into the pulmonary interstitium is opposed by lymphatic drainage, but as fluid accumulates, this drainage is overwhelmed. After a latent period, fluid reaches alveoli and peripheral airways, leading to increasingly severe dyspnea and pulmonary edema.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, phosgene exposure has resulted in liver or kidney damage, skin irritation, and respiratory damage. The inhalation LC_{50} is 1400 mg m^{-3} in rats, 1800 mg m^{-3} in mice, 4200 mg m^{-3} in dogs, 600 mg m^{-3} in monkeys, 1000 mg m^{-3} in rabbits, and 1300 mg m^{-3} in guinea pigs. The lowest observed lethal concentration in cats was 190 mg m^{-3} for 15 min. Acute (4 h) exposure of male rats to phosgene (0.125–1 ppm) led to changes in lung weights (wet and dry) and increased protein in lavage fluid. Total number of cells in lavage fluid was higher in phosgene-exposed rats. Increase in polymorphonuclear leukocytes was a sensitive indicator of phosgene toxicity. All parameters returned to near control levels within 3 days, indicating repair of damage and reversible lung damage.

Human

Exposure to high levels may cause death. Phosgene causes irritation to skin, eyes, nose, throat, and lungs. Phosgene exposure may be asymptomatic in the short term, with effects delayed for up to 48 h. High concentrations may cause accumulation of fluids in the lungs or pneumonia, and can produce choking, chest

constriction, pain in breathing, coughing, blood in sputum, and heart failure. Exposure to eyes and mucous membranes can be very irritating. Buildup of phosgene in the liver or kidneys may produce damage.

Chronic Toxicity (or Exposure)

Animal

Relatively little information is available on the long-term effects of chronic exposure to phosgene in animals. Studies in male rats exposed (6 h day^{-1}) to 0.1, 0.2, 0.5, or 1 ppm of phosgene, either acutely or repeatedly, for up to 12 weeks suggested that high concentrations with long exposure intervals led to more chronic pulmonary damage (increased bronchoalveolar lavage protein, hydroxyproline, and collagen). Chronic pneumonitis and fibrinous pneumonia was reported in one study with long-term phosgene exposure.

Human

Chronic inhalation to low levels of phosgene can lead to some degree of tolerance to acute effects noted in humans, but can also cause irreversible pulmonary changes, for example, emphysema and fibrosis. There appears to be no increased incidence of cancer in workers chronically exposed to phosgene.

Clinical Management

The exposed individual should be removed from exposure. Clothing should be removed carefully avoiding further exposure. The body should be washed rapidly with soap and water, and eyes flushed if needed Centers for Disease Control and Prevention (CDC). Individuals should be given immediate medical attention and monitored for 48 h for delayed effects (CDC). There is no antidote (CDC).

Environmental Fate

In air, the major route of phosgene degradation in air is hydrolysis. Even with very high humidity, however, phosgene is slowly degraded and is likely to persist and be transported long distances. In water, phosgene is efficiently degraded to hydrochloric acid and carbon dioxide. Phosgene is not likely to be detected in soil or vegetation.

Ecotoxicology

Relatively little is known regarding ecotoxicity of phosgene. It is estimated that common environmental levels would have little effect on aquatic or terrestrial species. Some damage to plants and aquatic organisms could occur, however, with accidental releases from release of hydrochloric acid upon hydrolysis.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit for phosgene is 0.1 ppm. The National Institute for Occupational Safety and Health immediately dangerous to life and health value is 2 ppm. The California Environmental Protection Agency chronic inhalation reference exposure level is 0.0003 mg m^{-3}. The US Army general population limit is 0.0025 mg m^{-3}.

See also: Pesticides; Respiratory Tract; Sensory Organs.

Further Reading

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http://chppm-www.apgea.army.mil – US Army Center for Health Promotion and Preventive Medicine. Detailed Facts About Choking Agent Phosgene (CG) (accessed September 3, 2004).

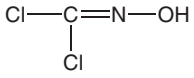
http://www.bt.cdc.gov – Centers for Disease Control (CDC) (2004) Facts about phosgene (accessed September 3, 2004).

http://www.epa.gov – US Environmental Protection Agency (EPA) (accessed September 3, 2004).

Phosgene Oxime

David R Wallace

- Chemical Abstracts Service Registry Number: CAS 1794-86-1
- Synonyms: Dichloroformoxime; CX
- Chemical/Pharmaceutical/Other Class: Urticant or nettle agent
- Chemical Formula: CCl_2NOH
- Chemical Structure:



Uses

Phosgene oxime was originally developed as a chemical warfare agent. It is sometimes grouped with the vesicant agents, but it is not a true vesicant in that it does not induce blisters. Phosgene oxime is an urticant or nettle agent that causes corrosive-type injuries. There is no evidence that this agent has ever been used as a chemical warfare agent.

Exposure Routes and Pathways

Phosgene oxime is a colorless solid or yellowish-brown liquid that can vaporize at room temperature. Due to its ability to rapidly change physical state, phosgene oxime can be absorbed through inhalation, dermal/ocular contact, or oral ingestion.

Toxicokinetics

Phosgene oxime appears to act directly and there have been no reported studies that have determined if any metabolism of phosgene *in vivo*.

Mechanism of Toxicity

The molecular mechanism of phosgene oxime toxicity is unknown.

Acute and Short-Term Toxicity (or Exposure)

Most studies on the action of phosgene oxime have utilized animal studies. Human information has been obtained from accidental exposure to the chemical. Health effects following phosgene oxime exposure are dependent on the route of exposure.

Since phosgene oxime is an urticant/nettle agent, common physical effects include erythema, wheals, and urticaria. Phosgene oxime is a highly corrosive agent and the response resembles wounds caused by strong acids. Ocular contact results in severe pain, conjunctivitis, and keratitis. Direct dermal exposure to phosgene oxime causes immediate pain and blanching with an erythematous ring. In ~ 0.5 h a wheal will form followed by tissue necrosis. Extreme pain can persist for days. Absorption of phosgene oxime through the skin can result in pulmonary edema. Inhalation of phosgene oxime vapor will produce immediate irritation to the airways. Pulmonary edema, necrotizing bronchiolitis, and pulmonary thrombosis can also occur following inhalation or systemic absorption of phosgene oxime. There has been no human data on effects of phosgene oxime following ingestion, but animal studies suggest that hemorrhagic inflammatory lesions may occur throughout the gastrointestinal tract.

Chronic Toxicity (or Exposure)

There have been no studies on chronic exposure to phosgene oxime. Thus, there is no data regarding the carcinogenicity or teratogenicity of phosgene oxime.

In Vitro Toxicity Data

No *in vitro* toxicity studies have been reported. The mechanism of phosgene oxime toxicity is unknown and long-term exposure effects have not been determined.

Clinical Management

Individuals who come in contact with phosgene oxime liquid or solid can contaminate those around them by release of vapor. Individuals who have been exposed to the vapor will not be able to contaminate others. Patients who come in contact with phosgene oxime will experience immediate pain and develop necrotic lesions. Since there is no antidote for phosgene oxime exposure, only supportive measures can be given. Patients arriving to the triage area must first be decontaminated to prevent cross-contamination. For inhalation exposures, the individual should be removed from the source of exposure. Oxygen must be administered to patients with significant respiratory symptoms. Artificial respiration should be given if necessary. For ocular treatment, eyes

should be flushed with copious amounts of water. Topical antibiotics should be applied to reduce the risk of infections and adhesions. Topical anticholinergics should be applied to reduce the risk of future synechiae formation. Skin contact will require decontamination with large amounts of water. Treatment should be in the same fashion as with a chemical burn. If phosgene oxime has been ingested, emesis should not be induced. Parental analgesics such as morphine or meperidine may be administered to reduce pain.

Environmental Fate

Phosgene oxime does not accumulate in the soil. Small amounts that may be present can vaporize into the air or be degraded by soil bacteria. Once in vapor form, phosgene oxime remains in vapor form and will be inactivated by compounds in the atmosphere or broken down by bacteria. There is no evidence that phosgene oxime will accumulate in groundwater.

Ecotoxicology

Phosgene oxime is rapidly cleared from the environment and poses little threat unless animals come directly in contact with the gas or liquid/solid.

Exposure Standards and Guidelines

No exposure standards and guidelines have been established.

See also: Diphosgene; Phosgene.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Phosgene Oxime.

http://www.biochemhazard.com – Biochemhazard.com, Dominion Research Center (2000) Chemical Weapon Agent: Phosgene Oxime (CX) – Blister Agent.

http://www.emedicine.com – eMedicine, CBRNE: Urticants, Phosgene Oxime (2003).

Phosphine

Danny Villalobos

- Chemical Abstracts Service Registry Number: CAS 7803-51-2
- Synonyms: Hydrogen phosphide; Phosphorus hydride; Phosphorus trihydride; Phosphoretted hydrogen; Aluminum phosphide (Celphos, Phostoxin, Quick Phos)
- Chemical/Pharmaceutical/Other Class: Fumigant
- Chemical Formula: PH_3

Uses

Phosphine is used as an insecticide for the fumigation of grains, animal feed, and leaf-stored tobacco, and as a rodenticide. Phosphine is also used as an intermediate in the synthesis of flame retardants for cotton fabrics, as a doping agent for *n*-type semiconductors, as a polymerization initiator, and as a condensation catalyst. Phosphine is used in the semiconductor industry to introduce phosphorus into silicon crystals.

Exposure Routes and Pathways

Inhalation is the major route of phosphine exposure. Phosphides may be absorbed through broken skin causing systemic toxicity. Phosphine gas produces little adverse effects on the skin or eyes. Contact with liquefied or compressed phosphine gas may cause frostbite. Ingestion of phosphine is rare due to its volatility. However, metallic phosphide ingestion can lead to systemic toxicity.

Toxicokinetics

Phosphine is absorbed readily through the lungs and produces early symptoms in the brain and liver, suggesting that it is rapidly distributed at least to these organs. After peak exposure, phosphine is excreted unchanged in expired air and some is oxidized to phosphite and hypophosphite ions, which are excreted in the urine. Metal phosphides may hydrolyze to produce phosphine, which may be absorbed through the intestine after ingestion. Some zinc phosphide has been shown to reach the liver and kidneys intact after ingestion and to hydrolyze slowly in the tissues to phosphine and zinc salts. Hydrolysis of metal phosphides on the skin could lead to the evolution of gaseous phosphine, which could then be absorbed by inhalation. Little percutaneous absorption of metal phosphides occurs.

Mechanism of Toxicity

Metallic salts can cause severe gastrointestinal irritation. Phosphine may be an *in vivo* inhibitor of

oxidative phosphorylation, via inhibition of cytochrome oxidase. As with other fumigants, sufficient phosphine in the atmosphere can lead to oxygen starvation, apnea, and cardiac arrest.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute animal tests in rats have demonstrated phosphine to have extreme acute toxicity via inhalation. Signs include early hypoactivity followed by restlessness, escape behaviors, ataxia, convulsions, and death within 30 min with high concentrations. Concentration–time studies demonstrated evidence of Haber's law, that is, within certain limits, the product of concentration and time of exposure to elicit lethality was remarkably constant. The lowest lethal concentration in rats was $7.5\,mg\,m^{-3}$. The acute oral LD_{50} for metallic salts (e.g., aluminum phosphide) is typically quite low ($\sim 10\,mg\,kg^{-1}$). In rabbits acutely exposed to high levels of phosphine via inhalation, dyspnea, paralysis, convulsions, hepatotoxicity and renal toxicity, and damage to the spleen were reported.

Human

Phosphine is a respiratory tract irritant that attacks primarily the cardiovascular and respiratory systems causing peripheral vascular collapse, cardiac arrest and failure, and pulmonary edema. Acute (short-term) inhalation exposure to phosphine may cause headaches, dizziness, fatigue, drowsiness, burning substernal pain, nausea, vomiting, cough, labored breathing, chest tightness, pulmonary irritation, pulmonary edema, and tremors in humans. In severe exposure, lung irritation with persistent coughing, ataxia, paraesthesia, tremor, diplopia, and jaundice may also occur. Very severe cases may progress to acute pulmonary edema, cardiac dysrhythmias, convulsions, cyanosis, and coma. Oliguria, proteinuria, and anuria may be induced. Delayed pulmonary edema with an onset of 72 h or more postexposure can occur. Convulsions may ensue after an apparent recovery. Ingestion of phosphine is unlikely because it is a gas at room temperature. Ingestion of metallic phosphides (e.g., aluminum phosphide) can produce phosphine intoxication when the solid phosphide contacts gastric acid. Deliberate ingestion of phosphides causes nausea, vomiting, and sometimes diarrhea, retrosternal and abdominal pain, tightness in the chest and coughing, headache, and dizziness. In severe cases, gastrointestinal hemorrhage, tachycardia, hypotension, shock, cardiac arrhythmias, hypothermia, metabolic acidosis, cyanosis, pulmonary edema, convulsions, hyperthermia, and coma may occur. Clinical features of renal insufficiency and hepatic damage including oliguria and jaundice may develop later, if the patient survives.

Chronic Toxicity (or Exposure)

Animal

There is relatively little information on effects of prolonged exposure to phosphine. Decreased body weight and kidney and liver effects have been reported in animals exposed repeatedly to phosphine via inhalation. Male and female rats exposed to phosphine ($1.5–15\,mg\,m^{-3}$) exhibited marked lethality (4/10) in females only with the highest dosage. Significant reductions in body weight and food consumption were noted across all treatment groups and sexes. Dose-related changes in blood urea nitrogen and other clinical parameters were also seen across exposure groups. Histopathological examinations revealed renal cortical lesions with the highest dosage, $15\,mg\,m^{-3}$, but not at lower exposure levels. All effects were apparently reversible within a month of termination of exposure. Phosphine does not appear to be a reproductive or developmental toxicant.

Human

Chronic (long-term) occupational exposure of workers to phosphine may cause inflammation of the nasal cavity and throat, weakness, dizziness, nausea, gastrointestinal, cardiorespiratory, and central nervous system symptomology, jaundice, liver effects, and increased bone density. Chronic exposure to very low concentrations may result in anemia, bronchitis, gastrointestinal disturbances, and visual, speech, and motor disturbances. Chronic exposure may be more serious for children because of their potential longer latency period. There is no evidence of cumulative effect in grain workers exposed for long periods to phosphine. Intermittent exposures for months led to headaches but no other symptoms.

The US Environmental Protection Agency has determined that phosphine is not classifiable as to its human carcinogenicity.

In Vitro Toxicity Data

Studies on isolated rat liver showed that mitochondrial oxygen uptake is inhibited by phosphine due to its reaction with cytochrome C and cytochrome C oxidase. Phosphine inhibits insect catalase, though this appears to be an indirect effect and might be a consequence, not a cause, of toxicity.

Clinical Management

Management depends on the route of exposure and proper first aid treatment must be performed. There is no antidote available.

First Aid

In case of phosphine inhalation, the patient must be removed from the exposure site and rested. Rescuers should follow full safety procedures. If a patient is unconscious, place him in the semiprone recovery position, otherwise maintain the airway and give oxygen if required. If breathing stops, immediately ventilate the patient artificially (mouth-to-mouth/nose or mechanically with oxygen if available). If the heart stops, begin cardiopulmonary resuscitation. The patient must then be referred to the nearest medical center for further treatment.

In case of ingestion of a metal phosphide, do not give milk, fats, or saline emetics by mouth. If the patient is conscious, consider induction of emesis. After vomiting, administer activated charcoal (50 g in water by mouth) if available.

Medical Treatment

1. Gastric lavage, endotracheal intubation to protect the airway, followed by activated charcoal.
2. Monitor and support vital functions, particularly cardiovascular, respiratory, hepatic, and renal functions.
3. Treat shock conventionally with appropriate vasopressors as needed.
4. Perform arterial blood gas analysis and correct respiratory dysfunction by clearing the airways, giving oxygen and perform artificial (mechanical) respiration if required. Metabolic acidosis must also be treated by giving sodium bicarbonate according to the results of arterial pH and blood gas analyses.
5. Hepatic and renal failure should be treated as required, with consultation with an experienced hepatologist and nephrologist.

There are no specific blood or urine tests for phosphine itself. Breakdown products of phosphine can be measured in urine, but the result of this test is generally not useful in the clinical management of patients.

Environmental Fate

In the air, phosphine will exist solely as a gas. Phosphine gas reacts with substances commonly found in the air. Half of the phosphine in the air degrades in ~ 1 day. At high concentrations, phosphine vapors may spontaneously combust in air. Phosphine is expected to react with water and be broken down into other products. Some of the phosphine that is not broken down may evaporate into air. When released to soil, phosphine is broken down very quickly. Phosphine does not accumulate in the food chain.

Ecotoxicology

Little information is available on the ecotoxicity of phosphine. Turkeys and chickens exposed to 211 and 224 mg m^{-3} for 74 and 59 min, respectively, showed dyspnea, convulsions, and death. Aluminum phosphide is very toxic to rainbow trout, with an acute LC_{50} of 4.1 μg l^{-1}.

Other Hazards

Phosphine reacts with air, oxidizers, chlorine, acids, moisture, halogenated hydrocarbons, and copper.

Exposure Standards and Guidelines

The reference dose for aluminum phosphide is 0.004 mg kg^{-1} day^{-1}.

The Occupational Safety and Health Administration permissible exposure limit for phosphine is 0.3 ppm (averaged over an 8 h work shift).

The National Institute for Occupational Safety and Health immediately dangerous to life or health value for phosphine is 50 ppm.

ERPG-2 (emergency response planning guideline) (maximum airborne concentration below which it is believed nearly all individuals could be exposed for up to 1 h without experiencing or developing irreversible or other serious adverse health effects or symptoms that could impair an individual's ability to take protective action) = 0.5 ppm.

Miscellaneous

Phosphine is a colorless gas with odor of garlic or decaying fish (1–3 ppm threshold). It is slightly soluble in water (0.3% at 68°F). Phosphine is extremely flammable and explosive; it may ignite spontaneously on contact with air.

See also: Aluminum Phosphide; Phosphorus.

Further Reading

Brautbar N and Howard J (2002) Phosphine toxicity: Report of two cases and review of the literature. *Toxicology and Industrial Health* 18: 71–75.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Phosphine.

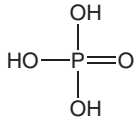
http://www.intox.org – Phosphine (Poisons Information Monograph 865 from the International Programme on Chemical Safety).

http://www.epa.gov – Phosphine (from the US Environmental Protection Agency's Air Toxics Website).

Phosphoric Acid

Samantha E Gad and Russell Barbare

- Chemical Abstracts Service Registry Number: CAS 7664-38-2
- Synonyms: Orthophosphoric acid; Hydrogen phosphate
- Chemical/Pharmaceutical/Other Class: Inorganic acids; Corrosive mineral acids
- Chemical Formula: H_3PO_4
- Chemical Structure:



Uses

Phosphoric acid is a component of fertilizers (80% of total use), detergents, and many household cleaning products. Dilute solutions have a pleasing acid taste; thus, it's also used as a food additive, lending acidic properties to soft drinks and other prepared foods, and in water treatment products. It is also used in rust proofing, engraving, and metal coating and is an intermediate or reagent in many manufacturing processes. Phosphoric acid also occurs naturally in many fruits and their juices. Apart from use of phosphoric acid itself, the greatest consumption of phosphoric acid is in the manufacture of phosphate salts. Taking advantage of its ability to lower blood pH, phosphoric acid has been used therapeutically to treat lead poisoning.

Exposure Routes and Pathways

Inhalation of mist, ingestion, and dermal, ocular, and mucous membrane contact are possible routes of exposure. Because phosphoric acid has a low vapor pressure, it must be aerosolized somehow and become airborne in order to affect the respiratory tract.

Toxicokinetics

Phosphoric acid is rapidly absorbed from the gastrointestinal tract and through the skin.

Mechanism of Toxicity

Because of its acidic properties, phosphoric acid produces toxicity much like any other acid. Excessive exposure causes corrosion on contact and disruption of internal pH balance (acidosis) when large concentrations are distributed systemically.

Acute and Short-Term Toxicity (or Exposure)

Animal

Phosphoric acid is irritating to the skin and eyes of rabbits. In rats the oral LD_{50} is $1530\,mg\,kg^{-1}$, the inhalation $LC_{50} > 850\,mg\,m^{-3}$, and the no-observed-adverse-effect level is $180\,mg\,m^{-3}$.

Human

Exposure to highly concentrated solutions can irritate the skin and mucous membranes. Phosphoric acid is highly corrosive. If ingested, corrosion damage may occur to the gastrointestinal tract and nausea and vomiting are possible. The inhalation of acid mist may cause irritation to the throat and lungs leading to reactive airway dysfunction and respiratory failure in extreme cases.

Chronic Toxicity (or Exposure)

Animal

Chronic inhalation studies have been conducted with combustion products of phosphorus with plastics or felt. These products produce phosphoric acid on contact with the water in tissue but also have effects from the other combustion products. Deaths and respiratory damage occurred in all species studied if the dose was high enough. Fetal effects were seen in rats exposed *in utero*. Effects included increased mortality and decreased pup body weights.

Human

Bronchiolar fibrosis has been reported with chronically high exposures. However, chronic inhalation exposure at the low levels most exposed individuals are likely to experience, produces no changes in pulmonary function, or in other effects occasionally

reported, including reduced leukocyte count, or reduced hand bone density.

Clinical Management

Gastric lavage and emetics should be avoided after exposure to phosphoric or other acids, should not be induced. An exposed area should be washed with copious amounts of water and a neutralizer such as magnesium oxide, lime water, or aluminum hydroxide gel. Eyes should be irrigated with large amounts of water.

Environmental Fate

Phosphoric acid quickly disperses in natural water sources. The acidity of this compound is eventually reduced but phosphate may persist indefinitely.

Ecotoxicology

Phosphoric acid percolates through soil and is harmful to aquatic life due to its acidity. If undiluted, it will destroy vegetation. When entering the water table, phosphate remaining from the reduction of phosphoric acid can stimulate marine and fresh water algae and plant growth, leading to algae blooms and eutrophication.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (TLV) and the Occupational Safety and Health Administration permissible limit value is $1\,mg\,m^{-3}$ of air; TLV – STEL (short-term exposure limit) is $3\,mg\,m^{-3}$. Phosphoric acid is listed by the US Food and Drug Administration on the 'Generally Recognized as Safe' list when used according to good manufacturing practices. The Food and Agriculture Organization considers less than $30\,mg\,kg^{-1}$ of body weight safe when ingested in foods.

See also: Acids; Corrosives.

Further Reading

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Relevant Websites

http://www.osha.gov – Chemical Sampling Information: Phosphoric Acid. US Department of Labor, Occupational Safety and Health Administration.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Phosphoric Acid.

http://www.ccohs.ca – Health Effects Phosphoric Acid. Canadian Center for Occupational Health and Safety.

http://www.oehha.ca.gov – Chronic Toxicity Summary: Phosphoric Acid. California Office of Environmental Health and Hazard Assessment.

Phosphorus

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 7723-14-0
- Synonyms: Elemental phosphorus; White phosphorus; Red phosphorus; Yellow phosphorus
- Chemical Formula: P

Uses

Phosphorus is used in the manufacture of weapons, insecticides, fertilizers, and rodenticides.

Background Information

Phosphorus is found in rocks, soil, plants, and animal tissues. Commercial preparations of phosphorus are either white or yellow. Yellow phosphorus is white phosphorus that contains small quantities of red phosphorus. Heating white phosphorus in the presence of an oxygen-free and inert atmosphere produces red phosphorus.

Phosphorus is an essential mineral element. Phosphorus homeostasis in the body is controlled by hormonal and renal control systems. Phosphorus intoxication from excessive consumption in food is not known. Toxic exposures have been reported to occur from its industrial use or from suicidal ingestion of phosphorus-containing materials. Phosphorus is highly toxic to humans and animals. The acute lethal dose in humans is $\sim 1\,mg\,kg^{-1}$.

Phosphorus has a garlic-like odor and, when exposed to air, it produces a white smoke and a greenish light. These physical properties can help the clinician in the diagnosis of phosphorus poisoning as

the vomitus and feces of patients who have ingested phosphorus may have a garlic-like odor, be luminescent, and give off what appear to be white fumes.

Toxicokinetics

Phosphorus can be absorbed into the systemic circulation from the skin, lungs, and intestinal tract. For all practical purposes, white and yellow phosphorus are readily absorbed while red phosphorus is not. The target organs of toxicity include the gastrointestinal tract, liver, kidney, bone, and the cardiovascular and central nervous systems.

Mechanism of Toxicity

Phosphorus is an oxidizing agent that, when exposed to air, may burn spontaneously. Thus, direct contact may result in both thermal and chemical burns. Second- and third-degree burns can be seen at the point of contact. When absorbed, phosphorus will act as a cellular poison by uncoupling oxidative phosphorylation.

Acute and Short-Term Toxicity (or Exposure)

Human

The major hazard associated with direct exposure to phosphorus is direct irritation and severe damage to skin, eye, or mucosal surfaces. Systemic absorption of high doses (normally $>0.2\,\mathrm{mg\,kg^{-1}}$) results in acute poisoning. Phosphorus poisoning presents three distinct phases: during the first phase, a painful burning sensation in the throat and stomach is present. Intense thirst, nausea, vomiting, and diarrhea accompany the abdominal pain. Breath, vomitus, and excreta may present the characteristic garlic odor. Feces and vomitus may be luminescent and appear to give off fumes. Severe poisoning may be accompanied by shock and death. During the second phase, poisoning symptoms disappear and the patient appears to be recovering. The second phase may last a few days. During the third phase, the gastric symptoms reappear with nausea, vomiting, and diarrhea. In addition, symptoms indicative of blood, liver, and kidney damage appear. Some of the symptoms include liver tenderness and enlargement, jaundice, oliguria, hematuria, albuminuria, anuria, skin itching and hemorrhages, inhibited blood clotting, and cardiovascular collapse. During advanced stages, the presence of convulsions, delirium, and coma are indicative of central nervous system damage. Death may occur within 4 to 8 days. Prognosis is good if the patient survives for more than 1 week after exposure.

Chronic Toxicity (or Exposure)

Human

Chronic ingestion and/or inhalation of phosphorus may result in osteomyelitis and bone necrosis. Signs and symptoms of this condition include bone inflammation, spontaneous bone fractures, anemia, and weight loss. A typical example of this condition is 'phossy jaw'. This condition is caused by the absorption of phosphorus fumes through teeth cavities. Once absorbed, phosphorus attacks and destroys the bones of the mandible and maxilla. The extent of facial bone loss can be so severe that the bone necrosis may extend from the maxilla to the eye orbits. Phossy jaw is an irreversible and usually fatal condition.

Clinical Management

Basic life-support measures should be implemented and further absorption should be prevented by removing contaminated clothing and washing the affected area. If ingested, the esophagus and digestive tract may be irritated and may be burned. Therefore, a careful examination should be performed and gastric lavage should be instituted only if the esophagus is not damaged and it is believed that lavage may be effective at removing the ingested material.

Medical examination should look for signs of skin, eye, gastric, liver, and kidney damage. Patients should be monitored and treated in an intensive care unit. Monitor vital signs and blood chemistry at least once a day. Life support should be instituted as needed.

Exposure Standards and Guidelines

Phosphorus is listed as a hazardous pollutant under the Clean Air Act and the Clean Water Act. Federal Drinking Water Guidelines: Environmental Protection Agency (EPA) $0.1\,\mu\mathrm{g\,l^{-1}}$ (white phosphorus); Occupational Safety and Health Administration: permissible exposure limit: Table Z – 1 8 h time-weighted average (TWA): $0.1\,\mathrm{mg\,m^{-3}}$; threshold limit values: 8 h TWA: $0.1\,\mathrm{mg\,m^{-3}}$ (yellow phosphorus).

See also: Gastrointestinal System; Organophosphates.

Further Reading

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Photoallergens

Shayne C Gad

Introduction

Photoallergy is an acquired immunologically mediated reaction to a chemical that is initiated by the formation of photoproducts. A photoallergen is a chemical that leads to this response. The occurrence of a photoallergic response to a chemical is sporadic and highly dependent on the specific immune reactivity of the host. Photoallergic responses are thought to be cell-mediated hypersensitivity reactions involving two distinct mechanisms.

In the first reaction type, light initiates the conversion of the hapten (synonymous with photosensitizer) to a complete allergen. Animal studies suggest that the photoreactive chemical in the skin absorbs light and is converted to a photoproduct that subsequently binds to tissue proteins producing a complete antigen:

$$\underset{}{\mathrm{PS}} \rightarrow \underset{(\mathrm{Hapten})}{\mathrm{Photoproduct}} + \mathrm{Tissue\ \ Protein} \rightarrow \underset{(\mathrm{Antigen})}{\mathrm{Allergen}}$$

Halogenated salicylanilide photoproducts are believed to be formed in this fashion.

In the second type of reaction, light absorbed by the photosensitizer results in its conversion to a photoproduct that is a more potent allergen than the parent compound:

$$\underset{(\mathrm{weak\ \ allergen})}{\mathrm{PS}} \rightarrow \underset{(\mathrm{strong\ \ allergen})}{\mathrm{Photoproduct}}$$

The photoproduct of sulfanilamide is thought to be formed by this second pathway in which the parent sulfanilamide compound is converted by ultraviolet (UV) light to the potent allergic sensitizer *p*-hydroxyaminobenzene sulfonamide. Patients with this type of photoallergy have demonstrated an allergic reaction to sulfanilamide in the dark.

Background

The first basic experimental work in photoallergy was done more than 50 years ago, when Epstein, in a straightforward and very perceptive study, demonstrated that sulfanilamide was both a phototoxin and a photoallergen. He and others had observed patients receiving sulfanilamide who developed dermatitis in sun-exposed areas. Six naive subjects (one of whom was himself) were chosen and skin sites were injected intradermally with sulfanilamide (0.1 ml of a 1% saline solution). Then these areas were irradiated with UV light from a mercury arc lamp (UVA and UVB). In all six subjects, the procedure induced a mild erythema leading to hyperpigmentation at the injected sites; that is, a sulfanilamide-mediated phototoxic reaction. Repetition of the protocol (intradermal sulfanilamide and then UV irradiation) at a different site, some days later, caused a marked dermatitis in two of the six individuals. These two subjects had been photosensitized to sulfanilamide, and with further phototesting they continued to show an altered reactivity to sulfanilamide followed by UV radiation (but not to sulfanilamide alone); their photoallergy persisted. In later work, Epstein induced photoallergic contact dermatitis to chlorpromazine in human subjects, utilizing the topical application of chlorpromazine for photosensitization and photochallenge. Those results paralleled his findings with sulfanilamide: Chlorpromazine was both a phototoxin and a photoallergen. Biopsies of positive chlorpromazine photoallergic reaction sites showed a histopathological picture consistent with that of delayed-type hypersensitivity; that is, reactions similar to those of classical experimental allergic contact dermatitis in humans.

Over the ensuing years, a considerable number of compounds have been tested in humans for their possible photoallergenicity, and many of the larger dermatology units have photo testing sections for evaluating patients for possible photoallergy to materials with which they come into contact. Experimental

work in humans sometimes followed the lead of clinical impressions, as was the case with chlorpromazine and tetrachlorosalysilanilide. Kaidbey and Kligman designed a prospective testing scheme in humans for evaluating possible photocontact allergens. Their method requires repeated photosensitizing exposures; that is, application of the test chemical to the skin followed by UV light, for photosensitization; photochallenge is done 10–14 days after the last photosensitization at an untreated skin site. This routine, which is a variant of the 'maximization' test in humans for classical contact allergens, has proven very useful for identifying the photoallergenicity of suspect materials.

Schwartz and Speck were the first to demonstrate photoallergy in an experimental animal, the guinea pig. Their initial investigation was with sulfanilamide and derivatives, and later experiments were with chlorpromazine. The common theme in tests is to photosensitize by the repeated successive application of prospective allergen, followed by UV radiation, to a clipped area (sometimes with the injection of complete Freund's adjuvant into the photosensitization site). Photochallenge is done at a different skin site some weeks later and reactions are evaluated by eye, as for classic allergic contact dermatitis in the guinea pig. The technique successfully identifies most known photocontact allergens, although the substance bisphenol-A, by clinical report a photosensitizer of humans, does not appear to photosensitize guinea pigs.

Test procedures designed to identify potentially photosensitizing chemicals evolved in the wake of the photosensitivity outbreak caused by the antimicrobial halogenated salicylanilides in the early 1960s. Photocontact allergy, although relatively uncommon, proved to be particularly troublesome. A minority of affected patients developed a persistent photodermatitis for many years despite avoidance of further contact with the offending chemical. While removal of the photosensitizing phenolic compounds from the marketplace reduced the incidence of photosensitivity, it quickly became apparent that other, chemically unrelated substances were also capable of inducing this adverse reaction. There was a clear need for a laboratory test to detect potentially photosensitizing agents.

Testing for photoallergy is similar to patch testing for allergic contact dermatitis. Duplicate allergens are placed on the back under occlusion with stainless steel chambers. Approximately 24 h later, one set of patches is removed and irradiated with UVA. All patches are removed and clinical assessments of patch test sites are made 48 h and then 1 week following placement. A reaction to an allergen solely on the irradiated side is deemed photocontact dermatitis. Reactions occurring simultaneously on the irradiated and nonirradiated sides are consistent with an allergic contact dermatitis. There is disagreement about the likelihood of coexisting allergic contact and photocontact dermatitis to the same agent since a photopatch test may occasionally exhibit greater reactivity on the irradiated side compared to the nonirradiated side. **Table 1** lists potential photoallergens used in photopatch testing.

Phototoxicity versus Photoallergenicity

From a mechanistic standpoint, light-induced dermatopathologic changes can be divided into phototoxic and photoallergic categories. Phototoxic skin damage results from the direct interaction of irradiation with subcellular targets, while photoallergic reactions involve immunomodulation of cutaneous photoreactivity. Both variants require initiation by exogenous light, but subsequent cytopathologic mechanisms may be substantially different.

With phototoxicity, light may originate directly from exogenous sources, such as the sun, artificial lighting, or photodynamic topical chemicals, or it

Table 1 Photoallergen series for photo-patch testing

p-Aminobenzoic acid
Bithionol (thiobis-dichlorophenol)
Butyl methoxydibenzolymethane
Chlorhexidine diacetate
Chlorpromazine hydrochloride
Cinoxate
Dichlorophen
4,5-Dibromosalicylanide
Diphenhydramine hydrochloride
Eusolex 8020 (1-(4-isopropylphenyl)-3-phenyl-1,2-propandione)
Eusolex 6300 (3-(4-methylbenzyliden)-camphor)
Fenticlor (thiobis-chlorophenol)
Hexachlorophene
Homosalate
Menthyl anthranilate
6-Methylcoumarin
Musk ambrette
Octyl dimethyl *p*-aminobenzoic acid
Octyl methoxycinnamate
Octyl salicylate
Oxybenzone
Petrolatum control
Promethazine
Sandalwood oil
Sulfanilamide
Sulisobenzone
Tetrachlorocarbanilide
Thiourea
Tribromosalicylanilide
Trichlorocarbanilide
Triclosan

may emanate from endogenous sources such as photodynamic drugs or chemicals following activation or excitation by percutaneous irradiation. Subcellular targets have not been completely characterized but may include the formation of thymine dimers, DNA–protein cross-links, or photodependent oxidations. Immunologic processes are not involved in this form of photosensitivity.

With photoallergic reactions, cytopathologic events are believed to be even more complex than with direct phototoxicity. Although many mechanistic features remain obscure, fundamental concepts include the photoactivation of endogenous or xenobiotic haptens so that they combine with cellular proteins and form a complete antigen. Subsequent immunologic reactions, especially cell-mediated hypersensitivity, complete the sensitivity process.

In contrast to phototoxicity, photoallergy represents a true type IV delayed hypersensitivity reaction. Hence, while phototoxic reactions can occur with the first exposure to the offending chemical, photoallergy requires prior sensitization. Induction and subsequent elicitation of reactions may result from topical or systemic exposure to the agent. If topical, the reactions are termed photocontact dermatitis, while systemic exposures are termed systemic photoallergy. In many situations, systemic photoallergy is the result of the administration of medications. Generally, the mechanisms of photocontact dermatitis and that of systemic photoallergy are the same as those for allergic contact dermatitis. In the context of photocontact dermatitis, however, UV light is necessary to convert a potential photosensitizing chemical into a hapten that elicits an allergic response.

Although precise cytopathologic mechanisms have not been established for many photosensitivity reactions, clinical and pathological features have been extensively documented. The following outline describes key diagnostic findings that serve to differentiate photosensitivity reactions from other dermatologic phenomena.

Photoallergy versus Contact Allergy

Photocontact allergic reactions are often compared with contact allergic reactions. Four pathogenetic features are present in both reaction types:

1. Compounds with a low molecular weight can act as haptens.
2. The antigen is produced by covalent binding of the hapten to skin components.
3. The immunological reactions are T cell dependent.
4. The histological pictures of contact and photocontact allergic reactions are similar.

Photoactivation of Molecules

The main difference between the two pathogenetic mechanisms is that in photoallergy light energy is necessary for the activation of the hapten or skin components to form covalent allergenic adducts. Besides photoactive exogenic or endogenic heteromolecules, the following skin components can be activated by photon energy: amino acids and proteins, blood components, lipoproteins, DNA, RNA, and so on. The reaction possibilities between hapten, light, and skin components can be classified in six different groups depending on the activated molecule:

1. Through the absorbed light quantum the prohaptene is transformed into the haptene.
2. Through the absorbed light quantum the active protein carrier is formed from the protein in the skin.
3. The haptene formed by irradiation combines with a skin protein to form an antigen.
4. The haptene combines with the protein changed by light to form an antigen.
5. The haptene altered by light combines with the protein changed by light, thus forming an antigen.
6. The haptene and light catalyze a chemical reaction on the protein, which leads to an autoantigen.

Distinct photochemical processes are now known for molecular photoactivation. Most of the photoactive molecules have X electrons. If a molecule is activated by light, two different energy levels can be attained. The molecule can be activated from the ground state to the singlet energy level to the triplet energy level. In the case of the singlet-state level, an electron reaches a higher orbital while the original spin configuration is maintained. In the case of the triplet-state level, the electron in the higher orbital changes the spin configuration so that the two electrons in the different orbitals have parallel spin configurations.

The activated singlet-state molecules are short-lived and return to their ground state in time periods of 10^{-1} to maximal 10^{-6} s. Fluorescence is one of the observed manifestations of the nascent energy. Triplet states are of longer duration, their lifetime can reach the range of 10^{-1}–100 s. Phosphorescence may be observed. Besides fluorescence and phosphorescence, nascent energy of activated molecules can also be released in the form of heat; electrical charges can be transferred to other molecules, and radicals can be formed or the molecule itself transformed.

In the case of photoallergic reactions, the formation of heteroadducts plays an important role. It comprises the combination of exogenous molecules

with autologous tissue or cell components. This is the main process for the formation of the complete antigen. The formation of heteroadducts is also the most important factor in the treatment of psoriasis with 8-methoxypsoralen (8-MOP) and UVA. The binding of 8-MOP to thymine molecules in the DNA is important not only for clinical treatment but also for possible late side effects (carcinogenicity).

An important complication of some of the chemicals inducing photoallergic responses is the development of persistent light reactions in which a marked sensitivity to light persists despite the apparent termination of exposure. Removal of the offending photoallergen in these cases does little to abate the condition and the action spectrum broadens to include the UVB as well as the UVA bands. As the phrase implies, this condition is long-lived and troublesome. This particular problem validates the importance of developing and utilizing screening tests for photoallergenicity to prevent exposure of a susceptible population of people to chemicals with this potential.

Clinical Findings

Usually, but not invariably, dermatologic lesions are restricted to light-exposed areas. Changes may vary from urticaria to papular and eczematous eruptions with subsequent exfoliation and lichenification. Microscopically, it is very difficult to distinguish photoallergic reactions from nummular eczema, atop dermatitis, eczematous drug eruptions, and, especially, allergic contact dermatitis.

Histopathologic Findings

Generally, microscopic findings do not provide an adequate basis for separating photoallergic reactions from the eczematous drug eruptions and allergic contact dermatitis previously discussed. Salient features include spongiosis with lymphocytic exocytosis, mild dermal edema, and mild to moderate dermal perivascular cuffing consisting of lymphocytes, histiocytes, and varying numbers of eosinophils. A feature that may distinguish photoallergy from contact allergy in human skin is that inflammatory cell infiltrations in light-induced allergic reactions may be both superficial and deep within the dermis, whereas with contact allergy they tend to be limited to the superficial dermis.

Assessment of Photosensitization

Photosensitivity reactions account for a very small percentage of the total number of undesirable effects from environmental chemicals. However, the increasing incidence and severe disability resulting from these types of skin changes suggest that additional photobiologic research efforts are needed, particularly when the photosensitivity response is of the persistent light reactor mechanism. Predictive testing is an obvious approach used to assess the photosensitizing potential of new chemicals entering the commercial market. These methods make it possible to identify and possibly minimize or eliminate exposures to those compounds demonstrating risk-benefit ratios that are undesirable for the general population or especially sensitive individuals.

In vitro and *in vivo* methods with predictive value for estimating the photosensitizing potential of new compounds have developed rapidly to meet the demanding requirements of today's society. *In vitro* methods for assessing photosensitization are desirable because they are usually rapid and inexpensive and therefore allow screening of a large number of compounds. Many of these methods are not very specific, however, and will generate a greater percentage of false positive results than *in vivo* tests using animal or human models. Complex *in vitro* test systems appear to be useful in identifying the site and mechanism of action in certain situations. Continued evolution of *in vitro* methodologies will add to the understanding of the photosensitization mechanism as better correlation is established with *in vivo* studies.

See also: Skin; Toxicity Testing, Dermal.

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Photochemical Oxidants

Shayne C Gad

Introduction

The oxidant of critical importance in the photochemical atmosphere is ozone (O_3). Several miles above the Earth's surface, in the troposphere, there is sufficient shortwave ultraviolet (UV) light to directly split molecular O_2 to atomic O to combine with O_2 to form O_3. These UV wavelengths do not reach the Earth's surface. In this region, nitrogen dioxide efficiently absorbs longer wavelength UV light, which leads to the following simplified series of reactions:

$$NO_2 + UV \rightarrow O + NO$$

$$O + O_2 \rightarrow O_3$$

$$O_3 + NO \rightarrow NO_2$$

This process is cyclic, with NO_2 regenerated by the reaction of NO and O. In the absence of hydrocarbons, this series of reactions would approach a steady state with no excess or buildup of O_3.

However, near the Earth's surface, the hydrocarbons, especially olefins and substituted aromatics, are attacked by the free atomic O, and with NO, produce more NO_2. Thus, the balance of the reactions shown in the above reactions is upset so that O_3 levels build up, particularly when the Sun's intensity is greatest at midday. The reactions with hydrocarbons are very complex and involve the formation of unstable intermediate free radicals that undergo a series of changes. Aldehydes are major products in these reactions. Formaldehyde and acrolein account for $\sim$50% and 5%, respectively, of the total aldehyde in urban atmospheres. Peroxyacetyl nitrate (CH_3COONO_2), often referred to as PAN, and its homologs, also arise in urban air, most likely from the reaction of the peroxyacyl radicals with NO_2.

Short-Term Exposures to Smog

The complexity of photochemical air pollution challenged toxicologists early on to ascertain its potential to affect human health adversely. Although ozone was quickly suspected as a primary toxicant because of its reactivity and abundance, a number of studies were undertaken with actual (outdoor-derived) smog or synthetic (photolyzed laboratory-prepared atmospheres) smog in an attempt to assess the potency of a more realistic pollution mix. When human subjects were exposed to actual photochemical air pollution (Los Angeles ambient air pumped into a laboratory exposure chamber), they experienced changes in lung function similar to those described in controlled clinical studies of ozone (i.e., reduction in spirometric lung volumes; see below), thus supporting the notion that ozone is of primary concern.

Acute animal studies utilized more easily controlled synthetic atmospheres (usually irradiated automobile exhaust) where the ozone target levels could be made to mimic high air pollution levels: <0.5 ppm. Again, very much like ozone alone, just a few hours of exposure to irradiated exhaust resulted in deep lung damage, primarily within the alveolar or small airway epithelium. In some of these studies, early evidence of edema appeared in the interstitium, particularly in older animals. Additionally, similarly exposed mice were found to be more susceptible to bacterial challenge and lung pneumonias. With time after the termination of exposure, the end-airway lesions recovered and the susceptibility to infection waned, although some of the pathology in the distal lung persisted for more than 24 h. While ozone appeared to be the prime toxicant in these studies, that was not always the case. When guinea pigs were exposed to irradiated automobile exhaust, airway resistance increased, indicating that a more soluble irritant probably was active, presumably reactive aldehydes. Thus, the array of effects of a complex atmosphere may be more diverse than would be predicted if it were assumed that ozone alone was responsible.

Chronic Exposures to Smog

Studies of both humans and animals exposed to smog have attempted to link chronic lung defects with photochemical air pollution. Cross-sectional and retrospective field studies have suggested an accelerated loss of lung function in people living in areas of high pollution compared to those living in area of low pollution, but most of these studies have been imprecise because of confounding factors (meteorological factors, exposure measurement imprecision, and population variables). Recently, there has been a rejuvenation of interest in what are sometimes called sentinel studies, which allow a detailed study of animals exposed to the same highly polluted urban air to which people are exposed. This approach has had a troubled past, but newer studies have attempted to minimize or at least control for the problems of infection, animal care, and lack of control of the exposure atmosphere.

Synthetic smog studies in animals were undertaken to eliminate some of the concerns about ambient smog exposure. The most extensive effort to evaluate the potential long-term health effects of synthetic smog was undertaken at the Cincinnati US EPA laboratory in the mid-1960s. Beagle dogs were exposed to synthetic atmospheres on a daily basis (16 h) for 68 months, followed by a clean air recovery period of ~3 years. The lungs of exposed dogs then underwent extensive morphological examination to correlate physiological and morphological observations. While the study did not show time-related lung function changes, all exposure groups had abnormalities, most of which persisted or worsened over the 3 year recovery period in clean air. Enlargement of air spaces and loss of interalveolar septa in proximal acinar regions were most severe in dogs that were exposed to oxides of nitrogen, oxides of sulfur, or oxides of sulfur with irradiated exhaust. Oxidants such as ozone arising from the irradiated exhaust would be expected to act on the distal lung. These studies elucidated a morphological lesion that was degenerative and progressive in nature, not unlike that of chronic obstructive pulmonary disease, the condition most often noted in the epidemiological studies.

See also: Nitric Oxide; Ozone; Pollution, Air; Respiratory Tract.

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Phthalate Ester Plasticizers

Shayne C Gad

- Representative Chemicals: Di-(2-ethylhexyl) phthalate (DEHP); Diisononyl phthalate (DINP); Diethyl phthalate (DEP); Di-*N*-butylphthalate; Dimethylphthalate; Methyl-glycol phthalate; Phthalic acid; Bis(2-methoxyethyl) ester. For the purposes of this article, the focus will be on DEHP, which is the most widely used phthalate, with some discussion of DINP since it has been the subject of controversy with regards to its possible adverse effects on children's health.
- Chemical Abstracts Service Registry Number: CAS 131-11-3 (Di-(2-ethylhexyl) phthalate)
- Synonyms: *o*-Dicarboxylic acid esters; Phthalic acid ester (PAE)

Uses

Phthalate esters are widely used in the production of plastics, particularly vinyl plastics, to add flexibility to products made with these materials. DEHP is commonly used in medical devices, including cardiac catheters, endotracheal tubes, and certain implanted devices, while DINP is more often found in wires and cables, hoses, and plastic toys. DEHP is also used in plastic containers, such as those used for food. These phthalates are not bound chemically to the plastic but are physically dissolved in it.

Exposure Routes and Pathways

The routes of exposure of most concern for DEHP are leaching into liquids administered intravenously in the course of medical treatment and leaching into foodstuffs (particularly lipophilic foods) stored in plastic containers, although exposures through the latter route are much smaller. For DINP, the main exposure concern is leaching as a result of children mouthing plastic toys.

Toxicokinetics

All phthalate esters are readily absorbed, but toxicokinetics vary based on the route of exposure. Once absorbed, they are quickly distributed to organs and other body tissues such as the liver (bile) or kidneys. Phthalate esters metabolize quickly to a monoester but do not progress further. From 4.5% to 15% of single doses of 10–30 g of DEHP are excreted as metabolites in the urine of man.

Mechanism of Toxicity

The mechanism by which DEHP causes at least some of its adverse effects appears to be through peroxisome proliferation in the liver of rodents. There is some controversy as to whether this effect would also occur in humans since they seem much less sensitive to this type of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Phthalates are not irritating or slightly irritating to eye or skin, and are not sensitizers. For DEHP, the intraperitoneal LD_{50} is 14–75 $g\,kg^{-1}$ and the oral LD_{50} is $>30\,g\,kg^{-1}$ in mice. In rats, the intraperitoneal LD_{50} is 30.7 $g\,kg^{-1}$ and the oral LD_{50} is $>25\,g\,kg^{-1}$.

Human

Phthalate esters may irritate eyes, skin, or mucous membranes.

Chronic Toxicity (or Exposure)

Animal

Repeat oral high doses of DEHP result in hepatomegaly (peroxisome proliferation) and tumorgenesis in rats. While DEHP appears to cause reproductive toxicity, similar effects have not been seen with DINP exposure. As a result of these and other effects, there has been concern that DEHP can cause endocrine effects. It appears that neither DEHP nor DINP are genotoxic.

Human

There are reports from patients receiving hemodialysis that DEHP may cause toxicity to the heart, lungs, and reproductive system. DEHP has been classified as a probable human carcinogen by the US Environmental Protection Agency (EPA).

Clinical Management

If ingested, gastric lavage should be performed and respiratory therapy administered, if needed. Emesis should be avoided. Treatment should be symptomatic.

Ecotoxicology

Phthalates are of low toxicity to aquatic life. For example, the LC_{50} for bluegill is $>770\,000\,\mu g\,l^{-1}$ per 96 h and the LC_{50} for *Daphnia magna* is 1000–5000 $\mu g\,l^{-1}$ per 48 h. Phthalates tend to persist to some degree in the fat of organisms in the environment.

Exposure Standards and Guidelines

The US Occupational Safety and Health Administration has established a permissible exposure limit of 5 $mg\,m^{-3}$. The US EPA has established a drinking water standard (maximum contaminant level) of 0.006 $mg\,l^{-1}$.

See also: Endocrine System; Polymers.

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Phthalates *See* Phthalate Ester Plasticizers.

Physical Hazards

Gene Rider

Introduction

Toxicologists are often required to assess the overall chemical- and design-related risks of reasonably foreseeable exposures to consumer products. Such a risk assessment would include the potential physical hazards associated with consumer products. Examples of potential physical hazards associated with products include designs that could lead to asphyxiation, aspiration, choking, strangulation, or suffocation incidents. Other examples of potential physical hazards include products that could create light (specifically, certain wavelengths of electromagnetic radiation), products creating very cold or hot temperatures, products with a high potential for flammability, products with an ability to create loud noise, products capable of forceful impact or with a sharp component that could lead to trauma, and products capable of generating strong vibration.

Analysis of the etiology of the physical injuries from consumer products includes three components: determination of the at-risk population (exposure to hazard), mechanism of injury (consequences of hazard), and characteristics of products (mitigation of hazard).

In order to understand the potential physical hazard-type risks associated with consumer products, it is important to utilize a multidisciplinary approach. In order to identify the population that is at risk, it is necessary to investigate injury and fatality incidents with similar products. Following this identification, characterization of the physical interaction of a consumer and a product reveals hazard and associated severity levels. Characterization of product attributes allows for the development of strategies that may mitigate product hazard and therefore reduce the probability of injury.

At-Risk Populations (Exposure to Hazard)

If a consumer can gain access or become exposed to hazardous product characteristics, the probability of this event must be determined. Probable exposure to the hazard may be determined using injury and fatality data analysis.

Learning from history through the analysis of variables associated with real-life injuries and fatalities allows for an understanding of the connections between product characteristics, child behavior, and injury. Statistical analysis and modeling reveal the critical characteristics associated with the risk of product-related injury.

Mechanism of Injury (Consequences of Hazard)

If a consumer is exposed to hazardous product characteristics, the severity level or potential consequence of this exposure must be evaluated. Human factors analysis is conducted to determine the consequences (i.e., potential product-related injuries) based on the foreseeable behaviors consumers will use when interacting with products. Virtual and physical models of the human anatomy are used to effectively diagnose and demonstrate hazardous product characteristics. (In contrast to a physical hazard such as those noted above, 'physical' in this human context relates to the usage of three-dimensional (3D) models of various parts of humans relevant to the exposures associated with use and/or misuse of a product.)

Human factors analysis utilizes accurate virtual and physical simulations of the human anatomy to identify the potential hazards posed by consumer products.

In order to determine the potential magnitude of a product-related physical hazard, both product characteristics and anatomical characteristics of likely consumers are examined. Virtual and physical human factor tools are used to conduct this research.

Characteristics of Products (Mitigation of Hazard)

The severity level of a physical hazard may be reduced by design characteristics that lead to reduced consequence or decreased time to effective treatment, and possibly by product labeling and/or usage instructions that could impact consumer behavior to help eliminate the hazardous condition, or at least mitigate the consequences of the exposure.

Airway Obstruction

At-Risk Populations

Children are driven to mouth objects as an inevitable part of their normal developmental process. This behavior has the potential to lead to choking, aspiration, insertion, or ingestion injuries. Small part injuries involve the unintentional entry of a foreign body into the aerodigestive system through the mouth or nose.

Children under the age of 4 represent the majority of airway obstruction injuries (**Figure 1**). Individuals of advanced age are also at greater risk for such injuries as they may be edentulous and suffer from

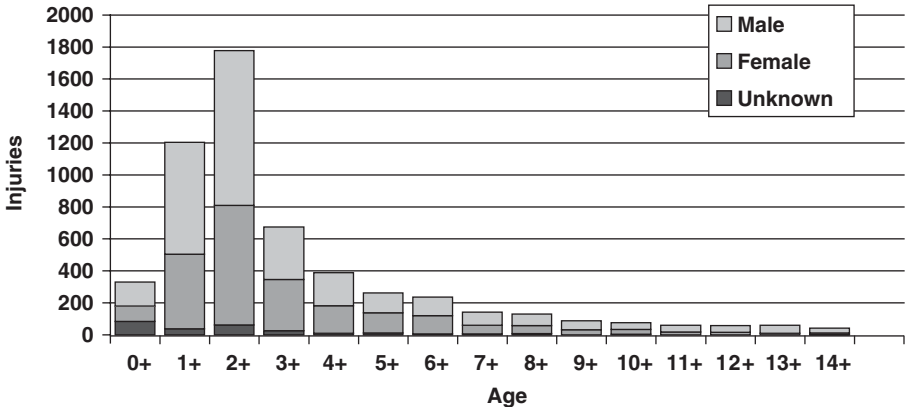

Figure 1 Age of victims of airway obstruction injury.

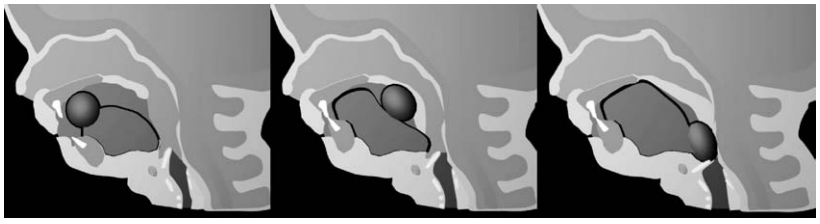

Figure 2 Compressible object lodging in pharynx.

decreased oral sensation. Alcohol or drug use and aging decrease the sensation of the nerves in the oral cavity increasing the likelihood of airway obstruction injuries.

Neurologically impaired people (estimated at 2–4% of the population often have greater difficulty during feeding and swallowing increasing the likelihood of airway obstruction. The term neurologically impaired is a blanket description that covers a multitude of different disorders. Persons suffering from these disorders may have them to widely varying degrees. Oral airway dysfunction in this group may present as diminished control, sensation, or comprehension. While most neurologically impaired persons have a normal airway, a segment of this population has abnormal airway anatomy altering their risk of airway obstruction.

Mechanism of Foreign Object Airway Obstruction

Direct Obstruction Several characteristics influence a foreign object's chance of penetrating the defenses of the mouth and pharynx. Foreign objects that are small, thin, smooth, or slick when wet may inadvertently slip through and enter the pharynx. Foreign objects that are round or cylindrical and pliable or compressible most effectively form a plug in the airway (**Figure 2**). A large bolus or foreign object mass is more likely to block the airway at the pharynx and cause asphyxia. When a premature or an inadvertent foreign object penetration occurs, a gag and or cough reflex may be triggered. Smooth, slick, and pliable food-like objects are less likely to trigger a timely gag reflex than textured or sharp objects. Gagging and coughing is frequently followed by rapid deep inhalation as the victim attempts to regain breath. This action may draw the object downward leading to physical impaction and obstruction. This consequence is facilitated by the temporary expansion of the pharyngeal and laryngeal chambers that occurs during vigorous inspiratory effort. This reaction is more intense in infants than in older children or adults. Pliable conforming objects (**Figure 3**) are less likely to be expelled from the airway than rigid objects.

Esophageal Wall Protrusion Foreign objects that are ingested and lodge beyond the upper esophageal sphincter may distend the wall of the esophagus into the volume of the airway along the length of the trachea resulting in asphyxia (**Figure 4**).

Oral Nasal Occlusion Rounded 3D objects that reach the rear of the oral cavity, posterior to the hard palate, may obstruct the flow of air into the lungs, leading to asphyxia (**Figure 5**). The mechanism of these injuries includes the following actions: these

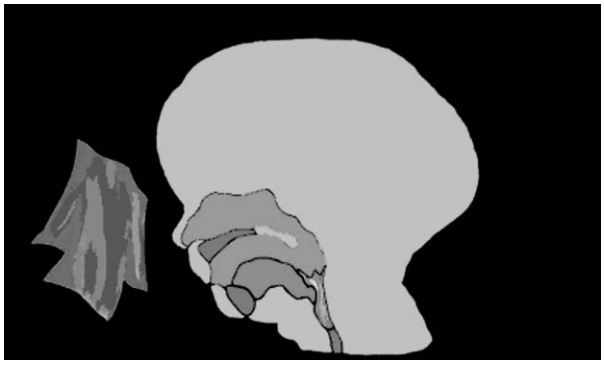

Figure 3 Conforming object lodging in pharynx.

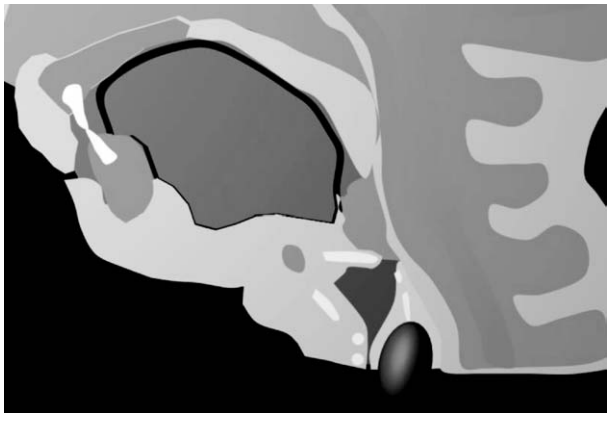

Figure 4 Esophageal wall protrusion.

objects may elevate the soft palate and prevent air passage through the nasopharynx (A). This action may diminish the size of the nasopharynx at this point, preventing the passage of air while simultaneously creating a seal against the soft tissue of the rear of the oral cavity (B). Objects that pass beyond the hard palate may be difficult to extricate due to the mechanical resistance to anterior motion created by the interference with the edge of this skeletal structure (C).

Object Characteristics Associated With Airway Obstruction

Size Airway (pharynx, larynx, trachea) sized objects will occlude the airway if other conditions are present. Small, lightweight nonwettable objects may be entrained in the inspiratory flow of air and consequently aspirated. Oral sensors can discriminate the size of solid materials, but children, the elderly, and the neurologically impaired may make poor decisions as to what they can successfully swallow.

Shape Previous studies of airway obstruction have classified foreign objects by shape. Square and

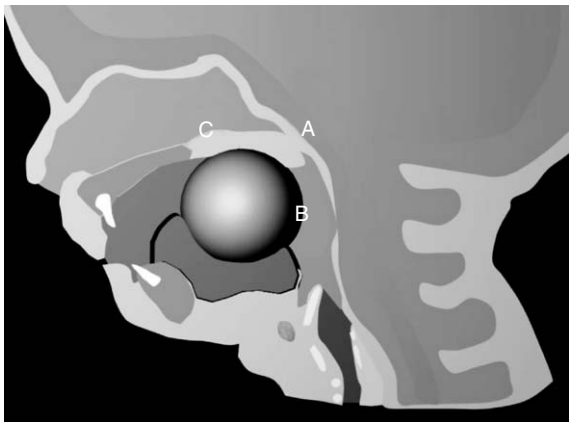

Figure 5 Oral nasal occlusion.

rectangular shapes make up less than 6% of the objects in this database, while a significant number, 22%, are spheres and ellipsoids. This illustrates that round objects make effective airtight seals against the flexible, rounded interior walls of the aerodigestive system. As expected, most of the incidents involving two-dimensional (2D) objects, such as coins, resulted in less severe symptoms, while 3D and conforming objects are more likely to produce more severe symptoms.

Consistency – Food Foreign Objects Penetration of the airway by food foreign objects is possible due to the combined oral airway functions of the oral cavity and pharynx. Humans must interrupt respiration during the pharyngeal phase of the swallow cycle. An asphyxiation hazard for a foreign object is a function of the degree of difficulty in processing the foreign objects into a bolus suitable for successful swallowing. Foreign objects that are difficult to process create a condition where the hazard of asphyxiation is present.

Compressible and nonfriable foods resist bolus formation. Compressible foods that do not break apart do not become well mixed with saliva. Compressible foods require transverse and rotational movement of the mandible to be effectively masticated. Compressible foods will deform to the shape of the airway.

Consistency – Nonfood Foreign Objects Conforming objects will, with certain flexibility and surface characteristics, adhere closely to the surface topographic features of the airway, making an effective seal. Expiratory effort will allow air to leave the lungs but inspiration will create a negative pressure on the distal surface of the conforming object that draws it further into the airway. Deformable materials (i.e., materials or objects whose shapes can be altered by

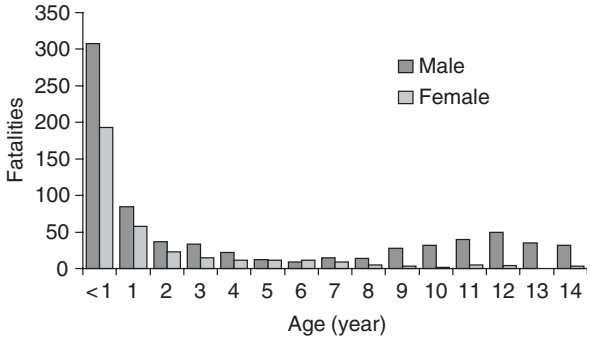

Figure 6 Age and gender of victims of strangulation fatalities.

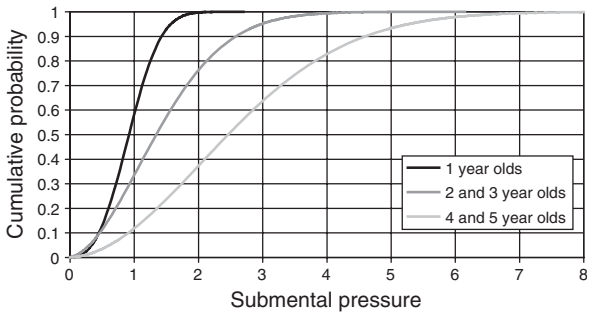

Figure 7 Strangulation force.

applying forces similar in magnitude to those which would be experienced in a child's mouth) also account for a significant number of serious injuries.

Strangulation

At-Risk Populations

There are 1160 strangulation fatalities involving children under age 15 documented in US Consumer Product Safety Commission (CPSC) death certificate files between 1994 and 2003. These 1160 strangulation fatalities constitute 14.1% of the total childhood fatalities (8224) (**Figure 6**).

Age: The number of strangulation fatalities was greatest among children under one, who accounted for approximately half (45%) of the childhood strangulation fatalities. The fatality trend declined until age seven, after which it rose until dropping again at age 13.

Gender: The majority (68%) of the incidents occurred to male children. The discrepancy in gender is more noticeable after the age of eight.

Mechanism of Injury

The neck region is a complex passageway for communication between the head and the trunk. The bony components in the neck are the vertebra, which enclose the spinal cord. The most important vessels are the carotid artery and the interior jugular vein that are contained in the carotid sheath along with the vagus nerve. The neck also contains the airway (larynx and the trachea); in children they are cartilaginous and highly mobile.

Strangulation is due to constriction of the neck causing direct airway closure. This often occurs as a result of suspension of all or a portion of the body weight by an object around the neck resulting in asphyxia. The constriction generally occurs above the larynx but below the angle of the jaw. The most common scenario in children is partial hanging, occluding the airway but not the jugular vein or carotid artery. Airway obstruction is currently believed to occur as the base of the tongue is pushed against the posterior wall and the epiglottis folds over the larynx.

In a previous study by the author, submental pressure (**Figure 7**) was noted to elevate the larynx, but occlusion occurred at the level of the nasopharynx and oropharynx. The soft tissues of the submental region pushed the tongue against the soft palate.

Suprahyoid pressure brought the epiglottis up and posterior compressing it against the posterior pharyngeal wall. The arytenoids compressed by the posterior wall of the hypopharynx overrode the true vocal cords to occlude the airway. The thyroid cartilage supported by its attachments to the hyoid was lifted superiorly and posteriorly (**Table 1**).

Characteristics of Objects Causing Strangulation

Apparel of children that contain components that can be caught on doorknobs, play ground equipment, or protrusions.

Continuous loops with a circumference greater than 13.94 in (35.4 cm) can be placed over a child's head.

Loose ends of strings, cords, or straps capable of forming a loop greater than 8.66 in (22.0 cm) can be placed around a child's neck.

Suffocation

At-Risk Populations

Approximately 300 infants have died over the past 4 years as a result of accidental suffocation associated with a variety of consumer products (plastic films, toys, and packing materials). Suffocation injuries and fatalities are most common to children under the age of 2 years (**Figures 8** and **9**).

There are two potential mechanisms involved in suffocation incidents, suffocation caused by mechanical

Table 1 Force required to occlude airway: Summary by percentile

Percentile	*Force(lbs)*					*Weight of child*			
	1 year old	*2 year old*	*3 year old*	*4 year old*	*5 year old*	*⩽10 kg*	*12.5 kg*	*15.0 kg*	*>15 kg*
10%	0.7	0.7	1.0	1.1	1.4	0.4	0.6	0.7	1.1
20%	0.9	0.9	1.2	1.4	1.6	0.6	0.8	0.9	1.4
30%	1.1	1.1	1.4	1.6	1.9	0.8	1.0	1.1	1.7
40%	1.3	1.3	1.6	1.8	2.1	1.0	1.2	1.3	2.0
50%	1.5	1.5	1.8	2.1	2.4	1.2	1.4	1.5	2.4
60%	1.7	1.8	2.1	2.3	2.8	1.6	1.8	1.8	2.9
70%	2.1	2.2	2.5	2.7	3.2	2.1	2.2	2.1	3.5
80%	2.6	2.9	3.0	3.3	3.9	3.0	3.0	2.7	4.6
90%	3.6	4.3	4.1	4.3	5.1	5.4	4.9	3.9	6.7
95%	4.8	6.1	5.3	5.5	6.4	9.3	7.6	5.4	9.6

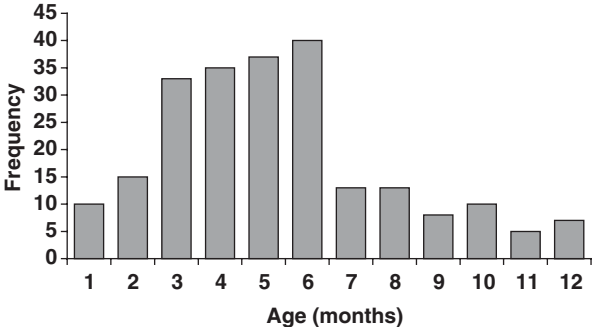

Figure 8 Age distribution in months – films.

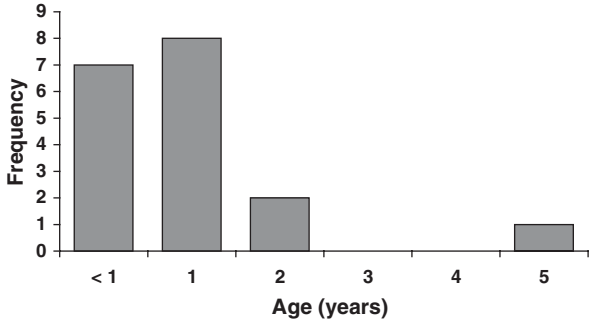

Figure 9 Age distribution in years – rigid container mechanisms of suffocation.

resistance to the passage of air and suffocation caused by physical responses to carbon dioxide (CO_2) rebreathing.

Mechanical Resistance Suffocation

Objects placed externally on the face of a child may lead to suffocation incidents. These objects are most commonly plastic films or bags, but may be any product that makes a seal against the face of a child, obstructing airflow to the mouth or nose. The faces of children in this age group have greater amounts of fat and undeveloped prominent bony structures and are consequently more likely to provide an effective seal, leading to suffocation injuries.

Respiration

Infants usually breathe through the nasal passages. However, during crying or in the event their nasal passages are blocked, infants may breath through their oral cavities. One-year-old children can produce a respiratory pressure up to 30 cm H_2O (positive for expiration and negative for inspiration) for a brief period of time. Young children can produce pressures of 15 cm H_2O for a more extended period of time.

Suffocation

Mechanical resistance suffocation takes place when the passage of air to the oral cavity and nasopharynx are both blocked externally by an object. When respiration is interrupted, CO_2 levels in the blood rise. The body's response to this elevation in CO_2 level is to attempt respiration. If the mechanical blockage is complete and the agent of suffocation is not removed, the incident will be fatal after 2–3 min. Partial blockage may be survived for longer periods of time, depending on the level of resistance and the strength of the child.

Protective Mechanisms

Very young children do not have effective defense mechanisms to protect themselves from suffocation injuries. In adults, raised CO_2 levels incite more and more strenuous attempts at respiration. In infants and young children, this response is not present. During suffocation incidents in infants, if the initial attempts at respiration fail, an increase in respiration effort and agitation is not observed.

Characteristics of Objects Causing Suffocation

Table 2 lists the respiration characteristics for both 6-month-old and 1-year-old children.

Table 2 Respiratory characteristics

Characteristics	*6 Month old*	*12 Month old*
Weight (lbs)	13	20
Flow resistance, R (cm H_2O per liter per second)	21	13
Peak flow rate (liter per minute)	22	34
Pressure for airway flow resistance, 'Peak flow rate $\times$ R' (cm H_2O)	7.7	7.4
Pressure for elastic recoil (cm H_2O)	4	4
Long-period sustainable pressure (cm H_2O)	14	18
Six-hour sustainable pressure (cm H_2O)	25	30
One-hour sustainable pressure (cm H_2O)	30	35

Table 3 Computed allowable pressure P_a

Time	*Pressure*	*6 Month old*	*12 Month old*
Long period	Allowable pressure (using peak flow rate)	2.3 (cm H_2O)	6.6 (cm H_2O)
Six hours	Allowable pressure (using peak flow rate)	13.3 (cm H_2O)	18.6 (cm H_2O)
One hour	Allowable pressure (using peak flow rate)	18.3 (cm H_2O)	23.6 (cm H_2O)

As a part of the sustainable pressure must be used to overcome normal airway flow resistance and elastic recoil of lung and chest, the amount of pressure (P_a) allowed to be used to overcome abnormal airway blockage, such as plastic film blockage, is smaller and can be found by

$$\begin{aligned} P_a = {} & \text{Sustainable pressure} \\ & - (\text{Pressure for flow resistance} \\ & + \text{Pressure for elastic recoil}) \end{aligned}$$

Table 3 gives the computed allowable pressure P_a for different time duration for both 6-month and 12-month-old children.

Thermal Burn Injury

Thermal burns are classified by the amount of damage done to the skin and other body tissue. The surface area of the skin ranges from 0.2 to 0.3 m^2 in an average newborn, and 1.5–2.0 m^2 in an adult. The skin consists of two layers: the epidermis, ranging from 0.05 mm thickness (in such areas as the eyelids) to over 1 mm thickness on the soles; and the dermis, usually at least 10 times thicker than the associated epidermis. An average total skin depth is 1–2 mm. Males generally have thicker skin than females. Skin is very thin in infants, increasing in thickness until age 30–40, and then progressively thinning with age.

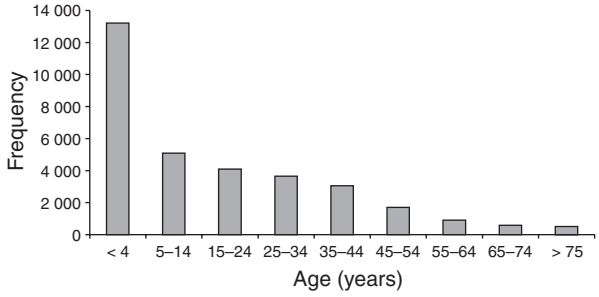

Figure 10 Age of victims of thermal burn injury.

Burn injury is tissue damage caused by thermally induced irreversible chemical reactions. Burns are often classified according to their severity as first degree, second degree, or third degree. First-degree burns, often referred to as surface burns, are minor burns that heal quickly. Second-degree burns, also referred to as partial thickness burns, are more serious injuries, which may require medical attention and possibly skin grafts to prevent permanent scarring. Third-degree burns, also referred to as full-thickness burns, extend deeply into tissue and are characterized by charring of the skin.

At-Risk Populations

Children constitute the most at-risk population for thermal burn injuries (**Figure 10**).

Mechanism of Injury

Thermal injuries to skin are assessed by measuring the amount of energy transferred to the skin by radiation, convection, and conduction.

These factors, and duration of exposure, which may be expressed as a dose function, are the primary determinants of the severity of thermal injury.

From an anatomical viewpoint, it is common to classify burns according to the depth of penetration into various skin layers. As seen in **Figure 11**, skin is composed primarily of a (usually) thin outer layer called the epidermis followed by a thicker dermis. Below the dermis is a layer of subcutaneous fat followed by skeletal muscle. Burns can be characterized according to the deepest layer of tissue penetrated and the depth to which that layer has been affected. In this scheme, two numbers are used to classify a burn: the first designates the lowest layer of tissue damaged, and the second the fractional depth to which that layer has been penetrated. Therefore, a 1.5-degree burn is one affecting only the epidermis (skin layer 1) in which half of the epidermis (0.5) has

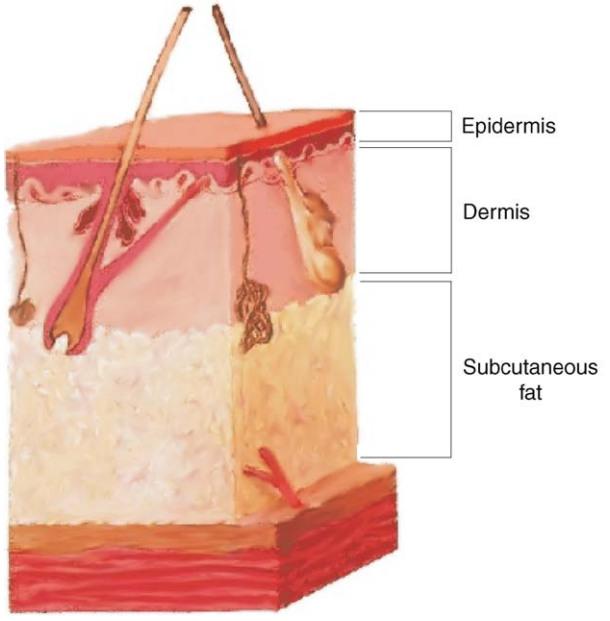

Figure 11 Diagram of skin showing epidermis, dermis, and fat.

been penetrated. By comparison, a 2.8-degree burn is one in which at least some portion of the injury extends 8/10ths of the way through the dermis (second skin layer), and a 3.4-degree burn is one in which the deepest portion of the injury extends through the epidermis and dermis until it has reached 4/10ths of the way through the subcutaneous fat (third layer).

The science of burn modeling effectively began in the late 1940s with a series of studies conducted at Harvard medical school by A.R. Moritz and F.C. Henriques. Motivated by the events of World War II, Henriques and Moritz conducted experimental studies of burn injuries from various heat sources to pigs and human volunteers. The studies of greatest relevance to modern-day burn modeling involved the use of flowing constant-temperature water to produce burn injuries on the backs of both pigs and humans. One hundred seventy-nine experiments were conducted on pigs at water temperatures between 44°C and 100°C and times ranging from 1 s to 7 h, and 33 exposures performed on volunteer soldiers at temperatures ranging from 44°C to 60°C and times between 3 s and 3 h. Although other experimental studies of contact or scald burn injury were conducted both before and after Henriques and Moritz, the combination of extent, high-temperatures/short times, and applicability to humans make their work unique.

One of Henriques' and Moritz' primary goals was to develop 'dose–response' curves designating the minimum exposure times at given temperatures yielding second-degree burns and the maximum exposure times at the same temperatures resulting in only first-degree burns. To obtain this information, they waited for up to a week following exposure and then medically examined each injury to determine its severity. Henriques subsequently developed a model based on this dose–response information that remains the basis for the vast majority of burn injury calculations performed today.

Characteristics of Objects Causing Thermal Burns (The Henriques Model)

Henriques developed a two-step method to calculate burn injury. The first step is a calculation of temperature distribution within the skin, while the second determines burn injury based on the time–temperature history. This general approach remains in use although modern burn modeling techniques generally involve sophisticated computer models of temperature distribution that were unavailable to Henriques and Moritz.

Henriques treated the skin as a semi-infinite body in which all skin layers have the same thermal properties, and the total skin thickness is far greater than that heated by the thermal source. Based on this assumption, he obtained the following formula for the temperature of the basal epidermal layer as a function of time:

$$T_t = T_s - (T_s - T_0)\mathrm{erf}(\gamma/\sqrt{t})$$

where t is time (seconds) after the start of heat exposure, T_t is the temperature of the basal epidermal layer at time t, T_s is the surface temperature of the skin during heat exposure, T_0 is the initial skin temperature (assumed constant 35°C throughout prior to heat exposure), erf is the error function, and γ is given by

$$\gamma = \frac{L}{2\sqrt{k/c_p\rho}}$$

where L, k, c_p, and ρ are, respectively, the thickness of the epidermis and the thermal conductivity, heat capacity, and density of the skin. Henriques used $\gamma = 0.15\ \mathrm{s}^{1/2}$ in his calculations.

Having approximated the temperature of the basal epidermal layer as a function of time, Henriques assumed that the burn injury process follows the kinetics of irreversible unimolecular reactions constant and obtained the following expression:

$$\Omega = P\int_0^t \exp\left(\frac{-\Delta E}{RT}\right)\,\mathrm{d}t$$

where Ω is a burn injury function indicative of the extent of burn injury. He arbitrarily set $\Omega = 1$ for

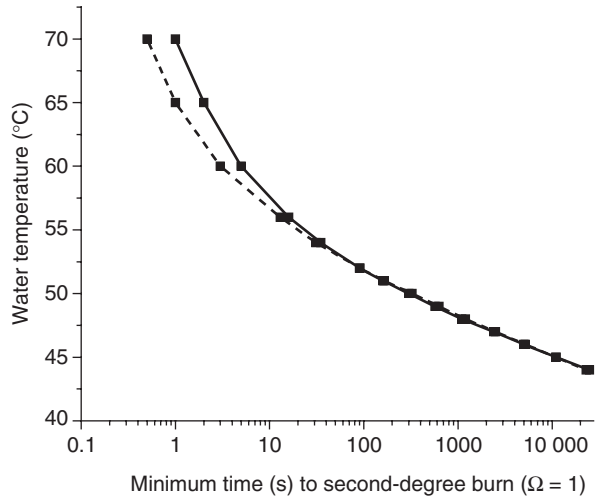

Figure 12 Henriques calculations (dashed line) vs. data (solid line) for minimal second-degree burn.

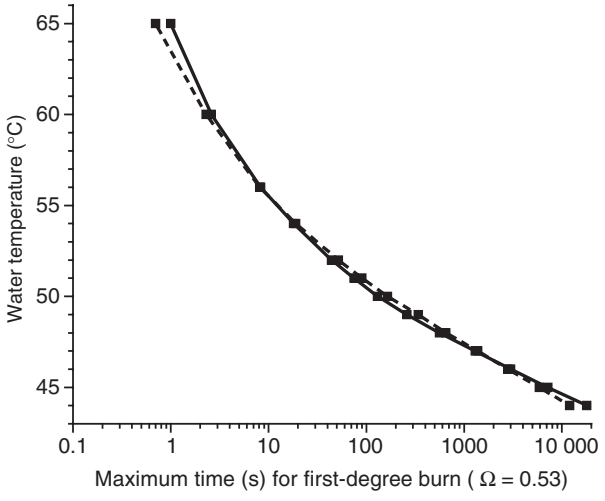

Figure 13 Henriques calculations (dashed line) vs. data (solid line) for maximal first-degree burn.

minimal second-degree injury (burn injury corresponding to the shortest time yielding a second-degree burn at a given temperature) and determined the values of the constants $\Delta E = 150\,000\,\text{cal mol}^{-1}$ ($\Delta E/R = 75\,000\,\text{K}$) and $P = 3.1 \times 10^{98}\,\text{s}^{-1}$ by fitting his equations to the burn injury data. Graphs showing Henriques' calculations along with Moritz and Henriques' data are shown in **Figures 12** and **13**.

Hazard Level

The data shown in **Figures 12** and **13** are based on measurements made on the backs of adult males having epidermal thickness of $\sim 80\,\mu\text{m}$. The time to produce burns can be significantly shorter for areas having thinner epidermis (e.g., eyelids), and for children and older adults. To prevent burns in at-risk groups, the American Burn Association recommends setting water heaters to 120°F and taking measures to prevent children and other at-risk populations from coming in contact with heat sources such as hot drinks, cooking surfaces, and hot appliances.

Light Toxicity

Introduction

This article reviews direct and indirect (e.g., afterimage, flash blindness) light hazards from common incoherent light sources. For direct hazards specific to lasers and other specialized coherent sources, the reader is referred to organizations such as the Laser Institute of America and the International Electrotechnical Commission.

Light is a form of electromagnetic radiation that is distinguished from other regions of the electromagnetic spectrum, such as radio waves, microwaves, X-rays, by its wavelength. From the standpoint of hazard, light is usually divided into three regions: ultraviolet (UV), visible, and infrared (IR). UV light ranges in wavelength from ~ 400 to 100 nm and is hazardous due to photochemical action. (A nanometer or nm is 1 billionth of a meter.) The most common source of UV is the sun. However, tanning lamps, black lights, and very hot objects such as welding torches can emit a significant amount of UV radiation. Visible light ranges in wavelength from ~ 700 to 400 nm. IR light ranges in wavelength from ~ 700 nm to 1 mm and is perceived by the body as heat. Common IR emitters include hot objects, heat lamps, light-emitting diodes in remote controls and other electronic equipment, and the sun. The hazards associated with light are reviewed by a number of international and national bodies, including the International Commission on Non-Ionizing Radiation Protection (ICNIRP), the International Electrotechnical Commission, the American Conference of Governmental Industrial Hygienists (ACGIH), the American National Standards Institute, and the National Radiological Protection Board.

Mechanism of Injury (UV Radiation)

Of the three regions of light discussed here, UV is most often associated with hazards. UV light primarily affects the skin and eyes. Sunburn (erythematic), premature skin aging, and skin cancer, are the best known effects of UV light; however, the skin incorporates part of the immune system, and UV exposure can accordingly decrease immune response to skin cancer, infectious agents, and other antigens. UV light can damage external ocular tissues including the cornea, iris, and conjunctiva, resulting in photokeratitis

Table 4 ICNIRP/ACGIH recommended limiting exposure limits

Exposure per day	*Effective irradiance E_{eff} ($W m^{-2}$)*
8 h	0.001
4 h	0.002
2 h	0.004
1 h	0.008
30 min	0.017
15 min	0.033
10 min	0.05
5 min	0.1
1 min	0.5
30 s	1
10 s	3
1 s	30
0.5 s	60
0.1 s	300

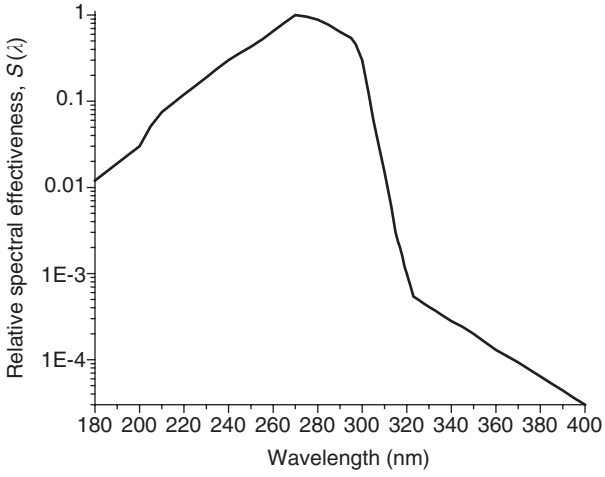

Figure 14 Relative spectral effectiveness for UV exposure.

or photokeratoconjunctivitis (more commonly known as snow blindness or welder's flash). UV light that penetrates through the cornea can result in opacities of the lens (cataracts) as well as retinal damage if not absorbed in the aqueous or vitreous humor. While it is difficult to quantify the extent of UV-related health problems, the World Health Organization believes solar UV exposure to be a significant contributor to the 2–3 million nonmelanoma skin cancers and 130 000 melanoma skin cancers occurring globally each year.

The nature and extent of UV toxicity is both wavelength and intensity dependent. In order to simplify wavelength dependence, the UV is divided into three sub regions: UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm). ICNIRP and ACGIH recommend that skin and eye exposure to UV-A radiation be limited to $10\,W\,m^{-2}$ for periods exceeding 1000 s, and $10\,kJ\,m^{-2}$ for periods less than 1000 s. For UV-B and UV-C, recommended exposures are given in **Table 4**. The effective irradiance E_{eff} is calculated as

$$E_{eff} = \sum E_\lambda \cdot S_\lambda \cdot \Delta_\lambda$$

where E_λ is spectral irradiance ($W\,m^{-2}\,nm^{-1}$) and S_λ is the relative spectral irradiance given in **Figure 14**.

The exposure limits in **Table 4** are meant to be absolute limits for the eye and advisory limits for skin. They are based on consideration of the Caucasian population, which has the greatest UV sensitivity. These limits do not apply to at-risk populations such as highly photosensitive adults, aphakic individuals, young children, or persons exposed to photosensitizing agents. A large number of agents can cause hypersensitivity to UV radiation including antibiotics such as tetracycline and sulfathiazole, antidepressants such as imipramine and sinequan, and some antipsychotic drugs, diuretics, dyes, cosmetics, and coal tar products. Further, intense UV light produced by lasers must be considered differently from more common incoherent light sources.

Mechanism of Injury (Visible Light)

Visible light is most commonly associated with damage to the eye. However, skin injury is possible, particularly in the presence of endogenous (e.g., bilirubin) or exogenous (e.g., phenothizine) photosensitizers. The ocular tissue most susceptible to visible light injury is the retina. (In the absence of cataracts, ocular structures anterior to the retina transmit visible light.) Visible-light-induced eye injury can occur through either a photochemical or a thermal mechanism. The photochemical mechanism is commonly known as 'blue light' hazard. The relative hazard level as a function of wavelength is known as the blue light hazard function, $B(\lambda)$, and is shown in **Figure 15**. The peak in this function at 440 nm is due, in large part, to absorption of shorter wavelength radiation by the lens and cornea. In the case of aphakic individuals, $\sim$80% of UV-A radiation reaches the retina. For these individuals and children under 2, whose lenses have enhanced UV transmission, the aphakic hazard function shown in **Figure 16** is more appropriate.

In order to assess blue light hazard, ICNIRP and ACGIH recommend calculating an average source radiance, L_B

$$L_B = \sum L_\lambda \cdot B(\lambda) \cdot \Delta\lambda$$

where L_λ is the spectral radiance in the wavelength interval $\Delta\lambda$, and $B(\lambda)$ is the blue light hazard function

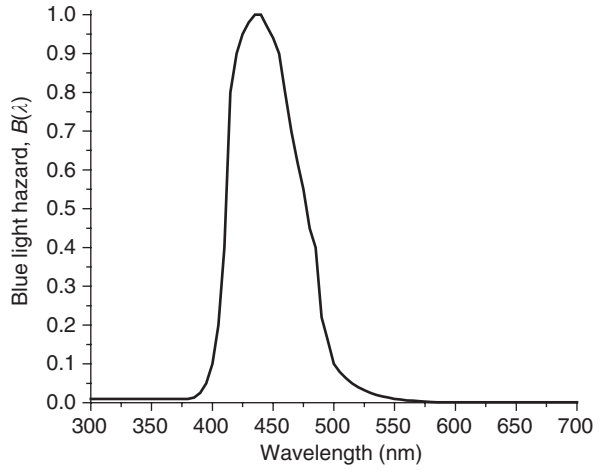

Figure 15 Blue light hazard function.

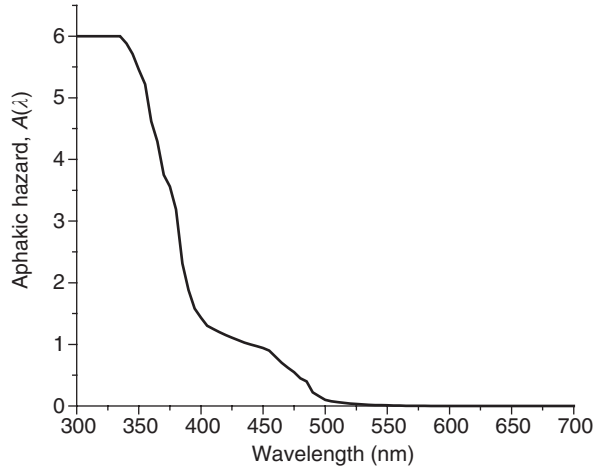

Figure 16 Aphakic hazard function.

shown in **Figure 15**. (The aphakic hazard function should be used for aphakic individuals and small children.)

For blue light and other photochemical hazards, the threshold dose is a product of dose rate and time. That is, injury can result from a short exposure to a very bright light or a longer exposure to a less intense light.

For exposures less than 10 000 s, the recommended exposure limit is

$$L_B \cdot t \leqslant 1 \text{ MJ m}^{-2} \text{ sr}^{-1}$$

(megajoule per square meter per steradian)

and for longer exposures

$$L_B \leqslant 100 \text{ W m}^{-2} \text{ sr}^{-1}$$

In addition to direct tissue damage, visible light can create indirect hazard through effects such as glare, flash blindness, and afterimage. The extent of this hazard depends not only on the qualities of the light source, but also on ambient light conditions and the nature of the activities in which individuals may be engaged. Perhaps the most stringent regulations concerning indirect light hazard are those developed to protect civilian pilots conducting terminal operations from hazards such as laser pointers. Here four flight safety exposure limits have been established: laser free zone ($0.0005\,\text{W m}^{-2}$), critical flight zone ($0.05\,\text{W m}^{-2}$), sensitive flight zone ($1\,\text{W m}^{-2}$), and normal flight zone ($25\,\text{W m}^{-2}$). (By contrast, normal direct terrestrial visible solar irradiance is in the range of several hundred watts per meter square.) Indirect light hazards from artificial light sources have been observed in other environments as well, with increasingly powerful light emitting diodes becoming a particular concern.

Mechanism of Injury (IR Light)

The most common effects to skin and ocular tissue associated with IR light are: (1) thermal injury to the retina; (2) thermal injury to the lens; (3) thermal burns to skin; and (4) thermal burns to cornea (1400 nm–1 mm).

Unlike photochemical injury, thermal injury does not exhibit reciprocity between intensity and length of exposure. Injury only occurs if the light intensity is sufficient to raise tissue temperature above $\sim 45^{\circ}\text{C}$. In the case of less intense light and longer exposure, normal heat transfer mechanisms within the body serve to cool the exposed tissue.

In the case of exposure to sources such as welding torches and arc welding equipment producing both IR and visible light, a small temperature rise caused by IR can work synergistically to increase blue light damage.

For sources that include visible light (wavelengths between 380 and 1400 nm) and viewing times between 10 μs and 10 s, ICNIRP and ACGIH recommend a maximum weighted source radiance of

$$\sum_{380}^{1400} L_\lambda \cdot R(\lambda) \cdot \Delta\lambda < 50/(\alpha \cdot t^{0.25}) \text{ kW m}^{-2} \text{ sr}^{-1}$$

where α is the angle subtended by the light source at the viewing distance, t is the viewing time, and $R(\lambda)$ is the retinal thermal hazard function shown in **Figure 17**. For times greater than 10 s, the 10 s value is used. For sources such as heat lamps that produce little visual light and therefore do not trigger pupillary contraction, ICNIRP recommends limiting the weighted radiance for exposures greater than 10 s to

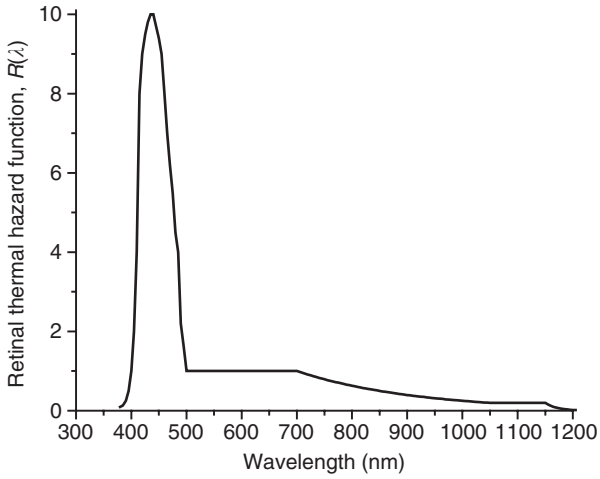

Figure 17 Retinal thermal hazard function.

$$\sum_{780}^{1400} L_\lambda \cdot R(\lambda) \cdot \Delta\lambda \leqslant 6000/\alpha \text{ W m}^{-2} \text{ sr}^{-1}$$

For protection of the cornea and lens, ICNIRP recommends limiting exposure to IR radiation (between 780 nm and 3 μm) to irradiances of less than $100\,\text{W m}^{-2}$ for long exposures (greater than 1000 s). For shorter exposures, ICNIRP recommends limiting irradiances given by

$$E_{\text{IR}} \leqslant 18t^{-3/4}(\text{kW m}^{-2})$$

where t is the exposure time in seconds. ICNIRP does allow relaxation of this limit in cold environments.

For short duration (less than 10 s) thermal injury to the skin, ICNIRP recommends limiting visible and IR exposure to below

$$20\,000t^{0.25} \text{ J m}^{-2}$$

Noise-Induced Hearing Loss

At-Risk Populations

Noise-induced hearing loss is a problem of epidemic proportion in modern society, and is currently the second most common form of hearing impairment in the United States (after age-related hearing loss). Although it is difficult to accurately assess the extent of the problem, the US Centers for Disease Control and Prevention has estimated that 12.5% of US children aged 6–19 years have some form of noise-induced hearing loss in one or both ears, and a 1990 Consensus Statement issued by the US National Institutes of Health estimated that over one-third of the 28 million people in the United States suffering from hearing impairment could attribute this problem at least in part to noise.

Sound Measurement

From the standpoint of sound toxicity, the most important properties of sound are power (or loudness) and frequency (or pitch). Sound power is usually expressed in terms of a logarithmic scale known as the decibel scale and is given by

$$L = 10 \log \frac{W}{W_0} \text{ dB}$$

where L is the sound power level in decibels (dB), W is the intensity of the sound in W m^{-2} and W_0 is a reference level equal to $10^{-12}\,\text{W m}^{-2}$ (approximately the softest audible sound). This somewhat unusual scale was chosen to accommodate human loudness perception. Increasing sound intensity by 10 dB is perceived as an approximate doubling in loudness even though the sound power is actually increased by a factor of 10.

Sound frequency is usually expressed in units of cycles per second or hertz (Hz). Humans have a useful hearing range of ~20–20 000 Hz, but are most sensitive to frequencies between about 1000 and 6000 Hz. (For reference, the lowest and highest notes on the piano are 27.5 and 4186 Hz, respectively.) This increased sensitivity is due in part to the shape of the external portion of the ear and the ear canal, which serve to amplify frequencies in this range.

Human loudness perception depends in a complex manner on both frequency and the overall loudness of sound. (For example, bass is more difficult to hear in music played at low volume than in the same music played at high volume.) To capture this behavior, two weighting scales have been developed for use in sound hazard analysis. The most common of these is the A weighting scale, which is commonly used to assess occupational and environmental noise. The A scale weights sounds in the 1000–6000 Hz range much more heavily than low-frequency sounds. The A-weighted intensities (dBA) of some common sounds are listed in **Table 5**. By contrast, the C weighting scale is used for very loud sounds and is a much flatter function of frequency.

Mechanism of Injury

The human hearing apparatus is commonly considered in three sections: the outer ear, middle, and inner ear. The outer ear consists of the pinna (generally called the ear) and the external auditory canal, which terminates in the tympanic membrane or eardrum. The outer ear collects sound, amplifying some frequencies and attenuating others. The eardrum

Table 5 Intensity and response for some common sounds

Sound	*Intensity (dBA)*	*Response*
	0	Threshold of hearing
Normal breathing	10	
Rustling leaves	20	
Soft whisper or ticking clock	30	
Quiet street at night	40	Quiet
Quite office	50	
Normal conversation	60	
Vacuum cleaner	70	Moderately loud
Loud speech or radio	80	
Heavy truck (50 ft)	90	
Pile driver (50 ft), ambulance siren (100 ft)	100	Very loud
Loud thunder	110	
Jet takeoff (200 ft), rock concert	120	Threshold of feeling and pain
Machine gun at close range	130	Painful
Aircraft carrier deck operations	140	

Table 6 Exposure times recommended by NIOSH and CDC

Noise level (dBA)	*Recommended permissible exposure time*
85	8 h
88	4 h
91	2 h
94	1 hr
97	30 min
100	15 min
103	$7\frac{1}{2}$ min
106	$3\frac{3}{4}$ min

transfers vibration to three small bones in the middle ear known as the ossicles, which in turn transfer vibration to the inner ear. The inner ear contains a helical organ called the cochlea in which sound vibrations are converted into nerve impulses by a series of small cells known as hair cells. It is the hair cells that are damaged by sound. Noise-induced hearing loss is usually divided into three classes. Noise-induced temporary threshold shift is a reversible loss of sensitivity over a range of frequencies. Noise-induced permanent threshold shift has a similar manifestation but is permanent. Both types of threshold shift generally result from relatively long exposure to loud noise. By contrast, acoustic trauma is hearing impairment associated with short-term exposure at extremely high levels.

Hazard Level

Numerous international standards have been developed to regulate noise exposure. Permissible exposure times recommended by the US National Institute for Occupational Safety and Health and the US Centers for Disease Control and Prevention are shown in **Table 6**.

The halving of recommended permissible exposure time with each 3 dBA increase in noise level reflects the doubling of sound power with each 3 dB increment.

See also: American Conference of Governmental Industrial Hygienists; American Industrial Hygiene Association; Consumer Product Safety Commission; National Institute for Occupational Safety and Health; Occupational Exposure Limits; Occupational Safety and Health Administration.

Further Reading

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Relevant Websites

http://www.icphso.org – International Consumer Product Health and Safety Organization (ICPHSO).
http://www.cpsc.gov – US Consumer Product Safety Commission (CPSC).
http://www.census.gov – US Department of Commerce, Bureau of the Census, Statistical Abstract of the United States 1998.
http://www.nsc.org – US National Safety Council (NSC).

Picloram

Richard A Parent

This article is a revision of the previous print edition article by Richard A Parent, T R Kline, and R E Sharp, volume 2, pp. 530–531, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 1918-02-1
- Synonyms: 4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid; 3,5,6-trichloro-4-aminopicolinic acid; 4-amino-3,5,6-trichloro-2-picolinic acid
- Chemical/Pharmaceutical/Other Class: Chlorinated amino-pyridine herbicide
- Chemical Formula: $C_6H_3C_{13}N_2O_2$

Uses

Picloram and its salts are systemic herbicides produced by chlorination of 2-methylpyridine followed by hydrolysis and reaction with ammonia. Most broadleaf crops, except crucifers, are sensitive; most grasses are resistant. Picloram is effective in controlling annual weeds, is used alone or in combination with 2,4-D against deep-rooted perennials on noncropland, and is used typically as pellets or in combination with 2,4-D or 2,4,5-T for brush control.

Exposure Routes and Pathways

Picloram is either a colorless powder or crystalline solid having very low vapor pressure, making inhalation exposure unlikely unless the dust is inhaled. Exposure to picloram occurs mainly through its manufacture and its use as a herbicide in forests. Environmental exposures in humans occur when forest visitors or others not directly involved in spray operations come in contact with spray or sprayed foliage, inhale spray mist, eat plants or animals contaminated with the herbicide, or drink water containing the herbicide. A suggested no-adverse-effect level is $1.05\,mg\,l^{-1}$.

Toxicokinetics

Picloram is readily translocated from foliage or from roots to other plant parts, accumulating primarily in the areas of most rapid growth. Picloram is rapidly absorbed from the gastrointestinal tract and is excreted virtually unchanged in the urine and feces of male Fischer 344 rats within 48 h. The fate of picloram was defined in six healthy male volunteers following single po doses of 5.0 and $0.5\,mg\,kg^{-1}$ and a dermal dose of $2.0\,mg\,kg^{-1}$. Picloram was administered orally as the sodium salt in grape juice. The dermal dose was applied to the backs of volunteers as the free acid dissolved in ethanol. The resulting data indicated that the compound was rapidly absorbed from the gastrointestinal tract and rapidly excreted unchanged in the urine. Over 90% of the oral dose was recovered as unchanged picloram in the urine excreted through 72 h. Most of the dose ($\geqslant$75%) was excreted within 6 h. By comparison, picloram was slowly absorbed through the skin, and only a small fraction (0.2%) of the picloram applied to the skin was absorbed.

Picloram is not readily metabolized and is rapidly excreted unchanged in the urine and feces of treated rats. Following a $10\,mg\,kg^{-1}$ [^{14}C]picloram intravenous dose, the isotope was cleared biophysically and excreted in the urine. Balance studies in rats indicated that 98.4% of the dose was recovered. Urinary excretion resulted in an 80–84% recovery, fecal excretion resulted in $\sim$ 15% recovery; less than 0.5% was recovered in the bile, and virtually no radioactivity was recovered as trapped $^{14}CO_2$ or as other volatile compounds. Studies with [^{14}C]picloram showed that 90% of the compound fed in the diet to dogs was excreted within 48 h in the urine, with small amounts appearing in the feces.

Mechanism of Toxicity

Little is known about the mechanism of toxicity of picloram.

Acute and Short-Term Toxicity (or Exposure)

Animal

Picloram is a relatively nontoxic pesticide. Rat LD_{50} (oral) is given as $8200\,mg\,kg^{-1}$. Other reports of oral LD_{50} values include $500\,mg\,kg^{-1}$ in rats, 200–$4000\,mg\,kg^{-1}$ in mice, and $\sim 2000\,mg\,kg^{-1}$ in rabbits. The reported dermal LD_{50} in rabbits is $\sim 4000\,mg\,kg^{-1}$. The technical grade of picloram is moderately toxic by inhalation with a reported 4 h LC_{50} of greater than $0.35\,mg\,l^{-1}$.

Signs of intoxication from acute oral administration may include skin rashes, eye irritation, hair loss, tachycardia, diarrhea, ataxia, leukopenia, vaginal bleeding, prostration, and, in cases of very high exposure, seizures. Liver and kidney lesions have also been reported.

Human

Picloram causes a mild skin irritation, although it is not a skin sensitizer in humans. Picloram is not likely to be absorbed readily through the skin. Contact with exposed eyes causes moderate irritation which heals readily; corneal injury is unlikely. Inhalation of contaminated dusts may be somewhat irritating but is not likely to cause illness. Possible nausea may result from ingestion of massive amounts. Some believe that picloram may have the ability to damage the central nervous system.

Chronic Toxicity (or Exposure)

Animal

Picloram administered orally at three dose levels (20, 200, and $2000\,mg\,kg^{-1}$ body weight) induced no cytogenic aberrations in bone marrow cells. B6C3F1 mice and Osborne–Mendel rats were fed picloram for 80 weeks. After treatment, mice were observed for 10 weeks. Upon death or sacrifice, major organs were examined. In rats there was a high incidence of follicular hyperplasia, C cell hyperplasia, and C cell adenoma of the thyroid. There was an increased incidence of hepatic neoplastic nodules considered to be benign in female and male rats. Both male and female rats showed lesions of the liver diagnosed as foci of cellular alteration. It was concluded that picloram was not carcinogenic in mice or male rats but did possess the ability to induce benign tumors in the livers of female Osborne–Mendel rats.

Lifetime daily exposure of rats and dogs to diets containing $150\,mg\,kg^{-1}$ body weight doses of picloram resulted in no observable gross or microscopic signs of toxicity. A 6 month dog study at doses as high as $175\,mg\,kg^{-1}\,day^{-1}$ did result in weight loss, increased relative and absolute liver weights with a calculated NOEL of $7\,mg\,kg^{-1}\,day^{-1}$. A 90 day feeding study in B6C3F1 mice also produced increases in absolute and relative liver weights in female mice at doses starting at $1000\,mg\,kg^{-1}\,day^{-1}$.

Human

Because of a lack of information for humans and animals, picloram is not classifiable with regard to its carcinogenicity in humans according to the IARC and the ACGIH. Little or no data is available relating to the chronic toxicity of picloram.

In Vitro Toxicity Data

Picloram was not mutagenic in gene mutation assays in bacteria and yeast, with or without metabolic activation. Using the forward mutation spot test picloram was mutagenic in *Stertomyces coelicolor*, which is not a widely accepted screen for mutagens.

Clinical Management

Emesis is not recommended after oral ingestion of picloram because of potential for seizures. Gastric lavage may be considered but a patent airway must be maintained. Activated charcoal may be administered but treatment should be supportive of symptomatology. For seizures, benzodiazepine should be considered with subsequent phenobarbital if seizures persist. Hypotension, dysrhythmias, respiratory depression, and need for endotracheal intubation should be monitored. Evaluation must be done for hypoglycemia, electrolyte disturbances, and hypoxia. If inhaled, patient should be removed to clean air and respiratory distress should be monitored. If cough or difficulty breathing occurs, evaluation for respiratory tract irritation, bronchitis, or pneumonitis should be done. Bronchospasm should be treated with inhaled β-2 agonist and oral or parenteral corticosteroids. For dermal contact, contaminated clothing should be removed and the skin washed with soapy water.

Environmental Fate

Picloram is a herbicide and is introduced directly into the earth. It does not absorb on soil, and does not hydrolyze or evaporate from soils or groundwater. It is subject to leaching and may biodegrade in soils and groundwater. In groundwater, it is not expected to adsorb on sediment, to bioconcentrate, to evaporate or hydrolyze significantly. Near surface photolysis is possible and as a result its half-life ranges from 2.3 to 41.3 days. Since it is an amine, its degradation could be accelerated through contact with oxidizing agents.

Release to the atmosphere would result in significant deposition and washout due its low vapor pressure. In soil, the half-life of picloram could exceed 5 years depending on the conditions.

Other Hazards

No teratogenic or embryotoxic effects have been found in rats fed up to 1000 mg kg^{-1} on gestational days 6–15. Similar findings were noted in rabbits. A multigeneration study in which rats were exposed to picloram from gestation through reproductive cycles to levels as high as 3000 ppm diet produced no evidence of effects on fertility, gestation, viability of pups, lactation, or skeletal development. Pregnant rats receiving doses of 1000 mg kg^{-1} day^{-1} during organogenesis were normal, but there was a slight increase in embryo resorption. A dose of 2000 mg kg^{-1} day^{-1} was toxic to the mothers but did not induce malformations in the pups. A negative response to an effort to induce embryotoxic and teratogenic effects in New Zealand white rabbits at doses as high as 400 mg kg^{-1} day^{-1} also failed to provide any indication of a dose–response relationship to the sporadic findings.

Multigeneration studies in rats dosed orally at 15 mg kg^{-1} day^{-1} did not have any effect on fertility, whereas rats showed no effects when dosed up to 180 mg kg^{-1} day^{-1}. Picloram does not appear to cause reproductive toxicity.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) 8 h time-weighted average (TWA) is 10 mg m^{-3}.

ACGIH exclusion limit for 30 min day^{-1}: 30 mg m^{-3}.

Environmental Protection Agency's (EPA's) permissible exposure limit (PEL) is 5 mg m^{-3} for the respirable fraction (8 h TWA).

ACGIH cannot classify picloram relative to human carcinogenicity.

EPA's Federal Drinking Water Standard is 500 μg l^{-1} while Arizona has a standard of 49 μg l^{-1}.

EPA's maximum contaminant level goal (MCLG) has been set at 0.5 ppm.

Picloram is a slightly toxic compound in EPA toxicity class III and products containing it must bear the signal word CAUTION on the label.

Miscellaneous

Picloram has a molecular weight of 241.48 and is a white crystalline solid at room temperature. It has a chlorine-like odor but has a very low vapor pressure (6.16×10^{-7} mmHg) at 35°C. It has some solubility in water (450 mg l^{-1}) but is a lot less soluble in nonpolar solvents such as benzene or ether.

See also: Pesticides; Pollution, Water.

Relevant Websites

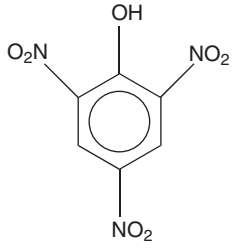
http://www.epa.gov – US Environmental Protection Agency.
http://ace.orst.edu – National Pesticide Information Center, Oregon State University, Corvallis, OR, USA.
http://infoventures.com – Information Ventures, Inc., Philadelphia, PA, USA.

Picric Acid

Samantha E Gad

This article is a revision of the previous print edition article by Jayne E Ash, volume 2, pp. 531–532, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 88-89-1
- Synonyms: 2,4,6-Trinitrophenol; Piconitric acid; Carbazotic acid; Nitroxanthic acid
- Chemical/Pharmaceutical/Other Class: Nitrogen-substituted phenols, a trinitro phenol
- Chemical Formula: $C_6H_3N_3O_7$
- Chemical Structure:



Uses

Picric acid is used in the production of explosives, matches, and electric batteries. It is also used in

etching copper and manufacturing colored glass, in the leather industry, and in the synthesis of dyes. Picric acid is very unstable and is a flammmable/combustible material. It may be ignited by heat, sparks, or flames. Dried-out picric acid may explode if expose to heat, flame, friction, or shock, and should be treated as an explosive. Picric acid can react vigorously with oxidizing materials, and it can form unstable salts with concrete, ammonia, bases, and metals.

Exposure Routes and Pathways

The most likely exposure to picric acid is in the workplace from use in explosives, matches, electric batteries, from etching copper, and making colored glass. These activities could lead to dermal contact or the inhalation of dust of picric acid or its salts.

Toxicokinetics

Picric acid is readily absorbed through the skin or through the respiratory tract. It is eliminated from humans as picric acid and as picramic acid.

Mechanism of Toxicity

Picric acid is an uncoupler of mitochondrial metabolism.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, picric acid is a dermal sensitizer and strong eye irritant. The oral LD_{50} in rats is $200\,mg\,kg^{-1}$, and the oral LD_{Lo} is $500\,mg\,kg^{-1}$ in cats, $120\,mg\,kg^{-1}$ in rabbits, and $100\,mg\,kg^{-1}$ in guinea pigs. Dogs receiving an acute lethal dose of picric acid die from respiratory paralysis, and necropsy found yellow staining of the subcutaneous fat, lung, intestines, and blood vessels. Liver swelling and glomerulitis were also observed. Sublethal doses in dogs, $\leqslant 50\,mg\,kg^{-1}$, caused transient changes in the kidneys, including glomerulitis and other changes in ultrastructure.

Human

In occupational exposures, for example, the manufacture of explosives, the main health issue has been the occurrence of skin disease. Systemic poisoning is rare. Picric acid is irritating to eyes and skin. Dermal exposure may cause local or generalized allergic reactions. It causes yellow staining of skin. Absorption into the skin or ingestion may cause nausea, vomiting, diarrhea, abdominal pain, olguria, anuria, staining of skin, pruritus, sudden acne, stupor, convulsions, and death. The CDC revised the IDLH (documentation for Immediately Dangerous to Life or Health concentrations) after ingestion of 1–2 g caused severe poisoning in man. During the 1920s and 1930s, picric acid was used alone and in combination with butyl aminobenzoate as an antiseptic surgical dressing for the treatment of burns; however, this was reported to be capable of leading to serious central nervous system problems. An outbreak of hematuria among US Navy personnel based in Japan was attributed to picric acid in drinking water that had been contaminated by confiscated Japanese ammunition; $2–20\,mg\,l^{-1}$ picric acid were found in the drinking water.

Chronic Toxicity (or Exposure)

Animal

Picric acid also causes liver and kidney damage and produces central nervous system effects. It has been a mutagen in some, but not all, studies.

Human

Liver, kidney, and blood are affected by prolonged or repeated exposure. Hair, skin, and conjunctiva of eye may become yellow, with matching yellow vision (symptoms not from jaundice). Delayed cataract formation may occur, as well as intravascular hemolysis.

In Vitro Toxicity Data

Picric acid has shown mutagenic properties *in vitro* in some, but not all, studies.

Clinical Management

The victim should be removed from exposure. Gastric lavage with water should be performed. Activated charcoal is also recommended.

Ecotoxicology

Juvenile rainbow trout (*Salmo gairdneri*) and American oyster (*Crassostrea virginica*) were exposed to sublethal doses of picric acid for 42 days. No significant inhibition of growth was observed for rainbow trout exposed to 0.45 and $0.05\,mg\,l^{-1}$ picric acid. American oysters exposed to 0.45 and $0.05\,mg\,l^{-1}$ picric acid showed significant inhibition of shell deposition during exposure period. Discoloration of the nacre layer of the shell and body mass was observed in oysters by the end of 42 days.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, as an 8 h time-weighted average (TWA), is 0.1 mg m^{-3}. The same value (0.1 mg m^{-3}) has also been recommended by the (US) Occupational Safety and Health Administration permissible exposure limit (PEL), 8 h TWA, with an added skin designation, and the (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level (REL), averaged over a 10 h work day. The NIOSH short-term exposure limit (STEL), for a 15 min exposure, is 3 mg m^{-3}.

See also: Acids.

Further Reading

Gosselin RE, Smith RP, and Hodge HC (1984) *Clinical Toxicology of Commercial Products*, 5th edn. Baltimore: Williams and Wilkins.

Nipper M, Carr RS, Biedenbach JM, *et al.* (2001) Development of marine toxicity data for ordinance compounds. *Archives of Environmental Contamination and Toxicology* 41: 308–318.

Nipper M, Carr RS, Biedenbach JM, Hooten RL, and Miller K (2002) Toxicological and chemical assessment of ordinance compounds in marine sediments and porewaters. *Marine Pollution Bulletin* 44: 789–806.

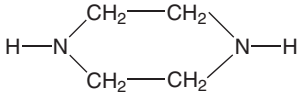
Relevant Website

http://www.cdc.gov – 2,4,6-Trinitrophenol (International Chemical Safety Card).

Piperazine

David Brandwene

- Chemical Abstracts Service Registry Number: CAS 110-85-0
- Synonyms: 1,4-Diazacyclohexane; 1,4-Piperazine; Diethylenediamine; Hexahydropyrazine; Piperazidine; Pyrazine hexahydride
- Chemical/Pharmaceutical/Other Class: Ethyleneamines
- Chemical Formula: $C_4H_{10}N_2$
- Chemical Structure:



Uses

Piperazine is an intermediate in the manufacture of insecticides, rubber chemicals, corrosion inhibitors, and urethane foam production catalysts. It is also used in gas-washer liquids to absorb carbon dioxide and as a hardener in prepolymers for adhesives. Piperazine is an intermediate in the production of human and veterinary pharmaceuticals and is also an active ingredient in veterinary antihelminthics. It is prescribed as a human antihelminthic in the United States.

Exposure Routes and Pathways

Occupational exposure may occur in industries where piperazine is manufactured or used as an intermediate. Routes of occupational exposure are primarily dermal and inhalation.

Consumer exposure from the diet may occur from residues present in eggs of treated hens.

Toxicokinetics

Animal studies show that piperazine is readily absorbed from the gastrointestinal tract, excreted primarily in the urine with the peak plasma concentration reported 1 h after dosing. Most of the parent compound is excreted unchanged during the first 48 h. *N*-Mononitrosopiperazine has been identified as the primary urinary metabolite. Limited human data indicate a similar toxicokinetic profile to animals. There are no data available on the toxicokinetics of piperazine following dermal or inhalation exposure.

Mechanism of Toxicity

The mechanism of the neurotoxicity induced by piperazine in mammals is unknown. Piperazine acts as a GABA agonist in invertebrates. The mechanism of liver and kidney toxicity seen in subchronic oral studies in laboratory animals has not been determined.

Acute and Short-Term Toxicity (or Exposure)

Piperazine is corrosive to skin and eyes and causes skin and respiratory sensitization. Piperazine has not been shown to be teratogenic or mutagenic.

Animal

Oral LD_{50} values in rodents range from 2.4 to 4.9 g kg^{-1}. The dermal LD_{50} in rabbits was reported

to be $4\,g\,kg^{-1}$. In skin irritation studies in rabbits, a 50% concentration of piperazine was corrosive following a 4 h exposure period. In an eye irritation study in rabbits, a 1–5% concentration of piperazine was corrosive.

Piperazine was evaluated in the local lymph node assay (LLNA) and the guinea pig maximization test. In both studies, piperazine was a mild sensitizer. In the LLNA, piperazine did not induce markers indicative of respiratory sensitization. In laboratory studies, piperazine has exhibited cross-sensitization reactions with diethylenetriamine.

Piperazine phosphate at dose levels of 250, 1000, or $5000\,mg\,kg^{-1}$ was orally administered to pregnant rats during days 6–15 of gestation. There were no teratogenic effects in any dose group. In a developmental toxicity study in rabbits, piperazine phosphate was orally administered during days 6–18 of pregnancy at dose levels of 100, 225, or $500\,mg\,kg^{-1}$. An increased incidence in embryotoxicity and malformations were seen at the highest dose. These effects were considered to be secondary to maternal toxicity.

In a micronucleus study, administration of piperazine phosphate orally to mice at doses up to $5000\,mg\,kg^{-1}$ did not result in an increase in the level of micronuclei in bone marrow erythrocytes.

Human

Transient side effects from therapeutic use of piperazine via the oral route include headaches, nausea, vomiting, lethargy, tremor, and vague ocular disturbances. EEG changes were observed in a study of children treated with piperazine hexahydrate. Although the mechanism of toxicity for EEG changes and several other case reports of neurotoxicity is unknown, it may be related to GABA agonism. This conclusion is based on laboratory data in invertebrates.

Piperazine causes primary dermal irritation and skin burns at high concentrations. Piperazine also causes eye irritation in humans.

Many case studies have shown that exposure to piperazine results in allergic contact dermatitis and occupational asthma. A recent study of 93 patients exposed dermally to a 1% piperazine solution showed 3.2% positive reactions. At a piperazine production facility, ~10% of the current and former factory workers were diagnosed with occupational asthma.

Chronic Toxicity (or Exposure)

In repeated dose studies, systemic toxicity was seen at dose levels of piperazine above $50\,mg\,kg^{-1}$. The results of a reproductive toxicity study suggest that piperazine can impair fertility.

Animal

In a 13 week oral study, piperazine dihydrochloride was administered to dogs (four per sex per dose) at concentrations up to 3692 ppm in the diet. Clinical chemistry changes indicative of mild liver effects were the only sign of systemic toxicity. The no-observed-adverse-effect level in the study was 1477 ppm, which is $\sim 25\,mg\,kg^{-1}$ of piperazine base. In another 13 week oral study, piperazine was administered to rats (10 per sex per dose) at concentrations of ~50, 150, or $500\,mg\,kg^{-1}$ in the diet. Histopathological changes were seen in the liver and kidneys in the two higher dose groups. The no-effect level in the study was $50\,mg\,kg^{-1}$.

In a two generation reproductive toxicity study, piperazine dihydrochloride was administered in the diet of rats at doses of 250, 600, or 1250 mg $kg^{-1}\,day^{-1}$. A dose–response effect for decreased litter size was seen in the 600 and 1250 mg kg^{-1} groups suggesting that piperazine exposure at these dose levels can impair fertility.

Human

There are no reports of long-term repeated exposure to piperazine. Therapeutic uses of piperazine via the oral route for ~1 week have resulted in symptoms of neurotoxicity in children and adults.

In Vitro Toxicity Data

Piperazine was not mutagenic in the Ames assay and did not produce chromosome aberrations in Chinese hamster ovary cells.

Clinical Management

If exposure occurs, medical attention should be sought. In general, the following are recommended. For ingestion, water should be provided but emesis must not be induced. For skin and eye exposure, the affected area should be flushed with water for at least 15 min but no attempts must be made to neutralize with chemical agents. For inhalation, the exposed individual should be removed to fresh air and artificial respiration given if necessary.

Environmental Fate

Piperazine is not readily biodegradable, does not rapidly hydrolyze, and has a low potential for bioaccumulation. The octanol–water partition coefficient is -1.24. Piperazine released to the environment would be expected to distribute primarily to soil and water.

Ecotoxicology

The acute 96 h LC_{50} in guppies and 72 h EC_{50} in algae are greater than 1000 ppm (relatively nontoxic). Piperazine is more hazardous to invertebrates than fish or algae. In *Daphnia magna*, the 48 h EC_{50} is 26 mg l^{-1} and adverse effects were seen in a 21 day study at concentrations of 25 mg l^{-1} and above with a no-adverse-effect level of 12.5 mg l^{-1}.

Exposure Standards and Guidelines

No US exposure standards or guidelines for piperazine were identified. The 8 h occupational exposure limit in Europe is 0.1 mg m^{-3}.

See also: Kidney; Liver; Neurotoxicity.

Further Reading

American Conference of Governmental Industrial Hygienists Inc. (1991) Piperazine dihydrochloride. In: *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th edn., vol. 3, pp. 1276–1277.

EU Final Draft Risk Assessment on Piperazine, November 2003.

Trochimowicz HJ, *et al.* (1994) Heterocyclic and miscellaneous nitrogen compounds. In: Clayton GD and Clayton FE (eds.) *Patty's Industrial Hygiene and Toxicology*, 4th edn., vol. 2, Part E, pp. 3315–3319. New York: Wiley.

Relevant Websites

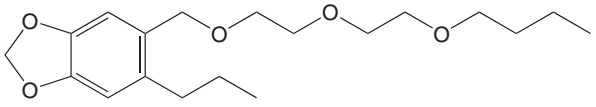
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Piperazine.

Piperonyl Butoxide

Marilyn Weber

This article is a revision of the previous print edition article by Sushmita M Chanda, volume 2, pp. 532–533, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 51-03-6
- Synonyms: Butacide; Butocide; Butoxide; Nusyn-noxfish; Prentox; Pybuthrin; Pyrenone; 5-[[2-(2-Butoxyetoxy) ethoxy]methyl]-6-propyl-1,3-benzodioxole
- Chemical/Pharmaceutical/Other Class: Methylenedioxyphenyl insecticide synergist
- Chemical Structure:



Uses

Piperonyl butoxide is a synergist for carbamates, pyrethrins, pyrethroids, and rotenone.

Exposure Routes and Pathways

Dermal exposure is the most common exposure pathway. Piperonyl butoxide is available as an aerosol, dust, emulsion, and solution.

Toxicokinetics

Piperonyl butoxyl inhibits detoxification of pesticides by insects. The synergists inhibit cytochrome P-450 dependent monooxygenases (cyp450 s), detoxifying enzymes found in both mammals and insects. These cyp450 s degrade selected foreign substances such as pyrethrum, allethrin, or resmethrin. Synergists simply bind the oxidative enzymes and prevent them from degrading the toxicant.

Mechanism of Toxicity

Piperonyl butoxide exerts toxicity by inhibiting mixed function oxidases. These enzymes are responsible for detoxifying pyrethrins and pyrethroids; their toxicity is therefore increased by piperonyl butoxide.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity in laboratory animals following exposure to piperonyl butoxide is low (the oral LD_{50} in rats is >6 g kg^{-1}). Repeated doses may also cause delayed onset of these same signs. A no-observed-adverse-effect level in dogs was 3 mg kg^{-1} day^{-1}.

Human

Piperonyl butoxide has a low incidence of acute toxicity. A single oral dose of piperonyl butoxide (50 mg, $\sim$0.71 mg kg^{-1} body weight) in adult volunteers did not elicit any signs of toxicity. A likely oral lethal dose

in humans was estimated at 5–15 g kg^{-1}, or between a pint and a quart for a 150 pound person. Laboratory findings in animal studies indicate that piperonyl butoxide may cause various anemias, hepatic changes, liver injury, and increased metabolic enzymes. No eye injuries have been reported with piperonyl butoxide/pyrethrin combination.

Chronic Toxicity (or Exposure)

Animal

Piperonyl butoxide was reported to increase liver tumor incidence at high exposure levels in mice but not rats. The primary target organ for chronic piperonyl butoxide exposures is the liver.

Human

Very little is known about the chronic effects of piperonyl butoxide. The US Environmental Protection Agency has categorized piperonyl butoxide as a group C carcinogen based on limited evidence of cancer in laboratory animals.

Clinical Management

Dermal decontamination should be accomplished by repeated washing with soap. Exposed eyes should be irrigated with copious amounts of water for at least 15 min. Piperonyl butoxide may be mixed with hydrocarbons; thus, emesis should be avoided. Activated charcoal can be administered following oral exposure. Treatment is symptomatic. No antidote is available.

Environmental Fate

Piperonyl butoxide is short-lived in the environment. It has a low to moderate potential for leaching into groundwater.

Ecotoxicology

Piperonyl butoxide has low to very low toxicity in birds. Researchers consider piperonyl butoxide moderately toxic to fish, although it is unlikely to accumulate. Some aquatic invertebrates may be highly sensitive to this compound.

Exposure Standards and Guidelines

The acute dermal and oral reference doses (RfDs) for piperonyl butoxide are 10 and 2 mg kg^{-1} day^{-1}, respectively. The chronic oral RfD is 0.0175 mg kg^{-1} day^{-1}.

See also: Pesticides; Pyrethrins/Pyrethroids.

Further Reading

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Piperonyl Butoxide.

Plants, Poisonous

Teresa Dodd-Butera and Molly Broderick

Edible and nonedible plants contain numerous naturally occurring chemical substances that can be toxic if exposures are excessive. Many of these chemicals act as natural 'pesticides' that help protect the plant from insects and other predators. As far as the edible plants are concerned, eating a varied diet containing fresh fruits and vegetables with beneficial properties usually avoids significant risks of poisoning by naturally occurring plant toxins.

The toxins selected for discussion in this section are found in plants that are not consumed as part of normal diets. The plants discussed are those most commonly identified in acute, systemic poisonings. These poisonings can be quite severe, although variability in the concentration of toxic chemicals may occur between and within plant species due to factors such as age and part of plant, season, and growth conditions. Historically, the use of plants has ranged from medicinal to homicidal purposes. The list of poisonous plants is extensive and varied, and can not be fully covered in this text. Human

exposures to many plants do not result in appreciable toxicity; however, some of the more notable toxic exposures will be mentioned.

Ackee (*Blighia sapida*)

Description

Ackee is a fruit-bearing tree distributed throughout Africa, the Caribbean, and tropical areas.

Uses

When ripe, the fruit is edible and a normal dietary constituent in some cultures.

Exposure Routes and Pathways

Ingestion of unripened fruit may result in poisoning.

Mechanism of Toxicity

The mechanism of toxicity is unclear; however, it contains the toxin hypoglycins A (β-methylene cyclopropyl-L-α-aminopropionic acid) and B (a dipeptide of hypoglycin A).

Symptoms of Toxicity

Symptoms of nausea, vomiting, and hypoglycemia may be delayed following ingestion. Liver abnormalities have occurred.

Clinical Management

Fatalities and seizures have been reported but can be avoided with good supportive measures. Glucose administration for hypoglycemia and symptomatic care are effective therapies for this toxin.

Apple Seeds (*Prunus* species)

Description

A perennial flowering herb, it comes in white, pink, and purple. Other examples include apricot and peach pits and choke cherry seeds.

Uses

Cyanogenic (cyanide producing) plants of the *Prunus* species are flowering and fruit trees. Seeds and pits are commonly ingested inadvertently along with the edible fruit.

Exposure Routes and Pathways

Poisoning may occur from ingestion of crushed seeds and pits. In addition, leaves and bark may be toxic. The toxin of this species is amygdalin.

Mechanism of Toxicity

The toxin, amygdalin, releases hydrocyanic acid. Thus, toxicity is similar to cyanide, and is dose-related. Cytochrome oxidase inhibition interrupts electron transport and oxygenation at the cellular level.

Symptoms of Toxicity

Symptoms may be delayed following ingestion and include headache, dizziness, coma, seizures, and potential death.

Clinical Management

Treatment involves the use of activated charcoal in order to prevent amygdalin metabolism. In addition, there is a cyanide antidote kit available.

Castor Bean (*Ricinus communis*)

Description

A large, leafy green plant which produces capsules containing three hard, shiny, brown seeds.

Uses

The plant is used for commercial production of castor oil, a lubricant and purgative. The seeds are uses in making jewelry and rosaries.

Exposure Routes and Pathways

Human toxicity can occur if the seeds are ingested from the plants or ornaments made from the plants. If the hard outer coat remains intact, no toxicity will occur. However, if the outer coating is damaged, then ricin is released.

Mechanism of Toxicity

How does toxalbumin relate to ricin? Toxalbumins consist of two subunits (A and B) which are joined by a disulfide bond. The A subunit irreversibly binds the 60S ribosomal subunit, which blocks protein synthesis. The B subunit allows for binding and penetration across the gastrointestinal cell wall. Sufficient doses of ricin can cause cell death due to continued inhibition of protein synthesis.

Symptoms of Toxicity

Symptoms develop within 2–10 h after toxalbumin ingestions. Abdominal pain, severe vomiting, and bloody diarrhea may occur. Significant fluid losses may ensue, accompanied by tachycardia and hypotension. Systemic poisoning can be seen within 24 h, with multiple organ involvement. Hepatic and renal failure, lethargy, seizures, coma, and death may occur in severe intoxications. Allergic reactions may

occur for the seeds, or occupational exposure to the oil from this plant.

Clinical Management

Though ricin can be deadly, most exposures result in uncomfortable but limited gastroenteritis and minimal systemic toxicity. Gastrointestinal decontamination should be considered, depending on the time of ingestion. Symptomatic and supportive measures are the mainstay of treatment. There is no specific antidote for this toxin.

Jimson Weed (*Datura atura stramonium*)

Description

Jimson weed is a malodorous, fruit-bearing plant with dark green pointed leaves and tubular white flowers. It grows 3–5 ft in height. Jimson weed is native to Asia; however, it is also found throughout the United States and elsewhere. Other names for this plant and related species with the same active substance are locoseed, locoweed, devil's trumpet, Tolguacha, apple of Peru, Jamestown weed, devil's apple, thorn apple, stinkweed, hyoscyamine (leaves, roots, seeds); hyoscine (roots).

Uses

Historically, this has been recognized as a toxin and has inadvertently caused poisoning.

Exposure Routes and Pathways

Poisoning may occur through ingestion, drinking tea, or smoking the plant. Seeds have the highest concentration of toxin. Exposure may be unintentional or for experimentation purposes due to the hallucinogenic properties of the plant.

Mechanism of Toxicity

Anticholinergic poisoning occurs from belladonna alkaloids in various plants. Toxins may include atropine, hyoscyamine, and scopolamine.

Symptoms of Toxicity

Symptoms may begin within 1–4 h after ingestion. Jimson weed causes the anticholinergic toxidrome characterized by tachycardia, mydriasis, dry flushed skin, decreased bowel sounds, urinary retention, sedation, and hallucinations. Symptom resolution may vary from 1 day to 2 weeks.

Clinical Management

Treatment is supportive, though physostigmine is potentially indicated for stupor, coma, seizures, high fever, and severe agitation unresponsive to other treatment. Mild symptoms will usually resolve in a calm environment.

Monkshood (*Aconitum mapellus*)

Description

A perennial flowering herb, aconitum comes in white, pink, and purple. It is distributed throughout the Northern Hemisphere.

Uses

Aconite is used in Eastern medicine as an herb for properties of analgesia and antiinflammation. It has also been used experimentally in Western medicine to study cardiac arrhythmias.

Exposure Routes and Pathways

Poisoning may occur through ingestion and dermal exposure. The roots and flowers contain the highest concentration of alkaloid and are the most poisonous parts of the plant.

Mechanism of Toxicity

This species contains diterpene and norditerpine alkaloids, which mainly affect the cardiovascular system. The mechanism of toxicity is unclear; however, it is assumed to be due to blockade of the voltage-sensitive sodium channels.

Symptoms of Toxicity

Symptoms of toxicity are related to the cardiovascular, gastrointestinal, and neurological systems. These include bradycardia, ventricular tachycardia, nausea, and vomiting. Paresthesias of the extremities and generalized weakness have been reported. Rarely, seizures may occur in humans. This symptom is more commonly seen in animal models.

Clinical Management

Treatment is supportive, as death may occur from cardiovascular collapse. No specific antidote is available, but bradycardia and hypersalivation may be managed with atropine.

Oleander (*Nerium oleander*)

Description

Oleander is a flowering tree in the summer, which grows to a height of 25 ft, with green leaves and thick sap. Oleander is native to the Mediterranean, but found in the southern United States and elsewhere in the world. In addition to oleander, other toxic

cardiac glycosides with similar effects are lily of the valley (*Convallaria majalis*), and yellow oleander (*Thevetia peruviana*).

Uses

The oleander is cultivated as a flowering shrub in gardens. Also, *Digitalis purpurea* and *Digitalis lantana* have been used medicinally.

Exposure Routes and Pathways

Poisoning may occur through ingestion and dermal exposure. All parts of the plant contain varying amounts of cardiac glycosides. Concentrations of toxins peak during flowering season, and are found in seeds, stems, roots, and red flowers, in particular. Leaves contain oleandrin.

Mechanism of Toxicity

Toxins similar to digoxin inhibit sodium–potassium ATPase and include oleandrin, digitoxigenin, nerium folinerium, and rosagenin.

Symptoms of Toxicity

Toxicity from the cardiac glycosides includes gastrointestinal and cardiovascular symptoms. Nausea, vomiting, and irregular heartbeat may occur.

Clinical Management

Symptomatic and supportive treatment, in addition to digoxin-specific antibody fragments, can be effective for oleander toxicity.

Poison Hemlock (*Conium maculatum*)

Description

Poison hemlock is a weed that grows along roadsides. It has large fern-like leaves, and resembles some wild edible plants. Poison hemlock is found in wooded areas.

Uses

This plant was used in ancient times as a means of execution. For example, Socrates' death is attributed to Poison hemlock.

Exposure Routes and Pathways

Poisoning may occur through ingestion. Unintentional ingestion can occur from coniine when similar plants are mistaken for parsley, anise (seeds), or carrot plant. It is tuberous, similar to turnip roots.

Mechanism of Toxicity

Piperidine alkaloid toxins, such as coniine, are structurally similar to nicotine, and contained in all parts of the plant.

Symptoms of Toxicity

Initially, toxicity is manifested by nausea, vomiting, abdominal pain, tachycardia, sweating, shaking, dilated pupils, and seizures. This is followed by bradycardia, paralysis, and coma. Death can occur from respiratory failure.

Clinical Management

Gastrointestinal decontamination and aggressive treatment of symptoms is needed since no specific antidote is available and toxicity may be severe.

Common Potato or Irish Potato (*Solanum tuberosum*)

Description

Potato is an edible tuberous plant, and is toxic under certain conditions due to solanine. The toxin can be found throughout the plant, in varying concentrations. Potatoes are cultivated in many areas of the world.

Uses

The potato is edible and used in various diets.

Exposure Routes and Pathways

Ingestion of 'green' potatoes is commonly responsible for poisoning from solanine. Factors that increase the amount of toxin in the plant include exposure to light, shallow planting, excessive time in storage, and extreme temperatures.

Mechanism of Toxicity

Solanine is a glycoalkaloid that contains three sugar molecules. In animal models, solanine acts as a cardiac glycoside and inhibits cholinesterase activity.

Symptoms of Toxicity

The toxin includes gastrointestinal symptoms, with potential to impact the central nervous system. Symptoms may be prolonged, depending on the severity of the poisoning.

Clinical Management

Fluid and electrolyte replacement and supportive care is the general approach to symptoms of poisoning.

Rhododendron (Ericacricaceae family)

Description

Rhododendron is a flowering, green, shrub-like plant. Various species are found throughout Europe and the United States.

Uses

Rhododendron has been used to make tea, which can result in toxicity.

Exposure Routes and Pathways

Exposures occur after drinking tea made from the plant or sucking nectar from the flowers. Ingestion of honey contaminated by nectar from the plants may also cause toxicity.

Mechanism of Toxicity

Andromedotoxin (Grayanotoxin I) is a diterpene found in all parts of the plant. It opens sodium channels in the myocardium and increases permeability.

Symptoms of Toxicity

Cardiovascular effects, such as hypotension and both bradydysrhythmias and tachydysrhythmias may occur. In addition, gastrointestinal symptoms, perioral numbness, drowsiness and weakness are possible. Seizures and coma have also been reported.

Clinical Management

Minimal toxicity occurs in the majority of cases of rhododendron exposure. In severe exposures, decontamination and supportive care are required.

Tobacco (*Nicotiana tabacum*)

Description

Nicotiana tabacum is the principal source of nicotine. The stems and leaves of the plant are used for commercial purposes. Tobacco is now cultivated in many countries of the world.

Uses

This plant is used in the production of cigars, cigarettes, chewing tobacco, and nicotine replacement products.

Exposure Routes and Pathways

Unintentional poisoning may occur, if tobacco-containing products are ingested (e.g., by young children). Additionally, occupational exposures may cause toxicity to workers harvesting the plant. Dermal exposure can cause toxicity, even with intact skin. Wet leaves from the plant may enhance absorption.

Mechanism of Toxicity

Nicotiana tabacum contains 0.5–9% nicotine, which is the primary toxin. Nicotine binds to select acetylcholine receptors throughout the body; known as nicotine receptors. This produces initial stimulation, but inhibition later, at the receptor sites throughout the nervous system. Low doses enhance the release of catecholamines and sympathetic stimulation. Higher doses produce parasympathetic stimulation.

Symptoms of Toxicity

Initially, toxicity is manifested by nausea, vomiting, abdominal pain, seizures, tachycardia, and hypertension, followed by bradycardia, hypotension, and respiratory difficulties. Death may occur.

Clinical Management

Gastrointestinal decontamination and supportive care are the mainstay of treatment. Atropine may be used for excessive bronchial secretions. Removal of clothes that have been exposed and washing the victim are important for treatment of dermal exposure.

Water Hemlock (*Cicuta maculata*)

Description

The water hemlock is a toxic weed, with thick hollow, tuberous roots, commonly found along lakes and streams.

Uses

Water hemlock may be ingested when mistaken for the edible species, *Daucus carota* (Queen Anne's lace).

Exposure Routes and Pathways

The oral route of exposure is of primary importance with water hemlock.

Mechanism of Toxicity

The alkaloid, cicutoxin, is distributed throughout the plant, though concentrated in the roots. Cicutoxin is an unsaturated aliphatic alcohol which is postulated to exert toxicity by central cholinergic stimulation. Specifically, stimulation of both nicotinic and muscarinic receptors occur.

Symptoms of Toxicity

Initial symptoms of toxicity include nausea and vomiting. Seizures, status epilepticus, occur in severe

toxicity. Hypotension and bradycardia are followed by hypertension and tachycardia.

Clinical Management

No specific antidote is currently available. Thus, symptomatic and supportive care, especially aggressive treatment of seizures, can be life saving.

See also: Aconitum Species; Castor Bean; Hemlock, Poison; Jimsonweed; Oleander; Proteomics.

Further Reading

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Specific Poisonous Plants.

Plasticizers *See* Phthalate Ester Plasticizers.

Platinum

Shayne C Gad

- Representative Chemicals: *cis*-Dichlorodiammine platinum (cisplatin); *cis*-Platinum chloride; Potassium chloropaletinite
- Chemical Abstracts Service Registry Number: CAS 7440-06-4
- Chemical Formulas: Pt^{2+}; Pt^{4+}

Uses

Platinum and its alloys are used in jewelry, dentistry, the chemical industry, and the electrical industry. Most automobile catalytic converters contain platinum. Certain platinum compounds that have the *cis* configuration and can combine with DNA are useful therapeutic agents for many cancers that do not respond readily to conventional chemotherapy (especially testicular, ovarian, bladder, prostate, and thyroid cancers). Testicular cancer, which was once always fatal, now responds to platinum-containing drugs.

Background Information

Platinum's abundance in the earth's crust is ~0.01 ppm. Platinum has been known since ancient times and is very resistant to corrosion.

Exposure Routes and Pathways

Inhalation of industrial platinum compounds may be a problem. The general population is exposed to platinum by the dermal route, especially from jewelry. The oral route is not significant because the absorption is very poor.

Toxicokinetics

Following inhalation, lung clearance of platinum metal is very slow. Approximately 1 week following ingestion of platinum-containing water, platinum is found in the kidneys and liver. Following injection of the cancer chemotherapeutic agent, cisplatin, platinum is found mainly in the gonads. In autopsy specimens, platinum is found in adipose tissue. Serum creatinine levels correlate with *cis*-platinum doses. Most platinum is excreted in the feces.

Mechanism of Toxicity

While the metal itself is systemically of little concern, its salts are very toxic. The *cis*-platinum compounds

can react with disulfides and amino groups and form adducts with some bases in nucleic acids. Platinum compounds inhibit a few enzymes, including leucine aminopeptidase, and the hydrogenases of malate, alcohol, and lactate. Cisplatin can form crosslinks between strands of DNA.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for platinum chloride in rats is $>3\,g\,kg^{-1}$ and the intraperitoneal LD_{50} is $>200\,mg\,kg^{-1}$. The oral and intraperitoneal LD_{50} for cisplatin in rats is considerably lower (25 and $7\,mg\,kg^{-1}$, respectively). Degenerative changes in proximal tubules including vacuolization and tubular dilation are noted within 5 days of a nephrotoxic dose of cisplatin in rats. A single exposure to cisplatin may lead to ototoxicity. Platinum is a dermal and pulmonary sensitizer.

Human

Inhalation of platinum dusts produces a pneumonitis characterized by coughing, wheezing, shortness of breath, and an asthma-like action. In some cases, cyanosis develops. Skin contact with various salts of platinum (especially the chlorides) can cause allergic dermatitis, characterized by eczematous patches. Type I hypersensitivity can be induced easily. Often the toxicity is not due to platinum itself but to its complexing with tissues. Exposure to salts can lead to cyanosis and lymphocytosis.

Chronic Toxicity (or Exposure)

Animal

Administered subcutaneously, local tumors appear. Rat (oral) TD_{Lo} is $9100\,mg\,kg^{-1}$, 26 weeks. Rats repeatedly treated with cisplatin ($2\,mg\,kg^{-1}$ twice a week for 4 weeks) exhibited neurophysiological and morphological indicators of peripheral neuropathy.

Human

Platinum salts (such as cisplatinum) produce a variety of serious side effects. It tends to deposit platinum in the corticomedullary area of the kidney and thus causes gastrointestinal upset (e.g., severe nausea and vomiting), nephrotoxicity (injuring both proximal and distal tubes), and blood changes (e.g., hypomagnesemia, leukopenia, and thrombocytopenia). Ototoxicity (e.g., tinnitus and hearing loss), peripheral neuropathy, and allergic reactions are also reported.

In Vitro Toxicity Data

Cisplatinum is an active mutagen in the Ames test and can cause sister chromatid exchanges.

Clinical Management

Symptoms of platinum inhalation usually abate soon after terminating exposure. Dermal exposure should be treated by washing the affected area immediately after exposure. To prevent skin allergic responses to platinum, it is best to control platinum dusts in the environment. The toxicity of the chemotherapeutic agent, cisplatin, can be reduced by prehydration using copious amounts of fluids.

Environmental Fate

Platinum can enter the environment through automobile emissions from the platinum-containing catalytic converter. Relatively high levels of platinum can be found along congested roadways. A number of chemotherapeutic agents contain platinum and thus their disposal can lead to environmental contamination. In industrialized regions, relatively high concentrations can be found in waterway sediments. Organic matter binds to the metal. In soil, mobility depends on pH, redox potential, and chloride concentration. Platinum will likely only mobilize under highly acidic conditions or in soil water with a high chloride content. Some platinum(IV) complexes, in the presence of platinum(II), may undergo methylation by microorganisms.

Ecotoxicology

Growth of the green alga *Euglena gracilis* was inhibited by hexachloroplatinic acid (250, 500, and $750\,\mu g\,l^{-1}$). Cisplatin inhibited growth in water hyacinth at $2.5\,mg\,l^{-1}$. The 3 week LC_{50} for hexachloroplatinic acid ($H_2[PtCl_6]$) in *Daphnia magna* was $520\,\mu g\,l^{-1}$. Reproduction was impaired at 14 and $82\,\mu g\,l^{-1}$. LC_{50} values (24, 48, and 96 h) for tetrachloroplatinic acid ($H_2[PtCl_4]$) in the Coho salmon were 15.5, 5.2, and $2.5\,mg\,l^{-1}$, respectively. Swimming behavior was affected at $0.3\,mg\,l^{-1}$.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value – time-weighted average is $1\,mg\,m^{-3}$ for elemental platinum and $0.002\,mg\,m^{-3}$ for soluble salts as platinum.

See also: Kidney; Metals.

Further Reading

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Relevant Website

http://www.inchem.org – International Programme on Chemical Safety.

Plutonium

Richard Belanger

- Representative Chemicals

  Plutonium oxide, PuO (CAS 12035-83-5)
  Plutonium dioxide, PuO_2 (CAS 12059-95-9)
  Plutonium nitride, PuN (CAS 12033-54-4)
  Plutonium tetrafluoride, PuF_4 (CAS 13709-56-3)
  Plutonium hexafluoride, PuF6 (CAS 13693-06-6)

- Chemical Abstracts Service Registry Number: CAS 7440-07-5
- Chemical/Pharmaceutical/Other Class: Metals, radioactive

Uses

The primary use for plutonium (Pu) is in nuclear power reactors, nuclear weapons, and radioisotopic thermoelectric generators (RTGs). Pu is formed as a by-product in nuclear reactors when uranium nuclei absorb neutrons. Most of this Pu is burned (fissioned) in place, but a significant fraction remains in the spent nuclear fuel. The primary plutonium isotope formed in reactors is the fissile Pu-239, which has a half-life of 24 400 years. In some nuclear programs (in Europe and Japan), Pu is recovered and blended with uranium (U) for reuse as a nuclear fuel. Since Pu and U are in oxide form, this blend is called mixed oxide or MOX fuel. Plutonium used in nuclear weapons ('weapons-grade') is metallic in form and made up primarily (>92%) of fissile Pu-239. The alpha decay of Pu-238 (half-life = 86 years) provides a heat source in RTGs, which are long-lived batteries used in some spacecraft, cardiac pacemakers, and other applications.

Background Information

The toxicity of plutonium is primarily due to its radioactive nature, and its effects have been extensively studied both in animals and in workers with occupational intakes. Shortly after its discovery in 1941, plutonium became a health concern by virtue of its property as an alpha emitter with a long half-life. The earliest attempts to predict toxicity focused on comparison to radium. Based on half-life and total alpha energy emitted, plutonium toxicity could be estimated at ~0.16 that of Ra-226. However, animal experiments quickly revealed that plutonium is much more toxic than radium.

Toxicity studies of plutonium compounds were initiated during World War II as nuclear weapons were developed. The pace was slow at first since only trace amounts of the element were made available for biological studies. In 1944, scientists at Berkeley, Rochester, and Chicago observed that plutonium deposits more unevenly in bone than radium, concentrating on areas of active bone growth. This led to the earliest safety guidelines, which were specified as maximum allowable body burden. A guideline of 1.0 μg (0.06 μCi) was generally used for the Manhattan Engineering District, while a more restrictive limit of 0.5 μg (0.03 μCi) was applied at Hanford Operations. In 1959, the International Commission on Radiological Protection (ICRP) specified a standard of 0.04 μCi, a value that stood until the system of dose limitation was revised in the late 1970s. (Current standards are specified in terms of absorbed radiation dose or radionuclide intake, as opposed to a quantity in the body.)

Exposure Routes and Pathways

The ionizing properties of Pu and other radioactive materials is one determinant of the level of hazard associated by different exposure routes. Radioactive elements are those that undergo spontaneous transformation (decay) in which energy is released either in the form of particles, such as alpha or beta particles, or waves, such as gamma or X-ray. Plutonium exists in several isomeric forms, the most important of which are Pu-238 and Pu-239. When these isotopes decay, they emit primarily alpha particles, which are densely ionizing and, therefore, damaging; however, the penetration of alpha particles into tissue is slight, so

biological damage is limited to cells in the immediate vicinity of the alpha-emitting radioactive material.

Alpha particles from plutonium cannot penetrate the epidermis, so toxicity is limited to conditions where the substance is present within the body. The primary routes of entry are inhalation, ingestion, or through wounds, cuts, or abrasions. The potential for adverse health effects caused by plutonium isotopes depends on the route of entry and subsequent deposition, redistribution, and retention, which in turn is highly influenced by the physical (e.g., particle size) and chemical forms of the isotope.

An analysis of 203 workers with internal deposits of plutonium showed that 131 were contaminated by inhalation, 48 through wounds, and eight by both routes. Most exposures to the general population involve minute quantities inhaled with ambient air or ingested in food and water. In the 1970s, a mean dietary intake of 1.6 pCi year^{-1} was estimated for New York City.

Toxicokinetics

There are several isotopes of plutonium (Pu-238 and Pu-239 being the most important), and it is the chemistry of the isotopes that determines the reactions within the environment as well their transport and reactions within the body. Ingested plutonium is primarily excreted in feces, as there is very poor absorption from the gastrointestinal tract. For inhalation, the regional deposition pattern depends primarily on particle size distribution. Within the first few days, a fraction of the deposited activity is rapidly cleared from the respiratory tract. The remaining fraction is cleared slowly, with retention half-time of months to years, depending on the chemical form (oxides, for example, tend to be cleared more slowly than nitrates). Materials absorbed from the respiratory tract are primarily deposited in bone and liver, where it is retained for many years. A very small fraction may also be deposited in testes or ovaries.

As a rule, isotopes of the same chemical form will have identical chemistry; however, Pu-238, which because of its shorter half-life is more intensely radioactive, has been shown to be more mobile in the body than the longer-lived Pu-239. Specifically, inhaled Pu-238 appears to be more rapidly cleared from the lung and transported to bone. An increased rate of radiolysis around deposited Pu-238 may account for this difference.

Mechanism of Toxicity

Plutonium is both toxic and carcinogenic. The primary mechanism responsible for both is the absorbed radiation dose delivered to cells at the sites of deposition.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute pulmonary toxicity of inhaled plutonium has been observed in experimental rodents and dogs that have inhaled large quantities of Pu-239. Changes leading to death within a year include edema, pneumonitis, and fibrosis. Bone fractures have also been observed in animals injected with large doses of Pu-239. The equivalent dose in man for these effects is more than 2.6 MBq (70 μCi). In addition, gross renal damage has been observed in animals following lethal doses of plutonium.

Human

In humans, acute pulmonary effects would only occur in extreme accident situations. It has been estimated that ~3.7 MBq (100 μCi) deposited in the lungs would be lethal to half the exposed population within 1 year.

Chronic Toxicity (or Exposure)

Animal

Chronic effects of plutonium exposure include life-shortening and cancer. These effects have been observed in numerous animal studies. The main late pulmonary effects of plutonium inhalation are pulmonary fibrosis and lung cancer. Lung cancers in animals have been reported for intakes equivalent to ~37 kBq (1 μCi) in man.

Studies in animals indicate that bone cancer is the most common form of malignancy induced by Pu-239 that has entered systemic circulation. The length of the latency period appears to depend on the amount of plutonium deposited in bone. Liver cancers have been observed in animals given Pu-239 injections, but they occur much less frequently than bone cancers.

Human

There is no evidence of life shortening or malignant disease in US workers with accidental intakes. Exposed workers in the former Soviet Union show biological effects, primarily pulmonary fibrosis and an increase in lung, bone, and liver cancers. Workers at the Mayak facility in the Russian Federation, who experienced far greater plutonium intakes than workers in other countries, have been reported to

have an excess lung cancer risk relative risk at age 60 of 0.6 per Sv of lung dose equivalent, assuming a radiation weighting factor of 20 for alpha particles. Excess bone and liver cancer mortality has also been reported in the Mayak workers with body burdens estimated to exceed 7.4 kBq (0.2 μCi), as well as among workers with unknown burdens. Although leukemia has been observed in humans exposed to relatively high levels of external radiation, it does not appear to be a significant effect of plutonium deposition in bone.

In Vitro Toxicity Data

Alpha radiation from plutonium produces cytotoxic and genotoxic effects in cultured cells. These can include cell death, chromosomal aberrations (dicentrics, translocations, and complex exchanges), and pretransformation molecular alterations such as upregulation of oncogene products coupled with inactivation of tumor suppressor genes.

Clinical Management

Clinical management can potentially reduce the effects of plutonium intake, although the effectiveness can be highly variable. Administration of the calcium salt of diethylenetriaminepentaacetic acid (DTPA) can accelerate removal of soluble forms of plutonium from body fluids and recent deposits. It is unable to remove intracellular deposits or activity buried in bone and must therefore be administered as soon as possible after an intake. In a review of 18 patients exposed to plutonium, americium, or curium, the US Food and Drug Administration concluded that administration of 1 g Ca–DTPA in 5 ml sterile aqueous solution, either by intravenous injection or as a nebulized inhalation dose, increased the rate of radioactivity elimination in urine by an average of 39-fold. Daily maintenance doses of Zn–DTPA resulted in continued elimination of radioactivity.

Bronchopulmonary lavage may also be effective for removal of inhaled plutonium, and has been recommended for occupational intakes of insoluble forms exceeding 100 times the annual limit on intake.

For wounds, any detectable plutonium in the wound or in spot urine samples should warrant considering administration of DTPA. If the activity in the wound is >5 nCi, excision of tissue should also be considered.

Environmental Fate

Although primarily a manmade substance, minute quantities of plutonium have existed and currently exist in nature. About 5000 kg of Pu-239 were dispersed into the environment by the atmospheric testing of nuclear weapons during the 1950s and 1960s, and trace amounts are present in most environmental media. Deposition (approximately three-quarters of which occurred in the northern hemisphere) reduced atmospheric levels to less than 20 kg by 1975, and as there have been few atmospheric tests conducted since that time, these levels have continued to decline. Measurable concentrations have been found in air, food, soils, and human and animal tissues. Plutonium has also reached the environment from routine and accidental releases from nuclear facilities, primarily those involved in the reprocessing of nuclear fuels.

The environmental behavior of plutonium is highly dependent on physicochemical properties of both the Pu compounds and the environmental media. As a rule, plutonium adsorbed on soil or sediment particles migrates very slowly, although the rate can be accelerated depending on Pu oxidation state and soil characteristics (mineral makeup, pH, presence of ligands). Uptake and concentration in edible plants is relatively low (concentration ratio on the order of 10^{-4} in vegetative parts).

Exposure Standards and Guidelines

Plutonium is regulated primarily as a radioactive material and the applicable standards and guidelines are set by entities involved in radiation protection. There are no US Occupational Safety and Health Administration or National Institute for Occupational Safety and Health exposure limits for plutonium.

United States of America

EPA National Primary Drinking Water Standard – 15 pCi l^{-1} (applicable standard for Pu is MCL for gross alpha particle activity, excluding uranium and radon).

NRC maximum concentration in effluent air at boundary of licensed facility (App. B to 10 CFR 20.1001-20.2401) for selected Pu isotopes:

- Pu-238 (all forms) – 2×10^{-14} μCi ml^{-1}; and
- Pu-239 (all forms) – 2×10^{-14} μCi ml^{-1}.

Annual limit on intake (occupational exposure) for inhalation of selected Pu isotopes:

- Pu-238, Class W – 7000 pCi (300 Bq);
- Pu-239, Class W – 6000 pCi (200 Bq);
- Pu-238, Class Y – 20 000 pCi (700 Bq); and
- Pu-239, Class Y – 20 000 pCi (600 Bq).

France

Plutonium in foodstuff intended for general consumption – 10 Bq kg^{-1}. Plutonium in baby foods or milk – 1 Bq kg^{-1}.

See also: Radiation Toxicology, Ionizing and Nonionizing.

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Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Plutonium.

Poinsettia

Allison A Muller

- Chemical Name: Poinsettia
- Synonyms: *Euphorbia pulcherrima*; Christmas star; Christmas flower; Painted leaf; Lobster plant; Mexican flame-leaf; Star of Bethlehem; Flower of nativity; Easter flower; Pappagallo

Exposure Routes and Pathways

Ingestion and dermal contact are possible routes of exposure.

Mechanism of Toxicity

Diterpene esters are primary dermal and gastrointestinal irritants. The amount of toxin found in the common greenhouse variety of poinsettia is minimal and very rarely causes symptoms. Irritation, whether dermal or oral, is rare. The poinsettia, despite its unfavorable reputation, appears for the most part to be innocuous.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals, particularly domestic animals, have shown a very low incidence of toxic effects. Of those that do develop symptoms, gastrointestinal irritation (nausea, vomiting, diarrhea, hypersalivation) is the most common effect seen after ingestion of poinsettia.

Human

Most human exposures occur in children with only small amounts ingested or in contact with skin. In these situations, symptoms are infrequent. Those symptoms that do occur are due to irritation of the affected area. Poinsettia ingestions may produce vomiting and diarrhea. Dermal exposures to the sap of the plant may cause irritation. Most exposures result in either no clinical effects or only minor, self-limited symptoms.

Clinical Management

For ingestion of plant material, symptomatic treatment consists of dilution with cool liquids. Ingestions rarely cause any symptoms aside from minor, self-limited gastrointestinal effects. Dermal exposures are treated with irrigation and local skin care.

See also: Gastrointestinal System.

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Poisoning Emergencies in Humans

Christopher P Holstege

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Introduction

Poisoning emergencies are a common occurrence. In 2002, The *Toxic Exposure Surveillance System* of the American Association of Poison Control Centers reported 2 380 028 toxic exposures and 1153 resultant fatalities. Of these total exposures, 548 093 (22.2%) were managed in a healthcare facility and 72 877 were admitted to a critical care unit (3.1%). The mortality rate associated with these overdose patients was less than 1%. Thorough evaluation, adequate supportive care, and the use of a few specific antidotes have resulted in lowered morbidity and mortality if the poisoned patient arrives at the hospital in time for the healthcare team to intervene. In select cases, decreasing further toxin absorption by various decontamination procedures may be of benefit.

Poisonings are among the most preventable public health problems. The majority of exposures are accidental (86%) and occur in children under 6 years of age. However, poisoning exposures are not limited to children and occur during every decade of life. Children aged 18–36 months are at the greatest risk due to excessive hand-to-mouth behavior and their innate curiosity, which results in extensive exploration of their environment. The carelessness by caretakers and the lack of adult awareness about what constitutes a poison also contributes to the risk of childhood exposure. Childhood exposures are largely preventable by recognizing the toxic potential of medications, herbals, household products, cosmetics, and plants and by keeping these agents out of the reach of children. Homes should clearly keep readily available the number of the local poison center. In the United States, all citizens can dial the toll free number 1-800-222-1222 and reach their local poison center. The American Association of Poison Control Center keeps an updated list of poison centers that can be located at their website.

Across all age groups, medications are involved in the majority of reported poisonings. Cosmetic products are the leading cause of pediatric poisoning exposures, followed closely by cleaning substances, analgesics, topical agents, and plants. These agents result in the majority of exposures because they are commonly found in households where children reside. Poisonings are associated with a relatively low mortality rate – only 0.048% of poisoned victims have fatal outcomes. Adults account for 98% of these fatalities and children account for only 2%. Most adult poisoning-related fatalities involve analgesics and psychiatric agents.

Management

All patients presenting with potential toxicity following exposure to various agents should be thoroughly assessed. The patient's airway should be patent and adequate ventilation assured. If necessary, endotracheal tube intubation should be performed. Too often physicians are lulled into a false sense of security when a patient's oxygen saturations are adequate on high flow oxygen. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk of subsequent CO_2 narcosis with worsening acidosis or pulmonary aspiration. The initial treatment of hypotension consists of intravenous fluids. There should be close monitoring of the patient's pulmonary parameters to ensure that pulmonary edema does not develop as fluids are infused. The healthcare providers should consider placing the potentially poisoned patient on continuous cardiac monitoring with pulse oximetry. Frequent neurological checks should be considered. In all patients with altered mental status, the patient's glucose should be checked. These patients should receive a large bore peripheral intravenous line and all critically ill patients should have a second line placed either peripherally or centrally.

Decontaminating the Poisoned Patient

Approximately 80% of all poisonings occur by ingestion and subsequently the most common type of decontamination performed is gastrointestinal decontamination using a variety of techniques including emesis, gastric lavage, activated charcoal, cathartics, and whole bowel irrigation (WBI). Poisonings may also occur by dermal and ocular routes, which necessitate external decontamination. Significant controversy exists concerning the need for routine gastric emptying in the poisoned patient. Current available evidence dissuades one from the routine use of gastric decontamination. Gastric decontamination may be considered in select cases and specific scenarios. Before performing gastrointestinal decontamination techniques, the clinician responsible for the care of the poisoned patient must clearly understand that these procedures are not without hazards, and any decision on their use must

consider whether the benefit of decontamination outweighs any potential harm.

Dermal Decontamination

Patients presenting to healthcare facilities with dermal contamination pose a potential risk to healthcare personnel. Contaminated patients should not gain entrance into the healthcare facility prior to decontamination. Personnel involved in the dermal decontamination may need to don personal protective equipment. Most chemical exposures do not pose a risk of secondary exposure. For exposures that occur in the workplace, Material Safety Data Sheets can be obtained and either the local poison center or the Agency for Toxic Substances and Disease Registry can be contacted to obtain advice on what level of protection is appropriate. Contaminated clothing and valuables should be placed in an impervious bag to avoid potential of gassing.

Dermal decontamination can be performed by using soap and copious warm water irrigation. Starting from head to toe, irrigate the exposed skin and hair for 10–15 min and scrub with a soft surgical sponge, taking care not to abrade the skin. Irrigate wounds for an additional 5–10 min with water or saline. Clean underneath the nails with a brush. Stiff brushes and abrasives should be avoided as they may enhance dermal absorption of the toxin and can produce skin lesions that may be mistaken for chemical injuries. Sponges and disposable towels are effective alternatives.

Ocular Decontamination

Ocular irrigation should be performed as rapidly as possible by instillation of a gentle stream of lukewarm tap water into the affected eye(s). The skin contiguous to the eye should also be irrigated. In minor ocular toxicity cases, this procedure can be conducted in the home. If irritation persists following home irrigation, referral to an emergency department may be necessary. In the emergency department, the patient should undergo ocular irrigation with sterile normal saline for a period of at least 1 h. Exposures to some corrosives may necessitate prolonged ocular irrigation. Irrigation of the eyes should be directed away from the medial canthus to avoid forcing contaminants into the lacrimal duct. Longer irrigation times may be needed with specific substances and the endpoint of irrigation should be normalization of the eye's pH.

Gastrointestinal Decontamination

Emesis, gastric lavage, activated charcoal, cathartics, and WBI are the most common means of gastrointestinal decontamination. With emerging evidence, gastric lavage and syrup of ipecac-induced emesis are rarely being utilized to decontaminate the poisoned patient. At this time, the documented risks associated with these procedures should be carefully weighed in light of the rare indications. Activated charcoal as the sole means of gastric decontamination is increasing in popularity, but its efficacy has specific limitations. The major issue currently facing the clinician is the choice of gastrointestinal decontamination in the significantly poisoned patient. The choice of decontamination method for these patients must be individualized using both evidence-based medicine and clinical acumen. No patient should undergo any of the available procedures unless it is anticipated that decontamination will provide clinical benefit.

Emesis

Numerous emetics have been advocated in the past for the treatment of the poisoned patient. Past emetics have included apomorphine, egg whites, saltwater, copper sulfate, and household dish-washing liquid. However, the use of these agents is fraught with ineffectiveness and potential harm to the patient. The only acceptable emetic that may be considered is syrup of ipecac.

Syrup of ipecac is available as a nonprescription product in many countries. It is derived from the dried rhizome and roots of the *Cephaelis ipecacuanha* or *Cephaelis acuminata* plant. These plants contain the potent emetic alkaloids emetine and cephaeline, which induce vomiting by both direct local gastrointestinal effects and central nervous system actions. Emesis following syrup of ipecac ingestion typically occurs within 20 min of ingestion and persists for 30–120 min.

There have been numerous animal and human volunteer studies examining both the efficacy of syrup of ipecac to expel specific ingested agents from the stomach and its ability to decrease serum drug levels. In these studies, the amount of marker removed by syrup of ipecac was highly variable and the efficacy at expelling experimental markers decreased as the administration time post-ingestion increased. Syrup of ipecac is of very limited benefit if more than 60–90 min have elapsed since the time of ingestion. No studies have demonstrated that syrup of ipecac improves patient outcome. In fact, recent studies suggest there is no reduction in resource utilization or improvement in patient outcome from the use of syrup of ipecac at home. In 2003, the American Academy of Pediatrics recommended that ipecac should no longer be used routinely as a home treatment strategy and that existing ipecac in the home

should be disposed of safely. Also in 2003, the US Federal Drug Administration (FDA) Nonprescription Drugs Advisory Committee met to discuss whether there was sufficient evidence of the benefits of ipecac syrup to outweigh the potential for misuse, abuse, and adverse effects associated with it as an over-the-counter (OTC) drug. At the conclusion of the meeting, the Committee recommended by a six-to-four vote that the FDA rescind ipecac's OTC status. The position statement written by the American Academy of Clinical Toxicologists and the European Association of Poison Centers and Clinical Toxicologists declared that the routine administration of ipecac should be abandoned.

In the rare cases where syrup of ipecac is administered to a patient, it should only be given to an alert, conscious patient who has ingested a potentially toxic amount of a poison no more than 60 min prior to administration. The administration of syrup of ipecac is contraindicated in any person who demonstrates compromised airway protective reflexes or has the potential to lose such protective reflexes. It should be avoided in persons who have ingested substances that could result in coma, seizures, cardiovascular collapse, or paralysis. Syrup of ipecac is also contraindicated in persons who have ingested corrosive substances (acids or alkalis), hydrocarbons, and foreign bodies that could potentially result in airway obstruction. Caution should be exercised in using syrup of ipecac in patients who possess medical conditions that could be further compromised by the induction of emesis, such as patients with bleeding diatheses. The most commonly reported complications of ipecac administration include diarrhea, lethargy, and prolonged vomiting. Other reported complications include pulmonary aspiration of gastric contents, bradycardia, cerebral hemorrhage, gastric rupture, gastric diaphragmatic herniation, Mallory–Weiss tear, and pneumomediastinum. The use of ipecac may both delay the administration and diminish the effectiveness of other methods of gastrointestinal decontamination.

Gastric Lavage

The efficiency of gastric lavage to remove a marker significantly decreases with increasing time following ingestion. This is due to the fact that the greater the time after ingestion, the more time there is for the marker to be absorbed and for the marker to pass out of the stomach. It is rare that gastric lavage can be performed within the first hour after toxic ingestion. Not only does it take time for these patients to present to the emergency department, but it also takes time for evaluation, stabilization, and for the gastric lavage to take place. Based on the available literature, gastric lavage should not be routinely employed in the management of poisoned patients. Oral charcoal alone is considered superior to gastric lavage if a drug is adsorbed by charcoal.

The performance of gastric lavage is contraindicated in any person who demonstrates compromised airway protective reflexes unless they are intubated. Gastric lavage is also contraindicated in persons who have ingested corrosive substances (acids or alkalis), hydrocarbons (unless containing highly toxic substances such as paraquat, pesticides, heavy metals, halogenated and aromatic compounds), have known esophageal strictures, and a history of gastric bypass surgery. Caution should be exercised in performing gastric lavage in patients who possess medical conditions that could be compromised by performing this procedure, such as patients with bleeding diatheses, and in combative patients.

Numerous complications have been reported in association with gastric lavage. Depending on the route selected for tube insertion, damage to the nasal mucosa, turbinates, pharynx, esophagus, and the stomach have all been reported. After tube insertion, it is imperative to confirm correct placement. Radiographic confirmation of tube placement should especially be considered in young children and intubated patients. Instillation of lavage fluid and charcoal into the lungs through tubes inadvertently misplaced within the airways has been reported. The large amount of fluid administered during lavage has been reported to cause patient fluid and electrolyte disturbances. These disturbances have been seen with both the use of hypertonic and hypotonic lavage fluids in the pediatric population. Hypothermia is a possible complication if the lavage fluid is not pre-warmed. Pulmonary aspiration of gastric contents or lavage fluid is the primary potential risk during gastric lavage, especially in patients with compromised airway protective reflexes.

Activated Charcoal

Activated charcoal acts both by adsorbing a wide range of toxins present in the gastrointestinal tract and by enhancing toxin elimination, if systemic absorption has already occurred. It enhances elimination by creating a concentration gradient between the contents of the bowel and the circulation, but it also has the potential of interrupting enterohepatic circulation if the particular toxin is secreted in the bile and enters the gastrointestinal tract prior to reabsorption. Oral activated charcoal is given as a single dose or in multiple doses.

Single dose activated charcoal is indicated if the clinician estimates that a clinically significant

fraction of the ingested substance remains in the gastrointestinal tract, the toxin is adsorbed by charcoal, and further absorption may result in clinical deterioration. It may also be administered by multiple dosing if the clinician anticipates that the charcoal will result in increased clearance of an already absorbed drug. In 1997, the American Academy of Clinical Toxicology released a position statement recommending that activated charcoal should not be routinely administered but should be reserved for cases in which serious toxicity is anticipated. It is most effective within the first 60 min after oral overdose and decreases in effectiveness over time.

The administration of charcoal is contraindicated in persons who demonstrate compromised airway protective reflexes, unless they are intubated. It is contraindicated in persons who have ingested corrosive substances (acids or alkalis). Charcoal not only provides no benefit in a corrosive ingestion, but its administration could precipitate vomiting, obscure endoscopic visualization, and lead to complications if a perforation develops and charcoal enters the mediastinum, peritoneum, or pleural space. Charcoal should be avoided in cases of pure aliphatic petroleum distillate ingestion. Caution should be exercised in using charcoal in patients who possess medical conditions that could be further compromised by charcoal ingestion, such as those with gastrointestinal perforation or bleeding. Since it is often impossible to determine the exact nature of an ingestion, a liberal use policy is advocated for potential mixed overdoses.

Charcoal is generally very safe and few adverse effects from the use of single dose activated charcoal have been reported despite its widespread use. There are no reports of gastrointestinal obstruction associated with single dose activated charcoal. The most common complications of charcoal administration include constipation, diarrhea, and vomiting. Pulmonary aspiration of activated charcoal is a dreaded complication that can result in pneumonitis, obstruction of the respiratory tree, and bronchiolitis obliterans. Aspiration of large amounts of charcoal can be fatal.

The use of multidose activated charcoal (MDAC) may be indicated in select cases. Its use has been advocated to prevent continued absorption of a drug that may still be present within the gastrointestinal tract and to increase the clearance of a drug that has already been absorbed. MDAC prevents continued absorption by binding a drug that may be either present throughout the gastrointestinal tract or one that exists as an extended-release or enteric-coated preparations. MDAC enhances elimination of a drug by interrupting enterobiliary recirculation or augmenting enterocapillary exsorption. By interrupting enterobiliary recirculation, charcoal binds to an active drug that is secreted by the biliary system, subsequently preventing reabsorption. By augmentation of enterocapillary exsorption, charcoal produces sink conditions that drive diffusion of the drug from the capillaries into the entraluminal space from where it is subsequently eliminated. This process is called 'intestinal dialysis'. MDAC is contraindicated if there is evidence of bowel obstruction. An ileus is a relative contraindication. The administration of MDAC is contraindicated in any patient who does not have an intact or protected airway. MDAC should be avoided in patients who have repetitive emesis, especially when associated with decreased mental status or a decreased gag reflex. The concurrent use of cathartics with MDAC remains unproven and is not recommended. The first dose of activated charcoal should be $1\,g\,kg^{-1}$ (maximum of 100 g). If a cathartic is used, it should be administered only with the first dose of charcoal to decrease the risk of cathartic-induced electrolyte abnormalities that can potentially develop, especially in children. The initial dose of charcoal is followed by $0.5\,g\,kg^{-1}$ (up to 50 g) of activated charcoal every 4 h. If repeat examination reveals an absence of bowel sounds or reveals a distended abdomen, MDAC should be terminated and the physician should consider placement of a nasogastric tube on low intermittent suction. The use of antiemetics may help decrease the incidence of vomiting associated with MDAC. Charcoal therapy should be continued until there is clinical improvement and plasma drug levels have fallen to acceptable levels. There have been reports of gastrointestinal obstruction and perforation from MDAC therapy, especially in conjunction with the ingestion of drugs with anticholinergic properties.

Cathartics

The use of cathartics is intended to decrease the absorption of substances by accelerating the expulsion of the poison from the gastrointestinal tract. However, most data suggest negligible clinical benefit from cathartic use. There is little evidence that a single dose of aqueous activated charcoal is significantly constipating; however, cathartics are often given for this potential problem. The routine administration of a cathartic in combination with activated charcoal is not endorsed by the American Academy of Clinical Toxicology or the European Association of Poison Centres and Clinical Toxicologists. In addition, the administration of a cathartic alone has no role in the management of the poisoned patient.

Cathartics are contraindicated if there is volume depletion, hypotension, significant electrolyte imbalance, corrosive ingestion, ileus, recent bowel surgery,

and intestinal obstruction or perforation. The administration of cathartics is also contraindicated with patients who do not have an intact or protected airway. They should be avoided in patients who have repetitive emesis, especially when associated with decreased mental status or a decreased gag reflex. Cathartics should be used cautiously in young children and the elderly because of the propensity of laxatives to cause fluid and electrolyte imbalance.

There are two types of osmotic cathartics: saccharide cathartics (sorbitol) and saline cathartics (magnesium citrate, magnesium sulfate, sodium sulfate). Many charcoal formulations come premixed with sorbitol, but there is considerable variation in the sorbitol content. Multiple doses of cathartics should be avoided. The administration of sorbitol has been associated with vomiting, abdominal cramps, nausea, diaphoresis, and transient hypotension. Multiple doses of sorbitol have been associated with volume depletion. Multiple doses of magnesium-containing cathartics have been associated with severe hypermagnesemia. Children are particularly susceptible to the adverse affects of cathartics, and therefore cathartics should be used with caution, or totally avoided, in children.

Whole Bowel Irrigation

Whole bowel irrigation (WBI) has emerged as the newest technique in gastrointestinal decontamination. It involves the enteral administration of an osmotically balanced polyethylene glycol-electrolyte solution (PEG-ES) in sufficient quantity and rate to physically flush ingested substances through the gastrointestinal tract, purging the toxin before absorption can occur. PEG-ES is isosmotic, is not systemically absorbed, and will not cause electrolyte or fluid shifts. Available data suggest that the large volumes of this solution needed to mechanically propel pills, drug packets, or other substances through the gastrointestinal tract are safe, including in pregnancy and in young children. WBI may be considered for ingestions of exceedingly large quantities of potentially toxic substances, ingestions of toxins that are poorly adsorbed to activated charcoal, ingestions of delayed-release formulations, late presentation after ingestion of a toxin, pharmacobezoars, and body stuffers or packers. The most common indication for WBI in the Emergency Department (ED) is for the treatment of toxic sustained-release medications (such as calcium channel blockers, theophylline, lithium) and iron tablets. WBI is contraindicated in patients with gastrointestinal obstruction, perforation, ileus, and corrosive ingestion. It should also be avoided in patients with hemodynamic instability or an unprotected airway. WBI should be avoided with patients who have repetitive emesis, especially when associated with decreased mental status or a decreased gag reflex. WBI should be used cautiously in debilitated patients.

Cooperative patients with intact airway protective reflexes may drink the solution. However, the large volume and taste often limit even the most motivated patient's ability to comply. If the patient is unable or unwilling to drink this solution, it should be administered through a small-bore nasogastric tube after placement is confirmed. Unconscious patients with protected airways may receive WBI. Prewarming the irrigant to a temperature of $\sim 37^{\circ}C$ avoids the potential complication of hypothermia. The endpoint of WBI is the arrival of clear rectal effluent and/or resolution of toxic effect.

There have been few reported complications from WBI therapy, especially pertaining to acute poisonings. Nausea, vomiting, abdominal cramps, and bloating have been described. Nausea and vomiting may make administration of WBI difficult. Antiemetics and a 15–30 min break followed by a slower rate may allow readministration. As discussed with the other methods of decontamination, attention should be directed to the airway and the potential for aspiration. Administration of a large amount of chilled or room temperature WBI fluid to pediatric patients could potentially cause hypothermia. Warmed fluid should be considered in these patients.

Pharmacologic Antagonists

The number of pharmacologic antagonists or *antidotes* is quite limited. There are few agents that will rapidly reverse toxic effects and restore a patient to a previously healthy baseline state. Administering some pharmacologic antagonists may actually worsen patient outcome compared to merely employing basic supportive care. As a result, antidotes should be used cautiously and with clearly understood indications and contraindications.

Atropine

Atropine is the initial drug of choice in symptomatic patients poisoned with organophosphates or carbamates. Atropine acts as a muscarinic receptor antagonist and blocks neuroeffector sites on smooth muscle, cardiac muscle, secretory gland cells, peripheral ganglia, and in the central nervous system. Atropine is therefore useful in alleviating bronchoconstriction and bronchorrhea, relieving tenesmus, abdominal cramps, nausea and vomiting, resolving

bradydysrythmias, and halting seizure activity. Atropine can be administered by either the intravenous, intramuscular, or endotracheal route. The dose varies with the type of exposure, but typically even the worst cases require less than 20 mg in the first 24 h. There are a few reports of severe organophosphate pesticide poisonings requiring hundreds of milligrams of atropine each day. For the mildly and moderately symptomatic patient, 2.0 mg kg^{-1} for adults and 0.02 mg kg^{-1} for children (minimum of 0.1 mg) is administered every 5 min. In the severely poisoned patient, dosages may need to be increased and given more rapidly. Tachycardia is not a contraindication to atropine administration in these patients. Drying of the respiratory secretions and resolution of bronchoconstriction are the therapeutic end-points used to determine the appropriate dose of atropine. Atropine has no effect on the nicotinic receptors and therefore has no effect on autonomic ganglia and neuromuscular junction. Therefore, muscle weakness, fasciculations, tremors, and paralysis are not an indication for further atropine dosing. Atropine does have a partial effect on the central nervous system and may be helpful in resolving seizures.

Deferoxamine

Deferoxamine is an effective chelator of iron. Deferoxamine chelates iron and converts it to a water-soluble complex, ferrioxamine, which is eliminated readily via the urine. Indications for deferoxamine infusion include significant clinical signs of iron toxicity, metabolic acidosis, shock, serum iron levels $> 500\ \mu g\ dl^{-1}$, and/or an X-ray positive for multiple pills. Deferoxamine should be infused intravenously at a starting rate of 15 mg $kg^{-1}\ h^{-1}$, not to exceed 1 g h^{-1}, over a total of 6 h and then reevaluated. Deferoxamine-induced hypotension may occur at fast rates, and adequate hydration should be assured before infusion initiation. As iron is chelated and excreted, urine may develop a characteristic rusty-red ('vin rose') appearance.

Crotalidae Antivenin

Currently in the United States, there are two available Crotalidae antivenoms: polyvalent IgG (Wyeth-Ayerst Laboratories) and polyvalent Fab immunoglobulin fragments (CroFab-® by Protherics Inc.). Use of antivenin in the appropriate doses can control local swelling and serious systemic effects (e.g., neurologic effects and coagulopathies) that occur in patients who have been envenomated. However, the antivenin should not be used prophylactically since a significant number of snake bites are dry bites. There are numerous dosage regimens that vary with the degree of systemic toxicity and regional treatment preferences. Consultation with a poison center or a clinical toxicologist is advised for the most contemporary treatment recommendations.

There have been numerous reports of immediate hypersensitivity reactions associated with the use of crotalidae antivenom polyvalent IgG (Wyeth-Ayerst Laboratories). Incidence rates for immediate hypersensitivity reactions associated with the use of this product range from 23% to 56%. This high reaction rate may be in part due to the large amount of non-venom neutralizing proteins within this partially purified horse antivenom. In addition, this product contains the Fc portion of the antibodies that may result in cross-linking on cell surface receptors and lead to mast cell and basophil degranulation. CroFab-® is reported to have a lower risk of immediate hypersensitivity reactions. The product contains reduced amounts of the immunogenic Fc portion of the antibody.

Digoxin Immune Fab

A milestone in the treatment of cardiac glycoside poisoning was the development of drug-specific antibodies. Digoxin-specific Fab fragments (Digibind or DigiTab) are antibody fragments produced by enzymatic cleavage of sheep immunoglobulin (IgG) antibodies to digoxin. Fab fragments can reverse digitalis-induced dysrhythmias, conduction disturbances, myocardial depression, and hyperkalemia in severely poisoned patients. Most patients have an initial response to cardiac glycoside toxic dysrhythmias within 30 min of Fab administration and those who responded had complete resolution by 4 h. Animal studies and case reports have demonstrated the efficacy of Fab fragments to the cardiac glycoside contained in plants. Adverse reactions to Fab administration have been few and include rare but mild hypersensitivity reactions, precipitous drops in serum potassium, and supraventricular tachydysrhythmias previously controlled by digoxin.

Fab fragment therapy should be administered for the following indications: (1) potassium $> 5.0\ mEq\ l^{-1}$ following acute ingestion, (2) serum digoxin concentration $> 10\ ng\ ml^{-1}$, (3) patients with potentially life-threatening dysrhythmias. Often, chronically poisoned patients can be managed by discontinuing digoxin and close monitoring. However, the threshold for treatment with Fab should be lower in chronically poisoned patients with signs of cardiac toxicity or those who have chronic pulmonary disease, hypokalemia, hypothyroidism, renal insufficiency, or underlying cardiac disease. If patients are managed conservatively the Fab dose to

be administered should be calculated and the Fab fragments made available at the bedside while the patient is monitored for worsening toxicity.

Although serum digoxin levels should not be the sole factor in determining the need to administer Fab, dosage calculations for Fab are based on the serum digoxin level or estimated body load of digoxin. It is assumed that equimolar doses of antibody fragments are required to achieve neutralization. Thirty-eight milligrams of Fab (one vial) will bind 0.5 mg of digoxin. A severely toxic patient in whom the quantity ingested acutely is unknown should be given 5–10 vials at a time and the clinical response observed. If cardiac arrest is imminent or has occurred, the dose can be given as a bolus. Otherwise, it should be infused over 30 min. In contrast, patients with chronic therapeutic overdose often have only mildly elevated digoxin levels and respond to one to two vials of Fab. The recommended dose for a given patient can be determined using the tables in the package insert or by contacting a regional poison center or toxicology consultant.

Free digoxin levels are decreased to zero within 1 min of Fab fragment administration, but total serum digoxin levels are markedly increased. Since most assay methods measure both bound and free digoxin (total), very high digoxin levels are seen after Fab fragment therapy, but they have no correlation with toxicity. Serum levels may be unreliable for several days after Fab treatment. The digoxin–Fab complex is excreted in the urine and in patients with renal failure; elimination of the digoxin–Fab complex is prolonged and free digoxin levels gradually increase over hours after Fab administration. Rebound cardiac glycoside toxicity is rare but has been reported. Hemodialysis does not enhance elimination of the digoxin–Fab complex.

Flumazenil

Benzodiazepines are involved in many intentional overdoses. While these overdoses are rarely fatal when a benzodiazepine is the sole ingestant, they often complicate overdoses with other central nervous system depressants (e.g., ethanol and sedatives) due to their synergistic activity. Flumazenil finds its greatest utility in the reversal of benzodiazepine-induced sedation from minor surgical procedures. The initial flumazenil dose is 0.2 mg and should be administered intravenously over 30 s. If no response occurs after an additional 30 s, a second dose is recommended. Additional incremental doses of 0.5 mg may be administered at 1 min intervals until the desired response is noted or until a total of 3 mg has been administered. Flumazenil should not be administered as a nonspecific coma-reversal drug and should be used with extreme caution after intentional benzodiazepine overdose since it has the potential to precipitate withdrawal in benzodiazepine-dependent individuals and/or induce seizures in those at risk.

Fomepizole

Fomepizole (4-methylpyrazole) is an alcohol dehydrogenase inhibitor. It is administered in cases of suspected or confirmed ingestion and intoxication with ethylene glycol or methanol. Fomepizole should be administered intravenously as a loading dose of $15\,mg\,kg^{-1}$, followed by doses of $10\,mg\,kg^{-1}$ every 12 h for four doses, then $15\,mg\,kg^{-1}$ every 12 h thereafter; all doses should be administered as a slow intravenous infusion over 30 min. During hemodialysis, the frequency of dosing should be increased to every 4 h. Therapy should be continued until ethylene glycol or methanol concentrations are less than $20\,mg\,dl^{-1}$ and the patient is asymptomatic.

Hydroxocobalamin

Hydroxocobalamin (vitamin B_{12a}), currently investigational in the United States, is a safe and effective alternative that is currently being used in Europe for the treatment of cyanide toxicity. It acts as a chelating agent for cyanide. The reaction of hydroxocobalamin with cyanide results in the displacement of a hydroxyl group by a cyano group to form cyanocobalamin (vitamin B_{12}), which is then excreted in the urine. One molecule of hydroxocobalamin binds one molecule of cyanide. Hydroxocobalamin is given intravenously in a 5% dextrose solution. The usual adult dose is 4 g, which may be increased in cases of massive cyanide poisoning. The most common side effect is an orange-red discoloration of the skin, mucous membranes, and urine, which lasts for $\sim$12 h.

N-Acetylcysteine

Acetaminophen overdosage, whether accidental or intentional, is the most common type of poisoning event reported to American poison centers. Most acetaminophen overdoses do not produce adverse effects because most of these are minor exposures in children. However, significant overdoses may need to be treated with *N*-acetylcysteine (NAC; Mucomyst) if the patient has a toxic serum acetaminophen concentration or is in hepatic failure. NAC increases glutathione levels and serve as a glutathione surrogate. An acetaminophen overdose may deplete glutathione, permitting the toxic metabolite to destroy hepatocytes. NAC is most effective if administered within 8 h of the acetaminophen ingestion; however, it is still effective

days after the ingestion when patients are already in hepatic failure and acetaminophen levels are no longer detectable.

NAC is approved for both oral and intravenous administration:

- *Oral*: 140 mg kg^{-1} loading dose followed by 70 mg kg^{-1} every 4 h for 17 doses.
- *Intravenous*: 150 mg kg^{-1} loading dose followed by 50 mg kg^{-1} over 4 h followed by 100 mg kg^{-1} infused over 16 h.

Parenteral administration of NAC eliminates compliance problems associated with oral therapy (very bad taste and odor due to the sulfhydryl groups) and circumvents the problems associated with acetaminophen-induced vomiting.

Naloxone

Opioid poisoning from the abuse of morphine derivatives or synthetic narcotic agents may be reversed with the opioid antagonist naloxone (Narcan). Naloxone is commonly used in comatose patients as a therapeutic and diagnostic agent. The standard dosage regimen is to administer from 0.4 to 2.0 mg slowly, preferably intravenously. Intramuscular administration is an alternative parenteral route, but if the patient is hypotensive, naloxone may not be absorbed rapidly from the intramuscular injection site. The intravenous dose should be readministered at 5 min intervals until the desired endpoint is achieved – restoration of respiratory function and an improved level of consciousness. If the intravenous route of administration is not viable, alternative routes in addition to intramuscular injection are administration via the endotracheal tube in intubated patients as well as intralingual and sublingual injection. Intraosseous administration may be an alternative route in pediatric patients.

A patient may not respond to naloxone administration for a variety of reasons: insufficient dose of naloxone, the absence of an opioid exposure, a mixed overdose with other central nervous and respiratory system depressants, or medical or traumatic reasons.

Physostigmine

Once touted as the medication of choice to treat lethal tricyclic antidepressant overdoses, physostigmine (antilirium^{TM}) has very limited uses today in overdose management. Physostigmine is a cholinesterase inhibitor and finds its primary application in the treatment of severe anticholinergic poisoning. When indicated, physostigmine is administered preferably in small incremental doses of 2 mg mixed in 10 cc of saline by slow intravenous infusion over 10 min. Rapid injection or the administration of large doses may produce a cholinergic crisis or seizure activity.

Pralidoxime Chloride

Pralidoxime chloride (2-PAMCL, Protopam^{TM} Chloride) reactivates acetylcholinesterase (AChE) by exerting a nucleophilic attack on the phosphorus resulting in an oxime–phosphate bond that splits from the AChE leaving the regenerated enzyme. This reactivation is clinically most apparent at skeletal neuromuscular junctions, with less activity at muscarinic sites. Pralidoxime must therefore be administered concurrently with adequate atropine doses. In addition, the process of aging will prevent pralidoxime from regenerating the AChE active site and, as a result, is ineffective after aging has occurred. Therefore, the sooner pralidoxime is administered, the greater the clinical effect. The recommended dose of pralidoxime is 1.0 gm for adults or 15–25 mg kg^{-1} for children by the intravenous route. Slow administration over 15–30 min has been advocated to minimize side effects. These side effects include hypertension, headache, blurred vision, epigastric discomfort, nausea, and vomiting. In multiple animal models, the pralidoxime serum concentration to achieve therapeutic efficacy was reported to be 4 mg l^{-1}. The above dose will attain these levels, but pralidoxime is rapidly excreted and the concentration falls below 4 mg l^{-1} within 2 h. Subsequently, repeat pralidoxime should be administered at hourly intervals if progressive worsening or serious signs of toxicity persist. In order to achieve a steady-state blood level of pralidoxime following loading, it has been recently recommended that a continuous intravenous infusion be administered. Continuous intravenous infusion for insecticide organophosphate poisoning has proven to be safe and effective. As pralidoxime is rapidly excreted in the urine, adequate hydration should be maintained during therapy. Theoretically, dosing should be lowered for patients with renal failure. If medical personnel are unable to initially obtain intravenous access, a solution for intramuscular use can be made by mixing the contents of a 1 g vial with 3 ml of sterile saline. Intramuscular administration to a patient with an adequate blood pressure will produce a therapeutic plasma concentration of 4 mg l^{-1} within 10 min.

Pyridoxine

Isoniazid, hydrazine, and the *Gyrometria* species of mushrooms can decrease the brain concentrations of gamma-aminobutyric acid by inhibiting pyridoxal-5-phosphate activity, resulting in the development of severe seizure activity. The administration of

pyridoxine (vitamin B_6) can prevent or actively treat the central nervous system toxicity associated with isoniazid poisoning. Pyridoxine is administered on a gram-for-gram basis with isoniazid (i.e., the amount of pyridoxine should equal the amount of isoniazid). If the ingested amount of the agent above is unknown, the dose of pyridoxine should be 5 g administered intravenously. This dose can be repeated.

See also: Anticholinergics; Atropine; Charcoal; Deferoxamine; Gastrointestinal System; Lithium; Pesticides; Polyethylene Glycol; Pyridoxine.

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Relevant Website

http://www.aapcc.org – American Association of Poison Control Centers.

Pokeweed

Ann P Slattery

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- Synonyms: *Phytolacca americana*; *P. pecandra*; *P. rigida*; American nightshade; Cancerroot; Crowberry; Indian polk; Inkberry; Pigeonberry; Pokeberry; Red ink plant; Red weed; Red wood; Scoke

Uses

Pokeweed is a tall perennial shrub growing up to 12 ft. This shrub can be found in damp fields, along fences, and wooded areas of southeastern Canada, eastern United States, as well as California and Hawaii. The stalks are reddish. There are small, greenish-white flowers and berries in opposite clusters. The berry is dark purple, almost black in color, and matures late summer to autumn. Pokeweed has been used in tea and as a herbal medicine. In folk medicine, pokeweed as a tincture has been used for arthritis and chronic rheumatism. Taken by mouth pokeweed was used as a purgative and as an emetic. The young leaves, if boiled and drained twice, are supposedly edible.

Exposure Routes and Pathways

Exposure to pokeweed is by ingestion and dermal contact.

Mechanism of Toxicity

The toxic activity of the plant is unclear. Pokeweed mitogen (PWM) noted in the plant fluids may initiate changes in the immune system that alter T- and B-lymphocytes. The gastrointestinal irritant properties of phytolaccinic acid and related triterpenoid glycosides (saponins) cause diarrhea and severe vomiting.

Acute and Short-Term Toxicity (or Exposure)

Human

Pokeweed contains phytolaccatoxin and related triterpenes. All plant parts are poisonous, especially the roots. Uncooked berries have been known to poison children. Toxic exposures have occurred from eating the uncooked leaves in salads or when the root is mistaken for horseradish, parsnip, or ginseng. Effects appear 30 min to 6 h after exposure. Symptoms include nausea, abdominal cramps, profuse sweating, and foamy diarrhea. Other effects include oral burning, a bitter taste in the mouth, dyspnea, weakness, tremors, and seizures. One case of Mobitz Type I heart block has been reported after ingesting pokeweed, but is believed to be secondary to parasympathetic effects from prolonged vomiting. Symptoms may last up to 48 h. As few as 10 berries can result in toxic effects. Dermal exposures result in irritation, pain, and the sensation of heat.

Clinical Management

Symptoms usually resolve within 24 h. In significant exposures, treatment should include gastric lavage (depending on time since exposure) followed by activated charcoal. Symptomatic and supportive care should include rehydration and correction of electrolyte imbalance. Promethazine may decrease gastrointestinal symptoms. There is no antidote for exposure to pokeweed.

See also: Charcoal; Gastrointestinal System.

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Pollutant Release and Transfer Registers (PRTRs)

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Background

Over the past decade, the public's right to know has moved to the forefront of environmental policy-making and action. Principle 10 of the Rio Declaration, articulated at the 1992 UN Conference on Environment and Development (UNCED), popularly known as the 'Earth Summit', the Organisation for Economic Cooperation and Development (OECD) Council Recommendation (1996), and the Aarhus Convention (1998) all emphasize the importance of providing public access to environmental information. In most OECD countries, the involvement of the public in environmental decision-making is regarded as an important component of sustainable development. A key tool that governments are using to provide data to the public about the releases and transfers of potentially hazardous pollutants is the Pollutant Release and Transfer Register (PRTR).

What is a PRTR?

A PRTR is an environmental database or inventory of potentially harmful chemicals and/or pollutants released to air, water, and soil, and transferred off-site for treatment. According to the OECD Council Recommendation (C(96)41/FINAL), as amended by (C(2003)87), and the PRTR Protocol under the Aarhus Convention, the core elements of a PRTR system are: (1) a listing of chemicals, groups of chemicals, and other relevant pollutants that are released to the environment or transferred off-site; (2) integrated multimedia reporting of releases and transfers (to air, water, and land); (3) reporting by source, covering point sources and nonpoint sources, where appropriate; (4) periodic reporting (preferably annually); and (5) making data available to the public. A PRTR brings together in one place information about what pollutants are being released, where, how much, and by whom.

Each country has its own set of requirements for reporting. However, releases from nonpoint sources, although contributing a large share to any industrialized country's pollution burden, are so far included only in a limited number of PRTR systems. These nonpoint, or diffuse, sources include area sources (e.g., residential wood combustion, dry cleaners), mobile sources (e.g., automobiles, aircrafts, trains), biogenic sources (e.g., vegetation and microbial activity), and geogenic sources (e.g., soil erosion and volcanoes).

PRTR History

The OECD Council Recommendation (1996) and the subsequent Guidance Manual (1996) provided a catalyst for the development of PRTRs across the OECD countries and elsewhere. Since 1996, the number of OECD countries with operating PRTR systems has more than doubled. By 2004 at least 14 OECD countries had an operational PRTR in place (Australia, Canada, Denmark, Hungary, Ireland, Japan, Korea, Mexico, the Netherlands, Norway, Slovak Republic, Sweden, United Kingdom, and the United States). Many more countries, within the OECD and beyond, have already taken concrete steps toward the establishment of a PRTR.

Since the 'Earth Summit', there has been a more general call for information exchange on toxic chemicals and chemical risks. The Aarhus Convention created a framework and process for the potential integration of current national PRTRs, cleaner production activities, and improvement of the 'right to know' processes in general. A Working Group on PRTRs under the Convention was established in 2000 and charged with the task of preparing a legally binding instrument, a Protocol on PRTRs. The Protocol would be open to all States,

*The opinions expressed in this paper are those of the authors and do not necessarily represent the views of the OECD or of the member governments.

whether or not Parties to the Convention. The Protocol was formally adopted and signed at the Fifth Ministerial Conference, 'Environment for Europe', in Kiev, Ukraine, May 21, 2003. More than 30 States took part in the negotiations and 36 countries and the European Community signed the Protocol. Also, a new Working Group on PRTRs was established at the Kiev meeting, and its first meeting took place in February 2004 in Geneva.

Some PRTRs have been up and running for many years and even predate the Earth Summit, others have been initiated more recently, and yet others are still in the planning stages. This overview takes a closer look at some of the more fully developed PRTRs, while making reference also to those in earlier phases of development.

North America

United States' Toxics Release Inventory (TRI)

In 1984, a deadly cloud of methyl isocyanate killed thousands of people in Bhopal, India. Shortly thereafter, there was a serious chemical release at a sister plant in West Virginia. These incidents underscored demands by industrial workers and communities in several states for information on hazardous materials. Public interest and environmental organizations around the United States accelerated demands for information on toxic chemicals being released to the environment. Against this backdrop, the Emergency Planning and Community Right-to-Know Act (EPCRA) was enacted in 1986.

EPCRA's primary purpose is to inform citizens of chemical hazards in their communities. Sections 311 and 312 of EPCRA require businesses to report the locations and quantities of chemicals stored on-site to State and local governments in order to help communities prepare to respond to chemical spills and similar emergencies. EPCRA Section 313 requires the US Environmental Protection Agency (EPA) and the States to annually collect data on releases and transfers of certain toxic chemicals from industrial facilities, and make the data available to the public in the Toxics Release Inventory (TRI).

Reporting year 2002 is the 16th year that TRI data has been collected (2002 data were released by the EPA in June 2004). The amount and nature of data have changed over the years. Its initial list of chemicals has more than doubled to some 650. Passage of the Pollution Prevention Act of 1990 broadened the information TRI collects to include off-site transfers to recycling and energy recovery as well as on-site management of toxic chemicals. Beginning with reporting year 2000, thresholds were lowered for persistent, bioaccumulative toxic (PBT) chemicals. The SIC codes (Standard Industrial Classification System) covered have been expanded to include other industry sectors not initially covered, such as metal and coal mining, electrical utilities that combust coal and oil, and hazardous waste treatment facilities, as well as federal facilities.

EPA offers TRI data back through 1988 via its TRI Explorer search engine. TRI data are also offered from 1995 onwards on the National Library of Medicine's (NLM) TOXNET system. Being part of TOXNET allows easy linkages between TRI and other NLM files, such as the Hazardous Substances Data Bank and TOXLINE, containing health and environmental effects information. NLM has also implemented a TRI mapping feature called TOX-MAP that allows for geographic visualization of TRI data. An interesting value-added version of TRI is Scorecard, a project of Environmental Defense. Scorecard uses the most current TRI data and integrates it with information from other databases, including EPA's National Emissions Trends (NET) database and the Canadian National Pollutant Release Inventory (see below). Scorecard actually covers ~7000 chemicals, including the TRI set. Their full complement includes high production, toxicity, or exposure US chemicals that are part of federal or California regulatory programs. Scorecard, however, only offers TRI data from the most recent reporting year.

Canada's National Pollutant Release Inventory (NPRI)

Canada's NPRI is an outgrowth of the government's Green Plan initiative and currently falls under the renewed Canadian Environmental Protection Act (CEPA). The year 2002 was the tenth reporting year for NPRI. The 1999 renewal of the Canadian Environmental Protection Act (CEPA) included provisions that require mandatory NPRI reporting and the annual publication of a summary report. Many of the reporting requirements and thresholds are similar to the United States' TRI. Neither system requires reporting on greenhouse gases. However, the NPRI includes releases from diffuse sources, while TRI does not include this requirement. The online database search screen permits entry of terms related to the facility, chemical name or CAS registry number, province/territory/city/postal code, and SIC code (Canadian or American).

NPRI covers some 260 chemicals (in 2001). However, only 204 substances were the same in NPRI and TRI in 2001. NPRI provides information on on-site releases and off-site transfers for final disposal and other treatment. Reporting on off-site transfers to recycling and energy recovery was made

mandatory in 1998. Also, reporting on pollution prevention activities has been mandatory since 1997. However, no quantitative estimates on the achieved pollution reduction are required.

Mexico's Registro de Emisiones y Transferencia de Contaminantes

Mexico has made great strides recently in the development of its PRTR program. Voluntary reporting began with the *Registro de Emisiones y Transferencia de Contaminantes* (RETC) program. In December 2001, legislation was passed providing for a mandatory, pulicly accessible PRTR. President Fox has now signed Mexico's mandatory reporting rule and it was formally published in Mexico's *Diario Oficial* on 3 June 2004. This puts Mexico and its North American partners at the forefront of international cooperation in promoting publicly accessible pollutant release and transfer registers. Much work still remains, however, as Mexico must now formally designate the substances to be reported. This will be based upon a list of 104 chemicals under the former voluntary reporting rule.

North American Pollutant Release and Transfer Register (Commission for Environmental Cooperation (CEC)

North America is well positioned to serve as a global leader in the development and use of PRTRs nationally and regionally. Each of the three North American countries, as discussed above, has a national PRTR program. First reporting years for the United States, Canada, and Mexico were 1987, 1993, and 1997, respectively.

The CEC's North American Pollutant Release and Transfer Register project tracks and publishes information on the amounts, sources, and handling of toxic chemicals from industrial activities in North America, including analyses of trends in pollutant releases and transfers since the early days of NAFTA. Each year the CEC publishes the '*Taking Stock*' report and website, which provides a unique regional picture of pollutant data in North America, based on available data from the national PRTR systems.

In May 2002, the CEC published *Taking Stock 1999*, the sixth in series. The report featured the first-ever 5 year look at trends in pollutant releases and transfers in North America. To date, *Taking Stock* includes data from Canada and the United States only. Comparable data from Mexico are not yet available. Since the start of the CEC PRTR project, there has been roughly a 50% increase in the amount of data that are comparable between the Canadian and US PRTRs. The most recent '*Taking Stock 2001*' was released in June 2004, focusing on the releases of toxic substances to the air and trends of releases and tansfers in 1995–2001.

Europe

The Netherlands

The Dutch PRTR is a bit different from other PRTRs and comprises the inventory, analysis, localization, and presentation of emission data of both industrial and nonindustrial sources in the Netherlands. The PRTR is used as the national instrument to monitor the emissions from all sources to air, water, soil, and off-site transfers as waste. In total, some 800 substances are included in the Dutch PRTR. Data cover industry, public utilities, traffic, households, agriculture, and natural sources, and it is to some extent open to public. The emission data are partly updated every year and some 170 most important substances are covered in a report that is published annually in close cooperation with all actors in the field.

Sweden

The Swedish PRTR system (KUR) contains annual information on emissions of a number of chemical substances and groups of substances by large facilities. The creation of a register is one step in the EPA's program to improve public access to information on national emissions and also to comply with international agreements entered into by Sweden. The figures in the PRTR are taken from annual facility reports and are mainly used by the supervisory authorities. Only IPPC (EC Directive on Integrated Pollution Prevention and Control) facilities report emissions of chemical substances and groups of substances. IPPC facilities are large facilities with a capacity above certain thresholds. Small and medium size enterprises (SMEs) are therefore not included in the register, neither are emissions from diffuse sources (e.g., the use of pesticides in agriculture). However, releases from products are to some extent covered in the KUR. The number of facilities currently listed in the register is 1050. All figures are reported as total emissions per year and are not related to production volumes. This implies that available emission figures cannot be the basis for a judgment of the environmental impact of a facility; neither can they be compared with other facilities operating under different conditions. The PRTR register contains 70 substances/groups of substances in total. The selection is based on the requirements of the EPER-reporting (European Pollutant Emission Register) and those substances prioritized by OSPAR (The Convention for the Protection of the Marine Environment

of the North-East Atlantic) in 2000 as substances of concern.

United Kingdom

The United Kingdom's Pollution Inventory (formerly Chemical Release Inventory) contains details on large industrial sites as designated in the 1990 Environmental Protection Act. Local authorities regulate smaller sites. Data collection started in 1991. Presently reporting covers some 180 chemicals, including greenhouse gases and releases from diffuse sources. Inclusion of emission data from landfill sites and waste transfer stations is a new feature for 2002 data. It is currently optional for emitters of radioactive substances to report to the pollution inventory. Over the next few years reporting to the inventory of activities involving radioactive substances is likely to become compulsory. The food and drink, surface coating, and intensive agriculture industries will also begin to provide emissions data to the inventory over the next few years. The pollution inventory has been adapted to meet the reporting requirements of the European Pollutant Emission Register.

European Pollutant Emission Register

In July 2000, the European Commission adopted a decision on the implementation of a European Pollutant Emission Register (EPER) according to Article 15 of Council Directive 96/61/EC concerning Integrated Pollution Prevention and Control (IPPC). The general purpose of the IPPC Directive is to reduce pollution by industry and to control emissions from larger facilities. National governments of all EU Member States are required to maintain inventories of emission data from specified industrial sources and to report emissions from individual facilities to the European Commission. The reported data will be made accessible in a public register (EPER), which is intended to provide environmental information on major industrial activities. EU Member States were required to submit their first report in June 2003 covering emissions in 2001. The next report will be delivered in June 2006 and will cover emissions in 2004. The present EPER can be considered as a first step toward the development of a fully integrated PRTR for Europe according to the requirements of the PRTR Protocol under the Aarhus Convention.

The objectives of the EPER are: (1) collection of comparable emission data from ~20 000 individual polluting industrial sources and activities as specified in the IPPC Directive; (2) storage of the reported data in a database or register (EPER), which is publicly accessible; the register relates to emissions to air and water for 50 major pollutants; and (3) dissemination of the registered data to the public by written reports and the Internet.

Every 3 years, the European Commission will publish a report on the inventoried emissions and their individual sources. For the first time, it will be possible for the public to compare emissions from individual facilities, industrial sectors, or countries. Governments will use the EPER to monitor progress of achievements by industry in meeting environmental targets in national or international agreements or protocols.

Other Countries

Australia's National Pollutant Inventory (NPI)

Australia's NPI is an Internet database designed to provide the community, industry, and government with information on the types and amounts of certain substances emitted to the environment. In total, 90 substances are reported to the NPI. A limited reporting started in 1998–99, but the coverage of all present 90 substances commenced in 2001–02. Greenhouse gases, ozone depleting substances, and transfers of waste/chemicals are not reported to the NPI. However, releases from diffuse sources will be included on the database.

Japan

Japan published in March 2003 its first PRTR report for 2001 data on 354 chemicals based on a legislative framework. The report includes release and transfer data submitted by industry for 35 000 facilities and estimated release data for diffuse sources. Information is available in English, also for 2002.

PRTRs under Development

The foregoing discussion has, for the most part, looked at fully developed PRTRs within a fairly strict context as defined by the OECD and various international conventions. It should be noted that a larger array of countries have in place pollution inventories or a PRTR under development or consideration. Among these are all European Union Member States, Brazil, Bulgaria, Chile, Croatia, Cuba, Egypt, Kazakhstan, Moldova, Romania, Russia, South Africa, Switzerland, and Thailand. These countries, and many others, may or may not ultimately adhere to the requirements set up for a full-scale PRTR, but their efforts to report to the public on pollution are clearly in the right direction.

See also: Environmental Protection Agency, US; National Library of Medicine/TEHIP; Organisation for Economic Cooperation and Development.

Relevant Websites

http://www.environment-agency.gov.uk – Environment Agency Pollution Inventory, UK.
http://europa.eu.int – European Pollutant Emission Register.
http://www.emissieregistratie.nl – Milieumonitor, The Netherlands.
http://www.npi.gov.au – National Pollutant Inventory, Australia.
http://www.ec.gc.ca – National Pollutant Release Inventory, Canada (NPRI).
http://www.cec.org – North American Pollutant Release and Transfer Register.
http://www.oecd.org – OECD Pollutant Release and Transfer Registers.
http://www.prtr.nite.go.jp – PRTR, Japan.
http://www.naturvardsverket.se – Sweden's PRTR (KUR).
http://www.epa.gov – Toxics Release Inventory, US (TRI).
http://www.unece.org – United Nations Economic Commission for Europe (UNECE), the Protocol on Pollutant Release and Transfer Registers to the Convention on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters (Aarhus Convention).

Pollution Prevention Act, US

Shayne C Gad

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- Agency: US Environmental Protection Agency
- Year Passed: 1990
- Groups Regulated: Industrial manufacturers

Synopsis of Law

Rather than continue to spend millions of dollars annually to control the millions of tons of pollution each year, Congress decided to encourage industry to reduce source pollution through cost-effective changes in production, operation, and use of raw materials. These actions prevent pollution, and can also reduce the amount of raw materials used, limit liabilities of compliant industries, and reduce risks to workers as well as to the environment.

Prior to passage of the Pollution Prevention Act (PPA), control efforts within industry were reactive, focusing on treatment and disposal of waste, with pollution prevention also referred to as 'P2'. The act proposed a front-end approach to pollution control, reducing the amount of materials entering the production process. It also suggested technical support to business in order to put source reduction into practice. The policy states the following:

The Congress hereby declares it is to be the national policy of the United States that pollution should be prevented or reduced at the source whenever feasible; pollution that cannot be prevented should be recycled in an environmentally safe manner, whenever feasible; and disposal or other release into the environment should be employed only as a last resort and should be conducted in an environmentally safe manner.

Under the PPA, the Environmental Protection Agency (EPA) established an office responsible for creating standards to measure source reduction, ensuring that EPA policy is consistent with this initiative, and providing the public with such information. The act also established a Source Reduction Clearinghouse to promote industry efforts by providing information and workshops, helping set measurable goals, and establishing incentive and reward systems for efforts or innovations. Incentive systems included matching grants to states to establish their own source reduction programs.

The PPA also included specific source reduction actions in conjunction with the businesses required to file an annual toxic chemical release form under the Superfund Amendments Reauthorization Act. The additional toxic chemical source reduction and recycling report documents the amount of the chemical entering the waste stream, the amount that is recycled, and efforts to reduce source use. In turn, EPA is required to provide a detailed evaluation report of the source reduction program to Congress every 2 years.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Comprehensive Environmental Response, Compensation, and Liability Act, US; National Environmental Policy Act, US; Toxic Substances Control Act, US.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency (EPA), Pollution Prevention. See also: Pollution Prevention Act of 1990.

Pollution, Air

Terry Gordon

Types and Sources of Air Pollutants

Both man-made and natural sources contribute to the particles and gases that pollute our ambient environment. Episodic natural events, such as fires, wind erosion, and volcanic eruptions, produce considerable amounts of particulate matter and gases including mineral ash, pyrolysis products of combustion, carbon monoxide, and carbon dioxide. Although these 'natural' particles and gases can have significant global effects such as short- and long-term alterations in weather conditions, little is known about the health effects resulting from inhalation of these materials. Obviously, little can be done to alter the contribution of pollutants from natural sources such as volcanic eruptions. A great deal more information is known regarding the generation, underlying chemistry, and adverse health effects of air pollutants from man-made (anthropogenic) sources. This continued interest in understanding the generation and health effects of man-made air pollutants stems largely from the fact that measures can be taken to control these pollutants and thus modify adverse health effects. Such control measures are often promulgated and regulated at the governmental level and are based on comparisons of the tangible (e.g., increased morbidity and mortality and financial) and intangible (e.g., quality of life) costs to society and the environment versus the cost of control measures.

Both stationary and mobile sources contribute to the particulate matter and gases that make up polluted urban and rural environments. Fossil fuel-powered electricity plants, heat generators, and waste incineration sites represent the major stationary point sources of pollution. Industrial processing plants such as smelters also produce a wide range of particulate matter and gases. Because these pollution sources are stationary, significant differences in both the quantity and the makeup of regional air pollution can occur. For example, sulfur is present in fossil fuels (primarily in coal) used for heat production in the northeastern United States. The resulting acid components of ambient particles in the northeastern United States are thus primarily sulfates, whereas the acidic fraction of airborne particles in California is primarily nitrates.

A major portion of ambient air pollution results from gasoline- and diesel-powered automobiles and trucks. The mobile and ubiquitous nature of motor vehicles makes their pollution products widespread. While the presence of lead in automobile exhaust has been virtually eliminated, the contribution of motor vehicles to ambient concentrations of nitrogen oxides, hydrocarbons, and carbon monoxide in urban atmospheres is great. Indeed, transportation sources are responsible for 45% of the nitrogen oxides and 80% of the carbon monoxide emissions in the United States. Sunlight can drive a series of chemical reactions involving nitrogen oxides and hydrocarbons (a process known as photochemical oxidation), which result in secondary pollutants such as ozone. Though a secondary pollutant, ambient ground-level ozone is a major health concern for both urban and rural dwellers and produces agricultural crop losses and tree damage approaching several billions of dollars each year.

Regardless of the source of primary and secondary air pollutants, meteorological conditions play a significant role in the formation and transport of gases and particulate matter. One well-documented example occurs when sulfur dioxide released from industrial point sources in the northeastern United States forms acidic particles. These acidic precursors undergo long-range transport resulting in adverse effects due to acid rain in southern Canada. Thus, movement of masses of air can reduce ambient levels of pollutants in one region at the expense of air quality in another region. Meteorologic conditions also influence the creation of photochemical smog. Inversions occur when cooler air is trapped beneath a blanket of warm air, resulting in stagnant weather patterns. In southern California, the combination of inversions, sunlight, and motor vehicle emissions drives the photochemical reaction of trapped precursors and results in high ozone concentrations.

Regulation of Air Pollution

Reductions in ambient concentrations of some but certainly not all air pollutants have taken place over the past decade. In the United States, the US Environmental Protection Agency (EPA) is the primary agency responsible for promulgating and regulating air pollution standards. National ambient air quality standards (NAAQS) have been established for six classes of outdoor pollutants: lead, carbon monoxide, ozone, nitrogen dioxide, sulfur dioxide, and particulate matter. These standards (**Table 1**) are periodically reviewed and updated based on currently available data regarding adverse health effects. Bases on new evidence, the standards for ozone and

Table 1 National ambient air quality standards (NAAQS)

Pollutant	*Primary standard*	*Type of average*	*Nonattainment population*[a]
Lead	$1.5\,\mu g\,m^{-3}$	Quarterly average	0.01
Carbon monoxide	35 ppm ($40\,mg\,m^{-3}$)	1 hour	19.0
	9 ppm ($10\,mg\,m^{-3}$)	8 hour	
Ozone	0.08 ppm	8 hour	>100
	0.12 ppm	1 hour	>100
Nitrogen dioxide	0.053 ppm ($100\,\mu g\,m^{-3}$)	1 year	0
Sulfur dioxide	0.14 ppm ($365\,\mu g\,m^{-3}$)	24 hour	2.7
	0.03 ppm ($80\,\mu g\,m^{-3}$)	1 year	
Particulate matter (PM)			
PM 10	$150\,\mu g\,m^{-3}$	24 hour	29.2
	$50\,\mu g\,m^{-3}$	1 year	
PM 2.5	$65\,\mu g\,m^{-3}$	24 hour	data not available
	$15\,\mu g\,m^{-3}$	1 year	data not available

[a] Millions of persons living in counties with air quality levels not meeting NAAQS in 1992.

particulate matter were recently changed. The ozone standard was amended to include an 8 h average to reflect results of clinical studies that demonstrated cellular and biochemical pulmonary changes in exercising human subjects exposed to 0.10 ppm ozone for several hours. The particulate matter standard was also changed to include a standard for fine particles. Those metropolitan sites in which a particular pollution level exceeds the NAAQS are designated as nonattainment areas. Currently, more than half of the population in the United States lives in a nonattainment area for at least one regulated air pollutants (**Table 1**). The Air Quality Index, a more general ranking of air quality, is used for the daily reporting of air quality to the layperson via newspapers and telecasts in most major cities (**Table 2**). It is noteworthy that five pollutants account for ~98% of the total mass of air pollution and that gaseous emissions make up the majority of air pollutants (**Table 3**). These figures do not take into account fugitive dust emissions that are not inhalable (i.e., coarse dust which is generated by wind erosion, farming, construction, and mining and quickly settles out due to its large size). Despite extensive legislation (three Clean Air Acts within the past 30 years) to set primary and secondary standards for the protection of human health and the environment, respectively, considerable numbers of air toxics are currently unregulated.

Control and reduction of ambient air pollutants in the United States has met with varying degrees of success. Unleaded gasoline now accounts for 99% of all gasoline sales. This change has virtually eliminated mobile sources as emitters of lead and reduced ambient lead levels by more than 75%. Likewise, stationary point sources of lead emissions, primarily industrial smelters, have dropped by more than 90% over the past three decades, although significant problems exist with individual smelters. Over the past decade, programs in reducing all Criteria pollutants have been successful. The reduction program for nitrogen dioxide has been partially successful only recently. A slight decrease in total nitrogen dioxide emissions occurred during a time period in which total motor vehicle miles in the United States increased substantially. A lack of major changes in ambient levels of nitrogen oxides and volatile organic compounds has resulted in only marginal success in reducing ambient levels of the secondary pollutant ozone. The long-term trend for ozone concentrations

Table 2 Air Quality index values

Index value color	*Descriptor*[a]	*Level of health concern*
301–500	Maroon	Hazardous
201–300	Purple	Very unhealthy
151–200	Red	Unhealthy
101–150	Orange	Unhealthy for sensitive groups
51–100	Yellow	Moderate
0–50	Green	Good

[a] General health descriptor used in the lay press and media.

Table 3 Emission estimates for the United States (2003)

Pollutant	*Total emissions*[a]	*10 Year trend*[b]
Carbon monoxide	93.7	21% decrease
Nitrogen dioxide	20.5	12% decrease
Sulfur dioxide	15.8	31% decrease
Volatile organics	15.4	25% decrease
Particulate matter (PM)		
PM 10	2.3	22% decrease
PM 2.5	1.8	17% decrease
Lead	0.003	5% decrease

[a] Millions of tons/year for 2003.
[b] Percentage change in estimated emissions between 1993 and 2003.

is downward, although meteorologic conditions appear to modify peak ozone levels monitored throughout the United States (high ozone levels have been measured during summers with hot, dry conditions and low levels measured during cool summers). In summary, legislative efforts have been successful in reducing ambient air pollution over the past three decades. Reduction of emissions from mobile sources such as motor vehicles has met with the greatest success, while reducing emissions from stationary point sources has often proven difficult as a result of conflicting interests of business, state and federal regulations, and enforcement agencies. While progress has been made in reducing ambient oxidant pollutants, it should be noted that a significant problem still exists and in the United States more than 100 million people lived in countries that exceeded the ozone standard in 2003.

Health Effects of Air Pollution

There is mounting evidence that a number of air pollutants play a causal role in adverse health effects and that copollutants such as acid aerosols, ozone, and nitrogen oxides can have synergistic effects with each other. The major challenges for environmental health scientists are to identify the acute and long-term adverse health effects of ambient air pollution, pinpoint the relevant concentrations at which these effects occur, and determine sensitive subpopulations. This latter point is important in developing risk assessment paradigms as current federal legislation in the United States acknowledges the importance of protecting the health and welfare of all individuals.

In general, a great deal more is known about the acute effects of ambient air pollutants than is known about the chronic effects. The following discussion will outline the findings of epidemiologic, controlled clinical and animal studies that have examined the adverse health effects of outdoor air pollutants. More detailed information can be found in the Further Reading section.

Ozone

Exposure to ozone in the ambient air is a major health concern in urban and rural communities throughout the United States. Current strategies to control the exposure of the general population to this highly reactive gas have been only marginally successful. Indeed, tens of millions of people reside in communities in which the 1 h and 8 h ozone NAAQS has been exceeded.

Substantial evidence from epidemiological and controlled clinical studies suggests that acute ozone exposure at current ambient levels is associated with adverse respiratory effects in human subjects. The functional and symptomatic response of human subjects to inhaled ozone, however, appears to be highly variable. After performing moderate exercise during a single 6.6 h exposure to 0.12 ppm ozone, the change in forced expiratory volume in 1 s (FEV1) ranged from no decrement to -39% in healthy adult volunteers. The decrement in FEV1 and the increase in respiratory symptoms were dose dependent, with some volunteers responding to as little as 0.08 ppm ozone. Significant increases in the airway responsiveness to inhaled methacholine have also been observed after exposure to near-ambient ozone concentrations in laboratory studies. These functional effects are accompanied by an inflammatory response that occurs shortly after exposure and persists for at least 1 day. An influx of neutrophils and an increase in a number of mediators, including eicosanoids, neutrophil elastase, and cytokines, were measured in bronchoalveolar lavage fluid recovered from subjects exposed to near-ambient concentrations of ozone.

The adverse functional effects observed in controlled clinical studies are similar to those reported during exposure to ambient air. Decrements in lung function have been noted in a series of camp studies in which children were exposed to ambient ozone during normal outdoor play activity. Compared to controlled chamber studies, greater decrements in lung function were observed in the camp studies when the data were normalized for ozone concentration. A number of factors may explain the greater response in the camp study, but the most likely reason is the simultaneous exposure to ambient copollutants such as acid aerosols. Epidemiologic studies have found strong correlations between respiratory symptoms, such as cough, throat irritation, and chest discomfort, and ambient ozone levels. Exacerbation of asthma, increases in hospital admissions for respiratory infections, and excess mortality have also been reported to be associated with oxidant air pollution episodes. Thus, a number of epidemiologic, field, and clinical studies provide evidence that adverse respiratory effects occur after acute exposure to ozone at or below the current NAAQS. Animal studies have corroborated these findings, although test animals in general appear to be less sensitive than human subjects to ozone.

Despite ample evidence for an acute response to ozone in human subjects, relatively little is known about the cumulative effects of acute injury and possible progression to adverse chronic lung

dysfunction. Many studies have found that the functional decrements and symptoms observed after a single exposure to ozone lessen or are absent upon repeated exposure. The phenomenon of tolerance to the acute effects of ozone was described decades ago in animal studies. Clinical studies examining ozone-induced tolerance have clearly demonstrated that functional and inflammatory changes that are typically observed after the first day of exposure are attenuated by the second or third day of exposure for both normal and asthmatic subjects. Interestingly, the development of tolerance after repeated ozone exposure appears to occur for some functional parameters but not for others. For example, it has been observed in healthy adults that despite the rapid development of tolerance to decrements in FEV1 following repeated ozone exposure, ozone-induced increases in airway responsiveness to methylcholine were sustained throughout the five daily exposures. In addition, increases in markers of inflammation, such as an influx of neutrophils, are attenuated after five daily exposures. Markers of cell injury, however, do not appear to adapt as readily to repeated ozone exposure. These latter findings and similar results observed in animal studies suggest that although the respiratory tract is able to adapt to a major portion of the acute effects of ozone, long-term consequences may occur.

The few population-based and animal toxicology studies examining the chronic pulmonary effects of ozone suggest that ozone may be associated with long-term reductions in lung function and pathological changes. Animal studies using concentrations above the current NAAQS reveal that the centriacinar region of the airways and the nasal cavity are the most sensitive to pathological changes induced by chronic ozone. Epidemiologic studies have demonstrated that chronic exposure to ozone is associated with decrements in lung function and increases in the incidence and severity of asthma. The ability of these epidemiologic studies to establish cause and effect is hampered by confounding factors such as copollutants. Thus, the question whether chronic adverse health effects are clearly associated with ambient ozone exposure has not been answered at this time.

Sulfur Oxides

Significant and, on occasion, disastrous adverse health effects have accompanied acute air pollution episodes involving reducing-type pollutants. In the middle of this century, meteorologic inversion conditions resulted in high levels of particulate matter and sulfur dioxide in the Meuse Valley in Belgium, Donora in Pennsylvania, and London. Excess mortality accompanied each of these pollution episodes and has been attributed to the smoke and sulfur dioxide generated by fossil fuel combustion. A number of recent epidemiologic, clinical, and animal studies have confirmed that both particulate matter and sulfur oxides produce adverse health effects. These adverse effects have been observed during pollution episodes in which the gas and particle concentrations do not approach the magnitude of the three incidences mentioned previously. Delineating the relative contribution of particulate matter and sulfur oxides to these adverse effects is difficult because of the chemicophysical association of sulfur oxides and particles. This section is limited to the current state of knowledge on sulfur oxides and acid aerosol-related health effects. The following section will discuss particulate matter-related effects.

Sulfur dioxide is generated during the combustion of fossil fuels (primarily coal) containing traces of sulfur. Controlled laboratory studies using human subjects and test animals have demonstrated that sulfur dioxide can produce functional and pathological changes. These changes include increases in airway resistance and in mucus production. In general, the concentrations of sulfur dioxide necessary to produce these changes are greater than those encountered in the ambient environment. A notable exception is the bronchoconstrictive effect of sulfur dioxide on atopic and asthmatic subjects. Inhalation of 0.4 or 0.5 ppm sulfur dioxide in combination with moderate exercise causes substantial bronchoconstriction, shortness of breath, and cough in these sensitive individuals. Similar changes occur in normal (nonatopic) individuals only after exposure to at least a magnitude greater concentration of sulfur dioxide.

Despite the clear evidence of a subpopulation of individuals sensitive to near-ambient peak levels of sulfur dioxide, the two-decade-old NAAQS for sulfur dioxide has not been changed nor has a short-term peak standard been instituted. A considerably greater amount of attention has been placed on the contribution of airborne particulates, particularly those associated with sulfur oxides, to adverse health effects. The carbon-, mineral-, and heavy metal-based particles produced during fossil fuel combustion and smelting promote the conversion of sulfur dioxide to sulfuric acid. Recognition of sulfur dioxide–particle interactions comes as a result of findings garnered from a number of animal studies and the characterization of sulfuric acid, ammonium sulfate, and ammonium bisulfate associated with atmospheric particles. The importance of the coexistence of sulfur oxides and particulate matter is reflected in the

difficulty of epidemiology studies to separate the contribution of each pollutant to adverse health effects.

Epidemiological evidence from both Europe and North America suggests that acid aerosols formed by gas–particle interactions in the atmosphere play a major role in the adverse health effects seen during severe and moderate pollution episodes. The increases in mortality observed in London from 1958 to 1972 were more closely associated with acid aerosol concentrations than other pollutants such as smoke and sulfur dioxide. In the United States and Canada, cross-sectional analyses have demonstrated that ambient sulfate concentrations are better than indices of particulate concentrations as a predictor of excess mortality and hospital admissions due to air pollution. A prospective cohort study, known as the Six Cities Study, has found that increased mortality from cardiopulmonary deaths and lung cancer were strongly associated with sulfate and particulate concentrations. This same study has demonstrated that the incidence of bronchitis in children is correlated with ambient levels of acid aerosols. Similarly, in northern Europe, an acidic pollution episode in 1985 has been linked with significant excesses in respiratory mortality and morbidity and with persistent decrements in pulmonary function in children. In summary, a large body of evidence suggests that acid aerosols play a significant role in the adverse health effects attributed to air pollution.

Epidemiology studies are limited in their ability to establish direct cause and effect relationships. Many confounding factors such as smoking, occupational exposure, and copollutants such as ozone may contribute to observed effects and, for this reason, investigators have exposed human volunteers and animals to acid aerosols under controlled conditions.

Animal studies have demonstrated that exposure to near-ambient concentrations of sulfuric acid produces both conducting airway and alveolar changes, including increased airway resistance, airway hyperresponsiveness, and alterations in clearance mechanisms and macrophage function. Controlled human exposures to acid aerosols, however, have demonstrated few pulmonary effects at concentrations below 500–1000 $\mu g\,m^{-3}$. The adverse effects reported to occur after acute exposures to sulfuric acid aerosols have largely been observed in atopic subjects, are small in magnitude, and are readily reversible. Therefore, a research need has developed to explain the difference between the results of epidemiological studies and the paucity of data demonstrating adverse health effects in controlled human studies. One possible cause of this discrepancy is the type of acid aerosols used in the laboratory studies. Although pure sulfuric acid droplets are used almost exclusively in controlled exposures, ambient acid aerosols are chemically complex and are proposed to be composed of a core consisting of carbon, minerals, or heavy metals surrounded by acidic (sulfuric or nitric acid) surface material. Thus, knowing which chemical species is responsible for acid aerosol-induced adverse health effects is fundamental in developing proper control strategies for reducing air pollutants at their source.

Particulate Matter

Particulate emissions are by-products of fuel combustion, industrial processes, and motor vehicles and are believed to have a significant potential for causing adverse health effects. Carbonaceous material present in atmospheric aerosols is a combination of elemental carbon and organic and inorganic compounds. Particulate matter may also consist of fly ash, minerals, or road dust and contain traces of a number of heavy metals. Population-based studies have consistently found that the association between adverse respiratory effects and particulate concentrations occurs in a number of regions throughout the United States. This association is strongest for PM_{10} and $PM_{2.5}$ indices (particulate matter less than 10 and 2.5 μm in diameter, respectively). The observed adverse effects include increases in total mortality, mortality due to respiratory and cardiovascular causes, chronic bronchitis, and hospital visits and admissions for asthma. Elderly or unhealthy individuals and infants appear to comprise subpopulations that are most sensitive to the adverse health effects of PM.

Because the chemical makeup of particles varies greatly from region to region, the identification of the factor(s) responsible for the adverse health effects associated with PM is merely conjecture at this time. Few controlled human studies have used realistic particles and thus have contributed little to our understanding of particle-induced injury. Animal studies have been somewhat more productive and have demonstrated that particle-induced lung injury may be dependent on particle size, the presence of transition metals, and surface acid content. Effects of exposure to carbonaceous particles have been reported in studies investigating the toxicological significance of automotive diesel engine exhaust and fly ash. Long-term exposures to automotive diesel engine exhaust were found to cause focal fibrotic and proliferative lung disease accompanied by a progressive accumulation of soot in the lungs and impaired alveolar clearance. Exposure of rats to high concentrations of diesel exhaust was also associated

with an increase in lung cancer. Only minimal lung injury and irritant potency have been noted after repeated exposure of test animals to resuspended fly ash. Animal studies using freshly formed fly ash suggest that physical, chemical, and especially surface characteristics of the fly ash change substantially during the collection, storage, and resuspension processes. More recent studies have used concentrated ambient PM to examine the biological plausibility and mechanisms underlying the adverse health effects of PM. These studies include both acute animal and human controlled exposure studies using individual components (e.g., ultrafine carbon particles) as well as concentrated ambient PM. Additionally, repeated exposure studies of concentrated ambient PM in test animals and *in vitro* studies have been performed to delineate the components of PM, which may contribute to the respiratory and cardiac effects observed in epidemiology studies. As yet, animal toxicology and controlled clinical studies have not yet provided clear answers to questions regarding the factor(s) responsible for the adverse health effects temporally associated with PM pollution episodes.

Nitrogen Oxides

Nitrogen oxide is produced in high-temperature combustion processes and is rapidly converted to nitrogen dioxide. Nitrogen dioxide is an irritant gas that produces oxidant lung injury similar to that produced by ozone. Nitrogen dioxide is far less potent than ozone and few functional or pathological changes have been observed in animals exposed to <0.5 ppm nitrogen dioxide. Pathological changes occur primarily in the terminal bronchioles and the alveolar region, although changes in mucociliary clearance have been observed during chronic exposures. Both long- and short-term exposures to nitrogen dioxide can increase the susceptibility of animals to respiratory infection. Studied in a number of animal species, this effect includes increased mortality, decreased survival time, and impaired clearance of instilled pathogens. These findings reflect those obtained in epidemiologic studies that have found an increased incidence of respiratory infections in homes with gas appliances.

Nitrogen oxides other than nitrogen dioxide have been studied for possible adverse health effects. Chemical analysis of ambient aerosols collected in southern California has revealed that nitrates exhibit particularly high values compared to other parts of the United States. These aerosols are generally acidic in nature and are composed of nitric acid and nitrate salts that are formed through photochemical reactions with nitrogen dioxide and other oxides of nitrogen. These forms of nitrogen oxides contribute to acid aerosol formation in the ambient air and result from particle surface–gas interactions similar to those which have been described for sulfuric acid generation. Unique to the conditions of the coastal regions of California, acid fog forms from the interaction of nitrogen oxides and fog water droplets. A paucity of toxicologic and epidemiologic data does not allow a clear assessment of the health effects of either nitric acid-based particle.

Although research has clearly demonstrated a potential for nitrogen oxides, particularly nitrogen dioxide, to have serious health consequences, few exceedances of the NAAQS occur (see **Table 1**). In general, health researchers are more concerned with (1) the key role nitrogen oxides play in the photochemical reactions which produce ozone and (2) the presence of nitrogen oxides indoors (both in occupational settings and in homes). Ambient concentrations of nitrogen oxides are generally lower than those found in grain silos and in homes using fossil fuel-consuming appliances.

Carbon Monoxide

Despite an increase in motor vehicle miles traveled over the period of 1990–2000, total emissions for carbon monoxide decreased by 29%. This dramatic change is attributed largely to controls initiated by the Federal Motor Vehicle Control Program. These figures do not reflect the additional decreases in carbon monoxide emissions that have resulted from the use of oxygenated fuels since 1992. Under the Clean Air Act of 1990, oxygenated fuels are required in all areas that do not meet the NAAQS for carbon monoxide during the winter months (when carbon monoxide levels are highest). Preliminary results of the oxygenated fuel program suggest that further decreases in carbon monoxide emissions will be achieved.

In general, ambient exposure to carbon monoxide is directly related to one's proximity to motor vehicle exhaust. Away from highways and industrial combustion processes, ambient carbon monoxide concentrations rarely exceed 1 ppm. Carbon monoxide levels can reach 3 or 4 ppm near roads and 5 ppm in the passenger compartment of automobiles. Heavier traffic conditions are typically associated with peak concentrations of 10–50 ppm. Even greater carbon monoxide concentrations can be encountered by workers in confined spaces such as tunnels. Significant exposures to carbon monoxide can also occur indoors. Levels as high as 10 000 ppm have been recorded in enclosed spaces in which a firefighter might enter. Operation of gasoline-powered equipment within a building can also result in significant

carbon monoxide levels with ill-effects (e.g., Zamboni ice cleaners in skating rinks). Importantly, significant amounts of carbon monoxide are present in cigarette smoke. In nonsmoking human subjects, carboxyhemoglobin levels do not exceed 0.4% if environmental carbon monoxide levels are zero. Carboxyhemoglobin levels in cigarette smokers, however, can range from 5% to 10%.

Carbon monoxide is classified as a chemical asphyxiant. Its detrimental effects are mediated by its ability to combine with hemoglobin and other oxygen-carrying or -utilizing proteins. By binding avidly to hemoglobin and causing the formation of carboxyhemoglobin, the carrying capacity of hemoglobin for oxygen is reduced proportionately. One of the most sensitive measures of ill-effects after carbon monoxide inhalation is neurological testing. As little as 4% carboxyhemoglobin impairs neurologic function in repetitive tasks. In patients with preexisting angina or chronic pulmonary obstruction, increases in carboxyhemoglobin levels of only 2% were found to produce quicker onset of angina and dyspnea, respectively, during exercise. Reduced night and peripheral vision accompany carboxyhemoglobin levels of 10%. As levels exceed 10%, headaches may occur and at carboxyhemoglobin levels of 20–30%, nausea and weakness ensue. Decreases in mental function, collapse, and coma are evident as carboxyhemoglobin exceeds 35%.

Thus, carbon monoxide can produce a wide range of adverse effects. The concentration of carbon monoxide encountered in urban environments is relatively low and may have little effect on normal individuals. Several subpopulations, however, may be sensitive to current ambient exposure levels of carbon monoxide. These groups include individuals with chronic obstructive pulmonary disease, exertional angina, and cardiac arrhythmias. Fetuses may also be affected by carbon monoxide. Carbon monoxide binds more tightly to fetal hemoglobin and is cleared more slowly. Animal studies have demonstrated that maternal carbon monoxide exposure can reduce birth weight and increase neonatal mortality. Epidemiologic findings appear to confirm this effect of environmental carbon monoxide exposure on fetuses, although the confounding influences of smoking and indoor sources of carbon monoxide are hard to eliminate.

Lead

Research on the health effects of chronic, low-level lead exposure is quite extensive and has been garnered from both epidemiologic and animal studies. The most critical of these adverse health effects have occurred in children and include deficits in physical and neurobehavioral development. In adults, small but consistent increases in blood pressure are significantly correlated with increases in blood lead concentrations. Acute, high-dose lead exposures result in more severe toxicological effects.

Exposure to lead can occur via a number of pathways including ingestion (drinking water, food, and soil) and inhalation. Although ingestion of lead contributes the majority of the average individual's body burden of lead, airborne lead has been estimated to be responsible for 7–40% of blood lead. The major sources of airborne lead are gasoline additives, metal smelters, and battery manufacturing/disposal. Total emission for lead has decreased dramatically over the past two decades and has reduced ambient air concentrations by 90% nationwide. The decrease in total emissions and ambient concentrations is a direct result of federal regulations issued by US EPA requiring the removal of lead from gasoline. The dramatic decrease in lead emissions has been paralleled by an equally impressive decrease in average blood lead levels, making this one of the most successful federal intervention programs in the field of environmental health. Over a 4 year period (1976–80), average blood levels decreased from ~ 15.5–$9.5\,\mu g\,dl^{-1}$. Despite the improvement in nationwide airborne lead concentrations, industrial point source release is still a problem. As of 2004, 3 areas in the United States were designated as nonattainment areas in regard to airborne lead and ~ 10 million people resided in counties that do not meet the NAAQS for lead.

Future Directions and Control Strategies

Improvements in air quality in the United States have occurred as a result of federal regulations promulgated by the Clean Air Acts of 1970 and 1990. While the decrease in emissions for some NAAQS pollutants has been impressive (e.g., lead and carbon monoxide), only minor changes have been documented for others (e.g., nitrogen and sulfur oxides and PM_{10}). Moreover, as of 2003, nonattainment regions have been identified for five of the six NAAQS pollutants. Thus, it is important to acknowledge that a major air pollution problem still exists. The lack of significant improvement in various pollutant categories occurs as a result of several factors including economics, technological limitations, inability to identify proper control strategies, and politics. Reduction of pollutant emissions from point sources, in particular, has proven to be difficult to regulate and enforce. The Clean Air Act is not a static regulation, and changes have been made. Recent promulgated changes to the New Source Review have been made to give the industry more flexibility

without damaging the environment, but opponents claim these changes will institute a delay in cleaning the nation's air. Such controversies will arise as cost/benefit analyses and politics enter into the quasi-science of risk assessment.

The reported emissions for the six pollutants with NAAQS are only a portion of the total amount of toxic substances released by mobile and point sources. While regulations and controls set in place to reduce the release of particulate matter, volatile organics, and nitrogen oxides will also reduce the emissions of many air toxics, it is estimated that 1 million tons of air toxics are released in the United States each year. Air toxics are generally defined as hazardous air pollutants, other than the six NAAQS pollutants, with the potential for causing increases in mortality or serious illnesses. The Clean Air Act Amendments of 1990 identify 189 substances requiring regulation. Regulation of these air toxics necessitates technology-based standards for reducing emissions and establishing an accidental release program. The top 10 air toxics, in terms of total emissions, are toluene, methanol, methyl ethyl ketone, xylene, chlorine, hydrochloric acid, carbon disulfide, and chlorinated alkanes and alkenes. Over a 9 year period (1987–95), a sustained downward trend in total emissions of these air toxics was obtained. It must be emphasized that provisions in the Clean Air Act Amendments of 1990 focus on point sources of air toxics emissions rather than individual substances. Thus, key source categories have been identified and are to undergo prompt regulation for reducing hazardous emissions. Examples of key emissions sources for which regulations have been developed include chemical manufacturing plants (which emit as many as 150 of the 189 hazardous air toxics), coke oven batteries, dry cleaning facilities, ethylene oxide sterilization facilities, industrial cooling towers, and chromium electroplating operations.

See also: Clean Air Act (CAA), US; Combustion Toxicology; Ecotoxicology; Environmental Toxicology; Lead; Ozone; Photochemical Oxidants; Pollution, Air Indoor; Pollution, Soil; Pollution, Water; Respiratory Tract.

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Pollution, Air Indoor

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Introduction

Indoor air quality is one of the key determinants of the acceptability of an indoor environment. Indoor air pollution in occupied buildings is often higher than outdoor air pollution. This is true for modern buildings in developed countries when significant sources of indoor air pollution exist, as well as for buildings in developing countries, particularly in rural areas, where people use open stoves for cooking and heating. The World Health Organization (WHO) has estimated that indoor air pollution from the use of solid fuels in 'high-mortality' developing countries is ranked fourth among the 10 leading risk factors as percentage causes of the global disease burden.

Half of the world's population, living mostly in developing countries, is exposed to indoor air pollution, which is estimated to cause 36% of all lower respiratory infections and 22% of chronic obstructive pulmonary disease (COPD). Indoor air pollution is a concern in the developed countries as well, where energy efficiency improvements sometimes make houses relatively airtight, reducing ventilation, and raising pollutant levels. The risk perception of people with respect to indoor versus outdoor air pollution is, however, often characterized by a lack of awareness that the indoor air environment may contain some of the same pollutants found outdoors and quite a number of different ones. Indoor air pollution is, in fact, not a new problem. When early humans discovered fire and used it to heat their shelters, they must have found that one of its undesirable side effects was production of and exposure to smoke. Attempts to provide adequate ventilation may have been made, but success was only partial in that mummified human lungs from the preindustrial age show considerable carbonaceous pigmentation.

It has become increasingly evident that the indoor environment is a significant source of personal exposure to various air contaminants, for example, formaldehyde and other volatile organic compounds (VOCs), some of which can reach fairly high concentrations. However, any health effects from exposure to indoor air pollutants are a function of the total exposure, which is related to the air pollutant concentration, exposure duration and frequency, the actual indoor compartment of exposure, and the population group exposed. For example, even when indoor concentrations are low, exposures may be of long duration and the total, or cumulative, dose can be quite high. This reflects the fact that people can spend upwards of 90% of their time indoors, be it at home, at the office, in a school, or in shopping malls or in cars, buses, trains, or other forms of public transit. In many instances, indoor sources actually provide the bulk of personal exposure to certain airborne toxicants (e.g., aldehydes) and the only source of exposure to others. Furthermore, the population exposed largely indoors is much more diverse than that exposed in occupational environments or even in ambient outdoor air. In addition to healthy adults, it can include infants, children, and people with medical conditions, all of whom may be especially vulnerable to certain toxicants.

For a long time, remaining indoors was considered to afford protection from air pollution, and early studies of indoor air quality were generally concerned with examining the ratios of indoor to outdoor concentrations of various contaminants since it was felt that indoor contaminant levels were controlled primarily by outdoor concentrations. Outdoor pollutants can indeed infiltrate indoors through cracks, windows, doors and other openings in buildings and through ventilation systems, or they be carried indoors by building occupants. However, the relative amount of an outdoor pollutant found indoors depends largely on its physicochemical properties. For example, highly reactive gases like ozone may be removed from the air in or prior to entering an indoor environment, and the resulting indoor concentrations would be much lower than those found outdoors. Indoor versus outdoor (I/O) ratios in homes reported in the literature for total suspended particulate matter (TSP) range from 0.2 to >1.0; for PM_{10} I/O ratios range from 0.4 to 1.5. In the smaller size fractions (the subscript for PM refers to the aerodynamic diameter of the particles, in microns, with the number being the high end of the aerodynamic particle diameter range of interest), annual average I/O ratios for fine particulate matter $<2.5\,\mu m$ ($PM_{2.5}$) was found close to unity in nonsmoking homes.

It is now very clear that indoor air contaminants are not totally derived from outdoor sources, but that numerous contaminants can be directly released into the indoor environment from local sources, such as cooking over an open flame, from smoking, from heating systems, from modern synthetic building and furnishing materials, from consumer products and clothing, and even from natural sources, including the normal biological activities of building occupants. Many of these contaminants have been found to occur in increasing levels over recent years due to the attempt to make homes, and other buildings, more airtight for energy conservation. This reduces the rate of air exchange between the outside (fresh air) and inside environments. For example, older homes may have air exchange rates which are 2 to 10 times greater than those found in newer houses. The result is that in newer houses, levels of contaminants can be many times higher than the concentration of these same materials in the outdoor environment, if they occur outdoors at all.

Buildings are not the only sources for exposure to indoor air contaminants. Many people spend a significant portion, often up to 5%, of their day in transit, and public transportation or walking, running, bicycle riding, etc., provide additional opportunities for exposure to various toxicants. Although the air exchange rate in most forms of public transportation is generally higher than in buildings, in many cases the number of occupants per unit volume of air is much greater. A good example of this is modern aircraft, which also may recirculate as much as 50% of the interior air, leading to enhanced concentrations of

contaminants such as ozone, carbon dioxide, and volatile constituents of heated oil and hydraulic fluids. Further, during ground operations, exhaust fumes and deicing fluids can enter into the air supply system.

The major sources of air pollutants found in buildings are described below. However, the mere presence of a potential contaminant source does not necessarily mean that exposure will ensue. This is because the extent of exposure, if any, often depends on the physical nature of a source or the manner in which it is used; an example of this is asbestos, as will be discussed later. Furthermore, the health significance of exposure to indoor pollutants may not always be clear. While many of these toxicants may have adverse effects under exposure conditions found in occupational and other environments, often much less is known about biological responses with prolonged exposures at concentrations common in indoor environments.

Sources of Indoor Air Contaminants

Combustion By-Products

One very common source of indoor air pollutants is the combustion of biomass or fossil fuels, such as in gas ranges (including pilot lights), wood-burning stoves and fireplaces, and gas and kerosene space heaters. These emit both particles and gases. Particles consist of fine ($PM_{2.5}$) and ultrafine ($PM_{0.1}$) particulate matter (PM) (the subscript number refers to the aerodynamic diameter of the particles, in microns, with the number being the high end of the aerodynamic particle diameter range of interest), carbon soot, various mineral constituents of the fuels, and organic compounds, while emitted gases include carbon monoxide, carbon dioxide, nitrogen dioxide, nitric oxide, and, depending on the fuel used, sulfur dioxide and various organics. The amounts of specific contaminants emitted vary depending on the fuel type, the combustion process used, and the nature of the appliance. For example, properly operated gas heaters and stoves emit little if any PM, while wood burning stoves emit much greater amounts. When properly used and vented, many potential contaminants from combustion sources do not remain within the indoor environment, thus becoming outdoor pollutants. However, because combustion activities tend to be episodic, short-term indoor concentrations can be quite high for unvented or improperly vented systems and interior spaces. This is particularly the case in rural areas of Southeast Asia, Africa and South America, where firewood, charcoal, and cow dung are widely used in open stoves for cooking and heating.

About half of all homes in the United States use natural gas for cooking, a typical example of unvented combustion and a major source of indoor nitrogen dioxide and carbon monoxide, especially in kitchen areas. Other generally unvented combustion sources are gas and kerosene space heaters, found in $\sim$10% of homes in the United States. Emissions from the latter are similar to those from gas-fueled devices, but particles from kerosene heaters consist of carbon onto which may be adsorbed organic chemicals (e.g., hydrocarbons), many of which show significant mutagenic activity. Wood stoves are also used for home heating in many areas, and while they are generally vented outdoors, improper venting or lack of proper seals can also result in significant indoor contamination by organic-coated carbon particles. The actual exposure to contaminants from any of these combustion sources depends on the degree of venting used while the appliance is in operation, and the extent and pattern of its use, but indoor concentrations of nitrogen dioxide and carbon monoxide are generally higher than those outdoors when significant sources are present, especially in the winter when interior ventilation tends to be reduced.

Particulate Matter

During the 1990s, suspended PM was identified as one of the most important indoor and outdoor air pollutants. Due to the different sizes of the particles that lead to different deposition patterns in the human airways, PM is mostly not a single compound like a gas but rather a mixture of compounds. Epidemiological studies of short-term exposures found that each $10\,\mu g\,m^{-3}$ increase in PM_{10} is associated with $\sim$0.4–0.6% increase in daily mortality. Somewhat larger associations are observed for cardiovascular mortality and considerably larger associations were found for respiratory mortality. PM_{10} is also associated with increased hospitalization and related health care visits for respiratory disease and, to a somewhat lesser degree, cardiovascular disease. Associations also exist with lower respiratory symptoms, exacerbation of asthma, and coughing. Small but usually statistically significant declines in lung function have been observed as well. The data used in these estimations were from American and European time series studies. A recent analysis of data in Asia has found a similar association between PM_{10} and total mortality. Similar but somewhat stronger associations have also been established for $PM_{2.5}$ and daily mortality.

A threshold for the onset of health effects due to PM could not be established from these studies. In its Guidelines for Air Quality, the WHO, for the first

time, did not derive guideline values as in past publications but quoted exposure–response relationships.

Evidence from long-term exposure studies indicates that cardiopulmonary health effects are associated with $PM_{2.5}$ and that $PM_{2.5}$ was more closely associated with health outcomes than PM_{10}. Total mortality, in general, is observed to be associated with long-term exposure to PM by ~2–4% per $5\,\mu g\,m^{-3}$ increase in $PM_{2.5}$.

These results were obtained from outdoor air investigations of PM-related health outcomes. In view of the indoor/outdoor ratios quoted above and the similarity of particle size distributions of indoor and outdoor sources, there is little doubt that these associations also apply for indoor environments. These epidemiological studies, although coherent and partially consistent, have important limitations that emerge from the investigation of people who are living in uncontrolled environments. As the biological mechanisms are poorly understood, a better understanding of the health effects due to PM requires contributions from toxicology, exposure assessment, and other disciplines.

Nitrogen Dioxide

Any high-temperature combustion process in air initially generates nitric oxide and some nitrogen dioxide, but the former becomes rapidly oxidized to the latter. Combustion processes may produce other forms of nitrogen-containing compounds, such as nitrous and nitric acid vapors; however, the toxicological significance of these is not certain.

When natural gas ranges are in operation for home cooking, indoor nitrogen dioxide levels are generally higher than those found outdoors and are always above those found in homes using electric ranges. The average daily concentration in homes using gas for cooking purposes can range from 0.05 to $0.95\,mg\,m^{-3}$, but short-term peaks of $1.9\,mg\,m^{-3}$ are not uncommon. The result is that personal exposures to nitrogen dioxide are primarily driven by indoor sources in homes using gas appliances. The highest nitrogen dioxide concentrations occur in inadequately ventilated skating rinks by emissions from gasoline or propane-powered resurfacing machines used to periodically smooth or groom the ice. Personal sampler measurements in children in Sweden showed peak hour concentrations up to 8 mg $NO_2\,m^{-3}$.

Nitrogen dioxide is an upper respiratory tract irritant and has, in some cases, been linked to a 20% increased incidence of acute respiratory infection in children residing in homes using gas for cooking. There is also some indication that nitrogen dioxide, at concentrations found in homes associated with the use of natural gas stoves, may increase symptoms of asthma, wheezing, colds, sore throats, hay fever, and absences from school in children. Studies of long-term exposure of adults to gas stoves found more frequent respiratory symptoms, including a reduction in lung function in women but not in men, with gas use. A follow-up of the consequences of gas stoves, wood stoves or fireplaces on nonsmoking asthmatics showed a close association between use of a gas stove and shortness of breath, cough, or restrictions in activity. However, there is still not enough evidence from epidemiological studies alone to establish whether the observed effects are causally related to NO_2 only. Following the precautionary principle, the WHO has derived a guideline value for NO_2 of $200\,\mu g\,m^{-3}$ for 1 h exposures, and an annual guideline value of $40\,\mu g\,m^{-3}$.

Carbon Monoxide

Carbon monoxide is produced during the incomplete combustion of carbon-containing fuels, such as natural gas, kerosene, and wood. Its production rate by gas ranges is actually greater than that for nitrogen dioxide, and indoor levels can be several times greater than those found outdoors. Concentrations ranging from 2.3 to $17\,mg\,m^{-3}$ have been found in homes using gas for cooking. In some cases, indoor levels are enhanced by carbon monoxide derived from automobiles housed in garages attached to residences or connected to office buildings.

Carbon monoxide binds very strongly to hemoglobin in red blood cells, resulting in the production of carboxyhemoglobin (COHb); this can actually be used as a marker for exposure to carbon monoxide. The presence of COHb impairs the normal transport of oxygen within the blood and can result in adverse effects on tissues, such as those in the cardiovascular and nervous systems, which have high oxygen needs.

Symptoms of acute carbon monoxide poisoning range from headache to death. Prolonged exposure can affect the body due to oxygen deprivation, a condition known as tissue hypoxia. Levels of carbon monoxide encountered indoors have been found to result in disorientation in exposed individuals. Other potential health effects of CO include neurological deficits, neurobehavioral changes, and increases in daily mortality and hospital admissions for cardiovascular diseases. Several studies showed small, statistically significant relationships between CO and daily mortality. Some studies appear to show that the association between ambient CO and mortality and hospital admissions due to cardiovascular diseases persists even at very low CO levels indicating no

threshold for the onset of these effects. It is possible that CO may have more serious health consequences than the COHb formation, and at lower levels than that mediated through elevated COHb levels. The effects of prolonged exposures to indoor concentrations of carbon monoxide on the health of normal individuals are, however, not certain.

The WHO guideline values for CO are 100, 60, 30, and 10 mg m^{-3} for exposure times of 15 min, 30 min, 1 h, and 8 h, respectively.

Smoke from Solid-Fuel Use

Solid fuels used in cooking and heating include wood, coal, lignite, peat, and dung. Half the world's population uses these burning materials. The use of wood for space heating in homes has also increased during the past 25 years in a number of areas in the United States. Smoke from solid fuels is a complex mixture of gases and particles, including PM, carbon monoxide, nitrogen dioxide, sulfur dioxide, and various organic compounds such as polycyclic aromatic hydrocarbons. The amount of each produced depends on burn rate, the type and quantity of wood used, and its moisture content. In developed countries, wood stoves are generally vented outdoors, and the newer ones even operate under negative pressure and should contribute little contamination to the indoor air environment. However, some indoor pollution may occur from faulty venting, leakage, or during nonairtight conditions such as during start-up, stoking, and reloading. There has not, however, been adequate characterization of the influence of wood combustion on indoor air quality. Furthermore, while individual constituents of wood smoke are irritants and carcinogens, the actual health hazards due to indoor exposure are unknown. There is some evidence of an increase in chronic respiratory symptoms, such as cough and wheeze, in children.

In developing countries, evidence on the health impacts of solid-fuel use has emerged. The apparent odds ratios (ORs) comparing the risk of these diseases between people living in houses using unvented biomass fuel and similar households not using such fuels are listed below. All the ORs reported are statistically significant results, mostly of multivariate analyses in which a number of potentially confounding variables were included.

Acute respiratory infections in children are the chief cause of childrens' ill health in the world and strongly associated with indoor use of solid fuels for cooking in a number of studies in Asia and Africa (OR = 2–6). COPD is strongly associated with use of solid fuels in nonsmoking women, often along with *cor pulmonale*, in studies from Latin America, South Asia, and Saudi Arabia (OR = 3.4–15). In many Chinese studies lung cancer was statistically associated with the use of coal for cooking and heating, but not biomass fuels (OR = 3–9).

There is some evidence from studies of solid-fuel use in developing countries indicating a relationship between adverse pregnancy outcomes and smoke exposure. After multivariate analyses, stillbirth has been associated with biomass fuel use by pregnant women in one Indian study (OR = 1.5) and with low birth weight in Guatemala. After multivariate analyses, TB and blindness (cataracts) have been shown to be related to use of biomass fuels in two national and two local studies in India. Unfortunately all these studies relied on the type of stove or fuel as the indicator of pollution.

Environmental Tobacco Smoke

One indoor air pollutant of concern since the 1950s in terms of preventable morbidity and mortality is tobacco smoke, which contains over 4000 different chemical compounds emitted as particles or gases. Tobacco smoke is the largest single source of air contamination in many indoor environments in developed countries and also in developing countries in indoor environments without use of open burning for cooking and heating. Environmental tobacco smoke (ETS) is the term used to describe the smoke found indoors and which consists of a combination of that emitted into air from the burning end of a cigarette, cigar, or pipe (side-stream smoke) plus the smoke that is exhaled by the smoker. Generally active smoking consumes half of a cigarette while smoldering consumes the other half.

The combustion conditions differ when a cigarette is puffed compared to when it smolders, so the actual ratios of chemical constituents in side-stream and mainstream smoke also differ, although qualitatively the materials are similar. Because the temperature of the burning cone is lower as the cigarette smolders than during active puffing, combustion is less complete in side-stream than in mainstream smoke. Consequently, side-stream smoke has higher concentrations of chemical compounds than mainstream smoke. The bulk of ETS actually consists of side-stream smoke. While the amount of these inhaled by the nonsmoker compared to the active smoker is reduced by dilution in room air, ETS is the source of numerous toxic and carcinogenic contaminants in indoor environments; some of the major ones are listed in **Table 1**. Respirable particle levels in the smoking areas of some buildings can be up to 25 times greater than those in nonsmoking areas, reaching concentrations above 300 μg m^{-3}. Nicotine,

Table 1 Major indoor air contaminants derived from environmental tobacco smoke

In vapor/gas phase	*In particulate phase*
Acetaldehyde	
Acetone	Aniline
Acrolein	Benzo(*a*)pyrene
Ammonia	Carbon
Carbon dioxide	Nicotine
Carbon monoxide	Metals (nickel, arsenic, cadmium)
Formaldehyde	Phenol
Hydrogen cyanide	
Nitrogen oxides	
Pyridine	

polycyclic aromatic hydrocarbons, carbon monoxide, formaldehyde, acetaldehyde, acrolein, nitrogen dioxide, and benzene are also significantly elevated in the homes of smokers compared to those of nonsmokers. For example, median nicotine levels monitored weekly in US smoker homes ranged between 1.4 and 3.0 $\mu g\,m^{-3}$, with maximal values between 4.4 and 28.6 $\mu g\,m^{-3}$.

Passive smoking, or involuntary smoking, is the term used to describe the inhalation of ETS by nonsmokers. The amount of smoke to which any individual is exposed is quite variable, depending on the number of sources (i.e., active smokers), the degree of building ventilation that affects dilution, and the presence of any air cleaning devices. The contribution of various indoor environments to personal exposure to ETS varies with the time-activity patterns and the individual susceptibility. For example, infants who do not attend day care are mainly exposed in the homes of smokers. Nonsmoking adults who work in offices together with smokers may be principally exposed in these offices. Studies have shown that exposures to ETS in the home are usually greater than those at the workplace.

Laboratory studies indicate that changes in ventilation rates simulating conditions expected in many residential and commercial environments during smoking do not have a significant influence on the air concentration levels of ETS components, for example, CO, NO_x, aromatic compounds, and nicotine. This suggests that efforts to reduce indoor air pollution through higher ventilation rates in buildings would not lead to a meaningful improvement of indoor air quality and is in agreement with the results of studies carried out in the United States at different hospitality venues.

Personal exposures to ETS can be assessed using biological markers in body fluids, such as saliva, blood, or urine. The presence of ETS components and their metabolites in body fluid of exposed nonsmokers strengthens the plausibility of associations between ETS exposure and disease. Biological markers of exposure to ETS have been used to estimate the prevalence of doses of potential toxic agents inhaled during involuntary smoking. At present, the most sensitive and specific of these markers are nicotine and its major metabolite, cotinine. Nicotine and cotinine are almost never present in body fluids in the absence of ETS exposure. Because the circulating half-life of nicotine is $\sim$30 min, nicotine concentrations in body fluids are representative of very recent exposures. Cotinine remains in the body for $\sim$20–30 h and, therefore, reflects an equilibrium reached by daily exposure to ETS. Its assessment clearly indicates that passive smoking is a significant source of exposure to cigarette smoke, with cotinine levels in nonsmokers approaching 10% of those found in active smokers.

Exposure to ETS has been linked to various diseases and symptoms, particularly in children of smoking parents. Some of the health effects result predominantly from transplacental exposure of the fetus to tobacco smoke components. Health effects on the fetus resulting from maternal smoking during pregnancy include decreased birth weight, growth retardation, or prematurity, spontaneous abortion, perinatal mortality, and congenital malformations. Postnatal health effects of ETS exposure include sudden infant death syndrome, and adverse effects on neuropsychological development and physical growth. ETS has also been evaluated as a risk factor for major childhood cancers. The evidence is, however, limited and does not yet support conclusions as to the causal nature of observed associations.

Studies of involuntary smoking and lower respiratory illnesses in childhood, including more severe episodes of pneumonia and bronchitis, have demonstrated dose–response relationships. Schoolchildren responses to parental ETS include increased acute respiratory infections; increased frequency of chronic respiratory symptoms (i.e., cough, phlegm, and wheezing); middle ear infections; and reduced lung function and rate of lung growth. Exposure to ETS might also cause asthma as a long-term consequence of the increased occurrence of lower respiratory infection in early childhood, other pathophysiological mechanisms including inflammation of the respiratory epithelium, or increased airways responsiveness developed shortly after birth from smoking mothers. While the underlying mechanisms remain to be identified, the epidemiological evidence associating ETS with childhood asthma is increasing.

Effects in adult nonsmokers are not as conclusive in terms of alterations in lung function, but irritation of the eyes and of the upper and lower respiratory tract do occur, and ETS both increases the risk of

developing cardiovascular disease and is a major preventable cause of cardiovascular disease and death.

ETS is a significant risk factor for lung cancer in nonsmokers, and it has been classified as a respiratory carcinogen by IARC. The increased individual risk can be 30–50% depending on the extent of exposure, and exposure to ETS is estimated to be responsible for lung cancer deaths among nonsmokers in the United States. The most recent meta-analysis estimated the excess risk of lung cancer for nonsmokers who lived with a smoker as 26% with a 95% confidence interval between 7% and 47%. This and other estimations illustrate that passive smoking must be considered an important cause of lung cancer death from a public health perspective. The WHO estimated the unit risk for ETS as 10^{-3} per $\mu g\,m^{-3}$, a risk for developing cancer during lifetime only below the unit risks of benzo(*a*)pyrene, chromium(VI), and arsenic.

Volatile and Semivolatile Organic Compounds

The WHO has categorized indoor vapor-phase organic compounds into classes given in the following table:

Category description	*Acronym*	*Boiling point range (°C)*
Very volatile (gaseous) organic compounds	VVOCs	<0 to 50–100
Volatile organic compounds	VOCs	50–100 to 240–260
Semivolatile organic compounds	SVOCs	240–260 to 380–400
Organic compounds associated with particulate matter, particle bound organic compounds	POMs	>380

Some VOCs can be malodorous pollutants, sensory irritants, or hazardous air pollutants. Hazardous VOC air pollutants include acetaldehyde, benzene, carbon tetrachloride, chloroform, ethylbenzene, formaldehyde, hexane, methylene chloride, naphthalene, paradichlorobenzene, pesticides (biocides), styrene, tetrachloroethylene, toluene, trichloroethylene, and xylenes. They are found in essentially all indoor locations, released by off gassing from numerous sources, such as construction and decorating materials, consumer products, paints, paint removers, furnishings, carpets, and from combustion of wood, kerosene, and tobacco. While more than 500 VOCs have been identified in indoor air, ~50 occur most commonly. The major sources for many of these are listed in **Table 2**. In older homes, the total concentration of all volatile organics can range from 0.02 to $1.7\,mg\,m^{-3}$, while in newer homes, levels of 0.5–$19\,mg\,m^{-3}$ have been found. Exposure to certain organic compounds indoors is much greater than that which occurs outdoors, with indoor concentrations of some substances being 10 times higher than those outdoors and with short-term peaks reaching 1000 times higher.

Semivolatile organic compounds, which are solids or liquids at room temperature, are also found in indoor air, derived from pesticides, wood preservatives, floor waxes and polishes, and from combustion sources. These have, however, not been as extensively investigated indoors.

Some VOCs are known human carcinogens (e.g., benzene, vinyl chloride). Others are animal carcinogens and may be human carcinogens (methylene chloride, trichloroethylene, tetrachloroethylene, chloroform, and *p*-dichlorobenzene). The unit risks for these compounds are shown in the table below: (Unit risks reflect the probability of attracting cancer in a hypothetical population during lifetime exposure to $1\,\mu g\,m^{-3}$ of VOCs.)

VOC	*Unit risk* $(\mu g\,m^{-3})^{-1}$	*Source*
Benzene	6×10^{-6}	WHO
Chloroform	4.2×10^{-7}	WHO
p-Dichlorobenzene	6.6×10^{-6}	US EPA
Methylene chloride	4.7×10^{-7}	US EPA
Tetrachloroethylene	6×10^{-6}	US EPA
Trichloroethylene	4.3×10^{-7}	WHO
Vinyl chloride	1×10^{-6}	WHO
Formaldehyde	1.3×10^{-5}	

Many more VOCs are respiratory tract irritants or can affect the central nervous system (e.g., toluene and xylene) at high (occupational) concentrations. Acute effects at lower environmental concentrations are often difficult to observe under controlled conditions. Furthermore, many organic chemicals have distinct odors, which can act as stressor agents affecting response. Exposure to volatile organics is generally assessed by measurement of the chemical in breath samples, but some can also be found in body fluids, such as mother's milk and blood. Other routes of VOC exposure are drinking water (chloroform, trihalomethanes), food and beverages (chloroform, trihalomethanes, tetrachloroethylene, trichloroethylene), and dermal absorption (chloroform).

One of the most common volatile organic contaminants found in indoor air is formaldehyde. It is

Table 2 Common indoor sources of volatile organic compounds

Chemical class	*Examples*	*Typical sources*
Aldehydes	Formaldehyde	See **Table 3**
Hydrocarbons		
Aliphatic	Propane, butane, undecane, pentane	Cooking and heating fuel; aerosol propellants; lubricants; perfume; glues
Aromatic	Benzene, styrene, toluene, xylene	Paint; varnish; glue; cleaners; lacquers; combustion sources, ETS
Halogenated	Chloroform, 1,1,1-trichloroethane, trichloroethylene, methylene chloride, *p*-dichlorobenzene	Pesticides; dry-cleaning solvents; aerosol propellants; degreasing agents; paint strippers
Alcohols	Methanol, hexanol	Window cleaners; paint; adhesives; cosmetics
Ketones	Acetone	Lacquers; polish removers; adhesives
Terpenes	Pinene, limonene	Air fresheners; polishes; fabric softeners

derived from various sources, as shown in **Table 3**, but its use as a bonding resin in pressed wood products, such as plywood, particle board, paneling, and fiberboard commonly found in home and furniture construction, represents the single largest current use. In past years, a major source for formaldehyde was urea–formaldehyde foam insulation injected into the walls of homes. While this use has generally ended in developed countries, very high indoor levels of formaldehyde are still found in homes with such insulation, where concentrations can range from 150 to 500 $\mu g\,m^{-3}$ compared to levels of 40–113 $\mu g\,m^{-3}$ in homes where it was not used. Homes that make extensive use of plywood, such as mobile and prefabricated houses, also have high levels, which have been measured at 1300–5000 $\mu g\,m^{-3}$. It is evident that formaldehyde concentrations vary widely; they depend on the age of the structure, potential sources, and indoor temperature and humidity (e.g., high temperatures enhance off gassing).

Significant quantities for formaldehyde are consumed in the production of other resins or polymers such as polyacetyls, melamine resins, and alkyl resins. Formaldehyde is also used in rubber/latex manufacture, textile treatment other than permanent-press fabrics, dye manufacture and use, photoprocessing chemicals, laboratory fixatives, embalming fluids, disinfectants, and preservatives. Formaldehyde can also be emitted by combustion appliances, wood fires, tobacco smoke, and in indoor chemistry.

Average concentrations of formaldehyde range between 30 and 60 $\mu g\,m^{-3}$ for conventional homes, are at 100 $\mu g\,m^{-3}$ in mobile homes, and range between 50 and 350 $\mu g\,m^{-3}$ in homes with exposure to ETS. At the workplace without occupational exposure similar concentrations of formaldehyde are observed. With occupational exposure, the concentrations may be as high as 1000 $\mu g\,m^{-3}$.

Table 3 Common indoor sources of formaldehyde

- Urea–formaldehyde foam insulation (UFFI)
- Resins used as bonding agents in pressed wood products
  - Particle board
  - Plywood
  - Paneling
- Resins used as water repellants, stiffeners, or wrinkle resistors
  - Paper products
    - Paper towels
    - Grocery bags
    - Waxed paper
  - Permanent press clothing
  - Carpeting
  - Linoleum
  - Plastics
  - Drapery
- Consumer products
  - Cosmetics
  - Shampoo
  - Deodorants
  - Dyes
- Combustion processes
  - Natural gas ranges and heaters
  - Kerosene heaters
  - Tobacco smoke

Formaldehyde can enter the body via the respiratory system, skin, or gastrointestinal tract, but it is primarily absorbed in the respiratory tract where it is rapidly metabolized. It is an upper respiratory tract and eye irritant; may cause respiratory symptoms, reductions in lung function, and headaches; may predispose to asthma; and can also affect the nervous system. It is carcinogenic in laboratory animals, but human carcinogenicity is still an open issue. The WHO guideline value for formaldehyde is 100 $\mu g\,m^{-3}$ as a 30 min average, intended to prevent significant sensory irritation. This guideline value represents an exposure level at which there is a negligible risk of upper respiratory tract cancer in humans. An IARC expert group recently classified formaldehyde as carcinogenic to humans, determining that there is

now sufficient evidence that formaldehyde causes nasopharyngeal cancer in humans.

Other aldehydes, which may be important in the indoor environment, include acetaldehyde, acrolein, and glutaraldehyde. Acetaldehyde is a major by-product of hydrocarbon oxidation when wood or kerosene is burned for heating and cooking in developing countries, and a combustion by-product from unvented gas and kerosene appliances. It is also the predominant aldehyde detected in mainstream and side-stream tobacco smoke. Acrolein is produced and released into the indoor environment as a combustion/chemical oxidation product from the heating of oils and fats containing glycerol, wood combustion, and cigarette smoke. Acrolein emissions in mainstream smoke are significantly lower than formaldehyde emissions but are significantly higher in side-stream smoke.

Acetaldehyde is a relatively mild irritant of the eyes and upper respiratory system. It is toxic to the cilia of respiratory epithelia and may interfere with respiratory clearance mechanisms. Acetaldehyde is also a central nervous system depressant and a proven carcinogen in animals and a potential carcinogen in humans. The tolerable concentration for acetaldehyde according to the Guidelines for Air Quality of the WHO is $2000\,\mu g\,m^{-3}$ for 24 h and $50\,\mu g\,m^{-3}$ as annual mean.

Acrolein is a very potent eye irritant, causing lacrimation at concentrations of $\sim 2.3\,mg\,m^{-3}$ and irritation at concentration as low as $58\,mg\,m^{-3}$. At high concentrations, acrolein can cause significant lung injury, including dyspnea, asthma, congestion, edema, and persistent respiratory insufficiency with decreased lung function. Acrolein is ciliatoxic like formaldehyde, and can suppress pulmonary killing of bacteria. On chronic skin exposure, acrolein can cause contact dermatitis and sensitization. Acrolein can also be a potential carcinogen at least as potent as formaldehyde. The WHO has derived a guideline value of $50\,\mu g\,m^{-3}$ as a 30 min average based on eye irritation in humans.

Exposure concentrations for glutaraldehyde range up to $2\,mg\,m^{-3}$. Exposure to glutaraldehyde can lead to significant prevalence rates of nasal and throat irritation, nausea, and headache. Pulmonary symptoms, such as chest tightening, asthma, and similar symptoms have also been reported for medical workers. Other effects include skin symptoms, reproductive effects, and cancer. An exposure value of $0.21\,mg\,m^{-3}$ has been proposed by the American Conference of Governmental Industrial Hygienists (ACGIH) as a time-weighted average concentration to be at or below in order to protect nearly all workers from adverse effects.

Indoor/Outdoor Air Concentrations and Exposure Estimates for Benzene and Formaldehyde in Europe

Benzene

The EXPOLIS and MACBETH studies were extensive measuring campaigns carried out at the pan-European level to determine indoor/outdoor concentrations for benzene and other pollutants, and to relate them to personal exposure estimates. The results clearly indicated that ambient air concentrations for benzene substantially vary between the northern and southern part of Europe, with higher ambient air levels measured in the cities of southern Europe. This is mainly due to climatic conditions (higher temperature, low wind speed regimes), heavy traffic and often the lack of infrastructure needed to facilitate the movement of the citizens to and from the city. While in Athens (Greece), Murcia (Spain), and Milan (Italy) outdoor concentrations up to $21\,\mu g\,m^{-3}$ were measured, in Copenhagen (Denmark), Helsinki (Finland), and Prague (Czech Republic) outdoor concentrations reach values up to $5\,\mu g\,m^{-3}$. In indoor environments (homes), mean benzene concentrations range from a low of 2.2 to a high of $13.2\,\mu g\,m^{-3}$. Personal exposure monitoring concentrations were found to be often higher compared to those from indoor and outdoor sampling. There is clear evidence that personal exposure to benzene is at least twice as high as the ambient air concentrations.

In the frame of the German Environmental Survey (GerES II), the personal exposure (mean) concentrations were $13.5\,\mu g\,m^{-3}$, similar to those obtained in other cities of Central Europe (Prague, Antwerp). Assuming a 24 h exposure to this concentration a daily intake of 270 μg of benzene is estimated, based on a breathing volume of $20\,m^{-3}$ daily. Another study reported on personal exposure concentrations in the city of Nancy ranging from 9.9 to $55.5\,\mu g\,m^{-3}$, with a mean value $\sim 23.8\,\mu g\,m^{-3}$, which is significantly higher than the (mean) indoor and outdoor concentrations of 10.8 and $4.4\,\mu g\,m^{-3}$, respectively. Using the mean personal exposure concentration of $23.8\,\mu g\,m^{-3}$, a daily intake up to 476 μg of benzene is estimated.

In the United Kingdom, ambient air concentrations of benzene are generally in the range of 1–$6\,\mu g\,m^{-3}$. Mean indoor air concentrations were estimated to be $8\,\mu g\,m^{-3}$ for homes. However, nonoccupational exposed adults receive very high daily doses of 74–528 μg of benzene, which corresponds to an average range of benzene in air of 3.7–$26.4\,\mu g\,m^{-3}$, an amount significantly higher than

the mean outdoor air benzene concentration. Other studies reported that the mean personal exposure for individuals in Hertfordshire, England, was 183.9 μg m^{-3} (24 h). Using the mean outdoor air concentration near homes to predict personal exposures a value of 92.6 μg m^{-3} (24 h) has been obtained. At the pan-European level, and in accordance with the studies carried out, the mean home-indoor concentration for benzene considering all cities included in EXPOLIS and MACBETH studies, is ~9.6 μg m^{-3}; the home-outdoor/urban concentration (mean) is ~7.4 μg m^{-3}. Taking into account the time people approximately spend indoors and in work places (85%) and outdoors (15%), a daily intake of 184.4 μg of benzene results from exposure to indoor and outdoor air. This value corresponds fairly well to those reported from the local measuring campaigns.

From all data available, it can be concluded that personal exposure cannot be estimated from ambient air concentrations. Reducing benzene emissions from mobile sources only will have a rather limited effect on total human air exposure to this compound.

Formaldehyde

Formaldehyde has been one of the most important pollutants in indoor nonindustrial environments. A large body of data exists on measurements for formaldehyde in homes and buildings in Europe. Indoor air concentration levels for formaldehyde range from a few μg m^{-3} up to 70 μg m^{-3}, while mean outdoor concentrations of ~10 μg m^{-3} were measured. In air pollution episodes formaldehyde concentrations can reach high values (up to 80 μg m^{-3}) even at locations far from emission sources. However, in almost all measurements formaldehyde indoor concentrations exceed by several times (5–20 times) the outdoor levels, indicating strong emission sources inside buildings and homes. According to a WHO study, exposure of humans to formaldehyde is mostly determined by its concentration indoors. A daily intake of 20 μg results from the exposure to ambient air, while indoor and workplace concentrations has been estimated to amount to ~0.5–2 mg day^{-1}.

Indoor/Outdoor Air Concentrations and Exposure Estimates for Benzene and Formaldehyde in the United States

Several studies have been carried out in the United States to determine indoor/outdoor air concentration levels for priority pollutants and to assess personal exposure estimates. They have shown higher indoor than outdoor concentrations for the main pollutants, especially for VOCs.

Indoor (mean) concentrations for benzene range from 8.2 to 17 μg m^{-3}. 'Typical values' for indoor as well as for outdoor environments were up to 5 μg m^{-3}. For formaldehyde mean indoor concentrations reach values up to 92 μg m^{-3}, while 'typical values' for outdoor air concentrations of 4 μg m^{-3} are reported. Indoor/outdoor (*I*/*O*) ratios, based on typical air concentration levels, of 2 and of 50 for benzene and formaldehyde, respectively, are calculated. Daily exposure estimates are based on the assumption that people spend ~90% of its time in indoor environments and 10% outdoors. For benzene daily personal exposures vary between 108 and 177 μg m^{-3} for 24 h periods, ~20% lower than the mean exposures estimated for European citizens. For formaldehyde personal exposures range from 1080 to 2000 μg m^{-3} over 24 h, rather similar to European exposure estimates.

Asbestos and Other Man-Made Vitreous Fibers

Asbestos is a class of fibrous silicate minerals, each type of which differs in fiber shape and chemical formulation. It was widely used for decades because of its properties as a heat and sound insulator and fireproofing material and can be found in older floor and ceiling tiles, roofing felt and shingles, dry wall patching compounds, fireproofing insulation sprayed around steel beams, and the insulation of boilers and pipes. While it can no longer be used for most applications in new buildings, it is still a major indoor contaminant in many older ones, including homes and schools. However, the mere presence of asbestos in an indoor environment does not indicate exposure. If the asbestos-containing item is intact and fibers do not escape into the air, there is no exposure, and in many cases it is better to leave the material in place if it is well contained. However, much asbestos-containing material is old and in poor condition or damaged and may be friable (i.e., sheds fibers into the air). Asbestos fibers can be released during renovation of older buildings.

Actual indoor air concentrations of asbestos range from below 100 to several thousand fibers per m^3. Exposure to certain types of asbestos fibers is associated with specific respiratory diseases. These are asbestosis, a form of lung fibrosis, and two types of malignancies, namely, mesotheliomas, which are tumors of the lung pleura or peritoneum, and bronchial carcinomas. There is a strong weight of evidence that asbestos shorter than 5 μm do not cause cancer in humans. Evidence from occupational case–control studies indicates that the relative risk of mesothelioma is related to asbestos fibers longer than 5–10 μm. For lung fibrosis or asbestosis, the role of

fibers below 5 μm is not as clear. WHO estimated that with a lifetime exposure to 1000 F m^{-3} in a population of whom 30% are smokers the excess risk due to lung cancer would be of the order 10^{-6}–10^{-5}. For the same lifetime exposure, the mesothelioma risk for the general population would be in the range 10^{-5}–10^{-4}.

Synthetic or man-made vitreous fibers, such as continuous filament fiber glass, glass wool fibers, rock wool fibers, slag wool fibers, refractory ceramic fibers, and glass microfibers, used as asbestos substitutes for many applications seem to pose much less of a public health risk. The potential for deep lung penetration is greatest for refractory ceramic fibers and glass microfibers; both of these are primarily used in industrial applications. In two large epidemiological studies in the 1980s, there have been excesses of lung cancer in rock/slag wool production, but not in glass wool, glass microfiber, or continuous filament fiberglass workers. Although concomitant exposure to other substances may have contributed to the observed increase in lung cancer, the fibers appeared to be the principal determinants of risk. More recent cohort and case–control studies have not found increased respiratory cancer risk for rock or slag wool exposure or for refractive ceramic fibers. In spite of many epidemiological and experimental studies, the debate on man-made vitreous fibers is still controversial.

Radon

Radon (Rn-222) is an odorless and colorless natural radioactive gas. It is produced during the radioactive decay of radium-226, itself a decay product of uranium-238 found in many types of crustal materials, that is, rocks and soils. Rn-222 has a short half-life (3.8 days) and decays into a series of solid particulate products, known as radon progeny or radon daughters, all of which have even shorter half-lives ($\sim$30 min or less). Other isotopes of radon also occur naturally, but due to differences in half-life and dosimetry their health significance is minimal compared to that from exposure to Rn-222.

The main source of indoor air radon is the soil and rock beneath a building, from which the gas penetrates indoors, primarily through cracks or openings in the foundation or basement, including drain and utility access areas. Some well (ground) water in areas having high soil radium content may also be a source of indoor radon, as may natural gas or building materials containing radium. Often radon levels indoors tend to be highest in the lowest levels of a building, from which the gas can then permeate the entire structure. Arithmetic mean radon concentrations in European countries range from $\sim$30 to 140 Bq m^{-3}. In Russia, radon levels range between 19 and 230 Bq m^{-3}; in the United States average levels are around 50 Bq m^{-3}. Because of the skewed distribution of radon levels the geometric mean concentrations range 20–50% lower. Many countries have set an action level of 200 Bq m^{-3} at which mitigation measures should be taken to reduce radon levels at home. In the European Community, the action level is 400 Bq m^{-3}. The highest acceptable level of residential radon has been set by the US EPA at 150 Bq m^{-3}, but $\sim$5–10% of homes in the United States exceed this benchmark.

The risk from radon exposure is essentially due to inhalation of its progeny, which can attach to abundant sources of particles in indoor air that then act as carriers of these radioactive particles into the respiratory tract. Radon accounts for up to 50% of the total internal dose from all natural background radiation sources and this, in turn, is due almost completely to two of its progeny, namely, polonium-218 and polonium-214, which decay via the release of α-particles. Alpha particles, while lodged in the airways of the lung can damage the cells lining the airways, thus inducing lung cancer.

Radon exposure in the home likely substantially increases lung cancer risk in either nonsmokers or smokers. According to a nationwide Swedish epidemiological study of lung cancer due to radon exposure, the attributable proportion of lung cancer related to residential radon exposure ranges between 2% and 5% for lifetime exposure to 25 Bq m^{-3}, 5–9% for lifetime exposure to 50 Bq m^{-3}, and 9–17% for lifetime exposure to 100 Bq m^{-3}. The WHO estimated that these attributable proportions of lung cancer correspond to a unit risk of $3–6 \times 10^{-5}$ per Bq m^{-3}. The (US) National Academy of Sciences, in 1998, has estimated that between 15 400 and 21 800 lung cancers per year in the United States can be attributed to radon exposure. Furthermore, the individual risk may increase if other cancer-associated factors, especially cigarette smoke, are also present.

Biological Agents

Indoor air can contain a wide variety of biological contaminants; some examples are presented in **Table 4**. While many of these are nonpathogenic, others induce disease by infection of the respiratory tract or by immunologic means, such as allergy.

Biological agents in indoor environments include fungi, bacteria, allergens from dust mites, cockroaches and animal dander, and toxic components such as endotoxins and mycotoxins. Biological agents and

Table 4 Common indoor biological contaminants

Bacteria
Bacillus subtilis
Escherichia coli
Klebsiella pneumoniae
Legionella pneumophila
Mycobacterium spp.
Pseudomonas aeroginosa
Salmonella typhosa
Staphylococcus aureus
Streptococcus albus
Streptococcus spp.
Viruses
Fungi
Alternarium
Aspergillus spp.
Penicilium funiculosum
Thermophilic actinomycetes
Insects and insect parts
Cockroach
Mites
Dander

some of the diseases they can cause are often associated with moisture and dampness in buildings, in heating, ventilation, and air conditioning (HVAC) system components, porous materials, gypsum boards, and other locations with inadequate humidity control. Diseases potentially caused by biological agents in indoor environments include:

- Legionnaire's disease (pneumonia);
- humidifier fever (acute influenza-like symptoms);
- hypersensitivity pneumonitis (acute fever and cough, fibrosis of lung);
- asthma (intermittent episodes of wheezing, coughing, difficulty in breathing);
- pulmonary hemosiderosis (bleeding in the lungs);
- acute febrile illness (influenza, cold);
- tuberculosis; and
- lung cancer.

Biological agents are disseminated in indoor air by various means. Depending on the organism, this includes human actions (such as sneezing and coughing); via mechanical devices which result in the aerosolization of water spray containing these agents, such as humidification systems and whirlpool baths; via air movement induced by ventilation systems and by air currents derived from convective radiant heating systems; or by dusting or vacuuming of contaminated carpets or furniture. The risk of developing an infection or allergy from exposure to indoor air is often greater than that from outdoor air due to reduced ventilation in confined spaces resulting in the buildup of microorganisms or allergens to effective localized levels.

Infectious Agents

Infectious agents found in indoor air include viruses, bacteria, fungi, and protozoans. Viruses are internal cell parasites and can exist outside living cells for only a short period. On the other hand, bacteria, fungi, and protozoans can exist for extended durations on nonliving material. While bacteria are primary pathogens for humans, fungi and protozoans are generally opportunistic, that is, they produce disease only in compromised individuals, such as those with reduced defenses due to concurrent disease or use of certain medications.

Infectious disease can be produced by any pathogen able to be aerosolized and subsequently transported into the respiratory tract at the appropriate concentration. Some common diseases which may result from airborne transmission in indoor environments are listed in **Table 5**. The rate of infection within any environment is a function of the viability and virulence of the pathogen, its concentration in the inhaled air, and characteristics, such as droplet size, of the carrier aerosol within which it is contained. Some biological agents produce disease at low concentrations, while others must accumulate to a higher level. Furthermore, individual susceptibility to infection depends on a number of factors, such as age and health, as well as concomitant exposure to chemical pollutants.

While there are a number of potential sources of infectious agents in the indoor environment, humans are the principal one for pathogens responsible for most airborne viral diseases and many bacterial diseases. Nonliving sources can also harbor infectious agents. A good example is the bacterium, *Legionella pneumophila*, which becomes airborne from contaminated cooling system water and is responsible for Legionnaire's disease. Another example is humidifier fever for which episodes have been associated with inhalation of aerosols from humidifiers contaminated with gram-negative bacteria and protozoa. Endotoxin from *Flavobacterium* and from a *Pseudomonas* species was shown to be the potential agent of two humidifier fever outbreaks.

Pathogenic fungi generally derive from outdoor air, but their spores are able to penetrate into buildings through air spaces or intake vents, and interior growth can then occur on damp surfaces. Fungi and bacteria growing in the HVAC system have been implicated in outbreaks of hypersensitivity pneumonitis. Causative agents included *Cladosporium*, thermophilic actinomycetes, *Bacillus subtilis*, and *Penicillium* species. In large buildings with complex HVAC systems and with many potential sites of

Table 5 Some diseases potentially spread by indoor air exposure

Viral
Chickenpox
Colds
Influenza
Smallpox
Measles
Bacterial
Legionnaire's disease
Tuberculosis
Brucellosis
Fungal
Histoplasmosis
Cryptococcosis
Coccidiomycosis
Protozoan
Pneumocystis carinii
Acanthameoebosis

microbial growth, outbreaks of the disease often cannot be ascribed to a single agent.

Allergens and Immunologic Agents

Indoor air may contain biological agents capable of eliciting an allergic response. An allergic response is characterized by production of a specific immunoglobulin (antibody) termed IgE. Allergic sensitization is an important risk factor for asthma, particularly in children and young adults. Occupational asthma can develop among adults exposed to sensitizers. Very common indoor allergens are dust mite, cockroaches, animal danders from pets, and mold. The indoor allergens, except for mold, are often present in greater concentrations in residential than in other buildings. Mold flourish in any building with inadequate moisture control.

Another type of immunologically mediated lung disease is hypersensitivity pneumonitis. This is acutely characterized by flu-like symptoms, including fevers, cough, and chills, but in a chronic state may result in a slow, progressive decline in pulmonary function. A number of antigenic materials can produce hypersensitivity pneumonitis. While they are mostly complex organic particles, a common indoor antigen involved in its pathogenesis is the thermophilic actinomycetes. These organisms are found in decomposing organic matter and contaminate indoor environments through ventilation and humidification systems.

Allergic asthma may be exacerbated by exposure to antigens found in indoor air, including house dust, fungal spores, and molds. The house dust mite (*Dermatophagoides farinae*), which exists in bedding and in the stuffing of upholstered furniture, contains a potent allergen which occurs at high concentrations in house dust and then becomes airborne during cleaning activities. Inhalation of dust contaminated with these mites can increase the severity of asthma or perhaps even the risk of its inception.

Pulmonary hemosiderosis or pulmonary hemorrhage has been observed in a number of young infants (most under 6 months old), in the eastern neighborhoods of Cleveland, who have been coughing up blood due to bleeding in their lungs. Some infants have died and more infants continue to get ill. This bleeding appears to be caused by something in their home environments, most likely toxins produced by an unusual fungus called *Stachybotrys chartarum* or similar fungi.

Tuberculosis (TB) is caused by any of the human, bovine, or avian types of the tubercle bacillus *Mycobacterium tuberculosis*. Indoor levels of *M. tuberculosis* are generally low, but since TB is infectious, the clinically relevant exposure may be only a few bacteria. Globally, TB is on the increase, especially in developing countries.

Lung cancer through exposure in the indoor environment can be caused by carcinogenic mycotoxins, secondary metabolites produced by fungi mycelium and spores. Molds include *Aspergillus flavus*, which produces aflatoxin, a potent carcinogen, and *Aspergillus versicolor*, which produces a precursor for aflatoxin. *Aspergillus versicolor* is common in buildings with poor humidity.

Sick Building Syndrome and Multiple Chemical Sensitivity

Most indoor environments are contaminated by a combination of both microorganisms and particles and gases, but little is known regarding health effects from exposure to such complex mixtures even though biological responses to the inhalation of contaminated indoor air may depend on interactions between individual substances. Examples of some potential interactions in the risk of developing lung cancer are those between radon and ETS and between asbestos and ETS; between ETS and PM in the induction and/or exacerbation of respiratory infection in children; and between allergens and ETS in the exacerbation of asthmatic symptoms. Exposure to mixtures of indoor air pollutants does appear to be associated with two clinical conditions, namely, sick building syndrome (SBS) and multiple chemical sensitivity.

There have been numerous reports of a spectrum of nonspecific health complaints from occupants of various buildings, including schools, hospitals, and, most often, modern offices. Complaints include respiratory tract infection, irritation of the eyes, nose,

and throat, headaches, neurological reactions, nausea, lethargy, and dizziness. The range and severity of the symptoms varied greatly depending on the sensitivity of exposed individuals. While a causative role of the indoor environment was strongly suggested when it became clear that the symptoms generally abated upon leaving the building, in most cases no specific cause for them has been found. The term used for this collection of clinical signs is SBS, or building-related symptoms (BRS) as suggested by the American Conference of Governmental Industrial Hygienists. BRS is estimated to occur in about one-third of all buildings in the United States, especially in those that have been made 'tight' for energy conservation. This, in turn, allowed for the accumulation of contaminants from numerous indoor sources.

A single chemical is most likely not responsible for SBS but, rather, it probably reflects exposures to various chemicals, which can differ at different sites. Because many VOCs produce similar symptoms to those noted in BRS, they are suspected to be potential causative agents. However, there seem to be some contributions from a wide range of other factors in the environment such as temperature, humidity, and cleanliness of offices, personal control over the environment, noise, and lighting. In addition, biological agents may contribute to BRS.

BRS are to be distinguished from what have been termed building-related illnesses (BRI). These latter have definite etiological agents and specific clinical manifestations, for example, hypersensitivity pneumonitis associated with molds and thermotolerant bacteria. Other examples of BRI are rhinitis induced by sensitizers or irritants, allergic fungal sinusitis, asthma exacerbated by VOCs, molds and bacteria, allergic or irritant contact dermatitis induced by molds and/or VOCs, allergic or irritant conjunctivitis induced by molds and/or VOCs, and CNS toxicity induced by CO, VOCs, heat, and noise. The symptoms of BRI do not abate when leaving the building, and medical treatment is generally necessary.

Multiple chemical sensitivity (MCS) or multiple chemical intolerance (MCI) is a term used to describe a variety of symptoms associated, in some cases, with exposure to indoor air contaminants. Individuals with this syndrome seem to respond to very low levels of chemicals, and the condition can involve various organ systems. It appears to be induced by a wide variety of agents, but once induced it can be triggered by low-concentration exposures to numerous other chemicals. Indoor air pollutants not only appear to set off symptoms in the chemically intolerant, but several studies suggest that some pollutants or pollutant mixtures may also initiate the condition. This phenomenon has been described in more than a dozen countries, including the United States, Canada, Australia, and nine European countries. Among the chemicals reported as initiating exposures were organophosphate and carbamate pesticides in the United States and organic solvents in Europe. The fact that people in different countries have different cultural practices and time-use patterns, live in buildings made out of different construction materials, have different ventilation practices and uses of chemicals indoors and yet share a toxicant-induced loss of tolerance (TILT) is a compelling anomaly that is still the cause of much debate.

Management of Indoor Air Quality

Control and improvement of indoor air quality can be achieved by proper design and construction of buildings. Design considerations include site selection for the building, building envelope design, ventilation, commissioning levels of pollutants, selection of materials, and combustion appliances. Indoor air pollution control in existing buildings includes the management of pollutant sources, operation and maintenance of ventilation systems, and air cleaning. Resolving indoor air-related problems includes addressing occupant complaints and symptoms, applying building diagnostic procedures, and conducting building and health surveys from the end of building managers. Governments can help to improve indoor environment quality by developing and implementing integrated strategies for the indoor environment, strengthening public education, and supporting research and technology development. Once an understanding of the nature of the problem is obtained, remediation generally involves some combination of the following: changes in ventilation; source removal, substitution, or modification; air purification; or changes in human behavior. While details are beyond the scope of this entry, some examples of these approaches will suffice. Increased ventilation to allow dilution of indoor air with fresh outdoor air or recirculated indoor air can reduce levels of combustion by-products, biological agents, and radon gas; removal of sources or substitution of less hazardous materials for asbestos insulation and organics in consumer products and furnishings can reduce contamination by these agents; source modifications, such as reduction of contaminant emission rates through design changes or containment of emissions by some barrier, can reduce levels of combustion by-products, radon, and volatile organics; and behavioral modifications can reduce cigarette smoke exposure to nonsmokers. Management of the quality of the indoor environment concerns not only indoor air quality but also thermal comfort, lighting, and noise protection.

In addition to the management procedure mentioned above, in developing countries, management of indoor air pollution is a very important task due to the use of open stove cooking and heating indoors. Two types of interventions play a role: technical interventions include change of kitchen layout, improvement of stoves and use of fuel alternatives. Social–behavioral interventions refer to change of behavior with respect to cooking traditions and practices, and cultural patterns; they also refer to issues in population groups (e.g., women's involvement, education, workload and time constraints) and the community (needs, training, sustainability of intervention, access to cleaner fuels).

See also: Asbestos; Combustion Toxicology; Diesel Fuel; Fuel Oils; Pollution, Air; Respiratory Tract; Tobacco Smoke; Volatile Organic Compounds (VOC).

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Pollution, Soil

Thomas E McKone

Introduction

Soil is the thin outer zone of the earth's crust that supports rooted plants and is the product of climate and living organisms acting on rock. A true soil is a mixture of air, water, mineral, and organic components. The relative mix of these components determines both the value of the soil for agricultural and other human uses and the extent to which chemicals or biological organisms added to soil will be transported and/or transformed within the soil. Soils are characteristically heterogeneous. A trench dug into the soil zone typically reveals several horizontal layers having different colors and textures. These layers and their generic structure are illustrated in **Figure 1**. These multiple layers are often divided into three major horizons – (1) the 'A' horizon, which encompasses the root zone and contains a high concentration of organic matter; (2) the 'B' horizon, which is unsaturated, is below the roots of most plants, and contains a much lower organic carbon content; and (3) the 'C' horizon, which is the unsaturated zone of weathered parent rock consisting of bedrock, alluvial material, glacial material, and/or soil of an earlier geological period.

In an ecological sense, soils exist where the atmosphere, the hydrosphere, the geosphere, and the biosphere all converge. Thus, contaminants in soil can impact human health and the environment through a complex web of interactions. The sections below provide an introduction to three issues related to toxicology and soil – (1) the potential for soil

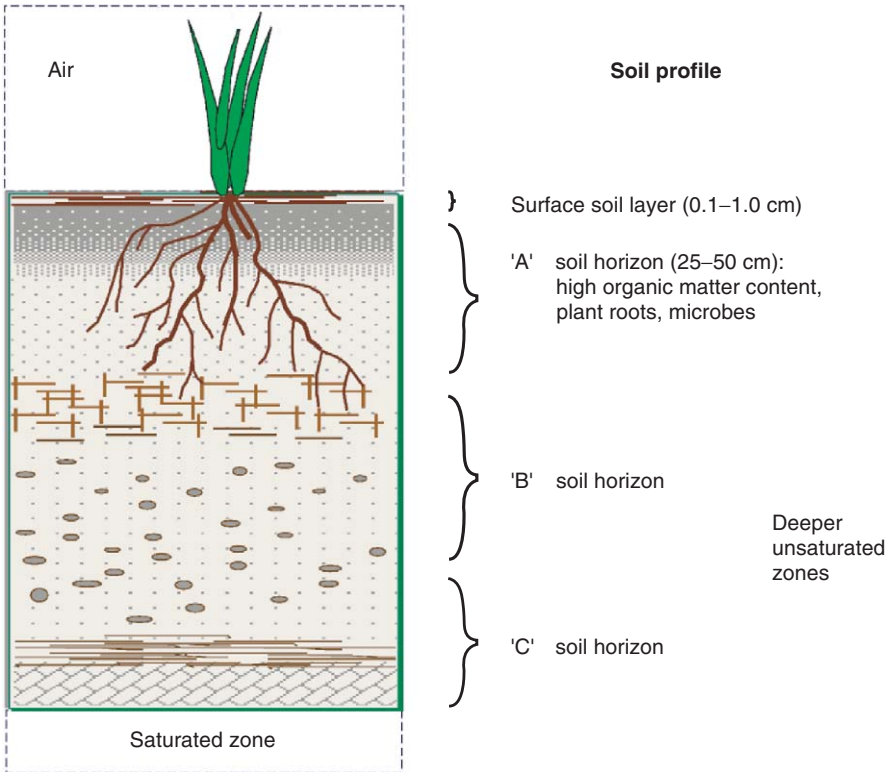

Figure 1 The typical horizontal profile and structure of soil in the unsaturated zone.

contamination by chemical, biological, and radioactive contaminants; (2) the potential fate, including both transport and transformation processes, for contaminants in soil; and (3) the types of direct and indirect human and animal contacts with soil contaminants that can result in risks to human health.

Soil Contamination

Throughout the world soils are contaminated to some extent from local, regional, and global pollution sources. Frequently, this contamination is the result of human and natural activities that involve the direct application of contaminants to soil. However, soil contamination also results from the transfer by rain and dry deposition of contaminants from air; by the transfer of contaminants through sewage-sludge (biosolids) applications; from the use of contaminated water for irrigating farms, gardens, or lawns; or by the soil itself through natural physical or biological agents that provide a source of contamination. Metal species and radionuclides released from combustion processes or from volcanoes and persistent organic pollutants migrate globally in the atmosphere and result in low levels of soil contamination as a result of deposition from the atmosphere. Pesticide use and the disposal of radioactive, biological, and chemical wastes can lead to much higher but localized levels of soil contamination. Some sources of contamination, such local high concentrations of toxic elements, the natural production of radon in soils, and the replication of toxic organisms are not external but internal to the soil. In the sections below, sources of soil contamination are identified and discussed.

Direct Application of Contaminants to Soil

Direct releases to soil occur in the form of pesticide, herbicide, and fertilizer applications; burial or land farming of domestic and industrial wastes; applications of sewer sludge to agricultural lands; and chronic releases from motor vehicles, resulting from the wear of brakes and tires as well as oil leaks. In addition, accidental discharges to the soil from storage tanks and miscellaneous spills during the transport of toxic substances can also occur. Contaminant releases to soil are normally quantified in terms of mass per unit area per unit time. For example, pesticide applications to agricultural fields can range from under 1 to over 20 $\mathrm{kg\,ha^{-1}}$.

Deposition from the Atmosphere

Contaminants in the atmosphere can be transferred to soil either directly through dry deposition, wet deposition, and vapor partitioning or indirectly

through deposition to plants, whose parts fall onto the soil. Dry deposition is the process by which particulate matter settles out of the atmosphere and onto soil and plant surfaces. Contaminants that are attached to these particles will be transferred to soil through this deposition process. Atmospheric contaminants on particles are also washed out of the air to soil with rain or snow in the wet deposition of the particles. Contaminants dissolved in the gas phase of air and not bound to particles can also be transferred to soil through a combination of wet deposition and chemical partitioning. Contaminants dissolved in air that are water soluble are easily washed out during rain and snow. This is wet deposition of a gas phase. In addition, contaminants that are water soluble can be transferred from air to soil through partitioning, which involves the diffusing of chemical from solution in air to solution in the soil water. Similarly, contaminants that are relatively insoluble in water but highly lipid soluble can be carried from air to soil through partitioning into the organic phases of soil. In this process, the contaminants diffuse from solution in air to solution in the organic phase of soil. Finally, contaminants in air can be transferred from air to vegetation surfaces by dry deposition, wet deposition, and by partitioning into the lipid and water phases of plants. When the plants decay, lose leaves, or are mowed, residual contamination is transferred to soil.

Use of Contaminated Water for Irrigation

The use of contaminated water supplies to irrigate farmlands, gardens, and lawns can result in the accumulation of persistent compounds in the irrigated soil. Organic contaminants with low water solubility, when introduced to the soil, will migrate to the organic carbon-phase of the soil where they can be retained for relatively long periods. Some metal species can also accumulate and persist in soil if their soil chemistry favors the binding of these contaminants into the mineral phase.

Use of Sewage Sludge on Agricultural Lands

A large fraction of the sewage sludge produced in many regions of the world is used as soil amendments often after treatment to reduce the content of harmful microorganisms. Sewage sludge is the semisolid residue from municipal wastewater treatment plants. Sewage sludge contains nutrients and organic matter that can improve soils. They also contain contaminants and pathogens that are discharged to the sewer system from homes, businesses, industries, and streets. Controversy surrounding both the practice of land application and the science behind the regulations as well as allegations of illness and even death resulting from use of sewage sludge prompted the US Environmental Protection Agency to commission a study by the National Research Council (NRC) of the US National Academy of Sciences on the health risks of sewage sludge. The NRC completed its report in 2002.

Contaminant Sources Internal to the Soil

In some cases the source of soil contamination is the soil itself. For example, soils rich in toxic elements such as arsenic, lead, mercury, and cadmium provide their own source of contamination. In addition, soils rich in uranium and its radioactive decay product radium provide continuous long-term sources of the radioactive gas radon in soil. The radon can diffuse from soil into the air of buildings or into groundwater, with resulting radiation exposures to human and animal populations. Other possible sources of contamination internal to soil itself are biological organisms, which are either themselves health threatening or which produce toxic chemicals.

Transport and Transformation of Soil Contaminants

There are a number of competing processes that impact the fate of a physical, chemical, or biological contaminant found in soils. When a contaminant is added to or formed in a soil column, there are a number of mechanisms by which it can be transported out of the soil column to other part of the environment, be destroyed, or be transformed into some other species. Therefore, once a contaminant has been identified in the soil column, one must also determine whether that substance will (1) remain or accumulate within the soil column, (2) be transported by dispersion or advection within the soil column, (3) be physically, chemically, or biologically transformed within the soil (i.e., by hydrolysis, oxidation, etc.), or (4) be transported to another part of the environment through a cross-media transfer (i.e., volatilization, runoff, groundwater infiltration, etc.). The purpose of this section is to provide an overview of the processes by which contaminants are transported in and out of soil layers and to provide a summary of typical transformation processes. **Table 1** summarizes processes by which contaminants are transferred to and from soils.

The Composition of Soil

In terms of their ability to transport, sequester, or transform harmful substances, we regard soils as composed of three major phases – gases, liquids, and

Table 1 Processes by which contaminants are transferred to and from soils

Gains	*Losses*
Deposition from air	Volatilization to air
Washout from air by rainfall	Resuspension of soil particles
Dry deposition of air particles	Mass transfer (diffusion and advection) downward to groundwater
Mass transfer (diffusion and advection) upward from groundwater	Transfers to vegetation
Contaminant sources	Soil solution runoff
	Erosion (mineral runoff) to surface water
	Chemical/physical transformation

solids. The fraction by volume that each of these phases contributes to total soil volume varies with soil type and with depth. The volume fraction of soil that is gas varies from a value of 10% typical in clay soils to 25% typical in sandy soils. The volume fraction of gas in soil decreases as one moves from the 'A' down through the 'C' horizon. The water phase of soil, the 'soil solution', consists mostly of water but includes dissolved minerals and nutrients. The volume fraction of soil that is liquid ranges from 10% typical of sandy soils to 40% typical of clay soils. The solid phase of soil makes up from 50% to 80% by volume of the soil composition and from 75% to 90% by mass of the soil. Soil solids include mineral (i.e., the parent rock) and organic components, including humic acids and decaying matter. The mineral component of soil is in the range of 70–90% by mass. The organic phase of soil is defined by the organic-carbon content of the soil. The organic-carbon content of soil ranges from much less than 1% by mass for desert and/or sandy soils to as much as 5% by mass for clay soils and even as high as 10% by mass for carbon rich soils such as peat bogs.

Transport Processes in the Soil Column

In order to understand how chemical species are transported in soil, it is important to recognized that the soil column needs to be viewed as having at least three distinct reservoirs for contaminants. These reservoirs are – (1) the surface-soil layer, (2) the rooting zone, and (3) the deeper unsaturated zone. The nature of these soil components is described below. These layers are illustrated in **Figure 1**.

The Ground-Surface-Soil Compartment Studies of radioactive fallout in agricultural land-management units reveal that, in the absence of tilling, particles deposited from the atmosphere accumulate in and are resuspended from a thin ground- or surface-soil layer with a thickness in the range 0.1–1 cm. The ground-surface-soil layer is at the top of the 'A' soil horizon. The ground-surface-soil layer has a lower water content and higher gas content than underlying layers. Contaminants in this surface-soil layer are more likely than deeper-soil contaminants to be transported horizontally by mechanical runoff and soil-solution runoff to nearby surface waters. Surface-soil contaminants are susceptible to wind erosion, volatilization, photolysis, biodegradation, and transfer to plant surfaces by rainsplash. In contrast to contaminants in deeper soil, surface-soil contaminants are susceptible to chemical transformation by sunlight. Surface-soil contaminants are transferred to and from air by diffusion and resuspension/deposition and transferred to and from the rooting-zone soil by diffusion and leaching.

The Rooting-Zone Soil Root-zone soil includes the 'A' horizon below the surface layer. The roots of most plants are confined within the first meter of soil depth. In agricultural lands, the depth of plowing is 15–25 cm. In addition, the diffusion depth, which is the depth below which a contaminant is unlikely to escape by diffusion, is on the order of a meter or less for all but the most volatile contaminants. Soil–water content in the root zone is somewhat higher than that in surface soils. The presence of clay in this layer serves to retain water. Contaminants in root-zone soil are transported upward by diffusion, volatilization, root uptake, and capillary motion of water; transported downward by diffusion and leaching; and transformed chemically primarily by biodegradation or hydrolysis.

The Deeper Unsaturated Soil The deeper unsaturated soil includes the soil layers below the root zone and above the saturated zone, where all pore spaces are filled with water. This compartment can encompass both the 'B' and the 'C' soil horizons. The soil in this layer typically has a lower organic carbon content and lower porosity than the root-zone soil. Contaminants in this layer move downward to the groundwater zone primarily by capillary motion of water and leaching. Chemical transformation in this layer is primarily by biodegradation.

Transformation

The transformation of toxic substances in soil can have a profound effect on their potential for human exposure and accumulation by biota. Transformation processes in soil include physical processes such

as radioactive decay; chemical processes such as photolysis, hydrolysis, and oxidation/reduction; and biological processes such as microbial transformations. All of these processes can significantly reduce the concentration of a substance or alter its structure in such a way as to enhance or diminish its toxicity.

Radioactive Decay Radioactive elements are made up of atoms whose nuclei are unstable and give off atomic radiation as part of a process of attaining stability. The emission of radiation transforms radioactive atoms into another chemical element, which may be stable or may be radioactive such that it undergoes further decay.

Photolysis Most organic contaminants are capable of undergoing photolytic decomposition. Such decompositions can be partial, resulting in the formation of stable by-products, or complete, resulting in the destruction of the compound or organism. Although the atmosphere attenuates solar radiation before it reaches the earth's surface, the solar radiation generally sufficient to break bonds in many compounds at this surface. Phototransformation in soil impacts only those contaminants on the soil surface. However, in agricultural lands that are tilled, contaminants in the tilling horizon ($\sim$15–20 cm) can be brought to the surface where phototransformation occurs. Phototransformations can result in relatively short half-lives (e.g., hours to days) for contaminants such as pesticides that are applied directly to crops or surface soils.

Hydrolysis Hydrolytic transformation of organic chemicals can be a significant destructive process for toxic compounds that are present in the aqueous phase of soils. Hydrolysis is most important for chemicals that have functional groups (e.g., amides, esters, carbamates, organophosphates), which can be rapidly altered (e.g., minutes to days) in the presence of water. For amides and carbamates, hydrolytic cleavage yields aromatic and aliphatic amines with increased likelihood of toxic activity. Conversely, hydrolytic degradation of compounds that contain stable constituents (e.g., halogenated compounds such as carbon tetrachloride) can have half-lives of several thousand years. Because hydrolytic reactions are driven by the availability of hydrogen and hydroxide ions, the pH of the soil can have a dramatic influence on the rate of hydrolysis for any given compound.

Oxidation and Reduction Many inorganic and organic chemicals can undergo oxidation or reduction reactions in soil. An indicator of a compound's ability to be oxidized or reduced is provided by its oxidation potential (E^o), which is the voltage at which it is transformed to its reduced state. A similar measure of a soil's ability to reduce a compound is provided by the redox potential (pE), which is a measure of electron activity. Redox potentials are relatively high and positive in oxidized environments (e.g., surface waters), and low and negative in reduced environments (e.g., aquatic sediments and the subsurface soil layers). These environmental conditions are especially important for inorganic chemicals that are rarely present in their elemental form in the environment. Arsenic, for example, exists primarily in its oxidized form (arsenate) in the atmosphere and in surface waters and in its reduced form (arsenite) in sediments.

Microbial Transformation Due to their broad range of enzymatic capabilities, microorganisms are capable destroying other microorganisms and transforming many inorganic and organic compounds. The chemical transformations can result in the partial degradation of a compound (e.g., conversion of trinitrotoluene to dinitrotoluene), mineralization (i.e., complete transformation to carbon dioxide and water), or synthesis of a stable product (e.g., formation of methyl arsenicals from arsenate). While these processes generally result in the detoxification of the parent compound, toxic products may also be formed. For example, the microbial metabolism of aromatic amines can result in the formation of toxic by-products.

Human Contact with Soil

Human contacts with soil can be multiple and complex. **Table 2** lists a matrix of potential human

Table 2 The matrix of exposure pathways that link humans with contaminated soils through direct and indirect contacts

Exposure routes	*Exposure pathways linking contaminated soil with human contact*
Ingestion	Direct soil ingestion by humans
	Ingestion of fruits, vegetables, and grains contaminated by transfer from soil
	Ingestion of meat, milk, and eggs contaminated by transfer from soil to plants to animals
	Ingestion of meat, milk, and eggs contaminated through soil ingestion by animals
	Ingestion of groundwater contaminated by soil
Inhalation	Inhalation of soil vapors that migrate to indoor air
	Inhalation of soil particles transferred to indoor air
Dermal contact	Dermal contact with soil

contacts with soils that can result in human uptake of soil contaminants through inhalation, ingestion, and dermal exposure routes. In the sections below we consider what is known about some of these exposure pathways and how they might be assessed in a risk assessment or other health-effects study.

Direct Soil Ingestion

Both adults and children continuously ingest small amounts of soil through inadvertent hand-to-mouth activities. Children who spend a great deal of time outdoors have been observed to contact and ingest soil through their repeated exploration and contact with surfaces and their frequent hand-to-mouth activities. But even adults through activities such as gardening, outdoor labor, and cleaning are also subject to inadvertent soil ingestion. Some individuals have been observed to intentionally ingest rather large quantities of soil. The ingestion of nonfood substances such as soil is called pica. Geophagia is the intentional, chronic, and often addictive consumption of earth. Although they are not activities common to the population at large, pica and geophagia can result in very large consumptions of soil contaminants and put the groups who engage in these activities at much higher risk of exposure to soil contaminants.

Several studies have been conducted to characterize ranges of soil ingestion by children. Some studies make use of measurements of soil levels on children's hands in combination with observations of hand-to-mouth activity to estimate soil uptake. The reliability of this method has improved recently by the introduction of videotaping combined with computer-based evaluation of the tapes to record hand-to-mouth activity. Another approach to soil ingestion measurement makes use of tracer elements in feces. Both feces of children and soil in their play yard are analyzed for elements such as aluminum, silicon, and titanium – elements thought to be poorly absorbed in the gut. Assuming no nonsoil sources of these elements, and a fecal excretion rate, soil ingestion for each child is estimated on the basis of the mass of each tracer element in feces relative to that in soil. Hospitalized children who have little contact with soil are often used as control groups.

Transfer of Soil Contaminants to Vegetation and Food Products

Soil contaminants in both the rooting zone and the surface-soil layer can be transferred to edible parts of vegetation by a number of processes. Contaminants in the rooting zone are transferred to plants through root uptake. The partitioning of contaminants between soil and root tends to increase with increasing contaminant concentration, since the root membrane on most plants restricts uptake to dissolved species. Contaminants in the rooting zone can be transferred to surface soil by plowing and tilling or by the activities of burrowing animals such as worms, ants, and rodents. Contaminants in surface soil can be transferred to edible plant parts through resuspension/deposition, rainsplash, and volatilization/partitioning. Resuspension/deposition is the process in which soil particles are blown by the wind up from the soil surface and then fall back onto the leaves of vegetation where the soil contaminants can be retained for some time on the leave surfaces or absorbed by the plant into the leaf tissues and possibly transported to other parts of the plant. Rainsplash is a process in which the impact of falling rain drops onto the soil surface causes soil particles to scattered into the air with impact onto plant surfaces. Volatilization/partitioning is a two-step process in which contaminants with a sufficiently high vapor pressure are volatilized from the soil and then collect into the waxy surface or the water portion of leaves through air/lipid or air/water exchange.

In the current scientific literature, plant/soil bioconcentration ratios (BCRs) are used to express a concentration ratio that relates the concentration measured in edible vegetation to a concentration in the soil supporting that vegetation. The plant–soil BCR expresses the ratio of contaminant concentration in plant tissues, roots, stems, leaves, seeds, and fruit, in milligram per kilogram (plant fresh mass) to concentration in soil. There are different protocols for expressing soil concentration among the different researchers who have measured plant–soil BCRs. Some express soil concentration in the soil solution, milligram per liter, whereas others use the soil dry mass concentration milligram per kilogram.

Contaminants in vegetation can be transferred to food products that are derived from the vegetation. The level of contamination of vegetative food products often depends on which part of a plant is being consumed. Translocation, which is the process by which a contaminant is transferred from one part of a plant to another, can result in significant differences in contaminant concentration between the total plant and the part of the plant being consumed; that is, the fruit or seeds. In addition, ingestion of contaminated soil and the ingestion of soil-contaminated pasture or grains by food producing animals can lead to the contamination of animal-based food products; that is, meat, milk, dairy products, and eggs.

Dermal Contact with Soil

Dermal exposure to contaminants in soil can occur during a variety of activities, such as construction

work, gardening, and recreation outdoors. Adults who work outdoors in activities such as construction, farming, or gardening can have rather high soil loadings on their skin. Children playing outdoors can also have rather large soil loadings on their skin. Lipid-soluble chemicals have a strong tendency to move from a soil layer on the skin surface to the lipid-rich outer layer of human skin. However, the rate at which this transfer takes place is often very slow and could require hours or even days to reach an equilibrium state. Estimating doses that result from dermal contact with a contaminated soil involves a number of often difficult-to-measure parameters, including the contaminant concentration in soil, the soil-to-skin adherence factor, the chemical-specific absorption factor for the skin–soil system, the exposure frequency, and the exposure time. The exposure frequency expresses how often, that is, days per year, an individual is involved in an activity the results in soil contact. The exposure time is a measure of how long, in hours, the soil is in contact with skin during an exposure activity.

Dose estimates for soil contact include a great deal of uncertainty. This uncertainty arises because we must deal with the transport of chemicals within the skin layer; the interaction of the soil layer on the skin with the skin surface; the dynamic conditions always involved in scenarios addressing interaction of the skin surface with chemicals, soil, air, and water; and addressing the level of protection provided by clothing.

Inhalation of Soil Particles Suspended as Dust

Soil contaminants that are bound to soil particles can be resuspended and inhaled along with the fine particles to which these contaminants are attached. The inhalation of suspended particles can take place both outdoors and inside buildings. Exposure assessors and toxicologists now recognize that fine and coarse particles in the indoor environment are attributable to both air and soil sources and enter the indoor environment by processes such as penetration through windows and cracks and soil tracking. Soil tracking is the process by which soil particles are carried into the indoor environment by shoes and clothing of human occupants as well as on the feet and fur of pets.

Contaminant Vapor Transport into Buildings

The vapors of volatile contaminants, such as radon and volatile organic compounds, can be transported through diffusion from the soil pore spaces into buildings. Three principal factors are needed to define the ratio of contaminant concentration in indoor air to observed contaminant concentration in soil gas. These are (1) the distance between the contaminant source and the building foundation, (2) the permeability of the soil, and (3) the area of cracks in the foundation relative to the total area of the foundation.

Groundwater Contamination

Soil contaminants can be transformed by physical, chemical, and/or biological processes. Those that are not transformed can be carried to groundwater in areas of net recharge. Once contaminants move from soil into groundwater these contaminants can contact humans through a number of exposure pathways – such as direct water ingestion, dermal uptake in showers/baths, irrigation of crops, feeding food-producing animals.

Summary

The purpose of this article is to consider the nature of soils, how soils are contaminated by human activities, how these contaminants are transported and transformed in the soil column, and the types of human activities that could result in human exposure to soil contaminants. Soils are complex systems that exist at the interface among atmosphere, biosphere, hydrosphere, and lithosphere. A true soil includes gas, water, mineral, and organic components. Potential human contacts with soil can result in inhalation, ingestion, and dermal uptake of soil contaminants through both direct and indirect exposure pathways. The magnitude and persistence of exposure depends not only on the level of soil contamination, but also on the physical and chemical properties of soil, the chemical properties of the contaminant, and the frequency and duration of human activities such as occupational and recreational activities or use of home-grown food, which result in direct and indirect soil contacts. Toxicologists should be aware of the complex nature of soils, of the potential of soil contamination, and of types of direct and indirect contacts that human populations have with soil.

See also: Pollution, Air; Pollution, Water.

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Pollution, Water

Ruth Custance

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Water is a major transporter of toxic chemicals in the environment. Our view of the hazards associated with water pollution varies considerably depending on how the water is to be used. Chemicals in water used for drinking or bathing result in direct exposure to humans and, if doses of chemicals derived from these activities are sufficiently high, these exposures can lead to toxic effects. However, chemicals introduced into streams and lakes frequently result in exposures by less direct means, for example, by eating fish obtained from these waters. The nature of chemicals that are involved in exposure via drinking water and that of chemicals involved in exposure through food derived from contaminated water are frequently quite different. Generally, chemicals that are found in drinking water tend to have significant water solubility and low affinities for clay and organic matter found in soils. Those chemicals that are obtained in food derived from water are generally much less soluble in water and have very high solubility in fats. These properties account for their accumulation in fish tissues. Many of the chemicals which bioaccumulate are also poorly degraded in the environment. This further contributes to their accumulation. In many cases, the impact of these latter chemicals on wildlife is more important than their effects on human health.

Chemicals are introduced into water in a variety of ways. In the past, the focus has been on industrial pollution. When industrial outfalls into bodies of water were less well-controlled than they generally are today, these point sources were important. While it is important to recognize that point sources of chemicals can still be responsible for local or regional problems in water quality, nonpoint sources of water pollution contribute much more to chemical contamination of water on a national basis. Chemical contamination of groundwater has occurred as a result of poor chemical disposal practices in the past or the leaking of storage vessels such as underground tanks and landfills. The types of chemicals that occur in groundwater are chemicals that are very mobile in soils. As with other point sources, the impact of these sites is local rather than national in scope. However, significant portions of entire groundwater basins used for drinking water have been contaminated in various regions of the country with the more mobile compounds especially the fuel oxygenate, methyl tertiary butyl ether (MTBE). Because, cleanup of groundwater is technically very difficult and expensive. Every effort must be made to prevent this type of contamination. While the actual impact on water used for drinking is relatively small when considered on a national basis compared to other types of water pollution, the problem of uncontrolled hazardous waste sites does occur in all regions of the country and produces a great deal of public concern. In addition, there have been instances where for more mobile compounds such as perchlorate, widespread contamination of groundwater and surface water used for drinking water has occurred as a result of releases from hazardous waste sites. Nonpoint sources would include chemicals used in

agriculture, such as fertilizers and various pesticides; fallout from products of incomplete combustion, such as the automobile; and chemicals that are washed into streams, rivers, and lakes by runoff of urban areas. Thus, the types and numbers of chemicals that can contaminate water from these sources are practically endless.

In recent years, efforts have been undertaken to conserve water due to the growing demand for a limited resource. As a result, water reuse is being employed, especially in the Western United States. Water reuse is when wastewater generated from a community is reclaimed for a beneficial use such as irrigation for ornamental or agricultural crops, decorative water features, industrial application and with advanced treatment potable water. The use of reclaimed water may expose the public to chemical and microbial contamination from the wastewater stream. Therefore, for each type of beneficial reuse, treatment standards are being established to protect public health.

Chemicals are also deliberately added to water. In the treatment of wastewater, and more particularly in the treatment of drinking water, a variety of chemicals are added for purposes of disinfecting, clarifying, and preventing corrosion of pipes. Moreover, as water is distributed to consumers, the surfaces it contacts have the potential of contaminating the water. These surfaces may be the water mains and pipes in a municipal distribution system, or they may be the surface of a plastic bottle in which the water is purchased in a supermarket.

Because of the complex sources of chemicals in water, contaminants of water will be discussed as they are introduced into water; those introduced into ambient water, chemicals introduced during the treatment of water, and contaminants associated with the distribution of water.

Contaminants of Ambient Water

Natural Contaminants

It is important to recognize that the bulk of the chemicals found in water are of natural origin. Many of these chemicals are innocuous at even the highest concentrations that might be found in freshwater. Some are essential minerals and metals that are important to the normal physiology of the body. These would include sodium, chloride, magnesium, calcium, bicarbonate, carbonate, sulfate, and iron. Occasionally, these materials are present at concentrations that will cause gastrointestinal disturbances (e.g., diarrhea induced by sulfates and nausea and vomiting due to copper).

Occasionally, water will come into contact with natural deposits of potentially hazardous chemicals. A relatively frequent contaminant of groundwaters in the Western United States is arsenic. Usually the concentrations are below $100\,\mu g\,l^{-1}$, but there are concerns that such concentrations may represent a cancer hazard. At higher doses, of course, arsenic is clearly toxic to a variety of organ systems. Less frequently, river water may erode deposits of asbestos. While asbestos is recognized as being carcinogenic when it is inhaled, there has been no convincing evidence that ingested asbestos presents such a hazard. This may be partly due to the small size of the asbestos fibers that are found in water. Fibers in excess of $5\,\mu m$ appear to be most dangerous.

Surface waters (i.e., streams and lakes) or groundwater influenced by surface water also contain a complex mixture of organic chemicals. These may range from a fraction of a $mg\,l^{-1}$ up to 10s of $mg\,l^{-1}$. Some of these chemicals are simple sugars, amino acids, and low-molecular-weight organic acids that are normal biological substrates. The bulk of these organic compounds, however, are humic substances. Humic substances consist of humic and fulvic acids which are polymers of small-molecular weight products of biological decay that form over time. The size of the humic acid molecules can be quite large and they can involve very complex and individual structures. Fulvic acids are significantly smaller and tend to be more soluble. The properties of these substances vary considerably in different climates. They are responsible for the dark color seen in many standing waters. In themselves, these chemicals do not pose health hazards. However, they do serve as substrates for reactions with various oxidant chemicals used in the treatment of drinking water.

Agricultural Chemicals

Agricultural chemicals have a high probability of affecting water supplies if they have a significant water solubility, are not rapidly degraded, and have a low affinity for soils. Fortunately, most chemicals currently used in agriculture do not fit this category. However, the large volume used of certain chemicals that are mobile in soils does result in adverse impacts on both surface water and groundwater. The most widespread example of this is nitrates derived from the use of fertilizers. The concentrations of nitrate in surface waters frequently exceed drinking water standards during certain times of the year. A more pervasive problem, however, is the relatively widespread contamination of groundwater by nitrate. These concentrations will remain high for years to

come, even if practices introducing them into the groundwater were stopped today.

Much of the public fear of agricultural products is focused on the use of various pesticides. Many of these compounds are highly toxic. Fortunately, those which are the most toxic and likely to contaminate water, the organophosphorus pesticides, are generally degraded in water. These chemicals would include parathion, methyl parathion, terbufos, and malathion. These chemicals have been found in water, but generally at low concentrations. On the other end of the spectra are the very water-insoluble compounds, such as DDT, chlordane, dieldrin, and lindane, that have high affinity for soils and are found primarily in particulate matter in water. Paraquat is a very dangerous contact herbicide that appears to be very immobile in soils and has rarely, if ever, been found in ambient waters. Generally, these particulates are removed from water before it is used for human consumption and any chemical remaining in the water is at very low concentrations ($<0.01\,\mu g\,l^{-1}$). In addition, the water-insoluble pesticide's affinity for soils minimizes their impact on groundwater. There are, however, a small number of pesticides, such as aldicarb (Temik) and diazinon, that are very mobile in soils and which can be significant contaminants of water. The other group of chemicals that are of concern are low-molecular weight halogenated compounds that are used as soil fumigants. These would include ethylene dibromide, dibromochloropropane, and 1,3-dichloropropene. In agricultural regions, these chemicals have been widely detected in groundwater, which is of concern due to their reproductive toxicity at low doses. The former two chemicals have recently been banned by the US Environmental Protection Agency (EPA). Herbicides such as atrazine, butylate, chloramben, DCPA (dacthal), MCPA, dicamba, metolachlor, metribuzin, picloram, prometon, pronamide, propachlor, propazine, simazine, and 2,4,5-T have also been detected in surface water and/or groundwater supplies. The last compound is no longer in use in the United States because it was contaminated with low levels of a very toxic chemical, 2,3,7,8-tetrachlorodibenzodioxin.

Industrial Chemicals

Industrial contamination of water occurs as the result of directly introducing contaminated wastewater into a body of water or lake or from improper disposal of chemicals to the land. The chemicals most frequently found in water from both of these activities are chemicals that are used in very high volume or are very mobile. The nature of surface waters contamination is more likely to depend on the nature of the industry impacting a particular body of water. The soil surrounding a disposal area frequently acts as an effective barrier to contamination to many chemicals found in hazardous waste sites.

Probably, the most frequent contaminants of water from these two sources are spilled liquid fuels, such as gasoline, kerosene, and diesel oil, and low-molecular-weight solvents such as trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane (methyl chloroform), benzene, toluene, various xylene isomers, MTBE and aliphatic hydrocarbons. Other solvents may also be found in high concentrations in groundwater where they have been disposed of in large quantities. These would include solvents no longer in common use such as carbon tetrachloride or chloroform. The toxicology of these chemicals varies widely. Acutely and at very high doses most solvents depress the central nervous system (CNS). It is very unlikely that such concentrations would be achieved as a result of environmental contamination. Some of these chemicals have a high probability of producing liver or kidney damage as delayed effects or under conditions of more chronic exposure (e.g., carbon tetrachloride, chloroform, 1,1,2,2-tetrachloroethane, and 1,1,2-trichloroethane). Others present a specific hazard of producing delayed and cumulative nervous system deficits (e.g., methyl butyl ketone, *n*-hexane, trichloroethylene, and toluene). Again, these effects may be observed at levels of exposure encountered occupationally but would be rare from generalized environmental contamination. Generally, the major concern of concentrations found in the aquatic environment is with those chemicals that produce cancer (e.g., benzene, vinyl chloride, dichloromethane, trichloroethylene, tetrachloroethylene, and carbon tetrachloride). Among these chemicals, only benzene and vinyl chloride have induced cancer in humans. Estimates of the cancer risks that arise from environmental exposures to these chemicals are quite controversial.

Around military bases and weapons laboratories of the US Department of Energy, there have been frequent incidents of groundwater contamination by explosives, specialized fuels, and radionuclides. Among the explosives identified in these circumstances are trinitrotoluene, HMX, and RDX. Radionuclides would include tritium, plutonium, and technetium.

Recently a group of chemicals have been identified called 'Emergent Chemicals' that are important regarding water quality. The term 'Emergent Chemicals' is used by the EPA and other regulatory agencies to identify a group of chemicals found in ground water and/or surface water that cause great concern with respect to drinking water supplies. Although detected at points of many monitoring wells, these chemicals

typically do not have State or Federal Regulatory standards due to their recent detection and the lack of health effects data. In addition, analytical methods to detect these compounds at sufficiently low concentrations may not be available or are under development. Finally, due to their chemical properties many of these compounds cannot be treated using standard water treatment processes. As a result, these chemicals pose a challenge to evaluate their occurrence and significance to public health.

One of the first chemicals to be identified in this group was MTBE, a fuel oxygenate that was added to gasoline to meet requirements of the Clean Air Act. Other chemicals that are used as fuel additives include tertiary butyl alcohol (TBA) and tertiary amyl methyl ether (TAME), however MTBE became the most popular due to production, blending, and cost considerations. While MTBE was considered less toxic than other fuel additives, MTBE and the other ethers are highly soluble and very mobile in water, and not readily biodegradable. The widespread use of these compounds resulted in significant and widespread groundwater contamination. Another important chemical that has been detected in drinking water sources is perchlorate. Perchlorate is an anion commonly associated with the solid salts of ammonium, potassium, and sodium perchlorate. The use of perchlorate includes the manufacture of air-bag inflators, matches and flares, paints photography, pyrotechnics, rubber, and tanning and finishing leather. Ammonium perchlorate has been used most significantly in Department of Defense (DoD) applications, as a component of explosives and rocket propellant. Because perchlorate salts readily dissolve in water, perchlorate contamination is being discovered in groundwater and surface water, especially near perchlorate production facilities and facilities that use large quantities in the manufacture of rocket propellants or devices such as flares. One of the more significant findings is the presence of perchlorate throughout the Lower Colorado River basin, a significant source of drinking water and irrigation water for the Southwestern United States. It is thought that the perchlorate is present due to releases from a former perchlorate-manufacturing plant near Lake Mead. Perchlorate can limit the uptake of iodide, an essential nutrient, by the thyroid gland. As a result, large doses of perchlorate were used therapeutically to treat hyperthyroidism. Current research indicates that at concentrations that may be present in the environment, perchlorate exposure can result in reduced levels of iodide in the thyroid which disrupts the thyroid hormones that regulate metabolism and growth. Certain populations are particularly susceptible, such as pregnant women and infants, to adverse health effects when this occurs. The toxicity of perchlorate and its effects on public health at concentrations typically measured in groundwater or surface water is under review by various regulatory agencies.

Attention is also being given to pharmaceuticals and endocrine disrupting chemicals (EDCs) that are being detected in water. EDCs are synthetic chemicals and natural plant compounds that may affect the endocrine system through mimicking the steroid hormones, estrogens and androgens, by binding to hormone receptors or influencing cell signaling pathways. Another way EDCs can act is by blocking or altering hormone binding to homornal receptors. Many of these substances have been associated with developmental and reproductive effects in wildlife and laboratory animals. Some proven environmental estrogens used as pesticides, most notably *o*,*p*′-DDT, toxaphene, and dicofol, have been banned from use in most western industrial countries but are still used in many developing nations. Other proven estrogenic compounds are still being used worldwide in plastics manufacturing (phthalates) and agriculture (endosulfan).

Chemicals Introduced during Treatment of Drinking Water

Chemicals are used for a variety of purposes in the production of drinking water. Also, chemicals are added to water for a variety of purposes. Reservoirs are frequently treated with herbicides to prevent overgrowth of vegetation. Disinfectants are added as barriers to the spread of waterborne infectious disease. Other oxidants (potassium permanganate and chlorine dioxide) are utilized to remove unwanted color or to remove or prevent the formation of chemicals that impart a bad taste or odor to the water. A variety of chemicals are utilized in the clarification of water. Alum and ferric chloride are utilized to aggregate particulate material in the water (i.e., coagulation). A variety of other polymeric chemicals are used to neutralize surface charge that prevents coagulation and settling of particulates and are referred to as coagulant aids. Lime is added to soften water and acids, bases, and buffers are added to adjust the pH and to control corrosion. It is impossible to catalog all the chemicals that are used in the treatment of water. For a more complete list, the interested reader is referred to the NSF International listing of chemicals that meet the NSF standards as drinking water additives (see Further Reading).

Disinfectants

In most of the world, disinfectants are reactive chemicals introduced into water to prevent the

spread of waterborne infectious diseases. In situations in which sanitation is poor, the need for disinfection is very obvious. However, the large amounts of water that are needed in metropolitan areas inevitably means that microbial contaminants are introduced into some of the source water. Chemical disinfectants provide an economical and simple technology for controlling these contaminants. On the other hand, disinfectants vary in their toxicological properties. Therefore, it is important to establish that these chemicals can be used safely at effective concentrations. Chlorine presents no specific toxicological problems at effective concentrations. Monochloramine (i.e., chlorine + ammonia) is relatively safe at the concentrations that are used, but it is a much poorer disinfectant than chlorine. Ozone presents no particular toxicological threat because it is not sufficiently stable in water to reach the taps at which the water is consumed. However, this is also a disadvantage because there is no residual disinfectant to prevent the outgrowth of microorganisms in the mains, service lines, and pipes that distribute the water. As a consequence, a second disinfectant is usually added after an initial treatment with ozone. Another chemical that has been proposed for use as a disinfectant is chlorine dioxide. This chemical is a very effective disinfectant, but it does produce thyroid disorders in experimental animals. Moreover, it degrades to two chemicals, chlorite and chlorate, that produce hemolytic anemia and methemoglobinemia. While it is probable that chlorine dioxide can be used safely for drinking water disinfection, there is less margin of safety with its use and the concentration needed for disinfection. Moreover, close attention must be paid to the amounts of chlorite and chlorate, which are inevitable by-products of this compound, that are produced in the distribution system and occur at the tap.

In 1974 it was discovered that the use of chlorine in the disinfection of water leads to the formation of a group of compounds referred to as the trihalomethanes. This group of compounds includes chloroform, bromodichloromethane, dibromochloromethane, and bromoform. The relative concentrations of the members of this class depend on the concentration of bromide in the water being disinfected. In recent studies, it has become clear that the trihalomethanes are only one class of by-products and that there are small concentrations of a wide variety of chemicals produced with chlorination. However, it should be recognized that all chemical disinfectants are reactive compounds and, as a consequence, all will produce unintended by-products as a result of their use.

Disinfectant by-products are produced by reaction of the disinfectant with other chemicals in the water. The bulk of these chemicals are of natural origin. Humic and fulvic acids are the most common organic chemicals present. These are formed by the natural decay of biological material and are in themselves harmless. As indicated previously, the bromide concentration in the water also influences the type of by-product that is formed. Chlorine and ozone oxidize bromide to hypobromous acid, which acts to add bromine to various chemicals. Under conditions of high pH (alkaline conditions), ozone can further react with hypobromite ion to produce bromate. Differences in pH also affect the levels of other chemicals that are produced. Acid pH results in the formation of a variety of mutagenic chemicals at very low concentrations when chlorine is utilized as the disinfectant, whereas high pH gives rise to higher concentrations of the trihalomethanes.

Epidemiological data suggest that chlorination of drinking water does increase the probability of developing cancer of the bladder and of the large intestine. The elevation of these cancers above background is relatively small. Consequently, the differences may be caused by other risk factors that were not identified. Animal studies do indicate that some of the chemicals that are produced with chlorination are capable of producing tumors, but the tumors have been more commonly found in the liver and kidney. Moreover, the actual risk predicted from the animal studies is much less than that suggested by the epidemiological studies. These differences may indicate that the results of the epidemiology studies were not correct. However, many of the chemicals produced by chlorination have yet to be evaluated in experimental animals. This is a very important question because many of the by-products that have yet to be studied in experimental animals are also produced by other disinfectants such as ozone. The modifications that should be made in the use of disinfectants will not be clear until the toxicological effects of these compounds have been established.

The types of chemicals produced by disinfectants and some specific examples are provided in **Table 1**. The reader should not be deceived by the fact that the list of by-products associated with chlorination is much longer than that of other disinfectants. This is the result of more thorough study, not necessarily an actual reflection of the numbers of by-products that are formed by each process.

Other Chemical Treatments of Water

An NSF International publication (NSF Listings, 1994) provides a complete list of products that have

Table 1 Classes of disinfectant by-products

Disinfectant	*Inorganic*	*Organic*	
		Halogenated	*Nonhalogenated*
Chlorine	Chlorate	Trihalomethanes Haloacetates Haloacetonitriles Haloaldehdydes Haloketones Halofuranones Chloropicrin	Aldehydes Carboxylic acids
Monochloramine		Cyanogen chloride	
		Others generally thought to be the same as chlorine, but of lower concentration	
Chlorine dioxide	Chlorite Chlorate	Not well-characterized	
Ozone	Bromate Hydrogen peroxide	Bromomethanes Bromoacetates Bromoaldehydes Bromoketones Iodinated analogs	Aldehydes Carboxylic acids

Table 2 Chemicals that are used as direct additives to drinking water

Chemicals	*Purpose*
Alum (aluminum salts) Iron (iron salts) Cationic polymers Nonionic polymers Anionic polymers Starch	Coagulation and flocculation for removal of particulate
Phosphates Polyphosphates Orthophosphates	Antiscalants, corrosion control, sequestering agents
Copper salts	Antifouling, algicides
Chlorine Calcium hypochlorite Sodium hypochlorite Ozone Chlorine dioxide Potassium permanganate Hydrogen peroxide	Oxidants (also disinfectants)
Calcium oxide (lime) Calcium hydroxide	Softening, pH adjustment
Potassium hydroxide Sodium hydroxide Hydrochloric acid Sodium bicarbonate	pH adjustment
Sodium flouride	Dietary supplement

been approved for use as direct additives to drinking water by NSF's certification program. The number of specific products used is too large to summarize easily in limited space. Consequently, a partial list of the active ingredients that are representative of products used for specific purposes is provided in **Table 2**.

Most chemicals that are direct additives to drinking water present little hazard to health. Many of these chemicals also have been used as food additives and have been subjected to appropriate levels of toxicological testing. Other additives, such as starch, are natural foodstuffs and would be generally regarded as safe, especially at the low concentrations that would be expected to reach the tap.

Polymeric chemicals are a somewhat special case. These are most frequently introduced as direct additives as coagulant aids. By virtue of their function, these polymers are almost quantitatively removed from the water during normal treatment. Even if applied inappropriately, these chemicals are of such high molecular weight that they would not be absorbed and are almost certainly not a threat to health if they have been properly tested. A potential difficulty with these chemicals is that they may contain varying amounts of the monomers used in their synthesis or other incompletely reacted material of lower molecular weight. Some of the monomeric compounds are quite toxic. Acrylamide is an example of one of these compounds that is neurotoxic, carcinogenic, and a reproductive toxin. Epichlorhydrin, vinyl chloride, and vinylidene chloride are additional examples of these chemicals. For this reason, the amount of unreacted monomer present in the product is closely regulated by certification agencies such as NSF International.

Chemicals Introduced during the Distribution of Water

Water used for human purposes is delivered in a variety of ways. It is placed in a container to be transported or it is forced by gravity into a system of mains, service lines, and pipes to deliver it to individual users. In both cases the water contacts a surface. Water is a very effective solvent and will invariably extract some chemicals from these surfaces. The surfaces that water contacts are metal, plastic, concrete, or a paint or other type of coating that is applied to the surface. In addition to pipes and containers, there are reservoirs and holding tanks in which similar problems are involved.

The chemicals leached from these surfaces depend on the corrosive properties of the particular water as well as the chemical nature of the surface. Hard water tends to deposit a mineral layer on the inside of pipes and on other surfaces that essentially limits the access of water to the surfaces. On the other hand, soft water, particularly at lower pHs, can actively dissolve toxic metals such as lead or cadmium from pipes or solder. Copper in pipes is also frequently leached from pipes at high concentrations when the water has corrosive properties. Asbestos-cement has been used widely in water mains. The extraction of the asbestos fibers from these surfaces is also very much increased at lower pH and with soft water. The use of lead pipe and solder in household pipes has pretty much been abandoned in the United States. However, alloys of lead are still utilized in many faucets and brass fixtures (e.g., submersible pumps). Rather high concentrations of lead can result if water stands in these fixtures overnight. As a result it is always wise to avoid using the water first drawn from the tap in the morning for human consumption. Low levels of lead exposure *in utero* or in the first few years of life have been associated with delayed CNS development in humans and experimental animals.

Plastic pipes are polymeric in nature (e.g., polyvinyl chloride). Within the pipe are traces of the monomers used in the manufacture of the pipe (e.g., vinyl chloride). In addition, there are a variety of other chemicals added during the manufacture of the pipe as lubricants to facilitate their manufacture or stabilizers to prevent the breakdown of the pipe. In Europe, lead has been used as the stabilizer for pipes, whereas various organic tin compounds have been utilized in the United States. Lead is widely recognized as being toxic. Inorganic tin has a very limited toxicity, but this is not the form of tin that is used. Some of the organic tin compounds are potent nervous system toxins (e.g., trimethyl or triethyl tin), while others appear to adversely affect the immune system (dioctyl tin). The forms of tin used in polyvinyl chloride pipe, however, are primarily monomethyl and dimethyl tin, which are much less active as neurotoxins than the trimethyl tin. There will be some extraction of all these chemicals from the pipe when it is first put into service. However, the concentrations that are found in the water decrease sharply with continued use of the pipe. This is only partially due to the depletion of the chemical from the pipe because continuous water flow will form an impermeable barrier (e.g., calcium carbonate) on the interior of the pipe that minimizes leaching from its surface.

Paints and coatings can be utilized on any surface in a distribution system all the way to the pipes in the consumer's home. However, most coatings are applied to storage tanks and water mains. In the past years, some rather dangerous coatings have been used. Coal tar paints were frequently utilized in the first several decades of this century. These paints contain very high concentrations of polycyclic aromatic hydrocarbons (PAHs). Generally, this does not pose much of a problem because the solubility of these compounds in water is quite limited. This is particularly true of most of those which are carcinogenic. However, when the coating begins to degrade with age, it tends to come off the surface as small particles. These very small particles can contain very high concentrations of benzo(*a*)pyrene and other PAHs and have been shown to be carcinogenic when introduced into the stomach of mice. Fortunately, the coal tar paints have been largely replaced by asphalt paints, which contain very much smaller concentrations of PAHs. However, many distribution systems throughout the country have mains which predate this conversion. Another suspect practice of the past was the use of red lead paint in water tanks. Fortunately, this product has also been abandoned.

Summary

The sources of water pollution are diverse. Some of this pollution occurs in the general environment and involves both point and nonpoint sources. Pollution of this kind can impact human health both directly, when the water is consumed for drinking purposes, and indirectly through accumulation of chemicals in foodstuffs derived from the water. The chemicals seen from these two sources have very different characteristics. There are new and emerging chemicals being detected in drinking water supplies that are important for public health. These chemicals are difficult to evaluate due to the lack of/or uncertainty regarding the available toxicity information. In addition, these chemicals can be difficult to treat using standard water treatment processes. Despite the fact that there is contamination of ambient water, most contamination of drinking water by chemicals occurs during its treatment and distribution. While there is no conclusive evidence that these sources of chemicals adversely affect health, it is important to keep this issue in mind in the development of new processes for treating drinking water and new materials for distributing drinking water.

See also: Ecotoxicology; Effluent Biomonitoring; Environmental Processes; Environmental Toxicology; Organophosphates; Pesticides; Pollution, Air; Pollution, Soil; Polycyclic Aromatic Hydrocarbons (PAHs); Polymers.

Further Reading

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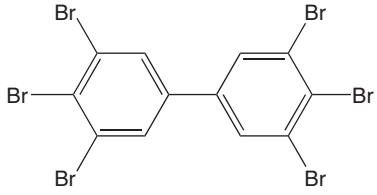
Relevant Website

http://www.epa.gov – Office of Water (US Environmental Protection Agency) – Activities, Documents, and News Online.

Polybrominated Biphenyls (PBBs)

Alan L Blankenship

- Representative Chemicals: Polybrominated biphenyls (PBBs) are members of the polyhalogenated diaromatic hydrocarbon (PHDH) class of compounds. PBBs are a complex mixture of individual compounds which are brominated with between one and 10 bromines in various combinations of positions to create a total of 209 possible congeners. The 10 positions are numbered 2–6 on one ring and 2′–6′ on the other ring. Positions 2, 2′, 6, and 6′, adjacent to the biphenyl bond are called *ortho* positions; 3, 3′, 5, and 5′, *meta* positions; 4 and 4′, *para* positions. Commercial products were mainly composed of hexa-, octa-, or deca-brominated homologs. Environmental contamination with PBBs is likely to have occurred mainly from two commercial products, FireMaster BP-6 and FireMaster FF-1. The principal components in both of these commercial products were 2,2′,4,4′,5,5′-hexabromobiphenyl or PBB-153 (54–68%) and 2,2′,3,4,4′,5,5′-heptabromobiphenyl or PBB-180 (7–27%)
- Chemical Abstracts Service Registry Numbers: CAS 67774-32-7; CAS 59536-65-1
- Synonyms and Commercial Products: Polybromobiphenyls; FireMaster BP-6; FireMaster FF-1; Bromkal 80; Flammex B 10; Adine 0102; Berkflam B 10
- Chemical Formula: $C_{12}H_nBR_m$ (where $n = 0$–9 and $m = 10$–n)
- Chemical Structure:



Uses

Polybrominated biphenyls (PBBs) are inert, stable chemicals used primarily as additive flame retardants to suppress or delay combustion. In their use as flame retardants, PBBs were added to polymer materials, but were not chemically incorporated into the polymer matrix and therefore could migrate out of the polymer matrix with time. Hexabromobiphenyl was used as a fire retardant mainly in thermoplastics in electronic equipment housings. Smaller amounts were used as a fire retardant in coating and lacquers, and in polyurethane foam for auto upholstery. After the voluntary ban of hexabromobiphenyl in the late 1970s, polybrominated diphenyl ethers (PBDEs) and other flame retardants were used as replacements.

Background Information

A significant incident of environmental contamination by PBBs occurred in 1973–74 when approximately 650 pounds (290 kg) of FireMaster BP-6 (principally composed of 2,2′,4,4′,5,5′-hexabromobiphenyl or P-153) were accidentally mixed with cattle

food that was distributed to a number of farms in the lower peninsula in Michigan. As a result, by June 1975, 412 farms had been quarantined. Use of PBBs as well as disposal of contaminated feed, animal carcasses (poultry, dairy cattle, swine), and animal products (dairy, meat, eggs) contributed to environmental contamination. About 50% of the original amount of PBBs mixed with feed was estimated to have been excreted in the feces of the exposed animals and remained on the farms in places of fecal deposition and manure disposal. PBB levels in surface soil samples from seven dairy farms in Michigan that spread contaminated manure on the fields ranged from 35 to 1260 $\mu g\,kg^{-1}$, while the concentrations in surface soil of control farms (that did not use contaminated manure) were $<25\,\mu g\,kg^{-1}$.

Exposure Routes and Pathways

In the past, PBBs were released to the environment during their manufacture and also during the disposal of commercial and consumer products containing PBBs. As a result, the general population may have been exposed to low levels of PBBs by inhaling contaminated air, ingesting contaminated water and food, and using consumer products containing PBBs. Prior to 1973, workers manufacturing fire retardants likely had the greatest potential for substantial exposure to PBBs. However, following the accidental contamination of cattle feed with PBBs in Michigan and the subsequent contamination of meat and dairy products, ingestion of contaminated foods became the exposure pathway of primary concern.

Although the episode in Michigan involving contaminated feed occurred in May 1973, incorporation of PBBs into foods was not identified until April 1974. Thus, PBB-containing meats, milk, butter, eggs, and cheese entered the human food chain for almost a year before the PBBs were identified. Concentrations of PBBs (on a fat basis) in milk samples collected from contaminated farms soon after PBBs were found ranged from 2.8 to 595 $mg\,kg^{-1}$. Concentrations of PBBs in other products processed from the contaminated milk were as follows: butter, 1–2 $mg\,kg^{-1}$; cheese, 1.4–15.0 $mg\,kg^{-1}$; and canned milk, 1.2–1.6 $mg\,kg^{-1}$. In 1974, the concentrations of PBBs in eggs from contaminated farm premises were as high as 59.7 $mg\,kg^{-1}$. The levels of PBBs in poultry and cattle tissues from a contaminated farm in 1974 were 4600 $mg\,kg^{-1}$ and up to 2700 $mg\,kg^{-1}$, respectively. With the seizure and destruction of the contaminated farm animals and products, the levels of PBBs in consumer products showed a steady decline. For example, in 1975, among 18 milk samples, 13 cheese samples, and 14 butter samples taken in Michigan, only three butter samples exceeded the FDA guidelines of 0.3 $mg\,kg^{-1}$ PBBs in fat. In 1975, PBBs were detected in 245/2040 meat samples collected in Michigan, with only 24 samples containing PBB levels $>0.3\,mg\,kg^{-1}$ fat. Although 95% of 1430 meat samples collected in Michigan in 1976 contained detectable PBBs, only one sample contained $>0.6\,mg\,kg^{-1}$, and a market basket survey in Michigan showed detectable PBBs in only 1/102 meat samples.

Currently, however, since PBBs are no longer produced, exposure of the general population to PBBs will likely only be from historical releases. Based on temporal data, it would appear that environmental levels have decreased substantially since the 1970s and current exposure, if any, will likely be at low levels.

Historical monitoring and body burden data indicate that low-level exposures to PBBs were limited to the population within the state of Michigan. The level of exposure to PBBs was slightly higher for the people residing in the lower peninsula of Michigan and highest among people residing in the immediate vicinity of the contaminated dairy farms, where people consumed contaminated meat, eggs, and dairy products. Consumer exposure from using PBB-containing plastic products (e.g., typewriters, calculators, projector housings, and movie equipment cases) is expected to be very low since the PBBs were incorporated into the plastic and their mobilization probably occurred only under conditions such as combustion.

Toxicokinetics

Data regarding the toxicokinetics of PBBs in humans are limited to information derived from cases of accidental ingestion of food contaminated with PBBs and cases of occupational exposure by the inhalation and dermal routes. These data provide qualitative evidence that PBBs are absorbed in humans by the inhalation, oral, and dermal routes. Absorption of PBBs from the gastrointestinal tract in animals can be inferred from the numerous reports of adverse effects and increased residue levels in tissues following oral administration of these compounds; however, few quantitative data exist. Limited quantitative data in animals indicate that some PBB congeners are well absorbed after oral exposure. For example, by comparing the amount of radioactivity in the feces of rats administered a single oral dose of 1 mg ^{14}C-2,2′,4,4′,5,5′-hexabromobiphenyl per kilogram with that monitored after a single intravenous injection of the compound, it was estimated that greater than 90% of the oral dose was absorbed over a 24 h

period. In contrast, with the high absorption rate for the hexabromobiphenyl congener, available data suggest that other congeners such as octabromobiphenyl may be less well absorbed by rats after administration of a single dose. For example, within the first 24 h after dosing with octabromobiphenyl, 61.9% of the dose was found in the feces, although it is unclear how much octabromobiphenyl may have been absorbed and undergone biliary excretion. Subsequent experiments in ruminants revealed that approximately half of an oral PBB dose is excreted unchanged in the feces 7 days after dosing and 23% is excreted in the milk within 95 days postdosing.

In blood, ~80% of PBBs are bound to protein and 20% are associated with lipids. The distribution pattern of PBBs does not appear to differ significantly between humans and animals and among animal species. Due to their lipophilic nature, PBBs, especially the highly brominated congeners, tend to accumulate in lipid-rich tissues. In general, relatively greater amounts of PBBs are usually found in the liver, adipose, skin, and breast milk. In rats treated by gavage with one or four daily doses of ^{14}C-2,2′,4,4′,5,5′-hexabromobiphenyl, initial concentrations of radioactivity were highest in muscle, liver, and adipose tissue, but later redistribution to adipose tissue (4–7 days after the last dosing) resulted in lower concentrations in liver and muscle. In rats dosed daily with ^{14}C-2,2′,4,4′,5,5′-hexabromobiphenyl over a 30 day period, the rank order of residue concentrations in fluids and tissues on day 31 were (in increasing order): blood, muscle, liver, skin, and adipose.

Certain components of PBB mixtures are metabolized by the microsomal monooxygenase system catalyzed by cytochrome P450 of the type induced by phenobarbital. The rate of metabolism of some PBB congeners depends on the bromine substitution pattern. PBB congeners of low bromine content are transformed into hydroxylated derivatives that are predominately eliminated in the urine. However, highly brominated congeners appear to undergo little or no metabolic transformation and are either retained or excreted unchanged in the feces.

Serum half-life values have been estimated using human data from the Michigan PBB cohort. A median half-life of 12–13 years was estimated. Just like polychlorinated biphenyls (PCBs), PBBs are capable of crossing the placental barrier and can concentrate in breast milk. Infants born to and nursing from PBB-exposed mothers may uptake and accumulate PBBs. Lactation constitutes the most important route of excretion of PBB in lactating women. Numerous studies reported PBB levels in breast milk from Michigan women. PBB levels in breast milk on a lipid basis ranged from undetected to 92 667 $\mu g\,kg^{-1}$, with a median of 250 $\mu g\,kg^{-1}$, in a group of parturient women from Michigan. Regression analysis of the data revealed that on a lipid basis, PBBs are 107–119 times more concentrated in milk than in serum.

There is limited information regarding excretion of PBBs in experimental animals. In rats gavaged with ^{14}C-2,2′,4,4′,5,5′-hexabromobiphenyl for 22 days, between 10% and 20% of the daily dose was excreted daily in the feces; this value was predominantly the result of elimination of unabsorbed PBB. In monkeys, the main route of excretion of hexabromobiphenyl residues was also in the feces. Between 60% and 70% of the administered dose was excreted in the feces in the first 11 days after dosing; urinary excretion was minimal.

Mechanism of Toxicity

The mechanism of toxicity for PBBs has been extensively studied, but is not completely understood. Many PBBs, PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and other structurally related halogenated aromatic hydrocarbons are believed to share a common mechanism of action strongly related to similarities in their structural configuration. Most of what is known regarding the mechanism of action of these compounds is based on structure–receptor binding relationships, structure–induction relationships, and structure–toxicity relationships. The mechanism for some congeners is related to the enhancement of gene expression triggered by initial binding to the cytosolic aryl hydrocarbon (Ah) receptor.

However, there are also likely physicochemical differences in PCBs, PBBs, and PBDEs due to the higher atomic weight and considerably larger molecular volume of bromine compared to chlorine. These differences contribute to dissimilar physical/chemical properties that can influence the relative bioavailability, absorption, tissue accumulation, receptor interactions, and toxicities of the chemicals.

Acute and Short-Term Toxicity (or Exposure)

The toxicological properties of PBBs are very similar to those of structurally related PCBs.

Animal

In a dairy cow herd that accidentally consumed FireMaster BP-6 in their diet, feed intake and milk production dropped to about half of normal levels. Initial symptoms noted in the herd included hematomas, hoof and hair abnormalities, and weight

loss. While the cause of these effects is generally attributed to PBB exposure, there is some controversy because these signs of toxicosis were not reproduced in controlled experiments with PBBs.

In rodents, oral doses of PBB cause liver hypertrophy, fatty liver, and scattered necrosis. In addition, neurological effects of PBB poisoning have been demonstrated in rats. Specifically, offspring from rats fed PBBs at a dose of 2 mg kg^{-1} during gestation and lactation showed signs of neurological damage and growth retardation.

The calculated 90 day LD_{50} for FireMaster FF-1 in rats is 149 and 65 mg kg^{-1} day^{-1} for male and female rats, respectively. However, in mink, the calculated dietary LD_{50} is 0.47 and 0.61 mg kg^{-1} day^{-1} for male and female minks, respectively, based on exposure to FireMaster FF-1 for 63–294 days.

Human

Epidemiological studies conducted following the Michigan incidence revealed no acute symptoms from the consumption of PBB-contaminated food. For long-term exposure the only symptoms that were at least partially attributed to PBB consumption included chloracne, blurred vision, and fatigue.

Chronic Toxicity (or Exposure)

Although available studies on chronic effects in humans are largely inconclusive, the animal data suggest that the PBBs can cause reproductive and developmental toxicity, and affect the liver, thyroid, and immune system. Hepatic effects in rodents and other laboratory animal species exposed orally to FireMaster PBBs in chronic-duration studies range from microsomal enzyme induction and liver enlargement to fatty changes and necrosis. Altered vitamin A homeostasis, primarily manifested as decreased hepatic storage of vitamin A, is another established effect of PBBs in animals. Thyroid effects, ranging from decreases in serum levels of serum T4 and serum triiodothyronine (T3) to histological and ultrastructural changes in the follicles, have been produced in rats in chronic-duration studies at doses as low as 1.3 mg kg day^{-1}.

Based on the results of the oral studies of FireMaster FF-1 in mice and rats, there is sufficient evidence to conclude that PBBs are carcinogenic in animals and potentially carcinogenic in humans. For example, in oral studies with mice and rats, hepatocellular adenomas, carcinomas, and/or liver neoplastic nodules were induced following single or repeated (intermediate- and chronic-duration) exposures. PBBs as a group have been classified as possibly carcinogenic to humans by IARC (Group 2B). This classification is based on sufficient evidence for carcinogenicity to animals and inadequate evidence of carcinogenesis in humans. The EPA has not classified the carcinogenicity of PBBs.

In Vitro Toxicity Data

PBBs induce cytochrome P450 isozymes from the CYP1A family. The ability to induce CYP1A enzymes is related to the binding affinity of congeners to the Ah receptor. In a study that attempted to utilize this biochemical effect as a biomarker of effect in humans, caffeine was used as a metabolic probe for induction of CYP1A activity. In a caffeine breath test of Michigan subjects from populations with varying concentrations of PBBs in serum, the correlation was poor between PBB concentration in serum and measures of caffeine metabolism. The authors concluded that there was substantial variability in enzyme activity possibly due to polymorphisms of the genes that regulate metabolizing enzymes and factors such as exposure to cigarette smoke, age, nutrition, hormone use, and hepatic disease.

Environmental Fate

Based on the similarity in structure and physicochemical properties between PBBs and PCBs, the environmental partitioning behavior of PBBs is generally very similar to that of PCBs, for which there are much more data. In general, PBBs are persistent, lipophilic, and tend to bind to particulate matter. The log octanol/water partition coefficients ($\log K_{ow}$) for PBB congeners vary by congener but are generally in the range of 5.53–8.58. The log carbon matter partition coefficients ($\log K_{oc}$) for PBB congeners vary by congener but are generally in the range of 3.33–5.09. Studies have shown that adsorption to and transport of sediments and particulates is a major transport mechanism of PBBs in aquatic systems. In sediments, PBBs can be reductively debrominated through a mechanism similar to that of reductive dechlorination of PCBs, dependent upon the presence of dehalogenating microorganisms. PBBs may be transported from water to aquatic organisms by direct bioconcentration as well as through diet. In fathead minnows (*Pimephales promelas*), experimentally derived bioconcentration factors BCFs of ~18 000 were derived for hexabromobiphenyl mixtures. Much less is known about the environmental fate of PBBs in terrestrial systems. Available data indicate that translocation of PBBs from soil into plants is not significant.

See also: Polychlorinated Biphenyls (PCBs).

Further Reading

Agency for Toxic Substances and Disease Registry (2002) *Toxicological Profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers*. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

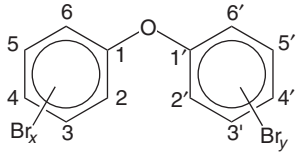
Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Polybrominated Biphenyls.

Polybrominated Diphenyl Ethers (PBDEs)

Alan L Blankenship, John Newsted, and Paul Jones

- Representative Chemicals: Heptabromodiphenylether (HepBDE); Nonabromodiphenyl ether (NoBDE); Tetrabromodiphenyl ether (TeBDE); Hexabromodiphenyl ether (HeBDE); Octabromodiphenyl Ether (OBDE); Pentabromodiphenyl ether (PeDBE); Decabromodiphenyl ether (DeBDE)
- Chemical Abstracts Service Registry Numbers: CAS 68928-80-3 (HepBDE); CAS 63936-56-1 (NoBDE); CAS 40088-47-9 (TeBDE); CAS 36483-60-0 (HeBDE); CAS 32536-52-0 (OBDE); CAS 32534-81-9 (PeDBE)
- Synonyms: Heptabromodiphenyl ether; Heptabromodiphenyl oxide; Nonabromodiphenyl ether, 2,3′,4′-Tribromodiphenyl ether; Tribromodiphenyl ether; Tetrabromodiphenyl ether; Hexabromodiphenyl ether; Octabromodiphenyl ether; Octabromodiphenyl oxide; Pentabromodiphenyl ether pentabromodiphenyl oxide; 4,4′-Dibromodiphenylether bis-*p*-bromophenyl ether; Dibromodiphenyl ether; *p*,*p*′-1,1′-oxybis (2,3,4,5,6-pentabromobenzene); Berkflam b 10E; Bis(pentabromophenyl) ether; BR 55N; Bromkal 82-ode; Bromkal 83-10de; DE 83R; DBDPO; Decabromodiphenyl ether; Pentabromodiphenyl ether; Saytex 102; Saytex 102E; Tardex 100
- Chemical/Pharmaceutical/Other Class: Brominated aromatic
- Chemical Formula: $C_{12}H_3Br_7O$
- Chemical Structure:



Uses

Polybrominated diphenyl ethers (PBDEs) are used as additive flame retardants in a wide range of products including thermoplastics. The most important limitations on their use are incompatibilities that affect the physical properties of the polymers and the tendency for additives to be fugitive. These flame retardants are added to polymer materials instead of being chemically incorporated into the matrices and as a result are much more prone to leaching or escape from the finished polymer product than reactive flame retardants. The major uses of the PBDEs are in: high-impact polystyrene, acrylonitrile butadiene styrene (ABS), flexible polyurethane foam, textile coatings (not clothing), wire and cable insulation, electrical/electronic connectors, and other interior parts. These applications account for 80–90% of the consumption of PBDEs in the United States. PBDEs are used in resins, polymers, and substrates at levels ranging from 5% to 30%. In consumer products, resins containing PBDE are typically used in interior parts, minimizing the potential for exposure of the public. Currently, technical DeBDE is the most widely used PBDE flame retardant worldwide, followed by ODBE.

Exposure Routes and Pathways

The widespread use of PBDEs has resulted in their ubiquitous presence in the environment. In occupational environments, exposure to DeBDE may occur through inhalation of dusts. Atmospheric inhalation exposure to PBDEs is expected to be low, since their vapor pressures are in the range of 10^{-7} mmHg. Particulates in the respirable range are expected to be formed during the grinding of solids. Dermal exposure may occur during filtration, drying, drumming/bagging, size reduction, and maintenance. Exposure to PBDEs can also take place during processing (incorporation into various polymers) and final production. Exposure of the general public may occur via inhalation of ambient air, ingestion of fish, and dermal contact with products such as television enclosures or textiles containing PBDEs.

Toxicokinetics

Studies conducted with rats suggest that PBDEs are poorly absorbed by oral, inhalation, or dermal

routes. In rats given oral doses of DeBDE and OBDE, ~91% and 62% of the administered dose, respectively, was found in the feces within 24 h indicating these compounds were not significantly absorbed by the rats. In rats fed DeBDE no organ or tissue contained more than 0.26% of the administered dose. However, absorption of PBDE is affected by degree of bromination; lower brominated PBDEs tend to be better absorbed. The elimination half-life of PeBDE from rats ranged from 19 to 110 days. However in rats given a mixture of OBDE and DeBDE, the elimination half-life of DeBDE in the feces was estimated to be <24 h while for OBDE there was an initial phase half-life of <24 h followed by a second phase half-life of >16 days. Little or no metabolism of PBDEs was observed in this study.

Mechanism of Toxicity

Some PBDEs have been shown to bind to the Ah receptor and as a result, may have some limited 'dioxin-like' activity. However, recent evidence suggests that trace levels of polybrominated dibenzo-p-dioxins, dibenzofurans, and biphenyls may have been present in PBDEs at sufficient concentrations to elicit the observed 'dioxin-like' toxicity in the experiments discussed below. In one Ah receptor activation study, the PBDEs with the greatest activity were penta- and hexa-BDE congeners while tri- and tetra-BDE congeners had the least potency. These results were similar to those observed in a receptor binding study where binding affinities of PBDEs ranged from 10^{-2} to 10^{-5} times that of dioxin. In this study, 2,3,4,4′-penta-BDE had the greatest affinity (2% of the TCDD affinity) for the Ah receptor while DeBDE did not bind to the receptor. The order of affinity of PBDE congeners to the Ah receptor was similar to that observed for several other end points including cytochrome P450 induction (EROD) and immunotoxicity as measured by splenic PFC responses to SRBC antigen. PBDEs have also been shown to disrupt thyroid function. Depending on dose, duration, and PBDE congener, the chemicals have been shown to disrupt production, transport, and disposition of thyroid hormones. Evidence for these effects include: (1) histological changes in the thyroid, (2) decreased serum thyroxine (T_4) levels with no changes in serum TSH, and (3) the structural similarity of several PBDEs to T_4. Estrogenic and antiestrogenic activities of several PBDE congeners and three hydroxylated PBDEs have been evaluated *in vitro*. Eleven of 17 PBDE congeners have showed estrogenic activity in the ER-CALUX assay. All PBDE congeners were at least 250 000 times less potent than 17β-estradiol (E_2). However, some hydroxylated PBDEs showed estrogenic potencies that exceeded E_2. These results indicate that pure and hydroxylated congeners of PBDEs can be agonists of estrogen receptors and that the metabolism of PBDEs may produce more potent pseudoestrogens.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of DeBDE for laboratory animals is low. It is not an irritant to skin or eyes in rabbits and is not chloracnegenic in rabbits and is not a human skin sensitizer. The combustion products of flame-retarded polystyrene containing DeBDE were tested for acute toxicity and comedogenicity. The rat oral LD_{50} of the soot and char was $>2000\,mg\,kg^{-1}$ body weight. In short-term toxicity studies on rats and mice, DeBDE at dietary levels of 100 or $50\,g\,kg^{-1}$ did not induce adverse effects. A one-generation reproduction study on rats showed no adverse effects with dose levels of $100\,mg\,kg^{-1}$ body weight. DeBDE did not cause any teratogenic effects in the fetuses of rats administered a dose of $100\,mg\,kg^{-1}$ body weight. Developmental toxicity studies have shown no evidence of teratogenicity of DeBDE, OBDE, and PeDBE in rats and rabbits, although fetotoxic effects that included variations in skeletal ossification were observed at maternally toxic doses.

Chronic Toxicity (or Exposure)

Animal

In a carcinogenicity study of DeBDE with rats and mice, an increase in the incidence of adenomas was found in the livers of male rats receiving $25\,g\,kg^{-1}$ and female rats receiving $50\,g\,kg^{-1}$. In male mice, increased incidences of hepatocellular adenomas and/or carcinomas were found as well as an increase in thyroid follicular cell adenomas/carcinomas (combined). Female mice did not show any increase in tumor incidence. There was equivocal evidence for carcinogenicity in male and female rats and male mice only at dose levels of $25–50\,g\,kg^{-1}$ diet. The International Agency for Research on Cancer (IARC) has concluded that there was limited evidence for the carcinogenicity of DeBDE in experimental animals. The very high dose levels, lack of genotoxicity, and minimal evidence for carcinogenicity indicate that DeBDE, at the present exposure levels, does not present a carcinogenic risk for humans.

Human

A morbidity study of extruder personnel blending polybutyl-eneterephthalate containing DeBDE during

an exposure period of about 13 years did not reveal any deleterious effects. Additional studies of this group showed that the immune system of the exposed persons was not adversely affected over this time.

In Vitro Toxicity Data

Cytogenetic examination of bone marrow cells showed no increase in aberrations in maternal and neonatal rats following maternal oral exposure to a DeBDE and NoBDE mixture. *In vitro* assays found that DeBDE did not induce gene mutations in several bacterial tests (Ames assays) or in mammalian cells. DeBDE also did not induce chromosomal aberrations in Chinese hamster ovary cells. However, exposure to the congeners 2,2′,4,4′-tetra-BDE, 3,4-diBDE, and 2-monoBDE caused increased recombinogenic activity at the HGPRT locus in several cell lines.

Environmental Fate

An estimated vapor pressure of 4.7×10^{-12} mmHg indicates DeBDE will exist solely in the particulate phase in the ambient atmosphere. Particulate-phase DeBDE will be removed from the atmosphere by wet and dry deposition. Direct photodegradation may be fairly rapid based upon studies with sunlight irradiation. If released into soil, DeBDE is expected to be immobile based upon an estimated K_{oc} of 692 000. Volatilization from moist soil surfaces is not expected to be an important fate process based upon an estimated Henry's law constant of 1.2×10^{-8} atm^3 mol^{-1}. No data were located showing the biodegradation of this compound in soil or water environments; this compound was not biodegraded over 14 days in a single screening biodegradation test. If released into water, DeBDE is expected to adsorb to suspended solids and sediment based upon the estimated K_{oc}. Volatilization from water surfaces is not expected to occur based upon this compound's estimated Henry's law constant. BCF values ranging from 0.3 to <50 suggest bioconcentration in aquatic organisms is low to moderate. The fate of DeBDE in the environment needs further study; in the absence of sunlight, the compound persists in soils and sediments while in sunlight, DeBDE readily degrades to the lower brominated congeners, such as tetra- and hexabrominated biphenyl ethers, which readily bioaccumulate.

Other Hazards

Formation of brominated dioxins and furans on combustion of PBDE containing products may be a hazard.

See also: Bromine; Polybrominated Biphenyls (PBBs).

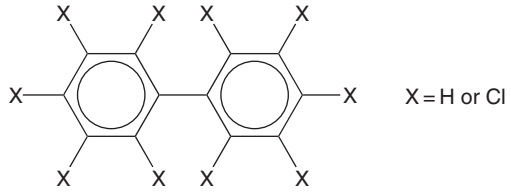
Further Reading

Agency for Toxic Substances and Disease Registry (2002) *Toxicological Profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers*. Atlanta, GA: US Department of Health and Human Services, Public Health Service.

Polychlorinated Biphenyls (PCBs)

Swarupa G Kulkarni and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 1336-36-3
- Synonyms: Arochlor; Chlorodiphenyls; Clophen; Fenchlor; Kanechlor; Phenochlor; Pyralene
- Chemical Structure:



Uses

Polychlorinated biphenyls (PCBs) were used in electrical capacitors, electrical transformers, vacuum pumps, and gas transmission tribunes. They were also used as hydraulic fluids, plasticizers, adhesives, fire retardants, wax extenders, lubricants and cutting oils, inks, dedusting agents, etc. PCBs are no longer commercially produced in the United States but are still found in the environment. PCB's have been found in at least 500 of the 1598 National Priorities List Sites identified by the Environmental Protection Agency (EPA).

Exposure Routes and Pathways

Most exposures are environmental or occupational with the delayed symptoms being the first indication that an intoxication has occurred.

Toxicokinetics

PCBs and polybromated biphenyls are absorbed by all routes. Dermal absorption varies depending on the compound, concentration, and species but is in the 15–56% range. PCBs are chemically inert and the more highly chlorinated compounds are resistant to metabolism. The liver is the primary site of metabolism and the primary mechanism is hydroxylation and conjugation with glucuronic acid and is inversely proportional to the chlorine content. PCBs are primarily distributed to the adipose tissues. During pregnancy, one-tenth of the maternal serum level can be found in cord blood and 107–119 times the serum level can be found in human milk. Excretion is variable depending on the species and inversely related to the chlorine content. PCBs are excreted in breast milk.

Mechanism of Toxicity

The exact mechanism of action by which PCBs cause their toxicity is unclear. They are potent enzyme inducers and affect thiamine utilization.

Acute and Short-Term Toxicity (or Exposure)

Animal

In laboratory animals exposed orally or cutaneously to sublethal levels of various PCB mixtures, common findings are severe atrophy of 1° and 2° lymphoid organs, lower circulatory immunoglobulin levels, and decreased specific antibody responses following immunization with antigens. Both augmentation and suppression of cell-mediated immunity on exposure to PCBs have been reported.

Human

PCBs have a low acute toxicity but they accumulate in the environment and in animal and human tissues; the potential for chronic or delayed toxicity is significant. The most dramatic case of PCB poisoning occurred in West Japan in 1968 (Yusho accident) when rice oil contaminated with PCBs poisoned more than 1600 people. Fatigue, headache, increased sweating of the palms, itching, visual disturbances, numbness of the extremities, subcutaneous facial edema, joint swelling and pain, cough, intermittent abdominal pain, and menstrual changes were noted. However, the symptoms may not be purely due to PCB toxicity since the oil also contained dibenzofurans and quaterphenyls, which are known to be toxic. Fifteen cases of reproductive and fetotoxic human effects were observed in the Yusho epidemic. Decreased immunoglobulin levels were observed.

PCBs are mildly irritating to the eyes and skin. Facial edema, eye discharge, swollen eyelids, conjunctival hyperemia, and visual and hearing disturbances may result. Increases in diastolic and systolic blood pressures are possible. Neurobehavioral and psychomotor impairment have been seen after occupational exposure. Gastrointestinal disturbances and diarrhea have been noted. Clinical hepatitis has been seen in the Yusho epidemic. PCB exposure can cause elevation of serum triglycerides. Chloracne, which may occur from either dermal contact or systemic absorption, is a specific skin reaction associated with cyclic halogenated compounds and is characterized by distinct cystic, skin-colored lesions and comedones, both of which may become inflamed and infected. Edematous swelling of the limbs has been reported. Pruritis was observed in 14% of the exposed persons following exposure to combustion products of PCBs. Small elevation in urinary uroporphyrin levels and decreased coproporphyrin levels in a small number of humans accidentally exposed to PCBs have been reported.

Chronic Toxicity (or Exposure)

Animal

Liver damage is a consistent finding in animal studies. PCBs are carcinogenic in animals causing liver tumors in rats.

Human

Long-term exposure to PCBs may cause embryo toxicity including fetal death, fetal resorption, cleft palate, dilated renal pelvis, and hypoplasia of the thymus. Males may be more susceptible to the teratogenic effects than females. It may cause reproductive and fetotoxic effects. Mammalian reproductive effects include changes in the estrus cycle, implantation failure, increased abortions, low birthweight offspring, and decreased postnatal survival. PCBs are considered potential human carcinogens. A slight increase in melanoma of the skin in men occupationally exposed to PCBs has been reported. Renal adenocarcinoma in workers chronically exposed to PCBs has occurred.

Clinical Management

Most exposures are environmental or occupational with the delayed symptoms being the first indication that intoxication has occurred. There is no specific treatment, only supportive treatment. Emesis is of no use since ingestion of PCBs will not be recognized until long after emesis is of any value. Vomiting may cause aspiration. On ingestion, activated charcoal

mixed with a saline cathartic or sorbitol may be used. On ocular exposure, the eyes should be flushed. On dermal exposure, multiple soap and water washings are necessary. On inhalation exposure, emergency airway support and 100% humidified supplemental oxygen with assisted ventilation may be needed. If a cough or difficulty in breathing develops, the victim should be evaluated for respiratory tract irritation, bronchitis, and pneumonitis.

Environmental Fate

PCBs have been identified in at least 500 of 1598 hazardous waste sites proposed for inclusion on the EPA National Priorities list. Before being banned and before the Clean Water Act regulated wastewater discharges, PCBs could be found, often at high levels, in wastewaters from industries handling PCB equipment. These wastewaters either were discharged directly to surface waters or sent to municipal sewage treatment plants. Urban industrial areas are more likely to have higher PCB contamination than rural areas. While not highly volatile, PCBs, especially the less chlorinated ones, will partition into the air. Atmospheric transport is the most important mechanism for dispersion of PCBs. Those PCBs with a high degree of chlorination are much more persistent in the environments than those with lower degrees of chlorination.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (ACGIH TLV) for chlorodiphenyl (42% chlorine) is $1\,mg\,m^{-3}$ time-weighted average (TWA). The ACGIH TLV for chlorodiphenyl (54% chlorine) is $0.5\,mg\,m^{-3}$ TWA. ACGIH has not established a short-term exposure limit for chlorodiphenyl.

The Occupational Safety and Health Administration permissible exposure limit (OSHA PEL) for chlorodiphenyl (42% chlorine) is $1\,mg\,m^{-3}$ PEL – TWA, with skin notation. The OSHA PEL for chlorodiphenyl (54% chlorine) is $0.5\,mg\,m^{-3}$ PEL – TWA, with skin notation.

The EPA has set a limit of 0.0005 mg of PCBs per liter of drinking water ($0.0005\,mg\,l^{-1}$). Discharges, spills, or accidental releases of 1 pound or more of PCBs into the environment must be reported to the EPA. The Food and Drug Administration (FDA) requires that infant foods, eggs, milk and other dairy products, fish and shellfish, poultry, and red meat contain no more than 0.2–3.0 parts of PCBs per million parts (0.2–3.0 ppm) of food. Many states have established fish and wildlife consumption advisories for PCBs.

Miscellaneous

PCBs are mixtures of different congeners of chlorobiphenyl. The arochlors are characterized by four-digit numbers. The first two digits indicate that the mixture contains biphenyl (12), triphenyls (54), or both (25 and 44); the last two digits give the weight percentage of chlorine in the mixture. For example, Arochlor 1242 contains biphenyl with ~42% chlorine.

Physical properties vary by product because of the varied composition. For example; Arochlor 1242 is a clear mobile liquid; Arochlor 1254 is a light yellow, viscous liquid; and Arochlor 1260 is a light yellow, soft sticky resin. PCBs are heat stable and resistant to biologic degradation as well as acids, bases, oxidation, and other chemical reactions.

See also: Environmental Hormone Disruptors; Neurotoxicity; Psychological Indices of Toxicity; Skin.

Further Reading

Faroon OM, Keith S, Jones D, and deRosa C (2001) Carcinogenic effects of polychlorinated biphenyls. *Toxicology and Industrial Health* 17: 41–62.

Faroon OM, Keith S, Jones D, and deRosa C (2001) Effects of polychlorinated biphenyls on development and reproduction. *Toxicology and Industrial Health* 17: 63–93.

Faroon OM, Jones D, and deRosa C (2001) Effects of polychlorinated biphenyls on the nervous system. *Toxicology and Industrial Health* 16: 305–333.

Relevant Website

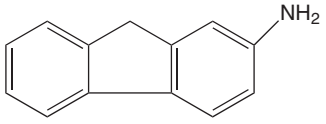
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Polychlorinated Biphenyls (PCBs).

Polycyclic Aromatic Amines

Shayne C Gad

This article is a revision of the previous print edition article by Jayne E Ash and Shayne C Gad, volume 2, pp. 576–577, © 1998, Elsevier Inc.

- Representative Chemicals: Flouren-2-amine; 3,3′-Dichlorobenzidine
- Chemical Abstracts Service Registry Number: CAS 153-78-6
- Synonyms: 2-Aminofluorene; Fluorene
- Chemical Structure



Uses

Polycyclic aromatic amines occur naturally in coal tar. They are by-products of the coal refining process. They were used in the 1930s as an insecticide.

Exposure Routes and Pathways

Dermal contact, ingestion, and inhalation are possible routes of exposure.

Toxicokinetics

Polycyclic aromatic amines are readily absorbed into the body via the gastrointestinal tract, where metabolic activation takes place. Aryl amines are *N*-hydroxylated and subsequently glucuronidated via uridine diphosphate (UDP)-glucuronosyl transferase or sulfated by sulfotransferases, *N*-acetylation of the amine, and *O*-acetylation of the *N*-hydroxy amine can occur.

Mechanism of Toxicity

N-Hydroxy metabolites within the gastrointestinal tract transform fluoren-2-amine into a mutagen or carcinogen. A number of polycyclic aromatic amines are potent bladder carcinogens. As noted above, sequential hydroxylation and glucuronidation leads to urinary excretion, with metabolites in the urinary bladder. While glucuronidation enhances excretion via the urine, a glucuronidase in the bladder hydrolyzes the glucuronide and under acidic conditions *N*-hydroxylarylamines are formed. A spontaneous conversion of the amine leads to an arylntrinium ion, which can initiate tumor formation. Sulfate esters can degrade to electrophilic nitrinium ion–carbonium ion, which can form adducts with macromolecules.

Acute and Short-Term Toxicity (or Exposure)

Animal

Polycyclic aromatic amines have relatively low acute toxicity potential. The oral and intraperitoneal values of LD_{50} of acetylaminofluorene in mice are 810 and 470 mg kg^{-1}, respectively.

Human

Little information is available regarding acute toxicity of polycyclic aromatic amines in humans.

Chronic Toxicity (or Exposure)

Animal

A number of polycyclic aromatic amines are carcinogens in animals. 2-Acetylaminfluorene is a teratogen, mutagen, and carcinogen. It is tumorigenic in rats at 2420 mg (TD, oral). Dietary exposure to 2-acetylaminofluorene in rats led to tumors of the liver, bladder, renal pelvis, ear canal, colon, lung, pancreas, and testis. Tumors of the liver, bladder, and kidney have been observed in mice exposed to dietary 2-acetylaminofluorene. Bladder and liver tumors have been observed in other laboratory animals exposed to 2-acetylaminofluorene.

Human

Carcinogenic properties are dependent on individual rates of acetylation. Persons who are slow acetylators are more susceptible to bladder cancer from aromatic amines, as generally are workers in industrialized countries. Nutrition is also implicated in the development of cancer by polycyclic aromatic amines.

Clinical Management

The victim should be removed from exposure.

Environmental Fate

Polycyclic aromatic amines may be transported as vapor or adsorbed onto particulates. Due to low water solubility, polycyclic aromatic amines are

not transported in water but adsorb onto soil and sediments. Leaching is negligible. Bioaccumulation is not considered a concern.

Ecotoxicology

Little information is available concerning the ecotoxicity of this class of chemicals.

See also: Carcinogenesis; Oil, Crude.

Further Reading

Hodgson E and Goldstein JA (2001) Metabolism of toxicants: Phase I reactions and pharmacogenetics. In: Hodgson E and Smart R (eds.) *Introduction to Biochemical Toxicology*, 2nd edn., pp. 67–113. New York: Wiley.
Kitchen KT (1999) *Carcinogenicity*. New York: Dekker.

Relevant Website

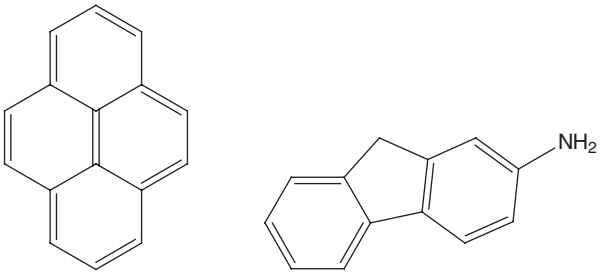
http://www.cdc.gov – National Institute of Occupational Safety and Health.

Polycyclic Aromatic Hydrocarbons (PAHs)

Shayne C Gad and Samantha E Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 2, p. 577, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: CAS 129-00-0 (Pyrene); CAS 153-78-6 (Fluoren-2-amine)
- Chemical Formula: $C_{16}H_{10}$ (for pyrene); $C_{13}H_9NH_2$ (for fluoren-2-amine)
- Other Compounds: Benzo[*a*]pyrene; 3-Methylcholanthrene
- Chemical Structures:



Uses

Pyrene is used in biochemical research. Polycyclic aromatic hydrocarbons (PAHs) occur naturally in coal tar, fossil fuel combustion, forest fires, and open flame grilled meats. PAHs are found in cigarette smoke and in diesel emissions, when asphalt surfacing and tar roofing, and also in aluminum and coke plants. Pyrene was used in the 1930s as an insecticide.

Exposure Routes and Pathways

Dermal contact, ingestion, and inhalation are possible exposure routes.

Toxicokinetics

PAHS are readily absorbed via the gastrointestinal tract and then metabolically transformed to more reactive forms. These toxicants are typically converted into more reactive metabolites through phase I biotransformations, and then converted into more readily excretable conjugates via phase II processes.

Mechanism of Toxicity

Pyrene increases photosensitivity and suppresses the immune system. P450 metabolism of a number of PAHs leads to carcinogenic and mutagenic potential. PAHs have different toxicity profiles; some are more toxic than others. However, the mechanism of toxicity often relies on adduct formation with macromolecules following biotransformation.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, pyrene is a mild dermal irritant and primary irritant. The oral LD_{50} is $2.7\,g\,kg^{-1}$ in rats and $800\,mg\,kg^{-1}$ in mice.

Human

Photosensitization of skin and eyes can be caused by dermal exposure and inhalation causing skin effects including erythema and lesions.

Chronic Toxicity (or Exposure)

Animal

Several PAHs have been shown to cause reproductive and developmental effects in rodents. Genotoxic properties have been found *in vitro* and *in vivo*.

Human

Toxic dermal effects are increased by exposure to ultraviolet light. Lesions on sun exposed skin may progress to skin cancer. Respiratory effects include cough, chronic bronchitis, and naematuria. Workers exposed to high airborne concentrations of some PAHs have shown increased rates of cancer and is therefore considered a probable carcinogen. Pyrene produces a carcinogenic effect from exposure to skin as well as a presence in bloodstream. It also produces immunodepression. Benzo[*a*]pyrene is found in relatively high levels in the environment and is a probable mutagen and teratogen; it has caused severe and long lasting hyperplasia and metaplasia as precancerous lesions.

Clinical Management

The victim should be removed from exposure. Exposed skin and eyes should be thoroughly flushed with tepid water. Supportive therapy should be provided.

Environmental Fate

The PAHs are produced by the incomplete combustion of fossil fuels, wood, and other organic material. These compounds are largely adsorbed onto smoke particles/aerosols and are a major component of industrial air pollution. Partitioning between water and air, between water and sediment, and between water and biota are the most important of the distribution processes. Even though most of these toxicants are released into the atmosphere, considerable amounts are found in water. These toxicants can enter the aquatic environment in many ways but mostly through large oil spills. Their affinity for organic matter in sediment, soil, and biota is high, and these compounds therefore accumulate in organisms in water and sediments. In *Daphnia*, accumulation of PAHs from water is correlated with their octanol–water partition coefficient. In organisms that actively metabolize these chemicals, absorbed concentrations are not correlated with the partition coefficient. Biomagnification is not observed with these toxicants. PAHs undergo photodegradation, microbial degradation, and metabolism in higher organisms. Hydrolysis plays essentially no role in their degradation. These chemicals are photooxidized in air and water in the presence of radicals; for example, OH, NO_3, and O_3. The reaction of two- to four-ring structures with NO_3 leads to nitro-derivatives, which are known mutagens.

PAHs exhibit toxic properties at low concentrations and several have been listed as priority pollutants to be monitored in industrial effluents, natural waters, soils, and sediments. They enter soil systems and natural waters via wastewater effluents from coke and petroleum refining industries, accidental spills and leakages, rainwater runoff from highways and roadways, or from intentional disposal in the past. Low aqueous solubilities of PAHs and high octanol–water partition coefficients (K_{OW}) often result in their accumulation in soils and sediments to levels several orders of magnitude above aqueous concentrations. PAHs can be potent carcinogens, and their presence in groundwater, streams, soil, and sediments may constitute a chronic human health hazard.

There has been tremendous interest in understanding the fate and transport of PAHs in subsurface environments that are largely microaerobic or anaerobic. Little is known about anaerobic biotransformation of these contaminants, particularly in the context of soil and ground water contamination. Aerobic transformation of PAHs associated with soil and groundwater often leads to rapid depletion of dissolved oxygen and this eventually decreases the redox potential (E_h). Such decrease in the redox potential can result in favorable growth environments for denitrifying, sulfate-reducing, or even methanogenic ($E_h < -0.3\,V$) microbial populations. Nearly 10–15% of the bacterial population in soil, water, and sediments consists of anaerobic organisms. Anaerobic transformations may, therefore, play a significant role in oxygen-depleted natural habitats.

Ecotoxicology

Marine organisms adsorb and accumulate PAHs from water. Concentrations up to $7\,mg\,kg^{-1}$ have been noted in organisms living near industrial effluents, and average levels in aquatic animals at contaminated sites were 10–500 $\mu g\,kg^{-1}$. Average levels of these toxicants in aquatic organisms at sites with unspecified sources of PAH were 1–100 $\mu g\,kg^{-1}$, but high concentrations (up to $1\,mg\,kg^{-1}$) were found in some species, for example, lobsters in Canada. Concentrations of PAHs in insects ranged from 0.7 to $5.5\,mg\,kg^{-1}$. In heavily contaminated locations, concentrations of benzo[*a*]pyrene in earthworm feces may reach $2\,mg\,kg^{-1}$.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit for benzo[*a*]pyrene is $0.2\,mg\,m^{-3}$.

See also: Absorption; Benz[*a*]anthracene; Carcinogenesis; Methylcholanthrene, 3-; Respiratory Tract.

Further Reading

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Klaassen CD (2001) *Casarett and Doulls Toxicology*, 6th edn. New York: Mcgraw-Hill.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs).
http://www.inchem.org – Selected Non-Heterocyclic Polycyclic Aromatic Hydrocarbons (Environmental Health Criteria 202 from the International Programme on Chemical Safety.

Polyethylene Glycol

Hon-Wing Leung

- Chemical Abstracts Service Registry Number: CAS 25322-68-3
- Synonyms: α-Hydro-ω-hydroxypoly-(oxy-1,2-ethanediyl); Macrogol; PEG; Carbowax; Jeffox; Nycolin; Pluracol E; Poly-G; Polyglycol E; Solbase; Polyox
- Chemical/Pharmaceutical/Other Class: A distribution of liquid and solid polymers of varying molecular weights (from 200 to several million) corresponding to an average number of oxyethylene groups
- Chemical Structure:

$$H(OCH_2-CH_2)_nOH$$

Where n = average number of oxyethylene groups

Uses

Polyethylene glycols are widely used in food, cosmetics, and topical pharmaceuticals (e.g., ointments and suppository base).

Exposure Routes and Pathways

Ingestion and skin contact are the most common routes of both accidental and intentional exposures.

Toxicokinetics

The absorption of orally administered polyethylene glycols is dependent on their molecular size. While 50–65% of liquid polyethylene glycols (molecular weight up to 600) are absorbed, only from 0% to 2% of solid polyethylene glycols (molecular weight more than 1000) are absorbed. High-molecular-weight polyethylene glycols are retained in the blood circulation for a longer period than low-molecular-weight polyethylene glycols.

Polyethylene glycols are not appreciably metabolized. Ethylene glycol is not known to be a metabolite. The distribution of the higher members of polyethylene glycols within the body is extracellular, whereas the lower-molecular-weight members of the series diffuse intracellularly to a considerable extent. Polyethylene glycols tend to accumulate in the muscle, skin, bone, and the liver to a higher extent than the other organs, irrespective of the molecular weight.

Liquid polyethylene glycols are rapidly excreted in the urine, while the higher-molecular-weight members are mainly eliminated in the feces.

Mechanism of Toxicity

Many years of human experience in the workplace and in the use of consumer products containing polyethylene glycols have not shown any adverse health effects, except for administering high doses to sensitive or unhealthy persons. Nephrotoxicity associated with the topical treatment of burn patients with polyethylene glycols may reflect the compromised function of the patients' kidneys rather than the direct toxic effects of polyethylene glycols.

Acute and Short-Term Toxicity (or Exposure)

Animal

Polyethylene glycols have a very low level of acute toxicity to animals. They do not produce appreciable irritation to the rabbit skin and are only mildly irritating to the rabbit eyes.

Human

There have not been any reports of acute toxic or irritative effects in humans exposed to polyethylene glycols. The lowest-molecular-weight members (200–300) have been observed to produce at most only a

mild sensitization reaction in a very small percentage of individuals in skin patch testing studies.

Chronic Toxicity (or Exposure)

Animal

Subchronic feeding and drinking water studies in rats and dogs revealed that polyethylene glycols have very low toxicity. Nephrotoxicity and hepatotoxicity have been observed in monkeys and dogs respectively, after continuous intravenous infusion of high doses of polyethylene glycol. Chronic feeding and skin painting studies in rat and mouse, respectively, do not indicate any significant incidence of tumor production.

Human

No epidemiological studies or case reports of ill effects in healthy humans attributable to chronic exposure to polyethylene glycols were found in the available literature.

In Vitro Toxicity Data

Polyethylene glycols are negative in a battery of genotoxicity tests.

Clinical Management

Since polyethylene glycols are of very low acute toxicity and nonirritating, emergency care is not anticipated. There is no specific antidote for polyethylene glycols. Treatment of overexposure should be directed at the control of symptoms and the clinical condition of the patient.

Environmental Fate

Like other polymeric substances, polyethylene glycols are not readily biodegradable. However, owing to their hydrophilicity, they have a low potential to bioaccumulate.

Ecotoxicology

Polyethylene glycols have a very low order of toxicity to aquatic organisms including daphnids and fish.

Exposure Standards and Guidelines

The American Industrial Hygiene Association has set a workplace environmental exposure limit of $10\,\mathrm{mg\,m^{-3}}$ as an aerosol for polyethylene glycol.

See also: Ethylene Glycol; Polymers.

Further Reading

Pang SNJ (1993) Final report on the Safety Assessment of Polyethylene Glycols (PEGs). *Journal of the American College of Toxicology* 12: 429–457.

Polymers

Samantha E Gad

Background

Polymers are macromolecules formed by the chemical bonding of five or more identical units called monomers. These monomers are then connected repeatedly to form isomers which are then strung together repeatedly to form polymers. In most cases the number of monomers is quite large (3500 for pure cellulose) and often is not precisely known. In synthetic polymers, this number can be controlled to a predetermined extent (e.g., by shortstopping agents). Combinations of two, three, or four monomers are called dimers, trimers, and tetramers respectively, and are known collectively as oligomers. Such oligomers are not polymers.

A partial list of polymers by type includes the following:

I. Inorganic: siloxane, sulfur chains, black phosphorus, boron–nitrogen, silicones.

II. Organic

1. Natural
a. Polysaccharides: starch, cellulose, pectin, seaweed gums (e.g., agar), vegetable gums (e.g., arabic)
b. Polypeptides (proteins): casein, albumin, globulin, keratin, insulin, DNA
c. Hydrocarbons: rubber and gutta percha (polyisoprene), also called elastomers
2. Synthetic
a. Thermoplastic polymers: nylon, polyvinyl chloride, polyethylene (linear), polystyrene,

polypropylene, fluorocarbon resins, polyurethane, acrylate resins
b. Thermosetting polymers: polyethylene (cross-linked) phenolics, alkyds, polyesters
3. Semisynthetic cellulosics (rayon, methylcellulose, cellulose acetate) and modified starches (e.g., starch acetate).

Further examples of natural polymers also include collagen, chitosan, and polyhydroxyalkanoates. Additional synthetic polymers include poly(glycolic acid) (PGA), poly(lactic acid) (PLA), copolymers of PGA and PLA, and polydioxananone.

Uses

In many materials, processing conditions can induce the polymer chains to link with each other along the length of the chain to produce a wide variety of mechanical properties, varied in order to suit current biomedical applications. Polyethylene (PE) is used in a variety of different applications. Depending on its linking, PE can be elastic and flexible, or hard and smooth. Low-density PE is used as tubing in catheters, while ultra-high-molecular-weight PE is commonly used in total hip or knee replacements; the smooth surface allows for low friction with other materials and therefore increases the durability of the artificial joint.

Polymers can be categorized in a number of ways. Homopolymers, for example, consist of only one repeating monomer unit. The most commonly encountered homopolymers are listed in **Table 1**.

Copolymers are produced by the simultaneous polymerization of two or more dissimilar molecules. Examples include polyvinyl acetate, polyesters, and polyamides. Synthetic elastomers (such as SBR synthetic rubber, made from styrene and butadiene) are also copolymers. This pattern continues with the terpolymers (such as acrylonitrile–butadiene–styrene (ABS)), which consist of three different monomers.

Hydrogels are another type of polymer structure comprised of a hydrophilic cross-linked network that swells in water. They can exist as homopolymers, copolymers, or multipolymers and are generally biocompatible and have low degradation. Hydrogels can be produced with a wide range of swelling characteristics determining solute diffusion rates, surface properties, refractive indexes, and mechanical characteristics. Cellulose derivatives swell to a higher degree than other polymers.

This ability of hydrogels to swell and dehydrate depending on composition and environment is used for the controlled release of drugs. Soft contact lenses also have hydrogel content which allows gas exchange to the eye. These may be used in future applications in blood contact applications and for wound-healing and artificial cartilage and skin.

Natural and synthetic biodegradable polymers are used for purposes that require only temporary stability to support tissue ingrowth. These polymers degrade when placed in the body while allowing functional tissue to grow in its place. This process takes advantage of polymers' properties of hydrolytic instability, hydration, molecular backbone cleavage, loss of molecular weight, and solubilization. These sutures degrade slowly into by-products that the body can remove itself through natural functions, allowing time for the wounded tissue to complete its own healing process and eliminating the need for a second operation to remove them.

Table 1 Commonly used homopolymers in medical devices

Polyacrylates	Polyamides
Polybutylene	Polychloroprene
Polyethylene	Polypropylene
Polysiloxanes	Polystyrene
Polysulfones	Polytetrafluoroethyene
Polyvinyl chloride	

Biocompatibility

For most devices, we are concerned only with the synthetic organic polymers. The principal class of natural polymers of concern is the elastomer class. The chief class of inorganic polymers of concern is the silicone class.

Partially as a reflection of their high molecular weights, true polymers themselves are not generally absorbed into the body, are not irritating, and are not sensitizers. As shown in **Table 2**, polymers themselves generally have very low toxicities.

The principal concerns with the biocompatability of polymers are additives, residual monomers, and contaminants that are leachable in the body. Plastic extractables include such chemicals as base polymers, fillers, lubricants, plasticizers, antioxidants, pigments, and slip agents. They may also include reaction

Table 2 Oral lethalities of common polymers

Polymer	*Rat LD_{50} value ($g\,kg^{-1}$ body weight)*
Polyethylene	>8
Polypropylene	>8
Polychloroprene latex	>40
Chlorosulfonated polyethylene	>20
Polyvinyl acetate	>25
Polyacrylonitrile	>3
Polyacrylamide	>8.2
Aromatic polyamides	>7.5

products or degradants formed during the device manufacturing process. They can reduce the purity or potency of a drug solution; create turbidity, precipitates, and particles; and even increase toxicity.

Residual monomers are those remaining individual building-block units in homopolymers, copolymers, terpolymers, etc. that are not successfully incorporated into the plastic during the synthesis process. Technically, we should also include dimers, trimers, and other small-chain fragments that are left in the polymer mass but are not chemically bound to it. Many factors help determine how much residual monomer will be left in a polymer and how available such residuals are to a surrounding biological matrix. Moreover, some of the monomers are quite active biologically. When testing a plastic for biocompatibility, biologically available (leachable) residual monomers are a significant concern. Examples of toxic monomers (and their principal toxicities) that can be found in polymers include the following:

- acrylonitrile: human carcinogen (liver, brain);
- vinyl chloride: human carcinogen (liver);
- formaldehyde: animal carcinogen (nasal); and
- methylene dianiline; suspect human carcinogen.

A wide variety of other chemical entities are specifically incorporated into plastics to achieve desired goals of structure, performance, and processing ease. A short list of the major categories of additives is provided in **Table 3**.

Such additives are available and significant biologically. A historical example is diethylhexylphthalate, a once widely used plasticizer that was found both to be an animal carcinogen and to migrate readily from plastic bags and tubing to the blood and intravenous solutions they contained.

The result of the additives and contaminants being in plastic is that a range of toxic materials may be leached from many plastics. A short list of some of the more significant toxic materials is provided in **Table 4**.

A number of tests are available for the chemical characterization of medical device materials to establish material safety and biocompatibility. These tests include infrared analysis, aqueous and nonaqueous physicochemical tests, high-performance liquid and gas chromatography, atomic absorption spectroscopy and inductively coupled plasma spectroscopy, and a variety of mechanical/physical tests.

The United States Pharmacopeia (USP) describes a group of tests used to characterize the plastic components of pharmaceutical containers and medical devices to avoid use of materials that may release water-soluble chemicals into the drug products or tissue fluids they contact. USP limits can be used to establish specifications for raw materials.

These aqueous physicochemical tests are designed to determine the presence of water-soluble substances without regard to their identity. Results are presented as a set of four values, showing the results for test type together with the corresponding USP limits (see **Table 5**). These aqueous extract tests are intended to serve as the basis for design specifications.

The USP recommends isopropyl alcohol (IPA) for conducting physicochemical tests of elastomeric closures used for pharmaceutical containers. IPA can dissolve many chemicals that are insoluble in water. The extract is analyzed for nonvolatile residue and residue on ignition. Turbidity and ultraviolet absorption tests are performed to detect the presence of extractables without specifically identifying their chemical makeup. Results are presented as a set of five values for each of the end points (see **Table 6**). USP limits do not yet exist for these tests, but they are not necessary for establishing specifications for the acceptance of materials.

Table 3 Additives used in plastics

Plasticizers	UV absorbers	Lubricants
Blowing agents	Antioxidants	Fillers
Colorants	Release agents	Emulsifiers
Flame and fire retardants	Stabilizers	Accelerators
Curing agents	Antistatic agents	

Table 4 Identified toxic materials in polymers

Aluminum	Ketones and hydrocarbons
Acrylonitrile (monomer)	Lead
Arsenic	Mercaptobenzothiazole
Benzene	Methyl chloride (monomer)
Benzoic peroxide	Methylene chloride
Bisphenol A	Methylene dinaline
Cadmium	Nickel
Carbon tetrachloride	PAHs on carbon black
Dibutyl tin	Pyrene
Epoxy curing agents	Tin
Ethylene dichloride	Tricresyl phosphate
Ethylene oxide	Triphenyl phosphate
Formaldehyde	

Pyrolysis

All plastics emit toxic and irritant fumes with increasing temperatures. However, the evolution rate and composition of the fumes emitted vary for different plastics and are strongly temperature dependent. Some common examples include thermoplastics such as polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS), ABS copolymer, and polytetrafluoroethylene (PTFE). When

Table 5 Testing for water-soluble substances in polymers

Polymer	*Nonvolatile residue (mg)*	*Residue on ignition (mg)*	*Heavy metals (ppm)*	*Buffering capacity (ml)*
ABS	1	<1	<1	<1
Polyurethane	1	⩽1	⩽1	<1
Polycarbonate	1	⩽1	⩽1	<1
Polyisoprene	9	⩽1	⩽1	<1
Polyvinyl chloride	1	<1	<1	1
Polyethylene	<1	<1	⩽1	<1
PTFE	<1	<1	⩽1	<1
Polystyrene	<1	<1	⩽1	<1
Polypropylene	<1	<1	⩽1	<1
Silicone	1	⩽1	⩽1	<1
USP limits	15	5	1	10

Note: Results of aqueous extraction physicochemical testing on polymers commonly used in medical devices.

Table 6 Physicochemical tests for polymer contaminants using isopropanol extraction

Polymer	*Nonvolatile residue (mg)*	*Residue on ignition (mg)*	*Turbidity (NTU)*	*Maximum optical density*	*Wavelength of max. optical density (nm)*
ABS	46	<1	4.18	>2.0	241
Polyurethane	119	<1	21.38	>2.0	244
Polycarbonate	<1	<1	0.04	>2.0	227
Polyisoprene	223	<1	24.38	>2.0	250
Polyvinyl chloride	123	1	0.24	>2.0	297
Polyethylene	20	<1	7.08	>2.0	241
PTFE	<1	<1	0.00	0.0	
Polystyrene	66	1	8.10	1.2	290
Polypropylene	20	<1	13.10	0.1	
Silicone	444	248	0.70	0.1	

Note: Results of alcohol extraction testing on polymers commonly used for medical devices.

Table 7 Inhalation lethalities of common polymers

Polymer	*Rat LC_{50} value (mg l^{-1}) 30 min*
Polyethylene	75.5
Polyacrylamide	45.7
Polystyrene	56.6
Nylon 6/6	58.1
Polysulfone	63.2
Chlorinated polyethylene	87.5

heated to destruction the parent monomers of a polymer are often one of the pyrolysis products. CO has been a concern, also PVCs since they can give off HCl during a fire, and polyurethane because of HCN. The health effects of hot-wire cutting of PS foams, and PVC and PE films have been studied. **Table 7** shows pyrolysis lethal concentrations of several polymers.

The manufacture of these polymers offers no opportunity for excessive heating. However, in the process of fabrication, reclaiming clad metal, wire coating and stripping, nonstick cookware, scavenging melts, coatings, fires, incineration, and machining, thermal exposure or thermal abuse might occur. When subject to the normal melt processing temperatures, most plastics would produce complex mixtures of small quantities of toxic vapors, usually at concentrations considerably below their exposure standards. However, irritant aerosols and gases can also be produced which may cause complaints of sensory irritation if the process is not controlled properly.

PTFE, also known as Teflon is a synthetic polymer (CF_2CF_2) with antistick (lubricant) properties that is used as a coating for nonstick cookware, domestic boilers, irons, ironing board covers, solid fuel burners, and heat lamps. Problems arise when pans boil dry or unfilled saucepans are heated. Frying temperatures normally range between 100°C and 200°C. Above 280°C, a polymer undergoes chemical decomposition (pyrolysis). PTFE, as well as butter or corn oil, can produce pyrolysis products that can cause death in birds. When PTFE undergoes pyrolysis, both gaseous and particulate materials are given off, including fluorinated compounds, which are toxic to animals and humans with birds being most susceptible. In humans, exposure to fumes can lead to a transient, febrile, flu-like syndrome called polymer-fume fever. Polymer-fume fever is caused by inhaling the fumes from a hot polymer and is characterized by typical flu symptoms (chills, spiking

fever, achy feeling, tightness of chest, headache, cough, weakness in legs, and malaise). These symptoms last 18–48 h before complete recovery without any residual effects or after effects. No animal species has yet been found that responds to PTFE or poly FEP fume the same way as humans. No similar events or fume fevers have been reported when other polymer fumes are inhaled. There are no known deaths from polymer-fume fever.

See also: Biocompatibility; Combustion Toxicology; Pollution, Water.

Further Reading

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Relevant Website

http://www.devicelink.com – Albert DE. The Growing Importance of Materials Characterization in Biocompatibility Testing, Canon Communications LLC © 2002. Originally published in *Medical Device & Diagnostic Industry*, March 2002.

Potassium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-09-7
- Synonyms: Kalium
- Chemical/Pharmaceutical/Other Class: Alkali earth metal
- Chemical Formula: K^+

Uses

An essential element for humans, potassium is used in foods and as a salt substitute. It is a major essential element for plants as well and is, therefore, a constituent of most fertilizers. Potassium and its compounds are used in specific medicinal preparations and in cement and glass manufacturing. Potassium bromate is used in hair waving products. Potassium permanganate, a powerful oxidizing agent, is used in the photographic and chemical industries. A dilute solution is used for special dermatological applications.

Background Information

Potassium forms 2.50% of earth's crust and was first isolated in 1807. It is one of the most reactive metals. Radioactive decay of ^{40}K to ^{40}Ar is used as a tool in geological dating.

Exposure Routes and Pathways

The primary exposure pathway is through ingestion of food; sources include milk, meat, and a variety of fruits. Many salt substitutes contain potassium chloride.

Toxicokinetics

Potassium salts are more than 90% absorbed, but blood levels are controlled by hemostatic mechanisms. Climate plays a role in potassium blood levels; people in warm climates have ~30% more potassium in their blood than people in very cold climates.

All tissues of the body contain potassium. It is found mainly in the muscle followed by the skeleton. Excretion of potassium via urine is also controlled by hemostatic mechanisms; the kidney regulates this so that there is normally no major loss of this essential element. The amount of potassium excreted depends on the chloride ion concentration and the adrenal hormone secretion level.

Mechanism of Toxicity

Potassium is a cofactor and activates a large variety of enzymes, including glycerol dehydrogenase, pyruvate kinase, L-threonine dehydrase, and ATPase. Its acute toxicity is primarily due to its action as an electrolyte. Excessive or diminished potassium levels can disrupt membrane excitability and influence muscle cell contractility and neuronal excitability.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral, intraperitoneal, and intravenous LD_{50} values of potassium chloride in rats are 2600, 660, and 142 mg kg^{-1}, respectively. Signs of acute toxicity may include convulsions and seizures, cardiac arrythmias, dyspnea, cyanosis, nausea, and vomiting.

Human

Excess intake of potassium, reduced renal excretion of potassium, or both can lead to hyperkalemia, which can lead to serious arrhythmia and death. The toxicity of excess potassium can be exacerbated by aldosterone antagonist drugs. Slow-release potassium tablets in overdose are a frequent cause.

Periodically, solutions containing a relatively high concentration of a potassium salt are sold as a nutritional supplement. In light of the fact that ingestion of additional potassium can upset the sodium–potassium ratio, potassium supplements are only indicated on the advice of a physician. Unusually high intake of potassium can cause abnormal EKG readings (T-waves will be evaluated and P-waves depressed). Ventricular fibrillation can result and lead to cardiac arrest. A large increase ($\sim$18 g day^{-1}) may produce neuromuscular weakness or paralysis.

Potassium permanganate is a mucous membrane irritant. Taken internally, it can be corrosive to the stomach. It is poorly absorbed, but it can cause nervous system symptoms and increased methemoglobin levels.

Chronic Toxicity (or Exposure)

Animal

Little is known regarding chronic effects of potassium exposures in animals.

Human

Potassium perchlorate can induce aplastic anemia, which can be fatal.

Clinical Management

Intravenous injection of calcium gluconate can antagonize the cardiac effects of excess potassium. Also, intravenous injection of sodium bicarbonate and glucose will help diminish the effects of potassium hemodialysis overdose, while dialysis can be used to remove excess serum potassium.

See also: Lye; Sodium.

Further Reading

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Potassium Iodide

Elizabeth J Scharman

- Chemical Abstracts Service Registry Number: CAS 7681-11-0
- Synonyms: SSKI, Iosat®, Thyro-Block®
- Chemical/Pharmaceutical/Other Class: Antithyroid agent; Antisporotrichotic agent
- Chemical Formula: KI

Uses

In patients with hyperthyroidism, potassium iodide is used in the treatment of thyrotoxicosis and to decrease the vascularity of the thyroid before the thyroid gland is surgically removed. Potassium iodide is also used to protect the thyroid by blocking the uptake of radioactive iodine, for example, during radionuclide therapy with I-131 or following an accident at a nuclear power facility. Potassium iodide can be given orally for the treatment of cutaneous sporotrichosis. Although used as an expectorant, clinical evidence regarding efficacy for this indication is lacking.

Exposure Routes and Pathways

Ingestion is the route of both accidental and intentional exposure to potassium iodide.

Toxicokinetics

Data on the percentage bioavailability, volume of distribution, and half-life of potassium iodide are not

available. Iodides are distributed in extracellular body water. Limited information reports that potassium iodide is readily absorbed and that iodide is concentrated in the thyroid and salivary glands, gastric mucosa, choroid plexus, placenta, and breast milk with 90% being renally excreted and the remainder being excreted in sweat, feces, and breast milk.

Mechanism of Toxicity

Adverse effects are the result of hypersensitivity reactions to the iodide component or the result of iodine accumulation following chronic administration. In patients with renal impairment, potassium concentrations may increase.

Acute and Short-Term Toxicity (or Exposure)

Human

Potassium iodide is unlikely to result in acute toxicity. Manifestations of a hypersensitivity reaction may include angioedema, cutaneous and mucosal hemorrhage, urticaria, fever, arthralgia, enlarged lymph nodes, and eosinophilia. In patients with chronic urticaria or systemic lupus erythematosus, hypocomplementemic vasculitis may be precipitated.

Chronic Toxicity (or Exposure)

Animal

Chronic feeding studies in minks showed shorter gestational periods and fewer animals per litter compared with controls. At the highest doses tested (1000 ppm), no animals whelped.

Human

Potassium concentrations may become elevated in patients with renal impairment. Signs of potassium excess include confusion, muscle weakness, and dysrhythmias. Chronic iodine toxicity, iodism, is manifested by symptoms that include stomatitis, laryngitis, metallic taste, salivation, tenderness of parotid and submaxillary glands, gastric irritation, diarrhea, headache, coryza, sneezing, productive cough, eye irritation, eyelid swelling, and acneiform eruptions.

Toxicity is usually the result of chronic administration.

In Vitro Toxicity Data

Studies using the alkaline comet assay have not found potassium iodide to produce DNA damage.

Clinical Management

Allergic reactions should be treated appropriately with supportive care, maintenance of airway, breathing, and circulation, and antihistamines plus steroids as needed. Discontinue potassium iodide administration and provide symptomatic and supportive care. The extent of iodide adsorption to activated charcoal has not been determined. Plasma iodide levels do not guide therapy; the potassium level should be checked.

See also: Charcoal; Gastrointestinal System.

Further Reading

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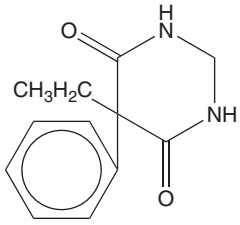
Potentiation *See* Chemical Interactions.

Primidone

S Rutherfoord Rose

- Chemical Abstracts Services Registry Number: CAS 125-33-7
- Synonyms: Primaclone; Hexamidinum; 2-Desoxyphenobarbital; 5-Ethylperhydro-5-phenylpyrimidine-4,6-dione; Mysoline®; Midone®; Dilon®; Mylepsin®; Liskantin®; Majsolin®; Sertan®
- Chemical/Pharmaceutical/Other Class: Primidone is a desoxybarbiturate; a congener of phenobarbital

- Chemical Formula: $C_{12}H_{14}N_2O_2$
- Chemical Structure:



Uses

An anticonvulsant, primidone is used in the treatment of generalized tonic–clonic seizures and partial focal seizures. It is often used in combination with phenytoin or carbamazepine.

Exposure Routes and Pathways

Ingestion is the route of exposure. Toxicity results from acute or chronic overdosage of tablets or oral suspension.

Toxicokinetics

Following therapeutic doses, primidone is usually well absorbed (bioavailability ranges from 70 to 95%) with peak plasma concentrations occurring in 3–6 h. Primidone is converted by the liver to two metabolites: phenobarbital and phenylethylmalonamide (PEMA). Both metabolites are active and phenobarbital is thought to be primarily responsible for primidone's anticonvulsant activity. Phenobarbital appears in the blood 2–4 days after beginning primidone therapy. The volume of distribution averages $0.6\,l\,kg^{-1}$, but there is much interindividual variation. Approximately 20% of primidone and PEMA are bound to plasma proteins, but the binding of phenobarbital is ~50%. Primidone crosses the placenta and is excreted in breast milk.

The majority of a dose is excreted in the urine as PEMA; ~15% as phenobarbital. The plasma elimination half-lives of primidone, PEMA, and phenobarbital are ~8–10, 24–36, and 100 h, respectively. The metabolism of primidone is enhanced with chronic therapy, with a reduced half-life of 4–7 h. An elimination half-life of 6.2 h has been documented following overdose.

Mechanism of Toxicity

Primidone and PEMA appear to have weak anticonvulsant activity compared to that of phenobarbital. Both primidone and phenobarbital contribute to central nervous system depression, probably through enhancement of GABA activity in the brain and the resulting decreased neuronal excitability.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute poisoning in animals may cause lethargy, incoordination, loss of reflexes, coma, or respiratory depression. Treatment is based on supportive care in consultation with a veterinarian.

Human

Acute intoxication resembles barbiturate toxicity. Clinical effects include dose-related central nervous system depression, nystagmus, ataxia, nausea and vomiting, dizziness, vertigo, and irritability.

Chronic Toxicity (or Exposure)

Animal

Toxic effects similar to those seen in humans are found when higher than therapeutic doses are used.

Human

With chronic exposure, side effects may include rash, thrombocytopenia, leukopenia, and a lupus-like disorder. Chronic therapy is likely to result in tolerance, and withdrawal symptoms if primidone therapy is abruptly stopped. Doses in excess of 1500 mg (twice the maximum recommended daily dose) should be considered toxic. Less common side effects are hypotension, hypothermia, and dermal bullae. Encephalopathy has been observed in an epileptic patient with high plasma levels and poor renal function. With plasma concentrations exceeding $80\,\mu g\,ml^{-1}$, primidone may precipitate and cause crystalluria. Plasma levels $>10\,\mu g\mu ml^{-1}$ are associated with toxic effects. The therapeutic range is reportedly $5–10\,\mu g\,ml^{-1}$, but clinical effects correlate more closely with phenobarbital blood levels.

In Vitro Toxicity Data

Mutagenicity studies using mammalian polychromatic erythrocytes have been positive.

Clinical Management

The most important aspect of treatment for acute overdose is provision of airway maintenance and ventilation. Hypotension and hypothermia should be

corrected if present. Decontamination should be accomplished with oral activated charcoal. There are no antidotes. Patients with high plasma phenobarbital levels may be treated with multiple oral doses of activated charcoal and/or urinary alkalinization to enhance phenobarbital excretion. At therapeutic levels, hemodialysis has been shown to increase primidone clearance from 30 to 98 ml min^{-1}. Phenobarbital is also removed by hemodialysis. Primidone, PEMA, and phenobarbital can be removed by hemoperfusion. There is no evidence that extracorporeal drug removal has a beneficial clinical effect with respect to morbidity or mortality.

See also: Charcoal.

Further Reading

Bertino JS Jr and Reed MD (1986) Barbiturate and non-barbiturate sedative hypnotic intoxication in children. *Pediatric Clinics of North America* 33(3): 703–722.

Sigg T and Leikin J (1999) Massive crystalluria in a patient taking primidone (letter). *Annals of Emergency Medicine* 33: 726–727.

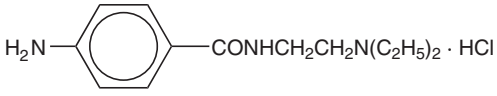
Probabilistic Analysis *See* Monte Carlo Analysis.

Procainamide

Christopher P Holstege

This article is a revision of the previous print edition article by Daniel J Cobaugh, volume 2, pp. 586–588, © 1998, Elsevier Inc.

- Chemical Name: Procainamide
- Chemical Abstracts Services Registry Number: CAS 51-06-9
- Synonyms: 4-Amino-*N*-[2-(diethylamino)ethyl] benzamide monohydrochloride; Amisalin; Novocamid; Procamide; Procanbid; Procan-SR; Procapan; Pronestyl
- Chemical/Pharmaceutical/Other Class: Class IA antiarrhythmic
- Chemical Structure:



Uses

Procainamide is used in the management of ventricular tachydysrhythmias.

Exposure Routes and Pathways

Reports have shown toxicity by both oral and parenteral routes.

Toxicokinetics

The bioavailability from immediate-release capsules ranges from 50% to 95%. Sustained-release formulations are designed to deliver release of procainamide over 12 h. In overdose, absorption may be delayed, especially if sustained-release preparations are involved. Procainamide's volume of distribution (V_d) is ~2 l kg^{-1}. Protein binding is minimal at 15–25%. Procainamide is metabolized to its active metabolite *N*-acetyl procainamide (NAPA). NAPA has a V_d of ~1.5 l kg^{-1} and is 10% protein bound. The elimination half-lives of procainamide and NAPA are ~3 and 6 h, respectively. Fifty per cent of procainamide is eliminated unchanged in the urine.

Mechanism of Toxicity

Cardiac voltage-gated sodium channels reside in the cardiac cell membrane and open in response to depolarization of the cell. Procainamide binds to the transmembrane Na^+ channels and decreases the number available for depolarization. This creates a delay in the entry of Na^+ into the cardiac myocyte during phase zero of depolarization. As a result, the upslope of depolarization is slowed and the electrocardiogram (EKG) QRS complex widens. Procainamide may also affect phase three of the action potential, resulting in prolongation of repolarization and subsequent QTc prolongation on the ECG. Vasodilation associated with procainamide toxicity is due to interference with ganglionic transmission of catecholamine neurotransmitters. A reflex tachycardia may occur in response to this vasodilation. Procainamide may also have weak anticholinergic effects that produce tachycardia. Negative inotropic effects may occur in toxicity. The NAPA metabolite of procainamide has pharmacologic and toxicologic effects similar to those of the parent compound.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rapid intravenous infusion of large doses of procainamide can produce central nervous system and respiratory depression as well as arrythmias.

Human

The primary toxicities observed with procainamide are cardiovascular in nature. Initially, a tachycardia may occur due to procainamide's anticholinergic properties or as a reflex response to vasodilation. Cardiac conduction disturbances may occur. On the ECG, these may be displayed as prolongation of the QRS and/or QTc duration. Heart block, bradycardia, and asystole have been reported. Procainamide can also cause ventricular tachycardia, ventricular fibrillation, and Torsades de Pointes. Severe hypotension due to decreases in cardiac output and/or vasodilation may be seen. Altered mental status and seizure activity can occur in procainamide toxicity.

Chronic Toxicity (or Exposure)

Animal

Procainamide is used in veterinary practice as an antiarrhythmic. Clinical effects seen in animals are similar to those seen in humans and include arrythmias, gastrointestinal complaints, and systemic lupus erythematosus-like syndrome.

Human

Procainamide may induce a syndrome similar to systemic lupus erythematosus. This syndrome consists of arthralgias, myalgias, pleurisy, rash, fever, and elevated nuclear antibodies. Patients who are slow acetylators are at increased risk for developing this syndrome. While some studies have reported that less than one in 500 on chronic procainamide therapy developed this syndrome, others have reported this syndrome in up to 30% of patients on long-term therapy. Other side effects with chronic use include development of neutropenia, thrombocytopenia, hemolytic anemia, agranulocytosis, liver failure, a myasthenia-like syndrome, and psychosis with hallucinations.

In Vitro Toxicity Data

Mutagenicity studies in rat and human hepatocytes as well as Chinese hamster ovaries have been negative.

Clinical Management

All patients presenting with toxicity or potential toxicity following ingestion of procainamide should be aggressively managed and monitored. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered. Because procainamide sustained-release preparations exist, multidose charcoal administration ($1\,g\,kg^{-1}$ first dose and then $1/2\,g\,kg^{-1}$ q 4 h) may be considered along with whole bowel irrigation (polyethylene glycol–electrolyte solutions at $500\,ml\,h^{-1}$ for children and $2\,l\,h^{-1}$ for adults). Gastric lavage has questionable efficacy, especially in late presenters, and can induce an unwanted vagal response.

The management of the Na^+ channel blocking activity of procainamide consists of administration of sodium and/or alkalosis. Infusion of sodium bicarbonate by either intermittent bolus or by continuous infusion has been advocated for symptomatic patients. Lidocaine has been suggested in the treatment of ventricular dysrhythmias, although clear evidence is lacking. Other class IA and IC antiarrhythmics should be avoided due to their ability to block cardiac sodium channels.

Hypotension not responsive to intravenous fluids should be managed with vasopressors, such as dopamine, norepinephrine, epinephrine, and/or phenylephrine. If seizures occur, benzodiazepines should be administered. Due to their pharmacokinetic characteristics, moderate volume of distribution, and low protein binding, procainamide and NAPA may be removed via hemodialysis and hemoperfusion. Both procainamide and NAPA serum concentrations should be obtained. Normal therapeutic ranges are: procainamide, $3–14\,\mu g\,ml^{-1}$; NAPA, $12–35\,\mu g\,ml^{-1}$. Measurement of electrolytes, renal function tests, and arterial blood gases should be considered.

See also: Benzodiazepines; Charcoal; Gastrointestinal System; Polyethylene Glycol.

Further Reading

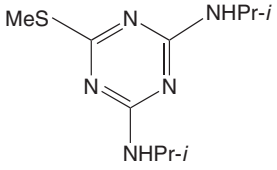
Smith WM and Gallagher JJ (1980) 'Les torsades de pointes': An unusual ventricular arrhythmia. *Annals of Internal Medicine* 93: 578–584.

White SR, Dy GL, and Wilson JM (2002) The case of the slandered Halloween cupcake: Survival after massive pediatric procainamide overdose. *Pediatric Emergency Care* 18: 185–188.

Prometryn

Larry J Dziuk

- Chemical Abstracts Service Registry Number: CAS 7287-19-6
- Synonyms: Prometryn(e) (preferred name); 2-Methylthio-4,6-bis(isopropylamino)-*s*-triazine; G 34161. Trade names include Caparol, Gesagard, Prometrex, Primatol Q, and Mercasin
- Chemical/Pharmaceutical/Other Class: Sulfur-substituted triazine pesticide
- Chemical Formula: $C_{10}H_{19}N_5S$
- Chemical Structure:



Uses

Prometryn is used as an agricultural herbicide.

Exposure Routes and Pathways

Ingestion is a possible route of exposure. Prometryn mixers, loaders, and applicators and field workers receive the most exposure by way of skin and eye contact, as well as from inhalation. The product is not available for use by the general public, so exposure to persons other than mixers, loaders, and applicators is limited to exposure by way of ingestion of food crops. However, risk due to ingestion of food is low because allowable residue limits on food crops are low.

Toxicokinetics

Triazine compounds are generally well absorbed in the gastrointestinal system. When administered by the oral route, the greatest concentrations of prometryn are found in the blood, spleen, and lungs. Dermal absorption is relatively high, with 7–15% of the material applied to skin being absorbed. Prometryn is excreted in urine or feces within 72 h. It is extensively metabolized, with less than 2% of the parent material appearing in the urine or feces.

Mechanism of Toxicity

On ingestion prometryn metabolizes, producing amine dealkylation and side chain oxidation. It affects the tricarboxylic acid cycle.

Acute and Short-Term Toxicity (or Exposure)

Animal

In toxicity studies evaluated in support of registration of the material for use as a pesticide, prometryn was regarded as being slightly to practically nontoxic. The rat oral LD_{50} is $1800\,mg\,kg^{-1}$ for male rats and $2076\,mg\,kg^{-1}$ for female rats. The dermal LD_{50} in the rat is greater than $3170\,mg\,kg^{-1}$. The 4 h inhalation LC_{50} value for rats was $4.96\,mg\,l^{-1}$.

Prometryn was not a sensitizer when applied to the skin of guinea pigs. It was determined to be a mild eye irritant to rabbits and produced only slight irritation when applied to the skin of rabbits.

Human

Prometryn has low acute toxicity. Symptoms include nausea or sore throat if swallowed.

Chronic Toxicity (or Exposure)

Animal

The US Environmental Protection Agency (EPA) has tested prometryn for carcinogenic potential and has classified the material as a group E constituent; that is, no evidence of human carcinogenic potential. In a 102 week feeding study with mice, chronic doses up to $429\,mg\,kg^{-1}$ were not associated with the production of cancer. There was no significant effect of dosing on clinical signs, mortality, gross pathology, or histopathology. Rats fed prometryn in the diet for 104 weeks at doses up to $80\,mg\,kg^{-1}$ developed concretions in the kidneys at the high dose. No evidence of carcinogenicity was found. Beagle dogs fed at dose equivalents of up to $37.5\,mg\,kg^{-1}$ over a 106 week period developed kidney effects at the high dose but no carcinogenicity was noted.

There was evidence of developmental toxicity in rats administered prometryn by gavage at a dose of $250\,mg\,kg^{-1}$ during gestational days 6–15. No developmental effects were noted in rats receiving a dose of $50\,mg\,kg^{-1}$. Rabbits receiving prometryn by gavage during gestational days 6–19 at a maximum dose of $72\,mg\,kg^{-1}$ experienced a slight but nonsignificant increase in abortions. In a two-generation reproductive toxicity study with rats, statistically significant decreases in body weight of the pups were noted in both generations at a dose of $\sim 50\,mg\,kg^{-1}$.

Human

No information was found relating to chronic toxicity in humans. According to the US EPA, systemic

toxicity of prometryn and other triazine herbicides is unlikely unless large doses are swallowed and acute toxicity develops.

In Vitro Toxicity Data

Using the Ames *Salmonella* test, prometryn was not mutagenic when tested up to the cytotoxic solubility limits. Prometryn was negative for bacterial DNA repair and gene mutation in an unscheduled DNA synthesis test using rat hepatocytes.

Clinical Management

Lavage and catharsis are recommended for ingestion. Oxygen therapy should be provided if needed. There is no specific antidote for prometryn.

Environmental Fate

Prometryn binds readily to organic matter in soil and tends to remain in the top 12 in. of soil after application. Degradation by soil microorganisms occurs in 1–3 months; the soil half-life is 60 days. In water, no hydrolysis occurred over a 28 day period.

Ecotoxicology

When tested in support of registration as an agricultural herbicide, prometryn was determined to be slightly toxic to amphibians, moderately toxic to fish, and slightly toxic to zooplankton. The 96 h LC_{50} to rainbow trout is 5.46 mg l^{-1}. The 96 h LC_{50} to bluegill sunfish is 7.95 mg l^{-1}. It is practically nontoxic to birds. The 8 day bobwhite quail and mallard duck dietary LC_{50} values are greater than 10 000 ppm. Prometryn is nontoxic to bees and earthworms.

Other Hazards

Thermal decomposition products may include oxides of carbon, nitrogen, and sulfur.

Exposure Standards and Guidelines

No specific occupational exposure limit has been established by Occupational Safety and Health Administration. Tolerance levels ranging from 0.1 to 0.5 ppm have been established for the presence of propachlor in seven crops.

See also: Pesticides.

Relevant Websites

http://www.elsevier-ecotox.com – Elsevier Science. *ECOTOX. Ecological Modelling and Ecotoxicology.* An Electronic Publication (7-150).

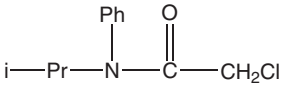
http://pmep.cce.cornell.edu – Pesticide Information Profile on Prometryn from EXTOXNET.

Propachlor

Larry J Dziuk

This article is a revision of the previous print edition article by Janye E Ash and Shayne C Gad, volume 2, pp. 588–589, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 1918-16-7
- Synonyms: 2-Chloro-*N*-isopropylacetanilide; 2-Chloro-*N*-(1-methylethyl)-*N*-phenylacetamide; Bexton; Bexton 4L; Kartex A; Niticid; Propachlore; Ramrod; Satecid; CP 31393; *N*-Isopropyl-α-chloroacetanilide
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbon pesticide. Specifically, a chloroacetanilide herbicide
- Chemical Formula: $C_{11}H_{14}ClNO$
- Chemical Structure:



Uses

Propachlor is used as an herbicide; pre-emergence herbicide.

Exposure Routes and Pathways

Nondietary exposure to propachlor by a farmer as an applicator during mixing, loading, spraying, and flagging is probable. Dermal contact, ocular contact, and ingestion are possible exposure routes. Inhalation of spray or mist is another possible route of exposure.

Exposure of humans to propachlor through contamination of groundwater and runoff contamination of surface water after heavy precipitation is probable.

The dietary exposure (milligram per kilogram per day) to propachlor by the US population from treated food crops is possible. Residual amounts ($0.04\,mg\,kg^{-1}$) of propachlor remained in tomatoes up to 85 days after application of $6\,kg\,ha^{-1}$.

Toxicokinetics

Propachlor is absorbed through the gastrointestinal tract, through intact skin, and through the respiratory system after inhalation of dust or spray mist. It is metabolized via the mercapturic acid pathway. The major fecal metabolite is a cysteine conjugate. Rats administered ^{14}C propachlor orally excreted 98.6% of the dose in the urine and feces within 48 h. Approximately 50% is excreted as metabolites through urine or feces within 24 h.

Mechanism of Toxicity

Propachlor inhibits production of cytochrome oxidase (brain and kidneys) and cholinesterase in the liver.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the oral LD_{50} is $710\,mg\,kg^{-1}$ and the dermal LD_{50} is $2000\,mg\,kg^{-1}$. In mice, the oral LD_{50} is $392\,mg\,kg^{-1}$ and the dermal LD_{50} is $380\,mg\,kg^{-1}$. In ducks, the oral LD_{50} is $512\,mg\,kg^{-1}$. The main symptoms in acute poisoning relate to the central nervous system and include a state of excitement, trembling, and light convulsions. Inhalation of dust for 4 h caused inflammation of tracheal mucosa, 25% mortality, and hemorrhagic secretions in lungs and bronchi of rats. The effective threshold concentration was 136–456 mg m^{-3}. Propachlor severe dermatitis, ulceration, and necrosis of the skin of rabbits and mice. Four- and six-month studies with rats at 1/20, 1/100, and 1/200 of the LD_{50} showed inhibition of spermatopoiesis at the phase of spermatid formation and histomorphologic changes in the spermatopoietic epithelium.

In subchronic toxicity studies with mice, a dose-related decrease in white blood cells was noted as well as an increase in the incidence of centrilobular hepatocellular hypertrophy in mice receiving up to $5000\,mg\,kg^{-1}$ in the diet.

Human

The probable oral lethal dose 5–15 $g\,kg^{-1}$. Exposure to propachlor for 8 days caused erythematopapular contact eczema on hands and forearms of workers. The substance is considered a skin irritant. There have been no published reports of poisoning. There have been no reports of symptoms or diseases among occupationally exposed workers. Propachlor has been produced since 1965.

Chronic Toxicity (or Exposure)

Animal

Propachlor causes dystrophic changes in the liver of rats accompanied by decreased enzyme activities. In a 2 year study with rats, propachlor administered at doses up to $27\,mg\,kg^{-1}$ developed changes in the liver such as centrilobular hypertrophy, clear cell cytoplasmic alteration, and eosinophilic cytoplasmic alteration. There were no other changes noted. Mice receiving doses up to $105\,mg\,kg^{-1}$ over a period of 18 months developed an increase in the ratio of liver weight to body weight and a decrease in the ratio of kidney weight to body weight. However, no histological changes were noted.

Propachlor was not teratogenic when administered orally to rats at doses up to $200\,mg\,kg^{-1}$ over days 9–15 of gestation. Mice administered up to $270\,mg\,kg^{-1}$ during days 1–21 of gestation developed offspring with a statistically significant increase in hydrocephaly.

Human

Propachlor is only slightly hazardous with normal handling. No reports of poisoning of the general population or of workers are available other than for sensitization studies. Of 79 workers engaged in manufacturing a formulation of propachlor, 19% showed evidence of contact dermatitis. However, 108 workers engaged in the same manufacturing process tested 3 years later exhibited no evidence of sensitization.

In Vitro Toxicity Data

Propachlor caused increased aberrant metaphases in mouse bone marrow cells. Propachlor did not produce evidence of mutagenicity in the Ames spot test with or without microsomal fortification. Results of an *in vivo/in vitro* hepatocyte DNA repair assay were negative with rats administered up to $1000\,mg\,kg^{-1}$.

Clinical Management

Symptoms of poisoning include irritation and inflammation of the skin, eyes, and mucous membranes. Except for dermatitis, no clinical or laboratory signs of toxicity to man are known. If a small amount has

been ingested, an emetic (i.e., ipecac) should be given within the first hour. If the propachlor has been combined with a hydrocarbon, ipecac should not be used. Gastric lavage should be provided if a large amount has been ingested. If ingested more than 1 h prior, activated charcoal or magnesium sulfate should be used. If the substance has entered the eyes, an isotonic saline or water should be used. Exposed eyes should be flushed with running water for 15 min. Exposed skin should be washed with soap and water.

There is no specific antidote for poisoning. If the acute toxic effect is survived, recovery will be uneventful.

Environmental Fate

Rapidly degraded in the environment under most conditions. Does not bioconcentrate or biomagnify. Microbial degradation is the primary means of breakdown in soil. Soil half-lives of up to 3 weeks have been reported. Water solubility is $700\,mg\,l^{-1}$, which suggests mobility in aquatic systems. The vapor pressure is very low 2.5×10^{-4} mmHg at 25°C, suggesting no appreciable volatility to air.

Ecotoxicology

Ecotoxicity is somewhat variable. The material has a low to moderate toxicity to birds, a high toxicity to many aquatic organisms, and a low toxicity potential to bees. Oral LD_{50} pheasants $735\,mg\,kg^{-1}$; bobwhite quail oral LD_{50} $91\,mg\,kg^{-1}$; mallard duck LD_{50} (8 days study) $>5000\,mg\,kg^{-1}$; 96 h threshold limit median (TLM) for bluegill fingerlings $30\,mg\,l^{-1}$. The LC_{50} for rainbow trout is $0.17\,mg\,l^{-1}$. Propachlor poses a low hazard to earthworms and honey bees.

Other Hazards

When heated to thermal decomposition, irritant and corrosive fumes may be present. Products of thermal decomposition are oxides of nitrogen, hydrogen chloride, and carbon monoxide.

Exposure Standards and Guidelines

No specific occupational exposure limit has been established for propachlor. Tolerance levels ranging from 0.02 to 5 ppm have been established for the presence of propachlor in 50 foods and food by-products.

See also: Pesticides.

Relevant Websites

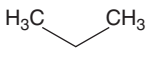
http://www.inchem.org – Environmental Health Criteria 147. Propachlor. International Programme on Chemical Safety. World Health Organization.

http://www.elsevier-ecotox.com – Elsevier Science. *ECOTOX. Ecological Modelling and Ecotoxicology.* An Electronic Publication (7-154).

Propane

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 74-98-6
- Synonyms: Dimethylmethane; Propyl hydride (UN1978, DOT); Bottled gas; LP-gas
- Chemical/Pharmaceutical/Other Class: Aliphatic hydrocarbon
- Chemical Formula: C_3H_8
- Chemical Structure:



Uses

Propane is used principally as a fuel source for homes (including indoor and outdoor cooking) and industries and as an aerosol propellant. It is also used in the synthesis of organic chemicals, in the manufacture of ethylene, as a refrigerant, and as an extractant.

Exposure Routes and Pathways

Because propane exists as a gas at normal temperature and pressure, exposure generally occurs by inhalation (trace amounts of propane have been measured in air expired by humans). Typical background concentrations detected at ground level in major US cities range from 0.050 to 0.4 ppm. It is possible to spill liquid propane from a pressurized tank, causing frostbite on skin contact due to rapid evaporation and loss of heat. Propane has also been detected in cigarette smoke (~0.83 mg per cigarette).

Mechanism of Toxicity

Some sources classify propane as a simple anesthetic, although it can principally be classified as a simple asphyxiant; and concentrations that are high enough to displace oxygen would be expected to cause lightheadedness, loss of consciousness, and possibly death from asphyxiation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Propane has been shown to have adverse effects on the cardiovascular system in the primate, dog, cat, and mouse. Guinea pigs exposed to 2.2–5.5% of the gas showed sniffing and chewing movements. In dogs, 1% caused hemodynamic changes, whereas 3.3% produced decreases in aortic pressure, stroke volume, and cardiac output and an increase in pulmonary resistance. Ten percent propane in the mouse and 15% in the dog did not produce arrhythmia but did produce weak cardiac sensitization.

Human

Propane is not considered to be inherently toxic to humans. Air concentrations up to 10 000 ppm (10%) for a few minutes will only produce slight dizziness in humans. At high concentrations, it may have a narcotic effect; but at concentrations below 100 ppm, propane causes no physiological effects in humans. However, it will cause chemical suffocation at concentrations that are high enough to displace oxygen.

Chronic Toxicity (or Exposure)

No information could be found on chronic toxicity of propane.

Clinical Management

Persons exposed to high concentrations of propane should vacate or be removed from the source of the gas and seek fresh air.

Ecotoxicology

There are no data in the US Environmental Protection Agency's ECOTOX database on propane. This is probably because highly volatile compounds such as propane, which exist as gases at normal environmental temperatures (e.g., $>0^{\circ}C$), would be expected to be found in air and not water. Some microbes can utilize propane as an energy source, whereas others are inhibited by its presence.

Other Hazards

Propane gas which is heavier than air is both an explosion and a fire hazard (the upper and lower explosive limits are 2.4% and 9.5% by volume, respectively). Extreme care must be taken to keep areas of expected high concentration free from ignition sources, such as sparks from static electricity. Explosion-proof equipment should be used in these areas.

Exposure Standards and Guidelines

The US Food and Drug Administration classifies propane as generally recognized as safe (GRAS). The Occupational Safety and Health Administration permissible exposure limit (time-weighted average) is 1000 ppm and the American Conference of Governmental Industrial Hygienists immediately dangerous to life and health level is 2100 ppm.

Miscellaneous

Propane is a colorless, highly flammable/explosive gas that is heavier than air. It occurs in natural gas at concentrations from 3% to 18%. It is emitted into the atmosphere from furnaces, automobile exhausts and sources of natural gas. With sufficient oxygen, it is combusted to carbon dioxide and water but carbon monoxide, a deadly gas, will be generated under leaner conditions. Some references state that propane is odorless while others provide an odor threshold of 22 000–36 000 mg m^{-3} (odor index = 425 at 20°C). In air, 1 ppm propane = 1.83 mg m^{-3}.

See also: Neurotoxicity.

Further Reading

Gosselin RE, Smith RP, and Hodge HC (1984) *Clinical Toxicology of Commercial Products*, 5th edn. Baltimore, MD: Williams and Wilkins.

Relevant Website

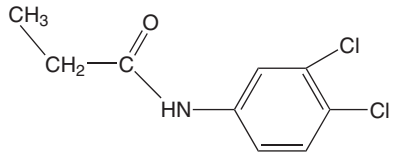
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Propane.

Propanil

Marcia D Howard

This article is a revision of the previous print edition article by Thuc Pham, volume 2, pp. 590–591, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 709-98-8
- Synonyms: *N*-(3,4-Dichlorophenyl)propionamide; 3,4-Dichloropropionanilide; Dipram; DCPA; Propanide; Grascide; Chem-Rice; Stampede; Stam
- Chemical/Pharmaceutical/Other Class: Herbicidal amide; Acetanilide
- Chemical Formula: $C_9H_9Cl_2NO$
- Chemical Structure:



Uses

Propanil is a postemergence herbicide used to control weeds in rice and potato crops.

Exposure Routes and Pathways

The general population may be exposed to propanil by inhalation and dermal contact from spraying or orally by consumption of contaminated food. Workers may also be exposed by dermal, ocular, or inhalation exposure. Exposures to crop workers may occur during application, contact with treated foliage, or pesticide-contaminated materials.

Toxicokinetics

Propanil is readily absorbed by ingestion, dermal, or inhalation exposure. Ocular exposure may also occur. Propanil is hydrolyzed by hepatic acylamidase forming 3,4-dichloroaniline and propionic acid. The major microsomal metabolites of 3,4-dichloroaniline are 6-hydroxy-3,4-dichloroaniline and *N*-hydroxy-3,4-dichloroaniline. Peak blood levels in rats occur after 1 h with acute oral exposure. Within 5 min of a single oral administration (650 and 1000 mg kg^{-1}), the compound is detected in blood and all tissues of the rat with maximum accumulation occurring within 1–6 h of treatment. Blood concentrations in rats are maintained 24 h after oral administration (1000 mg kg^{-1}, p.o.) but are undetectable 48–72 h later. When fed to cows for 4 days, 1.4% of the total dose of propanil was recovered in feces but was undetected in urine or milk.

Mechanism of Toxicity

In plants, propanil is toxic through inhibition of photosynthesis at the level of photosystem II. In animals, propanil induces methemoglobinemia resulting in tissue hypoxia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Propanil has relatively low toxicity in mammals. Acute oral LD_{50} values are 0.360 g kg^{-1} (mice), 0.367–2.5 g kg^{-1} (rats), and 1.3 g kg^{-1} (dogs). In both rats and dogs, death was characterized by central nervous system (CNS) depression occurring over a 3 day period. For dermal exposure, the LD_{50} for rabbits is >5000 mg kg^{-1}.

Human

Acute exposure results in CNS depression and methemoglobinemia. Chloracne has been reported in production facility workers following dermal exposure. Death may occur due to respiratory failure. Ingestion results in a burning sensation in the mouth, esophagus, and stomach, nausea and vomiting, fever, dizziness, and drowsiness. Inhalation may cause nose and throat irritation. Prolonged or repeated dermal contact may result in slight skin irritation. Repeated or excessive exposure by any route may result in cyanosis.

Chronic Toxicity (or Exposure)

Animal

A 2 year feeding study resulted in a no-observed-adverse-effect level (NOAEL) of 600 ppm (15 mg kg^{-1} day^{-1}) in dogs. For rats, the NOAEL was 300 ppm (15 mg kg^{-1} day^{-1}) in a three-generation reproductive study. Teratology studies in rats established an NOAEL of 20 mg kg^{-1} day^{-1} (decreased pup size, delayed ossification at 100 mgkg^{-1} day^{-1}). Reproductive effects were observed only in exaggerated doses that were fatal to the mothers. Chronic effects from propanil exposure include centilobular enlargement of the liver, methemoglobinemia, decreased hemoglobin, and cyanosis although the dose levels producing these effects were many times greater than those expected from normal usage or exposure to

the compound. Long-term exposure may result in kidney and liver damage. There is no evidence of carcinogenicity in mice and rats.

Humans

Little is known regarding long-term effects of propanil. Methemoglobinemia would be expected.

Clinical Management

For oral exposure, induced vomiting is not recommended. Activated charcoal can be administered or gastric lavage (within 1 h of exposure) can be performed. Seizures should be controlled first. Oxygen should be administered to symptomatic patients. Intravenous methylene blue can be administered to patients suffering methemoglobinemia. Inhalation exposure can be treated by moving the patient to fresh air. Medical attention should be sought if breathing difficulty persists. Oxygen should be administered and assisted ventilation provided as required. For dermal exposure, contaminated clothing should be removed and the affected areas washed with soap and water. Eyes should be flushed with copious amounts of fresh water for 15 min.

Environmental Fate

Propanil is rapidly metabolized under anaerobic and aerobic conditions in a water and soil matrix. It is unlikely to be persistent for a sufficient amount of time to leach into groundwater in measurable quantities. Propanil is rapidly metabolized in soil with a half-life of 1–3 days. It is stable to photodegradation and chemical degradative processes but susceptible to biodegradation.

Ecotoxicology

Propanil is toxic to aquatic invertebrates (e.g., crayfish, worms, snails) and to fish. It is moderately toxic to birds.

Exposure Standards and Guidelines

The reference dose for propanil is 0.03 mg kg^{-1} day^{-1} and the chronic population adjusted dose is 0.003 mg kg^{-1} day^{-1}.

See also: Blood; Pesticides.

Relevant Websites

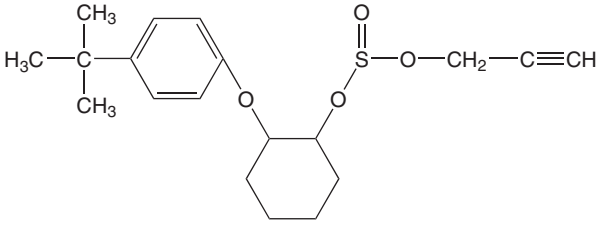
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Propanil.

http://www.intox.org – International Programme on Chemical Safety.

Propargite

Jing Liu

- Chemical Abstracts Service Registry Number: CAS 2312-35-8
- Synonyms: 2-[4-(1,1-Dimethylethyl)phenoxy] cyclohexyl-2-propynyl-sulfite; BPPS; Omite; Comite
- Chemical/Pharmaceutical/Other Class: Organic sulfite acaricide
- Chemical Formula: $C_{19}H_{26}O_4S$
- Chemical Structure:



Uses

Propargite is primarily used to control motile forms of mites.

Exposure Routes and Pathways

Dermal, oral, inhalation, and ocular exposures are all possible.

Toxicokinetics

Propargite can be absorbed from the gastrointestinal tract or skin after oral or dermal administration. It undergoes hydrolysis at the sulfite ester followed by oxidation/hydroxylation. Metabolites of propargite are eliminated in both urine and feces.

Mechanism of Toxicity

The mechanism of toxicity of propargite is unclear. Propargite is, however, an inhibitor of monoamine

oxidase and therefore can alter the metabolism of monoamines.

Acute and Short-Term Toxicity (or Exposure)

Animal

Propargite exhibits low acute toxicity via oral and dermal exposures. The oral LD_{50} value in rats is about 1–4 g kg^{-1}. With dermal exposure, the LD_{50} values are 250 mg kg^{-1} (male) and 680 mg kg^{-1} (female) in rats and >3 g kg^{-1} in rabbits. Propargite is, however, a strong eye and skin irritant that causes erythema, edema, and eschar formation in rabbits.

Human

Dermatitis is the major form of toxicity following dermal propargite exposure. Signs include erythema, burning, itching, exfoliation, and hyperpigmentation. Ocular exposure produces irritation. Changes in the chemical formulation have alleviated many of the acute irritant effects associated with propargite use.

Chronic Toxicity (or Exposure)

Animal

Both sexes of Crl:CD BR rats maintained on diets containing 50 or 100 mg kg^{-1} day^{-1} propargite for 13 weeks showed significantly lower body weights throughout the study. Various hematological and clinical chemical parameters such as erythrocyte count and hemoglobin level, urea nitrogen, and total protein were altered. Propargite was not genotoxic, mutagenic, or teratogenic but showed some carcinogenicity (jejunal sarcomas) in a species- and strain-specific manner in animal studies. Propargite caused increased incidence of intestinal tumors in CD (Crl:CDBR) and Sprague–Dawley rats but not in CD-1 mice or Wistar rats. No carcinogenicity was found in beagle dogs based on a 2 year feeding study.

Human

The US Environmental Protection Agency has listed propargite as a probable human carcinogen based on the appearance of intestinal tumors in animals.

Clinical Management

Treatment is symptomatic.

Environmental Fate

Propargite degrades rapidly under alkaline conditions in moist environments. It is, however, 'moderately persistent' to 'persistent' under neutral and acid conditions. Propargite has high affinity for soil and sediments and therefore has the potential of moving off the site of application.

Ecotoxicology

Propargite is highly to very highly toxic to freshwater aquatic organisms, fish, and invertebrates, with LC_{50} or EC_{50} values below 167 µg l^{-1}. Propargite is very highly toxic to estuarine/marine organisms, with LC_{50} values <100 µg l^{-1}. Propargite is expected to be highly toxic to amphibians, in particular early life stages. Propargite may pose a reproduction risk for avian species.

Exposure Standards and Guidelines

The reference dose for propargite is 0.04 mg kg^{-1} day^{-1} and the acceptable daily intake is 0.01 mg kg^{-1} day^{-1}.

See also: Pesticides.

Further Reading

Knowles CO (1991) Miscellaneous pesticides. In: Hayes WJ Jr. and Laws ER Jr. (eds.) *Handbook of Pesticide Toxicology*, ch. 22, p. 1473. San Diego, CA: Academic Press.

Relevant Websites

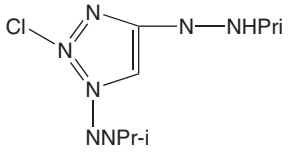
http://www.alternatives2toxics.org – Californians for Alternatives to Toxics.
http://www.inchem.org – FAO and WHO (1999). Pesticide Residues in Food (prepared by E. Bosshard).
http://www/agrimor.com – Propargite.
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Propargite.

Propazine

Raju Kacham

This article is a revision of the previous print edition article by Jayne E Ash and Shayne C Gad, volume 2, pp. 591–592, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 139-40-2
- Synonyms: 2,4-Bis(isopropylamino)-6-chloro-*s*-triazine; AI3-60348; BRN 0747081; CCRIS 1026; EINECS 205-359-9; G-30028; Geigy 30028; Gesamil; HSDB 1400; Maxx 90; Milocep; Milogard; Milo-pro; NSC 26002; Plantulin; Primatol P; Propasin; Propazin; Propinex; Prozinex
- Chemical/Pharmaceutical/Other Class: Triazine herbicide
- Chemical Formula: $C_9H_{16}N_5Cl$
- Chemical Structure:



Uses

Propazine is a selective preemergent herbicide for controlling broadleaf weeds and annual grasses (primarily pigweed) in sorghum. It is applied at the time of planting or immediately after. It is also used as a postemergence herbicide on carrots, celery, and fennel and is registered for use in greenhouses.

Background Information

The US Environmental Protection Agency (EPA) has determined that propazine belongs to a group of other triazines (including atrazine, simazine, and some related metabolites) that act through a common mechanism of toxicity based on ability to suppress the pituitary luteinizing hormone (LH) surge and elicit effects on reproductive function and development.

Exposure Routes and Pathways

Dermal and eye contact, inhalation of dust, and ingestion are possible routes of exposure.

Toxicokinetics

Propazine is readily absorbed and metabolized by amine-dealkylation and side-chain oxidation.

Seventy-two hours after oral administration of single dose of radiolabeled propazine to rats, 66% of the dose was excreted in the urine and 23% was excreted in feces. It may also accumulate in animal and human fatty tissues. Propazine and its metabolites were detected in lungs, spleen, heart, kidneys, and brain tissues of rat 8 days after dosing.

Mechanism of Toxicity

Propazine and its primary metabolite, diaminochlorotriazine, can attenuate the pituitary LH surge, leading to disruption of estrous cycle and certain reproductive and developmental processes. Propazine causes fatty degeneration. It also blocks metabolism of sugars and carbohydrates. It may also disturb the metabolism of some of the B vitamins (thiamine and riboflavin).

Acute and Short-Term Toxicity (or Exposure)

Animal

Propazine is slightly toxic by ingestion, inhalation, and by dermal contact. Oral LD_{50} values of propazine are 3850–7000 mg kg^{-1} in rats, 3180 mg kg^{-1} in mice, and 1200 mg kg^{-1} in guinea pigs.

Human

Symptoms of propazine exposure can include dizziness, dyspnea, muscle spasms, ataxia, anorexia, emaciation, diarrhea, coma, convulsions, and liver and kidney damage. It can also cause mild irritation to the skin, eyes, and upper respiratory tract.

Chronic Toxicity (or Exposure)

Animal

Similar to some other triazine herbicides, propazine induces mammary gland tumors in female (but not male) rats, likely through pituitary neuroendocrine disruption. No such tumors are noted in either sex of mice, however. Propazine delayed vaginal opening and affected testes weights in rat pups from exposed dams. After administering 500 mg kg^{-1} for 1–4 months, rabbits developed a type of anemia.

Human

Repeated exposure can lead to dermatitis. The US EPA considers propazine as a potential human carcinogen.

Clinical Management

Affected areas should be flushed with plenty of water for 15 min. Contaminated clothing should be

removed. In case of inhalation exposure, fresh air should be provided.

Environmental Fate

Propazine is highly persistent in soil and resistant to degradation by hydrolysis. The main degradation comes from microbial action. There is a possibility for contamination of ground water.

Ecotoxicology

It is practically nontoxic to slightly toxic to birds, and slightly toxic to fish.

Other Hazards

Propazine is combustible in open flames.

Exposure Standards and Guidelines

- Reference dose is $0.02\,mg\,kg^{-1}\,day^{-1}$.
- Acceptable daily intake of propazine is $0.0464\,mg\,kg^{-1}\,day^{-1}$.

The US EPA has established a Lifetime Health Advisory level of $10\,\mu g\,l^{-1}$ for propazine in drinking water.

See also: Common Mechanism of Toxicity; Pesticides; Pollution, Water.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Propene

Patricia J Beattie

- Chemical Abstracts Service Registry Number: CAS 115-07-1
- Synonyms: Propylene; 1-Propylene; Methylethylene; Liquid petroleum gas
- Chemical/Pharmaceutical/Other Class: Aliphatic alkene
- Chemical Formula: C_3H_6
- Chemical Structure: $H_2C=CH–CH_3$

Uses

Propene is used as a chemical intermediate in the production of polypropylene, acrylonitrile, propylene oxide, isopropanol, and cumene. Refineries use much of their production of propene internally as a refinery heating gas, to produce alkylates in gasoline, and to produce liquefied petroleum gas.

Exposure Routes and Pathways

Because propene is a gas, inhalation exposure is the primary route of entry.

Toxicokinetics

The toxicokinetics of propene have been studied in laboratory animals. In Sprague–Dawley rats, at steady state, 42% of inhaled propene is exhaled unchanged and is not absorbed into the bloodstream, with the remainder being metabolized and eliminated. Propene is metabolized to propene oxide, which reacts to form hemoglobin complexes at cysteine, histidine, and *N*-terminal valine. It is then further reacted to an alcohol and excreted.

Mechanism of Toxicity

Propene is classified as a simple asphyxiant and its toxicity is associated with the central nervous system effects associated with oxygen deprivation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation of high concentrations of propene by experimental animals results in anesthetic effects similar to those seen in humans. Anesthesia has been induced in cats after exposure to concentrations of propene of 20–31% without causing other signs of toxicity. At higher concentrations, from 40% to 80%, blood pressure decreased, pulse increased, and an unusual heartbeat was reported. Cardiac sensitization was reported following propene exposure in dogs.

Human

Propene is relatively nontoxic to humans and has been investigated for use as an anesthetic. Its flammability and explosivity, however, indicate this application is

inappropriate. Exposure to a concentration of 6.4% for 2.25 min resulted in mild intoxication, a sensation of numbness, and an inability to concentrate. At 12.8% for 1 min, these same symptoms were more pronounced, with 24% and 33% for 3 min resulting in unconsciousness. Exposures from 40% to 75% for a few minutes caused reddening of the eyelids, flushing of the face, tearing, and coughing. This is consistent with the fact that liquefied propene may cause skin burns on direct contact. As with any asphyxiant, high exposures for sufficient time, resulting in oxygen deprivation, can result in death.

Chronic Toxicity (or Exposure)

Animal

Subchronic propene exposure to rats and mice for 2–14 weeks at concentrations ranging from 625 to 10 000 ppm resulted in no reported toxicity. Male and female Sprague–Dawley rats were exposed to propene at concentrations of 200, 1000, and 5000 ppm for 7 h day^{-1}, 5 days week^{-1} for 104 weeks, and male and female Swiss mice were exposed for 78 weeks. The mortality rate of the male rats increased slightly after exposures of 1000 and 5000 ppm and that of male mice after exposure to the highest dose. No evidence of other toxicity was observed. In another long-term study with exposures up to 10 000 ppm, 6 h day^{-1}, 5 days week^{-1} for 103 weeks in rats and mice, nontumorigenic lesions were reported in the nasal cavity of male rats. It was concluded that these effects were due to inflammatory changes from local irritation. No exposure-related changes in tumor incidence were reported.

In Vitro Toxicity Data

Propene was tested for mutagenic potential in L5178Y mouse lymphoma cells at concentrations up to 30% for 4 h in the presence or absence of liver S9 mix. Propene was not cytotoxic or mutagenic in the absence of S9. Inconsistent, nonreproducible mutagenic responses occurred in the presence of S9 mix. Propene was not mutagenic when tested in *Escherichia coli*.

Clinical Management

Overexposure to propene is treated by simply moving the victim to fresh air. If skin or eye irritation has occurred, affected areas should be flushed with water for at least 15 min. Recovery is usually rapid and complete.

Environmental Fate

Propene degrades in the atmosphere by reaction with photochemically produced hydroxyl radicals with a half-life of 14.6 h. It also reacts in air with ozone and nitrate radicals with half-lives of 1 and 4 days, respectively. In soil, volatilization is expected to be the primary fate due to propene's high vapor pressure. Volatilization also occurs from water, while remaining propene is readily degraded by microorganisms. This results in propene being unlikely to bioaccumulate or bioconcentrate in soil or aquatic organisms.

Exposure Standards and Guidelines

American Conference of Governmental Industrial threshold limit value is 200 ppm (8 h time-weighted average).

See also: Ethane; Propylene Oxide.

Further Reading

Clayton GD and Clayton FE (eds.) (1981–1982) *Patty's Industrial Hygiene and Toxicology: Volumes 2A, 2B, 2C: Toxicology*, 3rd edn., p. 3200. New York: Wiley.

Propionic Acid

Shayne C Gad and Samantha E Gad

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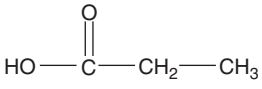
- Chemical Abstracts Service Registry Number: CAS 79-09-4
- Synonyms: Ethanecarboxylic acid; Ethylformic acid; Carboxyethane; Methylacetic acid
- Chemical/Pharmaceutical/Other Class: Antifungal
- Chemical Structure:



Uses

Propionic acid is used as a feed and corn preservative, and as a chemical intermediate. It is also used to control fungi and bacteria in drinking water for livestock. Propionic acid has been qualitatively detected as a volatile component of baked potatoes and cooked meats. It has also been detected in other foods and beverages, including dairy products. Propionic acid is a major constituent (100–300 μg per cigarette) of the gas phase of the mainstream smoke of unfiltered cigarettes.

Exposure Routes and Pathways

Occupational exposure to propionic acid may occur through inhalation and dermal contact. The general population may be exposed to propionic acid via inhalation of ambient air, ingestion of food and drinking water, and dermal contact with this compound and other consumer products containing propionic acid.

Toxicokinetics

Propionic acid is rapidly absorbed through the skin. It is excreted primarily through expired air (77%) and urine and feces (7%). It is carried by blood to the liver, where it is metabolized and removed.

Mechanism of Toxicity

If sodium propionate is ingested or applied topically in an acid media, it becomes propionic acid. It oxidizes fatty acids, lowers pH values, and facilitates the citric acid cycle through interaction with coenzyme A. There has been evidence of heightened production of insulin in cows and sheep; the insulin later settles to an overall lower level.

Propionic acid inhibits $^{14}CO_2$ production from palmitate in both control and methylmalonic fibroblasts; propionic acid also inhibited ureagenesis in rat liver slices. These findings may explain the fatty degeneration of the liver and hyperammonemia in propionic and methylmalonic acidemia. Propionic acidemia is an autosomal recessive disorder caused by a defect of propionyl–coenzyme A carboxylase. The main clinical findings are vomiting, lethargy, hypotonia, and metabolic ketoacidosis, and early clinical onset occurs during the neonatal period in ~80% of the patients.

Acute and Short-Term Toxicity (or Exposure)

Animal

In animals, the symptoms include polytropism. It also produces central nervous system, cardiovascular, respiratory, and blood effects. It has also proven to be a severe skin irritant and severe eye irritant. In a rabbit skin irritation test, tissue necrosis was observed after application of 10 mg of undiluted propionic acid for 24 h. Propionic acid is corrosive to the gastric lining and, upon oral intubation, results in desquamation and hemorrhage. The intravenous LD_{50} is 625 mg kg^{-1} in mice. The oral LD_{50} in rats and mouse is 2.60–5.16 g kg^{-1} and 5.10 g kg^{-1}, respectively. Propionic acid was tested using micronucleus test *in vivo*, with no evidence of genotoxicity.

Human

Propionic acid produces burning or inflammation from contact with skin, eye, and mucous membranes. It is less toxic when ingested or inhaled than it is when dermal or ocular exposure occurs.

Chronic Toxicity (or Exposure)

Animal

Rats, mice, and hamsters administered 4% propionic acid in the diet for 7 days showed evidence of damage and cellular proliferation in the epithelium of the forestomach, and a five- to sixfold increase in cell proliferation in the mid-region of the rat forestomach after 27 days of treatment.

In Vitro Toxicity Data

Propionic acid has been tested using the *Escherichia coli* DNA repair assay, the SOS chromotest, the *Salmonella*/microsome mutagenicity test, and the sister chromatid exchange test *in vitro*. All tests except the DNA repair assay with *E. coli* yielded negative results. These data support other evidence that propionic acid is not mutagenic and that genotoxic events are unlikely to be the cause of forestomach lesions in rats fed propionic acid in the diet.

Clinical Management

The victim should be removed from exposure. Treatment is symptomatic. If exposure is dermal or ocular the area should be flushed with excess amounts of water. If ingested vomiting should not be induced.

Environmental Fate

Propionic acid has been detected in wastewater from olive oil production as a result of breakdown and oxidation of fatty acids, and data suggest that propionic acid is produced by photooxidation of anthropogenic compounds during long-range transport. Propionic acid is released to the environment via

effluents from the manufacture and use of coal-derived and shale oil liquid fuels, and from the disposal of coal liquefaction and gasification waste by-products, and wood preserving chemical waste by-products. Propionic acid may also be released to the aquatic environment in wastewater discharges from textile mills and sewage treatment facilities. Municipal and industrial landfills and hazardous waste sites via leachates can release propionic acid to groundwater supplies. Propionic acid can be emitted to air as a component of exhaust from gasoline and diesel fueled engines, and has been identified as an organic degradation and emission product from shop primers, primers, and finishing paints used on steel ships.

Propionic acid should exist as a vapor in the ambient atmosphere, and will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 11 days. Photolysis of propionic acid is not expected to be important. Biodegradation is likely to be the most important removal mechanism of propionic acid from water and soil. Hydrolysis is not expected to occur due to the lack of hydrolyzable functional groups.

Exposure Standards and Guidelines

Joint Expert Committee on Food Additives does not give an acceptable daily intake level. The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average is 10 ppm.

See also: Acids.

Further Reading

Glasgow AM and Chase HP (1976) Effect of propionic acid on fatty acid oxidation and ureagenesis. *Pediatric Research* 10: 683–686.

Harrison PT (1992) Propionic acid and the phenomenon of rodent forestomach tumorigenesis: A review. *Food and Chemical Toxicology* 30: 333–340.

Henschel R, Agathos M, and Breit R (1999) Acute irritant contact dermatitis from propionic acid used in animal feed preservation. *Contact Dermatitis* 40: 328.

Lucke T, Perez-Cerda C, Baumgartner M, *et al.* (2004) Propionic acidemia: Unusual course with late onset and fatal outcome. *Metabolism* 53: 809–810.

Proposition 65, California

Samantha E Gad

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- Title: Safe Drinking Water and Toxic Enforcement Act
- Agency: State of California
- Year Passed: 1986
- Groups Regulated: Those doing business in California (except as preempted by Federal law)

Introduction

California voters in 1986 approved an initiative that reflected concerns about exposures to toxic chemicals via the environment, residence, and workplace. That initiative became the Safe Drinking Water and Toxic Enforcement Act of 1986, and is better known by its original name of Proposition 65. Proposition 65 requires the State to publish a list of chemicals known to cause cancer or birth defects or other reproductive harm. The list must be updated at least once a year, and includes over 700 chemicals.

Proposition 65 requires businesses to notify Californians about significant amounts of chemicals in the products they purchase, in their homes or workplaces, or that are released into the environment. The goal is that this information allows Californians to make informed decisions about protecting themselves from exposure to these chemicals. In addition, Proposition 65 prohibits California businesses from knowingly discharging significant amounts of listed chemicals into sources of drinking water. A business also includes any company outside of California that sells their products in California.

California Environmental Protection Agency's (Cal/EPA) Office of Environmental Health Hazard Assessment (OEHHA) administers the Proposition 65 program. OEHHA also evaluates all currently available scientific information on substances considered for placement on the Proposition 65 list.

What Types of Chemicals are on the Proposition 65 List?

Proposition 65 contains a wide range of naturally occurring and synthetic chemicals that are known to cause cancer or birth defects or other reproductive

harm. These chemicals include additives or ingredients in pesticides, common household products, food, drugs, dyes, or solvents. Listed chemicals may also be used in manufacturing and construction, or they may be by-products of chemical processes, such as motor vehicle exhaust.

How is a Chemical Added to the List?

There are three principal ways for a chemical to be added to the Proposition 65 list. A chemical can be listed if either of two independent committees of scientists and health professionals finds that the chemical has been clearly shown to cause cancer or birth defects or other reproductive harm. These two committees are the Carcinogen Identification Committee (CIC) and the Developmental and Reproductive Toxicant (DART) Identification Committee, and both are part of OEHHA's Science Advisory Board. The committee members are appointed by the Governor and are designated as the 'State's Qualified Experts' for evaluating chemicals under Proposition 65. When determining whether a chemical should be placed on the list, the committees base their decisions on the most current scientific information available. OEHHA staff scientists compile all relevant scientific evidence on various chemicals for the committees to review. The committees also consider comments from the public before making their decisions.

A second way for a chemical to be listed is if an organization designated as an 'authoritative body' by the CIC or DART Identification Committee has identified it as causing cancer or birth defects or other reproductive harm. The following organizations have been designated as authoritative bodies: the US Environmental Protection Agency, the US Food and Drug Administration (US FDA), the National Institute for Occupational Safety and Health, the National Toxicology Program, and the International Agency for Research on Cancer.

A third way for a chemical to be listed is if an agency of the state or federal government requires that it be labeled or identified as causing cancer or birth defects or other reproductive harm. Most chemicals listed in this manner are prescription drugs that are required by the US FDA to contain warnings relating to cancer or birth defects or other reproductive harm.

In addition to these three listing procedures, Proposition 65 also requires the listing of chemicals meeting certain scientific criteria and identified in the California Labor Code as causing cancer or birth defects or other reproductive harm. This method was used to establish the initial chemical list following voter approval of Proposition 65 in 1986.

What Requirements Does Proposition 65 Place on Companies Doing Business in California?

Businesses are required to provide a 'clear and reasonable' warning before knowingly and intentionally exposing anyone to a listed chemical. This warning can be given by a variety of means, such as by labeling a consumer product, posting signs at the workplace, distributing notices at a rental housing complex, or publishing notices in a newspaper. Once a chemical is listed, businesses have 12 months to comply with warning requirements.

Proposition 65 also prohibits companies that do business within California from knowingly discharging listed chemicals into sources of drinking water. Once a chemical is listed, businesses have 20 months to comply with the discharge prohibition.

Businesses with less than 10 employees and government agencies are exempt from Proposition 65's warning requirements and prohibition on discharges into drinking water sources. Businesses are also exempt from the warning requirement and discharge prohibition if the exposures they cause are so low as to create no significant risk of cancer or birth defects or other reproductive harm. Health risks are explained in more detail below.

What Does a Warning Mean?

If a warning is placed on a product label or posted or distributed at the workplace, a business, or in rental housing, the business issuing the warning is aware or believes that one or more listed chemicals is present. By law, a warning must be given for listed chemicals unless exposure is low enough to pose no significant risk of cancer or is significantly below levels observed to cause birth defects or other reproductive harm.

For a chemical that causes cancer, the 'no significant risk level' is defined as the level of exposure that would result in not more than one excess case of cancer in 100 000 individuals exposed to the chemical over a 70 year lifetime. In other words, a person exposed to the chemical at the 'no significant risk level' for 70 years would not have more than a 'one in 100 000' chance of developing cancer as a result of that exposure.

For chemicals that are listed as causing birth defects or reproductive harm, the 'no-observed-effect level' is determined by identifying the level of exposure that has been shown to not pose any harm to humans or laboratory animals. Proposition 65 then requires this 'no-observed-effect level' to be divided by 1000 in order to provide an ample margin of

safety. Businesses subject to Proposition 65 are required to provide a warning if they cause exposures to chemicals listed as causing birth defects or reproductive harm that exceed 1/1000th of the 'no-observed-effect level'.

To further assist businesses, OEHHA develops numerical guidance levels, known as 'safe harbor numbers' (described below) for determining whether a warning is necessary or whether discharges of a chemical into drinking water sources are prohibited. However, a business may choose to provide a warning simply based on its knowledge, or assumption, about the presence of a listed chemical without attempting to evaluate the levels of exposure. Because businesses do not file reports with OEHHA regarding what warnings they have issued and why, OEHHA is not able to provide further information about any particular warning. The business issuing the warning should be contacted for specific information, such as what chemicals are present, and at what levels, as well as how exposure to them may occur.

Safe Harbor Numbers

OEHHA has developed safe harbor numbers to guide businesses in determining whether a warning is necessary or whether discharges of a chemical into drinking water sources are prohibited. A business has 'safe harbor' from Proposition 65 warning requirements or discharge prohibitions if exposure to a chemical occurs at or below these levels. These safe harbor numbers consist of no significant risk levels for chemicals listed as causing cancer and maximum allowable dose levels for chemicals listed as causing birth defects or other reproductive harm. OEHHA has established safe harbor numbers for over 200 chemicals and is continuing to develop safe harbor numbers for listed chemicals.

Enforcement of Proposition 65

The California Attorney General's Office enforces Proposition 65. Any district attorney or city attorney (for cities whose population exceeds 750 000) may also enforce Proposition 65. In addition, any individual acting in the public interest may enforce Proposition 65 by filing a lawsuit against a business alleged to be in violation of this law. Lawsuits have been filed by the Attorney General's Office, district attorneys, consumer advocacy groups, and private citizens and law firms. Penalties for violating Proposition 65 by failing to provide notices can be as high as $2500 per violation per day.

How is Proposition 65 Meeting Its Goal of Reducing Exposure to Hazardous Chemicals in California?

Since it was passed in 1986, Proposition 65 has provided Californians with information they can use to reduce their exposures to listed chemicals that may not have been adequately controlled under other State or Federal laws. This law has also increased public awareness about the adverse effects of exposures to listed chemicals. For example, Proposition 65 has resulted in greater awareness of the dangers of alcoholic beverage consumption during pregnancy. Alcohol consumption warnings are perhaps the most visible health warnings issued as a result of Proposition 65.

Proposition 65's warning requirement has provided an incentive for manufacturers to remove listed chemicals from their products. For example, trichloroethylene, which causes cancer, is no longer used in most correction fluids; reformulated paint strippers do not contain the carcinogen methylene chloride; and toluene, which causes birth defects or other reproductive harm, has been removed from many nail care products. In addition, a Proposition 65 enforcement action prompted manufacturers to decrease the lead content in ceramic tableware and wineries to eliminate the use of lead-containing foil caps on wine bottles.

Proposition 65 has also succeeded in spurring significant reductions in California of air emissions of listed chemicals, such as ethylene oxide, hexavalent chromium, and chloroform.

Although Proposition 65 has benefited Californians, it has come at a cost for companies doing business in the state. They have incurred expenses to test products, develop alternatives to listed chemicals, reduce discharges, provide warnings, and otherwise comply with this law. Recognizing that compliance with Proposition 65 comes at a price, OEHHA is working to make the law's regulatory requirements as clear as possible and ensure that chemicals are listed in accordance with rigorous science in an open public process.

See also: Developmental Toxicology; Toxicity Testing, Reproductive.

Further Reading

Curry KK, Brookman DJ, Whitmyre GK, *et al.* (1994) Personal exposures to toluene during use of nail lacquers in residences: Description of the results of a preliminary study. *Journal of Exposure Analysis and Environmental Epidemiology* 4: 443–456.

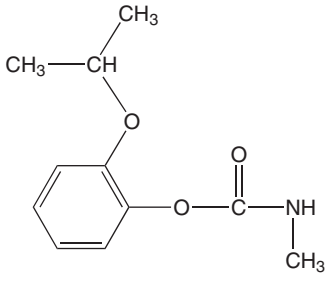
Relevant Website

http://www.oehha.ca.gov – State of California Environmental Protection Agency (Cal/EPA), Office of Environmental Health Hazard Assessment (OEHHA) Proposition 65. State of California's website for Proposition 65.

Propoxur

Paul R Harp

- Chemical Abstracts Service Registry Number: CAS 114-26-1
- Synonyms: 2-Isopropoxyphenyl-*N*-methylcarbamate; Baygon; Blattanex; IMPC; Invisi-Gard; IPMC; Propogon; Sendra; Sendran; Suncide; Tendex; Unden; Undene; BAY 39007; BAY 9010; BO 58 12315; ENT 25671; OMS 33; SHA 047802
- Chemical/Pharmaceutical/Other Class: *N*-Methylcarbamate insecticide
- Chemical Structure:



Uses

The main uses of propoxur include control of residential and commercial insect pests, domestic animal pests, and mosquitoes. It is available in a wide variety of formulations including aerosols, baits, dusts, emulsifiable concentrates, pest strips, pet flea collars, powders, ready-to-use solutions, and wettable powders.

Exposure Routes and Pathways

For both occupational and nonoccupational environments, dermal contact is the most common pathway but exposure has also occurred through ingestion and inhalation. The relatively high vapor pressure of propoxur (which is advantageous for mosquito control) has been implicated in several cases of inhalation exposure after recommended safety precautions were not observed. The rate of revolatization is influenced by temperature with applications in higher temperature, higher humidity environments presenting a greater risk of inhalation.

Toxicokinetics

Dermal absorption of propoxur in humans has been estimated to be ~ 16%; estimated absorption from the gastrointestinal tract in experimental studies with humans has been complicated due to propoxur-induced emesis. A biological half-life of 3.1 h has been determined and 2-isopropoxyphenol is the major metabolite. The majority of the dose undergoes urinary excretion within 48 h of exposure. In rats, both the parent compound and 2-isopropoxyphenol appear to be eliminated primarily in the urine as sulfate conjugates.

Mechanism of Toxicity

Propoxur binds and inhibits acetylcholinesterase, the enzyme responsible for metabolizing the neurotransmitter acetylcholine and terminating its action at cholinergic synapses. Exposure to propoxur results in synaptic accumulation of acetylcholine in both the central and peripheral nervous systems and hyperstimulation of muscarinic and nicotinic receptors leading to 'cholinergic crisis'. In contrast to the organophosphate anti-cholinesterases, acetylcholinesterase inhibition by the *N*-methylcarbamates is reversible with fairly rapid reactivation occurring through spontaneous decarbamoylation or via hydrolysis of the carbamate.

Acute and Short-Term Toxicity (or Exposure)

Animal

Signs of acute exposure in laboratory animals are similar to those described for humans and recovery from nonlethal exposures occurs rapidly. LD_{50} values (or ranges) reported for acute exposure in rats are 80–191 mg kg^{-1} (oral), from 1000 to >2400 mg kg^{-1} (dermal), and >1.44 mg l^{-1} (inhalation).

Human

The acute effects of exposure are due to cholinergic overstimulation and may include the SLUDGE (salivation, lacrimation, urination, diarrhea,

gastrointestinal cramping, and emesis) syndrome, respiratory depression, bronchospasms, increased bronchial secretions, pulmonary edema, blurred vision, miosis, headache, tremors, muscle fasciculations, convulsions, mental confusion, coma, and death (due to respiratory failure). Symptomatic recovery from nonlethal exposures occurs very rapidly (usually within a few hours).

Chronic Toxicity (or Exposure)

Currently, insufficient evidence exists to indicate any significant long-term health risk associated with propoxur exposure.

Clinical Management

Persons providing medical assistance should avoid contact with contaminated clothing. Contaminated clothing should be removed and either laundered or discarded. Contaminated leather garments such as shoes or gloves should be discarded. Exposed dermal areas should be cleaned thoroughly with soap and water. Exposed eyes should be flushed with generous amounts of clean water for at least 15 min. If necessary, an endotracheal tube should be used to maintain a clear airway, aspirate any secretions, and provide oxygen via mechanical ventilation.

For ingestion, if the patient is asymptomatic and can be treated soon after exposure, activated charcoal may be used to reduce absorption of the carbamate. If potentially life-threatening quantities have been ingested, gastric lavage should be considered if it can be conducted within $\sim$1 h of exposure. Charcoal and/or catharsis are contraindicated in presence of severe vomiting or diarrhea. Muscarinic effects (i.e., SLUDGE) may be reduced by intravenous or intramuscular administration of atropine. Seizures can be treated with intravenous benzodiazepines (diazepam or lorazepam); phenobarbital may be helpful for recurrent seizures. Pralidoxime is indicated in cases of mixed exposure to both carbamates and organophosphorus compounds but is contraindicated in cases of carbamate-only exposure. Furosemide may be useful for pulmonary edema that continues after full atropinization. Metabolite analysis of a urine sample may allow confirmation of the intoxicating agent.

Environmental Fate

Propoxur is of moderate to low persistence in the soil (half-life of 14–50 days). It does not bind with high affinity to soil and therefore tends to be mobile. It is highly water soluble and thus has potential for leaching into groundwater. Propoxur is very mobile in sandy loam, silt loam and silty clay soils. Propoxur degrades in water at a rate of $\sim$1.5% per day at neutral pH. Propoxur is well absorbed into plant tissues and can thereby be active against insects for up to 1 month.

Ecotoxicology

Propoxur is very highly toxic to many birds. The LD_{50} in quail, mourning doves, and finches was 25.9, 4, and 4 mg kg^{-1}, respectively. LD_{50} values were from 6 to 120 mg kg^{-1} in other bird species. Acute signs of toxicity in birds included tearing, salivation, muscle incoordination, diarrhea, and tremors. Death generally occurred rapidly (5–45 min) with severe acute poisoning and recovery was also rapid. Propoxur is moderately to slightly toxic to aquatic species. LC_{50} values (96 h) were 3.7–6.6 mg l^{-1} in trout and in bluegill. Propoxur does not markedly bioaccumulate. Propoxur is highly toxic to honeybees.

Exposure Standards and Guidelines

The reference dose for propoxur is 0.005 mg kg^{-1} day^{-1}. The acceptable daily intake is 0.02 mg kg^{-1} day^{-1}.

See also: Carbamate Pesticides; Cholinesterase Inhibition; Pesticides.

Further Reading

Ecobichon DJ (2000) Carbamates. In: Spencer PS and Schaumburg HH (eds.) *Experimental and Clinical Neurotoxicology*, 2nd edn., pp. 289–298. New York: Oxford University Press.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.
http://www.epa.gov – US Environmental Protection Agency.

Propoxyphene

Christopher P Holstege

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- Chemical Name: Propoxyphene
- Chemical Abstracts Service Registry Number: CAS 469-62-5
- Synonyms: Darvocet-N; Darvon; Darvon-N; Darvon pulvules; Propoxyphene hydrochloride; Propoxyphene napsylate; Wygesic
- Chemical/Pharmaceutical/Other Class: Synthetic opioid

Uses

Propoxyphene is used as an analgesic. It is also used as a drug of abuse.

Exposure Routes and Pathways

All propoxyphene preparations are for oral use; ingestion is the most common route of exposure. Propoxyphene-containing pharmaceuticals are also solubilized and injected for purposes of abuse.

Toxicokinetics

Propoxyphene is readily absorbed from the gastrointestinal tract with measurable levels within 5 min and peak plasma levels within 1–2 h following therapeutic dosing. Propoxyphene undergoes extensive first pass metabolism and is primarily metabolized in the liver by *N*-demethylation to norpropoxyphene. Propoxyphene and norpropoxyphene are widely distributed throughout the body. Concentrations in the tissues are reportedly 10–40 times greater than in the blood. Peak plasma levels of propoxyphene and norpropoxyphene occur within 1–2 h and 2–4 h, respectively. Propoxyphene and norpropoxyphene are highly protein bound (80%). The volume of distribution is 10–26 l kg^{-1}. Norpropoxyphene is a pharmacologically active metabolite. Serum norpropoxyphene levels are reportedly higher than propoxyphene due to norpropoxyphene's slower metabolism. Norpropoxyphene is further metabolized in the liver to a variety of inactive metabolites that include: *p*-hydroxypropoxyphene, norpropoxyphene carbinol, *p*-hydroxynorpropoxyphene, dinorpropoxyphene, cyclic dinorpropoxyphene, and dinorpropoxyphene carbinol. Propoxyphene and its metabolites are excreted in the urine, with less than 10% of a propoxyphene dose excreted unchanged in the urine. High concentrations of propoxyphene and norpropoxyphene have been found in the bile, suggesting that enterohepatic recirculation occurs. There is substantial individual variation in the elimination half-life of propoxyphene, with the elimination half-life of propoxyphene ranging from 8 to 46 h and norpropoxyphene from 6 to 54 h. The elimination half-life of propoxyphene is prolonged following over dosage, repetitive dosing, and shock.

Mechanism of Toxicity

Propoxyphene is an agonist of opioid μ receptors. It is this opioid effect that is responsible for the central nervous system and respiratory depression seen in overdose. Both propoxyphene and norpropoxyphene are potent blockers of myocardial sodium channels, an effect identical to type IA antidysrhythmic agents. This myocardial sodium channel blockade may result in prolongation of the electrocardiogram QRS complex, arrhythmias, and cardiovascular depression seen in propoxyphene poisoning.

Acute and Short-Term Toxicity (or Exposure)

Animal

Central nervous system and cardiovascular effects in animals are similar to those seen in humans.

Human

Acute toxicity with propoxyphene has been reported to cause nausea, vomiting, miosis, confusion, restlessness, somnolence, coma, apnea, seizures, hypotension, arrhythmias, pulmonary edema, and cardiac arrest.

Chronic Toxicity (or Exposure)

Animal

Pregnant rats fed up to 400 mg kg^{-1} day^{-1} demonstrated ~20% maternal mortality, decreased fertility, and some fetal death. No teratogenic effects were observed.

Human

Tolerance and dependence may occur with prolonged use. Abrupt cessation in patients utilizing propoxyphene chronically may result in an opioid withdrawal syndrome in both adults and neonates.

Clinical Management

In patients presenting with propoxyphene toxicity, the airway should be patent and adequate ventilation assured. If the patient has either inadequate ventilation or a poor gag reflex, then the patient may be at risk of subsequent CO_2 narcosis, worsening acidosis, and/or aspiration. If necessary, endotracheal tube intubation should be performed. The initial treatment of hypotension consists of intravenous fluids. There should be close monitoring of the patient's pulmonary parameters to ensure that pulmonary edema does not develop as fluids are infused. The patient should be placed on continuous cardiac monitoring with pulse oximetry. Frequent neurological checks should be made. Gastrointestinal decontamination should be considered only after initial supportive care has been provided and airway control has been assured. Activated charcoal ($1\,g\,kg^{-1}$) may be administered.

Naloxone may be of benefit in reversing the neurological and respiratory depressant effects of propoxyphene. Naloxone may also decrease the propensity for developing seizures after overdose. Naloxone has no effect on the potential myocardial sodium channel blocking properties of propoxyphene. The management of the sodium channel blocking effects of propoxyphene consists of administration of sodium and/or alkalosis. Infusion of sodium bicarbonate either by intermittent bolus or by continuous infusion has been advocated. The indications for sodium bicarbonate infusion include a QRS duration of >100 ms, persistent hypotension despite adequate hydration, and dysrhythmias. During infusions of sodium bicarbonate, close monitoring of electrolyte, pH, and fluid balance should be performed. Hyperventilation has also been shown to be effective in reversing myocardial sodium channel blocking activity due to the induced respiratory alkalosis. Lidocaine therapy has been suggested in the treatment of ventricular dysrhythmias, although clear evidence is lacking. Class IA and IC antiarrhythmics should be avoided due to their ability to block cardiac sodium channels.

See also: Charcoal; Gastrointestinal System; Poisoning Emergencies in Humans.

Further Reading

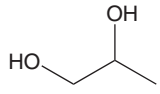
Hantson P, Evenepoel M, and Ziade D (1995) Adverse cardiac manifestations following dextropropoxyphene overdose: Can naloxone be helpful. *Annals of Emergency Medicine* 25: 263–266.

Sloth-Madsen PS, Strom J, and Reiz S (1984) Acute propoxyphene self-poisoning in 222 consecutive patients. *Acta Anaesthesiologica Scandinavica* 28: 661–665.

Propylene Glycol

Vijay M Vulava

- Chemical Abstracts Service Registry Number: CAS 57-55-6
- Synonyms: 1,2-Propanediol; α-Propylene glycol; Methyl glycol; Methylethyl glycol; Methylethylene glycol; Monopropylene glycol; PG 12; Sirlene; 1,2-Dihydroxypropane; 1,2-Propylene glycol; 2-Hydroxypropanol; 2,3-Propanediol; Propane-1,2-diol; Dowfrost; Propylene glycol usp; 1,2-Propylenglykol; Solar winter ban; Sentry propylene glycol; Isopropylene glycol; Ucar 35; Solargard P; Aliphatic alcohol; Chilisa FE; Ilexan P; Inhibited 1,2-propylene glycol; Prolugen; Propanediol; Trimethyl glycol
- Chemical/Pharmaceutical/Other Class: Glycols; 1,2-Diols
- Chemical Formula: $C_3H_8O_2$
- Chemical Structure:



Uses

Propylene glycol is one of the most commonly used humectants – substances that have a high affinity for water and have a stabilizing action on the water content of a material. Propylene glycol is used to maintain moisture within a narrow range in certain food products, such as coconut and marshmallows, in certain medicines and cosmetics as well as in tobacco, and is a solvent for food colors and flavors. The US Food and Drug Administration has classified propylene glycol as an additive that is 'generally recognized as safe' for use in food.

Propylene glycol is commonly used to make antifreeze and deicing solutions for cars, airplanes, and boats; to make polyester compounds; and as solvents in the paint and plastics industries. It is used as a substitute for ethylene glycol mono-alkyl ethers in all-purpose cleaners, coatings, inks, nail polish, lacquers, latex paints, and adhesives. It is also used to create artificial smoke or fog used in fire-fighting training and in theatrical productions.

The general population is exposed to propylene glycol by oral intake, dermal contact, and inhalation. The average daily intake of propylene glycol from food products in the United States has been estimated at 2400 mg day^{-1} (34 mg kg^{-1} body weight (bw) per day for a 70 kg person). Propylene glycol is an inert ingredient in some pharmaceutical preparations. Propylene glycol is also found in many pharmaceuticals that are administered intravenously, which represents a unique exposure route for certain subpopulations.

Exposure Routes and Pathways

As propylene glycol is ubiquitous in several foods, medical, and cosmetic products, humans are commonly exposed via several routes.

Toxicokinetics

The pharmacokinetics of propylene glycol is reasonably well understood in humans as well as animals. Propylene glycol is rapidly and extensively absorbed followed by rapid distribution into total body water. Dermal absorption studies in humans have shown that absorption of propylene glycol through intact skin is very limited. However, once the dermal layers are disturbed (such as with burns or irritation), dermal absorption can be a significant source of exposure. Except for the amount entering the nasopharynx and being swallowed, under normal exposure conditions propylene glycol exposure by inhalation is not toxicologically relevant due to its low vapor pressure (0.07 mmHg).

Total body clearance occurs by metabolic clearance and by renal excretion. The excretion of propylene glycol is species dependent. Humans clear ~45% of propylene glycol via kidney, and in dogs, up to 88%. In rats and rabbits, very little of the parent compound is excreted by the kidney until saturation of metabolism occurs. Inhibition of alcohol dehydrogenase by pyrazole increases urinary excretion of propylene glycol to 75% in rats.

The rate-determining step in the metabolic clearance of propylene glycol in humans and animals is NAD-dependent alcohol dehydrogenase. Humans clear propylene glycol similarly to rats and rabbits, but saturation of metabolic clearance occurs at lower doses (up to 8–10 times) in humans than in rats and rabbits. The lower dose required for saturation in humans is related to the expression of various isoforms of alcohol dehydrogenase in various species and in different tissues. By a NAD-dependent reaction, alcohol dehydrogenase converts propylene glycol to lactaldehyde, which is further metabolized to lactate. Since propylene glycol has a chiral center, technical grade propylene glycol results in the formation of 50/50 D,L-lactate. L-Lactate is indistinguishable from endogenous lactate, which is a good substrate for gluconeogenesis. D-Lactate is less readily converted to glucose than L-lactate, which prolongs its half-life leading, under conditions of prolonged exposure (e.g., intravenous infusion), to D-lactic acidosis. It is difficult to cause L-lactic acidosis even with very high doses of propylene glycol because of its efficient detoxification via gluconeogenesis.

The second reason for lack of development of L-lactic acidosis is the saturation of alcohol dehydrogenase, which results in a constant rate of lactate production. Due to removal of L-lactate by gluconeogenesis, a further increase in lactate levels is not possible after saturation of metabolism.

Mechanism of Toxicity

The absorption, distribution, metabolism, and excretion of propylene glycol have been studied in humans, cats, rats, mice, and rabbits. There are no identifiable differences between humans and animals in the toxicity of propylene glycol. Toxic effects of propylene glycol occur only at very high doses. Since propylene glycol has very low intrinsic toxicity, saturation of metabolism plays a protective role in its toxicity since the conversion of propylene glycol to the more toxic lactate (particularly D-lactate) is slowed. Because of low alcohol dehydrogenase activity in infants and children, this protective effect is more pronounced in infants and up to 5 years of age. High blood levels of propylene glycol during continuous therapeutic infusion in pediatric intensive care patients 15 months of age and younger were not associated with any acute toxicity. The knowledge that human metabolism of propylene glycol saturates at an 8–10 times lower dose than in rats or rabbits provides further confidence that human developmental or reproductive risks are of negligible concern.

Propylene glycol administered to mice in drinking water at up to 5% (w/v) had no effect on fertility of either males or females in either the first or second generation. Other data indicate that this compound is not a reproductive or developmental toxicant in mice, rats, hamsters, or rabbits. There are no major

differences in general toxicity between humans and most animals, and toxicity only occurs at very high doses (LD_{50} values of 8–46 g kg^{-1} in rats, and is estimated to be >15 g kg^{-1} in humans). Current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans.

No indications on mutagenicity or carcinogenicity have been found in laboratory animal studies. Subcutaneous injections in mice led to a small increase in fetal malformations, but experiments with oral exposure of mice over several generations did not show any effects of toxicity to reproduction.

Propylene glycol is mildly to moderately irritating to skin in concentrations above 10%. No irritation was seen in rabbit eyes. Several cases of allergy have been described, and concentrations above 10%, particularly if occluded, may give rise to allergic skin reactions. With skin affected by disease or damage the risk of irritation and allergic reaction is increased. Reactions have been described by 2% on eczematous skin. As propylene glycol is widely used, allergy cases are considered unusual. Propylene glycol may be absorbed through skin and increase the absorption of other substances.

Acute and Short-Term Toxicity (or Exposure)

There are sufficient data to characterize the acute and chronic toxicity of propylene glycol in laboratory animals, including nonhuman primates. In humans, information on toxicity is limited to medical case studies. However, because of the similarities in the toxicokinetic profile of propylene glycol across species, the toxicity data from the animal studies can be extrapolated to human exposures.

Propylene glycol has very low systemic toxicity in experimental animals and very high doses are required to determine a toxic level. Central nervous system (CNS), hematologic, hyperosmotic, and cardiovascular effects have been noted in humans and animals and high serum concentrations of propylene glycol may result in lactic acidosis and hyperosmotic changes in the blood.

Animal

Animals lethally intoxicated undergo CNS depression, narcosis, and respiratory arrest. Acute oral toxicity has been well characterized in the rat, mouse, rabbit, dog, and guinea pig with LD_{50} values, 8–46 g kg^{-1}, reported at very high oral doses. An average daily dose of 1.7 g kg^{-1} bw in male rats and 2.1 g kg^{-1} bw in female rats has been shown to have no adverse effect on body weight gain, mortality, hematology, urinary cell excretion, renal function, serum chemistry, or absolute and relative organ weights.

Human

In humans, a lethal oral dose has been estimated to be >15 g kg^{-1} for an adult. Mortality has occurred in hospitalized infants after repeated exposure to propylene glycol in medication.

Chronic Toxicity (or Exposure)

Animal

There are few studies that investigated chronic exposure of propylene glycol to animals. A propylene glycol diet at 2 and 5 g kg^{-1} bw per day fed to dogs for 2 years resulted in RBC destruction in dogs fed with higher amounts of propylene glycol while no effect was observed in dogs fed at lower concentration. In a continuous inhalation study chronic toxicity of propylene glycol (55–113 ppm) in Rhesus monkeys and rats were studied for up to 1 year. Both rats and monkeys inhaling propylene glycol gained more weight than the control group; no adverse effects were noted.

Results from animal studies indicate that intermediate and chronic exposure to propylene glycol may lead to changes in hematological parameters and hemolysis of RBCs. Cats exposed to oral administration of propylene glycol developed Heinz bodies in RBCs and decreased RBC survival. Doses as low as 0.424 g kg^{-1} bw per day have resulted in Heinz body formation in cat erythrocytes.

Human

Chronic occupational exposure to propylene glycol in humans may occur through dermal contact or through inhalation of airborne propylene glycol from heating or spraying processes. Propylene glycol occupational exposure data are limited to several small studies. An investigation measuring propylene glycol exposure in motor servicing workers did not detect normal urinary propylene glycol levels. Another study measured airborne propylene glycol exposure (geometric mean 350 μg m^{-3}, maximum 12 700 μg m^{-3}) among Swedish painters during indoor application of water-based paints. Elevated propylene glycol levels were measured in urine samples collected pre- and postshift from aircraft deicing workers (range: 0.72–13.44 mg l^{-1}; 0.41–10.58 mg g^{-1} creatinine); and in urine samples from a comparison group (range: 0.29–10.7 mg l^{-1}, 1.18 mg g^{-1} creatinine). In a National Institute for Occupational Safety and Health 'Health Hazard

Evaluation' of aircraft deicing workers, personal breathing zone air samples for propylene glycol over a 6 h period ranged from 10 to 21 mg m^{-3}, with a mean of 15 mg m^{-3}.

In Vitro Toxicity Data

Propylene glycol in a concentration of 0.5–1.0% has been shown to inhibit natural cytotoxicity and neutrophil chemiluminescence in human cells *in vitro* in one study.

In vitro studies of embryonic development suggest that propylene glycol alters the development of mouse zygotes. Treatment with propylene glycol caused cell membrane damage and altered pH, resulting in a decrease in embryonic development.

Clinical Management

Degree of injury must be considered when determining initial treatment. When large amounts of propylene glycol are ingested, copious amounts of water should be given to the patient to dilute stomach contents. In case of inhalation exposure, the patient should be removed to fresh air and be given supplemental oxygen in case breathing becomes difficult. In case of dermal exposure, the exposed skin should be immediately washed with copious water and soap. Upon exposure to propylene glycol, eyes should be flushed with copious water including areas under eye lids. In all cases, a physician should be consulted in case of serious injury.

Environmental Fate

Propylene glycol has a low vapor pressure (0.07 mmHg at 20°C) and is miscible with water. High solubility of propylene glycol in water ensures at least partial removal of the compound will occur by wet deposition when released to atmosphere as vapors. Relatively low Henry's law constant values for the compound suggest that releases to surface water will not partition to the atmosphere via volatilization. Adsorption to sediment or soil particulates is not significant due to low sorption partitioning coefficient (K_{oc}) value and hence propylene glycol can have a high mobility in soil and could leach into groundwater. Low octanol/water partition coefficient (K_{ow}) suggests that bioconcentration and biomagnification are also not likely to occur.

Propylene glycol released to the atmosphere is expected to undergo rapid photochemical oxidation via reaction with hydroxyl radicals. The half-life for the photochemical oxidation of propylene glycol has been estimated to be 20–32 h.

Biodegradation by a variety of acclimated and unacclimated microorganisms, under both aerobic and anaerobic conditions, is also the most important transformation process for propylene glycol in surface waters and soils. Half-lives for the biotransformation of propylene glycol generally range from 1 to 4 days under aerobic conditions and from 3 to 5 days under anaerobic conditions. The rates of biodegradation of propylene glycol in soils are significantly dependent on substrate concentrations, soil types, and ambient soil temperatures, but nutritional supplements had minimal effects. Propylene glycol rapidly disappears from culture flasks containing activated sludge microorganisms under both aerobic and anaerobic conditions.

Exposure Standards and Guidelines

Currently there are no published or recommended exposure standards for propylene glycol. However, the World Health Organization suggests an acceptable daily intake of 0–25 mg kg^{-1}.

See also: Food Additives.

Further Reading

Thomas JA, DeSesso JM, Fowler BA, *et al.* (2003) NTP-CERHR expert panel report on the reproductive and developmental toxicity of propylene glycol. National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction, US Department of Health and Human Services. Report No. NTP-CERHR-PG-03.

Relevant Websites

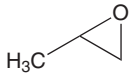
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Propylene Glycol.

http://cerhr.niehs.nih.gov – National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction, US Department of Health and Human Services.

Propylene Oxide

Ada Kolman and Siv Osterman-Golkar

- Chemical Abstracts Service Registry Number: CAS 75-56-9
- Synonyms: 1,2-Propylene oxide; 1,2-Epoxypropane; Methyloxacyclopropane; Methyloxirane; Methyl ethylene Oxide
- Chemical/Pharmaceutical/Other Class: Epoxides
- Chemical Formula: C_3H_6O
- Chemical Structure:



Uses

Propylene oxide is widely used in the chemical industry as an intermediate in the production of a broad spectrum of materials, such as polyether polyols, propylene glycol, and propylene glycol ethers. These products are further used in the manufacture of polyurethane, lubricants, and detergents. Propylene oxide is used for the fumigation of dried fruit and various other foodstuffs. Propylene oxide is also used for embedding tissues for electron microscopy.

Exposure Routes and Pathways

Propylene oxide is a volatile colorless liquid with an ethereal odor. Occupational exposure by inhalation of contaminated air, as well as contact with eyes and skin, provide the significant routes of exposure. It is not known to occur as a natural product.

Toxicokinetics

Studies in experimental animals have demonstrated that propylene oxide is readily absorbed and effectively metabolized. Only a minor fraction of the compound is exhaled unchanged. The main metabolic pathways are enzyme-catalyzed reactions with glutathione and water.

The distribution of propylene oxide within the body has been studied in experimental animals by means of measurement of its alkylation products (adducts) with DNA in various tissues and with hemoglobin in red blood cells. In rats, exposed to the compound by inhalation, the highest DNA adduct levels were found in the nasal epithelia, followed by lung, lymphocytes, spleen, liver, and testis. The adduct level in the respiratory mucosa was ~30-fold higher than in the testis. The dose response for adduct formation was linear up to 500 ppm of propylene oxide in the air. Propylene oxide is ~5–10 times less efficient than the related compound ethylene oxide concerning its alkylation capacity *in vivo*.

Mechanism of Toxicity

The toxic effects of propylene oxide are related to its ability to react directly, without metabolic activation, with various components of cells, including DNA, RNA, and proteins.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity of propylene oxide has been studied in several animal species. Acute oral LD_{50} values of 380 mg kg^{-1} in the rat and 660 mg kg^{-1} in the guinea pig have been reported. The 4 h LC_{50} value for inhalation is 4000 ppm for the rat and 1740 ppm for the mouse. Propylene oxide neuropathy has been demonstrated in rats. In a 7 week study, animals exposed to propylene oxide at a concentration of 1500 ppm in air, developed ataxia in the hind legs.

Human

Contact with propylene oxide may cause severe skin and eye irritation. Cases of allergic contact dermatitis and hand eczema have been described. Inhalation of propylene oxide may result in spasm, inflammation, and edema of the larynx and bronchi, as well as pulmonary edema leading to pneumonia. Symptoms of exposure may include burning sensation, coughing, wheezing, headache, nausea, and vomiting. Propylene oxide may cause central nervous system depression and other neurological disorders.

Chronic Toxicity (or Exposure)

Animal

Chronic and subchronic exposure of rats to propylene oxide by inhalation induced respiratory cell hyperplasia, irritation and toxicity in the nasal epithelium, at a concentration of 300 ppm or higher. No adverse effects on reproduction were observed in rats or rabbits exposed to propylene oxide at up to 500 ppm. *In vivo* studies in rodents of dominant lethal mutations, sperm abnormalities, micronuclei, chromosomal aberrations, and sister chromatid exchanges

have given negative results. Neither chromosomal aberrations nor sister chromatid exchanges were induced in monkeys exposed to 300 ppm. Long-term carcinogenicity studies in rodents, administered propylene oxide by different routes, demonstrated increased incidences of tumors mainly at the site of contact. At oral administration, tumors of the forestomach, which were mainly squamous-cell carcinomas, were produced in rats. In rats of both sexes exposed by inhalation, papillary adenomas of the nasal cavity were observed, as well as thyroid adenomas and carcinomas in females. Increased incidences of mammary fibroadenomas and adenocarcinomas have been observed in females. In mice exposed by inhalation, propylene oxide produced hemangioma and hemangiosarcoma of the nasal cavity and a few malignant nasal epithelial tumors. Subcutaneous administration of propylene oxide to mice produced local sarcoma.

Human

Convincing epidemiological data on cancer in humans are lacking for propylene oxide. However, based on the body of data including positive responses in tests for toxicity and carcinogenicity in experimental animals, DNA adduct formation, and also propylene oxide genotoxicity in several *in vitro* tests in mammalian cells, the International Agency for Research on Cancer has classified propylene oxide as 'possibly carcinogenic to humans'.

In Vitro Toxicity Data

Propylene oxide is mutagenic in several microorganisms and in *Drosophila*. It induces sister chromatid exchanges and chromosomal aberrations, as well as DNA damage (single- and double-strand breaks) in human cells. Propylene oxide induces neoplastic cell transformation in mouse embryo cells.

Clinical Management

If propylene oxide is swallowed, the mouth should be washed out with water. Vomiting should not be induced. In case of inhalation of the compound, the person should be moved to fresh air. If breathing is difficult, oxygen should be given. If not breathing, artificial respiration should be given. In case of contact with eyes or skin, contaminated areas should immediately be flushed with plenty of water for at least 15 min. In all cases of extensive exposure, immediate medical advice should be sought.

Environmental Fate

Propylene oxide is not expected to bioaccumulate. When released into water, it is hydrolyzed with a half-life between 10 and 30 days. Degradation of propylene oxide in the air may occur by reaction with photochemically produced hydroxyl radicals.

Other Hazards

Propylene oxide is extremely flammable. Propylene oxide–air mixtures may be explosive by contact with heat or by ignition. Propylene oxide is incompatible with acids, bases, oxidizing agents, polymerization catalysts, epoxy resins, and high temperatures. It reacts violently with acetylide-forming metals such as copper or copper alloys.

Exposure Standards and Guidelines

Recommendations regarding limits for occupational exposure to propylene oxide differ markedly. The current 8 h time-weighted average (TWA) established by the Occupational Safety and Health Administration in the United States is 100 ppm. The American Conference of Governmental Industrial Hygienists recommends a threshold limit value of 2 ppm as an 8 h TWA. In European countries, the limits of exposure are in the range 1–20 ppm (8 h TWA).

See also: Epichlorohydrin; Ethylene Oxide.

Further Reading

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Prostaglandins

Samantha E Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 2, pp. 596–597, © 1998, Elsevier Inc.

- CHEMICAL/PHARMACEUTICAL/OTHER CLASS: Unsaturated derivatives of arachidonic acid, a 20-carbon fatty acid

Uses

Prostaglandins are a family of naturally occurring compounds found mainly in animals, and are involved in numerous physical processes, both beneficial and pathological. These include inflammation (especially allergy related), cell growth regulation, and smooth-muscle contraction. They are hormone-like chemicals that are produced in the cell membranes of virtually every organ. Like all eicosanoids, they are derived from oxidized arachidonic acid, an essential polyunsaturated fatty acid. They are produced and metabolized in the kidney from essential fatty acids, and can be found in various tissues and body fluids. Perhaps the most commonly known therapeutic application related to prostaglandins is the use of aspirin to stop inflammation and pain. Aspirin works by inhibiting the synthesis of prostaglandins. Prostaglandins have a number of therapeutic uses, for example, preventing ulcers, reducing the potential size of myocardial infarctions, and to treat glaucoma. Another example is that prostaglandins are also used to promote cervical or uterine contractions in pregnant women, either as a labor inducer or as an abortifacient (i.e., RU486).

Exposure Routes and Pathways

Aside from endogenous production, prostaglandins can be absorbed into the body through ocular exposure, inhalation, ingestion, or injection.

Toxicokinetics

The prostaglandins as a group are known for having extremely short life span, sometimes as short as several minutes within an organism before enzymatic action breaks them down. They are produced on demand, and act in very small amounts to regulate the nervous, respiratory, cardiovascular, reproductive, endocrine, immunological, and renal systems.

In the case of glaucoma treatment, a prostaglandin derivative (S-1033) is rapidly absorbed through the eyes and into the plasma.

Drug Interactions

NSAIDs (nonsteroidal antiinflammatory drugs): Isolated cases of adverse neurological side effects have been seen with naproxen or phenylbutazone given with misoprostol. Misoprostol also increases the abdominal pain and other side effects of diclofenac and indometacin (indomethacin). Paracetamol (acetaminophen) intensifies pain if given with mifepristone and sulprostone used to induce abortion.

Digitalis glycosides: Epoprostenol caused a small predicted decrease in digoxin clearance in the short term; this may not be clinically significant.

Mechanism of Toxicity

Mast cell degranulation releases arachidonic acid, which is broken down by the enzyme cyclooxygenase to form prostaglandins, mediated by prostaglandin H synthase (PHS). PHS is a peroxidase involved in biotransformation, adding a peroxide oxygen to the xenobiotic. Interaction with some substances, such as benzo[*a*]pyrene or aflatoxin B1, may catalyze the release of teratogenic and tumorigenic metabolites. By oxidizing acetaminophen to *N*-acetyl-benzoquinoneimine, PHS may also contribute, along with cytochrome P450 and glutathione, to the nephrotoxic effects of the drug. PHS peroxidation may also suppress bone marrow by binding to proteins and DNA in the marrow. The role of PHS in biotransformation is mediated by the availability of archidonic acid, which may be the key to controlling its toxic effect.

Acute and Short-Term Toxicity (or Exposure)

Human

Some prostaglandins have vasoconstrictive and or bronchodilatory properties while others are vasodilatory and or bronchoconstrictive. Prostaglandins regulate smooth muscle function in the lungs, heart, and uterus. They contribute to changes in the oxygen flow to the heart causing rapid changes in coronary blood flow. Asthmatics exposed to prostaglandin F2a (or Dinoprost) may experience bronchospasm, arrhythmia, or hyperventilation. It may induce grand mat seizures in epileptics. Prostaglandins contribute to platelet aggregation of blood clots and NSAIDs such as aspirin or ibuprofen counteract that activity.

When used to induce labor, Dinoprost effects may include cervical laceration or rupture with retention of the placenta or hemorrhaging. It may affect the

alimentary tract as well, causing nausea, vomiting, and diarrhea. In two cases, women died of cardiovascular collapse following a 40 mg dose of Dinoprost. The TD_{Lo} is 20 $\mu g\,kg^{-1}$.

Chronic Toxicity (or Exposure)

Animal

PHS is suspected of contributing to bladder cancer in dogs by converting aromatic amines to reactive radicals through one-electron oxidation in the liver.

Human

PHS could be a human carcinogen, since dogs and humans have similar tumorigenic responses to aromatic amines.

Clinical Management

Since prostaglandins are rapidly metabolized in the body, discontinued use and supportive therapy are usually the recommended treatments for a toxic response. In cases of placental retention, blood transfusions may be necessary.

See also: Ethylene Glycol Monoethyl Ether.

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Relevant Website

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Proteomics

Udayan M Apte and Harihara M Mehendale

Introduction

Proteomics is the study of 'proteome' or the protein expression in the cell on a global level. It is known that each cell in the body of a living organism has a complete set of genes but the cell type and functions performed by the cells are governed by stringently controlled processes of transcription (DNA to mRNA) and translation (mRNA to protein). The revolution in genomic technology has enabled us to study gene expression of thousands of genes simultaneously using high-density microarray. Although microarray technology has revolutionized the way one studies gene expression in the cell, it has some obvious drawbacks. It has been noted that there is very poor correlation between the microarray data and the protein expression data, largely due to post-transcriptional control of gene expression. Only the genes with high 'codon bias' exhibit high correlation in mRNA and protein expression in the cell. Thus, even though the microarray technology remains a very powerful tool, proteomic techniques to study global protein expression are becoming increasingly popular.

For toxicology the advantages of proteomics goes beyond the ability to compare protein expression differences. Proteomics allows a researcher to study protein modifications due to toxic treatment and more importantly allows identification of toxicant-protein adducts. Extraordinary advances in technology during the last decade have made proteomics a highly accurate, relatively inexpensive, and, therefore, a routinely used technique in biomedicine and toxicology. Proteomics is applied in toxicology mainly for three purposes: protein expression profiling, that is, assessment of changes in protein expression following toxicant exposure (control vs. treatment type comparisons); protein modification studies, that is, identification and characterization of protein adducts formed due to interaction of reactive metabolites with proteins,

changes in structure and chemistry of proteins following toxicant exposure; and finally for predictive toxicology where protein expression patterns of model toxicants are used to predict toxic effects of novel compounds.

Analytical Approaches in Proteomics

There are four main steps in any proteomic analysis (**Figure 1**):

1. Obtaining protein samples from cell cultures or tissues.
2. Breakdown of proteins to peptide mixture and further resolution of peptides.
3. Mass spectrometric analysis of peptides.
4. Identification of protein from mass spectrometric analysis.

Isolation of Proteins

Any proteomic study starts with the collection of proteins from biological samples such as cell culture media, cultured cells, serum, or any biological fluid, and a variety of animal tissues. The first step is to obtain a protein sample under conditions of least protein degradation. This involves use of various protease inhibitors that stop the protein degradation. The use of protease inhibitors depends on the type of sample and the analytical technique used in the subsequent analysis. The selection of protease inhibitors used is critical since many protease inhibitors and detergents used in the preparation of tissue homogenates can interfere with mass spectrometry (MS) procedures, which are an integral part of the proteomic analysis.

Breakdown of Proteins to Peptides and Further Resolution of Peptides

The second step of proteomic analysis involves breaking down of the proteins into smaller peptides (typically six amino acids long) and separating them depending upon molecular weight and/or charge to increase the resolution of proteomic analysis. The protein sample obtained from cells or tissue is further digested using various proteases with known properties to obtain a mixture of peptides. While various enzymes or enzyme combinations such as trypsin, chymotrypsin (their mixture), and Gluc-C are used, trypsin remains by far the most popular choice for protein breakdown into peptides. Most proteins are extremely large and complex molecules, and have posttranslational modifications such as phosphorylation and glycosylation, which renders them unsuitable for mass spectrometric analysis. Digesting proteins into peptides enables accurate detection of their mass by MS.

Breaking the proteins into smaller peptides using proteases produces a large and complex mixture of peptides. This peptide mixture needs further separation, mostly depending upon size (molecular weight) of the peptides, in order to achieve higher resolution and accuracy in MS analysis. Therefore, an integral part of the second step in proteomic is separation of individual peptides contained in the mixtures of peptide using one of the two methods: preparative isoelectric focusing or high-performance liquid chromatography (HPLC). The peptides separated, using

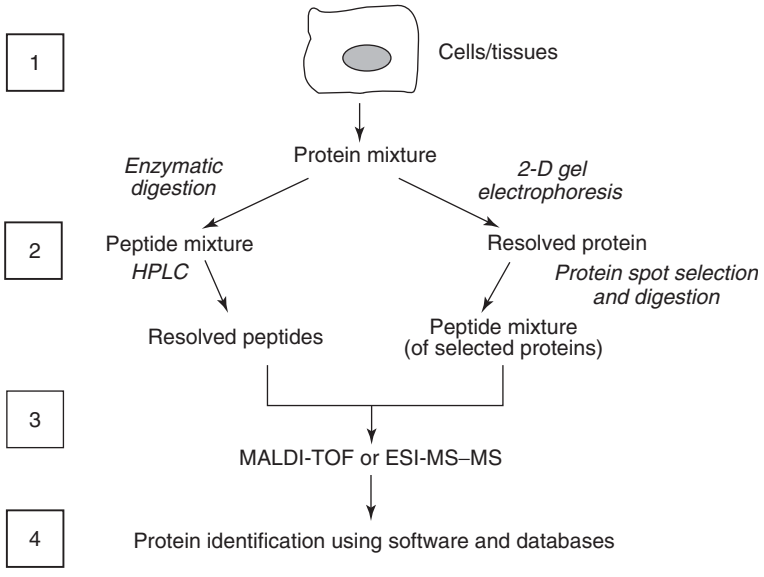

Figure 1 Schematic representation of the general analytical approach in a typical proteomic study.

one of these methods, are subjected to MS analysis in the final step of proteomic analysis.

Alternatively, one can separate the proteins before breaking them into peptides and then subject them to protein breakdown to obtain the peptide. This approach involves mainly the use of gel-based procedures such as one-dimensional or two-dimensional electrophoresis (2-DE) to resolve protein mixture into protein spots based on molecular weight and charge (isoelectric point or p*I*). Following separation, protein spots are cut out from the gel; protein is harvested from gel matrix, digested by peptides, and subjected for further mass spectrometric analysis.

Since both these approaches have their own advantages and disadvantages, the choice of method used is generally based on the type of analysis (expression analysis vs. adduct identification). Most of the proteomic studies use one of the two approaches that have evolved in recent years:

1. Proteins are resolved on 2-DE followed by protein spot excision from the gel and peptide digestion.
2. Enzymatic protein digestion to obtain peptide mixture, which is separated using HPLC.

The product of this peptide resolution is the subjected to matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF) in the case of 2-DE or electrospray ionization-mass spectrometry (ESI-MS) analysis in the case of HPLC separation.

Mass Spectrometric Analysis

MS is the heart of proteomic analysis and the success of proteomic experiments depends largely on the sensitivity and accuracy of MS equipment used to identify peptide sequences. MS machines have three main components (**Figure 2**): a source, which generates peptide ions, a mass analyzer, which separates peptide ions based on mass to charge ratio (*m/z*), and a detector that detects the ion resolved by the mass analyzer. All the modern MS machines are computer controlled and assisted by highly intelligent software.

There are two main types of MS equipment used in proteomic analysis, which function differently and provide different types of results. These are MALDI-TOF and ESI-MS–MS.

MALDI-TOF It stands for matrix-assisted laser desorption ionization-time-of-flight mass spectrometer. It is a very popular type of MS instrument used in proteomic analysis mainly because it is easy to use, robust, and has very high sensitivity. MALDI-TOF is generally used in combination with 2-DE based protein separation. Proteins are separated by 2-D gel and digested out of the gel to obtain a peptide mixture. This peptide mixture is then mixed with a chemical matrix (e.g., α-cyano-4-hydroxycinnamic acid), spotted on a chip, and allowed to evaporate in the air. This results in the formation of a crystal lattice containing the peptide mixture. This crystal lattice containing the sample peptides is then placed in the MALDI-TOF and excited using a laser. The chemical matrix is excited by absorbing photons from the laser; the extra energy is transmitted to the peptides converting the peptides into peptide ions. These peptide ions are then ejected into the gas phase from the crystal lattice. The matrix material aids in the formation of a crystal lattice and in turn in generation of peptide ions.

The peptide ions formed in the matrix-assisted source now enter the mass analyzer. The mass analyzer in MALDI-TOF is of time-of-flight (TOF) type. The peptide ions pass down the mass analyzer towards a detector, which is placed at the other end of the mass analyzer. The time of flight of each ion depends upon the *m/z* ratio of that particular ion. The general rule is that greater the *m/z* ratio, faster the movement of the ion. One of the problems with this process is that peptide ions with the same *m/z* value are poorly resolved. To overcome this problem, a device called as reflectron is used. The reflectron is placed at the end of the mass analyzer and focuses on ions with the same *m/z* ratio together and sends them to the detector. The addition of a reflectron has greatly increased the accuracy and sensitivity of the MALDI-TOF analysis.

ESI-MS–MS The electrospray ionization tandem MS (ESI-MS–MS) is generally used in combination with HPLC as method of separating digested peptide

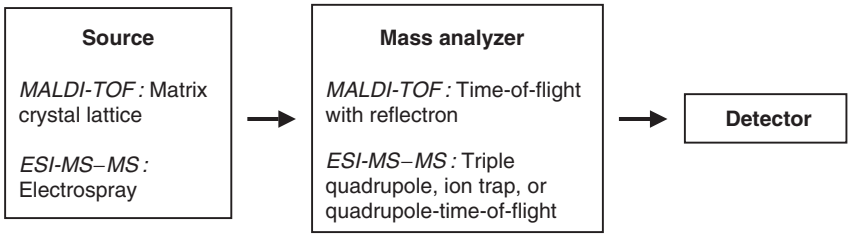

Figure 2 Schematic diagram depicting the main components of a mass spectrometry instrument.

mixtures rather than 2-DE. A major difference between MALDI-TOF-MS and ESI-MS–MS is that the peptide samples are in solution (liquid phase) in ESI-MS–MS as opposed to solid matrix in the case of MALDI-TOF. Proteins obtained from cells or tissues are digested using proteases and the resulting peptide mixture is resolved using tandem HPLC methods. Thus, separated peptides are in a solution of solvents (mostly the mobile phase chemicals such as acetonitrile) and water. The ESI source is a fine pointed needle of stainless steel maintained at very high voltage. The peptides separated from HPLC are directly introduced into the ESI source, which are sprayed inside the MS machine under extreme pressure and high voltage resulting in ionization of peptides. In the next step, called as desolvation, the HPLC solvents that enter MS instruments along with the peptides are removed and the remaining peptide ions are taken to a series of mass analyzers.

There are three main types of mass analyzers in ESI-MS–MS instruments: triple quadrupole, ion traps, and quadrupole-time-of-flight (Q-TOF). There are several differences between the mass analyzers in MALDI-TOF and in ESI-MS–MS. Unlike in MALDI-TOF-MS, in ESI-MS–MS two mass analyzers are used in tandem to increase the sensitivity of the technique. The peptide ions produced by the ESI sources are carried to the first mass analyzer and only peptides of a set *m/z* ratio are selected. The selected ions are then carried to a collision cell where they are subjected to additional fragmentation to produce smaller amino acid ions using a process called as collision induced dissociation (CID). The CID process employs inert gases such as argon for the dissociation of peptides. These smaller amino acid ions are then resolved in the second mass analyzer before sending to the detector. This process essentially enables highly sensitive detection of actual amino acid sequence of the peptides based on the *m/z* ratios of individual amino acids.

Protein Identification from MS Data

The final step in any proteomic study is the identification of proteins using the MS data. This is highly dependent on large databases of protein sequences and computer algorithms that can compare and interpret the MS spectra using these databases. Since the output of MALDI-TOF and ESI-MS–MS analyses are essentially different, they employ different types of computer software for the interpretation of MS spectra.

The databases used to analyze the MALDI-TOF MS data contain sequence information about thousands of proteins from a number of species. These sequences can be subjected to 'virtual digestion' using the same proteases used in the actual study (e.g., trypsin) to generate virtual peptide sequences. The algorithms can generate information about actual *m/z* values of these peptides. Then the peptide masses calculated from the MS spectra are compared with the virtual peptide masses to find accurate 'hits'. As one can imagine, the more the number of hits in such as query, higher the accuracy of the peptide identified. This process of identification of peptides based on masses is called as 'peptide mass fingerprinting' (PMF). The most popular database used for PMF is 'SWISS-PROT'. The software programs used to compare the MS data with the databases include PepSea, PeptIdent, MOWSE, ProFound, and Mascot.

In the case of the ESI-MS–MS data, actual amino acid sequence can be deduced. This is possible due to the CID processes, which breaks the peptides further into amino acid ions. Each amino acid ion has a specific mass and by calculating masses of specific amino acid from the MS spectra, the exact sequence of the peptide and in turn the protein can be deduced. The workhorse of such analysis is a program called 'Sequest'. Since the ESI-MS–MS analysis provides information about the actual amino acid sequence, it is also useful to obtain information about protein modifications (such as phosphorylation) and toxicant-induced protein adducts. This has become even easier with the advent of new software tools and highly intelligent algorithms such as 'SALSA'.

Advantages and Disadvantages of Proteomic Technologies

Extraordinary technical advancement in mass spectrometry in last 10 years has made proteomic analysis a routine technique used in toxicology in academic, government, and industry settings. The major advantage of proteomics is that it generates information about proteins in the cell, which are the functional entities in biology, as opposed to RNA expression, which has relatively poor correlation with physiological endpoints. Proteomics has also enabled identification of protein adducts induced by toxicant exposure in easily collectable human tissues such as serum. This will potentially introduce novel biomarkers of exposure and revolutionize the field of risk assessment. Since there are diverse proteomic approaches available, the choice of the method used depends upon the end result desired. The 2-DE combined with MALDI-TOF can be used for efficient, high throughput analysis of changes in protein expression between control and treatment groups, while the ESI-MS–MS analysis works better for identification of protein modifications and chemical nature of toxicant-induced protein adducts.

The highly technical nature of proteomic analysis is its major disadvantage. The success of a proteomic study is highly dependent upon various factors: the quality of protein samples, protein separation, enzymatic digestion to obtain peptide mixtures, and mainly the sensitivity and accuracy of MS instruments. Thus, proteomics demands implementation by highly skilled technicians and expensive instrumentation for generation of meaningful information. Secondly, protein expression in the cell is extremely dynamic and changes with physiological condition, nutritional status, and exposure to toxicants or drugs can change this dynamic process. Therefore, a proteomic study is generally a snapshot of protein expression in the cell at a given time under the given experimental conditions. Nevertheless, proteomic technologies have and are constantly maturing and have become an integral part of toxicological analysis.

See also: Microarray Analysis.

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Relevant Website

http://dir.niehs.nih.gov/proteomics – Proteomics Group, National Center for Toxicogenomics (of the National Institute of Environmental Health Sciences).

Prunus Species

Christopher P Holstege

- Chemical Name: *Prunus* species
- Synonyms: *Prunus armeniaca* (Apricot); *P. avium* (sweet cherry); *P. caroliniana* (cherry laurel); *P. cerasus* (sour cherry); *P. domestica* (common plum); *P. dulcis* (almond); *P. malus pumila* (common apple and crab apple); *P. persica* (peach); *P. serotina* (wild cherry); *P. virginiana* (chokecherry)
- Chemical/Pharmaceutical/Other Class: Cyanogenic glycosides

Background Information

The *Prunus* species includes a group of more than 400 trees and shrubs. They often find ornamental use because of their flowers, fruit, and nuts.

Exposure Routes and Pathways

Exposure is by ingestion of seeds, leaves, stems, roots, and fruit. Crushed seeds of some of the mentioned varieties are marketed as health foods. They are also marketed and sold surreptitiously as cancer remedies or vitamin supplements. Laetrile and Aprikern are some 'health' products consisting of crushed seeds from the *Prunus* species.

Toxicokinetics

Cyanogenic glycosides contain amygdalin. Amygdalin is erratically absorbed from most of the gastrointestinal tract but is effectively absorbed from the duodenum. Amygdalin is not toxic until it is metabolized by the enzyme emulsin to hydrocyanic acid. This metabolism may occur slowly and result in delayed clinical toxicity. Emulsin is found within the seeds of the *Prunus* species and in certain bacteria found within human intestinal flora. The presence of amygdalin in the seed kernels is not harmful unless the seed is crushed (masticated) and moistened, allowing release of emulsin. Amygdalin may result in cyanide toxicity in humans. Cyanide is converted to thiocyanate by an enzymatic reaction catalyzed by rhodanese. Thiocyanate is renally excreted.

Mechanism of Toxicity

Cyanide reversibly binds the ferric iron associated with the cytochrome oxidase system, thereby inhibiting the mitochondrial respiratory chain. This results in an inability to adequately utilize oxygen and causes 'internal asphyxia'. Cyanide combines with hemoglobin to form cyanhemoglobin, which does not transport oxygen. Cyanide also inhibits antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ingested *Prunus* plant material has not been associated with toxicity in mammals.

Human

Cyanide poisoning due to the accidental ingestion of these plants is rare. Acute symptoms are the same as in cyanide poisoning and include difficulty in breathing, dyspnea, muscular twitching, headache, muscle spasms, ataxia, seizures, coma, and death. The onset of symptoms may be very rapid with few premonitory signs, or may be delayed.

Clinical Management

Supportive therapy should be provided for all patients with *Prunus* exposures. Activated charcoal may be effective if administered early. Oxygen (100%) should be administered in symptomatic patients. Administration of the cyanide antidote kit may be necessary in symptomatic patients with metabolic acidosis. The cyanide antidote kit contains amyl nitrite inhalant, sodium nitrite, and sodium thiosulfate. Hydroxycobalamin has also been utilized as an effective antidote for cyanide toxicity. Diazepam may be used to control seizures. Acidosis should be treated with sodium bicarbonate.

See also: Amyl Nitrite; Charcoal; Cyanide; Diazepam.

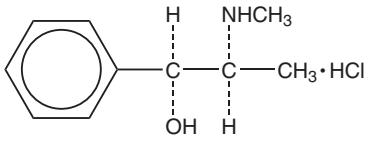
Further Reading

Hall AH and Rumack BH (1986) Clinical toxicology of cyanide. *Annals of Emergency Medicine* 15: 1067–1074.

Pseudoephedrine

Brenda Swanson-Biearman

- Chemical Abstracts Service Registry Number: CAS 670-40-6
- Synonyms: Pseudoephedrine hydrochloride; Pseudoephedrine sulfate; D-Isoephedrine; Isoephedrine; Sudafed®
- Chemical/Pharmaceutical/Other Class: Pseudoephedrine is a stereoisomer of ephedrine, in the drug class of sympathomimetics. It occurs naturally in plants of the genus *Ephedra*.
- Chemical Structure:



Uses

Pseudoephedrine is an orally active sympathomimetic amine exerting its decongestant action by acting directly on α-adrenergic receptors in the respiratory tract mucosa producing vasoconstriction resulting in shrinkage of swollen nasal mucous membranes, reduction of tissue hyperemia, edema, and nasal congestion, and an increase in nasal airway patency. Drainage of sinus secretions is increased and obstructed eustachian ostia may be opened. Relaxation of bronchial smooth muscle by stimulation of β-adrenergic receptors may also occur.

Exposure Routes and Pathways

Accidental and intentional exposures to pseudoephedrine occur most often by the oral route and involve either the pure form or multisymptom cold preparations containing pseudoephedrine in combination with antihistamines, analgesics, and antitussive agents.

Toxicokinetics

Pseudoephedrine is well absorbed from the gastrointestinal tract within 15–30 min, with peak effect between 30 and 60 min for prompt release dosage forms. Duration of action persists for 4–6 h for noncontrolled release formulations while extended release capsules may increase the duration of action to 12 h. Pseudoephedrine has been shown to have a mean elimination half-life of 4–6 h, which is dependent on urine pH. The elimination half-life is decreased at urine pH <6 and may be increased at urine pH >8, varying the half-life from 1.9 to 21 h. Approximately 55–75% of the parent drug is

excreted unchanged in the urine; the remainder is hepatically metabolized by *N*-demethylation to an inactive metabolite. A small amount is excreted as the active metabolite, norpseudoephedrine. Pseudoephedrine is 20% protein bound with a volume of distribution of 2.1–3.3 l kg^{-1}.

Mechanism of Toxicity

Pseudoephedrine is a weak base (p*K*, 9.4) that stimulates both α- and β-adrenergic receptors, as well as the release of neuronal norepinephrine. β1 stimulation produces increased heart rate and blood pressure. The α-adrenergic effects are believed to result from the reduced production of cyclic adenosine-3′,5′-monophosphate (cyclic 3′,5′-AMP) by inhibition of the enzyme adenyl cyclase, whereas β-adrenergic effects appear to be caused by the stimulation of adenyl cyclase activity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Following the ingestion of a large dose of pseudoephedrine, dogs and cats may exhibit hyperactivity, mydriasis, depression, vomiting, hyperthermia, disorientation, bradycardia, and tachycardia. Therapy is directed at prevention of absorption and control of tachyarrhythmias with lidocaine (dogs only) or procainamide (dogs only). Diazepam may be used for symptoms associated with central nervous system (CNS) stimulation.

Human

Hypertension and tachycardia are the primary toxic manifestations of pseudoephedrine overdose. An amount of more than three or four times the maximum daily dosage for adults or children may produce symptoms of β-adrenergic stimulation. In severe poisonings, cardiac dysrhythmias and cerebral hemorrhage due to hypertensive crisis may occur. Anxiety, muscle tremor, and seizures may result from CNS stimulation. Hallucinations, drowsiness, and/or irritability are more common symptoms exhibited by children. Hypokalemia and hyperglycemia may be noted. Acute renal failure and rhabdomyolysis have occurred in rare instances with large overdoses.

Chronic Toxicity (or Exposure)

Animal

Pseudoephedrine has been used in a dog model of allergic nasal congestion.

Human

Pseudoephedrine is generally well tolerated in therapeutic doses. Common adverse effects include CNS stimulant effects (e.g., tremor, restlessness, nervousness, irritability) and gastrointestinal effects (e.g., nausea, vomiting, dysgeusia).

Clinical Management

Basic and advanced life-support measures should be instituted as necessary. Activated charcoal may be considered for substantial recent ingestions. The cardiac and hemodynamic status should be carefully monitored. Prolonged observation (at least 24 h) may be necessary in patients ingesting sustained-release formulations. β-Adrenergic blocking agents and antiarrhythmic agents may be necessary to treat cardiac complications. Hypertension is generally transient, requiring only observation. Antihypertensive agents may be necessary in rare instances. Administration of benzodiazepines may result in decreased blood pressure in hypertensive pseudoephedrine overdose. Symptoms of CNS stimulation usually respond to a calm environment and supportive measures. Benzodiazepines can be administered for seizures. Treatment of exposure to products in which pseudoephedrine is combined with antihistamines, antitussives, analgesics, and/or alcohol must include toxicologic management of the concurrent drugs involved. In symptomatic patients, laboratory evaluation should include electrolytes and blood glucose, and creatine phosphokinase in more severe overdoses.

See also: Benzodiazepines; Charcoal; Diazepam; Ephedra; Hypoglycemics, Oral.

Further Reading

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Psilocybin *See* Mushrooms, psilocybin.

Psoralen (P) and Long-Wave Ultraviolet Radiation (UVA) *See* PUVA.

Psychological Indices of Toxicity

Bernard Weiss

The Emergence of Psychological Measures

Psychology is the science that strives to understand, measure, and modify our behavior: what we do, what we say, what we think, and what we feel. At its most basic level, behavior describes how we manipulate and respond to our environment. It is the ultimate output of the nervous system. Its domain ranges across the entire universe of human activities from simple reflexes to the creation of cosmological theories. Toxicology, however, at least in the formal sense, recognized the crucial role of behavioral neuroscience only recently. Perhaps behavior seemed somewhat exotic compared to the study and traditional endpoints of death and tissue damage. However, step back from the brink that these endpoints represent and toxicology swiftly becomes a more complex and subtle enterprise.

Behavior began to insinuate itself into toxicology in the late 1960s and early 1970s. It was not a total novice, though. It came with an impressive technology molded by the discipline of behavioral pharmacology, which had begun to emerge in the 1950s with the discovery of the tranquilizing drugs. These drugs, offering the prospect of chemotherapy for psychological disorders, needed a scientific support structure. Behavioral pharmacology provided the consummate scientific basis for appraising and discovering drugs designed to alter behavior. Neurochemistry blossomed at the same time, but only the patient's behavior, measured either in a clinical or in a laboratory setting, could be the arbiter of a successful search. The same technology transferred effortlessly to the study and measurement of adverse behavioral effects, the theme of the discipline of behavioral toxicology.

Acceptance of the notion that behavioral measures could yield evidence of toxicity also benefited from the insistence of Soviet scientists that central nervous system (CNS) function and behavior offered more sensitive and appropriate measures of toxicity than the criteria prevailing in the West. Because of its own scientific history, especially the influence of Pavlov, and its political doctrines, Soviet toxicology elevated the CNS to a dominant role. Soviet scientists maintained that their exposure standards, generally much lower than those prevailing in the West, derived from their reliance on indices of CNS function rather than detectable tissue damage. Although some of these claims proved scientifically equivocal, perhaps because they needed to comply with political doctrines, they aroused the interest and attention of Western scientists who then began to apply and develop a more sound behavioral technology.

Behavioral criteria had also been adopted by industrial hygienists to set exposure standards for inhaled materials. The short-term exposure limit prescribed by American Conference of Governmental Industrial Hygienists (ACGIH) singled out performance criteria, such as reduced work efficiency and impairment of self-rescue, as indications of excessive exposure. The courts also played a role. They had begun to accept complaints of defective psychological functioning as legitimate grounds for suits alleging excessive workplace exposure. Finally, the environmentalist movement, changing its focus from tangible pollution, such as filthy waterways and mass kills of wildlife, began to recognize the possibility of subtle functional effects arising from prolonged low-level exposure to environmental chemicals. Reductions in IQ scores stemming from lead exposure prompted the elimination of lead from gasoline. Proposed links between environmental chemicals and neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease further aroused the public's interest.

Data from both the laboratory and the field began to converge once behavior became a source of questions about adverse effects. Both information conduits offered evidence of widespread behavioral consequences of chemical exposure generally measurable only with the appropriate application of psychological assessment methods. Workplace surveys, for example, showed many more complaints of symptoms such as sleep disturbances, excitability, depression, irritability, restlessness, apathy, and nervousness in workers exposed to neurotoxicants than in unexposed workers. Animal experiments revealed deficits in critical functions such as learning, even in superficially healthy subjects. As the literature grew, the rationale for psychological testing became more

solidly entrenched. No other approach seemed comparable in unmasking changes that typically would go undetected, nor did other indicators seem as responsive to early health effects.

Occupational Sources of Adverse Psychological Responses

Much of what we have learned about the detrimental psychological effects of exposure to chemicals has come from the workplace. One reason is that greater hazards were tolerated in the workplace than in the communal environment. Another is that research protocols could be more specific about the chemicals because they could be identified with designated industrial processes.

Metals

Heavy metals are acknowledged inducers of behavioral toxicity. Lead, mercury, and manganese, especially, are associated with unique syndromes. Other metals, such as aluminum, selenium, thallium, and tin, have also been implicated in adverse behavioral effects. **Table 1** lists some of the symptoms ascribed to metal toxicity. They range in severity from subjective complaints, such as fatigue and depression, to clear neurological deficits such as tremor. Manganese is especially intriguing. Most identified victims of manganese poisoning have been miners exposed by breathing dust containing the ore. The earliest indications of toxicity typically consist of psychological signs such as extreme emotional lability marked by abnormal laughter and crying. In the South American mining communities where manganese intoxication is endemic, the syndrome is known as 'locura manganica', or manganese madness. Later, more direct neurological signs begin to loom. Some of these, such as abnormal gait and slowness of movement, are reminiscent of Parkinson's disease.

Other sources of manganese exposure, such as ferromanganese processing and ore-crushing plants, expose workers to much lower levels of inhaled manganese. Even in these workers, who give no indication of clinical deficits when examined by neurologists, psychological tests reveal an elevated incidence of fatigue, tinnitus, finger tremor, and increased irritability. Behavioral testing of animal subjects also unmasks subtle deficits at exposure levels too low to induce overt neurological signs. In monkeys trained to pull against a weight with a rowing motion, manganese treatment elicits long pauses between responses such as those that might be expected under conditions of fatigue. Such findings make many scientists wary of proposals to introduce manganese compounds as additives for gasoline. They fear that dispersal of manganese into the environment may create the same intractable health problems that followed the introduction of leaded gasoline.

Table 1 Symptoms ascribed to metal toxicity

Ansomia	Incoordination
Appetite loss	Irritability
Depression	Paresthesias
Disorientation	Polyneuritis
Dizziness	Somnolence
Fatigue	Tremor
Headache	Visual disturbances
Insomnia	Weakness

Metallic (elemental) mercury, the mercury found in thermometers, also generates a constellation of both behavioral and neurological signs. Mercury is extremely volatile so that it enters the body through inhalation. Mercury vapor readily passes from the lung into the blood and then penetrates into the brain, where, at high enough levels, it produces neurotoxicity. Such effects have been recognized for centuries. Bernardo Ramazzini, often called the father of occupational hygiene because of his celebrated work published in the eighteenth century, was keenly aware of mercury poisoning and its manifestations. His descriptions of the sufferings of Venetian mirror gilders and of workers in other occupations who came in contact with mercury are exquisitely detailed and vivid. High exposures to mercury were also experienced by mercury miners; even today, in the famed mercury mines of Almaden in Spain, miners work only a few hours each week to preclude mercury poisoning.

The cardinal neurological marker of mercury vapor intoxication is tremor. Workers in the hat industry frequently suffered from mercury poisoning. Mercury compounds helped convert the stiff, straight animal fur into a limp, flexible mat that could be shaped into a hat. Vapor escaped during the process, and, inhaled day after day by the workers, sometimes evoked tremor so severe that some found it difficult even to walk without support. A survey of hat factories conducted by the US Public Health Service in 1940 found a clear relationship between workplace mercury levels and the severity and incidence of tremor. Eventually, the mercury compounds were replaced.

However, even in persons exposed to much lower ambient levels, abnormalities in the frequency components of the tremor can be detected with appropriate instrumentation and mathematical analysis. A technique introduced in 1973 involved having the worker insert a finger into a slot connected to a transducer that converted tremor into an electrical signal. The worker was instructed to maintain a

pressure within limits signaled by a pair of lights. The tremor signal, fed directly into a digital computer, was then broken down into its frequency components. Unlike normal tremor, the mercury-induced tremor showed two peaks, at different frequencies, rather than one. This study of women exposed to mercury vapor in the course of calibrating pipettes was able to trace their recovery, after removal from the factory, by recording and analyzing the amplitude and frequency of the tremor. Another application of tremor measures, designed to guard against excessive exposure, was adopted in a study of chlor-alkali workers. Chlor-alkali processing plants convert brine into chlorine and caustic soda by electrolysis. Huge pools of elemental mercury serve as the electrodes in this process. Even with devices to restrict the emission of mercury vapor, enough may escape to induce incipient toxicity. In this application, the investigators monitored tremor by using these advanced signal processing techniques; one indication of excessive exposure was the appearance of two peaks in the frequency distribution of the tremor rather than a single peak, and this served as a criterion for transferring the worker to another part of the plant.

Tremor is often accompanied by a group of symptoms termed 'erethism', a term derived from the Greek root for irritation or redness. The symptoms include hyperirritability, labile temperament, timidity and shyness, blushing easily, depression, insomnia, and fatigue. One description of erethism, from a report on women in a factory in which mercury was released in the production of motor components, is virtually a classic example. The worker complained of dizzy spells, weakness, fatigue, forgetfulness, grouchiness, and 'a fluttery feeling like I was scared or floating in space'.

Because the Mad Hatter in *Alice in Wonderland* exhibited some of the symptoms of erethism, he is sometimes held to be a model for the afflictions suffered by workers in the hat industry; whether Lewis Carrol intended such a parallel is still disputed, but the resemblances are uncanny. Even in the absence of identifiable symptoms, psychological testing has revealed what could be called nascent erethism in workers exposed to mercury vapor but showing no overt signs of toxicity. Tests of coordination and reaction time reveal differences between exposed and unexposed workers. Performance on elements of adult intelligence tests, such as the ability to repeat strings of digits, also shows differences.

A source of mercury other than the workplace has now assumed ascendancy in driving public unease. Recent publicity has indicted mercury amalgam dental fillings as a source of adverse health effects. Although it is true that chewing can release mercury vapor from amalgam fillings, the quantities are typically too small to produce elevations in blood or urine mercury levels that are hazardous to adults. Risks posed to the fetus and children are of greater concern but are characterized by a paucity of data.

Perhaps no other metal has aroused as much public discussion and attention as lead. It was one of the earliest metals exploited for practical uses; the word plumbing comes from the Latin word for lead. The Romans constructed cisterns and cooking utensils from lead. Lead pigments are found in glazes, even those that are used to decorate pottery; acidic foods leach the lead from the glaze. Lead permeates our current environment because of its presence in paint and in industrial products such as storage batteries and because of the lead added to gasoline.

Ancient physicians, such as Galen, were aware of lead's toxicity. Alice Hamilton, who founded the specialty of occupational medicine in the United States, termed lead the oldest of the industrial poisons except for carbon monoxide. In England, regulations designed to protect workers against the consequences of clinical lead poisoning, such as convulsions and coma, were prescribed in the nineteenth century. However, incipient lead poisoning remained a more formidable problem because the classical signs are absent. Instead, the symptoms tend to be vague; the workers may act sluggish, achy, and fatigued and be prone to errors on the job. Recent assessments of lead workers, undertaken with psychological tests, show adverse effects even in the absence of clinically overt problems. These take the form of reduced scores on tests of memory, vigilance, spatial relations, and coordination. Standardized inventories of affect and personality also show disturbances in exposed workers. Lead can also impair hearing; rises in the concentration of lead in blood, a marker of recent lead exposure, elevate hearing thresholds. The most destructive of lead's neurotoxic effects, however, is interference with brain development. This aspect will be addressed later.

Pesticides

Pesticides are another class of chemicals capable of damaging the nervous system and, even at low levels, produce deficits detectable by psychological testing. The organophosphorus insecticides, which are chemical relatives of the most potent nerve gases, are notorious poisons and, carelessly handled, as often happens in underdeveloped countries, can prove lethal. Parathion, diazinon, and malathion are representatives of this class and are widely used in the United States. Acute poisoning episodes produce signs such as eye irritation, headache, dizziness, nausea, and visual

disturbances. These gross effects fade with time, usually in days or weeks. However, when farmworkers who had undergone an episode of acute poisoning were evaluated with psychological tests 1 year later, they showed persisting sequelae. Compared to controls, who were matched on age and education, they displayed lower scores on a widely used adult intelligence scale and on a test of coordination and higher scores on a battery of psychological tests designed to measure incipient neurological impairment. These results are consistent with those from similar studies and with experiments in monkeys showing enduring effects on the electrical activity of the brain.

Other studies demonstrate that effects of even rather modest exposures can be detected with psychological tests. In one example, even though they gave no indication of overt poisoning and seemed superficially healthy, farmworkers who had been exposed to organophosphorus insecticides displayed evidence of psychological disturbances on a test constructed to measure anxiety. They selected items indicating that they experienced more tension, more restlessness, more emotional instability, more nervousness, and more fitful sleep than a sample of unexposed workers.

The organochlorine insecticides such as DDT are also potent nervous system poisons. Like the organophosphorus compounds, they interfere with the nervous systems of insects, so it is no surprise that they exert similar effects in humans. At high doses, they cause convulsions. An epidemic of convulsions in an English town, in fact, was traced to flour inadvertently contaminated with an organochlorine insecticide. At lower doses, the effects are more subtle. One organochlorine insecticide, chlordecone, was responsible for an outbreak of poisoning in a Virginia factory. After workers began to complain of health problems to local physicians, public health officials began an investigation and confirmed that poor hygiene in the plant had exposed the employees to excessive amounts of the chemical. The most intriguing facet of this episode is that the earliest index of toxicity turned out to be complaints of excessive nervousness. Would even an alert plant physician be likely to consider chemical exposure as the source of such complaints? Would not a more likely diagnosis be personal problems either at home or at the place of employment? Episodes such as these have led some observers to recommend that workers in comparable environments undergo periodic psychological assessments to detect adverse effects.

Solvents

Among the chemicals evoking the most attention from psychologists are the volatile organic solvents. Carbon disulfide, toluene, xylene, styrene, trichloroethylene, and methylene chloride are representative members of this class. They may have evoked such attention because they are demonstrably neurotoxic. Due to their volatility, they are inhaled. ACGIH exposure standards for solvents are based on this property. Because they are soluble in fatty tissues, they easily reach the brain. At high ambient levels, they produce narcosis; in fact, some have been used as surgical anesthetics. The question posed to investigators is whether low concentrations, even those meeting current workplace exposure standards, produce adverse effects.

Scandinavian investigators pioneered studies of chronically exposed workers. On the basis of their research, they posited what has been called the organic solvent syndrome, toxic encephalopathy, or painter's syndrome (because painters often work in an environment suffused with solvents). They asserted that chronic exposure in the workplace to volatile organic solvents produced permanent deficits reflected by diminished performance on psychological tests. They pointed to lower scores on tests of intelligence, memory, learning, and other cognitive functions and to elevated reaction times and personality changes. These claims were vigorously debated, with critics arguing that necessary controls, such as matching exposed and unexposed workers for education and drinking habits, were lacking. They also argued that the generous worker compensation regulations of some Scandinavian countries encouraged claims of solvent-induced impairment. Later investigations, with more rigorous controls, and in other countries, supported the original claims and have even expanded them to include an impaired sense of smell and deficits in color vision.

Solvents are ubiquitous in the workplace and are produced in the millions of kilograms annually. They also appear in many household products such as cleaners, glues, and paint thinners. Because so many workers are exposed to solvents, and because their use is so common in other settings, the US Environmental Protection Agency (EPA) proposed that solvent manufacturers undertake a comprehensive evaluation of ten solvents with high production volumes. They specified four components in the evaluation: functional observation battery, motor activity, neuropathology, and schedule-controlled operant behavior.

In the functional observation battery, rats or mice are exposed acutely and subchronically to a solvent. Technicians then make a number of systematic observations such as the response to prodding, orienting to a click, resistance to pulling, and other simple responses. A numerical score, based on the individual components, is then calculated.

In evaluating motor activity, rats or mice are exposed acutely or subchronically and tested in a device that measures amount of movement. For example, the rat may be placed in a figure-eight maze equipped with photocells at the intersections. Motor activity is scored by the number of photobeams interrupted. Although motor activity can be influenced by many factors, it is especially responsive to chemicals acting on the CNS.

Neuropathology involves sacrificing rats or mice after subchronic exposure and inspecting for lesions in the brain.

Schedule-controlled operant behavior has come to play a prominent role in behavioral toxicology because it provides a supple, flexible scheme for assessing the capacity for complex behavior. The US EPA explained its choice of schedule-controlled operant behavior by focusing on its versatility.

Solvents may have neurotoxic effects on memory, learning, and performance which can be permanent. These effects are less well understood…. The schedule-controlled operant behavior test has typically been required as a second tier test…it is proposed as a first-tier test…because of EPA's desire to obtain data on the effects of solvents on learning, memory, and performance.

The origins of this proposal from the US EPA lie in the demonstrated efficacy of performance tests as measures of psychophysiological function and in their sensitivity to the effects of chemical exposures in the workplace. These tests come from two sources. One originated in the need to provide diagnostic guidance for psychologists evaluating clients or for personnel selection. Test design and construction comprise one of psychology's major specialties; its methods have evolved over at least eight decades. Psychometric techniques provide the basis for selection tools such as the Scholastic Achievement Test. The second was the experimental psychology laboratory, the site of fundamental research on all aspects of human performance including sensory function, motor function, and cognitive functions such as memory. Contributions from these two sources always overlapped and influenced one another, but they converged especially effectively to meet the growing interest in the measurement of performance stirred by evidence that such measures could uncover toxic effects that otherwise would remain concealed.

The demonstrated sensitivity of psychological test methods to solvent exposure led the World Health Organization (WHO) to call upon experts for the design of a test battery that could be applied even in underdeveloped countries lacking such a tradition. The basic WHO battery is shown in **Table 2**. More comprehensive batteries tend to be used in the advanced industrial countries, where psychologists have tried to exploit the potential of digital computers for test design, presentation, and analysis.

Table 2 WHO neurobehavioral core test battery

Functional domain	*Core test*
Motor speed	Aiming; dot placing
Attention	Simple reaction time
Perceptual-motor	WAIS digit-symbol
Manual dexterity	Santa Ana test
Visual memory	Benton test
Auditory memory	WAIS digit span

Psychological Measures of Impaired Development

Hardly any facet of psychology claims as much attention in toxicology as brain development. Teratology describes the discipline whose dominion is the study of congenital deformities, or birth defects. By analogy, some investigators appropriated the term to label a new area they called behavioral teratology. The label became accepted practice because it graphically described what these scientists viewed as a vital but previously neglected aspect of toxicology: the functional consequences, later in life, of exposure to neurotoxic agents during gestation or infancy. Although such functional consequences might include defects as severe as mental deficiency, most of the research in this area has taken the form of questions about less blatant outcomes. Learning disabilities, conduct disorders, slower than normal language acquisition, delayed motor development, and downward shifts in the distribution of IQ scores are among the outcomes reported in the scientific literature.

In response to overwhelming public anxieties, regulatory agencies in Japan and the United Kingdom began to insist on behavioral teratology information for new drugs. The US EPA has also been active in setting guidelines for developmental toxicity that also embrace potential behavioral effects. These guidelines prescribe a range of behavioral testing protocols ranging from simple locomotor activity to tests designed to measure learning and memory. The impetus for such protocols comes from the recognized vulnerability of the developing brain to neurotoxic chemicals. The fetal alcohol syndrome is one striking example. Three agents in particular have aroused the interest of toxicologists: lead, methylmercury, and the polychlorinated biphenyls.

Lead

Severe lead poisoning in children is now a much more infrequent event in the United States than even

in the recent past. The current focus of attention is the impact of much lower exposure levels on how well children function. Twenty-five years ago, a blood lead concentration below 40 $\mu g\,dl^{-1}$ was considered acceptable. By 1991, the Centers for Disease Control (CDC), weighing all the accumulated evidence, had concluded that levels exceeding 10 $\mu g\,dl^{-1}$ gave cause for concern. The primary motive for this change stemmed from depressed scores on intelligence tests.

Attempts to construct a metric of intelligence have occupied the energies of many psychologists from the middle of the nineteenth century to the present. Definitions of intelligence continue to elicit intense debate. Intelligence testing of children, however, beyond doctrinal disagreements, has come to rest on a forthright principle: measure the child against his or her peers. Intelligence tests vary widely in the items they choose for such comparisons but typically include an assessment of vocabulary, the ability to count and calculate, the ability to discern relationships among objects, and other markers of how well the child has mastered his or her environment. The components of a leading test, the Wechsler Intelligence Scale for Children-Revised, are listed in **Table 3**. As in all psychological tests, items basically represent stimuli for the elicitation of behavior samples. They are not absolute measures of some fundamental property.

From items such as those contained in the component subtests listed in **Table 3**, a test score, equivalent to a test age, is derived. The IQ is computed as the quotient of the test age, based on the performance of a standardized population of children, divided by the child's chronological age. A child who is average for his or her age will yield an IQ of 100. An above-average child will obtain an IQ above 100. There is some dispute about the interpretation of an IQ based on a standardized population significantly different in ethnic background and socioeconomic status from the child being tested; so that exposure conditions and the child's other environmental circumstances should not be confounded.

Table 3 Components of the Wechsler intelligence scale for children (revised)

Verbal IQ	*Performance IQ*
Information	Picture completion
Vocabulary	Picture arrangement
Digit span	Block design
Arithmetic	Object assembly
Comprehension	Coding
Similarities	Mazes

IQ scores began their ascendancy in assessing the risks of childhood lead exposure as long ago as the 1950s, but poorly focused investigations, inadequate measures of exposure, and the then unrecognized scope of lead toxicity yielded little more than a stream of ambiguous studies. A pioneering report in 1979 by Herbert Needleman and colleagues marked the first of many well-designed studies showing significant IQ reductions in young children ascribable to quite modest increments of lead exposure. It adopted the then novel strategy of estimating cumulative exposure to lead by relying on baby teeth, which, like bone, store lead. The findings were so compelling that they stimulated additional investigations in many parts of the world that built further support for the lead and IQ relationship.

Subsequent investigations adopted an even more forceful strategy; they undertook prospective studies in which children with documented prenatal lead exposures were followed from birth. These studies demonstrated that even lead levels so low that they would have been considered insignificant just a few years earlier could reduce scores on IQ and analogous developmental tests.

Some critics charge that such findings possess little practical significance. They argue that a difference of a few IQ points exercises negligible influence on how well a child functions. However, such an argument neglects the implications for the population as a whole. Because of the way in which IQ scores are distributed, in a population of 100 million in which the mean IQ is 100, 2.3 million individuals will score above 130, the superior range. If the mean is shifted downward by five IQ points (5%), which the critics deem insignificant, the mean IQ becomes 95 and only 990 000 individuals will score above 130. Most observers would contend that such an impact on a society cannot be considered negligible. This perspective, gained from the results of psychological tests, made a key contribution to the CDC decision to designate a lead level of 10 $\mu g\,dl^{-1}$ in blood as a level of concern.

Methylmercury

About 26 states now disseminate fish advisories for lakes and rivers based on methylmercury contamination. Methylmercury is an organic form of mercury and a potent nervous system poison. It is especially destructive to the developing brain. Although recognized as a poison for over 100 years, its impact on the fetal brain came to attention only in the 1950s, when the population of a small Japanese fishing village, Minamata, experienced widespread methylmercury poisoning. Fish and shellfish from

Minamata bay had been contaminated by effluent from a factory that used mercury as a catalyst in the production of acetaldehyde. Many inhabitants died. Even more suffered permanent neurological damage. In addition, a much higher incidence of retarded brain development was observed in Minamata than elsewhere in Japan but the population was too small to yield a cogent answer.

The final evidence came in the form of an outbreak of methylmercury poisoning in Iraq. Because grain crops had been decimated by a severe drought in 1971, the Iraqi government ordered over 80 000 tons of seed grain from Mexico and the United States. The order specified that the grain be treated with a methylmercury fungicide, which ordinarily would dissipate into the soil after planting. Despite warnings, many farming communities in the Iraqi countryside, facing food shortages, baked the treated grain into bread. The result was a mass poisoning episode, in the winter of 1971–72, that killed as many as 5000 people. It was the largest mass chemical disaster in history.

University of Rochester investigators, led by Dr. Thomas W. Clarkson, were called upon for assistance because of their research experience with mercury and with antidotes. They established a laboratory in Bagdhad and began a project to survey the countryside. One phenomenon struck them with singular force. Offspring of mothers who had consumed large amounts of the tainted bread displayed evidence of brain damage. Some seemed afflicted with cerebral palsy. Some were prone to seizures. Others were late in speech and motor development. Because Clarkson and colleagues had discovered that growing hair took up methylmercury from the blood, hair became the ultimate measure of exposure. Because scalp hair grows ~ 1 cm (~ 0.5 in.) per month, a 12 cm length of hair had engraved on it a year's history of methylmercury blood levels, which closely reflect consumption.

With this tool, the investigators were able to establish a relationship between maternal methylmercury exposure and indices of child development. Statistical analyses of the correlation between maternal hair levels and delayed walking, for example, suggested that even slightly elevated methylmercury consumption by a pregnant woman might pose a risk for fetal brain development.

The primary repository of methylmercury in the diet is fish. Natural sources of inorganic mercury, such as volcanoes, and human contributions from fossil fuel and waste combustion contribute to a global mercury cycle that deposits the mercury in waterways. Microorganisms in the bottom sediment convert the inorganic form into methylmercury, which ascends the food chain and concentrates in the predators at the apex of the food chain. Swordfish, shark, pike, snapper, and tuna are among these predatory species.

In New Zealand, comparisons among children, whose mothers consumed different amount of fish during gestation, indicated that higher consumption levels tended to depress scores on IQ and other psychological tests. Because a single study could not be definitive, other studies have been undertaken. Their results have now begun to appear and show little evidence of adverse outcomes. Studies of this kind must occupy several years, however, because some consequences of developmental damage, such as performance on certain components of IQ tests, cannot be assayed until the child is advanced enough to be tested. In the meantime, regulatory authorities have adopted a position of caution and advised against the consumption of certain species of fish from particular sites by pregnant women, and young children.

The concerns aroused by methylmercury in fish, arising from the susceptibility of the developing brain to this neurotoxicant, led to the design and execution of two large prospective studies. One was located in the Seychelle islands, which lie in the Indian Ocean. The other was located in the Faroe Islands, which lie in the North Sea. Both communities consume large quantities of seafood. In the Seychelles, it is almost exclusively in the form of fish. In the Faroes, virtually all the methylmercury comes from the consumption of pilot whales, which are also contaminated with polychlorinated biphenyls (PCBs). Both studies assayed maternal exposures to methylmercury. In the Seychelles, maternal hair was used as the index; it reflects the history of blood levels. The Faroes study relied primarily on cord blood.

Neurobehavioral testing of the Seychelles cohort of ~ 800 children has been carried out periodically from early development to 9 years of age. The Faroes cohort was assessed at 7 years of age with a variety of neurobehavioral tests, and supplemented by electrophysiological measures at 14 years of age. The Seychelles data show little indication of adverse effects attributable to prenatal methylmercury exposure. In contrast, the Faroes data point to subtle adverse effects. Because of such effects, the EPA has concluded that limiting methylmercury intake to $0.1\ \mu g\ kg^{-1}$ daily is necessary to provide an adequate margin of safety.

Polychlorinated Biphenyls

The PCBs are as ubiquitous in the environment as lead. They also share many properties in common with other organic halogen compounds such as the

organochlorine insecticides and dioxins. Their health risks until recently have been dominated by potential carcinogenicity. Newer data sources now suggest that their most serious risks may stem from actions on the developing brain. Two poisoning episodes, one in Japan and one in Taiwan, yielded the first clues. In both instances, cooking oil had been contaminated by PCBs, which enjoyed wide use as insulating material for transformers. They are dissolved in an oil base, so contamination cannot easily be detected. Children born to mothers who had consumed the contaminated oil, besides showing skin darkening and other signs of PCB toxicity, also suffered from mental retardation. Suspicions that problems might lurk in lower levels of exposure stimulated studies equipped to measure more accurately the correlation between maternal PCB exposure and offspring development. The resulting data indicate to many scientists that current levels of tissue PCBs are disturbingly close to levels that represent a hazard to optimal brain development.

One origin of this altered point of view is a series of studies based on correlations between maternal intake of PCB-contaminated fish during pregnancy and the performance of the offspring on psychological tests. The higher the maternal PCB level (measured in blood samples or fat biopsies), the lower the IQ score. IQ scores of children whose mothers consumed Lake Michigan fish suffered a 6% (6 point) decline at 11 years of age. Additional psychological tests confirmed this relationship. Normal infants shown two pictures, one of which is familiar and one of which is novel, will tend to spend more time gazing at the novel picture. The degree of bias in the direction of novelty apparently correlates to a surprisingly degree with later IQ scores. Children later shown to be at risk for developmental retardation show little novelty bias. Maternal PCB levels are significant predictors of novelty bias; the higher the PCB level, the lower the degree of bias. These two psychological indices – IQ scores and visual recognition memory – established the PCBs, even at levels that produce no obvious indications of toxicity, as hazards to brain and behavioral development. The implications of these findings are disturbing because so many women maintain body burdens of PCBs uncomfortably close to the levels associated with lowered scores on psychological tests of developmental outcome. They are also disturbing because PCBs represent a class of chemicals, including the dioxins and DDT, that have been labeled endocrine disruptors and that have the potential to interfere with sexual differentiation of the brain, with immune system function, and with thyroid development.

Food Additives

An instructive instance of the changed perspectives that psychological measures may impose on toxicity evaluation and risk assessment emerged from claims that some foods and food additives might elicit behavioral disturbances in children. The claims were formulated by Dr. Ben Feingold, a pioneering pediatric allergist in the Kaiser-Permanente system in California. Feingold asserted that some of the children labeled as hyperactive, or suffering from what is currently called attention deficit disorder, actually were exhibiting adverse responses to certain dietary constituents. Among the additives, he singled out synthetic colors and flavors for elimination from diets because, in addition to reports in the allergy literature linking them to adverse reactions, they lacked nutritional value in any case.

The US Food and Drug Administration (FDA) does not require testing of food additives for neurobehavioral toxicity and Feingold's claims were based on clinical experience rather than on controlled clinical trials. His claims, however, generated sharp public interest, particularly on the part of agonizing parents, and provoked a series of clinical trials designed to test his hypothesis. Although the investigators adopted a wide variety of approaches, and most focused on food dyes for experimental convenience, the total published literature converges to the conclusion that, in principle, Feingold's claims were valid. The major disagreements stem from the estimated proportion of children at risk, which range from $\sim$1% to 25%, and the scope of the dietary components evoking behavioral disturbances. Another source of disagreement arises from how risks are perceived. Some critics argue that a 1% prevalence of adverse responses to food dyes, for example, is not a reason to eliminate them from the food supply. At the same time, regulatory agencies such as the US EPA strive to establish exposure levels to ensure cancer risks below one per million persons. An incidence of 1% is hardly trivial.

One of the experiments indicating the potential of food dyes to induce adverse behavioral responses was conducted by the author and coinvestigators with a sample of young California children. These children had been designated by their parents as responders. That is, their behavior had been seen to improve on a diet that eliminated food additives and some other foods. The parents enrolled in a study in which they provided daily behavioral observations of their child's response to a soft drink containing either a blend of food dyes or innocuous colorings such as caramel. The two drinks were not distinguishable. During the 11 week experimental period, the daily

drink contained the blend of dyes on eight randomly assigned occasions.

Of the 22 enrolled children, two showed consistent responses to the blend of dyes. One of the children, a 34-month-old girl, gave highly elevated scores, after drinking the challenge drink, on the following items of a ten-item list: short attention span, acts as if driven by motor, runs away, throws and breaks things, and whines. She also showed elevated scores on a standardized rating scale of attention deficit disorder. **Table 4** shows the difference between the amount of US FDA-approved food dyes evoking behavioral disturbances in sensitive children and the acceptable daily intakes based on the standard 2 years feeding study in rodents (a study required by the US FDA). The differences between conventional assays, largely based on pathology, and those based on psychological measures are about 50–60-fold.

Psychological Measures of Enhanced Chemical Sensitivity

A new array of problems closely entwined with environmental toxicology is attached to labels such as sick building syndrome, multiple chemical sensitivity, chronic fatigue syndrome, and Gulf War syndrome. They have spawned a sizable literature and gripped public interest and anxieties. For all three labels, the primary clinical manifestations consist primarily of subjective complaints; conventional medical indices are lacking. Especially for multiple chemical sensitivity and sick building syndrome, the instigators are held to be toxic chemicals, but in most instances the offending agents lack clear identification.

Patients allegedly suffering from multiple chemical sensitivities complain of depression, excessive fatigue, sleep disorders, irritability, headaches, and symptoms, such as rhinitis, similar to those associated with allergies. Although immune system disorders are hypothesized as the most frequent underlying cause, compelling evidence in support of such a mechanism is lacking. Another puzzle is the emergence of such symptoms in response to chemical agents of widely divergent classes and, typically, at rather low concentrations. The vague, malleable contours of the syndrome and the absence of an identifiable etiology have engendered a countervailing skepticism about its validity on the part of many clinicians and scientists. The absence of sound investigational protocols and experiments, except for a sparse, scattered literature, has nourished such skepticism.

Table 4 Food dyes: doses (mg day^{-1}) eliciting behavioral responses vs. FDA acceptable daily intake (ADI)

Color	*Behavior*[a]	*ADI*
Yellow 5	9.07	300
Yellow 6	10.70	300
Red 40	13.80	420
Red 3	0.57	150
Blue 1	0.80	200
Blue 2	0.15	37
Green 3	0.11	150

[a] Modified from data in Weiss B, Williams JH, Margen S, *et al.* (1980) Behavioral response to artificial food colors. *Science* 207: 1487–1489.

The sick building syndrome is another victim of sparse empirical support. The contemporary emphasis on energy efficiency has produced buildings notable for poor air quality manifested as inadequate ventilation coupled with contaminating agents ranging from infectious microorganisms to common allergens to volatile organic chemicals. Outbreaks of illness, attributed to such environmental conditions, have made their way into the biomedical literature and the popular media.

Like multiple chemical sensitivity patients, those asserted to be afflicted with the sick building syndrome exhibit a collection of largely subjective complaints; headaches, fatigue, and lightheadedness are among symptoms. They are accompanied by complaints QP persistent cough, chest tightness, wheezing, and eye and throat irritation. Although better documented than the multiple chemical sensitivity syndromes because the complaints often can be traced to a specific site, it too has often aroused suspicions of its validity. Some critics contend that most reports of widespread illness in particular buildings are more likely instances of suggestibility than of authentic illness. Others, citing unsuccessful attempts to relate variations in air quality to the quantity of complaints, also tend to belittle the syndrome as a disease entity.

US EPA scientists are among the groups that have sought to view these two syndromes from an experimental perspective. The agency's unfortunate experience with its own building renovation program, which left a residue of sick building complaints, gave this effort a substantial impetus. They exposed healthy subjects to a mixture containing 22 volatile organic chemicals commonly detected in new or newly renovated buildings and asked the subjects to rate the intensity of various responses. During the 2.75 h exposure periods, perceived odor intensity diminished and air quality ratings improved. Ratings of eye and throat irritation, headache, and dizziness either increased or remained stable. Such results indicate that odors alone, as suggested by some observers, do not trigger the symptoms of multiple chemical sensitivity or sick building syndrome.

Concurrent assessments of neurobehavioral function found that subjects reported increased fatigue and confusion. At the same time, their performance on successive administrations of a battery of 13 psychological tests remained unimpaired, a finding the authors speculate may have been influenced by the tendency to improve with practice.

Chronic fatigue syndrome, although its reality is also debated, is more firmly established as a valid entity than the other two syndromes. The label is attached to patients who suffer prolonged feelings of fatigue, weakness, and even exhaustion. They report inability to concentrate, memory loss, depression, sleep disorders, and a variety of symptoms reminiscent of influenza. The prevailing view among those who accept the syndrome is that it reflects an immune system disorder, perhaps triggered by a viral infection, but its character and etiology remain equivocal.

When psychological testing of such patients has been undertaken, some are revealed to suffer reliable cognitive deficits. For example, they exhibit slowed reaction times, reduced accuracy in searching for target letters on a page of typed letters, impaired recall of a narrative, and lowered scores on various tests of memory. Such results are intriguing and perhaps significant. They are supported by data indicating that psychological test performance is impaired by experimentally induced viral or bacterial infections and that recovery of performance, despite recovery by clinical measures, may require a prolonged period. Another clue comes from animal experiments. In response to infections, the immune system releases substances called cytokines. Interleukins are members of this class. Interleukins also exert profound effects on behavior, and some scientists speculate that the nonspecific symptoms of infection, similar to the complaints vented by chronic fatigue syndrome patients, originate from the action of these and other cytokines.

Although these three syndromes are not intrinsically linked to the traditional domain of toxicology, they illustrate the role that psychological measures are increasingly assuming when adverse effects of environmental chemicals emerge as an issue. Clinical medicine prefers to deal with specific signs pointing to specific diseases. Environmental toxicants, however, far more often now than in the past, are being indicted as the sources responsible for diffuse aberrations of function such as conduct disorders, learning disabilities, memory and concentration difficulties, feelings of listlessness, fatigue, depression, and a galaxy of other disturbances beyond the catalog of accepted medical diagnoses. Psychological test methods, developed over a period of many decades, provide the tools for making the appropriate connections.

See also: Behavioral Toxicology; Food Additives; Lead; Mercury; Metals; Methylmercury; Pesticides; Polychlorinated Biphenyls (PCBs); Sick Building Syndrome.

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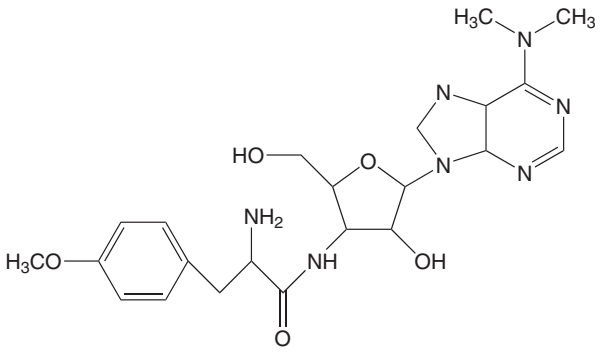
Puromycin

Midhun C Korrapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 53-79-2
- Synonyms: (*S*)-3′-((2-Amino-3-(4-methoxyphenyl)-1-oxopropyl)amino)-3′-deoxy-*N*,*N*-dimethyladenosine; Achromycin; Stillomycin
- Chemical/Pharmaceutical/Other Class: It is an antineoplastic and antiprotozoal (Trypanosoma) and used in research as a protein synthesis inhibitor

- Chemical Formula: $C_{22}H_{29}N_7O_5$
- Chemical Structure:



Uses

Puromycin has been widely used as a basic tool in research for studying protein synthesis. It is an antibiotic used by scientists in bioresearch to select cells modified by genetic engineering. It inhibits protein synthesis by binding to RNA. It is also an antineoplastic and antitrypanosomal agent.

Background Information

Puromycin is an aminonucleoside antibiotic produced by *Streptomyces alboniger*. It specifically inhibits peptidyl transfer on both prokaryotic and eukaryotic ribosomes. The antibiotic inhibits the growth of Gram positive bacteria and various animal and insect cells. Fungi and Gram negative bacteria are resistant due to the low permeability of the antibiotic. For more than 30 years, puromycin has been widely used as a basic tool for studying protein synthesis. Now, puromycin hydrochloride is particularly useful for the selection of cell types harboring plasmids carrying puromycin resistance genes.

Exposure Routes and Pathways

Most common exposure pathways to puromycin are via absorption through skin, inhalation, or by oral route when swallowed accidentally.

Toxicokinetics

Limited information indicates that puromycin is rapidly absorbed and distributed to the liver and is primarily excreted via the kidneys.

Mechanism of Toxicity

Puromycin is a specific metabolic inhibitor of protein synthesis and acts as an aminoacyl-tRNA analog and peptidyl acceptor. The latter causes premature chain termination of the protein and the release of nascent or growing polypeptide chains. In liver it has been shown to cause fat accumulation without causing death of the hepatocytes. Puromycin causes focal glomerular sclerosis, alters the morphology, localization of anionic sites, and metabolism of renal epithelial cells. This injury is attributable to the production of reactive oxygen species.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rabbit erythrocytes exposed to low concentrations of puromycin (7×10^{-4} mol l^{-1}) caused disruption of cell membrane indicating inhibition of protein synthesis by erythrocytes. Puromycin is shown to cause nephrosis in rats. It is a glomerular nephrotoxicant and is extensively used to study pathophysiology of glomerular nephritis and nephrotic syndrome.

Human

Puromycin may cause irritation and reddening of eyes. Prolonged or repeated exposure may cause cataract and severe, permanent damage to the eyes. Puromycin causes rash, blistering, and allergic reactions if contacted with skin, and may cause nasal, gastrointestinal, and lung irritation. Exposure of erythrocytes to puromycin led to cell membrane disruption. It is a possible mutagen in humans.

Chronic Toxicity (or Exposure)

It is a possible mutagen in humans.

Clinical Management

If inhaled, the patient should be move to fresh air. If not breathing, artificial respiration should be given. If breathing is difficult, oxygen should be given. Vomiting should not be induced unless directed to do so by medical personnel. Unconscious persons should not be given anything by mouth. If large quantities of this material are swallowed, a physician should be called immediately. Tight clothing such as a collar, tie, belt or waistband should be loosened. In case of dermal exposure, the skin should be flushed with plenty of water. Contaminated clothing and shoes should be removed and clothing removed before reuse. Shoes should be cleaned thoroughly before reuse. Contact lenses should be removed. In case of contact, eyes should be flushed immediately with plenty of water for at least 15 min and medical attention sought. Repeated or prolonged exposure is not known to aggravate medical condition.

Further Reading

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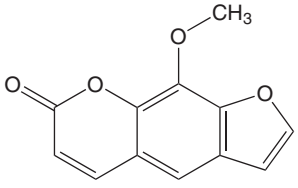
PUVA

Jean L Lim and Robert S Stern

- Chemical Abstracts Service Registry Number: CAS 298-81-7
- Synonyms: Psoralen and UVA; Photochemotherapy; Light therapy; 8-Methoxypsoralen (8-MOP); 5-Methoxypsoralen (5-MOP); 4,5′,8-Trimethylpsoralen (TMP)
- Chemical/Pharmaceutical/Other Class: Furocoumarin and UVA light
- Chemical Formula: $C_{12}H_8O_4$ (8-Methoxypsoralen)
- Chemical Structure: Psoralens are planar, tricyclic compounds, consisting of a furan ring fused to a coumarin moiety



Uses

PUVA is the acronym originally introduced to describe the combined administration of psoralen and subsequent exposure to high-intensity ultraviolet radiation from an artificial source (UVA). In the United States, orally administered 8-methoxypsoralen (8-MOP) is the psoralen most frequently used in combination with UVA light therapy and will be the focus of this discussion. However, the term PUVA also refers to therapy with other oral and topical psoralens, most commonly 4,5′,8-trimethylpsoralen (TMP), a synthetic psoralen, and 5-methoxypsoralen (5-MOP), not available in the United States.

PUVA is used most commonly in the treatment of psoriasis, vitiligo, and cutaneous T-cell lymphoma. However, up to 30 other skin diseases have been reported to be responsive to PUVA therapy. The most common of these include: palmarplantar pustulosis, polymorphous light eruption, dishydrotic eczema, atopic dermatitis, allergic contact dermatitis, actinic reticuloid, solar urticaria, pityriasis lichenoides, and graft versus host disease.

Background Information

Psoralen and psoralen derivatives are found in plants (*Psoralea corylifolia* and *Ammi majus*) and other vegetation such as limes, figs, parsnips, and certain fungi. Psoralen is a photosensitizing drug and was used with sunlight to treat skin diseases in Egypt and India as early as 1200–2000 BC. The ancient Egyptians and Indians applied plant extracts to the skin or ingested the extracts orally and then exposed themselves to sunlight to induce repigmentation in vitiligo. 8-Meythoxypsoralen, derived from *A. majus*, has been available in the United States since 1951 and was first combined with high intensity ultraviolet light from an artificial source in 1974. PUVA treatment involves the administration of psoralen (usually $0.6\,mg\,kg^{-1}$), followed by exposure to long-wave UV radiation (320–400 nm) ~1–2 h later. Clearing can usually be achieved in 8–12 weeks with two to three treatments per week. However, without maintenance therapy of up to once a week, disease will return.

Exposure Routes and Pathways

Therapeutic psoralen exposure occurs by the oral or topical route. UVA exposure occurs via epidermal contact. The patient stands upright in a vertical cylinder or similar large enclosure lined with fluorescent tubes to receive an exactly calculated UV radiation dose measured in joules per square centimeter. More than half of the energy emitted is of wavelengths in the range 340–370 nm.

Toxicokinetics

Depending on the patient, dose form, and ingestion of food, peak 8-MOP serum dose levels occur between 30 min and 4 h after ingestion, with a mean time of 90 min. Equilibrium between levels in the blood and in the skin is achieved in 1 h. Mean peak serum concentrations are $\sim 200\,\mu g\,l^{-1}$ (range: $0–500\,\mu g\,l^{-1}$) and is lower under postprandial in comparison to fasting conditions. At low doses (i.e., 40 mg or less), 8-MOP undergoes extensive first-pass elimination so that only a small amount of unchanged 8-MOP reaches the general circulation. At high doses, liver enzymes become saturated and serum concentrations

of 8-MOP increase quickly. Elimination half-life for 8-MOP ranges from 1.1 to 1.9 h and is not affected by the ingestion of food. Terminal half-life is 200 h. Psoralens are transformed to polar metabolites by hydroxylation and glucuronidation in the liver. In rats, radiolabeled 8-MOP has been recovered in high concentrations in the liver and kidney soon after oral and intravenous administration. In humans, 80% of orally administered 8-MOP is excreted in the urine within 8 h, and lesser amounts are found in the urine and feces over several days after ingestion.

Mechanism of Toxicity

The mechanisms of action and of toxicity of PUVA are not completely understood. Because of their planar structure and hydrophobicity, psoralens readily intercalate with nucleic acid basepairs when exposed to UVA light. Absorption of a single photon by psoralen results in the formation of a monofunctional adduct. A monofunctional adduct formed by the 4′5′-furan bond with the 5,6-bond of a pyrimidine can absorb a second photon leading to the formation of a bifunctional interstrand crosslink. Both monofunctional and bifunctional adducts are believed to inhibit DNA replication, which may be responsible for the antihyperproliferative effect of PUVA. In comparison to monofunctional adducts, bifunctional adducts are more strongly implicated in the toxic side effects of PUVA including irreversible damage to keratinocytes, resulting in apoptosis and cell necrosis leading to sunburn-like skin damage and blistering. Mutations arising from photoadducts in sensitive DNA sequences that encode for tumor suppressor genes may lead to the development of skin tumors. PUVA therapy has also been shown to have a suppressive effect on T cells, leading to inhibition of the delayed hypersensitivity response and decreased release of proinflammatory cytokines. This response is therapeutic for the treatment of various inflammatory and lymphoproliferative skin diseases, but the resulting immunosuppression may also explain the reported increased incidence of herpes simplex among PUVA-treated patients and may contribute to risk of squamous cell cancer.

Acute and Short-Term Toxicity (or Exposure)

Animal

In 1 day, 16 day, and 13 week studies, 8-MOP administered in high doses to rats can result in mortality, decreased body weight gain, dose-related increases in liver to body weight ratios, and fatty changes in the liver, and in male rats, atrophy of the testis, seminal vesicles, and prostate.

Human

The most common short-term side effects of PUVA are pruritus and transient nausea. Up to 25% of patients experience pruritus, which is UV dose-related and is associated with dryness of the skin. Usually, the pruritus responds well to emollients and antihistamines. Transient nausea affects $\sim$12% of patients taking 8-MOP and can be minimized by taking the medication with food or using antiemetics. PUVA pain is a rare, intermittent, severe burning pain that occurs 4–8 weeks after the onset of PUVA therapy. Because the pain worsens with ongoing therapy, PUVA must be discontinued and the pain usually resolves spontaneously in a few weeks. Other reported adverse effects include erythema and burning, maculopapular rash, exacerbation of photodermatoses, increased incidence of herpes simplex, and hepatotoxicity.

Chronic Toxicity (or Exposure)

Animal

Chronic 8-MOP administration of up to 75 mg kg^{-1} for 2 years increases the incidence of renal tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney and carcinomas of the zymbal gland in male rats. 8-MOP with the addition of UVA induces the development of squamous cell hyperplasia, squamous cell papilloma, squamous cell carcinoma, cutaneous melanoma, and cataracts in rats.

Human

Chronic PUVA exposure in humans results in premature skin aging, PUVA keratoses, PUVA lentigines, and nonmelanoma skin cancer (NMSC) typically in a dose-dependent fashion. The incidence of cataracts may also be higher among PUVA treated patients. Cardiovascular disease, noncutaneous neoplasms and teratogenic effects have not been shown to be associated with PUVA therapy.

Many studies have analyzed the effect of PUVA on the development of skin cancer. A multicenter study led by Stern and others involving 16 centers in the United States begun in 1975 has monitored the adverse effects of PUVA treatment over the past 27 years. This study has documented an increased risk of squamous cell cancer (SCC) and basal cell cancer (BCC) in a dose-dependent relationship with the number of PUVA treatments received. The overall risk of developing an NMSC among subjects in this cohort is 17 times and four times higher for the development of a first SCC and BCC, respectively, in comparison to the general population. In addition, for patients on high dose PUVA ($\geqslant$337 treatments)

compared to the general population, the risk of developing a first SCC and BCC is 104 times greater and 11 times greater, respectively. Comparable studies in Europe have shown a similar increase in the relative risk of NMSC. Japanese studies, on the other hand, report a much lower incidence of NMSC in psoriatic patients treated with PUVA. This finding might reflect the protective effect of moderately pigmented skin among Asians and greater use of topical rather than oral PUVA in Japan.

PUVA exposure also appears to be a risk factor for the development of malignant melanoma, but this finding remains somewhat controversial. A multicenter US study has shown a greater than fivefold increase in risk of melanoma among patients receiving high dose PUVA ($\geqslant$250 treatments) compared to the general population. However, a similar cohort study of Swedish patients did not show a statistically significant increase in melanoma. This difference in risk between the US and Swedish populations might be attributable to the use of different treatment protocols in the two countries, or more likely, the result of insufficient power in the Swedish study to show an increase in risk due to small numbers of patients with sufficiently large amounts of PUVA exposure.

Clinical Management

Short-term toxicity such as erythema and burning is managed by decreasing PUVA dosage, keeping sunburned areas covered by clothing while receiving PUVA, or if severe, discontinuing therapy. Long-term toxicity is prevented by the protection of genitalia in males, the protection of the face unless significant psoriasis is present, and the use of protective eyeware from the time of psoralen ingestion, in the treatment unit, and for the next 24 h if the patient is exposed to sunlight. In addition, PUVA should only be used when skin disease is a significant burden and after consideration of the risks and benefits of PUVA relative to other treatments for that patient. Efforts to limit the total number of lifetime treatments should be made, particularly in younger patients and those with a higher innate risk of skin cancer. Patients receiving PUVA or with past history of significant PUVA exposure should also have regular periodic follow-up with a dermatologist or other qualified health practitioner for skin examinations and tumor excision.

See also: Methoxypsoralen, 8-; Skin.

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Relevant Website

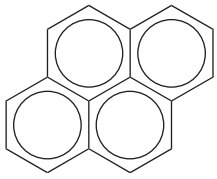
http://ntp-server.niehs.nih.gov – TR-359 Toxicology and carcinogenesis studies of 8-methoxypsoralen (CAS 298-81-7) in F344/N rats (gavage studies). National Toxicology Program. (Accessed October 9, 2003).

Pyrene

Lu Yu

- Chemical Abstracts Service Registry Number: CAS 129-00-0
- Synonyms: β-Pyrene; Coal tar pitch volatiles
- Chemical/Pharmaceutical/Other Class: Polycyclic aromatic hydrocarbon
- Chemical Formula: $C_{16}H_{10}$

- Chemical Structure:



Uses

Pyrene is used in the production of dyes and optical brighteners as a starting material. It is also produced as a result of incomplete combustion in the exhaust of motor vehicles and engines, in cigarette smoke, and in stoves and furnaces. It is found naturally in coal tar and fossil fuels.

Exposure Routes and Pathways

Inhalation and dermal contact are the major pathways for both occupational exposure and general population. Consumption of contaminated food and drinking water are also possible pathways for the general population. Persons with skin disorders are more susceptible to the toxicity of pyrene.

Toxicokinetics

Pyrene is highly lipophilic and easily penetrates cellular membranes. However, pyrene is readily metabolized to more water-soluble compounds such as 2-hydroxypyrene, 1,6-dihydroxypyrene, 1,8-dihydroxypyrene, and 4,5-dihydrodiol by liver microsomes. Two trihydroxy derivatives were also identified as metabolites of pyrene in a rat study. The high water solubility of the metabolites makes them readily excretable.

Mechanism of Toxicity

Oxidation of the rings of pyrene is an important step in its metabolism carried out by mixed function oxidases of the liver containing cytochromes P450 and P448. Epoxide intermediates are formed from oxidation. They are very reactive and can form covalent complexes with DNA and histones which serves as the ultimate carcinogenic form of pyrene.

Acute and Short-Term Toxicity (or Exposure)

Animal

It was reported that LD_{50} of rat exposed orally to pyrene was $2700\,mg\,kg^{-1}$. In a study, rats were dosed orally at concentrations near LD_{50} and succumbed in 2–5 days, and dosed through inhalation at LC_{50} in 1–2 days. Inhalation caused hepatic, pulmonary, and intragastric pathologic changes, although $10\,g\,kg^{-1}$ was considered to be less toxic through dermal contact. Cutaneous exposure for 10 days caused hyperemia, weight loss, and hepatopoietic changes. Cutaneous application for 30 days produced dermatitis and leukocytosis. In another study, four concentrations of pyrene (ranging from $5\,\mu mol\,l^{-1}$ to $5\,mmol\,l^{-1}$) were applied to the dorsal surface of six animals that received $1.0 \times 10^5\,J\,m^{-2}$ of UVA radiation. Erythematous response was evaluated after 20 h and pyrene was found to be strongly phototoxic.

Increasing dietary doses of pyrene ranging from $1000\,mg\,kg^{-1}$ food ($127\,mg\,kg^{-1}\,day^{-1}$) up to $25\,000\,mg\,kg^{-1}$ food ($917\,mg\,kg^{-1}\,day^{-1}$) for a mean dose of $426.6\,mg\,kg^{-1}\,day^{-1}$ over a 25 day period produced dilation of the renal tubules in an unspecified number of mice. The effect was not observed until the highest dose was administered, which limited the toxicological significance of this study.

Human

Pyrene is a skin, eye, and respiratory irritant. Coal tar pitch volatiles are reported to cause bronchitis.

Chronic Toxicity (or Exposure)

Animal

In a study, male and female CD-1 mice were gavaged with 0, 75, 125, or $250\,mg\,kg^{-1}\,day^{-1}$ pyrene in corn oil for 13 weeks. Minimal or mild kidney lesions (presence of multiple foci of renal tubular generation, accompanied by interstitial lymphocytic infiltrates and/or foci of renal fibrosis) were observed in all dose groups. The no-observed-adverse-effect level was obtained as $75\,ng\,kg^{-1}\,day^{-1}$, and the lowest-observed-adverse-effect level was $125\,mg\,kg^{-1}\,day^{-1}$ for nephropathy and decreased kidney weights. The animal carcinogenicity data are inadequate. Newborn male and female CD-1 mice received intraperitoneal injections of pyrene. The incidences of total liver tumors, lung tumors, or malignant lymphomas were not significantly different from control animals. Mouse skin-painting assays of pyrene as a carcinogen or as an initiator of carcinogenicity were either negative or inconclusive. Pyrene did not produce tumors in Jackson A mice 18 months after a subcutaneous injection.

Human

Pyrene is not classified as human carcinogen by the US Environmental Protection Agency (EPA) based on no human data and inadequate data from animal bioassays. Increased incidences of lung, skin, or genitourinary cancers were observed in workers exposed to a variety of polycyclic aromatic hydrocarbons. Prolonged exposure to coal tar pitch volatiles can cause dermatitis.

In Vitro Toxicity Data

Mixed results were observed for *in vitro* tests of pyrene. Negative results were obtained for pyrene in DNA damage assay in *Escherichia coli* and *Bacillus subtilis*. Both positive and negative results were observed in bacterial gene mutation test. Pyrene did not induce an increase in sex-linked recessive lethal gene in *Drosophila*. It increased the incidence of mitotic gene conversion but not other genetic endpoint in yeast.

Pyrene increased the frequency for sister chromatid exchange (SCE) in CHO cells at all treatments, but no apparent increase was observed when the concentration was increased 10-fold. Two negative results were reported for both SCE and chromosome aberrations in CHO cells at the same treatment levels. Another study also reported no increase of SCE frequency in Chinese hamster cells. Chromosome aberrations or SCE in bone marrow were not increased in several mouse strains receiving intraperitoneal injections of pyrene.

Clinical Management

The affected individual should be removed from the exposure source. A patent airway should be established. Signs of respiratory insufficiency should be monitored, and oxygen should be administered if necessary. The individual should be monitored for pulmonary edema and shock. The mouth should be rinsed and water given for dilution if patient has swallowed pyrene. Activated charcoal should be administered. Emetics should not be used. In case of eye contamination, the eyes should be flushed with water, and irrigated with normal saline during transportation.

Environmental Fate

Pyrene is immobile in soil. Volatilization from dry soil surface is extremely low with a half-life of 500 days. Biodegradation is expected to be very slow with estimated half-life of weeks to years. Photolytic degradation occurs in soil. When pyrene is disposed into the aquatic system, it is expected to adsorb to suspended soil and sediments strongly. Because of this strong adsorption, the expected volatilization from the water surface was severely attenuated. Photolytic degradation is significant at the surface of aquatic system. Biodegradation is also expected to be very slow in water. Bioaccumulation of pyrene in aquatic organisms is moderate to high. When pyrene is disposed into air, it is expected to exist in both vapor and particulate phases. The vapor-phase pyrene degrades by reaction with hydroxyl radicals and nitrate radicals in the atmosphere. Pyrene in particulate phase could be removed from air through deposition. The background concentration of pyrene in rural, agricultural, and urban soils is 1–19.7, 99–150, and 145–147 000 $\mu g\,kg^{-1}$, respectively.

Ecotoxicology

Pyrene has a moderate to high tendency to bioaccumulate in aquatic organisms from water, sediment, and food. The median threshold limit for Mosquito fish is 0.0026 $mg\,l^{-1}$ per 96 h in a static bioassay.

Other Hazards

Pyrene is a skin, eye, and respiratory irritant. It is flammable. When heated, it decomposes, and produces smoke and irritating fumes.

Exposure Standards and Guidelines

- The EPA IRIS reference dose is $0.03\,mg\,kg^{-1}\,day^{-1}$.
- The State drinking water guidelines are: Florida, 210 $\mu g\,l^{-1}$; Minnesota, 220 $\mu g\,l^{-1}$; and Wisconsin, 250 $\mu g\,l^{-1}$.
- Clean Water Act Requirements: For the maximum protection of human health from the potential carcinogenic effects due to exposure to pyrene, the ambient water criteria are 28.0, 2.8, and 0.28 $ng\,l^{-1}$, respectively, corresponding to the levels which may result in incremental increase of cancer risk over the lifetime at 1×10^{-5}, 1×10^{-6}, and 1×10^{-7}. The levels are 311, 31.1, and 3.11 $ng\,l^{-1}$, respectively, if the above estimates are made for consumption of aquatic organisms, excluding consumption of water.

See also: Polycyclic Aromatic Hydrocarbons (PAHs).

Relevant Websites

http://www.epa.gov – US EPA Integrated Risk Information System (IRIS) on pyrene. On the substance file list as of October 4, 2004.

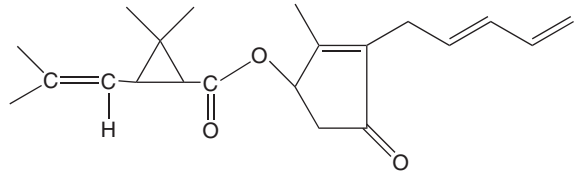
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Pyrene.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Pyrene.

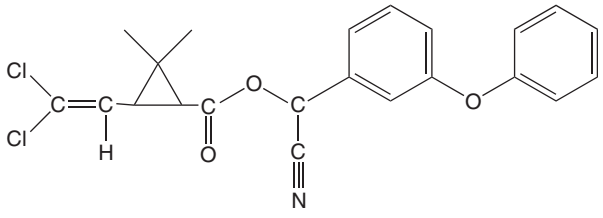
Pyrethrins/Pyrethroids

David E Ray

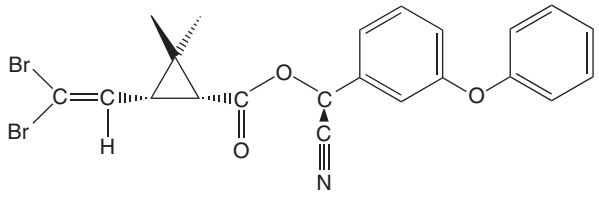
- Chemical Names: Synthetic Pyrethroids and Natural Pyrethrins
- Representative Chemicals: Pyrethrin I; Cypermethrin; Deltamethrin; Permethrin
- Chemical Abstracts Service Registry Numbers: CAS 121-21-1 (Pyrethrin I); CAS 52315-07-8 (Cypermethrin); CAS 52918-63-5 (Deltamethrin); CAS 52645-53-1 (Permethrin)
- Synonyms: The pyrethins are also known as pyrethrum
- Chemical/Pharmaceutical/Other Class: Ester insecticides
- Chemical Formulas: Pyrethrin I, $C_{21}H_{28}O_3$; Cypermethrin, $C_{22}H_{19}O_3NC1_2$; Deltamethrin, $C_{22}H_{19}Br_2NO_3$; Permethrin, $C_{21}H_{20}O_3Cl_2$
- Chemical Structures:



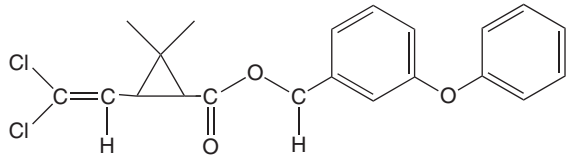
Pyrethrin I



Cypermethrin



Deltamethrin (a pure 1*R*,3,*RS* - α-isomer)



Permethrin

Uses

Both the naturally occurring pyrethrins and the synthetic pyrethroids are widely used as insecticides in agricultural, public health, and domestic applications. They are also used as ectoparisiticides in veterinary and human medicine. Attractive features are their low environmental persistence, and their rapid 'knock down' activity, whereby flying insects very quickly become uncoordinated and unable to fly before they are killed.

From the nineteenth century until the 1970s only pyrethrin mixtures obtained by solvent extraction of pyrethrum flowers (usually *Chrysanthemum cinerar-aefolum*) were available for use. However, the development by Martin Elliott of the cheaper and more light-stable synthetic pyrethroids from the 1970s led to their becoming a major pesticide class. Over a 1000 pyrethroid structures have been synthesized, some of which show considerable divergence from the original pyrethrins. The natural pyrethroids are now largely restricted to indoor domestic uses where light stability is less important. The pyrethroids are major agricultural pesticides in terms of treated land area (although not tonnage as they are more potent than most other pesticide classes). In UK arable farming, cypermethrin was the most widely used single pesticide in 2002.

Exposure Routes and Pathways

All pyrethroids and pyrethrins have low volatility (10^{-4}–10^{-8} mmHg), and so occupational and domestic use exposure is primarily via dermal contamination or inhalation/ingestion of spray droplets. Consumers are exposed to low-level residues of agricultural pyrethroids in many foods. Residues of domestic pyrethroids and pyrethrins have been found in house dust.

Toxicokinetics

Although the inherent toxic potential of pyrethroids and pyrethrins can be high (intravenous LD_{50}s range from 0.5 to $>250\,mg\,kg^{-1}$), this is limited in practice by rapid detoxification in skin, blood, and liver. The blood half-life of pyrethroids ranges from 19 min to 10 h (typically a few hours), and intoxication by

the oral route is correspondingly short lasting. The dermal toxicity of pyrethroids is further limited by low absorption through the skin and the capacity for local metabolic destruction of pyrethroids in the skin during absorption. In man the bioavailability of dermal pyrethroids is ~1%, compared to 36% for gastric absorption. Hence the dermal route of exposure presents relatively little risk of systemic poisoning, although in the few cases of very severe skin contamination that have been reported, intoxication has lasted for several weeks: possibly due to a large reservoir of pyrethroid bound to the epidermis. Once absorbed, pyrethroids rapidly distribute through the body, their high lipophylicity and lack of exclusion by the ABC multidrug transporters ensuring ready entry into the central nervous system (CNS).

The pyrethrins and allethrin are broken down mainly by oxidation of the isobutenyl side chain of the acid moiety and of the unsaturated side chain of the alcohol moiety, with ester hydrolysis playing an unimportant part, but for the other pyrethroids ester hydrolysis predominates. The acid and alcohol components of pyrethroids have very little toxic potential, so hydrolysis represents a one-step detoxification. These reactions can take place in both liver and plasma and are followed by hydroxylation and conjugation to glucuronides or sulfates, which are then excreted in urine. A number of factors modify the rate of breakdown, notably stereospecificity: with *trans* isomer hydrolysis being catalyzed by esterases but *cis* isomer hydrolysis being catalyzed at a rather lower rate by oxidases. This slower breakdown of *cis* isomers may contribute to their greater mammalian toxicity, but their higher inherent affinity for the sodium channel complex is thought to be the predominant factor. An additional influence on the rate of metabolism is the presence of an α-cyano group, which slows both hydrolysis and oxidation.

Neonatal rats are four to 17 times more vulnerable than adults to the acute lethality of both type I and II pyrethroids. This is entirely attributable to their lesser capacity for metabolic detoxification, and does not extrapolate to lower dose effects.

The relative sensitivity of insects to pyrethrins and pyrethroids is attributable (in roughly equal proportions) to their slower metabolic disposal, to their lower body temperature, and to the inherently higher sensitivity of their target sites. Although there are few, if any, toxic actions of the pyrethroids in insects that do not have their counterpart in man, these three quantitative factors combine to give insect–mammalian toxicity ratios of 2 or 3 orders of magnitude.

Mechanism of Toxicity

The pyrethrins and the pyrethroids are primarily functional toxins: causing death by hyper-excitation, and causing direct cytotoxicity in mammalian cells only at concentrations much higher than are reached in the brain of severely intoxicated animals.

The main target of the pyrethrins and the pyrethroids is the voltage-gated sodium channel family. This is responsible for the generation of the inward sodium current that produces the action potential in most cells, and is closed at normal resting potentials. Sodium channels consist of an α subunit, which forms the trans-membrane pore, resembles those of other voltage-gated ion channels, and can take several possible isoforms; and the β_1 and β_2 subunits which modify the basic function of the α subunit. There are many variant forms of the α subunit, 10 being characterized in the rat, and channels are also subject to glycosylation and phosphorylation which further modify function. These variant forms also show very different sensitivity to pyrethroids. Unfortunately there is no standard nomenclature for the many channel isoforms, and descriptions based on pharmacological properties (e.g., tetrodotoxin resistant) or tissue source (e.g., brain I, II, III) are widely used.

The interaction of pyrethroids with the sodium channel has the effect of slowing both its activation and its inactivation processes, overall causing the pyrethroid-modified channel to adopt a hyperexcitable state. This hyperexcitable condition is sustained until the pyrethroid is removed, when the channel returns to normal. Since there is a far higher density of expression of sodium channels in most cells than is needed to maintain normal excitability, only ~0.1% of sodium channels need to be modified by a pyrethroid in order for the extra current that they generate to render the whole cell hyper-excitable. This greatly increases the toxicity of pyrethroids, since they are effective well below their inherent ED_{50}s. Although activation is slowed at the single-channel level, the high density of sodium channels also means that sufficient unmodified channels are always present to ensure that the activation phase of the action potential is not appreciably delayed. However in the falling (inactivation) phase of the action potential even a low proportion of modified channels can generate enough extra current to delay inactivation. This slower rate of inactivation of pyrethroid-modified channels generates a prolonged depolarizing 'tail' current that follows the normal action potential. This 'tail' will trigger a second action potential if the current is large enough and lasts longer than the 0.5–1 ms needed for the normal

sodium channels to reactivate. In this situation, what would normally be single action potential can become multiple action potentials or a continuous uncontrolled discharge. Action potential amplitude normally remains constant, so this uncontrolled excitation produces marked functional disruption, although very high concentrations of pyrethroids or hyperactivity beyond that which the cell can sustain will eventually cause depolarization and conduction block. This depolarization is more readily produced by those pyrethroids that hold the sodium channel open longest.

An important characteristic of the pyrethroid-generated tail current is that its amplitude and duration are independent. The current amplitude is dependent only on the proportion of sodium channels modified, and hence shows a saturable relationship with pyrethroid concentration or dose. The current duration however is dependent only on the pyrethroid structure: some pyrethroids, such as permethrin holding the channel open for a few milliseconds and others, such as deltamethrin, holding it open for tens of milliseconds. Individual pyrethroids thus generate a characteristic time constant for prolongation of the sodium channel tail current that is virtually independent of dose.

Different forms of the sodium channel show differential sensitivity to pyrethroids. Pyrethroids are ~10 times more potent on the tetrodotoxin-resistant subtype of the sodium channel, which is expressed in the developing mammalian brain and in adult dorsal root ganglia. The different forms can show structure-specific sensitivity also: the rat brain IIa form being sensitive to type II, but not type I pyrethroids. Some of the selectivity of action of pyrethroids within the nervous system parallels the distribution of sensitive sodium channel subtypes, although there are at present only limited data to support this. Peripheral nerve (SNS/PN3) sodium channels are highly sensitive to pyrethroids, and action at these channels may be relevant to the production of paresthesia.

Pyrethroid action on the sodium channel shows a marked steriospecificity: the 1*R* and 1*S* *cis* isomers binding competitively to one site, and the 1*R* and 1*S* *trans* isomers binding noncompetitively to another. The 1*S* forms do not modify the channel function but do block the effect of the 1*R* isomers. In whole mammals the 1*R* isomers are thus active and the 1*S* isomers inactive and essentially nontoxic. Isomerism at the third carbon of the cyclopropane ring gives *cis* and *trans* isomers, with the *cis* isomers being about 10 times more potent than the *trans* ones. A final chiral centre is generated if a cyano substituent is added to the alcohol, giving eight possible isomers. Again this affects potency, with only the α-*S* and not the α-*R* forms being toxic to both insects and mammals. A practical consequence of this is that the toxicity of products such as permethrin, which are sold as variable isomeric mixtures, can vary from batch to batch. Thus the rat oral LD_{50} value of commercial samples of permethrin can vary from 430 to 8900 mg kg^{-1} depending on *cis* isomer content.

Many target sites other than the sodium channel have been suggested to be relevant to poisoning. Most show insufficient potency to be relevant at the concentrations of 1–30 nmol g^{-1} tissue that are seen during severe poisoning in rats, but the complex effects of pyrethroids on the CNS have led to suggestions that they also act via antagonism of γ-aminobutyric acid (GABA)-mediated inhibition, by modulation of nicotinic cholinergic transmission, or by enhancement of norepinephrine release. However, most neurotransmitter release is secondary to increased sodium entry. Actions at both voltage-dependent chloride channels and at calcium channels have also been proposed. Voltage-sensitive chloride channels are found in brain, nerve, muscle and salivary gland, and their function is to control cell excitability. The pyrethroids deltamethrin and fenvalerate decrease chloride channel currents at low enough concentrations to be relevant to mammalian poisoning, but others do not. These pyrethroids decrease chloride channel current which increases excitability, and so would indirectly synergize pyrethroid actions on the sodium channel. Voltage-dependent calcium channels are certainly pyrethroid targets in insects, and some mammalian calcium channels, such as those in the cardiac sinoatrial node, are also sensitive.

The mechanism whereby pyrethroids interact with ion channels is not yet understood, since their high lipophylicity makes investigation difficult, but type II pyrethroids have been shown to directly stimulate protein kinase C-dependent protein-phosphorylation at very low concentrations. Since ion channel activity is modulated by phosphorylation state, this is likely to be an important mechanism of action.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute effects of high oral or intravenous doses of the pyrethrins and most noncyano pyrethroids closely resemble those of DDT (which acts similarly on the sodium channel). They are characterized by fine continuous tremor, which may be severe enough to cause hyperthermia, and by marked reflex hyperexcitability. Consciousness is preserved up to the point

of death. This is variously described as T (tremor) or type I syndrome. However, with the development of the first cyano-substituted pyrethroids such as deltamethrin it was realized that these produced a very different acute poisoning syndrome in both insects and mammals known as chorea salivation (CS) or type II syndrome. This is characterized progressively by chewing, salivation, hind-limb rigidity, and finally by EEG spiking, choreoathetosis and tonic–clonic seizures with loss of consciousness. The last three are initially precipitated by sensory stimuli, but become spontaneous at near-lethal doses. In dogs, upper airway hypersecretion and gastrointestinal symptoms are more prominent than in rats. Some pyrethroids such as cyphenothrin produce mixed syndromes with both sets of symptoms superimposed, and for all pyrethroids at low doses it can be difficult to distinguish the two syndromes. Few pyrethroids have sufficient toxic potential to produce severe acute signs by the dermal route, although those with relatively long blood half-lives such as the type II pyrethroid cyhalothrin, can do so.

The nature of the poisoning syndrome can be predicted from the duration of the pyrethroid-induced sodium channel after-potential: type I pyrethroids producing shorter time constants, type II pyrethroids producing longer time constants, and mixed I/II pyrethroids having intermediate time constants. All type II pyrethroids have a cyano substituent, but not all type I pyrethroids lack one: the *trans* and *cis* isomers of flurocyphenothrin producing type I and II effects respectively. Both pyrethroid classes have a similar range of mammalian toxicity but of commercial pesticides the type II, such as deltamethrin and cypermethrin, are generally more toxic than the type I, such as permethrin. The extent to which the two classes of pyrethroids truly differ is not yet clear, but in isolated neonatal rat dorsal root ganglion cells the actions of the type I tetramethrin and the type II fenvalerate appear to be mutually exclusive.

Both type I and II pyrethroids cause marked adrenal activation in rats, probably by a direct activation of norepinephrine release. The type II pyrethroid deltamethrin causes increased corticosteroid secretion, and even moderate pyrethroid poisoning occurs against a background of profound adrenal activation – with corresponding behavioral and cardiovascular consequences. With only type II pyrethroids there is also a direct increase in cardiac contractility, although blood pressure remains well controlled.

Electrophysiological studies have shown that all of the type I motor signs of systemic pyrethroid intoxication are generated at the spinal level, although type II poisoning involves the whole CNS. Dermal application of pyrethroids can directly activate peripheral nerves however, producing paresthesia which is described in the next section.

Human

Fortunately there have been few reports of systemic poisoning, since use of adequate protective clothing will prevent intoxication even under tropical conditions. However systemic poisoning can occur under conditions of misuse or inadequate user protection.

Almost all reports of human poisoning relate to the more potent type II pyrethroids, so it is not certain how well the two syndromes seen in animals applies to man, although what has been seen in man fits quite well with the animal observations. Human type II poisoning seems to be characterized by paresthesia (if via the dermal route), dizziness, nausea, listlessness, and muscular fasciculations. More severe poisoning caused epigastric pain, nausea and vomiting (if via the oral route), hypersalivation and pulmonary edema, opisthotonos, seizures, and coma.

Given the common formulation of pyrethroids with volatile solvents such as xylene, symptoms of poisoning can be complicated by solvent toxicity, and solvents may also introduce additional skin effects. Mild poisoning symptoms may also be amplified by anxiety, which may itself be precipitated by fear or by the disconcerting paresthesia resulting from dermal contact with pyrethroids.

Although systemic toxicity is very rare, local effects are more commonly reported: skin contamination producing paresthesia, ingestion producing gastrointestinal irritation, and inhalation yielding upper respiratory tract irritation. All of these effects are reversible. The gastrointestinal irritation is rare (being limited to cases of ingestion) and has not been well studied, but presumably is a phenomenon similar to the more common dermal paresthesia. Respiratory tract irritation can be produced at comparable thresholds in rats and man, but is rarely reported. Dermal exposures far below the threshold for systemic poisoning can lead to a local paresthesia, which is evoked by all classes (the pyrethrins and type I and II pyrethroids), with a severity roughly in proportion to their systemic toxic potential. Paresthesia is by far the most commonly described toxic effect of pyrethroid exposure.

Pyrethroid-induced paresthesia is dose-dependent in severity and duration, lasting for 4–30 h after a single application. When mild the sensation is of continuous tingling or pricking, and when more severe, burning. Paresthesia is more commonly felt in the thinner skin of the face than the hands, even when the hands are the primary site of contamination. Erythema is not seen normally unless the sufferer scratches the area. Paresthesia is annoying

but not disabling and does not appear to be associated with any lasting ill effects. In animals, electrophysiological tests show peripheral nerve hyperexcitability lasting up to 24 h after exposure, and such tests have been used to monitor local pyrethroid effects in man. The mechanism of paresthesia has not been studied directly, but presumably results from abnormal pyrethroid-induced repetitive activity in skin nerve terminals.

Respiratory and dermal sensitization have both been reported after exposure to pyrethroids, but very rarely considering their widespread usage, and always in association with other potential allergens. However in the past impure pyrethrin extracts have given rise to contact dermatitis.

Chronic Toxicity (or Exposure)

Animal

Near-lethal doses of all classes of pyrethroids can give rise to an axonal degeneration in peripheral nerves closely resembling Wallerian degeneration, but this effect is inherently reversible and is only seen at dose levels which produce prolonged and severe motor signs. Central neuropathology has been described in one study of adult rats given repeated near-lethal doses of the type II pyrethroid deltamethrin, but others (including the present author) have found no such pathology. A study of repeated low doses of permethrin has also described central pathology when given in combination with other agents known to produce central neuropathology at higher doses.

A number of effects of exposure to pyrethroids during early development have been described in mice. The pyrethroids permethrin and deltamethrin (in addition to DDT, PCBs, nicotine, and paraquat) have also been reported to induce permanent changes in behavior and neurochemistry of adult mice when administered directly to the neonate. These effects were seen at dose levels, which were not acutely toxic. These results contrast with a lack of effect of longer term, dietary administration of pyrethroids in rat studies conducted for regulatory purposes using different end points, and at present the relevance of the mouse results to human health is uncertain. In other studies, delayed development of the blood–brain barrier has been reported in rat pups given pyrethroids at higher doses sufficient to reduce body weight, although it is likely that this effect was nonspecific in nature.

Human

Long-term ill-health of a variable and somewhat nonspecific nature has occasionally been ascribed to pyrethroid exposure, and this has been the subject of public concern and legal claims in Germany. Pyrethroids have been detected in domestic house dust at a low level, but no clear clinical or epidemiological studies have shown a causal relationship between pyrethrin or pyrethroid exposure and ill-health. The few descriptions of systemic acute pyrethroid poisoning and the larger number of paresthesia cases that have been reported all described complete recovery.

In Vitro Toxicity Data

Inexcitable mammalian cells are little affected by pyrethrins or pyrethroids. A number of pyrethroids produce growth inhibition at $10^{-5}\,\mathrm{mol\,l^{-1}}$ and decreased viability has been described after treatment with permethrin at $100\,\mathrm{ng}/10^6$ cells – a level which is approximately 10–100 times higher than that reached in the brain of lethally intoxicated animals. By contrast, excitable tissue (neurons, synaptosomes, and isolated nerve fibers) has proved invaluable for research into mechanisms of action: effects being seen from $10^{-10}\,\mathrm{mol\,l^{-1}}$. However the very low water solubility of pyrethroids ($\sim 10^{-9}\,\mathrm{mol\,l^{-1}}$) has meant that most such studies have been carried out using aqueous pyrethroid suspensions that then dissolve into tissue lipids, making absolute ED_{50}s difficult to determine. This solubility problem also causes most *in vitro* effects to be irreversible, since there is no route for removal of the pyrethroid – in marked contrast to the effects seen *in vivo*. Such irreversibility has been interpreted by some authors as providing potential for irreversible pyrethroid toxicity in man, but when toxicokinetic differences are taken into account, *in vitro* and *in vivo* data are generally in good agreement.

Clinical Management

The most commonly encountered sign of pyrethroid poisoning is dermal paresthesia. This can be treated by lavage of the contaminated skin with oils – but not by soap and water, as pyrethroids bind to skin and are poorly water soluble. Vitamin E cream has also been found effective for treating paresthesia in clinical trials. When applied to the skin from 29 h before to 15 min after the pyrethroid protection lasted for more than 5 h. The concentration required to give greater protection than that of the corn oil solvent alone was very high (50%) and similar relief can be obtained by use of presumably inert preparations such as corn oil or petroleum jelly. Topical treatment with local anesthetics has been described in humans and in animals, but anesthesia may be more inconvenient than the paresthesia.

If systemic toxicity does occur, the central signs of poisoning can be difficult to control and may be

confused with intoxication by other pesticides such as anticholinesterases, which also cause salivation and hyperexcitability – although pyrethroids do not inhibit acetylcholinesterase. Since pyrethroids produce no morphological damage and are rapidly removed from the body, only symptomatic treatment is needed. Two approaches are possible: to attempt to antagonize the primary ion channel effects of the pyrethroids, or to control the secondary consequences mediated by specific neurotransmitter systems. Since pyrethroids act via ion channels on multiple neurotransmitter systems, most successful attempts at therapy have been based on ion channel or membrane-stabilizing drugs. An ideal therapeutic agent would antagonize the abnormal, pyrethroid-evoked, sodium current but leave the normal one unchanged. *In vitro*, phenytoin, phenobarbitone, and valproate act equally on the pyrethroid-evoked and normal sodium current; and diazepam and mephenesin had less action on the abnormal pyrethroid-evoked current than on the normal one. Hence mephenesin and methocarbimol are effective in rats only at maximum tolerated doses, and diazepam was found to be ineffective in man. Pentobarbitone, which is both a membrane stabilizer and chloride channel agonist, was however effective against all the type II motor signs caused by deltamethrin at 25% of the anesthetic dose in rats. An equi-sedative dose of phenobarbitone (which does not act on chloride channels) was much less effective. Although phenobarbitone has been tried and found ineffective as a type II pyrethroid antidote in man, pentobarbitone does not appear to have been tested in man.

Since type II poisoning involves a combined action on the CNS, adrenals, autonomic system, and muscle, multi-drug therapy may be needed. The combination of methocarbimol and atropine prevented all deaths at LD_{80} doses of pyrethroids in rats.

Environmental Fate

Environmental residues of pyrethroids and pyrethrins are degraded by hydrolysis, and pyrethrins by photolysis, and so do not accumulate in most ecosystems. The main environmental hazard associated with pyrethroid use is contamination of freshwater by acute run-off after use as an agricultural pesticide or ectoparisitide near to water, which can lead to death of aquatic invertebrates or fish (which have very limited pyrethroid detoxification capacity).

Other Hazards

Pyrethroids and pyrethrins present no other hazards, but are usually formulated in organic solvents which may be inflammable.

Exposure Standards and Guidelines

The acceptable daily intakes set by the Joint Meeting on Pesticide Residues (JMPR) for cypermethrin, deltamethrin, and permethrin are 0–0.01 mg kg^{-1} body weight, with acute oral reference doses for deltamethrin or permethrin of 0.05 mg kg^{-1} bw. The National Institute for Occupational Safety and Health maximum allowable concentration (MAC) for pyrethrins at an 8 h time-weighted average is 5 mg m^{-3}.

Miscellaneous

The pyrethrins and some pyrethroids are commonly co-formulated with the synergist, piperonyl butoxide. This has limited toxic potential in itself but inhibits both oxidative and hydrolytic detoxification reactions and so enhances their toxicity – especially to insects.

See also: Organochlorine Insecticides.

Further Reading

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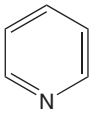
Pyridine

Kathryn J Kehoe

- Chemical Abstracts Service Registry Number: CAS 110-86-1
- Synonyms: Azabenzene; Azine
- Chemical/Pharmaceutical/Other Class: Heterocyclic nitrogen
- Chemical Formula: C_5H_5N
- Chemical Structure:



Uses

Pyridine is a solvent used in the synthesis of pharmaceuticals and other organic compounds. Approximately 50% of pyridine produced is used as an intermediate in insecticide and herbicide manufacture. Another 20% is used to produce piperidine. It can be introduced into the environment by the decomposition of many natural materials.

Exposure Routes and Pathways

Inhalation, dermal contact, and ingestion are possible routes of exposure.

Toxicokinetics

Pyridine is absorbed by the gastrointestinal tract, the skin, and the lungs. Pyridine can be excreted in the urine unchanged or it may be methylated at the N-position to form the urinary metabolite *N*-methylpyridinium hydroxide. Pyridine also may undergo oxygenation by liver microsomes (cytochrome P450) in the presence of NADPH and oxygen.

Acute and Short-Term Toxicity (or Exposure)

Animal

Dermal LD_{50}s include 1121 mg kg^{-1} for rabbits and 1 g kg^{-1} for guinea pigs. Oral LD_{50}s include 891 mg kg^{-1} for rats, 1500 mg kg^{-1} for mice, and 4 g kg^{-1} for guinea pigs. Intravenous LD_{50}s include 420 mg kg^{-1} for mice and 880 mg kg^{-1} for dogs. The LC_{Lo} (inhalation) in rats is 4000 ppm per 4 h.

Human

Despite widespread industrial use, reports of human injury as a result of pyridine are rare. The most important effect of pyridine exposure is hepatotoxicity. Acute exposures to pyridine result in irritation to the skin, nose, and throat. Central nervous system (CNS) depression results in dizziness and lightheadedness. Exposure to high concentrations may result in coma and death. Contact with the eyes causes burning and can lead to permanent damage. Ingestion of small amounts may produce narcotic effects including anorexia, nausea, fatigue, and mental depression. Larger quantities have resulted in systemic effects and death within 43 h.

Chronic Toxicity (or Exposure)

Animal

A 2 year drinking water study performed in rats and mice showed hepatocellular injury by week 13 and clear evidence of carcinogenic activity in all animals that survived 1 year or longer. Renal tubule neoplasms, mononuclear cell leukemia, hepatocellular neoplasms, and interstitial cell adenoma of the testis were noted.

Human

Chronic exposure at 6–16 ppm may result in severe liver damage and kidney injury. Permanent damage to the CNS may result and be accompanied by confusion and mental changes including headache, insomnia, and back pain. Chronic ingestion results in symptoms similar to inhalation. Chronic exposure causes liver and kidney damage.

Pyridine is an allergen and exposure may result in sensitization. It has no known human carcinogenic effects.

In Vitro Toxicity Data

In genetic toxicity screening pyridine was not mutagenic in both *Salmonella typhimurium* and mouse lymphoma cells. Negative results were also observed in sister chromatid exchange studies and no chromosomal aberrations were detected with Chinese hamster ovary cells. More recently it was noted that pyridine induced chromosomal malsegregation and increased nondisjunction in *Drosophilia melanogaster* females.

Clinical Management

The victim should be removed from the source of exposure. For inhalation exposures, fresh air should be supplied. Artificial respiration should be provided if breathing has stopped; oxygen should be administered if available. Treatment should be symptomatic, noting the narcotic effect of pyridine. Dermal exposure should be minimized by washing away all traces of the

chemical with soap or mild detergent and large amounts of water. Symptoms of dermatitis should be treated.

For ingestion, if the victim is conscious and not convulsing one or two glasses of water should be given to dilute the chemical and a hospital or poison control center called immediately. Activated charcoal may be administered. It should be noted that large doses could act as a heart poison.

Environmental Fate

Pyridine is biodegradable and not considered a threat to the environment.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit (time-weighted average) is 5 ppm.

See also: Pesticides.

Further Reading

National Toxicology Program (NTP) (2000) Toxicology and Carcinogenesis Studies of Pyridine (CAS No. 110-86-1) in F344/N Rats, Wistar Rats and B6C3F1 Mice (Drinking Water Studies). NTP Technical Report Series No. 470. NIH Publication No. 97-3960. Research Triangle Park, NC: US Department of Health and Human Services.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Pyridine.

Pyridostigmine

Teresa Dodd-Butera and Molly Broderick

- Chemical Abstracts Service Registry Number: CAS 101-26-8
- Synonyms: Mestinon; Regonol (trade names)
- Chemical/Pharmaceutical/Other Class: Anticholinesterase agent
- Chemical Formula: $C_9H_{13}BrN_2O_2$

Uses

Pyridostigmine is a carbamate that is used in the treatment of myasthenia gravis, a neuromuscular disease. It can also be used as a method of protection against nerve agent poisoning. Carbamates can occupy the catalytic site of acetylcholinesterase (AchE), which temporarily prevents phosphorylation.

Exposure Routes and Pathways

Pyridostigmine bromide is available for use by oral or parenteral routes.

Toxicokinetics

Pyridostigmine is absorbed poorly after oral administration; thus, oral doses must be higher than by the parenteral route. The drug is hydrolyzed by plasma esterases and is metabolized in the liver. Both the quarternary alcohols and parent compounds are excreted in the urine. The elimination half-life is increased with renal dysfunction.

Mechanism of Toxicity

Pyridostigmine bromide competitively binds to nerve tissue AchE. The binding is reversible and has been shown to protect AchE against irreversible inhibition by organophosphorus nerve agents. Pyridostigmine is a quarternary compound and does not readily cross the blood–brain barrier. Thus, it is not expected to affect or protect brain AchE. Cholinesterase inhibition, which is a mechanism of action, is also responsible for toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Pyridostigmine bromide studies have been performed in dogs, guinea pigs, monkeys, rabbits, rats, and mice. Diarrhea, salivation, tremors, and respiratory failure were seen prior to death. Side effects of the drug are related to muscarinic and nicotinic effects. Toxicity is also related to cholinergic stimulation. Effectiveness of pretreatment to reduce lethality after exposure to nerve agents (in particular, soman) is dependent on the administration of atropine and pralidoxime, postexposure.

Oral administration of nonlethal doses of pyridostigmine did not alter male or female reproductive indices. Administration of pyridostigmine bromide also did not result in significant increases in congenital malformations at low and comparable doses to those used therapeutically in humans. At the high dose level, delayed ossification and missing vertebrae were noted in animal studies.

Human

Acute side effects occur from therapeutic doses in ~1% of patients. However, an excessive dose of an anticholinesterase drug results in a cholinergic crisis. The condition results from stimulation of muscarinic receptors and depolarization of the motor end plate. Symptoms of salivation, lacrimation, diaphoresis, weakness, and respiratory failure may result. Therapeutic use of pyridostigmine should be discontinued in the presence of nerve agent poisoning, as it may exacerbate symptoms in certain exposures.

Chronic Toxicity (or Exposure)

Animal

Chronic administration of therapeutic levels of pyridostigmine in mice did not demonstrate alterations in heart rate and blood pressure. Long-term carcinogenicity studies have not adequately evaluated carcinogenicity of pyridostigmine in animals.

Human

Initial and long-term follow-up found that veterans of the first Persian Gulf War reported various, unexplained symptoms termed 'Persian Gulf War Syndrome'. It is characterized by chronic fatigue, ataxia, impaired cognition, weakness, incontinence, myoneuropathy, and adenopathy. Although prophylactic use of pyridostigmine has been suspected as the causative agent (see Uses above), this syndrome has not been noted in patients with myasthenia gravis using pyridostigmine in their treatment regimen. It has been proposed, but not proven, that the combination of pyridostigmine, combustion products of pesticides, insect repellants, and post-traumatic stress disorder may be responsible for the Persian Gulf War Syndrome.

In Vitro Toxicity Data

Pyridostigmine was mutagenic in mouse lymphoma cells with metabolic activation.

Clinical Management

Symptoms of toxicity should be managed to alleviate cholinergic symptoms, with special attention to respiratory support. Atropine and pralidoxime may be needed. In a military setting, symptoms should be distinguished from nerve agent poisoning to provide proper treatment.

Potential interactions of drugs in the clinical setting for consideration include mefloquine (antimalarial), narcotics, aminoglycoside antibiotics, anesthetics, and succinylcholine. Medical conditions that warrant precaution with the use of pyridostigmine include: glaucoma, bronchial asthma and obstructive lung disease, and cardiac arrhythmias. Allergic reactions may occur in persons with bromide sensitivity.

See also: Anticholinergics; Cholinesterase Inhibition; Nerve Agents; Organophosphates; Soman.

Further Reading

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Goldfrank L, Flomenbaum N, Lewin N, *et al*. (eds.) (2002) *Goldfrank's Toxicologic Emergencies*, 7th edn. New York: McGraw-Hill Medical Publishing Division.

Klaassen C (ed.) (2001) *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.

Relevant Website

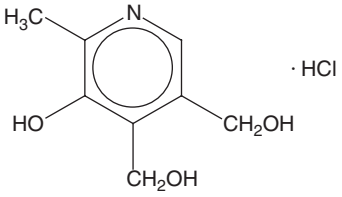
www.va.gov – Vetran's Administration (VA); search for Pyridostigmine.

Pyridoxine

Diana Ku

This article is a revision of the previous print edition article by Denise L Kurta, volume 2, pp. 612–613, © 1998, Elsevier Inc.

- Chemical Name: Pyridoxine
- Chemical Abstracts Services Registry Numbers: CAS 58-56-0; CAS 65-23-6
- Synonyms: Vitamin B_6; Pyridoxal; Pyridoxamine; Adermine hydrochloride; 3,4-Pyridinedimethanol; 5-Hydroxy-6-methyl hydrochloride
- Chemical/Pharmaceutical/Other Class: Water-soluble vitamin
- Chemical Formula: $C_8H_{12}ClNO_3$
- Chemical Structure:



Uses

Pyridoxine is a nutritional supplement used for the prophylaxis or treatment of pyridoxine deficiency resulting from conditions such as severe diarrhea, malabsorption, congenital metabolic dysfunction, hyperthyroidism, renal and hepatic disease, congestive heart failure, alcoholism, drug-induced conditions, and during pregnancy and lactation. Pyridoxine-dependent syndromes including pyridoxine-dependent seizures in infants, homocystinuria, pyridoxine-responsive anemia, and hyperoxaluria may require the clinical use of pyridoxine as well. Pyridoxine is also used as an antidote for isoniazid, hydrazine, and ethylene glycol toxicities.

Background Information

Pyridoxine deficiency was first identified in 1926; however, it was erroneously attributed to vitamin B_2. Ten years later, the active form of pyridoxine was identified and named vitamin B_6 (pyridoxal-5-phosphate, PLP).

Exposure Routes and Pathways

Routes of exposure are oral, intravenous, and intramuscular. Dietary sources of pyridoxine include bananas, potatoes, eggs, lentils, legumes, cereals, chicken, liver, and kidneys. Cooking destroys some amount of the vitamin.

Toxicokinetics

Pyridoxine is readily absorbed from the gastrointestinal tract mainly in the jejunum by passive diffusion. It is hepatically metabolized and stored mainly in the liver, muscle, and brain. Volume of distribution and protein binding are both low. The plasma half-life is 1.7 h and the biological half-life is 15–20 days. Pyridoxine is excreted renally almost entirely as metabolites. Excess amounts of pyridoxine (beyond daily needs) are excreted unchanged in the urine.

Mechanism of Toxicity

The exact mechanism of pyridoxine-induced neurotoxicity has not been established but may occur at the dorsal root and sensory ganglion.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity is not expected in animals.

Human

Acute toxic effects are not expected; however, a case report of a husband and wife who were mistakenly administered a single, large, intravenous dose ($2\,g\,kg^{-1}$) of pyridoxine shows that it resulted in permanent dorsal root and sensory ganglia deficits. Allergic reactions to the use of pyridoxine have also been reported.

Chronic Toxicity (or Exposure)

Animal

It would be unlikely for animals to be given a chronic pyridoxine overdose.

Human

Chronic doses of 200–6000 mg daily for several months may cause severe sensory neuropathy, ataxia, incoordination of hands, weakness, and paresthesias. Seizure and death have been reported with extremely large intravenous doses of pyridoxine.

In Vitro Toxicity Data

The fetus has the ability to concentrate pyridoxal phosphate 6.6 times above the maternal plasma concentration. Administration of larges doses of pyridoxine during pregnancy resulted in an infant with a requirement for supplemental vitamin B_6. In a case report, a woman administered $50\,mg\,day^{-1}$ for the

first 7 months of pregnancy was associated with an infant with phocomelia.

Clinical Management

Acute and chronic ingestions should be discontinued and any toxic effects treated symptomatically. A study on healthy volunteers reported neurotoxic symptoms to progress for 2–3 weeks upon discontinuation of pyridoxine.

See also: Ethylene Glycol; Folic Acid; Hydrazine; Isoniazid; Pyridoxine; Riboflavin; Thiamine.

Further Reading

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Pyriminil

Lynn Weber

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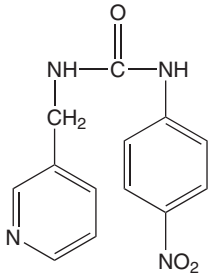
- Chemical Abstracts Service Registry Number: CAS 53558-25-1
- Synonyms: 1-(3-Pyridylmethyl-3-(4-nitrophenyl) urea; DLP-87; DLP-787; PNU; Pyrinuron; Vacor; RH-787
- Chemical/Pharmaceutical/Other Class: Substituted phenylurea
- Chemical Structure:



Uses

Pyriminil was first introduced in 1975 as a rodenticide to control rats and house mice. It is especially effective against rodents resistant to anticoagulant poisons. Pyriminil was marketed for indoor use only in the form of bait and tracking powder. Its use has been banned in the United States.

Exposure Routes and Pathways

Oral and dermal routes of exposure are possible.

Toxicokinetics

Pyriminil is absorbed from the gastrointestinal tract. Following absorption, pyriminil is distributed in the body and undergoes metabolism in the liver by cytochrome P450 1A-dependent monooxygenases. Different metabolites have been identified in the urine of poisoned rats, dogs, and humans. These metabolites include pyriminilglucuronide, aminopyriminil, acetamidopyriminil, *p*-aminophenyl urea, *p*-acetamidophenyl urea, *p*-nitroaniline, *p*-phenylenediamine, *p*-acetamidoaniline, nicotinic acid, nicotinuric acid, and nicotinamide. Dogs develop tolerance to pyriminil, which may be partially attributed to enhanced hepatic detoxification and excretion.

Mechanism of Toxicity

Pyriminil toxicity occurs primarily because it inhibits NADH:ubiquinone oxidoreductase activity of complex I in mammalian mitochondria resulting in preferential toxicity to high-energy-demanding cells such as nerves and pancreatic β-cells. However, pyriminil may also act as a nicotinamide antagonist and interfere with the synthesis of NADH/NADPH, furthering neural and β-cell toxicity. Inhibition of mitochondrial respiration in nerves causes somatic, autonomic, and central nervous system neuropathies while inhibition in β-cell causes an immediate, irreversible insulin-dependent diabetes mellitus condition. Pyriminil also acts as a noncompetitive inhibitor of rat acetylcholinesterase.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, the LD_{50} is very low ($\sim 5\,mg\,kg^{-1}$). Mice and cats are also relatively sensitive to pyriminil toxicity with LD_{50} values of 98 and $62\,mg\,kg^{-1}$, respectively. Other species are markedly less sensitive (LD_{50} values from 0.5 to $4\,g\,kg^{-1}$). A horse was reported to show

severe muscle fasciculations, sweating, dilated pupils, and tachycardia following ingestion of 0.25–0.5 kg of pyriminil. Other signs of toxicity in horses include colic, hind limb weakness, ataxia, and persistent loss of appetite. Pyriminil intoxication in other animals causes gastrointestinal disorders (e.g., vomiting and abdominal cramp), visual problems, cardiovascular disorders, ataxia, tremor, and coma.

Human

Pyriminil appears highly toxic to humans. The lowest acute toxic dose of pyriminil in humans was estimated at 5 mg kg^{-1}. Ingestion of one-half of one 39 g packet of Vacor (2% pyriminil) reportedly led to a fatality. A seven year-old child was found dead 1 day after ingesting one packet of Vacor (2% pyriminil). In another case, two of nine people died after ingestion of 39 g of Vacor; the remaining people developed chronic hypotension and permanent diabetes mellitus. Generally, the symptoms of acute poisoning were characterized by rapid onset of ketoacidosis-prone diabetes mellitus, severe orthostatic hypotension, autonomic dysfunction, autonomic neuropathy (dysphagia, impotence, urinary retention, constipation, or diarrhea), and peripheral neuropathy. Other symptoms included nausea, vomiting, abdominal cramp, diffuse myalgias, polyuria, polydipsia, dyspnea, malaise, and general weakness. Peripheral sensory and motor neuropathies are possible signs of pyriminil exposure. Neurological effects of pyriminil can occur within hours of ingestion and may persist for months.

Clinical Management

Because pyriminil can cause early onset seizures, induction of emesis is contraindicated. Gastric lavage may be useful, if performed soon after ingestion. Activated charcoal/cathartic therapy may be adopted to retard the absorption of pyriminil from the gastrointestinal tract. According to US Food and Drug Administration guidelines, 240 ml of diluent may be mixed with 30 g of charcoal. The usual charcoal dose is 30–100 g in adults and 15–30 g in children (1 or 2 g in infants).

Conventional anticonvulsants (e.g., diazepam, phenobarbital, and phenytoin) may be administered to treat pyriminil-induced seizures. Niacinamide has been demonstrated to be an effective antidote in pyriminil poisoning in rats but little information is available regarding its antidotal efficacy in humans. Insulin therapy could be instituted as a preventive measure for possible diabetes mellitus. Orthostatic hypotension due to pyriminil exposure may be treated with conventional mineralocorticoids.

See also: Pesticides.

Further Reading

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Pyrrolizidine Alkaloids

Gerardo Ibanez

- Chemical Abstracts Service Registry Number: CAS 643-20-9
- Synonyms: 1-Azabicyclo(3.3.0)octane; 1*H*-Pyrrolizine; Hexahydropyrrolizine
- Chemical/Pharmaceutical/Other Class: Alkaloid
- Chemical Formula: $C_7H_{13}N$

Background Information

Pyrrolizidine alkaloids (PAs) constitute a class of plant toxin associated with disease in humans and animals. They are found in a wide variety of plant species in the world and it is estimated that ~3% of the world's flowering plants contain toxic pyrrolizidine alkaloids. The toxin is present in more than 12 higher plant families, among which three families, Compositae (Asteracea), Boraginaceae, and Leguminosae (Fabaceae), contain most toxic PAs. The wide distribution of plants containing PAs around the globe makes it difficult to prevent human and animal exposure and every year animals and people suffer from acute and chronic PA exposures.

In 1920, there was a large outbreak of food poisoning in South Africa. This incident was due to the contamination of wheat flour with toxic PAs. Also, large outbreaks have been reported in Afghanistan, India, and the former USSR. These large outbreaks were possible because PA-containing plants grow in climatic conditions in which food sources such as wheat are usually grown.

In the last decade, dietary supplement consumption has increased in Europe and the United States. This has prompted regulatory agencies to enact

regulations to protect the health of consumers. In 1992, the Federal Health Department of Germany restricted the manufacture and use of pharmaceuticals containing PAs with an unsaturated necine skeleton. Also, in 1994, the US Congress passed the Dietary Supplement Health Education Act (DSHEA), which amended the US Federal Food, Drug, and Cosmetic Act (FFDCA) and created a new regulatory category for the Food and Drug Administration (FDA) to regulate dietary supplements. Furthermore, in 1997, the US FDA published Good Manufacturing Practice (GMP) regulations that manufacturers of herbal products must follow. These regulations are meant to improve the quality of dietary supplements and minimize the risk of poisoning due to the presence of PAs in dietary supplements.

Exposure Routes and Pathways

The main route of exposure of humans and animals to PAs is the oral pathway. Human exposure occurs through consumption of food contaminated by toxic plant products or by the ingestion of herbal medicines containing the toxin. PAs have been found in wheat, milk, honey, herbal medicines, and herbal teas at different concentrations. Livestock exposure to PAs is attributed to the consumption of PA-containing plants while grazing.

Toxicokinetics

Upon ingestion, PAs are absorbed mainly through the small intestine and are carried to the liver. The most hydrophobic PAs are readily excreted unchanged in the urine within a 24 h period while less hydrophobic PAs are metabolized by cytochrome P450s and flavin monooxygenase enzymes. The metabolites are eliminated as soluble glutathione and other conjugates in the bile and urine.

Mechanism of Toxicity

The target organ for PA toxicity in experimental animals and humans is the liver. PAs cause liver toxicity and venoocclusive disease. The mechanism of hepatotoxicity has been extensively investigated and it is well established that metabolic activation of PAs to reactive metabolites is responsible for causing liver toxicity. In animals, PAs exhibit a large variety of genotoxic effects, including DNA binding, DNA cross-linking, DNA protein cross-linking, sister chromatid exchange, chromosomal aberrations, mutagenicity, and carcinogenicity. However, these effects have not been observed in humans.

Acute and Short-Term Toxicity (or Exposure)

Animal

In acute toxicity, extensive hemorrhagic necrosis of the liver is observed, which results in the death of the animal. There is conclusive evidence from studies on experimental animals that the effects of a single exposure to PAs may result in liver disease and may lead to cirrhosis of the liver. Also, animal susceptibility to PA poisoning varies among animal species depending on how fast they metabolize the parent compound. Animals more resistant to PAs, such as sheep, hamsters, and rabbits, have a higher capability toward metabolizing PAs, whereas susceptible species such as cattle, chickens, and rats have a low capability toward metabolizing the parent compound.

Human

In humans, acute poisoning causes severe liver toxicity with hemorrhagic necrosis. Acute human cases following the brief ingestion of PAs have been known to progress to cirrhosis. The most common signs of acute PA poisoning are lassitude, anorexia, nausea, vomiting, diarrhea, edema, emaciation, hepatomegaly, splenomegaly, and jaundice.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure results in a number of sublethal effects on the liver, eventually leading to venoocclusive disease. The signs of chronic PA poisoning are sluggishness, loss of appetite, wasting ascites, jaundice, photosensitization, and behavioral changes. Also, chronic exposure to PAs leads to cancer in experimental animals.

Human

Chronic exposure to PAs results in hepatic damage and venoocclusive disease. Chronic poisoning takes place mainly in the liver, lungs, blood vessels, and, in some instances, kidneys, pancreas, gastrointestinal tract, bone marrow, and the brain. Some common signs of chronic exposure to PAs are cell enlargement (megalocytosis), venoocclusion in liver and lungs, fatty acid degradation, nuclei enlargement with increasing nuclear chromatin, loss of metabolic function, inhibition of mitosis, fatty acid degeneration, proliferation of biliary tract epithelium, liver cirrhosis, and hyperplasia. Liver failure due to cirrhosis and venoocclusive disease may occur months to years after the last episode of PA exposure.

Clinical Management

There is no known method to prevent PA liver damage once a hepatotoxic dose of the alkaloid has been ingested. Reversibility of chronic damage is uncertain and unpredictable. In man, it is reported that following a poisoning outbreak in which significant acute toxicity is observed, ~50% of patients will recover completely and 20% will die rapidly. Of the survivors, ~20% will appear to recover clinically but may go on to develop cirrhosis and liver failure years later. There is no clinical treatment for venoocclusive disease and the prognosis depends on the extent of damage and whether exposure to PAs recurs.

See also: Belladonna Alkaloids; Liver; Plants, Poisonous.

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Q

QT Interval

Russell Barbare

Definition

The QT interval is a measure of the portion of time in a heartbeat starting with depolarization of the ventricles and ending with their repolarization. It is called this because standard electrocardiogram (ECG) notation uses the letters P through U to indicate various phases of the heartbeat, with the QRS complex being various stages of ventricular depolarization and T used to designate the period of repolarization (**Figure 1**). Depolarization is a lowering of the electrical potential that is normally maintained across nerve and muscle cell membranes and repolarization is the reestablishment of that potential. These changes cause various phases of the heartbeat and are caused by the exchange of calcium, potassium, sodium, and chloride ions through specific channels across the cell membranes.

Significance

The QT interval is important because notable change in it, most especially lengthening, is the most accepted indicator of potentially fatal cardiac arrhythmias. For example, excessive extension of the QT interval is strongly associated with ventricular arrhythmias including Torsade de Pointes (TdP), an acceleration in cardiac rhythm that can lead to heart attack. The root causes of heart rate and rhythm abnormalities are diverse, complex, and often difficult to evaluate. Since QT interval changes can be checked using portable, noninvasive techniques and are associated with a wide variety of cardiac problems they are a valuable indicator of cardiac function. The degree of QT interval prolongation is generally proportional to the risk.

Risk Factors

Risk factors for QT interval prolongation include genetic factors, medical history, metabolic imbalances, age, and use of pharmaceuticals. Genetically, females

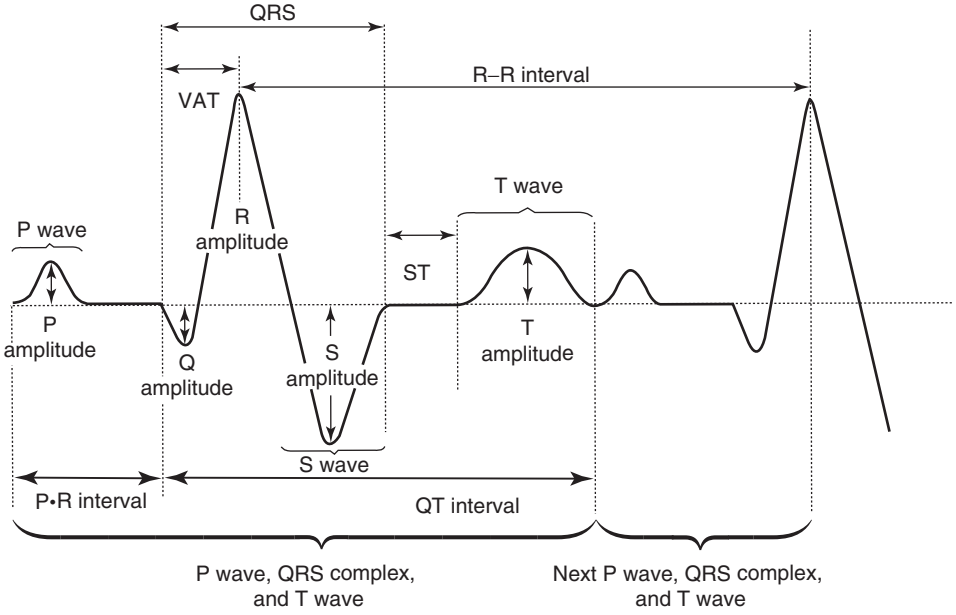

Figure 1 A typical ECG signal consists of the P wave, QRS complex, and the T wave. The time between the start of the QRS complex and the end of the T wave is called the QT interval.

are twice as likely to show prolongation as males and congenital long QT syndrome, which is actually one of six genetic defects in cellular ion channels, affects ~1 in 5000 people independent of their gender. Factors in medical history that raise the likelihood are incidences of cardiac disease, central nervous system trauma, and liver disorders. Metabolic imbalances that are risks include electrolyte imbalances, endocrine disorders, and diabetes. Using two or more QT interval-prolonging drugs together raises the chance of heart arrhythmias as well as using a QT-altering drug with another that changes the metabolism of that drug.

Measurement Standardization

Intersubject QT intervals vary noticeably, with a standard deviation of ~40 ms, or over 10% of the total. Single subject standard deviations are around 10 ms, which is still over 2.5% of the total. This is partly because the heart's rhythm is partially a hysteresis loop (i.e., previous patterns affect successive patterns) and also because of the influence by many other factors, including heart physiology, body electrolyte balance, genetics, disease, age, nutrition, and exercise.

The QT interval has an inverse relationship to the heart rate, so as the heart rate increases, QT interval decreases. The changes are not proportional to the change in heart rate, so QT measurements are often standardized by various formulas to the corrected QT interval, or QTc interval. Unfortunately, there is no standard formula that is used for the correction. Over thirty corrections have been proposed and several, including Bazette's, Fridericia's, and Van de Water's are in common use. In an ECG, both the QT interval and the QTc interval are usually examined for abnormalities.

There is also some debate as to how to interpret the exact start and end points of the various cardiac phases from an ECG. The actual changes are based on altered ionic balances across cell membranes, which move in cyclic patterns through the heart tissue and are manifested externally as minute changes in skin electrical potential. An ECG uses multiple leads to pick up the pattern of changes and so is an indirect observation that must be interpreted by a professional.

Changes in the QT interval are best interpreted using multiple measurements over time and should include the baseline or prechange observations, but data that extensive and accurate are expensive to obtain and hard to get without a controlled environment. There are portable products that digitally capture heartbeats but they have not been fully accepted by the various regulatory agencies and the data still require professional interpretation.

Various organizations, including the Food and Drug Administration (FDA), the International Conference for Harmonization, and the Committee for Proprietary Medical Products have all published guidelines for interpretation of QT interval changes. Due to the personal variability in QT interval and the uncertainties of measurement and interpretation, these are in the form of strong recommendations rather than absolutes. For example, the FDA recommends looking at both absolute QT interval length and changes from the baseline as indicators of concern in the testing of new drugs prior to any introduction into humans. In their standards, the highest levels of risk are indicated by absolute lengths greater than 500 ms, individual increases from baseline greater than 60 ms, or mean group changes from baseline greater than 20 ms.

Drugs Affecting the QT Interval

Since cardiac potential is controlled by the exchange of Ca^{2+}, K^+, Na^+, and Cl^- ions across the cellular membrane, any drugs altering the action of these ion channels directly or indirectly (for example, causing altered metabolism of another drug) can cause QT prolongation. All drugs withdrawn for TdP so far have shown inhibiting effects on the rapid potassium channel (I_{Kr}) and there are suggestions that many other drugs that cause QT interval prolongation affect K^+ exchange. This makes potassium flow inhibition the most studied basis for drug-related QT changes but not the only possible cause.

There are several classes of drugs that are specifically designed to be antiarrhythmics, to regularize or control the heart beat, and will therefore almost certainly affect the QT interval. These include over forty compounds in subclasses based on their effects on ion channels (Na, Ca, or K) or receptors (α-adrenergic, β-adrenergic, cholinergic, or adenosinergic). The mechanisms of action vary considerably and most have multiple effects so a compound designed to correct one problem might cause another.

Over 130 nonantiarrhythmic drugs now on the market have published or unpublished evidence of prolongation and ~60 of those have official warnings attached, but the same compound may have different warning levels in different countries. In one multicountry study of drugs dispensed from pharmacies, an estimated 13–20 doses per 1000 persons per day had the potential to lengthen the QT interval. Most of the drug withdrawals from marketplace since 1990 have been due either to

Table 1 Nonantiarrhythmic drugs associated with QT prolongation

Class name	*Reports*[a,b]	*Class name*	*Reports*[a,b]	*Class name*	*Reports*[a,b]
GI prokinetics		Opioids		Antiasthmatics	
Cispride	P, N, O	Levacetylmethadol	O	Fenoterol	P, O
Domperidone	*P, N*	Methadone	*N*	Procaterol	P
		Pethidine	*N*	Salbutamol	P, O
				Salmaterol	P, O
Antiemetics		Antimigraine agents			
Dolasetron	P, N, O	Naratriptan	O	Antihistamines	
Granisetron	P, N	Sumatriptan	O	Astemizole	P, N, O
Ondansetron	P, N, O	Zolmitriptan	O	Azelastine	*O*
				Cetirizine	N
Cardiovascular Drugs		Antipsychotics		Chlorpheniramine	*N*
Bepridil	P, N, O	Amisulpride	*P, O*	Clemastine	*N*
Diltiazem	N	Chlorpromazine	P, N, O	Cyproheptadine	*N*
Indapamide	P, N	Clozapine	P, N	Diphenhydramine	*P, N*
Indoramin	*P*	Droperidol	P, N, O	Ebastine	P, N
Isoprenaline	P, N	Haloperidol	P, N, O	Emedastine	*O*
Isradipine	P, N, O	Mesoridazine	*O*	Epinastine	*P, N*
Ketanserin	P, N	Olanzapine	*P, N, O*	Fexofenadine	P
Lidoflazine	N	Pimozide	P, N, O	Loratidine	N
Losartan	*N*	Prochlorperazine	*P, O*	Mizolastine	P, N, O
Methoxamine	*P, N, O*	Quetiapine	*P, O*	Oxatomide	*P*
Mibefradil	O	Risperidone	N, O	Promethazine	*P, N*
Nicardipine	*N*	Sertindole	P, N, O	Pyrilamine	*N*
Perhexiline maleate	*P, N*	Sultopride	*P, N, O*	Terfenadine	P, N, O
Prenylamine	*P, N*	Thioridazine	P, N, O		
Triamterene	P	Tiapride	*P*	Miscellanea	
Trimetaphan	*P, N, O*	Trifluoperazine	*N*	Amantadine	*P*
Verapamil	N	Ziprasidone	P, O	Antimony sodium gluconate	*P*
Vincamine	*P*	Zotepine	*O*	Arsenic trioxide	P, O
				Bupropion	*P*
Antibacterials		Antidepressants		Chloral hydrate	*P*
Clarithromycin	P, N	Amitryptyline	P, N, O	Dexfenfluramine	*N*
Clindamycin	*P*	Citalopram	*P, N*	Famotidine	*P*
Cotrimoxazole	*P, N*	Clomipramine	*P*	Felbamate	*O*
Erythromycin	P, N, O	Desipramine	P, O	Fenoxedil	*P*
Gatifloxacin	N, O	Doxepin	P, N	Foscarnet	*O*
Grepafloxacin	P, N, O	Fluoxetine	*P, O*	Fosphenytoin	*O*
Levofloxacin	P, N	Imipramine	P, N	Glibenclamide	*P, N*
Moxifloxacin	P, N, O	Maprotiline	*P*	Hydroxazine	*P, N*
Roxithromycin	P, N	Mianserin	*P, N*	Mitoxantrone	*N*
Sparfloxacin	P, N, O	Nortriptyline	P, N	Octreotide	*O*
Spiramycin	*P*	Paroxetine	*P*	Papaverine	*P*
		Protriptyline	*P*	Pentamidine	P, O
Systemic Antimycotics		Trazodone	*P*	Probucol	P, O
Fluconazole	P	Vanlafaxine	*O*	Radiographic contrast media	P, N
Ketoconazole	P, N	Zimeldine	*P, N*	Ritanserin	*P*
				Sildenafil	*N*
Agents used in general anaesthesia		Antimalarials		Tacrolimus	P, N
Enflurane	P, N	Chloroquine	P	Tamoxifen (high doses)	P, N, O
Fentanyl	*N*	Halofantrine	P, N, O	Terodiline	P, N
Halothane	P, N	Mefloquine	P, O	Tizanidine	*O*
Isoflurane	P, N	Quinine	P, O	Vasopressin	*P*
Ketamine	*N*			Vesnarinone	*N*
Pentobarbital	*N*				
Propofol	P, N				
Sevoflurane	P, N				
Sufentanil	P, N				
Thiopental	P, N				

[a] Italics indicate poorly documented evidence.

[b] P, published clinical evidence; N, published nonclinical evidence; O, official warning (from Public Assessment Reports by the European Agency for the Evaluation of Medicinal Products, the Physician Desk Reference, Dear Doctor letters from the FDA, and the British Formulary.

From information compiled by Fabrio De Ponti, Elisabetta Poluzzi, Andrea Cavalli, Maurizio Recanatini, and Nicolo Montanero.

indications of QT interval prolongation or to actual cardiac problems. Categorization shows the compounds belong to many different pharmacological classes and the mechanisms of action are diverse. The compounds themselves must be examined on an individual basis; some have QT prolongation and associated cardiac-related fatalities (terfenadine/Seldane®) while some had noticeable prolongation without any associated fatalities (moxifloxacin/Avelox®). Validated reports show that QT prolongation or cardiac arrhythmia with these other implicated compounds most often happen in combination with other compounds that also increase the risk.

The time it takes for a compound to affect the QT interval or to cease its effects upon discontinuation depends greatly on the compound. For example lidocaine and amiodarone are both drugs with antiarrhythmic properties but lidocaine's effects on the QT interval start within minutes when administered intravenously and end in a few hours as the drug clears the body, while the effects of amiodarone generally take 1–3 weeks to manifest and as long (or longer) to subside when discontinued.

QT interval testing is included in the trials of most new drugs entering the marketplace, with exceptions being mostly for compounds closely related to known compounds with no effect. The considerations for risk include the chemical and/or pharmacological class, an ion channel assay, an *in vivo* QT assay in a nonrodent species, and a repolarization assay or other follow-up studies as warranted. Despite extensive testing and consideration, it is often not clear in clinical studies whether a compound may have significant cardiotoxicity, first because the numbers used in clinical trials are often too low for the needed statistical power, and second because clinical trials do not involve the drug interactions usually associated with adverse events.

Summary

Examination of the QT interval is an important part of studies of cardiac health because of its ability to indicate potentially fatal cardiac arrhythmias before they happen even though the causes of the arrhythmias are diverse. Due to this diversity and significant normal variations in the QT interval, there is no generally accepted exacting standard of measurement or interpretation of changes in the QT interval but there are several guidelines that are in general agreement. The drug industry and its regulators regularly examine the potential of candidate drugs and released compounds to alter the QT interval.

Drugs Withdrawn for Correlation with Torsade de Pointes

Terfenadine/Seldane® (antihistamine, February 1998), Cisapride/Propulsid® (GI prokinetic, July 2000), Grepafloxacin/Raxar® (antibiotic, November 1999), Sertindole (antipsychotic, December 1998), and Astemizole (antihistamine, June 1999). See **Table 1** for a list of nonantiarrhythmic drugs associated with QT interval prolongation.

See also: Digitalis Glycosides; *hERG* (Human Ether-a-Go-Go Related Gene); Potassium; Safety Pharmacology; Sodium.

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http://www.hc-sc.gc.ca – The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrythmic drugs. US Food and Drug Administration's Center for Drug Evaluation and Research (CDER) and Health Canada's Health Product and Food Branch (HPFB).

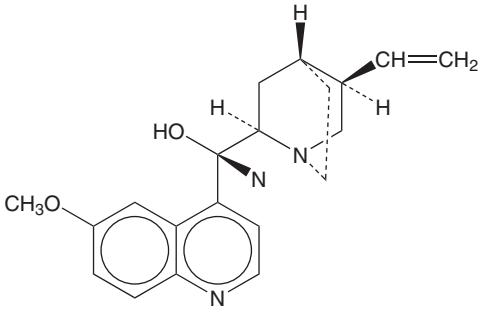
Quantitative Structure–Activity Relationship *See* Toxicity Testing, Modeling.

Quinidine

Dennis J Naas

This article is a revision of the previous print edition article by Bridget Flaherty, volume 3, pp. 2–3, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 56-54-2
- Synonyms: Cin-Quin; Quinidine sulfate; Quinidine gluconate (CAS 7054-25-3); Quinidine polygalacturanate (CAS 27555-34-6); Quinaglute; Chinidin; Cinchonan-9-ol, 6′-methoxy-; Conchinin; Conquinine; (9*s*)-6′-Methoxycinchonan-9-ol; α-(6-methoxy-4-quinolyl)-5-vinyl-2-quinuclidinemethanol; NCI-c56246; Pitayine; *β*-quinine; Quinidine hydrate (CAS 63717-04-4); Quinidex
- Chemical/Pharmaceutical/Other Class: Class IA (membrane stabilizing) antiarrhythmic agent; The dextrorotary isomer of quinine
- Chemical Structure:



Uses

Quinidine is used to treat and control atrial fibrillation and atrial flutter. Quinidine is also approved to treat premature ventricular contractions and to treat paroxysmal atrial tachycardia or paroxysmal atrioventricular junctional rhythm. It may also be used to treat malaria, although quinine is preferred.

Exposure Routes and Pathways

Ingestion is the most common route of exposure in both accidental and intentional poisonings. Quinidine is also available in intravenous and intramuscular forms.

Toxicokinetics

Quinidine undergoes extensive hepatic oxidative metabolism. The bioavailability of quinidine is up to 90%. Peak plasma effects occur in 1–3 h. Sustained-release preparations produce peak plasma levels in 5 or 6 h. Quinidine is up to 90% protein bound, but it is lower in pregnant women, and in infats and neonates it may be as low as 50–70%. The volume of distribution is 2 or $3\,l\,kg^{-1}$. Congestive heart failure can lower the volume of distribution to $0.5\,l\,kg^{-1}$ while in patients with cirrhosis of the liver this can be increased to 3 or $5\,l\,kg^{-1}$. Up to 80% of quinidine undergoes hepatic hydroxylation. The remainder ($\sim$20% of a therapeutic dose) is eliminated unchanged in the urine. Quinidine generally has a plasma half-life of 6–8 h in healthy individuals, but half-life may range from 3 to 16 h or longer. Longer half-lives are reported for malaria patients and those with chronic liver disease. Quinidine crosses the placenta and is distributed into milk.

Mechanism of Toxicity

Quinidine has direct and indirect, or antimuscarinic, effects on cardiac tissue. Quinidine decreases myocardial excitability, conduction velocity, and contractility. As quinidine concentrations increase, conduction velocity progressively decreases. This is evident in an increase in PR interval, an increase in QRS duration, and an increase in QT interval. The effective refractory period is prolonged by quinidine. The anticholinergic effect on the heart is a decrease in vagal tone. In overdose, sinus node automaticity may be depressed. It is the α-adrenergic blocking properties of quinidine that cause vasodilatation and hypotension.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute overdosage can result in both cardiovascular and neurologic effects. Ventricular dysrhythmias and hypotension are the most serious toxicities. Cardiac effects occur as a result of myocardial depression and depression of atrial, atrioventricular, and ventricular conduction. EKG changes will be evident. These EKG changes include a widening of the QT, PR, and QRS complexes; ST depression; and T inversion. Myocardial depression and vasodilation can cause hypotension to develop. Syncope can result from transient Torsade de Pointes (i.e., bursts of atypical ventricular tachycardia). Ventricular tachycardia and ventricular fibrillation may develop. Possible central nervous system (CNS) effects include lethargy, seizures, and coma. Other acute effects can include apnea. Signs of toxicity are expected to occur in

adults ingesting a gram or more. Therapeutic plasma levels of quinidine range from 1 to 4 $\mu g\,ml^{-1}$. Cardiac toxicity can occur with levels of at least 14–16 $\mu g\,ml^{-1}$.

Chronic Toxicity (or Exposure)

Human

With chronic toxicity, gastrointestinal symptoms are common. Nausea, vomiting, and diarrhea are generally seen. The toxidrome known as cinchonism can occur in chronic toxicity. Effects include headache, fever, visual disturbances, mydriasis, decreased hearing or tinnitus, nausea, vomiting, hot flushed skin, rash, and CNS impairment (lethargy, memory impairment, delirium, hallucinations) and may present without cardiotoxicity, other than QT prolongation. Cinchonism can occur when quinidine plasma levels are at least 5 $\mu g\,ml^{-1}$. Loss of vision can occur when levels are at least 10 $\mu g\,ml^{-1}$.

Clinical Management

Basic and advanced life-support measures should be used as needed. Induction of emesis is not recommended due to the potential for a decreased level of consciousness, seizures, and arrhythmias. Gastric lavage followed by activated charcoal is recommended. Repeated doses of activated charcoal may enhance elimination. Serum electrolytes should be monitored in all serious exposures. Intravenous administration of sodium bicarbonate may decrease toxicity. Hypotension can be treated with fluids and vasopressors if needed. Ventricular dysrhythmias can be treated with class IB antiarrhythmics such as phenytoin or lidocaine. Persistent bradycardia and third-degree heart block are indications for insertion of a temporary pacemaker. Seizures can be treated with diazepam. If seizures are uncontrolled, phenobarbital or phenytoin can be administered.

See also: Cardiovascular System; Quinine.

Further Reading

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Goldfrank L, Flomenbaum N, Lewin N, Howland M, and Nelson L (eds.) (2002) *Goldfrank's Toxicologic Emergencies*, 7th edn. New York: McGraw-Hill Professional.

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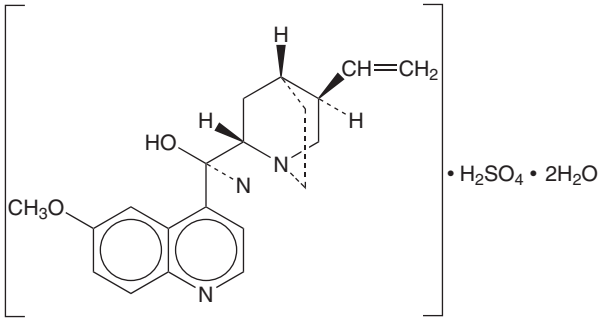
Klaassen C (ed.) (2001) *Cassarett and Doull's Toxicology, The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill Professional.

Quinine

Dennis J Naas

This article is a revision of the previous print edition article by Linda Hart, volume 3, pp. 3–4, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 130-95-0
- Synonyms: Quinine sulfate (preferred name); Chininum; Quinina; Quinine bisulfate; Chinine; Cinchonan-9-ol, 6′-methoxy-; (8α,9r), chinin; 6′-Methoxycinchonan-9-ol; 6-Methoxycinchonine; α-(6-Methoxy-4-quinolyl)-5-vinyl-2-quinuclidinemethanol; α-(6-Methoxy-4-quinoyl)-5-vinyl-2-quinclidinemethanol; 6-Methoxy-α-(5-vinyl-2-quinuclidinyl)-4-quinolinemethanol; 2-Quinuclidinemethanol; α-(6-Methoxy-4-quinolyl)-5-vinyl
- Chemical/Pharmaceutical/Other Class: Antimalarial
- Chemical Structure:



Uses

Quinine is the drug of choice for the treatment of malaria; it is also used in the treatment of nocturnal leg cramps. It is often misused as an abortifacient.

Exposure Routes and Pathways

Quinine is available in oral dosage forms. Ingestion is the most common exposure pathway.

Toxicokinetics

Quinine is rapidly absorbed orally. It is metabolized in the liver by oxidation to several polar hydroxy metabolites. The volume of distribution is 1 or $2\,l\,kg^{-1}$ and protein binding is 70%, although plasma binding of 90% or more has been reported in malaria patients. Quinine is excreted by the kidneys; ~10% is excreted as unchanged drug. The therapeutic half-life of quinine is 11.1 ± 4.1 h, and may be longer in malaria patients due to hepatic impairment. The half-life can more than double at toxic doses to 26.5 ± 5.8 h.

Mechanism of Toxicity

The exact mechanism of toxicity is unknown. Quinine acts on all body muscle groups, most notably cardiac, uterine, and skeletal muscles.

Acute and Short-Term Toxicity (or Exposure)

Human

Accidental and intentional ingestions have resulted in headache, deafness, blindness, tachycardia, respiratory arrest, and death. Reversible renal failure can occur. The adult toxic dose can be as low as 2 g. Death from intentional or accidental overdose generally follows renal failure, acute hemolytic anemia, and respiratory arrest.

Chronic Toxicity (or Exposure)

Animal

Quinine has not been found to be carcinogenic in mammalian studies. Developmental effects were found when the drug was used in rabbits and guinea pigs, but not when drug was used in mice, rats, dogs, and monkeys.

Human

Cinchonism can arise from cumulative dosing. Clinical symptoms associated with the syndrome of cinchonism include headache, dizziness, tinnitus, rash, cardiovascular effects, intestinal cramping, vomiting, diarrhea, fever, confusion, and seizures. The symptoms resolve with cessation and elimination of the drug. Sensitization to quinine and quinidine have been observed (eczema, itching). Even at therapeutic dosages, an enhanced pseudohemophiliac effect can occur through the triggering of thrombocytopenia.

In Vitro Toxicity Data

There was no evidence of mutagenicity in animal studies in mice or *Salmonella typhimurium*; however, positive results were observed in studies in *S. typhimurium* when mammalian liver homogenate was added.

Clinical Management

Quinine should not be used during pregnancy. Recommended treatment includes gastric decontamination with gastric lavage and repeated doses of activated charcoal. Intensive monitoring of vital signs and the EKG are important. Quinine levels may be useful to confirm exposure. Following gastric lavage, the symptomatic therapy for acute poisonings includes atropine for bradycardia and phenytoin in the presence of tachycardic heart rhythm disorders. Forced diuresis and hemodialysis are not suitable as therapeutic measures. Monitor plasma and serum potassium levels. If refractory arrhythmia develops, assess calcium and magnesium. Other treatment may include administering a Stellate block for quinine-induced blindness and the use of vasodilators for residual visual impairment. Hemolytic–uremic syndrome following quinine ingestion is a newly described phenomenon. The reaction may be mediated by the presence of antibodies reactive against platelets in the presence of quinine. Treatment has included use of plasma exchange, prednisone, aspirin, and dipyridamole. Patients have all regained some degree of renal function. However, it is unclear whether pharmacological treatment or spontaneous resolution is responsible for the improvement.

See also: Chloramphenicol; Chloroquine.

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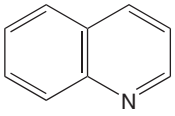
Quinoline

David R Wallace

- Chemical Abstracts Service Registry Number: CAS 91-22-5
- Synonyms: l-Azanaphthalene; l-Benzazine; Benzo(*b*)pyridine
- Chemical/Pharmaceutical/Other Class: Solvent and by-product of petroleum processing
- Chemical Formula: C_9H_7N
- Chemical Structure:



Uses

Quinoline is used as an intermediate in the production of quinoline-related compounds (e.g., 8-hydroxyquinoline). It is a solvent for resins and terpenes, and is used in the production of paint. Quinoline is also an antimalarial agent. Sources of quinoline include petroleum and coal processing, wood preservation and the use of shale oil.

Exposure Routes and Pathways

The major routes of exposure for quinoline are inhalation and oral ingestion. Contaminated air from petroleum distillation, coal mining, coking, and release from shale oil can lead to inhalation exposures. Quinoline is also found in cigarette smoke.

Toxicokinetics

Quinoline can undergo either detoxification (major pathway) or bioactivation (minor pathway). Less than 1% of administered quinoline is excreted unchanged in the urine.

Mechanism of Toxicity

Quinoline undergoes phase I metabolism to form an enamine oxide, a rapid transitional epoxide, which can then form DNA adducts. This epoxide is formed on the pyridine moiety of quinoline. Fluorination at position 3 completely prevents the mutagenicity of quinoline. The major metabolic enzyme is the CYP2E1 isoform with the primary end-product from this reaction being 3-hydroxyquinoline.

Acute and Short-Term Toxicity (or Exposure)

Available data have described acute or subchronic exposure. Due to higher mortality in long-term studies, little direct evidence is available to describe the actions of quinoline following chronic exposure.

Animal

Animals fed a diet that contained 0.05%, 0.1%, and 0.25% quinoline exhibit robust tumor formation and significant early mortality, which was concentration-dependent. Mean survival time decreased ~50% between the lowest and highest concentrations. Additional studies have confirmed these findings and extended them with the inclusion of proper control groups. There was an increase in the incidence of hepatic and lung tumors as well as hemangioendotheliomas. It is believed that these tumors are due to the formation of the reactive metabolic intermediate. Further investigation has shown that quinoline itself can act as a promoter of liver carcinogenicity. Treatment with quinoline in conjuction with the hepatic carcinogen diethylnitrosamine elicited significantly greater tumor formation compared to diethylnitrosamine treatment alone. Quinoline also appears to promote skin tumor formation.

The effects of quinoline on the central nervous system have also been examined. There is a structural similarity between the basic backbone of quinoline and the dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), but there was no evidence that quinoline was a dopaminergic neurotoxin.

Human

No acute studies of humans exposed to quinoline have been reported.

Chronic Toxicity (or Exposure)

There have been no studies of chronic quinoline effects which have exceeded 40 weeks in animals. These studies were terminated prior to this time due to the early identification of tumors and premature mortality. The studies described here relate to the carcinogenic effects of quinoline. No reports of chronic exposures in humans are available.

In Vitro Toxicity Data

In vitro mutagenicity of quinoline has been reported in *Salmonella typhimurium*, possibly through base

pair substitution. It has been determined that the cytochrome P450 monooxygenase system, particularly the CYP2E1 isoform, is responsible for the formation of the reactive epoxide intermediate. Recently, data have indicated that not only is quinoline a tumor-promoter, but is also an initiator of tumor formation. Quinoline appears to exert its toxic effects in both a genotoxic and mitogenic fashion.

Clinical Management

There are no antidotes for quinoline exposure. The patient should be removed from the source of quinoline exposure and symptomatic treatment given if necessary. Individuals exposed to quinoline may complain of severe eye irritation. Persons in occupations with possible exposure to quinoline should take measures to minimize their exposure.

Environmental Fate

If quinoline is released into water it will degrade dependent on the temperature and microbial conditions. Complete degradation can be expected to occur in less than a week. If ground soil is contaminated with quinoline, it will quickly partition to groundwater. Less than 0.5% of the quinoline will be expected to remain in the soil.

Exposure Standards and Guidelines

Quinoline has been labeled as a group B2 agent, 'probable human carcinogen, which is likely to be carcinogenic in humans based on animal data', due to significant evidence in animal models. Establishment of reference concentrations and allowable levels of exposure have been difficult to obtain due to the limited nature of animal studies (oral exposure, no inhalation studies). Computational modeling, using the Weibull model, yielded a potency of $3.07\,mg^{-1}kg^{-1}day^{-1}$ from an LED_{10} of $32.55\,\mu g\,kg^{-1}day^{-1}$. Animal experiments have suggested an oral LD_{50} of $331\,mg\,kg^{-1}$ in the rat and a dermal LD_{50} of $540\,mg\,kg^{-1}$ in rabbit.

See Also: Quinidine; Quinine.

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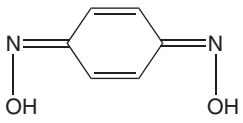
Saeki K, Kadoi M, and Kawazoe Y (1993) Metabolism of mutagenicity deprived 3-fluoroquinoline: Comparison with mutagenic quinoline. *Biological & Pharmaceutical Bulletin* 16: 232–234.

Quinone

Sachin S Devi and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 106-51-4
- Synonyms: Benzoquinone; *p*-Benzoquinone; Cyclohexadienedione; 1,4-Cyclohexadienedione; 2,5-Cyclohexadiene-1,4-dione; 1,4-Dioxybenzene; 1,4-Benzoquinone
- Chemical/Pharmaceutical/Other Class: Organic compounds
- Chemical Formula: $C_6H_4O_2$
- Chemical Structure:



Uses

Quinone is used as a chemical intermediate, a polymerization inhibitor, an oxidizing agent, a photographic chemical, a tanning agent, and a chemical reagent. It is also used in the manufacture of hydroquinone, in fungicides, as an analytical reagent, in photography, as a chemical intermediate, and as an oxidizing agent.

Background Information

Quinone is formed as yellow crystals and has a characteristic irritating odor like that of chlorine. It is slightly soluble in water, alcohol, ether, hot petroleum ether, and alkalis. Quinone is an oxidizing agent and is reduced to hydroquinone. It has been declared a federal hazardous air pollutant and was identified as a toxic air contaminant in April 1993 under AB 2728.

Exposure Routes and Pathways

Quinone may be released to the environment in effluents during its commercial production and use; and in wastewaters from the coal industry. If released to soil it is likely to leach and may volatilize and photodegrade on soil surfaces. A single degradation study found that quinone rapidly degraded in a chernozem soil to stable metabolites. If released to the aquatic environment, it may be degraded by photolysis as it absorbs ultraviolet radiation. In water, it is not expected to volatilize, adsorb to particulate matter or sediment, or bioaccumulate in aquatic organisms. Biodegradation in water may be important based upon the rapid degradation of quinone in soil. If released to the atmosphere, it will react rapidly in the vapor phase with both hydroxyl radicals and ozone with half-lives of 3.6 and 3.3 days, respectively, and may be susceptible to direct photolysis.

Toxicokinetics

There is complete reduction of a *p*- or *o*-quinone to the corresponding hydroquinone or catechol, respectively. In the human liver, carbonyl reductase may play a role in the reduction of some quinones. Catechols are primary substrates for catechol *o*-methyl transferase, but also undergo sulfation. However, for the antitumor quinones, mitomycin C, adriamycin, and daunomycin, two-electron reduction serves as an efficient bioactivation mechanism, elegantly affirming the concept of 'bioreductive alkylation' for the preferential bioactivation of antitumor prodrugs with oxygen deficient tumors.

Mechanism of Toxicity

The acute narcotic effects are due to the physical interaction of quinone itself on the cells of the central nervous system (CNS). The long-term effects are most likely due to the production of an unstable reactive intermediate during biotransformation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure of quinone leads to dermatitis, hypomelanosis, and delayed hyperpigmentation. It is a skin irritant, which may cause redness, swelling, and necrosis.

Human

Vomiting and gastrointestinal tract irritation have been seen with quinones. Nonspecific liver changes and jaundice have been reported. Ingestion of quinone results in dyspnea, anoxia, and respiratory failure. It also causes asphyxia and pulmonary damage. Cyanosis and cardiovascular collapse have also been reported.

Chronic Toxicity (or Exposure)

Animal

Quinones may lead to paralysis of the medullary centers and coma. Albuminuria and hematuria may occur. Chronic exposure of quinones to laboratory animals has resulted in skin irritation and has also caused redness of the skin.

Human

Quinone is known to cause eye irritation with chronic dust or vapor exposure. Keratitis, corneal ulceration, and discoloration of the conjunctiva may occur. Workers exposed chronically to these compounds may develop a reddish discoloration of the hair. No epidemiological data relevant to the carcinogenicity of quinone are available. There is inadequate evidence in experimental animals for the carcinogenicity of 1,4-benzoquinone. Overall evaluation: 1,4-benzoquinone is not classifiable as to its carcinogenicity to humans (group 3).

Clinical Management

1. *Emesis*: Ipecac-induced emesis is not recommended because of the potential for CNS depression and seizures.
2. *Activated charcoal*: Charcoal should be administered as a slurry (240 ml water per 30 g charcoal). Usual dose: 25–100 g in adults/adolescents, 25–50 g in children (1–12 years), and 1 g kg^{-1} in infants less than 1 year old.
3. *Gastric lavage*: Lavage should be considered after ingestion of a potentially life-threatening amount of quinones if it can be performed soon after ingestion (generally within 1 h).
4. *Eye exposure*: Decontamination: Exposed eyes should be irrigated with copious amounts of room temperature water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should be seen in a healthcare facility.
5. *Dermal exposure*: Decontamination: Contaminated clothing should be removed and the exposed area washed thoroughly with soap and water. A physician may need to examine the area if irritation or pain persists.

Environmental Fate

Quinone exists in the atmosphere in the gas phase. The dominant atmospheric loss process for quinone is expected to be by reaction with the hydroxyl (OH) radical (reaction with ozone is expected to be slow because of the $>C(O)$ substituent groups). The estimated half-life and lifetime of quinone in the atmosphere due to reaction with the OH radical are ~ 3 and 4 h, respectively.

See also: Hydroquinone.

Further Reading

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Quintozene *See* Pentachloronitrobenzene.

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Radiation Toxicology, Ionizing and Nonionizing

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Radiation toxicology is a specialized area of toxicology that relates to both characterizing radiation exposure of humans and evaluating the expected health consequences of the radiation exposure. It is a well-researched discipline that provides a wealth of knowledge about both the adverse and beneficial effects of radiation exposure. The indicated research covers the molecular, cellular, systemic, organism, and population levels.

Because everyone is exposed to low radiation doses from natural and human-generated ionizing radiation sources during their lifetimes, it is important to understand the potential adverse effects from such exposures as well as those that could occur as a result of high-level radiation exposures associated with infrequent events such as nuclear accidents, radiotherapy for cancer, or exposure as a result of use of a radiological (dirty bomb) or nuclear weapon.

This section provides a brief summary of the current knowledge of radiation-induced health effects together with certain basic elements of radiation toxicology needed to understand the concepts and terminology that are associated with this discipline.

Ionizing and Nonionizing Radiation

Radiation is energy in the form of waves or particles. The energy associated with any radiation can be transferred to matter. This transfer of energy can remove electrons from the orbit of atoms leading to the formation of ions. The types of radiation capable of producing ions in matter are collectively called 'ionizing radiation'. There are two general types of ionizing radiation: particulate and electromagnetic. Particulate ionizing radiations include alpha (α) particles, beta (β^-) particles, positrons (β^+; positive electrons), neutrons, protons, and heavy ions (i.e., charged heavy nuclei). Ionizing electromagnetic radiation includes X-rays and gamma rays.

Electromagnetic radiation with insufficient energy-causing ionization is called nonionizing radiation. Examples are ultraviolet (UV) radiation, radio frequency radiation (which includes microwaves), and extremely low frequency (ELF) radiation (associated with electric power lines).

The field of radiation toxicology has mainly focused on ionizing radiation. The sections that follow relate only to ionizing radiations.

Physical Characteristics of Ionizing Electromagnetic Radiation

Electromagnetic ionizing radiation energy is generally expressed in units such as kilo electron volts (keV) or million electron volts (MeV). Because gamma rays and X-rays have similar energies, they produce similar biological damage when delivered in the same manner and evaluated at the same dose. The different terminology reflects the difference in their origin, that is, gamma rays occur as a result of energy released from unstable atoms seeking a lower nuclear energy state, whereas X-rays occur as a result of energy releases by over-energized atomic electrons.

When rearrangement of orbital electrons occur as a result of ionization of an atom, the X-rays produced are called characteristic X-rays and have discrete energy values unique to the atom from which they arise. Gamma rays also have a discrete energy that is characteristic of the nuclide from which they are emitted.

When electrons that have been accelerated by a positive, high-voltage field collide with a target such as tungsten (as in an X-ray machine), the electron loses energy in the form of electromagnetic radiation (called bremsstrahlung radiation). In this type of interaction, the emitted radiation can assume a continuum of energy values up to a maximum value. Bremsstrahlung radiation is frequently encountered by astronauts in space travel as a result of the interaction of space radiation with the transport vehicle.

Gamma rays and X-rays are among the most penetrating types of radiations. Gamma rays are produced in the nuclear reactions that take place in nuclear power plants. They were also associated with the nuclear weapons that were detonated in Hiroshima and Nagasaki, Japan.

X-ray machines are used at most hospitals for diagnostic purposes. Since both X-rays and gamma

rays are not charged, they interact with matter primarily by the photoelectric effect (dislodging electrons), Compton scattering (bouncing off of an electron which is then released from its atom), and pair (negative and positive electron) production.

With pair production all of the energy of the incident photon (component of which X-rays and γ-rays are comprised) is lost in the production of the electron/positron pair. In the interaction of X-rays or γ-ray photons with atoms, electrons can therefore be ejected (e.g., via Compton scattering) from irradiated media atoms and carry with them energy transferred to them by the incidence photon. These 'secondary electrons' then interact with the surrounding media producing additional ionizations in the same manner as beta particles.

As γ- and X-ray photons pass through matter, the photon intensity decreases exponentially because of energy losses associated with interactions with electrons. The photon intensity (I) at a distance of penetration d is expressed as a fraction of the initial intensity I_0 using the following equation:

$$\frac{I}{I_0} = e^{-\mu d}$$

where μ is the media density-dependent, attenuation coefficient in the medium for the energy considered (in units of $cm^2\,g^{-1}$). The penetration distance d is expressed here in $g\,cm^{-2}$ (equal to distance in cm $\times$ density of the absorbing media).

The penetrating ability of photons depends on both the photon energy and the composition of the absorbing media, with penetration increasing with increasing energy, and decreasing density and effective atomic number of the medium. The penetrating ability of photons is sometimes described by a half thickness or half-value layer (HVL), which is the thickness of an absorbing medium that decreases the photon intensity by one-half. For example, the HVL for 1 MeV gamma rays is $\sim$9 m of air, 9.6 cm of water, 4 cm of aluminum, and 9 mm of lead.

Physical Characteristics of Ionizing Particulate Radiation

Of the various ionizing particulate radiations, the most important in terms of likelihood for human exposure are alpha particles, beta particles, protons, and neutrons. Alpha and beta particles occur as a result of the radioactive decay of unstable atoms. Neutrons generally result from nuclear reactions, such as nuclear fission (as in nuclear reactors and fission-based nuclear weapons) and charged-particle activation of target atoms (as with some accelerator-produced radioisotopes). Protons arise from atomic interactions of neutrons.

Alpha Particles

Alpha particles have a positive charge and are identical with helium nuclei and consist of two protons and two neutrons. They result from the radioactive decay of heavy elements such as radium, thorium, uranium, and plutonium. Because of their double-positive charge, alpha particles have great ionizing power, but their large mass results in very little penetration. For example, alpha particles from 4 to 10 MeV have ranges in air of 5–11 cm; the corresponding range for alpha particles in water would be from 20 to 100 μm.

Beta Particles

Beta particles are equivalent to electrons but arise from radioactive decay of unstable atoms. They are emitted with a continuous range of energies up to a maximum that is characteristic of each radionuclide. Electrons have a greater range and penetrating power but much less ionizing potential compared to alpha particles. The range of beta particles in air is $\sim$4 m per MeV of energy. In water the range in cm is approximately one-half the maximum beta energy when expressed in MeV. For example, the range of the energetic beta particles from yttrium-90 (maximum energy 2.27 MeV) is $\sim$1.15 cm in water and similarly in soft tissue.

Neutrons

Neutrons are neutrally charged particles with a mass slightly larger than that of a proton. Because they are neutrally charged, they produce ionizations indirectly. In biological material, neutrons can eject protons from the nuclei of hydrogen atoms by nuclear collisions, which in turn are charged and directly ionizing. Neutrons can also activate hydrogen and other elements by neutron capture, which results in the release of gamma rays, and sometimes radioactive by-products. Neutrons that are produced by fission or by special sources such as americium–beryllium sources have a spectrum of energies that range over many orders of magnitude. Since their ranges depend on both neutron energy and the composition of the absorbing material, it often requires complex calculations to describe the ranges of neutrons. However, as simple examples, very low-energy neutrons (called thermal neutrons) have a range of $\sim$30 cm in soft tissue, whereas high-energy or fast neutrons can penetrate tissue to about the same extent as can highly penetrating gamma rays.

Energy Loss by Electromagnetic and Particulate Ionizing Radiations

Ionization Patterns

A single initial or secondary ionizing particle passing through matter deposits its energy in a stochastic (random) and nonuniform manner on a microscopic scale. This deposited energy creates positively and negatively charged molecules and atoms (called ion pairs) along the path (or track) traveled by the ionizing particle. The density of ion pairs produced along a track varies significantly depending on the ionizing particle and the medium through which it passes and is proportional to the average energy deposited per unit path length. For example, secondary electrons produced by 200 kV X-rays create ~ 80 ions per μm path length; protons generated from interactions with 12 MeV neutrons produce ~ 300 ions μm^{-1}; and alpha particles from the decay of radium-226 produce ~ 3700 ions μm^{-1}.

Linear Energy Transfer

The ionization patterns produced by different charge particles (e.g., electrons, protons, helium ions, heavy charged nuclei) relate to their linear energy transfer (LET). LET is the average energy loss in traversing a small thickness of the target medium of interest and depends on the energy of the charged particles. Gamma rays, X-rays and neutrons have no charge. Their energy loss is mainly associated with secondary charged particles they produce. For typical X-rays (which produce electrons), LET ranges from ~ 0.2 to 15 keV μm^{-1}; for fast neutrons (which produce protons) from ~ 8 to 40 keV μm^{-1}; and for alpha particles greater than ~ 260 keV μm^{-1}. As mentioned previously, the efficiency with which a particular type of radiation produces biological effects depends strongly on LET. Further, when biological cells are hit by charged particles associated with a given radiation source, the spatial distribution of the hit cells depends strongly on LET. Because intercellular signaling can have an important influence on the outcome of radiation exposure, it is important to consider the spatial distribution of hit cells when explaining observed biological effects. The field of microdosimetry deals with the frequency and spatial distribution of cells hit by primary- and secondary-charge particles associated with ionizing radiation sources.

Dosimetric Quantities and Units

Radiotoxicology, like other disciplines of toxicology, has specialized quantities that define the relationships between exposure to radiation and the resulting dose received by specific biological entities. Some of these quantities are based on measurements and/or calculations; others, particularly those used in radiation protection, consist of theoretical quantities that include modifying factors designed to allow comparison of risks to people exposed to a variety of radiation types and with widely varying spatial patterns of dose.

Units that Apply to Radioactivity

The defining event of a radioactive nuclide is the transformation of its nucleus into the nucleus of another species, that is, radioactive decay. The number of nuclear transformations occurring per unit of time is called 'activity'. Sometimes 'radioactivity' is used instead of 'activity'. The traditional unit of activity has been the Curie (Ci), which is equal to 3.7×10^{10} nuclear transformations per second. The conversion of radiation units to the international system (Syst̨me International d'Unit or SI) has now taken place in the United States. The more fundamental unit of activity, the Becquerel (Bq), equal to 1 nuclear transformation per second, has replaced the Curie. Both units of activity are modified by prefixes such as kilo-, milli-, and micro- to achieve standard multiples of the fundamental unit. A listing of the most commonly used prefixes is given in **Table 1**.

Units that Apply to Radiation Exposure

In radiation physics, the term 'exposure' is used to describe the amount of ionization caused in air by

Table 1 Standard multiples used with radiation units

Prefix	*Multiplication factor*	*Symbol*
exa	10^{18}	E
peta	10^{15}	P
tera	10^{12}	T
giga	10^{9}	G
mega	10^{6}	M
kilo	10^{3}	k
hecto	10^{2}	h[a]
deca	10^{1}	da[a]
deci	10^{-1}	d[a]
centi	10^{-2}	c[a]
milli	10^{-3}	M
micro	10^{-6}	μ
nano	10^{-9}	n
pico	10^{-12}	p
femto	10^{-15}	f
atto	10^{-18}	a

[a] It has been suggested that all SI units be expressed in 'preferred standard form' in which the multiplier is 10^{3n} where n is a positive or negative whole number. Consequently the use of hecto, deca, deci, and centi is to be avoided wherever possible.

gamma and X-rays. The unit of exposure is the Roentgen (R), which is equal to 2.58×10^{-4} coulomb kg^{-1} of air. This quantity is most often used in diagnostic radiology and does not apply to ionizations produced by either particulate radiations or high-energy (>3 MeV) X-rays or gamma rays. For radiobiological applications, the exposure rate (e.g., in R min^{-1}) is most commonly used.

Absorbed Dose Units

The most commonly used quantity describing radiation dose is the absorbed dose (D), which is defined as the mean energy, e, imparted by ionizing radiation to matter of mass m divided by the mass, that is, $D = e/m$. This quantity is a measurement of the deposition of energy in any substance by all types of ionizing radiation. It applies to macroscopic but not to microscopic masses. The traditional unit of absorbed dose is the rad, which is equal to 100 ergs g^{-1} or 0.01 J kg^{-1}; the corresponding SI unit is the Gray (Gy), which is equal to 1 J kg^{-1} (therefore, 1 Gy is equivalent to 100 rad). The absorbed dose should be used in preference to the exposure whenever the former can be measured or reliably calculated. In this section we have used the rad unit to be consistent with our use in the 1998 publication of this section. However, in many of the current research journals, the mGy or Gy unit is preferred over the rad.

Microdosimetric Units

When viewed at the microscopic level of a cell, or smaller biological subunit, the dose D is replaced by what is called specific energy (z). While D is a macroscopic dose, z is a microscopic dose. For a single absorbed dose D to an organ or tissue, there can be many different microscopic doses z to cells in that organ or tissue. In addition, a cell may have no dose (i.e., $z = 0$) at all, while another cell in the same tissue may have a very large dose. However, when z is averaged over the microscopic targets in the macroscopic mass of interest (e.g., organ), the average value obtained should equal the absorbed dose D as defined previously.

LET Units

As previously indicated, the LET is the average rate of energy loss in traversing a small thickness of the target medium. The unit generally attributed to LET is keV μm^{-1} path length.

Quality Factor

The quality factor (Q) is a dimensionless quantity used to make adjustments for differing qualities for different radiations in producing biological damage. The factor Q takes into account the type of radiation and other factors. It also relates to LET. The greater the value for Q, the greater the biological damage produced by a given type of radiation.

Dosimetric Units Used in Radiation Protection

For radiation protection purposes, several theoretical dosimetric quantities have been created that attempt to 'normalize' the responses of different tissues and organs of the body from irradiation by different types of ionizing radiation so that uniform radiation protection guidelines can be promulgated that are insensitive to the particulars of any given irradiation scenario. The traditionally used quantity has been the dose equivalent (DE), which is defined as the absorbed dose (D) multiplied by the quality factor Q. The unit of dose equivalent has been the rem, which is dimensionally the same as the rad; the SI unit is the Sievert (Sv). Recently, the DE has been replaced by a similar concept called the equivalent dose. The equivalent dose depends on the relative biological effectiveness rather than on Q.

Current radiation protection guidelines are specified in terms of a quantity called the effective dose (E). The effective dose is presumed to have associated with it the same probability of occurrence of cancer and genetic effects whether received by the whole-body, via uniform irradiation, or by partial-body or individual-organ irradiation. To take into account the observed varying radiosensitivities of the different organ systems of the body and to adjust for nonuniformity of irradiation, a tissue weighting factor, W_t, is used. An additional radiation weighting factor, W_r, is used to adjust for the biological effectiveness of different radiations. The current weighting factors, as stated by the National Council on Radiation Protection and Measurements, are summarized in **Table 2**.

Relative Biological Effectiveness

Since equal doses of different types of ionizing radiations do not produce equivalent biological effects, a quantity called the relative biological effect (RBE) was developed to allow comparison of effects produced in identical biological systems from different types of radiations. The RBE is customarily defined as the ratio of two doses (a reference dose divided by a test dose) for producing a given level of biological effect under a given condition. The reference is often taken to be X-rays. For example, if 90% cell killing is produced by 10 Gy of X-rays (D_x), but only 0.5 Gy of neutrons (D_n) is needed for 90% killing, then the RBE in this case would be $D_x/D_n = 10\,Gy/0.5\,Gy = 20$. Thus, RBE has no units. The RBE influences both the equivalent dose and the effective dose used in radiation protection.

Table 2 Radiation and tissue weighting factors used in radiation protection guidelines[a]

Radiation type and energy range	W_r
X-rays and gamma rays, electrons and positrons	1
Neutrons, energy	5
<10 keV	10
10–100 keV	10
>100 keV to 2 MeV	20
>2–20 MeV	10
>20 MeV	5
Protons	2
Alpha particles, fission fragments	20
Organ or tissue	W_t
Gonads	0.20
Red bone marrow	0.12
Colon	0.12
Lung	0.12
Stomach	0.12
Bladder	0.05
Breast	0.05
Liver	0.05
Esophagus	0.05
Thyroid	0.05
Skin	0.01
Bone surface	0.01
Remainder	0.05

[a] W_r is the radiation weighting factor, and W_t is the tissue weighting factor.

Sources of Ionizing Radiation Exposure

Humans are routinely exposed to ionizing radiation. Some of the sources are naturally occurring, and others are due to man-made uses of radiation and radioactive materials. In general the radiation from natural sources includes cosmic radiation, external radiation from radionuclides in the earth's crust, and internal radiation from radionuclides inhaled or ingested and retained in the body. Man-made sources of radiation include X-ray equipment, particle accelerators and nuclear reactors used in the generation of nuclear energy, radionuclides used in nuclear medicine, radionuclides released to the environment as a result of nuclear weapons testing or a nuclear accident, and occupational exposure to both external and internal radiation. The magnitude of the exposure to natural sources depends mostly on geographical location, whereas exposure to man-made sources depends on human activities.

Natural Background Radiation

Exposure to natural sources of external ionizing radiation results from the levels of cosmic and terrestrial X and gamma radiation present in the environment. Cosmic radiation at the earth's surface is affected by altitude, geomagnetic latitude, and solar modulation. For example, the dose rate at 1800 m is about double that at sea level. Within the United States, the effect of latitude and solar modulation on cosmic ray dose rate is $<10\%$. Because cosmic radiation is highly penetrating, it results in relatively uniform whole-body irradiation. The average dose rate from cosmic irradiation in the United States has been estimated to be ~ 28 mrem year^{-1}.

Humans are also exposed to external gamma radiation from concentrations of naturally occurring radioactive materials in soils and rocks. These radioactive elements include uranium and thorium radionuclides plus their radioactive progeny and potassium-40, and result in widely varying dose rates that depend on the geology of the particular region. Estimates of the annual dose rate for this type of exposure in the United States averages 28 mrem year^{-1}.

Internally deposited naturally occurring radionuclides also contribute to the natural radiation dose from inhalation and ingestion of these materials when contained in air, food, and water. Included are radionuclides of lead, polonium, bismuth, radium, potassium, carbon, hydrogen, uranium, and thorium. Potassium-40 is the most prominent radionuclide in normal foods and human tissues. The dose to the total body from these internally deposited radionuclides has been estimated to be ~ 39 mrem year^{-1}.

The major exposure of the population to natural radiation arises from inhalation of the short-lived radioactive progeny of the radioactive noble gas radon-222, which in turn is a sixth-generation radioactive decay product of natural uranium. The amount of radon-222 present in the air depends on many factors (e.g., gas permeability in soil and rock, relative humidity, and barometric pressure) but is necessarily linked to the geological concentration of the uranium parent radionuclide. There is about an eightfold range of concentrations of uranium in different types of rocks and soils.

Most of the early measurements of radon levels were made outdoors; however, it has become apparent that the indoor concentrations are generally several times higher than those outdoors. Because people in Western countries spend only $\sim 15\%$ of their time outdoors, most of the exposure to radon therefore occurs indoors. Additionally, the trend toward the construction of more energy-efficient housing (more air-tight) has also enhanced the concentrations of radon-222 indoors.

The average annual radiation dose to the general population due to inhalation of radon and its progeny is estimated to be ~ 200 mrem. However, this dose can range upward by 1 or 2 orders of magnitude in cases in which the indoor radon concentrations are very high. Because of the short

half-lives of the radon progeny, and the fact that the most important radionuclides decay by α-particle emission, their radiation dose is delivered primarily to the tissues of the respiratory tract.

Man-Made Radiation Sources

Several human activities involving the production and use of radionuclides as well as the development of nuclear weapons have resulted in releases of radioactive materials into the environment. Such activities include past atmospheric testing of nuclear weapons, production of nuclear weapon materials, production of electricity by nuclear reactors, radioisotope production and use in industry and medicine, accidental releases of radionuclides at both civilian (Three Mile Island and Chernobyl) and military (Kyshtym and Windscale) nuclear installations, and intentional releases (Mayak Plutonium Production Facility). Additionally, there has been a significant increase in the types of quantities of sources of potential radiation exposure from consumer products. These include radioluminescent devices containing tritium, promethium-147, or radium-226; smoke detectors containing americium-241; static eliminators containing polonium-210; and airport X-ray baggage inspection systems. In other cases, radiation emissions are incidental or extraneous to the purpose for which the consumer product was designed, for example, television receivers, tobacco products containing polonium-210 and lead-210, combustible fuels and building materials containing uranium- and thorium-series radionuclides, and gas mantles, camera lenses, and welding rods containing thorium.

A summary of the contributions of the various natural and man-made radiation sources to our radiation background is given in **Table 3**. It can be seen that natural sources contribute ~82% of the total, with radon being the largest single source (67% of natural radiation dose). Of the 18% contributed by man-made sources, medical exposure is the most prominent (83%). Attempts to significantly reduce population radiation doses would most likely be focused on the largest contributors, that is, indoor radon and medical radiation.

Now there is growing realization of the possibility of the use of radiological weapons (dirty bombs) by terrorist organizations. With such weapons, radioactive material could be dispersed in populated areas. Such acts could lead to a variety of problematic health and environmental consequences. The National Council on Radiation Protection and Measurement has recently published a valuable reference entitled 'Management of Terrorist Events Involving Radioactive Material'. The report provides guidance on the management of radiation casualties in such circumstances.

Table 3 Radiation exposure of the US general population

Radiation source	*Per capita annual effective dose equivalent (mrem)*
Natural radiation	
Cosmic rays	28
Terrestrial, external	28
Internally deposited radionuclides (except radon)	39
Inhaled radon and progeny	200
Sources due to or enhanced by human activity	
Medical uses	53
Nuclear power[a]	0.05
Consumer products	8
Weapons fallout (averaged to year 2000)	5
Total	361

[a] Includes contributions from uranium mining and milling, fuel fabrication, power plant operation, reprocessing of spent fuel, and transportation.

Radiobiological Effects

Radiation-induced biological effects include alterations in expressed genes and proteins, oncogene activation, suppressor gene inactivation, chromosomal aberrations, mutations, cancer, genetic effects, and loss of normal tissue and organ functions. Although the biological effects of concern from exposure to ionizing radiation are described typically at the tissue or organ level, it has long been recognized that an understanding of the mechanisms by which radiation produces effects such as cancer, genetic changes, and tissue destruction are best obtained from studies performed at the cellular and molecular levels.

Direct and Indirect Radiation Effects

At the chemical level, a solute molecule (DNA, RNA, and protein) in a biological system can be affected by radiation in two different ways. When an ionization track passes either directly through a molecule or close enough so that the created ions can drift to and interact chemically with the molecule before they recombine and neutralize in solution, the phenomenon is called a direct radiation effect. On the other hand, since the largest fraction of almost any biological system consists of water (e.g., 70–80% of a typical cell), the most frequent initial radiation interactions will be with water molecules. When this occurs, ion radicals and free radicals are created.

When irradiated, water molecules become ionized in a two-step process:

$$H_2O + \text{radiation} \rightarrow H_2O^+ + e^-$$

$$e^- + H_2O \rightarrow H_2O^-$$

However, the charged water molecules are unstable, having lifetimes less than 10–15 s, and almost immediately dissociate into one smaller ion and a free radical:

$H_2O^+ \rightarrow H^+ + OH^\bullet$ (the dot symbolizes the unpaired electron of the OH free radical)

$$H_2O^- \rightarrow H^\bullet + OH^-$$

The free radicals thus produced are very reactive and diffuse through the solvent system interacting in a fairly indiscriminate manner with other free radicals, with molecules previously damaged by radiation, or, most important, with intact solute molecules previously unchanged by the radiation. Free radical reactions may also produce other more or less reactive chemical species, such as H_2, H_2O, and H_2O_2, they may react with oxygen to enhance the effect of the radiation, or they may interact with organic molecules creating organic free radicals:

$$H_2O^- + RH \rightarrow R^\bullet + H_2O_2$$

$$RH + HO_2^\bullet \rightarrow RO^\bullet + H_2O$$

This latter phenomenon is called an indirect radiation effect. If RH is an important molecule (e.g., DNA and RNA) then these interactions can affect cell functions.

Effects of Radiation on Cells

Depending on type and quantity, radiation can produce a variety of effects on cells. These effects include chromosomal aberrations, apoptosis (programmed cell death), necrotic cell death, mutations, neoplastic transformation (an early step in cancer induction), altered cell-cycle regulation, alterations of metabolic functions, and changes in intercellular signaling characteristics. As more studies are done using the tools of molecular biology, more insights into the mechanisms of radiation action are being found. This knowledge plays a key role in the development of biologically based risk models that are used to extrapolate results from studies performed at relatively high radiation doses and dose rate to low-radiation doses and dose rate. Establishing a reliable low dose and dose-rate cancer risk model is of greatest interest and importance for the protection of people and the environment from radiation damage.

Deleterious and Protective Bystander Effects

Recently it has been learned that bystander cells not directly hit by radiation can also be impacted via other mechanisms in addition to reactive oxygen species. Since the nonhit cells are bystanders, the effect has been given the special name 'bystander effect'. In the case of high-LET α-particle research, new devices called microbeams have been developed that allow a single cell to be hit by charged helium ions (same as alpha particles) and for the nearby and distant nonhit cells to be examined for possible biological effects. These studies show that some unhit cells can also develop chromosomal aberrations, mutations, and can be neoplastically transformed. Biological substances released by the hit cells appear to play an important role in occurrence of bystander effects. Simply irradiating the medium containing the cells does not lead to bystander effects.

In contrast to the observations with high-LET radiation, other studies using low dose, low-LET gamma rays (>0–10 rad) have shown that such low doses can turn on a protective process that removes spontaneous transformants (neoplastically transformed cells), presumably reducing the risk of cancer *in vivo*. This protective process has been studied in detail by German researchers at the University of Freiburg related to their cancer prevention research and involves cross-talk (signaling) between nontransformed and transformed cells. Transforming growth factor beta and reactive oxygen species are involved in cross talking. In the case of transformed cells, the net effect is that at least some neoplastically transformed cells that are present are selectively eliminated via apoptosis (i.e., the cells undergo self destruction). This process was recently given the name Protective Apoptosis-Mediated (PAM) process. This process is discussed in an in-press paper by the first author of this section and colleagues. The PAM process is a form of natural protection against problematic cells (e.g., mutant cells, neoplastically transformed cells) and seems quite important in protecting humans from cancer occurrence. However, the PAM process does not appear to be turned on by low doses of high-LET alpha radiation.

Cell Survival

Cell survival curves are used to describe the relationship between radiation dose and the proportion of cells that survive. Usually, mathematical models are used to describe cell survival data. Survival of

normal cells is an important consideration in radiation therapy. It is the cancer cells that the therapist wants to destroy, while to the extent possible, protecting normal cells.

The endpoint of survival can have two different meanings depending on whether the cell populations studied have proliferative potential. In the case of nondividing, terminally differentiated cells, survival is generally related to the ability of the cells to maintain function. In general, relatively large radiation doses are required to inactivate cell function for terminally differentiated cells. For cells that proliferate either in tissue or in culture, cell death or survival is more related to ability of that cell to continue to divide and produce clones of cells. Thus, a cell that is able to undergo no more than one or two cell divisions after irradiation would still be considered 'dead'. Doses on the order of a few rad are generally required to kill the most sensitive proliferating cells, although there is a range of radiosensitivities to this effect. For example, normal human fibroblasts irradiated with X-rays have a D_0 of 120 rad (D_0 is the dose at which survival is reduced to 37% of its original value); in comparison, cells from a patient with the disease ataxia-telangiectasia (AT) have a D_0 of $\sim$50 rad and are therefore more radiosensitive – a hallmark of AT. The heightened radiosensitivity relates to a deficiency in DNA repair for persons with AT.

Studies using experimental designs in which only certain selected portions of a cell were irradiated have shown that the sensitive sites for radiation-induced cell killing are in the nucleus as opposed to the cytoplasm. Furthermore, there is strong circumstantial evidence that the DNA in the chromosomes is the primary target for radiation-induced cell killing.

Cell and Tissue Radiosensitivity

The sensitivity of various cells and cell lines to radiation-induced lethality can differ significantly, and different organs consist of different cell populations (e.g., connective tissue and vascular, parenchymal). As early as 1906, Bergonié and Tribondeau studied cellular radiosensitivity and postulated that actively proliferating cells were most radiosensitive, that the degree of cellular differentiation was inversely related to radiosensitivity, and that radiosensitivity of cells was proportional to the duration of mitotic and developmental activity. In general, this 'law' is valid for different cell types, although there are exceptions. For example, the small lymphocyte, which is highly differentiated and has little if any proliferative potential, is one of the most radiosensitive mammalian cells. One scheme for categorizing the sensitivity of normal cells to cell killing is the following:

- *Very high*: Lymphocytes, immature hematopoietic cells, intestinal epithelium, spermatogonia, and ovarian follicular cells.
- *High*: Urinary bladder epithelium, esophageal epithelium, gastric mucosa, and mucus membranes.
- *Intermediate*: Endothelium, growing bone and cartilage, fibroblasts, glandular epithelium of breast, pulmonary, renal, pancreatic, thyroid, and hepatic epithelia.
- *Low*: Erythrocytes, muscle cells, mature connective tissue, osteocytes, chondrocytes, and ganglion cells.

The effects of radiation on more complex organ systems will depend on the effects produced on the different subpopulations of cells that comprise the organ. For example, if the parenchymal cells are most radiosensitive (as in intestinal mucosa), then loss of function of the organ may occur at the lowest doses, followed perhaps by vascular damage at higher doses. If the parenchymal cells are normally nondividing (as in the brain), then the reverse may occur, with damage to the microcirculation predominating at lower doses.

Genetic Effects

Radiation Effects on Inheritance

Radiations such as X-rays, gamma rays, and beta particles can damage genetic material in reproductive cells and cause mutations that can be transmitted from one generation to another. In the 1920s, researchers using fruit flies (*Drosophila*) found that chromosomes could be easily injured by radiation and such injury could lead to mutations that were expressed in subsequent generations. This finding was quickly confirmed in numerous species of plants and animals. Today it is known that relatively small radiation doses ($<$10 rad) can cause alterations in nucleotides and visible breaks in chromosomes of germ cells that can lead to genomic instability that can be passed on to subsequent generations.

In evaluating the effect of radiation on heredity of germ cells, two specific germ-cell stages are considered important: (1) the stem-cell spermatogonia in males and (2) the oocytes, primarily the immature ones, in the female. The spermatogonia continue to multiply throughout the reproductive lifespan of an individual. However, oocytes are not replaced during adult life.

Because of the lack of information for humans, most genetic studies have been carried out with

experimental organisms, especially mice. Radiation has been found to cause mutations in all nonhuman experimental organisms studied and therefore such effects are also expected to arise in humans.

The genetic effects that could be caused by radiation are too numerous to be considered individually. For nuclear accident risk assessment, genetic disorders have been grouped as (1) dominant and X-linked single-gene disorders, (2) chromosome disorders, and (3) multifactorial disorders.

Dominant and X-Linked Single-Gene Disorders

Most cells from humans contain two sets of chromosomes with matched pairs of genes, one gene from each parent. The matched genes can differ, with one gene being dominant over its recessive counterpart. A recessive gene can only show its effect if both matching chromosomes carry that gene. If an altered gene is present on the X chromosome it will invariably produce an effect in boys, who have only one X chromosome, but will behave as recessive in girls, who have two X chromosomes. Single-gene disorders related to damage to the X chromosome are called X-linked effects.

Dominant gene disorders that could be caused by radiation include traits such as Huntington's chorea, hypercholesterolemia, and achondroplastic dwarfism. The X-linked traits include traits such as muscular dystrophy, hemophilia, and agammaglobulinemia. However, there is no direct evidence that these diseases have been induced in humans by irradiation.

Chromosome Disorders

Two forms of genomic damage that depend on radiation quality (i.e., LET) are the induction of single-strand (SS) and double-strand breaks (DSBs). The two types of damage are considered to be important because SSBs are more easily and accurately repaired by the cell than are DSBs. Thus, DSBs result in damage that is both more lethal and more able to result in chromosome disorders. For low-LET radiation, increased production of DSBs is a function of dose rate, as single tracks are so sparsely ionizing that breaking more than one chromosome with a single track is unlikely, especially at low radiation doses; therefore, DSBs arise as a consequence of multiple tracks occurring sufficiently close in time and space. On the other hand, high-LET radiation produces a high enough ionization density within its tracks that DSBs can occur from single traversals of a cell nucleus. This in part is responsible for the greater RBE for high-LET radiation for cell killing, mutation induction, cell transformation, and cancer induction.

Damage that is produced by radiation can be chromosomal or chromatid, depending on whether the cell is in a pre- or postreplication state. In either case, sufficient energy is imparted to break a chromosome or chromatid, usually into a major and a minor fragment. Once this has occurred, (1) the broken ends may rejoin to restore the original configuration of the chromosome; (2) a fragment may fail to rejoin, resulting in a deletion, which is sometimes large enough to be scored as a micronucleus; or (3) broken ends may rejoin with other broken ends to yield abnormal forms that are subsequently scored at the following mitosis as rings, dicentrics, anaphase bridges, or symmetric and asymmetric translocations.

Chromosome anomalies and aberrations can influence heredity. Most somatic cells of humans contain 23 pairs of chromosomes, with one member of each pair contributed by the sperm and the other contributed by the egg. When the process of sperm or egg cell production goes awry as a result of radiation damage, abnormal chromosome numbers (aneuploidy) can arise. Aneuploidy is a form of genetic instability.

It has been estimated that in $\sim$90% of cases, aneuploidy will result in spontaneous loss of pregnancy. In the remaining 10% of cases, a severely affected child would be expected because of the inherited genomic instability. Conditions such as Down's syndrome and both Klinefelter and Turner anomalies are the result of genomic instability associated with aneuploidy. These defects are relatively severe – in terms of both life expectancy ($\sim$45 years) and level of disability ($\sim$50%). Persons born with aneuploidy usually are physiologically and morphologically abnormal and do not have children. Thus, their genomic instability tends not to be passed on to other generations.

Chromosomes can be easily broken by radiation which can lead to a structural rearrangement (called a translocation). Translocations are also a form of genomic instability. When translocations occur in germ cells, they can be transmitted to the offspring. Translocations normally yield chromosomes with too little or too much genetic information. If a child is born with a balanced translocation (not too little or too much information) he or she would not normally be affected but could pass on genomic instability to future generations. Those born with such genomic instability could suffer from severe physical and mental disabilities.

Multifactorial Disorders

Multifactorial diseases involve complex patterns of inheritance and represent a very large class of genetic

diseases. For such diseases to arise, a specific combination of mutant genes must be present. Environmental factors can also be important. Examples of multifactorial diseases include congenital malformations (e.g., spina bifida and cleft palate), constitutional diseases, and degenerative diseases.

Genetic Effects in Irradiated Populations

Epidemiology has not detected hereditary effects of radiation in humans with a statistically significant degree of confidence. Nevertheless, there can be no doubt of the existence of hereditary effects in man. Following radiation exposure of a large population (e.g., as occurred in the former Soviet Union (FSU) after the Chernobyl nuclear accident in 1986), an increase in the incidence of genetic disease would be expected to occur. The genetic damage would show up both early (as an increased incidence of birth defects among some children of the exposed population) and late (through latent mutations expressed in their grandchildren, great-grandchildren, and subsequent generations). It has been estimated that $\sim 50\%$ of all genetic damage introduced by radiation exposure following a major nuclear accident will be manifest within the first three to five subsequent generations, with the remaining damage dispersed over future generations.

Early and Continuing Deterministic Effects

If a person is exposed to a large amount of radiation (i.e., large radiation dose) delivered to the entire body, cells in tissues can be destroyed in large numbers. Because tissues have important functions, the destruction of significant numbers of cells can lead to impairment in one or more of these functions. The biological effects that arise when large numbers of cells are destroyed by radiation are called 'acute somatic effects' if they occur in a relatively short period of time (e.g., within a few weeks) after brief exposure. Acute somatic effects are a subset of what is now formally called 'early and continuing deterministic effects' (once called nonstochastic effects).

Deterministic effects are those that increase in severity as the radiation dose increases and for which a threshold is presumed to exist. Besides acute somatic effects, deterministic effects also include radiation effects (other than cancer and genetic effects) that continue to occur after an extended period (e.g., years) of chronic exposure. Such chronic exposures can arise from long-lived radionuclides (e.g., isotopes of plutonium and cesium) ingested via contaminated food or inhaled via contaminated air and retained in the body. Populations in Russia, Ukraine, and Belarus continue to ingest and inhale long-lived radionuclides that were released during the 1986 nuclear accident at Chernobyl. Firemen who fought the reactor fire during the Chernobyl accident and plant workers present at the time of the accident were chronically exposed to large radiation doses from inhaled radionuclides.

Examples of deterministic effects are hypothyroidism arising from large radiation doses to the thyroid gland; skin burns arising from exposure of small or large areas of the skin; permanent suppression of ovulation in females; temporary suppression of sperm production in males; growth and mental retardation caused by exposure of a fetus during pregnancy; and death from severe damage to critical organs such as the bone marrow, lung, or small intestine.

Thresholds arise for deterministic effects because large numbers of cells usually must be simultaneously destroyed to produce such effects, which is highly unlikely at low doses. The threshold dose for a specific deterministic effect depends on the type of radiation, on the rate at which the dose is delivered (dose rate), and, for some effects, on other factors.

Factors Affecting the Production of Deterministic Effects

The type of radiation is important because different types of radiation interact with body tissue differently. Gamma rays and X-rays can easily penetrate into body tissue and therefore can produce deterministic effects in all body organs if the dose and amount of tissue irradiated are both large enough. Beta radiation can cause skin burns and ulcers when beta-emitting hot particles (highly radioactive, very small particles) are deposited on the skin, but little damage is likely to be done to other tissue unless the beta-emitting particles are taken into the body in large amounts (e.g., by inhalation or ingestion). Alpha radiation does not cause skin burns or ulcers when alpha-emitting particles are deposited on the skin because alpha radiation does not have enough energy to penetrate the dead layer of tissue that covers the skin surface. However, when taken into the body in large amounts, alpha-emitting particles can cause deterministic effects.

For total-body exposure to X-rays or gamma rays, organs and tissue at risk include all organs and tissue in the body. For inhalation or ingestion exposure to beta-emitting materials, organs and tissue at risk include the lungs and gastrointestinal tract as well as other sites depending on the metabolic fate of the radionuclide of concern. For example, strontium isotopes preferentially irradiate the skeleton, while

Table 4 Threshold gamma or X radiation doses (lower, central, and upper estimates) for specific deterministic effects[a]

Effect	*Organ/tissue*	*Lower bound (rad)*	*Central (rad)*	*Upper bound (rad)*
Vomiting	Upper abdomen	Not estimated	50	Not estimated
Diarrhea	Upper abdomen	Not estimated	100	Not estimated
Erythema	Skin[b]	200	300	400
Moist desquamation	Skin[b]	800	1000	1200
Permanently suppressed ovulation	Ovum in females	20	60	100
Suppressed sperm counts[c]	Testes in males	20	30	40
Cataracts	Lens of eye	0[d]	100	150
Hypothyroidism	Thyroid	Not estimated	200	Not estimated
Radiation pneumonitis	Lung	400	500	600
Hematopoietic death[e]	Bone marrow	120	150	180

[a] Applies to gamma rays or X-rays delivered to the indicated organ or tissue in less than 1 h.
[b] For 50–100 cm^2 area of skin and the dose evaluated at a depth of 0.1 mm.
[c] Two-year suppression of sperm counts in males.
[d] Used to include the possibility that cataracts may be a stochastic effect with no threshold.
[e] Death from lethal injury to the sensitive bone marrow.

iodine isotopes preferentially irradiate the thyroid. When considering possible deterministic effects from inhaled radionuclides, organs other than the lung should also be considered because radionuclides can translocate from the lung to other organs such as the liver and skeleton.

Radiation dose and dose rate are important because the larger the dose, the larger the amount of potentially destructive radiation energy deposited in tissue, which can lead to extensive cell death and concomitant impairment in important tissue functions. A significant impairment can lead to morbidity and lethality. Likewise, radiation dose rate is important because when it is sufficiently high, radiation can overwhelm cell repair mechanisms and organs cannot recover from tissue injury. Most efficient recovery occurs when the radiation dose rate is low and when the amount of tissue that the radiation interacts with is small. In the administration of radiation therapy to cancer patients, physicians try to minimize damage to healthy tissue by delivering the radiation in a number of fractions over a number of days or weeks. This allows damaged normal tissue to recover during the periods between the fractionated exposures. The rate of recovery differs for different organs.

Other factors that can be important in determining the impact of radiation exposure include a person's age and sex, how healthy they are, and the type of medical support received from physicians after being injured by radiation.

Thresholds Doses for Specific Deterministic Effects

For nuclear accident risk assessment, organs of primary interest because of their high sensitivity or their potential for receiving large radiation doses are bone marrow, gastrointestinal tract, thyroid gland, lungs, skin, gonads, and eyes. **Table 4** shows estimates (central, lower bound, and upper bound) of threshold doses for a variety of deterministic effects of exposure to gamma rays when the dose is delivered quickly (within 1 h). Larger doses would apply when the dose is delivered over hours, days, weeks, or longer. For example, the central estimate of the γ-ray threshold for acute lethality from radiation-induced injury to the hematopoietic system is 150 rad (see **Table 4**) when the dose is delivered within an hour. However, when the dose is delivered continuously over several years, individuals have survived γ-ray doses as high as 600–1000 rad (which would be fatal if received within a few hours). Nuclear workers in the former Soviet Union (Mayak workers) who participated, during the late 1940s through mid-1950s, in the production of plutonium for nuclear weapons received large γ-ray doses (up to $\sim$1000 rad in some cases) over several years and survived.

Table 5 shows estimates (lower bound, central, and upper bound) of thresholds for specific deterministic effects of exposure of the unborn embryo or fetus to X or gamma rays delivered quickly (within 1 h).

Late Somatic Effects

Late somatic effects are those that occur long after exposure to a DNA-damaging agent in progeny of cells other than germ cells. The late somatic effect that is of most concern is cancer.

Induction of Cancer by Ionizing Radiation

One of the first observations of cancer following irradiation was the appearance of skin cancer on the

Table 5 Thresholds (lower, central and upper estimates) for deterministic effects of exposure of the unborn embryo or fetus[a]

Effect	*Time/period* [b]	*Lower bound (rad)*	*Central (rad)*	*Upper bound (rad)*
Small head size	0–17 weeks	5	10	Not estimated
Severe mental retardation	8–15 weeks	0	10	20
	16–25 weeks	0	20	50
Death of embryo or fetus	0–18 days	0	10	50
	18–150 days	20	40	50
	150–term (days)	120	150	180

[a] Applies to X or gamma rays delivered within 1 h.
[b] Refers to time after conception in days or weeks.

hands of some of the early workers who used X-rays. Since that time, animal and epidemiological studies have shown that radiation can cause an increase in the incidence of specific cancers. They have also shown that cancer does not appear immediately after exposure to radiation but only after a delay (latent period). For humans, the latent period may be quite long (many years) for some cancers.

Mechanisms that may be involved in the induction of cancer by radiation have been proposed. These mechanisms include (1) the induction of mutations, (2) the activation of oncogenes, (3) the inactivation of tumor suppressor genes, and (4) the induction of cancer-causing viruses. Although the relative importance of the various mechanisms in the induction of cancer is not clear, more than one mechanism could be involved for a given type of cancer.

For both humans and laboratory animals, one cannot currently distinguish between a radiation-induced cancer and a spontaneously occurring cancer (i.e., from an unknown cause). Therefore, statistical methods are used to determine whether radiation exposure is associated with an increase in cancer in a given study population. There have been several epidemiological studies in which definite dose–response relationships have been established for radiation-induced cancers. The best studied populations include atomic bomb survivors, *Tinea capitis* irradiation patients, ankylosing spondylitis irradiation patients, radium dial painters, radium therapy radium-224 patients, Thorotrast patients, uranium miners, Chernobyl fallout victims, and Mayak plutonium facility workers.

Atomic Bomb Survivors

Within a 3 day period in August 1945, atomic bombs were dropped on the Japanese cities of Hiroshima and Nagasaki, killing a total of 64 000 people within 1 km of the explosions as a result of blast, thermal effects, and instantaneous gamma and neutron irradiation. Since that time, a prospective epidemiological study has been conducted by a joint group of United States and Japanese scientists (the Radiation Effects Research Foundation; RERF) on $\sim$92 000 survivors who were within 10 km of the center of the respective blasts and $\sim$27 000 others who were not in either city at the time of the explosions. The study includes detailed dose reconstruction for $\sim$76 000 individuals and medical follow-up on as many of the survivors as possible. As the follow-up has continued, the RERF has periodically published updates of the cancer incidence and mortality data for these populations. Data as of 1988 showed a total of 3435 cancers, of which 357 were radiation induced. From these data, excess cancer risks are calculated which form the basis for many of the current radiation risk factors in use today. It should be noted that a large fraction of the atomic bomb survivors are still alive, particularly those who were irradiated as children, so that additional information can be anticipated as this population continues to be studied.

Tinea capitis Irradiation

From 1905 to 1960, X-ray irradiation of the scalp for treating ringworm, *T. capitis*, was regularly performed on as many as 200 000 children worldwide. For a typical series of X-ray treatments, doses of 220–540 rad were received by the scalp, 140 rad to the brain, 380 rad to the cranial marrow, and $<$100 rad to other organs and tissues of the head and neck. Cancers of the thyroid and skin (basal cell carcinoma) were the major consequences of irradiation.

Ankylosing Spondylitis Irradiation

About 14 000 patients with the disease ankylosing spondylitis received X-ray therapy between 1935 and 1954 in Great Britain and Northern Ireland. In irradiating the spine, doses of 300–700 rad were received by tissues in the thoracic region. The major radiation-related outcome has been an excess of leukemia due to irradiation of bone marrow progenitor cells within the ribs and vertebrae and, recently, an indication of excess solid tumors in the lungs,

esophagus, and breast. The importance of this study has been in the health effects from partial-body irradiation and in the temporal pattern of appearance of solid tumors.

Radium Dial Painters

Radium, as radium-226 and radium-228, was used in luminous paints during 1920–50. Large amounts of radium were ingested by painters of watch and instrument dials as they tipped their brushes by mouth to achieve a fine point. The radium, once ingested, behaves chemically like calcium and, therefore, deposits in significant quantities in bone mineral, where it is retained for a very long time. Being an alpha-emitting radionuclide, the radium irradiates bone surface-lining cells and has resulted in an excess incidence of osteogenic sarcomas. Of interest in these patients has been the observation of a 'practical threshold' of dose and dose rate from radium-226, below which bone cancers do not appear to occur. This has also been observed in some experimental animal studies.

Radium Therapy (^{224}Ra)

In Europe, the short-lived radionuclide (3.6 day half-life) radium-224 was used for more than 40 years in the early 1900s in treating tuberculosis and ankylosing spondylitis. Because of its effectiveness as an analgesic in treating debilitating bone pain from the latter, its use has continued. Radium-224, being an alpha-emitting radionuclide that deposits on bone surfaces, delivers its radiation dose effectively to bone-lining cells, inducing an excess of osteogenic sarcomas, similar to those found in the radium dial painters. Interestingly, no excess of leukemia cases has been found in this population, even though portions of the hematopoietic precursor cell populations are purportedly within range of the alpha-particle irradiation.

Thorotrast Patients From 1928 to the 1950s, a preparation of the radioactive, colloidal thorium dioxide (Thorotrast) was used extensively as an X-ray contrast medium in angiographic studies. Because of the very high density of thorium to X-rays and the tendency of the colloidal particles to be taken up by the fixed phagocytes within the liver and spleen, it was effective in diagnostic imaging of these organs. However, because Thorotrast is chemically insoluble *in vivo* and is retained tenaciously for long times, long-term alpha irradiation of liver, spleen, and bone marrow tissues occurred, with a resultant large increased incidence of various liver carcinomas and sarcomas. In this case and unlike the results from radium exposure, an excess incidence of leukemia has been observed.

Uranium Miners

As part of the radioactive decay series of uranium, radon-222, a radioactive noble gas, emanates from geological deposits. During underground mining, this gas was released to the work space, and miners inhaled both this gas and its radioactive progeny in significant amounts. Epidemiological studies have been done on mining populations from the United States, Canada, Australia, Czechoslovakia, France, China, and Sweden. Their results have shown conclusively that inhalation exposure to radon and progeny is a strong risk factor for lung cancer, both with and without concurrent exposure to cigarette smoke. This database is used to project lung cancer risk for exposure of the general population to radon in indoor environments.

Chernobyl Fallout Victims

The nuclear reactor accident that occurred in Chernobyl in April 1986 released large quantities of radionuclides to the environment. The contamination was highest near the reactor, with significant fallout also occurring in the western part of the former Soviet Union and spreading to many parts of western Europe. At this point, the medical follow-up of the populations who lived near the reactor has found only one significant disease attributable to the radiation from the accident, that is, thyroid cancer in persons who were children at the time of the accident. The radiation dose to the thyroid was due to inhalation and ingestion of radioactive iodine isotopes released when the reactor core was breached; estimates indicate that the doses to children's thyroids ranged upward to as high as 1000 rad. The relatively high incidence of thyroid cancer is significant in that it was not expected based on extrapolation from the results of the atom bomb survivor study.

Mayak Plutonium Facility Workers

In the southern Ural Mountains region of Russia are the cities of Yekaterinburg, with a population of $\sim$1.4 million and 125 miles south, Chelyabinsk with a population of about 1.1 million. Shortly after the end of World War II, in the former Soviet Union construction was begun on a nuclear weapons production complex (called the Mayak Production Association (PA)) in the region. Established nearby to house workers was a secret, closed city. Because of the secrecy, the city was originally known by its postal destination, Chelyabinsk-40 (later Chelyabinsk-65). The city is no longer a secret and is called

Ozyorsk and has a population of ~90 000 persons. The main purpose of the Mayak PA was plutonium production for nuclear weapons. The Mayak PA complex included nuclear reactors, radiochemical and plutonium plants, and associated nuclear waste facilities. In the earliest years (1948–52) of operations at the facility, massive quantities of radioactive materials were released into the nearby Techa River leading to ingestion of mainly beta- and gamma-emitting radionuclides by inhabitants of villages along the river. In addition, workers at the Mayak facility inhaled large amounts of plutonium over a period of years. These workers were also exposed chronically to large doses of gamma rays.

International studies are being conducted to investigate cancer occurrences among the thousands of plutonium-239-exposed Mayak workers. Plutonium-239 is an alpha-emitting radionuclide. For the Mayak workers exposed to alpha radiation from inhaled plutonium-239 along with external gamma rays, excess cases of lung, liver, and bone cancers have been demonstrated. Several studies are ongoing that relate to a variety of health effects among the Mayak PA workers as well as to dose reconstruction.

Excess radiation-induced cancers have also been demonstrated in well-controlled studies using laboratory animals (e.g., mice, rats, and dogs). The data from animal studies are being used to supplement the dose–response information obtained from epidemiological studies in humans and are providing model systems for the investigations of the mechanisms of radiation-induced diseases such as cancer.

Cancer Risk Estimation

Models Used to Demonstrate Excess Cancers in Populations

Specific risk-assessment models are used to demonstrate an excess in radiation-induced cancer by relating the risk of cancer induction to radiation dose and to other variables and factors such as sex, genetic makeup, the presence of cigarette smoking, and the type of radiation considered. For example, smokers exposed in uranium mines to alpha radiation from inhaling radon and its progeny have a higher risk of lung cancer than do nonsmokers. In addition, alpha radiation is ~20 times more effective than gamma rays in producing lung cancer.

Important variables used in risk-assessment models include radiation dose and dose rate, age, and follow-up time. For example, very high dose rates of gamma or X-rays are thought to be about two times more effective in causing cancer in humans than are very low dose rates. There is also some evidence that a very low dose rate of alpha radiation can be more effective in producing lung cancer than somewhat higher dose rates. However, this phenomenon may be related to changes in the susceptibility of lung cancer induction with age. It is now known that the ability to repair DNA damage declines with increasing age.

Application of Absolute and Relative Risk Models

Two types of models are often used for conducting statistical analysis of cancer risks: (1) absolute-risk models and (2) relative-risk models. With absolute-risk models, the excess risk due to exposure to radiation does not depend on the normal risk that would arise when there is no radiation exposure. With relative-risk models, the relative risk is a multiple of the normal risk. Unlike absolute risk, which is measured on a scale that starts at 0 and goes to 1, relative risk values begin at 1 and go to infinity (i.e., very large numbers). A value of 1 for the relative risk means that there is no excess risk.

As an example of application of absolute risk, if the normal risk over the lifetime is 0.001 for a specific type of cancer, and radiation adds an additional risk of 0.01, then the absolute risk of cancer over the lifetime is $0.001 + 0.01$, or 0.011.

The relative risk takes into consideration how the normal risk changes with age. For example, if the normal risk of developing a given type of cancer between the ages of 50 and 51 years is 0.001, and radiation exposure leads to a relative risk of 2; then, the relative risk is used to multiply the normal risk so one has to calculate the product 2×0.001, or 0.002. Thus, instead of having a normal risk of 0.001 for cancer in the age interval 50–51 years, the risk is increased to 0.002 because of exposure to radiation. Similar calculations are carried out for other age intervals depending on the age of the person at exposure and the latent period for the cancer type of interest. The risk for the different age intervals would then be added to obtain a lifetime risk. However, no radiation-related risk would be counted during the latent period. Currently used lifetime risk estimates for cancer induction are largely based on either relative-risk or absolute-risk models.

Current Lifetime Risk Estimates

On the basis of available evidence, the Committee on the Biological Effects of Ionizing Radiations (called BEIR V Committee) has recommended use of a lifetime excess risk (i.e., normal risk has been subtracted) of 0.08 per 100 rad for death from γ-ray- or X-ray-induced cancer. This risk applies to the average person in the United States population (all ages considered) exposed to doses up to 10 rad, when

delivered in a short time (e.g., minutes to a few hours). When the same dose is delivered over weeks or months, the risk is expected to be reduced, possibly by a factor of 2 or more. The risk for exposure during childhood is estimated to be about twice as large as that for adults. Males and females are judged to have similar risks. However, all of the cited risk estimates should be regarded as uncertain. These same risks would apply to other radiation sources (e.g., neutrons, beta particles, and alpha particles) if the absorbed dose in rads or Gy were replaced by effective dose in rem or Sv (see Dosimetric Quantities and Units for an explanation of effective dose). The cited risk estimates do not apply to the known subpopulations that are highly sensitive to radiation.

See also: Carcinogenesis; Chromosome Aberrations; Developmental Toxicology; Gastrointestinal System; Metals; Molecular Toxicology–Recombinant DNA Technology; Occupational Toxicology; Pollution, Air Indoor; Respiratory Tract; Risk Assessment, Human Health; Skeletal System; Skin.

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Radium

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- Representative Chemicals: Radium bromide and radium chloride, both soluble in water, are two of the common forms of radium with public health concerns
- Chemical Abstracts Service Registry Number: CAS 7440-14-4
- Chemical/Pharmaceutical/Other Class: Radioactive alkaline earth metals
- Chemical Formula: Ra^{2+} (radium isotopes with molecular weights of 226 and 228 are the most common isotopic forms found in the environment)

Uses

Intentional uses of radium today are primarily in the treatment of cancer using a radiation source and as a neutron source in research and instrument calibration. Earlier uses of radium in paints and as a treatment for other illnesses and health-rejuvenating tonics were halted after its toxicity was recognized (see below).

Background Information

Radium has a particularly interesting history. It was isolated from pitchblende by the Curies in 1898, who in 1903 jointly won the Nobel Prize in physics for their studies of radiation. Many people experienced significant exposures to radium before its harmful health effects were known. In the early twentieth century, luminous paint was developed that contained radium. Workers painted 'glow in the dark' watch dials, clocks, compasses, and other instruments starting in the early part of the century through the 1960s. Many developed cancer (bone sarcoma) by ingesting significant amounts of radium if they used unsafe work practices; for example, moistening and shaping the thin tips of their delicate brushes by insertion into the mouth. Also around this time the American Medical Association accepted radium therapy as a treatment for rheumatism, mental instability, and a variety of other disorders. Over-the-counter solutions containing radium (e.g., 'Radithor') were ingested by thousands of people seeking cures for these illnesses. Other patients sought relief by exposing themselves to natural water sources containing significant amounts of radium. In post-World War I Germany, injections were given to treat tuberculosis, ankylosing spondylitis, and other

diseases. Some of these worker and patient cohorts continue to be studied in order to gain a better understanding of the long-term effects of radium exposure.

Exposure Routes and Pathways

Radium is a silvery-white radioactive metal found in most soils and rocks, although usually present in small quantities. Virtually everyone is exposed to low levels of radium in inhaled air and ingested water and food. The concentrations of radium-226 and radium-228 in drinking water are generally low, but there are specific geographic regions where high concentrations of radium occur due to geologic sources. Radium is a product of uranium and thorium breakdown and present in all uranium ores. It undergoes spontaneous disintegration to form radon. People living near industries that burn coal or other fuels or in areas where uranium is abundant can expect to have higher exposures to both radium and radon. Miners and people living or working around radioactive waste disposal sites are also exposed to higher levels of radium than the general public.

Toxicokinetics

Radium is like calcium in that it deposits in bones and teeth when taken into body. Following an accidental acute inhalation of radium, the substance deposited first in lungs, some amount was detected for a short time into soft tissues, and most of the remaining amount lodged in the skeleton. The biological half-life was determined in this case to be $\sim$120 days. When ingested, a $\sim$80% can be expected to be excreted in feces and 20% retained and distributed in the body, primarily in the skeleton. Radium is not metabolized by the body; it only decays over time. The toxicokinetics of dermal exposures to radium have not be well characterized, although it is known that the predominant radioactive α- and β-decay products of radium do not penetrate appreciably into the body following skin exposures.

Mechanism of Toxicity

The radioactive properties of radium are the greatest concern and overwhelm all else. All radioactive materials may cause harm when decay particles are released that disrupt many critical cell functions, including DNA replication. Radioactive materials may also produce toxicity not related to their radioactive behavior. Like barium compounds, radium enters teeth and bones, altering growth and causing them to be weak and brittle.

Acute and Short-Term Toxicity (or Exposure)

Injection of single high doses of radium (2000–4000 μCi kg^{-1} or 74 000–148 000 Bq kg^{-1}) into mice has caused death with a few weeks. Theoretically, similarly high acute exposures could also produce the same effect in humans.

Chronic Toxicity (or Exposure)

Responses of laboratory animals to chronic radium exposure have been studied extensively and are similar to human responses. The large amount of human exposure data available is probably most relevant. Potential symptoms of overexposure to radium include anemia, cataracts, fractured teeth, excess cavities, and cancer. Bone cancer is the most common cancer site associated with high-level radium exposure, but increased risks of liver and breast cancer have also been reported.

Ecotoxicology

Radium is almost ubiquitous in soils, water, geologic materials, plants, and foods at low concentrations. The utilization of radium, uranium, and fossil fuels has resulted in the redistribution of radium in the environment by way of air, water, and land releases. The concentration of radium in natural water is usually controlled by adsorption–desorption reactions with minerals and rocks and by the solubility of radium-containing minerals. In addition, radium is constantly being produced by the radioactive decay of its precursors, uranium and thorium. Radium does not degrade other than by radioactive decay at rates which are specific to each of four naturally occurring isotopes. Radium may be bioconcentrated and bioaccumulated by plants and animals, and it is transferred through food chains from lower trophic levels to humans. The radioactive decay half-lives of the more common radium isotopes range from 3.6 days, 11.4 days, 5.7 years, and 1600 years for 224, 223, 228, and 226, respectively.

Exposure Standards and Guidelines

The US Environmental Protection Agency (EPA) drinking water limit for radium-226 and radium-228 combined is 5 pCi l^{-1}. EPA's limit for maximum soil concentration for radium-226 in uranium and

thorium mill tailings is 5 pCi g^{-1} in the top 15 cm of soil and 15 pCi g^{-1} in deeper soil.

See also: Radon; Uranium.

Further Reading

Cotton FA, Wilkinson G, Murillo CA, and Bochmann M, (1999) *Advanced Inorganic Chemistry*, 6th edn., pp. 111–130. New York: Wiley.

Macklis RM (1990) Radiation and the era of mild radium therapy. *Journal of the American Medical Association* 264: 614–616.

Stebbings JH (2001) Health risks from radium in workplaces: An unfinished story. *Occupational Medicine* 16(2): 259–270.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Radium.

Radon

Richard A Parent

This article is a revision of the previous print edition article by Richard A Parent, T R Kline, and D E Sharp, volume 3, pp. 19–20, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 10043-92-2
- Synonyms: Radon-222; Nitron; Alphatron
- Chemical/Pharmaceutical/Other Class: Unstable radioisotope
- Chemical Formula: ^{222}Ra

Uses

Radon is used in cancer treatment, as a tracer in leak detection, in flow rate measurement, in radiography, and in chemical research.

Exposure Routes and Pathways

Radon and its decay products enter the body by inhalation, dermal absorption, and ingestion. The extent to which the population is exposed to radon-222 and its daughters (polonium-218 and polonium-214) in the air, especially indoors, has recently received increased attention. Indoor radon-222 and daughter concentrations arise from outside air, building materials, water supplies, and the soil and rock underlying the building. Ventilation rates may be altered to obviate unacceptable levels of radon. Persons working with radium and its compounds are also exposed to radon.

Toxicokinetics

Radon is transported in the air by absorption on dust particles that are easily deposited in the bronchiolar areas of the pulmonary system. Deposition on the sticky surface of the bronchial epithelial tissue allows for the irradiation of that tissue with α-particles and consequent transformation to cancer tissue. Radon daughters are also easily absorbed on solid surfaces, especially colloids and dust particles present in the atmosphere.

Short-lived and long-lived radon daughters, produced within the atmosphere and the body, may become selectively distributed to various organs via the bloodstream.

The major systemic threat of these materials is to the kidneys from biotransformed radon daughters. Radon transported by the blood reaches various tissues and organs. Its distribution depends chiefly on the fat content of organs and tissues since it is lipid soluble. From 50% to 90% of the radon body burden is located in the fatty tissues. Radon daughters taken in become localized largely in active deposits in the lungs, to which they represent a grave threat.

Radon is eliminated mainly in exhaled air (~90% in the first hour and the remainder within 6 or 7 h), whereas radon daughters are eliminated mainly by excretion in feces and urine. The biological half-life of radon is reported to be 3.823 days.

Mechanism of Toxicity

Radon gas has demonstrated carcinogenicity attributed chiefly to its radioactive properties. Radon gas has been implicated in the occurrence of lung cancer in individuals engaged in mining ores. Miners who smoke cigarettes are at higher risk, indicating a possible synergistic effect between ore dust, radiation, and cigarette smoking. This situation leads to a high risk of cancer in the respiratory tract. Occupancy of radon-containing homes, particularly in the lower floor levels, might also be a cause of lung cancer. Deliberate or inadvertent intake of radioactive

elements or their compounds that concentrate in certain organs or tissues may be a cancer risk.

Radon itself is chemically inert and electrically uncharged but it is radioactive, which means it undergoes a decay process and can change into other atoms. These other atoms are called radon daughters or progeny and they are electrically charged. As a result of their being charged, they can attach to tiny dust particles in indoor air. These dust particles are respirable and can deposit in the lungs and conducting airways. Because the radon daughters are also unstable, they decay producing α-radiation, which irradiates proximate tissue causing damage to the cellular DNA and in turn can lead to cancer.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute lethal effects of radon and its daughters have been studied in mice and a 30 day LD_{50} was estimated based on a single exposure at a concentration of $2.2 \times 10^8\,pCl^{-1}$ of air for 5–40 h of exposure. All of the exposed mice died within 2 weeks after 40 h of exposure but no animals died after 26 h of exposure or less.

Human

Acute radiation syndrome involves extreme cases of radiation exposure and it is difficult to envision such exposure from environmentally generated radon and its daughters. When it does occur, however, it appears to progress in four stages: prodome, latent, manifest illness, and recovery. The prodome phase occurs $\sim$48–72 h postexposure and is characterized by nausea, vomiting, diarrhea, intestinal cramps, salivation, and dehydration with accompanying neurovascular dysfunction, which includes fatigue, weakness, apathy, fever, and hypotension. During the latency period, exposure of the bone marrow results in decreased cell counts that are dose dependent. This period lasts from 1 to 2.5 weeks. Major organ damage can occur during this phase and extends into the manifest illness phase that results in either recovery or death.

Radon is not acutely irritating to the eye or mucous membranes.

Chronic Toxicity (or Exposure)

Animal

Sprague–Dawley rats were exposed to radon progeny up to 82 days. The lung cancer incidence in rats was directly proportional to the lifetime cumulative exposure to radon progeny. Mixed adenosquamous carcinomas, bronchiolar/alveolar carcinomas, and squamous cell carcinomas were observed in treated animals and were significantly elevated above control animals. Exposed hamsters also showed increased incidences of squamous cell carcinomas. Chromosomal aberrations have also been demonstrated in animals exposed to radon. Radon and its daughters are considered to be carcinogenic in animals as exemplified by the numerous radon exposure studies resulting in bronchogenic cancers.

Human

Inhalation of dust particles contaminated with radon and its daughters represents the major hazard to human health from these materials. The absorbed material is deposited in the bronchial area of the lung. Before the dust can be cleared from the lung, some of it is absorbed and all of it has irradiated the epithelial surface of the bronchial region of the lung with α-particles, creating a significant risk of cell transformation to cancer foci. An increased risk of lung cancer has been associated with radon exposure in uranium miners. This increased risk of the development of respiratory cancer has been well documented. An additive, rather than multiplicative, model has been gaining support to illustrate the connection between smoking and radon daughter-induced lung cancer. Mostly bronchogenic cancers are produced, including squamous cell carcinomas, mixed adenocarcinomas, and, in miners, mostly oat cell carcinomas.

A case–referent study of exposure to radon from the ground and bronchial cancer was carried out on 292 female lung cancer cases and 584 controls who had lived in Stockholm for 30 or more years. Lung cancer cases were diagnosed as oat cell and other types of anaplastic pulmonary carcinomas and the study concluded that radon and daughters were a significant etiologic factor in the cancers noted.

A case–control study of 27 lung cancer subjects in Ontario, Canada, resulted in a marginally significant association between radon exposures and the lung cancers but a strong association with smoking.

Other human consequences of radon exposure include cataracts, nephritis, and dermatitis. Congenital malformations and spontaneous abortions have also been reported in miners exposed to significant concentrations of radon.

Chromosomal aberrations in peripheral lymphocytes from underground miners have also been reported at significantly increased incidence levels. Peripheral lymphocyte chromosomes from 80 underground uranium miners were studied. Significantly,

more chromosomal aberrations were observed among workers with markedly atypical bronchial cell cytology, suspected carcinoma, or carcinoma *in situ* than among miners with regular or mildly atypical cells.

Radon and its daughters have been classified by the International Agency for Research on Cancer as being carcinogenic to humans and animals based on extensive data. The Environmental Protection Agency (EPA) and the National Cancer Institute have estimated that there are 15 000 deaths annually in the United States from radon-induced lung cancer. EPA's recommended exposure guideline of $4\,\mathrm{pCi\,l^{-1}}$ of air is estimated to pose a 1–5% risk from developing lung cancer if a person is a smoker or nonsmoker. The risk of lung cancer in radon exposed individuals is 10 times greater in smokers than in nonsmokers. The National Research Council estimates lung cancer risk at from 0.8% to 1.4% in persons exposed to the EPA's lifetime exposure guideline.

In Vitro Toxicity Data

Ionizing radiation is genotoxic, causing chromosomal damage, DNA fragmentation, and large-scale changes in the DNA structure and function.

Clinical Management

Although it is imperative to provide medical surveillance for those subjected to elevated exposures to radon and its decomposition products such as miners, once a cancer has developed its treatment depends a lot on the extent of the neoplasm and its location. Cytogenetics can be used as a biological monitoring tool for exposed populations.

Most treatments are symptomatic and supportive. Prevention of infection from bone marrow depletion of cellular components is imperative. Bone marrow depression must be treated and fluids and electrolytes must be replaced as needed. Seizures can be managed with benzodiazepines or phenobarbital.

Environmental Fate

Escape of radon and its daughters from soils into the atmosphere is highly dependent on meteorological conditions and the types of soils in the particular area. Release from rock, soils, and other materials is not well understood. Radon and its daughters are readily adsorbed on various surfaces and surface waters are known to contain some amount of radon. In the atmosphere, one part of radon is thought to be present in 1×10^{21} parts of air but these concentrations vary daily and seasonally.

Other Hazards

High rates of congenital malformations and spontaneous abortions have been reported in uranium mining areas.

Exposure Standards and Guidelines

EPA proposed drinking water standard: $300\,\mathrm{pCi\,l^{-1}}$ (see Federal Register: November 2, 1999 (vol. 64, no. 211, pp. 59245–59294).

Massachusetts has a drinking water guideline of $10\,000\,\mathrm{pCi\,l^{-1}}$ of water. Radionucleotides have been designated as hazardous air pollutants under section 112 of the Clean Air Act.

Miscellaneous

Radon is a naturally occurring radioactive gas and environmental contaminant resulting from the radioactive decay of radium. It is considered an inert gas and has an atomic weight of 222. It is a colorless, tasteless, odorless, and extremely dense gas that phosphoresces when condensed into liquid. It boils at $-61.7^{\circ}\mathrm{C}$ and has a density of $9.72\,\mathrm{g\,l^{-1}}$ at $0^{\circ}\mathrm{C}$. The half-life of radon-222 is ~ 3.8 days, decaying to polonium-218 and polonium-214 among other materials. Exposures to radon and its daughters are measured in working-level months. A working-level month is defined as a 170 h working month exposure to alpha radiation from radon daughters equal to $1.3 \times 10^{+5}\,\mathrm{MeV}$ emitted in 1 l of air. There is no method for detecting radon other than laboratory sampling and measurement. Radon is quite soluble in water and is the heaviest gas known to man.

Radon in houses can come from building materials, the soil under the house, the water, and the domestic gas. Some materials such as alum shale and phospho-gypsum have significantly higher radium concentrations than others and can thus cause increased internal radon concentrations to increase. Ventilation rates in basements and in houses in general can reduce exposure significantly.

See also: Pollution, Air Indoor; Pollution, Soil; Pyrrolizidine Alkaloids.

Further Reading

Borak TB and Johnson JA (1988) Estimating the risk of lung cancer from inhalation of radon daughters indoors: Review and evaluation. *Government Reports Announcements and Index 18*; pp. 1–131.

Field RW, Steck DL, Smith BJ, *et al.* (2000) Residential radon gas exposure and lung cancer: The Iowa radon lung cancer study. *American Journal of Epidemiology* 151(11): 1091–1102.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Radon.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Radon.

http://www.nsc.org – Biological Effects of Ionizing Radiation(BEIR) VI Report: 'The Health Effects of Exposure to Indoor Radon', Public Summary.

http://www.cheec.uiowa.edu – Field RW *et al.* (2000) Residential Radon and Lung Cancer Case–Control Study; See also 'Iowa Radon Lung Cancer Study Abbreviated Methodology', May 25.

Ranitidine

Alexander B Baer and Christopher P Holstege

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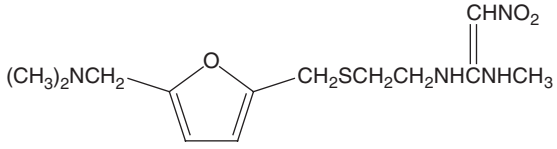
- Chemical Name: Ranitidine
- Chemical Abstracts Services Registry Number: CAS 6637-35-5
- Synonyms: Zantac; Histamine blocker; Antacid; *N*,*N*-Dimethyl-5-(2-(1-methylamino-2-nitrovinylamino)-ethylthiomethyl)furfurylamine
- Chemical/Pharmaceutical/Other Class: A competitive inhibitor of the H2 receptor located on the gastric parietal cells
- Chemical Structure:



Uses

Ranitidine is indicated for therapy of peptic ulcer disease, gastroesophageal reflux disease, pathological hypersecretory conditions (e.g., Zollinger–Ellison syndrome), erosive esophagitis, and adjunctive treatment of acute allergic reactions.

Exposure Routes and Pathways

Both injection and ingestion are the routes of both accidental and intentional exposures to ranitidine.

Toxicokinetics

Ranitidine is absorbed rapidly from the gastrointestinal tract and undergoes extensive first-pass metabolism. The absolute bioavailability of orally administered ranitidine is 39–87%. Mean peak serum concentrations occur within 2 or 3 h following oral doses of 150 mg. Ranitidine is metabolized in the liver to *N*-oxide, desmethyl ranitidine, and ranitidine *s*-oxide. Ranitidine is widely distributed throughout the body and is 10–19% protein bound. The apparent volume of distribution is $1.2–1.9\,l\,kg^{-1}$. Ranitidine is excreted principally in urine via glomerular filtration and tubular excretion. Approximately 30% of an oral dose and 70% of the parenteral dose is excreted unchanged in the urine. The elimination half-life of ranitidine is 2–2.5 h in healthy children and adults. Its half-life is prolonged in patients with renal failure (5.9–8.9 h).

Mechanism of Toxicity

Rare adverse cardiac effects have been reported secondary to ranitidine. These effects may be either due to direct ranitidine blockade of cardiac H2 receptors or due to potentiation of acetylcholine activity on the heart by ranitidine-induced inhibition of acetylcholinesterases. Ranitidine-induced hepatic injury is thought to be secondary to an idiosyncratic reaction or a hypersensitivity reaction.

Acute and Short-Term Toxicity (or Exposure)

Animal

Antihistamine toxicity in animals is usually mild, causing sedation and ataxia. Treatment consists mainly of basic supportive measures.

Human

Reports of toxicity by ranitidine are extremely rare and are primarily based on individual case reports.

Chronic Toxicity (or Exposure)

Animal

Feeding studies in mice and rats at up to $2\,g\,kg^{-1}$ have not shown evidence of carcinogenicity.

Human

Ranitidine is generally well tolerated in therapeutic doses. Ranitidine has less central nervous system (CNS) penetration, endocrine effects, and cardiovascular effects than cimetidine. Reported CNS effects associated with ranitidine include hallucinations, depression, delirium, headaches, dystonia, and choreoathetosis. Cardiac arrest during infusion, bradycardia, and progressive AV block with syncope have been reported in association with ranitidine. Abnormal liver enzymes, interstitial nephritis, parotitis, leukopenia, granulocytopenia, thrombocytopenia, pancytopenia, eosinophilia, vasculitis, dermatitis, toxic epidermal necrolysis, sexual impotence, gynecomastia, and polymyositis have also been reported in association with ranitidine therapy.

In Vitro Toxicity Data

Mutagenicity studies of ranitidine and its metabolites have not demonstrated positive effects.

Clinical Management

In addition to general supportive measures directed to the airway, breathing, and circulation, the clinician may consider measures to decrease gastrointestinal absorption in the alert patient they suspect has been exposed to a potentially toxic dose of ranitidine. Among the appropriate tests that should be obtained are an electrocardiogram, transaminases, and a complete blood count. In the vast majority of cases of ranitidine exposures, patients develop minor symptoms and no therapy is necessary.

See also: Cimetidine.

Further Reading

MacMahon B, Bakshi M, and Walash MJ (1981) Cardiac arrhythmias after intravenous cimetidine. *New England Journal of Medicine* 305: 832–833.

Price W, Coli L, and Brandstetter RD (1985) Ranitidine-associated hallucinations. *European Journal of Clinical Pharmacology* 29: 375–376.

Read Across Analysis *See* Toxicity Testing, 'Read Across Analysis'.

Recombinant DNA *See* Molecular Toxicology–Recombinant DNA Technology.

Recommended Exposure Limits (REL)

Alan J Weinrich

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Recommended exposure limit (REL) is the name used by the US National Institute for Occupational Safety and Health (NIOSH) for the occupational exposure limits (OELs) it recommends to protect workers from hazardous substances and conditions in the workplace. RELs are not regulations. While they are intended primarily as recommendations to the US Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA) for use in promulgating legal standards, they also may help employers, workers, and health professionals to recognize and control occupational hazards. Most RELs have been developed for chemical air contaminants, usually represented as numerical values for airborne concentrations (expressed as ppm, $mg\,m^{-3}$, or $fibers\,cm^{-3}$). However, NIOSH has developed RELs for other hazards, including physical agents such as noise, heat, and ultraviolet radiation. Like other OELs, NIOSH expresses most RELs as time-weighted average (TWA) exposures, for up to $10\,h\,day^{-1}$ during a 40 h workweek. An REL also may be expressed as a:

- short-term exposure limit (ST) that should never be exceeded and is to be measured in a specified sampling time (usually 15 min), or
- ceiling limit (C) that should never be exceeded even instantaneously, unless specified over a given time period.

In addition to quantitative exposure recommendations, NIOSH occasionally assigns one or more

notations to selected RELs. Most prominent of these is the 'skin' designation for chemical substance RELs, indicating the potential for dermal absorption and implying a recommendation that skin exposure be prevented by using good work practices and gloves, coveralls, goggles, and other appropriate equipment.

The US Occupational Safety and Health Act of 1970, in addition to creating OSHA and NIOSH, mandated NIOSH to develop objective safety and health criteria describing RELs "at which no employee will suffer impaired health or functional capacities or diminished life expectancy as a result of his work experience." Most RELs that were independently developed by NIOSH resulted from exhaustive reviews of available data, called criteria documents, in the 1970s. However, the criteria document process almost stopped after the 1970s. NIOSH generated hundreds of RELs in the 1980s through other processes, mainly by accepting most 1989 proposed updates of the OSHA permissible exposure limits (PELs), which generally were derived indirectly from the then-current American Conference of Governmental Industrial Hygienists (ACGIH®) threshold limit values (TLVs®). NIOSH continues to review and develop RELs in much smaller numbers and to publish the RELs in various documents, most notably the frequently updated NIOSH Pocket Guide to Chemical Hazards.

NIOSH develops most RELs from qualitative and semiquantitative risk assessments, using expert judgments based on comprehensive reviews of relevant scientific literature. However, a number of RELs have been based on limits of sampling capabilities or on limits of technological feasibility. In response to an OSHA rule on carcinogens (29 CFR 1990.103), NIOSH had subscribed to a policy calling for 'no detectable exposure levels for proven carcinogenic substances'. NIOSH bases recently developed RELs for carcinogens and other chemical substances on risk evaluations using human and animal health effects data, and on feasibility assessments for engineering controls and analytical methods.

See also: American Conference of Governmental Industrial Hygienists; Occupational Exposure Limits; Occupational Safety and Health Act, US; Occupational Safety and Health Administration; Occupational Toxicology.

Further Reading

Fairchild EJ (1976) Guidelines for a NIOSH policy on occupational carcinogenesis. *Annals of the New York Academy of Sciences* 271: 200–207.

NIOSH (1992) *Recommendations for Occupational Safety and Health: Compendium of Policy Documents and Statements*. Cincinnati, OH: US Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 92-100.

NIOSH (2004) *Pocket Guide to Chemical Hazards (NPG)*. Cincinnati, OH: US Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 97-140, Fourth printing with changes and updates (2004).

US Public Law 91-596, 91st Congress, S.2193. Occupational Safety and Health Act of 1970.

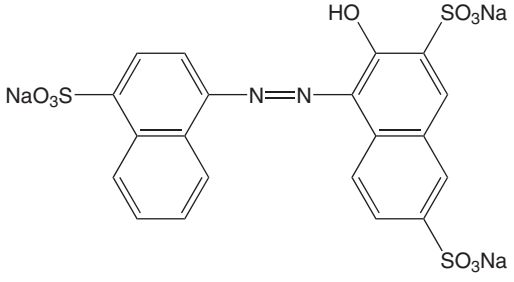
Relevant Website

http://www.cdc.gov – The current version of the NIOSH *Pocket Guide to Chemical Hazards (NPG)* is available online at an extension on this webpage.

Red Dye No. 2

Janice McKee

- Chemical Abstracts Service Registry Number: CAS 915-67-3
- Synonyms: Amaranth; FD&C No. 2; Whortleberry red; 3-Hydroxy-4-[(4-sulfo-1-naphthalenyl) azo]-2,7-naphthalenedisulfonic acid trisodium salt
- Chemical/Pharmaceutical/Other Class: Azo dye
- Chemical Formula: 20-$H_{11}N_2O10S_3Na_3$
- Chemical Structure:



Uses

Red Dye No. 2 was formerly used in food, drugs, and cosmetics but was banned by the US Food and Drug Administration (FDA) in 1976. It is currently used in the United States for dyeing wool, silk, and other textiles as well as paper, wood, and leather products. It is also used as an indicator in hydrazine titrations and is used in color photography and in the manufacture of phenol–formaldehyde resins. Red Dye No. 2 continues to be widely used in food, drugs, and cosmetics in other countries.

Background Information

In 1960, amendments to the Food, Drug, and Cosmetic Act of 1938 added the so-called Delaney anticancer clause to FDA's legal mandate. Among other things, the clause prohibits marketing any color additive the agency has found to cause cancer in animals or humans, regardless of amount. In the early 1970s, data from Russian studies raised questions about Red Dye No. 2's safety. FDA conducted its own tests, which were inconclusive. The consumer-based Health Research Group petitioned FDA to ban the color. FDA turned the matter over to its Toxicology Advisory Committee, which evaluated numerous reports and decided there was no evidence of a hazard. The committee then asked FDA to conduct follow-up analyses. Agency scientists evaluated data and concluded that "it appears that feeding FD&C Red No. 2 at a high dosage results in a statistically significant increase" in malignant tumors in female rats. FDA ultimately decided to ban the color because it had not been shown to be safe. Industry could petition FDA to list Red Dye No. 2 as a certifiable color again if animal study data adequately show safety.

Exposure Routes and Pathways

Exposure may occur through oral or dermal routes. Inhalation routes of exposure are unlikely. Occupational exposure may occur during its production and use as a dye.

Toxicokinetics

When given orally, 8% of the amaranth is absorbed from the intestinal tract. Intestinal flora have a reducing effect on amaranth, and azo reduction is also mediated by the hepatic monooxygenase system. Aromatic amine metabolites, including 1-amino-4-naphthalene sulfonic acid and 1-amino-2-hydroxy-3,6-naphthalene disulfonic acid, are excreted in the urine and bile.

Mechanism of Toxicity

Dietary amaranth in animals results in an exfoliating or solubilizing effect on the brush border membrane of the small intestine. Amaranth stimulates *in vitro* RNA synthesis by causing the dissociation of chromatin. Amaranth has also been shown to increase kidney malate dehydrogenase activity after intramuscular dosing.

Acute and Short-Term Toxicity (or Exposure)

Animal

Amaranth did not cause sensitization in guinea pigs, nor was there any significant dermal or systemic toxicity related to dermal treatment in rabbits. An allergic response was observed after intradermal stimulation in the guinea pig. The acute toxicity of amaranth is low: in rats, the intraperitoneal and intravenous LD_{50} is $1000\,mg\,kg^{-1}$, and the oral LD_{50} in mice is $10\,000\,mg\,kg^{-1}$.

Human

Some persons are sensitive to azo dyes, with reactions including recurrent urticaria. Children with sensitivity to amaranth have exhibited behavioral changes.

Chronic Toxicity (or Exposure)

Animal

Numerous chronic and transgenerational studies have shown no statistically significant increase in reproductive, developmental, or teratogenic effects due to dietary amaranth, although some behavioral changes in male mice pups have been observed. Chronic feeding studies in rats have shown increased mortality, growth inhibition, vacuolar dystrophy, granular deposits in the intestinal tract, increased kidney weight, decreased vitamin A content of the liver, and fatty degeneration of liver cells. However, no histopathological or other effects were noted in beagle dogs fed amaranth for 7 years. Carcinogenicity studies on amaranth have mixed results, with some studies showing skin carcinoma, intestinal carcinoma, lymphosarcoma, mammary tumors, hepatoma, and adenofibroma. Many other studies showed no statistically significant increase in tumors.

Human

There are no data on the carcinogenicity or chronic effects of amaranth in humans.

In Vitro Toxicity Data

Amaranth has not tested positive in a variety of *in vitro* mutagenicity studies; however, its metabolites have tested positive in some assays. DNA damage was induced in gastrointestinal organs and clastogenicity has been observed during at least three recent *in vivo* mouse mutagenicity studies.

Clinical Management

Patients exhibiting toxicity or sensitivity should be treated symptomatically. Phenobarbital has been shown to increase plasma disappearance and biliary excretion of amaranth but is not recommended for clinical treatment on a routine basis.

Environmental Fate

Red Dye No. 2 will exist in air solely in the particulate phase due to an extremely low vapor pressure. Particulate-phase Red Dye No. 2 may be physically removed from the air by wet and dry depositions. Red Dye No. 2 may have very high mobility in soil as it does not adsorb to organic carbon; however, its ionic nature may result in ion-exchange processes with clay that would retard leaching. Volatilization from dry and moist soil surfaces is not expected to be a major fate process. Red Dye No. 2 may be biodegraded anaerobically as a wide variety of anaerobic bacteria have the ability to cleave the azo linkage to produce aromatic amines. Red Dye No. 2 is not expected to volatilize from water surfaces, but it may undergo photodegradation in water. Red Dye No. 2 is not expected to bioconcentrate in aquatic organisms.

Exposure Standards and Guidelines

Red Dye No. 2 was banned by the US FDA in 1976 for use in foods, drugs, and cosmetics in the United States.

See also: Food Additives; Food and Drug Administration.

Further Reading

US Food and Drug Administration (FDA) (2001) *Color Additives Fact Sheet*. Center for Food Safety and Applied Nutrition, July 30.

World Health Organization, International Agency for Research on Cancer (IARC) (1975) *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, vol. 8. Geneva: WHO.

Red Phosphorus

S Satheesh Anand and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS: 7723-14-0
- Chemical Formula: P_n

Uses

Red phosphorus (RP) is a component of matchbox strike plates and is used as an ingredient in certain commercial rat and cockroach poisons. RP is used in the manufacture of pyrotechnics, semiconductors, fertilizers, incendiary shells, smoke bombs (in combination with butyl rubber), and tracer bullets. It is also used in organic synthesis reactions and in the manufacture of phosphoric acid, phosphine, phosphoric anhydride, phosphorus pentachloride, phosphorus trichloride, and in electroluminescent coatings. RP (2–10%) is also used as a flame-retardant additive for plastics such as polyamides, polyesters, and polyurethanes. RP is combined with elemental iodine to produce hydriodic acid, which is used to reduce ephedrine or pseudoephedrine to methamphetamine.

Background Information

Phosphorus is all about fire, and means 'light bearing' in Greek. Phosphorus was first isolated in 1669 from urine and is now primarily obtained from phosphate rock ($Ca_3(PO_4)_2$). Phosphorus is the 11th most abundant element in the Earth's crust. Phosphorus is an essential nutrient in the formation of structural biomolecules, such as membrane phospholipids; functional macromolecules such as nucleic acids, and high energy storing biomolecules such as adenosine triphosphate; and metabolic intermediates, such as sugar phosphates.

Depending upon the nature of interatomic bonds established during its formation, solid elemental phosphorus could occur in three allotropic forms: black, white (yellow), or red. Other forms of phosphorus are

derived from these allotropes. Only white phosphorus (WP) and RP are of industrial importance. WP literally glows in the dark because it is always reacting with the air around it and is known to cause severe toxicity. RP is exactly the same but in a different crystalline form. RP is entirely stable and safe to keep around. Although phosphorus was isolated in 1669, it was mainly during the first part of the nineteenth century that numerous accidental or criminal poisonings by this metalloid were observed, linked to the extensive use of the WP in making matchsticks.

RP is an amorphous phosphorus polymer; it is more resistant to oxidation, less reactive, and less toxic than WP. It is denser than air and melting point ranges from 585°C to 610°C. Also, it is nonvolatile and insoluble in water. RP is manufactured in seven countries, the largest producers being Germany, India, and China.

Exposure Routes and Pathways

Exposure to RP may occur through ingestion, inhalation, and dermal contact.

Toxicokinetics

No information available.

Mechanism of Toxicity

No information available.

Reactivity

In reactivity, the red allotropic form of elemental phosphorus is intermediate to the black and white varieties. It does not ignite spontaneously. The autoignition temperature for RP is 260°C (500°F). When RP burns, the evaporated phosphorus condenses as WP, which creates fire (reignition) and is a health hazard. For this reason, RP fires should be thoroughly cleaned up.

Although it is stable at normal temperature and pressure, the substance is classified as highly flammable and may explode when exposed to heat or by chemical reaction with oxidizers. RP can also react with reducing materials and represents a moderate explosion hazard by chemical reaction or on contact with organic materials. It reacts with oxygen and water vapor to produce the toxic phosphine.

Health Hazards

Phosphorus poisoning has been a known cause of hepatic injury for more than a century. It was used as a classical hepatotoxicant along with CCl_4 to understand the mechanisms of injury and is a direct hepatotoxin. Among the three allotropic forms, only WP is shown to cause the aforementioned effects. RP, on the other hand, is poorly absorbed and does not usually represent a significant health hazard. Thus, it can be assumed as completely safe. However, RP is often contaminated with WP and poses a health hazard. Therefore, exposure to contaminated RP may result in adverse effects on health, including irritation of the skin, eyes, lungs, and gastrointestinal tract.

Individuals with preexisting skin disorders, eye problems, jaw or tooth abnormalities, or impaired liver, kidney, or respiratory function may be more susceptible to the effects of RP. There are no reports concerning the health effects of RP in children.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of RP was reported to be greater than $10\,g\,kg^{-1}$ in Fischer 344 rats. RP caused 90% and 20% mortality in rats when exposed to $4.3\,mg\,l^{-1}$ for 1 h and $1.5\,mg\,l^{-1}$ for 4 h, respectively. The LC_{50} in Sprague–Dawley rats that were exposed to RP-BR smoke for 1 h on five consecutive days was estimated to be $2320\,mg\,m^{-3}$.

High amounts of RP may cause irritation of the skin, bronchitis, stomach pains, vomiting, and diarrhea and may affect eyes, upper respiratory tract, gastrointestinal tract, and mucous membranes if absorbed through skin, ingested, or inhaled. Effects may vary from mild irritation to severe destruction of tissue depending on the intensity and duration of exposure. Acute exposure may cause liver or kidney impairment if contaminated with WP.

No dermal and eye irritation was observed following RP exposure in rabbits. However, interdermal injection resulted in slight irritation.

One hour exposure to the combustion products of 95% RP/5% butyl rubber produced epiglottal deformation, laryngeal edema, and laryngeal and tracheal lesions in rats. A 4 h exposure produced more severe effects of a similar nature plus some hemorrhaging. Acute exposure to 97% RP/3% butadiene styrene smoke resulted in pulmonary congestion.

Human

Health effects of RP in humans in either environmental or occupational setup have not been reported. However, it is assumed that $\sim 2000\,mg\,m^{-3}$ for more than 15 min might result in death and that $700\,mg\,m^{-3}$ is the highest tolerable concentration.

Chronic Toxicity (or Exposure)

Animal

Prolonged and/or repeated skin contact may result in dermatitis and may cause eye irritation and corneal injury. Chronic exposure may cause kidney and liver damage, anemia, stomach pains, vomiting, diarrhea, blood disorders, and cardiovascular effects if RP is contaminated with WP.

Transient ocular irritation, and reddening and swelling of the eyelids were noted in rats exposed to RP-BR smoke for 5 days per week, for 12 weeks. These effects subsided by the end of the exposure.

Chronic exposure to the combustion products of 95% RP and 5% butyl rubber in male Sprague–Dawley rats for 13 weeks caused 10% mortality at high dose (1200 mg m^{-3}). Surviving animals showed dose-dependent decreases in weight gain and fibrosis of the terminal bronchioles. Guinea pigs are particularly intolerant to the effects of the smoke. Mice showed dose-dependent accumulation of alveolar macrophages.

If RP is contaminated with WP, chronic ingestion may cause necrosis of the jaw bone (Phossy jaw).

Human

According to the National Institute for Occupational Safety and Health (NIOSH), 10 occupations use RP and total number of employees exposed is 2924. There have been no studies available for the chronic effects of RP in humans. No classification data on carcinogenic properties of this material is available from the Environmental Protection Agency (EPA), International Agency for Research on Cancer (IARC), National Toxicology Program (NTP), Occupational Health and Safety Administration (OSHA), or the American Conference of Governmental Industrial Hygienists (ACGIH). There are no relevant data available for mutagenicity, genotoxicity, carcinogenicity, reproductive toxicity, and teratogenicity.

Exposure Standards and Guidelines

As a result of inadequate toxicity data, no occupational exposure limits have been set by NIOSH. Also, there are no acute or chronic reference exposure levels.

See also: Phosphorus.

Further Reading

Cal/EPA (2003) Red phosphorus. In: *Technical Support Document*: *Toxicology Clandestine Drug Labs/Methamphetamine*, vol. 1, No. 12, pp. 1–11. Office of Environmental Health Hazard Assessment.

National Research Council (1997) *Toxicity of Military Smoke and Obstructants*, vol. 1, pp. 98–126. Washington, DC: National Academy Press.

Relevant Website

http://www.cefic-efra.com – European Flame Retardants Association. Red phosphorus.

Red Squill

Alexander B Baer and Christopher P Holstege

- Chemical Name: Red squill
- Synonyms: Red squill (*Urginea maritima*); Sea onion; Oignon marin; Meerzwiebel; El-Ansal; Rat's onion; Bassel-El-Far; Wild boar's onion; Bassel-El-Khanzir; Scille; Oignon d'Egypte; Oignon de Pharaon; Scillae bulbus; Dethdiet®; Roding®

Uses

Red squill historically was used as a rodenticide, but has been replaced by newer, more effective agents. It is no longer used in medicine but may be used in folk remedies to treat cardiac insufficiency, arrhythmia, nervous heart complaints, venous complaints, edema, bronchitis, asthma, whooping cough, pain, wounds, and fractures.

Background Information

Red squill is native to the Mediterranean but has been transplanted and cultivated elsewhere. It is a member of the family Liliaceae and like most lilies produces a pear-shaped bulb that can be quite large. The bulb may be red or white in color, and this differentiates the red and white squill varieties. It produces white star-shaped flowers. It has been described as having a bitter and acrid taste. Squill was known and used by the ancients for many

purposes including treatment of coughs and arthritis, and has also been used as a diuretic, a heart tonic, and as an emetic. During the nineteenth century, medicinal use of red squill began to decline as foxglove was revealed to be both a more effective and safer alternative. Unfortunately, deaths have occurred and occur even today when humans use red squill for medicinal purposes in folk remedies. The entire plant may be toxic, but it is the bulb that is usually used and contains the greatest quantity of active compounds.

Exposure Routes and Pathways

Exposure occurs through ingestion of the plant, especially the bulb. The bulb also produces an irritating juice that can cause inflammation if rubbed onto the skin or splashed into the eye.

Mechanism of Toxicity

Red squill contains many cardiac glycosides, the most prominent being scillaren A and scilliroside. These glycosides produce digitalis-like effects when ingested and, like digitalis, inhibit Na^+/K^+ ATPase, block AV conduction, and cause sinus bradycardia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Mouse LD_{50}: 0.440 mg kg^{-1}; rat LD_{50}: 0.7 mg kg^{-1}; cat LD_{50}: 100 mg kg^{-1}; dog LD_{50}: 145 mg kg^{-1}; sheep LD_{50}: 250 mg kg^{-1}. Diarrhea and vomiting are among the first signs of toxicity. Lethargy, fatigue, and anorexia are also common. Nearly any form of cardiac arrhythmia may be seen. When ingested by mice and rats, the scilliroside may also lead to seizures.

Human

Nausea, vomiting, headaches, bradycardia, almost any form of cardiac dysrhythmias, visual disturbances, and hyperkalemia may be expected to be seen in severe overdoses.

Chronic Toxicity (or Exposure)

Animal

Vomiting, diarrhea, weakness, and eventually death may be seen after exposure.

Human

Anorexia, nausea, vomiting, visual disturbances, and the cardiac effects seen in acute toxicity may be seen. In chronic exposures, patients may not demonstrate the classic finding of hyperkalemia, which is frequently seen in acute exposures. Decreased renal function may interfere with clearance of the glycosides. Patients may be more sensitive to the effects of their squill remedies if they are on medication that also slows AV conduction such as quinine, beta blockers, or calcium channel blockers.

Clinical Management

Supportive care should be provided for all cases of squill exposure. Exposures to squill can usually be confirmed by obtaining a digoxin blood level. While levels are not linear between red squill and digoxin, and cannot be used to determine toxicity, enough cross-sensitivity exists to confirm the exposure. For significant recent exposures, activated charcoal should be considered. Atropine is recommended for treatment of bradycardia. If cardiac effects are resistant to these treatments or if hyperkalemia greater than 5 mEq l^{-1} is present, treatment with digoxin-specific Fab should be considered. If inflammation occurs due to exposure of the eyes or skin to the bulb, thorough flushing should be sufficient to resolve irritant symptoms.

See also: Atropine; Charcoal; Digitalis Glycosides.

Further Reading

El Bahri L, Djegham M, and Maklouf M (2000) *Uriginea maritima* (squill): A poisonous plant of North Africa. *Veterinary Human Toxicology* 42(2): 108–110.

Tuncok Y, Kozan O, Cavdar C, *et al*. *Urginea maritima* (squill) toxicity. *Clinical Toxicology* 33(1): 83–89.

Red Tide

Robin C Guy

Background Information

Red tide is a marine event where protistas, including algae and dinoflagellates, go through a tremendous growth period, called bloom. In a 2–3 week period, it is possible for each algal cell to produce one million daughter cells. This usually takes place in the spring and summer in response to an increase in light intensity. During this time, the warm, shallow seawater tends to become discolored by the enormous concentration of algae. This discoloration is dependent on the species of algae and may be the result of the various pigments, including orange, yellow, blue, green, brown, or red. Some may not be visible at all. As red is the most common pigment, the phenomenon is called 'red tide'.

Red tides occur throughout the world, causing a negative impact to natural resources and humans. Red tides can drastically affect Scandinavian and Japanese fisheries, Caribbean and South Pacific reef fishes, and shell fishing along US coasts. The Florida red tide is caused by blooms of a type of microalgae known as a dinoflagellate. This is a single-celled alga called *Karenia brevis* and it is usually found in warm saltwater, but it can exist at lower temperatures. *K. brevis* is the new (*c.* 2000) taxonomic name for the reclassified *Gymnodinium breve* and is found almost exclusively in the Gulf of Mexico.

Most species contributing to algal blooms are harmless; however, some of the toxins produced by certain species are highly toxic. Often, the algae and the shellfish that consume them are unaffected. However, further up the food chain, these toxins can be fatal. Man, dolphins, manatees, and reptiles are potentially exposed to aerosolized toxins. Brevetoxins are potent ichthyotoxins and have been responsible for the death of billions of fish over the years. Brevetoxin is absorbed directly across the gill membranes of fish or through ingestion of *K. brevis* cells. Some of these toxicity differences will depend on the differential susceptibility of fish species to exposure to *K. brevis* strains involved, toxic components and concentration, stability of extracellular toxins, and exposure routes. Mortality typically occurs at cell concentrations of 2.5×10^5 *K. brevis* cells per liter, which is often considered to be a lethal concentration.

The symptoms of shellfish poisoning, start as soon as the victim's digestive system starts to digest the infected shellfish. Cooking does not destroy the toxins. There are different types of poisonings, with a wide variety of symptoms, depending upon the toxins(s) present, their concentrations in the shellfish and the amount of contaminated shellfish consumed. These include paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and neurotoxic shellfish poisoning (NSP).

In PSP, the toxin attacks the nervous system and causes effects that are primarily neurological and include tingling, burning, numbness, drowsiness, incoherent speech, and respiratory paralysis. Symptoms of the disease develop fairly rapidly, within 0.5–2 h after ingestion of the shellfish (generally mussels, clams, cockles, and scallops), depending on the amount of toxin consumed. In severe cases respiratory paralysis is common, and death may occur if respiratory support is not provided. There is no antidote. When such support is applied within 12 h of exposure, recovery usually is complete, with no lasting side effects. In unusual cases, because of the weak hypotensive action of the toxin, death may occur from cardiovascular collapse despite respiratory support.

DSP causes extreme gastrointestinal upset; DSP is less dangerous than PSP, but failure to treat the diarrhea may lead to death from dehydration or other complications. DSP is primarily observed as a generally mild gastrointestinal disorder, that is, nausea, vomiting, diarrhea, and abdominal pain accompanied by chills, headache, and fever. Onset of the disease, depending on the dose of toxin ingested, may be as little as 30 min to 2–3 h, with symptoms of the illness lasting as long as 2–3 days. Recovery is complete with no after effects; the disease is generally not life threatening. DSP is presumably caused by a group of high molecular weight polyethers, including okadaic acid, the dinophysis toxins, the pectenotoxins, and yessotoxin. DSP is generally associated with mussels, oysters, and scallops.

ASP is caused by domoic acid toxicity, which causes amnesic shellfish poisoning, binds to chemical receptors in brain cells and causes their dysfunction. The poisoning begins with gastrointestinal disorders (vomiting, diarrhea, abdominal pain) within 24 h, rapidly followed by dizziness, disorientation, and memory loss within 48 h; the symptoms may persist indefinitely and also result in seizure and coma. During a 1987 outbreak on Prince Edward Island, 1% of the reported poisonings resulted in death from brain damage. ASP is associated with the injection of

mussels. The toxicosis is particularly serious in elderly patients, and includes symptoms reminiscent of Alzheimer's disease.

NSP is the result of exposure to a group of polyethers called brevetoxins. Both gastrointestinal and neurological symptoms characterize NSP, including tingling and numbness of lips, tongue, and throat, muscular aches, dizziness, reversal of the sensations of hot and cold, diarrhea, and vomiting. Onset occurs within a few minutes to a few hours; duration is fairly short, from a few hours to several days. Recovery is complete with few after effects; no fatalities have been reported. NSP is associated with the ingestion of shellfish harvested along the Florida coast and the Gulf of Mexico.

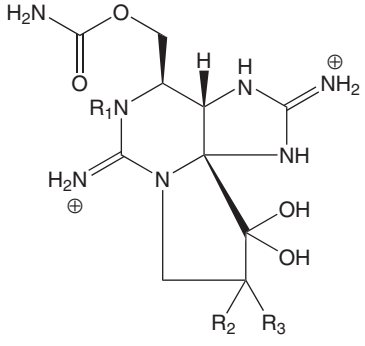
Cases are frequently misdiagnosed and, in general, infrequently reported. Of these toxicities, the most serious from a public health perspective appears to be PSP. The extreme potency of the PSP toxins has, in the past, resulted in an unusually high mortality rate.

Red tide can also pose a serious problem for public health in that the presence of airborne toxins may have an impact on the human respiratory system. Symptoms including irritation of the eyes, nose, throat, tingling lips, and tongue are common during red tides. Waves, wind, and boat propellers in high concentrations of red tides disperse toxin particles into the air causing these problems for people along the shoreline. People suffering from severe or chronic respiratory conditions such as emphysema or asthma, should try to avoid red tide areas. Symptoms usually disappear within 24 h once the exposure is discontinued. Shellfish poisoning is caused by a group of toxins elaborated by planktonic algae (dinoflagellates, in most cases) upon which the shellfish feed. The toxins are accumulated and sometimes metabolized by the shellfish. The 20 toxins responsible for PSPs are all derivatives of saxitoxin.

Since the 1950s, the Canadian–United States Conference on Shellfish Toxicology endorsed a mouse bioassay that was based on the use of purified toxins and has historically been the most universally applied technique for examining shellfish (especially for PSP); other bioassay procedures have been developed but not generally applied. The intraperitoneal minimal lethal dose of the toxin for the mouse was $\sim 9\,\mu g\,kg^{-1}$ body weight. The intravenous minimal lethal dose for the rabbit was ~ 3–$4\,\mu g\,kg^{-1}$ body weight. The minimal lethal dose of the toxin for humans is estimated to be between 1 and 4 mg.

Unfortunately, the dose-survival times for the DSP toxins in the mouse assay fluctuate considerably and fatty acids interfere with the assay, giving false-positive results; consequently, a suckling mouse assay that has been developed and used for control of DSP measures fluid accumulation after injection of the shellfish extract. Considerable effort has been applied recently to development of chemical assays to replace these bioassays. As a result a good high performance liquid chromatography (HPLC) procedure has been developed to identify individual PSP toxins (detection limit for saxitoxin = 20 fg per 100 g of meats; 0.2 ppm), an excellent HPLC procedure (detection limit for okadaic acid $= 400\,ng\,g^{-1}$; 0.4 ppm), a commercially available immunoassay (detection limit for okadaic acid = 1 fg per 100 g of meats; 0.01 ppm) for DSP, and a totally satisfactory HPLC procedure for ASP (detection limit for domoic acid $= 750\,ng\,g^{-1}$; 0.75 ppm).

Some red tides can take up several hundred square miles of water. Red tides are affected by many variables such as weather and currents; therefore, no one can predict when or where red tides will appear or how long they will last. As they tend to occur more in the spring and summer months, there may be a good reason to the folklore that warns us not to eat shellfish in months without an 'r' in their names!



STX	R_1	R_2	R_3
STX	H	H	H
GTX-II	H	H	OSO_3^-
GTX-III	H	OSO_3^-	H
NeoSTX	OH	H	H
GTX-I	OH	H	OSO_3^-
GTX-IV	OH	OSO_3^-	H

See also: Pollution, Water.

Further Reading

Kirkpatrick B, Fleming LE, Squicciarini D, *et al.* (2004) Literature review of Florida red tide: Implications for human health effects. *Harmful Algae* 3: 99–115.

Pierce RH and Kirkpatrick GJ (2001) Innovative techniques for harmful algal toxin analysis. *Environmental Toxicology and Chemistry* 20: 107–114.

Tibbetts J (1998) Toxic tides. *Environmental Health Perspectives* 106: A326–A331.

Relevant Websites

http://museum.gov.ns.ca – Red Tide (from the Nova Scotia Museum).

http://vm.cfsan.fda.gov – US Food and Drug Administration, Center for Food Safety & Applied Nutrition, Foodborne Pathogenic Microorganisms and Natural Toxins Handbook.

Redbook

Robin C Guy

History

The *Redbook 2000* provides guidance for the safety of food ingredients, and is produced by the US Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN). The first part of this guidance was issued in July 2000. It is available electronically at the website listed at the end of the article.

The completed sections now substitute for, or supplement, guidance available in the *Redbook I* and the Draft *Redbook II*. The *Redbook I* titled *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*, and was prepared by the US FDA, Bureau of Foods (now CFSAN), in 1982. The 1993 Draft *Redbook II* has the same title and was published on March 29, 1993 (Notice of Availability, 58 *Federal Register* 16536).

Earlier versions of the *Redbook* focused on direct food additives and color additives used in food. The *Redbook 2000* provides guidance for the safety assessment of food ingredients, including direct food additives, color additives used in food, generally recognized as safe substances, food contact substances and constituents, or impurities of any of the above.

Current Progress

At this time, the *Redbook 2000* is an incomplete document. Sections are continuously being written and are added to the website after finalization. The current table of contents is listed in **Table 1**. Topics that have been completed are in **Table 2**.

Table 1 *Redbook 2000* table of contents

Table 1 Continued

- 8. *In Utero* Exposure Phase for Addition to Carcinogenicity Studies with Rodents
- 9. Reproduction and Developmental Toxicity Studies
  - a. Guidelines for Reproduction Studies
  - b. Guidelines for Developmental Toxicity Studies
- 10. Neurotoxicity Studies

V. Additional Studies
- A. Introduction
- B. Metabolism and Pharmacokinetic Studies
  - 1. Recommended Metabolism and Pharmacokinetic Studies
  - 2. Considerations in the Design of Pharmacokinetic Studies
    - a. Test Substance
    - b. Animals
    - c. Route of Administration
    - d. Dosage Regimen
    - e. Sampling
    - f. *In Vitro* Studies: Dose Response, Mechanism
    - g. Pregnancy/Lactation/Reproductive Studies
  - 3. Analysis and Use of Data from Pharmacokinetic Studies
    - a. Data Reporting and Parameter Estimation
    - b. Pharmacokinetic Models: Data Interpretation and Predicting Effects
  - 4. Use of Pharmacokinetic Results for Study Design and Risk Assessment
    - a. Design of Toxicity Studies
    - b. Setting Dose Levels
    - c. Determining Mechanisms of Toxicity
    - d. Improving the Risk Assessment Process/Safety Assessment
  - 5. References
- C. Immunotoxicity Studies
  - 1. Immunity: A Brief Review
  - 2. Key Concepts in Immunotoxicity Testing
  - 3. Indicators of Possible Immune Toxicity
  - 4. Expanded Type 1 Immunotoxicity Tests
  - 5. Type 2 Immunotoxicity Tests
  - 6. Relevance of Primary Indicators of Immune Toxicity to Health
  - 7. Adequacy and Reliability of Primary Indicators of Immune Toxicity
  - 8. Recommendations for Further Immunotoxicity Testing when Primary Indicators are Positive
  - 9. Animal Models for Immunotoxicity Tests
  - 10. Recommended Strategy for Assessing the Immunotoxic Potential of Food Ingredients
  - 11. Conclusion

VI. Human Studies
- A. Clinical Evaluation of Food Ingredients
  - 1. General Considerations for Clinical Studies of Food Ingredients
  - 2. Specific Considerations for Clinical Studies of Food Ingredients
  - 3. Sequence of Clinical Studies for Food Ingredients
  - 4. Submitting Reports of Clinical Studies on Food Ingredients
  - 5. Appendix A – Principles of Institutional Review and Informed Consent
- B. Epidemiology Studies

Table 1 Continued

VII. Emerging Issues
- A. Introduction
- B. Macro-Additives
- C. Safety of Food Ingredients Developed by Biotechnology
- D. Enzymes
- E. Microbially Derived Food Ingredients
- F. Advances in the Development of Alternative Toxicity Testing
- G. Heritable and Somatic Genetic Toxicity

VIII. Glossary: Acronyms and Definitions

Table 2 *Redbook 2000* completed guidelines

Preclinical Toxicity Studies
- 1. General Guidelines for Designing and Conducting Toxicity Studies
- 2. Guidelines for Reporting Results of Toxicity Studies
- 3. Pathology Considerations in Toxicity Studies
- 4. Statistical Considerations in Toxicity Studies
- 5. Short-Term Tests for Genetic Toxicity
  - a. Bacterial Reverse Mutation Test
  - b. *In Vitro* Mammalian Chromosomal Aberration Test
  - c. *In Vitro* Mouse Lymphoma $TK^{+/-}$ Gene Mutation Assay
  - d. *In Vivo* Mammalian Erythrocyte Micronucleus Test
- 6. Short-Term Toxicity Studies
  - a. Short-Term Toxicity Studies with Rodents
  - b. Short-Term Toxicity Studies with Non-Rodents
- 7. Subchronic Toxicity Studies
  - a. Subchronic Toxicity Studies with Rodents
  - b. Subchronic Toxicity Studies with Non-Rodents
- 8. One-Year Toxicity Studies with Non-Rodents
- 9. Reproduction and Developmental Toxicity Studies
  - a. Guidelines for Reproduction Studies
  - b. Guidelines for Developmental Toxicity Studies
- 10. Neurotoxicity Studies

Human Studies
- 1. Epidemiology Studies

Glossary: Acronyms and Definitions

See also: Food Additives; Food and Drug Administration; Immune System; Generally Recognized as Safe (GRAS); Good Laboratory Practices (GLP).

Relevant Website

http://www.cfsan.fda.gov – US Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN). *The Redbook*.

Reference Concentration (RfC)

Patricia M Nance

The reference concentration (RfC) methodology to estimate benchmark values for noncancer toxicity of inhaled chemicals was adapted for inhalation studies from the reference dose methodology used for oral exposure assessment. The same general principles were used, but the RfC methodology was expanded to account for the dynamics of the respiratory system as a portal of entry. The reference dose (RfD) methodology included dosimetric adjustments to account for species-specific relationships of exposure concentrations to deposited or delivered doses. Particles and gases are treated separately, and the type of toxicity observed influences the dosimetric adjustment applied to score the exposure concentration for animals to a human equivalent concentration.

The RfC can be defined as an estimate of continuous inhalation exposure to the human population, including some level of uncertainty (perhaps spanning an order of magnitude), and sensitive subpopulations that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or benchmark concentration, with uncertainty factors generally applied to reflect limitations of the data used. The RfC is generally used in the US Environmental Protection Agency's (EPA) noncancer health assessments. The formula for calculating the RfC is as follows:

$$\mathrm{RfC} = \frac{\mathrm{NOAEL(or\ LOAEL)}}{\mathrm{UF} \times \mathrm{MF}}$$

The NOAEL is the highest experimental dose at which there is no statistically or biologically significant increase in frequency or severity of adverse health effects, as seen in the exposed population compared with an appropriate unexposed population. Effects may be produced at this level, but they are not considered to be adverse. If there is no suitable NOAEL available, then an LOAEL can be used instead. The LOAEL is the lowest dose or exposure level of a compound in a study that there is no statistically or biologically significant increase in frequency or severity of adverse health effect in the expose population as compared with an appropriate unexposed population.

The uncertainty factors used in the development of the RfC represent a specific area of uncertainty inherent in the extrapolation from the available data. The basis for the application of these uncertainty factors are (1) human variability; (2) animal to human extrapolation; (3) subchronic to chronic extrapolation; (4) use of an LOAEL instead of an NOAEL; and (5) database confidence. These uncertainty factors can be 1, 3, or 10, depending on the amount of uncertainty.

The modifying factor (MF) ranging from 0 to 10 is included to reflect a qualitative professional assessment of additional uncertainties in the critical study and in the entire database for the chemical not explicitly addressed by the uncertainty factors. The default value for the MF is 1.

The confidence in the RfC can be high, medium, or low. High confidence indicates the judgment that the RfC is unlikely to change in the future because there is consistency among the toxic responses observed in different sexes, species, study designs, or in dose–response relationships, or that the reasons for existing differences are well understood. High confidence is often given to RfCs that are based on human data for the exposure route of concern, since in such cases the problems of interspecies extrapolation have been avoided. Low confidence indicates the judgment that the data supporting the RfD may be of limited quality and or quantity and that additional information could result in a change in the RfC.

Occupational exposure limits (OELs) are standards based on toxicological, epidemiological, and clinical information pertaining to human exposure of airborne contaminants. OELs are generally time-weighted average concentrations of airborne substances to which a health worker can be exposed during defined work periods and under specific work conditions throughout a working lifetime, without material impairment of health. OELs also are based on a variety of assumptions and considerations, such as industrial hygienists can control workplace environments, as well as reflect the cost of controlling these environments. Because of these assumptions and considerations, the use of OELs for the derivation of RfCs is generally not done. The OELs often are not based on chronic effects and may differ in regard to the severity of the effects used for RfCs. The OELs further assume intermittent exposure, whereas RfCs are set to protect against continuous exposure. The evaluation process of toxicity data by agencies deriving OELs may differ from the US EPA's process with respect to weight-of-evidence classification, application of uncertainty factors, and other

issues. It is not recommended to use OELs in the derivation of RfCs.

See also: Occupational Exposure Limits; Reference Dose (RfD); Respiratory Tract; Risk Assessment, Human Health; Uncertainty Factors.

Relevant Website

http://www.epa.gov – US EPA (1994) *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*, EPA/600/8-90/066F, October 1994. See also US EPA (2002) *A Review of the Reference Dose and Reference Concentration Processes. Prepared for the Risk Assessment Forum*, EPA/630/P-02/002F.

Reference Dose (RfD)

Patricia M Nance

Systemic effects have traditionally been evaluated using such terms as 'acceptable daily intake' (ADI), 'safety factor' (SF), and 'margin of safety' (MOS), concepts that are associated with certain limitations. The US Environmental Protection Agency (EPA) developed a methodology for evaluating available data pertaining to xenobiotics for purposes of developing oral reference doses (RfDs). Although similar to the intent of deriving regulatory levels of ADIs to protect exposed populations from adverse effects, RfDs were based upon a more rigorously defined methodology that adhered to the principles proposed by the US National Academy of Sciences paradigm (1983) and included guidance on the consistent application of uncertainty factors for prescribed areas of extrapolation required in the operational derivation. The RfD methodology represents a quantitative approach to assess toxicity data in order to derive a dose–response estimate.

An RfD is an estimate of the oral daily exposure to the human population, including some level of uncertainty (perhaps spanning an order of magnitude) and sensitive subpopulations that are likely to be without an appreciable risk of deleterious effects during a lifetime. It is generally expressed in units of milligrams of a compound per kilograms of body weight per day ($mg\,kg^{-1}\,day^{-1}$). The RfD is useful as a reference point from which to gauge the potential effects of the compound at other doses for long-term exposure to a compound. It is generally used in the US EPA's noncancer health assessments. The RfD is calculated by dividing the no-observed-adverse-effect level (NOAEL) (or lowest-observed-adverse-effect level, LOAEL or benchmark dose) by the product of the total amount of uncertainty factors and the modifying factor applied reflecting the limitations of the data used. The formula for calculating the RfD is as follows:

$$\mathrm{RfD} = \frac{\mathrm{NOAEL\ (or\ LOAEL)}}{\mathrm{UF} \times \mathrm{MF}}$$

The NOAEL is the highest experimental dose at which there is no statistically or biologically significant increase in frequency or severity of adverse health effects, as seen in the exposed population compared with an appropriate unexposed population. Effects may be produced at this level, but they are not considered to be adverse. If there is not a suitable NOAEL available, then a LOAEL can be used instead. The LOAEL is the lowest dose or exposure level of a compound in a study in which there is no statistically or biologically significant increase in frequency or severity of adverse health effect in the exposed population as compared with an appropriate unexposed population.

The UFs (uncertainty factors) used in the development of the RfD represent a specific area of uncertainty inherent in the extrapolation from the available data. The basis for the application of these uncertainty factors are (1) human variability; (2) animal to human extrapolation; (3) subchronic to chronic extrapolation; (4) use of a LOAEL instead of a NOAEL; and (5) database confidence. These uncertainty factors can be 1, 3, or 10, depending on the amount of uncertainty.

The MF (modifying factor) ranging from 0 to 10 is included to reflect a qualitative professional assessment of additional uncertainties in the critical study and in the entire database for the chemical not explicitly addressed by the uncertainty factors. The default value for the MF is 1.

The confidence in the RfD can be high, medium, or low. High confidence indicates the judgment that the RfD is unlikely to change in the future because there is consistency among the toxic responses observed in different sexes, species, study designs, or in dose–response relationships, or that the reasons for existing differences are well understood. High confidence is often given to RfDs that are base on human data

for the exposure route of concern, since in such cases the problems of interspecies extrapolation have been avoided. Low confidence indicates the judgment that the data supporting the RfD may be of limited quality and or quantity and that additional information could result in a change in the RfD.

See also: Reference Concentration (RfC); Risk Assessment, Human Health; Uncertainty Factors.

Further Reading

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Refrigerants *See* Freons.

Regulation, Toxicology and

Michael A Kamrin

Introduction

During the last half century, due to increasing concerns about possible adverse human and environmental effects of chemicals detected in food, air, and water, a number of new laws and regulations were enacted, and old ones amended, in the United States and many other countries. In the United States, these statutes, designed to mitigate such possible adverse impacts, include the Federal Fungicide, Insecticide and Rodenticide Act, the Clean Air Act, the Clean Water Act, the Safe Drinking Water Act, the Federal Food, Drug, and Cosmetic Act, and the Toxic Substances Control Act. (Detailed descriptions of each of these can be found elsewhere in this encyclopedia.)

As can be seen from the names of the legislation, they are designed to address problems related to a particular environmental medium, such as the air or water, or to a specific type of chemical such as a drug or pesticide. In general, the regulations promulgated to carry out the intent of each of these pieces of legislation were developed independently of each other; that is, they do not consider that humans and other organisms may be simultaneously exposed to the same chemicals in a variety of media. For example, acceptable levels of a chemical in food may be calculated without consideration of concomitant exposures through air or soil – exposures that may be governed by other agencies carrying out other legislative mandates. In addition, nonregulated exposures such as those resulting from inhalation of indoor air, are also generally left out of the calculations.

There are a number of possible consequences of this medium by medium and chemical by chemical approach. One consequence might be that regulations governing a chemical in one medium are not stringent enough because humans and other organisms may also be exposed to comparable amounts of this same chemical in several other media – leading to a combined exposure that may be too high. It is also possible that this approach may have the opposite impact. For example, the regulations governing a chemical in one medium may be so stringent that they only permit exposures that are orders of magnitude lower than those allowed in other media or that result from unregulated exposures. Thus, these regulations are too stringent since they have essentially no impact on overall exposure. A good example of this is the very stringent US limits on benzene in drinking water while indoor air exposures to benzene are often orders of magnitude higher than those occurring from drinking water consumption, especially if smoking is occurring in this indoor environment.

Recognition of the problem of possible over or under regulation raises the questions of why regulations are so narrowly focused on specific chemicals or environmental media and how such large media-specific differences in allowable exposures have come about. These questions have come into greater prominence in recent decades as legislation governing environmental contaminants has been enacted in more and more countries. With the inception of multinational units, such as the European Union, it has become clear that acceptable exposures differ not only among different types of legislation within countries but also among countries – the degree of regulatory stringency can vary significantly from country to country even when dealing with the same exposures in the same environmental media.

In the case of the European Union, recognition of these problems in regulatory consistency has resulted in extensive discussions among the member states with the goal of establishing a common regulatory

metric that could apply to all. In the absence of such a metric, citizens are faced with conflicting information about what is 'safe', a conflict that is most evident when they live near borders separating countries with regulations characterized by differing stringency. In addition, with the internationalization of business and commerce, the lack of regulatory uniformity has even wider relevance and economic impact. As a result, there has been pressure for countries around the globe to harmonize environmental regulations. However, progress is slow for a variety of reasons, one of the most important being the role of policy considerations and cultural values in the application of toxicological knowledge to the generation of regulatory limits and strategies.

Role of Toxicology in Environmental Regulation

Whatever the environmental medium or chemical involved, the critical question in regulation is how to determine the maximum allowable levels of a chemical in a particular environmental medium. Answering this question is often thought of as determining the 'safe' level for that chemical. Once these 'safe' values are calculated, they are then used to promulgate legislation and regulations aimed at reducing existing 'unsafe' environmental levels and preventing the introduction of 'unsafe' levels into food, air, water, etc.

However, 'safe' and 'unsafe' are not scientific terms and so it is not obvious how to best determine them. Government agencies have responded to this problem by defining 'safe' through the issuance of guidance documents that specify what toxicological and exposure data should be considered and how these data should be interpreted. The determination of 'safe' limits is based on a process known as risk assessment. While assessments of both ecological and human health risks are performed as part of the development of environmental regulation, the discussion here will focus on human health risks. Human health risk assessment is a multistep process that combines experimental and epidemiological evidence as to the levels of a toxicant that are required to cause adverse effects with data and assumptions as to the amount, frequency and duration of exposures to that agent through each environmental medium.

While this simple description might suggest that risk assessment is a fairly straightforward process that can be performed by simply following commonly accepted guidance documents, this is not the case. One fundamental problem arises from the unavailability of toxicity data obtained directly from humans. In the absence of this most relevant scientific information, toxicological data from other species must be used in assessing risk. These data are generally collected using rodents and experiments are most often performed by administering very high doses over long periods of time to ensure an effect will be produced. To apply such animal data to humans, it is necessary to extrapolate both from high to low doses and also from other species to humans. In most cases, there is not enough scientific information and understanding to perform these extrapolations confidently. As a result, a variety of assumptions must be made in translating the risk assessment data collected from experimental animals into numbers that are applicable to humans.

To appreciate the regulatory problems this approach leads to, it is important to understand that risk assessment was developed as a tool for carrying out risk management, rather than a scientific process for understanding risk. Thus, both the selection of the data to be used and the way these data are extrapolated to the usual human exposure situation, reflect both scientific and policy considerations. As a result, risk assessment results do not represent the best scientific estimates of risk, estimates that are subject to scientific consensus, but rather 'prudent' values that incorporate margins of safety. These margins of safety are included to increase the likelihood that regulations based on these risk assessments will successfully protect the public and the environment.

However, since each governmental entity is responsible for carrying out its own unique set of legislative mandates and has a unique regulatory history, definitions of 'prudence' are often agency-specific. As a result of the differences in definition, risk assessment procedures and results often differ among agencies within countries as well as among countries. The application of such divergent procedures has contributed strongly to the diversity in risk limits established by various agencies and governments.

Another factor also contributes to the variability in limits promulgated by various agencies. This is the importance that risk is given relative to other factors, such as cost, in setting regulations. In some cases, legislation requires that risk be the only consideration; others require that risk must be balanced against benefit; still others specify that economic factors must also be taken into account. Such differences can lead to great diversity in regulatory limits even if the data utilized are the same and application of the risk assessment methodologies lead to the same result.

However, within this diversity, there are some commonalities. For example, one common element of risk assessment across agencies is the division of environmental toxicants into two categories; carcinogens and

noncarcinogens. Under most risk assessment schemes, carcinogens are evaluated under a paradigm that leads to probabilistic risk numbers; that is, the incidence of cancer expected per unit of administered dose of agent. By combining this cancer risk number with policy choices, particularly the acceptable upper bound for cancer incidence, maximum allowable limits for carcinogens in the environment can be set.

In contrast, the procedures for evaluating the risk from noncarcinogens leads to single value estimates of allowable exposures – not probabilistic risk values. These estimates have often been misinterpreted as bright lines separating 'safe' from 'unsafe'. However, a careful examination of the origins of these noncarcinogen risk values reveals that they represent prudent numbers below which adverse effects are not expected. The assessment procedure does not provide information as to what the risk will be if these values are exceeded but it is expected that the risk will be insignificant until exceedences are significantly above the established 'safe' value. Once calculated, this noncarcinogen risk value is then converted into a regulatory number by the appropriate agency, utilizing a variety of assumptions and policy judgments.

For example, in the United States, the Environmental Protection Agency's Office of Water uses both carcinogen and noncarcinogen risk numbers as inputs for establishing drinking water standards that represent the maximum acceptable levels of a variety of agents in public drinking water systems. Similarly, states in the United States may use this type of information to set maximum allowable levels of agents in soil or air, standards that apply only in that state. These national or state risk values may also be used in determining 'how clean is clean'; that is, the maximum allowed environmental levels in various media; for example, air, water, soil, at hazardous waste sites.

Summary

In summary, many environmental regulations require the generation and interpretation of toxicological data. However, the way that these data are used can be influenced greatly by a variety of factors. These include the stipulations in the legislation as to how the risk numbers are to be utilized, the degree of prudence adopted by the agency promulgating the regulation, the data available and whether the agent is labeled a carcinogen or noncarcinogen. A number of intra- and international groups are addressing the regulatory inconsistencies that have arisen as a result of these factors and it is expected that at least some degree of regulatory harmonization will result.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food, Drug, and Cosmetic Act, US; Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Risk Management; Safe Drinking Water Act, US; Toxic Substances Control Act, US.

Further Reading

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Renal Toxicology *See* Kidney.

Reproductive System, Female

Bill L Lasley

Introduction

Modern technology now creates and applies new chemicals faster than they can be tested for their potential adverse health effects. At the same time, more women work outside the home in expanding job classifications, many of which have a potential for chemical exposure that is increased above the typical exposures in the home. In addition, the 'reproductive years' for women today have been widened with the development of new medical technologies that permit older women to bear children and younger women to delay their pregnancies. This, in turn, has broadened the age that must be considered at risk for reproductive impairments. In addition, health risks associated with reproductive failure in women are now recognized to extend beyond the issues of family

planning and fertility. Reduced bone accumulation in the second and third decade, bone loss, and increased risk of heart disease in later life are just three of the nonreproductive problems which may result from abnormal ovarian function.

The need for a better understanding of female reproductive toxicology, then, is driven by issues associated with women's health in the context of today's changing society and our current knowledge of women's general health. A woman's reproductive function is often considered to be more sensitive to environmental perturbations than that of a man's. This concept is supported by studies that demonstrate that reproductive failure in women can be induced by strenuous exercise, marginal nutrition, as well as by acute physical or emotional stress. Despite concerns that women may be more adversely affected than men when confronted by the same exposures, very little information is available in terms of female reproductive toxicology. Recent reference books on reproductive toxicology generally provide much more information relating to male- than female-related issues because more information is available for male compared to female toxicology. The reason for this disparity is most likely attributable to the practical considerations in experimental design and the availability of critical research materials that make the studies of males more attractive to research programs. For example, in many experimental designs, viable gametes are the ultimate object of evaluation. Spermatozoa are plentiful and more easily collected compared to ova. Although semen collection and analyses are not simple procedures, sperm production provides a constant and quantifiable end point for male fertility. Ova, in contrast, are irregularly produced and seldom available for study except in animal studies or under complex clinical management protocols. Even when ova are made available for scientific study, there is little technology available to evaluate their quality in contrast to spermatozoa.

While it is increasingly evident that gender differences probably exist in reproductive toxicity, progress in documenting male and female differences has been slow. Perceived and real difficulties in using female animal models in controlled studies have been the major impediments to progress in this area of research. The result of these difficulties is that much more information exists for the male than the female in terms of reproductive toxicology. The inequities in the database will change only when methods are developed that permit the female system to be studied as efficiently and effectively as the male is studied. Fortunately, toxicological investigations of female reproduction are becoming more common, new techniques are being developed to monitor women's reproductive health, and the information relating to female reproductive toxicology is growing. As a consequence, the gender gap in information relating to the effects of toxic exposures is closing.

Reproductive Epidemiology

Infertility in humans is usually defined as the failure to conceive following natural attempts to achieve pregnancy for a year or more. Individuals and couples not wishing to have children are seldom evaluated or even surveyed in terms of their reproductive health. In poor economic times the desire for children decreases in developed countries and reproductive health in terms of fertility becomes a less important issue. Thus infertility rates may be higher than current estimates due to insufficient and/or inaccurate information. There is a general lack of epidemiologic information regarding reproductive function in human populations and most estimates relating to fertility are biased toward the clinic population. Most of the existing information concerning human reproduction is obtained from clinical records and does not adequately represent the general population. Only recently have tools been developed to design and evaluate population-based prospective studies assessing basic reproductive physiology.

Recognized exposures of humans to reproductive toxicants have been infrequent and, when detected, the toxicants were eliminated as quickly as possible rather than studied. Controlled and/or prospective experiments with humans have moral and ethical ramifications; therefore, most of the information relating to the mechanism of action of recognized or putative toxicants on human reproduction is limited. The lack of information relating to real-world exposures combined with the recognition that adequate animal models for human reproductive processes make the study of spontaneous reproductive failures an attractive approach to predict sites and mechanisms of action of putative reproductive toxicants. This approach assumes that induced reproductive failures resulting from toxic exposures will mimic spontaneous events since there is a finite number of control mechanisms that can fail. The spontaneous failures are thought to reveal 'weak links' and are the most susceptible targets for the adverse effects of toxic exposure.

The primary problem in studying human reproduction is that many aspects of the reproductive process occur without the knowledge of either the woman or her physician. Ovulation, fertilization, and implantation all occur as concealed events. Reproductive biologists and epidemiologists, however, have recently developed new and incisive tools to associate exposures to reproductive health. While it will be years

before the 'baseline' information is available to apply these broadly, the promise for the future is that reproductive health can be monitored as well as any other aspect of health. Meanwhile, toxicologists use animal models in invasive or terminal experiments to demonstrate the effects of documented and putative toxicants to gain insight regarding their basic mechanisms of action. Because of species differences in the expression of reproductive function, the direct application of these kinds of information and the validity of certain end points to human reproductive health are not altogether clear.

What are the risks to women in regard to reproductive toxicants at home or at work? Overall increases in reported cases of infertility are difficult to evaluate. The increasing median age of most societies and delays in family planning lead to an increase in age-related infertility, which is a natural phenomenon. However, fertility rates of young couples have decreased during the past 10 years and experts speculate that as much as 37% of the reproductive failure seen in modern society could be related to environmental factors. There is no direct evidence that women have been affected more or less than men but this is often assumed. Sperm counts have been assessed and show a decline over this same time period. No such similar assessment can be made on the potential of female fertility for comparison. It is doubtful, therefore, that the entire decline in overall fertility can be attributed to men. Traditional epidemiological studies used to monitor menstrual function do not provide information to permit an assessment of fecundity or explain the trends in female fertility. This lack of information regarding female fecundity does underscore the basic theme of this review: female reproductive toxicology has been, perhaps, one of the most neglected area of toxicology.

Problems Associated with Female Reproductive Toxicology

Female reproductive toxicology is a challenging study area for several reasons. One of the purposes of this review is to delineate some of the problems that reproductive toxicologists face when attempting to investigate putative female reproductive toxicants. This will be approached by focusing on three broad aspects or qualities of female reproduction that most limit progress in this field. These areas include the sensitivity of the female reproductive system to environmental factors, the complexity of the female reproductive system compared to that of the male, and the species specificity in regard to ovarian function. Since it is essential that human and animal female reproductive physiology be understood for the effects of toxic exposures to be studied, each overview of female reproductive toxicology must provide a brief yet detailed description of normal female reproductive physiology and types of reproductive failures. Finally, examples of known reproductive toxicants and their mechanism of action are presented.

Sensitivity of Female Reproduction to Environmental Stressors

The most obvious quality of female reproductive system that influences the way it must be studied is its sensitivity to environmental influences. As mentioned previously, the female reproductive system appears to be more sensitive to environmental perturbations and is more complex in its organization than most other physiologic systems or when compared to male reproductive physiology. Reproductive failure in female animals is often considered to be the first and only detriment resulting from nonlethal adverse environmental impacts despite the fact that the capacity to reproduce (fecundity) is considered the essential quality of an animal's fitness in its current location. Chemical or physical stressors that are not adequate to perturb other physiologic functions can interrupt or delay reproductive function. In times of acute stress, or in response to chronic challenges to the survival of the organism, reproductive function may be selectively suppressed as a short-term adaptive process. Thus, as a nonessential mechanism for short-term survival, reproductive function is sacrificed as an immediate fail-safe strategy for long-term survival. This preferential selection to curtail reproduction in response to nonlethal stressors and the sensitivity of reproduction to nonspecific physical, chemical, and emotional stressors make it difficult to identify nonreproductive toxins as being distinctly separate from specific reproductive toxicants. Agents that cause reproductive failure directly and may not be specific reproductive toxicants can lead to reduced fertility. In both males and females (but probably to a higher degree in females) the general health of the individual is likely to be reflected in reproductive capacity. Toxicants which have nonreproductive organs as targets may affect sexual development or influence reproductive processes sooner and more noticeably than they affect other physiologic processes.

Complexities of Reproductive Processes

The second issue regarding the study of female reproductive toxicology is that of the complexities within the female reproductive system. The degree that the female reproductive system is considered to be more complex, compared to the male, is not necessarily the issue since this supposition is debatable.

Female reproduction may be recognized to be complex because it has been studied in greater detail and many more aspects of the female reproductive system are defined than for the male. Certainly the female reproductive system is overtly more dynamic and, perhaps because of this dynamicism, more susceptible to physical, chemical, and emotional stressors. The discrete series of events of the ovarian cycle which requires precise coordination between the central nervous system, hypothalamus, and pituitary in order for gametogenesis and ovulation to take place provides the opportunity for environmental changes to adversely influence normal processes. If these events are delayed or altered appreciably, some form of short-term infertility will most likely result. When this is compared to the male, the relatively monotonous production of hormone and gametes is not as likely to be overtly influenced by short-term events.

Female reproductive function, in general, is intermittently expressed, cannot be completely assessed in a single individual, and is often influenced by normal environmental factors. In contrast, most other organ functions are expressed continuously, can be appraised equally well at any point of time, and respond predictably to changes in the environment. Most other physiologic processes can be studied in an individual and therefore can be characterized in terms of the biological variation within one individual. In contrast, reproduction can only be evaluated completely when pairs of individuals are studied for prolonged time periods and, in some cases, when more than one generation is studied serially. Reproductive failure can vary from reduced sexual drive to complete sterility. Infertility can be the result of either functional or organic defects and subfertility may be the result of defects at one of several levels of reproductive function (e.g., menstrual dysfunction, anovulation, early fetal loss, and pregnancy loss).

Both the nervous system and the endocrine system are involved in reproductive processes and any number of metabolic processes are essential for normal reproductive function. Both the synthesis and metabolism of neural transmitters and endocrine messengers (glycoprotein and steroid hormones) are critical for normal fertility. Reproduction is a process that includes growth and development of organ systems, gametogenesis, courtship behavior, coitus, gamete transport/interaction, internal fertilization, implantation, gestation, and nurture. Perturbations and/or derangements at any stage in this process can reduce or eliminate fertility. The complete reliance on the endocrine mechanism makes the reproductive system susceptible to the downstream effects of vascular, hepatic, and renal dysfunction.

While reproductive biology is a progressive research field, many of the physiologic mechanisms involved with gamete transport, fertilization, implantation, and gestation are still poorly defined. As much as 20% of the clinically described subfertility is classified as unexplained; this may indicate that a large portion of infertility is the result of yet undefined environmental hazards. Subfertility in human populations is estimated at frequencies as high as 20% in married couples of child-bearing age and over 10% in married noncontracepting women between 20 and 35 years of age.

Experimental designs that involve the female reproductive tract must consider the influence of changing hormonal events. Portions of the female reproductive tract are sequentially modified under the influence of pituitary and ovarian hormones. Many female tissues are induced to proliferate and then differentiate in response to steroid and protein hormone patterns. There are clearly time periods of increased sensitivity and time intervals that are specific for different toxicants in one species or for the same toxicant in different species to exert its maximal effects. The concept of precise 'sensitive' periods for toxic effects is well established for developmental toxins (teratogen) and this same concept is likely to be true for toxicants that impact female reproductive functions such as follicle recruitment, folliculogenesis, gamete transport, and endometrial maturation. Very few studies have addressed these issues directly due to the complexities of the experimental design as well as concerns relating to the adequacy of the animal models that are available to study. Long-term testing with sublethal doses is currently the approach used to ensure that exposures are delivered at all possible sensitive time periods. This kind of design may have very little relevance to real-world exposures which generally occur acutely.

Historically, toxicologists have viewed the developmental aspects of reproductive toxicology (e.g., teratogenicity and growth retardation) as the fundamental or basic component of reproductive toxicology. The effects of toxicants on adult reproductive processes and organs (those aspects which limit or perturb fertility) are often considered as less important. Because of this oversight, much of female reproductive toxicology has been focused on effects of agents that target conception and/or pregnancy. In terms of the potential for life-threatening exposures and our responsibility to safeguard the fetus, the emphasis on teratology is understandable. However, it should be recognized that the opportunity for exposure and the potential for adverse effects on reproductive processes is as great if not greater for the nonpregnant woman as it is for the fetus. Currently,

the amount of scientific information available and the degree that we understand developmental reproductive toxins is greater than those for nondevelopmental reproductive toxins. For this reason, the following discussion will be limited to perturbations to reproduction success in the adult human female.

Species Specificity of Female Reproductive Physiology

The third issue is that of species specificity of reproductive physiology. The great variation in reproductive function between species creates the greatest challenge for reproductive toxicologists who study the female. Whereas the basic events of female reproductive cycles can be compared between species, the organization of the components of these events is more varied than any other of the physiologic systems. When compared to the kinds of differences observed between species for the other systems (e.g., the cardiovascular, digestive, integumental, muscular, skeletal, immune, and respiratory), the physiologic mechanism and expression of reproductive function is more diverse than any other. Even if an abundance of good and practical models existed for human reproductive toxicology, there would still be concerns regarding species specificity of toxicants because of differences in metabolic mechanisms through other organ systems, such as the liver or kidney, and in the expression of reproduction function, that is, reproductive performance.

Extrapolating data relating to ovarian function from females of one species to females of another is of limited use. Each species of mammal (there are more than 4000 species) has developed and retained a unique organization of the physiologic processes that make up the complex set of processes that are essential for reproduction to take place. Of the more than 4000 patterns of ovarian cycle organization that we might expect, less than 100 have actually been characterized. This represents the limited numbers of species that have been domesticated or adapted to captivity. It is important to remember that most of our domestic and laboratory species have been artificially selected for reproductive performance and may be more tolerant of environmental influences on their reproductive processes. Very little is understood regarding the effects of multiple stressors on any physiologic system and for most systems this can be justified because of the substantial independence of most systems from others. The reproductive system is clearly one for which this simple logic does not apply, particularly when animal modeling is involved.

When the most basic components of female reproductive physiology are compared, such as neural and pituitary control of ovarian function and ovarian morphology and endocrinology of the ovarian cycle, the diversity becomes clear. Differences between species are most easily discussed in terms of the higher nervous center control over gonadal function and organization of the ovarian cycle. Changes in photoperiod, temperature, conditions of the substrate, nutrition, and even nonspecific stressors can modulate gonadal activity through highly species-specific control mechanisms at the level of the hypothalamus. Each species has adapted to reproduce optimally in response to unique environmental conditions that artificial enclosures cannot duplicate. These factors make interspecific comparisons of reproductive performance within a controlled setting difficult.

The laboratory macaque represents the best potential model for the human female; however, the expense of maintaining the monkey model, difficulties in handling and manipulating mature monkeys, insufficient baseline data, a limitation of animal resources, the time required to perform multigeneration studies, and inadequate experimental tools make the use of monkeys as a model severely limited. Since less than the ideal model is usually used, model selection for human reproductive toxicology must be built on a solid understanding of female reproductive physiology and modern trends in reproductive medicine and pharmacology.

Female Reproductive Development and Physiology

Development

Unlike the male phenotype, the female mammal requires little additional directing force beyond the correct genotype. Thus, fetal development in the female is similar in the presence or absence of the normal fetal gonads. Since the same somatic substrates are present in male and female fetuses, the introduction of androgenic substances to a female fetus will produce the inappropriate development of male-type secondary sex characteristics. This can occur as a result of endogenous adrenal production of weak androgens (congenital adrenal hyperplasia) or exogenous androgenic agents (anabolic steroids) which can cause the development of ambiguous or male-type genitalia, and, in the most severe cases, infertility. This ability to respond inappropriately to androgens persists throughout life and females can be virilized at any time, although the sequelae of virilization generally decrease with age.

All aspects of ovarian function and adult reproductive normalcy in the female are ultimately dependent on the process of germ cell maturation in the

adolescent and adult. Ovarian steroids are responsible for the development of secondary sex characteristics as well as the function and maintenance of the reproductive tract. The ovary can be compared to an undifferentiated organ that retains its embryonic capacity to differentiate throughout the reproductive years. Ovarian stroma cells derive their function only under the direction of a competent hypothalamic-pituitary drive and the presence of developing primary oocytes. If the oocytes are depleted, by accident or age, the ovary ceases to function and all aspects of reproductive function that depend on sex steroid support will regress. The preponderance of reproductive functions is either driven or modulated by sex steroid hormones. For this reason a clear understanding of steroid hormone production and action is essential to understand either reproductive physiology or reproductive toxicology.

Germ Cells

Germ cell numbers are finite in females and are present in the early embryo. Having multiplied by mitosis and migrated to the genital ridge, they initiate, but do not complete, meiosis immediately. Unlike spermatogonia, which continue to be replenished throughout adult life, all of the germ cells are present in a resting stage from the early fetal period to the end of the reproductive life of the female. Usually the oocytes remain in a suspended stage of meiosis which is complete just prior to fertilization. If these original germ cells are lost they cannot be replaced. If the oocytes are all lost then ovarian function and all reproductive function will irreversibly cease. Unlike the testis, in which the endocrine and gametogenic activities of the gonad are physically separated, the individual ovarian follicle comprises the combined endocrine and gametic functional unit of the ovary. This is an important difference between the sexes and is reflected in the approaches that are used to assess reproductive health in each.

The 'resting' stage of germ cells (primary oocytes) and their vestments of follicle cells are tightly clustered in the cortical portion of the ovary in what is termed the germinal epithelium. The counterpart to this in the male would be the lining of the seminiferous tubules behind the 'testis–blood barrier'. While the presence of the ovary and its hormonal products are not essential for embryonic development of the female phenotype up to the neonatal stage, the presence of viable germ cells together with their surrounding differentiated gonadal tissue is essential for complete sexual maturation. Just prior to sexual maturity and under the control of higher nervous centers that control gonadotropin secretion, increased pituitary secretion of gonadotropins stimulates some of the resting oocytes and their surrounding primitive follicle cells to mature. Both the resting oocytes and the undifferentiated follicular cells must be present in the germinal epithelium for this earliest phase of normal ovarian function to occur. In the absence of viable oocytes, follicle cells will not develop and, as a consequence, there will be no response to gonadotropin stimulation.

Ovarian Function

At the onset of puberty, the undifferentiated ovarian stromal cells in the immature ovary, in response to gonadotropin secretion and their close proximity to a viable, resting primary oocyte, differentiate and develop the capacity to produce sex steroid hormones (primarily androgens and estrogens). These sex steroids are directly responsible for the development and maturation of the secondary sex organs and complete the process of sexual maturation (puberty) at the appropriate time. The follicular events associated with this follicle activation require the differentiation of the previously undifferentiated primitive follicle cells into two separate cell types, the theca and the granulosa cells. The theca cells produce androgens (androstenedione and testosterone) from acetate and circulating cholesterol, and depend on the granulosa cells to convert the androgens to estrogens. Increased production of androgens from the theca cells or decreased ability to aromatize these androgens by the granulosa calls can lead to virilization (masculinization) and infertility.

In the earliest stages of puberty, ovarian follicles develop to the stage of producing estrogen but do not ovulate. This period of 'adolescent sterility' in primate species is associated with adequate estrogen stimulation for the development of secondary sex characteristics, general sexual development, and sexual maturity. Inappropriate release of gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland) prior to the age of normal puberty will induce follicular development and precocious sexual development since all of the components of adult ovarian function are present at birth and only lack gonadotropin stimulation for complete, normal function.

The Ovarian Cycle

The hormonal events associated with ovulation are incompletely understood. Simplistically, a feedback loop is established between the hypothalamus-pituitary and the ovary at the onset of sexual maturity. Pituitary gonadotropins function to stimulate primitive follicles to secrete estrogen, progesterone, and

peptide hormones which modulate both the pituitary and the hypothalamic control of reproduction through positive and negative feedback loops. Collectively this is referred to the hypothalamus-pituitary-ovarian axis (HPO axis). The maturing follicle secretes increasing amounts of estrogen prior to ovulation, which modulates the secretion of gonadotropins, orchestrates sexual behavior (in some species), and prepares the reproductive tract for mating, gamete transport, and potential conception. Ovulation occurs during a limited time period within the complete ovarian cycle and the extruded ova are fertilizable for only a short time period. Thus, the synchrony of ovulation and mating is very important. Precise synchrony of these events is achieved by the action of estrogen from the maturing follicle acting upon the central nervous system, the pituitary, and the reproductive tract.

Gametogenesis in the female (folliculogenesis and ovulation) is intimately associated with hormone production patterns and can be monitored by measuring changes in hormone production rates. Although the periovulatory LH surge requires increasing estrogen in order to be elicited, the final release mechanisms vary between species. For some rodents the hypothalamic release of neural peptides (catecholamines, indolamines, and specific gonadotropin-releasing factors) is closely associated with the diurnal light–dark exposure; in other species copulation is an absolute requirement; and in yet others, such as primates and most domestic and laboratory animals, it occurs as a result of follicular maturation only. For all species, the coordination between the higher nervous centers, the pituitary, and the ovary is mediated through hormone signals from the maturing follicle cells. Thus, in the female, hormone patterns that represent HPO activity are precise and appropriate indicators of reproductive status and potential fertility. This should be contrasted to the male in which the gamete itself is usually evaluated and endocrine parameters have limited clinical significance.

Ovulation is associated with a surge of gonadotropin release that is the result of estrogen-positive feedback. This massive release of gonadotropins probably functions primarily as a fail-safe mechanism to complete the ovulatory process, which is initiated through follicle maturation and the synergism of gonadotropins and estrogen acting upon the mural granulosum. The LH surge secondarily functions to convert the original vestments of the oocyte (follicle cells) into a different cell type (the corpus luteum) that will secrete another sex steroid (progesterone) during the pregestational period. If conception does not occur, progesterone secretion by the ovary is limited to the time interval following ovulation and may be produced for as short as 2 days or as long as 3 weeks depending on the species. Progesterone action serves to prepare the reproductive tract, primarily the lining of the uterus (endometrium), for the embryo implantation and also acts centrally to prevent additional follicles from developing during this time interval.

Following ovulation the ova that are extruded onto the surface of the ovary (either into the body cavity or within a bursa) are picked up by the oviducts. The oviducts serve as conduits that transfer both fertilizable ova toward the uterus and allow selected spermatozoa from the lower reproductive tract to meet and fertilize the ova. Following fertilization the resulting zygote is transported to the uterus where successful implantation can take place. The function of the oviducts, which are responsible for gamete and zygote transport, is controlled by the ovary through estrogen and progesterone production throughout the ovarian cycle. The secretion of these sex steroids is dependent on pituitary gonadotropin support. When implantation does occur, it is usually 2–6 days following ovulation and fertilization; however, delays of implantation can be as long as several months in species such as bears, seals, most mustelids, and some edentates.

The embryo survives unattached to the uterine lining for 5 days to 7 weeks in laboratory and domestic species. During this preimplantation period, nutrient requirements are absorbed from the materials within the uterine lumen. After this time some form of stable attachment is formed between the trophoblast (primitive placenta) and the endometrium. This can range from a very superficial apposition with many cell layers separating maternal and fetal circulations to true implantation with only one cell layer separating the two vascular beds.

Hormone Action

Neuropeptides and polypeptides derived from neural tissue are largely responsible for pituitary function. The neurotransmitters (catechols, indoles, endorphins, and dopamine) act directly or indirectly to cause the release of the gonadotropins or prolactin and exert their action through synaptic junctions to alter neural activity in the hypothalamus and other areas of the brain. The polypeptide hormones (gonadotropin hormone-releasing hormone, adrenocorticoid-releasing hormone, and thyroid-releasing hormone) act through membrane receptors and transduce their signal by intracellular second messengers such as cAMP, calcium, and/or phosphoinositol. The fetal pituitary is capable of responding to higher nervous centers by midgestation but does not do so until these centers 'awake' at the time of puberty long after birth. Premature 'awakening' of these centers leads to premature sexual

development and arrest of the normal somatic growth pattern through the action of sex steroids on bone growth. The absence of the awakening of the central nervous centers that control pituitary function leads to a failure to undergo sexual development.

The primary pituitary hormones that influence female reproductive function are two glycoproteins (LH and FSH) and one protein hormone (prolactin). The glycoprotein hormones (LH and FSH) are stimulated to be synthesized and released by a single polypeptide neurohormone, gonadotropin-releasing hormone (GnRH), and are modulated by both ovarian steroids (estrogen and progesterone) as well as ovarian and pituitary peptides through positive and negative feedback loops which impinge both at the level of the hypothalamus and the pituitary. Derangements which lead to spontaneous or early release of the gonadotropins are rare as the GnRH 'drive' is an essential stimulation. The opposite is true for prolactin because it is controlled primarily for the negative actions of dopamine. Any action which decreases the domaminergic drive to the pituitary will lead to hyperprolactinemia and reproductive dysfunction relating to prolactin excess. Failure of the pituitary to release gonadotropins is common particularly in women and can be the result of inadequate hypothalamic support or inability of the pituitary to manufacture and release gonadotropins. When this occurs prior to puberty delayed maturity and infantilism are the result. If this occurs following puberty, then ovarian function and menstrual periods cease.

All aspects of female reproduction are regulated directly or indirectly by ovarian sex steroids. These small lipids, which are ubiquitous to all species, act to both develop and differentiate all secondary sex characters. The principal female sex steroids are estradiol and progesterone. Estradiol is mitogenic in estrogen-sensitive tissues, and is responsible for end organ growth and proliferation, and acts through estrogen receptors that are constitutive in all estrogen-sensitive tissue. Progesterone can be mitogenic and/or differentiating, depending on the tissue. In the uterine endometrium progesterone differentiates the 'proliferated' endometrium and decreases the action of estrogen by decreasing estrogen receptors. In the breast progesterone complements the proliferative action of estrogen. All progestational cells require the antecedent action of estrogen in order to express progesterone receptors. Exogenous compounds, either natural or synthetic, can mimic endogenous hormones and cause infertility, inappropriate somatic changes, or induce hyperplastic disease. Examples include the synthetic steroid hormones and oral contraceptives, which have been produced to artificially regulate female fertility. These compounds were created to be easily ingested or absorbed and possess unusually long biological half-lives. If an exogenous ligand has androgenic activity, then the results of female exposure to this ligand are similar to those of excessive adrenal androgen production as described previously. Estrogenic or pure progestational agents have different, nonmasculinizing effects but still can result in infertility by interfering with the normal signals that are sent by the ovary to the hypothalamus, pituitary, and reproductive organs.

The direct effects of steroid hormones are limited to cells that contain steroid hormone receptors. Each steroid has at least one specific receptor but each steroid receptor has a strong structural and functional relationship to other compounds with a similar structure. While each steroid has specific effects on specific target organs, these effects can generally be divided into three categories: mitogenic (proliferative), differentiating, or regulatory. Estrogen has all three effects, is the principal female sex steroid hormone, and will be discussed in detail.

Because of the pivotal importance of estrogens it is somewhat surprising that many naturally occurring compounds, other than hormonal estrogens, have estrogenic potential due to structural similarities to the steroidal estrogens (estrone, estradiol, and estriol) and their ability to bind estrogen receptors. It is important that toxicologists understand that many xenobiotics have estrogenic properties for the same reason. Since estradiol is the primary estrogenic hormone for all species, the estrogen receptors that transduce the 'estrogenic message' have been conserved to a great degree. Conservation of both the ligand and the receptor should permit a great deal of uniformity of estrogenic activity of different compounds between species. This, however, is not the case. Estrogens of all types have different effects in different species. In terms of toxicants, for example, 'clover disease' in sheep which results in sterility, is caused by phytoestrogens found in certain legumes and tubers does not occur in other species even though the same phytoestrogen is found at the same circulating concentrations.

In humans, estrogens are primarily responsible for the development of female sex characteristics. The development of vagina, uterus, and fallopian tubes as well as the breasts, fat deposition for body contours, the pubertal growth spurt, pubic/axillary hair, and pigmentation of the genital region and areolae are all a part of estrogen action, although androgens are also probably involved. Some overlap exists between the action of estrogen and androgen in terms of anabolic effects, but, in general, estrogens oppose or have opposite effects of androgens. The loss of estrogen at menopause leads to decreased bone deposition,

decreased turgor of the skin, and sclerosis of the blood vessels. Relative immunity to coronary disease and gout is also dependent on estrogen and is lost at menopause. In animals, estrogens are psychogenic and the desire to mate or become receptive to males is a direct effect of estrogens. The effects of estrogens on emotions are not well defined in humans, although changes in emotion can be striking following menopause.

The toxic effects of estrogens are generally considered to be the adverse effects observed when estrogen is given in supraphysiologic doses or for inappropriate time intervals. Since the normal pattern of estrogen production is quite variable and since the effects of even normal patterns have a wide range of effects in different individuals, it is difficult to separate some adverse estrogenic effects from 'normal effects', that is, swelling and soreness of the breast, morning sickness in early pregnancy, and dysfunctional uterine bleeding. In very high doses, estrogens can cause water retention, thus edema related to heart failure or renal disease could be accentuated by extremely high doses of estrogen. The effect of estrogen on liver function varies tremendously between species. In birds and fish, for instance, estrogens mobilize large amounts of lipids for egg production to the point that the serum becomes milky in appearance. In humans, estrogens change the pattern of circulating lipids (they can be considered 'protective' for circulatory diseases) and this effect is of great interest to those who study heart disease and atherosclerosis.

In women, menstrual function disturbances can be an early and accurate indicator of estrogen imbalance. In other species which do not slough the endometrium, other end points need to be assessed. Assessments of estrogen deficiency can be particularly difficult to detect in particular effects of estrogen antagonists or antiestrogens. Estrogen-induced changes are not only different between species but also different within the same species at different levels of the reproductive system. In some species a carcinogenic action of estrogens has been described as for diethylstilbestrol (a nonsteroidal estrogen). In most studies the ability of estrogens to cause tumors is largely attributed to a genetic predisposition for tumor formation and has caused unnecessary fears for its use therapeutically; the exception, however, is diethylstilbestrol, which, when taken by pregnant women, leads to hyperplastic disease in female children. Estrogens are mitogenic and tend to increase their own receptor numbers and these effects are counteracted by progesterone. Thus, prolonged exposure to estrogens in the absence of progesterone can cause abnormal growth of proliferative tissues like the endometrium.

Progestins is the generic term for compounds that exert an effect similar to that of progesterone. Progesterone is the second most important steroid hormone for female reproductive function. Progesterone receptors are induced by the action of estrogen, thus progesterone has little effect in the absence of estrogen priming. The primary role of progesterone in the uterus is to create an implantation site for the embryo by causing the final differentiation of endometrial cells. Insufficient progesterone leads to an inadequate implantation site and to implantation failure. Progesterone plays a key role in pregnancy by reducing uterine contractile tone and preserving pregnancy. Within the uterus, progesterone causes a decrease in estrogen receptors, thus attenuating the mitogenic effect of estrogen on this tissue. Similarly, progestins reverse the actions of estrogen on cervical mucus, labial color and swelling, and sexual behavior. In the breast, however, progesterone acts to augment the mitogenic effect of estrogen and acts to proliferate the epithelium of the ducts in preparation for milk production.

Female Reproductive Failure

Reproductive failure can be induced by environmental hazards at any level, although most types of infertility have not been linked to environmental factors. Since it is not ethically possible to observe or impose reproductive toxic exposures on human subjects, much of what we believe about the effects of putative toxins are based on the assumption that well-defined spontaneous reproductive failures are accurate surrogates for environmentally induced defects. Spontaneous reproductive failures, together with results from animal experiments, are used as models to predict or to understand the impact of reproductive toxicants on the human system.

Ovarian senescence due to increasing age (menopause) is another form of infertility that is a normal consequence of the aging process and can be compared to the effects of an ovarian toxicant. As oocytes are depleted through the normal process of atresia, the reproductive system responds in much the same way as it would to an ovarian toxicant that acts to destroy oocytes. In both cases, the absence of gonadal hormones results in increased pituitary drive in compensation for the decreased negative feedback from the ovaries which, without germ cells, cannot produce steroid hormones. The increased gonadotropins (hypergonadotropism) cannot, however, compensate for the irretrievable gonadal deficiency (hypogonadism).

Defects that act to suppress hypothalamic or pituitary function also lead to infertility but are

expressed differently. Kallman's syndrome (anosmia with isolated gonadotropin deficiency), for example, is a form of hypothalamic deficiency in which the pituitary is not stimulated to release gonadotropins due to a defect at the level of the hypothalamus and higher nerve centers. The gonads of affected individuals remain in a preadolescent condition, and sexual maturity is never achieved. In such case the disease simulates a central nervous system toxicant that leads to reduced gonadotropin release (hypogonadotropic) and a subsequent reduced ovarian activity (hypogonadism). Since the pituitary is not compromised in this condition the appropriate administration of the hypothalamic factor which causes the release of gonadotropins (GnRH) will restore pituitary and subsequently gonadal function as well. Physical, nutrition, or even emotional stress can lead to different degrees of 'hypothalamic amenorrhea', which is a general term for hypogonadotropic hypogonadism in women. Professional dancers, athletes, and overzealous dieters can exhibit this reversible form of infertility at any stage in life and, as a consequence, this type of reproductive failure occurs relatively frequently. Such cases can be used to model theoretical toxicants which block normal hypothalamic or pituitary function.

Many kinds of organic or functional defects can lead to postovulatory reductions in fertility. For instance, anatomical impediments to gamete transport can prevent fertilization. Poorly developed or insufficient endometria due to end organ insensitivity to steroids, insufficient steroid hormone production, or impediments to steroid action at the level of the endometrium will not adequately support the implantation site of an otherwise healthy embryo. Previous reproductive tract infections are responsible for the majority of these kinds of reproductive failures; however, developmental defects and alterations in organ function caused by inappropriate stimulation or response also contribute.

Reproductive Toxicants

In recent years public concern regarding toxicant exposure has focused on the potential of reproductive hazards resulting from exposure to agricultural and industrial chemicals. This has led to the suggestion that a significant amount of the recognized reproductive failure among humans and animals can be attributed to increased toxic exposures. The increasing number of female workers in industry as well as the recent recognition of hazards to female reproduction in the workplace has heightened concerns relating to female reproductive toxicology. Such concerns, however documented, have resulted in an increase in risk assessments of both putative and real reproductive toxicants as well as in the creation of regulations concerning disposal of and exposure to xenobiotics. Progress in this area, however, has been slow for a number of reasons.

Reproductive toxicants obey the rules of other toxicants and their effects can usually be linked to some interruption of normal physiologic mechanisms such as errors in metabolism, interfering with ligand/receptor interaction or alterations of signal transduction. They can act directly by inducing a change through their inherent chemical activity. For example, the purine analogs interfere with the normal process of oogenesis and have greatly different effects at different stages of reproductive development. Some reproductive toxicants mimic or block hormone action by virtue of their structural similarity to these hormones and mimic or antagonize endogenous messengers. Other toxicants act through receptors that may or may not have well-defined physiologic functions and interact with hormone transduction signals, trans-activating factors or response elements. Some toxicants act directly while others must be metabolized to an active form before they can exert their adverse effects. Some compounds have dissimilar adverse reactions prior to and following metabolism. Other reproductive toxicants act indirectly after being metabolized from an inert compound to a form that is chemically or biologically active. Polycyclic aromatic hydrocarbons can exert their effects indirectly by inducing hepatic and ovarian enzymes, which govern steroid production and metabolism, and act by transducing adverse signals or signals that impede normal physiologic functions. Lipophilic compounds can be sequestered in adipose tissues and exert adverse effects years after a single exposure.

The identification of chemical compounds of high concern as human reproductive and developmental toxicants was provided by a report from the Government Accounting Office (GAO) in 1991. That report reviewed the evidence that identified compounds as male, female, or developmental toxicants and what safeguards were in place to protect the public. The report lists 30 compounds, 21 of which have adverse reproductive effects in women or female animals. These compounds include industrial solvents (toluene, ethylene glycol monoethyl, and monomethyl ethers); metals (cadmium and lead), pesticides, fungicides, and fumigants (clordecone and its metabolite mirex, DDT, ethylene dibromide, ethylene oxide, hexachlorobenzene, and the pesticide contaminant dioxin); halogenated hydrocarbons (vinyl chloride, PBBs, and PCBs); products of combustion (carbon disulfide, carbon monoxide, and tobacco smoke); as well as arsenic, diethylstilbestrol, and warfarin.

The GAO list of reproductive toxicants does not include some putative reproductive toxicants which are currently highly regulated or banned for industrial use such as benzene, benzamine, chloroprene, formaldehyde, styrene, and xylene. More are added to the list of putative toxicants every year. Of the toxicants listed as female reproductive hazards, approximately half have strong evidence of direct adverse effects on human (or nonhuman primates) female reproduction separate from their action as developmental toxicants and teratogens. A number of compounds such as the glycol ethers are only now being recognized as reproductive toxicants and reports demonstrating this effect are beginning to appear in the literature. Thus, the list of compounds for which there is strong evidence of adverse effects on fertility, menstrual function, or other gynecological disorders in nonpregnant women can be theoretically condensed to the 14–16 individual or groups of compounds. These are listed in **Table 1** along with the adverse effects that are associated with each. The actual number of compounds that have adverse effects on female reproduction is undoubtedly much greater than this list indicates, and will grow as new chemicals are developed. Many of the compounds which have documented effects on males will likely have adverse effects on females once they are investigated properly. However, until the adverse effects of exposures of these are observed for women and are documented, they cannot be included.

Table 1 not only illustrates the relatively small number of documented human female reproductive toxicants but also underscores the difficulty of investigating exposures to reproductive toxicants in human populations. There is a lack of specific knowledge in terms of the targets and mechanisms of action of most reproductive toxicants because the end point for recognizing the adverse effect is 'downstream' of the actual target. The literature lists more than half of the adverse effects as only 'menstrual dysfunction', which provides little help in identifying a specific site or action. This general outcome is reported because it is the only relevant end point that is usually available during the study periods that usually follow the actual exposure. Assessment of fertility, for instance, would need to include a relatively large number of women who were simultaneously exposed to the possibility of pregnancy over an interval of time that would permit adequate pregnancies to occur and be completed.

Table 1 A condensed list of human female reproductive toxicants and their adverse effects

Benzene	Menstrual dysfunction
Benzamine	Menstrual dysfunction
Chloroprene	Menstrual dysfunction
Formaldehyde	Menstrual dysfunction
Mercury	Menstrual dysfunction
Halogenated hydrocarbons	Menstrual dysfunction
Anesthetic gases	Infertility
Toluene	Menstrual dysfunction
Styrene	Menstrual dysfunction
Diethylstilbestrol	Infertility
Ethyl oxide	Abortion
Lead	Abortion
Vinyl chloride	Ovarian dysfunction
Dioxin (TCDD)	Infertility

Regardless of the number of women exposed and the time of exposure, most reproductive toxicity data are collected in retrospect, using the subjects' recall as the source of information relating to reproduction. Practical end points for assessing the target of toxicity other than the woman's menstrual calendar have not been available historically and only general symptoms such as menstrual function can be recalled and reported. As reviewed earlier, menstruation is the normal result of an ovulatory ovarian cycle and ovulatory cycles can be quite variable in length and regularity. Irregular menstrual cycles may be typical for some women and not for others and a woman's recall of her previous 'regularity' may not be accurate. In addition, vaginal bleeding for other reasons that have characteristics of true menstruation may occur in the absence of ovulation, for example, breakthrough bleeding as a result of unopposed estrogen stimulation. While the listing of adverse effects as menstrual dysfunction may be adequate to indicate that female reproduction has been perturbed, it provides very little information as to the target or mechanism of action, nor does it provide information as to the health risk except in the most severe cases.

A deeper understanding of the site of toxicity and the mechanism of action can come only from controlled animal studies in which basic hypotheses are tested using laboratory rodents or primates. As indicated previously, there are concerns of species specificity in terms of sensitivity or response to reproductive toxicants, routes of exposure, and relevant dosage that make this less than a perfect science. However, knowledge of the similarities and differences in the reproductive physiology of the model species compared to human function as presented earlier in this section, together with a knowledge of human reproductive health and disease, permits a great deal of information to be obtained from laboratory animal studies. The complete understanding of basic reproductive physiology allows the toxicologist to focus on specific targets of toxic action. It is from the understanding of basic reproductive physiology, the experiments of nature provided by spontaneous reproductive diseases, and

laboratory experiments with animal models that targets of toxicity on functional and anatomical bases are appreciated. These targets are defined in the following sections.

Central Targets

The organs, nuclei, and organelles that are required for normal pituitary secretion of gonadotropins are considered to be the central targets of toxicity. They are generally divided into the neural tissues, nerve tracts, specific nuclei in the brain, and their organelles (including the hypothalamus and higher nervous centers) and the anterior pituitary gland. In some cases, the pineal gland would also be considered a target because of its direct effect on pituitary function.

Hypothalamus and Higher Brain Centers Toxicants that disrupt the synthesis of GnRH or its normal pulsatile release will cause reproductive failure by way of pituitary dysfunction. There are two general mechanisms for this to occur. The direct effect is one in which neural transmission is altered by other neurotransmitters or their analogs. Anesthetics, anticonvulsants, and recreational drugs are examples of agents that can cause hypothalamic dysfunction that, in most cases, decrease GnRH pulse and amplitude. These kinds of toxicants can reduce neuronal firing rate and reduce either the baseline gonadotropin secretion or block the midcycle periovulatory surge in laboratory animals. In human subjects decreased nutrition, increased exercise, as well as physical or emotional stress can lead to similar derangements of the hypothalamic–pituitary axis by increasing catecholamine, indolamine, and endorphin levels with oligomenorrhea or amenorrhea as a result. Some compounds such as the ergot derivatives can mimic dopamine action and reduce prolactin secretion. The indirect effect is one in which the normal 'long-loop' feedback mechanisms are altered. Bioactive steroid hormones or their analogs can inappropriately increase or decrease hypothalamic drive leading to alterations in pituitary gonadotropin secretion. Increased adrenal glucocorticoid, for example, is thought to decrease gonadotropin secretion although the precise mechanism is not known. Diethylstilbestrol is a model for a toxicant that might decrease hypothalamic drive because it is a potent estrogen agonist. In contrast, tamoxifen or clomiphene citrate, which are estrogen antagonists, would have the opposite effect. Some of the halogenated hydrocarbons are thought to act as estrogen antagonists and may influence hypothalamic function by acting through estrogen receptors.

Anterior Pituitary The anterior pituitary can also be adversely influenced by two separate but general mechanisms. It can be directly affected by changes in the stimulatory effect of GnRH from the hypothalamus and it can be modulated by ovarian steroid and peptide hormones from the ovary (as discussed previously). Perturbations of the hypothalamus are transduced directly to the pituitary through the primary GnRH signal; thus, normal pituitary function is unlikely when the hypothalamic drive is perturbed. Because of the location of the hypothalamus and pituitary at the base of the brain and the intimate vascular and neuronal connections between them, it is difficult to separate actions that occur at this level. Therefore, hypothalamic and pituitary failure are often considered together as simple 'central effects' as opposed to actions at the level of the ovary, reproductive tract, or related reproductive tract organs.

Inappropriate circulating levels of bioactive steroid hormones or their analogs can lead to perturbations of pituitary function. Increased blood concentrations of bioactive estrogen, progesterone, androgen, or their analogs will lead to decreased secretion of gonadotropins. Steroid antagonists will open this feedback loop and cause increased amounts of gonadotropins to be secreted. The therapeutic bases of oral contraception and one aspect of fertility enhancement are based on these principles. Many halogenated hydrocarbons are thought to act as estrogen analogs and act to either transduce false signals through the estrogen receptor or block endogenous estrogen from exerting normal action. The latter case is well defined for DDT, which causes thin egg shells in birds exposed to DDT. Some PCBs have agonistic and antagonistic action in different animal species and different organs within the same species. It is not clear how many chlorinated biphenyls have estrogenic effects or if any of these compounds are serious potential hazards to women. In many cases they are estrogen agonists when acting in the absence of steroidal estrogen and estrogen antagonists in the presence of steroidal estrogen. It is also difficult to separate actions which occur at the hypothalamus and pituitary; therefore, these are often considered collectively as 'central' effects as opposed to effects that occur downstream such as at the level of the ovary or reproductive tract organs.

New evidence is now emerging that some halogenated hydrocarbons exert their effects through receptors other than the classic estrogen receptor. Beyond the identification of alpha and beta (possibly gamma?) forms of the original estrogen receptor, other nonrelated receptors are now thought to interact with the classic estrogen receptor hormone signally, besides membrane-bound forms of the estrogen receptor as well as with nonrelated orphan

receptors. One such orphan receptor is the arylhydrocarbon (Ah) receptor and has no known physiologic role but acts much like the receptors of the steroid hormone superfamily of receptors. The binding of the Ah receptor to its ligand, which can be dioxin or related coplanar, chlorinated biphenyls, elicits transcription of new proteins and/or blockage of other proteins such as estrogen receptors.

Ovarian Targets

Ovarian tissue can be compared to embryonic tissue in that most of its functional elements are still in various stages of development. All of the endocrine aspects of the ovary are differentiated at the time that a subpopulation of germ cells matures. Both the endocrine and the germ cell populations are transient populations that must be renewed with each reproductive cycle. The ovarian targets of toxicity are therefore ever-changing populations of different cell types. For this reason it has been difficult to identify cytotoxic agents that have specific ovarian cell types as their unique target. In general, ovarian targets can be divided into two categories. The most important category is the germ cells, which are primarily primary oocytes in a resting stage of meiosis. The second category is represented by the cells which produce steroid and peptide hormones.

Germ Cells Unlike the testes, in which steroid production can proceed in the absence of spermatogenesis, the ovary can function as an endocrine organ only if viable germ cells are in residence. Toxicants that eliminate the resting germ cells automatically eliminate all endocrine function. Since all aspects of female reproduction are dependent on ovarian steroids, the growth, development, and integrity of the entire reproductive system will be disrupted by loss of the germ cells. A complete loss of ovarian function would ensue and, in humans, menstrual function would cease as it would with complete hypothalamic; pituitary dysfunction. In contrast, toxicants that adversely affect only the oocytes which have ended their resting phase and begun to mature will interrupt only the current ovarian cycles as additional oocytes can be recruited from the resting germs cells. Such compounds may be 'silent' hazards having the effect of delaying conception only slightly. Exposure to such toxicants would most likely be recognized through menstrual dysfunction, long menstrual cycles, and possibly as a delay to conception.

Ovarian Steroid-Secreting Cells Steroidogenic cells within the ovary are also transient cell populations. While the development of gonadotropin receptors and steroidogenic machinery are dependent on the proximity and continued viability of a healthy oocyte, the mature cell will survive only the length of the reproductive cycle and possibly through one pregnancy. The steroidogenic cells of the ovary are recruited in each cycle from undifferentiated ovarian stroma. Agents that arrest differentiation, block the expression of gonadotropin receptors, or block the production of steroid hormones will have adverse effects on ovarian function. Steroidogenesis can be blocked by either blocking the transport and availability of cholesterol, compromising the reducing capacity of the cell, or by direct block of steroidogenic enzymes. Such disruptions would be recognized as menstrual dysfunction ranging from irregular menstrual cycles to complete amenorrhea if steroidogenesis is completely stopped.

Reproductive Tract Targets

The female reproductive tract is completely dependent on the functioning ovary to provide estrogen and progesterone for its growth, development, and function. Reduced steroid production, increased clearance of circulating steroid hormones, or antagonism of steroid action at the level of the steroid hormone receptor will lead to decreased size and function of all aspects of the female reproductive tract. Increased circulating concentrations of sex steroids or their agonist generally lead to hypertrophy, hyperplasia, and dysfunction. Sex steroids or their analogs at relatively high circulating concentrations will disrupt pituitary function through the long-loop feedback. However, exposure to low levels of steroid analogs for prolonged time periods may have adverse effects on the reproductive tract without disrupting the HPO axis. Such theoretical toxicants could cause infertility with no other overt signs. An example of this kind of toxicant is low-dose progestin therapy which, in some women, is an effective contraceptive although relatively normal menstrual cycles are observed, suggesting adverse effects at the level of the endometrium while exerting no demonstrable effect at the level of the HPO axis. Similarly, weak sex steroids could act locally to alter cervical secretion, reducing sperm survival and transport through the reproductive tract as observed in sheep exposed to plant estrogens.

Over 300 different plants contain either compounds with estrogenic activity or precursors for the formation of nonsteroidal estrogens. Coumestrol, equol, and zearalenone are examples of phytoestrogens that are found in legumes, tubers, and fungi that infest grains. These substances clearly act as reproductive toxins in sheep (equol in clover disease) and

pigs (zearalenone in moldy corn syndrome). Evidence is not as convincing for carnivores fed commercial diets with plant 'fillers'. In humans there is some evidence that Asian diets act as a protectant for some forms of hyperplastic disease. Some claims for precocious puberty being the result of contamination with environmental estrogens have been made. Although there are structural similarities between the parent phytoestrogen molecules and DES, it is speculated that only phenolic metabolites of these compounds are active compounds since pretreatment with carbon tetrachloride (to inhibit the mixed function oxidase in the liver) reduces the estrogenic *in vivo* potency of *o*,*p*′-DDT in rats. However, *in vitro* studies indicate that *in vitro* competition of compounds, such as *o*,*p*′-DDT and methoxychlor with estradiol for binding the rat uterine estrogen receptor, is positively correlated with *in vivo* estrogenicity. The fact that the estrogenicity of either the parent compound or its metabolite competitively competes with estradiol for receptor binding may limit the action of circulating steroidal estrogens. By limiting the action of the more potent steroidal estrogens, the weaker nonsteroidal estrogens may act as antiestrogens. In addition, some DDT analogs such as *p*,*p*′-DDT are thought to act by inducing liver enzymes that metabolize endogenous steroidal estrogen, thus reducing normal estrogen delivery to the target tissue.

Substances that increase or decrease smooth muscle activity can cause adverse reproductive effects. Nicotine, for example, acting through epinephrine and oxytocin can influence tubal and uterine contractions. Theoretically, such agents could cause mistiming of gamete and/or embryo transport and failure of fertilization or implantation, respectively. Hemotoxic agents can alter menstrual flow and result in menstrual irregularities (as indicated previously) at the level of the endometrium without having any effect on the reproductive system directly.

Nonreproductive Organ Targets

Key nonreproductive organs are essential for normal reproduction. An example of this kind of interaction is the production of binding proteins that are essential for steroid hormone transport by the liver. Hepatotoxins such as ethanol can limit binding protein production and adversely alter the ability of sex steroids to be transported to their binding sites. The liver also plays the primary role in deactivating and eliminating steroid hormones. Hepatotoxins, such as the halogenated hydrocarbons, barbiturates, and anticonvulsants which alter enzymes that either conjugate or metabolize steroid hormones, can also adversely affect reproductive function. Normal thyroid function is important for normal reproduction. Thyroid hormone is essential for normal cell function in general, and thyroid disease is often associated with reproductive failure.

Summary and Conclusion

In summary, in the broadest view reproductive toxicants can impinge on the female system through changing normal sexual development, obliterating gametes, causing dysfunction of reproductive organs, interfering with the differentiation of cell types, or interrupting the hormone messages through which the processes of hormone synthesis, transduction, or metabolism occur. Reproductive toxicants can influence reproductive performance by affecting sexual or social behavior, embryo survival and development, as well as affecting reproduction indirectly by influencing general health. The primary difficulties faced in identifying reproductive toxicants are the sensitivity of female reproductive processes to normal environmental change, the lack of baseline data, the complexities of the ovarian cycle, and the species-specific nature of female reproductive physiology.

A great deal of progress is currently being made in this discipline in response to pressures exerted by the public. Real concerns are now being expressed that environmental factors are causing an increase in female infertility while more women are exposed to chemicals through the workplace. Perhaps the greatest impediment to progress in this area is adequate animal models or an *in vitro* screening test for human sensitivity to the large number of chemicals being produced.

See also: Androgens; Carcinogen–DNA Adduct Formation and DNA Repair; Chromosome Aberrations; Developmental Toxicology; Dose–Response Relationship; Endocrine System; Epidemiology; Reproductive System, Male; Risk Assessment, Human Health; Sister Chromatid Exchanges; Toxicity Testing, Developmental; Toxicity Testing, Reproductive.

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Reproductive System, Male

Marion G Miller and Shelley Brown DuTeaux

Introduction

Both the public and the scientific community have become increasingly aware of the potential for chemicals to adversely affect the male reproductive system. It has been estimated that as many as 15% of couples in the United States are infertile. For ~40% of those couples, infertility is associated with the male partner. Recent reports suggest that the rates of prostate and testicular cancer, prostatic hyperplasia, and cryptorchidism (undescended testes) are also increasing. Although a continuing subject of debate, an analysis of human sperm counts from 1938 to 1990 provided data indicating that the average sperm density in males has declined in the last 50 years. It has been proposed that exposure to estrogens and other endocrine disruptors in the environment could be associated with adverse effects on the male reproductive system.

Evidence for male reproductive toxicity in humans generally surfaces only when the outcomes are severe. For example, pesticide formulators exposed to high levels of the nematocide dibromochloropropane developed testicular atrophy and infertility. The loss of sperm production in the testes of several of the men studied became permanent. The opportunity to study such overt and devastating effects in humans is rare. It is more likely that a chemical will affect the reproductive tract in more subtle and complicated ways. Animal models are generally used to test the ability of chemicals to cause reproductive toxicity. Data generated from such experiments can supplement human epidemiological studies to help determine the safety of chemicals in the workplace and the environment. However, male reproductive toxicity testing has only been done on a fraction of the over 500 000 chemicals and mixtures currently used in commerce and industry. These data gaps have created an awareness of the need to improve the adequacy of our testing procedures and to better understand the events underlying male reproductive toxicity.

Reproduction is biologically complex. Perturbations in any number of biological processes could result in changes in the organs of the reproductive system and increase the potential for passing along development defects to children. An adequate evaluation of reproductive toxicity should consider the multitude of effects and how chemicals interact at the level of cells and tissues to result in dysfunction. The following discussion of male reproductive toxicology includes (1) male reproductive tract physiology, (2) methods for reproductive toxicity testing, (3) a description of specific targets, (4) examples of male reproductive toxicants, and (5) issues involving chemical regulation and safety.

Physiology of the Male Reproductive System

A series of tightly orchestrated events must occur for a male to produce viable sperm capable of fertilization and producing normal offspring (**Figure 1**). The process of spermatogenesis in the testis is subject to neuroendocrine controls via the hypothalamic-pituitary axis (I), and indirect influences arising from nutritional status, liver metabolism, and vascularization (II). Within the testis (III), endocrine, autocrine, and paracrine controls are required for the proliferation and differentiation of the stem cell spermatogonia into the mature spermatid that is released into the lumen of the seminiferous tubule. The released spermatozoa travel through the rete testis and efferent ducts to the head (caput) of the epididymis. As the spermatozoa pass through the middle (corpus) and tail (cauda) of the epididymis, they undergo maturation (IV) and gain motility as well as the ability to fertilize oocytes. Toxicants could affect any of these steps or have direct effects on sperm cell viability (V) or on the ability to penetrate and fertilize an oocyte (VI). To date, little is known about male mediated developmental toxicity, whereby the male gamete transmits inheritable defects to offspring (VII). This possibility has received more attention in recent years.

The Testis and Spermatogenesis

The testis is made up of tightly packed seminiferous tubules surrounded by a vascularized interstitium.

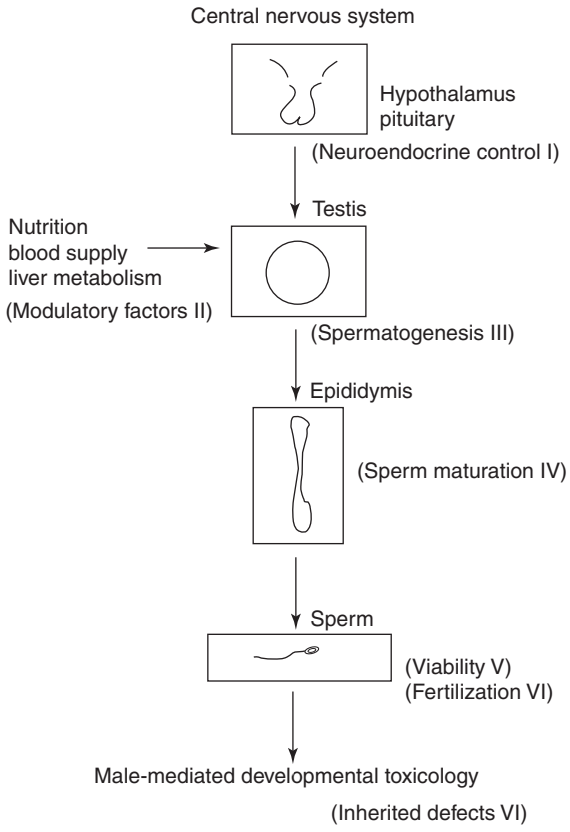

Figure 1 Overview of the male reproductive system.

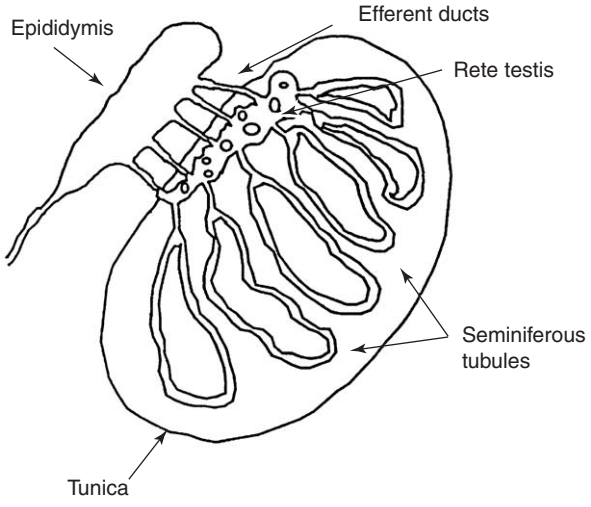

Figure 2 Schematic representation of the structure of the testis. (Reproduced from Working PK (1989) *Toxicology of the Male and Female Reproductive Systems*. New York: Hemisphere, with permission from Taylor and Francis, Inc.)

It is enclosed in a tough fibrous capsule called the tunica albuginea (**Figure 2**). Within the seminiferous tubules, germ cells develop into spermatozoa in a process called spermatogenesis. The Leydig cells located between tubules in the testis interstitium carry out the synthesis of steroids. Steroidogenesis is essential both for spermatogenesis and for developing and maintaining secondary sexual characteristics. (For more about steroids, see section 'Hypothalamic–Pituitary–Gonadal Axis'.)

The production of gametes in mammals (spermatogenesis, oogenesis) requires the process of meiosis to reduce the number of chromosomes in each cell from 46 (diploid) to 23 (haploid) so that a fertilized zygote will contain 46 chromosomes, half from each parent. In the male, the process of spermatogenesis involves mitosis and meiosis and three germ cell types: (1) spermatogonia, (2) spermatocytes in various stages of meiosis, and (3) postmeiotic spermatids undergoing elongation prior to release as spermatozoa. The first germ cell type, spermatogonia, undergo stepwise mitotic proliferation and differentiation and are classified by their stage of development (types A_1–A_4, intermediate, and type B). Type B spermatogonia ultimately divide into primary spermatocytes that enter meiosis. The process of reducing chromosomes from 46 in diploid spermatocytes to 23 in haploid spermatids starts in the preleptotene phase of meiosis I. Each primary spermatocyte enters meiotic prophase, forming distinct cell types at each phase (leptotene, zygotene, pachytene, and diplotene). At the end of meiosis I, two secondary spermatocytes are produced which enter meiosis II and rapidly divide to produce a total of four haploid round spermatids with 23 chromosomes each.

The metamorphosis of round spermatids into spermatozoa is described as spermiogenesis. Initially, the round spermatid develops an acrosome derived from the intracellular Golgi complex. The acrosome starts as a small vesicle and develops into a pronounced cap on the sperm head. The acrosome is necessary for oocyte fertilization and contains the lysosomal enzymes required to penetrate the vestments surrounding the egg. Early in spermiogenesis, a microtubule-containing flagellum begins to develop. Nuclear DNA undergoes condensation and is no longer synthesized before the nuclei elongate around a microtubule structure called the manchette. As the spermatid elongates, mitochondria collect in a sheath behind the sperm head and around the flagellum in what will form the midpiece. The mitochondria will supply energy for sperm movement. Release of mature spermatids into the tubular lumen (spermiation) is accompanied by the loss of the spermatid cytoplasm. The residual cytoplasm is endocytosed and forms residual bodies within the Sertoli cell. The final spermatozoa are ideally designed to transport DNA from the male to the oocyte, with little cytoplasmic baggage, a good mitochondrial engine, and a large tail for propulsion.

Immature germ cells develop in the basal area around the circumference of the seminiferous tubule. As spermatogenesis progresses, developing sperm advance toward the central lumen (**Figure 3**). The Sertoli cell, the 'nurse cell' of the testis, supports, nourishes, and protects the developing germ cells that it surrounds. Sertoli cell tight junctions form a 'blood-tubule' barrier that prevents the entry of blood-borne materials and maintains a specific tubular milieu necessary for germ cell development. In the rat, it takes ~56 days for spermatogonia to complete spermatogenesis and be released from the testis. Spermatogonial differentiation is initiated every ~12.9 days within the seminiferous tubules. At any given time there will be germ cells from successive generations and at different phases of development within the seminiferous tubules. Fourteen stages of spermatogenesis have been defined based on nuclear morphology and the appearance of the acrosome in the spermatid. The seminiferous epithelium cycles through the stages of spermatogenesis in a time-dependent manner. Different stages follow one another along the length of the seminiferous tubule in what is known as the 'wave of spermatogenesis'. This progression is necessary to maintain continuous sperm production. If there were no 'wave', spermatogonia throughout the testis would enter spermatogenesis at the same time and fertility would become episodic.

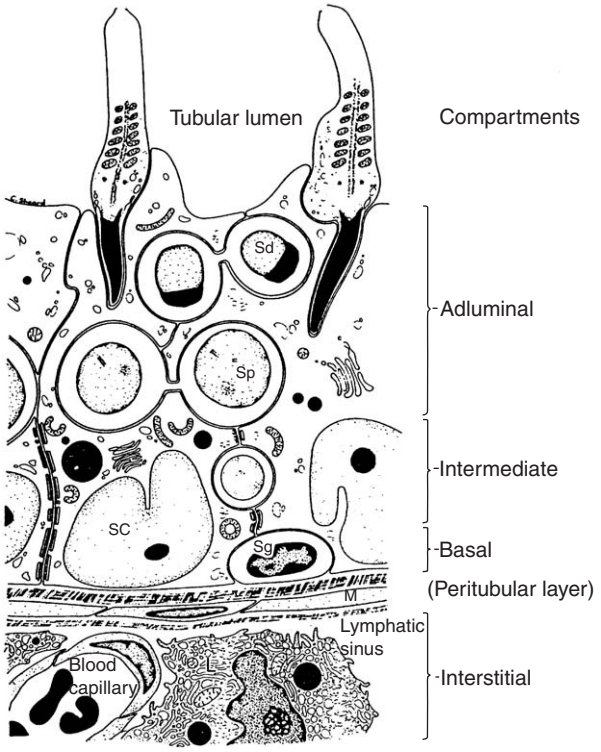

Figure 3 Diagrammatic representation of a portion of a seminiferous tubule. L, Leydig cell; M, myoepithelial peritubular cell; SC, Sertoli cell; Sg, spermatogonium; Sp, spermatocyte; Sd, spermatid. (Reproduced from Lamb JC, IV and Foster PMD (1988) *Physiology and Toxicology of Male Reproduction*. San Diego: Academic Press, with permission from Elsevier.)

The stages of spermatogenesis differ between species. Therefore, the duration and cycle length of spermatogenesis also differs between species. In the human, it is thought that there are six stages of spermatogenesis defined by specific cellular associations. This is in comparison to 14 stages in the rat. Interestingly, the human male may have no clearly defined 'wave' of spermatogenesis arranged consecutively along the length of the seminiferous tubule. While some researchers believe that human germ cell development occurs along helical and longitudinal axes, others believe that the arrangement of stages of spermatogenesis may simply be a random occurrence. From a toxicological point of view, germ cells at different stages of development and differentiation may have different susceptibility to toxicants.

The Excurrent Ducts and Sperm Maturation

Spermatozoa leave the testis by first passing through the rete testis, then flowing through the efferent ductules to the epididymis. These reproductive structures are collectively known as the excurrent ducts (**Figure 4**). In the rodent, the efferent ductules connect to the initial segment of epididymis. In humans, however, the efferent ductules are embedded within the head (caput) of the epididymis. Efferent ductules are comprised of epithelial cells that surround an open lumen. The ductule epithelial cells are specialized for reabsorption, with the portion adjacent to the testis absorbing the majority of fluid and the portion adjacent to the epididymis absorbing small proteins and other macromolecules released with sperm. From the efferent ductule, the concentrated sperm enter the epididymis.

The mammalian epididymis is a highly coiled duct where sperm undergo maturation and are stored prior to ejaculation. The epididymis is comprised of a head (caput), a body (corpus), and a tail (cauda), which can be defined by their relative location, tissue characteristics, and cell types. Within a connective tissue sheath, the epididymis is a complex of tubules lined with columnar epithelial cells attached to a basement membrane. Epithelial cell height decreases and luminal diameter increases from the initial segment to the cauda of the epididymis. There are several distinct epithelial cell types found in the mammalian epididymis, including the principal, narrow, basal, clear, and halo cells. The principal cells

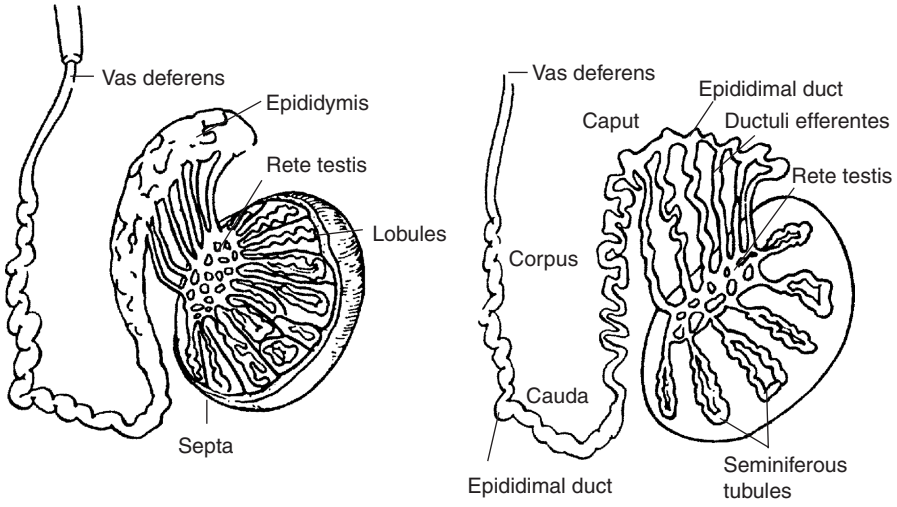

Figure 4 Structural relationships between the testis and the epididymis. (Reproduced from Zaneveld LJD and Chatterton RT (eds.) (1982) *Biochemistry of Mammalian Reproduction* © New York: Wiley. This material is used by permission of John Wiley & Sons, Inc.)

represent between 65% and 80% of the entire epithelial cell population and are involved in absorptive and secretory processes.

Sperm entering the epididymis from the testis are functionally immature and require further differentiation within the epididymis to become motile and to gain the ability to fertilize oocytes in the female reproductive tract. Sperm maturation events have not been completely elucidated. However, research indicates that sperm maturation is a complex process that involves the remodeling of the sperm plasma membrane within a changing luminal environment. The composition of the epididymal milieu is controlled in part by the epididymis–blood barrier and the active uptake and release of specific macromolecules. As sperm transit the epididymis, they are exposed to different epididymal 'microenvironments' that are important for sperm maturation. As sperm mature, they are distinguished by the loss of the cytoplasmic droplet, acrosomal and nuclear changes, and alterations to lipid and protein composition, all of which may be important to sperm gaining their fertilizing ability.

When mature spermatozoa reach the cauda of the epididymis they are stored until ejaculatory release via the vas deferens. Spermatozoa are discharged through the ejaculatory duct. The major portion of ejaculate volume is made up of products secreted by the accessory sex glands: the seminal vesicles, the prostate, and the bulbourethral glands. Rodents also have coagulating glands and preputial glands. Using mature spermatozoa from the cauda epididymis, it has been demonstrated that the secretions of the rodent accessory glands are not important for successful *in vitro* fertilization. However, there is a reduction in *in vivo* fertility when accessory gland products are not present in semen, indicating the importance of these components to successful reproduction.

Recently, it has been demonstrated that an immature human spermatid can be injected directly into an oocyte, resulting in successful pregnancy and birth. In practice, the success rates of intracytoplasmic sperm injection (ICSI) vary from 0% to 68%, depending on the number of oocytes injected, the age of the mother, and the quality of sperm. Currently there are no standardized indications for the use of ICSI for infertile couples. However, there is general agreement that ICSI should be used when male infertility (as diagnosed by semen analysis) is a factor. Some severe cases of male infertility are associated with chromosomal aberrations (e.g., aneuploidies, deletions). Therefore, the use of ICSI has raised concerns about the risk of transmission of chromosomal or genetic defects to embryos and negative consequences during development. Recent data have also suggested that the technique of ICSI, which bypasses the normal barriers of fertilization, may itself be responsible for alterations in the viability and health of fertilized embryos. Notwithstanding these concerns, the health of a majority of children delivered after ICSI has been normal.

Hypothalamic–Pituitary–Gonadal Axis

Neuroendocrine control of gonadal function is regulated through the hypothalamus in the brain and the closely associated anterior pituitary gland (**Figure 5**). Gonadotropin releasing hormone (GnRH) is released from the hypothalamus in a pulsatile

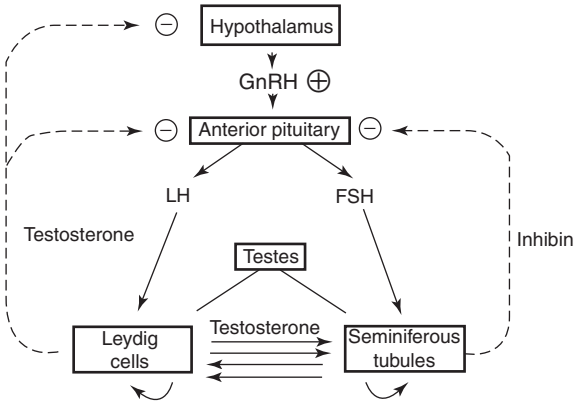

Figure 5 Neuroendocrine control of the male reproductive system. (Reproduced from Heindel JJ and Treinen KA (1989) Physiology of the male reproductive system: Endocrine, paracrine, and autocrine regulation. *Toxicology Pathology* 17 (2): 411–445, with permission from Society of Toxicologic Pathology.)

manner, and carried in the blood supply directly to the anterior pituitary. After being stimulated by GnRH, the pituitary releases the gonadotrophins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH circulate in the blood and reach the testis where they play a central role in regulation of testicular function. Like GnRH, LH and FSH release are most likely pulsatile in nature. In the testis, LH targets the Leydig cells, where it binds to receptors and stimulates steroidogenesis. Testosterone production is episodic, coincident with the pulsatile release of LH. The Sertoli cell is the testicular target for FSH. The complete role of FSH in spermatogenesis is as yet unknown. However, FSH is necessary for spermatogenesis presumably due to its involvement in Sertoli cell function.

Hormonal regulation is through a series of feedback mechanisms taking place at both central and peripheral sites. To complete the endocrine feedback loops, testosterone regulates LH production, while inhibin and other Sertoli cell products regulate FSH secretion. These feedback loops modulate the release of GnRH from the hypothalamus as well as LH and FSH from the anterior pituitary. Within these loops, factors that perturb one component may alter regulatory influences on another. For example, if the Leydig cells were damaged, there could be a decrease in testosterone production. In response to low circulating levels of testosterone, LH release would increase in an attempt to restore testosterone production.

Approaches to Male Reproductive Toxicity Testing

A variety of tools are available to assess the male reproductive toxicity of a chemical and potential mechanisms underlying toxicity. The most important physiological endpoint is fertility. Therefore, the effect of a toxicant on fertility should be included in most assessments. However, impaired fertility is also the most severe effect. Therefore, toxicity testing should also be designed to detect subtle compromises, such as alterations in histology, testicular function, epididymal function, and sperm assessment. For humans, the most compelling reproductive toxicity data are collected from epidemiological studies and case reports. When human data are not available, toxicity testing may be conducted on experimental animals. Because experimental species such as rats, rabbits, and mice are very fecund and have far larger sperm reserves than humans, these animals generally need to be exposed to high doses of chemicals before an effect on fertility is observed.

Human Studies

Generally, it is difficult to assess the adverse effects of a chemical or exposure on the male reproductive tract unless the man is actively trying to conceive. Therefore, a chemical may be affecting fertility in a man and go unnoticed. The 'hidden' nature of male reproductive toxicity may underrepresent the effects of reproductive toxicants on the human population.

Human data may be collected from clinical studies of subfertile men or from epidemiological studies. Epidemiological case–control and historical cohort studies have successfully identified the male reproductive toxicity of several solvents and pesticides. (See section 'Examples of Male Reproductive Toxicants'.) Clinical studies might bring to light specific exposures or risk factors affecting the male's ability to conceive with his female partner. The first step is generally a detailed semen analysis, including assessment of sperm concentration, semen volume, percent motile sperm, sperm viability, and morphology. A more detailed analysis of sperm pattern and vigor can be done with computer-assisted sperm analysis. Other specialized tests, designed to assess the acrosome reaction or the fertilizability of sperm through the hamster egg-sperm penetration assay, may also be performed. Certain chemicals may affect sexual potency (the ability to achieve erection), ejaculation, and libido. Testing hormone levels, testicular size and rarely histology (through biopsy) can be important.

Animal Studies

The majority of information about male reproductive toxicants has been obtained from studies carried out in the rat, the most common animal model used for reproductive toxicity. Animal studies allow for controlled experimentation where events

underlying reproductive toxicity can be better understood. In a typical study, age- and weight-matched male animals are randomly placed into treatment and control groups. Treatment animals are dosed with a specific amount of the chemical for a given duration. The control group undergoes the same type of dosing regimen with an identical formulation minus the toxicant. Dosing is generally by oral gavage; however, other routes of dosing may be used to reflect the expected route of exposure. Various endpoints are assessed, including testis and epididymis size and weight, histology, seminiferous tubule diameter, *in vitro* fertilization, natural mating, mating behavior, and function of the accessory sex glands. Additionally, these endpoints may be studied in a group of animals that have entered a recovery phase after dosing to assess reversibility of effects.

A substantial literature exists emphasizing the use of mating trials for testing male reproductive toxicants. These studies are useful for determining not only the ability of a toxicant to affect reproductive performance, but the subsequent health and development of offspring. In a single mating trail, male animals undergo extended dosing to insure exposure throughout all phases of spermatogenesis and sperm maturation. Males are then mated with untreated females. Following successful mating, the pregnant females are necropsied and the level of gestational success is determined. Endpoints include comparing the number of live implants in females in the treated group versus the control group. Postimplantation loss, or the ratio of dead to total implants from the treated groups compared to the same ratio from the control group, can be measured along with preimplantation loss, which is based on the number of corpora lutea counts and the total implants per female in treated and control groups. Females may be mated with treated males at various times during dosing to determine which sperm cell type might be affected by the toxicant. For example, if a chemical targets the spermatocytes, fertility would decrease coincident with 4–5 weeks of dosing. This delay reflects the time required for the damaged cell population to complete spermatogenesis and pass through the epididymis. However, if the toxicant affects mature spermatozoa, the onset of fertility changes may occur very rapidly after dosing.

Multigenerational reproductive toxicity studies are established testing paradigms used by agencies such as the US Environmental Protection Agency (EPA). Typically, both males and females are dosed prior to mating, and the females are dosed throughout gestation, birth, and lactation. A group of the pregnant dams is sacrificed and the gestational success is determined, as outlined above. Another group of pregnant dams is continually dosed and allowed to deliver and nurse live pups. In order to determine if *in utero* exposure alters reproductive capability, a group of the F_1 pups is dosed from weaning to sexual maturity using the same protocol as their parents. These F_1 pups are eventually mated. Animals in each generation are necropsied and evaluated for systemic and reproductive toxicity, including the F_1 animals, which have been exposed to the toxicant through all stages of development. Endpoints include fertility and histopathology in the parent generation, litter size, and offspring weight, external abnormalities, and subsequent growth. This type of toxicity testing protocol is necessary for pesticide registrations and for other regulatory purposes, but it cannot supply detailed information about the mechanism of toxic action.

Fetal malformations are generally believed to arise *in utero*. However, there exists the possibility that adverse developmental outcomes in the fetus might arise because of paternal exposures to environmental agents. For example, exposure to a particular agent may genetically damage sperm without affecting the ability of the sperm to fertilize an oocyte. However, sperm from the exposed male may be compromised such that the fertilized oocyte will die during cell division. The dominant lethal test is designed to look for loss of the conceptus resulting from alterations in the normal chromosomal complement (e.g., aneuploidy, polyploidy, nondisjunction). The mouse is the predominant animal model for testing dominant lethality. Generally, the male is treated for an extended period and mated with an untreated female. The pregnant dam is sacrificed and pre- and postimplantation losses are calculated. In addition, the recovered embryos may be tested for the presence of chromosomal aberrations.

In addition to genetic alterations, nongenetic developmental defects might arise in the fetus because of paternal exposures to environmental agents. Male-mediated developmental toxicity (either genetic or nongenetic in origin) could result in alterations to fertilization, growth, and development in the absence of maternal exposures. Testing for male-mediated developmental toxicity in animals generally involves dosing the male animal and then mating with untreated females. The pregnant dams are typically sacrificed near the end of pregnancy and the pups are examined for teratological defects, such as bone, brain, and other organ deformities. Another group of females may be allowed to rear their young so that the effects of the paternal exposure can be evaluated on the stages of physiological and behavioral development. Exposure of the father to the anticancer drug cyclophosphamide may result in

male-mediated developmental toxicity. Studies of cyclophosphamide have shown malformations and retardation of growth in the surviving fetuses and a high frequency of fetal death, while causing only minimal alterations to fertility.

Innovative Strategies

In recent years, there has been increasing interest in toxicity tests that minimize the use of animals and allow for high throughput testing of multiple chemicals. While there is no substitute for the whole animal, integration of data from such studies is especially helpful to define mechanisms. Recently, the US EPA developed the Endocrine Disruptor Screening Program (EDSP) to determine whether pesticides, other chemical products, and environmental contaminants could affect the endocrine system. The US EPA is currently developing assays designed to detect chemical substances capable of interacting with the estrogen, androgen, and thyroid hormonal systems. One of the assays under development, the Androgen Receptor Binding assay, is designed to determine if a chemical can bind like testosterone to the androgen hormone receptor *in vitro*, and mimic the action of the natural hormone or block access of the hormone to the site, thereby affecting androgen-dependent activities of cells and tissues. Such high throughput assays may provide cost- and time-efficient ways of gathering reproductive toxicity data with minimal resources.

Specific Targets of Toxic Action

Many compounds have been implicated as male reproductive toxicants, but their sites and mechanisms of action are not well understood. The classification of male reproductive toxicants as direct or indirect is useful to help define the primary site of toxicity (**Figure 6**). A direct toxicant would primarily target the testicular cells, the excurrent duct system of the male reproductive tract, or mature spermatozoa. An indirect toxicant would cause reproductive toxicity by acting on hypothalamic/pituitary neuroendocrine controls or on extragonadal systems. Since the testis is subject to hormonal control and feedback loops, the action of indirect toxicants on endocrine homeostasis can ultimately damage testicular cell types.

Adsorption, Distribution, and Metabolism of Reproductive Toxicants

The absorption, distribution, and metabolism of a chemical can be important in determining potential reproductive toxicity. First, the amount of a chemical absorbed into the body will affect its potential to cause toxicity. Different chemicals are differentially absorbed via the skin, the gastrointestinal tract, and the lungs. Some compounds can pass through the gut or be exhaled virtually unchanged. However, once in the body, a chemical can be distributed to tissues and organs via the circulatory system. Once a toxicant reaches the liver by gastrointestinal adsorption or by circulation, it can be rapidly metabolized and detoxified. The levels of the chemical in the body would decline and there may be less opportunity for the chemical to cause reproductive toxicity. Enzymes present in the liver may not only detoxify chemicals in this manner, but also can bioactivate the parent compound into more reactive intermediates. These metabolites, if sufficiently stable, can reenter the circulation and be delivered to the reproductive tract where toxicity can result. Recent studies have also shown that the testis, efferent ductules, and epididymis contain metabolizing enzymes capable of bioactivating chemicals *in situ*. The rate of metabolism of these enzymes within the reproductive tract is far lower than metabolic rates in the liver. However, the close proximity of these testicular and epididymal enzyme activities to developing and maturing germ cells could be of particular importance for toxicity.

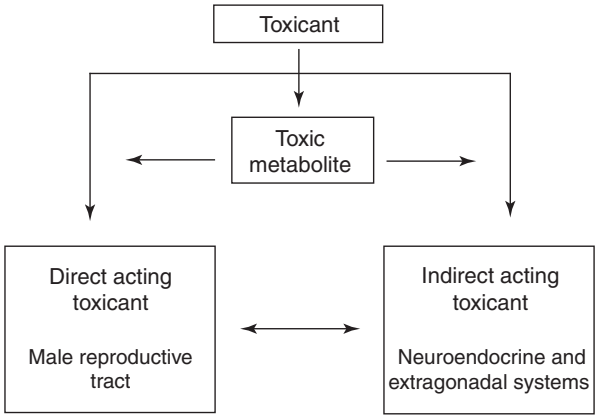

Figure 6 Classification of male reproductive toxicants as direct or indirect acting.

Another factor that may predict a chemical's ability to move about the body is its lipophilicity. The reproductive tract may be a potential target of toxicants that are fat-soluble. Like the blood barriers of the placenta and brain, the testis– and epididymal–blood barriers do not restrict the flow of compounds that are highly lipophilic. Importantly, a large fat pad surrounds the epididymis and efferent ductules. The proximity of the fat pad to these organs can potentially increase the exposure of these organs and maturing spermatozoa to lipophilic toxicants that tend to accumulate in adipose tissue. For example, the highly lipophilic organochlorine pesticides dieldrin

and aldrin have been detected in the male reproductive tract of animals after exposure.

Germ Cell Targets

The germ cells of the testis are continually undergoing renewal. Because of the diversity of events occurring in spermatogenesis, the germ cells of the testis have differing susceptibilities to the action of toxicants. However, because of the interrelationships of the developing germ cells to one another and to the supporting Sertoli cell, it is often difficult to discern which cell type was affected first.

Spermatogonia The rapidly dividing spermatogonia may be susceptible to toxicity induced by agents that affect cell division. For example, radiation and some anticancer drugs such as busulfan and procarbazine have been shown to cause genotoxicity in spermatogonia. These agents are used in cancer therapy because of their potential to damage rapidly dividing tumor cells. It is not surprising that they could also target rapidly dividing germ cells.

Spermatocytes Ethylene glycol monomethyl ether (EGME) was formerly used in the semiconductor industry and has been shown to elicit a relatively specific toxicity primarily to pachytene spermatocytes. It is not EGME, but its metabolite methoxyacetic acid, that is thought to be responsible for damaging spermatocytes. It has been found that the methoxyacetic acid disrupts protein kinase activities in dividing mitotic cells, and that cotreatment of seminiferous tubules with EGME and protein kinase inhibitors blocked the cytotoxic effects to spermatocytes.

Spermatids Few agents have been specifically implicated in spermatid toxicity. Exposure to methyl chloride (once used as a fumigant) caused a delayed release of mature spermatids from the testis. In addition, spermatids were present at much later stages than would be expected. Another discontinued fumigant, ethylene dibromide, also directly affects spermatids, although other germ cell types were also affected.

Spermatozoa α-Chlorohydrin directly affects spermatozoa with a resultant diminution in motility. Since this direct effect is reversible, α-chlorohydrin was once considered a candidate for male contraception. However, because irreversible toxicity was found in the epididymis, the potential for drug development of α-chlorohydrin was not explored further. The sulfonamide drug sulfasalazine may affect mature spermatozoa in the epididymis, resulting in decreased fertility. However, historical data on the toxicity of sulfasalazine were collected from patients being treated for inflammatory bowel disease. Therefore, it is possible that the spermatotoxicity and reproductive dysfunction may be a consequence of the disease state rather than the drug.

Testicular (Nongerm Cell) Targets

The testes are specialized for the development of germ cells into spermatozoa and the production of testosterone. The two major nongerm cell types supporting these functions are the Sertoli cell and the Leydig cell. Various agents that target the Leydig or Sertoli cells can disrupt spermatogenesis directly by affecting cell function or indirectly by interfering with the hormonal regulation of spermatogenesis. Because these somatic cells are integral to the processes of spermatogenesis, it is important to consider each cell type as a vulnerable target.

The Leydig Cell

Leydig cells have a central role in the synthesis and secretion of testosterone. LH, released from the anterior pituitary, stimulates production of testosterone by the Leydig cell. Many chemicals alter Leydig cell function and some can cause Leydig cell death. For example, ethylene dimethanesulfonate, an agent formerly used for cancer treatment, causes Leydig cell death with subsequent loss of testosterone biosynthesis. A chemical may also disrupt steroidogenesis by its action on the testosterone biosynthetic pathway without causing cell death. For example, δ-9-tetrahydrocannabinol (THC), the active ingredient in cannabis, causes a decline in the release of FSH and LH, resulting in decreased serum levels of testosterone in both experimental animals and humans. Besides disruption to the hypothalamic–pituitary–gonadal axis, THC and its water soluble metabolites can directly reduce cAMP-stimulated testosterone production in Leydig cells.

The Sertoli Cell

The Sertoli cell performs a pivotal role in spermatogenesis, orchestrating and nurturing the developing germ cells. The Sertoli cell has many functions. It plays a protective role through Sertoli–Sertoli cell tight junctions, which compartmentalize the developing germ cells away from the extratesticular milieu. This barrier means that the nutritive and hormonal requirements of germ cells must pass through or be generated within the Sertoli cell. The Sertoli cell cytoskeleton also performs specialized transport and support functions. Microtubule networks track through the cell, carrying a multitude of hormonal and nutritive factors essential for germ cell development. The

cytoskeleton also provides the scaffolding and physical support for the developing germ cell.

The Sertoli cell plays a key metabolic role in the processes of germ cell development. Compounds that disrupt Sertoli cell metabolism would be expected to cause testicular toxicity. For example, 1,3-dinitrobenzene and other nitroaromatic compounds cause testicular toxicity apparently by disruption of Sertoli cell function. These compounds can undergo reductive metabolism to toxic nitroso intermediates, which may be ultimately responsible for the Sertoli cell toxicity. As indicated above, microtubules play an important role in support and transport processes. Hexanedione has been studied extensively as an agent capable of altering testicular microtubules. Other compounds that disrupt microtubule assembly and Sertoli cell function include the fungicide benomyl and the antiinflammatory agent colchicine, both of which prevent the assembly of testicular tubulin into microtubules.

The Epididymis as a Target Organ

The potential for the epididymis to be a target organ may depend, in part, on it being unique from many other components of the male reproductive tract. The specialized processes involved in sperm maturation (i.e., ion and fluid regulation, protein secretion) and its distinct cell types may make the epididymis more or less vulnerable to the effects of toxic action. Several male reproductive toxicants target the epididymis and alter specific cell functions. For example, the immunosuppressive agent cyclosporine alters the number and size of specific epithelial cell types within the epididymis and affects epididymal sperm morphology. α-Chlorohydrin and its chlorolactaldehyde metabolite are thought to cause a reversible vacuolization of the tubular epithelium in the caput epididymis, lead to the formation of epididymal sperm granulomas, and increase the number of morphologically abnormal spermatozoa. Any agent that alters testosterone production may also perturb epididymal structure and function and the androgen-dependent processes of sperm maturation. Toxicity in the epididymis could be overshadowed by toxicity in the testis, especially if there are profound changes. It may be difficult to distinguish the direct effect of a toxicant on the epididymis from indirect effects that arise from testicular toxicity.

Another feature that may influence the epididymal toxicity of an agent is the presence of metabolizing enzymes within the epididymis. The basal cells of the epididymis contain alcohol dehydrogenases capable of oxidizing small alcohols (i.e., methanol and ethanol) to aldehydes (i.e., formaldehyde and acetaldehyde). Glutathione *S*-transferases, a family of isozymes that catalyze the detoxification of electrophilic compounds by conjugation with glutathione, have been localized in several epididymal cell types. Recently, cytochrome P450 2E1, which is important for the metabolism of chlorinated solvents, has been localized to portions of the epididymis and efferent ductules. While metabolizing enzymes in the epididymis could potentially play a protective role, these enzymes can also bioactivate chemicals, producing intermediates that in some cases are more toxic than the parent compound. Under such conditions, the presence of metabolizing enzymes within the epididymis can increase tissue toxicity.

Hypothalamic–Pituitary–Gonadal Targets

Agents that alter the central nervous system control of the hypothalamic release of GnRH have the potential to disrupt the hypothalamic–pituitary–gonadal axis. Hypothalamic release of GnRH is stimulated by α-adrenergic receptors. Therefore, agents that alter α-adrenergic function may alter GnRH release. For example, the insecticide chlordimeform may decrease GnRH release through an adrenergic mechanism. Conversely, endogenous opioids inhibit GnRH release and morphine and morphine-like drugs can suppress GnRH-mediated secretion of LH.

In recent years, scientists have suggested that certain chemicals might disrupt the endocrine system of humans and wildlife. A variety of chemicals have been found to disrupt the endocrine systems of laboratory animals. There is also compelling evidence showing that endocrine systems of some fish and wildlife species have been affected by environmental contaminants, resulting in developmental and reproductive problems. Agents that act in place of endogenous steroid hormones or disrupt receptor or enzyme action have the potential to disrupt the hypothalamic–pituitary–gonadal axis. For example, DDT, chlorodecone (Kepone), and polychlorinated biphenyls (PCBs) are classified as endocrine disruptors. Exposure of wildlife to PCBs has been associated with feminization and decreased levels of testosterone in males. The mechanism of endocrine disruption may be a change in the binding of natural hormones to their receptors or the increased binding of environmental chemicals to the hormone receptors, both of which might result in an inappropriate hormonal response. In addition, these agents might interfere with steroid biosynthesis by altering the function of enzymes along the hypothalamic–pituitary–gonadal axis. In the male, blocking the action of testosterone can result in an inappropriate release of GnRH, LH, FSH, and disruption of gonadal function.

Examples of Male Reproductive Toxicants

The following describes the male reproductive toxicity of the pesticides dibromochloropropane and carbendazim, and finasteride, a drug used for male-pattern baldness and prostate enlargement. While our knowledge is still evolving, these examples show ways in which animal and human data combine to give a broad understanding of the mechanisms underlying male reproductive tract disruption. More complete listings of male reproductive toxicants are available from a variety of sources, including the State of California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (see Relevant Website).

Dibromochloropropane (DBCP)

In 1977, a group of men working as pesticide formulators and applicators in Central California noticed that few of them had recently fathered children. These men worked with DBCP, a brominated organochlorine nematocide first produced in the 1950s. Significant fertility problems came to light when the full cohort of DBCP production workers was studied. The epidemiological studies showed a significant relationship between DBCP and failed spermatogenesis. All men were exposed by inhalation and/or dermal routes, and the severity of effects increased with the length of exposure to DBCP. Testicular biopsies showed that the seminiferous tubules, and hence the site of spermatogenesis, were severely affected. Several samples showed that the cells that make up the seminiferous tubules were atrophic and contained few or no sperm cells. Several workers recovered many years after their exposure ended, and went on to father healthy children. However, some men who were initially characterized as having no sperm (azoospermic) never recovered. Ongoing epidemiological studies in California's Central Valley are considering the association between DBCP exposure via contaminated drinking water and adverse reproductive outcomes in surrounding communities; however, no clear associations have been found.

As yet, DBCP has no clearly defined mechanism of male reproductive toxicity. However, data suggest that spermatogenesis is an important target of DCBP. There is some evidence that cytochrome P450-dependent metabolism may be less important that glutathione conjugation in the toxicity of DBCP. Studies have shown that depletion of testicular glutathione with diethylmaleate protects against testicular cell damage caused by DBCP. There is also evidence that an episulfonium ion is formed following glutathione conjugation, and that this reactive intermediate may covalently bind and damage DNA. Interestingly, there are species differences in the sensitivity to the toxic effects of DBCP. The rat and the guinea pig appear to be the most sensitive test species, while the mouse and hamster are much less sensitive to the adverse effects of DBCP. If mouse or hamster data were relied upon in setting safety standards, we would have failed to properly characterize the reproductive hazards associated with human exposure to DBCP.

Benomyl

Benomyl is a benzimidazole fungicide that has been used effectively for many years on a variety of food crops and ornamental plants. Benomyl is metabolized primarily into carbendazim. It is suggested that benomyl and its metabolite carbendazim cause testicular toxicity by the same mechanism by which they act as fungicides. Benomyl and carbendazim inhibit the assembly of microtubules in fungi while leaving plant microtubules unharmed. However, they also disrupt the assembly of Sertoli cell microtubules in the testis. Microtubule organization, as mentioned before, is important to maintain the function of the Sertoli cells. Interestingly, Sertoli cell microtubules are most sensitive to the microtubule disruption caused by the fungicides, as neither microtubules involved in cell division or neuronal transport are affected at dose levels that disrupt Sertoli cell microtubules.

Early events in benomyl toxicity include the sloughing of germ cells into the seminiferous tubule lumen. This is followed by the disappearance of microtubules within the Sertoli cell. There are also reports that benomyl metabolism may affect the morphology of developing spermatids. At higher dosages, benomyl may increase fluid reabsorption in the efferent ductules and cause sloughing of ductule epithelial cells. The resulting ductule occlusion blocks passage of sperm from the testis to the epididymis. If the occlusion is severe enough, it can result in a rapid increase in testicular backpressure, leading to swelling of the testis, and, ultimately, seminiferous tubular atrophy and infertility. Both benomyl and carbendazim cause adverse effects in the testis. However, it appears that carbendazim produces more severe, longer-term damage, suggesting that the carbendazim metabolite is responsible for the adverse effects seen after benomyl exposure.

Finasteride

Finasteride is a synthetic polycyclic steroid prescribed for the systemic treatment of male pattern baldness. It is also promising in the treatment of

prostate enlargement. Finasteride selectively inhibits type II 5α-reductase that catalyzes the formation of dihydrotestosterone (DHT) from testosterone. There are two distinct isozymes of 5α-reductase, but type II is primarily expressed in the epididymis, prostate, and seminal vesicles. This enzyme is also found in the hair follicles. Over time, hair follicles produce thinner, finer hairs, and eventually senesce due to the action of DHT. Finasteride works by inhibiting DHT formation, thereby reducing serum and scalp DHT levels and slowing the progression of androgenic alopecia.

Several critical organs within the reproductive tract maintain their function because of DHT, including the epididymis, which forms DHT from testosterone by 5α-reductase. Studies in rats have shown that oral administration of finasteride causes an ~30% decrease in fertility, decreased fecundity, and related decreases in the seminal vesicle and prostate weight. The latter may actually be a beneficial effect from finasteride treatment, in that there is evidence that finasteride can help reduce prostate enlargement. In men undergoing treatment with finasteride, there are reports of slight but nonsignificant alterations in circulating levels of LH and FSH and increased circulating levels of testosterone and estradiol. In addition, men undergoing treatment with finasteride have also experienced decreased libido, erectile dysfunction, ejaculation disorders, and reductions in sperm count. However, because finasteride works by reversible competitive inhibition, several of these effects were resolved after discontinuation of therapy.

Assessing the Risks Associated with Reproductive Toxicants

Currently, there are a limited number of chemicals classified as male reproductive toxicants. As scientific, public, and regulatory interest in this field increases, the result will most certainly be an increased knowledge base of the type and number of chemicals that can adversely affect the male reproductive tract. Once a chemical is considered a potential reproductive toxicant, there are measures in place to protect humans from occupational and environmental exposure. For example, when the results from a risk assessment indicate that the potential exists for adverse reproductive effects in humans, a regulatory agency such as the US EPA may impose restrictions on the availability or uses of certain compounds. Reproductive toxicity testing is fundamental to this type of risk-based decision-making, and will hopefully lead to the development of safer chemicals and drugs.

When no human or epidemiological data exist, data from animal models can be used to predict human effects. Much of the data used for regulatory purposes is derived from animal toxicity testing. One approach is to obtain dose levels at which no-observed-adverse effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) occurs in the animal model. To determine an exposure level acceptable for the human population a safety factor or multiple safety factors (often totaling (×) 1000) can be incorporated. The US EPA has published specific guidelines for reproductive toxicity risk assessment. Toxicokinetic and metabolic data are beginning to be incorporated into risk assessments. How a chemical enters the body, in what organs it is metabolized, and how easily the parent and reactive metabolites can reach the reproductive system are all important. The transport and metabolism of a chemical is often given considerable weight in determining the risk that a chemical poses to humans, and is generally incorporated in risk models.

While the processes of reproduction in humans and animals could be expected to have broad similarities, many species differences do exist. For example, it is well documented that rodents have sperm reserves that are much greater than man. In the rat, epididymal sperm counts can be reduced by as much as 90% without a significant affect on fertility. Since rodents have such large sperm reserves, rodent breeding studies may not detect subtle changes in reproductive capacity. To complement breeding studies, information about the effect of toxicant exposure on sperm numbers, motility, and morphology would be desirable. Mechanistic insights about causative events underlying changes in sperm parameters and their relationship to fertility would improve our ability to devise sensitive and specific toxicity tests which predict those chemicals most likely to cause reproductive harm.

See also: Benomyl; Dibromochloropropane; Glycol Ethers; Proposition 65, California; Reproductive System, Female; Toxicity Testing, Reproductive.

Further Reading

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Thomas JA (1996) Toxic responses of the reproductive system. In: Klaassen CD (ed.) *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 5th edn., pp. 547–581. New York: McGraw-Hill.

Relevant Website

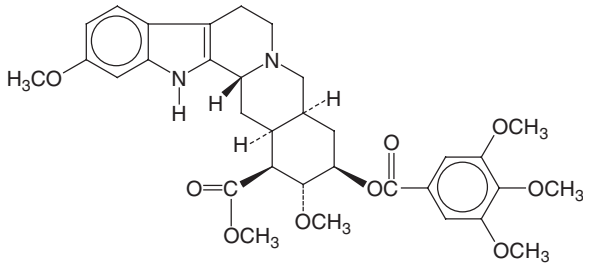
http://www.oehha.ca.gov – Proposition 65: Chemicals Known to the State of Cause Cancer or Reproductive Toxicity, State of California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Safe Drinking Water and Toxic Enforcement Act of 1986.

Reproductive Toxicity Testing *See* Toxicity Testing, Reproductive.

Reserpine

Elizabeth J Scharman

- Chemical Name: Reserpine
- Chemical Abstracts Services Registry Number: CAS 50-55-5
- Synonyms: Reserpinum; 3,4,5-Trimethoxybenzoyl methyl reserpate; (3β, 16β, 17α, 18β, 20α)-11, 17-Dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy] yohimban-16-carboxylic acid methyl ester; Serpasil®
- Chemical/Pharmaceutical/Other Class: Rauwolfia alkaloid hypotensive agent
- Chemical Formula: $C_{33}H_{40}N_2O_9$
- Chemical Structure:



Uses

Reserpine has been used in the management of mild to moderate hypertension, the treatment of agitated psychotic states, as adjunctive therapy, second-line, for treating thyrotoxicosis, and to decrease the number and severity of vasospastic attacks caused by Raynaud's phenomenon and similar peripheral vascular disorders.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to reserpine. It is available in an oral dosage form either alone or in combination with a thiazide diuretic, with or without hydralazine.

Toxicokinetics

The bioavailability of reserpine is ~40%. When given orally, peak blood levels occur within 1–3 h; however, the onset and duration of reserpine's pharmacologic effects are not related to drug concentrations in the blood or brain. The onset of action is within 3–6 days. Complete effects may be delayed by 2–3 weeks. Over 90% of the drug is metabolized in the liver to inactive metabolites. Sixty per cent of an oral dose is recovered in the feces in 4 days when given orally; 30% is recovered in the feces during the same time period when given intramuscularly. Reserpine is highly distributed into tissues, especially adipose tissue. The volume of distribution has not been determined. Reserpine crosses the blood–brain barrier, the placenta, and appears in breast milk. Protein binding is 96%. The elimination of reserpine is biphasic; the half-life is 50–100 h. The half-life may be longer in obese patients and is significantly longer if the creatinine clearance is $<10\,\text{ml}\,\text{min}^{-1}$. Pharmacodynamic effects may last from days to weeks after chronic use is stopped.

Mechanism of Toxicity

The exact mechanism of this peripheral adrenergic neuron blocking agent is not well defined. Reserpine administration results in depleted stores of norepinephrine, dopamine, and serotonin in multiple organs. The decreased peripheral resistance and cardiac output that results is manifested as a decrease in blood pressure. A central nervous system (CNS) effect may also play a role in decreasing blood

pressure. Depletion of catecholamines in the brain may explain the drug's adverse effects on the CNS.

Acute and Short-Term Toxicity (or Exposure)

Animal

Reserpine is used in horses as a tranquilizer but its use is not well studied. Toxic effects in horses are extensions of side effects and include colic and drowsiness. Dogs are extremely sensitive to the effects of reserpine. A single $10\,mg\,kg^{-1}$ dose can be fatal.

Human

Little experience exists to define a minimum toxic dose. Most of the reported cases have been in the pediatric population; amounts ingested in overdoses substantially larger than adult therapeutic doses have not resulted in fatalities. Manifestations of toxicity may include hypertension and tachycardia followed by hypotension and bradycardia. Ataxia, drowsiness, lethargy, or coma may be noted. Pupils may be pinpoint and not reactive to light. Diarrhea may occur. Extrapyramidal symptoms and cardiac dysrhythmias have been documented.

Chronic Toxicity (or Exposure)

Animal

Dogs administered reserpine daily for 1 year showed signs of CNS depression, muscle tremors, and parkinsonian symptoms. Lower doses administered to dogs resulted in prolapsed nictitating membranes, miosis, diarrhea, CNS depression, and changes in hematocrit.

Human

Side effects seen with chronic therapy may include drowsiness, depression (which can be severe), headache, dizziness, flushing, anxiety, nasal congestion, dry mouth, gastrointestinal upset, sodium and water retention, and an increase in appetite, dreaming, and nightmares. Depression is most likely with doses $>0.25\,mg\,day^{-1}$ and usually occurs 2–8 months after starting therapy.

In Vitro Toxicity Data

Mutagenicity studies in Ames Salmonella, *Escherichia coli*, and rat hepatocyte assays have been negative.

Clinical Management

Reserpine is adsorbed by activated charcoal. Treatment is largely symptomatic and supportive. Standard supportive therapies, such as vasopressors, should be utilized as clinically indicated. Because symptoms may be delayed, observation for up to 72 h may be indicated.

See also: Charcoal; *E. coli* (*Escherichia coli*).

Further Reading

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Resistance to Toxicants

Stephen R Clough

In toxicology, the term resistance may be defined as an inherent genetic capability of an organism to oppose any adverse effects, manifest in either potency or dose, of a toxicant. Others have defined resistance as the ability of an organism to tolerate toxic doses of a substance that would be lethal to most in a normal population of the same species. It is important to distinguish the phenomenon of resistance from tolerance, which is the ability of an organism to adapt to the adverse effects of a toxicant with each successive dose of that toxicant. Resistance can also be a relative term with regard to the population or species that may oppose a toxic effect. For example, in a typical toxicology study the number of animals responding to a range of doses of a chemical usually reveals a small percentage of the population showing adverse effects in the lower dose range and a small percentage of the population showing no adverse effects in the higher dose range. The animals responding at the lower doses are typically categorized as susceptible individuals, whereas the animals showing little or no response at the higher doses are categorized as resistant individuals. Similarly, some species of bacteria are resistant to penicillin, whereas others are susceptible.

Microorganisms probably provide the best examples of the phenomenon of resistance. Although the science of toxicology generally addresses higher levels of organisms, such as fish or mammals, bacteria may serve as a good illustration of resistance to toxic effects because antibiotics, generally derived from microorganisms, evolved in nature as a form of 'toxic warfare' allowing one microorganism to gain a competitive advantage over another. Humans have taken advantage of this by developing drugs, based on the structures of these natural antibiotics, which are effective in curing infectious diseases. Bacteria may be resistant to certain antimicrobial agents because (1) the drug fails to reach its target, (2) the drug is detoxified, or (3) the intended target is changed in a way that the drug cannot affect it. Some bacteria have cell walls that will not allow a drug to cross it, thus providing resistance. Other species or strains have enzymes on or within the cell wall that are capable of inactivating the drug. The physical and/or chemical composition of the cell wall may also resist the diffusion of a drug that may be dependent on certain environmental conditions such as a certain pH or the presence of oxygen.

Because bacteria can produce hundreds to thousands of generations within a very small time frame, they can acquire resistance through natural selection, that is, a small mutation may change a cellular process to allow resistance to a drug, and the subpopulation, cloned from the cell that acquired the mutation, now has an advantage in the presence of drug treatment and can cause infection even in the presence of the drug. Bacteria may also acquire resistance through a transfer of a resistant gene to another strain or even a different species. This can occur through conjugation (direct transfer of genes through a sex pilus or bridge), transduction (transfer via a bacteriophage), or transformation (envelopments and incorporation into the bacteria of resistant-encoded DNA that is free in the environment into the bacteria).

Resistance is a phenomenon that can also be observed in the higher animals, although whether or not it applies to a specific situation is a matter of dispute (i.e., whether an animal is simply less sensitive versus more resistant). Factors that may impart resistance include age, sex, species, and/or strain; of these, species is probably the most common factor imparting resistance to a toxicant. For example, a human cannot eat acorns because of the presence of toxic alkaloids present in the meat of the nut. Squirrels, however, are resistant to the toxic effects of these alkaloids because they possess liver enzymes capable of detoxifying these natural toxins. Another example is the classic resistance of certain strains of mice to oppose the effects of cadmium on the male reproductive system. It has been known for decades that most strains of mice will show severe testicular hemorrhage, followed by necrosis and sterility, after the parenteral injection of small amounts of cadmium chloride. Some strains, however, are remarkably resistant to this toxic phenomenon, being able to endure lethal doses with little or no effect on the testis. This resistance is also seen in some species of animals that have testis that are located within the abdominal cavity.

An age-related effect of resistance to metal toxicity can be seen following exposure to lead in humans. Although adults usually have higher blood lead concentrations than children, they are apparently more resistant to the neurotoxic effects of lead poisoning than a child. This is probably due to age-related differences in neurological development, as well as the permeability of the gastrointestinal tract and the blood–brain barrier to lead. Some may argue that children are simply more susceptible to lead poisoning than adults, but the change in resistance with age deserves some attention.

See also: Immune System; Modifying Factors of Toxicity.

Further Reading

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Resource Conservation and Recovery Act, US

Mario Mangino

- Title: RCRA
- Agency: US Environmental Protection Agency (EPA)
- Year Passed: 1976
- Groups Regulated: Chemical industry

Synopsis of Law

Several statutes administered by the US EPA regulate the treatment, storage, and disposal of hazardous

waste and hazardous materials. The principal law is the Resource Conservation and Recovery Act (RCRA), enacted in 1976. The term RCRA is often used interchangeably to refer to the original statute, the implementing of the US EPA regulations, and EPA policy and guidance. RCRA regulations have a wide reaching authority affecting thousands of commercial facilities that generate or handle hazardous chemicals and chemical wastes. The goals of RCRA are: to protect human health and the environment from the hazards posed by waste disposal; to conserve energy and natural resources through waste recycling and recovery; to reduce or eliminate, as expeditiously as possible, the amount of waste generated, including hazardous waste; and to ensure that wastes are managed in a manner that is protective of human health and the environment.

To achieve these goals, RCRA established three distinct yet interrelated programs. RCRA Subtitle D, the 'solid waste program', encourages all the US states to develop comprehensive plans to manage nonhazardous industrial solid waste and municipal solid waste, establishes criteria for municipal solid waste landfills and other solid waste disposal facilities, and prohibits the open dumping of solid waste; RCRA Subtitle C, the 'hazardous waste program', establishes a system for controlling hazardous waste from the time it is generated until ultimate disposal – essentially from 'cradle to grave'; RCRA Subtitle I, the 'underground storage tank (UST) program', regulates underground tanks storing nonwaste materials, mainly gasoline and other petroleum products. (Although RCRA creates the framework for the proper management of hazardous waste and nonhazardous solid waste, it does not address the problems of hazardous waste found at inactive or abandoned sites or those resulting from spills that require emergency response. These problems are addressed by a different act, the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly called Superfund, which was enacted in 1980.)

Under Subtitle C, RCRA established a comprehensive Federal scheme for identifying and managing hazardous waste. Directed to promulgate criteria for identifying hazardous wastes, the US EPA has specified these criteria as ignitability, corrosivity, reactivity, and toxicity. The agency has identified acceptable protocols for determining these characteristics and established a list of chemical substances whose presence will make a waste hazardous.

RCRA directs the US EPA to regulate the activities of generators, transporters, and those who treat, store, or dispose of hazardous wastes. Standards applicable to generators, transporters, and handlers of hazardous wastes must 'protect human health and the environment'. The US EPA's regulations applicable to generators and transporters establish a manifest system that is designed to create a paper trail for every shipment of waste, from generator to the final destination, to ensure proper authority over persons who own or operate hazardous waste treatment, storage, or disposal facilities. Pursuant to RCRA, the US EPA issued regulations prescribing methods of treating, storing, and disposing of waste; governing the location, design, and construction of facilities; mandating contingency plans to minimize negative impacts from such facilities; setting qualifications for ownership, training, and financial responsibility; and requiring permits for all such facilities. The permits cover a myriad of different operations including wastewater treatment plants, solvent recyclers, hazardous waste landfills, and hazardous waste combustors (e.g., incinerators, cement kilns, boilers, industrial furnaces). In addition, the Subtitle C program contains provisions that allow the US EPA to authorize state governments to implement and enforce the hazardous waste regulatory program. State programs must be at least as stringent as the federal program. Because the state authorization process normally takes place in a piecemeal fashion, a specific state may be authorized to implement and enforce some RCRA regulations and not others. Currently, the US EPA has authorized 48 states to implement their own regulatory programs in place of all or substantial portions of the RCRA federal hazardous waste program.

RCRA has been amended several times since 1976, and continues to evolve as Congress revises it to reflect changing waste management needs and concerns. The Act was amended significantly in 1984, by the Hazardous and Solid Waste Amendments (HSWA), which expanded the scope and requirements of RCRA. HSWA was created largely in response to citizen concerns that the historical methods of hazardous waste disposal, particularly land disposal, were not safe as a long-term solution. The Congress also revised RCRA in 1992 by passing the Federal Facility Compliance Act, which strengthened the US EPA's authority to enforce RCRA at federal facilities. In addition, the Land Disposal Program Flexibility Act of 1996 amended RCRA to provide regulatory flexibility for the land disposal of certain wastes. Of these amendments, HSWA is the most significant because it directed EPA to establish the Land Disposal Restrictions (LDR) program and the Corrective Action program.

The LDR program requires that protective treatment standards must be met before hazardous waste is land disposed. As soon as a waste is generated, it is

subject to three LDR prohibitions: (1) The Disposal Prohibition – before a hazardous waste can be land disposed, treatment standards designed for that specific waste material must be met. An operator may meet such standards by either treating hazardous chemical constituents in the waste to meet required treatment levels by any available method other than dilution, or treating hazardous waste using a treatment technology specified by EPA. Once the waste is treated with the technology required under LDR, it can be land disposed. (2) The Dilution Prohibition – waste must be properly treated and not simply diluted in concentration by adding large amounts of water, soil, or nonhazardous waste. (3) The Storage Prohibition – waste must be treated and cannot be stored indefinitely. This prevents generators and treatment facilities from storing hazardous waste for long periods merely to avoid treatment. Waste may be stored only for the purpose of accumulating quantities necessary to facilitate proper recovery, treatment, or disposal.

The Corrective Action program requires corrective action (e.g., removal, stabilization, engineering controls) for all releases of hazardous waste or chemical constituents from any solid waste management unit at a facility seeking a permit under Subtitle C, regardless of the time at which the waste was placed in the unit. If such corrective action cannot be completed prior to issuance of the permit, the permit must contain a schedule for completion of the corrective action and provisions for financial assurance. In addition, corrective action can be required through the US EPA or state 'enforcement order' if an agency finds evidence that chemical constituent releases occurred from a waste management unit that is not identified under an existing RCRA permit. When releases of hazardous constituents are documented, the corrective requirements could apply to several environmental media, including soil, groundwater, surface water, and sediments. The facility must also take corrective action measures beyond facility boundaries to protect human health and the environment when necessary.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Hazardous Waste; Toxic Substances Control Act, US.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency.

Respiratory Tract

Donald E Gardner and Daniel T Kirkpatrick

Introduction

The route by which a chemical enters the body is a major factor in determining whether a substance is toxic. More than 100 years ago it was noted that the air we exhaled was less dusty than the air we inhaled, demonstrating that airborne substances were removed from the inhaled air and deposited in the respiratory tract. When toxic chemicals are inhaled and deposited on sensitive tissues, normal respiratory functions required to maintain the morphological and physiological viability of the respiratory system may be significantly impaired, increasing an individual's risk of disease. With each breath, our body is potentially exposed to numerous gases, vapors, and airborne viable and nonviable particles that could adversely affect the vital function of this system. The lung is a most vulnerable target organ since it has nearly four times the total surface area interfacing with the environment as does the total combined surface area of the gastrointestinal tract and the skin. Because of this large surface area ($70\,m^2$), inhalation becomes a major route for entry into the body of toxic substances from occupational and environmental exposure. It has been calculated that at rest, the average adult breathes $\sim 15\,kg$ of air each day. This is significantly more than the daily intake of food and water, which is ~ 1.5 and $2.0\,kg\,day^{-1}$, respectively. Breathing is a function that must be continuous on a minute-by-minute basis, whereas extended intervals without exposure occur between periods of water and food intake. In addition, the dose of polluted air reaching the respiratory tract is dependent on the state of exercise with minute ventilation varying by up to a factor of 30 between sleep and exercise.

To maintain its primary function as an organ of gas exchange, the mammalian respiratory system must be able to defend itself from constant assault of hazardous agents that enter the body by this route of exposure. When these normal pulmonary defenses are compromised, inhaled toxic substances have the potential for initiating or aggravating existing lung disease. The health effects associated with airborne

contaminants are not limited to the respiratory tract. This route of exposure may also be the portal of entry for substances that can then be translocated from the respiratory tract to systemic sites. Because the blood leaving the lung is rapidly distributed to all parts of the body, deposited contaminants may be transported to the entire body. To produce an effect that is beyond the pulmonary system, it is necessary that the chemical, its metabolite(s), or a reactive product(s) be transported to some specific susceptible target site. There is also evidence that lung tissue can be damaged when toxic chemicals enter the body by other routes and are then transported by the bloodstream to the lung. For example, interperitoneal injection of butylated hydroxytoluene or ingestion of the pesticide, paraquat, produces acute lung damage.

This entry presents a discussion of the principles of respiratory toxicology including (1) an historical perspective, (2) approaches used to evaluate respiratory responses to inhaled chemicals, (3) classification of airborne chemicals, (4) concepts of dose–time relationships, (5) factors influencing toxicity of airborne substances, (6) the basic biology of the respiratory system with emphasis on those structures and functions that are involved in toxicological responses, (7) biomarkers of pulmonary effects, (8) toxicological response associated with inhaled chemicals, and (9) assessing the human risk of airborne chemicals.

Historical Perspective: Respiratory Morbidity and Mortality

The consequences of breathing contaminated air have long been known. The public awareness of and concern for the nature and degree of health and welfare risk associated with exposure to airborne chemicals have varied considerably over history. Concern for air pollution may have begun with human's first use of fire for heating and cooking and was accelerated following the wide use of coal as an energy source. As early as the thirteenth century the public began to complain of impaired visibility, soiling, odor, and health effects associated with coal smoke. As a consequence of the need for more energy to support the industrial revolution, a number of serious air pollution episodes began to occur. In 1930, pollution in the Meuse Valley of Belgium reached levels sufficient to cause over 60 deaths and hundreds of illnesses. In 1952, the high levels of air pollution in London combined with fog, resulting in an atmosphere that caused over 4000 deaths. Other serious air pollution problems occurring in Donora, Pennsylvania, in 1948, in Tokyo in 1970, and in New York in 1953 and 1963, brought public attention to the hazards associated with uncontrolled emissions. These most serious life-threatening episodes usually were of an acute nature and produced the most serious effects among the old, infirm, and those with respiratory disease. Meterologic conditions (inversions) over the polluted area typically led to an increase in mortality and morbidity.

The 1950s and 1960s were periods during which the public became increasingly aware of environmental pollution with industrial chemicals. Rachel Carson's book *Silent Spring* was a milestone in arousing public concern over environmental contaminants that produced human health effects. Increased health risk from accidental release of massive amounts of extremely hazardous substances into the environment from chemical spills, industrial explosions, fires, or accidents involving railroad cars and trucks transporting these chemicals have recently become a major concern. People living in communities surrounding these spills are at considerable risk of being exposed. The possibility of such sudden exposure at sites where hazardous substances are produced, stored, or used became very evident following the release of methyl isocyanate from a pesticide manufacturing plant in Bhopal, India, in 1984. The incident caused over 2000 deaths and $\sim$20 000 more suffered irreversible damage to their eyes and lungs. In another case in which the exposure was chronic rather than acute, levels of beryllium released from a manufacturing plant were sufficient to cause beryllium disease in people residing near the plant. These most serious incidents brought an increased public and scientific awareness of air pollution and its sources, the health and welfare effects associated with such exposure, and the need to develop sound strategies for elimination of such risk. Guidelines have now been developed by the National Research Council to be used to develop community emergency exposure levels for such extremely hazardous airborne substances.

While today's health effects due to such air pollution episodes may be less dramatic, the scientific community is greatly concerned that other natural and man-made substances may be released into the environment at very low levels that may still result in serious long-term effects. For some chemicals, a threshold level for effects might not exist. Four of the leading causes of death (cancer, pneumonia and flu, chronic obstructive pulmonary disease (COPD), and emphysema) involve the respiratory system and may be related to exposure to airborne chemicals. It has been estimated that at least 11%, and possibly as much as 21%, of the lung cancers may be attributed to air pollution. Air monitoring studies have revealed a large number of contaminants, including carcinogens such as benzene, vinyl chloride, and chloroform. Such airborne pollutants may be associated

with the occurrence of a higher incidence of lung cancer in urban populations. In the United States, as in most other developed countries, regulations have been established to control pollutant concentrations in outdoor air and in the workplace. New pollutants are regularly introduced into the environment and identifying and understanding the association between such contaminants and any resulting disease states remains a challenge for the toxicologist.

Exposure to airborne contaminants is not only limited to the outdoor air or to the working environment, but may also occur in the home. In addition voluntary exposures may occur through personal activities such as cigarette smoking and certain hobbies. The health effects associated with the indoor environment, where an individual may spend as much as 90% of his/her time, have become a major concern. Families of workers have developed documented illness associated with contact with clothing that is contaminated with industrial dusts. The US Environmental Protection Agency (US EPA) has ranked the American home as fourth on its list of serious health hazards. New building materials, emissions from wood and gas stoves, heaters, furnishings, air-conditioning systems, insulation, tobacco smoke, and household products such as pesticides that are used indoors have been linked to serious health risk. Nonspecific symptoms in occupants of modern office buildings, often referred as sick building syndrome (SBS), have been widely reported but have not been clearly linked to a specific air contaminant. SBS symptoms include (1) eye, nose, and throat irritation; (2) sensation of dry mucous membranes; (3) erythema (skin irritation and redness); (4) mental fatigue and headaches; (5) high frequency of airway infections and cough; (6) hoarseness and wheezing; (7) itching and unspecific hypersensitivity; and (8) nausea and dizziness. Young children may be unusually sensitive to the toxic effects of these chemicals.

Approaches Used to Evaluate Respiratory System Response to Airborne Chemicals

Supporting data for evaluation of adverse biological responses to chemical exposure and subsequent prediction of human health risk of a particular level and pattern of exposure are generated using epidemiology, studies of controlled clinical exposures, laboratory animal toxicology, and *in vitro* studies. Each category of study has certain intrinsic advantages and limitations and, in general, a database including results from multiple study categories is required to overcome the individual shortcomings (**Table 1**).

Epidemiological studies may show an association between exposure and mortality, morbidity, or a specific disease and may allow direct inference of human risks since actual human exposure conditions, such as the presence of appropriate chemical mixtures, are involved. Examples of strong associations include lung cancer with cigarette smoking or with inhalation of asbestos or metallic compounds of arsenic, chromium, or nickel; liver tumors with occupational exposure to vinyl chloride; and leukemia with occupational exposure to benzene. Studies involving environmental exposures of the general population have the advantage of including sensitive subpopulations. For example, the elderly and individuals with preexisting cardiopulmonary disease were a sensitive cohort during the previously described London air pollution episode of 1952 and, recently, children were found to be more sensitive than adults to NO_2 emissions from gas cooking stoves. Epidemiologic studies of urban regions in the United States and Europe have demonstrated associations between ambient particulate matter (PM) levels and acute increases in cardiovascular and respiratory morbidity (e.g., increased hospital admissions, respiratory symptoms, and cardiovascular episodes) and mortality or decrements in lung function. (See section on 'Classification of Airborne Chemicals' for definitions of PM fractions.) Statistical relationships have tended to be stronger when the fine particle ($PM_{2.5}$) or ultrafine fractions of ambient PM were used as the exposure parameter or susceptible subpopulations were considered (the elderly, young and those with pre-existing pulmonary or cardiovascular disease). An important example is the Harvard Six Cities Studies, in which PM and mortality data from six eastern US cities were analyzed using complex multivariant statistical methods. The strongest associations with mortality were for the concentrations of PM_{10}, $PM_{2.5}$, and sulfate particles, but not aerosol acidity. Studies of asthmatics in the United States, Germany, and the Czech Republic produced significant associations between concentrations of fine or ultrafine particles and peak expiratory flow, respiratory symptoms and/or use of medication.

A prominent shortcoming of most such studies is limited or incomplete exposure information, including both the exact chemicals involved and the airborne concentrations. As a result, evaluation of dose–response relationships and determination of acceptable exposure limits is difficult. Even when strong associations can be demonstrated for high levels of exposure, as in the case of benzene exposure, the low statistical sensitivity of epidemiological methods makes it difficult to assess the risk to individuals with a history of long-term exposure at lower levels. Confounding variable bias is usually a significant problem since exposure histories typically

Table 1 Advantages and limitations of study approaches used to assess pulmonary response to inhaled toxicants

	Epidemiological studies	*Controlled clinical studies*	*Animal studies*
Exposure conditions	+ Realistic concentrations + Real chemical interactions − Definition of exposure difficult − Confounding agents interfere	+ Well-defined exposures − Limited to low concentrations	+ Well-defined exposures + Wide concentration range possible + Easy exposure manipulation
Exposure time frame	+ Realistic, acute to chronic	− Short-term only	+ Acute to chronic − Relevance of pattern/length of exposure is questionable
Toxicologic effects	− Limited to severe or crude effects (mortality, morbidity) − Insensitive, twofold change required to detect effect − Disease present, prevention not addressed	+ Subtle, less severe effects measurable − Only mild, reversible effects, questionable toxicological significance	+ Wide range of responses may be evaluated − Relevance of subtle effects to human is uncertain
Population characteristics	+ Measured in humans + Large population size possible + Full range of sensitive subpopulations possible	+ Measured in humans − Limited number of subjects + Possible to study sensitive subpopulations	− Extrapolation to humans + Large group size possible − Homogeneity of animal model population and environmental factors-relevance to human?
Utility	− Assessment of dose response is difficult − No information on mechanism of action − Costly and time consuming	+ Dose response may be tested (limited range) − Limited information on mechanism of action − High cost	+ Dose response may be tested over a wide range + Possible to investigate mechanism of action + Relatively lower cost

+, Advantage; −, limitation.

include multiple chemicals. This is particularly important for both cancer and nonneoplastic disease of the respiratory tract. For example, inaccurately reported cigarette smoking or work history can greatly distort findings. Such bias may be involved in the highly controversial finding of an association between environmental tobacco smoke exposures and lung cancer. Studies of worker populations in the synthetic rubber, plastic resin, and coating industries provide additional examples of confounding exposures. Synthetic rubber and thermoplastic workers may have complex exposure histories with prominent exposures to styrene, butadiene, and, in some cases, acrylonitrile. Coating industry workers typically have histories of exposure to a broad spectrum of chemicals including styrene, epoxy compounds, acrylic monomers, isocyanates, and anhydrides. Finally, exposure to wood dust may have been a confounding factor in studies reporting an association between formaldehyde exposure and sinonasal tumors.

Epidemiology also suffers from the fact that effects are generally counted when significant disease, morbidity, or mortality has occurred and, thus, protection from disease is not addressed. The use of validated biomarkers for early effects may improve the utility of these studies. As an example, studies have found a clear link between occupational beryllium exposure and the presence of a sensitization-dependent, progressive, and incurable granulomatous disease of the lung (chronic beryllium disease). Development of beryllium-dependent transformation tests for bronchoalveolar lavage and peripheral blood lymphocytes may lead to early diagnosis of sensitization and subsequent removal of workers from further exposure and/or corticosteroid treatment to limit progression of the disease.

Studies showing associations between ambient PM levels and parameters of human health illustrate many of the weaknesses of epidemiological studies. Statistical methods are inconsistent and result in high levels of uncertainty. Air pollution is typically a complex mixture of particles and gaseous materials for which concentrations are often correlated. Eliminating the effects of gaseous components to show an association for PM is difficult.

Controlled clinical studies using volunteers have most frequently been used to evaluate human effects of exposure to low levels of air pollutants, including sulfur dioxide, nitrogen dioxide, ozone, carbon monoxide, PM fractions, and acid aerosols of sulfates and nitrates. Major advantages of this approach are that humans make up the exposure population and that it is possible to closely define and control the exposure concentration. To a limited extent, sensitive subpopulations may be tested. For example, airway hyperreactivity to sulfur dioxide and sulfuric acid aerosols have been demonstrated in asthmatics (asymptomatic at the time of testing). Individuals with heart disease are especially at risk when exposed to carbon monoxide. When patients with histories of angina pectoris were exposed to low levels of carbon monoxide, they experienced reduced time to onset of chest pain as a result of insufficient oxygen supply to the heart muscle. Since the safety of the experimental subjects must be a primary concern, only short-term exposures to low concentrations that produce only mild and transient responses may be used. The effects of chronic exposures cannot be tested and the range of end points that can be assessed is often limited to pulmonary function measurements and blood clinical chemistry assays. In some university hospital settings, additional evaluation procedures are used, including examination of bronchoalveolar or nasal lavage fluids and evaluation of effects on mucociliary clearance and epithelial permeability using inhalation of radiolabeled aerosols. In general, the reversible changes that are observed following single human exposures are of uncertain clinical significance in predicting long-term effects.

Assessments of chemicals or chemical mixtures for risk to workers and/or the general population clearly require a database obtained from intact, living organisms. *In vitro* methods cannot be used to model the complex interactions and feedback processes between cells, tissues, and organ systems of a functioning mammalian organism or the complex deposition, uptake, and clearance processes of the respiratory system. Alternatively, *in vitro* studies can be very useful for screening a large number of chemicals for a specific effect, for example, genotoxicity or cytotoxicity, and for development of information on mechanism of action. In terms of risk assessment, *in vitro* study data may provide information that aids in the interpretation of the database derived from animal and human exposure.

In regulatory decisions, the primary standard *in vitro* methods are the genotoxicity assays, including the Ames test and the mouse lymphoma cell mutagenesis assay. Animal and human respiratory tract cells or tissues in culture are frequently used for screening and mechanistic studies. For example, alveolar type II cells in culture have been used to evaluate xenobiotic chemical metabolism; alveolar macrophages in culture have been used to test for cytotoxicity, macrophage activation; and the effects of exposure on macrophage function (e.g., phagocytosis and bacterial or virus inactivation), and tracheal explant cultures have been used to model the preneoplastic action of airway carcinogens.

The driving philosophy behind an aggressive strategy of toxicity testing in laboratory animals is the conviction that human beings should not have to suffer from avoidable, debilitating, or lethal chemical-induced toxicity or cancer when the effect of the chemical can be demonstrated in a test animal species. Historical examples such as benzene, asbestos, and vinyl chloride for which animal models were developed after the association of disease with exposure was demonstrated in humans, show the need for well-designed safety testing in animals. Animal studies allow maximal flexibility in choice of chemical agents, exposure concentrations and regimens, biological end points, and test species. Exposure conditions can be tightly controlled and readily manipulated and exposures can be acute, subchronic, or chronic. Studies can be designed to help elucidate the mechanism of action and the existence and basis for species differences in response. A broad range of biological responses can be evaluated, including target organ histopathology, changes in hematological and blood chemistry parameters, changes in organ system function, changes in immunological responses, effects on neurobehavioral parameters, and reproductive/developmental effects. Large chemical testing programs, such as the hazardous air pollutant (HAPs) and high production volume (HPV) testing programs in the United States frequently include studies of neurotoxic and reproductive/developmental effects following inhalation exposure since available information regarding such effects is insufficient to evaluate human risk. Of particular importance to evaluate respiratory tract toxicity are histopathology, lung function, and bronchoalveolar lavage fluid cytology and chemistry.

Many examples of the use of animal exposures to study the respiratory tract toxicity of inhaled chemicals are discussed in portions of this entry describing indicators of respiratory tract response. Examples cited here demonstrate ways in which animal studies are used to help protect human populations and guide assessment of human risk. For most chemicals that pose a potential inhalation risk to workers, there are insufficient human data to set safe occupational exposure limits. Using inorganic nickel compounds as an example, epidemiological data indicate an

increased risk for nasal and lung cancer in workers involved in nickel sulfide ore smelting and refining processes, and lung tumors have been found in rodents chronically exposed to nickel compounds. However, the current occupational threshold limit value (TLV) for soluble nickel salts has been set based on studies in which nonneoplastic lesions, including epithelial hyperplasia, inflammation, and fibrosis, have been evaluated in laboratory animals.

Animal models have been developed for chronic pulmonary effects of asbestos fibers and silica. The insolubility and cytotoxicity of the chemicals and the inability of alveolar macrophages to normally phagocytize and clear the chemicals are important features of the models of asbestos-induced fibrosis and cancer and silica-induced fibrosis. These validated animal models have been used to evaluate the potential for man-made fibers to cause cancer or other crystalline materials to cause fibrosis. Tested using this approach, exposure to glass fibers has produced both positive and negative results. Using interpleural and intraperitoneal instillation of very thin glass fibers, researchers in Germany produced tumors in rats and hamsters. In a recent article supporting the need for inhalation testing, data cited indicated that chronic whole body inhalation of glass fibers failed to induce lung cancer in rats, even at very high fiber loads in the lung. In parallel studies using chrysotile asbestos, 18.9% of the animals had tumors and about half of those were malignant or carcinomas. From these studies, the author concluded that even with levels of exposure 1000 times higher than seen in a typical exposure situation, there was no evidence of tumors. Such information indicates the importance of using a natural route of exposure when assessing the risk of inhaled substances.

Effects of long-term exposure to air pollutants are difficult to evaluate using human data since, in epidemiology, exposure history and confounding factors cannot be controlled or, in clinical studies, only short-term exposures are possible. Long-term exposure studies using laboratory animals provide information that can be used to predict human effects with several models suggesting changes that correspond to well-documented human disease states. Long-term exposure of rats to sulfur dioxide produces thickening of the tracheal mucous layer and hypertrophy of goblet cells, both features of human chronic bronchitis. Repeated sulfur dioxide exposure of rats also interferes with clearance of inert particles. Rabbits that have been exposed to sulfuric acid aerosols have a slowing of mucociliary clearance, goblet cell hyperplasia, decreased pH of intracellular mucous, decreased airway diameter, and increased airway reactivity to acetylcholine. This pattern of response is similar to the pathology observed in patients with chronic bronchitis and asthma. US EPA scientists have exposed rats to ozone using a diurnal concentration pattern (range 0.06–0.25 ppm), producing alveolar epithelial hyperplasia within 12 weeks, which resulted in a slowing of the clearance rate of asbestos after 6 weeks and functional changes indicative of a stiffer lung after 12 months. Use of such an exposure regimen considered to be realistic (for an urban area of high pollution) suggests possible relevance to human toxicity.

The use of data derived from laboratory animal exposures to assess human risk is complicated by issues concerning extrapolation from animals to humans. Differences between animal and human biochemical and pharmacokinetic processes may diminish or negate the relevance of a particular animal model. Xenobiotic metabolizing capacities and patterns of distribution of these activities within the respiratory tract may differ between species. A biochemical that is specific for male rats (and is not found in humans), α_{2u}-globular protein, appears to be required for susceptibility to renal tubular nephropathy and tumors induced by inhalation of unleaded gasoline vapors. In the respiratory tract, species differences in three-dimensional airway structure may result in differences in toxic effects. For example, the complexity and relative surface area of the nasal turbinates are very different in rodents and humans. Respiratory tract detoxification processes may also differ between species. In addition, the genetic homogeneity of laboratory animal strains and the closely controlled environmental conditions (e.g., diet) used in laboratory animal studies may affect the relevance of such studies to humans. In laboratory rodent carcinogenicity studies, high background incidence rates for certain tumors appear to be related to unrestricted food availability. There is concern that this rodent model might also have heightened susceptibility to chemically induced tumors or that resultant life-shortening for the model might interfere with detection of tumors. All of these potential differences highlight the importance of animal and *in vitro* studies to provide pharmacokinetic data and information on the mechanism of action.

Extrapolation from high-dose exposures in animals to realistic human exposure levels is also a serious concern for risk assessment. For example, metabolic and detoxification processes may be dependent on exposure level. A commonly cited example involves the increased incidence of lung tumors in rats following particulate exposure regimens that produce high lung particle loads (e.g., chronic, high level diesel exposure). Macrophage-based clearance mechanisms become overwhelmed with chronic

exposure at high concentrations and this may be associated with tumor development that would not be seen at ambient levels. The relevance of tumor incidence under these conditions to prediction of human risk has been questioned.

Classification of Airborne Chemicals

Airborne substances that are of interest to inhalation toxicology include gases, vapors, aerosols, and complex mixtures in various combinations. Aerosols may exist as mists, fogs, smokes, fumes, or dusts. Physical properties of airborne chemicals are most frequently used by the inhalation toxicologist for primary classification, with the first division based on whether the material is a gas, vapor, or aerosol (particulate material). For materials that are not highly reactive, movement and behavior in the respiratory airstream, the sites of deposition and/or uptake, the fraction retained, and the rate of interaction with airway tissues and cells are highly dependent on the physical state (see section on Factors Affecting Toxicity). In addition, this approach provides the inhalation toxicologist with valuable information on the nature of the material to which a population at risk is exposed, information that can be used to decide on the best methodology for generation of test atmospheres of a material for toxicological studies. Although classification by chemical type is used for applications such as industrial hygiene, this approach has important limitations. Materials with very different chemical structures may have similar toxic effects and materials with similar chemical properties or even a single chemical may have different toxic effects depending on whether the form inhaled is a gas or an aerosol/gas mixture.

Gases and vapors are usually grouped together since a vapor is the gaseous fraction of a chemical that is a liquid at ambient temperature and atmospheric pressure. Two properties, solubility and chemical reactivity, are particularly important determinants of the toxic actions of inhaled gases and are also used for classification. In general, the solubility of a gas/vapor is important in determining the primary sites of deposition and injury (see section on Factors Influencing Toxicity). Reactive gases interact chemically with components of cells at the site of deposition, producing direct injury that is typically followed quickly by inflammation and edema but can progress to cause a variety of toxic effects. Following deposition, nonreactive materials may undergo activation to a reactive intermediate or may interact with the cellular oxidation–reduction machinery to be activated or to deplete critical cellular reducing substances or antioxidants (e.g., NADPH and glutathione).

An aerosol may be defined as a suspension in air of solid particles, as in dusts, fumes, and smokes, or liquid droplets, as in fogs, mists, and liquid aerosols of organic materials. Dusts are formed by milling or grinding of larger masses of a parent material, while fumes and smokes are formed by combustion, sublimation, or condensation usually with a chemical change in the material. In fibrous aerosols, the solid particles have a length along one axis that is at least three times greater than that along either of the other two axes (i.e., an aspect ratio of greater than 3:1). Examples include asbestos, glass and plastic fibers, and mineral wool. Mists and fogs are typically formed by condensation of water on microscopic particles. Liquid aerosols are also produced by nebulization or spraying in the use of man-made products (e.g., pesticides and paints). Particles in aerosols may also consist of viable agents, including bacteria and viruses, as well as fungal spores and pollen. Thus, inhaled biological aerosols may produce infectious diseases such as influenza, viral and bacterial pneumonia and tuberculosis, and allergic reactions. Other properties of inhaled aerosols that are used for classification include particle size, which is the primary determinant of regional airway deposition, electrical charge, solubility, and rate of dissolution in aqueous media, and hygroscopicity.

Ambient air PM is one of the seven air pollutants regulated under the US EPA National Ambient Air Quality Standards (NAAQS) and has become a significant focus for air pollution research. PM_{10} is defined as PM with a mass median aerodynamic diameter (aerodynamic particle size) of $<10\,\mu m$ and is appropriately used to represent all ambient particles that are inhalable and, therefore, are a potential human health concern. $PM_{2.5}$ and $PM_{10–2.5}$ represent fine particles with aerodynamic diameters $\leqslant 2.5\,\mu m$ and coarse particles with aerodynamic diameters between 10 and $2.5\,\mu m$, respectively. The $PM_{2.5}$ class includes the ultrafine particle subclass made up of particles with diameters $<0.1\,\mu m$. Fine particles result from combustion of fuels and atmospheric reactions of primary pollutants, tend to have greater deposition in the pulmonary region of the airways (deep lung) and represent a greater health concern than coarse particles. $PM_{2.5}$ includes a broad range of particle types, including sulfuric and nitric acid aerosols, ammonium salts, organic and elemental carbon, metals and metal salts, and biological material. In addition, PM may include carbonaceous particles with adsorbed gaseous pollutants or constituent transition metal compounds.

Most human inhalation exposures in the workplace, home, or outdoor environment involve airborne mixtures of chemicals, which frequently

include both gaseous and particulate material. In addition, gases and vapors may be adsorbed onto the surface of aerosol particles and be carried to potential sites of injury in the lungs by the respirable particles. Three environmental mixtures of continuing concern for air pollution are photochemical smog, diesel exhaust, and environmental tobacco smoke. Smog is typically a complex mixture of gaseous combustion products, including oxides of carbon, sulfur and nitrogen, ozone, hydrocarbons, reaction products of ozone, and other pollutants and particulate aerosols of carbon and various metal oxides. Diesel exhaust and environmental tobacco smoke are also mixtures of particulate and gaseous combustion products. Of particular concern are potential carcinogens, including polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines, that may reach sensitive regions of the airways adsorbed to particles.

Exposure, Concentration, and Dose–Time Concepts

Confusion often occurs with the use of the terms 'exposure', 'concentration', and 'dose'. Dose is the amount of contaminant that is deposited or absorbed in the body of an exposed individual over a specific duration. Dose occurs as a result of exposure. Concentration is that level of contaminant present in the air potentially available to be inhaled. The atmospheric concentration of a chemical by itself does not define the total dose of a chemical delivered or the specific sites of potential injury. For a substance present in inhaled air to be toxic, a significant dose must first be removed from the inhaled air and be deposited on sensitive tissue. Knowledge of the dose to initial target sites provides a critical link between exposure and the subsequent biological response. Understanding the disposition of inhaled xenobiotics is complex and, due to space limitations, cannot be described in detail here. However, certain basic concepts need to be presented to provide information on the various factors related to exposure, dose, and response that are fundamental to understanding the potential human risk from inhaled chemical agents.

The prediction of biologic effects from inhaled pollutants is often based on the study of concentration–time. However, in inhalation toxicology, the concept of dose is most important to the understanding of the relationship between exposure concentration and the body's response. Actually dose can be apportioned into two components: internal dose and biological effective dose. Internal dose is the amount of a contaminant that is absorbed into the body over a given time. Biological effective dose is the amount of contaminant or its metabolites that has interacted with a target site over a given period of time so as to alter a physiological function. The consequence of the chemical reaching the target tissue is governed by its pharmacokinetic behavior, which includes the processes of absorption, distribution, metabolism, and elimination. The effective dose to the respiratory tract, for example, for inhaled particles, is proportional to particle retention and integrated particle retention is derived from the balance of two processes: deposition and clearance.

Because of the difficulties in determining actual dose in inhalation studies, the toxicologist must assess the extent to which the concentration (C) of a given chemical and the duration of exposure (T) interrelate to determine the magnitude of the biological response (K). Often the formula, $C \times T = K$ will be used to relate the toxic effect of certain inhaled substances to its concentration and time of exposure. This formula, referred to as Haber's law, is valid only for certain combinations of concentrations and exposure time and for only a limited number of substances. While it may be necessary to use Haber's formula in certain conditions, caution must be exercised in using the general expression, $C \times T = K$, when comparing exposure conditions that are to be used in extrapolating from effects seen in laboratory animals to humans. A more appropriate general expression for estimating $C \times T = K$ would be given by $C^a \times T^b = K$, where the exponents a and b are estimated from the data. Such a formula allows for the fact that C and T do not always contribute equally to the observed toxicity. Haber's law may be inappropriate for certain materials such as ammonia and nitrogen dioxide which are more toxic with high concentration over shorter exposure periods.

Factors Influencing Toxicity

Scientists who seek an understanding of the toxicological hazards associated with inhalation of airborne substances need a basic knowledge of the structure and normal functioning of the respiratory system. This information is essential to understanding how this system responds to inhaled substances and the possible health consequences resulting from exposure to toxic substances. Various regions of the respiratory system can be sensitive target sites for inhaled xenobiotics. However, the potential hazard associated with such exposure will depend on many interacting factors that will be discussed in the following section. In each region of the respiratory tract there are certain defense systems capable of coping with an insult. However, when these defenses are compromised or overwhelmed, the potential for disease is significantly increased. A number of factors

can significantly influence the deposition, retention, and redistribution of these inhaled substances, which in turn can directly affect the toxicity of the inhaled substance.

The factors and processes affecting the deposition of airborne substances in all regions of the respiratory tract can be broadly categorized as those related to the (1) structure of the respiratory system, (2) chemical and physical properties of the airborne substance, and (3) ventilatory functions including route of breathing (nasal, oral, and oronasal).

The morphology of the specific respiratory tract region at both the gross anatomical and the microscopic levels is an important factor. In extrapolating animal effects to the human, one must be aware that the respiratory tract structure will vary both within individuals and between species at each level of anatomy.

In all regions of the respiratory tract, the specific anatomy, dimensions, composition, flow, and thickness of the mucous or fluid lining layers and regional differences in tissue types and metabolic capabilities all have a major effect on that region's dosimetry. Dosimetry refers to estimating or measuring the amount of a compound or its metabolite or reactive product that reaches a specific target site after exposure to a given concentration.

Whenever the airborne substance is deposited on the linings of the respiratory tract, its new biological environment will react to it. For inhaled particles, a major factor that influences deposition is size. A particle's characteristics may alter its size; for example, if the particle is hygroscopic, it can be expected to grow substantially while still airborne within the respiratory tract and will be deposited based on its hydrated size. The deposition probability for particles with geometric diameter $\geqslant 0.5\,\mu m$ is governed largely by their equivalent aerodynamic diameter. Smaller particles are deposited based on their actual diameter. Since particles are generally inhaled as aerosols rather than as a single particle, the mass median aerodynamic diameter is the most appropriate parameter to use with aerosols in which the particles have actual diameter $\geqslant 0.5\,\mu m$. Aerosols containing particles with diameters less than this should be expressed in terms of diffusion diameter or geometric size. **Figure 1** shows the range of deposition variations in the various respiratory regions. Particle deposition at various sites within the respiratory tract is dependent on several mechanisms. These include impaction, sedimentation, Brownian diffusion, interception, and electrostatic precipitation (**Figure 2**). The most important are impaction, sedimentation, and diffusion. Impaction is the inertial deposition of a particle onto an airway surface. It is the main mechanism by which particles having a diameter $\geqslant 0.5\,\mu m$ are deposited in the upper respiratory tract. The probability of impaction increases with increasing air velocity, rate of breathing, and particle size and density. Sedimentation is deposition due to gravity and is an important mechanism for particles with a diameter $\geqslant 0.5\,\mu m$ that penetrate to those airways when air velocity is relatively low. Submicrometer-size particles are deposited due to a random motion owning to their bombardment by surrounding air molecules (Browning diffusion) that results in particle contact with the nearest airway wall. This is a major mechanism in airways where the airflow is very low (e.g., bronchioles and alveoli).

Physical and chemical properties of the ultrafine ($< 100\,nm$) fraction of ambient PM give this fraction the highest potential for toxic effects. These particles readily reach the alveolar region, have a high deposited fraction, are present in high numbers with high

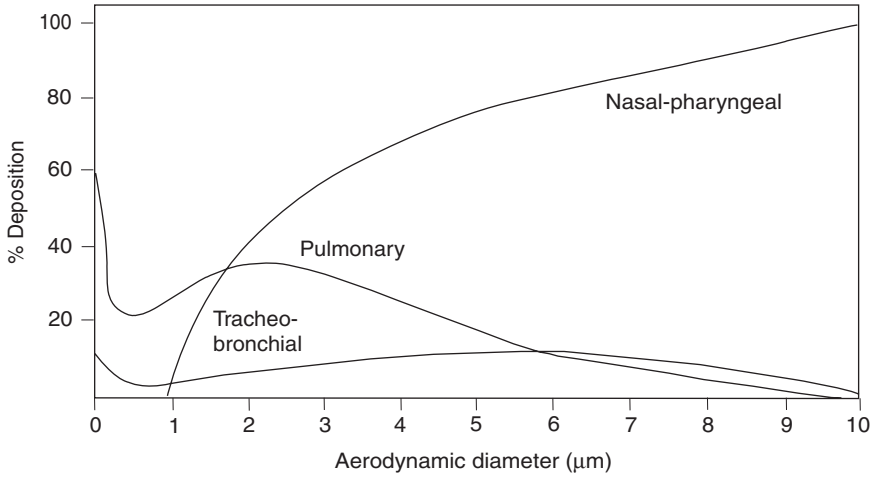

Figure 1 Regional deposition of inhaled aerosols as a function of particle size. (Reproduced from Hayes AW (ed.) (1989) *Principles and Methods of Toxicology*, 2nd edn., p. 364. New York: Raven Press.)

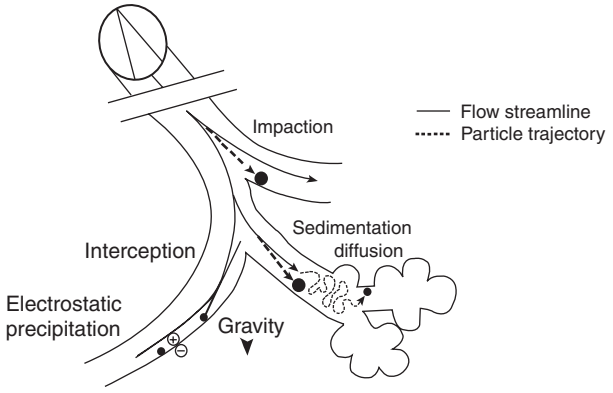

Figure 2 Mechanisms by which particles may be deposited in the respiratory tract. (Adapted from Schlesinger RB (1989) Deposition and clearance of inhaled particles. In: McClellan RO and Henderson RF (eds.) *Concepts in Inhalation Toxicology*, p. 164. New York: Hemisphere, with permission from Taylor and Francis, Inc.)

relative surface area and often include biologically active transition metal components.

Respirable fibers may be quite long and extend beyond 50 μm. However, the most important factor in the deposition of fibers, such as asbestos, is the diameter of the fiber, not the length. Fibers of small diameter (0.5 μm) will remain suspended in the airway and drift with the airflow to be deposited in the airspace.

Particle size, lengths, and configurations not only influence the site of deposition, which in turn affects the mode by which the particle is cleared, but also influence the metabolic fate of the chemical. All segments of the respiratory tract, from the nasal cavity to the periphery of the pulmonary compartment, contain enzymes that are capable of metabolizing xenobiotic compounds. These enzymes are capable of metabolizing some compounds to products that are less toxic, while other metabolites may be more toxic than the original inhaled chemical. There are significant differences in rates of metabolism at the different sites in the respiratory tract. In general, the nasal and the pulmonary regions have a higher metabolic activity than other regions. Metabolic capability is an important factor that plays a crucial role in defining species susceptibility to toxicants.

The solubility of an inhaled contaminant influences the disposition of gases, vapors, and particulates. In general, those substances that are highly water soluble, such as ammonia, formaldehyde, and hydrogen chloride, will be removed by the upper respiratory tract. Formaldehyde is concentrated in the nasal mucosa and is a nasal carcinogen in the rat. Chemicals with intermediate solubility, such as halogens and ozone, deposit in both the upper respiratory tract and the lung, while chemicals with low solubility, such as phosgene and nitrogen dioxide, deposit in and affect mainly the lung. To understand the kinetics related to solubility and to predict the toxic response one must be able to establish the solubility of the chemical not only in water but also in other media including mucus, blood, or tissue. Some particles, such as fogs, mists, and therapeutic aerosols, are aqueous droplets that rapidly merge with the mucus or liquid lining layer, greatly increasing their bioavailability for absorption.

The absorption of gases is dependent on the solubility of the gas in the blood. For example, chloroform has high solubility and is nearly completely absorbed. Respiration rate is the limiting factor. However, ethylene has low solubility and only a small percentage is absorbed – blood flow limited absorption. It is of interest to note that as a generalization, there is a pattern of relative absorption rates that extends between the different routes of exposure. This order of absorption (by rate from fastest to slowest and in degree of absorption from most to least) is intravenous $\geqslant$ inhalation $\geqslant$ intramuscular $\geqslant$ intraperitoneal $\geqslant$ subcutaneous $\geqslant$ oral intradermal $\geqslant$ other dermal. It should be remembered that because of the arrangement of the body's circulatory system, compounds inhaled and absorbed initially enter the systemic circulation without any 'first-pass' metabolism by the liver.

The depth and rate of breathing influences the dose and site of deposition of airborne substances. The process of ventilation is controlled by a variety of internal and external physical and chemical stimuli which can be affected by airborne chemicals. For many inhaled agents, deviation from normal breathing pattern serves as the earliest indicator of response. Assessing the breathing patterns, lung volumes, and lung mechanical properties are frequently used techniques in evaluating the toxicology of inhaled materials. These tests can provide useful information on whether or not pulmonary function has been impaired, the type of impairment, and the extent or magnitude of the function loss. These are not only excellent methods for assessing toxicity but are also useful in characterizing the pathogenesis of lung disease and for extrapolation of such data from animal to humans. There have been numerous studies documenting the importance of measuring respiratory parameters such as respiratory rate and tidal volume in animals exposed to inhaled toxicants. Significant alterations (depression) in these functions have been associated with exposure to methyl chloride, methylene chloride, methyl bromide, and formaldehyde. In such cases, the predicted delivered dose would have been overestimated had respiratory measurements not been recorded.

Structural Factors Influencing Toxicity

Because of the complexity of the respiratory system, it is frequently described by dividing the system into three general regions or compartments based on the anatomical structure and the corresponding physiological functions attributed to that region. **Figure 3** is a schematic showing these various compartmental areas.

Nasopharyngeal Region

The nasopharyngeal (NP) region is the most proximal region of the respiratory system and is the first potential target for airborne substances. The specific structures making up this region include the anterior nares, the turbinates, the epiglottis, the glottis, the pharynx, and the larynx. The nose is the normal portal of entry for all inhaled material. In addition to being an organ for smell, the nose has other functions including the conditioning and transporting of inhaled air and providing an effective filtering system that serves to protect the upper respiratory system against toxic chemicals and biological agents. This mechanical barrier, while being fundamentally nonspecific, can be quite effective. For example, under normal conditions ~100% of the sulfur dioxide, 20–80% of the ozone, and 73% of the nitrogen dioxide drawn through the nose are trapped in this region, preventing the pollutant from reaching the lower areas of the lung. However, under certain conditions, such as at times of high physical activity, individuals resort to mouth breathing and, as a result, the inhaled air bypasses these defenses. This significantly changes the deposition pattern of the inhaled gases or particulates and possibly their toxicity. The concentration of inhaled pollutants at the NP region can be expected to be higher than the level delivered to the lower respiratory airways and is most similar to the ambient concentration.

While the removal of airborne contaminants by the nose is effective, this action also renders this organ susceptible to toxic damage. The behavior of the inhaled substances in the NP airways and the ultimate determination of whether they are deposited or exhaled depends on numerous factors; for example, breathing patterns that influence nasal airflow rates and the chemical and physical properties of the airborne material, such as size, shape, water solubility, and reactivity. Soluble particles may, once deposited, rapidly enter the blood circulation and be transported systemically. Thus, the effective dose of toxicant delivered to the target tissue depends on factors other than the environmental concentration.

In the anterior one-third of the nose, where particles larger than 5 μm are deposited, the principal means of clearance is by blowing, sneezing, or wiping. However, following some exposures, certain

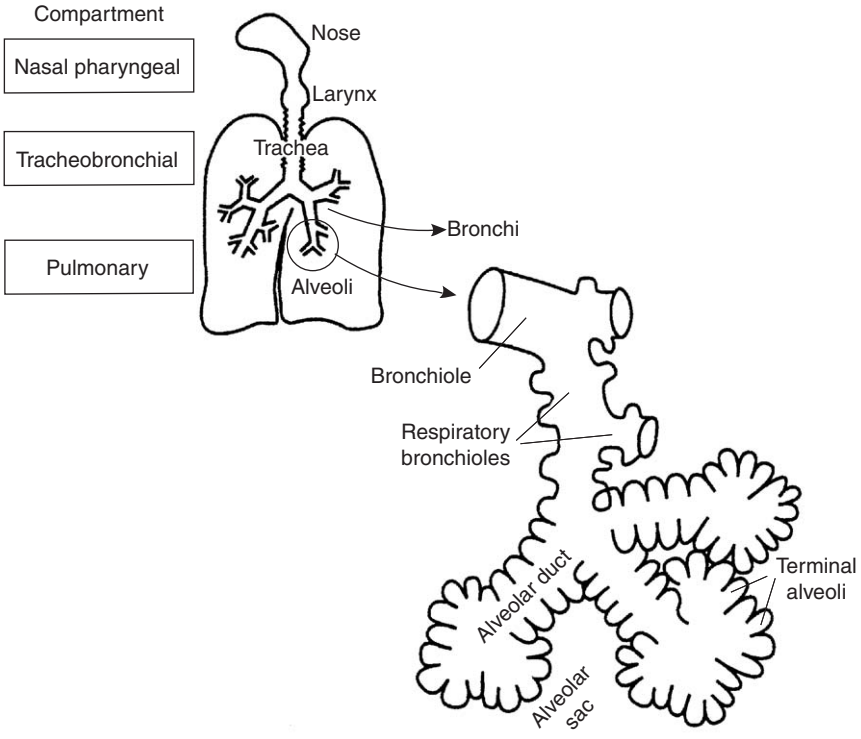

Figure 3 Compartment model of the respiratory tract. (Reproduced from Witorsch P and Spagnolo S (eds.) (1994) *Air Pollution and Lung Disease in Adults*, 1st edn., p. 22. Boca Raton, FL: CRC Press, with permission from CRC Press.)

particles may actually remain in this area for several days after deposition. Such retention patterns may be responsible for serious health effects. For example, nasal cancer identified in machinists was shown to be related to the high nasal collection efficiency for those particles above 10 μm in diameter. Such large-size particles are made airborne by various grinding and sandblasting activities. Particles approximating 3 μm in diameter are deposited in the NP region primarily through inertial impaction that occurs at airway branching. Breathing patterns involving higher air flow rates tend to increase deposition of particles as small as 1 or 2 μm in diameter. In all cases, deposition can be expected to increase with duration of breathing and depth of breathing. Deposition occurs during both inhalation and expiration. Chemical and biological agents deposited in this region may lead to inflammation (rhinitis), congestion, impairment of the sense of smell, ulceration, and cancer. To prevent a buildup or uptake of these deposited substances and possible long-term health effects, it is important that they are removed quickly. The mucus layer lining the NP epithelium plays an important role in clearance of deposited material by providing a moist, sticky surface that entraps inhaled particles and gases. Particles are then transported mouthward, due to the beating of the underlying cilia, where they are swallowed or expectorated. Various disease states, such as chronic sinusitis, bronchiolectasis, rhinitis, and cystic fibrosis, adversely alter mucociliary clearance from this region. It has been estimated that normal physical clearance from the NP region has a half-life of $\sim$4 min.

Nasal metabolism also plays a role in the response of this organ to xenobiotics. The nose contains a large number of enzymes including cytochromes P450, dehydrogenases, esterases, transferases, and hydrolases. Nasal metabolism can be responsible for both the protection of and the damage to the nose. For example, the breakdown of formaldehyde by dehydrogenases may be protective, whereas the toxicity of certain nitrosamines has been attributed to metabolic activation to toxic metabolites by nasal cytochromes P450.

Because the nose is directly exposed to a wide variety of infectious and antigenic agents, the nasal immune system also plays an important role in defending this region from such agents. Nasal secretions contain locally produced antibodies that can be monitored in both humans and test animals by nasal lavage. This is a technique that permits the detection of both immune mediators and the influx of inflammatory cells in this region, providing useful biomarkers of effects.

Tracheobronchial Region

Inhaled air together with all of the airborne substances not removed in the NP region enter the tracheobronchial (TB) region. The TB region of the respiratory system consists of the trachea and the bronchial tree down to and including the terminal bronchioles. In the mammalian TB trees, there are two forms of branching, monopodial and regular dichotomous. In most nonprimate species the branching is monopodial; that is, there are long, tapering airways with small lateral branches that come off of the main airway at an angle of $\sim 60^{\circ}$. In a human lung the branching is symmetric (dichotomous) involving the division of a tube into two daughters, each with nearly equal diameters and nearly equal angles of branching with respect to its parent tube. The major function of this TB region is to transport the inhaled air into the pulmonary region for gas exchange and to remove it during exhalation. At the entry into this region, the proximal end of the trachea is continuous with the larynx and extends distally into the thoracic cavity to the carina where it bifurcates to form two primary branches called bronchi. The tracheal airway is maintained during breathing by cartilaginous rings that prevent it from collapsing. As the bronchi continue to divide into smaller and smaller diameters there is a point where the cartilage is no longer present and the airways are composed of smooth muscle and loose connective tissue. At this region they are referred to as nonrespiratory bronchioles. There are several generations of such bronchioles. The most distal conducting and nonrespiratory airways in the respiratory tract are the terminal bronchioles. It is this distal end of the conducting airways that connects to the respiratory bronchioles.

Since the TB region contains both very large and very small conductive airways, substances of all sizes and chemical compositions can be expected to be deposited in this region. The many bifurcations in the TB region are vulnerable sites of high regional deposition. For example, the centriacinus, which is the site of the junction between the most distal conducting airways and the gas exchange area, is a common site of injury from a variety of airborne chemicals including diesel exhaust, oxidant air pollutants, and asbestos fibers. With physical exertion and mouth breathing, the beneficial defenses of the NP region are significantly reduced and a greater number of larger particles can be expected to be deposited in this region. Toxic substances deposited in this region in sufficient quantities may lead to bronchospasm, allergic reactions, congestion, bronchitis, and cancer. The more rapidly these materials are cleared, the less time for injury.

The primary clearance mechanism for this region is similar to that of the NP region; that is, by mucociliary transport to the glottis with subsequent expectoration or swallowing. The TB region is

equipped with both ciliated and secreting cells for removal of deposited material. The airway epithelium has secretory capabilities for synthesis and release of mucus, ions, and water. In certain diseases (e.g., bronchitis, cystic fibrosis, and asthma) an excess of secretions may result, causing airflow obstruction, increasing the residence time of inhaled substances, and thus increasing the dose to the airways. The ciliated cell is one of the major cell types and is probably a nonproliferative, terminally differentiated cell. The ciliated cells have on their mucosal surface ~200 cilia per cell. Their length is ~6 μm in the large airways and 5 μm in the smaller airways. Groups of cilia beat spontaneously with metachromal waves in a coordinated fashion independent of nervous control. The nonciliated cells consist of various secretory cells (mucous, clara, and serous) depending on species and a nonsecretory basal cell. The mucous and clara cells may differentiate into ciliated cells. The ciliated cells can actually retract their cilia and become a secretory cell. Because of the difference in the airway size, the clearance rate differs significantly within the region. Since smaller sized particles are deposited deeper in the airways, the clearance of such particles from the TB tree is slower when compared with those larger sized particles deposited higher up in the airways. Within the large airways, the half-time for clearance is ~0.5 h. For the intermediate airways the half-time is 2.5 h and in the finer airways the removal half-time is 5 h. Exposure to inhaled toxicants and viral and bacterial microorganisms can significantly decrease the efficiency and speed of TB clearance and hence increase the potential health risk. Certain human disease states are also associated with alteration of clearance from this region. Bronchial mucus transport may be impaired in people with bronchial carcinoma, chronic bronchitis, asthma, and acute infections.

Pulmonary Region

Ultimately, the air with its remaining contaminants reaches the pulmonary region, which is the most distal and includes the respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli. The main purpose of the alveolar architecture of the mammalian lung is to expose blood to gas over a large surface area within a comparatively small volume.

The pulmonary region includes the functional gas exchange sites of the lung. The terminal bronchioles of the TB tree branch to form the respiratory bronchioles. The major structural elements of the parenchyma of this region of the lung include the alveolar ducts, alveolar sacs, alveolar capillaries, and pulmonary lymphatics. The walls of the tubular alveolar ducts are covered with alveoli. As these branch, they exhibit increasing number of alveoli opening into their lumina. The human alveolus (which number ~300 million) is a polyhedral structure ~250–300 μm in diameter. These alveoli are thin walled and surrounded by blood capillaries for ease of gas exchange. The total thickness of this air-to-blood interface has been demonstrated by electron microscopy to range from 0.36 to 2.5 μm in the human. Gas exchange in the lung must be very efficient since the average human consumes ~2.5 l of O_2 per minute. To be transported through this air–blood barrier and reach a red blood cell, a molecule such as oxygen must penetrate this tissue at the alveolar surface, transverse the tissue barrier, enter the capillary blood at the capillary surface, move through the blood to a red blood cell, and finally penetrate the red blood cell to bind with hemoglobin. The greater the exchange surface, the more area available for oxygen to diffuse. In addition, the thinner the air–blood barrier, the smaller the resistance to the oxygen diffusion, and the more blood flow, the greater the amount of oxygen that can be bound to hemoglobin. The air–blood tissue barrier is sealed toward both blood and the air space by continuous cell layers, the blood capillary endothelial cell layer and the alveolar epithelium. The endothelial cells function to control the passage of fluid, proteins, and other blood components from the vessel lumen into the interstitium and the air spaces of the lung. These endothelial cells also function in a wide variety of specific metabolic activities important in the pulmonary processing of vasoactive substances – for example, enzyme inhibitors, receptors, and transport systems of these endothelial cells can determine the level of biogenic amines, kinin, angiotensins, and prostaglandins entering the circulation. Airborne toxic substances are capable of causing many types of injury to this gas exchange region, possibly increasing the thickness of the epithelial lining or changing the permeability and resulting in an influx of cellular and acellular fluids into the alveolar spaces. Such changes can have an adverse effect on normal gas exchange.

The most prominent cell making up the epithelial lining layer in the pulmonary region is the type I cell. It makes up more than 93% of the alveolar surface area. These squamous epithelial cells line the alveolar surface and are only ~0.1–0.3 μm or less in thickness, minimizing the barrier for gas exchange. The thicker type II cell is cuboidal and covers only ~7% of the alveolar surface area. The airway surfaces of these cells are covered with microvilli, greatly increasing the surface area. Together, these two cell types function as a permeability barrier to limit the movement of molecules between the alveolar space and the interstitium. The type II cells are the progenitors of the

type I cell and proliferate to reestablish the epithelial surface when the type I cells are injured. The type II cells also function as a source of an essential alveolar lining fluid, surfactant. A deficiency of surfactant may lead to alveolar collapse, resulting in hypoxemia and decrease in lung compliance. It is speculated that the alveolar macrophages release certain factors that may promote growth of the type II cells.

Due to its anatomic location, the alveolar epithelial surface is often directly exposed to inhaled gases and particulates. Injury to alveolar cells has been associated with a number of pulmonary toxicants, for example, silica, ozone, NO_2 herbicides, trace metals, and a number of organic vapors. It is of interest that the chemicals paraquat and diquat both cause type I cell damage but only paraquat damages type II cells, indicating that these two cells may differ in their sensitivity to chemical insults. This alveolar region is the site of several pathological lesions including centrilobular emphysema, fibrosis, and a variety of cellular injuries due to oxidant gases. Loss of or damage to alveolar tissue adversely affects the efficiency of gas exchange in this region.

The alveolar macrophages are large, nucleated cells that are found on the surface of the alveoli. These cells are not a fixed part of the alveolar epithelial wall but are mobile and possess the ability to engulf (phagocytize) and remove foreign material from the region. Macrophages, in addition to being responsible for clearing the lung of debris, play a major role in initiating and modulating the primary immune response in this area of the lung and are also effective in maintaining the sterility of the lung by killing or inactivating viable microorganisms. These cells locate the material by either random motion or are directed to the site by certain chemotactic substances. Under normal conditions the number of macrophages is estimated to be $\sim$3% of the total alveolar cells, but this number can be significantly increased with an increase in deposition of particles in the lung. These cells can ingest more than 10 times their weight in particles without any measurable loss in mobility or phagocytic ability. A number of host and environmental factors can modify the rates of pulmonary clearance by this mechanism. Individuals with chronic obstructive lung disease, viral infections, asthma, interstitial fibrosis, and inflammation, as well as individuals exposed to numerous inhaled gases and particulates, have reduced numbers and impaired function of these cells, resulting in a concomitant increased risk of pulmonary disease. Certain kinds of particles may be difficult to clear due to their particular shape. Long fibers such as asbestos may be cleared more slowly and may induce biochemical changes that can ultimately be toxic to the macrophage.

Once loaded with particles the macrophage may be cleared from this region by a number of pathways. The primary route out of the alveoli is via the mucociliary escalator. The macrophages reach the distal terminus of the mucus blanket and then are swept distally by ciliary beating within the airways. Macrophages may also migrate within the interstitium to the lymphatic system. There is also evidence that macrophages may enter the blood directly where they, together with their engulfed particles, can travel to extrapulmonary sites. If the ingested substance is toxic to the macrophage, it may be lysed while still in the alveolar region and the particle released to be taken up by another macrophage. Such cytotoxic substances may thus remain in the lung for considerable time. Clearance kinetics indicates that the successful removal of insoluble particles by macrophages consists of two phases. The first has a half-life measured in days and the second in hundreds of days. Clearance routes and kinetics are a function of lung burden, the physicochemical and toxicological properties of the material to be transported, and the health of the individual.

An actively functioning pulmonary immune system is critical for defense of the lung. Immune activity has been shown in both the conducting airways and in the lung parenchyma. All major immunoglobulins – IgA, IgG, IgM, and IgE – are present in the bronchial secretions. These are derived from local synthesis and by transudation from serum. In the parenchyma of the lung, the pulmonary macrophage participates in the generation, expression, and regulation of the immune response. These cells serve as antigen-presenting cells, as effector cells for T-cell immunity, and as regulatory cells that serve either as promoters or suppressors of pulmonary immune response. A detailed description of the mechanism of the immune response in the lung is complex and beyond the scope of this entry.

Briefly, antigenic materials deposited on pulmonary tissue initiate and stimulate the immune process. Antigens are taken up by and processed by the macrophage. Antigens in alveolar spaces that escape this phagocytic action and other clearance mechanisms may still gain access to the pulmonary interstitium, where they may be subsequently transported to nearby lymphoid tissue where immune stimulation can occur. The pulmonary macrophage presents the antigen to local lymphatic tissue that ultimately produces cell-mediated or humoral immune response. Lymphatic tissue and lymphocytes are present at or near the air–tissue interface at all levels of the respiratory tract from the nasopharynx to the alveolar spaces of the pulmonary racemus. These tissues are important in ensuring pulmonary immune response

Table 2 Examples of immunomodulation by various inhaled chemicals

Classification	*Symptoms*	*Chemical agents*
Immediate (type I) hypersensitivity	Bronchial asthma, asthmatic bronchitis, urticara, rhinitis, atopy	Beryllium, chloramine, ethylenediamine, ethylene oxide formaldehyde, isocyanates, platinum, nickel
Cytolytic (type II) hypersensitivity	Chemically induced hemolytic anemia, bone marrow depression, thrombocytopenia	Trimellitic anhydride, mercury
Arthus-immune complex (type III) hypersensitivity	Hypersensitivity pneumonitis, rheumatoid disease, sarcoidosis, vasculitis	Trimellitic anhydride, mercury
Cell-mediated (type IV) hypersensitivity	Contact dermatitis, sarcoidosis, anergy, delayed hypersensitivity	Beryllium, chromium, isocyanates, mercury, phthalic anhydride, trimellitic anhydride
Immunosuppression	Altered immune responses and host resistance following inhalation exposure	Asbestos, silica, metals, toluene, oxidant gases, tobacco smoke, benzene, toluene
Irritancy or nonimmunological	Pseudoallergic symptoms of bronchial asthma and asthmatic bronchitis	Formaldehyde, isocyanates, ethylenediamine

since they contain antigen-presenting cells and the full repertoire of antigen-reactive T and B lymphocytes needed to react with the antigen.

Cell-mediated response begins with the macrophages but is then mediated through the thymus-derived lymphocytes (T cells). These T cells regulate the immune system. T cells interact with B cells for antibody production. T cells can not only kill cells presenting antigen but can also release cytokines that modulate the immune response. Humoral immune responses are the end result of antigen interacting with marrow-derived or bursal cell-equivalent lymphocytes (B cell). B cells secrete antibodies that inactivate antigens in the body. The B-cell function is regulated by two subpopulations of T cells: helper T cells that are required for optimal production of antibody and suppressor T cells that are active in modulating the humoral response once initiated. A wide array of substances that can affect the immune response is discussed in the section on effects on pulmonary defenses. **Table 2** lists examples of immunomodulation by various inhaled chemicals.

Macrophages also release substantial amounts of diverse substances that exhibit a broad range of biological activities. Examples of such mediators include (1) interleukins, which play an important role as mediators of inflammation, are chemotactic for neutrophils, promote the differentiation of natural killer (NK) cells, and function in the maturation of helper T cells; (2) monokines, which regulate the growth and activation of other cells such as fibroblasts and endothelial cells; and (3) interferon, which represents a group of antiviral proteins that function to inhibit the intracellular replication of many viruses and the proliferation of malignant cells and promote NK cell functions. These NK cells do not play a role in the antigen-specific antibody response but are critical components of the general, nonspecific immune defenses.

From this brief description it is evident that the immune system is very complex and proper functioning depends on the interaction of several components. Each of these steps is a potential target for a toxic chemical.

Biomarkers of Pulmonary Effects

There is a growing need for development of sensitive assays that can be used in inhalation toxicology as biological markers of adverse health effects associated with pulmonary injury. A pulmonary biomarker should be able to reflect a change in a biological system that can be related to a specific effect or an exposure to a specific toxic substance. Such a marker should be an indicator of early biological response of the respiratory system, indicating alterations in cellular, biochemical, or immunological processes or functional or structural changes. An ideal biomarker of an effect should be unique to a specific disease, capable of quantitatively relating to a particular stage of the disease, reproducible, sensitive to small changes due to an exposure, and specific to a particular test substance. A number of such markers have been developed and are discussed in the 1989 National Research Council monograph, *Biological Markers of Pulmonary Toxicity*. The major focus of this section is to provide examples of biomarkers of pulmonary response and to discuss how these indicators can help to improve our understanding of the respiratory system in normal and disease states.

Markers of physiological effects can be useful in identifying early changes in respiratory functions of the lung due to inhaled material. Biomarkers are available to measure lung mechanical properties, ventilation, expiratory flow, intrapulmonary gas distribution, alveolar–capillary gas exchange, and perfusion. Such measurements have been used to test the effects of exposure to an array of inhaled toxicants. These assays can reveal functional manifestation of structural changes in the respiratory system, whether

these changes are transient, resulting from bronchoconstriction, inflammation, or edema, or irreversible, such as from fibrosis, emphysema, or chronic obstructive lung disease. While the current functional tests are useful in evaluating clinical lung disease, they, by themselves, are not sensitive markers of the lung injury. The lung responds to air contaminants in much the same way regardless of the specific toxic nature of the toxicant. Increased efforts are being devoted to the development of functional tests that will be better indicators of specific alteration, focusing on certain regions of the respiratory system, such as the terminal bronchioles and respiratory bronchioles. Alterations at these sites, which are likely targets of several types of airborne toxicants, would be indicative of small airway disease.

Airway hyperreactivity is a useful marker that can be assessed by measuring increased bronchoconstriction (i.e., contraction of airway smooth muscle). Hyperreactivity can be measured in the pollutant-exposed subject by following exposure with a challenge of (1) a variety of pharmacological chemicals such as methacholine, carbachol, histamine; (2) a physical stimuli such as cold or dry air or exercise; or (3) air pollutants such as sulfur dioxide. An exposed individual may develop bronchoconstriction after inhaling a lower concentration of a provoking agent than is needed to cause a similar degree of change in the airway in a normal subject. Airway hyperreactivity has proved to be useful in assessing airway responsiveness following exposure to a low concentration of pollutants, such as ozone, nitrogen dioxide, sulfuric acid aerosols, allergens, and certain irritant gases. Evidence indicates that these tests constitute markers that are useful for detecting risk of accelerated loss of lung function, which may be indicative of the development of chronic lung disease.

Since the mechanisms of clearance of particles from the respiratory tract are similar in most mammals, markers measuring alterations in the effectiveness of these defenses have been used to predict respiratory tract disease and for extrapolating animal data to humans. Both human and animal studies have shown that exposure to certain gases and PM may significantly alter bronchial mucociliary clearance rate. Relating these changes to specific health effects remains speculative. However, there is a predisposition to respiratory infections (e.g., chronic bronchitis), with retarded clearance from the airways. By increasing the residence time of carcinogens, altered mucociliary clearance may also be a factor in the development of bronchial cancer.

More noninvasive markers are needed for assessing early alterations in lung structure. The cells of the nasal, tracheal, and bronchial regions can be relatively accessible with bronchoscopy, brushing, and biopsy. In the TB region, markers of differentiated phenotypes are useful in providing a direct indication of cellular damage. Mucous glycoproteins are markers for alterations of mucous cells and specific histochemical staining techniques are used to characterize secretory cell products. A low-molecular-weight protein appears to be a specific marker of clara cell secretory products. The presence of dynein appears to be a good marker for structural changes in the ciliated cells. Other biochemical and immunological markers (keratin expression, transglutaminase, and sulfotransferase) may reflect differentiation of TB epithelial cells. Measuring such changes could provide early indication of pathologic changes in easily accessible airway lining cells.

Inhalation of many types of toxic chemicals can cause selective injury to the more proximal portions of the gas exchange region of the lung. Markers focusing on specific focal patterns of injury that may be caused by different pollutants would be useful. For the alveolar region, specific markers of injury or disease are even less developed. Because an early response to cell injury from airborne pollutants is likely to result in proliferation of airway cells (e.g., epithelial, fibroblast, and macrophages) markers have been used to measure these responses following exposure to cigarette smoke, asbestos fibers, and oxidant gases. Using morphometrics, the total number of cells in the lung and the distribution of cells among the various types of alveolar cells have been determined in both humans and animals. Such techniques, although difficult to apply, offer promise for the development of sensitive markers of early structural changes.

Cellular and biochemical markers have been widely used to detect changes in the acellular and cellular content of nasal, bronchial, or bronchoalveolar lavages. The response of these regions to several inhaled substances, such as ozone, nitrogen dioxide, ambient PM, fibrogenic material, and several trace metals, can be measured by examining the lavage fluid to assess any variation from normal. Indicators being used include the presence of blood neutrophils and mast cells (markers of permeability changes and influx of inflammatory cells), influx of eosinophils and basophils (indicators of allergic reaction), serum protein (marker of increased permeability of alveolar–capillary barrier), lactate dehydrogenase (marker of cytotoxicity), and lysosomal enzymes (markers of activation or lysis of macrophages). Other markers of effect have also been measured in lavage fluid, including growth factor, interleukins, arachidonate metabolites, and increase in prostaglandins. There is still a need to develop reliable markers to detect specific cell responses at the molecular level. Molecular type

markers to characterize changes in DNA and RNA, changes in DNA sequences, and changes in the extent or pattern of gene expression would be of most value since they might aid the scientist in identifying individual susceptibility to pulmonary disease.

Toxicological Response to Inhaled Chemicals

The toxicology literature is extensive in the documentation of many human and animal studies that have been conducted to detect the health effects associated with airborne pollutants. Causal relationships between exposure to an agent and various forms of toxicity can be readily established using controlled animal studies. Animal studies suffer the obvious drawback of requiring extrapolation of these responses to humans. Such studies are nevertheless commonly used to identify toxic properties of chemical agents because of the shortcomings of human epidemiological and clinical studies. Unfortunately, many of the available animal studies were designed and conducted to study the responses at relatively high concentrations, making it difficult to directly relate such responses to the relatively low levels found in the ambient environment. It is not the intent of this entry to provide a complete overview of all treatment-induced effects associated with inhaling airborne chemicals; instead, this entry provides a toxicity profile that is focused on an array of health effects caused by exposure and relates these observed responses to the potential health risk of the population.

The objective of any toxicological study is to determine the relationship between an appropriate exposure and a measured biological response in a susceptible species by the most valid and sensitive technique. Current research continues to focus on identifying that portion of the respiratory system that experiences the greatest effect of an inhaled toxicant. However, the point of maximal injury can be expected to vary with the nature of the toxicant, its concentration and duration of the exposure, the effectiveness of local defense mechanisms, and the inherent susceptibility to damage of the cells at risk. The size and complexity of the respiratory system in humans and animals provides numerous sites of potential damage caused by inhaled gases and particulates. To fully understand the toxicological consequences resulting from exposures, testing procedures must apply multiple end points and varying durations of exposure and must evaluate a variety of target tissues for injury. In evaluating the significance of the available database, the toxicologist needs to understand the various relationships that may exist between the measured response and the exposure. With multiple end points of toxicity and a given concentration of the agent, an infinite number of linear and curvilinear relationships could be generated. The dose–effect relationship may be steep, indicating that a small increase in concentration (dosage) elicits a dramatic increase in the effect, or the slope may be shallow, indicative of only a small change in the altered state accompanying a large increase in the dosage of the toxicant. Frequently, a toxicant may elicit effects on more than one target organ, giving rise to dose–effect curves of different configurations. Such information is vital in predicting dose–response relationships.

Irritation and Inflammatory Response

Although irritation often suggests a relatively mild, transient effect, respiratory tract irritation is one of the most significant airway responses for the inhalation toxicologist. Irritation is frequently the first observable adverse response of the airways following exposure to airborne materials. In addition, irritation often occurs at relatively low concentrations that may be realistic for typical human exposures. The number of chemicals and common mixtures that are known to be respiratory irritants is far greater than that for any other respiratory system response. Many common components of air pollution, including sulfur dioxide, H_2SO_4, nitrogen dioxide, ozone, and various metal oxides, are respiratory irritants. This, along with the fact that many people have personal experience with such irritation, for example, by household ammonia, cigarette smoke, or photochemical smog, produces a high public awareness and concern for the irritancy of airborne chemicals. Agents that produce an irritant response on contact with airway tissues are termed direct irritants. The responses may be mild to severe, with typical concentration dependence, and they are usually reversible. Many organic vapors that are potential workplace hazards are sufficiently reactive to produce irritant injury to the airways. Examples include aldehydes (e.g., acrolein), epoxy compounds (e.g., ethylene oxide and propylene oxide), halogenated alkanes (e.g., bromotrichloromethane), aliphatic isocyanates (e.g., methyl isocyanate), and aliphatic nitro compounds (e.g., tetranitromethane). Many of these chemicals are also capable of producing respiratory tract neoplasms in laboratory animals. Respiratory irritancy is the most frequently used basis for setting occupational exposure limits, such as American Conference of Governmental Industrial Hygienists TLVs.

The mouse respiratory depression model of Alarie, which is described in more detail in the section on physiological assessment, provides a lung function-based

system for classifying and describing the relative potency of respiratory tract irritants. Upper respiratory tract irritants, the 'sensory' irritants of the Alarie model, are usually water-soluble chemicals, such as formaldehyde, ammonia, sulfur dioxide, and acrolein. The early effects produced by such chemicals, including burning sensations of the eyes and upper airways and the cough and bronchoconstriction caused by irritation of conducting airways including the larynx, as well as the decreased respiratory frequency in mice, are neurally mediated reflex responses. Irritant receptors in the conducting airways also respond to mediators, such as histamine, serotonin, and prostaglandins, and produce bronchoconstriction via a reflex increase in vagal efferent activity. Some human populations, such as asthmatics and the young, may be especially sensitive to the effects of upper airway irritants, responding at lower concentrations than the general population. Irritants that penetrate to the deeper regions of the lung, the pulmonary irritants of the Alarie model, are generally less water soluble or, in the case of aerosols, have small particle diameters. Examples include ozone, nitrogen dioxide, phosgene, and oxides of metals such as cadmium and beryllium. Again, the early responses – cough, chest tightness, and substernal soreness in humans, rapid, shallow breathing in rats, and respiratory depression in mice – appear to be neurally mediated reflexes.

Although the initial responses to irritants are reflexes mediated by irritant nerve endings, prolonged and/or repeated exposures result in cellular and tissue injury, edema, and inflammation. Such irritant-induced structural effects have been demonstrated for most sensory and pulmonary irritants, including chlorine, sulfuric acid, methyl isocyanate, formaldehyde, ozone, and nitrogen dioxide. It is generally believed that materials that produce primary respiratory irritation have the potential to produce long-term effects following repeated exposure. An important question concerns the potential role of the irritant response in the pathogenesis of chronic disease and cancer.

Under normal conditions, the alveolar epithelial and endothelial cell layers that make up the air–blood barrier control the passage of fluids and cells between the air spaces of the lung and the interstitium. Damage to this delicate barrier can cause an inflammatory response and the impairment of lung function. Changes in the permeability of the alveolar–capillary barrier lead to an infusion of proteinaceous serous fluid (edema) and blood cells (neutrophils, macrophages, and eosinophils). This influx of cells usually peaks within the first 3–7 days of the inflammatory response. If the inflammation is sustained it is usually accompanied by a specific immune response mediated by pulmonary lymphocytes.

This is the normal reaction and may be the lung's first response against the insult. However, after entering the lung, inflammatory cells can actually enhance the effect of the original insult and may be causally related to certain chronic lung diseases. These cells respond to injury by producing a number of potent chemicals, such as cytokines, chemotactic factors, prostaglandins, lysosomal enzymes, active oxygen radical species, and leukotaxines. Involvement of oxygen radicals has been hypothesized for a number of pulmonary diseases related to exposures to numerous agents, including asbestos, paraquat, cigarette smoke, ozone, nitrogen dioxide, and ionizing radiation. In normal circumstances, the generation of oxidants by defense cells is essential for effective host defense against invading microorganisms. If the inhaled substance causes subsequent lysis of these cells, these highly active cellular products would be released into the lung where they could act directly on the pulmonary tissue. Macrophages, for example, release proteolytic enzymes that can degrade intercellular components of lung connective tissue and also interact with certain constituents of serum such as complement. These agents may, alone or in combination, cause functional impairment of epithelial cells, mesothelial cells, and fibroblasts, resulting in disease.

The analysis of isolated bronchoalveolar lavage fluid is an effective means for the detection of inflammatory responses in the lung. In both animals and humans, cell counts and cell distributions can be determined, along with measures of protein and bioactive mediators.

Asphyxiation

By definition, asphyxiants are chemicals that deprive the tissues of oxygen when inhaled. Any physiologically inert gas, including hydrogen, nitrogen, helium, and methane, that is inhaled at a high enough concentration to exclude an adequate concentration of oxygen acts as a simple asphyxiant. Chemical asphyxiants such as carbon monoxide, cyanide, hydrogen sulfide, and nitrites block the use of oxygen, causing asphyxiation when inhaled along with an adequate concentration of oxygen. Carbon monoxide is an odorless and tasteless by-product of incomplete combustion of carbonaceous materials. Carbon monoxide poisoning continues to be a significant public health concern both because of its use in suicides and because of accidental poisonings caused by faulty ventilation of home-heating devices. Since the binding affinity of red blood cell hemoglobin is 200 times

greater for carbon monoxide than for oxygen, carboxyhemoglobin formed at a relatively low concentration of this gas can block oxygen transport by a large proportion of hemoglobin. Full dissociation of carbon monoxide from hemoglobin occurs following removal from the carbon monoxide-containing environment. Therefore, poisoning is not cumulative. Carbon monoxide is an air pollutant and component of cigarette smoke, and smokers, parking garage workers, and traffic policemen are repeatedly exposed at low levels. Although asphyxiation is not a concern with such exposures, transient neurobehavioral deficits may develop and there may be an increased risk to individuals with heart disease. Other chemicals, such as sodium nitrite, interfere with transport of oxygen by oxidizing the iron moiety of hemoglobin, producing methemoglobin. Cyanide does not block oxygen transport but is a classic tissue-level poison, inhibiting cytochrome oxidase and blocking energy production.

Morphological and Structural Effects

Morphological studies are often the cornerstones of toxicity experiments. Pathological evaluation of exposed tissue permits the identification and characterization of structural damage to the respiratory system. Animal studies have been effective in improving our understanding of the pathologic sequelae of chemical deposition at specific sites in the respiratory system. The difference in the structure of the respiratory system of humans and experimental animals may complicate but does not necessarily prevent qualitative extrapolation of risk to humans. Since the lesions resulting from a particular exposure can be similar in several mammalian species of test animals, it would appear likely that the biological processes responsible for the lesions in animals could also occur in humans. However, it should be understood that different exposure levels may be required to produce a similar response in humans. The concentration at which effects become evident in humans can be influenced by a number of factors such as preexisting disease, dietary factors, combination with other pollutants, and the presence of other stresses.

A wide variety of morphological changes have been associated with inhalation of airborne contaminants. Both acute and chronic exposures directly affect the structural integrity of the respiratory system. Acute studies are conducted primarily to define the intrinsic toxicity of the chemical, to identify the target organs, and provide information for the design and selection of doses for long-term studies.

The epithelium of the conducting airways represents a tissue that is uniquely sensitive to a number of inhaled toxicants and that shows early histopathological damage when injured. Such injury in turn often elicits a variety of acute inflammatory responses. The ciliated cells, which are distributed throughout much of the length of the conducting airways, often exhibit morphological damage causing ciliary dysfunction, slowing of transport rate, and excessive mucus production. It appears that these ciliated conducting airway cells are the most sensitive to direct-acting toxicants and that cells with the most secretory capacity are less sensitive (i.e., mucous and clara cells). Cilia may be reduced in length or diameter and exhibit reduced density, and the cells may exhibit a variety of cytoplasmic changes including dilated endoplasmic reticulum, swollen mitochondria, and condensed nuclei. Tests for clearance of marker substances have been used to demonstrate that morphological effects on the cilia can result in a significant reduction in mucociliary clearance. Cigarette smoke, sulfur dioxide, alcohol, H_2SO_4, ozone, nitrogen dioxide, trace metals, and certain bacterial infections are toxic to the cilia and lead to impairment of mucociliary clearance. Individuals with bronchial carcinomas, cystic fibrosis, chronic bronchitis, and certain infectious diseases, such as influenza, atypical pneumonia, and tuberculosis, have impairment of lung clearance. Disruption or impairment of this defense system may result in greater accumulation of and potential injury by various airborne substances and increase the susceptibility to bacterial and viral infections. Continued chemical exposure can cause necrosis and the subsequent sloughing off of ciliated epithelial cells. The epithelial tissue may be repaired by the proliferation of the secretory cells. In areas of repair, the nonciliated cells often appear to be more numerous. In this type of injury, the repair process is initiated soon after the test animals are removed from the exposure atmosphere.

The respiratory alveolar epithelial response to toxic injury can be rapid, resulting in necrosis and subsequently sloughing of the sensitive type I cells. This type of response is seen with exposure to such toxicants as ozone, nitrogen dioxide, and butylated hydroxytoluene. This injury stimulates the proliferation of the more resistant type II cells. This proliferative response typically peaks at $\sim$48 h after onset of the initial injury to the type I cells. The increase in number of type II cells can be expected to alter the diffusion capacity of the pulmonary region through populating this membrane with these thicker cells.

Following lung injury, recovery depends on prompt and orderly repair. The type and extent of the injury determine whether cell replication results in the restoration of the normal structure or in

abnormal remodeling that may lead to profound anatomic distortion due to an exuberant fibroproliferation response. Reepithelization of any damaged respiratory area is critical to maintenance of normal lung function. For example, shortly after an injury, the alveolar surface may be denuded with only type II cells remaining. These type II cells begin to replicate, resulting in the repopulation of alveolar basement membrane. Eventually, the replacement cells flatten and begin to acquire the morphological features of the type I cells as the air–lung interface is reconstituted. However, it is also possible that in this repair process, a rapid migration of fibroblasts into the damaged area may occur. When this happens, these cells begin to replicate and deposit connective tissue. This obliterates the air space architecture, resulting in alveolar fibrosis. Pulmonary fibrosis results in decrease in diffusion capacity and a decrease in lung volume and compliance. Inhaled agents causing such fibrosis in humans include silica, asbestos, organic dust, cadmium fumes, paraquat, and some infectious microorganisms. The proliferation of epithelial cells, fibroblasts, and other lung cells following exposure can be measured *in vivo* and is useful in studying the pathogenesis of pulmonary disease. Cell proliferation assays are designed to quantify the relative rates of cell division within such target tissues using specialized immunohistochemical staining techniques to detect proliferating cells.

Alterations in capillary permeability are often associated with structural injury to endothelial cells. The endothelial defects are less evident at low concentrations but include cell swelling and disruption of the basement membrane. The difference in the extent of epithelial and endothelial damage can be explained by the different repair potential of these two lining layers rather than the dissimilar reaction to the injury. The pulmonary endothelium is susceptible to injury by oxygen-based free radicals. It is especially sensitive to the effects of high oxygen tension. Numerous studies have shown that such lung oxygen damage is the result of a direct toxic effect through intracellularly generated O_2 intermediates and not solely by the recruited polymorphonuclear cells. Paraquat, nitrofurantoin, cyclophosphamide, and bleomycin are among substances known to injure endothelial cells.

Three-dimensional reconstruction of cells and tissues is now being used to study subtle changes in intracellular organelles and cell-to-cell relationships that are affected by exposure. Developing such techniques has required advances in computer processing power to supply the memory and appropriate algorithms necessary to make this process technically feasible. Together with time-lapse photography and high-voltage electron microscopy, computer-time reconstructions can be used to study the effects of chemicals on cell function and cell regulation.

The main types of noncarcinogenic response of lung cells to chronic exposure are hyperplasia, hypertrophy, and metaplasia. In human studies, it is difficult to identify the chemical(s) causing a chronic pulmonary disease that is associated with morphological alterations due to the long latency period involved. In many cases, the disease symptoms may fail to be evident until after 20 or more years of exposure. Chronic lung disease can be conveniently classified into three broad groups: restrictive lung disease, chronic obstructive lung disease, and cancer.

Both restrictive and obstructive lung disease are associated with serious impairment of the flow of gases into the gas exchange regions of the lung. Pulmonary function tests are used to distinguish between these two diseases. Chronic COPD includes three major types – asthma, chronic bronchitis, and emphysema. Existing chronic bronchitis and asthma result in greater susceptibility to the effects of air pollutants, including SO_2, acid aerosols, and other PM_{10} components. Forced peak expiratory flow is reduced by greater bronchoconstriction and respiratory symptoms increase during episodes of high ambient PM_{10}. In humans, a clear distinction between emphysema and bronchitis is not possible. Most patients who have chronic bronchitis also have emphysema. The resulting gas trapping and persistent slowing of airflow make expiration difficult. The bronchial wall thickness may be 50–100% greater than normal. Individuals with COPD can be recognized by their difficulty in performing more than light to moderate exercise and nonuniform distribution of ventilation. They frequently have associated cardiovascular disease, chronic cough, and recurrent expectoration.

Chronic bronchitis is a major health problem that is associated with long-term cigarette smoking, dusty environments such as grain elevators and coal mines, trace metal exposure (vanadium, arsenic, and iron oxide), phosgene exposure, and the exposure to ambient air heavily polluted with sulfur oxides and combustion products. Chronic bronchitis is clinically evident as excessive bronchial mucus production. Histological examination of human bronchial airways shows hypertrophy of mucus glands in the large bronchi; chronic inflammatory changes, including cellular infiltration and an accumulation of fibroblasts and connective tissue; edema; and possibly increases in smooth muscle in the airways. In the early stages, these effects are potentially reversible, but in advanced stages they are irreversible. Additional features of chronic bronchitis include inflammation of

the mucous membranes of the bronchial airways and a reduction in the number of ciliated cells together with increased secretions having abnormal physicochemical properties. These effects ultimately result in grossly impaired mucociliary transport. Cough aids as the clearance mechanism for excess mucus.

Asthma is defined clinically by recurrent episodes of airway obstruction that reverse either spontaneously or with bronchodilator therapy. The airway obstruction is accompanied by increase in airway resistance due to bronchospasm, inflammation, and excessive mucus production. Bronchoconstriction, airway closure, and gas trapping may eventually lead to respiratory failure. Hyperresponsiveness is considered a hallmark of asthma, making these individuals uniquely sensitive to exposure to airborne chemicals such as isocyanates.

Emphysema differs from the other two conditions in that there is evidence of anatomic alterations of the lung characterized by abnormal, uneven, permanent enlargement of the air spaces distal to the terminal bronchioles, resulting from the destruction/distension of the alveolar walls. Airway restriction or collapse results from loss of supporting tissue that normally maintains airway patency. Such structural changes are associated with various pulmonary functional abnormalities related to loss in lung elasticity and decreases in normal diffusion capacity and forced expiratory volume. Emphysema has been associated with long-term exposure to coal dusts, cigarette smoke, osmium tetroxide, cadmium oxide, and some common atmospheric pollutants (e.g., ozone). When such chemicals are inhaled they cause cell injury and an inflammatory response. During this process, proteases, lysosomal enzymes, and oxidants are released during phagocytosis, cell injury, and cell death. To maintain structural integrity under such conditions, the lung can respond with biochemical modifiers such as antiproteases. A balance between these two responses must be maintained since these reactive substances can degrade pulmonary elastin and collagen, resulting in a destruction of the supporting structure of the alveoli. With this destruction of lung tissue, there is a subsequent loss in total lung surface area and reduction in the ability of the lung to meet gas exchange demands.

Restrictive lung disease occurs when the elastic properties of the lung are so impaired that the lung becomes stiff as in fibrotic diseases related to silicosis, pneumonia, and asbestosis. This disease condition is characterized by increased lung recoil and a decrease in lung volumes, such as vital and total lung capacity. Such restriction decreases the normal ability of the lung to expand, making inflation of the lung more difficult.

When the lung is chronically exposed to a contaminant that is not easily removed or degraded, the lung may undergo a process referred to as granuloma formation. This lesion is characterized by accumulation of mononuclear cells (macrophages, lymphocytes, and giant cells) into a relatively discrete structure. These granulomas may distort the interstitial architecture, interfering with the normal process of gas exchange, and can cause tissue damage and fibrosis that may result in permanent dysfunction and morbidity. Granulomas are dynamic structures in that freshly recruited monocytes are continually entering the lesion and replacing mature cells. Ultimately, these granulomas may resolve or become fibrotic due to the influx and proliferation of fibroblasts. These lesions may be initiated by infectious agents (mycobacteria, fungi, and viruses) and inorganic substances like beryllium. A common property of all such agents is their low biodegradability and persistence, often within the macrophage. Individuals exposed to beryllium fumes may develop acute pulmonary edema and pneumonia. While most of these individuals recover, some develop chronic granulomatous lesions appearing years after the initial exposure. Generally, such chronic disease results from prolonged exposure to low concentrations of beryllium.

Fibrotic lung disease is directly associated with chronic inflammation in which the inflammatory process in the lower respiratory tract injures the lung and modulates the proliferation of mesenchymal cells to form a fibrotic scar. Practically any chronic injury that is capable of sustaining a continued inflammation will produce some degree of interstitial fibrosis. Such effects reflect a chronic, ongoing process and may ultimately involve the entire organ. The fibrotic process involves damage to the normal alveolar architecture, which in turn leads to activation of the macrophage and release of potent growth factors. These factors cause the mesenchymal cells to proliferate and produce large amounts of collagen that then accumulates in the interstitial space. This excess collagen deposition leads to pulmonary fibrosis. It is interesting to note that in the postexposure period, the fibrotic process tends to continue. Once fibrosis occurs within a group of alveoli, it is unlikely that those alveoli will ever recover. Fibrosis-producing agents include inorganic particulates (silica, beryllium, coal dust, iron oxide, chromium, and asbestos), toxic gases (ozone, nitrogen dioxide, and high concentrations of oxygen), cigarette smoke, paraquat, and a variety of immunotoxicants.

Pulmonary Function: Physiological Assessment

Although pulmonary injury by a toxic agent and/or disease process is normally defined by morphological

Table 3 Common measurements for assessment of changes in pulmonary function

Test category	*Individual test/parameter*	*Definition/functional significance*
Ventilatory pattern	Respiration rate	Breathing frequency (breaths min^{-1})
	Tidal volume	Volume of breath
	Minute volume	Total volume inspired/expired per minute
Static lung volumes	Vital capacity	Maximum volume that can be expelled from the lungs by forced effort following maximum inspiration
	Total lung capacity	Volume of gas in lungs at end of maximum inspiration
	Residual volume (RV)	Volume of gas in lungs at end of maximum expiration
	Functional residual capacity (FRC)	Volume of gas remaining in lungs at end of tidal expiration
	Inspiratory capacity	Maximum volume of gas that can be inhaled from FRC level
	Expiratory reserve volume	Maximum volume of gas that can be expired below FRC level
Respiratory Mechanics	Air flow resistance	
	Total lung flow resistance	Flow resistance of airways
	Static lung compliance	
	Dynamic lung compliance	Stiffness (elasticity) of the lung
	Maximum forced expiratory maneuver	'Stress' test for obstruction of airflow in peripheral airways
	Forced vital capacity (FVC)	
	Forced expiratory volume	
	Peak expiratory flow rate	
	Expiratory flow at 50%, 25%, and 10% of FVC	
Distribution of ventilation	Single and multiple breath nitrogen washout	Homogeneity of ventilation in lungs – airflow obstruction and gas trapping causes greater variability of ventilation
	Closing volume	Volume difference from RV representing onset of closure of small airways; increases with air flow obstruction
Diffusion	Carbon monoxide diffusing capacity	Measurement of efficiency of alveolar gas exchange; decreases with thickening of alveolar blood–air barrier
Blood gases	Measurements of arterial pO_2, pCO_2, and pH	Evaluates adequacy of ventilation; changes typically require severe functional deficits
Pulmonary circulation	Edema: marker radioisotope movement to airways; wet/dry lung weight ratios	Evaluation for transudation of fluid into airways
	Cardiovascular pressures	Hyper- or hypotension in vascular system
	Cardiovascular volumes, flow resistance	Cardiovascular function

change, the functional manifestations of these structural effects have proved to be sensitive indicators of toxic response and lung disease. Pulmonary function testing provides a safe, noninvasive approach for clinical evaluation of the presence, type, and severity of pulmonary impairment. When workplace conditions include a risk of inhalation exposure to toxicants, preemployment and periodic, repeated lung function testing can be a key element in health effect screening and disease prevention. For many lung function tests, repeated testing is also possible in laboratory animals and progression of or recovery from disease may be evaluated in animals and individuals. Evaluation of pulmonary function in both humans and animals complements evaluation of structural changes caused by inhaled chemicals. In addition, specific lung function tests may detect significant respiratory tract effects or disease states that do not produce lasting or detectable structural changes. Finally, a large body of experimental evidence from animal models of specific pulmonary diseases and animal toxicology studies suggests that similar lung insults and/or structural changes produce similar functional effects in humans and animals. Therefore, effects observed in animals may be used to predict human pulmonary effects. **Table 3** provides examples of pulmonary function measurements that have been used for evaluation of impairment by airborne toxicants.

In interpreting pulmonary function data, several key points should be understood. (1) A specific functional effect is not diagnostic for a single structural change. For example, reduced vital capacity or compliance may be caused by several structural changes, including fibrosis, edema, hemorrhage, cellular hyperplasia, and heavy particle loading. (2) The respiratory system has a large functional reserve. Therefore, a relatively diffuse and extensive lung lesion may be required to produce a detectable effect on lung function. (3) A useful approach to pulmonary function testing is the use of a battery of measurements to develop patterns of functional change that are consistent with a particular disease such as fibrosis or emphysema. (4) Restrictive lesions (e.g., fibrosis) are characterized by a lung that is less elastic, while obstructive lesions (e.g., emphysema) are characterized by changes that obstruct the movement of air in the airways. (5) Methods used in animals often require the use of anesthesia or restraint and the potential impact of such procedures on measurements must be considered.

The lung function tests that are most frequently used in animals evaluate breathing, patterns, lung volumes, lung mechanical properties (including compliance, airway resistance, and flow rates), and diffusing capacity. Breathing pattern measures include respiratory frequency, tidal volume (the volume of a single, normal breath), and minute ventilation. A useful screening approach for evaluation of acute respiratory irritancy of inhaled chemicals has been developed by Alarie and co-workers. Using a head-only exposure system for mice, the effect of chemical exposure on respiratory frequency is monitored and, to allow comparison of irritancy between chemicals, the concentration that depresses the frequency by 50% is calculated. Two patterns of irritancy have been described. 'Sensory' or upper airway irritants are usually highly water-soluble chemicals, such as formaldehyde and ammonia, and cause a reflex depression in respiratory frequency with a slow expiratory phase. Pulmonary or peripheral airway irritants are typically less soluble chemicals, such as phosgene, ozone, and nitrogen dioxide, and cause a respiratory depression marked by pauses between breaths. In rats, pulmonary irritants produce tachypnea (rapid shallow breathing).

Measures of breathing pattern tend to be relatively insensitive to early restrictive or obstructive lesions, but with advanced chronic disease, restrictive lesions produce rapid, shallow breathing, and obstructive lesions cause slow, deeper breathing. Fibrosis produced by subchronic inhalation exposures to metal oxides of cadmium or vanadium produce tachypnea. 'Stress test' methods developed to enhance the sensitivity of these ventilatory end points in unanesthetized animals employ exercise-induced or carbon dioxide-induced hyperventilation. The latter approach, which has been used in guinea pigs and restrained rats, has been used to detect lung injury by several agents, including methyl isocyanate, sulfuric acid, quartz dust, cotton dust, and wood smoke.

Figure 4 depicts the physiologically defined lung volumes of humans and animals. Inhalation exposures that cause restrictive lung lesions, including exposure to silica, cadmium compounds, ozone, and diesel exhaust, produce decreases in total lung capacity and vital capacity. Obstructive lesions lead to breathing at higher lung inflation (due to gas trapping), with increased total lung capacity, residual volume, and functional residual capacity. Inhalation

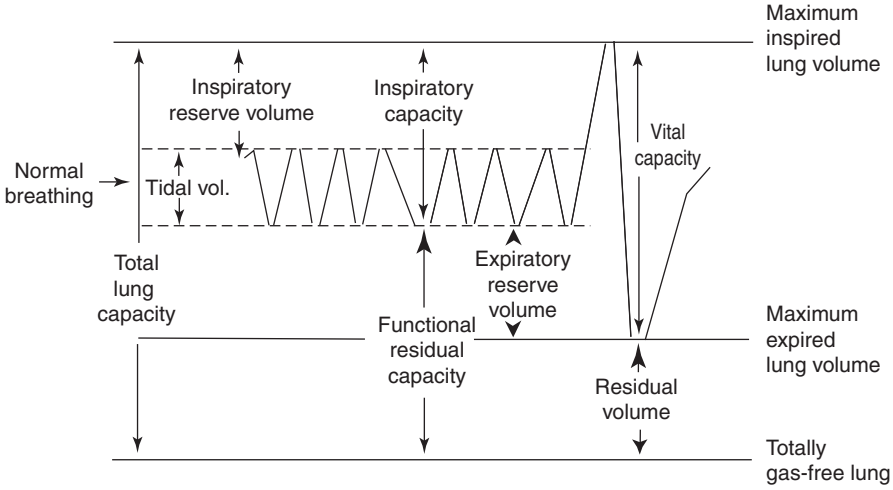

Figure 4 Capacities and lung volumes of the lung. (Adapted from McClellan RO and Henderson RF (eds.) (1989) *Concepts in Inhalation Toxicology*, p. 364. New York: Hemisphere, with permission.)

exposure to ozone and acrolein may produce this type of response.

Mechanical properties of the lung may be tested using static or dynamic testing modes. The static test can be conducted in living animals or using excised lungs and is based on information derived from the pressure–volume curve produced during lung deflation. Static compliance, a measure of lung elasticity derived from this curve, is decreased following exposure to fibrogenic agents, such as mineral dusts, or agents that cause edema, inflammation, and cellular hyperplasia, such as oxidants and irritants. It is increased following subchronic exposures to agents that cause emphysema-like lesions, such as ozone and nitrogen dioxide. Tests for dynamic lung mechanics require monitoring of flow, volume, and pressure and provide measures of total lung resistance, which is most dependent on large airway obstruction, and dynamic compliance, which is a measure of elasticity that is also sensitive to peripheral airway obstruction. Because of their sensitivity to bronchoconstrictors, guinea pigs are frequently used for evaluation of irritant effects, nonspecific airway hyperreactivity to bronchoconstrictors (e.g., histamine), and immunologically determined airway hypersensitivity. Many irritants that cause decreased dynamic compliance and/or lung resistance, such as ozone, sulfur dioxide, sulfuric acid, acrolein, and toluene diisocyanate, also cause nonspecific increased airway reactivity. Specific immunoglobulin-dependent sensitization to inhaled proteinaceous materials (e.g., ragweed pollen, animal dander, and grain dust) has been demonstrated in animals and humans using lung function measurements. Asthmatic responses with pulmonary sensitization to low-molecular-weight chemicals (haptens), such as isocyanates and anhydrides, have been observed in workers and have been modeled in guinea pigs using lung function tests. The role of the immune system in responses to many haptens remains in question since it has not been possible to consistently demonstrate the presence of an antigen-specific antibody.

The approach most commonly used to evaluate effects on distal airways in clinical and occupational medicine is the maximum forced expiratory maneuver, which allows measurement of airflows as a function of lung volume from total lung capacity to residual volume. Typically, the forced vital capacity (FVC) and the forced expiratory volume at 1 s (as a % of FVC) (FEV_1) are measured. Peak expiratory flow is a frequently used measure since simple portable devices permit self-evaluation by patients with obstructive disease. Decreased airflow rates are seen with emphysema, chronic bronchitis, and following acute exposures to bronchoconstricting irritants or agents that produce asthmatic responses following previous sensitization. When exercise is superimposed on certain pollutants, for example, with an exposure of humans to certain pollutants (e.g., ozone), decrements for FVC and FEV_1 have been observed at realistic air pollution levels (0.08–0.12 ppm). Animal models of forced expiratory maneuvers require the use of anesthetics and/or paralytic agents. The forced expiration produced in an apneic animal by applying a steady negative airway pressure following inflation to total lung capacity is measured. FVC, peak expiratory flow rate, mean mid-expiratory flow, and expiratory flow at 50%, 25%, and 10% of FVC may be derived from the maximum expiratory flow–volume curve. Using such methods, it has been demonstrated that the qualitative patterns of flow–volume curves are similar in humans and small laboratory animals when values are normalized for lung volume. As expected, decrements in airflow rates are seen in rats with elastase-induced emphysema. However, for demonstration of effects of more realistic exposures to toxicants and/or more subtle injury, the utility of measurements from forced expiratory tests in small animals may be limited.

Since the efficiency of the diffusion of O_2 and carbon dioxide at the alveoli is directly related to the primary function of the lung, tests of diffusing capacity are important components of a lung function testing battery. Diffusing capacity for carbon monoxide (DL_{CO}) is usually measured and normalized for lung volume. Diffusing capacity can be reduced as a result of structural changes in the alveolar region, as in thickening of the air–blood barrier (restrictive disease/fibrosis) or destruction of alveolar epithelium, or to an effective reduction in alveolar surface, as in obstructive disease. Thus, decrements in DL_{CO} may be difficult to interpret without correlation with other functional end points or histopathological evidence. In addition, rodents appear to compensate with hypertrophic or hyperplastic lung changes resulting in unexpectedly normal DL_{CO} values.

Pulmonary Carcinogenicity

Testing for the potential carcinogenic effects of airborne chemicals has received high priority in an effort to protect human health. Lung cancers are the most rapidly increasing cancers in Western Europe and North America. In the United States, cancers of the lung or bronchus were diagnosed in more than 170 000 people in 1993. Because of the long latency period associated with chemical carcinogenesis, it is

often difficult to identify, in the human population, the specific causative agent. Although there are a number of short-term tests for determining genotoxicity, these are generally used in screening and have not replaced the need for long-term animal testing. The basic premise of carcinogenesis research is that a substance that affects animal cells in such a way as to cause cancer is highly likely to affect human cells in the same way. Positive results from such testing are useful in that they demonstrate that a specific chemical is carcinogenic to animals under the conditions of the test. This information can be used as an indicator of the potential carcinogenic hazard to humans. Pulmonary cancers, like other forms, can be caused by both external factors (chemicals, radiation, viruses, and diet) or internal factors (hormones, inherited genes, and immune conditions) or the interaction of these factors. Evidence of pulmonary carcinogenicity in animals can be based on (1) an increase in the incidence of a specific tumor type, (2) the development of a specific tumor type earlier than seen in controls, (3) the presence of types of tumors normally not seen in control groups, and (4) an increase in multiplicity of tumors. In the absence of adequate human data, it appears reasonable and appropriate to predict that, with sufficient evidence of carcinogenicity in animals, the chemical presents a similar risk to humans. Nearly all known human carcinogens have been shown to be carcinogenic in some animal model. Recent reviews of chemical carcinogenesis, mechanisms of carcinogenesis, and pathophysiology of induced tumors provide current information on all aspects of carcinogenesis in each organ system.

Carcinogens can be divided into two general types: those that act directly and those that act indirectly. Direct-acting carcinogens are those that interact with cellular constituents such as protein, lipids, and nucleic acids. There are relatively few direct-acting carcinogens (e.g., bis(chloromethyl)ether, ethylene oxide, and nitrogen mustard).

The indirect-acting carcinogens are all substances that require metabolic activation before they interact with cellular macromolecules. These agents are often referred to as pro- or precarcinogens and include certain cyclic and polycyclic aromatic hydrocarbons (benzene and benzo(*a*)pyrene), aliphatic hydrocarbons (methylene chloride and pesticides), nitrosamines, and other chemicals such as urethane, formaldehyde, and acrylonitrile. Both the indirect- and direct-acting carcinogens can ultimately react with the genetic material of the cell. Such substances are referred to as genotoxic carcinogens, which differ from nongenotoxic or epigenetic carcinogens that do not appear to bind with the DNA of the cell but have other mechanisms of action. Agents included in this latter group include fibers, trichloracetic acid, and certain plasticizers. These chemicals do not damage DNA nor are they mutagenic in the standard short-term screening assays.

Examples of inorganic carcinogens include arsenic, asbestos, chromium, and nickel. The chemical form is important in determining the dose response. For example, beryllium sulfate is more carcinogenic than beryllium oxide. The difference may relate to the solubility of these compounds in the lung and the actual dose of the chemical to the target tissue. Although beryllium, lead, cadmium, and silica are carcinogenic in animals, the evidence in humans is less substantial. Epidemiological studies have implicated nickel as a carcinogen for cancer of the nasal cavity, lung, and possibly larynx. Carcinogenicity of chromium is associated with slightly soluble chromates (CR^{+6}), while the insoluble and very soluble salts of chromic acid or trivalent forms show little or no carcinogenicity. Mesotheliomas, tumors of the pleural lining of the lung and thoracic cavity, result from inhaled asbestos. Quartz and silica are generally not considered to be carcinogenic in humans, but some recent studies have demonstrated that these substances may cause lung cancer in rats. Multiple mechanisms may be involved in the carcinogenicity of asbestos. Asbestos is considered to be a cocarcinogen or tumor promoter. The hypothesis is that asbestos may cause generation of active oxygen species that lead to lipid peroxidation and DNA strand breakage. High levels of arsenic increase the risk of lung cancer. Trivalent arsenic appears to be the most active form and affects DNA synthesis and repair.

The induction of nasal carcinomas following inhalation exposure to several chemicals, including benzene, acetaldehyde, diallylnitrosamine, formaldehyde, hydrazine, and vinyl chloride has been reported. It is of interest that some chemicals, such as epichlorohydrin and bis(chloromethyl)ether, that produce nasal carcinomas in rats also produce lung cancer in humans. However, there is no epidemiological evidence indicating an increased incidence of nasal cancer in workers exposed to these industrial chemicals. Moreover, agents such as nitrosamines that are delivered by other routes of exposure can also induce tumors in the nose. For example, the consumption of salted fish having high concentrations of volatile nitrosamines may cause nasal tumors in experimental animals. Consumption of alcohol has also been associated with laryngeal cancers in humans.

Tumors of the nasal passages can vary from small papillomas and adenomas to large carcinomas that have the potential to metastasize to other parts of the body. While most of these neoplasms arise within the

epithelium of the nose, chemical carcinogens have also induced mesenchymal and neuroectodermal neoplasms. Nasal tumors are encountered most frequently. Laryngeal tumors are relatively rare but have been reported following exposure to smoke and acetaldehyde vapors.

Numerous chemicals have been identified as capable of causing pulmonary cancer in both animals and humans. The International Agency for Research on Cancer (IARC) states that there are adequate experimental inhalation studies in animals for several chemicals. **Table 4** lists chemicals that cause lung

Table 4 Carcinogenic agents associated with lung or pleural cancer in laboratory animals and humans

Agents causing lung tumors in animal models	*Agents associated with human lung cancer*
Organic chemicals	Industrial processes
Gases	Aluminum production
Benzene[a]	Coal gasification
Bis(chloromethyl)ether[a]	Coke production
Bromomethane (ethyl bromide)[a]	Hermatite mining, underground with exposure to radon
1,3-Butadiene[a]	Iron and steel founding
1,2-Dibromo-3-chloropropane	Painter, occupational exposure
1,2-Dibromoethane	Rubber industry
Dimethyl sulfate[a]	Chemicals for which exposure has been occupational
1,2-Epoxybutane	Asbestos[a]
Ethylene oxide[a]	Bis(chloromethyl)ether[a]
Formaldehyde[a]	Chromium compounds, hexavalent[a]
Methylene chloride	Coal tars[a]
3-Nitro-3-hexene	Coal tar pitches
1,2-Propylene oxide[a]	Mustard gas[a]
Tetrachloroethylene	Nickel and nickel compounds[a]
Tetranitromethane	Soots[a]
Urethan	Talc containing asbestiform fibers[a]
Vinyl chloride[a]	Vinyl chloride[a]
Particles	Environmental agents and cultural risk factors
Benzo(a)pyrene[a]	Erionite
Polyurethan dust	Radon and its decay products[a]
Inorganic compounds	Tobacco smoke[a]
Metallic	
Antimony compounds	
Beryllium compounds[a]	
Cadmium chloride[a]	
Chromium dioxide[a]	
Nickel compounds[a]	
Titanium compounds	
Nonmetallic	
Asbestos fibers[a]	
Zeolite fibers	
Ceramic aluminosilicate fibers	
Kelvar aramid fibers	
Silica[a]	
Oil shale dust[a]	
Quartz	
Volcanic ash	
Radionuclides	
α-emitting radionuclide particles	
β-emitting radionuclide particles	
Radon and its decay products[a]	
Complex mixture	
Cigarette smoke[a]	
Diesel engine exhaust	
Gasoline engine exhaust	
Coal tar aerosols[a]	
Artificial smog	

[a] Identified by IARC as chemical causing lung cancer in humans and respiratory cancers in animals.

Reproduced from Gardner DE, Crapo JD, and McClellan RO (eds.) (1993) *Toxicology of the Lung*. New York, NY: Raven Press.

neoplasia in laboratory animals and humans following inhalation. Studies investigating the carcinogenic potential of airborne chemicals usually focus on the morphological examination of tissue to determine the number of various types of tumors, the number of tumor-bearing animals, the number of tumors per animal, and the time of onset of the tumor. In such studies, few biochemical or physiological assessments are performed except for periodic hematological assays.

Inhalation of certain durable natural mineral fibers of amphibole asbestos, such as amosite and crocidolite, can lead to the development of inflammation, fibroproliferation, pulmonary neoplasms, and cancer of the serosal lining of the body cavities or mesothelioma. Administration of fibrous particles to laboratory animals has included, in addition to inhalation, intratracheal instillation and intracavitary implantation and instillation. Inhalation studies are difficult to conduct due to the problems associated with the generation and characterization of the fibers during all phases of the assay.

While many studies have been conducted using rats, there has been concern that the rat may not be an appropriate model for studying particulate-induced pulmonary tumorigenesis. One problem involves the finding that tumors can be induced under conditions of so-called 'pulmonary overload' even with 'inert' particles. These overload tumors may arise via mechanisms distinct from those normally associated with pulmonary carcinogenesis.

Another problem with inhalation studies conducted with rats is a lack of sensitivity for detecting the induction of fiber-inducted neoplasms, particularly mesotheliomas. Rats develop a low incidence of such tumors following exposure to amosite or crocidolite, which are known to cause tumors in humans.

In an effort to increase sensitivity, investigators have used other exposure methods including intratracheal, intrapleural, and intraperitoneal methods. There is significant concern over the induction of cancers by these nonphysiological exposure routes. Cancers induced by intracavity instillation may be due more to chronic inflammation and fibrosis from the 'bolus effect' rather than to the mechanisms of fiber-induced proliferative disease that normally occurs following the inhalation route of exposure. With intratracheal instillation, the distribution in the lung is not uniform and the resulting lesions differ from those reported in inhalation studies. It is generally agreed that long-term rodent inhalation studies provide the most definitive animal data for extrapolation to human assessment.

While many carcinogenicity studies on individual chemicals have been conducted, the study of complex mixtures presents a formidable scientific challenge for the toxicologist. One of the most difficult tasks is related to finding the primary causative agents of the effects. Examples of complex mixtures that have been studied for carcinogenicity include tobacco smoke and diesel engine emissions.

Cigarette smoke has been extensively studied due to its association with human lung cancer. However, it is not an impressive inducer of lung tumors in experimental animals. Lung tumors have been observed following long-term exposure using special strains of mice. Strains of mice such as A strain are known to have a high incidence ($\geqslant 70\%$) of spontaneous tumors. While there have been many studies of tobacco smoke using the laboratory rat, only one study showed an increase in lung tumors. Syrian hamsters exposed to whole smoke have developed laryngeal cancer but not lung tumors. This may be the result of an unusual increase in deposition of the inhaled smoke at this site in the hamster.

Several studies have shown that diesel exhaust is carcinogenic to the rat following long-term exposure. In these studies two basic types of tumors were found – bronchoalveolar tumors and squamous cell tumors – both arising from the alveolar parenchyma. Diesel exhaust represents complex mixtures of numerous organic and inorganic chemicals as well as various gases that may be toxic or carcinogenic. The complex interaction of organic hydrocarbons and carbon particles may be responsible for the tumors seen in these studies. The organic hydrocarbons present initiate the process and the particles, with adsorbed hydrocarbons, promote the initiated cells. A number of epidemiologic studies in London, the United States, and Canada did not detect significant health risk or indicated only a small increase in lung cancer incidence among workers exposed chronically to diesel exhaust. However, the animal studies together with supporting *in vitro* (e.g., mutagenic to bacteria and mammalian cells) data taken in aggregate led to the conclusion that diesel engine exhaust is a potential human carcinogen but probably represents a low level of risk.

While various types of lung cancer have been noted in humans, all known human pulmonary carcinogens are taken into the body by inhalation. It is equally important that inhaled chemicals can cause neoplasms at sites elsewhere in the body. For example, exposure to vinyl chloride, butadiene, acrylonitrile, and ethylene oxide by inhalation may produce a significant increase in cancer incidence in other organs (liver, brain, and blood) as well as in the

lung. Often, the neoplasms in other organs have a higher incidence and are a more serious health risk than the lung tumors.

Effects on Normal Pulmonary Defenses

The host defense system is one of the prime targets for which function is adversely affected by exposure to a wide range of environmental chemicals. During the air pollution episodes of this century (Meuse Valley, Belgium, London, and Donora, Pennsylvania) excess deaths were recorded from lower respiratory tract infections. The American Thoracic Society has published guidelines on what constitutes an adverse respiratory health effect. Among the five most important adverse respiratory effects are a greater incidence of lower respiratory infections. Because of the importance and the complexity of this system many *in vivo* and *in vitro* assay systems have been used to assess the integrity and biological activity of both the cellular and acellular components of the lung defenses. Any breach in these defenses should be considered as a possible indicator of an increased risk of pulmonary disease. This section is intended to familiarize the reader with the various defense system responses that have been studied, the measurements made, and the gaps in the information database. The host defense parameters which have been used most widely to examine the association between airborne toxicants and lung disease include mucociliary clearance dysfunction, functional and biochemical activity of the alveolar macrophages, immunological competency, and susceptibility to infectious disease. Increases in respiratory morbidity and impairment of lung clearance occur at ambient levels of air pollution and are associated with susceptibility to pathogenic microorganisms.

As discussed earlier, a major component of the respiratory defense system is the mucociliary clearance mechanism of the conducting airways. Mechanisms for clearance of deposited substances appear to be quite similar in most mammals, including humans. The effectiveness of this defense has been determined by measuring the rate of transport of deposited particles, the frequency of ciliary beating, the integrity of the ciliated cells, the physical–chemical properties of the mucus blanket, and the rate of mucus production and transport. Exposure to a variety of inhaled agents, such as formaldehyde, cigarette smoke, ozone, nitrogen dioxide, airborne PM, including trace metals (cadmium and nickel), and sulfuric acid, causes ciliary damage and dysfunction, such as slowing of the frequency of ciliary beating, resulting in a significant reduction in transport rates.

Impairment of alveolar macrophage function alters the ability of the cell and/or the lung to (1) maintain sterility within the gas exchange regions of the lung, (2) provide an effective clearance mechanism from the lung for inhaled particles and cellular debris phagocytized by these cells, (3) interact with lymphocytes, and (4) release immunologically active soluble mediators. To fully meet these functional responsibilities, these cells must maintain mobility, a high degree of phagocytic activity, an integrated membrane structure, and a well-developed functional enzyme system.

As the first line of defense, the resident macrophage must (1) isolate ingested particles by phagocytosis, (2) act as a vehicle for physical movement from the lung, and (3) inactivate or detoxify inhaled and ingested microbes or chemicals. The sequence of events that must take place for this defense system to function is complex and involves a number of intricate and interrelated biological functions. Chemicals can interfere with this function at many sites. Alterations in the ability of any of these functions could be expected to significantly increase the host's risk of pulmonary disease. A number of assays have been developed to identify functional changes in alveolar macrophages and have been used to demonstrate effects following exposure to agents such as carbon, diesel exhaust, PbO, nickel chloride, CO, Pb_2O_3, cigarette smoke, cotton dust, and quartz. These chemicals also promote the influx of new macrophages into the lung. These new cells may be derived from (1) an influx of interstitial macrophages, (2) proliferation of interstitial macrophages with subsequent migration of the progeny into the airspace, (3) migration of blood monocytes, or (4) division of free lung macrophages. While such an accumulation of macrophages may appear to be a necessary response to the immediate insult, a possible consequence of this mass recruitment may be the development of chronic pulmonary disease, as was discussed earlier.

Not just macrophages migrate into the lung during pulmonary insults. Polymorphonuclear leukocytes (PMN) also accumulate following exposure to such agents as diesel exhaust, ozone, nitrogen dioxide, iron oxide, cotton dust, cigarette smoke, HCl, and cadmium chloride. A large pool of PMN normally remains within the microvessels and few are found in the air spaces. However, following injury these migrate out of the vascular space. These cells migrate through the endothelium to the inflammatory site, where they attempt to phagocytose and destroy foreign material and may sometimes damage the host tissue in the process. While in the lung, powerful oxidants (oxygen radicals) and enzymes can be

released and produce tissue injury. For example, there is evidence that cigarette smoke can activate the PMNs and produce a shift in favor of proteolysis by the release of elastase from its lysosomal granules and by the generation of oxidants with the NADPH oxidase system and myeloperoxidase. Such a proteolytic imbalance may cause lung tissue destruction leading to emphysema.

Not all exposure to chemicals results in an increase in the number of available macrophages in the lung. Exposure to lead sequioxide, silica, asbestos, Pb_2O_3, cadmium fumes, MnO_2, Mn_3O_4, ozone, crysotile, amosite, cadmium oxide, acrolein, and nickel chloride actually causes a reduction in the number of these defense cells. These chemicals are cytotoxic and result in a lysis of the macrophage upon exposure. Some chemicals, such as fly ash, carbon monoxide, and certain trace metals, may affect cellular viability but not cause lysis. In some cases, the same chemical may, at different concentrations, elicit a variety of measurable and significant effects. For this reason, the most appropriate approach is to use a battery of functional assays. Parameters such as total number, stability, viability, morphology, phagocytic and bacterial function, and biochemical metabolism are useful measurements of total functional capacity of the macrophage.

The efficiency of the phagocytic and lytic system of the macrophage determines the sterility and health of the lung. Marked changes in phagocytic efficiency of these cells are found following exposure to nickel chloride, nitrogen dioxide, ozone, sulfur dioxide, CH_2O, cadmium chloride, and cigarette smoke. Depressed bactericidal function of macrophages has been reported following exposure to many of the previous chemicals and to H_2SO_4, ethanol, and lead chloride.

The activity of a number of macrophage enzymes (e.g., acid phosphatase, β-glucuronidase, β-*N*-acetylglucosaminidase, peroxidase, and lysozyme), which function to combat infectious disease, has been significantly depressed following exposure to a number of toxicants. Depression in the ability of the macrophage to produce interferon, a substance that is involved in host defense against viral infection, has been identified following exposure to ozone, irradiated auto exhaust, and nitrogen dioxide.

Alterations in the previous respiratory defenses would be expected to make the lung more vulnerable to infectious disease. Animal models have served to demonstrate the effects of airborne chemicals and to establish associations between these effects and actual increases in susceptibility to respiratory disease. These *in vivo* models combine the adverse effects of the toxicant with the added stress induced by an infectious microorganism to measure the effectiveness of the host defenses after exposure to the toxicant. The test animals, and in some cases humans, are challenged with a laboratory-induced respiratory infection (bacterial, viral, or mycoplasma) following exposure to the test chemical. If the host defense mechanisms are functioning normally, there is a rapid inactivation of inhaled organisms that have been deposited in the lung. However, if the pollutant exposure has caused a dysfunction(s) in these defenses, the microbes will proliferate rapidly and a measurable increase in pulmonary infection can be identified. While exposure to a test substance alone may not be life-threatening, association with other environmental stresses, such as infection, could prove critical in the promotion or exacerbation of a particular disease. In a recent study by New York University researchers of the effects of ambient PM on host resistance in aged rats, $PM_{2.5}$ exposure did not appear to increase the susceptibility to a post-PM exposure bacterial challenge. However, a $PM_{2.5}$ exposure of previously infected rats at levels close to the NAAQS of $65\,\mu g\,m^{-3}$ resulted in higher bacterial burdens and lower lavageable neutrophils (%) and inflammatory cytokine levels compared to filtered-air-exposed, infected controls. This may model ambient PM-induced increased mortality and morbidity in elderly humans and an association of PM with pre-existing pneumonia.

The lung is an active immunologic organ which, when exposed to toxicants, can exhibit specific local immunologic effects as well as play a role in systemic alterations, such as changes in circulating immunoglobulins. Wheezing, chest tightness, rhinitis, and asthma are symptoms of a sensitization response to a foreign material by the pulmonary immune system. Immunity can be defined as all of the physiological mechanisms that enable an individual's body to recognize materials as foreign and to neutralize, eliminate, or metabolize them without injury to its own tissue. Over the past decade, data have been accumulated to clearly substantiate cases in which lung immunoregulatory functions of humoral and/or cell-mediated immunity have been compromised by inhaled chemicals.

While the immune system is highly regulated by complex interactions, both between components of the system and between immune and nonimmune organ systems, xenobiotics can modulate the immune system effecting either 'up'- or 'down'-regulation of the process. Inhaled chemicals may provoke a variety of different responses, including (1) reduction of normal immune response – immunosuppression resulting in an increased incidence of

infection or tumors (e.g., benzene, malathion, lead, cadmium, nickel, and nitrogen dioxide); (2) overactivation of the immune system or exaggeration of the response causing hypersensitivity reactions (beryllium, mercaptans, chromates, diiocyanates); and (3) promotion of an autoimmune reaction, a pathological condition, in which there is a failure of the body to distinguish between 'self' and 'nonself' and resultant production of structural and/or functional damage to tissues and organs (e.g., mercury, cadmium, vinyl chloride, and methyl cholanthrene). Over 100 xenobiotics have been associated with such autoimmunological effects.

Inhaled substances exacerbate various immune-mediated disorders including asthma, hypersensitivity, pneumonitis, allergic rhinitis, and workers' pneumoconiosis. **Table 5** gives examples of the chemical agents that, when inhaled, are capable of eliciting an immunotoxic effect.

It is of interest that the same person may have an immediate-onset response on one occasion, a delayed-onset reaction on another, and, under other exposure conditions, exhibit a dual response starting with immediate-onset symptoms that resolve within an hour and followed several hours later by a second set of symptoms. The underlying mechanisms for such effects are not known. However, clinical and experimental evidence has indicated that this process is like many other toxicologic effects in that the response is related to concentration, duration, and frequency of exposure.

Table 5 Examples of immunotoxins

Halogenated aromatic hydrocarbons
Polychlorinated biphenyls
Polybrominated biphenyls
Dioxins
Pesticides
Organophosphates
Organochlorides
Carbamates
Polycyclic aromatic hydrocarbons
Benzo(*a*)pyrenes
Methylcholanthrene
Dimethylbenz(*a*)anthracene
Solvents
Benzene
Heavy metals
Beryllium
Manganese
Nickel
Cadmium
Platinum
Air pollutants
Ozone
Nitrogen dioxide
Cigarette smoke

Assessing the Risk of Airborne Chemicals

Risk assessment has been defined as the process whereby the most relevant biological, dose–response, and exposure data are used to identify and characterize risk. This information is used to produce a qualitative and/or quantitative estimate of the probable hazard to human health resulting from exposure to the airborne chemical(s). This process was significantly improved when the National Research Council evaluated the role of risk assessment as it relates to toxicology and developed uniform guidelines for federal agencies to use in assessing risk. They categorized the process into four steps: hazard identification, dose–response assessment, exposure assessment, and risk characterization. The process, summarized in **Figure 5**, is now widely used in evaluating the risk of inhaled chemicals. The important question in risk analysis is not simply what is the specific toxic response to some chemical, but rather what is the likelihood that the chemical may actually produce, under conditions of human exposure, significant health effects. There are multiple reasons for conducting risk assessment for airborne material. In addition to using the information for establishing federal, state, and local governmental regulations necessary to protect the worker or the general public, the risk assessment is also of value in identifying data gaps and planning future research. It also provides a useful integration of our existing knowledge database. Since risk analysis is being used to establish air standards by policymakers, the toxicologist must play a key role in this process. Because of the public health and economic implications of risk assessment, the US Congress has requested a survey and analysis of the risk literature and a federally funded program on health risk assessment.

While each assessment is unique, there are certain basic principles that are common to all. This review concentrates on those that are appropriate for airborne chemicals. In utilizing data for risk assessment, certain assumptions have to be made. For noncarcinogenic effects, it is assumed that adverse effects will not occur below a certain level of exposure, even if the exposure continues over a lifetime. This threshold assumption is supported by the fact that the toxicity of many chemicals, including airborne materials, is manifested only after the depletion of a physiological reserve and that the various host biological repair and defense capabilities can accommodate a certain degree of damage. In such cases, the objective of the toxicological risk assessment is to establish, with best scientific certainty, a threshold dose below which adverse health effects are not expected to occur. For carcinogenic effects, especially

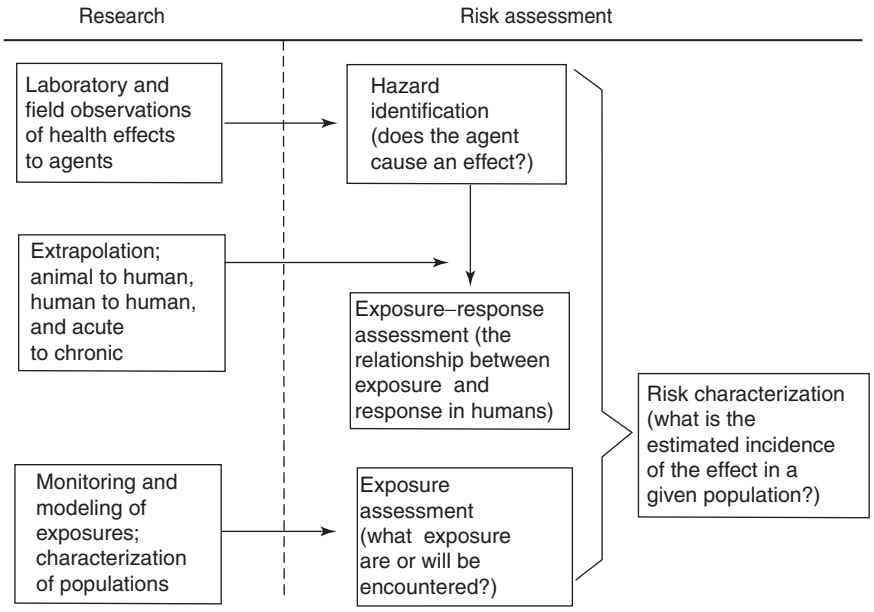

Figure 5 Summary of the process used in evaluating the risk of inhaled chemicals.

those considered to be due to genotoxic events (e.g., mutations), a threshold may not exist. Regulatory agencies consider that exposure to carcinogens pose a finite risk at all doses and that the probability of developing cancer increases with increased dose. The US EPA, in assessing the risk of carcinogens, assumes that the same total daily body burden will give the same tumor incidence, regardless of the route of exposure. This approach does not consider that some tumors at the site of contact (e.g., following inhalation) may be site specific or that the dose to a target organ may be modulated by the route of exposure. An important implication of this is that all levels of exposure, however small, add to the background risk and thus the experimental data are usually never adequate to exclude the possibility of added risk for the exposed population.

Effectively predicting the human health risk from exposure to airborne contaminants is complex and requires reliable data for hazard identification, an understanding of the dose–response relationships, and an analysis of the human exposure. Significant advances have been made in inhalation toxicology in accumulating useful data to support hazard identification and dose–response assessment. The development of sensitive analytical instrumentation permits the exposure to and monitoring of chemicals at increasingly lower concentrations. This is important not only in conducting laboratory studies but also is of value in developing techniques useful for assessing total human exposure of individuals or populations. Exposure assessment includes the identification of the contaminant, contaminant sources, environmental media of exposure, chemical and physical properties of the airborne substance, and the intensity and frequency of the exposure. Important improvements have been made in developing and validating reliable biomarkers of health effects and of exposure. Traditional analysis of body samples, such as urine, blood, exhaled air, and tissue, is useful for determination of dose. Detailed toxicokinetic studies in animals have provided information on the dose–response relationship. Physiology-based mathematical modeling of toxicokinetic parameters measured in animal studies has allowed easier interpretation and extrapolation of these animal data to human exposures.

The first step in risk assessment is to identify the potential deleterious effects of the substance. Frequently, risk assessment is conducted based on limited data. In cases in which the most relevant data are not available, attempts are often made to extrapolate from other toxicological information regardless of the route of administration or the concentration/dose used to produce a certain effect. Using such information may not ensure the protection of human health and may result in either overregulating or underregulating the risk of the chemical in question. The most reliable and scientifically defensible risk assessment analysis should be based on toxicological data collected under exposure conditions that are realistic and relevant to the human exposure; that is, by the same route of exposure, for similar duration, and in quantities that mimic the expected human exposure. All these factors are known to modulate the dose of the inhaled pollutant and/or its metabolites and hence the toxicological effects resulting

from the exposure. Currently, scientists are attempting to develop mathematical models that would permit appropriate extrapolation of high-dose studies to low-dose studies, short-term effects to lifetime health risk, and the ability to make appropriate route-to-route and species-to-species extrapolation from one compound to another and from *in vitro* to *in vivo*.

Developing reliable dose–response data from well-conducted inhalation studies is an essential step in the overall risk assessment process. With inhalation exposure, more so than with other routes of exposure, special attention needs to be paid to the difference between exposure and dose. In assessing health effects, exposure is often used as a surrogate for dose. However, when this is done important factors that may significantly modify the predicted effect (these factors include physical–chemical characteristics of the material, protective mechanisms, metabolism, and biological characteristics of the subject) are ignored. Defining the appropriate dose resulting from a certain exposure becomes a more difficult task when the exposure involves complex mixtures of airborne material such as automobile emissions, cigarette smoke, and atmospheric pollutants.

Being able to determine whether people exposed to airborne chemicals are at significant risk and the magnitude of the risk requires meaningful exposure assessment analysis. This analysis should include all exposures a person has to a specific contaminant, regardless of environmental medium (air, water, food, or soil) or the route of entry (inhalation, ingestion, or dermal contact). Exposure assessment can provide information on the distribution of the contaminant exposure within a population, the dose received, and routes of entry into the body. Exposure can be assessed using personal monitors near the breathing zone of the individuals. Passive samplers for air contaminants frequently use diffusion or permeation to concentrate the airborne material on a collecting medium, which is then returned to the laboratory for analysis. These samplers have been used for volatile organics, nicotine, formaldehyde, nitrogen dioxide, and carbon monoxide. Active samplers use small pumps to draw contaminated air through some collecting medium for analysis or through some form of direct-reading detector. When appropriate biomarkers are used in combination with the personal exposure data, an indication of internal dose can be estimated. For example, blood and urinary cotinine levels can be linked to air nicotine concentration and blood carboxyhemoglobin levels can be related to air carbon monoxide concentrations. The National Academy of Science has published an extensive discussion of the various approaches being used for assessing human exposure to airborne pollutants.

The final step, risk characterization, involves the integration and analysis of the existing database to provide a numerical estimate of the incidence of the adverse effect in a given population, assuming specific conditions of exposure.

The existing methods available for scientifically defensible risk characterization are not yet ideal since each step has an associated uncertainty resulting from data limitation and incomplete knowledge on exact mechanism of action of the toxic chemical on the human body. For noncancer end points, safety factors or uncertainty factors are applied since these effects are assumed to have a threshold below which no adverse effect is expected to be observed. US EPA has used the concept of a reference concentration (RfC) to estimate acceptable daily human exposure from HAPs. The RfC was adapted for inhalation studies based on a reference dose (RfD) method previously used for oral exposure assessment. The derivation of the RfC differs from that for the RfD in the use of dosimetric adjustment to extrapolate the exposure concentration for animals to a human equivalent concentration. Both are estimates, with uncertainty spanning perhaps an order of magnitude, of a daily exposure to the human population, including sensitive subgroups, which would be without appreciable risk of deleterious effects over a lifetime.

The RfC is estimated based on available knowledge of the toxic response of both humans and animals. Appropriate uncertainty factors (UF) and modifying factors (MF) are incorporated into the equation:

$$\mathrm{RfC} = \mathrm{NOAEL}/(\mathrm{UF}) \times (\mathrm{MF})$$

The NOAEL is the no-observed-adverse-effect level. **Table 6** indicates how these factors are used in deriving appropriate risk characterization.

For carcinogens, risk is estimated based on human and experimental animal data and other supporting evidence of carcinogenicity (e.g., structure–activity correlations, kinetics, and *in vitro* data). Decisions on the carcinogenicity of chemicals in humans need to be based on considerations of all relevant data, whether they are indicative of a positive or negative response, and should embody sound biological and statistical principles.

However, because animal carcinogens are not the same with respect to potency, target organs, mechanism, and so forth, and thus are not equally relevant to humans, hazard evaluation is on a weight-of-evidence basis. The weight-of-evidence evaluation of carcinogenic hazard to humans provides a basis for

Table 6 Application of uncertainty factors in deriving RfC

Type	*Magnitude*	*Purpose*
Interindividual	10	Intended to account for the variation in sensitivity among the human population
Interspecies	10	Used to account for uncertainty in extrapolating results from animals to average human population
Subchronic to chronic	5–10	Used to account for uncertainty in extrapolating less than chronic exposure results on animals or humans when no long-term human data are available
LOAEL to NOAEL	5–10	Accounts for the uncertainty inherent in extrapolation downward from LOAEL to a NOAEL
Incomplete to complete data	10	Used when experimental data are incomplete. This factor is intended to account for the inability of any single study to adequately address all possible adverse effects in humans. Depends on scientific judgment of the uncertainties of the study and data base

carcinogen classification and potency estimation. These assessments involve fitting mathematical models to experimental data and extrapolating from these models to predict risk at doses well below the experimental range. A range of risks can be produced using different models and assumptions about dose–response curves and the susceptibility of humans and animals to the test agent. Both IARC and US EPA have established a weight-of-evidence classification for carcinogens. They are similar but the IARC method does not address the potency of carcinogens, whereas the US EPA approach offers a means for developing quantitative estimate of carcinogen potency.

Space Flight and Respiratory Toxicology

Being able to predict the human health risk from exposure to airborne chemicals can be complex, requiring reliable analysis of human exposure. While the basic principles of risk assessment are applicable to various conditions of exposure, characterizing how an individual's health status can significantly influence the threshold for effects can be a most challenging component of the risk assessment process. One needs to consider the overall scientific weight-of-evidence to predict whether or not an individual may be uniquely susceptible to certain exposures in a specific exposure environment. This can be illustrated by examining the many factors facing individuals who have the responsibility for establishing safe levels of exposure necessary for ensuring the health and welfare of astronauts during space travel. The successful exploration of space not only depends on a high degree of excellence in engineering technology but also on maintaining a healthful environment for the space explorers. More than 400 air contaminants have been identified and their concentrations measured in the spacecraft atmosphere. Atmospheric chemicals detected during space missions include both gaseous (alcohols, aldehydes, aromatic hydrocarbons, ketenes, organic nitrogens, ammonia, carbon monoxide, etc.) and PM including microbes, cabin materials that have become airborne, ultra-fine particles, and pyrolysis products from small fires that are known to occur during flight.

Using the available toxicological database developed for exposure on earth may not be appropriate for assessing health risk in the unique living environment of outer space.

Several decades of human space flight have shown numerous changes in the health status of astronauts during and following space travel. As a consequence of the altered physiological status caused by the body's adaptation to microgravity, confinement, and stress, the human's response to airborne contaminants may be quite different from what has been learned from earth-based studies. Changes that occur from being in weightlessness include alterations in normal functioning of the human respiratory, cardiovascular, muscoskeletal, neurophysiological, renal, endocrine, hematological, and immune systems. Alterations in pulmonary function include changes in gas exchange, regional differences in blood flow, ventilation, diffusion capacity, residual volume, and intrapleural pressure.

Although the mechanisms of space flight-induced changes are not well understood, such changes during space flight can undoubtedly alter the normal physiological response to inhaled contaminants. In assessing potential health risks from being in such a space environment the toxicologist needs to consider: (1) microgravity does not permit the settling of airborne particles as experienced on Earth, and (2) physiological adaptations that occur from being in a weightless environment, can make a significant difference in deposition, retention and removal of inhaled particles which can influence the respiratory system response to inhaled chemicals.

See also: Absorption; Ames Test; Animal Models; Biomarkers, Human Health; Carcinogenesis; Clean Air Act; Combustion Toxicology; Donora: Air Pollution Episode; Dose–Response Relationship; Emergency Response and

Preparedness; International Agency for Research on Cancer; Mouse Lymphoma Assay; Occupational Toxicology; Pharmacokinetics/Toxicokinetics; Photochemical Oxidants; Pollution, Air; Pollution, Air Indoor; Polycyclic Aromatic Hydrocarbons (PAHs); Radiation Toxicology, Ionizing and Nonionizing; Risk Assessment, Human Health; Risk Characterization; Sick Building Syndrome; Silent Spring; Tissue Repair; Toxicity Testing, Inhalation.

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Retino *See* Vitamin A.

Rhodium

Shayne C Gad

- Representative Chemicals: Rhodium chloride ($RhCl_3$); Rhodium carbonyl chloride ($C_4Cl_2O_4Rh_2$)
- Chemical Abstracts Service Registry Number: CAS 7440-16-6
- Chemical/Pharmaceutical/Other Class: Platinum group metals; Precious metals; Transition metals
- Chemical Formula: Rh^{3+}

Uses

Rhodium is used as an alloy with platinum, and as a catalyst. It is also used as a corrosion-resistant electroplate for protecting silverware from tarnishing, for making high-reflectivity mirrors for cinema projectors, and searchlights. It can be used as a catalyst for chemical reactions, and in jewelry. In fact, rhodium is a very common plating for inexpensive jewelry because it is extremely shiny and tarnish resistant. It is actually a very expensive metal; however, only need a microscopically thin layer is needed.

Background Information

Rhodium is one of the platinum group elements, and is found at very low concentrations in the Earth's crust. Rhodium was discovered by William Hyde Wollaston (England) in 1804. The origin of the name comes from the Greek word *rhodon* meaning rose. The plated solid is very corrosion resistant and exceptionally hard. It is inert in air and acids. However, it can produce a violent reaction to chlorine, bromine pentafluoride, bromine trifluoride, and fluorine monoxide.

Exposure Routes and Pathways

The common routes of exposure are by inhalation and ingestion.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oral toxicity studies in rats showed that rhodium trichloride, sodium chlororhodite, and chloropentamine rhodium chloride(III) are of low systemic toxicity.

Human

No toxic effects of rhodium have been reported from observations of human beings. Workers wearing rings coated with rhodium showed negative rhodium patch tests while all other metals in the rings gave positive results. It has been reported that inhalation of excessive amounts of fine rhodium metal powder or dust may cause irritation of the respiratory system, and that eye contact with fine powder or dust may cause irritation (mechanical irritation).

Chronic Toxicity (or Exposure)

Animal

Rhodium's carcinogenic potential has not been fully established.

Human

Rhodium is listed as A4 (not classifiable as a human carcinogen) according to the American Conference of Governmental Industrial Hygienists (ACGIH). A potential symptom of overexposure to metal fumes and insoluble compounds is respiratory sensitization. A variety of rhodium compounds have been tested against various types of tumors and have been shown to have antitumor activities. However, the toxic effects of most of the compounds studied have prevented detailed examination (e.g., clinical trials), and their mechanism of action has not been studied systematically. Recent structural studies suggest that the antitumor activity of the dirhodium(II) carboxylates may be similar to that of cisplatin, that is, by binding to adjacent guanines on DNA.

In Vitro Toxicity Data

Water-soluble complex salts of rhodium have been shown to have mutagenic potential in the *Salmonella typhimurium*/microsome test system (Ames test).

Exposure Standards and Guidelines

The ACGIH threshold limit value, 8 h time-weighted average (TWA) is $1.0\,mg\,m^{-3}$ (as the metal and insoluble compounds), and the US National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day is $0.1\,mg\,m^{-3}$. NIOSH's immediately dangerous to life or health value is $100.0\,mg\,m^{-3}$ (as the metal fume and insoluble compounds).

See also: Platinum.

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Rhododendron Genus

Alexander B Baer and Christopher P Holstege

- Representative Chemicals: Azalea; *Rhododendron*
- Synonyms: *Rhododendron catabiense*, Ericaceae (heath) family; *Catawba rhododendron*; Mountain rosebay; Purple laurel; Rhodora; Rosa Laurel; Rosebay

Exposure Routes and Pathway

Exposure is through ingestion of leaves, flowers, nectar, tea brewed from the leaves, or honey produced exclusively from the nectar.

Mechanism of Toxicity

Rhododendrons contain grayanotoxins that bind to cell membrane sodium channels and increase sodium conduction. Nerve and muscle cells are subsequently kept in a state of depolarization.

Acute and Short-Term Toxicity (or Exposure)

Animal

There are several cases of animal poisonings from Rhododendrons. Goats that ingested branches of an azalea plant presented with profuse vomiting, central nervous system depression, and fasciculations. Other animals with reported poisoning include donkeys, dogs, and kangaroos.

Human

The entire plant is potentially toxic to humans. Onset of action typically occurs within 2 h of ingestion with complete resolution by 24 h. Most exposures result in no toxicity. Grayanotoxin poisoning may result from consumption of honey acquired from a hive where

the bees obtained nectar primarily from rhododendron. Commercial vendors mix honey from several different hives thus ensuring dilution of any potential grayanotoxin contaminated honey. Consumption of the plant, tea, or contaminated honey may cause burning of the mouth followed by salivation, vomiting, abdominal pain, diarrhea, ataxia, and weakness. Hypotension, bradycardia, progressive paralysis of the limbs, and seizures have also been reported.

Clinical Management

Minimal ingestions usually do not require treatment. Activated charcoal may be considered in substantial, recent ingestions. The care of the rhododendron-poisoned patient is largely supportive. Symptomatic bradycardia and hypotension should be treated with atropine and intravenous fluids, respectively. When hypotension is refractory, vasopressors should be considered. Complete heart blocks may require cardiac pacing. Seizures should be treated with benzodiazepines.

See also: Plants, Poisonous.

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Rhubarb

Ann P Slattery

- SYNONYMS: *Rheum rhabarbarium*; *Rheum rhapontieum*; Garden rhubarb; Pie plant; Wine plant (the herbal rhubarb is *Rheum officinale*)

Uses

Rhubarb is a perennial plant with stalks that grow 1–3 ft in length and become reddened when ripe. The leaves are large and wrinkled with wavy margins. Rhubarb is cultivated as a food source and for medicinal purposes in many parts of the world.

Exposure Routes and Pathways

The routes of exposure are ingestion and dermal contact.

Toxicokinetics

Oxalate absorption varies greatly with an oral absorption range of 1–22%. Oxalates are excreted unchanged in the urine within 24–36 h.

Mechanism of Toxicity

Soluble oxalates in the leaves are absorbed via the gastrointestinal tract. Once absorbed, oxalates bind with calcium producing secondary hypocalcemia. Once bound to calcium, oxalate salts become insoluble and may precipitate in the renal system resulting in kidney malfunction and electrolyte imbalance. Renal damage may be due to vascular stasis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Oxalate-containing plants can be a source of poisoning for grazing animals. In ruminants, a large acute exposure results in hypocalcemia and death. In chronic exposures, renal damage and urolithiasis from calcium oxalate deposition result. Other animals display gastrointestinal symptoms and renal damage. Treatment includes activated charcoal and intravenous electrolyte and calcium treatment. With rest animals become ambulatory after 1 than 8 h of treatment. Notable gastrointestinal tract mucosal edema and hemorrhage, abdominal ascites, and hyperemia have occurred.

Human

Rhubarb stalks are edible. Soluble oxalates are primarily found in the leaf blades and in much lower concentrations in the stalk. Anthraquinone glycosides are present in lesser amounts in rhubarb grown in the United States; therefore, exposures do not result in the expected cathartic effects as seen with *Rheum officinale*. Acute fatal poisonings due to ingestion of leaves are rare. Large amounts of the leaf must be ingested before symptoms develop. With ingestion of large amounts, symptoms include

abdominal pain, nausea, vomiting, weakness and drowsiness, seizures, possible liver damage, and kidney damage. Because digestion of the plant is slow, effects may be delayed several days. The ingestion of stalks and small leaf exposures are unlikely to cause serious problems.

Chronic Toxicity (or Exposure)

Animal

Animals may develop subacute toxicity if enough plant material is ingested to produce hypocalcemia and kidney damage, but no so much that the animal dies. With larger or more prolonged exposures, animals may experience larger deposits of calcium oxalate crystals that result in renal fibrosis or renal failure which can ultimately lead to death.

Human

Rhubarb is used therapeutically in many parts of the world as a stimulant laxative as well as a homeopathic remedy. Chronic stimulant laxative use may result in structural changes to the colon.

Clinical Management

For patients who present soon after substantial ingestion, treatment with activated charcoal may be useful. Low calcium levels and tetany should be treated with intravenous calcium gluconate. Serum blood urea nitrogen and creatinine should be measured, and urine should be checked for the presence of oxalates. The patient should be hydrated with fluids and electrolytes as needed. Hemodialysis may be indicated if anuria develops.

See also: Plants, Poisonous; Oxalates.

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Riboflavin

Diana Ku

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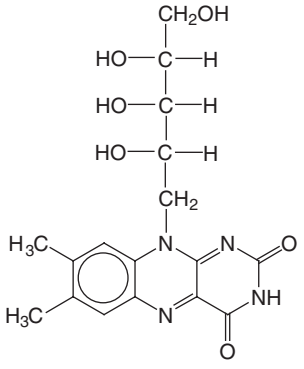
- Chemical Abstracts Service Registry Number: CAS 83-88-5
- Synonyms: Vitamin B_2; Beflavine; Flavaxin; Flaxain; Lactoflavin; Isoalloxazine; 7,8-dimethyl-10-(D-ribo-2,3,4,5-tetrahydroxyphentyl)
- Chemical/Pharmaceutical/Other Class: Water-soluble vitamin
- Chemical Formula: $C_{17}H_2ON_4O_6$
- Chemical Structure:



Uses

Riboflavin is a nutritional supplement used during periods of deficiency known as ariboflavinosis. Riboflavin deficiency usually occurs in association with malabsorption, alcoholism, or protein-calorie deficiency, and is rarely the sole vitamin deficiency. Riboflavin needs are increased during chronic debilitative stress to the body such as malabsorption diseases of the small intestine, liver disease, hyperthyroidism, alcoholism, and during pregnancy and lactation. Neonates undergoing phototherapy for hyperbilirubinema also have increased nutritional needs.

Exposure Routes and Pathways

The route of exposure is oral. Dietary sources of riboflavin include broccoli, spinach, asparagus, enriched flour, yeast, eggs, milk, cheese, mackerel, trout, poultry, liver, and kidneys.

Toxicokinetics

Riboflavin is readily absorbed from the gastrointestinal tract mainly in the duodenum. It is hepatically metabolized, moderately protein bound, and widely

distributed to tissue; however, little is stored in the liver, spleen, heart, and kidneys. Riboflavin is excreted renally almost entirely as metabolites. All riboflavin in excess of daily body needs is excreted unchanged in the urine. Riboflavin exhibits biphasic pharmacokinetics with an initial half-life of 1.4 h and a terminal half-life of 14 h.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity is not expected.

Human

Acute toxicity is unlikely following even 100 times the recommended daily allowance. There are no reports of acute toxicity from exposures to riboflavin.

Chronic Toxicity (or Exposure)

Animal

It would be unlikely for animals to be given chronic riboflavin overdoses.

Human

Chronic exposure to large doses of riboflavin may cause a bright yellow discoloration of the urine.

In Vitro Toxicity Data

There are no reports of congenital anomalies among children born to mothers who used large doses of pyridoxine during pregnancy.

Clinical Management

In cases of chronic excessive use, the patient should be instructed to discontinue the supplement.

See also: Vitamin A; Vitamin D; Vitamin E.

Further Reading

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Ricin and Other Toxalbumins

Mark A Hostetler

- Representative Chemicals: Ricin; Other toxalbumins with similar ricin-like properties: *Abrus precatorius* (jequirty pea, rosary pea), *Trichosanthes* spp. (Chinese cucumber), *Robinia pseudoacacia* (black locust), *Phoradendron* spp. (American mistletoe), *Viscum* spp. (European mistletoe), and *Wisteria* spp. (wisteria)
- Chemical Abstracts Service Registry Number: CAS 9009-86-3
- Synonym: *Ricinus communis* (castor bean)
- Chemical/Pharmaceutical/Other Class: Type 2 ribosome inactivating proteins
- Chemical Structure: Ricin is a heterodimeric ribosome inhibiting protein consisting of an A-chain (RTA), linked by a disulfide bond to the B-chain (RTB). The total molecular weight is 66 000 Da, with the A-chain contributing 32 kDa and the B-chain 34 kDa. The A-chain is a globular protein composed of 267 amino acids containing eight alpha helices and eight beta sheets, with the toxin's substrate binding site located within the cleft. The B-chain is the binding lectin composed of 262 amino acids, shaped like a barbell, and has a binding site specific for membrane sugars at each end (galactose and *N*-acetyl galactosamine)

Uses

Ricin is the most well known, and ubiquitous, of the toxalbumins. Castor beans are used in the production of castor oil, a major constituent in lubricants, brake, and hydraulic fluids. During the production process, 5–10% of the aqueous phase, also known as 'waste mash', is recoverable as ricin. In addition, castor beans and jequirty peas are also used extensively throughout Mexico and Central America for ornamental purposes in items such as necklaces, prayer or rosary beads, and the rattles in musical shakers (maracas).

Exposure Routes and Pathways

Once produced, ricin remains stable in powder, aerosol, solid pellet, or liquid form. Ricin may be

dissolved in water or weak acid, and remains stable at even the most extremes of temperatures. It is believed to be one of the most toxic naturally occurring substances in the world. The toxins are present in all parts of the plant but are most concentrated in the beans or seeds. The beans are covered by a hard, relatively impervious outer shell that must be chewed or broken in some way in order for the toxalbumin to be released.

Possible routes of exposure include cutaneous, mucosal, gastrointestinal, inhalation, and parenteral (intravenous or intramuscular). Gastrointestinal exposures are usually accidental and occur most commonly when castor (ricin) or jequirty (abrin) beans are chewed or swallowed. Cutaneous exposures are limited primarily to castor beans, which are unusually allergenic and may cause severe cutaneous hypersensitivity and systemic allergic reactions. Inhalation and parenteral exposures are generally limited to intentional, usually malicious, exposures.

Toxicokinetics

Although acute hypersensitivity and allergic reactions can be triggered by casual dermal contact, cutaneous absorption of the toxin through intact skin is negligible. Ricin powder is extremely irritating to the eyes and may result in severe inflammation and hemorrhagic conjunctivitis; however, very little is absorbed systemically. Ricin is inefficiently absorbed via the gastrointestinal tract ($LD_{50} = 30\,\mu g\,kg^{-1}$). Absorption via inhalation is much more efficient ($LD_{50} = 3\,\mu g\,kg^{-1}$). The overall lethality of an inhalational exposure is directly related to the particle size of the aerosol carrying the toxin. The smaller the particle, the higher the lethality. The highest potential lethality is seen with direct parenteral exposures ($LD_{50} = 1\,\mu g\,kg^{-1}$). Ricin does not undergo any significant hepatic or renal metabolism.

Mechanism of Toxicity

Ricin contains the two basic components necessary for it to enter cells and inhibit protein synthesis. The ligand portions of the B-chain act to bind to galactose moieties of the cell membrane and facilitate endocytosis of the entire ricin molecule into the cell where it is transported via endosomes to the Golgi apparatus and endoplasmic reticulum. Once there, the A-chain is translocated into the cytosol where *N*-glycosidase modifies a base (A_{4324}) in an exposed loop of the 28S rRNA fragment of the 60S RNA chain. Requiring no energy or cofactors, it catalytically and irreversibly inactivates the 60S ribosomal subunit halting all further protein synthesis, thereby causing severe cytotoxic effects on multiple organ systems.

Acute and Short-Term Toxicity (or Exposure)

Animal

Ricin has been used with a variety of different lectins and different specificities to map cellular patterns of glycosylation and intracellular transport in animals. Studies have confirmed the importance of *N*-acetyl galactosamine residues on the cell surface. In studies mapping the effects of ricin, ultrastructural analysis reveals that ricin binds to galactose on the cell membrane, is endocytosed, and then sequentially causes the dispersion of polyribosomes as the rough endoplasmic reticulum disorganizes into smooth vesicles. Finally, the cell bodies (perikaryon) swell, the nuclei degenerate, and the entire cell disintegrates.

Human

Although fatalities have been reported following ingestion of chewed castor beans, severe toxicity secondary to accidental exposure is rare. According to the American Association of Poison Control Centers, of the reported toxalbumin cases with known outcomes, 65% have no symptoms, and 31% have only minor symptoms. Although chewing and swallowing one bean may produce symptoms in an adult, swallowing an intact bean without chewing is unlikely to cause any serious sequelae.

Toxic effects of ricin have latent periods ranging from 2 to 24 h. Symptoms include delayed gastroenteritis, which may be severe and hemorrhagic, followed by delirium, seizures, coma, and death. Toxic effects include intestinal hemorrhage, diffuse nephritis, hepatic necrosis, and on a cellular level pyknosis of the nuclei and karyorrhexis. Patients may experience hypoglycemia, severe dehydration, and shock. Severe exposures may also result in the development of pulmonary edema, respiratory failure, and death within 36–72 h. If death has not occurred in 3–5 days, the victim usually recovers.

Clinical Management

Clinical management begins with an assessment of the most important features associated with toxic environmental exposures: identification of the substance; time, type, and duration of exposure; symptoms; treatment thus far; associated injuries; and preexisting conditions. Chief among these is to determine if any of the beans have been chewed or

swallowed. All exposures should be reported to the regional poison control center. Decontamination is essential to minimize the risk of further harm to the patient, and to reduce the risk of secondary contamination to others. Decontamination begins by removing all clothing and washing the patient's entire dermal surface with copious amounts of soap and water. An alternative is to use a dilute bleach mixture with a contact time of 15 min (0.5% sodium hypochlorite solution – approximately one part bleach to nine parts water).

Treatment options are largely supportive. An assessment should first be made for airway patency and adequacy of breathing. Circulation may become affected as shock develops secondary to severe gastroenteritis. The following laboratory studies are recommended for all symptomatic patients: computerized blood count, electrolytes, and coagulation studies (prothrobin time, activated partial thromboplastin time). In cases of uncertain or unknown exposure, there is an enzyme-linked immunosorption assay test available for the detection and verification of the presence of ricin

Patients may develop severe cutaneous hypersensivity or systemic allergic reactions. Signs may include the development of an urticarial hive-type reaction, facial or tongue swelling, bronchospasm, and acute upper airway obstruction. Treatment includes antihistamines, corticosteroids, and, if necessary, epinephrine.

Any further definitive treatment options are limited. Neither induced vomiting with syrup of ipecac nor gastric lavage is believed to be beneficial, or is recommended. Administration of activated charcoal has been suggested as a possible treatment to absorb toxin; however, the potential benefit (if any) remains unproven. Whole bowel irrigation has also been suggested as a possible treatment option to ensure rapid and complete decontamination of the gastrointestinal tract; however, the potential benefit (if any) remains unproven. There are no antidotes, and the toxins are not dialyzable. The mainstay of treatment remains, therefore, largely supportive with attention to fluid, glucose, and electrolyte replacement. Symptomatic patients should be admitted to the hospital; asymptomatic patients may be discharged safely after observation for at least 4–6 h.

Other Hazards – Ricin as a Biological Weapon

As one of the most toxic and easily produced toxins available, ricin was initially investigated as a biological weapon by the US military in World War I. Although it has never been used in battle, it has been used successfully in several small-scale killings, the most notorious of which includes the very well known assassination of a Bulgarian defector in 1978 (Georgi Markov). Although it is easy and inexpensive to produce, highly toxic, and stable in a variety of conditions, the amount required to cause a high level of lethality on an entire population is probably too massive so as to make it truly practical as a weapon of mass destruction. Its potential as a major cause for morbidity and mortality as an aerosolized agent, however, especially in enclosed environments, should not be underestimated. It could also easily be used as a food or water contaminant on a relatively large scale such that it could easily incapacitate or overwhelm an area's healthcare resources by nature of the amount of illness it would produce.

See also: Castor Bean; Plants, Poisonous; Wisteria.

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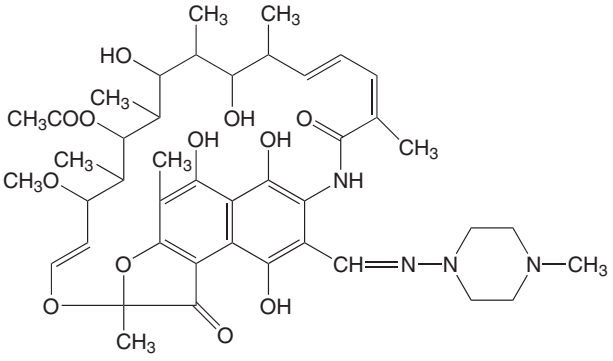
Rifampin

Christopher P Holstege

This article is a revision of the previous print edition article by Michael J Hodgman, volume 3, pp. 88–89, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 13292-46-1
- Synonym: Rifampicin
- Chemical/Pharmaceutical/Other Class: Antibiotic
- Chemical Structure:



Uses

Rifampin is used as an antibiotic. It is a semisynthetic derivative of rifamycin B, a macrocyclic antibiotic produced by the mold *Streptomyces mediterranei*.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Rifampin is available in oral and parenteral forms.

Toxicokinetics

Rifampin is rapidly and nearly completely absorbed from the gastrointestinal tract. Peak serum levels are seen within 2–4 h. Food, antacids, ketoconazole, and aminosalicylic acid interfere with absorption and delay peak levels. If these agents are used concurrently, they should be administered separately at an interval of at least 8 h. Massive ingestions in the overdose setting may delay absorption. Protein binding is 75–90%. The volume of distribution is $\sim 1\,l\,kg^{-1}$. Rifampin undergoes hepatic deacetylation to an active metabolite. Both rifampin and its deacetylated metabolite are excreted into the bile. Rifampin, and to a lesser extent its deacetylated metabolite, undergo enterohepatic recirculation. The half-life of therapeutic doses of rifampin is 1.5–5 h. The half-life is shortened after regular use due to induction of hepatic enzymes. Chronic liver disease increases the half-life. The kinetics is not well described in the overdose setting. In one case, the half-life was 4.4 h.

Mechanism of Toxicity

In the acute overdose setting, the mechanism of toxicity is not defined. A number of the toxic reactions occurring with intermittent dosing schedules or on reexposure are postulated to be due to the presence of antirifampin antibodies.

Acute and Short-Term Toxicity (or Exposure)

Human

Intentional overdoses of rifampin rarely lead to significant morbidity and fatalities are exceedingly uncommon. The few deaths that have been associated with rifampin have all been in individuals with a history of alcoholism or concomitant ethanol ingestion. Acute overdose with rifampin may cause a red to orange discoloration of the skin seen within 2 h of exposure, 'the red man syndrome'. Body fluids are also discolored and urine, feces, sweat, tears, and saliva may exhibit a red to orange discoloration. Symptoms associated with rifampin overdose include headache, abdominal pain, nausea, vomiting, and flushing. Pruritus, which may be limited to the scalp, may be seen, and a cutaneous burning sensation maybe noted. Lethargy and obtundation have been reported. Facial or periorbital edema may be seen. Minor and transient elevations of hepatic transaminases, bilirubin, and amylase have been reported. Rifampin may inhibit bilirubin excretion and may interfere with the bilirubin assay. An acute ingestion of 60 g was fatal in an alcoholic. Overdoses of 12 g in otherwise healthy individuals have been tolerated, as has 2 g in an 18-month-old. Because of the small number of cases, correlation of dose with severity is not possible, and serum levels are not useful.

Chronic Toxicity (or Exposure)

Animal

Offspring of rodents dosed at 150–250 $mg\,kg^{-1}\,day^{-1}$ during pregnancy have had greater than expected findings of cleft palate and spina bifida.

Human

Rifampin used daily at therapeutic doses is associated with facial flushing and itching in less than 5% of patients. More rarely, hepatotoxicity is seen and may lead to complete hepatic failure requiring liver transplantation. The risk of hepatotoxicity is increased with chronic liver disease, alcoholism, and old age. Acute renal failure, interstitial nephritis, nephrogenic diabetes insipidus, and thrombocytopenic purpura are rare complications of continuous daily use. The use of rifampin on an intermittent dosing schedule, two or three times weekly or less, is associated with a higher incidence of toxic side effects. These include a flu-like syndrome with that lasts up to 8 h following each dose of rifampin. More serious toxic effects associated with an intermittent dosing schedule include hemolytic anemia, thrombocytopenia, hepatitis, nephritis, acute renal failure, and shock. These reactions are believed to be hypersensitivity reactions and related to antirifampin antibodies. Rifampin is also a potent inducer of hepatic microsomal enzymes. Its administration may result in decreasing the half-life of numerous compounds.

In Vitro Toxicity Data

Mutagenicity studies using *Drosophila* have been inconclusive.

Clinical Management

Acute overdoses of rifampin are rarely serious. Supportive care, gastric decontamination with activated charcoal for substantial recent ingestions are all that is usually necessary. Given the extensive enterohepatic circulation of rifampin, repeated doses of activated charcoal may enhance elimination; however, the clinical utility of the procedure is questionable. Systemic toxicity associated with the chronic administration of rifampin is an indication to discontinue the drug.

See also: Liver.

Further Reading

Meisel S and Brower R (1980) Rifampin: A suicidal dose. *Annals of Internal Medicine* 92: 262–263.

Riot Control Agents

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General Considerations

Synonyms

Tear Gas, less than lethal, nonlethal, immobilizers, irritants, lacrimators, harassing agents, RCAs, crowd control agents (**Table 1**).

Table 1 Agents considered in this article

Military designation	*Chemical name*	*CAS number*
CN	1-Chloroacetophenone	532-27-4
CS	2-Chlorobenzylidene malononitrile	2698-41-1
CR	Dibenz(*b,f*)1:4-oxazepine	257-07-8
DM (Adamsite)	Diphenylaminochloroarsine	579-94-9
OC	Oleoresin capsicum	
Capsaicin		404-86-4
PAVA		
Fentanyl	*N*-Phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]propamide	437-38-7

Pharmacological Actions

Riot control agents (RCAs) cause disabling physiological effects when they come into contact with the eyes and/or skin, or when inhaled by unprotected individuals, by interacting with sensory nerve receptors in the skin and mucosal surfaces at the site of contamination, resulting in local pain and discomfort with associated reflexes. The Kratschmer reflex causes apnea, bradycardia, and a biphasic fall and rise in aortic blood pressure, which is mediated via the olfactory (I), trigeminal (V), and glossopharyngeal (IX) cranial nerves. This reflex has been demonstrated in both animals and humans following exposure to inhaled RCAs.

Pharmacological Classes

Irritants (peripheral sensory irritants), lacrimators, sternutators, emetics, sedatives, hypnotics, serotonin antagonists, hypotensives, thermoregulator disruptors, nauseants, vision disruptors, neuromuscular blockers, malodorous substances, centrally acting anesthetics, immobilizers, tranquillizers.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

The US Military has developed a basic military classification of Class Indices for chemical and biological warfare agents. The Class Indices divides RCAs into indices: incapacitating agents (fentanyl) tear agents – halogenated (CN and CS); tear agents – nonhalogenated (CR); tear agents in solvents (OC, Capsaicin, and PAVA); and vomiting agents (DM). While the acute physiological impacts from the various agents within each of these classes are essentially the same, there are variations in the physical/chemical properties and decomposition products.

Uses

RCAs are used as nonlethal or less-than-lethal materials in riot peacekeeping operations to temporarily distract, deter, incapacitate, disorient, or disable disorderly people, to clear facilities, areas, deny areas, or for hostage rescue. They are also used in military training as a confidence builder for the protective mask. Some of these agents can be used as agents in their respective pharmacologic class, that is, fentanyl is used as a short acting central nervous system (CNS) general anesthetic and capsaicin is used as a topical analgesic. Capsaicin spray is also used in the pharmaceutical industry to induce cough for testing antitussive candidates.

Physiochemical Characteristics

RCAs are solids with low vapor pressures. They can be dispersed as fine powders; foams, as coherent jets or streams of solutions from small or large spray cans, large spray tanks, or larger weapons; and as aerosols or smokes by pyrotechnic generation (**Table 2**).

Mechanisms of Action

RCAs are considered less than lethal and nonlethal because they have a very large safety ratio. That is, their effective concentration (EC_{50}) is very low compared to their lethal dose or concentration (LCt_{50}).

CS and CN are SN2-alkylating agents with activated halogen groups that react readily at nucleophilic sites. The prime targets include sulfhydryl-containing enzymes such as lactic dehydrogenase. In particular, CS reacts rapidly with the disulfhydryl form of lipoic acid, a coenzyme in the pyruvate decarboxylase system. It has been suggested that tissue injury may be related to inactivation of certain of these enzyme systems. CS causes the release of bradykinin that can produce pain without tissue injury. The initial response to the inhalation of CS or other sensory irritants is consistent with the Kratschmer reflex and the Sherrington pseudoaffective response. It is theorized that CR aerosols also stimulate the pulmonary irritant receptors to produce bronchoconstriction and increased pulmonary blood volume by augmenting sympathetic tone. The chlorine atoms released from CS on contact with skin and mucus membranes are reduced to hydrochloride acid, which can cause local irritation and burns.

Capsaicin, like the other irritant RCAs, also causes bronchoconstriction, but the mechanism is uncertain. Capsaicin releases substance P that can cause bronchoconstriction directly by activation of specific receptors or by release of histamine or other mediators. It may also cause reflex bronchoconstriction by stimulating C fibers in both pulmonary and bronchial circulation. Therefore, bronchoconstriction may be secondary to substance P release, or to a vagal reflex. The altered neurophysiology of sensory neurons in the airway mucosa induces the release of tachykinins and neurokinin A, which causes neuromediated inflammation of the epithelium, airway, blood vessels, glands, and smooth muscles. This leads to bronchoconstriction, mucus secretion, enhanced vascular permeability, and neutrophil chemotaxis.

DM is among the group of compounds including diphenylchloroarsine (DA), diphenylcyanoarsine (DC), and chloropicrin, which are classified militarily as vomiting agents. DM has been characterized as both a vomiting agent as well as a sneezing agent (sternutator), and was used in World War I. The estimated human LCt_{50} was reported to be 11 000 mg min m^{-3}. DM effects, unlike those of CN, CS and CR, have a slightly delayed onset and have a relatively long recovery period. DM effects occur in $\sim$3 min after inhalation exposure and may last for several hours.

Table 2 Physical properties of some selected RCAs

	CS	*CR*	*CN*	*DM*	*Capsaicin*
Molecular weight	188.5	195.3	154.5	277.5	305
Melting point (°C)	93	72	54	195	64
Vapor pressure (mmHg at 20°C)	0.00034	0.00059	0.0054	2×10^{-13}	0.011
Volatility (mg m^{-3} °C^{-1})	0.71/25°	0.63/25°	1.06/52°		
Solubility[a]	loc	loc	loc	lo	loc

[a] Solubility: l = limited in water; o = soluble in organics; c = soluble in chlorinated organics.

Also unlike the tear agents, DM is more likely to cause prolonged systemic effects. Signs and symptoms of DM exposure include eye irritation, upper respiratory tract irritation, uncontrolled sneezing and coughing, choking, headache, acute pain, tightness in the chest, nausea, and vomiting as well as unsteady gait, weakness in the limbs, and trembling. Mental depression might result after exposure to DM. Inhaled high concentrations can result in serious illness and death as a result of pulmonary damage.

Fentanyls

General Pharmacology

During the Cold War (1945–91), a great deal of research was directed to chemicals that were not necessarily lethal, but would merely temporarily incapacitate enemy personnel. In particular, the United States and the former Soviet Union investigated a wide number of pharmacological agents such as depressants, hallucinogens, belladonna drugs, and opiate derivatives for their potentials as incapacitants.

A major breakthrough in opiate drugs for use in medicine was the synthesis of fentanyl in Belgium in the late 1950s and was first patented by Janssen in France in 1963. Its primary use in medicine was for anesthesia. However, its major complication is respiratory depression, which can be monitored and reversed in an operating room, but can be a problem if used operationally in the field. Since 1996, a number of different analogs of fentanyl have been introduced for use in anesthesia such as carfentanil, sufentanil, alfentanil, and remifentanil. Their pharmacological activity is characteristic of opiates and they produce all of the effects of heroin, including analgesia, euphoria, miosis, and respiratory depression. Due to their high lipid solubility, regardless of the route of administration, fentanyls reach the brain very quickly, thus providing a very fast onset of action. Some of the analogs have been synthesized specifically for sale as Persian white, China white, Mexican brown, and synthetic heroin in the illicit drug market and to circumvent regulations on controlled substances. These illicit drugs are also called designer fentanyls and are used by abusers via intravenous injection, or smoked or snorted.

Fentanyls are synthetic opiates recognized for their short acting and highly potent narcotic analgesic, anesthetic, and immobilizing properties in both animals and humans. Fentanyl is also used as an adjunct to general anesthesia, and as an anesthetic for induction and maintenance. It is primarily a mu-opioid agonist. Abuse of this drug leads to habituation or addiction. The Chemical Abstracts Service Registry numbers of some of the analogs of fentanyl are: sufentanil, CAS 56030-54-7; carfentanil, CAS 59708-52-0; and remifentanil, CAS 132875-61-7.

The feasibility of dissociating the respiratory depressant effect from the opiate-induced sedative activity of alfentanil and fentanyl with naloxone was studied. Naloxone was more effective as an antagonist to alfentanil than to fentanyl. Later studies also suggested that in the rat and ferret, dissociation of the opiate-induced sedation and respiratory depression was feasible. This was accomplished by co-administration of the opiate agonist with antagonists. The opiate-induced effects were akinesia, catalepsy, loss of righting reflex, light anesthesia, and apnea. The pharmacodynamic mechanism of the co-administration may involve competitive displacement of the opiate agonist by the antagonist at their common receptor sites within the CNS. A pharmacokinetic mechanism may also be involved such that the opiate uptake, distribution, and clearance are affected, either directly or indirectly, by the antagonist. Changes in respiratory frequency, oxygen consumption, and apnea were monitored in ferrets following the intravenous co-administration of the opiate agonist sufentanil and the antagonist nalmefene. These studies demonstrated a dissociation of the sufentanil-induced sedation/anesthesia and severe respiratory depression. Nalmefine co-administration shortened the duration, but did not significantly delay the onset of the opiate-induced sedative/anesthetic effect. Narcotic antagonists such as nalmefene, naltrexone, and naloxone have clinical application in the diagnosis of addiction, prophylactic treatment of narcotic abuse, and emergency treatment of narcotic over dosage. These antagonists displace either previously assimilated opiates from their receptor sites, or if administered prior to the narcotic, will preclude the narcotic agonist from acting at these sites.

It has been reported that the serious adverse effects of opiate analgesia such as depression of breathing are caused by direct inhibition of rhythm-generating respiratory neurons in the Pre-Boetzinger complex (PBC) of the brainstem. Serotonin 4(a) or 5-HT4(a) receptors are strongly expressed in these neurons and their selective activation protects spontaneous respiratory activity. Rats treated with a 5-HT4 receptor specific agonist overcame the fentanyl-induced respiratory depression, and reestablished stable respiratory rhythm without loss of fentanyl's analgesic effect.

Opiate effects are mediated via multiple opioid receptors such as the mu, kappa, delta, and sigma. The mu receptors mediate analgesia, euphoria, physical dependence, and depression of ventilation,

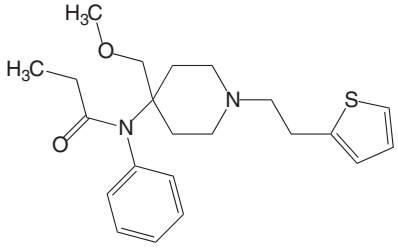
whereas kappa receptors mediate sedation and dieresis. Drugs may act at more than one opiate receptor with varying effect.

Human Toxicity

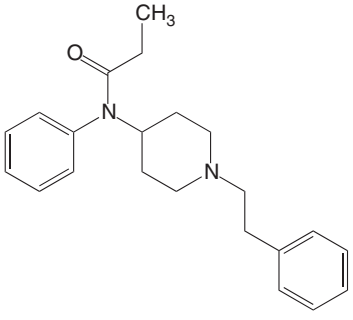
Since fentanyl is not listed in any schedules of the Chemical Warfare Convention (CWC), and is traditionally characterized by the rapid onset and short duration of action of 15–30 min of analgesia, it can be legally considered an RCA according to the definition set forth in the CWC. On October 23, 2002, at least 129 of the ~800 hostages died in the Moscow Dubrovka Theatre Center when Russian authorities subdued the hostage-takers there by pumping what many believe was fentanyl into the building; some believe that a mixture of fentanyl and halothane was used.

The Chechnian terrorists, who held the hostages, were from a particularly extreme and violent group who were on a martyrdom mission. It was also considered that the Russians might have used remifentanil since it is rather unique and extremely potent with relative fast action and short duration. The chemical structure of remifentanil also allows the body to quickly metabolize the substance into nontoxic and water-soluble forms, thus minimizing risks for both hostages and hostage-takers. Although the Russian authorities insisted that emergency personnel were prepared with 1000 antidotes in anticipation of the raid, there is still controversy whether local hospitals and physicians were adequately informed about the gas used during the operation. It has also been suggested that the Russian government revealed that a mixture of fentanyl and halothane was used to incapacitate the Chechnian terrorists in the attempt to liberate the hostages in Moscow. They further suggested that it was likely that massive doses of carfentanil were used to saturate the theatre so that maximal effect by inhalation could be achieved. Carfentanil is a potent opioid use to rapidly immobilize large, wild animals, horses, and goats. It produces rapid catatonic immobilization, characterized by limb and neck hyperextension. Adverse effects include muscle rigidity, bradypnea, and oxygen desaturation. Recycling and renarcotization have been reported as possible causes of death when low doses of antagonists are used. Although there were naloxone syringes found in the theatre, it is possible that the doses were insufficient to reverse the respiratory depression.

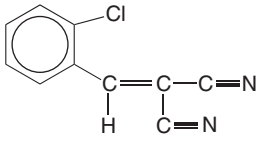
Chemical Structures



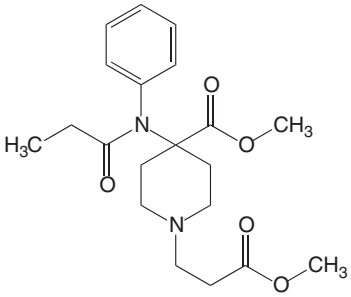
Sufentanil (SAN:BAN:INN) (RN: 56030-54-7)



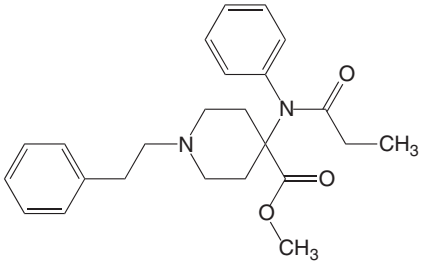
Fentanyl (RN: 437-38-7)



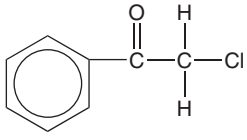
2-Chlorobenzylidene malononitrile (CS)



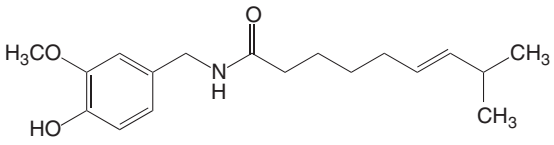
Remifentanil (RN: 132875-61-7)



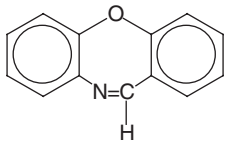
Carfentanil (RN: 59708-52-0)



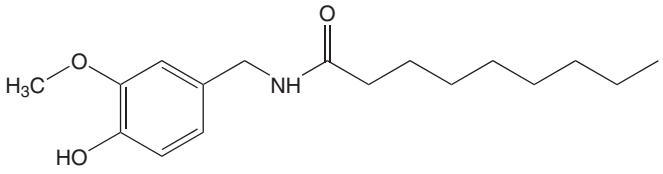
1-Chloroacetophenone (CN)



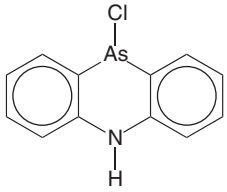
Capsaicin



Dibenz (b,f)–1:4-oxazepine (CR)



PAVA or Nonivamide



10-Chloro-5,10-diphenylarsazine (DM)

1-Chloroacetophenone

Animal Toxicology

Toxicological studies were conducted on 1-Chloroacetophenone (CN) dispersed from commercially available thermal grenades or from acetone solutions. Acute and sublethal effects following aerosol exposure in experimental animals were lacrimation, conjunctivitis, copious nasal secretions, salivation, hyperactivity, lethargy, and dyspnea, which occurred in all animals. Effects on the skin of exposed animals were primarily erythema. The estimated LCt_{50} values calculated for CN in the various animal species were 8878 mg min m^{-3} in the rat, 7984 mg min m^{-3} in the guinea pig, and 7033 mg min m^{-3} in the dog. The pathological findings in the animals that died from inhalation of CN consisted of congestion of the alveolar capillaries, alveolar hemorrhages, and excessive secretions in the bronchi and bronchioles, as well as areas of acute inflammatory cell infiltration of the trachea, bronchi, and bronchioles. It was also reported that CN was from three- to tenfold more toxic than CS in mice, rats, rabbits, and guinea pigs. The early deaths exhibited lesions of the upper respiratory tract, with marked pseudomembrane formation, excessive salivation, and nasal secretion. The animals that died later exhibited edema and hemorrhage of the lungs. In repeated exposures to lower individual concentrations than in the acute exposures for 10 consecutive days in guinea pigs, dogs, and monkeys, the toxicity of CN was found to be considerably less when administered in divided doses. Overall, studies demonstrated a lack of cumulative toxicity. Changes in biochemical endpoints measured following multiple exposures of CN and CR in mice were a decrease in hepatic glutathione and increased lipid peroxidation. Hepatic acid phosphatase increased after a 5 day exposure to CN, and the glutathione levels decreased after a 10 day CN exposure. CN-induced elevation in acid phosphatase levels reflected the release of lysosomal enzymes from the liver, indicative of tissue injury. CR exposure did not produce significant alterations in hepatic biochemical parameters. Additionally, hyperglycemia was observed after exposure to CN. Stress-mediated release of epinephrine is known to elevate glucose levels and thus may be responsible for the hyperglycemia. Significant decreases in body weight gain were also noted on exposure to these compounds with CN having a more prominent effect on body weight. These findings were consistent with results on the repeated dose effects of orally administered CR in various animal species. Histopathologic changes following CN exposures included hemorrhage, perivascular edema, congestion of the alveolar capillaries, occluded bronchioles, and alveolitis. Renal histopathology demonstrated congestion and coagulative necrosis in the cortical tubules in CN-exposed mice. Hepatic histopathology consisted of cloudy swelling and lobular and centrolobular necrosis of hepatocytes following CN exposures.

CN, particularly in solutions, is more likely to cause more serious eye effects than CS. At high concentrations, CN may result in chemical injury to the eye with corneal and conjunctival edema, erosion or ulceration, chemosis, and focal hemorrhages. CN-induced ocular effects on the rabbit eye following treatment with various formulations included lacrimation, chemosis, iritis, blepharitis, and keratitis, with severity dependent on the formulation.

CN is also a potent skin irritant, more likely to cause more serious injury to the skin than CS. These effects include diffuse and intense erythema, severe

edema, and vesication. CN is considered a more potent skin irritant and sensitizer than CS.

In 2 year carcinogenicity inhalation bioassays in rats and mice, there was no indication of carcinogenicity in male rats, while equivocal evidence was found in female rats. These findings were evidenced by increased fibroadenomas of the mammary gland. In these 2 year studies in mice, there was no evidence of carcinogenic activity in males and females.

In the body, CN is converted to an electrophilic metabolite. It is an SN2 alkylating agent that reacts with SH groups and other nucleophilic sites of biomolecules. Alkylation of SH-containing enzymes leads to enzyme inhibition with disruption of cellular processes. CN was found to inhibit human plasma cholinesterase via a non-SH interaction, and some of the toxic effects may be due to alkylation of SH-containing enzymes.

Human Toxicology

The incapacitating effects of CN in human volunteers during exposure include lacrimation, some blurring of vision, and conjunctivitis. On the nose and throat, CN causes a tingling sensation, irritation, pain, and some increases in secretions, while on the respiratory tract it causes irritation, burning, and pain. CN on the skin causes burning in the periorbital area, and other areas of tender skin, especially where sweating is present. Occasionally, nausea and gagging occur during and soon after exposure. Most of these effects disappear within 20 min after exposure, but conjunctivitis and blepharospasm usually disappear after a few days leaving no after effect. Incapacitating concentrations (IC_{50}) of CN have ranged from 20 to 50 mg min m^{-3}. The IC_{50} for CN is comparable to that for adamsite (DM), which was an early RCA that was replaced by CN. The IC_{50} values for CN and DM are greater than for CS. The estimates of human LCt_{50} values, extrapolated from animals exposed to CN dispersed from a solvent, is 7000 mg min m^{-3}, and 14 000 mg min m^{-3} when dispersed from commercially available grenades. Other estimates range from 8500 to 25 000 mg min m^{-3}. The maximum safe inhalation dosage of CN for humans has been estimated to be 500 mg min m^{-3}.

Human volunteers were tested in a wind tunnel at an air speed of five miles per hour (mph) to establish the length of time a subject could remain in the CN-containing air stream. The tolerance time varied with the subject. CN aerosols were generated from acetone solutions at a Ct of 350 mg min m^{-3} with a mass median diameter of about 0.6 μm. The immediate effects of exposure were tingling of the nose and rhinorrhea, burning of the eyes and throat, lacrimation, and blurred vision. Some subjects suffered dyspnea. These effects disappeared rapidly when the subjects left the wind tunnel. Acute injuries to the eyes, primarily from the effects of blast and missiles, may occur from tear gas weapons, such as pen guns. The immediate effects of these injuries include swelling and edema of the lids with penetration of skin, conjunctivitis, cornea, sclera, or globe by gunpowder and CN. Conjunctival ischemia and chemosis, corneal edema, erosion, inflammation or ulceration, and focal hemorrhage have also been reported just as injuries resulting from accidental discharges of tear gas guns at close range have been. Surgery was required in these cases to relieve pain and to remove foreign material. They all suffered continuing pain and some loss of sensation, apparently from the toxic action of CN on nerves.

2-Chlorobenzylidene Malononitrile

General Considerations

In addition to the nonpersistent form of 2-chlorobenzylidene malononitrile (CS), two hydrophobic variations were created, CS1 and CS2. CS1 is a micronized powder formulation containing 5% hydrophobic silica aerogel, which can persist for up to 2 weeks in normal weather conditions and CS2 is a siliconized microencapsulated form of CS1 with a long shelf life, persistence, resistant to degradation, and ability to float on water which could restrict or deny the use of water for military operations. CS is commonly used as an RCA and a simulant for training. Members of military organizations and law enforcement agencies are routinely exposed to heated CS during training. The heat vaporizes the CS for dispersion, which thus condenses to form an aerosol.

Repeated exposures of thermally dispersed CS were conduced in rats and dogs. They were exposed from 4 to 5 min day^{-1}, 5 days $week^{-1}$ for 5 weeks. The 25 day cumulative dosage (Ct) which the rats were exposed to was 91 000 mg min m^{-3} (3640 mg min m^{-3} per day), while the dogs were exposed to a cumulative dosage of 17 000 mg min m^{-3} (680 mg min m^{-3} per day). No lethality occurred in the dogs, while the rats became hyperactive and aggressive, biting noses and tails of other rats, and scratching their own noses. No changes were found in blood values for sodium, potassium, protein, albumin, or creatinine throughout the tests. Five of the 30 rats exposed died, two following the cumulative dosage of 25 000 mg min m^{-3}, and three died after 68 000 mg min m^{-3}. Gross pathological

examination of the rats that died was negative, as were those of six other rats that were sacrificed after 5 weeks of exposure. The exposed rats lost ~1% of body weight, while unexposed rats gained ~20% during the 5 weeks. There were no significant differences in organ to body weight ratios for heart, kidneys, lungs, liver, or spleen following the 5 week exposures. It was concluded that repeated exposures did not make the animals more sensitive to the lethal effects of CS. The animals that died after exposure to CS showed increased numbers of goblet cells in the respiratory and gastrointestinal tracts and conjunctiva, as well as necrosis in the respiratory and gastrointestinal tracts, pulmonary edema, and occasionally hemorrhage in the adrenals. Death appeared to result from poor transfer of oxygen from the lungs to the blood stream, probably because of edema, and hemorrhage in the lungs, and obstruction of the airways. The effects of repeated exposures to CS were studied in mice, rats, and guinea pigs to neat CS aerosols for 1 h day^{-1}, 5 days week^{-1} for 120 days. High concentrations of CS were fatal to the animals after only a few exposures, while mortality in the low and medium concentrations did not differ significantly from the controls. It was concluded that CS concentrations below 30 mg m^{-3} were without deleterious effects. Acute inhalation toxicity of CS which was generated in smoke and as an aerosol was studied in several animal species. The LCt_{50} data are presented in **Table 3**.

Other lethality estimates for CS are given below. From acute exposures to CS dispersed from a 10% CS in methylene dichloride the LCt_{50}s were as follows: mice, 627 000 mg min m^{-3}; rats, 1 004 000 mg min m^{-3}; and guinea pigs, 46 000 mg min m^{-3}. No deaths occurred in rabbits exposed to up to 47 000 mg min m^{-3}. CS at dosages up to 30 000 mg min m^{-3} did not cause any deaths in any of the monkeys with pulmonary tularemia. The combined LCt_{50} for mice, rats, guinea pigs, and rabbits was calculated to be 1 230 000 mg min m^{-3} for CS dispersed from methylene dichloride. Goats, pigs, and sheep did not exhibit hyperactivity on exposure to CS, and they were also resistant to its lethal effect. Therefore, no LCt_{50} values could be calculated for goats, pigs, or sheep. However, a combined LCt_{50} was calculated for all of the species tested, mice, rats, guinea pigs, rabbits, dogs, monkey, goats, pigs, and sheep, and was estimated to be 300 000 mg min m^{-3}. LCt_{50}s were also calculated for CS dispersed from M18 and M7A3 thermal grenades. These were 164 000 mg min m^{-3} for rats and 36 000 mg min m^{-3} for guinea pigs exposed to the M18 thermal grenade dissemination, and for the M7A3 thermal grenade the values were as follows: rats, 94 000 mg min m^{-3}; guinea pigs, 66 000 mg min m^{-3}; rabbit, 38 000 mg min m^{-3}; goat, 48 000 mg min m^{-3}; pigs, 17 000 mg min m^{-3}; dog, 30 000 mg min m^{-3}; monkey, 120 000 mg min m^{-3}. All of the acute exposure results were combined and LCt_{50} values were calculated for all rodents to be 79 000 mg min m^{-3}, and for all nonrodent species tested the value was calculated to be 36 000 mg min m^{-3}, and for all the species it was 61 000 mg min m^{-3}. The LCt_{50} values for CS2 was also calculated. CS2 is 95% CS, 5% Cal-o-Sil R, and 1% hexamethyldisilazane, and the LCt_{50} values are: rats, 68 000 mg min m^{-3}; guinea pigs, 49 000 mg min m^{-3}; dogs, 70 000 mg min m^{-3}; and monkeys, 74 000 mg min m^{-3}. The lethal effects in animals following inhalation exposures is caused by lung damage leading to asphyxia and circulatory failure, or bronchopneumonia secondary to respiratory tract injury. Pathology involving the liver and kidneys following inhalation of high dosages of CS is also secondary to respiratory and circulatory failure.

Table 3 Acute inhalation toxicity LCt_{50} (mg min m^{-3}) values for CS smoke and aerosols to various species

	CS smoke	*CS aerosol*
Guinea pig	35 800	67 000
Rabbit	63 600	54 090
Rat	69 800	88 480
Mouse	70 000	50 110

Various experimental animal species were exposed to aerosols of CS generated by various methods of exposure from 5 to 90 min. The toxic signs observed in mice, rats, guinea pigs, rabbits, dogs, and monkeys were immediate, and included hyperactivity, followed by copious lacrimation, and salivation within 30 s of exposure in all species except the rabbit. The initial level of heightened activity subsided, and within 5–15 min following initiation of the exposure, exhibited lethargy and pulmonary stress, which continued for about 1 h following cessation of the exposure. All other signs had disappeared within 5 min following removal from the exposure. When toxic signs were observed, they occurred following exposure by all of the dispersion methods.

The effects of CS inhalation were studied on embryonic development in rats and rabbits at concentrations consistent with those expected in riot control situations (~10 mg m^{-3}). Although the concentrations were low and the duration of exposure (5 min) may not have been adequate to assess the fetotoxic and teratogenic potential of CS, no significant increase in the numbers of abnormal fetuses or resorptions were noted. The mutagenic potential of CS and CS2 were studied in microbial and mammalian

bioassays. CS was positive in the Ames assay, while others reported questionable genotoxicity for *Salmonella typhimurium*, and negative when tested in *S. typhimurium* strains TA 98, TA 1535, and TA 1537 with and without metabolic activation. The mutagenic potential for CS and CS2 in mammalian assays such as the Chinese hamster ovary (CHO) test for the induction of sister chromatid exchange (SCE) and chromosomal aberration (CA), and the mouse lymphoma L5178Y assay for induction of trifluorothymidine (Tfi) resistance indicated that CS2 induced sister chromatid exchange, chromosomal aberrations, and Tfi resistance. The Committee on Toxicology of the National Research Council reported that taken in their totality, the test of CS for gene mutation and chromosomal damage provide no clear evidence of mutagenicity. Although most of the evidence is consistent with a lack of mutagenic potential, in the committee's judgment it is unlikely that CS poses a genotoxic hazard to humans. CS2 was evaluated for carcinogenicity in the NTP 2 year rodent bioassay. Compound related nonneoplastic lesions of the respiratory tract were observed. The pathologic changes observed in the exposed rats included squamous metaplasia of the olfactory epithelium, hyperplasia, and metaplasia of the respiratory epithelium. In mice, hyperplasia and squamous metaplasia of the respiratory epithelium was observed. Neoplastic effects were not observed in either rats or mice, and it was concluded that the findings suggests that CS2 is not carcinogenic to rats and mice CS in methylene chloride was also tested in mice and rats for carcinogenicity in a 2 year study, and no tumorigenic effects were observed in the CS exposed animals.

CS is absorbed very rapidly from the respiratory tract, and the half-lives of CS and its principal metabolic products are extremely short. The disappearance of CS follows first-order kinetics and spontaneously hydrolyses to malononitrile, which is transformed to cyanide in animal tissues. Metabolically, CS undergoes conversion to 2-chlorobenzyl malononitrile (CSH2), 2-chlorobenzaldehyde (oCB), 2-chlorohippuric acid, and thiocyanate. CS and its metabolites can be detected in the blood after inhalation exposure, but only after large doses. Following inhalation exposure of CS in rodent and nonrodent species, CS and two of its metabolites, 2-chlorobenzaldehyde and 2-chlorobenzyl malononitrile, were detected in the blood. In another study, human uptake by the respiratory tract, only 2-chlorobenzyl malononitrile was detected in trace amounts in the blood. CS and 2-chlorobenzaldehyde were not detected, even after high doses of CS of up to $90\,mg\,min\,m^{-3}$. This finding is consistent with the CS uptake studies in animals, and with the maximum tolerable concentration in humans, which is below $10\,mg\,m^{-3}$, it is unlikely that significant amounts of CS would be absorbed by the inhalation route at or near the tolerable concentrations. Experiments were conducted to determine the CS metabolite thiocyanate in humans exposed to amounts of CS that are intolerable. In dogs, exposure to $48\,000\,mg\,min\,m^{-3}$ of CS aerosol showed an unimpressive increase in plasma and urine thiocyanate concentration 24 h after exposure. These were lower than those observed in human subjects who smoked cigarettes. Smoking and nonsmoking human volunteers were exposed to doses up to $1.1\,mg\,min\,m^{-3}$ of CS (intolerable). Plasma and urine levels were significantly higher in smokers than in nonsmokers, and exposure to CS did not cause any significant increases in plasma and urine thiocyanate levels. Plasma and urine thiocyanate levels were measured in human volunteers following exposure to intolerable airborne concentrations of CS. Since cigarette smoking also increases thiocyanate in body fluids, levels in nonsmokers, light smokers, and heavy smokers, before and after CS exposure, were compared. There was no statistical difference in plasma or urine thiocyanate concentration between nonexposed and CS exposed volunteers. However, both light and heavy smokers' concentrations were significantly higher than nonsmokers. Thus, it was concluded that plasma and urine levels of thiocyanate CS metabolite are not high enough to detect following human exposure to intolerable levels of CS.

Human Toxicology

Exposure to CS is highly irritating to the mucous membranes that line the tissues of the eyes, nose, throat, and respiratory and gastrointestinal tracts. Irritation of the eyes may cause pain, excessive tearing, conjunctivitis, and blepharospasm (uncontrolled blinking). The nose and mouth may perceive a stinging or burning sensation with excessive rhino rhea or discharge of nasal mucous. Irritation of the respiratory tract may cause tightness of the chest, sneezing, and coughing, as well as increased respiratory secretions. Severe lung injury and subsequent respiratory and circulatory failure characterize death in experimental animals following inhalation of very high dosages of CS. Irritation of the gastrointestinal tract may cause vomiting and/or diarrhea. Following exposure of the skin, a burning sensation may be experienced, with subsequent inflammation and redness. Six minutes following exposure to CS, the irritation during exposure is so intense that the individual exposed seeks to escape.

When exposed to CS aerosols generated from solutions in acetone or methylene chloride or from thermal grenades at 3.0, 1.0, 0.5 μm MMAD, many untrained subjects were unable to don and retain their masks at low concentrations of CS, but at high concentrations were able to mask well enough to remain in the contaminated atmosphere. When properly fitted these masks will fully protect against CS. In those who were unable to mask rapidly, panic was evident. Concentrations of 9–10 $mg\,m^{-3}$ forced 50% of the subjects to leave the chamber within 30 s, 99% left at ~17 $mg\,m^{-3}$, and 100% left and were considered incapacitated at 40 $mg\,m^{-3}$ or greater. Persons who had been exposed previously to a high concentration developed a fear of the agent, and even though subsequently exposed to a lower concentration, the time to incapacitation for trained men was shorter than expected. There were no significant differences noted in the time to incapacitation in subjects exposed to CS at 0–95°F, although it appeared that the subjects appeared unable to tolerate the agent as well as those exposed at ambient temperature. At 95°F and relative humidity of 35% and 97% the skin-burning effects were much more prominent, possibly because of the excessive diaphoresis. Hypertensive subjects reacted similarly to and tolerated CS as well as normotensive individuals. However, their blood pressure elevation was greater and lasted longer than in normotensives, possibly because of the stress of exposure. The hypertensive subjects recovered as rapidly as the normotensives. Subjects with a history of peptic ulcer, jaundice, or hepatitis, and those between the ages of 50 and 60 reacted similarly to normal subjects. Persons with a history of drug allergy, hay fever, asthma, or drug sensitivity were able to tolerate CS exposure as well as the normal subjects; however, a higher percentage of this group had more severe chest symptoms than the normals. Although many of them lay prostrate on the ground for several minutes, no wheezing or ronchi were heard on ausculation, and recovery time was as rapid as for any other group tested. Hyperventilating subjects were incapacitated at much lower concentrations than normally breathing subjects, and recovery time was slightly prolonged, but only by 1–2 min. Although not significantly different, subjects exposed to CS disseminated from methylene dichloride appeared to tolerate the agent for a slightly longer period that those subjected to CS in acetone solution, nor was there any difference in CS disseminated from the miniature M18 CS smoke grenade. There was also a group exposed to a combination of CS and DM. The effects of DM were negligible when CS was effective within 30 s.

In experiments where only the eyes and respiratory tract of human volunteers were exposed to small (0.9 μm) and large (60 μm) particles of CS, the small particle size was more effective in producing eye irritation. Only two of the five men exposed to the 0.9 μm aerosol were able to tolerate the CS for 60 s, while all six men exposed to the large sized aerosol remained in the cloud for at least 60 s. Following exposure, all subjects had difficulty in seeing. Recovery times were based on the subjects' ability to sort and arrange cards. Recovery following exposure of the eyes to small particles averaged 90 s, while it took ~280 s following exposure to the large particle. The respiratory effects of exposure of the small particles were more dramatic. None of the six men could tolerate the small particles for longer than 30 s while four of the six men tolerated the larger particle exposure for at least 60 s.

A group of seven volunteers given 10 exposures of CS from 1 to 13 $mg\,m^{-3}$ in a period of 15 days revealed no clinical abnormalities. The dominant effect of the first exposure remained the dominant effect on subsequent exposures. None of the volunteers developed a tolerance to CS during the 10 exposures.

The immediate effects upon exposure to aerosols of CS were on the eyes, and were demonstrated by severe conjunctivitis accompanied by a burning sensation and pain that persisted from 2 to 5 min and usually disappeared abruptly rather than gradually. The conjunctivitis remained intense for up to 25–30 min. Erythema of the eyelids was generally present, persisted for 1 h, and was occasionally accompanied by blepharospasm. Lacrimation was invariably present, tended to be profuse and lingering for up to 12–15 min. The occasional 'tired feeling' in their eyes lasted for about 24 h. Photophobia, which was quite marked in 5–10% of the volunteers, remained for up to 1 h. On repeated exposures, the eye effects were reproduced. Rhinorrhea and salivation were profuse and persisted for up to 12 h.

The effects on the respiratory system appeared to be dependent on the duration of exposure and the depth of respiration. The first symptom was usually a burning sensation beginning in the nares and throat and then progressing down the respiratory tract, sometimes associated with coughing. As the exposure continued, the burning became painful and was rapidly followed by a 'constricting sensation' throughout the chest, which caused incapacitation for several minutes. Panic usually accompanied and accentuated this symptom, and these volunteers appeared unable to inhale or exhale. Fresh air and encouragement abated these effects. Auscultation of the chest immediately after exposure did not reveal wheezing, rales, or ronchi. Airway resistance

measured by an Asthmometer showed no significant changes, and a portable breath recording apparatus measured breathing patterns of exposed individuals. The patterns indicated that when the aerosol was inhaled, the subjects involuntarily gasped, and then held their breath or breathed slowly and shallowly. This was followed by short paroxysms of coughing that forced the individual to exit the exposure. An irregular respiratory rhythm was noted for several minutes after exposure was terminated. Many of the exposed individuals were aphonic for 1–2 min post-exposure, and several were hoarse for 24 h. It was concluded that the incapacitation cause by CS was due to the effects on the eyes, respiratory tract, or both, but regarded the effects on the respiratory system as potentially the most capable of causing incapacitation. A group of volunteers in a wind tunnel, wearing a self-contained remotely controlled breath-recording system, was exposed to 5–150 μg CS per liter for 110–120 s. Although the breathing patterns were disrupted by the CS exposure, adequate ventilation of the lungs was maintained, so they concluded that incapacitation is attributed to the unpleasant sensations rather than to any degree of respiratory failure. The apnea and cardiovascular changes observed following inhalation exposure to CS is not inconsistent with the Kratschmer reflex. These investigators also reported that sneezing was common among the observers exposed to small concentrations of CS at some distance from the exposure chamber.

Inhalation toxicity studies by aerosol dispersions of melted agents sprayed in the molten form, dry powder dispersion, sprayed from solutions of acetone or methylene dichloride, or dispersed from grenades by liberation of hot gases have been performed since World War I. Prior to the research on CS in 1958 and 1959, no toxicity studies were performed using munitions. In 1965, munitions studies were conducted with CN and DM. All of these studies demonstrated that munitions dispersed agents were less toxic than dispersion by other methods. The human LD_{50} value, based on the combined animal species toxicity data, is 52 000 mg min m^{-3} for CS by molten dispersion, and 61 000 mg min m^{-3} dispersed by the M7A3 grenade.

Although no fatalities have been validated following exposure to CS, there have been several cases of serious consequences. A documented case of pneumonia is reported in a normal 4-month-old white male infant exposed to CS gas for 2–3 h. Immediately when taken to the emergency room he was observed to have copious nasal and oral secretions, sneezed and coughed frequently, and required suction to relieve upper airway obstruction. The pneumonitis was treated aggressively and the patient was discharged from hospital on the 12th day. However, within 24 h the infant was returned to the emergency room and was rehospitalized. A repeat chest roentgenogram demonstrated a progression of the pulmonary infiltrates. Following treatment with antibiotics the chest roentgenogram was clear on the 17th day, and improvement continued and the patient was discharged after 28 days of hospitalization.

Another reported case of serious intoxication with CS tear gas was 11 days following a thorough internal medical examination that revealed no clinical or pathological findings, when a 43-year-old male was in a room in a cloud of fumes from a CS canister that a friend had ignited as a joke. Immediately he suffered from burning pains in the eyes and in his upper respiratory tract, lacrimation, and pains in his chest with dyspnea and coughing. This unusual exposure led to serious long-term complications such as toxic pulmonary edema, gastrointestinal difficulties, and indications of liver damage and passing right heart insufficiency. After 3 months of hospitalization, all tests were negative, and the patient was discharged to his home in a condition capable of work.

A reported case of major hepatitis attributable to CS inhalation exposure was described where a 30-year-old incarcerated male was sprayed with CS and was hospitalized 8 days later with erythroderma, wheezing, pneumonitis with hypoxemia, hepatitis with jaundice, and hypereosinophilia. For months he continued to suffer from generalized dermatitis, recurrent cough and wheezing consistent with reactive airway dysfunction syndrome, and eosinophilia. Systemic corticosteroids were successful, but abnormalities recurred off treatment. Although the dermatitis resolved gradually over 6–7 months, the asthma-like symptoms persisted a year after exposure. Patch testing confirmed sensitization to CS. The mechanism of the prolonged reaction is unknown, but may involve cell-mediated hypersensitivity, perhaps to adducts of CS, or a metabolite, and tissue proteins. The investigators reported this as the first documented case in which CS apparently caused a severe, multisystem illness by hypersensitivity rather than direct tissue toxicity.

Other human exposures were reported on the alleged use of tear gas in almost every major city in South Korea in June of 1987, where over 350 000 uses of CS tear gas was carried out by the government against civilians who exhibited cough and shortness of breath for several weeks. Hospitalized patients with asthma and chronic bronchitis, exposed to CS wafting through hospital wards through open windows, experienced deterioration in lung function. Persons close to the exploding tear gas

canisters and grenades sustained penetrating trauma from plastic fragments that was exacerbated by the tear gas. Lack of information and objective as well as epidemiological studies was due to fear of serious government reprisals.

There were also allegations that exposures to tear gas in Gaza and the West Bank of Israel have been associated with increases in miscarriages and stillbirths. Inquiries, by groups such as Amnesty International and Physicians for Human Rights, prompted a Government Accounting Office (GAO) investigation requested by Congressman Ronald Dellums. The GAO Report (1989) concluded that the Physicians for Human Rights fact-finding trip could not confirm any deaths linked to tear gas inhalation, nor could they substantiate the rumors of increased miscarriages. There was also no verifiable evidence available to conclude linking tear gas exposure to fetal deaths. In addition, the US State Department reported that they did not have any medical evidence to support a direct causation between tear gas inhalation and the number of deaths and miscarriages alleged. The exaggerated number of almost 400 deaths attributed to the use of tear gas by the Israeli Defense Forces (IDF) has also been repudiated by the State Department. They have concluded that at least four deaths had resulted from tear gas use in enclosed areas, and that the IDF was using primarily CN at the time.

The use of CS by the US forces in Vietnam in the years 1964–72 was to flush the enemy from bunkers and tunnels, reduce the ability of the enemy to deliver aimed fire while attacking, and to deny fighting positions and infiltration routes for extended periods of time.

Interest and possible concern developed about the adverse effects of chemicals employed in peacekeeping operations in the United Kingdom following the use of CS by the Ulster Constabulary in Londonderry, Northern Ireland, on 13 and 14 August 1969. As a result of this first use of CS for crowd control, a Committee of Inquiry was established to determine the medical effects, if any, in persons exposed to CS. Their report known as the Himsworth Reports, described that on exposure to various concentrations of CS, the effects vary from a slight prickly or peppery sensation in the eyes and nasal passages up to the maximum symptoms of profuse lacrimation, and salivation from the eyes and nose, spasm of the eyelids, retching and sometimes vomiting, burning of the mouth and throat, cough, and gripping pain in the chest. Even at low concentrations, the onset of symptoms is immediate, and they disappear when removed from the exposure. Of the many tens of thousands of military personnel in the United Kingdom who were exposed to CS in the course of their training, as well as those of the US military who undergo similar training, the signs and symptoms were similar to those described above, and there were no significant after effects. All the US military personnel exposed to CS in training and under field conditions reported similar effects. They also reported that a cluster of nine US Marine Corps Amphibious Reconnaissance students required hospitalization with pulmonary edema after strenuous exercise following exposure to CS. These patients did not become symptomatic until 36–40 h after the CS exposure and did not demonstrate evidence of airway dysfunction. It was proposed that these cases attributed to the acute pulmonary effects of CS more likely represented a cluster of incidents of either water aspiration or swimming induced pulmonary edema. Water aspiration is a well-described cause of pulmonary edema. No details were provided in the report as to whether the symptomatic marines aspirated pool or sea water or whether they were breath-hold-diving, but all became symptomatic immediately after pool or open ocean 1000–1500 m swims. Even when patients do not recall specific aspiration incidents while in the water, pulmonary edema has been described in divers and swimmers who have been immersed in cold water and strenuous swimming alone has been reported as a cause of pulmonary edema. Similar cases of pulmonary edema associated with immersion occurred at the US Basic Underwater Demolition/Seal School as well as at the Israel Naval Medical Institute. The case definition of pulmonary edema associated with immersion includes hypoxemia and radiograph air space filling that occurs during or immediately after swimming, followed by resolution of symptoms or radiographic improvement by greater than 50% within 48 h. On exposure, the eyes are red, but this disappears on leaving the contaminated atmosphere. On the skin CS causes a burning sensation on the exposed parts that can be followed by redness or the appearance of small blisters or vesicles at the points of friction. These effects are more prevalent in fair skinned persons especially if the skin is hot and moist. The Himsworth Committee reported that infants asleep in rooms where CS entered via broken windows were sufficiently distressed to awaken them crying from sleep. On snatching them out of the contaminated atmosphere, they quieted rapidly and required no hospitalization. They also found no special susceptibility to CS associated with old age. Human volunteers and members of the Himsworth committee over 50 years of age were exposed to $35\,\mathrm{mg\,m^{-3}}$ and the symptoms experienced and the time to recover from these were no

different from those in young adults. Exposure to CS was determined not to have had any effect on pregnancy since comparison of the 9 months following exposure compared to the 9 months of the previous year demonstrated no difference in abortions, stillbirths, or congenital abnormalities. Middle aged and elderly people who had chronic bronchitis and had been significantly exposed to CS did not show exacerbation different than that caused by natural causes. Following the riots of 1969, there was no increase in the death rate from chronic bronchitis and asthma. Asthmatics, especially children who were exposed to CS, did not show any difference in the number of attacks from their experience prior to the exposure. The committee reported that there is ample evidence that if CS causes unconsciousness in humans, it can do so only rarely and that many, if not all of the cases reported are more probably the result of other conditions that occur in riot situations. In animals, unconsciousness does not occur after inhaling CS. The Himsworth reports, considered to be the most extensive study of the use of CS agent on humans, by United Kingdom forces in Northern Ireland in the late 1960s, found that no deaths and no long-term injuries resulted from the widespread use of CS agent there.

Contamination of the skin with solutions of CS causes transient rises in both systolic and diastolic blood pressure. Contamination of the eye with solutions of CS also causes increases in blood pressure, together with transient rises in intraocular pressure.

Dibenz(*b*,*f*)-1:4-Oxazepine

Animal Toxicology

The mammalian toxicology in various animal species indicates that the acute toxicity (LD_{50} and LCt_{50}) of dibenz(*b*,*f*)-1:4-Oxazepine (CR) is less than that of CS and CN by all routes of exposure. Animals exposed to CR exhibited ataxia or incoordination, spasms, convulsions, and tachypnea or rapid breathing. In the animals that survived, these effects gradually subsided over a period of 15–60 min. Increasing respiratory distress preceded death. The animals that died following intravenous and oral administration demonstrated congestion of liver sinusoids and alveolar capillaries. At necropsy, the surviving animals did not show any gross or histological abnormalities. The toxic signs following intraperitoneal administration included muscle weakness and heightened sensitivity to handling. These effects persisted throughout the first day of exposure. Some animals also exhibited central nervous system effects. Surviving animals did not exhibit any gross or histological abnormalities at necropsy. Several animal species were exposed to the acute inhalation of CR aerosols and smokes for various time periods. Rats exposed to aerosol concentrations from 13 050 to 428 400 mg min m^{-3} manifested nasal secretions and blepharospasm or uncontrollable closure of the eyelids, which subsided within 1 h after termination of the exposure. There were no deaths during or following these exposures. There were also no deaths in rabbits, guinea pigs, or mice exposed to CR aerosols of up to 68 000 mg min m^{-3}. Animals exposed to CR smoke generated pyrotechnically, had alveolar capillary congestion, and intra-alveolar hemorrhage, as well as kidney and liver congestions.

The potential of CR aerosols to produce physiological and ultrastructural changes in the lungs was evaluated by electron microscopy. Rats exposed to CR aerosols of 115 000 mg min m^{-3} did not reveal any effects on organelles such as lamellated osmiophilic bodies. Lungs of animals exposed to aerosols of CR at dosages of 78 200, 140 900, and 161 300 mg min m^{-3} were found to appear normal on gross examination. On microscopic examination, however, the lungs revealed mild congestion, hemorrhage, and emphysema. Electron microscopy showed isolated swelling and thickening of the epithelium, as well as early capillary damage, as evidenced by ballooning of the endothelium. It was concluded that these very high dosages of CR aerosols produced only minimal pulmonary damage.

Repeated inhalation exposures in mice and hamsters to concentrations of 204, 236, and 267 mg m^{-3} CR for 5 days $week^{-1}$ for 18 weeks produced death in both species at the high concentrations, but no single cause of death could be ascertained, although pneumonitis was present in many cases. Chronic inflammation of the larynx was observed in mice. Although alveologenic carcinoma was found in a single low dose and a single high dose group of mice, the findings and conclusions were questioned because the spontaneous occurrence of alveologenic carcinoma is high in many mouse strains. Further, this tumor type differs in many respects from human lung tumors. No lung tumors and no lesions were found in hamsters exposed to CR aerosols. Histopathology revealed hepatic lesions in mice, but these were of infectious origin, and not CR related. CR exposures at high concentrations reduced survivability and produced minimal organ specific toxicity at many times the intolerable human dose, which has been reported as 0.7 mg m^{-3} (IC_{50}) within 1 min and 0.15 mg m^{-3} (IC_{50}) within a minute. The effects in rats of CR and CN aerosols on plasma glutamic oxaloacetic transaminase (GOT), plasma glutamic pyruvate transaminase (GPT), acid phosphatase, and alkaline

Table 4 Comparative acute animal toxicity LCt_{50} (mg min m^{-3}) values for CR, CS, and CN to various species

	Pyrotechnically generated			*Aerosol*		
	CR	*CS*	*CN*	*CR*	*CS*	*CN*
Mouse	203 600	76 000	No data	169 500	67 200	18 200–73 500
Rat	139 000	68 000	23 000	428 400	88 460	3 700–18 800
Rabbit	160 000	63 000	15 800	169 000	54 100	5 840–11 480
Guinea pig	ND[a]	ND	ND	169 500	50 010	3 500–13 140

[a] ND = no data.

phosphatase exhibited no change in any of these parameters for CR, while there were significant increases in all of these parameters in rats exposed to CN, suggesting that CN could cause tissue damage.

Comparative data for acute inhalation toxicity to various animal species for CR, CS, and CN are presented in **Table 4**.

The cardiovascular effects of CR administered intravenously demonstrated a dose-dependent increase in blood pressure of short duration and an increased heart rate and arterial catecholamines. The cardiovascular effects of CR were postulated to be related to sympathetic nervous system effects as evidenced by the abolition of CR induced presser effects by phentolamine and 6-hydroxydopamine.

Repeated cutaneous application of CR was conducted in experimental animals 5 days week^{-1} for 12 weeks with little effect on the skin. In view of the absence of any specific organ effects, it was postulated that absorption of even substantial amounts of CR would have little effect.

Mild and transitory eye effects such as mild redness and mild chemosis were observed in rabbits and monkeys after a single dose of 1% CR solution. Multiple doses over a 5 day period, of 1% CR solution to the eye produced only minimal effects. No signs of eye irritation in animals following single or multiple dose applications of 1% CR solutions was reported while moderate conjunctivitis following the application of 5% CR solution to the eyes of rabbits was reported. Although histological examination revealed normal corneal and eyelid tissues, aerosol exposures of 10 800 and 17 130 mg min m^{-3} resulted in mild lacrimation and conjunctival injection, which cleared in 1 h, while in solution, produced reversible dose-related increases in corneal thickness. It was concluded that CR produced considerably less damage to the eye than CN, and that there was a much greater degree of safety for CR than for CN. On skin it was reported to produce only transient erythema, but did not induce vesication or sensitization, and did not delay the healing of skin injuries.

The reproductive and developmental effects of CR were studied on rabbits and rats exposed to inhalation of aerosolized CR at concentrations of 2, 20, and 200 mg m^{-3} for 5 and 7 min. Groups of animals were also dosed intragastrically on days 6, 8, 10, 12, 14, 16, and 18 of pregnancy. No dose-related effects of CS were observed in any of the parameters measured and the number and types of malformations observed. No externally visible malformations were seen in any group and no dose-related effects of CR were noted in any of the fetuses in any group. Based on the overall observations, the author concluded that CR was neither teratogenic nor embryotoxic to rabbits and rats.

The mutagenic potential of technical grade CR and its precursor (2-aminodiphenyl ether) in the various strains of *S. typhimurium* as well as in mammalian assay systems were negative in all the assays, suggesting that CR is not mutagenic. Further testing is required to exclude the genetic threat to humans, as well as to determine the carcinogenic potential and its ability to cause other chronic health effects.

CR aerosols are very quickly absorbed from the respiratory tract following inhalation. The plasma half-life ($t_{1/2}$) is about 5 min, which is about the same following intravenous administration. CR metabolism *in vitro* and *in vivo* supported the conclusions that the major metabolic fate of CR in the rat is the oxidation to the lactam, subsequent ring hydroxylation, sulfate conjugation, and urinary excretion.

Human Toxicology

Studies at the Edgewood Arsenal and other research centers have been conducted to assess the effects of CR on humans following aerosol exposures, drenches, and local application. The human aerosol and cutaneous studies conducted at Edgewood Arsenal have been summarized by the National Academy of Sciences. The respiratory effects following aerosol exposures included respiratory irritation with choking and difficulty in breathing or dyspnea, while the ocular effects consisted of lacrimation, irritation, and conjunctivitis. The effects of dilute CR solutions on humans following splash contamination of the face, or facial drench, were an immediate increase in blood pressure, concomitant with decreased heart

rate. Humans exposed to whole body drenches also faced the same effects of immediate hypertension and bradycardia. Although it was theorized that insufficient amount was absorbed to cause systemic effects, it was suggested that the cardiovascular effects resulted via the sympathetic nervous system. Additionally, solutions of CR contaminating the eye caused a transient increase in intraocular pressure. Following aerosol exposure to a mean concentration of $0.25\,mg\,m^{-3}$ CR with a particle size of $1–2\,\mu m\,h^{-1}$, the expiratory flow rate decreased ~ 20 min after the onset of exposure. The investigators postulated that CR stimulated the pulmonary irritant receptors to produce bronchoconstriction and increased pulmonary blood volume by augmenting sympathetic tone. The human LCt_{50} was estimated to be $100\,000\,mg\,min\,m^{-3}$, while the incapacitant IC_{50} was estimated to be $\sim 1\,mg\,m^{-3}$.

Oleoresin Capsicum

General Considerations

Oleoresin capsicum (OC), pelargonic acid vallinylamide (PAVA), and capsaicin are derived from the pepper plant. The ingredients in hot peppers that are responsible for 'the heat' are called capsaicinoids. Capsaicinoids are a family of chemicals and they come with various heat qualities. The mixture used contains the active ingredient capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) as well as other compounds. PAVA is a pepper derivative that is extremely hot. PAVA (capsaicin II) is the hottest of the capsaicin family.

OC is a reddish-brown oily liquid obtained by extracting dried, ripe fruit of chili peppers, usually *Capsicum annuum* or *Capsicum fruitescenes*. The oleoresin is a mixture of many compounds. Its composition is variable and depends on factors such as maturity of the fruit and the environment in which the plants are grown, as well as the conditions of the extraction. More than 100 compounds have been identified in oleoresin capsicum. Among the branched-and straight-chain alkyl vanillylamides isolated from oleoresin capsicum, capsaicin is the major pungent component in many peppers, and it is particularly noted for its irritant properties. Depending on the variety of chili pepper, the oleoresin contains from 0.01% to 1.0% capsaicinoids on a dry mass basis. Other components of the oleoresin such as phenolic compounds, acids, and esters may also possess irritant properties.

OC is considered a highly effective irritant that has received much attention as a less-than-lethal agent in civilian, governmental, and military sectors. OC spray or pepper spray has gained popularity as a law enforcement weapon in recent years. Since OC is a natural product, it is considered safe – a viewpoint not necessarily accurate.

Animal Toxicology

Not much is known about the toxicology of OC, but because it is a natural product and much utilized food component, it is considered to be relatively safe, with a low order of toxicity. The pharmacology and toxicology of capsaicin, on the other hand, have been well characterized in both animal and human studies. The acute toxicity of capsaicin in several species found capsaicin to be highly toxic by all routes of administration except gastric, rectal, and dermal. The intravenous doses of capsaicin caused convulsions within 5 s and times to death were from 2 to 5 min. The toxic signs observed included excitement, convulsions with limbs extended, dyspnea, and death due to respiratory failure. A comparison of intravenous LD_{50}s in mice with other well-known chemicals is presented in **Table 5**. This was done since there was no known comparative inhalation LD_{50}s in mice, as all of these chemicals and the intravenous route has been considered very close to the inhalation route of exposure. This demonstrates that capsaicin's acute toxicity in mice is between that of nicotine and strychnine, two well-known potent poisons. Additionally, the intraperitoneal acute toxicity of capsaicin to the oleoresin capsicum in female mice indicated the extract to be four times more toxic than the capsaicin with LD_{50}s of 1.51 and $6.50\,mg\,kg^{-1}$, respectively. Guinea pigs appeared to be more susceptible than mice and rats, while hamsters and rabbits were less vulnerable to the toxic actions of capsaicin.

The pulmonary pharmacology and toxicology of capsaicin has been studied in some detail. Inhalation of capsaicin is consistent with the induction of the Kratschmer reflex, which is apnea, bradycardia, and a biphasic fall and rise in aortic blood pressure.

Table 5 Mouse intravenous LD_{50} values ($mg\,kg^{-1}$) for various RCAs

Botulinum toxin	0.00001	Fentanyl	11.2
Ricin	0.005	Parathion	13
VX	0.012	DM	35
GB	0.10	CR	37
Nicotine	0.30	CS	48
Capsaicin	0.40	Caffeine	62
Strychnine	0.41	CN	81
Potassium cyanide	2.60	Cocaine	161
Mustard gas	3.30	Isopropyl alcohol	1509
Methamphetamine	10	Ethyl alcohol	1973

Exposure to capsaicin causes bronchoconstriction in animals and humans, the release of substance P, a neuropeptide, from sensory nerve terminals, as well as mucosal edema. The pulmonary effects of capsaicin appear to be species related. In guinea pigs, intravenous and intraarterial administration causes bronchoconstriction. The bronchoconstriction in the dog and cat following intravenous capsaicin is dependent on a vagal cholinergic reflex, as is the bronchoconstriction in the cat following aerosol exposure. In guinea pigs, the bronchoconstriction following aerosol exposure suggests both a vagal-cholinergic and noncholinergic local axon reflex. The cardio respiratory effects following intravenous administration resulted in a triphasic effect on blood pressure and altered cardiac parameters. The complex effects on the cardiovascular system consist of tachypnea, hypotension (Bezhold–Jarish reflex), bradycardia, and apnea.

If capsaicin or pepper spray is the preferred agent of choice for self-defense or riot control, it has been suggested that much research is required, and preferably compared in parallel to CS and CR. Alternatively, capsaicin or an analog could be synthesized and evaluated as a single agent, rather than a mixture with undetermined interactions of the multiple components. These components vary qualitatively and quantitatively dependent on their natural origin (variety, species, and maturity of fruit, hydrology, geology, and meteorology). Such a synthetic equivalent pelargonyl vanillamide (PAVA or Nonivamide), a potent sensory stimulant, has become available and is being used by police forces in the United Kingdom and other countries. The available data are very limited. PAVA is also used as a food flavor in quantities up to 10 ppm, and in human medicine as a topically applied rubifacient, as is capsaicin. In the United States, PAVA, as a food flavor, has been given GRAS (generally recognized as safe) status by the Food and Drug Administration.

Human Toxicology

OC has been incorporated into a variety of formulations and marketed as pepper gas, pepper mace, and pepper spray for self-defense, criminal incapacitation, law enforcement, and riot control purposes. It has also been formulated in combination with CS and CN for the same purposes. OC exposure induces involuntary closing of the eyes and lacrimation. It also causes respiratory related effects such as severe coughing and sneezing, nasal irritation, bronchoconstriction, and shortness of breath. It causes burning sensations of the skin and loss of motor control. As a result, many exposed individuals can be easily subdued. Acute effects of capsaicin and capsaicinoids cause edema, hypertensive crisis, and hypothermia. Since 1990, there have been over 100 deaths reported following the use of OC spray. Although a causal relationship has not been established, most of the reported deaths had occurred within 1 h following exposure. The causes of these in-custody deaths remain controversial, but the most common explanation is death from positional asphyxia. Other suggested causes that should be considered include excited delirium, heat prostration, drug interactions, cardiopulmonary sensitization, and the compromised Kratschmer reflex.

Capsaicin was prepared and being evaluated by the military as early as the 1920s in the United States. Interest in its development waned when CS was synthesized, and research efforts were redirected to the development of CS as an RCA. Unlike the other RCAs such as CS, CR and CN, which have definite chemical compositions, OC is a mixture of compounds containing capsaicinoids, various acids and esters, alcohols, aldehydes, ketones, and carotenoid pigments. Capsaicin as the major component is considered to be the active ingredient without consideration as to the activity or interactions of the other capsaicinoids or components. Although the activity of the other capsaicinoids is similar, they differ in potency.

PAVA or nonivamide, a synthetic equivalent of capsaicin, which is pelargonyl vanillamide, is a potent sensory stimulant and has become available and is being used by police forces in the United Kingdom and other countries. Although it is being used as a food flavor in quantities up to 10 ppm and in human medicine as a topically applied rubifacient, as is capsaicin, there are limited data available for its use as a self-defense spray or RCA. Following a pilot exercise by the Sussex police force in the United Kingdom, they and the Northampton police force, as well as some police forces in other European countries and in North American, are now using PAVA spray as an alternative chemical incapacitant to CS spray. The spray used is a 0.3% solution of PAVA in 50% aqueous ethanol and is dispersed from handheld canisters by a nitrogen propellant. The coarse liquid stream spray pattern is considered to be directional and precise. The maximum effective range is 8–15 ft, aimed at the subject's face, especially the eyes. Users are cautioned not to use it at a distance of less than 3 f in order to avoid pressure injury to the eyes. The particle size of the spray indicates that the bulk of the droplets are over 100 μm, but a small proportion is in the range of 2–10 μm, with trace amounts below 2 μm. Thus it is unlikely that large amounts of PAVA will reach the respiratory system.

Volunteers, including mild asthmatics, were exposed to aerosols of PAVA generated using a nebulizer that provides respiratory particles to study the effects on the respiratory and cardiovascular systems. The normal volunteers experienced transient cough on exposure, and minimal effects on FEV_1 (forced expiratory volume in one second (1% reduction)), heart rate (15% increase), and blood pressure (8% increase). Similar results were noted in mild asthmatics also exposed to 0.1% PAVA. These were 3% reduction in FEV_1, 5% increase in heart rate, and 5% increase in blood pressure. It was noted that, in actual use, subjects might experience a high level of stress that could lead to clinically significant bronchospasm. Experience did not indicate any significant adverse effects or any persistent harm to skin or eyes of those exposed. However, based on the animal experiments, it is an eye irritant, and thus might cause marked effects in subjects wearing contact lenses. In view of the limited data available, a complete assessment of its adverse health effects is not possible.

Diphenylaminochlorarsine

Animal Toxicology

Various animal species including monkeys have been exposed to diphenylaminochlorarsine (DM). Following acute exposures the animals exhibited ocular and nasal irritation, hyperactivity, salivation, labored breathing, ataxia, and convulsions. Histopathology did not reveal any abnormalities at exposure dosages of below $500\,mg\,min\,m^{-3}$. At higher dosages, animals that died or were killed demonstrated hyperemia of the trachea, pulmonary congestion and edema, and pneumonia. These effects were consistent to exposure to pulmonary irritants. DM toxicity values are presented in **Table 6**.

Monkeys were exposed to varying concentrations and durations. At a Ct dosage of $2565\,mg\,min\,m^{-3}$, only one animal responded, and that was with oral and nasal discharge, and diminished response to stimuli. A Ct of $8540\,mg\,min\,m^{-3}$ resulted in ocular and nasal conjunctival congestion, facial erythema, and decreased responses, all of which were resolved within 24 h. Exposure to the high dosage of $28\,765\,mg\,min\,m^{-3}$ resulted in hyperactivity, copious nasal discharge, conjunctival congestion, marked respiratory distress, as well as gasping and gagging in all of the exposed monkeys. Eight of these exposed monkeys died within 24 h of exposure. Necropsy of these animals revealed congestion and extremely edematous lungs. Microscopic examination revealed ulceration of the tracheobronchial tree and pulmonary edema. Studies were also conducted in which monkeys were exposed to low target concentrations of 100 and $300\,mg\,m^{-3}$ DM for 2–60 and 2–40 min, respectively. As the exposure duration increased toxic signs increased, characteristic of exposure to irritants. At the maximum dosage of $13\,200\,mg\,min\,m^{-3}$, the animals exhibited nausea and vomiting, oral and nasal discharge, and conjunctival congestion. Below $1296\,mg\,min\,m^{-3}$, the only signs were blinking.

Table 6 Acute toxicity of DM

Species	*LCt_{50} (mg min m^{-3})*	*LD (mg kg^{-1})*[a]
Mice	22 400	17.9
Rats	3 700	14.1
Guinea pigs	7 900	2.4

[a] Theoretical dose calculated from respiratory volume, LCt_{50}, and estimated percent retention.

The effects of DM on the gastrointestinal tract were suggested as a possible cause of death. Dogs were dosed both intravenously and orally with lethal doses of DM, while the following parameters were monitored: central venous pressure, right ventricular pressure, cortical electric activity, alveolar CO_2, respiratory rate, heart rate, electrocardiogram, and gastric activity. DM caused a marked elevation of both amplitude and rate of gastric activity for 15–20 min and then returned to normal. Pretreatment with trimethobenzamide, an effective antiemetic for peripheral and centrally acting emetics did not prevent DM gastric activity, but chlorpromazine was effective. The authors concluded that DM affects the stomach directly, and that the primary cause of death following exposure to DM is its effects on the lungs.

The effects of DM on the eyes and skin of rabbits were studied with DM suspended in corn oil instilled into the eyes of rabbits in doses of 0.1, 0.2, 0.5, 1.0, and 5.0 mg. No effect was observed at 0.1 mg, but at 0.2 mg, mild conjunctivitis was observed. At 0.5 mg, mild blepharitis was also seen. Corneal opacity persisted over the 14 day observation period in rabbit eyes that were dosed with 1.0 and 5.0 mg. Corn oil suspensions of DM ($100\,mg\,ml^{-1}$) were placed on the clipped backs of rabbits at doses of 1, 10, 50, 75, and 100 mg. At 10 mg and higher, necrosis of the skin was observed. The skin sensitization potential of DM in guinea pigs was negative.

Human Toxicology

The earliest human studies describing the effects of DM inhalation exposures date back to 1922. The effects begin with acute pain in the nose and sinuses followed by pain in the throat and chest, with

sneezing and violent coughing. Then there is eye pain, lacrimation, blepharospasm, rhinorrhea, salivation, nausea, and vomiting. Recovery is usually complete in 1–2 h after exposure. The onset of signs and symptoms is delayed for several minutes, unlike the onset for CS and CN, which is almost immediate. The slow onset for DM allows for the absorption of much more DM before a warning is perceived. Threshold concentrations were estimated for irritation of the throat, lower respiratory tract, and initiation of the cough reflex to be 0.38, 0.5, and 0.75 mg m^{-3}, respectively. Varying concentrations were tested on human subjects, and it was agreed that men could tolerate concentrations of 22–92 mg m^{-3} for 1 min or more, and with concentrations in a range of 22–220 mg m^{-3}, it appears to be intolerable to 50% of a population for 1 min. Dosages from 49 to 370 mg min m^{-3} have been estimated to cause nausea and vomiting. Inhalation of high concentrations has resulted in severe pulmonary damage and death. DM is considered less effective as a riot control or incapacitating agent than CS and CN, and it was conjectured that there is greater differences in susceptibility among people to DM than to the other agents. DM, like CS, is considered to be a cholinesterase inhibitor, which may be responsible for its lacrimatory effect. DM also has a direct effect on gastric activity, but evidence suggests that the lethal effect is respiratory.

Essential Summary

The toxicological effects, which are actually the pharmacological effects of RCAs, but are perceived as adverse or toxicological effects, can be local or topical as well as systemic following absorption. In addition, the effects can be acute or long term. Also, the exposure can be acute, long, or repeated. The disposition of the agent in the exposed individual also needs to be considered. That is, absorption, distribution, metabolism (biotransformation), and excretion (ADME). RCAs have been described as nonlethal or less-than-lethal agents. Exposure to these compounds involve ocular, dermal, and inhalation effects, and indirectly oral or gastrointestinal. Their primary action is the local or topical effect on the eye, which appears to be the most sensitive target organ. They also act on the skin and respiratory tract. The immediate effects on exposure to these irritants include intense irritation of the eyes, marked irritation of the nose, throat, and lungs, as well as irritation of the skin. The margin of safety or the safety ratio between the dose eliciting the intolerable effect and that dose which causes serious adverse effects is large. Examples of these are presented as human estimates for incapacitation concentrations (IC_{50}) and lethal dosages (LCt_{50}) in **Table 7**.

Table 7 Estimates of IC_{50} values (mg m^{-3}) and LCt_{50} values (mg min m^{-3}) for various RCAs

Agent	*LCt_{50}*	*IC_{50}*	*Safety ratio (LCt_{50}/IC_{50})*
CN	8 500–25 000	20–50	425–500
CR	>100 000	−1	100 000
CS	25 000–150 000	5	5 000–30 000
DM	11 000–35 000	20–150	550–233

Relevant Ocular and Cutaneous Effects

Exposure to RCAs causes an immediate stinging sensation in the eyes and tearing, resulting in a temporary disabling effect. These effects are reversible and noninjurious at low concentrations. At high concentrations, however, some irritants can cause ocular damage. Moderate injury to the eye following exposure results in corneal edema, which is reversible. Most serious injury may include corneal opacification, vascularization, scarring of the cornea, and corneal ulceration. Ocular injuries are more prevalent following use of explosive- or thermal-type tear gas devices as contrasted with solvent spray-type devices (**Table 8**).

RCAs at low concentrations also produce a tingling or burning sensation and transient erythema of the skin. At higher concentrations, agents such as CN, CS, and DM can cause edema and blistering. They can also induce an allergic contact dermatitis after an initial exposure. These effects are successfully treated

Table 8 Estimates for human ocular sensory irritancy

Compound	*Onset/action*	*Threshold concentration (mg m^{-3})*	*Intolerable concentration (mg m^{-3})*	*10 min exposure lethal concentration (mg m^{-3})*
CN	Immediate	0.3	5–30	850
CR	Immediate	0.002	1	10 000
CS	Immediate	0.004	3	2 500
DM	Rapid	1	5	650
Acrolein	Rapid	2–7	50	350
OC	Rapid			

with topical steroid preparations and oral administration of antihistamines for itching. Appropriate antibiotics can be administered to treat secondary infections.

RCAs do not usually cause long-term or permanent toxic effects, although the risk for serious toxic effects, long-term sequelae, or even death increases with higher exposure concentrations and greater exposure durations, in enclosed spaces or in susceptible individuals. Overall, however, the toxicity of acute and short-term repeated exposures to RCAs is well characterized.

See also: Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents; Blister Agents/Vesicants; Chemical Warfare During WW1; G-Series Nerve Agents; Nerve Agents; V-Series Nerve Agents: Other than VX.

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Risk Assessment, Ecological

Steven Bartell

Introduction

Managers and decision makers face daunting challenges in solving complex environmental issues associated with the ever increasing pressures that humans place on valued natural resources and their life-sustaining ecosystems. These challenges are made difficult by the large number and diversity of human disturbances and perhaps even more difficult by the complexity and dynamics of imperfectly understood natural ecological systems. The process of ecological risk assessment (ERA) was designed to address ecological complexity and incorporate uncertainty in assessing the impacts of disturbances on ecological resources.

ERA applies methods of systems analysis to integrate ecology, environmental chemistry, environmental toxicology, geochemistry, hydrology, and other fundamental sciences in estimating the probabilities of undesired ecological impacts. In theory, ERA applies to both human-induced and natural disturbances. ERA can be viewed as a subset of basic disturbance ecology. However, in practice, most of the ERAs derive from specific needs to assess human-induced impacts on the environment. Many ERAs conducted in the United States are motivated by legislation, including the National Environmental Policy Act (NEPA), the Toxic Substances Control Act (TSCA), and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA or 'Superfund'). Other ERAs are undertaken by private industry to determine future ecological risks and potential liabilities associated with the development, use, and disposal (i.e., life-cycle) of new or existing products (e.g., herbicides, pesticides, industrial chemicals).

Several different approaches for performing an ERA have been proposed both nationally and internationally. While no single methodology has been officially sanctioned, the approach outlined by the US Environmental Protection Agency is being increasingly used to guide ERAs in the United States. The following discussion focuses, therefore, on the US EPA methodology, which consists of four basic components: problem formulation, exposure analysis, effects assessment, and risk characterization. This presentation will attempt to minimize the use of jargon that has proliferated during the evolution of the ERA methodology. Thus, the discussion might

not correspond in exact detail to the ERA methodology, although the major aspects of this approach will be described.

Problem Formulation

This initial and perhaps most important part of the assessment defines the nature and scope of the ERA, describes the sources of potential risk ('stressors'), identifies the ecological resources at risk ('endpoints'), considers the nature of the ecological impacts in relation to the stressors, and produces a conceptual model of the overall assessment. Thus, problem formulation essentially encapsulates the entire ERA process. Execution of this step requires collaboration among risk managers and risk assessors to define the assessment objectives and develop the corresponding conceptual model. This model, like most, should be viewed as dynamic and subject to change throughout the ERA in relation to modifications to the objectives and the development of new data and information.

Much of the original emphasis on ERA concerned the ecological effects of toxic chemicals. Importantly, the continuing evolution of ERA frameworks reflects the recognition that physical, geological, hydrologic, and biological stressors can produce undesired ecological effects. Examples of these kinds of stressors include physical habitat degradation, erosion of soils or sediments, drought/floods, and introductions of exotic species. Therefore, the set of stressors addressed by ERA has expanded to include other kinds of disturbance, some of which are influenced by human activities (e.g., introduction of exotic species, hydrology, climate change).

In assessing risks posed by toxic chemicals, 'exposure' (see exposure analysis below) refers to mechanisms of direct contact, ingestion, inhalation, or indirect accumulation through the consumption of contaminated food. The concept of exposure has been expanded to include analogous pathways and mechanisms that define the intersection in space and time of other stressors with individual organisms or their ecological support systems.

The large number and different kinds of ecological effects that are of potential concern distinguish, in part, ERA from more traditional human health risk assessment. An ERA might address alterations in basic physiological processes (e.g., photosynthesis, respiration), lethal or sublethal (i.e., growth) effects on individual organisms, changes in population dynamics (e.g., growth rate, fluctuations, local extinction), alterations in community structure, species diversity, ecosystem function (e.g., primary production, total system respiration, decomposition, nutrient cycling), and landscape-level impacts (e.g., habitat distribution). ERAs commonly identify more than one kind of ecological effect of concern in problem formulation. The ecological effects of concern identified during problem formulation should be ecologically important, sensitive to the stressor(s), and relevant to risk management.

Following construction of the conceptual model, problem formulation continues by developing a plan to implement the conceptual model of the ERA. The resulting analysis plan further characterizes the stressors, identifies specific ecological effects of concern, and identifies applicable data, as well as measures or models that can be used to quantitatively relate the stressors to the expected ecological effects.

The initial interactions between risk managers and risk assessors might also involve other organizations and concerned members of the public ('stakeholders'). Initial discussions can help ensure that all important aspects of the assessment are identified, included as part of the problem formulation, and represented in the conceptual model. Such interactions can also ensure that the kinds of results produced by the ERA can be used effectively in the process of risk management and decision-making. The US EPA approach argues for the separation of risk managers and risk assessors during the course of risk estimation. Upon completion of risk estimation, risk managers, risk assessors, and stakeholders may reconvene to discuss the nature and interpretation (e.g., conclusions, assumptions, caveats) of the results in the context of the overall assessment objectives. Possible outcomes of these interactions include revisions to the conceptual model, collection of new data, and subsequent iterations of risk estimation until the ERA needs of risk managers and decision-makers are fulfilled.

Exposure Analysis

Exposure is analyzed through characterizing the processes and mechanisms that bring organisms into contact with the stressor(s) of concern and quantifying the frequency, magnitude, and duration of such contact. Clearly, the nature of the stressor(s) and the kinds of ecological effects of concern will strongly influence the exposure analysis. Each identified stressor will suggest a relevant spatial and temporal scale for analysis. The scales might be local and relatively short-term, as for accidental spills of toxic, yet readily degraded or volatilized chemicals that result from hazardous waste management. Conversely, some stressors (e.g., fire, climate change) can exert ecological impacts over large expanses and for durations that greatly exceed the generation time of most

organisms. Stressors are also evident at intermediate scales, for example, major oil spills (e.g., Exxon Valdez) and certain exotic species (e.g., gypsy moth, zebra mussel, Asian long-horned beetle).

The nature of specific stressor(s) can provide information concerning the processes or mechanisms of exposure that will have to be evaluated in an ERA. Chemical contaminants introduced into the environment are naturally transported by the movements of wind and water. Certain chemicals can accumulate in organisms and be transmitted throughout complex food webs. Some organic chemicals are rapidly sorbed to soils and sediments, while others effectively remain in solution. Natural movements of biological stressors might be augmented by private and commercial transportation (e.g., cars, trucks, ships, airplanes) systems.

The kinds of ecological effects included in the conceptual model can also provide insights into exposure analysis for an ERA. Organisms occupy certain dimensions in space and time. Habitats have measurable spatial extent; ecological processes exhibit characteristic rates. Such observations can guide the analysis of exposure. For example, knowledge of the timing and duration of a sensitive life stage (e.g., eggs, larvae) can focus the corresponding measurement of stressors of concern and provide more meaningful quantification of exposure than longer term averages or monitoring that might completely miss the necessary time period for measurement. Seasonal changes in light, temperature, precipitation, and other physical factors can result in spatial–temporal variability in exposure. The important point is that variability in both the processes that influence the stressor and the characteristics of the ecological entities should be addressed in developing a meaningful analysis of exposure.

Alternative approaches can be used in a sequential manner to assess exposure. Worse-case scenarios can be developed that assume maximum values of the stressor. For example, 'end-of-pipe' concentrations of toxic chemicals can be used without accounting for physical dilution, chemical alterations, or biological degradation that would otherwise reduce the concentrations experienced by the organisms of concern. This approach can overestimate risk. If acceptable risks were estimated using these extreme exposures, the assessment process might reasonably be terminated. As an alternative to worse-case scenarios, exposures might be measured. Actual measures of exposure are undoubtedly the most easily defended scientifically (presuming competent sampling and analysis) and the most realistic inputs to an ERA. Finally, exposures might be estimated using physical (e.g., microcosms, mesocosms) or mathematical models. For example, several models have been developed and used to simulate the transport, fate, and distribution of toxic chemicals in the environment.

The product of exposure analysis is an exposure profile. For chemicals, the profile should include the nature of the source; pathways of exposure; environmental media of concern (e.g., soils, water, sediments, contaminated biota); exposure concentrations (magnitude, timing, duration, recurrence); and uncertainties associated with these exposures. Analogous exposure profiles would be developed for nonchemical stressors included in an ERA.

Effects Assessment

This component of the overall ERA methodology develops the functional relationships between the stressors and the selected ecological responses. The stressor–response functions are central to ERA. In short, ERA can be described as the development and application of uncertain stressor–response functions in assessing ecological impacts. The functions should estimate the severity of the ecological response in relation to the magnitude, frequency, and duration of the exposure. The derivation of stressor–response functions depends on the quantity and quality of available data.

Sources of data that might be used in the construction of stressor–response functions include: the results of toxicity tests (lethal, chronic) performed under controlled laboratory conditions, direct measures of exposure and response in controlled field experiments, and the application of statistical relationships that estimate the biological effects of chemicals based on physical or chemical properties of specific toxicants. The order of preference among these sources of data lists field observations as the most valuable, followed by laboratory toxicity tests, and finally by the use of empirical relationships. In the absence of directly relevant data, the development of stressor–response functions may require the use of extrapolations among similar stressors or ecological effects for which data are available. For example, effects might have to be extrapolated from the available test species to an untested species of concern in an ERA. Similarly, toxicity data might be available only for a chemical similar to the specific chemical stressor of concern in an ERA, and thereby require an extrapolation from one chemical to another to perform the assessment.

Risk Characterization

Risk characterization combines the exposure profiles with the stressor–response relationships to estimate

ecological risks in ERA. A variety of methods and tools are available for risk estimation. For assessing risks posed by toxic chemicals, one simple method simply divides the exposure concentrations by the toxicity reference values. Quotients equal to or greater than 1.0 imply risk; quotients less than 1.0 suggest minimal or no risk. Such quotients can prove useful in initial screening-level assessments to reduce the number of stressors that should be analyzed in greater detail. The screening assessments may be particularly effective if exposure estimates used in risk characterization are biased toward overestimating risk. This approach is limited in the context of using single-value estimates of exposure and toxicity to estimate risk.

Depending on the availability of data, distributions of exposure and toxicity can be constructed and compared. Risk can be estimated by comparing the degree of overlap between these distributions: the greater the overlap, the higher the risk. Using comparisons of distributions in screening-level assessments can extend the single-value quotient approach by including more information, including uncertainty, in the estimation of risk.

Experiments under field conditions or more controlled conditions in the laboratory (e.g., microcosms, mesocosms) can be used to characterize ecological risks. Experimental systems provide opportunities to physically impose the stressors of interest on the ecological resources of concern. Such experiments may be the only practical method for assessing risks posed by stressors not intended to be introduced into the environment. This approach may also prove essential in assessing risks posed by stressors that are virtually unknown or whose attributes are proprietary.

Mathematical and computer simulation models can be used to estimate ecological risks. There was a comprehensive review and evaluation of the existing ecological models for potential application in assessing ecological risks. Following decades of model construction in support of basic research and development, it stands to reason that some of these models might prove useful in estimating ecological risks posed by various stressors on individual organisms, populations, communities, and ecosystems. The critical aspect in adapting these models for assessing risk is the ability to derive a stressor–response relationship for the stressor(s) and ecological impacts of interest.

Uncertainty

Risk implies uncertainty. ERA was designed expressly to include uncertainty as an integral component of the assessment process. Sources of uncertainty include natural variability in ecological and environmental phenomena, as well as bias and imprecision associated with the stressor–response functions. This latter source of uncertainty can be exacerbated if extrapolations were involved in the derivation of the functions (e.g., laboratory to field, across species).

Uncertainties inherent to the risk assessment process can be quantitatively described using, for example, statistical distributions, fuzzy numbers, or intervals. Corresponding methods are available for propagating these kinds of uncertainties through the process of risk estimation, including Monte Carlo simulation, fuzzy arithmetic, and interval analysis. Computationally intensive methods (e.g., the bootstrap) that work directly from the data to characterize and propagate uncertainties can also be applied in ERA. Implementation of these methods for incorporating uncertainty can lead to risk estimates that are consistent with a probabilistic definition of risk.

Methods of numerical sensitivity and uncertainty analysis can be used to examine uncertainty and identify the key sources of bias and imprecision in quantitative estimates of risk. Once identified, limited resources (e.g., time, funding) can be efficiently allocated to obtain new information and data for those major sources of uncertainty and reduce it. These analyses can be repeated until uncertainties associated with the risk estimates are of an acceptable degree or until uncertainties cannot be further reduced.

Risk Communication and Management

If carefully crafted during problem formulation, the risk estimates derived from the previous step will provide information compatible with the process of risk management. The nature of the risk estimates should also facilitate their description and interpretation to stakeholders. Successful risk communication and management will likely require the risk assessors to again collaborate with managers and decision-makers to ensure proper interpretation of the risk estimates. Such collaboration can importantly help in developing and evaluating alternative management actions directed at the original goals and objectives of the ERA. Finally, discussions among risk managers and risk assessors can also lead to revision of goals and objectives, modifications of the conceptual model, and subsequent iteration of the risk assessment process.

See also: Cumulative Risk Assessment; Ecotoxicology.

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Risk Assessment, Human Health

Betty J Locey

Risk assessment is a process that can be used to qualitatively and/or quantitatively evaluate the potential for an event or events to occur. The process can be used to gain a better understanding of potential risks associated with a broad range conditions, including the following:

- risk of adverse health effects occurring after chemical or radiation exposure;
- risk of injury and/or death during air, highway, or rail travel; and
- risk of certain catastrophic events occurring, such as nuclear accident, industrial accident, or earthquake.

The following discussion focuses on the use of the process to better understand the potential for chemicals and agents to adversely impact human health. Typically, this process integrates science, science policy, and specific methodologies as defined under policy or regulatory mandate to identify and characterize risks. Used as a predictive tool, the risk assessment process generates information that can support risk management and decision-making. Typically, risk management is regarded as distinct from the risk assessment process, and decision-making is

based on an integration of the results of the risk assessment and other considerations. Other considerations may include engineering data; potential social, economic, and political impact; general feasibility; and cost–benefit analysis.

Evaluating the potential for exposure to a chemical or chemicals to pose an unacceptable risk of causing harm to people can be complex. The environment is composed of chemicals (e.g., soil, water, air) and living things are composed of chemicals and sustained by chemicals (e.g., food, drink, air). The bottom line is balance. An old toxicological adage states, the dose makes the poison. Almost any chemical can cause harm if the dose is high enough. For example, chromium at very low concentrations is a micronutrient and necessary for good health. At high enough concentrations it can cause a broad range of adverse effects and has been associated with an increase in the risk of cancer. The likelihood that chemical exposure will cause harm and the severity of effects depends on the inherent toxicity of the chemical, the individual' sensitivity to the effects of the chemical, the level of exposure (concentration in contact medium and/or amount absorbed into the body), and how long the individual is exposed (duration of exposure). Generally, the shorter the duration of exposure, the higher the concentration needs to be to cause harm. 'Poisons' are generally chemicals that can cause harm at very low doses in a relatively short time frame.

Generally, human health risk assessment is used to evaluate circumstances where the 'normal condition' has been changed or may change and there is a need to understand the potential health consequences of the change. Risk assessments may be used by regulators as well as the regulated community to more effectively manage benefits and risks and can answer questions like the following:

- Is it likely that the use a of particular pesticide at levels needed to control pests on particular crops will cause harm to end users (e.g., use on food crops and use on plants as tobacco and cotton).
- Could exposure to a specific chemical over a short term (e.g., accidental exposure, large-scale incidents) or over longer periods (such as residual impacts to environmental media like soil, air, and groundwater) cause significant health problems?
- How much of the chemical would someone have to be exposed to before it is likely to cause harm?
- What additional lifetime risk of cancer is associated with exposure to a specific chemical or chemical mixture?
- What levels of particular chemicals in drinking water are acceptable within a particular regulatory context?

Risk assessments may need to be completed by a team of specialists, including toxicologists, epidemiologists, physicians, biologists, chemists, fate and transport specialists, and engineers. Typically, a regulatory mandate will dictate in what circumstances and which chemicals need to be evaluated and controlled. For example, at the federal level US Environmental Protection Agency (US EPA) administers the Safe Drinking Water Act (SDWA), which provides for limits on certain chemical contaminants in drinking water, the Clean Air Act, which regulates chemical emissions into the ambient air, and the Resource Conservation and Recovery Act, which regulates hazardous waste handling, storage, and disposal. The Occupational Safety and Health Administration (OSHA) administers the Occupational Safety and Health Act (OSHAct) and regulates human exposure to chemicals in the work environment. The regulatory framework generally defines what is acceptable and what is not.

There is an ongoing effort to improve the risk assessment process. Changes often provide for integration of more of the underlying science to reduce uncertainty as well as exploring new approaches to evaluating risk. The following are examples of areas of current interest and activity.

- The use of physiologically based pharmacokinetic models (PBPK) to translate applied dose in animal studies to predict dose and risk in humans is increasing. Many regulatory programs recommend use of PBPK modeling when data and information are adequate.
- Consideration of effects of chemical exposure in sensitive populations with a focus on children's health is currently being evaluated by a number of regulatory agencies. There is new guidance and recommendations for addressing these issues in the risk assessment process.
- The standard approaches for using dose–response relationship information in the risk assessment process is the focus of ongoing efforts. Guidance provides for use of a point of departure when data are appropriate. The benchmark dose approach is commonly used.
- The consideration of mode of action in carcinogen risk assessment is becoming standard practice. When data are adequate to demonstrate use of the standard default low dose extrapolation models such as the 'linearized multistage model is not appropriate, alternate approaches, including threshold approaches are now being used.
- New guidance is available for addressing cumulative risk and looking at the potential consequences of exposure to mixtures.

National Academy of Science's Paradigm

The risk assessment process, as used to evaluate the impact of chemicals on human health, was formally defined in the National Academy of Science (NAS) 1983 report, *Risk Assessment in the Federal Government: Managing the Process* (the 'Red Book'). The study on which the report was based was carried out by a committee of the National Research Council (NRC) Commission on Life Sciences with support from the Food and Drug Administration. The NRC is a principal operating agency of the NAS. The report was developed to provide the federal government with a systematic approach for evaluating risks to human health associated with chemical exposure. The NAS framework was designed to strengthen the reliability and objectivity of the scientific basis of risk assessment as well as ensure that the best scientific data were integrated into the process and to ensure that there was consistency in the approach used by federal agencies. It was intended to minimize controversy and allow for more consistent and rational decision-making with regard to human health. The process was defined in broad and general terms and has also been applied to evaluation of risks associated with other organisms (e.g., wildlife) and the environment. The NAS divided the process into four major steps: hazard identification, dose–response assessment, exposure assessment, and risk characterization.

Hazard Identification

Hazard identification is the step in the risk assessment that qualitatively characterizes the inherent toxicity of a chemical. Scientific data are evaluated to establish a possible causal relationship between the occurrence of adverse health effects and chemical exposure. This step includes characterization of acute, subchronic, and chronic effects; the potential for local versus systemic effects; the influence of the route of exposure; the relevance, to humans, of effects seen in animals; an evaluation of the biological importance of the observed effects; the likelihood of the effects occurring under certain conditions; and the potential implications for public health. This step should be based on a thorough review of all the data that may provide information that is relevant to evaluating the potential chemical hazard. This may include data describing the effects on a variety of test animals, *in vitro* studies that characterize mechanisms of toxicity, metabolism, physiologically based pharmacokinetic studies, structure–activity relationships, short-term human studies, and epidemiological studies. Animal studies may focus on particular types of effects and may include reproductive toxicity studies, immunotoxicity studies, neurotoxicity studies, genotoxicity studies, and cancer bioassays. Each study must be evaluated with respect to quality, design, interpretation of the data, and statistical considerations to ensure that conclusions are valid before they can be integrated into the assessment.

Dose–Response Assessment

Dose–response assessment characterizes the quantitative relationship between exposure (usually determined in toxicity studies) and the occurrence of adverse health effects. Typically applied or administered dose, rather than effective tissue dose, is used to develop the dose–response relationship. As a rule, the higher the dose, the greater the frequency or intensity of the adverse reaction to a chemical. Often, different effects are observed at high and low doses. The approaches used to extrapolate the dose–response relationship from high experimental doses administered to relatively few animals used in laboratory animal studies to relatively low-dose human exposure anticipated to occur in the environment (e.g., via ambient media) vary and are critical to assessing potential risks. For noncarcinogenic effects and certain nongenotoxic carcinogens the lowest dose or doses at which no adverse effects are identified (or, if not available, the lowest dose at which adverse effects are observed) in the most sensitive species are commonly used as the basis for setting what are anticipated to be reasonably safe exposure levels (doses associated with acceptable risk) under certain conditions. For carcinogens regulated based on excess lifetime cancer risk, extrapolations are made from the incidence of cancer found at the high experimental doses to the doses expected to be associated with human environmental exposures.

In general, under US regulatory programs the results of the dose–response assessment usually differ depending on whether carcinogenic or noncarcinogenic effects are being assessed. For carcinogens, the outcome is an estimate of the potency of the chemical; that is, the probability of a certain incidence of cancer associated with a given dose. In contrast, for noncarcinogens, the assessment leads to an acceptable daily intake value. In the United States, this is a 'reference dose' (RfD) or 'reference concentration' (RfC); in other countries, this may be a 'tolerable daily intake' or 'acceptable daily intake'. In many regulatory programs around the world both carcinogens and noncarcinogens are evaluated using a 'threshold' approach.

Practices are constantly changing to provide for the use of as much scientific information in the risk assessments as possible. Current practice provides for

using a 'threshold' approach to developing exposure criteria if there is an adequate understanding of the mode of action. If the chemical is not mutagenic and does not cause cancer by heritable genotoxic lesions, a nonthreshold approach is deemed more appropriate. More detail is provided in US EPA's updated guidance for carcinogen risk assessment.

Toxicity values developed in the above described process are used to develop exposure criteria. Exposure criteria in a particular media represent levels of exposure at which the risk of adverse effects occurring are determined to be acceptable under a particular regulatory framework or as defined by a particular body (e.g., World Health Organization). Exposure criteria may be expressed as media concentrations (e.g., milligrams of chemical per kilogram of soil, liters of drinking water, or cubic meters of breathing zone air) or in terms of dose (e.g., milligrams of chemical per kilogram of body weight of the animal per day).

Exposure Assessment

Exposure assessment qualitatively and quantitatively characterizes the potential for exposure to occur in particular circumstances and includes an estimate of dose when possible. The assessment includes an estimation or measurement of chemical concentration in the contact media (e.g., soil, water, air, a particular food crop, a consumer product), an estimation of the length of time over which contact will occur, characterization of potential routes of exposure (inhalation, ingestion, and skin contact), and the likelihood for a chemical to be absorbed through those routes. In certain circumstances, direct measurements or fate and transport modeling may be used to estimate chemical concentrations in ambient media. For certain assessments, a quantitative estimate of the total dose of a chemical over a particular time frame and in the given circumstances is made.

Risk Characterization

Risk characterization provides for both qualitative and quantitative descriptions of risk. The step involves integrating the results of the hazard identification, dose–response assessment, and exposure assessment to characterize risk. Often, a direct comparison between exposure criteria developed in the first two steps and the results of the exposure assessment (concentration in the environmental media or the estimated dose, as appropriate) provide a basis for determining whether risks are acceptable. Typically, if criteria are exceeded, the risk is not acceptable. What is defined as acceptable, as well as the way risk is expressed, is often a function of the agency, law, and/or regulation that drives the analysis. Risk may be expressed as excess cancer risk, hazard index, or in terms of a margin of safety. The risk characterization must incorporate considerations of the uncertainties in the assessment. Each step of the risk assessment process contributes uncertainty and uncertainties must be clearly defined and integrated into the conclusions of the assessment.

Risk Management

Risk management was defined in the NAS report as the process of weighing policy alternatives and selecting the most appropriate regulatory actions. It is considered to be separate from the risk assessment process. Risk management decisions are based on the results of the risk assessment and other concerns that are relevant to the situation.

Human chemical exposure is regulated under a number of different laws in the United States, including SDWA, OSHAct, CERCLA, Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Toxic Substance Control Act, Hazardous Substance Act (HSA) (TSCA), Clean Air Act, and many state laws. These laws are administered by different agencies (e.g., US FDA, US EPA, OSHA, Consumer Product Safety Commission, and US Department of Agriculture). The approaches and problems associated with estimating exposure and risk, defining acceptable risk, and developing exposure criteria differ both under different regulatory mandates and the circumstances in which exposure is likely to occur.

The risk assessment process as described in the NAS paradigm identifies the critical information needed to evaluate risk in the broad sense; however, the resources expended to obtain the information defined in each step may vary in different circumstances. For example, for a new drug, a dose that provides therapeutic benefits and has an acceptably low risk of significant side effects must be identified prior to acceptance for use on the general public. The hazard identification and dose–response assessment steps are likely to be resource intensive. Drugs must be tested in a series of animal tests and clinical trials. The risks of side effects occurring (severity and likelihood of occurrence) have to be well characterized and balanced against the benefit derived from use of the drug. The exposure assessment portion of a risk assessment conducted to evaluate risks associated with environmental contamination regulated under certain state or federal laws (e.g., CERCLA) may be much more resource intensive than the hazard identification and dose–response assessments. The nature and extent of contamination in each environmental

media (e.g., soil, groundwater, and water) and the likelihood of human exposure has to be evaluated. Characterization of chemical concentrations in environmental media may require extensive environmental sampling and complex fate and transport modeling.

Exposure criteria are used in many regulatory programs and may be used as legally binding standards or provide guidance. A number of government agencies and other organizations develop criteria that are useful in different circumstances. These include OSHA (permissible exposure levels), American Congress of Governmental Industrial Hygienists (ACGIIH) (threshold limit values), Agency for Toxic Substances and Disease Registry (ATSDR) (minimal risk levels), US EPA (maximum contaminant levels), Health Canada, and the World Health Organization's International Programme on Chemical Safety.

Risk Assessment at Sites of Environmental Concern

US EPA's Approach to Assessing Human Health Risks Associated with Environmental Contamination under Superfund

Chemicals may be introduced into the environment (e.g., air, soil, surface water, and groundwater) during ongoing industrial and commercial activities, improper chemical handling, accidental spills and releases, as well as the routine disposal of chemical wastes by communities (e.g., household cleaners and solvents).

The risk assessment process has been used to identify situations in which contamination of the environment results in significant risks. CERCLA (Public L. No. 96-510, 40 CFR 300) was enacted on December 11, 1980, and gave the federal government the authority to act when hazardous substances were released (or could be released) into the environment in quantities that had the potential to endanger the public health. It provided for a system to clean up hazardous waste (e.g., spills, leaks, and abandoned dumpsites), for immediate reporting of chemical releases over specific amounts (reportable quantities), for fines and penalties, for a trust fund to pay for remediation at contaminated sites, and for recovering costs of remediation when the responsible party or parties can be identified. The Superfund Amendments and Reauthorization Act (SARA) to CERCLA was passed in 1986 and provided for more stringent and permanent remedies at sites regulated under the law.

Under CERCLA (sometimes referred to as the 'Superfund Law'), actions taken to remediate environmental contamination must protect both human health and the environment. US EPA administers CERCLA and has used the risk assessment process to characterize potential risks at sites of environmental concern. US EPA has developed guidance for conducting quantitative human health risk assessments at Superfund sites. Guidance documents include: *Risk Assessment Guidance for Superfund* (RAGS) originally published in 1989, *Human Health Evaluation Manual and Supplemental Guidance*, and *Exposure Factors Handbook*. This guidance was intended for use during the remedial investigation/feasibility study process at Superfund sites but has been widely used to address sites regulated under other environmental laws. The guidance continues to be updated and expanded to reflect changes in policy and approaches to evaluating risk.

US EPA's approach, as defined in RAGS, incorporates the principles defined in 1983 by NAS. The US EPA RAGS identifies four steps in an environmental risk assessment: data collection and evaluation, exposure assessment, toxicity assessment, and risk characterization. Tasks involved in characterizing the environmental media have greater emphasis because they often require tremendous resources and time.

Data collection and evaluation involves characterization of the concentration of contaminants in the media (e.g., soil, groundwater, and air) at the site in question. It includes the collection of samples to characterize soil and groundwater at contaminated property. This phase of a risk assessment may be complex and require significant resources but it is critical to providing the data needed to support the exposure assessment.

Exposure assessment includes both qualitative and quantitative evaluations of the potential for exposure to site-related chemicals to occur. Assessments commonly address both current and likely future uses of the property (e.g., residential, commercial, industrial, and agricultural). Typically, a conceptual model is developed that summarizes how site-related chemicals may contact receptors (e.g., humans, wildlife, and ecological). The model includes identification of chemical sources, impacted media, potential movement through the environment, identification of the appropriate exposure scenarios, and identification of the points at which contact between receptors and site-related chemicals are likely to occur. Chemical concentrations in environmental media may be estimated based on site data and using statistical analyses and/or fate and transport modeling. An estimate of the dose (intake) attributable to contact with environmental media through significant and completed pathways is made for chemicals of concern at

the site. This estimate is based on an estimation of the amount of time over which contact will occur, characterization of potential routes of exposure (ingestion, inhalation, and skin contact), and the likelihood for the chemical to be absorbed from the contaminated media through those routes.

Toxicity assessment includes characterization of the toxicity of a chemical, development of a dose–response relationship, and ultimately the development of exposure criteria. Toxicity values express a dose that is associated with either a given risk of cancer occurring over a lifetime of exposure (e.g., slope factors and unit risks) or a dose that is not expected to cause harm (e.g., RfDs). Some toxicity values are used as the basis for developing exposure criteria (RfDs) and some can be used as exposure criteria (e.g., RfCs). US EPA has developed toxicity values for many chemicals commonly associated with environmental contamination. Verified US EPA criteria are available in the Integrated Risk Information System (IRIS).

The toxicity values and criteria developed in the toxicity assessment combine the approaches and procedures described in the hazard identification and response steps described in the NAS paradigms. Because criteria are typically provided by regulatory agencies, for chemicals commonly identified as a concern the effort associated with this step for a given site may be limited. However, if conditions on the site appear to be unacceptable, these may be refined as part of the process.

Risk characterization includes a comparison between toxicity values and/or exposure criteria and exposure (dose or media concentration) to determine whether the exposure is acceptable. US EPA developed a formalized system that is commonly used to determine whether chemicals are likely to present an unacceptable risk based on current and likely future use of the property. The estimated dose is used to calculate an additional lifetime cancer risk for each chemical regulated as a carcinogen. Typically, a total site risk (sum of the risk associated with all carcinogens identified at the site) is presented. Acceptable risk is defined by the agency, in the appropriate laws, or by regulations that govern the site. Acceptable risk is a function of policy or law but is supposed to be rooted in science.

The contamination levels at which noncarcinogenic effects are likely to occur are also evaluated. Total dose is compared to a dose that is considered likely to be safe (exposure criteria; e.g., RfD). In a quantitative assessment the site-related dose is divided by the criteria and the resulting fraction is defined as the hazard quotient. This simply indicates whether the hypothetical dose exceeds the threshold criteria identified as likely to be safe. The hazard quotients for all chemicals at the site are summed and presented as a hazard index for the site. If the total does not exceed 1, it is assumed that the dose attributed to the site will not exceed the criteria and the potential exposure is deemed acceptable. If the hazard index for a site exceeds 1 (hypothetical dose exceeds criteria), a more refined analysis can be completed. Chemicals can be grouped according to target organ and a refined set of indexes can be developed for the site. The sum of the fractions for each target should not exceed 1. Uncertainties associated with each step in the process must be clearly defined and integrated into the conclusions of the assessment.

US EPA's approach is essentially an application of NAS', tailored to provide guidance for assessing risk associated with contamination in environmental media. Adverse health impacts associated with exposure to the chemical of concern are identified through the hazard identification, dose response, and toxicity assessment. Exposure is evaluated during the exposure assessment and data collection and evaluation steps.

Risk management is the decision-making process that follows the completion of a risk assessment. The risk assessment provides important information that supports decision-making and is integrated with other factors, including economic, feasibility, and cost–benefit analysis, in the risk management process.

See also: Carcinogen Classification Schemes; Dose-Response Relationship; Exposure Assessment; Exposure Criteria; Hazard Identification; Risk Assessment, Ecological; Risk Based Corrective Action (RBCA); Risk Characterization; Risk Communication; Risk Management; Uncertainty Analysis.

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Relevant Website

http://www.epa.gov – US Environmental Protection Agency.

Risk Based Corrective Action (RBCA)

Shawn L Sager

Introduction

Risk-based decision-making can be utilized to focus both remedial measures and limited funds while being protective of human health and the environment (i.e., prioritize) and to facilitate timely closure of impacted sites. The approach combines the information gathered during a site investigation together with data on the health effects of the site-related constituents to evaluate whether a particular site requires remedial action. A risk assessment demonstrating protection of human health and the environment can be helpful in determining if, and to what degree, active remediation is warranted at a site, and whether active remediation may be discontinued prior to removing all constituents from a medium at a site. Therefore, considerable savings can be realized while protecting human health and the environment.

A risk-based decision-making approach toward remediation is increasingly becoming an integral component in most regulatory programs under supervision by both federal and state agency personnel. American Society for Testing and Materials (ASTM) International committees have prepared Risk Based Corrective Action (RBCA) standards for evaluating petroleum sites (E 1739-95), chemical release sites (E 2081-00), and ecological resources (E 2205-02). RBCA is a process that quantifies: (1) the potential risks to identified receptors associated with exposure to site-related constituents, or (2) site-specific remediation goals for impacted media that are protective of human health if exposure to the identified receptors occurs. The process integrates components of the site assessment, risk assessment, risk management, and corrective action into a holistic site-specific approach that is consistent and technically defensible while still being practical and cost effective.

Sites with limited impacts do not require an extreme level of analysis. Utilizing RBCA to identify the project termination point minimally involves conducting an exposure assessment to identify complete exposure pathways by which receptors (people and/or the environment) could potentially be exposed to site-related constituents. Sites with a minimum amount of contamination can be handled through a Tier 1 Lookup Table, representing a health-protective generic screening approach. A majority of service station sites probably will be addressed through a Tier 2 quantitative approach involving assumptions of realistic current and future site use, simplistic fate and transport analysis, and health-protective site-specific exposure parameters. For those sites where multiple sources exist in a complex setting, a more detailed and comprehensive risk assessment may be warranted (Tier 3).

Background

RBCA was developed initially for underground storage tank (UST) programs. State programs developed

or adopted regulatory cleanup standards based on the needs of other programs. In some states, a standard for total petroleum hydrocarbons (TPH) was adopted based on intuition rather than on protection of human health and the environment explicitly. Finally, state agencies were finding that the procedures adopted for other programs did not necessarily fit UST programs.

There is a federal statutory requirement requiring evidence of ability to pay the costs of remediation at sites with UST releases. The costs of corrective action are borne by many state financial assurance funds or through the purchase of insurance policies by owners and operators. Many of these funds have experienced revenue shortfalls as the money paid out for corrective action is not covered by the money paid in. When funds start experiencing these shortfalls, corrective action slows down.

Each of these issues contributed to the establishment of a task group within ASTM to develop a standard for UST release sites. The goal of the ASTM RBCA process was to develop a procedure that would protect human health and the environment while at the same time being cost-effective. This meant that cleanup goals would be targeted toward actual exposures, not necessarily toward protection of unrealistic uses of the property. The vision of the ASTM RBCA standard was to provide a framework that state agencies could adopt to be consistent with state regulations and practices already in place.

ASTM RBCA Process

The key to the ASTM RBCA process is a step-by-step process from site investigation through decision-making to take a site to closure. The RBCA process is not very different from how site investigations have been conducted previously. The only additional information required focuses on the information required to make risk-based decisions. Briefly, the steps involved are described below.

Site Characterization

A site assessment is the process designed to collect data necessary to evaluate whether contamination is present at the site and, if necessary, direct future work at the site. Under the ASTM RBCA process, limited data are collected to characterize the site with respect to the source area, location of actual or potential receptors, and any other information necessary to perform the Tier 1 screening analysis. By only collecting limited data, the site assessment focuses only on the information required to make an initial decision on the site. Additional data can be collected during the Tier 2 or Tier 3 assessments to fill data gaps, if necessary.

The site characterization provides site-specific background information essential to visualizing site conditions and potential receptors and pathways. Characteristics of the site, such as history, climate, topography, local land use and populations, soil type, depth to groundwater, groundwater flow direction and rate, and distance to groundwater discharge, are considered. While all of these data are not required for the Tier 1 assessment, this information commonly is collected during site investigations and activities designed to delineate the extent of the hydrocarbon impacts. Further, this information will be readily available if a Tier 2 assessment becomes necessary. Site characterization data provide the basis for a realistic assessment of exposure pathways and are used as input in any models utilized.

A key component in the initial site assessment is the identification of human and environmental receptors potentially impacted by the site. An exposure pathway analysis relies on transport information to identify receptor or exposure points. For example, potentially significant transport and exposure pathways may include groundwater transport, vapor migration into buildings or utilities, etc. Current and potential future land use is identified as well as the potential for future installation of groundwater drinking water wells. If surface water has been impacted by the release, then appropriate surface-water exposure pathways will be identified.

Site Classification

Using a risk-based decision-making approach to determine if residual constituent concentrations are protective of human health and the environment can be cost-effectively conducted using a tiered approach. Under RBCA, site-specific data may be used to prioritize sites (in the case of limited remedial funds and resources). The ASTM classification scheme utilizes four classes. Class 1 sites are those posing an immediate threat to human health or the environment. For these sites, an interim response can be required to reduce this threat. Class 2 sites are those that may pose a short-term threat within 2 years or less. A Class 2 site may be one in which there are less than 2 years until impacted groundwater reaches a downgradient receptor. Class 3 sites are those that pose a longer term threat. For example, an aquifer may have been impacted, but the groundwater flow is such that the travel time to the nearest receptor is greater than 2 years. Some states have incorporated a classification or prioritization scheme into their RBCA process. States with an existing

classification have integrated it into their RBCA program, rather than starting with a new system. A number of states did not incorporate a classification system into their new RBCA program.

Tier 1

Tier 1 uses conservative, health-protective levels to compare to site-specific data to eliminate those sites not requiring remediation beyond monitoring (the obvious monitoring-only sites). The Tier 1 risk-based screening levels (RBSLs) typically incorporate very conservative or worst-case exposure assumptions. However, many sites with concentrations exceeding the RBSLs do not necessarily pose a threat to human health. Therefore, exceeding values of RBSLs by themselves do not indicate that remedial action should be actively undertaken to protect human health and the environment.

To pose a risk, a constituent must be present in the environment at a concentration high enough to cause a toxic effect if exposure occurs. Complete pathways must exist, and exposure to a constituent at that concentration must occur. Without the potential for exposure to a constituent, there is no risk; therefore, the driving force behind any remediation would not be the protection of human health. If no complete exposure pathways exist now or are not anticipated to exist in the foreseeable future at a site, the RBCA process technically can be terminated at this point as there is no risk. One example of this concept is the lack of exposure to impacted subsurface soil beneath a building that houses an operating business. However, it is often difficult to determine the future use of a site, and it may be necessary to evaluate potential risks associated with hypothetical future exposure. A key to the Tier 1 evaluation is the development of a conceptual site model that evaluates the possible exposure pathways for the site under current and hypothetical future conditions.

The data evaluation step in Tier 1 identifies the constituents of potential concern and the concentrations at which they occur in impacted media as determined by investigations conducted at the site. Most states do not require evaluation of historical data as part of the Tier 1 evaluation, although they may require submission of these data in the RBCA report as a basis for comparison (e.g., to demonstrate the decrease in constituent concentrations over time and/or demonstrate plume stability). However, the data that best represent the current environmental conditions at the site should be used in the risk-based decision-making approach.

A decision based on the Tier 1 evaluation may be to go to a monitoring program to verify that the conditions at the site, in fact, do not pose a threat to human health and the environment, to move to Tier 2, or to remediate to the Tier 1 levels. The costs required to collect additional data, along with the cost to perform the Tier 2 assessment and the benefits of higher cleanup goals, will be weighed against the remediation costs to meet Tier 1 levels to determine the best approach for sites with concentrations exceeding the Tier 1 RBSLs. UST sites with minimal impacts most likely will be addressed by this level of effort and sophistication. Estimates are that $\sim$10–15% of the sites will be closed under Tier 1.

Tier 2

Tier 2 produces site-specific target levels (SSTLs) that are protective of human health and the environment, but utilizes more site-specific data than Tier 1. This level of sophistication should address $\sim$70–80% of the sites. This more site-specific assessment involves the assumption of reasonable use exposure assumptions, considers actual beneficial uses of resources, and provides a tool for determining points of compliance.

The Tier 2 evaluation tends to focus on more realistic exposure pathways and may include fate and transport modeling to project whether site-specific concentrations will reach receptors where exposure could occur. Tier 2 will allow the exclusion of those pathways not expected to occur at the site. Groundwater flow models can be used in conjunction with contaminant transport models to describe the flow paths and rate of hydrocarbon movement and, thus, estimate exposure points concentrations. For example, if monitoring data indicate that a groundwater plume at a site has not migrated to a well or other exposure points where contact could occur, groundwater transport modeling may be used to predict if the plume will, in time, reach the exposure point at concentrations greater than regulatory standards or health-based concentrations. In Tier 2, generic assumptions used in Tier 1 modeling are replaced with site-specific data to ensure that the results accurately represent actual site conditions. In many cases, verification of the modeling results through monitoring is required by the state.

Tier 3

Tier 3 is more complex and detailed. It relies on more site-specific data. While Tier 2 may have relied on simple and relatively uncomplicated fate and transport models, Tier 3 will utilize more sophisticated models and will include additional site-specific data. Tier 3 also may rely on site-specific exposure assumptions, if appropriate. Some states allow the

incorporation of Monte Carlo techniques into the Tier 3 process. As the site evaluation and risk assessment process increases in tier level and complexity, the costs, data requirements, and level of sophistication required to complete the process also increase. It is anticipated that only ~5–10% of the UST sites will be closed under Tier 3.

Corrective Action

Corrective action can be a combination of passive and aggressive actions designed to reduce constituent concentrations to levels considered protective of human health and the environment. These can include natural attenuation, soil vapor extraction, source removal, etc. In each case, the type of corrective action is selected to meet the remediation goals (i.e., RBSLs or SSTLs) developed under the appropriate tier for the site. This allows the project to focus only on those areas or media posing a threat to human health or the environment.

No Further Action

The final step in the RBCA process is to take the site to closure. This may involve a monitoring program for a period of 1–2 years to verify that the assumptions included in the RBCA analysis were appropriate for the site. It is anticipated that RBCA will allow for closure of sites in a consistent and cost-effective manner. This will allow resources to be focused on those sites posing the greatest threat to human health and the environment. It will also allow sites posing little risk to potentially be placed in a monitoring program with the goal being to justify closure using natural attenuation and no active remediation.

Summary

RBCA and the use of risk-based decision-making to establish health-protective remedial measures and controls at a site is a process that is producing health-protective and cost-effective corrective action sites. RBCA is used to decide the level of corrective action necessary at a site to protect human health and the environment, site-specific remediation goals (RBSLs or SSTLs), and the concentrations of constituents that can remain at the site because they will not impact human health and the environment. For sites already undergoing remediation, the RBSLs or SSTLs can be used to determine when the site no longer poses a threat to human health and the environment. Therefore, RBCA is useful both for newly discovered releases as well as for old releases that may have treatment systems with constituent concentrations reaching asymptotic levels.

See also: Pollution, Air; Pollution, Soil; Pollution, Water; Risk Assessment, Ecological.

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Risk Characterization

Michael A Kamrin

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Risk characterization is the final step of the risk assessment process as laid out in the classic National Research Council report: *Risk Assessment in the Federal Government: Managing the Process*. In this step, the risk from a specific agent (chemical or physical) or group of agents in a particular setting is evaluated. This evaluation is based on a comparison of the results of the dose–response assessment for these agents with the outcome of the exposure assessment for these same agents in the situation of interest. For example, a risk characterization may address the risk from chemicals at a hazardous waste site to those living near the site. While a risk characterization can provide either qualitative or quantitative evaluations of risk, quantitative outcomes are generally most useful and so will be the focus of this article.

Because the dose–response assessment step is performed differently for agents considered to be carcinogens compared to those classified as non-carcinogens, the risk characterization process for these two kinds of agents differs as well. The result of the dose–response assessment for carcinogens is some measure of the potency of the agent; for example, the

daily dose that produces a specific number of additional cancers in a given population. In the case of noncarcinogens, the toxicity is expressed as an acceptable daily intake; that is, the maximum daily dose unlikely to be associated with adverse health effects. This acceptable daily intake may be described in a variety of ways. In the United States, the Environmental Protection Agency (EPA) calls this value a reference dose (RfD) or reference concentration (RfC).

Since the cancer potency and/or acceptable daily intake values are characteristics of the agent, they do not vary from situation to situation. Exposure, however, does. Exposure assessments provide an estimate of the dose to which individuals may be exposed via all possible routes in a specific circumstance. The result of the exposure assessment is usually expressed as a single number; for example, the average daily dose. However, since no two individuals are likely to have the same exposure it may also be expressed as a distribution. This distribution provides estimates of the exposures of particular segments of the population; for example, the top 95% of exposed individuals.

Quantitative risk characterization thus results in either an estimate of the additional cancers expected (for carcinogens) or an estimate of whether or not individuals will be exposed to doses that exceed the acceptable daily intake – and perhaps the magnitude of this exceedence (for noncarcinogens). However, as can be seen from the US EPA definitions of cancer potency: "Cancer potency is estimated as the 95% upper confidence limits of the slope of the dose response in the low dose region. This method provides an upper estimate of the risk; the actual risk may be significantly lower and may actually be zero." and the RfD: "An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime," there are considerable uncertainties associated with the dose–response estimates. In addition, because of difficulties in estimating exposure due to lack of information about environmental levels of agents and about human behaviors, uncertainties in exposure assessments may also be large.

Thus, while the results of a risk characterization may be expressed as a single value; for example, X additional cancers per million people exposed, a full description of the risk must include a discussion of the uncertainties in the risk estimate. Unfortunately, many of these uncertainties are hidden in the design of the dose–response studies and, in addition, there are many implicit value judgments that are part of both the dose–response and exposure assessment processes. Ideally, all of these uncertainties and judgments would be part of the risk characterization results but in reality many are not included. While a full description of all of the uncertainties and value judgments would lead to a cumbersome risk characterization result that would be difficult to use in making risk management decisions, including more information of this type than is presently used would aid those who manage risk.

Guidelines for performing and reporting the results of risk characterizations have been promulgated by governmental bodies. In the United States, the EPA is the agency that is responsible for risk assessment guidance for environmental contaminants and it has developed and issued such guidelines. These documents are written primarily for the use of federal risk assessors and risk managers. However, state governments also must deal with risks from environmental agents and they generally utilize these same guidelines although they are often not as well equipped as the federal government to appreciate the implicit uncertainties and value judgments in the risk characterization. Thus, their ability to comprehensively characterize risk and make sound risk management decisions may be compromised.

Perhaps more importantly, each citizen is a risk assessor and risk manager with regard to his or her own health and the health of loved ones. Members of the public make daily decisions about risk and may influence government decisions on these same risks but they are generally poorly equipped to appreciate the uncertainties and value judgments in risk characterizations. They are also most likely to be presented with single value risk characterization results tempered little by attendant discussion of uncertainties. This not only limits their ability to make the best management choices but also makes them vulnerable to unfounded and misleading claims made by advocacy groups.

In sum, risk characterization is the last and critical step in risk assessment. While it is often portrayed as providing objective, scientific best estimates of risk, the value judgments that are integral parts of the characterization process clearly reflect that this is not the case. Greater appreciation of this aspect of the risk characterization process would provide agency and citizen risk managers with a better understanding of the meaning of risk values and thus how to improve risk management decisions.

See also: Dose–Response Relationship; Exposure Assessment; Risk Assessment, Human Health; Risk Management.

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Risk Communication

Michael A Kamrin

Although communication about risk has a long history, risk communication has been formalized as an area of study for only ~15 years. It is an area that draws from a number of disciplines in the natural and social sciences. Research in this field includes studies of human perception and decision-making as well as investigations of a socioeconomic nature. Risk communication practitioners include government officials, business and industry representatives, public interest group members, academics, media professionals, and the general public.

Because of its short history, the concept of risk communication is still evolving. In its landmark report in 1989, *Improving Risk Communication*, a National Academy of Sciences panel defined risk communication in part as "An interactive process of exchange of information and opinion among individuals, groups and institutions." However, this definition describes one group's consensus as to what risk communication ought to be and does not reflect either the diversity of opinion on this topic or the reality of risk communication as it is currently practised.

Reduced to its essentials, risk communication is just one example of communication in general, a process by which information is transferred by one party to another through a variety of channels. The components of the process are the risk communicator or source, the message, the message channel, and the recipient of the message. While this description suggests a linear series of events, communication is often, but not always, an interactive process which includes feedback among the components. For example, the responses of the recipient may lead to alterations in the source, message, and/or channel and may result in a change in outcome.

Based on this conceptual framework, and using a variety of experimental approaches and case studies, risk communication professionals have been able to identify and, in some cases, roughly quantify many of the factors that determine the effectiveness or success of risk communication. One fundamental problem is, however, that effectiveness can be defined in a number of ways.

From one point of view, success can be measured as increased understanding of risk on the part of the message recipient, whether or not this results in changes in behavior. However, from another perspective, effectiveness is determined by the degree of behavioral change brought about by the risk message being transmitted. There are some who have argued that increased understanding is directly linked to changes in behavior but research suggests that this is not necessarily the case; for example, smokers who become aware of the risk do not always quit.

Many recent communication efforts about public health risks reflect the latter view. For instance, the success of risk communication programs about radon have been judged by the number of people who test for radon or take remedial action to reduce radon levels. Similarly, campaigns on smoking are assessed by the number of individuals who stop smoking. When behavior change is the criterion of success, the accuracy of the risk communication is not as important as its impact.

However, other risk communication efforts reflect an educational focus rather than a persuasive one. Understanding is considered the most important goal and a lack of change in behavior is not judged as a failure in communication; rather it is the result of decisions by the information recipient who may take into consideration a variety of other factors, such as offsetting benefits. Accuracy is considered critical so that the recipient can make an informed decision based on the best information.

The factors that affect the success of risk communication efforts, irrespective of the criteria for judgment, can be considered in the context of the components of the communication process. Studies of the risk messenger (source) have focused on the issue of trust and, not surprisingly, reveal that the effectiveness of the communication increases as trust in the communicator increases. This trust may be related to the degree of expertise of the communicator but more often is determined by other factors such as the messenger's perceived objectivity or social class and the accuracy of previous communications by this messenger or other messengers representing the same organization. For example, state agencies may have difficulties in effective risk communication for decades after taking actions or making statements seen as untrustworthy.

The content and form of the message are also critical and it has been demonstrated that the same risk expressed or framed in different ways can have different impacts. For example, describing the risk from a disease or surgery as 60% chance of survival will lead some people to different decisions than if it is stated as 40% chance of death. Similarly, a risk presented in terms of the total number of fatalities may be perceived differently than if it is presented as a probability.

Furthermore, there are a number of factors that come into play if the risk is presented in comparison to other risks – a communication strategy that is often employed. Research suggests that audiences are most receptive to comparisons of the same risk at one time or place to another time or place. For example, an effective message may describe the risk as high 2 years ago but having decreased continuously and now less than one-tenth of what it was. Risk comparisons that are not as successful involve comparisons between hazards that are thought of as having incomparable qualities (e.g., smoking and water contaminated with bacteria). Smoking is controllable and the effects delayed, while bacterial contamination of water is involuntary and the effects immediate.

The channel of communication is also an important variable in the communication process. Survey researchers have examined the relative credibility of various channels, including print media, radio and television, magazines, and advertising, and have found differences in the degree of trust people have in each. These differences depend not only on the class of channel but also on the specific representative of that class. For example, coverage of a risk issue in a local newspaper may be viewed differently from stories on the same issue in the national press.

Last, the perception of the recipient is critical to the process and is an aspect of risk communication that has been studied very intensively. This research has clearly shown that this perception depends both on the way that humans tend to internalize knowledge and on the background of the information recipient. For example, a scientist may perceive risk as a specific quantity and compare risks based on a quantitative approach (e.g., a risk of one in a million is much lower than a risk of one in a thousand).

However, potentially affected citizens may look at these quantitative descriptors as only partially describing the risk. They may consider a number of other factors such as the voluntariness, catastrophic potential, familiarity, and controllability of the hazard. Thus, contrary to the scientist, they may consider the one in a million risk to be more serious than the one in a thousand risk. Government officials are often reminded of this at public meetings, when people who smoke express great concern about very low levels of environmental contaminants which pose much lower health risks than smoking.

The results of research on the factors that influence successful risk communication are the bases for sets of risk communication principles that have been developed. One well-known set of principles is titled *Seven Cardinal Rules of Risk Communication*. This guide includes steps to increase trust such as to 'coordinate and collaborate with other credible sources', steps to increase the recipients' control such as to 'accept and involve the public as a legitimate partner', and steps to increase the interactive nature of the process such as to 'listen to the public's specific concerns'.

In the main, research to date has focused on understanding individual differences in risk perception and on the interactions between sources and recipients. However, recently, increasing attention has been paid to the social context in which risk communication is performed. Research in this area has led to a greater awareness of the sociocultural factors that affect the transmission of risk information.

These factors include the ethnic and socioeconomic characteristics of populations as well as the structure and history of communities that must deal with risk concerns. For example, a 'company' town may react quite differently than a rural agricultural village or an inner-city neighborhood to information about risk from an environmental chemical.

Another important social issue that influences risk communication is environmental justice and the perceived fairness of the risk to a community or subculture. For example, the perceived risk of adverse health effects from a landfill may depend on whether the waste deposited there is generated locally or transported from somewhere else. Similarly, the perception of risks associated with locating new

facilities in an area may be colored by environmental equity concerns.

These sociocultural factors can affect all aspects of the communication process. The credibility of a particular source may vary greatly depending on the cultural experience of the recipient with the organization the source represents, for example, industry or state government. In addition, the way that the message is framed may have quite different impacts in different cultural settings; an effective comparison in one setting may be an ineffective one in another. Communication channels may be seen as more or less reliable by different socioeconomic classes, for example, certain media may be seen as more trustworthy by different social groups. Furthermore, the relative importance of the factors that influence perception, for instance, controllability and immediacy, may be different in various cultural settings.

Research in this area, while in the formative stage, has been increasing. One aspect of this expanded effort involves studies aimed at understanding why certain communities have responded to risk, for example, from Superfund sites, quite differently than other communities, or why media focus on some risks has led to increased awareness and concern while attention to other risks has been met with little reaction. It is hoped that research into the reasons behind these differences will help in identifying the roles of various sociocultural factors in risk communication.

It is clear that there are many gaps in our current understanding of the risk communication process. For example, how exactly do age and gender affect the way risks are perceived? Other questions that are yet to be answered include exactly how the framing of the message affects its impact; how uncertainty in the risk message affects the perception of the risk and risk communicator; and why some risks that appear to share the same characteristics are perceived differently by individuals and/or communities.

Future research will address these questions and our understanding of the influence of various factors on the risk communication process will undoubtedly increase. In addition, innovative ways to present risk messages will certainly be developed. However, some fundamental issues with respect to the goals of risk communication will likely remain.

See also: Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Risk Management.

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Risk Management

Xuannga Mahini

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Risk management, in simplified terms, is the decision-making process by which risk assessment results are integrated with other information to arrive at decisions about the need for, method of, and extent of risk reduction. This definition covers many levels of decision-making. At one level, it deals with the question of what programs should be undertaken to reduce risk to the population of the country. At another level, it is the allocation of tax dollars to improve the quality of life in a city. At yet another level, it is the decision-making process for mitigating risk from a specific chemical in a specific use or at a specific contaminated site.

This latter level of risk management is discussed in some detail in the 1983 National Academy of Sciences (NAS)/National Research Council (NRC) report on risk assessment (*Risk Assessment in the Federal Government: Managing the Process* – the so-called Red Book). In that report, NAS advocated a clear conceptual distinction between risk assessment and risk management, since this distinction would help prevent, for example, the tailoring of risk

assessments to the political feasibility of regulating the substance in question. According to the Red Book, risk assessment is a four-step process: (1) Hazard assessment – Does the chemical have the intrinsic potential to cause an adverse health effect, for example, cancer? (2) Dose–response assessment – What is the relationship between low doses in humans (generally estimated from high-dose studies in laboratory rodents) and the incidence and severity of the potential adverse health effect? (3) Exposure assessment – What is the actual or hypothetical exposure of humans to the chemical in question? (4) Risk characterization – What is the estimate of probability or likelihood of specific harm to an exposed individual or population? Risk management – How do we mitigate this risk? – is typically the logical next step following the risk assessment process. This is "the process by which the results of risk assessment are integrated with other information – such as political, social, economic, and engineering considerations – to arrive at decisions about the need and methods for risk reduction" (NAS definition). In the 1994 *Science and Judgment in Risk Assessment*, NAS further recommended an iterative approach to risk assessment that would allow improvements to be made in the risk estimates (more realistic risk estimates with lesser degrees of uncertainties) and that would lead to an improved relationship between risk assessment and risk management.

Risk management decisions are based on the technical risk assessment information, but they also incorporate other information. Specifically, risk managers consider costs, benefits, risk of alternative actions, and risk of no actions. For example, some drugs have a very small safety margin between the level that is effective and the level that is toxic, but the benefits of the drug are judged to outweigh the risks; therefore, it is decided that these drugs can be used. At the other end of the spectrum, some food-coloring additives have very large safety margins between what is toxic and what is in the diet, but the benefits of food coloring are minimal and alternatives exist, so this risk would generally face a higher level of scrutiny in a risk management decision.

These examples emphasize that risk management decisions are based in part on level of risk, but other factors are also considered to most effectively protect the public. In making risk management decisions, it is also necessary to understand the level of uncertainty in the technical data. For example, there is considerable uncertainty in predictions of potential impacts of global warming, but the consequences are potentially very large. It is often the case in risk assessment that there is considerable uncertainty in the predictions of risk. These uncertainties need to be carried forward into the hands of the risk manager so that they are weighed in the final decision. The necessary level of public protection and the magnitude of margins of safety properly belong in the domain of risk management.

At a more global level, risk management refers to the activity of allocating resources (time and money) to reduce risk in general to the public. For example, the US Congress does this when they allocate money to US federal agencies. The trade-off in agency funding addresses the question of whether more resources should be allocated to improve air and water quality (US Environmental Protection Agency, EPA), ensure that our food is more strictly inspected and regulated (US Food and Drug Administration (FDA) and USDA, improve highway safety (NHSTA), improve air safety (FTA), or bolster national defense systems (armed forces). We do not have unlimited resources to expend, so Congress makes these allocations roughly proportional to where it thinks the money is most effectively spent. These allocations reflect risk management on a global level.

The level of risk management closest to what most individuals experience is risk reduction at a city management level. Cities are responsible for ensuring that their drinking water is tested and is safe relative to bacteria, disease, and chemicals. They are also responsible for disposing of garbage in either landfills or incinerators, for keeping beaches and swimming places safe, and for minimizing vehicle risk to pedestrians and vehicle occupants. A number of cities have a significant problem with keeping their drinking water and beaches clean because of storm water overflowing into sanitary water systems. Risk management at the local level involves deciding whether solving this storm water issue should be the highest priority for the next tax dollars being spent or whether road repair or expanding the capabilities of the local ambulance and hospital system should be highest priority.

The 1997 Presidential/Congressional Commission's Risk Management Framework

In the 1997 *Framework for Environmental Health Risk Management – Final Report* published by The Presidential/Congressional Commission on Risk Assessment and Risk Management (Commission), risk management was defined as "the process of identifying, evaluating, selecting, and implementing actions to reduce risk to human health and to

ecosystems." The goal of risk management is scientifically sound, cost-effective, integrated action that reduces or prevents risks while taking into account social, *cultural*, *ethical*, political, and *legal* considerations (italicized words are new considerations since the 1983 Red Book). This new definition of risk management is broader than the traditional definition, which is restricted to the process of evaluating alternative regulatory actions and decision-making only by regulatory agencies.

There are two reasons for the broadened scope of risk management beyond regulatory actions typically taken previously by federal, state, and local government agencies:

- Government risk managers now often consider both regulatory and voluntary approaches to reducing risks, as society is challenged to solve more complex risk problems with limited resources.
- Risk management is being conducted outside of government arenas, by 'stakeholders' (individual citizens, businesses, workers, industries, farmers, fishermen, etc.). So the decision-making process needs to be improved by the involvement of those affected by risk problems.

To help meet these needs, the Commission developed a systematic, comprehensive Risk Management Framework that has six stages:

1. Define the problem and put it in the appropriate public health or ecological context.
2. Analyze the risks associated with the problem in context (e.g., multisource, multimedia, multichemical, multirisk) based on identified risk management goals.
3. Examine options for addressing the risks (e.g., comparative risk analysis, expected effectiveness, feasibility, potential adverse consequences, priority to prevent risks not just controlling them).
4. Make decisions about which options to implement.
5. Take actions to implement the decisions.
6. Conduct an evaluation of the actions.

The Commission's proposed Risk Management Framework is conducted: (1) in collaboration with stakeholders and (2) using iterations if new information is developed that changes the need for or nature of risk assessment, while avoiding 'paralysis by analysis'. By emphasizing the importance of collaboration, communication, and negotiation among stakeholders (so that public values can influence risk management strategies), the new Risk Management Framework is designed to produce risk management decisions that are more likely to be successful than decisions made without adequate and early stakeholder involvement.

The most important tools to carry out the Commission's proposed Risk Management Framework are effective risk communication, comparative risk analysis, and benefit/cost analysis (BCA) and cost/effectiveness analysis (CEA). Effective risk communication is critical to successful implementation of the Risk Management Framework. Risk communication is the process of informing people about the risks or hazards. Like all communication, communicating risk is a two-way exchange to inform the stakeholders or target community about possible hazards, but also to gather information about those affected by the risk. The purpose of risk communication is to help stakeholders understand risk assessment and risk management, form scientifically valid perceptions of the likely hazards, and participate in making decisions about how the risk should be managed. Effective risk communication include knowledge and tools on how to communicate to the public/stakeholders the risks involved in a particular situation, and how to make day-to-day decisions regarding that risk.

Comparative risk analysis is a methodology that uses sound science, policy, economic analysis, and stakeholder participation to identify and address the areas of greatest environmental risks and provide a framework for prioritizing environmental problems. The results of a comparative risk analysis can be used to provide a technical basis for targeting activities, management priorities, and resource allocation. In this analysis, different risk management strategies beside 'command and control' can be explored: (1) education; (2) incentives (e.g., market-based, subsidies, alternative compliance); (3) monitoring; (4) surveillance; and (5) research.

BCA and CEA are together referred as economic analysis. The Commission recommended that where practicable, environmental equity needs to be considered in economic analysis. BCA has the advantage of helping make choices among policies and actions with quite different benefits and costs. It is guided by what stakeholders or members of society are thought to be willing to pay to reduce risks. CEA, in contrast, does not require that benefits be monetized. It defines some physical measures (e.g., tons of pollutants to be reduced or number of cancer deaths avoided) as effectiveness of risk management actions. These nonmonetized benefits cannot, however, be aggregated.

Successful examples of the Risk Management Framework cited by the Commission are: (1) stakeholders and EPA identified risk management options for the pulp and paper and the steel industries;

(2) environmental management plan to control pollution in the San Francisco Bay; (3) transportation policy that considered alternatives to highway expansion in Maine.

See also: Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Risk Communication; Sensitivity Analysis; Uncertainty Analysis.

Further Reading

EPA (2003) Considerations in Risk Communication: A Digest of Risk Communication as a Risk Management Tool. EPA 625/R-02/004; March.

National Research Council (1994) *Science and Judgment in Risk Assessment*.

The US Presidential/Congressional Commission on Risk Assessment and Risk Management (1997) *Framework for Environmental Health Risk Management – Final Report*.

Risk Perception

Patricia M Nance

Risk perception can play a critical role in the daily behavior of humans, and needs to be considered for developing effective risk communication. Risk perception is the apprehension or opinion of the likelihood of risk(s) associated with performing a certain activity or living a certain lifestyle. Many factors play a role in perception of risk. Some of these factors are personal experience with the risk, perceived importance of the risk, the credibility of the communicator and their organization, and the language and presentation format. Each individual has their own way of thinking and decision-making ability, which can make risk communication a challenge. Dramatic and memorable risks are less acceptable than uninteresting and forgettable ones. The factors that make a risk dramatic and memorable, such as airline crashes, may distort risk perceptions. Events that are highly publicized in the media become well remembered and appear to have happened more frequently than normal, hence creating a larger perceived risk.

Familiarity with a risk also skews the perception. Unfamiliar risks are not as acceptable and tend to be perceived to be as higher risks than familiar ones. The public tends to overestimate the risks of seldom occurring events and underestimate the risks of common, everyday risks. For example, the perceived risk of being in an automobile crash is perceived to be low compared to the risk of being in an airplane crash. In an automobile, the individual has a feeling of control, which allows the individual to feel safer than in an airplane where someone else is in control.

Trust and accuracy are two very important factors in risk perception and risk communication. If the public does not trust the experts, the perceived level of risk may be high. To build this trust, accurate information must be given to the public. No potentially important information should be left out and the public should not perceive the experts as hiding the key facts. There are two basic situations when dealing with trust: high trust, low concern and low trust, high concern.

The awareness of the risk also plays a crucial role in risk perception. If the public lacks the knowledge to understand the risk, then the risk can be overestimated or underestimated. Researchers have shown that experts and lay people are typically overconfident about their risk estimates. The role of experience is related to the knowledge of the risk. Individuals who have previous experience with the specific risk or those having a direct economic relationship to the risk usually have a more accurate perception of the risk. Experience does not mean that the individual must have personally been involved in the risk but has awareness of the risk's affects. Experience can also be influenced by the risk frequency. If an individual is exposed to a similar risk more frequently, it can create an overestimate of the risk due to the frequency of exposure.

There has been an increasing amount of research done in the area of risk perception in a variety of fields, such as sociology, political science, psychology, anthropology, and even geology. This research is leading to a better understanding of how individuals perceive a variety of risks in different situations. One expert (Paul Slovic) has stated, "Perhaps the most important message from this research is that there is wisdom as well as error in public attitudes and perceptions. Each side, expert and public, has something valid to contribute. Each side must respect the insights and intelligence of the other."

See also: Chemical Hazard Communication and Material Safety Data Sheets; Risk Communication; Risk Management.

Further Reading

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about four presentation formats for fish consumption health advisory information. *Risk Analysis* 18(5): 649–659.
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Covello V, McCallum D, and Pavlova M (1989) *Effective Risk Communication*. New York: Plenum.
Sandman P (1985) Getting to maybe: Some communication aspects of siting hazardous waste facilities. *Seton Hall Legislative Journal* 9: 437–465.
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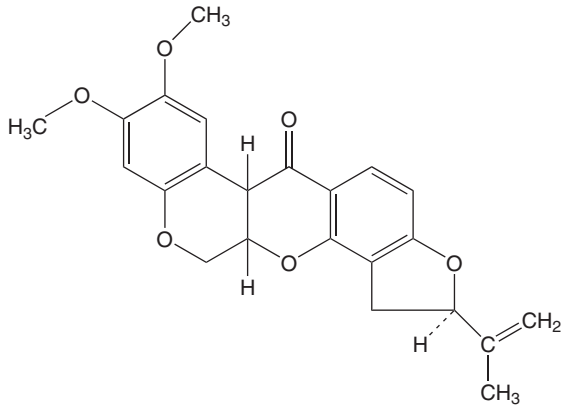
Rotenone

Carey N Pope

This article is a revision of the previous print edition article by Janice Reeves and Carey N Pope, volume 3, pp. 114–115, © 1998, Elsevier Inc.

- Chemical Name: Benzopyrano(3,4-*b*)furo(2,3-*h*)(1)benzopyran-6(6*aH*)-one,1,2,12,12*a*-tetrahydro-2-α-isopropenyl-8,9-dimethoxy
- Chemical Abstracts Service Registry Number: CAS 83-79-4
- Synonyms: Barbasco; Mexide; Ro-Ko; Fish-Tox; Chem Fish; Cube'; Derrin; Derris root; Nicoulins; Nusyn Nox fish; Prentox; Noxfish; Rotenone dust; Timbo powder
- Chemical Structure:



Uses

Rotenone has been used for centuries as a fish poison. Rotenone is used as an insecticide around the garden to control chewing insects on vegetables, fruits, and forage crops. Rotenone is also used as a dust on cattle and in dog and sheep dip formulations for scabies, chiggers, fleas, ticks, lice, and mange.

Background Information

Rotenone is a naturally occurring alkaloid (rotenoid) extracted from the roots, leaves, seeds, and barks of certain tropical plants, such as the Jewel Vine or Flame tree (*Derris* spp.), Lacepod (*Lonchocarpus* spp.), or hoary pea (*Tephrosia* spp.).

Exposure Routes and Pathways

Dermal and ocular exposures are most common, but rotenone may also be ingested or inhaled.

Toxicokinetics

Parenteral exposure is more hazardous than oral exposure; gastrointestinal absorption is slow and incomplete. Fats and oils increase rotenone absorption from the gastrointestinal tract. Rotenone exhibits a significant first-pass effect following oral exposure. Biotransformation of rotenone in rats leads to hydroxylated metabolites (rotenolones). In addition, *O*-demethylation inactivates rotenone. Rotenone distributes to lipid-rich tissues, including the nervous system. Elimination of rotenone from the body is primarily through the fecal route.

Mechanism of Toxicity

Rotenone inhibits the electron transport chain by blocking transport between the flavoprotein and ubiquinone. The oxidation of pyruvate in rat mitochondria is virtually completely blocked by rotenone *in vitro* ($<1\,\mu mol\,l^{-1}$ concentration).

Acute and Short-Term Toxicity (or Exposure)

Animal

Depression of the respiratory center appears to be the primary cause of death. The acute oral LD_{50} values in laboratory rats range from about 130 to 1500 mg kg^{-1}. Death following high oral doses in animals can occur within 2 days or as long as 2 weeks after exposure. Rotenone is more toxic by inhalation and intraperitoneal exposure. Rabbits appear markedly

less sensitive than rodents to oral rotenone exposures ($LD_{50} < 1\,g\,kg^{-1}$).

Human

The estimated oral LD_{50} in humans is 300–500 mg kg^{-1}. Ocular exposure to rotenone dusts can cause severe irritation. Inhalation exposure can cause irritation of the nose and throat, and a temporary anesthetic effect may occur. Significant exposures may cause nausea, vomiting, cramps, muscle tremors, loss of coordination, dyspnea, and seizures.

Chronic Toxicity (or Exposure)

Animal

Dogs given dietary rotenone for 6 months at dosages up to 10 mg kg^{-1} day^{-1} showed reduced food consumption and reduced weight gain. Lesions of the gastrointestinal tract were noted. Reproductive and developmental effects were only noted at maternally toxic levels of exposure. Hamsters given oral dosages as high as 120 mg kg^{-1} day^{-1} for 18 months showed no increased tumor incidence.

Human

Long-term exposure to rotenone may cause fatty liver and kidney damage.

In Vitro Toxicity Data

Rotenone was negative in bacterial mutagenesis assays.

Clinical Management

Respiratory and cardiovascular function should be supported with oxygen, assisted ventilation, and parenteral fluids. If eyes or skin are contaminated, they should be washed immediately. Gastrointestinal decontamination procedures should be used appropriately depending on the patient's level of consciousness and the amount of rotenone ingested. Oils or fats should not be administered because they can promote rotenone absorption. Activated charcoal should be used to block absorption with oral exposure. In animals, 10 mg of menadione (intravenously) reversed rotenone's blocking of mitochondrial oxidative phosphorylation; however, it is not known if this has been tried in humans.

Environmental Fate

Rotenone is rapidly degraded in soil and water, with half-lives of 1 and 3 days, respectively. Rotenone does not substantially leach into groundwater. Photodegradation also occurs such that little residue is left within 2–3 days of summer sunlight. It is also sensitive to heat, with much of the rotenone quickly lost at high temperatures.

Ecotoxicology

Rotenone is only slightly toxic in birds. The LD_{50} for rotenone in mallards and pheasants are $> 1.5\,g\,kg^{-1}$. A dietary LC_{50} of 4500–7000 ppm was reported in Japanese quail. Rotenone is highly toxic to fish whereas aquatic invertebrates have a wide range of sensitivity. Rotenone does not appreciably bioaccumulate. Rotenone is practically nontoxic to honey bees.

Exposure Standards and Guidelines

The reference dose for rotenone is 0.004 mg kg^{-1} day^{-1}. The 8 h permissible exposure limit for rotenone is 5 mg m^{-3}.

See also: Plants, Poisonous.

Further Reading

Hollingworth RM (2001) Inhibitors and uncouplers of mitochondrial oxidative phosphorylation. In: Krieger R (ed.) *Handbook of Pesticide Toxicology*, 2nd edn., pp. 1169–1261. San Diego, CA: Academic Press.

Relevant Websites

http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Rotenone.

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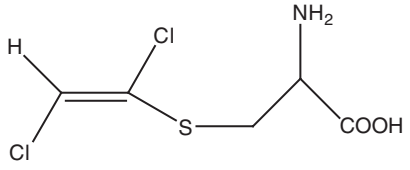
S

S-(1,2-Dichlorovinyl)-L-Cysteine

Vishal S Vaidya and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 627-72-5
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbons
- Chemical Formula: $C_5H_7Cl_2NO_2S$
- Chemical Structure:



Uses

S-(1,2-Dichlorovinyl)-L-cysteine (DCVC) is a model nephrotoxicant and cataractogen used to induce acute renal failure and cataracts in experimental animals to study the biochemical, physiological, and molecular mechanisms underlying the disease.

Exposure Routes and Pathways

The only way humans are potentially exposed to DCVC is through trichloroethylene (TCE) because it is a known metabolite of TCE, which is a common industrial solvent used for degreasing metals. TCE is produced in the United States at ~130 000 metric tons per year and is the most commonly found chemical contaminant of groundwater at many chemical waste sites. It is in the Agency of Toxic Substances and Disease Registry's National Priority List of Hazardous Chemicals and is an established animal carcinogen. Toxic and carcinogenic effects of TCE in the kidneys are hypothesized to be due to its metabolism by glutathione (GSH) conjugation, subsequent metabolism to the cysteine conjugation, DCVC, and metabolism of DCVC by the cysteine conjugate β-lyase to form reactive compounds. This chapter will not focus on the exposure pathways and potential toxic effects of TCE because that is discussed in a separate chapter on TCE.

Toxicokinetics

TCE conjugates with GSH yielding *S*-(1,2-dichlorovinyl)-glutathione, and which upon further metabolism yields DCVC. DCVC further undergoes *N*-acetylation to yield mercapturate, which because of its polarity, is readily excreted in the urine. The *N*-acetylation reaction is catalyzed by a cysteine *S*-conjugate *N*-acetyltransferase found in the endoplasmic reticulum (**Figure 1**). The mercapturate can also be deacetylated intracellularly, thus regenerating the cysteine conjugate. When [^{35}S]- and [^{14}C]-DCVC was administered intraperitoneally or intravenously to male Fisher 344 rats, a rapid initial half-life of 2.0 and 2.8 h, respectively was observed. The major plasma metabolite identified was inorganic sulfate, followed by pyruvate and *N*-acetyl-DCVC (NAcDCVC). Metabolite formation was rapid and peak plasma concentrations reached a maximum at 30 min, remained elevated for a few hours, and then decreased. In contrast to plasma the major urinary metabolite was NAcDCVC, followed by inorganic sulfate and pyruvate. The NAcDCVC that is secreted into the tubular lumen of the kidney or which arrives there by glomerular filtration and is not transported back into the renal epithelial cell for deacetylation is excreted into the urine. NAcDCVC has been recovered from rats, mice, and humans after exposure to TCE.

Mechanism of Toxicity

Cell death is initiated by the metabolism of DCVC via renal cysteine conjugate β-lyase, to a sulfur-containing reactive thiol radical that covalently binds to macromolecules (**Figure 1**). The findings that the nephrotoxicity and cataractogenesis of DCVC can be blocked by aminooxyacetic acid (a selective inhibitor of β-lyase) and probenecid (organic anion transport inhibitor) provide evidence for the roles of cysteine conjugate β-lyase and the organic anion transport system, respectively, in

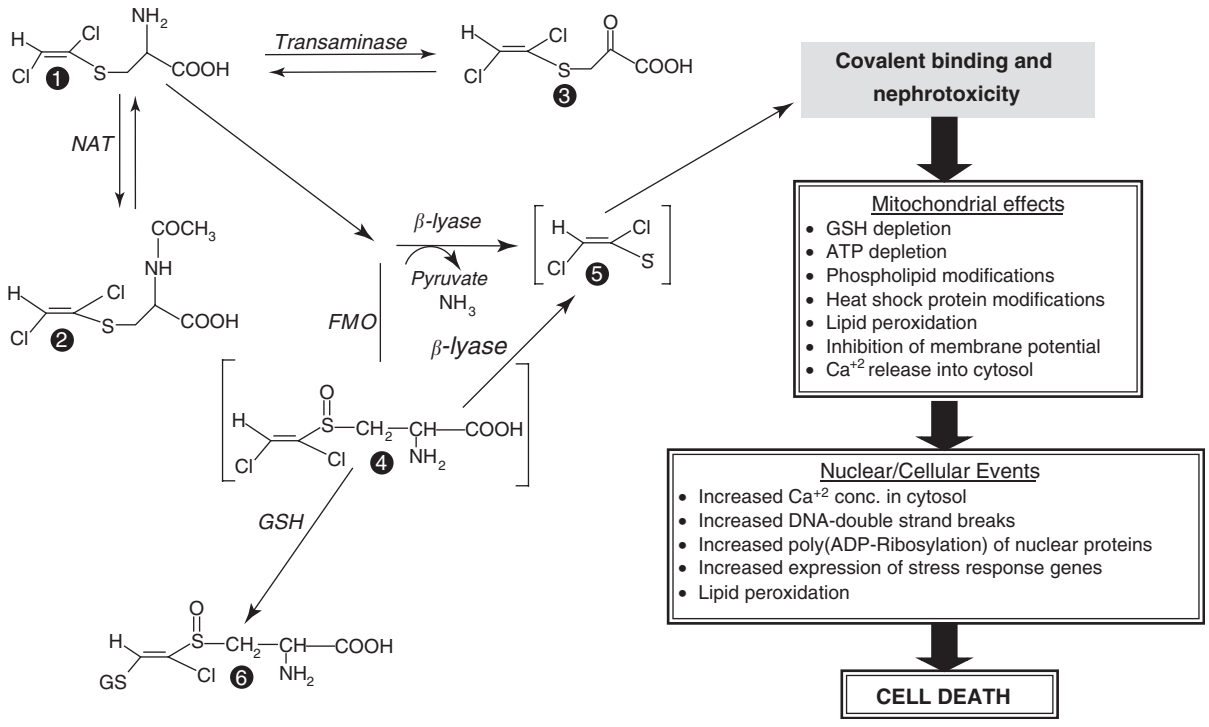


Figure 1 Metabolism of DCVC and mechanism of nephrotoxicity. ❶ *S*-(1,2-dichlorovinyl)-L-cysteine; ❷ *S*-(1,2-dichlorovinyl)-*N*-acetyl-L-cysteine; ❸ the α-keto acid metabolite of *S*-(1,2-dichlorovinyl)-L-cysteine; ❹ *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide; ❺ 1,2-dichlorovinylthiol; ❻ *S*-[1-chloro-2-(*S*-glutathionyl)vinyl]-L-cysteine sulfoxide.

DCVC-induced nephrotoxicity. Although the β-lyase enzyme is considered to be the major bioactivating enzyme for DCVC (**Figure 1**), other bioactivating enzyme activities have been described, and some of these may have relevance to risk assessment. Studies have shown that renal FMO3 can also metabolize DCVC to form DCVC sulfoxide thereby causing nephrotoxicity. Reports from several laboratories indicate that the cytotoxicity of DCVC is mediated at the mitochondrial level (**Figure 1**). Depletion of GSH, mitochondrial lipid peroxidation and GSSG formation, inhibition of mitochondrial lipoyl dehydrogenase activity, release of Ca^{2+} from mitochondria, and inhibition of mitochondrial membrane potential have been observed prior to renal cell death and correlated well with cytotoxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of DCVC is well characterized in various animal models including young calves, dogs, cats, rabbits, guinea pigs, turkeys, rats, and mice. With the possible exception of young calves, the primary target for DCVC is proximal tubules in the kidney. It selectively damages the S3 portion of the proximal tubule and causes a focal necrosis of the tubular epithelium in the outer stripe of the outer medulla. Various factors modulating DCVC nephrotoxicity *in vivo* and *in vitro* are summarized in **Table 1**. DCVC nephrotoxicity is usually followed by a nephrogenic repair response, which is characterized by an early stage of increase in proliferation of cells at the wound site by 24 h exposure, loss of differentiated character in the regenerative epithelium, and cessation of cell growth and redifferentiation between days 5 and 13. Studies also suggest that tissue repair is a critical determinant of the ultimate outcome of nephrotoxicity.

Human

Acute toxicity data for humans in unavailable and is unlikely to occur because this compound is a metabolite of TCE.

Chronic Toxicity (or Exposure)

Animal

When male Swiss Webster mice were given $0.1\,mg\,ml^{-1}$ of DCVC in drinking water for 37 or 46 weeks, by 26 weeks all the mice developed cortical cataracts. Cytomegaly, nuclear hyperchromatism and multiple nucleoli were noted in the cells of pars recta

Table 1 Factors modulating DCVC nephrotoxicity

	Effect	*Proposed Mechanism*
Age	Similar renal histological damage in mice of age 5, 15, 25 days old as compared with adult mice in spite of having an increasing accumulation of ^{14}C-DCVC in kidney with respect to age	Unknown
		No change in kidney β-lyase activity with respect to age
Sex	Higher toxicity in female mice as compared to male mice when administered a low dose (5 mg kg^{-1}, p.o.)	Higher β-lyase activity in the female mouse as compared to male mouse. However, this hypothesis is not consistent with the observed reversal in toxicity with respect to high dose
	Higher toxicity in male mice as compared to female mice when administered a higher dose (25 mg kg^{-1}, p.o.)	Sex differences in gastrointestinal biotransformation and/or absorption of DCVC
Species	Higher sensitivity of guinea pigs to DCVC-induced nephrotoxicity as compared to rats	Higher *in vivo* β-lyase activity in guinea pigs as compared to rats
		Lower cysteine *S*-conjugate *N*-acetyltransferase activity as compared to rats
Aminooxyacetic acid	Significantly reduces toxicity of DCVC *in vivo* and *in vitro*	Inhibits β-lyase activity
α-Ketoacids	Potentiate toxicity of DCVC *in vitro* and *in vivo*	α-Ketoacids increase the β-lyase activity
Probenecid	Inhibits both kidney binding and toxicity of DCVC	Inhibitor of organic anion transport in kidney

region of the kidney by 4 weeks and these mice later developed renal tubular atrophy and early interstitial fibrosis. This suggested that chronic DCVC ingestion in drinking water results in cataract formation and severe kidney injury but no incidence of renal tumors. These studies also indicate that the mutagenic potential of DCVC may not be expressed *in vivo*, perhaps due to natural repair and defense mechanisms. To put these findings into perspective, a 2 year bioassay in rats/mice is required.

Human

Some epidemiologic studies suggested a correlation between long-term, occupational exposures to high doses of TCE and the development of kidney tumors, whereas acute or chronic exposures to high levels may induce tubular necrosis in humans. However, others have failed to find any correlation between TCE exposure and renal cancer in humans. Conjugation of TCE with GSH yields *S*-(1,2-dichlorovinyl)-glutathione, and which upon further metabolism yields DCVC. Although GSH conjugation is not the predominant metabolic pathway for TCE, it may be the most relevant to the development of nephrotoxicity. GSH conjugation of TCE has been demonstrated *in vivo* in rats and humans exposed to TCE by detection of mercapturic acid in urine although the amount excreted as the mercapturate is very low. That the mercapturic acid and/or the corresponding GSH and cysteine S-conjugates are potent nephrotoxicants *in vivo* and cytotoxic *in vitro* is evidence that GSH conjugation is important in the development of the nephrotoxicity of TCE.

In Vitro Toxicity Data

Isolated proximal tubular cells from rat kidneys are susceptible to DCVC-induced necrosis at relatively high doses (>0.2 mmol l^{-1}). Similarly, high concentrations (>0.2 mmol l^{-1}) of DCVC are also required to produce significant necrosis in suspensions of freshly isolated human proximal tubular cells. DCVC has also been shown to induce apoptosis in primary cultures of rat proximal tubular cells and in the LLC-PK1 cells.

Clinical Management, Environmental Fate

These sections are the same for TCE and DCVC because human exposure to DCVC occurs only via potential formation of DCVC *in vivo* following TCE exposure.

See also: Kidney; Trichloroethylene.

Further Reading

Elfarra AA (1997) Halogenated hydrocarbons. In: Goldstein RS (ed.) *Comprehensive Toxicology – Renal Toxicology*, pp. 601–616. New York: Elsevier.

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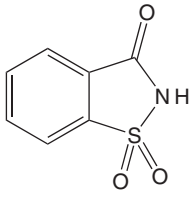
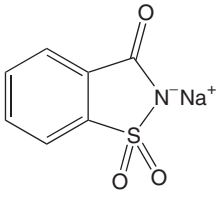
Saccharin

Robin C Guy

- Chemical Abstracts Service Registry Numbers: CAS 81-07-2; CAS 128-44-9 (sodium saccharin); CAS 6485-34-3 (calcium saccharin)
- Synonyms: 1,2-Benzisothiazol-3(2*H*)-one; 1,1-Dioxide benzosulfimide; 1,2-Benzisothiazol-3(2*H*)-one, 1,1-dioxide, sodium salt; 1,2-Benzisothiazol-3(2*H*)-one, 1,1-dioxide, calcium salt
- Chemical/Pharmaceutical/Other Class: Artificial sweetener
- Chemical Formula: $C_7H_5NO_3S$
- Chemical Structures:



Saccharin



Saccharin sodium salt

Uses

Saccharin is used as an artificial sweetener in foods, beverages, and personal care products.

Background Information

Saccharin has been produced commercially produced commercially in the United States for over 80 years. In the 8th report, 1998 National Toxicology Program (NTP) Report on Carcinogens from the (US) National Institute of Environmental Health Sciences (NIEHS), and until 2002, saccharin was classified as a 'reasonably anticipated carcinogen' in the United States. The Calorie Control Council submitted a nomination to the NTP to consider removing saccharin from the Report on Carcinogens based upon mechanistic data related to development of urinary bladder cancers in rats. Following a formal review by NTP, saccharin was delisted from the 10th Report on Carcinogens, because the rodent cancer data are not sufficient to meet the current criteria to list this chemical as 'reasonably anticipated to be a human carcinogen'. This is based on the observed bladder tumors in rats that arise by mechanisms not relevant to humans, and the lack of data in humans suggesting a carcinogenic hazard. Saccharin was finally removed from the carcinogen list in 2002.

The Human Health Assessment Group in the US Environmental Protection Agency's (EPA's) Office of Health and Environmental Assessment has evaluated saccharin for carcinogenicity. According to their analysis, the weight-of-evidence for saccharin is group C, which is based on inadequate evidence in humans and limited evidence in animals. As a group C chemical, saccharin is considered to be possibly carcinogenic to humans.

The International Agency for Research on Cancer (IARC) has judged that there is inadequate evidence in humans for the carcinogenicity of saccharin salts used as sweeteners; however, there is sufficient evidence in experimental animals for the carcinogenicity of sodium saccharin, and there is inadequate evidence in experimental animals for the carcinogenicity of saccharin (acid form) and calcium saccharin. Overall evaluation: in making the evaluation, the working group concluded that sodium saccharin produced urothelial bladder tumors in rats by a non-DNA reactive mechanism that involves the formation of a urinary calcium phosphate containing precipitate, cytotoxicity, and enhanced cell proliferation. The mechanism is not relative to humans because of critical interspecies differences in urine composition. Saccharin and its salts are not classifiable as to the carcinogenicity to humans (group 3).

The European Commission Scientific Committee for Food in 1997 established 1% sodium saccharin in the diet as a clear no-observed-effect level (NOEL) in relation to male rat bladder tumors and for other non-neoplastic effects of saccharin. In response to primarily updated experimental data and the extensive epidemiological data with no evidence of any relationship between saccharin intake and bladder cancer in humans, the Committee set a full acceptable daily intake (ADI) for sodium saccharin of 0–5 mg kg^{-1} body weight. If the ADIs were expressed in terms of the free acid, since sodium saccharin is not the only salt used, and taking into account of the molecular weight difference between sodium saccharin (molecular weight 241) and the free acid (molecular weight 183), then ADI expressed as the free acid is 0–3.8 mg kg^{-1} body weight.

Exposure Routes and Pathways

Oral: Occupational exposure to saccharin may occur through inhalation of dust particles and dermal contact with this compound at workplaces where saccharin is produced or used. The general population

may be exposed through the ingestion of food products such as soft drinks, table sweeteners, and candy that contain this product.

Toxicokinetics

Saccharin is excreted primarily unchanged in the urine.

Mechanism of Toxicity

There is evidence that saccharin is a promoter for bladder cancer, primarily in rodents. It has been delisted from the US NTP Report on Carcinogens, because the rodent cancer data are not sufficient to meet the current criteria to list this chemical as 'reasonably anticipated to be a human carcinogen'. This is based on the judgment perception that the observed bladder tumors in rats arise by mechanisms not relevant to humans, and the lack of data in humans suggesting a carcinogenic hazard. Studies indicate that the observed urinary bladder cancers in rats are related to the physiology of the rat urinary system including urinary pH (>6.5), decreased urine osmolality, increased urine volume, and the presence of urinary crystals or precipitate, and urothelial damage triggering a hyperplasia following consumption of dietary concentrations of 3% or higher with inconsistent findings at lower dietary concentrations. The factors thought to contribute to tumor induction by high doses of sodium saccharin in rats would not be expected to occur in humans.

Acute and Short-Term Toxicity (or Exposure)

Animal

The mouse oral LD_{50} is $17\,g\,kg^{-1}$.

Human

The effects found range from headaches, dizziness, severe depression, and palpitations. Tachycardia has been reported.

Chronic Toxicity (or Exposure)

Animal

In four studies of up to 30 months duration, sodium saccharin was carcinogenic in Dawley male rats as evidenced by a dose-related increased incidence of benign or malignant urinary bladder neoplasms at dietary concentrations greater than 1%. Slight increases (not statistically significant) in urinary bladder cancer have also been observed in female rats from studies showing a positive effect in males. In addition, several initiation/promotion studies in different rat strains have shown a reduced latency and/or increased incidence of similar urinary bladder cancers in male and female rats fed sodium saccharin subsequent to treatment with different urinary bladder initiators. The mouse data are inconsistent and require verification by additional studies.

Human

Results of several epidemiology studies indicate no clear association between saccharin consumption and urinary bladder cancer. Although it is impossible to absolutely conclude that it poses no threat to human health, sodium saccharin is not reasonably anticipated to be a human carcinogen under conditions of general usage as an artificial sweetener. Most of the relevant human epidemiology studies have examined associations between urinary bladder cancer and artificial sweeteners, rather than saccharin *per se*. The time trend data for bladder cancer show no clear indication that the increased use of saccharin or artificial sweeteners commencing in the 1940s is associated with a general increase in bladder cancer when controlled for confounding factors, chiefly smoking. Risks of bladder cancer in diabetics, who presumably consume greater amounts of artificial sweeteners compared to the general population, are not greater than risks in the general population.

In Vitro Toxicity Data

Studies of the genotoxicity of saccharin have shown generally negative but occasionally conflicting results. Sodium saccharin is essentially nonmutagenic in conventional bacterial systems but is weakly clastogenic or genotoxic in short-term *in vitro* and in some *in vivo* test systems. Urine from mice treated with sodium saccharin was mutagenic in the Ames test in one study. Saccharin does not covalently bind to DNA and does not induce unscheduled DNA synthesis in bladder urothelium.

Environmental Fate

Saccharin's production and use as a nonnutritive sweetener may result in its release to the environment through various waste streams. Saccharin will exist in both the vapor and particulate phases in air, and vapor-phase saccharin will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 3 days. Particulate-phase

saccharin will be removed from the atmosphere by wet and dry deposition. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's law constant. An estimated bioconcentration factor (BCF) of 3 suggests bioconcentration in aquatic organisms is low. This compound has the potential to chemically hydrolyze in aqueous environments to *o*-sulfamoylbenzoic acid and ammonium *o*-sulfobenzoic acid, but the kinetics of the potential hydrolysis is unknown. The importance of biodegradation in soil and water is unknown, but amides are usually susceptible to microbial metabolism.

Ecotoxicology

No ecotoxicity issues exist.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, 8 h time-weighted average (TWA) is $10\,mg\,m^{-3}$. The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA is $15\,mg\,m^{-3}$.

See also: Carcinogenesis.

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Whysner J and Williams GM (1996) Saccharin mechanistic data and risk assessment: Urine composition, enhanced cell proliferation, and tumor promotion. *Pharmacology & Therapeutics* 71: 225–252.

Relevant Website

http://ehp.niehs.nih.gov – US National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program. Report on Carcinogens from the National Toxicology Program. Agents, substances, mixtures, or exposure circumstances delisted from report on carcinogens.

Safe Drinking Water Act, US

Robert Kapp

- AGENCY: US Environmental Protection Agency (EPA)
- YEAR OF ENACTMENT: 1974; amended 1986 and 1996

Background

The first known public water system in the United States was built in Philadelphia as early as 1799. Over 400 additional water systems had been built in major cities throughout the nation by 1860, and this increased to over 3000 by the early 1900s. With these systems came several major outbreaks of disease because when the supply water was contaminated, the system was an efficient way to spread the contamination throughout the community. In 1849, there were cholera epidemics in New York City that claimed 8000 lives and another in New Orleans that claimed 5000, which were clearly linked to the water distribution system.

Federal legislation on the quality of drinking water began in 1914 when the US Public Health Service (USPHS) set standards for bacterial quality. These original standards applied to contaminants that could cause contagious disease in water systems, which provided drinking water to interstate carriers such as ships, trains, and buses. The USPHS subsequently revised and expanded these standards in 1925, 1946, and 1962. It is estimated that there were over 19 000 public water systems by 1960 growing to ~62 000 operating water systems by 1980. The 1962 standards regulated 28 different substances. By 1962 nearly all of the 50 states adopted the USPHS standards as either standards or guidelines. However, as society developed and became more sophisticated utilizing many man-made chemicals in agriculture and industry, many of these new substances began to appear in water supplies. Further, it was believed that many of these new chemicals were suspected of

causing health problems. The USPHS conducted a survey in 1969 which disclosed that only 60% of the nation's public water systems met the 1962 water standards. There were major deficiencies in over 50% of the systems. In addition, the water systems serving 500 people or less had the most deficiencies. These and other findings led to the passage of the Water Quality Improvement Act of 1970 (Public Law 91-224), which expanded the federal government's authority to set water quality standards.

Continuing concerns over water pollution led to the enactment of the Federal Water Pollution Control Act of 1972 (Public Law 92-500), which contained comprehensive provisions for restoring and maintaining all bodies of surface water. Another USPHS study conducted in 1972 found 36 chemicals, including trihalomethanes, detected in the treated water from treatment plants that were drawing water from the Mississippi River in Louisiana. Based upon the concerns over the findings in these and other studies, several legislative proposals were introduced to Congress in 1973. The Safe Drinking Water Act (Public Law 93-523) was signed into law by President Ford in 1974 to protect human health from contaminants in drinking water and to prevent contamination of existing groundwater supplies.

Overview of the Safe Drinking Water Act

The primary focus of the Safe Drinking Water Act (SDWA) was to set national contaminant-based drinking water standards. These included primary standards intended to address adverse health effects and consist of maximum contaminant level goals (MCLGs), which are nonenforceable goals, and maximum contaminant levels (MCLs) which are enforceable limits set as close as possible to the MCLGs. The MCLs represent an upper limit on the permissible concentrations of regulated contaminants in public drinking water supplies. The MCLGs are the maximum concentrations below which no negative human health effects are known to exist.

Also included in the legislation were secondary standards such as odor and appearance of the drinking water, which were not enforceable. In this law, public water systems were defined to include any water system that serves water to more than 25 people (or 15 service connections). The SDWA required EPA to promulgate interim national primary drinking water standards in order to "protect health to the extent feasible taking costs into consideration." Each contaminant was to determine an MCL or a treatment technique for its control. The interim regulations were replaced with recommendations for the MCLs based upon peer-reviewed science from the (US) National Academy of Sciences.

1986 SDWA Amendments

In 1986, the SDWA Amendments were passed to move EPA closer to enforcing the original Act. Only 23 contaminants had been established since the 1974 legislation and no treatment techniques had been established for any of the contaminants. The 1986 legislation required EPA to set standards (MCLs and MCLGs) for a total of 83 contaminants in the next 3 years. EPA was also directed to prescribe regulations for two treatment techniques for public water systems – namely filtration and disinfection. The 1986 Amendment also gave EPA the authority to fine violators as much as $25 000 per day per violation.

1996 SDWA Amendments

On August 6, 1996, the SDWA was amended again with the goals of establishing scientifically based programs there are flexible with technical and infrastructure assistance. The 1996 Amendments established water contamination prevention requirements including source water protection, capacity development, and operator certification.

The SDWA required formalization of the procedure to set enforceable health-based drinking water standards as follows:

1. Determine whether a contaminant should be regulated based upon peer-reviewed science.
2. Set an MCLG. These goals do not take into account available technology and therefore are sometimes set at levels which public water systems cannot attain. These levels are not enforceable.
3. Propose an enforceable standard in the form of an MCL or a treatment technique (TT). MCLs are set as close to the MCLGs as feasible considering available technology and cost. Required monitoring schedules are part of the enforceable standard. Upon determination of a proposed MCL or TT that is close to the MCLG as possible based upon affordable technology, EPA must perform a cost/benefit analysis to determine whether or not the benefits justify the costs.
4. EPA sets an enforceable MCT or TT. Upon review of all of the data, EPA sets an enforceable MCT or TT level including required testing and reporting schedules.
5. States are authorized to grant variances from EPA standards for water systems serving less than 3301 people if the systems cannot afford to comply with

the ruling. State variances to systems with 3301–10 000 people need EPA approval. No systems are permitted to have variances for microbial contaminants.

Also included were consumer information requirements comprising the development of consumer confidence reports and new notification requirements. The final right-to-know SDWA legislation requires specific information on the following:

1. What contaminants are found in the tap water distributed by the water system.
2. What the water source is for the water system in question.
3. Any known pollution sources responsible for detected contaminants.
4. Listing and details of any violations during the previous 12 months.

In addition, the water system is responsible for the following:

1. Sending a report to all water system customers.
2. For making a good-faith effort to get the report to tenants and others who would not receive a water bill, but who would otherwise use the water.
3. The report must not be cluttered or obscured with extraneous data, which is not directly critical to the purpose of the report.
4. Tabular data cannot be obscured with irrelevant information or presented in a way that is difficult for the recipient to interpret.

The 1996 Amendments further require EPA to establish a mechanism to identify and select new contaminants, as well as specific efforts to establish criteria for arsenic, sulfates, radon, and disinfection by-products. The SDWA required EPA to establish a list of contaminants every five years that are known or anticipated to occur in public water systems and may require further investigation and possible regulation under SDWA. The list is divided into those materials that are candidates for additional research, those that need additional occurrence data, and those that are priorities for consideration in rulemaking. The EPA then must prioritize the critical substances in each category and develop a plan of action for making regulatory decision for the most appropriate candidates.

The National Contaminant Occurrence Database, which stores data on the occurrence of both regulated and unregulated materials, was established by EPA. The monitoring data provides the basis for identifying contaminants that may be placed on future Contaminant Candidate Lists and support the Agency's decisions to regulate contaminants in the future.

See also: Clean Water Act (CWA), US; Pollution, Water.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency (EPA), the Safe Drinking Water website.

Safety Pharmacology

S Satheesh Anand and Harihara M Mehendale

Definition

Safety pharmacology is defined as the field that investigates the potentially undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above. It is also called functional safety.

Background

Successful drug development requires precise drug safety assessment. The importance of evaluating the safety of medicinal products before they are allowed into the market was realized following unacceptable levels of unanticipated deaths after drugs have entered the market. The eventual regulatory requirements were reached at different times in different regions. In the United States, a tragic mistake in the formulation of a children's syrup in the 1930s and in Europe, the thalidomide tragedy in the 1960s, are some examples that triggered the regulations requiring product authorization. Safety pharmacology has been an emerging discipline within the pharmaceutical industry in which unanticipated effects of new drug candidates on major organ function are critically assessed in a variety of animal models. Traditionally, drug safety studies are designed to examine effects other than the primary therapeutic effect of a drug candidate. While these studies evaluate the toxic profile of the drug candidate at

maximum tolerated dose and in selected organs, the effects on normal physiological functions at therapeutic doses have long been neglected. Deaths and adverse effects of drugs have been reported in patients and clinical trial participants due to failure in physiologic functions. Pharmacoepidemiology studies in Europe and the United States show that adverse drug reactions now may account up to 10% of admissions in hospitals. In the United States from 1954 until 1980, 7 000 000 people participated in clinical trials. Safety pharmacology studies are developed to help protect clinical trial participants and patients receiving marketed products from potential adverse effects of pharmaceuticals, while avoiding unnecessary use of animals and other resources. Until recently, there have been no internationally accepted definitions, objectives, or recommendations on the design and conduct of safety pharmacology studies. The harmonization in regulation was impelled by concerns over rising costs of healthcare, escalation of the cost of R&D, and the need to meet the public expectation that there should be a minimum delay in making safe and efficacious new treatments available to patients in need.

In 1990, representatives of the regulatory agencies and industry associations of Europe, Japan, and the United States proposed International Conference on Harmonisation (ICH) to develop harmonized guidance on technical issues aimed at ensuring that good quality, safe, and effective medicines are developed and registered in the most efficient and cost-effective manner. These activities are pursued in the interest of the consumer and public health, to prevent unnecessary duplication of clinical trials in humans and to minimize the use of animal testing without compromising the regulatory obligations of safety and effectiveness. The ICH guideline on safety pharmacology (ICHS7A) was recommended for adoption by regulatory bodies in the European Union, the United States, and Japan in November 2000 and it has become effective worldwide since 2001. However, the efforts are in preliminary stage. With this guideline calling for tests on the effects of compounds on vital functions of the human body, the data providing specific information on the safety profile of a new potential therapeutic agent used by clinicians designing clinical studies and by regulatory agencies in their assessment of the safety of a new product is crucial. A brief description of the safety pharmacology studies is presented in this article.

Types of Pharmacological Studies

Pharmacology studies can be divided into three categories: primary pharmacodynamic, secondary pharmacodynamic, and safety pharmacology studies. Studies on the mode of action and/or effects of a substance in relation to its desired therapeutic target are primary pharmacodynamic studies. Studies on the mode of action and/or effects of a substance not related to its desired therapeutic target are secondary pharmacodynamic studies. These have sometimes been referred to as part of general pharmacology studies.

Safety pharmacology studies investigate adverse effects of drugs on vital functions in mammalian species at therapeutic concentrations. Primary evaluations are cardiovascular, respiratory, and central nervous system assessment. The supplemental evaluations are renal/urinary function, gastrointestinal function, and immune function assessment. In addition to these batteries of tests, knowledge of potential harmful interactions or interactions between medications that may neutralize the beneficial effects is becoming increasingly important to evaluate the coadministration of drugs.

Objective

The objectives of safety pharmacology studies are: (1) to identify undesirable pharmacodynamic properties of a substance that may have relevance to its human safety; (2) to evaluate adverse pharmacodynamic and/or pathophysiological effects of a substance observed in toxicology and/or clinical studies; and (3) to investigate the mechanism of the adverse pharmacodynamic effects observed and/or suspected.

Study Design

It is important to adopt a rational approach when selecting and conducting safety pharmacology studies. The specific studies that should be conducted and their design will vary based on the individual properties and intended uses of the pharmaceuticals. Some safety pharmacology endpoints can be incorporated in the design of toxicology, kinetic, clinical studies, etc., while in other cases these endpoints should be evaluated in specific safety pharmacology studies. Although the list is neither exhaustive, nor comprehensive, the following list serves as an example of factors considered:

1. effects related to the therapeutic class of the test substance, since the mechanism of action may suggest specific adverse effects (e.g., proarrhythmia is a common feature of antiarrhythmic agents);
2. adverse effects associated with members of the chemical or therapeutic class, but independent of

the primary pharmacodynamic effects (e.g., antipsychotics and QT prolongation);
3. ligand binding or enzyme assay data suggesting a potential for adverse effects;
4. harmful effects or neutralization of intended effect of a therapeutic substance as a result of interactions (e.g., barbiturates);
5. results from previous safety pharmacology studies, from secondary pharmacodynamic studies, from toxicology studies, or from human use that warrant further investigation to establish and characterize the relevance of these findings to potential adverse effects in humans.

During early development, sufficient information (e.g., comparative metabolism) may not always be available to rationally select or design the studies in accordance with the points stated above; in such circumstances, a more general approach in safety pharmacology investigations can be applied. The methods used must be validated, well established, and should be in common use and should give reliable, reproducible results every time.

A hierarchy of organ systems can be developed according to their importance with respect to life-supporting functions. Vital organs or systems, the functions of which are acutely critical for life, such as the cardiovascular, respiratory, and central nervous systems, are considered to be the most important ones to assess in safety pharmacology studies. Other organ systems, such as the renal or gastrointestinal system, the functions of which can be transiently disrupted by adverse pharmacodynamic effects without causing irreversible harm, are of less immediate investigative concern. Safety pharmacology evaluation of effects on these other systems may be of particular importance when considering factors such as the likely clinical trial or patient population, for example, gastrointestinal tract in Crohn's disease, renal function in primary renal hypertension, and immune system in immunocompromised patients.

Test Systems

Consideration should be given to the selection of relevant animal models or other test systems so that scientifically valid information can be derived. Selection factors can include the pharmacodynamic responsiveness of the model, pharmacokinetic profile, species, strain, gender, and age of the experimental animals, the susceptibility, sensitivity, and reproducibility of the test system and available background data on the substance. Data from humans (e.g., *in vitro* metabolism), when available, should also be considered in the test system selection. The time points for the measurements should be based on pharmacodynamic and pharmacokinetic considerations. Justification should be provided for the selection of the particular animal model or test system. Animal models as well as *ex vivo* and *in vitro* preparations can be used as test systems. *Ex vivo* and *in vitro* systems can include, but are not limited to: isolated organs and tissues, cell cultures, cellular fragments, subcellular organelles, receptors, ion channels, transporters, and enzymes. *In vitro* systems can be used in supportive studies, for example, to obtain a profile of the activity of the substance or to investigate the mechanism of effects observed *in vivo*. In conducting *in vivo* studies, the use of unanesthetized animals is preferred. In the use of unanesthetized animals, the avoidance of discomfort or pain is a foremost consideration. The use of the same species is preferred for *in vivo* tests as those used in drug metabolism, pharmacokinetics, and toxicology – generally, rats and dogs.

Appropriate negative and positive control groups are included in the experimental design. The expected clinical route of administration should be used when feasible. Regardless of the route of administration, exposure to the parent substance and its major metabolites should be similar to or greater than that achieved in humans when such information is available.

Dose Levels or Concentrations of Test Substances and Metabolites

Safety pharmacology studies are intended to define the dose–response relationship of the adverse effect observed. The time course (e.g., onset and duration of response) of the adverse effects should be investigated, when feasible. Generally, the doses eliciting the adverse effects should be compared to the doses eliciting the primary pharmacodynamic effect in the test species or the proposed therapeutic effect in humans, if feasible. Since there are species differences in pharmacodynamic sensitivity, doses should include and exceed the primary pharmacodynamic or therapeutic range. In the absence of an adverse effect on the safety pharmacology parameter(s) evaluated in the study, the highest tested dose should be a dose that produces moderate adverse effects in this or in other studies using similar route and duration.

In vitro studies should be designed to establish a concentration–effect relationship. The range of concentrations used should be selected to increase the likelihood of detecting an effect on the test system. The upper limit of this range may be influenced by

physicochemical properties of the test substance and other assay specific factors. In the absence of an effect, the range of concentrations selected should be justified.

Safety pharmacology studies are generally performed by a single dose administration. When pharmacodynamic effects occur only after a certain duration of treatment, or when it results from repeat dose nonclinical studies, or results from use in humans, give rise to concerns about safety pharmacological effects, the duration of the safety pharmacology studies to address these effects should be rationally based.

Generally, any parent compound and its major metabolite(s) that achieve, or are expected to achieve systemic exposure in humans should be evaluated in safety pharmacology studies. Evaluation of major metabolites is often accomplished through studies of the parent compound in animals. Additionally, if metabolites from humans are known to substantially contribute to the pharmacological actions of the therapeutic agent, it may be important to test such active metabolites.

Safety Pharmacology Core Battery of Tests

The purpose of the safety pharmacology core battery of tests is to investigate the effects of the test substance on vital functions. In this regard, the cardiovascular, respiratory, and central nervous systems are usually considered the vital organ systems that should be studied in the core battery of tests. In some instances, based on scientific rationale, the core battery may be supplemented by other tests, or some of the tests may become unnecessary.

Central Nervous System

Effects of the test substance on the central nervous system should be assessed appropriately. Motor activity, behavioral changes, coordination, sensory/motor reflex responses, analgesia test (hot plate), proconvulsant activity, barbiturate-induced sleeping time, and body temperature should be evaluated. For example, a functional observation battery, modified Irwin's, or other appropriate tests can be used.

Cardiovascular System

Effects of the test substance on the cardiovascular system should be assessed appropriately. Blood pressure, heart rate, and the electrocardiogram should be evaluated. *In vivo*, *in vitro*, and/or *ex vivo* evaluations, including methods for repolarization and conductance abnormalities, should also be considered.

Respiratory System

Effects of the test substance on the respiratory system should be assessed appropriately. Respiratory rate and other measures of respiratory function (e.g., tidal volume or hemoglobin oxygen saturation) should be evaluated. Clinical observation of animals is generally not adequate to assess respiratory function, and thus these parameters should be quantified by using appropriate methodologies.

Supplemental Safety Pharmacology Studies

Supplemental studies are meant to evaluate potential adverse pharmacodynamic effects on organ system functions not addressed by the core battery of tests or repeated dose toxicity studies when there is a cause for concern.

Renal/Urinary System

Effects of the test substance on renal parameters should be assessed. For example, urinary volume, specific gravity, osmolality, pH, fluid/electrolyte balance, proteins, cytology, and blood chemistry determinations such as blood urea nitrogen, creatinine, and plasma proteins can be used.

Autonomic Nervous System

Effects of the test substance on the autonomic nervous system should be assessed. For example, binding to receptors relevant for the autonomic nervous system, functional responses to agonists or antagonists *in vivo* or *in vitro*, direct stimulation of autonomic nerves and measurement of cardiovascular responses, baroreflex testing, and heart rate variability can be used.

Gastrointestinal System

Effects of the test substance on the gastrointestinal system should be assessed. For example, gastric secretion, gastrointestinal injury potential, bile secretion, transit time *in vivo*, ileal contraction *in vitro*, and gastric pH measurement can be used.

Other Organ Systems

Effects of the test substance on organ systems not investigated elsewhere should be assessed when there is a reason for concern. For example, dependency potential or skeletal muscle, immune and endocrine functions can be investigated.

Adverse effects may be suspected based on the pharmacological properties or chemical class safety pharmacology core battery, clinical trials, pharmacovigilance, or from literature reports. When such

potential adverse effects raise concern for human safety, these should be explored in follow-up or supplemental safety pharmacology studies, as appropriate. Follow-up studies are meant to provide a greater depth of understanding, or additional knowledge to, than that are provided by the core battery of tests on vital functions. The following studies may be conducted to further evaluate these organ systems for potential adverse pharmacodynamic effects. These lists are not meant to be comprehensive or prescriptive, and the studies should be selected on a case-by-case basis after considering factors such as existing nonclinical or human data.

Central Nervous System

The studies on the central nervous system include those on behavioral pharmacology, learning and memory, ligand-specific binding, neurochemistry, visual, auditory, and/or electrophysiology examinations, etc.

Cardiovascular System

These studies concern with cardiac output, ventricular contractility, vascular resistance, the effects of endogenous and/or exogenous substances on the cardiovascular responses, etc.

Respiratory System

Airway resistance, compliance, pulmonary arterial pressure, blood gases, blood pH, etc. are studied.

The core battery of tests as well as the supplementary safety pharmacology studies can be conducted at the very beginning of *in vivo* screening and, at the latest, before first studies in man. The safety pharmacology core battery of tests as well as follow-up and supplemental studies should be conducted in compliance with good laboratory practice (GLP). Any study or study component not conducted in compliance with GLP should be adequately justified, and the potential impact on evaluation of the safety pharmacology endpoints should be explained.

Conditions Under which Studies are not Necessary

Safety pharmacology studies may not be needed for locally applied agents (e.g., dermal or ocular) where the pharmacology of the test substance is well characterized, and where systemic exposure or distribution to other organs or tissues is demonstrated to be low. For biotechnology-derived products that achieve highly specific receptor targeting, it is often sufficient to evaluate safety pharmacology endpoints as a part of toxicology and/or pharmacodynamic studies, and therefore safety pharmacology studies can be reduced or eliminated for these products. In addition, testing is not required for new salts having similar pharmacokinetics and pharmacodynamics, and cytotoxic agents for treatment of end-stage cancer patients.

Conclusions

Pharmacology studies have been performed worldwide for many years as a part of the nonclinical evaluation of pharmaceuticals for human use. Safety pharmacology studies are focused on identifying adverse effects on physiological functions at therapeutic doses. These studies are necessary not only to protect the patients treated with drugs, but also the healthy volunteers participating in the clinical trials. The 1960s and 1970s saw a rapid increase in laws, regulations, and guidelines for reporting and evaluating the data on safety, quality, and efficacy of new medicinal products. Until recently, although different regulatory systems were based on the same fundamental obligations to evaluate the quality, safety, and efficacy, the detailed technical requirements were different from each other. Because the pharmaceutical industries have to deliver the safe therapeutics rapidly due to many factors, the ICH was established in 1990 as a joint regulatory/industry project to improve, through harmonization, the efficiency of the process for developing and registering new medicinal products in Europe, Japan, and the United States. The ICH guideline on safety pharmacology has become effective worldwide since 2001. The effects of a test substance on the functions listed in the safety pharmacology core battery should be investigated prior to first administration in humans. Any follow-up or supplemental studies identified as appropriate, based on a cause for concern should also be conducted. No simple formula or set of tests is ideal for safety pharmacology studies for all kind of therapeutic compounds. Knowledge of the pharmacology of the compound and any knowledge gained from traditional toxicity can help to better determine and assess the safety of compounds. Although the ICH guideline for safety pharmacology is in place, it is in an early stage and the actual implementation of requirements and the use of resulting data in risk/benefit decision will require time to be fully worked out and understood.

See also: Safety Testing, Clinical Studies; Toxicity Testing, Validation.

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Safety Testing, Clinical Studies

Alessandra Pagnoni

Clinical safety studies are conducted in human volunteers to determine the safety of chemicals, drugs, formulations, devices, or other products, which may come in contact with the human body. Clinical safety testing should always be conducted prior to efficacy investigations in order to determine the benefit/risk ratio of efficacy trials. When designing a study, "benefits and risks shall be balanced and shown to be in a favorable ratio" (The Belmont Report). "The rights, safety and well-being of the trial subjects are the most important considerations…." (ICH Guidelines 2.3).

The approach to clinical safety studies differentiates between drugs and cosmetics (or their ingredients), and between topical and systemic formulations. The initial safety clinical studies for systemic drugs are usually conducted in a small number of humans. In the United States, the Food and Drug Administration (FDA) categorizes these under phase I studies, which usually involve as few as 10 healthy volunteers. These may include pharmacokinetic (PK) studies and dose escalation studies to determine possible adverse events. For topical drugs, trials may include skin studies for irritation (primary and cumulative applications), allergy (repeated insult patch testing – RIPT), PK and bioavailability (dermatopharmacokinetic – DPK), phototoxicity, and photoallergy. The FDA Guidance for Photosafety Testing states that "…photoirritation and photoallergy studies in humans should be considered for all drug substances and formulation components that absorb UVB, UVA, or visible radiation (290–700 nm) and are directly applied to the [sun exposed] skin or eyes, or significantly partition to one of these areas when administered systemically..."

If the drug passes the initial safety tests, then its safety is evaluated more rigorously against a placebo within larger efficacy trials.

Safety testing for cosmetics includes usually cumulative irritation, RIPT, phototoxicity, photoallergy, and finally, exaggerated use or home use studies.

Suggested guidelines for safety testing of topical materials are explained below. Skin reactions that develop under these skin tests, with a comparison to the controls where applicable, are the bases for conclusions on safety. Skin responses can range from mild erythema to bullous or edematous reactions.

Primary irritation: This test is designed to give information on the short-term irritation potential of the formulation, and usually consists of a controlled patch test involving 15–30 subjects with a one-time (24 or 48 h) application.

Cumulative irritation: The scope of this study is to determine long-term irritation potentials under exaggerated conditions or to assess and compare the mildness of formulations. It consists of a controlled patch test involving 15–30 subjects using 14–21 repetitive 24 h applications. The FDA recommends a 21 day patch test in a 30-subject panel. For more irritating cosmetics, a 14 day application would satisfy the objective of the study.

Repeated insult patch test (RIPT): RIPT is a key investigation for evaluating the potential of a topical to induce delayed contact allergy. It consists of a repetitive patch test study involving 100–200 subjects. It is divided into an Induction (of sensitization) Phase (9×24 or 48/72 h applications for 3 weeks), a Rest Phase (10–21 days), and a Challenge Phase (single 24 or 48 h applications with evaluations at 48 and 72/96 h postapplication to a naïve site). The FDA recommends 48/72 h repeated exposures (Jordan-King design) in a 200-subject panel. For hypoallergenic claims, a 200-subject panel is also recommended. Observations at the naive site during challenge and the patterns of reactivity during the induction period provide a basis for determining if the formulation is a contact sensitizer. In general, while irritant responses occur in a large number of subjects and appear also during the induction phase, allergic responses tend to occur only in a few individuals and only at challenge, unless the subject has been presensitized to the allergen. Additionally, a contact allergen induces a reaction that tends to escalate at 72–96 h postexposure, while an irritant response tends to improve after removal of the offending agent. The exaggeration of

conditions under patch application is important since it appears that to elicit a skin sensitization response, the antigen dose should produce an adequate irritation or 'danger' signal as described by McFadden in *Contact Dermatitis*, 2000. When assessing the incidence of responses, it should be noted that a zero sensitization in a 100-subject panel simply indicates that the rate of sensitization in the population is not likely to exceed approximately 2.95% as reported by Henderson and Riley – *Certain Statistical Considerations in Patch Testing* in 1945.

Phototoxicity and photoallergy tests: Photosensitivity is a term used to describe an adverse reaction, irritant or allergic in nature, elicited by light. Most phototoxic/photoallergic agents are activated by UVA radiations, although some reactions can occur at different wavelengths. Radiations absorbed by the chemical in the skin create a photochemical reaction, which may produce direct cellular/tissue damage (phototoxicity) or may induce an immune, cell-mediated, delayed hypersensitivity reaction (photoallergy) through the creation of a photoproduct that binds to proteins and forms an allergen. Phototoxic reactions are more frequent than photoallergic ones.

Tests to study phototoxicity and/or photoallergy usually involve 25 subjects with test material bearing patches applied in duplicate: one set does not receive radiations while the second set is irradiated. In phototoxicity, patches are applied for 24 h. The irradiated patch is exposed to $16\,J\,cm^{-2}$ UVA and 0.75 MED (minimum erythema dose) UVB. All sites are evaluated at 1, 24, 48, and 72 h following exposure.

As with RIPT, photoallergy testing is conducted in three phases. The Induction Phase consists of 6×6–24 h applications (for 3 weeks), each followed by 2 MEDs UVB exposures and evaluations at 24/72 h postexposure. A second set of patches remains unexposed. This is followed by a Rest Phase of 10–21 days and then by a Challenge Phase (one duplicate 6–24 h application, followed by irradiation with $16\,J\,cm^{-2}$ UVA and 0.75 MED UVB to one set of patches and evaluations at 1, 24, 48, and 72 h postexposure). No reaction at an unirradiated site but a reaction at an irradiated site is indication of a phototoxic or photoallergic response.

Exaggerated use test: After the appropriate preliminary safety testing, the final or prototype formulations may be tested under exaggerated use condition or on compromised skin/disease state to address safety concerns. Examples of these test designs include exaggerated controlled washing over a 5 day period, facial formulation applied under controlled sweating conditions and use test on atopic skin. The panel size for these studies will depend upon the test design and type of formulation. Visual reactions as well self-assessed sensory responses are important endpoints for both exaggerated and normal Use Tests.

Use test under normal use: This is usually the last step in the safety assessment of the final formulation and it is often combined within an efficacy investigation. It simulates normal use conditions during an extended period of at-home applications (4, 12 week, etc.). It is conducted on the intended use population with a panel of 50–100 subjects per cell (cosmetic formulations).

See also: Cosmetics and Personal Care Products; Photoallergens; Risk Assessment, Human Health; Skin.

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Saint John's Wort

Molly Broderick and Teresa Dodd-Butera

- Common Names: *Hypericum perforatum*; Goat weed; Klamath; Klamath weed; Sho-ren-gyo
- Active Derivative: Hypericum, a napthodianthrone flavinoid, and several bioflavinoids
- Description: Perennial herb 1–5 ft tall with yellow flowers in various shades. The petals have black dots. The flowers are displayed in flat topped clusters
- Distribution: Native to Europe, but distributed throughout the United States and Canada. Also

found in Australia, New Zealand, and North Africa

Uses

Herbal formulation derived from the plant extract is traditionally used in the management of depression, anxiety, and insomnia, and gastritis. It appears to provide a tranquilizing effect. Medicinally, it has been used as a hepatoprotective agent as well as a diuretic. Topically it has been used as an astringent. Hypericum extracts are licensed in Germany for the treatment of depression, anxiety, and insomnia. In the United States it is considered a 'dietary supplement' and is not classified by the Food and Drug Administration as a drug. The plant extract is being investigated in clinical trials for the use in AIDS patients, as hypericin may be synergistic with another AIDS drug, AZT (3′-azido-3′-deoxythymidine or Retrovir or Ziduvodin).

Exposure Routes and Pathways

The oral and dermal routes are the most common exposure pathways.

Mechanism of Toxicity

Saint John's wort is a serotonin reuptake inhibitor and to a lesser degree appears to inhibit monoamine oxidase. The toxin is hypericin, an anthraquinone dimer, which is present throughout the plant. It also contains tannin, rutin, and flavinoids.

Acute and Short-Term Toxicity (or Exposure)

Animal

Photosensitization has occurred.

Human

Minimal toxicity data is available. Photosensitization is possible. Acute overdose may cause increased heart rate, diarrhea, fever, erythema, and pruritus.

Chronic Toxicity (or Exposure)

Animal

Minimal data available in either laboratory animal studies or controlled clinical trials. However, toxicity to livestock, especially sheep, has been reported. Common symptoms include edematous lesions of the skin, especially exposed areas.

Human

Adverse effects may include nausea, fatigue, and, confusion. Neuropathy has also been reported.

Clinical Management

Treatment is symptomatic and supportive. Concerns have been noted in the literature about the potential for adverse interactions with other drugs, especially antidepressants and sympathomimetics.

See also: Fluoxetine.

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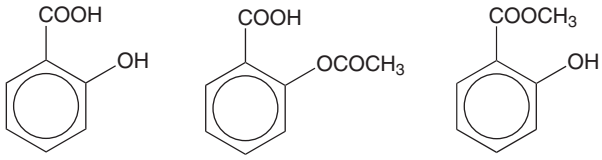
Salicylates

Alexander B Baer and Christopher P Holstege

- Chemical Abstracts Service Registry Number: CAS 50-78-2
- Synonyms: Aspirin; Acetylsalicylic acid; Acetaminosalol; Aluminum aspirin; Bismuth subsalicylate; Choline salicylate; Magnesium salicylate; Methyl salicylate; Phenyl salicylate; Potassium salicylate; Salsate; Salicylsalicylic acid; Sodium salicylate; Sodium thiosalicylate; Triethanolamine salicylate; Willow extract
- Chemical/Pharmaceutical/Other Class: Nonsteroidal synthetic derivatives of salicylic acid

- Chemical Structures:



Salicylic acid | Aspirin | Methyl salicylate

Uses

Salicylates are used for their analgesic, antipyretic, antiinflammatory, and keratolytic properties.

Exposure Routes and Pathways

Oral ingestion is the most common route of both accidental and intentional exposure to salicylates. Salicylates are also available in topical and rectal dosage forms.

Toxicokinetics

After ingestion, absorption occurs primarily from the upper small intestine via passive diffusion of unionized molecules. Serum salicylates are detected within 5–30 min after oral administration of rapidly absorbed dosage forms (aqueous solutions and uncoated or film-coated tablets). The rate of absorption may be slower with large, potentially lethal salicylate doses due to delayed gastric emptying, impaired dispersion of the drug in gastrointestinal fluids, and the possible formation of concretions. Absorption may also be delayed following ingestion with sustained release or enteric-coated preparations. Topical application of salicylic acid may result in systemic toxicity, especially in use with infants or in areas where the epidermis is disrupted. Rectal absorption is slow and unreliable.

Salicylates are metabolized principally in the liver by the microsomal enzyme system and are predominately conjugated with glycine to form salicyluric acid. Salicylates are also conjugated with glucuronic acid to form salicylphenolic glucuronide and salicylacyl glucuronide. In addition, small amounts of salicylates are hydrolyzed to form gentisic acid, which is an active metabolite and a potent inhibitor of prostaglandin synthesis. Salicylates rapidly distribute throughout extracellular fluid and into body tissues. Under normal physiologic acid–base conditions, salicylates cross the blood–brain barrier slowly because the ionized form predominates. However, systemic acidosis results in formation of the un-ionized salicylate which more easily distributes into tissues, especially the central nervous system. The volume of distribution (V_d) of salicylate at therapeutic levels is $0.2\,l\,kg^{-1}$, with ~80% of salicylate protein bound. As salicylate levels increase, the proportion bound to plasma protein decreases, and the V_d increases to $\sim 0.6\,l\,kg^{-1}$.

Salicylate and its metabolites are rapidly and almost completely excreted in the urine by glomerular filtration and by renal tubular secretion. Passive reabsorption of salicylate occurs in the distal tubules. Salicylate elimination is saturable and characterized by Michaelis–Menton kinetics where the elimination half-life is dependent on the dose. Since the pK_a of salicylic acid is 3, its renal clearance is greatly influenced by changes in urinary pH. Increasing urinary pH can significantly increase the overall salicylate elimination rate via ion trapping.

Mechanism of Toxicity

In acute salicylate toxicity, nausea, vomiting, and abdominal discomfort occur due to both local gastric irritation and stimulation of the medullary chemoreceptor trigger zone. Salicylates increase sensitivity to carbon dioxide in the medulla oblongata, thereby inducing hyperventilation, decreasing PCO_2, and causing respiratory alkalosis. A compensatory increase in the renal excretion of bicarbonate leads to the loss of potassium and sodium in the urine. A metabolic acidosis may follow due to the accumulation of organic acids. As a result, salicylate poisoning may produce a mixed acid–base abnormality consisting of both respiratory alkalosis and metabolic acidosis. In very large overdoses, salicylates uncouple oxidative phosphorylation, resulting in a failure to produce adenosine triphosphate while at the same time increasing oxygen utilization and carbon dioxide production. This results in an increase in heat production. Salicylates also interfere with glucose metabolism and gluconeogenesis. Salicylates may also profoundly decrease brain glucose concentrations despite normoglycemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may manifest toxicity to salicylates with signs and symptoms similar to those seen in humans. These may include fever, hyperpnea, seizures, respiratory alkalosis, metabolic acidosis, gastric hemorrhage, and kidney damage. Methemoglobinemia has also been seen in animals following salicylate toxicity. Activated charcoal has been used in animals. Methylene blue or ascorbic acid may be utilized for the treatment of methemoglobinemia.

Human

Acute ingestions of over 150 mg kg^{-1} may result in toxic effects. Nausea and vomiting are seen early in toxicity. Tinnitus and hyperventilation also commonly occur early in toxicity. As severity of toxicity increases, intractable vomiting, hyperthermia, confusion, coma, seizures, pulmonary edema, acute renal failure, and death may occur. Hyperglycemia may be seen early, whereas hypoglycemia may occur later in toxicity. Acid–base disturbances such as respiratory alkalosis and/or metabolic acidosis may be noted. Toxic salicylate blood levels appear over 30 mg dl^{-1}. In overdose, the formation of concretions, slow absorption of enteric coated tablets, and delayed gastric emptying may delay toxic reactions and cause salicylate levels to rise over the first 12–24 h. The Done nomogram provides no value in the assessment of acute ingestions and should not be used.

Chronic Toxicity (or Exposure)

Animal

Cats are particularly susceptible to the effects of salicylate due to a lack of ability to rapidly metabolize the drug. Low doses (33–63 mg kg^{-1} day^{-1}) in cats can produce hepatic damage, central nervous system (CNS) depression, vomiting, and weight loss. In rats, high doses (300 mg kg^{-1} day^{-1}) inhibits ovulation.

Human

Patients with chronic salicylism may present with symptoms clinically similar to those seen in the acute situation. However, some patients with chronic salicylate overdose may present with CNS effects as their primary complaint, and typically have a higher morbidity and mortality than patients with acute salicylate overdose. Chronic salicylism is more often associated with pronounced hyperventilation, dehydration, pulmonary edema, renal failure, coma, seizures, and acidosis. Chronic salicylism patients will have more profound clinical effects at lower serum salicylate levels compared to patients with acute overdoses. Patients have developed toxicity with chronic salicylate serum levels as low as 15 mg dl^{-1}.

In Vitro Toxicity Data

Salicylates have been negative in Ames *Salmonella* assays for mutagenicity.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastrointestinal decontamination procedures should be considered in the patient with appropriate airway protection. In general, a single dose of activated charcoal should be considered in patients that have substantial ingestions. Salicylate ingestions can result in substantial delays in absorption; therefore, charcoal may be given even up to 8 h postingestion and more than one dose of charcoal may be considered to prevent further drug absorption. Careful correction of fluid and electrolyte abnormalities is essential. The clinician should insure adequate urine output, but forced diuresis should be avoided. Administration of intravenous sodium bicarbonate should be considered in patients manifesting signs and symptoms of salicylate toxicity. Hemodialysis effectively increases clearance and improves fluid/electrolyte balance. This extracorporeal method of elimination should be considered in patients with acute mental status changes, renal failure, intractable acidosis, pulmonary edema, severe fluid imbalance, or acute serum salicylate levels over 100 mg dl^{-1} or patients with chronic salicylate overdose who have symptoms and serum levels >60 mg dl^{-1}.

See also: Acetylsalicylic Acid; Gastrointestinal System.

Further Reading

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Temple AR (1981) Acute and chronic effects of aspirin toxicity and their treatment. *Archives of Internal Medicine* 141: 364–369.

Salmonella

Melanie J Karst

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Description

Salmonella is a genus of gram-negative, facultatively anaerobic, rod-shaped bacteria that utilizes citrate as a sole carbon source. It is pathogenic for humans, causing enteric fevers, gastroenteritis, and bacteremia. Food poisoning is the most common clinical manifestation. Organisms within this genus are separated on the basis of antigenic characteristics, sugar fermentation patterns, and bacteriophage susceptibility. About 2200 types of *Salmonella* species are known.

Source of Exposure and Transmission

Salmonellosis in humans is contracted mainly through the consumption of raw or undercooked contaminated food of animal origin (mainly meat, poultry, eggs, and milk) although many other foods have been implicated in its transmission. The primary route by which humans acquire infection is by consumption of contaminated food of animal origin. Unlike *Salmonella enteritidis*, which is mainly associated with poultry and eggs, multidrug resistant *S. typhimurium* DT 104 can be found in a broad range of foodstuffs. The organisms pass through the food chain from primary production or through cross-contamination from food products in households or food-service establishments and institutions such as hospitals. In developed countries, human to human transmission is uncommon but can occur, notably in institutions; for instance, special-care baby units and residential homes for the elderly. Little is known about the epidemiology in developing countries but spread within hospitals and health centers has been reported.

A total of 2213 different *Salmonella* strains have been identified. *Salmonella* infects the digestive tracts of many domestic and wild animals, birds, and reptiles. They can be classified according to their adaptation to human and animal hosts:

- Group 1, for example, *S. typhi* and *S. paratyphi*, causes enteric fever only in humans and in higher primates.
- Group 2 causes disease in certain animals: *S. dublin* in cattle, *S. cholerae-suis* in pigs, but only infrequently in humans. However, when these strains do cause disease in humans, it is often invasive and can be life threatening.
- Group 3 includes the remaining strains. Typically, such strains cause gastroenteritis that is often mild and self-limiting but can be severe in the young, the elderly, and patients with weakened resistance against infectious diseases. This group includes *S. enteriditis* and *S. typhimurium*, the two most important strains for salmonellosis (transmitted from animals to humans).

Exposure Routes and Pathways

Outbreaks in the United Kingdom have been linked to poultry, a variety of meats and meat products, and unpasteurized milk. In addition to acquiring infection from contaminated food, human cases have also occurred where individuals have had contact with infected cattle. A small proportion of infected individuals may have contracted infection from pets such as cats and dogs, which can also be infected with some strains of *Salmonella*. These pets probably acquire the infection like humans, in other words through consumption of contaminated raw meat, poultry or poultry-derived products.

The evolution of specific *Salmonella* serotypes in intensive animal husbandry and subsequently in humans has been observed over the last three decades. The most recent epidemic was caused by *S. enteritidis*, which peaked in humans in 1992 in many European countries. Its current slight decline sets the scene for re-emergence of *S. typhimurium* as – epidemiologically – the most important serotype in human salmonellosis.

Dose

An infective dose may be as few as 15–20 cells depending on the age and condition of the host. The time of onset of symptoms depends on host factors, ingested dose, and strain characteristics.

Mechanism of Toxicity

The *Salmonella* organisms pass from the gut lumen and penetrate the epithelium in the small intestine where inflammation occurs. There is some evidence that an enterotoxin may be produced in some strains. *Salmonella* strains may produce a heat labile

enterotoxin related to the *Escherichia coli* heat labile enterotoxin or cholera toxin. Cytotoxins related to but distinct from those produced by *E. coli* or *Shigella* may also be produced.

Recent work indicates that a major virulence mechanism for *Salmonella* may involve type III secretion systems, which are encoded on plasmids and allows direct transfer of bacterial proteins to eukaryotic cells through a contact-dependent secretion mechanism. These effector proteins are capable of enhancing virulence and epithelial cell invasion.

Diagnosis of Human Infection/Illness

Diagnosis requires serological identification of culture isolated from a stool sample.

Nature of the Disease

The clinical course of human salmonellosis is usually characterized by acute onset of fever, abdominal pain, diarrhea, nausea, and sometimes vomiting. In some cases, particularly in the very young and in the elderly, dehydration can become severe and life threatening. Antibiotic treatment is necessary in less than 2% of the clinical cases. Serious complications occur in a small proportion of cases. The incidence is particularly high in children and the elderly, accounting for up to 60% of all reported laboratory confirmed cases. Studies in developed countries indicate that more than 80% of all salmonellosis cases occur individually rather than as outbreaks.

The onset of symptoms of *Salmonella* gastroenteritis is usually 6–72 h. Acute symptoms may last for 1–2 days or may be prolonged depending on host factors, ingested dose, and strain. Arthritic symptoms may occur 3–4 weeks after onset of acute symptoms. Symptoms are more severe in the elderly, infants, and immunocompromised individuals. *S. typhi* and *S. paratyphi* A, B, and C produce typhoid and typhoid-like symptoms in humans. Enteric fever (typhoid fever) may develop; other symptoms include anorexia, abdominal pain, malaise, myalgias, headache, cough, diarrhea or constipation, and delirium. Subsequent *Salmonella* septicemia may affect virtually every organ system.

Clinical Management

Severe forms of *Salmonella* infection may require hospitalization and isolation from other people. Patients with less severe infection and those who are recovering may be treated at home.

Antibiotics generally are not recommended unless the infection has spread from the intestines, because such medication can prolong rather than reduce the period of bacterial shedding in the intestine. Treatment involves monitoring hydration status and intravenous therapy to correct electrolyte imbalance. For individuals at high risk for invasive disease the recommended antibiotics include ampicillin, amoxicillin, trimethoprim–sulfamethoxazole, cefotaxime, and ceftriaxone.

Salmonella usually remains in the intestines for up to 5 weeks – and in some cases for many months. Be aware that some individuals can become chronic carriers of *Salmonella* bacteria and ~2% may develop chronic arthritis. Good personal hygiene and handwashing techniques would prevent the majority of transmissions. Wash hands thoroughly with warm, soapy water after visits to the restroom and before food preparation.

See also: Ecotoxicology, Genetic; Food and Drug Administration, US; Food Safety and Toxicology

Further Reading

Humphrey T (2004) *Salmonella*, stress responses and food safety. *Nature Reviews in Microbiology* 2(6): 504–509.

Relevant Websites

http://vm.cfsan.fda.gov – US Food and Drug Administration. Center for Food Safety and Applied Nutrition. Forborne Pathogenic. Microorganisms and Natural Toxins Handbook. *Salmonella* spp.

http://www.cdc.gov – CDC. Division of Bacterial and Mycotic Diseases. Salmonellosis.

Sarin

Harry Salem and Frederick P Sidell*

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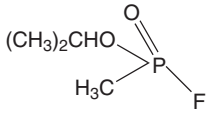
- Chemical Abstracts Service Registry Number: CAS 107-44-8
- Synonyms: GB; *o*-Isopropyl methyl phosphonofluoridate; G agent; Nerve gas
- Chemical/Pharmaceutical/Other Class: Nonpersistent anticholinesterase compound or organophosphate (OP) nerve agent, colorless to black liquid with no odor
- Chemical Formula: $C_4H_{10}FO_2P$
- Chemical Structure:



Uses

Sarin is a human-made nerve (gas) agent used in chemical warfare. It is an irreversible cholinesterase inhibitor.

Exposure Routes and Pathways

Casualties are caused primarily by inhalation, but can occur following percutaneous and ocular exposure, as well as by ingestion and injection. Sarin mixes easily with water, and people could be exposed by drinking contaminated water or via dermal contact with contaminated water. People could be exposed by eating contaminated food. Clothing can release sarin for ~30 min, which could lead to exposure of other people. Sarin vapor is heavier than air, and can sink to low-lying areas.

Toxicokinetics

Sarin is absorbed both through the skin and via respiration. It is more soluble in water than the other nerve agents (soman (GD) and VX); its solubility is directly related to temperature. The half-life of sarin, however, is inversely related to temperature and pH. In water the half-life of sarin is 15 min at 30°C and at pH 7.6. Nerve agents inhaled as vapors or aerosols enter the systemic circulation, resulting in toxic manifestations within seconds to 5 min of inhalation.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Mechanism of Toxicity

Sarin and the other nerve agents are organophosphorus cholinesterase inhibitors. They inhibit the enzymes butyrylcholinesterase in the plasma, acetylcholinesterase on the red blood cell, and acetylcholinesterase at cholinergic receptor sites in tissues. These three enzymes are not identical. Even the two acetylcholinesterases have slightly different properties, although they have a high affinity for acetylcholine. The blood enzymes reflect tissue enzyme activity. Following acute nerve agent exposure, the red blood cell enzyme activity most closely reflects tissue enzyme activity. During recovery, however, the plasma enzyme activity more closely parallels tissue enzyme activity.

Following nerve agent exposure, inhibition of the tissue enzyme blocks its ability to hydrolyze the neurotransmitter acetylcholine at the cholinergic receptor sites. Thus, acetylcholine accumulates and continues to stimulate the affected organ. The clinical effects of nerve agent exposure are caused by excess acetylcholine.

The binding of nerve agent to the enzymes is considered irreversible unless removed by therapy. The accumulation of acetylcholine in the peripheral and central nervous systems leads to depression of the respiratory center in the brain, followed by peripheral neuromuscular blockade causing respiratory depression and death.

The pharmacologic and toxicologic effects of the nerve agents are dependent on their stability, rates of absorption by the various routes of exposure, distribution, ability to cross the blood–brain barrier, rate of reaction and selectivity with the enzyme at specific foci, and their behavior at the active site on the enzyme.

Red blood cell enzyme activity returns at the rate of red blood cell turnover, which is ~1% per day. Tissue and plasma activities return with synthesis of new enzymes. The rates of return of these enzymes are not identical. However, the nerve agent can be removed from the enzymes. This removal is called reactivation, which can be accomplished therapeutically by the use of oximes prior to aging. Aging is the biochemical process by which the agent–enzyme complex becomes refractory to oxime reactivation. The toxicity of nerve agents may include direct action on nicotinic acetylcholine receptors (skeletal muscle and ganglia) as well as on muscarinic acetylcholine receptors and the central nervous system (CNS).

Recently, investigations have focused on organophosphate nerve agent poisoning secondary to

acetylcholine effects. These include the effects of nerve agents on γ-amino butyric acid neurons and cyclic nucleotides. In addition, changes in brain neurotransmitters such as dopamine, serotonin, noradrenaline, and acetylcholine following inhibition of brain cholinesterase activity have been reported. These changes may be due in part to a compensatory mechanism in response to overstimulation of the cholinergic system or could result from direct action of nerve agent on the enzymes responsible for noncholinergic neurotransmission.

Human Toxicity

Toxic effects occur within seconds to 5 min of nerve agent vapor or aerosol inhalation. The muscarinic effects include ocular (miosis, conjunctival congestion, ciliary spasm), nasal discharge, respiratory (bronchoconstriction and increased bronchial secretion), gastrointestinal (anorexia, vomiting, abdominal cramps, and diarrhea), sweating, salivation, and cardiovascular (bradycardia and hypotension) effects. The nicotinic effects include muscular fasciculation and paralysis. CNS effects can include ataxia, confusion, loss of reflexes, slurred speech, coma, and paralysis.

Following inhalation of sarin, the median lethal dosage (LCt_{50}) in humans has been estimated to be $70\,mg\,min\,m^{-3}$ at a respiratory minute volume (RMV) of $15\,l\,min^{-1}$, $100\,mg\,min\,m^{-3}$ at an RMV of $10\,l\,min^{-1}$ (resting) for a duration of 0.5–2 min.

Following percutaneous exposure of bare skin to sarin vapor, the LCt_{50} has been estimated at $12\,000\,mg\,min\,m^{-3}$ for a 70 kg human. For liquid percutaneous exposure, the LD_{50} has been estimated as 1.7 g for a 70 kg human, and for intravenous injection the LD_{50} has been estimated as 1 mg for a 70 kg human.

Median incapacitation doses estimated for humans following inhalation of sarin for an RMV of $15\,l\,min^{-1}$ for a 10 min exposure are as follows: $40\,mg\,min\,m^{-3}$ for moderate incapacitation, $56\,mg\,min\,m^{-3}$ for severe incapacitation, and $72\,mg\,min\,m^{-3}$ for very severe incapacitation. The symptoms for moderate incapacitation include maximal miosis, eye pain, headache, twitching eyelids, difficulty in ocular accommodation, tightness of chest, runny nose, salivation, sneezing and coughing, anorexia, nausea, heartburn, fatigue, weakness, muscle fasciculation, anxiety, and insomnia. Severe incapacitation includes all of the above plus diarrhea, frequent urination, dysphoria, and ataxia. For very severe incapacitation, the principal effects are convulsions, collapse, and paralysis.

The minimum effective dosage for miosis in man has been estimated between 2 and $4\,mg\,min\,m^{-3}$. The permissible airborne exposure concentration of sarin for an 8 h workday or a 40 h work week is an 8 h time-weighted average (TWA) of $0.00003\,mg\,m^{-3}$.

Sarin is the nerve agent studied most thoroughly in humans. At an estimated concentration of 3–5 mg $min\,m^{-3}$ in humans, it will produce miosis, rhinorrhea, and a feeling of tightness in the throat or chest. Exposure to small amounts of nerve agent vapor causes effects in the eyes, nose, and airways. These effects are from local contact and are not indicative of systemic absorption. Small amounts of liquid agent on the skin cause systemic effects initially in the gastrointestinal tract. Lethal amounts of vapor or liquid cause a rapid cascade of events resulting, within 1 or 2 min, in loss of consciousness and convulsive activity followed by apnea and muscular flaccidity.

Although miosis is a characteristic sign of exposure to the nerve agent, rhinorrhea may be the first indication. It severity is dose dependent.

Miosis occurs from direct contact of vapor with the eyes. It may also occur from moderate to severe exposure of skin to liquid agent or from a liquid droplet near the eye. Miosis will begin with seconds or minutes following vapor exposure and may not be complete for many minutes if the exposure concentration is low. In unprotected individuals, miosis is bilateral and is often accompanied by complaints of pain, dim and blurred vision, conjunctival injection, nausea, and occasionally vomiting. On occasion, subconjunctival hemorrhage is also present.

Inhalation of nerve agent vapor causes bronchoconstriction and increased secretions of the glands in the airways, which is dose related. Small amounts of the nerve agent will produce a feeling of slight tightness in the chest to severe respiratory distress following large amounts. Large amounts will cause cessation of respiration (apnea) within minutes after the onset. Both CNS effects and peripheral effects (skeletal muscle weakness and bronchoconstriction) may contribute to the apnea.

Systemic absorption of the nerve agent will cause increased motility of the gastrointestinal tract and an increase in glandular secretions. Nausea and vomiting are early signs of liquid exposure on the skin and diarrhea may occur following large amounts of agent.

Nerve agent exposure to glands increases their secretions. These glands include lacrimal, nasal, salivary, and bronchial. Localized sweating will occur at the site of liquid agent on the skin, and after large liquid or vapor exposure generalized sweating is common.

Stimulation of skeletal muscles by nerve agents will produce muscular fasciculation and twitching large amounts of the agent will cause fatigue and muscle weakness followed by muscular flaccidity.

Large amounts of the nerve agent in the CNS will cause loss of consciousness, seizure activity, and apnea. CNS effects of smaller amounts of the agent vary and are nonspecific. However, they may include forgetfulness, inability to concentrate, insomnia, bad dreams, irritability, impaired judgment, and depression. These effects may persist up to 6 weeks.

Nerve agent exposure may cause bradycardia due to vagal stimulation or it may often cause the reverse tachycardia due to fright and hypoxia and adrenergic stimulation secondary to ganglionic stimulation. Bradyarrhythmias such as first-, second-, or third-degree heart block may also occur. Blood pressure may also be elevated because of adrenergic stimulation, but it is usually normal until the terminal decline.

Clinical Management

Management of nerve agent intoxication consists of decontamination, ventilation, administration of antidotes, and supportive therapy.

The three therapeutic drugs for treatment of nerve agent intoxication are atropine, pralidoxime chloride, and diazepam.

Atropine, a cholinergic blocking or anticholinergic drug, is effective in blocking the effects of excess acetylcholine at peripheral muscarinic sites. The usual dose is 2 mg, which may be repeated at 3–5 min intervals. Pralidoxime chloride (protopam chloride; 2-PAM CL) is an oxime used to break the agent–enzyme bond and restore the normal activity of the enzyme. Abnormal activity decreases and normal strength returns to skeletal muscles, but no decrease in secretions is seen following oxime treatment. The usual dose is 1000 mg (iv or im), which may be repeated 2 or 3 times at hourly intervals, intravenously or intramuscularly. Diazepam, an anticonvulsant drug is used to decrease convulsive activity and reduce brain damage that may occur from prolonged seizure activity. It is suggested that all three of these drugs be administered at the onset of severe effects from nerve agent exposure, whether or not seizures occur. The usual dose of diazepam is 10 mg (im).

Miosis, pain, dim vision, and nausea can be relieved by topical atropine in the eye. Pretreatment with carbamates may protect the cholinesterase enzymes before nerve agent exposure.

Supportive therapy may include ventilation via an endotracheal airway if possible and suctioning of excess secretions from the airways.

Animal Toxicity

Small doses of nerve agents in animals can produce tolerance in additional to their classical cholinergic effects. In rats, acute administration of nerve agents in subconvulsive doses produced tumors and hind-limb adduction. In animals, nerve agents can also cause behavioral as well as cardiac effects.

The cause of death is attributed to anoxia resulting from a combination of central respiratory paralysis, severe bronchoconstriction, and weakness or paralysis of the accessory muscles for respiration.

Table 1 Inhalation LCt_{50} values of sarin in various species

Species	*LCt_{50} (mg min m^{-3})*	*Exposure duration (min)*
Mouse	150	30
Rat	1500	10
Guinea pig	256	2
Rabbit	1200	10
Cat	1000	10
Dog	1000	10
Monkey	1000	10

Table 2 Acute toxicities of sarin in various species by various routes of exposure

Route of exposure/species		*LD_{50} (μg kg^{-1})*
Percutaneous		
	Mouse	1 080
	Rabbit	925
Intravenous		
	Mouse	109
	Rat	39
	Rabbit	15
	Cat	22
	Dog	19
	Monkey	22 300
Intramuscular		
	Rat	108
	Mouse	164
Intraperitoneal		
	Mouse	283
	Rat	218
Oral		
	Rat	550
Subcutaneous		
	Rat	103
	Mouse	60
	Rabbit	30
	Guinea pig	30
	Hamster	95

Signs of nerve agent toxicity vary in rapidity of onset, severity, and duration of exposure. These are dependent on the specific agent, route of exposure, and dose. At the higher doses, convulsions and seizures indicate CNS toxicity.

Following nerve agent exposure, animals exhibit hypothermia resulting from the cholinergic activation of the hypothalamic thermoregulatory center. In addition, plasma concentrations of pituitary, gonadal, thyroid, and adrenal hormones are increased during organophosphate intoxication.

The LCt_{50} values ($mg\,min\,m^{-3}$) reported following the inhalation of sarin are presented in **Table 1**. The acute toxicities by other routes of exposure in various animal species are presented in **Table 2**.

See also: Nerve Agents; Soman; Tabun; V-Series Nerve Agents: Other than VX; VX.

Relevant Websites

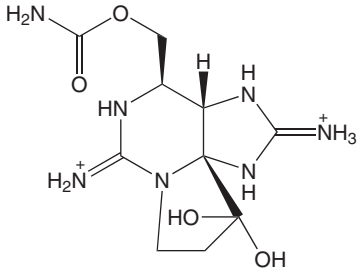
http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Saxitoxin

Samantha E Gad and Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 35523-89-8
- Synonyms: Mussel poison; Clam poison; Paralytic shellfish poison; Gonyaulax toxin; STX
- Chemical/Pharmaceutical/Other Class: Complex of amino acids – a tetrahydropurine
- Chemical Formula: $C_{10}H_{17}N_7O_4$
- Chemical Structure:



Background Information

Saxitoxin is a naturally occurring toxin that is synthesized by various marine dinoflagellates. It is used in neurochemical and molecular biology research. Saxitoxin causes paralytic shellfish poisoning. It is far more potent than the classic puffer fish toxin, tetrodotoxin. Saxitoxin is one of only two naturally occurring schedule 1 chemical warfare agents (the other is ricin).

Exposure Routes and Pathways

Ingestion of shellfish containing saxitoxin is the primary route of exposure.

Toxicokinetics

Saxitoxin is readily absorbed from the gastrointestinal tract and through mucous membranes.

Mechanism of Toxicity

Saxitoxin binds to the sodium channels in the membranes of excitable cells (neurons and muscle cells) blocking synaptic transmission. Saxitoxin is connected to red tides. Saxitoxin reduces nerve conduction velocities.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} values in mice are $10\,\mu g\,kg^{-1}$ (intraperitoneal), $263\,\mu g\,kg^{-1}$ (oral), and $3.4\,\mu g\,kg^{-1}$ (intravenous). One milligram is estimated to be sufficient to kill 5500 mice.

Human

Saxitoxin paralyzes the peripheral nervous system and alters cardiac chronotrophy. Symptoms include gastrointestinal complaints and paresthesias of the face, followed by (in severe cases) muscle paralysis. The estimated lethal dose in humans is 0.3–1 mg. A single contaminated shellfish may contain 50 lethal doses. Mortality for reported cases is 5.9%, but has a

higher rate in children. Clinical effects develop over 30 min to 3 h.

Chronic Toxicity (or Exposure)

Animal

Little information is available on the chronic effects of saxitoxin in animals.

Human

There are no reports on the chronic effects of saxitoxin in humans.

Clinical Management

The gut should be decontaminated and the patient observed carefully for signs of respiratory depression. Treatment is primarily supportive. Artificial respiration may be necessary.

Environmental Fate

Saxitoxin is a naturally occurring substance in dinoflagellates and taken up by shellfish. Consumption of the shellfish leads to toxicity. Aside from the knowledge that these organisms serve as a source of exposure, the environmental fate of the chemical itself has not been studied. It is heat-stable but sensitive to strong alkali.

Ecotoxicology

Red tides (containing saxitoxin) have been known to kill fish since antiquity. Humpback whales have died shortly after consuming fish that were contaminated with saxitoxin. Thus, accumulation of saxitoxin up the food chain may occur.

See also: Neurotoxicity; Red Tide; Shellfish Poisoning, Paralytic; Toxicity Testing, Aquatic.

Further Reading

Dart RC (2004) *Medical Toxicology*, 3rd edn. Baltimore: Lippincott.

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Relevant Websites

http://vm.cfsan.fda.gov – FDA/CFSAN Bad Bug Book.

http://www.bris.ac.uk – Saxitoxin (by Edwards N, The Chemical Laboratories at the University of Sussex at Brighton).

Scombroid

F Lee Cantrell

This article is a revision of the previous print edition article by Gaylord P Lopez, volume 3, pp. 125–126, © 1998, Elsevier Inc.

- SYNONYMS: Scombroidtoxicosis; Form of Ichthyosarcotoxicosi

Background Information

Scombroid refers to a complex of clinical symptoms associated with ingestion of improperly handled or stored fish from the Scombroidae family: mahi mahi, bluefish, Bombay duck, kahawai, kingfish, swordfish, pacific amberjack, salmon, tuna, bonito.

Exposure Routes and Pathways

The toxin (histamine) is created within the flesh of certain fish under specific environmental conditions. Histidine is present in the muscle protein of Scombrodiae. In the presence of certain bacteria, histadine gets broken down to histamine. Ingestion of the flesh of improperly handled fish causes ingestion of large amounts of histamine and development of symptoms of histamine poisoning. Histamine is not destroyed or inactivated by heating or cooking. Contaminated fish often looks and smells normal, but is periodically described as having a peppery taste. Proper refrigeration/freezing of fresh fish will dramatically reduce the risk of scombroid poisoning.

Toxicokinetics

Ingestion of histamine and saurine, when present in large amounts, results in histaminic effects. Absorption is rapid with clinical effects generally being seen within 5–90 min. The duration of untreated scombroid poisoning is generally 12–24 h.

Mechanism of Toxicity

The previously mentioned types of fish contain free histidine in their musculature. During spoilage, bacteria on the surface of the fish enzymatically convert histidine to histamine and saurine, which are responsible for the symptoms. The Food and Drug Administration considers levels of histamine >50 mg per 100 g of fish potentially toxic.

Acute and Short-Term Toxicity (or Exposure)

Human

Initial symptoms are those of a histamine reaction and typically occur within 5–90 min of ingestion. Common symptoms include dermal flushing especially of the face, neck, and upper torso, headache, nausea, vomiting, and diarrhea. Facial edema, burning of the mouth and throat, palpitations, dizziness, and rash has also been noted. Bronchospasm, urticaria, shock, and death are rare. Symptoms usually resolve within 3–24 h.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Treatment is generally symptomatic and supportive. Gastrointestinal decontamination procedures maybe considered for substantial recent ingestions. Other therapy is directed at limiting histaminic symptoms. Administration of oral or parenteral H_1 and H_2 receptor antagonists is appropriate. Inhaled beta-adrenergic agonists and epinephrine can be used for patients experiencing significant bronchospasm.

See also: Disulfiram; Fish Consumption Advisory.

Further Reading

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Morrow JD, Margolies GR, and Rowland J (1991) Evidence that histamine is the causative toxin of scombroid-fish poisoning. *New England Journal of Medicine* 324: 716–720.

Scorpions

Gary W Everson

- SYNONYMS: *Centruroides* species; *Vejovis* species; *Hadrurus* species

Exposure Routes and Pathways

Scorpions inflict a sting and inject their venom subcutaneously with a stinger located at the end (telson) of their multisegmented tail.

Toxicokinetics

Scorpion venom may reach systemic circulation through lymphatic transport following a sting. Of those scorpions located in the United States, *Centruroides exilicauda*, found in southeastern California, Arizona, Nevada, southern Utah, and southwestern New Mexico, is an example of a scorpion that can produce significant systemic toxicity following envenomation. Onset of systemic symptoms typically occurs within 4 h of the sting. The metabolism of venom components is not well understood. Tissue distribution of venom is complex. Venom components differ among the multitude of scorpion species and thus venom distributes to different tissue sites.

Mechanism of Toxicity

Scorpion venom is composed of many different fractions that can vary among the different scorpion species. These venom fractions act at different tissue receptor sites. Local tissue reaction is a result of the inflammatory response to the injected foreign proteins and enzymes making up the venom. The venom of *Centruroides* species contains several different neurotoxins. These toxins block the transmission of nerve impulses in the central nervous system and in muscles by blocking the transport of ions through sodium and potassium channels at the cellular level. Other venom components may decrease the heart rate by causing the release of acetylcholine.

Acute and Short-Term Toxicity (or Exposure)

Human

Most scorpion stings produce some local tissue reaction that is characterized by mild to moderate

burning pain. Usually there is minimal swelling and redness. In the United States, this is the limit of the reaction following stings of *Vejovis* species, *Hadrurus* species, and several other common scorpions. Wound infection is also possible following the sting. The more poisonous *Centruroides* scorpions, represented by *C. exilicauda*, may also produce systemic symptoms following significant envenomation. However, even these scorpions often produce only pain and other localized reaction at the sting site. When systemic symptoms do develop, they include increased heart rate, hypertension, dilated pupils, sweating, and increased blood glucose. Also, salivation, tearing, diarrhea, and bradycardia may develop when parasympathetic nerve stimulation predominates from acetylcholine release. Other clinical effects may include blurred vision, nystagmus, opisthotonus, muscle fasciculations, convulsions, breathing difficulty, respiratory failure, and cardiac arrhythmias. Young children, the elderly, and those with pre-existing cardiovascular disease are at greater risk for severe systemic symptoms. Occasionally, poisonous exotic species make their way into the United States either by illegal importation or along with agricultural product shipments from abroad. Signs and symptoms following envenomation vary depending on the species of scorpion involved.

In Vitro Toxicity Data

Scorpion venom is used in a variety of research settings because of its ability to block sodium channels. A new class of toxin (tetrapandins) has recently been identified within the venom of *Pandinus imperator*. These toxins have been shown to have inhibitory effects on store-operated calcium entry in human embryonic kidney-293 cells.

Clinical Management

Basic and advanced clinical life support may be required following severe envenomation by several *Centruroides* species. However, most scorpion stings require only local wound care. Ice may be applied to the sting site for 10–15 min to help decrease pain. Acetaminophen, aspirin, or ibuprofen may be helpful for mild pain. Applying ice for long periods of time or immersing the sting site in an ice bath (cryotherapy) is not recommended since this procedure decreases blood flow at the site causing tissue damage. The majority of patients presenting with systemic symptoms can be managed at the hospital with supportive care, pain management, and observation. Careful monitoring of heart rate, blood pressure, and respiratory function are essential. Muscle spasms may respond to diazepam or calcium gluconate. Occasionally, hypertension is sufficiently severe or prolonged to require treatment. Depending on the severity of hypertension, nitroprusside, labetalol, or nifedipine may be indicated. Respiratory failure due to neuromuscular blockade is a rare but possible complication. Mechanical ventilation may be required.

C. exilicauda-specific antivenin has been available in Arizona for the treatment of severe stings of *Centruroides exilicauda* (bark) scorpions. These preparations, are not generally available outside Arizona, are not approved by the Federal Drug Administration and complete scientific data regarding their efficacy are lacking. However, anecdotal reports indicate that the antivenin has reversed symptoms associated with neuromuscular blockade within 30–60 min. The local regional poison information center may be contacted to locate antivenin and assist in determining whether clinical indications exist for its use.

See also: Acetylcholine; Animals, Poisonous and Venomous.

Further Reading

Amitai Y, Mines Y, and Aker M (1985) Scorpion sting in children. A review of 51 cases. *Clinical Pediatrics* 24: 136–140.

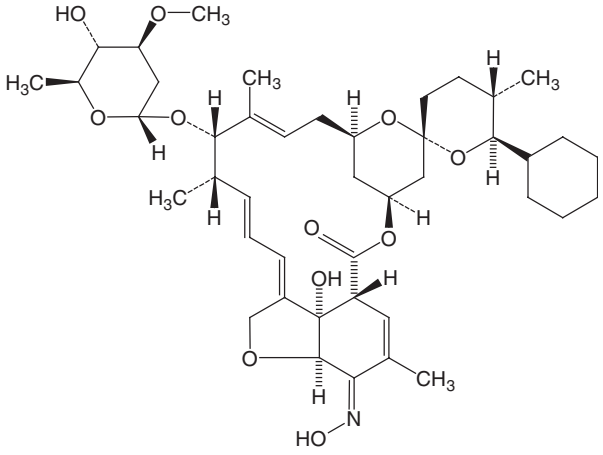
Sofer S, Shahak E, and Gueron M (1994) Scorpion envenomation and antivenom therapy. *Journal of Pediatrics* 124: 973–978.

Selamectin

Ramesh C Gupta

- Chemical Name: (5*Z*,25*S*)-25-cyclohexyl-4′-*O*-de(2,6-dideoxy-3-*O*-methyl-α-L-arabino-hexopyranosyl)-5-demethoxy-25-de(1-methylpropyl)-22,23-dihydro-5-(hydroxyimino)avermectin A_{1a}
- Chemical Abstracts Service Registry Number: CAS 220119-17-5
- Synonym: Revolution

- Chemical/Pharmaceutical/Other Class: Selamectin is a novel, semisynthetic avermectin, which has a molecular weight of 769.96. Selamectin is colorless to yellow and is flammable.
- Chemical Formula: $C_{43}H_{63}NO_{11}$
- Chemical Structure:



Uses

Selamectin is marketed as Revolution, which is a product of Pfizer, Inc. This is a topical parasiticide preparation recommended for use in dogs and cats 6 weeks of age and older. Selamectin is used to kill adult fleas and ear mites in dogs and cats. It also kills *Sarcoptes scabiei*, a mite that causes sarcoptic mange (scabies), certain ticks in dogs, and hookworms and roundworms in cats. Selamectin is also used to prevent heartworm disease and flea infestation in dogs and cats. The recommended dose is $6\,mg\,kg^{-1}$.

Exposure Routes and Pathways

Veterinarians, technicians, and pet-owners are exposed to selamectin through the dermal route.

Toxicokinetics

In dogs and cats, selamectin is rapidly absorbed from the skin into the bloodstream, where it kills heartworm microfilaria. Selamectin is excreted into the intestinal tract where it kills intestinal parasites. Finally, selamectin is selectively distributed from the bloodstream into the sebaceous glands of the skin, forming reservoirs that provide persistent efficacy against fleas, ear mites, and sarcoptic mites. Active concentrations of selamectin are found in the plasma for at least 30 days. It is excreted mostly in the feces and a small unmetabolized amount in the urine.

Mechanism of Toxicity

Selamectin binds to glutamate gated chloride channels in the parasite's nervous system, causing them to remain open. This causes chloride ions to continuously flow into the nerve cell, changing the charge of the cell membrane. The continuous flow of chloride ions blocks neurotransmission, and transmission of stimuli to muscles is prevented. Selemectin has no such effect in the mammalian nervous system, and therefore, it is much safer than common insecticides.

Acute and Short-Term Toxicity (or Exposure)

Animal

Hair loss at the site of application, vomiting, diarrhea, anorexia, lethargy, salivation, tachypnea, pruritis, urticaria, erythema, ataxia, lethargy, fever, and rare instances of death may occur with overt acute overdose.

Human

Selamectin may cause irritation to the skin and eyes. Reactions such as hives, itching, and skin redness have been observed in rare instances. Selamectin has a reported safety factor range of 279.

Chronic Toxicity (or Exposure)

Animal

In dogs and cats, seven monthly treatments of $60\,mg\,kg^{-1}$ (10 times the recommended dose) produced no adverse reactions when given to 6-week-old kittens or puppies. An exposure of $18\,mg\,kg^{-1}$ ($3\times$ dose) produced no effect on reproduction in females or males. Three monthly doses of $30\,mg\,kg^{-1}$ produced no adverse effects in ivermectin sensitive collies. There have also been rare reports of muscle spasms, seizures, ataxia, and other neurological signs.

Human

Little is known regarding effects of long-term exposure to selamectin in humans but experimental data indicate little potential for chronic toxicity.

Clinical Management

Individuals with known hypersensitivity to selamectin (Revolution) should use the product with caution. Wash hands after use and wash off any product in contact with the skin immediately with soap and water. If the product comes in contact with eyes, then

flush eyes with copious amounts of water. In case of ingestion, contact a physician immediately.

See also: Avermectins; Pesticides; Veterinary Toxicology.

Relevant Websites

http://cal.vet.upenn.edu – University of Pennsylvania.
http://www.vspn.org – Tina Wismer, ASPCA Animal Poison Control Center.

Selenium

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst and Shirley B Radding, volume 3, pp. 127–128, © 1998, Elsevier Inc.

- Selected Compounds: Hydrogen selenide (H_2Se); Sodium selenate (Na_2SeO_4); Sodium selenite (Na_2SeO_3); Selenium chloride (Se_2Cl_2). The toxicity of compounds varies substantially
- Chemical Abstracts Service Registry Number: CAS 7782-49-2
- Synonyms: Vandex; C.I. 77805
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Se^{4+}; Se^{6+}; Se^{2-}

Uses

Selenium is used in a wide variety of industries, including electronics, glass, ceramics, glass coloring, steel, pigment manufacturing, and rubber production. Medicinally, selenium is used in antidandruff shampoos and as a dietary supplement.

Background Information

Selenium was discovered in 1817. It is an essential trace element at $\sim$0.1 ppm in diets.

Exposure Routes and Pathways

For the general population, ingestion is the primary exposure pathway; sources include dietary supplements and various foods including seafood, meats, milk products, and grains. Trace amounts are found in drinking water. Selenium is not absorbed from shampoos.

In industrial settings, inhalation may be a significant exposure pathway. Airborne concentrations of selenium are higher in the vicinity of metallurgical industries. Selenium is present in most sulfide ores and is generally a by-product of the roasting of copper pyrite.

Toxicokinetics

Selenium and most of its compounds are rather insoluble and thus not absorbed orally. Soluble selenium compounds (e.g., sodium selenate and sodium selenite) are readily absorbed (up to 90%). Blood concentrations depend on the amount of selenium ingested. After blood levels of 200–240 $\mu g\,ml^{-1}$ are obtained, homeostatic controls take over. The greatest amount of absorbed selenium concentrates in the liver and kidneys, a lesser amount in the heart and lungs, and the least in the muscles.

Selenium is an essential trace element and an integral component of heme oxidase. It appears to augment the antioxidant action of vitamin E to protect membrane lipids from oxidation. The exact mechanism of this interaction is not known; however, selenium compounds are found in the selenium analogs of the sulfur-containing amino acids, such as cysteine and methionine. Se-cysteine is found in the active sites of the enzyme glutathione peroxidase, which acts to use glutathione to reduce organic hydroperoxides.

Selenium is rapidly excreted in the urine; some is incorporated into proteins. Elemental selenium and its oxides can be methylated. Trimethyl selenium is excreted rapidly in the urine; some is exhaled.

Mechanism of Toxicity

Excess selenium results in liver atrophy, necrosis, and hemorrhage. The mechanism of toxicity is unknown but may involve redox cycling. Sulfhydryl enzymes are attacked by soluble selenium compounds.

Acute and Short-Term Toxicity (or Exposure)

Animal

The toxicity depends on the molecular form. More soluble compounds, for example, sodium selenite, are more toxic than the less soluble elemental selenium, selenium sulfide, or selenium disulfide. Selenium dioxide is highly to moderately toxic, with oral

LD_{50} values in rodents from 20 to 70 mg kg^{-1}. The dermal LD_{50} for selenium dioxide in rabbits is 4 mg kg^{-1}. Oral LD_{50} values for sodium selenite range from 14 to 7 mg kg^{-1}, whereas an LD_{50} of 138mg kg^{-1} was noted for selenium disulfide, and an LD_{50} of >6 g kg^{-1} was noted for elemental selenium.

Respiratory effects include pulmonary congestion, hemorrhage and edema, dyspnea, weakness, and asphyxial convulsions. Acute exposures can also result in altered hematocrit, liver toxicity, and hemorrhage of the kidneys.

Human

Selenium is irritating to the eyes, skin, nose, and throat. The difference between an essential dose and a toxic dose for selenium is quite narrow. Normal intake can range from 50 to 200 μg; in the milligram range, toxicity is noted. Acute selenium poisoning results in nonspecific symptoms (e.g., eye irritation and coughing) and can affect the central nervous system and lead to convulsions. Liver and spleen damage has also been noted.

Inhalation of hydrogen selenide, a gas, may produce irritation of the upper respiratory tract and reduced respiratory flow rates, which can persist for a few years.

Chronic Toxicity (or Exposure)

Animal

Livestock and other animals are particularly affected by either selenium deficiency or excess selenium. In animals with selenium-deficient diets, liver necrosis arises. In areas with deficient selenium concentrations in soil, calves and lambs develop muscle atrophy, which is referred to as either 'white' muscle disease or 'stiff' muscle disease. Selenium supplementation (often injections) prevents these symptoms.

In areas with unusually high levels of selenium in the soil, livestock develop 'blind-stagger' disease, which is characterized by loss of vision, weakness of the limbs, and possible respiratory failure. Runoff from heavily fertilized farms causes excess selenium in ponds, which results in malformation of birds.

There are two interesting paradoxes concerning selenium. The first is that excess selenium is toxic; however, at lower levels it is a protective agent against the toxicity of cadmium, methylmercury, arsenic, copper, and thalium. The second paradox involves carcinogenicity. The US National Cancer Institute found selenium monosulfide (administered orally) to be carcinogenic in rodents; however, many epidemiological studies associate selenium intake with lower cancer rates in humans. Moreover, in the laboratory, selenium somewhat negates the carcinogenic action of carcinogenic aromatic hydrocarbons, acetylaminofluorene, and azo dyes, and it protects against spontaneous mammary tumors in various species of rodents.

Selenium is teratogenic in chickens and sheep; the evidence for humans is equivocal.

Human

Toxic manifestations of selenium poisoning include decaying and discoloring of teeth, gastrointestinal tract distress, skin lesions, and loss of hair and nails. In some cases, the skin on the fingertips and toes peels constantly. Excess selenium is metabolized to the dimethyl derivative, which is volatile and produces the 'garlic' or 'rotten' breath characteristic of selenium toxicity. Target organs are the respiratory tract, liver, kidneys, blood, skin, and eyes. Threshold limit value (TLV) = 0.2 mg m^{-1}.

Clinical Management

Currently, there are no antidotes of choice for selenium toxicity. Ethylenediaminetetraacetic acid and BAL (British antilewisite; 2,3-dimercaptopropanol) should not be used because they may enhance selenium toxicity. Treatment is symptomatic (e.g., cardiopulmonary). Often, supplemental oxygen is needed. Corrosive selenious acid (in gun-bluing solution) should be treated similar to other agents that cause esophageal burns.

Environmental Fate

Although selenium occurs naturally in the environment, it also can be released by both natural and manufacturing processes. As an element, selenium cannot be created or destroyed. However, forms of selenium can be transformed (changed) in the environment. Weathering of rocks to soil may cause low levels of selenium in water or it may cause it to be taken up by plants and naturally released into the air. Volcanic eruptions are suspected of contributing to selenium in air, and soils in the areas around volcanoes tend to have enriched amounts of selenium.

More commonly, selenium enters the air from burning coal or oil. Most of the selenium in air is bound to fly ash and to suspended particles. The elemental selenium that may be present in fossil fuels forms selenium dioxide during combustion (burning). Selenium dioxide can then form selenious acid with water or sweat. Selenium anhydride is released

during the heating of copper, lead, and zinc ores when there is selenium in them. Hydrogen selenide decomposes rapidly in air to form elemental selenium and water, thus eliminating the danger from this compound for most people, except those who are exposed to it in their workplace.

Airborne particles of selenium, such as in coal ash, can settle on soil or surface water. Disposal of selenium in commercial products and waste could also contribute to selenium levels in soil. But the amount of selenium released to soil from fly ash and hazardous waste sites has not been measured. The forms and fate of selenium in soil depend largely on the acidity of the surroundings and its interaction with oxygen. In theory, at equilibrium with no oxygen present, deep-soil selenium may be present as elemental selenium. In the absence of oxygen when the soil is acidic, the amount of biologically available selenium should be low. Elemental selenium that cannot dissolve in water and other insoluble forms of selenium (such as selenium sulfide and heavy metal selenides) are less mobile and will usually remain in the soil, posing less of a risk for exposure. Active agricultural or industrial processes may increase the amount of biologically available selenium by decreasing the acidity of the soil and increasing the oxygen and the soluble selenium compounds. Selenium compounds that can dissolve in water are very mobile. For example, selenates and selenites are water-soluble, and thus mobile, so there is an increased chance of exposure to them. Irrigation drainage waters may result in increased selenium entering the surface water. Other factors that may affect the rates at which selenium moves through the soil are temperature, moisture, time, season of year, concentration of water-soluble selenium, organic matter content, and microbiological activity.

Ecotoxicology

Selenium is implicated in the poisoning of birds in enclosed saline lakes. There is some evidence that selenium can be taken up in tissues of organisms ('bioaccumulate') and possibly increase in concentration ('biomagnify') in aquatic organisms as it is passed up through the food chain. Selenium concentrations in aquatic organisms have been a problem as a result of irrigation runoff in some dry areas of the United States. It is important to remember that selenium's behavior in the environment is largely affected by its surrounding conditions and by how it interacts with other compounds.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) TLV – time-weighted average (TWA) for hydrogen selenide is $0.05\,mg\,m^{-3}$. The ACGIH TLV – TWA for selenium and its compounds is $0.2\,mg\,m^{-3}$. Symptoms of overexposure include headache, chills, fever, bronchitis, garlic breath, gastrointestinal disturbance, and dermatitis.

See also: Metals; Veterinary Toxicology.

Further Reading

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology.* San Diego, CA: Academic Press.

Hamilton SJ (2004) Review of selenium toxicity in the aquatic food chain. *The Science of the Total Environment* 326(1–3): 1–31.

Whanger PD (2004) Selenium and its relationship to cancer: An update dagger. *British Journal of Nutrition* 91(1): 11–28.

Relevant Websites

http://risk.lsd.ornl.gov – Toxicity Summary for Selenium (from the Risk Assessment Information System).

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Selenium.

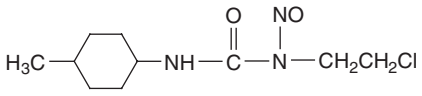
Semustine

Roberta Turci

- Chemical Abstracts Service Registry Number: CAS 13909-09-6
- Synonyms: Methyl-CCNU; *trans*-Methyl-CCNU; Lomustine, methyl; Methyl CCNU; MeCCNU; Me CCNU; Me-CCNU; NSC-95441; NSC 95441; NCI-C04955 Urea, *N*-(2-chloroethyl)-*N*′-(4-methylcyclohexyl)-*N*-nitroso-, *trans*-(9CI); Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-, *trans*-(8CI); 1-(2-Chloroethyl)-3-(*trans*-4-methyl-

cyclohexane)-1-nitrosourea; 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea

- Chemical/Pharmaceutical/Other Class: Nitrosoureas; Alkylating agents; Anticancer drugs
- Chemical Formula: $C_{10}H_{18}ClN_3O_2$
- Chemical Structure:



Uses

Semustine is an investigational drug used as an antineoplastic. It is the methyl analog of lomustine, a cytotoxic alkylating agent of the nitrosourea group, and has been used mainly for treatment of brain tumors, gastrointestinal tract adenocarcinomas, primary liver cancer, Hodgkin's disease and non-Hodgkin's lymphoma, melanoma, cervical cancer, head and neck cancer, breast and lung carcinomas. Occasionally, it is used in the treatment of solid tumors such as lung, carcinoma, colorectal, and breast carcinomas in combination with other anticancer agents.

Exposure Routes and Pathways

Cancer patients are exposed during chemotherapy. Semustine is available in 10, 50, and 100 mg capsules. The recommended adult oral dose ranges from 125 to 200 mg m^{-2}, given as a single dose every 6 weeks, as directed by the investigational study. Dosage may vary depending on the type of cancer and body weight and size of the individual. When used simultaneously with other antineoplastic drugs, the dose is usually reduced by 25–50%.

Health-care personnel preparing and administering anticancer therapies may be occupationally exposed to semustine by inhalation, dermal contact, or accidental ingestion.

Toxicokinetics

Semustine is well absorbed from the gastrointestinal tract following oral administration. It is rapidly metabolized and a number of active metabolites have been identified. Semustine as such is not detectable in either plasma or urine. The major route of elimination is urinary excretion. Up to 60% of a dose is eliminated renally within 48 h.

Peak plasma levels are produced within 1–6 h. The metabolites are reported to possess prolonged plasma half-lives, and can rapidly penetrate the blood–brain barrier, producing significant concentrations within 30 min in the cerebral spinal fluid. Small amounts may be excreted in feces and via the lungs as carbon dioxide.

Mechanism of Toxicity

Semustine produces interstrand cross-linking in DNA, generates carbonium ions, and may inhibit several vital enzymatic processes. Alkylation and carbamoylation by semustine metabolites interfere with synthesis and function of DNA, RNA, and proteins.

As all nitrosoureas, this agent rapidly and spontaneously decomposes into two highly reactive intermediates: chloroethyl diazohydroxide and organic isocyanate. This can result in a reaction that inactivates specific DNA repair enzymes. Semustine is cell cycle nonspecific.

Acute and Short-Term Toxicity (or Exposure)

Semustine is toxic by inhalation, if swallowed or absorbed through skin. It is irritating to the eyes, upper respiratory tract, and skin. Target organs are kidneys and bone marrow.

The more common side effects in cancer patients undergoing chemotherapies are decreased white blood cell count with increased risk of infection, decreased platelet count with increased risk of bleeding, nausea, and vomiting. Fetal abnormalities are observed if pregnancy occurs while taking this drug. Tiredness is a less common effect. Rare side effects are sores in mouth or on lips, liver problems, kidney problems, blurred vision or change in vision, scarring of lung tissue.

Animal

Ingestion of semustine affects behavior in mice. The LD_{50} in mice was 49.9 mg kg^{-1}. Prior to death, changes in motor activity were observed.

The lowest published lethal dose after oral or intravenous administration in dogs was 25 or 14 mg kg^{-1}, respectively. Hypermotility, diarrhea, agranulocytosis, and body temperature decrease were shown after intravenous exposure. Similar effects were observed in monkeys.

Human

Delayed myelosuppression, as evident by thrombocytopenia and leucopenia is a dose-limiting factor of semustine therapy. The nadir for thrombocytopenia and leucopenia is ~4–8 and 6 weeks, respectively, following administration. Myelosuppression tends to be cumulative with repeated doses. For this reason, the second or third dose is reduced by 25–50%.

Nausea and vomiting are frequently observed ~4–6 h after ingestion. Delayed nephrotoxicity, including renal failure, is often observed, especially in children. It seems to be total cumulative dose-related. About 25% of adults receiving semustine 1400 mg m^{-2} develop renal abnormalities.

Chronic Toxicity (or Exposure)

Animal

According to an International Agency for Research on Cancer (IARC) report, there is limited evidence of carcinogenicity in experimental animals. Semustine was tested for carcinogenicity by intraperitoneal injection in Sprague–Dawley and Swiss mice, together with a large number of other anticancer agents. The incidence of tumors increased in male rats, whereas the incidence of leukemia and lymphosarcomas in female mice increased only slightly. When administered by intravenous injection, semustine induced lung tumors in rats. It was not teratogenic in mice, although embryo viability was impaired. Semustine given to male mice caused temporary inhibition of spermatogenesis.

Human

Among the nitrosoureas, semustine has proved to be the most nephrotoxic compound. This has been a factor limiting more widespread use. Toxicity appears to be dose-dependent. Evidence of renal damage is often not apparent until 18–24 months following the completion of therapy. When it occurs, renal failure is usually progressive and irreversible. Nephrotoxicity is commonly heralded by increased serum creatinine levels, uremia, and proteinuria.

Semustine is a mutagen and a human carcinogen (group 1 according to IARC), based on sufficient evidence of carcinogenicity in humans. Adjuvant treatment with semustine has been evaluated in patients with gastrointestinal cancer, and a cumulative risk for the onset of acute nonlymphocytic leukemia (ANLL), of 4% at 6 years was observed. This percentage was not affected by concomitant radiotherapy or immunotherapy. A strong dose–response relationship was described, giving a relative risk of almost 40-fold among patients who had received the highest dose.

In Vitro Toxicity Data

Semustine was tested for *in vitro* effects on sister chromatid exchanges (SCE), cellular kinetics, and chromosome aberrations. Increase in SCE values was highly significant for all the concentrations tested (ranging from 1 to 10 mg l^{-1}). It also delayed cell cycle progression. Inhibition of DNA synthesis resulted in increased frequency of chromosomal aberrations. Therapeutic semustine concentrations had genotoxic effects on human peripheral blood lymphocytes and leukocytes studied *in vitro*.

Clinical Management

After inhalation exposure, the victim should be moved to fresh air. If not breathing artificial respiration should be given. If breathing is difficult, oxygen should be given.

After accidental ingestion, mouth should be washed out with water provided the person is conscious. A physician must be consulted.

Other Hazards

Hazardous combustion or decomposition products include carbon monoxide, carbon dioxide, hydrogen chloride gas, and nitrogen oxides.

Exposure Standards and Guidelines

According to the European Unit directives, semustine is classified as T (toxic).

Risk phrases: 45 46 23/24/25 36/37/38 (May cause cancer. May cause heritable genetic damage. Toxic by inhalation, in contact with skin and if swallowed. Irritating to eyes, respiratory system, and skin).

Safety phrases: 53 22 26 36/37/39 45 (Avoid exposure – obtain special instructions before use. Do not breathe dust. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)).

According to the US NTP 10th Report on Carcinogens (2000), semustine is known to be a human carcinogen.

OSHA regulates semustine under the Hazard Communication Standard and as a chemical hazard in laboratories. OSHA publication "Work practice guidelines for personnel dealing with cytotoxic (antineoplastic) drugs" and its revisions are among regulations in connection with occupational exposure to semustine in health care settings.

Miscellaneous

Semustine is a light yellow powder, stable under normal conditions. It should be protected from moisture and is incompatible with strong oxidizing agents and strong bases. It is slightly soluble in water

($<1\,\mathrm{mg\,ml^{-1}}$), and soluble in ethanol, acetone, and DMSO. Semustine is not available commercially. It has been only used in investigational studies, often producing low response rates.

See Also: BCNU (Bischloroethyl Nitrosourea); LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Methylnitrosourea.

Further Reading

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Sensitivity Analysis

Virginia Lau

Sensitivity analysis is a method used to evaluate the impact of a single variable or a group of variables on the results from a model calculation. Sensitivity analysis may be used to determine which parameters in a calculation have the greatest influence on the results such that greater emphasis is placed on characterizing these parameters. Moreover, the results from these analyses may be used to identify ways to improve the overall predictive capability of the model by reducing the uncertainty in the parameters that have the greatest influence on the outcome. Sensitivity analysis may be applied to the risk assessment process in order to identify those variables that dominate risk estimates as well as those that are relatively unimportant.

Sensitivity analyses are typically conducted at two levels: local and global. The local analysis generally assesses the effect of small perturbations of a single variable on the calculation. The primary rationale for using this technique is that the anticipated response to these slight changes is assumed to be nonlinear for at least one parameter in the calculation. Thus, increasing a specific parameter value by 10% is not predicted to produce a corresponding 10% increase in the result for all the parameters. The model is considered sensitive to a particular variable when small variations in the value produce large changes to the model predictions. There are several methods for calculating the effect of changes in inputs on model predictions. Perhaps the simplest method is to produce slight changes in a single variable in relative terms based on a fractional change from the base case value using deterministic single-point estimates while keeping the remaining parameters constant. Incrementally changing each parameter value by a set percentage essentially normalizes any differences between parameters that may occur due to deviations in units. A general case is to produce a $\pm 10\%$ change in the single variable to determine the resulting change in the output. Any arbitrary percentage may be used as long as the value is consistently applied to all the variables in the simulation. The results from the local sensitivity analysis may be used to determine places where additional parameters need to be better defined.

In mathematical terms, the local sensitivity analysis is analogous to determining the partial derivative for the calculation with respect to each parameter. Although the local sensitivity analysis normalizes differences between parameters such that the calculation is not sensitive to unit differences, this method does not address the effect on the results of using the full range of possible values for each parameter. This is an important limitation of conducting a local sensitivity analysis since the analysis is likely to produce misleading results for complicated calculations where parameter values have large uncertainties. For instance, a 10% increase above the default adult body weight in a standard risk calculation will produce a corresponding 10% reduction in risk since the individual has more mass available to equally distribute the pollutant concentration. Thus, the conclusion may be drawn that the risk calculation is not extremely sensitive to changes in body weight; however, this is not completely true since body weights are known to vary by as much as 20% from the default value. By accounting for this uncertainty, the actual risk may decrease by as much as 20%, which may be significant in certain cases. To resolve this problem, a global sensitivity analysis may be performed that explicitly evaluates the parameters in calculations where the uncertainty is large. It should be noted that the local sensitivity analysis is fairly accurate in cases in which the uncertainties are expected to be small.

The purpose of a global sensitivity analysis is to assess the effect a single parameter has on the results over the full range of possible values for that parameter. The range of values is often based on the frequency distribution assigned to the variable. Global sensitivity analysis identifies the parameters that contribute the most to the overall variance of the result by evaluating the uncertainty for each parameter individually. This approach generally follows the same Monte Carlo method used in uncertainty analysis to quantify uncertainty associated with all parameters defined in a calculation. The Monte Carlo method involves choosing values from a random selection scheme drawn from probability density functions based on a range of data that characterize the parameter of interest. A simple method of performing a global sensitivity analysis is to run the Monte Carlo simulation by incorporating the uncertainty for a single parameter and keeping all other values constant. However, this usually leads to a labor-intensive task if the calculation is complex and involves numerous parameters. Instead, the simulation may be performed by entering all the uncertainties for each parameter and somehow assigning the variances in the results to key parameters that are anticipated to be the most sensitive. Statistics such as regression analysis, partial correlations, fractional factorials, or partial rank correlations are often used to estimate which parameter is the most sensitive based on the variance of the result. This approach has the advantage that the same Monte Carlo simulations used to estimate uncertainty can be used to estimate the global sensitivity of the model predictions to the inputs and parameters used in the assessment.

See also: Hazard Identification; Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Risk Communication; Risk Management; Uncertainty Analysis.

Further Reading

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Sensitization Testing *See* Toxicity Testing, Sensitization.

Sensory Organs

Lewis Nelson

Due to their complexity, the special sense organs are both protected from and susceptible to toxic insult. Most toxic substances are excluded from the inner milieu of the eye and ear by specialized blood vessel barriers, selective membrane channels, or controlled pH gradients. However, some toxins elude these protective mechanisms and consistently produce toxic effects. Although classification is imperfect, differentiation must be made between agents capable of producing acute toxicity (single exposure) from those only toxic after chronic exposure. Although most of these agents are capable of producing multiple toxic effects in humans, the sensory organs' effects are often the most unique, identifiable, or disabling.

The Eye

The eye may be our most highly specialized organ. As an outgrowth of the central nervous system (CNS), it is susceptible to many of the same toxins as is the brain. However, several toxins stand out in their ability to produce nearly isolated ocular toxicity. In order to understand ocular toxicity, certain ophthalmologic principles need to be reviewed.

Vision has been called the 'vital sign' of the eye. Normal visual acuity implies intact light transmission through the optic system (cornea, lens, and vitreous humor) to the retina (the light sensing organ). The retina converts the light into nerve impulses which are sent to the occipital cortex via the optic tracts. Abnormalities in any of these components may diminish visual acuity or produce blindness.

Cornea

The cornea is the clear, external layer of the eye over the visual axis. The most common toxicologic disorder of the cornea results from irritant or caustic injury and results in edema (swelling) or erosion of the surface cells (abrasion). Patients with only mild corneal edema or erosion may experience 'halos' around bright objects and pain. This typically heals rapidly

over a few days with no demonstrable functional defects. With more severe injury, inspection of the external surface of the eye should reveal obvious injury to the cornea and surrounding ocular structures. Healing is imperfect and may result in extensive scarring and visual loss that may be amenable to surgical therapy.

Conjunctiva

The remainder of the exposed surface of the eye is covered by a thin mucosal layer known as conjunctiva. Mild irritation results in dilation of small blood vessels in the conjunctiva ('bloodshot'), pain, and a foreign body sensation. Chemosis, or swelling of the conjunctiva, is the typical response to more severe irritant or caustic exposure. Vision is not generally affected unless ocular perforation occurs or if scarring distorts the shape of the globe.

Pupil

The pupil is the window into the internal eye. The iris, or the colored border of the pupil, opens and closes to vary the amount of light admitted through the pupil into the eye. The pupillary size and reactivity are very useful clinical parameters by which toxic exposures are assessed. Control of pupillary size is complex and is dependent on the interaction of the opposing sympathetic and parasympathetic nervous systems. The pupil enlarges with sympathetic stimulation and constricts with parasympathetic stimulation. Conversely, sympathetic system blockade results in small pupils (miosis), while parasympathetic blockade produces large pupils (mydriasis). Light hitting the retina reduces sympathetic nervous signaling to the pupil and results in pupillary constriction. The opposite occurs in dark situations. Cholinergic agents, such as organophosphorus cholinesterase inhibitors, produce miosis (**Table 1**).

Lens

The lens is deep within the eye and is responsible for focusing an image of an object on the retina. Lens opacification (cataracts) may produce similar visual abnormalities as corneal damage but is often undetectable without careful inspection of the inside of the eye. Amiodarone, a commonly used antiarrhythmic agent, produces deposits or spotting in the cornea.

Table 1 Agents associated with pupillary changes

Miosis
Cholinergics: Organophosphorus pesticides, pilocarpine, nicotine
Opioids
Sedative-hypnotics
Clonidine
Mydriasis
Anticholinergics: atropine, diphenhydramine
Cocaine, amphetamines
Sedative or opioid withdrawal

Vitreous Humor

The majority of the eye is filled with a viscous, clear substance known as vitreous humor. The vitreous humor is rarely directly altered by toxic exposure, but bleeding into the vitreous may be a secondary effect of a systemic toxin such as an anticoagulant.

Retina

The retina is a true neural structure and as such is highly complex and not fully understood. It is responsible for converting light and colors into neural signals. Retinal toxicity results in blurry vision, often described as 'walking in a snowstorm'. Retinal toxicity may be due to a direct effect on the retinal cells or it may occur indirectly through a reduction in blood flow or oxygen delivery to the retina. Although medical or surgical restoration of sight is routinely available for corneal, lenticular, or vitreal damage, therapeutic interventions are much more limited for patients with retinal toxicity.

Brain

In addition to a normal eye, vision requires an intact circuit to and from the brain itself. The optic nerve (cranial nerve II), for example, carries retinal information to the occipital cortex in the posterior aspects of the brain. In addition, information concerning pupil size, direction of gaze, simultaneous movement of the eyes (conjugate gaze), and focus clarity is relayed from the brain back to the eye through multiple nerves. Ethambutol, an antituberculous medication, affects the optic nerves (optic neuropathy) whereas organic mercury compounds have been historically associated with toxic effects on the occipital cortex (cortical blindness).

Specific Ocular Toxins

Many agents are capable of producing visual loss in humans. **Table 2** lists selected agents capable of producing chronic toxicity to various portions of the eye. In the following sections, several common or important toxins are discussed.

Caustics and Irritants Acid and alkali burns to the eye often result in severe corneal injury and require aggressive intervention. It is often difficult to predict at the outset the amount of damage any given

Table 2 Agents associated with chronic visual changes

Corneal
Metals
Amiodarone
Chlorpromazine
Cataracts (Lens)
Dinitrophenol
Corticosteroids
Retinal injury
Carbon disulfide
Quinine
Vincristine
Neurologic (optic nerve, brain)
Ethambutol
Lead
Methylmercury

chemical will inflict on the eye. Generally, agents that have extreme pH, particularly alkali, and those which are solid produce the greatest corneal damage. Irritants, such as hydrocarbons and detergents, tend to be less problematic, producing primarily irritation and corneal erosion, but exceptions abound. The clinical effects of caustics range from corneal and conjunctival irritation to ocular perforation and destruction. Immediate treatment should consist of copious irrigation with water or saline. Measurement of the pH of the ocular surface is useful to guide therapy, but chemical neutralization should never be attempted due to the potential for additional irritant or thermal injury. Even with immaculate initial care, severe caustic injuries may produce corneal scarring and visual loss.

Methanol Of all substances consistently reported to produce direct ocular toxicity in humans, methanol is most frequently responsible. Methanol is locally available as a gasoline additive and as windshield cleaning fluid and is used widely in industry as a solvent. Several well-documented epidemic poisonings have occurred in the recent past resulting from the consumption of methanol in place of ethanol, and isolated cases are common. Ocular symptoms often take several hours to develop and are actually due to the metabolite of methanol, formic acid, formed by alcohol dehydrogenase (ADH). Formic acid also produces profound systemic acidosis which may be fatal. Management consists of inhibiting the metabolism of methanol by providing ADH with ethanol, its preferred substrate. Fomepizole, an ADH inhibitor, is equally as effective and significantly easier to use than ethanol, but it is more costly. Following either antidote, the remaining methanol, which is poorly excreted without metabolism, is generally removed by hemodialysis.

Quinine The antimalarial agent quinine is derived from the bark of the cinchona tree along with several other alkaloids and salicylate (aspirin). Many of these agents produce similar toxic features (cinchonism) in patients with excessive intake, but only quinine produces blindness. Cinchonism consists of abdominal pain and vomiting, ringing in the ears (tinnitus), and confusion. Visual loss after quinine overdose is due to direct retinal toxicity, although until recently it was believed to be due to spasm of the arterial blood supply to the retina. Treatment is difficult, but limited evidence suggests charcoal hemoperfusion may be beneficial (hemoperfusion is similar to hemodialysis, except in place of a semipermeable membrane to filter the toxin from the blood, charcoal is used to bind the toxin).

Agents Capable of Indirect Retinal Toxicity

Indirect retinal toxins produce retinal ischemia or reduced oxygen delivery by the blood. Cocaine, amphetamines, and ergot alkaloids (used in the treatment of migraine headaches) may produce retinal ischemia by reducing the caliber of the retinal arteries and thereby reduce blood flow. Many of these toxins vasoconstrict by stimulating the α-adrenergic receptors on peripheral arteries. Treatment focuses on reducing the vasoconstrictive effect of the primary toxin and may include sedatives (e.g., diazepam), direct acting vasodilators (e.g., nitroglycerine) or α-adrenergic antagonists (e.g., phentolamine). Foreign bodies, such as talc, introduced by use of impure intravenous drug can result in embolization (mechanical blockade) of the retinal arteries with resultant ischemia. Retinal ischemia can also occur in patients with poor retinal blood flow due to hypotension (low systemic blood pressure). Toxic causes of hypotension reported to induce blindness include calcium channel blockers, β-adrenergic blockers, and nitrates. Additionally, by preventing hemoglobin, the main oxygen transport protein in the blood, from binding and delivering oxygen, carbon monoxide can produce retinal ischemia.

Occupational Exposures

Although occupational inhalation of methanol may rarely produce ocular toxicity, the vast majority of occupational eye toxicity results from exposure to irritant chemicals. Highly water-soluble gases, such as ammonia or hydrogen sulfide, produce immediate pain and tearing upon exposure. Gases that are poorly soluble in the water of the eye only produce irritation after prolonged exposure (e.g., phosgene and ethylene oxide).

The Ear

The ear is nearly as complex as the eye and is also an outgrowth of the CNS. The ear converts sound waves into neural impulses which are transmitted to the brain for processing. Unlike the eye, toxins produce adverse effects only at limited sites in the ear. Like the eye, however, free entry of drugs into the inner ear is prevented by a selective filtering mechanism.

External Ear and Ear Canal

While the external ear and ear canal serve as a pathway for the entrance of sound into the internal ear, they are infrequently affected by toxic exposure. Caustics and irritants are the only agents commonly producing toxicity at this level.

Middle Ear

Several tiny bones in the middle ear amplify and convert sound waves from the eardrum into fluid waves in the inner ear. There are no significant toxic exposures affecting the middle ear.

Inner Ear

Two functions are served by the inner ear: hearing (cochlear system) and balance (vestibular system). The cochlea is capable of converting fluid waves into neural impulses. The cochlea is a fluid-filled, snail-shaped organ containing specialized nerve endings known as hair cells. Vibrations transmitted by the middle ear to the cochlear fluid cause movement of the hair cells, triggering signal production which is carried by the auditory nerve (cranial nerve VIII) to the brain. The electrolyte content of the fluid within the inner ear is closely regulated by specialized transport systems. The kidney contains a nearly identical system, which it uses to regulate the electrolyte composition of the blood, explaining why ototoxic agents are frequently also nephrotoxic.

Vestibular System

The vestibular system serves to balance the body. Dysfunction results in ataxia (incoordination), nystagmus (abnormal eye movements), and spatial disorientation. Few toxins produce isolated vestibulotoxicity, and most patients demonstrate hearing abnormalities concurrently.

Specific Otic Toxins

Aminoglycoside Antibiotics The aminoglycoside antibiotics are well known for their toxic side effects, renal and inner ear toxicity. Destruction of the hair cells of the cochlea produces hearing loss, beginning with high frequencies and progressing toward the lower frequencies. All of the aminoglycosides have the potential for such toxicity, but the relative toxicities differ. Newer techniques of administration such as bolus dosing may prove to reduce the frequency of ototoxicity. Aminoglycosides are also vestibular toxins, and such toxicity often precedes hearing loss.

Salicylates Aspirin and other salicylic acid derivatives are used as analgesic and antiinflammatory agents. Tinnitus, or high-frequency ringing in the ears, is the most common sign of toxicity and is variably accompanied by hearing loss. The ability of aspirin to cause ototoxicity was so widely known that in the early part of this century, tinnitus was used as a clinical marker for therapeutic dosing. Aspirin-induced tinnitus, which is almost always reversible, is probably due to interruption of the normal metabolic processes of the sensory hair cells. Several structurally unrelated nonsteroidal antiinflammatory agents are capable of producing toxic symptoms similar to aspirin, suggesting involvement of the prostaglandin system.

Diuretics The toxic effect of diuretics on the inner ear is related to the alteration of the fluid contained within. Changes in the electrolyte composition of the inner ear fluid causes swelling of the structures of the inner ear and tinnitus. However, this cannot be the only toxic mechanism since symptoms may occur immediately upon large exposure, at which time the fluid composition has not yet been altered.

Occupational Exposures

Surprisingly little research has been performed on the otic effects of chemicals on workers. However, several widely used chemicals are known to be ototoxic. However, the combination of toxin exposure and noise may be additive or synergistic in the production of hearing loss. This has made investigation of the isolated toxic effects on exposed workers difficult (**Table 3**).

Olfaction

The sense of smell is our most sensitive special sense. We are able to detect the odor of certain chemicals in the parts per million range. However, some chemicals are undetectable altogether, and others cause olfactory fatigue, in which exposure to a substance reduces our ability to detect its odor (e.g., hydrogen sulfide). Even more interesting, certain people are unable to detect certain odors, while others can

Table 3 Agents associated with hearing loss

Occupational
Bromates
Carbon disulfide
Carbon monoxide
Lead
Mercury
Styrene
Toluene
Trichloroethylene
Xylene
Drugs
Aminoglycosides
Diuretics
Chemotherapeutic agents
Salicylates

Table 4 Agents associated with smell disorders

Occupational exposures
Acrylate and derivatives
Cadmium dust
Carbon disulfide
Formaldehyde
Hydrogen sulfide
Solvents (volatile hydrocarbons)
Drugs
Antithyroid medications: methimazole, methylthiouracil
Antihypertensives: beta blockers, captopril, enalapril
Levo-dopa
Opioids: morphine, codeine
Cigarette smoking
Cocaine insufflation

Table 5 Agents associated with taste disorders

ACE inhibitors: captopril, enalapril
Carbamazepine
Chemotherapeutic agents
Cigarette smoking
Diuretics
Levo-dopa
Phenylbutazone
Metallic taste
Allopurinol
Ciguatoxin
Coprinus mushrooms
Disulfiram
Ethambutol
Heavy metals
Lithium
Metronidazole
Methotrexate
Penicillamine
Penicillin
Tetracycline

detect the same odor at tiny concentrations (e.g., detecting cyanide's almond odor seems to be genetically determined). The most common toxic olfactory insult is cigarette smoking, and this must be considered in all patients with olfactory or taste dysfunction.

Olfactory receptors, numbering about 20 million per nares, are receptor ends of neurons that form the olfactory nerve (cranial nerve I). This nerve sends projections to many parts of the brain, possibly explaining why smells often elicit profound emotional responses or memories. In addition, irritant receptors exist within the nasal cavity, which are unrelated to smell. This is sometimes called the 'common chemical sense' and connects to the brain via the trigeminal nerve (cranial nerve V). This differential recognition of irritants from odors is clinically useful. Patients complaining of dysfunctional odor recognition should have normal recognition of ammonia and other pungents. Failure to recognize irritant effects raises the possibility of malingering.

There are several syndromes of altered sense of smell (dysosmia). Hyposmia or anosmia is the reduction in intensity or complete lack of the ability to smell, respectively. Troposmia is the distortion of an odor compared to a previous exposure. Phantosmia is the perception of an odor when there is none present.

Few agents are acutely toxic to the olfactory receptors. Hydrogen sulfide, as mentioned earlier, causes rapid olfactory fatigue, and the normal 'rotten egg' odor quickly vanishes allowing prolonged exposure to this potentially fatal mitochondrial toxin. Occupational exposure to several solvents and metals has been associated with olfactory dysfunction (**Table 4**).

Gustation

Disorders of taste, like that of smell, are generally of limited toxicologic interest. The sensation of taste, like smell, is receptor mediated. Most abnormalities of gustation involve detection of abnormal tastes, not reduction in overall function of the sense.

Taste receptors reside within taste buds on the tongue, the larynx, and the palate. There are four primary taste sensations: sour, sweet, bitter, and salty. By mixing these primary taste sensations, the brain can identify many specific tastes (analogous to primary color mixing). Impulses from the taste buds are carried through the facial, glossopharyngeal, and vagus nerves (cranial nerves VII, IX, and X, respectively) to the brain. Taste is modified by the presence of odor, and in the absence of olfactory ability, taste is virtually eliminated.

A metallic taste is often noted with exposure to metals or metal-containing compounds, tetracycline, mushrooms (e.g., Coprinus sp.), snake venom, and others. Metal fume fever, a febrile immunologically mediated reaction to metal oxides volatilized during welding, also produces a metallic taste. An abnormal

garlic sensation is experienced after exposure to dimethylsulfoxide, organophosphate insecticides, and arsenic. Such an abnormal taste sensation is likely due to cross-recognition of certain chemical agents by specific taste receptors (**Table 5**).

See also: Acids; Aminoglycosides; Behavioral Toxicology; Corrosives; Eye Irritancy Testing; Metals; Methanol; Occupational Toxicology; Organophosphates; Physical Hazards; Quinine; Salicylates.

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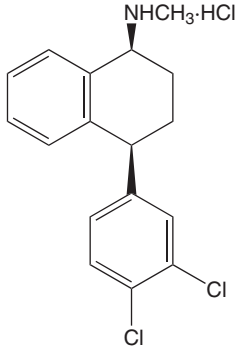
Sertraline Hydrochloride

Bruce Ruck

- Chemical Name: (1*S*-*cis*)-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine hydrochloride
- Chemical Abstracts Service Registry Number: CAS 79559-97-0
- Synonym: Zoloft
- Chemical/Pharmaceutical/Other Class: Antidepressant agent; Selective serotonin reuptake inhibitor
- Chemical Formula: $C_{17}H_{17}NCl_2 \cdot HCl$
- Chemical Structure:



Uses

Sertraline hydrochloride is used in the management of depression, obsessive–compulsive disorder (OCD), panic disorder, posttraumatic stress disorder (PTSD), premenstrual dysphoric disorder (PMDD), and social anxiety disorder.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposure to sertraline hydrochloride. Sertraline is available as an oral tablet and as an oral concentrate (liquid).

Toxicokinetics

Pharmacokinetic data is derived from ingestion of doses considered to be in the therapeutic range; hence, no data are included on the pharmacokinetics of sertraline after overdose.

After oral doses of 50–200 mg, sertraline reaches a maximum plasma concentration in ~4.5–8.4 h. Administration of the oral concentrate results in a higher area under the curve (AUC) and C_{max} compared to the oral tablet. Peak absorption may be delayed with large ingestions. When food was administered with therapeutic doses of tablet formulation, both the peak and total plasma levels (AUC) increased; however, the time to reach peak plasma concentration decreased. With repeated dosing, a steady-state plasma level should be achieved within 7 days.

Sertraline's bioavailability is reduced by extensive first-pass metabolism. The principal metabolite of this first-pass metabolism is *N*-desmethylsertraline. *N*-Desmethylsertraline has a half-life of 62–104 h and is substantially less active than the parent compound. Sertraline and its metabolite *N*-desmethylsertraline both undergo further biotransformation to nonactive compounds. Clearance of sertraline may be faster in children than in young adults and slowest in the elderly.

Sertraline is highly protein bound (~98%). Its volume of distribution is estimated at $20\,l\,kg^{-1}$.

Mechanism of Toxicity

Sertraline is a potent and highly selective serotonin reuptake inhibitor (SSRI) that increases the availability of this neurotransmitter in the synaptic cleft. Sertraline has minimal effects on norepinephrine and dopamine reuptake. It shows no significant affinity for adrenergic, cholinergic, γ-aminobutyric acid, dopaminergic, histaminergic, serotonergic, or benzodiazepine receptors. Sertraline has no effects on monoamine oxidase.

Acute and Short-Term Toxicity (or Exposure)

Human

Sertraline has a relatively low risk of toxicity. It is less sedating and has fewer cardiovascular effects than the tricyclic antidepressants. It has a high therapeutic index, which is consistent with other serotonin uptake inhibitors.

In general, ingestions of up to 4500 mg have been tolerated without significant toxicity. However, one patient ingesting 2500 mg had a fatal outcome. Patients may develop symptoms including nausea, vomiting, drowsiness, tachycardia, dilated pupils, slurred speech, and ataxia. Therapeutic and toxic plasma concentrations have not been well defined.

Sertraline has the potential to cause 'serotonin syndrome'. Most commonly, this syndrome occurs when two or more drugs capable of enhancing serotonin activity are used concomitantly. This syndrome can occur in the overdose situation.

Manifestations of serotonin syndrome include: altered mental status, restlessness, myoclonus, hyperreflexia, diaphoresis, shivering, tremor, incoordination, and/or fever.

Chronic Toxicity (or Exposure)

Animal

Mice receiving 10–40 $\mathrm{mg\,kg^{-1}\,day^{-1}}$ developed increased rates of hepatic adenomas compared to unexposed controls.

Human

Therapeutic chronic use of sertraline has reportedly caused visual defects, cardiac toxicity, gastrointestinal irritation, renal pathology, and loss of appetite. However, causality of these effects still requires confirmation.

Like other SSRIs, sertraline should not be used within 2 weeks of discontinuing monoamine oxidase inhibitors (MAOIs) and MAOIs should not be started for at least 2 weeks after stopping sertraline.

In Vitro Toxicity Data

Studies using RBL-2H3 cells demonstrated an increase in mRNA and protein levels of tryptophan hydroxylase due to sertraline.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastric decontamination with activated charcoal should be considered for substantial recent ingestions. Treatment recommended after decontamination is symptomatic and supportive. There is no antidotal therapy. Because patients taking an overdose of sertraline may also have access to other medications/chemicals, the patient should be evaluated and treated as appropriate for other substances that may have been ingested.

It should be noted that the abrupt discontinuation of an SSRI can cause a 'withdrawal' syndrome. This syndrome is often referred to as 'discontinuation syndrome'. SSRI discontinuation syndrome is often manifested by symptoms of fatigue, gastrointestinal complaints (nausea, vomiting, diarrhea, cramping), shortness of breath, memory impairment, dizziness, insomnia, chills, headache, eye discomfort, tinnitus, ataxia, and abnormal sensations (e.g., 'electric shocks', skin tingling sensations, and involuntary movements).

See also: SSRIs (Selective Serotonin Reuptake Inhibitors); Tricyclic Antidepressants.

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Seveso Disaster, and the Seveso and Seveso II Directives

Pertti J Hakkinen

The Seveso Disaster

The Seveso disaster began on July 10, 1976 at the Industrie Chimiche Meda Società Azionaria (ICMESA) chemical plant in Meda, Italy. This event became internationally known as the Seveso disaster, after the name of the most severely affected community. An increase in pressure due to an exothermic reaction in a 2,4,5-trichlorophenol-production reactor caused the rupture disk of the safety valve to burst. About 3000 kg of chemicals were released into the air. The release included 2,4,5-trichlorophenol, used in the manufacture of herbicides, and possibly up to 30 kg of the dioxin TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). Dioxin first came to widespread public notice during the Vietnam War, when it was identified as a component of the defoliant Agent Orange. Dioxin has also been considered to be the most toxic human-made substance.

The chemicals released into the air from the chemical plant near Milan in northern Italy were carried southeast by the wind towards Lake Como. The dioxin cloud contaminated a densely populated area about 6 km long and 1 km wide downwind from the site. The four most impacted municipalities included Seveso (a 1976 population of 17 000), Meda (19 000), Desio (33 000), and Cesano Maderno (34 000). Two other municipalities, Barlassina (6000) and Bovisio Masciago (11 000), were subject to postaccident restrictions. Health monitoring was extended to a further five municipalities. The entire affected area is part of the Brianza, one of the wealthiest and most industrialized regions of Italy. The economy of this area at the time of the disaster depended on small workshops and industries, mainly engaged in manufacturing furniture.

The Seveso disaster had a traumatic effect on the minds of the local populations because its seriousness was recognized only gradually. People elsewhere in the world also experienced heightened concern about chemical exposures and risks and the need for tighter regulation of hazardous chemical manufacturing. The accident was not immediately noticed since no one was at the plant when it happened. One day later, ICMESA managers informed local authorities of the escape of a "cloud of herbicide that causes harm to agriculture," stating that "in all likelihood the aerosol mixture which escaped consists of sodium trichlorphenate, caustic soda, and solvent, but possibly other toxic substances as well." They requested the authorities to warn the population, and samples were sent by courier for examination to a company, Givaudan SA, in Switzerland. Givaudan SA, once one of ICMESA's main customers, had taken over ICMESA as a subsidiary in 1969.

Two days after the disaster, nearby residents were warned not to eat any vegetables from their gardens. Four days after the disaster, the Technical Director of Givaudan in Geneva informed the Technical Director of ICMESA that the samples contained traces of TCDD. Authorities were told much later about the TCDD. The Seveso disaster resulted in the highest known TCDD exposure to residential populations, and has possibly been the most systematically studied dioxin contamination incident in history.

The first sign of human health problems was burn-like skin lesions, appearing on children after the accident. Beginning in September of 1976, chloracne, a severe skin disorder usually associated with dioxin, broke out on some people who were most exposed. Authorities began an investigation five days after the accident, when large numbers of local animals such as rabbits began to die. After a linkage to dioxin was made, over 700 hundred people living closest to the plant were evacuated within 3 weeks after the accident. In all, up to 2000 people were treated for dioxin poisoning. Approximately 4% of the local farm animals died, and $\sim$80 000 additional animals were killed to prevent contamination from filtering up the food chain.

The Seveso disaster areas were divided and subdivided based on soil contamination levels. Zone A, the most contaminated area with more than 50 μg of TCDD per square meter and covering 110 ha, was completely evacuated and fenced-off with entry prohibited. Zone A was later turned into a park, the Seveso Oak Forest. In the next-most contaminated areas, zone B (between 5 and 50 $\mu g\,m^{-2}$) and zone R (below 5 $\mu g\,m^{-2}$), farming as well as consumption of local agricultural goods and meats were strictly prohibited.

Professor Paolo Mocarelli of the University of Milano-Bicocca's Hospital of Desio was put in charge of a laboratory setup two weeks after the accident to test people for health problems. Dr. Mocarelli's laboratory has conducted neurological, obstetric, and other tests that have surpassed 1 million in number, and Dr. Mocarelli decided to save one sample of blood from each person just in case it would be possible to measure very low levels of TCDD in small blood samples someday. This became possible in 1987, and Dr. Mocarelli has worked with the US

Centers for Disease Control and Prevention to analyze the thousands of samples and to try to associate the levels with health effects. Reconstruction of the disaster using the samples taken over time has helped clarify how long dioxin stays in the human body, and the different effects it has on children and adults.

One toxic effect of the Seveso dioxin exposure was on reproduction. In the first 7 years after the accident, a very high proportion of females (46 females compared to only 28 males) were born to parents who were exposed to the chemical cloud. This was the first time a chemical had been observed to change the sex ratio, implicating dioxin as a hormone disrupter. TCDD is associated with increased fetal loss and reduced birth weight in animal studies.

The Seveso Women's Health Study (SWHS) is a retrospective cohort study of people who resided in the most contaminated areas, zones A and B. Serum samples collected near the time of the explosion were analyzed for TCDD. Also analyzed were pooled serum samples collected in 1976 from females who resided in the "unexposed" zone to assess concurrent background exposures to other dioxins, furans, and polychlorinated biphenyls (PCBs). The youngest children had the highest TCDD levels, which decreased with age at explosion until $\sim$13 years of age and were essentially constant thereafter. The zone of residence and age were the strongest predictors of TCDD level. Chloracne, nearby animal mortality, location (outdoors versus indoors) at the time of explosion, and consumption of homegrown food were also related to serum TCDD levels. The serum pools from the 'unexposed' zone residents had TCDD concentrations and average total toxic equivalent (TEQ) concentrations that suggested that the background exposure to dioxins, furans, and PCBs unrelated to the explosion may have been substantial. Therefore, the early SWHS studies that considered only TCDD exposure may have underestimated health effects due to total TEQ concentrations.

The early part of the SWHS looked at the relation of pregnancy outcome to maternal TCDD levels measured in serum collected shortly after the explosion. Ninety-seven pregnancies (10.9%) ended as spontaneous abortions. TCDD was associated with a nonsignificant adjusted decrease in gestational age and a 20–50% nonsignificant increase in the odds of preterm delivery. The exposed population also reported symptoms of immune system and neurological disorders; however, studies found no link to dioxin. Increases in some forms of cancer found in the exposed population have suggested a link between dioxin and some cancers.

Further research on the children of victims of the disaster is being conducted, as is research focusing on dioxin's long-term carcinogenic properties. For example, 25 years after the Seveso disaster, human milk from mothers in Seveso was found to have TCDD concentrations more than twice as high as those in central Milan and elsewhere in the areas near Seveso. This suggests that breastfed infants in Seveso might have appreciable amounts of TCDD in their body fat; however, the health consequences remain to be determined.

In addition to monitoring victims and offspring of the accident, another type of monitoring that continues concerns the Seveso Oak Forest's two large concrete tanks lying beneath the surface. These tanks are the resting place of the top 40 cm of soil removed after the disaster, and also are the final resting place of the contaminated animals that were slaughtered, and the factory (disassembled brick by brick by workers in protective suits) and other buildings exposed to the cloud. The area around the tanks is monitored for leaks, and the soil is said to now have lower dioxin levels than in average areas. The area is now a place where families can gather and animals have returned to the park and adjacent nature reserve.

After the Seveso disaster, investigation of the potential emission sources in the area and studies of people not exposed to the cloud indicated that combustion of wood residues from furniture factories might be an additional and perhaps substantial local source of dioxins, furans, and PCBs.

The Seveso Directive

In 1982, the European Union's Council Directive 82/501/EEC on the major-accident hazards of certain industrial activities, also known as the Seveso Directive, was adopted. The Directive was mostly designed to promote information flow and created the requirement that each Member State (i.e., each country belonging to the European Union) appoint a Competent Authority to oversee safety issues. The Seveso Directive was amended twice, following major accidents at the Union Carbide chemical factory in Bhopal, India in 1984 (a leak of methyl isocyanate caused thousands of deaths), and at the Sandoz chemical warehouse in Basel, Switzerland in 1986 (fire-fighting water contaminated with mercury, organophosphate pesticides and other chemicals caused massive pollution of the Rhine River and the death of hundreds of thousands of fish). Both amendments, broadened the scope of the Directive, in particular to include the storage of dangerous substances.

The Seveso Directive covered all European Union Member States, and held them responsible for ensuring that the relevant national institutions do what is required for adequate risk management. The entire Directive was also driven by a concern for prevention, including those parts that relate to post-accident activities. For example, terms such as 'industrial activity, manufacturer, major accident, and dangerous substances' were defined, the types of production, operations, and storage activities that are subject to regulation were described, and the dangers that are anticipated were noted.

Member States were required to ensure that manufacturers identify existing major accident hazards, and that they adopted all appropriate safety measures, including information, training, and equipment for workers. Further, Member States must set up Competent Authorities that will take responsibility for receiving such a notification, examining the information provided, organizing inspections or other measures of control, and ensuring that off-site emergency plans are prepared. The manufacturers were also required to provide the Competent Authorities with a notification containing detailed and updated information on safety precautions and other matters. In addition, Member States were held responsible for assuring that "persons liable to be affected by a major accident were informed in an appropriate manner of the safety measures and of the correct behaviour to adopt in the event of an accident."

Article 8 of the Seveso Directive was noteworthy in its content because the safety of people outside hazardous installations was taken into account for the first time in Europe (before this, only workers might have had the right to be informed). Information that had previously been 'for experts alone' was opened-up to inspection by, and input from, the public. Another article of the Directive required Member States to take the necessary measures to ensure that the manufacturer immediately provided full and detailed information about an accident to the competent authorities; they must in turn were to ensure that all necessary measures were taken and that full analysis of the accident was accomplished whenever possible. The European Commission was put in charge of setting up a register containing a summary of major accidents that occur within the European Union, including an analysis of causes, experience gained, and measures taken to enable Member States to use this information for prevention purposes.

The Seveso Directive also led the way to similar initiatives on other issues, for example, in environmental management and public health. This included the mandating of measures to encourage improvements in occupational safety and health; minimum safety and health requirements for the workplace, measures related to biotechnology; the freedom of access to environmental information; and public information about radioactive emergencies. Beyond Europe, the Seveso Directive was important for many international organizations, for example, the World Bank, the United Nations Environment Programme, the Council of Europe, the International Atomic Energy Agency, the Office of the United Nations Disaster Relief Coordinator (UNDRO), the World Health Organization, and the International Labour Organization (ILO). Further, the Organization for Economic Cooperation and Development (OECD) has focused much attention to accident prevention and response and has published a number of recommendations, some of which are specifically addressed to public information and public participation in decision-making.

The Seveso II Directive

In 1996, Council Directive 96/82/EC on the control of major-accident hazards, also known as the Seveso II Directive, was adopted. The Seveso II Directive replaced the original Seveso Directive, and Member States had up to 2 years to bring into force the national laws, regulations and administrative provisions to comply. Important changes and new concepts included a revision and extension of the scope of the Seveso Directive, the introduction of new requirements relating to safety management systems, emergency planning and land-use planning, and a reinforcement of the provisions on inspections to be carried out by Member States. Further, Member States can maintain or adopt stricter measures than those contained in the Seveso II Directive.

The Seveso II Directive covers industrial 'activities' and the storage of dangerous chemicals, with larger quantities of a chemcial leading to more control measures. A company holding a quantity of dangerous chemical less than Seveso II's lower threshold levels is not covered by this legislation, but will be proportionately controlled by general provisions on health, safety and the environment provided by other legislation not specific to major-accident hazards. Important areas excluded from the scope of the Seveso II Directive include nuclear safety, the transport of dangerous substances and intermediate temporary storage outside establishments and the transport of dangerous substances by pipelines.

All operators of establishments coming under the scope of the Seveso II Directive need to send a notification to the competent authority, and need to

establish a Major-Accident Prevention Policy. In addition, operators of 'upper tier establishments' (i.e., holders of high levels of a dangerous chemical) need to establish a Safety Report, a Safety Management System, and an Emergency Plan.

Member States have the obligation to report major accidents to the Commission. In order to fulfill its information obligations toward the Member States, the European Commission has created the Major Accident Reporting System (MARS) database to store and retrieve accident information reported by the Member States, and a Community Documentation Centre on Industrial Risks (CDCIR) was established to collect, classify, and review materials relevant to industrial risks and safety.

In order to assist Member States with the interpretation of certain provisions of the Seveso II Directive, the European Commission in co-operation with the Member States developed documents on the preparation of a safety report, guidelines on a major accident prevention policy and safety management system, explanations and guidelines on harmonized criteria for dispensations, guidance on land-use planning, guidance for the content of information to the public, and guidance on inspections. In addition, a series of answers to frequently asked questions has been published and regularly updated. These guidance documents and the answers to frequently asked questions have no legal status, but do provide valuable guidance to industrial operators as well as enforcement authorities within the European Union.

See also: Bhopal; Dioxins; European Union and Its European Commission; International Labour Organization (ILO); Organisation for Economic Cooperation and Development.

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Relevant Website

http://www.europa.eu.int – European Commission, Chemical Accident Prevention, Preparedness and Response.

Shampoo

Paul Sterchele

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- Trade Names: Neutrogena; Head and Shoulders; Prell; Pert; Johnson's; Pantene
- Chemical/Pharmaceutical/Other Class: Combination of nonionic, amphoteric, and anionic surfactants

Uses

Shampoos are rinse-off products used to cleanse the hair and scalp; they are available in noncoloring and coloring formulations. Lindane shampoos are available for the treatment of lice; antidandruff formulations are also available to control the symptoms of dandruff and seborrheic dermatitis.

Exposure Routes and Pathways

Ingestion is a common route of exposure. Ocular and dermal exposures occur as well.

Toxicokinetics

There is minimal absorption of anionic, nonionic, and amphoteric surfactants. Antidandruff shampoos may contain zinc pyridinethione and selenium

sulfide. The pharmacokinetics of zinc pyridinethione have been evaluated via multiple routes (percutaneous, oral, intravenous, intraperitoneal) and in several species (rats, rabbits, monkeys, dogs).. Selenium sulfide is poorly absorbed. Peak serum levels of lindane (an ingredient in shampoos used to treat lice infestation) occur ~6 h after a single dermal application. Lindane is highly lipid soluble and is stored in adipose tissue. Lindane is metabolized in the liver to chlorophenols.

Amphoteric, anionic, and nonionic surfactants are eliminated in the urine and feces. Selenium salts are excreted in the urine. Lindane has a half-life of ~18–21 h following dermal application.

Mechanism of Toxicity

The surfactants and other adjuvants in shampoo are primarily irritants, and most dermal, ocular, or gastrointestinal toxicity is a consequence of the irritant properties.

Acute and Short-Term Toxicity (or Exposure)

Animal

In general, nonlindane shampoos do not produce toxicity. Transient irritant effects are expected, especially in the event of ocular exposure. Exposure to lindane shampoos can produce vomiting, tremors, increased salivation, and seizures. Treatment is aimed at appropriate gastrointestinal decontamination and control of seizures.

Human

Nonionic and anionic surfactants and selenium and zinc pyrithione shampoos are irritants by nature. Nausea and vomiting can occur following ingestion in large volumes. Spontaneous emesis is common. Persistent vomiting has the potential to cause fluid and electrolyte imbalance. In general, gastrointestinal irritation is self-limiting.

Acute ingestion of lindane shampoo does have the potential to cause central nervous system excitation. Toxicity can occur when children ingest one teaspoon or more of 1% lindane shampoo. Ingestion of one tablespoon or more of lindane shampoo may result in significant toxicity. Symptoms of lindane toxicity include agitation, tremors, seizures, and respiratory depression.

Chronic Toxicity (or Exposure)

Human

Chronic dermal application of 1% lindane shampoo does have the potential to cause lindane toxicity, so it is not uncommon for products to contain precautionary labeling to avoid the reapplication of lindane products within at least a few months after use.

Clinical Management

Dilution is generally all that is required in exposures to nonlindane-containing shampoos. If spontaneous emesis does not occur, then it is unlikely that a large ingestion occurred, and mild to moderate, transient gastrointestinal distress is likely to be the only sequelae. If persistent vomiting occurs, then fluid and electrolytes should be monitored.

In toxic exposures to lindane shampoos, basic and advanced life-support measures should be utilized as needed. Emesis is not recommended in oral exposures to lindane. Gastric lavage utilizing saline cathartics is recommended; milk and fatty foods should not be administered in oral lindane exposures since this may enhance absorption.

See also: Lindane; Surfactants, Anionic and Nonionic.

Relevant Websites

http://www.ctfa.org – US Cosmetic, Toiletry, and Fragrance Association (CTFA).

http://hpd.nlm.nih.gov – US National Library of Medicine, 'Household Product Database' and 'ToxTown'. The Household Products Database links several thousand US consumer brands to health effects from Material Safety Data Sheets (MSDSs) provided by the manufacturers, and allows scientists and consumers to research products based on chemical ingredients.

Shellfish Poisoning, Paralytic

F Lee Cantrell

Background Information

Paralytic shellfish poisoning is a constellation of clinical effects caused by ingestion of contaminated shellfish found on the East and West coasts of the United States and Canada, the coasts around Japan, and coastal areas from southern Norway to Spain.

Implicated sources (shellfish) are mussels, clams, scallops, univalve mollusks, starfish, xanthid crabs, sand crabs, and turban shells.

Exposure Routes and Pathways

Ingestion of toxin-infected bivalve shellfish is the route of exposure. There is no reliable taste, smell, or color to detect contaminated shellfish. The toxin is not destroyed or inactivated by heating or cooking.

Toxicokinetics

The toxin is water soluble and absorbed through the oral mucosa and small intestine.

Mechanism of Toxicity

Neosaxitoxin, saxitoxin, and gongantoxin I–IV block transmission of impulses between nerve and muscle. They also block sodium channels in nerve and skeletal muscle, inhibiting the nerve and muscle action potential, thereby blocking nerve conduction and muscle contraction.

Acute and Short-Term Toxicity (or Exposure)

Animal

Shags, terns, and cormorants may develop inflammation of the gastrointestinal tract, hemorrhages in the base of the brain, and other hemorrhages.

Human

Common initial effects include numbness in the lips, tongue, and fingertips within a few minutes of ingestion. This numbness may spread to the extremities and then to the remainder of the body causing weakness and even muscle paralysis. Gastrointestinal symptoms are less common and consist of nausea and vomiting. Other symptoms include nystagmus, temporary blindness, irregular heartbeats, drops in blood pressure, headache, dizziness, difficulty in swallowing, and loss of gag reflex. Symptoms may persist for weeks.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Treatment is entirely symptomatic and supportive. Gastrointestinal decontamination with activated charcoal may be used depending upon the patient's clinical status, the history of the ingestion, and the time since the ingestion. Mechanical ventilation may be required for patients with decreased respiratory function.

See also: Red Tide; Saxitoxin.

Further Reading

Gessner BD and Middaugh JP (1995) Paralytic shellfish poisoning in Alaska: A 20-year retrospective analysis. *American Journal of Epidemiology* 141: 766–770.

Morbidity and Mortality Weekly Report (1991) Paralytic shellfish poisoning – Massachusetts and Alaska, 1990. MMWR 40: 157–161.

Shigella

Melanie J Karst

Name of the Organism

Shigella spp. (*Shigella sonnei*, *S. boydii*, *S. flexneri*, and *S. dysenteriae*).

Description

Shigella are Gram-negative, nonmotile, nonsporeforming, facultatively anaerobic, rod-shaped bacteria. *Shigella* are differentiated from the closely related *Escherichia coli* on the basis of pathogenicity, physiology (failure to ferment lactose or decarboxylate lysine), and serology. The genus is divided into four serogroups with multiple serotypes: A (*S. dysenteriae*, 12 serotypes); B (*S. flexneri*, 6 serotypes); C (*S. boydii*, 18 serotypes); and D (*S. sonnei*, 1 serotype).

Sources of Exposure/Transmission

Shigellosis, also known as bacillary dysentery, is caused by several bacteria of the genus *Shigella*. Contamination of foods is through the fecal-oral route. Fecally contaminated water and unsanitary handling of food are the most common causes of contamination. Foods that are frequently associated with shigellosis include salads (potato, tuna, shrimp, macaroni, and chicken), raw vegetables, milk and dairy products, and poultry. Any foods that require considerable handling during preparation are often involved in shigellosis.

Epidemiology

Shigellosis accounts for less than 10% of foodborne illness in the United States. Bacillary dysentery constitutes a significant proportion of acute intestinal disease in the children of developing countries. Shigellosis is endemic in developing countries where sanitation is poor. Typically 10–20% of enteric disease, and 50% of the bloody diarrhea or dysentery of young children, can be characterized as shigellosis. In developed countries, single-source, food, or waterborne outbreaks occur sporadically, while cases of endemic shigellosis can be found in some areas with substandard sanitary facilities.

Dose

An infective dose may be as few as 10 cells depending on the age and condition of the host. The time of onset of symptoms is somewhat influenced by the size of the challenge.

Mechanism of Toxicity

The disease is caused when virulent *Shigella* organisms attach to, and penetrate, epithelial cells in the intestinal mucosa. The cells then multiply intracellularly and spread to neighboring epithelial cells resulting in tissue destruction. Some strains produce enterotoxin and Shiga toxin. The hallmarks of shigellosis are bacterial invasion of the colonic epithelium and inflammatory colitis. These conditions are interdependent processes amplified by local release of cytokines and by the infiltration of inflammatory elements. Virulence involves both chromosomal- and plasmid-coded genes: including (1) siderophores that are iron-chelating compounds; (2) cytotoxins that cause cell necrosis; (3) Shiga toxins, a family of potent cytotoxins that inhibit protein synthesis and may play a role in progression of mucosal lesions; and (4) chromosomal genes that control lipopolysaccharide antigens in cell walls which may enhance cytotoxicity of Shiga toxins on endothelial cells.

Host Defenses

Inflammation, copious mucus secretion, and regeneration of the damaged colonic epithelium limit the spread of colitis and promote spontaneous recovery. Serotype-specific immunity is induced by a primary infection, suggesting a protective role as antibodies recognize the lipopolysaccharide somatic antigen. Other *Shigella* antigens include enterotoxins, cytotoxin, and plasmid-encoded proteins that induce bacterial invasion of the epithelium. The protective role of immune responses against these antigens is unclear.

Diagnosis of Human Infection/Illness

Colitis in the rectosigmoid mucosa, with concomitant malabsorption, results in the characteristic sign of bacillary dysentery: diarrhea tinged with blood and mucus. Shigellosis can be correctly diagnosed in most patients on the basis of fresh blood in the stool; however, watery, mucoid diarrhea may be the only symptom of many *Shigella* infections. This disease differs from profuse watery diarrhea, as is commonly seen in choleraic diarrhea or in enterotoxigenic

E. coli diarrhea, in that the dysenteric stool is scant and contains blood, mucus, and inflammatory cells. Any clinical diagnosis should be confirmed by serological identification of a culture isolated from stool. It is difficult to detect organisms in foods with current methods.

Nature of the Disease

The onset of symptoms of shigellosis is usually 12–50 h. The most common symptoms are abdominal pain, cramps, vomiting, and blood or mucus in stools. Infections may be associated with mucosal ulceration, rectal bleeding, and drastic dehydration. Death from Shigellosis may be as high as 10–15% with some strains. Sensitive populations such as the elderly, infants, and immunocompromised individuals are more susceptible to complications from the disease. The average duration of symptoms in untreated adults is 7 days, and the organism may be cultivated from stools for 30 days or longer.

Other complications may include; lethargy, delirium, seizure, encephalopathy, hemolytic-uremic syndrome, septicemia, Reiter syndrome, hepatitis, rectal prolapse, myocarditis, and toxic mega colon.

Clinical Management

Prevention of fecal–oral transmission is the most effective control strategy. Severe dysentery is treated with ampicillin, trimethoprim-sulfamethoxazole, or a 4-fluorquinolone such as ciprofloxacin.

Vaccines are not currently available. Dehydration is the most common complication of shigellosis. Supportive care with fluids and electrolyte replacement may be required.

See also: Food and Drug Administration, US; Food Safety and Toxicology; Gastrointestinal System.

Relevant Websites

http://vm.cfsan.fda.gov – US Food and Drug Administration. Center for Food Safety & Applied Nutrition. Foodborne Pathogenic. Microorganisms. and Natural Toxins Handbook. *Shigella* spp.

http://www.who.int – World Health Organization. Initiative for Vaccine Research (IVR). *Shigella*.

http://www.amm.co.uk – Association of Medical Microbiologists. The Facts about Shigella Infection and Bacillary Dysentery. What is dysentery?

Short-Term Exposure Limit *See* Occupational Exposure Limits.

Sick Building Syndrome

Michael Hodgson

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Sick building syndrome (SBS) is a term used to describe office worker discomfort and medical symptoms related to buildings and pollutant exposures, work organization, and personal risk factors. A wide range of definitions exists. Symptoms commonly considered integral parts of the syndrome are listed in **Table 1**. In recent years, with increased understanding, odors have generally been dropped from the list and chest symptoms have been included under mucous membrane irritation.

The problem may be viewed from the perspectives of (1) medicine and health sciences, to define symptoms related to work indoors and their associated pathophysiologic mechanisms; (2) engineering, based on design, commissioning, operations, and maintenance strategies and difficulties; and (3) exposure assessment, the formal measurement of specific pollutants.

Health and People

Since the mid-1970s, increasingly voiced office worker discomfort has been studied in formal ways

Table 1 Sick building syndrome

Mucous membrane irritation	Eye, nose, and throat itching and irritation
Central nervous system symptoms	Headaches, fatigue, difficulty concentrating, lethargy
Chest tightness and asthma-like symptoms (without true wheezing)	
Skin itching and irritation	
Odors	
Diarrhea	

including field epidemiologic studies using buildings or workstations as the sampling unit to identify risk factors and causes, population-based surveys to define prevalence, chamber studies of humans to define effects and mechanisms, and field intervention studies.

Cross-Sectional and Case–Control Studies

Approximately 30 cross-sectional surveys have been published and were reviewed by Mendell in 1993. Many of these have included primarily 'nonproblem' buildings, selected at random. These consistently demonstrate an association between mechanical ventilation and increasing levels of symptoms. Additional risk factors have been defined in several case–control studies. **Table 2** presents a grouping of widely recognized factors.

Many of these factors overlap. For some, pathophysiologic explanations exist. Women are considered more likely to voice discomfort at any given level of exposure and are exposed, on average, to higher levels of pollutants, such as volatile organic compounds (VOCs) and particulates, associated with symptoms.

Factor and principal components analyses of questionnaire responses in cross-sectional surveys have explored the interrelationship of various symptoms. Consistently, symptoms related to a single organ system have clustered together more strongly than symptoms relating different organ systems. That is, eye irritation, eye tearing, eye dryness, and eye itching all appear to correlate very strongly, and little benefit is obtained from looking at multiple symptoms.

Table 2 Risk factors for and causes of the sick building syndrome

Personal	Atopy (allergies, asthma, eczema) seborrheic dermatitis
	Work stress
	Gender
	Lower job status and pay
	Increased tear-film break-up time
Work activities	More time spent at photoduplication
	Carbonless copy paper
	More time at video display terminals
	Increasing amounts of time spent at workstation
Building factors	Mechanical ventilation
	Inadequate maintenance
	High-fleecing surfaces (high surface area surfaces such as carpets and drapes)
	Carpets
	Recent renovation
	Inadequate operations strategies

Data suggest that irritation among office workers does not represent a single distribution, but that susceptible subpopulations exist. Experimental studies, in chambers, show that atopic individuals usually have much lower irritation thresholds for agents commonly found indoors, even when their atopy is not active. In addition, the presence of tear-film instability, from Sicca complex, aging, or other underlying causes, poses an increased risk for irritation.

Controlled Exposure Studies

Animal testing to determine irritant properties and thresholds has become standard. A consensus method, American Society for Testing and Materials E, is widely regarded as the basis. This method has been used to develop structure–activity relationships, to demonstrate that more than one irritant receptor may exist in the trigeminal nerve, and to explore the interaction of multiple exposures. Recently, it has been used to demonstrate the irritating properties of office equipment offgassing.

In keeping with this method, several approaches have been developed to document methods and dose–response relationships for irritation in humans. This work suggests that, at least for 'nonreactive' compounds such esters, aldehydes, ketones, alcohols, carboxylic acids, aromatic hydrocarbons, and pyridine, the percentage of vapor pressure saturation of a compound is a reasonable predictor of its irritant potency. Specific physical properties of molecules predict overall irritation potential. This work is based on the identification of irritant thresholds for homologous series of specific agents. Quantitative structure–activity relationships derived from such work suggests a reasonable model to explain mucosal irritation.

Controlled exposure studies of volunteers in stainless steel chambers have been performed. Most have involved exposure to one specific mixture of VOCs; for example, the work of Molhave and Nielsen in 1992. These studies consistently document relationships between symptoms and increasing exposure levels. Office workers who perceived themselves as 'susceptible' to the effects of usual levels of VOCs indoors demonstrated some impairment on standard tests of neuropsychological performance. Healthy volunteers, on the other hand, demonstrated mucous membrane irritation and headaches at exposures in the range of 10–25 $\mathrm{mg\,m^{-3}}$ but no changes on neuropsychological performance. Recently, office workers demonstrated similar symptoms after simulated work in environments where pollutants were

generated from commonly used office equipment. Animals, using a standardized test of irritant potency, reacted similarly.

Population-Based Studies

At least three population-based studies have been published in Sweden, Germany, and the United States. The questionnaires differed considerably, and the studies do not allow prevalence estimate comparisons. Nevertheless, between 20% and 35% of respondents were thought to have complaints.

Mechanisms

A number of potential mechanisms and objective measures to explain and examine symptoms within specific organ systems have been identified. None of these have a high predictive value for the presence of disease and are not suitable for clinical diagnostic use. They are useful in field and laboratory investigations. These mechanisms and measures were reviewed by Doty and co-workers in 2004.

Both allergic and irritant mechanisms have been proposed as explanations for eye symptoms. More rapid tear-film break-up time, a measure of tear film instability, is associated with increased levels of symptoms. 'Fat-foam thickness' measurement and photography for documentation of ocular erythema have also been used. Some authors attribute eye symptoms at least in part to increased individual susceptibility based on those factors. In addition, office workers with ocular symptoms have been demonstrated to blink less frequently when working at video display terminals. Conjunctival staining with fluorescent dyes is a common clinical test for conjunctivitis sicca.

Nose

Both allergic and irritant mechanisms have been proposed as explanations for nasal symptoms. Measures that have successfully been used include nasal swabs (eosinophils), nasal lavage or biopsy, acoustic rhinometry (nasal volume), anterior and posterior rhinomanometry (plethysmography), and measures of nasal hyperreactivity (visual, using a dental prosthesis as a head fixative, and using an ear surgery microscope to measure distances and swelling).

Central Nervous System

Neuropsychological tests have been used to document decreased performance on standardized tests both as a function of controlled exposure and as a function of symptom presence.

Engineering and Sources

Beginning in the late 1970s, the National Institute for Occupational Safety and Health responded to requests for help in identifying causes of occupant discomfort in buildings. Although no standard investigative protocol was used, the primary cause of problems was attributed to ventilation systems ~50%), microbiological contamination (3–5%), strong indoor pollution sources (tobacco, 3%; others, 14%), pollutants entrained from the outside (15%), and the remainder unknown. On the other hand, Woods and Robertson published two well-known series of engineering analyses of problem buildings, documenting on average three problems that could be the source (**Table 3**).

The current professional ventilation standard (ASHRAE 62-89) suggests two approaches to ventilation: a ventilation rate procedure and an air quality procedure. The former provides a tabular approach to ventilation requirements: office buildings require $20\,ft^3$ of outside air per occupant per minute to maintain occupant complaint rates of environmental discomfort at below 20%. This assumes relatively weak pollution sources. When stronger sources are present, the same rate will provide less satisfaction. For example, when smoking is permitted at usual rates (according to data from the early 1980s), ~30% of occupants will complain of environmental discomfort. The second approach requires the selection of a target concentration in air (e.g., particulates, VOCs, and formaldehyde), information on emission rates (pollutant per time per mass or surface), and

Table 3 Defined engineering problems in series of problem buildings

Problem category	*Physical cause*	*Frequency*	
		Woods	*Robertson*
System design	Inadequate outdoor air	75	64
	Inadequate distribution	75	46
Equipment	Inadequate filtration	65	57
	Inadequate drain lines and pans	60	63
	Contaminated ducts and liners	45	38
	Humidifier malfunction	20	16
Operations	Inappropriate control strategies	90	–
	Inadequate maintenance	75	–
	Thermal and contaminant load charges	60	–

derives the ventilation requirements. Although this is an intellectually much more satisfying procedure, it remains elusive because of inadequate emissions data and disagreement on target concentrations.

In the past, odors were included under the etiologic list of SBS. A recent publication by Boswell and co-workers provide at least an overview of common odor sources. The single largest source was plumbing, such as dried-up traps (16%), followed by maintenance supplies (14%), renovations (11%), and ventilation (8%).

Pollutants

Environmental scientists have generally defined exposure and health effects on a pollutant-by-pollutant basis. In indoor environments these include multiple air pollutants (i.e., 20–50 different VOCs, including formaldehyde and other aldehydes), microbial products (including spores, cell fragments, viable organisms, and secretion products), and reactive agents such as ozone, fibers, and others. The American Thoracic Society defined six important categories listed in **Table 4**.

Environmental criteria have been established for many of these, but the utility and applicability of such criteria for indoor environments is controversial for at least four reasons. For example, the goals of the threshold limit values often do not include preventing irritation, a primary concern in indoor environments with requirements for close eye work at video display terminals. For most of the pollutant categories, the problem of interactions, commonly termed the 'multiple contaminants problem', remains inadequately defined. Even for agents that are thought to affect the same receptor, such as aldehydes, alcohols, and ketones, no prediction models are well established. Finally, the definition of 'representative compounds' for measurement is unclear. That is, pollutants must be measurable, but complex mixtures vary in their composition. It is unclear whether the chronic residual odor annoyance from environmental tobacco smoke correlates better with nicotine, particulates, carbon monoxide, or other pollutants. The measure 'total volatile organic compounds' is meanwhile considered an interesting concept but is not considered for practical purposes because the various components have such radically different effects. Particulates found indoors may differ in composition from those found outdoors because filter sizes affect entrained concentrations and indoor sources may differ from outdoor sources.

Finally, emerging data suggest that reactive indoor pollutants may interact with other pollutants and lead to new compounds. For example, Wechsler has shown that ozone, either from office machines or entrained from outdoors, may interact with 4-phenylcyclohexene and generate adlehydes.

Table 4 Principal pollutant categories (American Thoracic Society)

Bioaerosols
Combustion
Environmental tobacco smoke
Radon
Volatile organic compounds
Fibers

Primary Etiologic Theories

Volatile Organic Compounds

Buildings have always relied on general dilution strategies for pollutant removal, but designers have assumed that humans were the primary source of pollutants. Emissions from 'solid materials' (e.g., particle board desks, carpeting, and other furniture), from wet products (e.g., glues, wall paints, and office machine toners), and personal products (perfumes) have been recognized as contributors to a complex mixture of very low levels of individual pollutants as described by Hodgson and co-workers.

Several studies suggest that the presence of reactive VOCs, such as aldehydes and halogenated hydrocarbons, is associated with increasing levels of symptoms. Offices with higher complaint rates showed greater 'loss' of VOCs between incoming and outgoing air than did offices with lower complaints. In a prospective study of schools, short-chain VOCs were associated with symptom development. In another survey, higher personal samples for VOCs using a screening sampler that 'overreacts' to reactive VOCs such as aldehydes and halogenated hydrocarbons were associated with higher symptom levels. In that study, women had higher levels of VOCs in their breathing zone, suggesting another potential explanation for the increased rate of complaints among women. VOCs might adsorb onto sinks, such as fleecy surfaces, and be reemitted from secondary sources. The interaction of ozone and relatively nonirritant VOCs to form aldehydes is also consistent with this hypothesis. Although many individual agents are usually present, they are present only at concentrations well below those needed to cause irritation.

Modeling the irritant effects of such complex mixtures, such as that reported by Alarie and co-workers, has led to the development of a quantitative structure–activity relationship for irritation. Under

normal conditions, with usual sources and standard ventilation rates, concentrations should not reach irritant levels. On the other hand, in the presence of unusual sources (renovation, unusual office conditions) or inadequate ventilation, and especially in the setting of reactive species coexposure, concentrations are very likely to reach irritating levels.

Bioaerosols

Bioaerosols grow in moisture films on surfaces. Several studies have suggested that bioaerosols may contribute to occupant discomfort through several different mechanisms: irritant emissions; release of fragments, spores, or viable organisms leading to allergy; and secretion of complex toxins. Several studies suggest a relationship between symptoms indoors and airborne endotoxin levels. The endotoxin concentrations on cooling coils themselves are better predictors of irritation than airborne bacteria or endotoxin measurements. This suggests that some bacterial component, possibly endotoxin, perhaps in combination with other bioaerosols, plays an important role in generating symptoms and that moisture and growth on the coils rather than in the air or ductwork plays a role in generating symptoms. Clearly, heating, ventilating, and air-conditioning systems may be sources for microorganisms. Fundamentally, the presence of moisture always raises the suspicion of potential cause. Bulk moisture incursion, through roof defects, wall penetration, below-grade seepage, or internal leaks, may generate problems. The presence of moisture barriers in perimeter walls also presents an opportunity for condensation. Especially in the setting of negative pressure in the building, pulling moisture towards such barriers (such as vinyl wall paper), bioaerosols may grow and cause problems. They have also been noted in building construction materials (as a result of improper curing) and in office dust. The presence of sensitizers in the office environment, such as dust mites or cat danders brought in from home on clothing, presents another interesting exposure though not likely one of substantial importance.

Psychosocial Aspects of Work

In all studies in which it has been examined, 'worker stress' was clearly associated with SBS symptoms. Workers' reactions to job pressures, such as task conflicts, and outside pressures, such as spousal or parental demands, may lead to the subjective experience of 'stronger' irritation. At times, such experiences may result at least in part from poor supervisory practices. In addition, the persistence of irritants leading to subjective irritation may reinforce work stress.

Conclusion

The SBS is a phenomenon experienced by individuals, usually seen in groups, associated with engineering deficiencies, and likely caused by a combination of pollutants representing a variety of pollutant categories. As with all 'disease', a component of personal psychology serves as an effect modifier contributing to varying degrees of symptom intensity at any given level of distress.

See also: Behavioral Toxicology; Dose–Response Relationship; Exposure Assessment; Mixtures, Toxicology and Risk Assessment; Multiple Chemical Sensitivities; Neurotoxicity; Pollution, Air Indoor; Psychological Indices of Toxicity; Respiratory Tract; Sensory Organs.

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Silent Spring

Michael A Kamrin

Introduction

In the period following World War II, there was a great increase in industrial production in the developed world. Not only were existing products manufactured in larger numbers, but also a large number of new synthetic chemicals were developed. These included innovative structural materials, such as plastics, new and more effective pharmaceuticals and more effective pest control chemicals. Many of these new compounds rapidly gained widespread use in the developing as well as the developed world.

This increase in industrial production was soon accompanied by concerns about the environmental consequences of the effluents from production facilities as well as the new chemicals themselves. Graphic pictures alerted the public to the problems of decreasing air quality, and increasing amounts of waste effluents reaching surface waters. These included images of thick smoke bellowing from factories and fires in rivers, such as the Cuyahoga. The problem was international in scope as was illustrated by the discovery of serious human health impacts around Minamata Bay, Japan due to ingestion of fish contaminated with methylmercury formed from effluents emitted by an industrial facility on the bay.

Complementing this boom in industrial production was an great increase in the use of agricultural chemicals, both fertilizers and pesticides. In addition to an increase in the amounts applied, the types of pesticide chemicals used changed to those that were long lasting, such as the organochlorines. The most prominent example of this class of pesticides is DDT.

There were two main types of concerns about these persistent pesticides – one was their impact on birds and wildlife and the other their impact on human health. The former was illustrated by the reports in the late 1950s that DDT usage was linked to the decimation of the robin population on the Michigan State University campus. The latter concern was illustrated by the great cranberry scare that occurred right before Thanksgiving in 1958 when it was thought that pesticide contamination of cranberries would lead to cancer in people who ate them. It was also the year that the Congress passed the so-called Delaney Amendment that forbid the use of any food additive that might be linked to cancer in laboratory or epidemiological studies.

Silent Spring

It is against this background that *Silent Spring*, written by Rachel Carson, was published in 1962. The book was a polemic that focused on the impacts or potential impacts of pesticides on both humans and their environment. It was a call for people to be much more aware of the seriousness of the problems caused by these synthetic chemicals and to take actions to minimize and/or eliminate their use. The author recommended that chemical control of pests be replaced by biological controls and that persistent chemicals, such as DDT, be taken off the market.

The impact of this book was enormous as it seemed to coalesce the diverse and growing concerns of the public about damage to the environment and public health by industry. It contributed strongly to the rise and expansion of the environmental movement in the mid- to late 1960s and to the establishment of a number of environmental protection laws and policies in the United States and elsewhere in the 1970s. A very important result of this environmental movement was the creation of the US Environmental Protection Agency. In addition to catalyzing organizational change, it also led to specific actions that were called for in *Silent Spring*, particularly the banning of DDT use in the United States – which occurred in 1972.

While the focus of *Silent Spring* was pesticides, the environmental movement that grew out of it was

much broader and had the goal of limiting the use and disposal of a wide variety of industrial chemicals. Signal events, such as Love Canal, led to efforts in particular directions other than limiting pesticide use. In the case of Love Canal, this direction was the clean-up of hazardous wastes from the past. However, efforts to force a reduction in pesticide usage also continued unabated.

These efforts have led to increased reliance on a combination of methods for pest control including both chemical and biological controls, a technique known as integrated pest management (IPM). The increasing use of IPM has led to a decreasing use of pesticides. The continuing public concern about pesticides and other chemicals used in food production has been the impetus for a growing organic food movement. At least in the United States, a significant number of people are willing to pay a premium for foods that are certified as having been grown without the use of pesticides or other commercial chemicals.

Indirectly, this movement has also led to the development of agricultural biotechnology, a field that focuses on altering crop plants to reduce the need for pesticide applications. This includes research to develop plants that produce their own natural pesticide as well as crop plants that are resistant to synthetic pesticides. Plantings of bioengineered crops have rapidly increased in recent years and a majority of some crops grown in the United States are products of this technology.

Summary

The publication of *Silent Spring* was a seminal event in the environmental movement in the United States and, later, abroad. Prior to the book there was slowly increasing public recognition of environmental problems due to industrial effluents and use of certain synthetic chemicals. Afterwards, the environment became an overriding issue to many Americans and an environmental movement arose that is still going strong. While there are still questions about the wisdom of some of the recommendations that were made in *Silent Spring*, there is no question that the book has led to a different way of looking at our environment and the effects of some aspects of human progress on this environment.

From the toxicological perspective, it is clear that much of the research that has been performed in the past four decades has resulted from concerns that were raised in Silent Spring. These include studies of the adverse effects on humans and other organisms of pesticides and other chemicals in our environment as well as basic research on mechanisms of toxicity. It is evident that this type of research will continue as questions still remain about well known as well as newly discovered chemicals in our environment.

See also: DDT (Dichlorodiphenyltrichloroethane); Pesticides.

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Silica, Crystalline

Kent E Pinkerton and Randal J Southard

- Representative Chemicals: Quartz; Cristobalite; Stishovite; Tridymite; Coesite
- Synonym: Silicon dioxide
- Chemical Formula: SiO_2 – Crystalline silica, also called 'free silica', is defined as silicon dioxide (SiO_2). This chemical formula represents a very stable form of silicon, wherein the Si is completely polymerized through Si–O bonds in three dimensions
- Chemical Structure: Crystalline silica represents a form of silica, which is in a highly organized, framework pattern. The term 'crystalline' refers to the orientation of SiO_2 molecules in a fixed pattern as opposed to random molecular arrangement defined as amorphous, noncrystalline, or short-range order. The oxygen and silicon atoms of silicon dioxide are arranged in a three-dimensional pattern repeated indefinitely in three directions, forming the crystalline structure

Uses/Occurrence in Nature

The most common hazard for exposure to crystalline silica occurs with sandblasters who use sand for cleaning of surfaces, thus generating dust clouds of freshly fractured crystalline silica. Other occupations include farm labor where mineral dusts are generated

in field preparation and processing of crops, particularly in arid to semiarid regions where irrigation is required for crop production. Although silica is only one constituent of mineral dust, it can represent up to 15% of the respirable dust present in agricultural settings because quartz is so abundant in most soils. The proportion of quartz in respirable dust will thus be related to soil mineralogy and the relative abundances of the sand, silt, and clay fractions in the soil. Other compounds in which crystalline silica may be found include gravel, slate, diatomaceous earth, concrete, mortar, plaster, refractory materials, pottery clay, limestone, shale, bricks, and abrasives. The three most common crystalline forms of silica encountered are cristobalite, tridymite, and quartz.

Quartz is the most common crystalline form of silica encountered in nature. Quartz is present as alpha and beta (high temperature) forms. Alpha quartz is the most common form, and is found in large quantities in rocks and soils worldwide. That quartz is among the most abundant minerals in many, if not most, soils is a reflection of its chemical stability and resistance to weathering. In fact, quartz is so prevalent that the term 'quartz' is often used in place of crystalline silica. Coesite and stishtovite are formed at high pressure (e.g., meteorite impact craters), whereas tridymite and cristobalite form at high temperature (e.g., volcanic rocks). Other than alpha quartz, all of these forms are metastable at earth surface temperatures and pressures and will slowly convert to alpha quartz given enough time. Microcrystalline varieties of silica also include small grains of this material, possibly combined with amorphous silica. Tripoli, flint, chalcedony, agate, onyx, and silica flour are examples.

Background Information

Silicosis is the oldest known occupational lung disease. Ancient Greeks were familiar with lung disease in quarry workers (Hippocrates) and the fact that respirators could prevent the disease (Pliny). Agricola (1566) described disease in stone cutters as later did Ramazini (1713). By 1917, the US Public Health Service identified sand blasters and foundry workers to be at high risk of silicosis. As the twentieth century progressed, silicosis was the reference to which newer diseases were compared.

Exposure Routes and Pathways

Exposure routes are primarily by inhalation. The greatest hazards for exposure to crystalline silica are typically found in the workplace. When crystalline silica becomes small enough (i.e., $<10\,\mu m$ in diameter), these materials can become aerosolized and are able to enter the respiratory tract where they can deposit along the tracheobronchial tree or into the deep recesses of the lung where gas exchange takes place (i.e., alveoli). Bulk crystalline silica is defined by the size of the individual particles. In soils terminology, for example, 'sand' is composed of grains 50–2000 μm in diameter, 'silt' is in the 2–50 μm range, while 'clay'-size particles are less than 2 μm in diameter. Crystalline silica is considered respirable or inhalable when particles are less than 10 μm in diameter (i.e., in the silt and clay sizes).

Mechanism of Toxicity

Although a number of theories exist to explain the potential mechanism of toxicity to crystalline silica, the primary cause is described as membrane damage occurring to cells that ingest these tiny particles. It is thought that once crystalline silica is ingested into a cell, free radical oxygen species can be generated from the surface of the particle leading to lipid peroxidation and membrane damage followed by release of lysosomal contents and lysis of the cell, resulting in cell death. This process creates a vicious cycle where crystalline particles are taken up again into other cells that will undergo the same sequence of events. Although the precise events to drive this cell injury process are unclear, it has been observed that freshly fractured surfaces of crystalline silica more readily generate free radicals to damage the membrane of cells taking up these particles, thus producing greater and more rapid cell injury. This may explain, in part, the hazardous condition created in sandblasting where silica particles may be further fractured in the cleaning process. The acute toxicity of exposure to crystalline silica for both animals and humans follows a similar mechanism with rapid ingestion of particles into cells, primarily alveolar macrophages, and subsequent damage to lipid membranes and the lytic death of these cells. This repetitive process results in inflammatory events leading to the influx of numerous macrophages into the alveolar air spaces.

Pulmonary silicosis occurs by way of breathing these particles over short to long periods of time. The underlying mechanism for this disease involves the ingestion of silica particles by macrophages in an attempt to remove them from the lungs. However, silica particles produce membrane damage and death to these cells. The repetitive process of uptake and release from macrophages leads to the further release of hydrolytic enzymes and mediators that stimulate the influx of inflammatory cells and laying down of collagen by fibroblasts in an abnormal pattern to

produce diffuse interstitial lung disease or pulmonary fibrosis. Fibrotic changes in the lungs are a reflection of a prolonged injury process to macrophages with the accompanying deposition of collagen by fibroblasts to bring about scar tissue formation. This scarring process leads to the loss of alveolar airspace with excessive amounts of collagen fibers forming wherever quartz particles have been deposited and/or translocated in the lungs. The pattern of scarring associated with silicosis is typically found to be more prevalent in the upper lobes of the lung in a nodular pattern, leading to the complete obliteration of alveolar air spaces in affected sites.

Acute and Short-Term Toxicity (or Exposure)

In both animal and human studies, the toxicity of crystalline silica is manifested acutely as an inflammatory process with the influx of a large number of macrophages into the alveolar airspaces of the lungs.

Chronic Toxicity (or Exposure)

Chronic toxicity of crystalline silica in both animals and humans results in a patchy nodular disease known as pulmonary silicosis. Both animal and human studies demonstrate the persistence of lung inflammation associated with excess collagen deposition to form nodular as well as diffuse fibrotic lesions throughout the lungs. The disease process of silicosis is incurable and nonreversible. the disease progress, breathing becomes labored and more difficult, and can result in death in extreme cases. Symptoms of silicosis include cough, shortness of breath, wheezing, and repeated chest illnesses. The diagnosis of a chronic disease due to silicosis is determined through pulmonary function tests, chest X-rays, and history of occupational exposure to silica.

In addition to the disease process of silicosis, inhalation of crystalline silica has been associated with other diseases such as bronchitis and tuberculosis. There is also some indication of an association with lung cancer.

In Vitro Toxicity Data

Crystalline silica is toxic to cells *in vitro*, and it is commonly used as a positive control material in cytotoxicity testing in cell culture systems.

Exposure Standards and Guidelines

The US Occupational Safety and Health Administration mineral dust standards for occupational exposure to crystalline silica depend on the actual composition of the sample.

As outlined in the 1974 Center for Disease Control/National Institute for Occupational Safety and Health publication, *Occupational Exposure to Crystalline Silica*, employees who are exposed to free silica must be apprised at the beginning of their employment of the hazards, relevant symptoms, appropriate emergency procedures, proper conditions and precautions for safe use or exposure. The following warning is required to be posted in both English and the predominant language of non-English-speaking workers potentially exposed to free silica dust:

> WARNING!
> CONTAINS FREE SILICA
> DO NOT BREATHE DUST
> May Cause Delayed Lung Injury (Silicosis)

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Silver

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst and Shirley B Radding, volume 3, p. 144, © 1998, Elsevier Inc.

- Representative Chemicals: Silver chloride (AgCl); Silver nitrate ($AgNO_3$); Silver cyanide (AgCN)
- Chemical Abstracts Service Registry Number: CAS 7440-22-4
- Synonym: Plata
- Chemical/Pharmaceutical/Other Class: Precious metals
- Chemical Formula: Ag^+

Uses

Silver is used extensively in jewelry, eating utensils, coins, batteries, and dental amalgams. Silver solutions are used as antiseptics, astringents, and germicides. In some domestic water purifiers, silver is used to remove chlorine and kill bacteria. It has also been used in hair dyes. Medicinal use includes silver nitrate eye drops for newborns (a legal requirement in some states) and use as an antimicrobial on some implantable medical devices. The main industrial use of silver is in the form of silver halide for the photographic industry. Silver halide is photosensitive, making it an ideal coating for photographic plates.

Background Information

Silver is one of the earliest known metals. Silver has no known physiologic of biologic function, though colloidal silver is widely sold in health food stores.

Exposure Routes and Pathways

Ingestion and inhalation are possible routes of exposure; dermal absorption of silver is unlikely. Silver is not a normal constituent of foodstuff. Very little, if any, silver is detected in domestic drinking water; however, some domestic water-purifying systems contain silver.

Toxicokinetics

Approximately 10% of ingested silver is absorbed. Inhaled silver can be absorbed from the lungs. Silver can be absorbed across oral mucosa. Once absorbed, silver tends to precipitate in various tissues, as the affinity for sulfide by silver is immense. Silver tends to complex with sulfhydryl groups on macromolecules. It is carried by globulins in the serum and forms complexes with the serum proteins, mainly albumin, which accumulate in the liver. Silver is excreted in the feces (primarily) and urine.

Mechanism of Toxicity

While specific mechanisms of toxicity are unclear, high affinity for sulfhydryl groups on proteins could lead to alteration of a number of cellular processes.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of metallic silver and water-soluble compounds is moderate. The oral LD_{50} in mice for colloidal silver was $100\,mg\,kg^{-1}$ and relatively similar for the water-soluble compounds silver nitrate ($50–129\,mg\,kg^{-1}$) and silver cyanide (LD_{50} in rats, $125\,mg\,kg^{-1}$). Silver nitrate appears much less toxic in rabbits by the oral route ($800\,mg\,kg^{-1}$). The insoluble silver oxide was reported to exhibit an LD_{Lo} of $>2\,g\,kg^{-1}$ in rats.

Human

Acute oral exposure to silver nitrate has led to irritation and corrosion in the gastrointestinal tract, abdominal pain, diarrhea, vomiting, shock, convulsions, and death in humans. Silver or silver nitrate can lead to respiratory irritation with inhalation exposures. Silver nitrate is highly irritating to the skin, mucous membranes, and eyes. Insoluble silver compounds (e.g., silver chloride, silver iodide, and silver oxide) are relatively benign.

Chronic Toxicity (or Exposure)

Animal

With selenium or Vitamin E deficient diets, repeated exposure to silver (76 ppm in the drinking water for 52 days) in rats elicited hepatic necrosis and ultrastructural changes in the liver indicative of oxidative damage. This toxicity may be related to a silver-induced selenium deficiency and impairment of synthesis of the enzyme glutathione peroxidase. Dietary supplementation with selenium or Vitamin E prevented such changes. Mice exposed to silver nitrate in the drinking water for 4 months exhibited

silver-containing deposits in the central nervous system and reduced motor activity.

Human

Workers chronically exposed to silver have experienced industrial argyria, an occupational disease characterized by discoloring of the skin. Blue–gray patches are noted on the skin and possibly the conjunctiva of the eye or the mucous membranes. Long-term exposure can result in extensive skin discoloration, mainly on the parts of the body that are exposed to light (e.g., the face). Light may decompose the silver complex, resulting in extremely fine silver that gives the skin a metallic sheen. In some cases, the dark patches turn black. Chronic bronchitis has been reported following medicinal use of colloidal silver. Potential symptoms of overexposure are blue–gray eyes, nasal septum, throat, and skin. The discoloration can be permanent.

Clinical Management

Clinical management is supportive and there no known active treatment. Administration of table salt will help precipitate soluble silver as the insoluble silver chloride. British antilewisite (2,3-dimercaptopropanol) has not proven useful.

Environmental Fate

Silver exists in four oxidation states (0, 1+, 2+, and 3+). Silver occurs primarily as sulfides with iron, lead, tellurides, and with gold. Silver is found in surface waters as sulfide, bicarbonate, or sulfate salts, as part of complex ions with chlorides and sulfates and adsorbed onto particulate matter. Silver is released through natural processes, for example, erosion of soils. Sources of atmospheric contamination arise from processing of ores, steel refining, cement manufacture, fossil fuel combustion, and municipal waste incineration. Of anthropomorphic release, over 75% was estimated to be from disposal of solid waste. Ore smelting and fossil fuel combustion can emit fine particulates that may be transported long distances and deposited with precipitation. The major source of release to surface waters is effluent from photographic processing. Releases from the photographic industry and from disposal of sewage sludge and refuse are the major sources of soil contamination with silver. Silver can leach into groundwater; acidic conditions increases leaching. Silver can be bioconcentrated in fish and invertebrates.

Ecotoxicology

The no-observed-effect concentration of silver nitrate in a 28 day toxicity test using a marine invertebrate (*Americamysis bahia*) was $34\,\mu g\,l^{-1}$. The 96 h LC_{50} value was $260\,\mu g\,l^{-1}$. In a 21 day toxicity study using the freshwater invertebrate *Daphnia magna*, $5\,\mu g\,Ag\,l^{-1}$ (as silver nitrate) under static conditions, 20% mortality was noted (0% in controls). Silver caused a significant reduction of reproductive performance (14% decrease in the number of neonates). Silver caused a 65% decrease in whole body sodium concentration and a 60% increase in whole body Na^+,K^+-ATPase activity.

Silver nitrate ($10\,\mu g\,l^{-1}$) elicited a 35% reduction in whole body sodium and increases in daily mortality in developing rainbow trout. Exposure to $0.1\,\mu g\,l^{-1}$ silver led to reduction in growth.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (TLV) time-weighted average is $0.1\,mg\,m^{-3}$ for silver metal; the TLV is $0.01\,mg\,m^{-3}$ for soluble silver compounds.

See also: Metals.

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Sister Chromatid Exchanges

David A Eastmond

Sister chromatid exchanges (SCEs) are reciprocal exchanges of segments of chromatids: chromatids are the subunits of chromosomes, as visualized in metaphase, which become daughter chromosomes upon completion of cell division. Sister chromatid exchanges were discovered by J.H. Taylor in the late 1950s in experiments using the pulsed uptake of ^{3}H-labeled thymidine (TdR) in growing *Vicia faba* root tips, followed by autoradiography, to define the pattern of DNA replication in chromosomes.

Before Taylor's experiments, it was thought that one chromatid might be composed of newly synthesized DNA and the other of preexisting DNA. However, Taylor found that the DNA replicated semiconservatively, that is, in the first metaphase after [^{3}H]TdR incorporation (M_1) each chromatid was ^{3}H-labeled, which demonstrated that the chromatid was duplex, containing both preexisting and newly synthesized DNA. Furthermore, in the second division after ^{3}H incorporation (M_2) one chromatid was labeled and the other was unlabeled, that is, both the preexisting and the newly synthesized DNA in each daughter chromosome had served as a template for the next round of DNA replication, again resulting in two sister chromatids. Taylor also noted that occasionally in M_2 one otherwise labeled chromatid had an unlabeled segment and, when this occurred, the corresponding segment of the otherwise unlabeled chromatid was labeled with [^{3}H]TdR, indicating a reciprocal exchange of segments between the two sister chromatids, that is, a sister chromatid exchange.

The development of SCE assays for genetic toxicology research and testing did not occur until the early 1970s, a time when a plethora of approaches were identified for assessing the potential genetic hazards of chemical exposure. Rather than using [^{3}H]TdR and autoradiography to visualize SCEs, the defined approaches are usually based on the more precise and efficient incorporation of bromodeoxyuridine (BrdU), an analog of thymidine, in two rounds of replication followed by Giemsa, or fluorescence-plus-Giemsa, staining of the chromosomes. Because of semiconservative DNA replication, the chromatids are equally stained in M_1 chromosomes, and the M_2 chromosomes possess one chromatid that is half-BrdU-substituted and one fully substituted chromatid that is stained more lightly than the other. SCEs are revealed in M_2 chromosomes by an alternating or 'harlequin' pattern of darkly and lightly staining chromatid segments.

This approach has also been used to reveal chemical- and concentration-related delays in the progression of cells through the cell cycle as a preliminary test for selecting exposure conditions for chromosomal aberration assays. The objective of such preliminary tests is to define exposure conditions and harvest times that will yield sufficient numbers of first division, M_1, cells for cytogenetic analysis. This is beneficial because a high percentage of chromosomally damaged cells are often unable to progress to the second and following metaphases.

In vitro SCE assays are routinely conducted in cultured Chinese hamster ovary (CHO) cells or human lymphocytes, and assessments of SCEs in human lymphocytes have been used for human population monitoring. Following *in vivo* exposure, SCEs are usually visualized in bone marrow cells from mice implanted with BrdU-containing tablets (or pumps). Such SCE assays have been used to test several hundred chemicals and have been shown to be highly sensitive and, in comparison to conventional assays for chromosomal aberrations, to be more rapid, less subjective, and capable of detecting effects at lower dose levels.

SCE assays would, therefore, appear to be uniquely suited for inclusion in initial batteries of tests to assess genotoxicity. However, while this was initially perceived to be the case, the use of SCE assays for genotoxicity testing has been greatly reduced for several reasons. First, it was found that the use of BrdU (or [^{3}H]TdR) can induce SCEs; thus, there was concern that when SCE frequencies were elevated following chemical exposure, synergistic effects were being measured, which might not be as appropriate for risk assessment as the measurement of direct effects. Second, although there is strong evidence that SCEs result from misreplication of a damaged DNA template, probably from recombination at a stalled replication fork, there was uncertainty concerning whether to classify SCE assays as cytogenetic tests, as a measure of the repair of DNA damage, or as an independent category of test. Third, alarm was expressed when common chemicals such as NaCl (i.e., table salt) were found to be positive in *in vitro* SCE assays. It was subsequently shown that in *in vitro* assays, particularly in the presence of exogenous metabolic activation, such false-positive results could be eliminated if exposure conditions are monitored

and adjusted to preclude acidic pH shifts and high osmolality.

However, the most significant reason for an absence of regulatory requirements for the routine use of SCE tests and their discontinuation by industry was the outcome of a National Toxicology Program (NTP) comparison of the concordance of results from four *in vitro* tests with results from rodent carcinogenicity bioassays. Specifically, the NTP studies conducted by Tennant and colleagues found that, although few positive results were obtained for noncarcinogens in the *Salmonella typhimurium* reverse mutation assay (Ames test) or in the test for chromosomal aberrations in CHO cells, an unacceptably high number of false-positive results were obtained in the *in vitro* SCE assay.

Thus, SCE tests have been largely discontinued by industry and are recommended by regulatory agencies on a limited basis. However, this assay continues to be used as a research tool and in some regulatory settings. For example, despite its poor concordance, the SCE assay continues to be used by the NTP, in part because SCE techniques are sufficiently similar to those used for *in vitro* chromosomal aberration assays so that the two tests can efficiently be used in parallel by cytogenetic testing laboratories.

Similarly the measurement of SCEs for population monitoring has also diminished in recent years as a result of several prospective studies whose objective was to determine if cytogenetic assays had predictive value for future cancer risk. In these studies, no correlation was seen between SCE levels and future cancer risk whereas good correlations were seen between the frequency of chromosomal aberrations and the subsequent development of cancer in the study groups.

Recent evidence indicates that SCEs are primarily formed as the result of homologous recombination so that SCE frequencies represent a measure, not only of mutagen exposure but also of the efficiency of DNA repair. As a result, a direct correlation between SCEs and cancer incidence may not be expected. In spite of the recent developments, there continues to be uncertainty about the underlying mechanisms by which SCEs are formed, and how DNA damage or disturbances in DNA synthesis stimulate SCE formation. Based on our current understanding, SCEs are probably best regarded as a general indicator of mutagen exposure rather than as a specific measure of mutagenic effects.

See also: Ames Test; Analytical Toxicology; Aneuploidy; Carcinogen–DNA Adduct Formation and DNA Repair; Chromosome Aberrations; Developmental Toxicology; Dominant Lethal Tests; Host-Mediated Assay; Molecular Toxicology – Recombinant DNA Technology; Mouse Lymphoma Assay; Toxicity Testing, Mutagenicity.

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Skeletal System

M Joseph Fedoruk and Tee L Guidotti

This article describes the structure and function of the musculoskeletal system and provides an overview of the categories of toxic effects that can affect this body system. The article is divided into two principle parts, which form the main components of the musculoskeletal system: bone and skeletal muscle.

Bone

Bone, a form of connective tissue, composes the skeletal system. The skeletal system provides mechanical support for the body and protects internal organs such as the brain and heart, which are contained in

skull and the chest wall cavity, respectively. The human skeleton is composed of 206 bones that vary in size and shape and include flat, trabecular, and cuboid bones. The body size and shape are determined by the skeletal system.

Bone serves other functions. It is a dynamic tissue that plays a vital role in mineral homeostasis and is a reservoir for several essential minerals including calcium, phosphorus, magnesium, and sodium. Bone houses the delicate bone marrow that forms blood from hematopoietic cells. Bone is an extremely vascular tissue and receives up to 10% of the cardiac output.

Joints form the sites where bones come together or articulate. Joints are classified by the type of tissue that lies between the bones. Joints with fibrous tissue between the articulating surfaces are called fibrous joints and include the sutures of the skull. Cartilaginous joints are united by hyaline cartilage and are classified into primary and secondary cartilaginous joints. Primary cartilaginous joints do not allow any movement.

Bone is composed of live cells interspersed in an organic matrix. Inorganic elements or minerals (65%) are deposited into this organic matrix (35%), which makes bone one of the few tissues that normally mineralize. The principal inorganic element in bone is calcium hydroxyapatite ($Ca_{10}(PO_4)OH_2$), which accounts for $\sim$99% of the calcium and 80% of the stores of these respective minerals in the body. Calcium hydroxyapatite provides bone with strength and hardness. The organic matrix provides a degree of elasticity to bone.

The cellular elements of bone include osteoprogenitor cells, which are pluripotential cells derived from mesenchymal tissue. Osteoprogenitor cells produce offspring cells that can differentiate into osteoblasts. Osteoblasts are responsible for the formation of the organic matrix of the bone into which the mineral elements can be deposited. Groups of several hundred osteoblastic cells coordinate activities to facilitate the formation of the organic matrix. The organic matrix is principally composed of type I collagen (90%) and several other noncollagenous proteins, including (1) osteocalcin, which serves to translate mechanical stresses or signals into local bone activity; (2) osteonectin, a calcium-binding protein; (3) osteopontin, a protein that facilitates cell adhesion; (4) cytokines; and (5) growth factors, which help control cell proliferation, mineralization, and metabolism. Osteoblasts have several different types of receptors including those for hormones (e.g., parathyroid hormone and estrogen) as well as other receptors for cytokines and growth factors.

Osteoblastic activity initiates the process of mineralization. Unmineralized bone is known as osteoid. Minerals are deposited in specific holes that are located between collagen fibrils produced by the osteoblast. The architecture of the fibrils is designed to withstand external stress. Mineralization begins shortly after the formation of the secreted matrix. This process occurs in osteons, also referred to as Haversian systems, and is completed in several weeks. Blood vessels penetrate bond through channels known as Haversian canals.

Osteoblasts which have become encased in bone are called osteocytes. The bony covering is not complete and osteocytes maintain communication with other cells and the general circulation through a network of tunnels located in the bony matrix, which are called canaliculi. Osteocytes play several roles in body homeostasis, including maintaining normal levels of serum calcium and phosphorus.

Bone tissue also contains osteoclasts, which are multinucleated cells that are derived from the hematopoietic (granulocyte–monocyte) cell line located in bone marrow. Osteoclasts are primarily responsible for bone resorption and they secrete enzymes and hydrochloric acid that break down collagen matrix and help dissolve the bone. The area where osteoclast cell membrane lies adjacent to bony tissue is known as a Howship's lacunae. The osteoclast cell membrane that lies in close proximity to bone can contain numerous villous extensions and form a ruffled border. These areas are also known as resorption pits. The plasmalemma border of the osteoclast cell in this region forms a specialized seal with the underlying bone to prevent the release of enzymes and hydrochloric acid. This process also results in the release of growth factors previously deposited in bone by osteoblasts, which are responsible for maintaining the process of regenerating new bone.

Bone is developed by two methods. Membranous development involves bone formation directly from cartilaginous tissue. Osteoblasts directly deposit calcium and other mineral in this mesenchymal-derived tissue. The skull and portions of the clavicles (collar bone) are formed by this method and, at birth, portions of the membrane persist in the skull and are referred to as 'soft spots'.

The other method of bone formation involves endochondral ossification and is not completed until the 18th year or later. The long bones of the limbs are formed by endochondral ossification. Mesenchymal-derived cartilaginous tissue, which is formed during early fetal development, contains chondrocytes and provides a model for future bone. By the eighth week of gestation, this cartilaginous tissue undergoes a series of changes, which initiates the process of bone

formation. The center of this cartilage undergoes degradative changes that involve mineralization and later resorption by osteoclast-type cells. This process moves up and down the cartilaginous tissue and is accompanied by the ingrowth of blood vessels and osteoprogenitor cells, which will become new bone-forming cells. The remnants of the mineralized cartilage, also known as the primary spongiosa, serve as a framework for new bone deposition. Similar changes occur in the epiphyses of the bone. These changes produce an area of cartilage that lies between two centers of bone formation which is known as the growth plate.

The growth plate chondrocytes undergo proliferation, growth, degradation, mineralization, and resorption and provide the support structure for new bone formation. Bones can increase in length and width through this process. Endochondral ossification occurs near the base of the articular cartilage at the joints.

Bone undergoes continual remodeling through bone resorption and formation. The balance between formation and resorption determines the mass of bone during growth of the skeleton. In childhood, bone formation predominates. Peak bone mass is reached in early adulthood. During adulthood, ~10–15% of the skeletal mass undergoes remodeling and resorption yearly and this process remains balanced. The amount of resorbed bone starts to exceed the amount of newly deposited bone by the third or fourth decade.

The osteoblast and osteoclast can be considered to be the basic multicellular units of bone. The osteoblast plays an important role in mediating local osteoclast activity through the release of chemical messengers. The principal factors responsible for stimulation of bone resorption, such as parathyroid hormone, interleukin-1 (Il-1), and IL-6, have minimal effects on osteoclasts, but osteoblasts have receptors for these substances.

Increased resorption of bone relative to new bone formation leads to osteoporosis. Osteoporosis is characterized by a net reduction in the mass of bone with no significant decrease in the ratio of mineral components to organic matrix. The bone can be thought of as having increased porosity. Osteoporosis starts to occur in both sexes at the age of 46–50 years. Trabecular bone loss probably occurs earlier. On an average 0.7% of bone is lost on an annual basis. Bone loss accompanies aging for several reasons. Aging is associated with decreased activity of osteoprogenitor cells, decreased synthetic capability of osteoblasts, and the lessened biologic activity of growth factors contained in the organic bone matrix. Diminished physical activity associated with aging acts to reduce bone growth since exercise acts to stimulate the new bone formation. Bone loss occurs rapidly in astronauts who are in a weightless environment, with bed rest, or with the immobilization or paralysis of an extremity. Bone growth is stimulated by skeletal loading and muscle contraction associated with resistive exercises such as weight training.

Postmenopausal women are vulnerable to osteoporosis, which largely involves trabecular bones including the spinal vertebrae. Estrogen deficiency plays a major role since estrogen replacement reduces the rate of bone loss. The mechanism for this effect has not been fully characterized but decreased estrogen resulted in increased IL-1 secretion from blood monocytes. IL-1 stimulates osteoclastic activity and bone resorption. Other risk factors include excessive alcohol consumption and smoking.

Bone is a target tissue for several xenobiotics. Several metals can effect the development of bone. Radiographs of bone in children with significant lead exposure can reveal lead lines, which are areas of increased bone density in the metaphyseal bone region. Lead lines are characteristically seen in rapidly growing tubular bones including the distal femur and proximal tibula and fibula (knee joint), but the vertebral bodies and iliac wing can also be affected. This effect has been attributed to the action of lead on the remodeling of calcified cartilage in the zone of provisional calcification of the metaphyseal bone. Bismuth and yellow phosphorus can also produce similar metaphyseal bands.

Chronic ingestion of high concentrations of fluoride can produce fluorosis, whose clinical picture can include osteosclerosis. Osteosclerosis is a painful condition characterized by an increased density in the bones. This is thought to occur because hydroxyapatite is replaced with fluorapatite. Fluoride also accumulates in ligaments where X-rays can demonstrate increased bone density; mineral deposits in ligaments, tendons, and muscles; and periosteal outgrowths. Osteosclerotic changes have been observed among aluminum workers with fluoride exposures and among persons with prolonged use of water containing high concentrations of fluoride.

Hypervitaminosis A and D have also been associated with bone abnormalities. Vitamin D can cause resorption of calcium from bone. Chronic vitamin D intoxication may result in increased mineralization on bone and metastatic calcifications including joints, periarticular, and the kidney. Excessive vitamin D intake can cause demineralization of bone resulting in multiple fractures from very slight trauma.

Osteomalacia and likely osteoporosis among Japanese woman has been linked with ingestion of

cadmium-contaminated food, including shellfish. This painful condition, known as 'itai-itai byo' (ouch-ouch disease), has occurred primarily in postmenopausal multiparous women. Chronic exposure to cadmium has been associated with microfractures, osteomalacia, radiological decreases in bone density, and disturbances in calcium metabolism. One possible mechanism to account for this finding is increased serum parathyroid hormone and decreased serum vitamin D levels from cadmium-induced renal damage.

The mineral structure of bone also incorporates metals and metalloids that resemble calcium, including lead and a variety of elements some isotopes of which emit alpha radiation, including strontium-90, uranium-235, and plutonium-239. Bone acts as an important storage depot for these elements and the high local concentration in bone is responsible for the high risk of bone marrow effects and of bone cancers from α-emitting radionuclides.

Skeletal Muscle

Skeletal muscle is a major component of body tissue and accounts for 40–50% of the body weight. Skeletal tissue is composed of specialized striated cells, which function to convert chemical energy to mechanical work. Skeletal muscle plays a central role in body metabolism and serves as a source of body heat and a storage depot for energy-rich compounds, protein, and intracellular ions (e.g., potassium). It also contains up to 80% of the body water content. In contrast to cardiac and smooth visceral muscle tissue, skeletal muscle is under voluntary control.

Skeletal muscle is composed of individual muscle fibers or cells that are contained in connective tissue. The muscle fibers are composed of hundreds or thousands of myoblasts. Muscle fibers are consequently multinucleated cells that have lengths of up to 10 cm and diameters ranging from 10 to 100 μm. They seldom are as long as the length of muscle which they compose and form interlocking irregular polygons. The size of muscle fiber is influenced by several factors. Proximal large muscles have large-diameter fibers, while smaller distal muscles contain more smaller diameter fibers. Physical activity can increase the size of muscle fibers in both sexes, although men of comparable age have larger fibers than woman. Children have smaller fibers.

Striated skeletal muscle fibers are bound together by collagenous connective tissue to form individual muscles. The connective tissue covering a muscle is known as the epimysium. This forms a resilient elastic sheath covering that separates the muscle from surrounding structures such as tendons and bone. The connective tissue extends into muscle fibers and separates groups of individual muscle fibers or fasiculi. This connective tissue is known as the perimysium. Each muscle cell is surrounded by connective tissue known as the endomysium. This collagenous membrane combined with the adjacent muscle cell membrane is termed the sarcolemma. This tissue serves to maintain a framework for striated muscle cells. As long as the connective tissue remains intact, skeletal muscle can regenerate following injury and grow in the pattern provided by this connective tissue.

The muscle cell membrane is termed the plasmalemma. The cytoplasm of the muscle cell is filled with myofilaments, which form the myofibrils. Myofibrils are composed of sarcomeres, which consist of longitudinally directed thin and thick filaments and perpendicularly disposed z bands that are α-actin filaments. The myofibrils form the contractile apparatus of the muscle.

The sarcolemmal membrane has invaginations which run parallel to the z bands. These invaginations are also known as the T system and are involved in the release of calcium into the cell. The release of calcium leads to a contraction of the myofibrils. Sarcoplasm accounts for $\sim$40% of the volume of the fiber and contains glycogen, mitochondria, and lipid vacuoles.

There are two principal types of skeletal muscle fibers in humans: type 1 and type 2 fibers. They can be thought of as corresponding to red and white muscle. Type 1 fibers, or dark fibers, have more myoglobin and have the capacity for maintaining sustained force and weight bearing. They contain large numbers of mitochondria and maintain activity through sustained aerobic glycolysis. Type 2 fibers, or white fibers, are important in performing sudden and rapid movements. They have abundant glycogen but scant mitochondria and are not able to maintain sustained activity because they accumulate lactic acid. Strength training increases the number and size of type 2 fibers. Aerobic training involves hypertrophy of type 1 fibers.

A number of pathological processes can affect skeletal muscle. Since individual muscle fiber is formed by numerous myoblasts, any injury and pathological changes may only affect a small part of a muscle fiber. This has clinical significance since biopsy of a small segment of muscle may provide a nonrepresentative sample of muscle for assessment of a myopathy. Handling of specimens can be difficult and lead to artefactual lesions from fractures rising from the processing of the muscle. In general, the reactions of muscle are not specific to any disease or toxic agent.

Skeletal muscle can undergo atrophy due to several factors. Loss of innervation from anterior horn cell or a peripheral neuropathy can affect type 1 and type 2 fibers. This is characterized by a diminution of synthesis of myosin and actin and a decrease in size and resorption of the myofibrils; the cells, however, remain viable. Type 2 fiber atrophy can occur in several types of situations including inflammatory disorders involving muscle (e.g., polymyositis and polymyalgia rheumatica), metabolic disorders, corticosteriod myopathy, Cushings disease, and general cachectic states. Disuse of the muscle associated with inactivity (such as the placement of a cast) can lead to atrophy of muscle fibers, especially type 2 fibers. The cellular mechanism of the atrophy is poorly understood but could involve an increase in the rate of protein degradation, a reduction in protein synthesis, or a combination of both factors.

Hypertrophy, an increase in size of muscle cells, can occur in response to several situations. This is largely caused by an increase in the number of myofibrils. Hypertrophy is seen in more slowly progressive muscular dystrophies, a heterogeneous group of inherited muscle disorders (e.g., Becker's muscular dystrophy). Clinically they result in muscle wasting and weakness. Becker's muscular dystrophy is an X-linked disorder. Endocrine disorders including hypothyroidism and an increase in growth hormone or acromegaly can also be associated with hypertrophy.

Necrosis is the other principal muscular pathological process that muscle fibers can undergo. Usually the entire muscle fiber does not undergo necrosis. Segmental necrosis is the term used to describe necrosis confined to a segment of variable length of fiber rather then the entire fiber. The clinical spectrum of persons with systemic necrotizing myopathy typically includes proximal muscle limb weakness and elevated serum creatine kinase. Myoglobinuria can sometimes also be observed.

There are several potential causes for segmental necrosis of muscle fiber. The cause of this type of necrosis is not well understood but could be due to effects on the plasma membrane or the outer boundary of the muscle fiber. Aminocaproic acid (an antifibrinolytic medication used in the treatment of a subarachnoid hemorrhage), clofibrate (used to treat hyperlipedemia), emetine (found in ipecac syrup), cardiac glycosides, heroin, and phencyclidine have been associated with necrotizing myopathies.

Select drugs injected by the intramuscular route can produce focal necrotizing myopathic changes. Paraldehyde, chlorpromazine, and a number of antibiotics have produced this type of reaction.

Interference with homeostasis of mitochondrial DNA has been linked to segmental necrosis. Medications associated with this type of effect include zidovidine, which is used to treat HIV. Electron microscopic findings include marked increases in mitochondrial enlargement with vacuolation. Chemicals that block aerobic metabolism (e.g., 2,4-dinitrophenol) have been used to experimentally produce a mitochondrial myopathy characterized in some instances by segmental necrosis.

Several drugs that share the chemical property of being a large cationic amhiphillic molecule that has a hydrophobic and hydrophilic region with a primary or substituted amine group with a net positive charge have been shown to produce a necrotizing myopathy. The mechanism of action is that these drugs interfere with lysosomal digestion and lead to autophagic degeneration and accumulation of phospholipds. Autophagic membrane-bound vacuoles containing membranous debris and curvilinear bodies with short, curved membrane structures with light and dark areas are seen. Drugs in this class include chloproquiin, vincristine, colchicine, and amiodarone.

Corticosteroids have produced necrotizing muscle changes. Severity is variable and not always associated with the steroid level or therapeutic regimen but is most likely to occur in persons taking over 40 mg of prednisone per day. Pathologically, degeneration of type 2 fibers is often seen.

Myopathies can also occur as a result of secondary effects. Hypokalemia has been associated with myopathy. Myopathy has been observed in persons consuming large quantities of licorice extract, in persons taking diuretic and some other medications, and in persons with purgative abuse.

Myopathies can be associated with immunologically based reactions that have features of polymyositis or dermatomyosotis. The clinical features of eosinophylia–myalgia syndrome include the abrupt onset of muscle pain. This syndrome was thought to be due to a contaminant in l-tryptophan introduced by the manufacturing process. Certain medications are associated with necrotizing myositis, which has similar features. Acute rhabdomyolysis is a severe form of necrotizing myopathy.

See also: Blood; Cadmium; Eosinophilia–Myalgia Syndrome; Lead; Radiation Toxicology, Ionizing and Nonionizing; Tissue Repair; Vitamin D.

Skin

Peter Robinson

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Introduction

Skin is the largest organ in the body. As the primary interface between the body and its external environment, it serves as a living, protective envelope that prevents the entry of foreign chemicals and microbes, as well as preventing the evaporative loss of body fluids and heat. Although skin is an effective barrier, it is not a complete one in that it is becoming increasingly apparent that skin is an important portal of entry for chemicals into the body. Not all chemicals penetrate equally well, however, and an important part of the study of the toxicology of the skin is to understand and predict the differential penetration of potentially toxic materials into the skin, and through the skin into the general systemic circulation. We need to understand how the structure of the skin interacts with different chemical species in order to limit their entry into the body. We also need to understand how the skin itself reacts to the presence of toxic chemicals, and how this manifests itself in terms of various familiar local skin reactions.

Not long ago, studies in skin toxicology were primarily concerned with developing methods to produce and evaluate irritation and allergic reaction in both animal and human skin. However, significant recent advances in tissue culture techniques, cellular and molecular biology, and the understanding of toxicokinetic principles have enormously expanded our horizons in studies of skin function and toxicity, and we are just beginning to appreciate some of the more novel, but important, biochemical, physiological, and metabolic capabilities of this organ.

The purpose of this entry is to provide a general overview of cutaneous toxicology. Current knowledge of the etiology and mechanisms of skin toxicity will be summarized and some of the more obvious and typical skin responses to toxic insults will be described. Furthermore, current concepts regarding skin absorption and metabolism will be discussed and, together, it is hoped that a review of these topics will provide a better understanding of the toxicology of the skin.

We will not be discussing dermal exposure in any great detail, although this is, of course, crucial when incorporating the principles of dermal toxicity outlined here into an overall assessment of the hazards and risks of chemicals in the environment or in the workplace. In many cases, in fact, the uncertainties associated with quantifying dermal exposure may drive the overall uncertainty in the risk assessment. For example, exposure to chemicals in the soil may be a common pathway for many environmental contaminants, particularly for children and agricultural workers, but the contact of soil with the skin, both in terms of the overall amount and the exposed surface area is very difficult to quantify. Added to that, the availability of chemicals adsorbed to the soil particles for dermal absorption in the complex environment of the skin surface is also fraught with uncertainty. Similar situations exist for dermal exposure of chemicals from consumer products, household surfaces, fabrics, etc. These limitations should be borne in mind in what follows.

Skin Structure and Function

Mammalian skin can be described as a multilayer heterogeneous organ that forms the external covering of the body. It is the largest organ in the body and is continuous with the lining of orifices that open onto the body surface. In an adult man, the skin has a total surface area of $\sim 2\,m^2$ and in most places it is no more than 2 mm in thickness, yet it can account for 10–20% of total body weight. The basic structure of mammalian skin can be divided into three main components: (1) a superficial lining of epithelial cells, the epidermis, supported by (2) a subepithelial connective tissue stroma and vasculature, the dermis, which in turn is supported on (3) a layer of subcutaneous fat of varying thickness, called the hypodermis. Impregnated within the epidermis and dermis are specialized 'adnexa', which include hair follicles, sebaceous glands, sweat glands, and a complex neural network (**Figure 1**).

The epidermis, which develops from the embryonic ectoderm, comprises ~5% of full-thickness skin by weight. It is avascular and is composed primarily of keratinocytes. Based mostly on structural criteria, the epidermis can be subdivided into several layers. The basal layer consists of germinative cells, which retain the capability to undergo cell division and are extremely metabolically active. The daughter cells from the dividing basal layers migrate upward and undergo terminal differentiation to form the next two viable cell layers of the epidermis, the spinus and granular cell layers. During this process, the keratinocytes become flatter and lose many of their cytoplasmic organelles. Their nuclei condense and this is

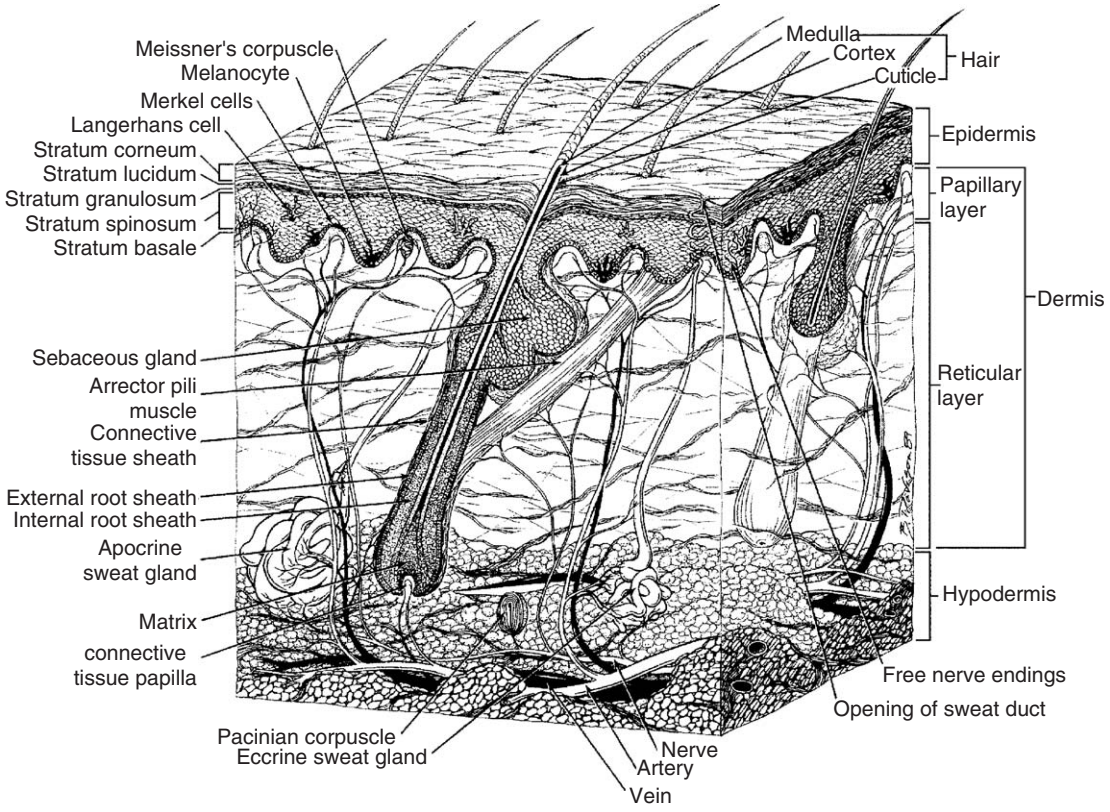

Figure 1 A composite representation of the structure of the integument found in typical skin in various regions of the body. (Reproduced from Hobson DW (ed.) (1991) *Dermal and Ocular Toxicology: Fundamentals and Methods*. Boca Raton, FL: CRC Press, with permission from CRC Press.)

accompanied by the appearance of granules that ultimately form keratin filaments. The endproduct of this terminal differentiation process is the strateum corneum, the outermost layer of the epidermis. The cells of this layer are essentially flat, anucleuated, and devoid of any metabolic activity. These cells are eventually sloughed off to be replaced by terminally differentiating cells from the basal layer. This process of differentiation and outward migration in the epidermis is continuous. The average turnover time varies greatly from species to species: in humans, it has been estimated to be about 28 days, but there is considerable variation, depending on anatomical site and disease state.

In addition to keratinocytes, the epidermis contains several 'dendritic' cell types. Langerhan's cells, which account for $\sim$5–10% of all cells found in the epidermis, are bone marrow mesenchyme-derived cells. These cells are involved in antigen recognition and processing during induction of immune responses in skin. Melanocytes are melanin-synthesizing cells of neural crest origin. These cells are found adjacent to the basal cells and supply them with melanin, the principal pigment in skin, localized in specialized organelles, called melasomes. Merkel cells are the third type of dendritic cell found in the epidermis. They are of neuroectoderm origin and are believed to have a neuroendocrine function in the skin.

The basal lamina, with its characteristic ridge-shaped appearance, forms the epidermal–dermal junction and is the interphase that separates the epidermis from the underlying dermis. The dermis, which originates from the embryonic endoderm, consists of connective tissues and covers the internal organs of the body in a strong, flexible envelope. This envelope can be divided into two anatomical layers – the thin outer papillary layer and the thick inner reticular layer. The papillary layer consists of loose connective tissue, whereas dense connective tissue is found in the reticular layer. The elasticity and strength of this envelope are due to the constituent materials forming the dermal matrix. This matrix contains fibrous proteins, such as collagens, elastin, and reticulin, which are embedded in an amorphous material known as the ground substance, consisting of proteins and the glycosaminoglycans chondroitin A sulfate and hyaluronic acid. Fibroblasts are the most important and most numerous cell types found in the dermis. They are motile, capable of mitosis, and are responsible for the synthesis and secretion of

the proteins and fibers that make up the bulk of connective tissue. In addition to fibroblasts, the dermis also contains a variety of cells scattered throughout this tissue, including macrophages, lymphocytes, adipocytes, and mast cells. The mast cells are of special interest and are most numerous in areas adjacent to skin appendages (hair follicles), blood vessels, and nerves. Their function in skin homeostatis is unclear; however, it appears mast cells are involved in the pathogenesis of some inflammatory conditions. They are generally indistinguishable from fibroblasts, except they have special intracellular granules containing histamine, heparin, and other vasoactive agents that are released in response to certain chemical irritants.

Within the dermis, there are a number of epithelial structures, known collectively as cutaneous adnexa or epidermal appendages. They are all various types of extensions of modified epidermal cell structures into the dermis. The pilosebaceous units are located over the entire surface of the body, consisting of hair follicles and their associated sebacous glands, together with the accompanying arrector pili muscle, capillary plexus, and nerve fibers. The hair follicles are composed of three layers: the inner root sheath of keratinized cells, which form the hair shaft; the outer root sheath, which is a continuation of the epidermis; and the connective tissue layer. This latter layer merges with the papillary layer of the dermis and is continuous with the dermal papilla, located at the base of the hair follicles. The dermal papilla contains the germinative epithelial cells that give rise to hair proper through a complex process of growth, differentiation, and regression. Hair follicles undergo a continuous cycle of growth, called anagen, where new hair shafts are formed, followed by a short period known as catagen, where mitotic activity of the germinative cells in the dermal papilla ceases and the papilla atrophies. At telogen, the resting phase of hair cycle, the hair produced during anagen remains anchored to the skin, and at the next anagen phase, the newly synthesized hair replaces this hair. The sebaceous glands develop from the infundibulum of the hair follicle and are found surrounding the hair follicles. They contain differentiating cells that are active in lipid synthesis. The lipids, which form droplets in the fully differentiated cells, are released into the sebaceous ducts and onto the surface of the skin as sebum. This release of sebum is accompanied by the total disintegration of the lipid-laden cells. Sebum, a complex mixture of lipids, has antibacterial and waterproofing functions on the skin's surface.

Eccrine and apocrine glands, the sweat glands, are distributed throughout the skin. They are situated deep within the dermis and are simple coiled tubular structures composed of a long coiled secretory tubule and a long connecting excretory duct that traverses the epidermis and opens directly onto the surface of the skin. The eccrine glands are responsible for producing and secreting aqueous sweat (i.e., water and salts) and participate in thermoregulation. The apocrine glands secrete a viscous material containing proteins, pheromones, sugars, and ammonia. Their function in humans is unclear, but in animals they are believed to be associated with communication, probably acting in a sex-attractant or territorial marker role. The mammary glands are merely enlarged and modified apocrine glands. In man, the apocrine glands are found only in specific body regions, such as the axilla, the areola, the pubis, the perianal region, the eyelids, and the external auditory meatus. They secrete odorless secretions that are decomposed by surface bacteria to form characteristic odiferous products. Due to the combined secretions of the sweat glands and the sebaceous glands, the outer surface of the skin is generally always coated by an acidic film (pH 4–6), composed of various lipids, including triglycerides, phospholipids, and esterified cholesterol, together with salts and water. This film is frequently colonized by certain species of bacteria (e.g., *Micrococciae* and *Corynebacterium*) and the overall composition of this film may vary, depending on such factors as the disease state of the skin or occlusion. Any changes in the composition of the film will have dramatic effects on the makeup of the microflora present on the skin surface.

The dermis is separated from the underlying fiscia of muscle by the subcutis. The subcutis is a layer of adipose tissue of varying thickness, which, in humans, depends on the body region, sex, age, and nutritional status. The extensive dermal vasculature arises from the subcutis and consists of networks of vascular plexuses that are found in the transitional zone of the dermis and subcutis, adjacent to and surrounding the adnexa (eccrine sweat glands, hair follicles, and sebaceous glands). Arterioles branch out from these areas, forming anastomoses generally immediately under the epidermis, in both the reticular dermis and the papillary dermis. The dermal blood supply is usually substantially greater than that required merely to nourish the skin and skin blood flow can vary by orders of magnitude; thus, the dermal vasculature has an additional role in thermoregulation by controlling the dissipation of heat to the body surface.

Intertwined with the dermal vasculature is a complex network of nerve plexuses consisting of both encapsulated and free nerve endings. These sensory and sensorimotor nerves ramify throughout the skin

and are of extreme importance in the perception sensory stimuli. The skin also has a plexuses of lymphatics that drains into the regional lymph nodes.

This is a generalized and simplified description of mammalian skin. The basic architecture of skin is similar in all mammals. However, there are substantial species differences for aspects such as thickness, blood supply, and types or amounts of the various adnexa, as well as regional differences within each species. The most obvious species difference is hair follicle density in the skin. In lower mammals (rodents, dogs, cats, etc.), hair density is relatively high, whereas hair coat covering in humans and pigs is typically rather sparse. Skin thickness is another parameter that shows extensive species and regional differences, varying from a few micrometers to several millimeters thick (**Table 1**). Furthermore, in humans, the predominant sweat gland is the eccrine sweat gland, which opens directly onto the surface of the skin, whereas in animals the apocrine sweat glands, emptying into hair follicles, predominate. In general, it can be said that the density and distribution of the adnexal structures provide the basis for species differences in skin structure and function while also contributing to the regional differences of skin anatomy noted for each particular species. Such species differences are important considerations when using experimental animals in percutaneous penetration experiments, either *in vivo* or *in vivo*, and extrapolating the results to man.

Table 1 Comparative epidermal thicknesses

Species	*Epidermis* (mm)	*Stratum corneum* (mm)
Mouse	9.7±2.3[a]	3.0±0.3
Rat	11.6±1.0	4.6±0.6
Rat	32.1±1.3[b]	18.4±0.5
Rabbit	15.1±1.4	4.9±0.8
Monkey	17.1±2.2	5.3±0.4
Dog	22.5±2.4	8.6±1.9
Cat	23.4±9.9	4.3±1.0
Cow	27.4±2.6	8.1±0.6
Horse	29.1±5.0	7.0±1.1
Pig	46.8±2.0	14.9±1.9
Pig	65.8±1.8[c]	26.4±0.4
Human	46.9±2.3[d]	16.8±0.7
Human	(60–120)[e]	(20–25)

[a] Mean±s.e. ($n=6$); histologically determined in skin from the ventral abdomen.
[b] Mean±s.e. ($n=9$); histologically determined in skin from the back.
[c] Mean±s.e. ($n=35$); histologically determined in skin from the back.
[d] Mean±s.e. ($n=16$); histologically determined in skin from ventral abdomen.
[e] Range; histologically determined using skin taken from the ankle.

Percutaneous Absorption

General Concepts

Mechanisms of Percutaneous Absorption In order to understand drug and chemical toxicity in the skin, the process whereby the various responsive cell types within skin are exposed to these agents must be examined. Percutaneous, or 'via the skin', absorption may be defined as the translocation of surface-applied agents through the various layers of the skin to a location where they can enter systemic circulation via the dermal microvasculature and lymphatics or remain in the deeper layers of the skin. Based on our current knowledge, the important steps involved in skin absorption have been identified as the partitioning of the compound from the delivery vehicle to the stratum corneum, transport through the stratum corneum, partitioning from the lipophilic stratum corneum into the more aqueous viable epidermis, transport across the epidermis, and uptake by the cutaneous microvasculature with subsequent systemic distribution. This process, therefore, is the sum of the penetration and permeation of a chemical into and through the different strata of the skin.

Assessment of this process following topical application of drugs and environmental chemicals is becoming an increasingly important aspect of both toxicological and pharmaceutical investigations. Relative to toxicology, the ultimate aims of skin absorption studies are to identify and quantify the potential cutaneous toxicity, estimate the relative risk, and develop the appropriate strategies to minimize this risk resulting from topical exposure. In contrast, percutaneous absorption studies in transdermal delivery are designed primarily to assess and manipulate the rates of transport of drugs across the skin and ultimately to determine if such rates are sufficient to achieve the desired exposure and provide optimal therapeutic response. The aim is to identify or design the therapeutic compound and its vehicle and/or its delivery system with the appropriate properties for commercial development. Closely associated with these studies are investigations that are designed to assess the cutaneous and systemic bioavailability and bioequivalence of the compounds under development. Thus, depending on one's perspective, the focus of skin absorption studies may be to increase penetration or to reduce absorption.

The rate-limiting barrier to skin absorption is generally considered to be the outermost layer, the nonviable stratum corneum. Consequently, the skin is frequently thought of as a passive, inert barrier and percutaneous absorption of chemicals was thought to be dominated by laws of mass action and physical diffusion. This reduction of percutaneous absorption

to diffusion equations and mass transfer coefficients has overshadowed any considerations of the possible contribution of biochemical factors that may influence the percutaneous fate of topically applied substances. In general, the diffusional theories and the assumption that the skin is merely a physical barrier persist, despite the fact that the skin is an organ active in many essential biochemical and physiological functions. Moreover, for certain lipophilic chemicals, it is clear that the stratum corneum is no barrier at all. The lipid-rich stratum corneum and skin appendages may act as a reservoir for topically applied lipid materials, thus functioning more as a sponge, capable of absorbing a quantity of material that is limited only by the solubility of the substances in the sebaceous and intrinsic epidermal lipids. For such lipophilic chemicals the viable epidermal membrane may be the more important barrier.

Skin appendages, which include sebaceous glands, hair follicles, and sweat glands, are often regarded as channels that bypass the stratum corneum barrier as such, they are generally thought to facilitate the dermal absorption of topical agents. Because they occupy only a small fraction of the skin's surface area in humans (0.01–0.1%), their overall effect on the extent of percutaneous absorption will be minimal for most compounds. Moreover, it is often overlooked that these appendageal structures are not open pores through the skin, but are usually plugged with hair shafts, dead cells, sebum oils, stratum corneum lipids, and/or aqueous salt solutions (sweat). Thus, this pathway is probably only important immediately after a substance is applied to the skin as a rapid shunt. The bulk of skin absorption takes place via the diffusion processes described previously and later. However, the significance of this follicular pathway in skin absorption remains to be experimentally assessed. The correlation between permeation and hair density of the different rat skin phenotypes (haired, fuzzy, and hairless) tends to support the hypothesis that the transfollicular pathway may be the more dominant route for the skin absorption of certain highly lipophilic chemicals such as polycyclic aromatic hydrocarbons and coal tars. In addition, these appendages may be important for highly polar molecules, which generally penetrate the stratum corneum very slowly, if at all (see the discussion of the 'polar pathway' in the next section).

Systemic and Local Skin Effects: Exposure Considerations Even without exploring directly the mechanisms of dermal absorption, external dermal exposure has different implications for compounds that may produce systemic effects (such as cancer or toxic effects in remote tissues) and for those that act locally in the skin itself. In the latter category, contact sensitization and irritation are two areas of concern (as well as skin cancer). Whether one is considering local or systemic effects (together with the dermal penetration potential of the compound) has a profound impact on what measures of applied dose are most relevant to consider in an exposure analysis. A few highly simplified examples will make this clear.

First consider a freely or moderately penetrating compound applied to the skin in a leave-on formulation. If there is little loss via evaporation or rub-off, it is reasonable to assume 100% absorption in such cases. For systemic effects, the most relevant exposure parameter is the total applied dose, regardless of how it is applied (all of which is absorbed and becomes available to the systemic circulation). On the other hand, if a local skin effect is indicated, the relevant parameter is likely to be the local concentration in the skin itself, or some other measure of local skin exposure. This is more likely to be related to the applied dose per unit area of the skin, rather than the total applied dose. In broad terms, this is the reason why the 'dose per unit area' is crucial in the elicitation of contact sensitization responses to allergens.

Now consider a poorly penetrating dermally applied compound, or one that is applied for such a short time T that only a small fraction of the applied dose is absorbed. In this case, it is the concentration c of the material in the formulation (as well as other factors that determine the flux across the stratum corneum, such as the dermal penetration coefficient K_p – see below), rather than the total applied amount of material, that determines the amount absorbed both into and through the skin. In simplest terms, the total amount Q absorbed into the systemic circulation is given by the equation:

$$Q\ (\text{mg}) = K_p\ (\text{cm h}^{-1}) \times c\ (\text{mg ml}^{-1}) \times T\ (\text{h}) \times A\ (\text{cm}^2)$$

where A is the applied surface area of skin. In terms of external exposure, the important dose parameter in this case is the product $c \times A$. (This in turn is equivalent to the total dose D divided by the film thickness h). In other words, both the applied concentration and the exposed surface area are relevant for systemic effects. Unlike the case of a rapidly penetrating compound, the total dose alone is not sufficient to characterize exposure: also needed is an additional parameter (such as the film thickness) that indicates how the dose is distributed over the skin surface. In addition, the amount absorbed also depends on the exposure time T.

The case is again different for local skin effects. In this case, it is the amount absorbed per unit area of skin that again is most relevant for local effects. From the equation given above, $Q/A = K_p \times c \times T$. Thus, the most relevant exposure parameter is the applied concentration c (as well as the exposure time T). In this case, at least to a first approximation, how the material is distributed over the skin surface is not as important as the nature of the material itself (i.e., its concentration).

Thus, to a first approximation, the most relevant exposure parameters for a specific situation is given in **Table 2**.

Mathematical Models Diffusion principles have been traditionally recognized as the most important determinants in skin absorption. Thus, Fick's law of diffusion provided the mathematical basis for early kinetic descriptions of percutaneous absorption. Fick's law simply states that

$$J_s = K_p \Delta c_s$$

where J_s is net flux of substance s ($\mu g\,cm^{-2}\,h^{-1}$), K_p is the permeability constant ($cm\,h^{-1}$), and Δc_s is the concentration gradient of s across the diffusion membrane ($\mu g\,cm^{-3}$). The validity of applying mass diffusion principles to skin absorption rests on at least two main assumptions. First, penetration of chemicals into and permeation within the various layers of skin are passive diffusional processes. Second, the diffusional resistance of the skin layers can be formulated into one or more equations describing diffusion of small particles or molecules across thin layers or barriers, and the layers or barriers must simulate as closely as possible the behavior of dilute polymeric solutions.

Because diffusional resistance of the outermost region of skin, that is, the stratum corneum, is generally far greater than that of other cutaneous substructures, models of skin absorption are frequently simplified to involve diffusion across only one layer, the stratum corneum. This equation forms the simplest mathematical framework describing many percutaneous absorption investigations.

The dermal penetration coefficient K_p in this simplest case depends on both the partitioning of the chemical from its vehicle (usually water) into the stratum corneum, and its diffusion through the stratum corneum. Both of these quantities can be estimated from a chemical's properties or structure. Partitioning from water into the stratum corneum can be estimated from a chemical's octanol–water partition coefficient, K_{ow}. Diffusion through the stratum corneum is dependent on the molecular volume of the chemical, which is in turn a function of its molecular weight (MW). Perhaps the most widely used expression of the dependence of stratum corneum permeability on readily available physicochemical properties is the 'Potts-Guy' equation:

$$\log K_{sc} = -2.72 + 0.71 \log K_{ow} - 0.0061\ MW$$

in which the parameters have been fitted to an empirical dataset of (usually *in vitro*) measured stratum corneum penetration coefficients K_{sc} from aqueous vehicle.

Unfortunately, percutaneous absorption is in reality a complex phenomenon involving a myriad of diffusional and metabolic processes that are proceeding either concurrently or sequentially. Consequently, theoretical models describing the overall process will be approximations and will reflect our current knowledge concerning the most relevant events. Neglecting cutaneous metabolism for the moment, but going beyond the simple stratum corneum model, the skin may be modeled as a number of serial and parallel barriers and pathways.

For compounds that have either a very low diffusion coefficient or a very low lipid–water partition coefficient, the lipid barrier of the stratum corneum is a formidable impediment to penetration through the skin. However, for such compounds it has been observed that there is no longer a correlation between skin permeation and lipid solubility; further, there also appears to be little dependence on molecular weight. It has therefore been hypothesized that such compounds make use of an alternative, low-permeability, and essentially aqueous pathway through the stratum corneum. Although direct physical evidence for such pores is lacking, the notion of a

Table 2 Relevant applied doses for systemic and local effects for rapidly and poorly penetrating materials (K_p is the dermal penetration coefficient)

	Rapidly penetrating compound	*Poorly penetrating compound*
Local skin effects predominate	Total applied dose (mg)	Concentration × surface area × exposure time (× K_p)
Systemic effects predominate	Dose per unit area of exposed skin ($mg\,cm^{-2}$)	Concentration × exposure time (× K_p)

different functional pathway (with a different (and minimal) functional dependence on K_{ow} and on MW) for such compounds is important. Estimates of the permeability coefficient K_{pol} of such a pathway for neutral molecules in human skin range from about (1 to 10) $\times 10^{-6}$ cm h^{-1}; taking into account a (weak) molecular weight dependence based on diffusion through a liquid, we obtain the following expression as representative of K_{pol} for healthy human skin:

$$K_{pol} \sim 1 \times 10^{-6}(300/MW)^{1/2}(\text{cm h}^{-1})$$

Note that as lipid solubility increases, the relative contribution of K_{pol} to overall passage through the stratum corneum progressively decreases.

Additional barriers include the viable epidermis and dermis, which can together be considered to constitute an aqueous layer beneath the stratum corneum, which may act as a significant barrier to the passage of more lipophilic materials from the surface of the skin to the systemic circulation. Finally, once they have penetrated sufficiently deeply into the skin, materials must also actually enter the circulation itself by passing through the capillary wall and partitioning into the blood (often with the help of plasma protein binding).

A schematic representation of the multicomponent structure of the skin that takes these multiple barriers/pathways into account is shown in **Figure 2**. When penetration through the stratum corneum (including the parallel polar pathways) and through the aqueous layers in series with the stratum corneum are taken into account and combined with clearance into the bloodstream, the overall dermal penetration coefficient K_p (cm h^{-1}) for such a (simplified) composite system is given (by analogy with electrical resistances in series and in parallel) by

$$K_p = \frac{1}{1/(K_{sc} + K_{pol}) + 1/K_{aq} + 1/K_{cap}}$$

where K_{sc} represents the penetration coefficient for the stratum corneum; K_{pol} represents that of the polar pathway in parallel with the stratum corneum; K_{aq} represents the permeability of the aqueous layer of the viable dermis, in series with the stratum corneum; and K_{cap} represents the effective penetration coefficient for clearance into the vasculature via the capillary network of the skin (in series with the stratum corneum and the aqueous layer). Note that K_{cap} will in principle depend on the rate of blood flow through the capillary bed of the skin. All four K parameters have units of, for example, cm h^{-1}.

As the lipophilicity of compounds continues to increase (as estimated by their octanol–water partition coefficients), dermal penetration does not increase indefinitely. In fact, it is limited by a number of factors. As we have seen above, penetration into the systemic circulation for lipophilic compounds is limited by their very modest capacities to penetrate the aqueous layers of the skin (dermis and viable epidermis), as well as their relative reluctance to enter the bloodstream. These factors set a natural upper limit to the dermal penetration coefficients for very lipophilic compounds of $\sim 10^{-1}$ cm h^{-1}. In many cases, however, it is not the penetration coefficient itself in which we are interested, but rather the flux of a compound through the skin. This flux is the product of a penetration coefficient and a driving concentration. Since the aqueous layers of the skin are the major impediment for lipophilic compounds, solubility in water plays a major role in determining the maximum flux of such compounds through the skin. Like other parameters we have

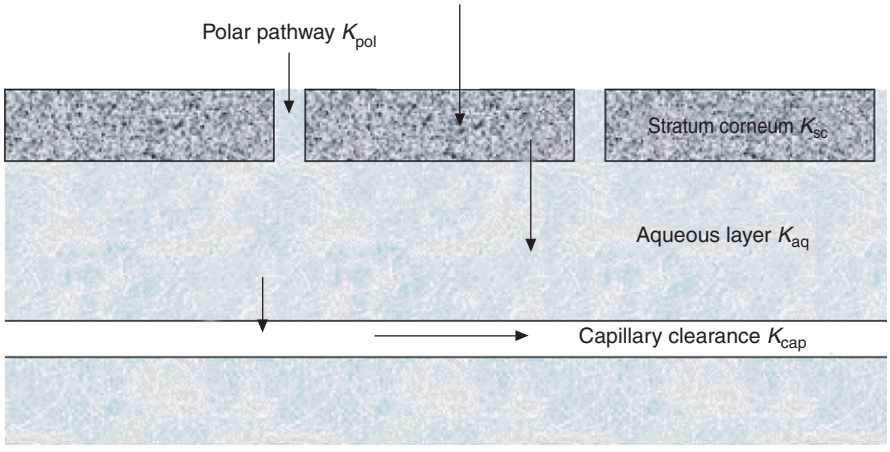

Figure 2 Schematic representation of a composite model of the skin.

been considering, water solubility (WS) depends on the physicochemical properties of compounds, particularly the octanol–water partition coefficient and molecular weight:

$$\log \mathrm{WS} = (3.25 - \log K_{\mathrm{ow}}) \times \mathrm{MW}/1000 \ (\mathrm{mg\ cm^{-3}})$$

The maximum flux $J_{\max}$ is then given by the product $K_{\mathrm{p}} \times \mathrm{WS}$. The maximum flux estimated in this way is a very useful conservative estimate of dermal penetration, particularly when the applied concentration of a compound or the precise nature of its vehicle is not known. It also applies to exposures of neat materials (liquids).

Conceptual models of percutaneous absorption which are rigidly adherent to general solutions of Fick's equation are not always applicable to *in vivo* conditions, primarily because such models may not always be physiologically relevant. Linear kinetic models describing percutaneous absorption in terms of mathematical compartments that have approximate physical or anatomical correlates have been proposed. In these models, the various relevant events, including cutaneous metabolism, considered to be important in the overall process of skin absorption are characterized by first-order rate constants. The rate constants associated with diffusional events in the skin are assumed to be proportional to mass transfer parameters. Constants associated with the systemic distribution and elimination processes are estimated from pharmacokinetic parameters derived from plasma concentration–time profiles obtained following intravenous administration of the penetrant.

These linear kinetic models and diffusion models of skin absorption kinetics have a number of features in common; they are subject to similar constraints and have a similar theoretical basis. The kinetic models, however, are more versatile and are potentially powerful predictive tools used to simulate various aspects of percutaneous absorption. Techniques for simulating multiple-dose behavior; evaporation, cutaneous metabolism, microbial degradation, and other surface-loss processes; dermal risk assessment; transdermal drug delivery; and vehicle effects have all been described. Recently, more sophisticated approaches involving physiologically relevant perfusion-limited models for simulating skin absorption pharmacokinetics have been described. These advanced models provide the conceptual framework from which experiments may be designed to simultaneously assess the role of the cutaneous vasculature and cutaneous metabolism in percutaneous absorption.

Due to the deficiencies of current experimental and analytical methods, our ability to appreciate and fully utilize these sophisticated models is limited. As noted previously, the model parameters (e.g., rate constants) are usually based on assumed partitioning phenomena or kinetic behavior. These assumptions are limited by the paucity of kinetic information provided by current experimental methods. For example, little is known about how volatility affects absorption of the applied dose and the concept of mass-balance studies following topical doses has only recently been addressed. From the perspective of absorption, the skin is a portal of entry for a variety of topically applied chemicals, a drug-metabolizing organ, and a target organ for local toxicity. When skin contact with a chemical results in local effects, pathological changes in the skin may be expected to affect its barrier properties and, hence, influence the fate of surface-applied chemicals. The integrity of the stratum corneum is therefore of primary importance. However, biochemical changes in the skin in response to topical exposure to biologically active chemicals may also influence the metabolic capabilities and metabolic status of the skin and thereby modulate the cutaneous disposition of topically applied substances.

Thus, knowledge of the processes involved in the translocation of chemicals through the skin into systemic circulation and the response of the skin to such chemicals are important aspects of skin pharmacology and toxicology. Research in this area is in its infancy and offers many opportunities. Mechanistic and functional approaches to skin absorption need to be developed. **Table 3** presents a fairly comprehensive list of the known factors affecting percutaneous absorption of topically applied drugs and chemicals. It is anticipated that future research will increase our knowledge of skin absorption, and exploitation of such knowledge would greatly facilitate the continual development of new strategies in reducing the skin absorption of hazardous industrial chemicals. Also, it would provide the basis for improving topical therapy and the transdermal delivery of drugs and prodrugs.

Metabolic Fate of Topically Applied Substances It is now generally recognized that skin is an organ capable of performing a variety of metabolic functions, including those involved in the metabolism of hormones, carcinogens, drugs, and environmental chemicals. Since skin contains enzymes capable of metabolizing xenobiotics, any chemicals that are applied to the surface of the skin will, during the course of penetration and translocation through this organ, be exposed to available biotransformation systems that are present in the skin. Consequently, the ability of the skin to function as an organ of xenobiotic

Table 3 Factors affecting percutaneous absorption

Factor	*Specific examples or other contributing factors*
Vehicle	Suspensions, emulsions, lotions, creams, ointments, pastes, PEG and PPG, demulcents, emollients, pH
Solvents	Water, acetone, ethanol, methanol, chloroform, THF
Enhancers	Dimethylformamide (DMF), DMSO, dimethylacetamide, urea, azone, 2-pyrrolidone, surfactants
Species	Skin thickness, hair density, quantity and types of glands, cutaneous vasculature, and blood flow
Application site	Epidermal and stratum corneum thickness, keratinization, blood flow, hair follicles/glands, skin condition (dermatoses, damage, hydration state, occlusion, pH)
Environment	Ambient temperature and humidity, air flow
Dose applied	Surface area, concentration, contact time, vehicle
Physicochemical	Partition coefficients, molecular weight, particle size/shape, dissolution characteristics, absolute aqueous solubility
Miscellaneous	Humans (age, sex, race); metabolic capacity of the skin

metabolism is of considerable interest, and questions are raised concerning the functional significance of skin metabolism in the percutaneous fate of chemicals. Is skin metabolism important? Can cutaneous metabolism influence dermal absorption? What, if any, is the functional significance of skin metabolism in the cutaneous and systemic disposition of chemicals, and can it be an important determinant in the development of local and systemic toxicity? What are the modulating factors that may affect skin metabolism and, consequently, the disposition of topically applied chemicals? What are the implications of skin metabolism in dermatotoxicity and dermatopharmaceutics? Indeed, will the ability of the skin to metabolize drug prove to be a desirable advantage or a confounding factor that complicates the development of novel transcutaneous therapeutic devices? Perhaps more important, what conceptual and experimental approaches are readily available for use in evaluating the functional significance of skin metabolism on the percutaneous fate of topically applied chemicals?

Numerous investigations with tissue slices, isolated cell preparations, and subcellular fractions from skin have shown this organ to possess a variety of enzyme activities including those involved in the metabolism of xenobiotics. Although the biotransformation of only two classes of compounds, steroids and polycyclic hydrocarbons, has been studied extensively in the skin, it is evident that a full complement of drug-metabolizing enzyme activities is present in the skin. These drug-metabolizing enzymes are generally thought to be associated with the epidermal cells. Recent reports have suggested that high drug metabolizing activities are also localized in the differentiated cells of the hair follicles and adjacent sebaceous glands. It is possible that the resident microorganisms of the skin may also contribute to the metabolism of the topically applied compounds. However, during the development of methods for assessing skin graft viability, it was determined that the metabolic contributions due to skin microorganisms were negligible. Furthermore, experience with metabolism in human skin preparations which have been thoroughly scrubbed with antimicrobial disinfectants support a conclusion that it is the constituent skin cells themselves, rather than surface microbes, which are responsible for the observed biotransformations. Finally, since it is known that the metabolizing activities of the skin readily respond to modulation by inducers and inhibitors, such enzyme modulation could have important implications on cutaneous absorption and disposition experiments.

Experimental Models In the past, percutaneous absorption investigations have usually concentrated on the physicochemical and biophysical factors that influence skin penetration and permeation of chemicals. There are a wide variety of experimental approaches that have been developed to assess skin absorption; however, it should be noted at the outset that currently there is no generally accepted technique. Debates and conflicting opinions continue to revolve around the various factors that are important in influencing percutaneous absorption. Consequently, the rationales by which experimental models are selected and developed are continually being modified and revised. Fundamentally, skin absorption investigations are concerned with how much, how fast, and what are the modulating factors that may influence the penetration and percutaneous fate of the topically applied agents. To answer these questions the primary methods available are based on *in vivo* and *in vitro* studies. The former is from the Latin phrase for 'in life', that is, using a living organism, while the latter means literally 'in glass', or done in the test tube. Both approaches have their advantages and limitations, as will be discussed below.

In Vivo Techniques It is generally recognized that the most reliable method for learning about skin

absorption is to measure it *in vivo* using the appropriate animal model or human volunteers. In principle, the *in vivo* approach is simple, but in practice it is often fraught with experimental and ethical difficulties, particularly when studies are conducted in man. Typically, *in vivo* studies are performed by applying the compound of interest, in a suitable vehicle, to the surface of a defined area of skin. To protect the application site, occlusive or nonocclusive covering is often placed over the treated skin area, and absorption is then monitored by various procedures. However, the techniques used to monitor *in vivo* skin absorption often assess absorption indirectly, and frequently measurements are based on nonspecific assays. The validity of the *in vivo* determination will depend, therefore, on the validity of the method used.

When a topically applied compound induces a biological response following skin absorption, the quantitation of that response may provide a basis for assessing skin absorption. Indeed, such physiological or pharmacological responses have been employed as endpoints in assessing skin absorption *in vivo*, and perhaps the most successful example is the vasoconstrictor response to topical corticosteroids. However, while these pharmacodynamic endpoints may be very sensitive and selective for defined classes of compounds, it should be noted that the parameter measured is the product of both the quantity and the potency of the compound under investigation and may not necessarily reflect the extent of skin absorption, cutaneous metabolism, or disposition.

Ideally skin absorption and metabolism should be assessed based on the analysis of the compound and metabolites of interest in the body following topical application, and such analysis should be performed using sensitive, selective, and specific assays. Although it has been possible in some select cases to determine a plasma concentration–time profile of the compound following topical application, such specific analyses in body fluids are not routinely feasible because the low absolute amount normally absorbed via the skin is often too small to quantitate. It is for this reason that radiolabeled compounds are frequently used, and the extent of absorption is typically assessed by monitoring the elimination of radioactivity in excreta over a period of several days. For small laboratory animals, the absorbed radioactivity that may be retained in the animal and not eliminated in the excreta can be determined directly by analysis of the carcass, following removal of the application site and homogenization of the appropriate tissues. However, in larger animals and in humans, such an approach is impractical, and a correction is required to adjust for such pharmacokinetic factors as absorption, distribution, metabolism, and excretion. This correction has often been made by injecting intravenously a single dose of the radiolabeled compound and monitoring radioactivity in the excreta. A correction factor can be obtained which represents the fraction of dose that would be excreted during the time course of the percutaneous absorption study if it were instantly absorbed upon topical application. This has been the standard approach by which the vast majority of *in vivo* skin absorption studies is conducted and has provided invaluable information concerning percutaneous absorption in humans.

When measurements from intravenous dosing are applied as a correction, the validity is dependent on the underlying assumption that metabolism and disposition of the applied compound are not route dependent and that pharmacokinetic behavior of the intravenous and topical doses are similar. Unfortunately, there is little or no experimental basis for substantiating this assumption, and often the pharmacokinetic profile of the compound under investigation has not been fully characterized. Kinetically, skin absorption resembles a slow infusion, but the intravenous dose for correction is often given as a single bolus injection. Subcutaneous injection or a slow intravenous infusion may be the more appropriate delivery method for correction. Moreover, the selection of the size of the intravenous dose is often not rationalized. When differences in the relative amount of radioactivity excreted in the urine and feces following intravenous and topical administration are observed, these differences may be the consequence of route of administration or they may be related to differences in extent of systemic exposure. Furthermore, when metabolites are found in the excreta following topical application, it is difficult, if not impossible, to differentiate between skin metabolism and systemic metabolism. As a result, the significance of cutaneous metabolism in skin absorption cannot be readily established from *in vivo* investigations.

More direct approaches for monitoring skin absorption have been proposed – for example, measuring the rate of disappearance of the chemical at the application site. However, the generally low permeability of the skin means that the rate of disappearance is often very slow, and the accuracy of the measurement will depend on analytical techniques that are capable of accurately quantifying minute differences. Reliable results can only be obtained with chemicals that are rapidly absorbed and/or easily quantitated analytically. The main use of this technique is monitoring the loss of radioactivity from the skin surface, but it should be appreciated that

measurements using high-energy emitters whose transmission range may be similar to or greater than the thicknesses of the skin could result in erroneous estimates of skin absorption. Other methods, such as those based on histochemical and fluorescence techniques, are highly specialized and cannot be used with all compounds. Another approach involves correlating the extent of percutaneous absorption with the reservoir function of the stratum corneum, as measured by tape-stripping the application site and extracting to determine the amount taken up by the stratum corneum after a short exposure period (30 min).

In most toxicological and pharmacological investigations, the dose administered is precisely defined and dose–response relationships are usually carefully evaluated. In percutaneous absorption studies, however, this is not always the case. A great deal of absorption information in the literature may be of questionable validity since the dose applied was frequently not clearly defined or reported, even though the extent of skin absorption is usually reported in terms of a percentage of the dose applied. Dose application in skin absorption studies conducted *in vivo* is relatively straightforward. The compound of interest is prepared in an appropriate vehicle that may be liquid or semisolid, and an appropriate amount of this preparation is then applied uniformly onto the surface of the skin. Uniformity of application is important but often difficult to assess and is generally assumed without supporting evidence. Furthermore, very little is known concerning the potential influence of local toxicity on cutaneous metabolism and skin absorption and it is suggested that whenever possible a 'no-effect' level of the compound should be used in these types of studies.

Defining the amount of the topical dose applied that is available for absorption is particularly challenging when the compound under investigation is volatile or semivolatile as in the case of solvents and insect repellents. Following topical application, some of the applied dose will penetrate the skin and be absorbed. At the same time, some fraction will evaporate slowly from the surface of the skin and be lost, unavailable for percutaneous absorption. It has been demonstrated that the rate of evaporation, and consequently the relationship between evaporation and skin penetration, can influence the quantity of chemical absorbed dermally. The extent of evaporation from the skin surface is a function of the dose applied, airflow, and temperature at the skin surface. The extent to which these variables may be controlled or monitored can have a major impact on the results of *in vivo* skin absorption studies. Furthermore, consideration of the evaporative loss of the applied dose will be particularly important when surface disappearance or stratum corneum concentrations are employed as methods for assessing *in vivo* skin absorption.

Vehicle as a modulating factor that can influence skin absorption has been discussed in great detail, particularly from a standpoint of increasing absorption in the delivery dermatopharmaceutics, and there is much interest in solvents such as dimethylsulfoxide and azone as vehicles because they act as penetrant enhancers. Postapplication loss of volatile components in the vehicles can also alter the permeation characteristics of the applied chemicals. For example, if a highly volatile vehicle is used this may result in the compound under investigation being deposited as a thin film of solid onto the surface of the skin. On the other hand, a nonvolatile vehicle, such as an ointment, may be occlusive and change the diffusional properties of the stratum corneum. Both of these scenarios can greatly influence the extent of percutaneous absorption. Therefore, the rationale used to justify the selection of an appropriate vehicle for dose application will have important bearing on the significance and validity of the *in vivo* observations.

The extent of skin absorption is greatly dependent on the concentration of the applied dose and the surface area of exposure. Increasing the concentration of the applied dose has been shown to result in a decrease in the percentage of the applied dose being absorbed, but total absorption is increased. This effect may be compound specific and may depend on the dose range under investigation. Moreover, increasing the surface area of exposure will also result in increases in the extent of absorption. In defining the dose applied, therefore, one must consider not only the amount of chemical applied per unit area but also the total surface area of application and the total dose applied. The frequency of application and the duration of exposure have also been shown to influence the extent of skin absorption. In the few times that it has been investigated, the results have shown that washing of the application site to remove the applied dose may enhance, reduce, or have no effect on absorption. Studies on the interrelationship and influence of the various parameters pertaining to dose application in skin absorption are in their infancy. How these parameters may influence the extent of skin absorption is being explored, and it is clear that the current knowledge in this area is far from complete.

Many of the variabilities associated with the lack of standardization of dosing techniques (dosing concentration, volume, applied surface area, application time, etc.) can be somewhat compensated for by

calculating a dermal penetration coefficient K_p from the data wherever possible. The advantage of estimating K_p (as opposed to, say, the percent absorbed) is that values from different experiments (and for different compounds) can be directly compared, since this is a normalized measure of dermal penetration capacity. (It is in fact the flux per unit applied concentration and per unit exposed skin area.) In addition, the extent of dermal absorption can also be readily extrapolated to other exposure conditions (different concentrations, doses, surface areas, application times, etc.). A disadvantage is that K_p is essentially a steady-state parameter, and care must be taken to ensure that a steady state with constant influx rate of the material at a constant applied concentration is in fact achieved (e.g., under 'infinite dose' conditions – see *In Vitro* Techniques below), or is at least compensated for by taking into account dose depletion.

In Vitro Techniques From a cursory review of the literature on percutaneous absorption it is evident that much of our current understanding of the mechanism of percutaneous absorption was derived from *in vitro* investigations. *In vitro* experiments generally afford the investigator the ability to manipulate and control the experimental conditions, and the approach provides the unique opportunity to monitor the rate and extent of percutaneous absorption in skin tissues removed from the confounding influences of the rest of the body. *In vitro* methods, primarily those involving excised skin mounted in diffusion chambers, are the most frequently employed techniques used in the assessment of skin absorption.

Generically, these diffusion chambers consist of a donor and a receptor compartment. Skin absorption is then determined based on the assumption that recovery of the compound of interest in the receptor compartment, following application to the skin surface in the donor compartment, will provide an accurate measure of penetration and permeation. The success and popularity of the *in vitro* approach stem from the fact that the techniques are relatively simple. In these experiments the investigator is provided with the ability to monitor the rate and extent of absorption through skin removed from the influence of other bodily organs. Experimental conditions can be readily manipulated and controlled and, compared to *in vivo* studies, *in vitro* results can be obtained relatively quickly. Furthermore, it is recognized that *in vitro* methodology has contributed significantly in defining the important physiochemical parameters underlying percutaneous penetration and is responsible for much of our current understanding on the mechanisms involved.

Typically, an appropriate fluid (ideally to mimic the properties of blood) is placed into the receptor compartment and an appropriate formulation of the compound under investigation (often radiolabeled) is placed in contact with the skin surface in the donor compartment. The recovery of radioactivity over time in the receptor fluid then provides an estimate of skin absorption. The justification of this methodology centers upon the generally accepted assumption that the passive barrier properties of the skin limit skin absorption. Since the outermost layer of the skin is composed essentially of nonliving tissues and is considered the principal barrier for most compounds, it is reasoned that biochemical processes are unlikely to influence the diffusional characteristics of the rate-limiting membrane and, hence, *in vitro* diffusion studies will accurately measure skin penetration and absorption. The preparation of the skin sample itself has some bearing here. Typically, skin samples are either 'full thickness' in which the skin, stripped merely of its underlying fat deposits, is mounted in the chamber, or 'split thickness', in which the skin is first dermatomed, or sliced parallel to its surface to a specific thickness (typically a few hundred micrometers). The advantage of full thickness skin is that it is less likely to be damaged, but it suffers from the great disadvantage that more lipophilic materials in particular are forced to penetrate through the primarily aqueous layers of the viable epidermis and the whole dermis, which provides a much more formidable barrier to these compounds than they would encounter on their way to the capillary bed *in vivo*. In the case of split thickness skin, a compromise is attempted between trying to simulate a thickness representative of the depth of skin capillaries and the tendency for the thinner preparations to suffer skin barrier damage due to the dermatoming process itself.

In vitro diffusion chamber experiments are based on measuring the compound under investigation in the receptor fluid and much of the research activity has naturally focused in this area. Therefore, recovery of material in the skin itself has received only limited attention. Cutaneous distribution, metabolism, and binding of the topically applied agent are integral parts of the percutaneous absorption process, however, implying that assessing the disposition of the applied chemical in the skin tissue should be an important measurement in the evaluations of skin absorption. Indeed, the amount of a chemical that passes through the stratum corneum into the viable epidermis and dermis is an important parameter for assessing local bioavailability, and it also contributes to the overall estimate of *in vitro* percutaneous absorption. Furthermore, analysis of the skin following

permeation studies would assist in determining mass balance and dose accountability. Such measurements are often not reported or conducted even though they are important in establishing the validity and the interpretation of *in vitro* observations. Because of obvious advantages, radiolabeled chemicals are routinely used in skin absorption studies, and frequently liquid scintillation spectrometry is the sole method used for detecting the penetrating substances in the receptor fluid. However, skin absorption may be accompanied by cutaneous metabolism; therefore, the radioactivity recovered in the receptor medium reflects not only the permeation of the parent substance but also its metabolites.

Experimental designs of *in vitro* studies utilize one of two main strategies. In the traditional steady state or 'infinite dose' technique, a well-stirred donor solution of the compound of interest, at a defined and constant concentration, is used to deliver the compound across the skin preparation. The absorbed compound is subsequently delivered into a well-stirred receptor compartment. The most important design feature of these studies is that the quantity of compound that penetrates the membrane must be kept small relative to the total amount available – there must be no appreciable reduction in the concentration of the compound in the donor compartment. This is so that steady-state flux conditions are not significantly violated and the studies are performed with rigorous compliance to the laws of diffusion. The conventional approach to presenting data from this type of study is to plot the cumulative amount of drug reaching the receptor as a function of time. From the linear portion of this plot, we obtain the most important piece of information, that is, steady-state flux of the compound across the skin membrane (slope $= J_s$). This value is generally normalized with respect to the area of the skin membrane and is usually expressed as amount of drug per unit area per unit time. The intercept on the x-axis, obtained by extrapolating the linear part of the curve, gives a measure of the time required to establish a linear concentration gradient across the skin membrane and is referred to as the lag time, or τ. From these two parameters it is relatively simple, using the diffusion equations, to calculate the permeability coefficient ($K_p = \text{slope}/\Delta c_s$) and derive the other mass transfer parameters such as the diffusion coefficient of the drug across the skin, the partition coefficient of drug between the skin and the receptor fluid, and the diffusional thickness of the membrane. Additional useful parameters can be determined from the earlier nonlinear portion of the curve, but this requires a detailed and often complex model of the kinetics of the absorption process.

While the infinite dose technique has been invaluable in the determination of important skin permeability parameters such as dermal penetration coefficients and in the development of transdermal drug delivery concepts, to mimic *in vivo* conditions, the so-called 'finite dose' technique was developed. This is essentially a modification of the traditional steady-state method. The important difference is that the skin preparation is supported over the receptor so that the epidermal surface is exposed in a manner that mimics the real-life exposure scenario, and the compound of interest is applied to the surface of the skin in a manner also similar to exposure *in vivo*. Although the results of such studies may give valuable information about the absorption of materials under specific exposure conditions, they are generally not amenable to extrapolation to other exposures since no invariant skin properties such as penetration coefficients can be readily calculated.

The techniques described for maintaining viability of the excised skin used in these studies are relatively simple. Basically, the skin preparations are maintained under appropriate conditions as short-term organ culture. They are supported over the culture medium so that their epidermal surfaces are not covered. Material of interest can be applied topically in a manner similar to exposure *in vivo*. This material reaches the epidermal cells by diffusion where it may be metabolized, and recovery of both metabolites and parent compounds in the culture fluid then provides a measure of skin permeation and the extent of cutaneous first-pass metabolism. Two systems have been described. In the 'static' system discs of freshly excised skin are maintained, epidermal side up, on filter paper on a stainless-steel ring support within individual culture dishes containing a suitable culture fluid. In the flowthrough system the skin discs, supported on a stainless grid, form the upper seal of tissue wells of a compact, water-jacketed, multisample skin penetration chamber. Fresh, oxygenated culture medium is continuously perfused through the tissue wells and the well effluents may be collected at timed intervals. The skin absorption and metabolism studies described previously utilizing this methodology demonstrated that, by maintaining the metabolic viability of the excised skin under appropriate culture conditions, the *in vitro* approach provided the means whereby the potential influence of skin metabolism may be evaluated in conjunction with the diffusional aspect of percutaneous absorption. This methodology, therefore, offers a possible approach with which an estimate for the contribution by skin to the percutaneous fate of topically applied chemicals may be determined. It has also been suggested that the metabolites found in the

perfusion medium result from metabolism of the parent compound after its permeation into the receptor fluid, and enzymes that have leaked into the receptor fluid from the cultured tissue mediate this biotransformation. Currently, there is no evidence to support this hypothesis.

The underlying assumptions inherent in the utility of using excised skin for diffusion experiments are (1) skin condition, particularly that of the stratum corneum, is comparable to that found *in situ* (i.e., a key variable is the barrier function of the skin sample once it is removed from the animal or man); (2) recovery of the applied substances in the receptor fluid provides a true reflection of the rates and extent of percutaneous absorption (i.e., tissue binding and partitioning into the receptor fluid are not confounding factors); (3) living processes have little or no effect on percutaneous absorption mechanism or kinetics (although some investigators have maintained the metabolic viability of the skin by using flow-through systems with a receptor solution rich in oxygen – see above); and (4) penetration through dermis is not rate limiting. Some of these premises are becoming increasingly difficult to justify in light of what we now know about active metabolism of some drugs within the skin and the influence of cutaneous blood flow on the clearance of drugs and their metabolites from the skin. For some other chemicals, on the other hand, *in vitro* data sets are becoming invaluable tools to predict their dermal penetration, particularly from aqueous vehicle. **Table 4** lists a number of important design considerations when assessing percutaneous absorption using in *in vitro* diffusion chamber experiments. As can be seen, many of these potential sources of error *in vitro* experiments could be predicted from the list of factors affecting skin absorption *in vivo* (**Table 3**). Others, such as skin thickness, barrier integrity/viability, and receptor fluid content, are true artifacts of the *in vitro* system.

Table 4 Experimental design considerations with *in vitro* assessments of percutaneous absorption

Factor	*Contributing factors*
Skin source	Animal species differences, fresh human vs. cadaver skin
Membrane thickness	Full-thickness vs. split-thickness (no dermis) vs. stratum corneum alone
Barrier integrity	Preparation and storage conditions, follicles and other holes, viability (metabolic capacity)
Receptor fluid	Solubility, maintenance of skin viability/barrier integrity
Dosing method	Finite vs. infinite, dose formulation
Environment	Ambient temperature and humidity, hydration state

In Vivo–In Vitro Correlation In the skin absorption literature there are only a few instances in which studies were designed specifically to correlate *in vivo* and *in vitro* observations. This is probably because such comparative experiments involve many variables that need to be controlled or monitored and are difficult to perform well. Meaningful comparisons can only be made when experimental parameters of the *in vitro* studies closely resemble those of the *in vivo* study or vice versa. Moreover, ethical and safety concerns often limit the extent to which *in vivo* experiments may be conducted in humans. Thus, *in vivo–in vitro* correlations using human skin are practically nonexistent. In addition, in humans the site of application is frequently the ventral forearm, whereas in animals the back is often used and potentially damaging pretreatments of the animal skin such as shaving, clipping, or chemical depilation (hair removal creams) are frequently necessary before skin absorption experiments can be conducted. Since these are treatment variables that can influence percutaneous absorption, the reported species differences and similarities in skin absorption may reflect the net result of many competing variables, and understanding the significance of these variables would provide additional insights into the mechanism of percutaneous absorption.

A general consensus among investigators in percutaneous absorption is that human skin is preferred and should be used for *in vitro* assessments. On the other hand, it is also recognized that a major liability of human skin as a research tissue *in vitro* is its notoriously high variability in barrier properties. The source of human skin is frequently from cadavers, and since the investigator often has little or no control over the source and characteristics of the donor skin, the high variability observed with human skin preparations is to be expected. Characteristics such as treatment of the cadaver, elapsed time from death to harvest of tissue, skin site, age, health, sex, race, and skin care habits are examples of variables which may bias the *in vitro* penetration studies. Also, when skin samples are derived from elective surgery, the preoperative procedures such as scrubbing with antimicrobial disinfectants, the surgical manipulations, and the manner in which the membrane is prepared from the excised tissue are important details of concern. Again, these variables may influence the *in vitro* penetration observations. It has been recommended that where possible, in an *in vitro* study with human skin, such information should be routinely collected and carefully documented.

Although human skin is the tissue of choice, its limited accessibility to many investigators and the variability experienced with human skin have led many researchers to explore skin from various animals as models for skin absorption. However, species differ considerably in the structure and function of their skin and it is unlikely that animal skin will have barrier properties that are identical to those of human skin. Nevertheless, animal skin is routinely used for evaluating dermal toxicity and percutaneous absorption. Histological evidence and physiochemical studies have concluded that animal skin can provide reasonable percutaneous absorption models that approximate human skin; however, the debate concerning the appropriate animal model continues. Numerous comparative studies, both *in vivo* and *in vitro*, have been conducted to identify the ideal animal model. From the results obtained thus far, it would appear that the choice of animal model will depend on the preference of the investigators and the compound under investigation. The pig, the monkey, hairless mice, hairless guinea pigs, and, recently, the fuzzy or hairless rat have been described as species with the potentials to be good candidates as predictive models of skin absorption in humans.

Because passive physical diffusion is assumed to be the principal determinant in skin absorption, and since the selection of an appropriate animal model remains controversial, artificial barrier systems have been explored as potential models for evaluating absorption in human skin. These systems offer some advantages over biological models in that they are reproducible, easily prepared, and the composition of the membrane can be readily manipulated. Such membranes offer a defined matrix with which basic physical concepts regulating permeation may be examined. Various materials have been used in the construction of artificial membranes, and they include chemicals such as silastic (silicone rubber), cellulose acetate, isopropyl myristate, mineral oil, and dimethyl polysiloxane. Materials such as collagen and egg shell membrane which are of biological origin have also been used. In general, the construction of these artificial membranes attempts to mimic the stratum corneum barrier, and their use in diffusion studies has provided some useful information on the underlying mechanisms governing the physiochemical properties of chemicals and the relative abilities of the chemicals to diffuse through lipid membranes. Artificial membranes have been used as models for evaluating potential drug formulations during the development of topical preparations and transdermal delivery systems, and they have proven particularly useful for exploring the bioavailability of chemicals from different vehicles. In cases in which the partitioning of the chemical from the vehicle into the skin and into the artificial membrane is rate limiting, the effect of changing the vehicle on dermal penetration can be predicted from artificial membrane studies. Indeed, the experimental procedure can be even further simplified, since it is only necessary in these cases to determine the relative partition coefficients *into* the artificial material from different vehicles (and not necessarily the relative steady-state fluxes), and simple probes or 'dip sticks' fashioned from the appropriate material will suffice for such purposes.

While there is no doubt that *in vitro* diffusion chamber experiments have made substantial contributions to the current knowledge concerning the diffusional aspects of skin absorption, there are limitations to their usefulness, particularly for developing predictive pharmacokinetic models of skin absorption. Standardized versions of these diffusion chambers are readily available commercially, and these systems are easy to use and are well designed, having incorporated many of the desirable features of diffusion chambers described by investigators with many years of research experience in the field of skin absorption.

Advanced Models: Isolated Organ Perfusion Methods A major limitation of all diffusion chamber experiments is the lack of normal vascular uptake mechanisms. One consequence of this inadequacy has been a large effort aimed at better diffusion chamber designs, particularly through the use of flowthrough devices mentioned previously. It should be noted that the recovery of material in the receptor fluid, which provides an overall measure of *in vitro* permeation (i.e., the net penetration through the various layers of the skin into the receptor), does not necessarily provide an accurate measure of percutaneous absorption. Material that permeates the skin and remains in the tissue is absorbed material that would not be included when receptor fluid only is assessed (although this does not matter if a steady-state dermal penetration coefficient for systemic absorption is being determined). Furthermore, various tissue slicing techniques or apparatus design considerations have failed to totally resolve the possibility that the thick dermis may represent an artificial and selective barrier limiting the permeation of lipophilic penetrants *in vitro*. There is no consensus on what constitutes an ideal receptor fluid. The selection of the optimum receptor fluid for a particular compound of interest is frequently empirical and often reflects the biases of the investigator. Therefore, caution should be exercised and *in vitro* observations should not always be considered to be true and

accurate representations of the *in vivo* situation with respect to cutaneous absorption and metabolism.

Given the obvious physiological limitations of the previously discussed organ culture and diffusion cell approaches, perfused skin preparations, with an intact and functional cutaneous microcirculation, appear to represent an ideal experimental methodology for investigating the pharmacokinetics and mechanisms of percutaneous absorption and metabolism. Following the development of the perfused rabbit ear model in the 1930s and the subsequent demonstration of its potential as a tool for studying skin absorption, surprisingly little progress was made over the next half century. Reports of perfused feline and canine skin flaps, both *in situ* (meaning 'on site,' or still attached to the animal) and *in vitro*, appeared sporadically in the literature. These models have provided useful information in studies of skin physiology and the basic pathways of cutaneous respiration and energy production. However, they have not been widely used to study skin absorption. In addition, although early attempts to develop human skin perfusion models have been documented, progress has been slow.

Recently, techniques for creating and maintaining isolated arterial sandwich skin flaps *in situ* in rats have been described. This rat skin flap is created on athymic nude rats by surgically raising a small area of skin, perfused by the superficial epigastric artery, and grafting a split-thickness skin sample from syngenetic rats (i.e., from the same breeding stock) onto the underside. Although athymic rats reject foreign skin grafts at a relatively high rate (up to 90%), some success has been achieved in creating and maintaining a hybrid rat–human sandwich flap (RHSF) on this animal by repeated low-dose cyclosporine therapy. It has been proposed that the RHSF might be useful for studying percutaneous absorption in human skin if it can be shown that the absorption mechanisms are unaffected by the surgical manipulations and cyclosporine treatments. The experimental advantages afforded by such human-grafted skin flaps, in addition to the fact that they are reusable, are intuitive. Unfortunately, the complicated surgical procedures, costly animal and housing requirements, and expensive cyclosporine therapy and its confounding effects on skin absorption, coupled with the apparently high variability in xenobiotic flux through the xenografts, place severe limitations on their utility as experimental models for studying cutaneous metabolism and skin absorption.

Since it has long been known that there is a high degree of anatomical and physiological similarity between skin obtained from certain pale-skinned porcine species and that of man, it is not surprising that various pig skin flaps have been pursued. The biochemistry and utility of pig buttock flaps, created surgically in several different patterns, for dermatological purposes have been extensively investigated. Proposed advantages for using pig skin flaps include the availability of large surface areas, similar vasculature and anatomic structure, and the ease and similarity in the types of clinical observations which can be made. Perhaps the most promising perfused skin preparation is the isolated perfused porcine skin flap (IPPSF), which has been developed recently and provides a novel *in vitro* approach for examining percutaneous absorption processes in intact, living skin. The biochemistry and morphology of the IPPSF, maintained using an isolated organ perfusion technique for skin which is essentially analogous to methods developed for other organs such as liver, lung, and kidneys, have been examined in great detail and appear to be consistent with that found in porcine integument *in vivo*. The absorption of a wide variety of topically applied xenobiotics has already been demonstrated using the IPPSF, including such diverse chemicals as organic acids and bases, organophosphate insecticides, and steroid hormones and organochlorines. In addition, the effects of applied surface concentration and coadministration of vasoactive drugs (tolazoline and norepinephrine) on lidocaine iontophoresis (electrically driven drug transport across biological membranes), as well as the iontophoretic transport of small peptides and proteins (insulin), have been examined using the IPPSF, demonstrating its potential for testing novel transdermal drug delivery systems. Cutaneous biotransformation of xenobiotics during percutaneous absorption has been demonstrated using the IPPSF with the chlorinated hydrocarbon, chlorbenzilate, and with the organophosphate, parathion. Among the limitations of this method are persistent issues with the choice of appropriate perfusion/receptor fluids (whole blood, plasma, artificial blood cocktails, etc.) and perfusion rates, and the often staggering complexity of the mathematical models needed to interpret some of the time-dependent absorption results.

Preliminary studies using the IPPSF have shown that compounds such as the cancer chemotherapeutic agents cisplatin and carboplatin and the antibiotics tetracycline and doxycycline readily distribute into the skin following intravascular administration. Also, compounds such as parathion, 1-aminobenzotriazole (ABT), and 25-hydroxyvitamin D are bioactivated in the skin following intravascular administration in the IPPSF. This demonstrates a role for the IPPSF as an ideal experimental model for studying the disposition of xenobiotics that are

distributed to skin from the systemic circulation. Interest in the so-called outward transdermal migration or reverse penetration concept, namely, that skin may function as a clearance organ following delivery of systemically administered substances via the cutaneous vasculature, has been stimulated by the development of noninvasive techniques for measuring and analyzing the pharmacokinetics of the distribution of substances to skin *in vivo*. The absence of confounding, extracutaneous metabolizing organs, such as the liver, lungs, and kidneys, is a distinct advantage in IPPSF investigations of this reverse penetration phenomenon.

In conclusion, there are many fundamental questions concerning skin absorption and metabolism that remain to be addressed. The potential role of the dermal vasculature, the contribution of skin appendages such as hair follicles and sebaceous glands, the influence of skin condition, age, disease state, and anatomic sites are just a few examples of questions that need to be resolved. When topical exposure results in local effects, pathological changes in the skin may be expected to affect its barrier functions. These changes may involve alteration of the physical barrier as well as the biochemical properties, such as the metabolic status of the skin. Such local changes may have important implications on the outcome of percutaneous absorption and fate of topically applied xenobiotics. The experimental techniques necessary to address these questions are available, and productive research in these areas will provide means whereby species differences in skin absorption and metabolism may be investigated. These studies should provide not only a better understanding of the mechanisms important in the percutaneous fate of topically applied chemicals but also a rational basis for cross-species extrapolation and, therefore, more predictive estimates for skin absorption and metabolism in man.

Etiology of Skin Toxicity

General Concepts

Because the skin is in direct contact with the external environment, it is constantly being exposed to drugs, chemicals, electromagnetic radiation, and physical materials capable of producing toxic responses in this organ. In addition, many drugs are delivered into the skin via the systemic circulation, which also may result in cutaneous toxicity. It is the purpose of this section to review and categorize the extensive list of agents that exert toxic effects within the skin. Without discussing specific mechanisms, which are described in detail later in this entry, it is necessary here to make a distinction between those agents that produce a direct irritant response and those that act via a systemic, immune-mediated pathway. The former is called irritant contact dermatitis (ICD), while the latter is called allergic contact dermatitis (ACD). Both ICD and ACD involve the participation of many immune cell types found in the skin and are often histologically and biochemically indistinguishable from each other. Moreover, a third category of skin reactions has emerged, called contact uticaria. Unfortunately, the mechanistic distinction (discussed later) between this syndrome and ICD is even more blurred than the ACD versus ICD comparison. Because the list of urticariants (**Table 5**) appears to be a subset of the contact irritants, representing materials from every chemical class, these agents will not be described separately in the categories developed for this section.

Table 5 Contact urticariants

Category	*Examples*
Natural agents	Birch bark, butter, cabbage, capsaicin, chicken, cinnamon, cobalt chloride, copper, cotton oils, eggs, fish, fruits (kiwi, strawberry), hawthorn, honey, horse saliva, laboratory animals, mahogany, milk, nickel, papain, prawn crust, seminal fluid, sorbic acid, spices, spider mites
Industrial sources	Alcohols, benzoates, BHT, carbamates, carbonless copying paper, chloramine, chlorhexidine, diethyl fumarate, DEETS, DMSO, formaldehydes, *p*-phenylenediamine, phosphorus sesquisulfide, plastics, rouge, rubber, sorbitan monolaureate
Pharmaceuticals	Aminophenazone, benzocaine, benzoyl peroxide, penicillins

Direct cutaneous irritation, or ICD, is one of the most common maladies in industrialized society. The symptoms of ICD are the classical inflammatory response markers: redness, swelling, pain, and loss of function. Although ICD is not often fatal, this disease does involve significant morbidity and takes a heavy economic toll due to its sheer prevalence. The incidence of ICD in the general populations of the United States and Western Europe has been variously estimated at between 1% and 10%; however, counting undiagnosed cases the true incidence may lie closer to 25%. It is well documented that ICD is the single most common occupational disease seen in the United States, with over 5000 man-made and natural chemicals known to be capable of irritating the skin. A simplistic classification of these irritants includes such agents as desiccants, abrasive materials, organic solvents, acids and alkalis, concentrated metallic salt

solutions, oxidizing/reducing agents, enzymes, plant extracts, and surfactants. The latter group of agents represents the various soaps and detergents used in the form of complex mixtures and marketed extensively as cleansers in personal, fabric, and hard surface care products. As such, surfactants are primarily responsible for ICD of household origin and are considered second only to organic solvents in producing occupational dermatitis.

Equally important from a dermatological viewpoint, although not nearly as prevalent, are the immune-mediated skin reactions, which can be broadly categorized as ACD. Whereas ICD is commonly thought to account for 60–80% of clinically recognized human contact dermatitis, ACD accounts for most of the remainder (20–30%). As will be discussed later in this entry, ACD is clinically and histologically indistinguishable from ICD in most cases. However, the presence of two etiologic factors renders this condition perhaps even more dangerous than ICD. First, once an individual has become sensitized to contact allergens, quite low amounts of the offending agent can subsequently elicit massive skin responses. Second, once induced, this hypersensitivity may persist for a long and varied period of time, possibly even for the rest of one's life.

Like ICD, ACD may also occur from a very large number of chemicals, but not from electromagnetic radiation or physical stimuli alone. Most substances are rarely allergenic and there is a great range in allergenic potency, with a small number of known strong sensitizers having been identified experimentally in man. These strong allergens are often aromatic substances with molecular weights less than 500, highly lipid soluble, and quite reactive with proteins (a mechanistic requirement, as will be detailed later). The simplistic classification of the principal ACD agents includes metallic salts, plant polyunsaturated alcohols and ketones, acrylates, plasticizers, antibiotics, aliphatic amines and phenols, and formaldehyde. The possibilities for human exposure to both contact allergens and contact irritants can be divided among four broad categories: consumer products, occupational or industrial chemicals, environmental agents, and pharmaceuticals.

Root Causes of ICD and ACD

Consumer Products As mentioned earlier, the soaps and detergents in cleaning products and cosmetics comprise the bulk of the household materials that are irritating to human skin. The molecules responsible for this type of ICD are called surface-active agents, or surfactants. There are four main classes of surfactants, which are listed in decreasing order of their irritancy: anionics (used as industrial-strength cleaners and fat-based soaps), cationics (mostly disinfectant cleaners), and nonionics and amphoterics (fabric cleaners, cosmetics, shampoos, and mild cleansers). The irritancy of surfactants is roughly correlated to their cleaning power and their ability to foam when mixed with water and air. Other consumer products likely to cause ICD are wool and fiberglass due to mechanical action of the fibers on the skin surface; formaldehyde residues found in newspaper inks, building materials, and clothing; and dry cleaning fluid residues of polychloroethylene. Diaper dermatitis is also a form of consumer product-induced ICD caused by the combination of enzymes in urine/feces and disinfectant cleansers used on the skin.

The largest group of agents capable of causing ACD in the household are perfumes and dyes used in cosmetics, toiletries, and clothing (**Table 6**). Metal salts, such as nickel salts, chromium salts, and cobalt;

Table 6 Chemical agents associated with ACD

Category	*Specific examples*
Plants	Barley dust, lichens (D-usnic acid), hops (colophony), hetzil, sawdust, sesquiterpene lactones (*compositae*, *frullania* spp.), tulips (tulipalin A), poison ivy (urushiol)
Plastics	Cyanoacrylate, epoxy resins, polyacrylates, phenolformaldehyde resins, polyurethane, rubber additives (thiuram, carbamates)
Metals	Nickel, cobalt, mercury, silver, chromates (welding fumes and cement), beryllium
Industrial chemicals	Bis-(4-chlorophenyl)-methylchloride, 3-bromo-3(4-chlorobenzoyl)-propionic acid, 4-bromomethyl-(6,8)-dimethyl-2(1 *H*) quinolone, bromomethyl-4-nitrobenzene, bromophthalide, 2-chlror-6-fluorobenzaldehyde-chlorooxime, hydrogen sulfide, *n*-hydroxyphthalimide, trimethyl hexamethylene diisocyanate, Solvents—formaldehyde, turpentine, persulfate, phosphorus sesquisulfide, thioureas, allylphenoxyacetate, dimethoxane, chloracetamides (paints, wood shavings)
Pharmaceuticals	Chloroquine sulfate, benzocaine, chlorpromazine, cytosine arabinoside, 4,7-dichloroquinoline, 2,6-dichloropurine, streptomycin, neomycin, vincamine tartarate, 2[4(5) methyl-5(4) imidazolyl-methyl-thio] C_{13} pyritinol hydrochloride
Pesticides	Calcium lignosulfate, captafol, captan, carbamates, dithianone, ethoxyquin, naled, pyrethrum, spiramycin, tetrachloroisophthalonitrile, thiuram, tylosine, virginiamycin
Cosmetics	Perfumes, deodorants, hair sprays, sunscreens, skin lotions/creams, nail polish, dyes, shampoos

organomercurials; and formalin are all sometimes used as preservatives in household products and cosmetics and can also become allergenic. In fact, reactions to nickel (jewelry) and nickel salts are typically the most prevalent response in diagnostic patch test studies involving a wide variety of known allergens. Certain pesticides (e.g., isothiazolone-containing biocides like Kathon) and sunscreens also produce ACD, although the more potent sensitizers, such as *p*-aminobenzoic acid, are no longer in general use as sunscreens. Finally, the component monomers from certain rubber and plastic materials may also leach out and cause ACD, although humans are more likely to be exposed to these molecules in the workplace.

Industrial Chemicals With the possible exception of consumer products, this category represents the largest and most widely studied group of irritants and sensitizers. Certainly, it consists of the widest range of chemical classes to which humans are routinely exposed. It has been estimated that occupational skin disease accounts for 40–60% of all lost work days and ~95% of the cost, with ICD being more prevalent than ACD. Moreover, 25% or more of the general population is considered to be atopic or predisposed to skin eruptions despite the lack of visual, or even histologic, evidence that the skin is compromised. Chronic exposure to damaging consumer products or environmental agents no doubt contributes to occupationally induced skin disease.

Table 7 lists high-risk occupations for developing ICD and ACD. A common factor in these occupations is the presence of water ('wet work') and exposures to organic solvents and surfactants. While water itself is not considered an irritant, continual wetting and drying of the skin usually produces many of the hallmark symptoms of ICD. Organic solvents are by far the chemical class most responsible for occupationally induced ICD. These chemicals are used as degreasing agents and lubricants in many processes in the electronics, manufacturing, and construction industries. They include, in decreasing order of their irritancy, chlorinated aliphatics (e.g., trichloroethylene and polychlorinated biphenyls), aromatics (benzene/toluene), aliphatics (*n*-hexanes), ketones (acetone), and alcohols. Surfactants, discussed earlier in the context of consumer products, represent the second most important class of industrial irritants.

Miscellaneous industrial irritants include alkalis, such as caustic soda, NaOH, cement, and lime used in mining, dying, tanning, and construction, as well as strong acids (sulfuric, chromic, nitric, hydrochloric, and hydrofluoric) used in ironworks, glass etching, and masonry. Hydrogen peroxide and organic peroxides in plastic manufacture and reducing agents, such as phenols, hydrazines, aldehydes, and thioglycollates, may also produce ICD in the workplace. Moreover, enzymes released from meats and fish have been known to cause ICD in processing/packing plants. Besides the rubber and plastics industries (monomers), the primary source of occupationally induced ACD is the manufacture of consumer products, or the raw materials thereof, containing perfumes, dyes, preservatives, biocides, and other specialty chemicals.

Table 7 High-risk occupations for ICD

Occupation	*Specific exposures of interest*
Baker	Soaps and detergents, fruit juices, spices, enzymes
Construction worker	Cement, chalk, acids, wood preservatives, glues, detergents, industrial solvents
Canner, food service industry	Soaps and detergents, brine, syrup, fruit and vegetable juices, fish, meat, poultry
Dental technicians	Soaps and detergents, soldering fluxes, adhesives, acrylics, solvents, mercury
Electricians	Soldering fluxes, metal cleaners (solvents), epoxy resins, PCBs and PBBs
Hairdressers	Soaps and detergents, shampoos, permanent wave liquids, bleaches, and dyes
Horticulture	Manure, fertilizers, pesticides, irritating plants
Mechanics	Detergents, degreasers (solvents), lubricants, petroleum products, battery acids, soldering fluxes, cooling system fluids (PEG), metal shavings
Nurses	Soaps and detergents, alcohols, disinfectants, hand creams
Printer	Solvents, acrylates, formaldehyde, phthalate esters (inks)
Agriculture	Pesticides, fertilizers, disinfectants, detergents, petroleum products, irritating plants, animal secretions

Environmental Agents Many plants contain rough hairs or large calcium oxalate crystals (*Dieffenbachia*, *Caladium*, and *Philodendron* spp.), both of which are capable of producing mechanical damage to the skin. In addition, enzymes like bromelin (pineapples) or mucanain (cowhage) and chemicals like capsaicin (nightshade) or polycyclic diterpene alcohols (spurges) are also somewhat irritating. Nettles produce a contact urticaria by direct injection of the inflammatory mediators acetylcholine, histamine, and 5-hydroxytryptamine (serotonin). Anthralin, a synthetic drug, but originally isolated from the araroba tree, is also an important environmental contact irritant. The primary

plant allergens are catechols present in the *Toxicodendron* genus which are responsible for the most common form of plant-induced ACD: poison ivy (urushiol)/oak/sumac. ACD is also caused by butryo- and sesquiterpene-lactones found in the *Primula obconica* and *Compositae* (ragweed and Australian bush) plant families. Finally, atmospheric changes can also cause or predispose certain individuals to have ICD since it has long been known that low ambient humidity (more common in winter) can impair the barrier function of skin.

Ultraviolet (UV) light, a principal toxic component of solar radiation, interacts with skin in a variety of different ways which deserve special mention here. Visible light, having wavelengths of 400–760 nm, is relatively harmless, but shorter wavelengths can produce devastating effects alone or in combination with 'photoreactive' drugs and chemicals (described below). The three important divisions of UV light are UVA (320–400 nm), UVB (280–320 nm), and UVC (220–280 nm). UVC is of little natural concern because these shorter wavelengths are almost entirely absorbed, or blocked, by the stratospheric ozone layer. UVB is the part of the solar spectrum responsible for the most damaging effects on the skin, although UVA is now felt to play a more prominent role in certain types of skin disorders. The more serious effects of UV exposure are pigmentation defects, actinic elastosis (premature skin aging), selective defects in immune function, actinic keratosis, squamous/basal cell cancers, and malignant melanomas. UVB alone produces a characteristic, ICD-like inflammatory response (sunburn) or can react with chemical agents in and on the skin to produce photoirritation, or photo-ICD. A list of common phototoxic chemicals is shown in **Table 8**. Most of the recognized photoirritants are drugs delivered into the skin from systemic, not topical, administration, although plant-derived phototoxins are also known. For example, a pigment from St. John's wort is delivered to the skin upon ingestion, reacts with sunlight, and causes a massive vascular leakage that may progress to sloughing of large patches of dead skin.

Photo-induced ACD, or photosensitization, is also a consequence of combined exposure to sunlight and certain chemicals. The vast majority of these reactions appear to result from UVA wavelengths acting on topical agents, although isolated and incompletely documented reports of photosensitization resulting from systemic administration have appeared in the literature. A list of selected photoallergens is shown in **Table 9**. All are substances that absorb UV light and most have a resonating structure, that is, aromatic ring(s). An important complication of photo-ACD is the development of persistent light reaction, seen with phenothiazines, wherein a marked sensitivity to light persists long after exposure to the photoallergenic chemical has ended.

Table 8 List of common photoirritants

Class	*Chemical agents*
Coumarins	8-Methoxypsoralen, 5-methoxypsoralen, trimethoxypsoralen
Polycyclic aromatic hydrocarbons	Anthracene, fluoranthene, acridine, phenanthrene
Pharmaceuticals	Tetracycline, sulfonamides, chlorpromazine, nalidixic acid, NSAIDs[a] (benoxaprofen)
Dyes	Eosin, acridine orange
Miscellaneous	Porphyrins, amyl-*O*-dimethylaminobenzoate

[a] Nonsteroidal antiinflammatory drugs.

Table 9 List of common photoallergens

Class	*Chemical agents*
Halogenated salicylanides	Tetrachlorosalicylanide, bithional, dibromosalicylanide, tribromosalicylanide, 4-chloro-2-hydroxybenzoic acid, *N*-butylamide (JADIT)
Coumarins	6-Methylcoumarin, 4-methyl-7-ethoxycoumarin, 7-methylcoumarin
Plants	*Compositae* family (ragweed, Australian bush)
Sunscreens	*p*-Aminobenzoate (PABA), glyceryl-PABA
Miscellaneous	Sulfonamides, phenothiazides, 4,6-dichlorophenylphenol, quinoxaline-1,4-di-*N*-oxide, musk ambrette

Pharmaceuticals Adverse drug reactions account for 3–5% of hospital admissions and occur in as many as 5% of patients who are already hospitalized. Cutaneous involvement is particularly common in these circumstances, especially in children, in part because these so-called skin rashes are easily identified. Although many of these conditions are relatively harmless, cutaneous adverse drug reactions (CADRs) may be only one symptom of a much larger, and potentially life-threatening, immune response to a drug, or CADRs may be severe in and of themselves. Moreover, except for the occasional irritancies produced by topical ointments or transdermal drug delivery devices (patches), which are often not drug related but are due to other chemicals/materials present in the formulation/device, CADRs are almost always a form of ACD.

The most common CADRs are the less severe exanthem-like (characterized by a small papular rash

which can cover large surface areas) and urticarial reactions, together accounting for over two-thirds of drug-induced skin rashes. **Table 10** presents a list of drugs that are often associated with CADRs. Antibiotics are the drug class most likely to produce skin reactions, particularly in children, in which they account for more than 50% of all prescriptions. In addition, this drug class is mostly responsible for the non-life-threatening skin rashes. However, as can be seen from **Table 10**, the situation is complex in that most of the drug-induced skin diseases have multiple causes, and many of the drugs are capable of causing more than one type of skin lesion. The probability that an individual drug will cause a particular CADR is under the control of many 'host' factors, such as genetics, age, sex, the presence of other drugs (interactions), and concurrent diseases (liver or kidney failure, for example). Thus, generalizations are not very useful in the case of CADRs.

Other types of skin reactions to drugs (**Table 11**) are less frequent, but some are much more severe and deserve special mention. These CADRs fall into three major classes: severe and life-threatening dermatoses, skin malignancies, and other skin reactions. The severe dermatoses are the erythrodermas, erythema multiforme, toxic epidermal necrolysis, and bullous or blistering diseases. The dangerous symptom common to most of these CADRs is the sloughing off of large areas of epidermis, leaving the underlying dermis unprotected from bacterial infection. Although exceedingly rare, the mortality of toxic epidermal necrolysis has been estimated at 34%. The other dermatoses generally respond better to withdrawal of therapy. Phototoxicity and photoallergic reactions to common drugs were described previously. Drug-induced skin tumors have provided increasing evidence for the role of the immune system in the inhibition of malignancy due to the observed higher frequency of skin tumors of patients receiving immunosuppressants. It is also possible that certain drug-induced dermatoses result in greater propensity toward skin malignancies. Although ideopathic lichen planus does not appear to result in greater incidence of skin tumors, lichenoid eruptions due to quinicrine appear to have predisposed some individuals to a subsequent squamous cell epithelioma. Since the latency period for skin cancers can be many years to decades, more examples of drug rashes leading to skin malignancies may be forthcoming. Finally, although not generally life-threatening, but often severely and socially debilitating, are pigmentation, hair, and nail changes, acne, and vascular inflammation, all of which are listed in **Table 11** under 'other lesions.'

Table 10 List of drugs often associated with CADRs

Class	*Specific drugs involved*
Antibiotics	Penicillins, cephalosporins, sulfatrimethoprim, sulfonamides, nitrofurantoin, isoniazid, rifampin
Anticonvulsants	Phenytoin, carbamazepine, barbiturates
Corticosteroids, gold, NSAIDs	Antiinflammatories
Others	Antineoplastics, allopurinol, diuretics (sulfa derivatives)

Skin's Response to Toxic Insult

General Considerations It is axiomatic that the body's reaction to injury is limited and it is often impossible to identify the causal agent based solely

Table 11 Types of CADRs

Category	*Subclass*	*Examples of drugs involved*
Exanthem-like erythemas (46%)[a]		Antibiotics, anticonvulsants
Urticarias (23%)		Antibiotics, antiinflammatories, opiate analgesics
Other erythemas (<20%)	SLE[b]	Antibiotics, anticonvulsants, oral contraceptives
	Erythrodermas	Sulfonamides, gold, isoniazid, streptomycin
	Lichenoid photosensitivity	quinicrine antimalarials (see **Table 5**)
Blistering diseases (<10%)	Erythema multiforme	Sulfonamides, penicillins, diclofenac, oxyphenbutazone, piroxicam, phenytoin, carbamazepine
	Ten[c] pemphigus	(Same as for erythema multiforme) penicillamine, captopril, piroxicam, penicillins, rifampicin
	Bullous pemphigoid	Frusemide, penicillamine, penicillin, PUVA therapy
Skin cancer (<1%)		Immunosuppressants, mexiletine, thioridazine, penicillamine, moduretic®, atenolol, quinacrine

[a] Where percentages are noted, this is the approximate frequency among all patients experiencing CADRs.
[b] Systemic lupus erythematosis.
[c] Toxic epidermal necrolysis.

on the observed responses. Toxic insults on the skin can result in a combination of functional, biochemical, and morphological changes. These alterations induced by toxicants do not differ, in general terms, from changes caused by physical or biological agents, but the magnitude of the changes that are observed at any point in time depends on the nature, rate, extent, depth, and duration of the insult. From a mechanistic viewpoint, toxic insults to the skin can be classified into two main categories, namely, direct injury (i.e., ICD or contact urticaria) and immune injury (i.e., ACD). However, as mentioned earlier, the basic pathological lesions and clinical features that are encountered in all inflammatory skin responses are essentially indistinguishable. Thus, irrespective of the mechanism, the manifestations of toxic responses of the skin to an insult are basically the same and are similar to those following any other cause of cell injury in other organs and tissues: degeneration, proliferation and repair, or any combination of these basic dynamic responses.

Degenerations are regressive changes within a cell or cell population in response to injury. They range from reversible changes such as atrophy, which may be considered an adaptative homeostatic response to an adverse environment, to irreversible changes such as necrosis or cell death, while still forming part of the living organ. In between are a range of cellular alterations, including hydropic changes, fatty changes, and other inclusions, resulting from cytoplasmic accumulation of water, lipids, and granular materials, respectively, all of which are derived from breakdown of intracellular components.

Proliferation, in contrast to degeneration, involves increased growth in response to an injurious stress. The hypertrophy and hyperplasia experienced may range from adaptative homeostatic responses to irreversible proliferation of a cell population, leading to cancer. Inflammation and repair are extracellular responses that often accompany degeneration and proliferation. They represent tissue responses that attempt to contain or remove the injurious agent and revitalize the damage tissue. The extent and nature of the inflammatory response varies according to the nature, extent, and duration of the injury and include vascular, neurological, humoral, and cellular responses at the site of injury. Acute inflammation is typically an immediate and early response to an injurious agent. The vascular and connective tissues adjacent to the injured cells are usually involved and may include local vasodilation with transient increased blood flow and increased vascular permeability, with egress of white blood cells into the injured tissue. These processes are coordinated and integrated by numerous inflammatory mediators (e.g., histamine and bradykinin) that are produced or released at the site of injury. Where injury persists, chronic inflammation ensues and is characterized by the accumulation or proliferation of macrophages, lymphocytes vascular endothelium, and fibroblasts at the damage site.

The goal of the inflammatory process is to rapidly effect the elimination of the causal agent and removal of debris from damaged cells by dilution and phagocytosis, as well as to initiate the repair process. Repair of the damaged tissue may be achieved by a process of regeneration, which involves the replacement of damaged cells with viable cells of the same type through proliferation of adjacent healthy cells. Where the intrinsic regenerative capacity of cells of the damaged tissue is limited or tissue damage is severe, repair will involve fibrosis, a process in which fibroblasts from adjacent connective tissue mediate the replacement of damaged cells, with a characteristic scar tissue formation as the inevitable consequence.

Nonneoplastic Lesions

Epidermal Lesions Since the skin is composed of various structures, the extent and degree of involvement of each component will depend on the agent itself and on the severity of the exposure. However, because of its location, the epidermis is always first exposed to externally applied toxicants. Consequently, many skin responses to adverse reactions are epidermal in nature and usually involve inflammation. In the mildest form of superficial skin injury, where damage is restricted solely to the epidermis and there is some degree of epidermal destruction, hyperplasia is generally the dominant response. The epidermal destruction ranges from focal keratinocyte swelling (e.g., spongiosis) to hydropic degeneration of the basal layers and focal cellular necrosis. Under these conditions, the basal cells typically respond by increasing cell division and the epidermis quickly regenerates to normal. However, when the insult is sustained the proliferative response continues and ultimately results in a thickening of the epidermis. A good example of such proliferative response is that observed in the thickened skin on the palms of manual workers and it is the result of a continued low-level abrasive injury. Depending on the particular cell layers of the epidermis that are affected, these hyperplasias are described as hyperkeratosis, hypergranulosis, and acanthosis for thickening of the stratum corneum, stratum granulosum, and stratum spinosum, respectively.

In severe injuries (e.g., corrosions), extensive epidermal necrosis, with accompanying damage to the

cells of the basement membrane as well the superficial dermis, is frequently encountered. In this case, the extensive epidermal necrosis may lead to various degrees of ulcerations and be seen as devitalized epithelial layers with pyknotic nuclei that loosely line the dermis. Alternatively, the epidermis itself has sloughed off leaving a denuded dermal surface exposed to the external environment. These ulcerations are frequently accompanied by inflammatory changes, with migration of inflammatory cells such as polymorphonuclear leukocytes to the site of ulceration at the junction of the necrotic and viable tissues. This is followed by regenerative and proliferative changes involving the surrounding viable epithelial and connective tissue elements in an attempt to repair the damage. The undamaged adnexal components (e.g., hair follicles) are generally the source of precursor cells involved in the regeneration of the epithelial layers, and fibroblasts from the surrounding dermis are responsible for repair by fibrosis. As the damaged epidermis is repaired, the dead layers are sloughed, eventually leaving a scar.

These scenarios represent the two extremes, with most forms of dermatitis falling somewhere in between. Mild to moderate injuries usually produce clinical conditions described as eczema and they represent a wide range of responses essentially involving various combinations of degeneration, proliferation, and inflammation. Inflammatory responses often dominate during the early stages and are characterized by erythema, exudation, and leukocyte migration. These responses are sometimes accompanied by bullae, or blisters, and abscesses, or pustule formation, resulting from epidermal accumulation of fluids and cellular debris, respectively. With chronic or protracted exposure to mild irritants, proliferation of the epithelium increases. The skin becomes thickened, fissures may develop, and the proliferating keratinocytes begin to differentiate abnormally in a process known as parakeratosis, where the nuclei are retained in the stratum corneum. Although proliferation, involving hyperplasia and/or hypertrophy, is the usual pattern of epidermal response to toxicant exposure, on rare occasions epidermal atrophy is observed wherein the epidermis responds with decreases in cell size or decreases in number of epidermal layers.

Dermal Lesions As alluded to previously, dermal responses to toxic insults can be elicited by direct penetration of the toxicant through the epidermis to the dermis and this may occur with or without the destruction of the epidermis. Furthermore, reactive processes, initiated in the epidermis as a consequence of epidermal exposure, while not injuring the dermis directly can also elicit dermal responses. In addition, dermal exposure to toxicants of systemic origin via diffusion through dermal capillaries may produce toxic responses in the dermis in the absence of associated injuries to the epidermis. As previously described, the extent and nature of the toxic response will depend largely on the severity of the insult and will likely involve a combination of mechanisms. Mild acute injuries can produce focal necrosis that may be accompanied by localized inflammatory infiltrations and possibly abscesses. On the other hand, severe injuries resulting from exposure to corrosive substances can produce dermal and eventual subcutaneous coagulative necrosis that may be very painful. Edema and congestion in both the dermis and the epidermis, with eventual formation of vesicles, often accompany allergic reactions in the dermis that result from either systemic or local exposure to toxicants. Prolonged dermal exposure to mild toxicants can result in chronic dermatitis and this is often associated with extensive subepidermal mononuclear infiltrates or with perivascular infiltrates. The presence of secondary infections often complicates the overall picture of the toxic response. Finally, proliferation of dermal fibroblasts accompanied by angioblastic activity completes the repair process and this frequently culminates in fibrosis, or dermal scarring.

Adnexal Lesions In response to toxicologic insults, the cutaneous adnexa (appendages) will also undergo the dynamic changes of degeneration, proliferation, inflammation, and repair in a manner similar to that described. Thus, during toxicant exposure, typical destructive and involutional changes (e.g., focal necrosis, edema, hypertrophy, and hyperplasia) are evident. However, severe acute or chronic injuries can result in the partial or, in certain instances, complete loss of skin appendages from the exposed area. This is due to the fact that although the epidermis can regenerate completely by cell migration from unaffected sites, the newly formed epidermis is unable to reconstitute the adnexal elements. When hair is the target of the toxic insult, alopecia (hair loss) is the main consequence. Hair is susceptible to damage by both external agents and agents reaching the hair matrix through the dermis. Two major types of injury are experienced, namely, matrix cell damage and keratolytic damage. Keratolysis, the dissolution of hair keratin, is generally associated with local or surface contact of the toxic agent with hair. The resulting hair loss, due to the increased fragility of the hair shaft, may involve local patches or extensive areas, depending on the extent of the exposure. Regrowth of hair generally occurs following removal

of the toxic agent as the hair matrix cells are not damaged.

Agents that damage the hair matrix cells may affect hair follicles at a specific phase of hair cycle, that is, during anagen or telogen. The effect of anagen toxicity is typically hair loss (anagen effluvium). The mechanism of toxicity involves interference of the rapid mitotic activity of the follicular cells, leading to either a cessation of growth and the loss of the hair or the later loss of excessively brittle hair at the site of a weak, constricted area in the hair shaft. Anagen effluvium can occur within one or two weeks of exposure to the toxic agent and a number of common cancer chemotherapeutic agents are known to be anagen toxicants. Hair loss is also a consequence of telogen toxicity. The onset of telogen toxicity is slower and occurs over months of exposure and may involve a variety of mechanisms. Anagen and telogen toxicity can occur simultaneously and typical early histological signs of toxicity may include the vacuolization, disappearance of mitosis, pyknosis of the nuclei in the follicular matrix, or the presence of nuclear and other debris in the hair shaft. When damage to the hair follicles is severe, there is the potential for complete and irreversible loss of hair follicles resulting in permanent alopecia. As indicated previously, although the epidermis has full regenerative capacity, the newly formed epithelium usually cannot regenerate the skin adnexa.

Another class of lesions of adnexal origin that is frequently seen as a result to exposure to a variety of agents, including grease, oils, coal tar, and cosmetic preparations, is acne. These acneiform lesions originate from the sebaceous glands and typically start with comedones and inflammatory folliculitis on the skin surface that is in direct contact with the causal agents. The resultant proliferation of the sebaceous gland follicular epithelium leads to the formation of lipid-filled keratin cysts, similar to those observed in acne vulgaris. Chloracne is a somewhat specific type of acneiform eruption which occurs after exposure to a group of halogenated aromatic hydrocarbons (e.g., polyhalogenated dibenzofurans, polychlorinated dioxins, polychlorinated naphthalenes, and polychlorinated biphenyls). Chloracne is characterized by small, straw-colored cysts, comedones, and, in severe cases, inflammatory pustules or abscesses may be seen. Histologically the changes that are seen during the development of chloracne begin with keratinization of the sebaceous gland epithelial duct and the outer root sheath of the hair follicle. The sebaceous gland is eventually replaced by a keratinous cyst and the typical fully developed lesion consists of a dilation of the upper third of the hair follicle, which is usually bottle shaped. No differentiation can be seen between the epithelia of the infundibulum and the sebaceous glands. Edema and mononuclear perivascular infiltrates are sometimes seen in the papillary dermis and late manifestations of chloracne often include mild fibrosis of the dermis, hypotrichosis, and hyperpigmentation. The affected areas are usually those located in the malar crescent of the face and behind the ears. The external genitalia, axillae, shoulders, chest, back, abdomen, and buttocks are sometimes involved, but lesions are rarely seen in the extremities. Chloracne often continues to appear even after exposure to the chemical agent responsible has ceased, possibly as a consequence of release from tissue depots since most chloracnegens tend to be highly lipophilic. Experimental chloracne has been produced in rabbits, monkeys, and hairless mice. This latter species is thought to be the most useful animal model for the disease, but the occasional presence of degenerative cystic hair follicles in normal hairless mice is a confounding factor with this model.

Selective local damage to other skin adnexa, such as the sweat glands, can occur with exposure to a number of cytostatic agents, such as cytarabine and bleomycin, which are used in human cancer therapy. The condition is characterized by necrosis of the epithelium lining the eccrine sweat duct, accompanied by acute inflammation and squamous metaplasia of the remaining cells of the eccrine apparatus. The mechanism for the selective toxicity is unknown, although high concentrations of these compounds in sweat may provide an explanation. Other chemicals that are toxic to the sweat gland include formaldehyde, arsenic, lead, fluorine, and thallium, all of which produce generalized anhidrosis (loss of the sweating mechanism) due to partial or total destruction of the eccrine system.

Neoplastic Lesions Cellular proliferation is one of the ways in which cells and tissues respond to an injurious insult, and the result is neoplasia, or cancerous growth when these proliferations show partial or complete loss of responsiveness to normal growth controls. Neoplastic lesions induced in the skin of experimental animals have played an important role in understanding the multistage process of chemical carcinogenesis. Tumors produced in this multistage process are initially benign exophytic lesions (e.g., papillomas), some of which may regress while others gradually convert into fully invasive, malignant, endophytic tumors (i.e., carcinomas). The mechanisms by which chemicals may lead to uncontrolled cell proliferation are outside the scope of this entry, but suffice it to say that chemical carcinogens may be divided into two categories based on their proposed

mechanisms of action: (1) genototoxic, or those acting intracellularly, usually directly damaging to DNA, and (2) nongenotoxic, or those which act via regulatory factors in the extracellular environment.

Papillomas are the most common neoplastic lesions occurring in rodent skin after exposure to chemical carcinogens. They generally arise from the infundibular region of metaplastic or hyperplastic hair follicles. They are composed of a series of folds, united by common stalks to the underlying skin, and have a cauliflower-like structure and appearance. The folds of a papilloma consist of a central connective tissue core covered by a thick layer of epidermis-like epithelium. The germinative layers of the epithelium contain numerous mitoses and there are distinct spinous and granular layers as well as a thick, fully keratinized stratum corneum. Papillomas may regress or continue their progression toward carcinomas, and confluency into larger malignant tumors can also occur.

Keratocanthomas are benign neoplastic skin lesions often found after exposure to UV radiation or complete carcinogens in various species, including humans. They originate in the hair follicles as an intradermal growth of epithelial prolongations. They have a cup-shaped architecture with a central horny crater that has a papillomatous exophytic component and an endophytic component of deeply penetrating epithelial cords, which appear not to invade the subcutaneous tissues. In mice, keratocanthomas generally progress to squamous carcinomas and regression is uncommon. In humans, however, they are generally considered to be abortive neoplasias that usually regress. Preneoplastic, intraepithelial lesions are commonly found in humans as the result of exposure to sunlight or arsenicals, but such lesions are not frequently inducible in animal models of chemical carcinogenesis. These preneoplastic lesions have the potential to progress to carcinoma.

Carcinomas of various types, for example, squamous cell and basal cell carcinomas, have been induced in many different laboratory species using UV light, other forms of ionizing radiation, and chemical carcinogens. Generally these tumors arise from existing papillomas, keratocanthomas, or intraepidermal preneoplastic lesions (in humans), as well as from otherwise normal or hyperplastic epidermis. In humans, cutaneous squamous cell and basal cell carcinomas are extremely common clinical problems and the major etiologic agent is generally considered to be chronic sun exposure. Fortunately, these tumors rarely metastasize and thus have low mortality, but they are locally destructive and can be associated with considerable morbidity. Melanomas, arising from the pigment-producing melanocytes in the epidermis, have been produced using chemical carcinogens in experimental animals. These melanotic tumors, which include both benign and malignant types, have generated considerable concern, particularly in relation to skin cancer in man. Melanomas, which metastasize widely, are responsible for more deaths than any other type of skin cancer. Chronic sun exposure is believed to be a major risk factor and the implication that UV radiation is a major causative agent in the pathogenesis of melanoma remains controversial. In experimental species, chemically induced melanotic tumors are less aggressive than the human malignant melanomas, thus they tend not to metastasize readily.

Other Responses Urticarias, or 'wheal and flare' reactions, are common skin responses produced by topical exposure to a variety of topical agents (**Table 5**), especially biogenic polymers released from plants and insects. The response generally occurs within one hour of exposure and involves the local release of vasoactive substances including histamine. Frequently, urticaria is associated with immunologic responses and is often an integral part of immediate hypersenstivity reactions to ingested agents (e.g., drugs involved in CADRs). Undesirable color or pigmentary changes are also encountered as adverse cutaneous responses to topical agents. Chemicals which show structural similarities to tyrosine, the major building block of melanin, are known to cause local loss of pigmentation, whereas increased pigmentation may result as a secondary consequence to a phototoxic response. Color changes in the skin may also occur as the result of cutaneous accumulation of endogenous (e.g., carotenemia from eating too many carrots) as well as exogenous (argyria from contacting silver) pigments. Subjective reactions such as itching, burning, or stinging sensations are often encountered by sensitive individuals following exposure to a variety of topical agents, primarily cosmetics and detergents. These reactions are entirely subjective and do not have any obvious manifestations that can be perceived by the outside observer. Nevertheless, they are considered by the affected individuals to be completely undesirable.

Mechanisms and Methods for Assessing Skin Toxicity

General Considerations

The classic signs of the inflammatory response in skin were recognized long ago in ancient Rome by the physician Celsus, who coined the Latin phrase, *Rubor et tumor, cum calore et dolor*, roughly

meaning redness and swelling, resulting in heat and pain. The underlying mechanisms whereby these processes take place in ICD, ACD, and contact urticaria were, of course, unknown at the time. Much work has been done to help clarify this mystery, and inflammation is now best described within the paradigm of two major phases: the vascular phase and the cellular phase. Although a third, more immediate, 'neurologic' phase has been identified recently, it is such a transient and poorly understood component of the inflammatory response that it bears little mention here.

The vascular phase represents the most acute response of the skin to the presence of an irritating chemical or to a potential allergen, taking place within minutes and generally lasting only a few hours. This phase is induced by several systems, first and foremost of which is the nonspecific release of inflammatory mediators by epidermal keratinocytes and dermal fibroblasts. Such vasoactive materials as IL-1β, other cytokines, and the arachidonic acid metabolites prostaglandin E_2, leukotriene D_4, and prostacyclin initiate a cascade of events resulting in vasodilation, increased vascular permeability, and the influx of blood cell constituents. A good analogy would be the situation presented by an overturned fuel tanker on a major highway. The roads would become swelled with traffic and the influx of police, fire trucks, ambulances, and onlookers would spill over into the surrounding countryside. Other systems involved in this early phase are the complement pathways, primarily C3a and C5a; the coagulation system (fibrin split-products, Factor XIIa, and thrombin); plasma bradykinin; and an immunological reaction mediated by mast cells, which are abundant in the dermis (7000–10 000 cells mm^{-3}). This latter component is the principal mechanism in contact urticaria (mentioned earlier) and the release of histamine, serotonin, heparin, and chemotactic factors from these mast cells is also important in initiating the cellular phase of the inflammatory response.

The cellular phase takes place over a period of several days and begins with leukocyte margination (contact with vascular walls) and the release of chemotactic factors causing the migration of neutrophils into the injured tissue. Neutrophils contain granules which provide microbicidal enzymes (myeloperoxidase and lysozyme), neutral serine proteinases (e.g., elastase and cathepsin G), β-glucuronidase, α-mannosidase, vitamin B_{12}-binding proteins, and collagenase. The net effect of these mediators is increased tissue oxygen consumption and the generation of reactive oxygen species, or free radicals (e.g., superoxide anions, peroxide radicals, and halide acids), all of which are lethal to invading pathogens and are somewhat responsible for the heat and pain which accompany the inflammatory response.

Table 12 Lymphocyte products acting on other cell types

Cell type affected	*Lymphocyte products involved*
Macrophage	Migration inhibitory factor (MAF), macrophage activating factor, macrophage aggregating factor, chemotactic factor, AG-dependent MIF
Nuetrophil	Chemotactic factor, leukocyte inhibitory factor (LIF)
Lymphocyte	Interleukins (IL-) 2, 3, 4, and 5; chemotactic factors
Eosinophil	AG-AB-dependent chemotactic factor, IL-5, migration stimulation factor
Basophil	Histamine releasing factor, IL-3
Other cells	Lymphotoxin, growth inhibitory factors, osteoclast activating factor (OAF)

Basophils and eosinophils may also be involved, especially in ACD. Basophils are similar to mast cells and play a role in delayed-type hypersensitivity, whereas eosinophilic migration is dependent on complement and chemotactic factors released early upon exposure to a contact allergen. Langerhan's cells and other macrophagic monocytes release IL-1 and other cytokines early and are important in antigen presentation to lymphocytes. The latter white blood cell type then proceeds to influence a number of other cellular responses (**Table 12**). Overall, there are three types of allergic reactions in skin: type I (anaphylaxis), typified by the 'wheal and flare' produced by IgA- and IgE-responsive mast cells; type III (immune complex), which is an anitgen–antibody response involving complement; and type IV (delayed-type hypersensitivity). The latter is by far the most prominent type of chemically induced ACD and begins with Langerhan's cell and lymphocyte presentation of antigen to regional lymph nodes, followed by a vascular phase 24–48 h later. As mentioned earlier, it is prerequisite for a molecule to produce ACD that it reacts chemically with proteins in the antigen presenting cells.

Experimental Models

***In Vivo* Techniques** Determination of eye and skin irritation potential is mandated for proper labeling of all consumer products, and is needed to meet various regulatory requirements (e.g., for chemicals or products to be transported across state lines in the United States, as required by the US Food and Drug Administration (FDA) and Department of Transportation (DOT), respectively). Animal testing for skin irritation (ICD) is almost exclusively restricted to modifications of the test first proposed by John

Draize at the FDA in 1944. The rabbit primary dermal irritation (PDI) bioassay, as recommended by the CPSC in 1981 (in the Federal Hazard Substances Act), provides the basis for the most modern version of this model. Slight modifications proposed by the Organisation for Economic Cooperation and Development (1981) and the US Environmental Protection Agency (1983) have recently been incorporated to reduce total animal use and eliminate the unnecessary discomfort of abraded test sites and overly long exposure periods. Briefly, 0.5 ml (liquids) or 0.5 g (solids) of each test substance are applied to unabraded sites only on three New Zealand White rabbits, under a 1×1 in. gauze pad, and the site is occluded with gauze and tape wrappings. Following a 4 h exposure period, the wrappings and patches are removed and the sites are gently swabbed free of residual test material. Scores ranging from 1 to 4 for both erythema and edema are based on visual observation of the test site immediately after patch removal and at 24 and 48 h postexposure. These scores are summed and divided by the total number of scores to calculate the PDI index, which serves as the *in vivo* response variable for each test substance. Occasionally, the guinea pig is used in place of rabbits in this assay or in full immersion studies and cumulative irritation (multiple doses) tests, while other species are used very infrequently. An exception to this rule may be the mouse ear swelling test (MEST), which has been undergoing extensive evaluation and validation in the past few years. Nevertheless, despite clear evidence that these animal models may not be relevant to the human condition, Draize-type testing is still a rather standard practice in the consumer products and cosmetics industries.

Human testing for ICD involves either single application patches or cumulative patching, usually fresh doses of the chemical every 48 h over a 21 day period, for most compounds. For soaps and detergents, specialized assays, called soap chamber tests and arm wash tests, are utilized. In the former, a modified Franz diffusion cell-type donor chamber is affixed to the forearm and the soap solution is left in contact with the skin surface for a few hours. Arm wash tests were devised to mimic actual use conditions. This test has been further modified to include multiple washes over a short time period, or an 'exaggerated' arm wash test, to help discriminate among milder irritants, which produce little or no response in the standard soap chamber, arm wash, or patch tests. The major limitation to all human tests is the large intersubject variability coupled with the heavy influence of environmental conditions on the skin's initial condition, which ultimately affects its ability to respond to irritant challenge. It is for this latter reason that most of these clinical assessments of ICD are performed in the summer months because cold, dry air alone can be very damaging to skin.

The situation with animal testing for ACD is somewhat more complicated than that for ICD tests, probably because the disease process and underlying mechanisms are more complex. The guinea pig is the standard animal model for ACD, based on the original intradermal injection studies of the nitro- and chlorobenzene classes of sensitizers in 1935. The following modifications of the original protocol are now in routine use: occluded patch test, ear-flank test, guinea pig maximization test, split-adjuvant test, guinea pig optimization or Freund's complete adjuvant test, and open epicutaneous test. The common feature to all these tests is that they are biphasic, employing an induction phase followed by a challenge phase. Their major limitations are the subjective nature of the visual scoring system and the fact that these are rather costly, time-consuming bioassays compared to the ICD counterparts. In addition, there are ethical concerns with the use of adjuvants, which are basically allergenic materials added to the assay to increase the response. Adjuvants alone, when injected intradermally, can cause considerable redness, swelling, and intense pain. Finally, there are two newer models under evaluation: a variation on the MEST and the local lymph node assay (LLNA). The LLNA is based on measurement of cellular proliferation and other parameters in white blood cells (lymphocytes) collected from the lymph node draining the site of exposure and has been accepted as an alternative to the guinea pig maximization test for assessing ACD by the US EPA, FDA and OSHA.

Human ACD assays are of two basic types: the so-called prophetic patch test or single-induction dose, which is insensitive and rarely used, and the repeat insult patch test (RIPT). The latter involves multiple applications (every other day for 2 or 3 weeks) of low concentrations of the test article during the induction phase, followed by a single 24 h exposure to a higher dose and visual scoring over a 3–7 day period during the challenge phase. A modification of the RIPT is Kligman's maximization procedure, which utilizes the irritating surfactant sodium lauryl sulfate to increase the skin's responsiveness to the test material. Besides the interfering factors cited previously for human ICD tests, a major limitation of the RIPT is the selection of nonirritating induction doses, vehicle effects, and the inability to properly evaluate the skin reactions. In addition, the results of RIPTs are the least quantitative of all the *in vivo* irritation and sensitization tests. This issue of quantitation is particularly important in human tests for both ICD and

Table 13 Instrumental methods for assessing cutaneous toxicity

Category	Instrument used (measured response)
Spectrophotometry	Dia-Stron erythema meter®, Minolta Chromameter®, Cortex Dermaspectrometer® (all measure a 'redness' index of erythema); laser Doppler Velocimeter (blood flow)
Evaporimetry	Servo-Med evaporimeter® (transepidermal or skin surface water loss)
Electrical properties	Skicon®, Corneometer®, Nova Dermal Phase Meter® (all measure conductance/capacitance to assess hydration state)
Calorimetry	Skin surface temperature, thermography
Mechanical properties	Dia-Stron Dermal Torque Meter®, rheometers, SEM 474 Cutometer®, gas-bearing electrodynamometer (all measure elasticity); Newcastle Friction Meter® (roughness); Cortex Dermascan®, and other ultrasound equipment (epidermal thickness)
Surface features	Anjinomoto Scopeman® or Microwatcher® image analyzers, ultrasound equipment, profilometers (roughness, flakiness, scaliness, etc.)
Miscellaneous	Differential scanning calorimetry, Fourier transform infrared spectrometry (changes in stratum corneum lipid structure/function)

Table 14 *In vitro* endpoints for predicting ICD

Class	*Specific examples of proposed markers*
Membrane integrity	Vital dyes (trypan blue, eosin); fluorescence (Hoechst, fluoresceins, rhodamine, ethidium bromide, propidium iodide); exogenous (^{51}Cr release); endogenous (LDH or alkaline phosphatase leakage, intracellular K^+, lipid peroxidation)
Subcellular function	Mitochondrial (MTT, XTT, Alamar blue, ATP); ribosomal (^{14}C-Leu or -URI incorporation); lysosomal (neutral red uptake/release); nuclear (^{3}H-Thy incorporation, DNA binding)
Cellular metabolism	Glucose utilization, O_2 consumption, growth inhibition, lactate/pyruvate ratios, glutathione/redox status
Inflammatory mediators	Arachidonic acid cascade (^{3}H-AA release, PGE_2 release, leukotrienes and HETEs); cytokine release (IL-1β, TNFα)
Morphology	Light and electron microscopic changes
Unknown mechanisms	Collagen swelling, Skintex®, Coumassie blue dye extraction from gelatin, quantitative structure activity relationships (QSAR computer models)

ACD, which normally depend entirely on subjective, visual assessments of erythema and edema. This need has led, in turn, to a large effort to develop instrumental methods for measuring the vast array of skin responses to toxic compounds (**Table 13**). Besides providing quantitative data for such diverse responses to cutaneous toxins as inflammation, altered hydration state (e.g., dryness and 'tight feel'), changes in elastic or mechanical properties, or altered surface morphology (e.g., roughness, scaliness, and flaking), these biophysical methods are much more sensitive than visual techniques. Moreover, some of these instruments have demonstrated utility in animal or *in vitro* studies of cutaneous toxicity.

***In Vitro* Techniques** During the past two decades, public pressure to reduce the use of animals in all areas of biomedical research has resulted in animal experimentation coming under close scrutiny and increased governmental regulation. One area in which alternatives to animal systems seem both feasible and justified is that of early screens in premarket safety evaluations. In fact, there are *in vitro* alternatives which have undergone large multiinstitutional validation studies and which are being extensively utilized for mutagenicity and ocular irritancy testing in the industrial setting. Furthermore, a number of alternative assays have been proposed as screens for cutaneous irritation, although the validation process has been much slower. Nevertheless, assays based on disruption of cell membrane integrity, metabolic activity, or growth; incorporation of radiolabeled nucleotides and amino acids; cellular release of inflammatory mediators; or induction of morphological alterations at the cellular level are all currently under evaluation (**Table 14**). These types of assays may be performed using human and nonhuman fibroblast and keratinocyte cell cultures or using the more complex, organotypic skin tissue and organ culture models. In addition, there are a number of techniques which do not involve tissue cultures, operate via unknown mechanisms or mechanisms that are unrelated to the ICD response *in vivo*, or which are known to be entirely correlative in nature. Many of the commonly used biochemical markers or endpoints associated with these alternative methods share significant limitations: (1) they often require high test substance concentrations, effectively killing a large fraction of the exposed cells, and there is no clear evidence that this degree of cytotoxicity is mechanistically relevant in ICD; (2) they produce extremely variable and unreliable results for diverse sets of test materials and are sometimes more costly than animal or human patch tests; and (3) they were primarily validated against *in vivo*

ocular irritation data. Since it is well documented that the potential for a chemical to produce eye irritancy is not well correlated with its irritability to the skin, the latter point is an important distinction to make in the validation of alternative models for predicting ICD.

The situation with *in vitro* models for predicting ACD, unlike its *in vivo* counterpart, is less complicated than that for ICD because there are very few *in vitro* systems which have even been proposed for ACD testing. This is also a consequence of the complexity of this disease since ACD involves the interaction of many organ systems, which cannot be properly simulated in any currently available cell or tissue culture model. Nevertheless, two assays that have shown some promise for predicting ACD with certain classes of allergens are the lymphocyte transformation test and the macrophage migratory inhibition test.

Conclusions

It is clear that skin is not just an inert, protective barrier that surrounds the body's internal organs, but rather is an active participant in the overall outcome of exposure to potentially injurious materials in the external environment. The significance of cutaneous reactions to topical agents, particularly the inflammatory response and carcinogenesis, is the subject of an increasing number of scientific investigations. From the perspective of the skin absorption process, this organ is at once a portal of entry for a variety of topically applied chemicals, a drug-metabolizing organ, and a target organ for local toxicity. Thus, knowledge of the mechanisms involved in translocating chemicals into and through the skin, coupled with its effect on the physiological disposition or availability of topically delivered chemicals to interact with skin and other body organs, is key to understanding cutaneous pharmacology and toxicology.

Local skin effects are not the only consideration for dermal toxicity. The role of the skin as a barrier preventing the free penetration of exogenous chemicals into the systemic circulation is equally important. Indeed, it is becoming apparent that the dermal route of exposure is in many cases comparable to inhalation and oral absorption as a potential source of potentially toxic chemicals in the body and forms an integral part of many multi-media multi-pathway risk assessments. In this context, for example, the (US) National Institute of Occupational Safety and Health is currently revising its current skin notations (which identify chemicals likely to present dermal hazards in the workplace) to take into account a compound's potential for dermal absorption, as well as its capacity to sensitize and damage the skin.

In this review, some of the theoretical models and experimental methodologies employed in dermatotoxicity studies, both *in vivo* and *in vitro*, have been described. It is suggested that a combination of these techniques may provide the basis for future experimental approaches toward increasing knowledge of the mechanisms of cutaneous toxicity. It should be emphasized that research in this area is evolving, such techniques are being developed, and the rationale by which *in vivo* or *in vitro* models are selected and utilized is under continual scrutiny. Further development in this area will necessitate improvements in bioanalytical techniques and a better understanding of the interplay between skin penetration, permeation, and metabolism, as well as the role of modulating factors that may influence the structure, function, and toxicology of the skin. As the underlying mechanisms are further elucidated, and experimental databases become more comprehensive, we will be seeing a greater role being played by quantitative structure–activity relationships and other physicochemical tools (including simple chemical measurements) in being able to more accurately predict the penetration and interaction of chemicals with the skin. Mathematical models that embody these mechanisms will allow predictions of dermal penetration for new compounds to be made from their physicochemical properties that will move from mere screening tools to increasingly powerful and useful predictors of skin penetration and potential local and systemic health effects.

See also: Acids; Alkalies; Carcinogen–DNA Adduct Formation and DNA Repair; Dioxins; Dyes; Fragrances and Perfumes; Hair; Hypersensitivity, Delayed Type; Nails (of the Fingers and Toes); Organophosphates; Photoallergens; Poisoning Emergencies in Humans; Safety Testing, Clinical Studies; Tissue Repair; Toxicity Testing, Alternatives; Toxicity Testing, Dermal; Toxicity Testing, Irritation.

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Smog *See* Great Smog of London.

Snake, Crotalinae

Gary W Everson

- SYNONYMS: Pit viper; Members of the Crotalinae subfamily in the United States include *Crotalus* species (rattlesnakes), *Agkistrodon* species (copperhead, cottonmouth), *Sistrurus* species (pigmy rattlesnake, massasauga)

Exposure Routes and Pathways

Most frequently, envenomation by North American pitvipers occurs subcutaneously. However, envenomation directly into an artery or vein has been documented and is associated with a rapid progression of life-threatening symptoms such as shock and cardiovascular collapse.

Toxicokinetics

Systemic absorption of venom is dependent on lymphatic transport following subcutaneous envenomation. The onset of local symptoms such as swelling and ecchymosis occurs within several hours. Cardiovascular, neurological, or hematological compromise varies in onset but may occur within 10–15 min following intravenous or intraarterial envenomation. The metabolism of venom components is not well understood. It is likely that venom components are inactivated by enzymes within tissues where the venom is ultimately distributed. The distribution of venom is variable and complex and possibly reaches different tissue sites unevenly. The biological half-life of Crotalinae venom has not been determined. Metabolized venom fractions are primarily eliminated by the kidney.

Mechanism of Toxicity

Snake venoms are complex mixtures of several different components or 'fractions' that can vary considerably within Crotalinae members. A complete review of venom components is beyond the scope of this review. Depending on the content of the venom, multiple organ systems may be affected. Historically, Crotalinae venom was classified as neurotoxic, hemotoxic, cardiotoxic, or myotoxic, depending on the species of snake involved in the envenomation. This oversimplifies the complex nature of Crotalinae venom. Clinically, a patient may develop such multisystem disorders as platelet destruction, internal bleeding, hypotension, paresthesias, and rhabdomyolysis.

Acute and Short-Term Toxicity (or Exposure)

Human

The severity of envenomation varies greatly and is dependent on various factors including the species involved, amount of venom injected, depth of envenomation (subcutaneous, venous, arterial), and the age of the victim. In general, Crotalinae venom initially produces local tissue changes that manifest

as swelling, ecchymosis, bruising, petechiae, pain, and erythema. Swelling may progress to involve the entire affected limb. These local symptoms commonly develop within minutes to several hours following envenomation. Because of poor tissue perfusion, local skin sloughing, and tissue necrosis may occur.

Crotalinae bites rarely penetrate the muscular fascial plane, consequently swelling of an envenomated extremity may be severe but rarely involves muscle compartments. In ~25% or more of Crotalinae bites, no venom is injected (dry bite). However, patients with dry bites may exhibit symptoms of erythema and slight swelling at the bite site due to trauma. Symptoms of dry bites are usually limited to the immediate area of the bite and require only wound management and follow-up care if necessary.

Systemic symptoms following envenomation may include paresthesias, coagulation disorders, thrombocytopenia, active bleeding, decreased hemoglobin, disseminated intravascular coagulation, hypotension, EKG changes, decreased level of consciousness, and rhabdomyolysis. In contrast to the venom of most rattlesnakes, the venom components of the Mohave Green rattlesnake (*Crotalus scutulatus scutulatus*) possess significant neurotoxic properties. Following Crotalinae envenomation, there is little local swelling and edema, which is normally used to measure extent of envenomation. Symptoms of envenomation from neurotoxic venom components may include paresthesias of the face and tongue and cranial nerve dysfunction resulting in ptosis and diplopia. The patient may experience confusion, disorientation, and coma. Generalized muscle weakness and shallow respirations may require intubation.

Clinical Management

Most first-aid measures that have been historically employed are of little value and some are dangerous and worsen medical outcome. The use of ice to prevent the spread of venom has been linked to an increased frequency of limb amputations and should never be employed. The incision of fang marks to relieve venom is ineffective and can result in nerve or artery damage. Tourniquet use may impede blood flow in the affected limb and contribute to local tissue damage. The application of electric shock at the bite site has shown to be ineffective in clinical trials and is also dangerous. However, the use of a properly applied constriction band as opposed to a tourniquet may possibly be effective in slowing the lymphatic distribution of venom. Anyone bitten by an unidentified snake requires evaluation in an emergency facility equipped to provide basic and advanced clinical life support. Aggressive supportive care is at least as important as the proper administration of antivenom in the outcome of a patient bitten by a venomous snake.

It is important to evaluate the clinical presentation of the patient as well as laboratory data to determine and guide the administration of antivenom. However, antivenom is not required in all patients who are envenomated and may not be necessary if there is no significant tissue swelling, systemic symptoms are absent, and laboratory parameters are normal.

When symptoms develop, a decision to start antivenom should be made. Patient response to the antivenom must be evaluated at frequent time intervals following administration of the initial dose to determine if further antivenom is required. CroFab™, a polyvalent sheep-derived (ovine) antivenom produced by Protherics, was approved by the Food and Drug Administration and is preferred over the older equine-based, Antivenin (Crotalidae) Polyvalent™ (Wyeth), because it is less antigenic and is now distributed much more widely than the Wyeth product. CroFab™ is marketed by Savage Laboratories. CroFab™ consists of highly purified ovine Fab fragments capable of neutralizing the toxic effects of most North American Crotalinae venoms. It contains a mixture of venom-specific Fab fragments and contains few of the proteins responsible for the allergic reactions associated with the Antivenin (Crotalinae) Polyvalent™ (Wyeth). Clinical trials to date have shown CroFab™ to be effective and safe in the treatment of Crotalinae envenomations. Acute reactions in the preliminary studies were minimal and serum sickness was virtually nonexistent. The initial dose of CroFab™ is four to six vials. Further doses may be given if symptoms continue to progress. Patients exhibiting life-threatening symptoms may require 30 or more vials of antivenom. The effectiveness of antivenom is directly related to its timely administration and the provision of adequate dosing. Clearly, the effectiveness of antivenom decreases as administration time is delayed.

See also: Animals, Poisonous and Venomous; Snake, Elapidae; Snakes.

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Snake, Elapidae

Gary W Everson

- SYNONYMS: *Coral snake*; *Micruroides euryxanthus*; *Micrurus fulvius*; *Micruroides euryxanthus*

Exposure Routes and Pathways

Envenomation by North American species of Elapidae occurs subcutaneously. The bite differs from Crotalinae species in that coral snakes possess smaller fangs and tend to grasp and hold on rather than strike and release. Venom is discharged through hollow fangs and the chewing motion allows more venom to be injected into the bite site. Due to the relatively small fang size, envenomation into an artery or vein is not likely.

Toxicokinetics

Systemic absorption of Elapidae venom is dependent on lymphatic transport following subcutaneous envenomation. The onset of neurotoxic symptoms usually occurs within 4 h but can be delayed up to 10 h following a bite. The metabolism of venom components is not well understood. It is likely that venom components are inactivated by enzymes within tissues where the venom is ultimately distributed. The distribution of venom is variable and complex and possibly reaches different tissue sites unevenly. The biological half-life of Elapidae venom has not been determined. It is likely that metabolized venom fractions are eliminated primarily by the kidneys.

Mechanism of Toxicity

Elapidae venom is composed of different components that vary among species. The venom of North American species contains fractions that are primarily neurotoxic. The venom results in a bulbar-type cranial nerve paralysis. In contrast to Crotalinae species, venom from North American elapids lacks most of the enzymes and spreading factors that cause local tissue destruction. Elapids from countries other than the United States can contain venom components different than that of North American coral snakes.

Acute and Short-Term Toxicity (or Exposure)

Human

The severity of an envenomation varies greatly and is dependent on various factors including the species of snake, amount of venom injected, and the age of the victim. Envenomation may occur despite the absence of identifiable fang marks at the bite site. Coral snake venom causes very little local tissue changes. Mild swelling, pain, and redness at the immediate bite site are generally the limit of the local reaction. The degree of local tissue reaction does not correlate with the degree of systemic symptoms, which may appear much later. Typically, lightheadedness, dizziness, or drowsiness marks the onset of systemic toxicity. Generalized muscle weakness, fasciculation, and tremor may develop. Increased salivation, nausea, and vomiting are also common. Neurological symptoms may progress to include slurred speech, ptosis, dysphagia, visual disturbances, muscle paralysis, and respiratory depression. Neurological symptoms may be delayed for up to 12 h after envenomation. Seizures may occur, especially in children. Death results from respiratory depression, hypotension, and cardiovascular collapse. The bite of the Arizona coral snake is associated with less severe progression of symptoms than that of the Eastern or Texas coral snakes. Headaches, blurred vision, and ataxia may be the limit of neurological symptoms following envenomation by the Arizona coral snake. Venom from exotic Elapidae species (cobras, kraits, mambas, and allies) may contain toxins that target the heart, coagulation factors, and other sites in addition to the central nervous system. Although rare, bites from exotic species may occur within the United States (e.g., zoo employees and herpetoculturists). In these cases, contacting the local poison center is essential in determining the nearest location of specific antivenom.

Clinical Management

Basic and advanced clinical life support is essential in the successful management of any coral snake envenomation. A coral snake bite is a medical emergency and requires immediate transport to the emergency department. Aggressive respiratory and cardiovascular support can be life saving. Establishing intravenous fluid support should be started soon after the bite. Early administration of *Micrurus fulvius* (Equine)® antivenin is essential following envenomation by the Eastern and Texas coral snakes. The antivenom is not effective for bites of the Arizona coral snake. Since local symptoms do not correlate with the severity of the envenomation, antivenom should be administered as soon as possible following envenomation, despite the absence of neurological symptoms. Three to five vials of

antivenom should be diluted in 100–500 cc of 0.9% sodium chloride. This should be infused intravenously over 30 min. This antivenom is equine based; anaphylaxis and delayed hypersensitivity reactions are not uncommon. A small amount of diluted antivenom should be administered as a test dose to check for allergic response. This procedure is outlined in the package insert included with the antivenom. Patients who exhibit a negative allergic response following the test dose may still develop an anaphylactic reaction. Therefore, one should be prepared at all times to treat an allergic reaction to the antivenom. Epinephrine, intravenous antihistamines, and corticosteroids should be readily available. Close observation of the patient is required to determine the patient's response to the antivenom. Additional doses may be required should neurological symptoms progress. Most envenomations require from three to ten vials of antivenom and further doses may be required in those patients exhibiting life-threatening symptoms. As in any snakebite, infection is possible and a broad-spectrum antibiotic could be considered. In addition, tetanus prophylaxis should be provided. Serum sickness may occur following the use of the equine-based antivenom. Although serum sickness is usually mild, an outpatient course of corticosteroid may be required in some cases.

Most first-aid measures are of little value and some are dangerous. The use of ice to prevent the spread of the venom has been linked to an increased frequency of limb amputations and should never be employed. Field procedures such as fang mark incisions may result in vein or artery damage and improperly placed tourniquets may impede blood flow. Electric shock directed at the site of envenomation has not been proven effective and is a dangerous procedure.

See also: Animals, Poisonous and Venomous; Snake, Crotalinae; Snakes.

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Snakes

Randy Powell

Introduction

There are over 2950 species of snakes currently recognized. Although all are limbless ectotherms, snakes occupy a variety of ecosystems and environments and exhibit a wide assortment of morphological, physiological, behavioral, and ecological adaptations. These adaptations have enabled snakes to occupy terrestrial, fossorial, arboreal, aquatic, and marine niches in almost every ecosystem on every continent except for Antarctica. Size variation among snakes is considerable, ranging from the smallest, less than 150 mm blind snakes and shield-tail snakes to the largest species of pythons and boas, which can reach lengths of over 8 m. Because of several anatomical adaptations to the skull and head, snakes are able to swallow prey items much larger in relationship to their own size. All snakes are carnivores and they employ different methods to overpower and kill their prey including: seize and swallow, constriction, and the use of venom. Smaller snakes that eat invertebrates and small vertebrates (earthworms, insects, frogs, etc.) utilize the seize and swallow technique, which entails grabbing the prey item with their mouth and swallowing it whole, usually while it is alive. Snakes that constrict also grasp the prey item first with their mouths and then quickly wrap their body around it and contract their muscles. The prey item is incapacitated as blood flow and respiration is increasingly restricted until death occurs.

Approximately 30–40% of snake species produce some type of buccal toxins or venom. The major adaptive functions of venoms and buccal toxins in snakes have been associated with food acquisition, defense, and predigestion of prey items. Venomous species possess specialized structures (venom glands or Duvernoy's glands), which produce venom or toxins and enlarged teeth or fangs, which inject or introduce the venom into the prey item. Venom contains enzymes and proteins that break down cellular structures and interferes with critical functions such

as circulation, ventilation, and nerve impulses. When a prey item is envenomated, pronounced physiological responses occur within the animal resulting in immobilization and ultimately death. In the case of human envenomation, the effects are a very serious medical emergency that can result in permanent scarring, dysfunction, the loss of a limb, or death. While the majority of snakes are nonvenomous and harmless there are numerous species that are medically important and dangerous to humans. Venomous snakebite remains a public health problem in many areas throughout the world and the incidence of global mortality from snake envenomation is estimated at 125 000 cases per year (**Table 1**).

Venomous Species

All venomous snake species occur within the superfamily Colubroidea 'advanced snakes', a large and ecologically diverse group distributed worldwide. Within Colubroidea there are five families that have species (all or in part), which are considered venomous: Elapidae, Viperidae, Hydrophiidae, Atractaspididae, and Colubridae.

The Elapidae family (cobras, coral snakes, and mambas) are a group of fast moving alert snakes. They have fixed nonretractable fangs located anteriorly on each maxillary bone (proteroglyphs). The majority of elapids are terrestrial with a few arboreal, semifossorial, or aquatic species. Elapids are distributed throughout Australia, Africa, the Americas, and southern Asia. Most elapids are small to moderate in size (0.5–2 m) with a few species reaching 3 m or more (the king cobra, *Ophiophagus hannah*, reaches lengths of over 5 m). Other noteworthy examples include the black mamba, *Dendroaspis polylepis*, a large aggressive and extremely fast snake from central Africa, numerous species of brightly colored coral snakes found throughout the Americas, and the ringhal or ring-necked spitting cobra, *Hemachatus haemachatus*, one of several species that have the ability to project or spray their venom.

Table 1 Annual deaths from snakebite worldwide

Country	*Estimated number of human fatalities*
Africa	20 000
Asia	100 000
Australia	<10
Central America (including Mexico)	1000
Europe	<40
Middle East	100
North America	<20
Oceania	200
South America	4000
Total	125 000

The Viperidae family (vipers and pit vipers) members are robust bodied with a distinct neck and somewhat triangular or club-shaped heads. Their moveable fangs located anteriorly on the maxillary bone are retractable and are folded against the roof of the mouth when not in use (solenoglyphs). Most viperids are terrestrial with a few arboreal and semiaquatic species. They are distributed worldwide with the exception of Australia and oceanic islands. Viperids can range in size from less than 25 cm to the large bushmasters, *Lachesis* spp., which can reach 3 m in length. Examples of viperids include the sawscaled viper, *Echis carinatus*, a wildly distributed species responsible for thousands of human fatalities each year, the gaboon viper, *Bitis gabonica*, a large tropical African species whose fangs can measure up to 50 mm, and the rattlesnakes (genera *Crotalus* and *Sistrurus*) found throughout the Americas.

The family Hydrophiidae (sea snakes and allies) includes both aquatic and terrestrial species. The aquatic species have laterally compressed bodies and paddle-like tails with many terrestrial taxa resembling viperids in body and head shape. All hydrophiines are proteroglyphs with fixed fangs located anteriorly on each maxillary bone. Their distribution includes tropical Indian and Pacific oceans, Australia, and New Guinea. Their size ranges from 30 cm to over 2.5 m. Some of the most toxic venoms are found in hydrophiines (**Table 2**). Noteworthy examples include the inland taipan or fierce snake, *Oxyuranus microlepidotus*, from central Australia, which has some of the most toxic venom of any terrestrial snake, the death adders, *Acanthophis* spp., and the strikingly colored yellowbelly sea snake, *Pelamis platura*.

The Atractaspididae family (burrowing asps, mole vipers, and stiletto snakes) are a group of terrestrial and fossorial snakes with short heads, cylindrical bodies, blunt noses, and rather small eyes. They are distributed throughout sub-Sahara Africa and the Arabian peninsular coast. Atractaspidids can range in size from less than 25 cm up to 1 m in length. The movable fangs are located on the maxillary bone, either anteriorly or posteriorly. They are unique in that each fang can be rotated laterally allowing it to be extended out from the side of the snake's closed mouth. Most atractaspidids are not considered dangerous to humans. However, some of the larger *Atractaspis* spp. should be regarded as dangerous and deaths have resulted from bites.

The family Colubridae (rat snakes, water snakes, racers, and allies) represents by far the most speciose

Table 2 Comparative toxicities of snake venoms

Species	*Family*	LD_{50}
Inland taipan, *Oxyuranus microlepidotus*	Hydrophiidae	0.025
Eastern brown snake, *Pseudonaja textiles*	Hydrophiidae	0.037
Reef shallows sea snake, *Aipysurus duboisii*	Hydrophiidae	0.044
Yellow bellied sea snake, *Pelamis platurus*	Hydrophiidae	0.067
Spiny-headed sea snake, *Acalyptophis peronii*	Hydrophiidae	0.079
Northern taipan, *Oxyuranus scutellatus*	Hydrophiidae	0.106
Black mamba, *Dendroaspis polylepis*	Elapidae	0.32
Eastern coral snake, *Micrurus fulvius*	Elapidae	1.30
King cobra, *Ophiophagus hannah*	Elapidae	1.8
Sidewinder, *Crotalus cerastes*	Viperidae	4.00
Puff adder, *Bitis arietans*	Viperidae	7.75
Boomslang, *Dispholidus typus*	Colubridae	10.00
Gaboon viper, *Bitis gabonica*	Viperidae	12.5
Copperhead, *Agkistrodon contortrix*	Viperidae	25.6

LD_{50} values equal milligram per kilogram administered subcutaneously in mice. LD_{50} values can be useful in establishing relative toxicity but do not necessarily extrapolate to other species. Venom toxicities within the same species can vary considerably across geographic ranges.

family of snakes. They are a diverse group both in body form and ecology with species inhabiting terrestrial, arboreal, fossorial, and aquatic habitats. Colubrids are distributed worldwide with the exception of oceanic islands and range in size from 20 cm to 3.5 m or more. Most of the colubrids have teeth without any groove or canal (aglyphic) and are considered harmless. However, many colubrids possess enlarged posterior maxillary teeth (opisthoglyphs) and specialized oral glands (Duvernoy's gland). Buccal toxins or 'venom' are secreted from Duvernoy's gland under low pressure from around the base of the enlarged teeth. The venom is not directly injected but is introduced more indirectly into the bite wound. Most of the 'venomous' colubrids are not considered dangerous to humans. However, there are several larger species that are 'mildly toxic' and bites can result in localized pain, edema, and ecchymosis and a few species are regarded as quite dangerous. Noteworthy examples in which human fatalities have been attributed to include the boomslang, *Dipholidus typus*, the tiger keelback, *Rhabdophis tigrinus*, and the vine snake, *Thelotornis capensis*.

Snake Venom

Snake venoms are complex mixtures of enzymes and proteins of various sizes, amines, lipids, nucleosides, and carbohydrates. Venoms also contain various metal ions that are presumed to act as cofactors and include sodium, calcium, potassium, magnesium, and zinc. Snake venoms have been studied much more thoroughly in members of the families Elapidae, Hydrophiidae, and Viperidae, with considerably less knowledge regarding venoms from Atractaspididae and Colubridae. There is a large degree of variability in venom composition at all taxonomic levels. In addition, within the same species, venom components have been shown to vary considerably among populations and across geographical areas. Venoms act on a variety of cells and tissues with pronounced physiological responses. Some of the actions of venom components include the digestion of cells and cell membranes, disruption of procoagulant and anticoagulant activities of blood, production of oxidizing agents, breakdown of collagen and the intercellular matrix between cells, and the disruption of nerve tissue. Snake toxins with defined actions include neurotoxins, hemotoxins, cardiotoxins, cytotoxins, and myotoxins.

Snake venom components can be grouped by their molecular weight. Low-molecular weight components (<1500 Da) are usually considered the least physiologically active and includes peptides, lipids, nucleosides, carbohydrates, amines, and metal ions. Larger venom components (mol. wt. 4500–10 000 Da) include polypeptide toxins such as postsynaptically acting neurotoxins and myotoxins. The largest components, the enzymes (mol. wt. 13 000–150 000 Da), comprise a diverse group and produce marked physiological effects. The percentage of enzymes in snake venom can vary widely and can constitute as much as 90% or more in some of the viperid venoms and as little as 25% in some elapid venoms. There are over 30 enzymes that have been identified in snake venoms (**Table 3**) including some that are common to all venomous snake families.

Venom and Research

Venomous snakes and venom have always been of interest to biologists. Historically, snake venoms were viewed as a valuable aid and were frequently used in early medical therapies. Ancient Egyptian and Chinese physicians utilized snake venoms as treatment for a variety of ailments and diseases. For over a century, snake venom has been used to develop antivenoms to treat snakebite envenomation. Currently, there are over 30 facilities worldwide that produce $\sim$120 different commercially available antivenoms. These antivenoms include both monovalent forms (effective for a specific species) and polyvalent forms (generally effective for several species that occur

Table 3 Enzymes found in snake venom

Family	*Enzymes*
Common in all families: Atractaspididae, Elapidae, Viperidae, Hydrophiidae, Colubridae	Adenosine triphosphate, L-amino acid oxidase, amylase, catalase, deoxyribonuclease, hyaluronidase, NAD-nucleosidase, 5′-nucleotidase, peptidase, phosphodiesterase, phospholipase A_2, phosphomonoesterase, ribonuclease
Prominent in Elapidae and Hydrophiidae	Acetylcholinerase, dehydrogenase lactate, glycerophosphatase, phospholipase B
Prominent in Viperidae	Argine ester hydrolase, collagenase, endopeptidase, factor X activator, fibrinogenase, kininogenase, metalloproteinase, prothrombin activator, serine protease, thrombin-like enzyme
Found in some Viperidae and Elapidae	Alkaline phosphatase, acid phosphatase, heparinase, lysophospholipase

within a limited region or country). Currently, there is an intensified interest in venom, particularly venom components or fractions. Numerous bioactive components and enzymes are present in snake venom. The modes of action and interaction on cells and tissues produced by these bioactive components make snake venom a tremendous interest to researchers. Venom components are used in basic research in physiology and biochemistry to delay or increase biochemical and cellular processes. Snake venom components are being used in a variety of medications and diagnostic tests. Examples of snake venom derived medications include Captopril (an angiotensin-converting enzyme inhibitor used for the treatment of hypertension and other cardiovascular disorders), Ancrod (an anticoagulant used in stroke patients), Reptilase (used to measure blood plasma clotting time and to diagnose dysfibrinogenemia), Cobroxin (an analgesic drug used to block nerve transmission), Nyloxin (used for severe arthritis pain), and Integrelin (used to treat acute coronary syndrome). In addition, preliminary data suggests snake venom components may yield new drugs to treat a variety of conditions from strokes and cancer to hypertension, heart disease, and neuromusculoskeletal disorders.

See also: Animals, Poisonous and Venomous; Snake, Crotalidae; Snake, Elapidae.

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Sodium

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst and Shirley B Radding, volume 3, pp. 185–186, © 1998, Elsevier Inc.

- Representative Compounds: Sodium chloride (NaCl); Sodium azide (NaN_3)
- Chemical Abstracts Service Registry Number: CAS 7440-23-5
- Synonym: Natrium
- Chemical/Pharmaceutical/Other Class: Alkali metals
- Chemical Formula: Na^+

Uses

Numerous industries use sodium compounds. They are used in detergents, hair straighteners, glass, paper, textiles, and wood pulp. Sodium chloride (table salt) is used in ion exchangers to soften water, and sodium bicarbonate is used in beverages, baking soda, and antacid pills. Sodium azide is used in

insecticides, and was investigated as an antihypertensive. Sodium violently decomposes in contact with water, forming sodium hydroxide and hydrogen, which may ignite spontaneously.

Background Information

Sodium is 2.83% of the crust of the earth. It is extremely water reactive, forming explosive hydrogen gas and Lye (NaOH).

Exposure Routes and Pathways

Ingestion is the primary route of exposure to sodium. Many foods contain sodium chloride naturally (e.g., milk, cheese, shellfish, and, to a lesser extent, meat and poultry). Nonetheless, most people add extra table salt to their food to the extent of 2000–7000 mg day^{-1}. In addition, all water supplies tested and nearly all carbonated beverages contain sodium. Inhalation of sodium is a minor route of exposure except in some industrial environments. Sodium in the air comes from the oceans. Dermal absorption is not normally considered an important exposure pathway.

Toxicokinetics

Ingested sodium compounds are usually completely absorbed. Once absorbed, sodium is distributed throughout all tissues in the body. Most sodium is found in the plasma. Urine and perspiration are the major routes of excretion. Heat and hard physical labor can contribute to excessive loss of sodium.

Mechanism of Toxicity

Very little is known about sodium's mechanism of toxicity. There is practically no information on the effect of sodium on enzymes. No information is available on metabolic alterations of the sodium ion.

Acute and Short-Term Toxicity (or Exposure)

Human

The metal itself is very corrosive to eye or skin. Sodium is associated with hypertension. Excess sodium results in an increase of extracellular fluid volume. Under these conditions the plasma protein concentration decreases. Sodium is an emetic; intake of excess sodium leads to nausea and vomiting. The accidental substitution of table salt for sugar has resulted in sodium poisoning in infants. These infants experienced increased body temperature, muscle twitching, and convulsions; in some cases, their kidneys were damaged. Sodium compounds with high pH values in solution (e.g., sodium hydroxide) are extremely corrosive to the skin and mucous membranes.

Chronic Toxicity (or Exposure)

Animal

Laboratory animals given a high salt diet develop hypertension.

Clinical Management

For extremely high sodium intake, peritoneal dialysis is the treatment of choice to lower the plasma sodium concentration. For exposure to sodium hydroxide, clinical management of skin corrosion is indicated.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists short-term exposure ceiling limit is 2 mg m^{-3}.

See also: Iodine; Potassium.

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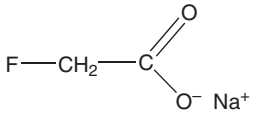
Sodium Fluoroacetate

David R Wallace

This article is a revision of the previous print edition article by Janice Reeves and Carey Pope, volume 3, pp. 186–187, © 1998, Elsevier Inc.

- Chemical Abstracts Services Registry Number: CAS 62-74-8
- Synonyms: 1080; SFA; Sodium monofluoro-acetate; FAA; Gifblaar poison; FAA
- Chemical/Pharmaceutical/Other Class: Rodenticide; Predacide
- Chemical Formula: $C_2H_2FNaO_2$
- Chemical Structure:



Uses

Fluoroacetate is primarily used as a rodenticide and is only available to licensed pesticide applicators. It is also used as a predacide against coyotes.

Exposure Routes and Pathways

Ingestion, inhalation, and dermal exposures are all possible, but ingestion is the major route of exposure. Sodium fluoroacetate is rapidly absorbed from the gastrointestinal tract and by the lungs.

Toxicokinetics

Fluoroacetate is rapidly absorbed by the gastrointestinal tract but not well absorbed dermally. Fluoroacetate is converted to the ultimate toxicant, fluorocitrate. Fluoroacetate is distributed to lipid-rich organs, such as the liver, brain, and kidneys. Fluoroacetate is primarily eliminated through urine. Up to 50% of the fluoroacetate is excreted unchanged in the urine by 72 h following administration. The kinetic half-life for sodium fluoroacetate is species dependent. Reported half-lives in rabbits, goats, and sheep are 1.1, 4–7, and 13.3 h, respectively.

Mechanism of Toxicity

Fluoroacetate produces its toxic action (after conversion to fluorocitrate) by inhibiting the Kreb's cycle. The compound is incorporated into fluoroacetyl coenzyme A, which condenses with oxaloacetate to form fluorocitrate. This inhibits the enzyme aconitase, which inhibits conversion of citrate to *cis*-aconitic acid/isocitrate. This inhibition will lead to a buildup of citric acid resulting in convulsions and death from cardiac failure or respiratory arrest. Mitochondrial uptake of acetate may also be affected. The heart and central nervous system (CNS) are the tissues most affected by this inhibition of oxidative energy metabolism. Oxygen consumption is markedly reduced. In addition to blockade of energy production, depletion of calcium may also be involved in the clinical manifestations associated with sodium fluoroacetate toxicity.

Acute and Short-Term Toxicity (or Exposure)

According to EPA RED facts about sodium fluoroacetate is characterized as a Toxicity Category I compound, the highest level of toxicity, with acute oral administration. It is a Toxicity Category II (moderate toxicity) for acute dermal exposure, Toxicity Category III (slightly toxic) as an eye irritant, and Toxicity Category IV (virtually nontoxic) for acute dermal exposure. Acute systemic toxicity resembles that of a metabolic poison with the target organs being the cardiovascular system, lungs, kidneys, and CNS. Generally, cold-blooded animals are more resistant to the effects of sodium fluoroacetate than are warm-blooded animals. There are also differences QJ;between herbivores and carnivores, with herbivores exhibiting more cardiovascular toxicity and carnivores more CNS effects. Omnivores show mixed effects.

Animal

Fluoroacetate is a compound of very high acute toxicity. Oral LD_{50} values in laboratory rodents range from 0.2 to 2 mg kg^{-1}. In mid- to high doses, testicular atrophy and renal tubule degeneration were observed in subchronic studies. There is a large variation in toxicity of fluoroacetate which is not due to differences in size of animal, type of digestive system, or basal metabolic rate. The variation may be due to the rate of elimination or rate of condensation of the poison with oxaloacetate. The oral LD_{50} in mammals is 110 μg kg^{-1}. In a 13-week oral gavage study in rats the no observed adverse effect level and the lowest observed adverse effect level were determined to be 0.05 and 0.20 mg kg^{-1} day^{-1}, respectively. The signs associated with this study were increased heart rate in both males and females,

decreased testis weight and altered spermatogenesis in males.

Human

Acute fluoroacetate poisoning can result in nausea, vomiting, cardiac arrhythmia, cyanosis, generalized convulsions, hypotension, and death from ventricular fibrillation or respiratory failure. Residual effects are uncommon if the patient survives the acute toxicity.

The LD_{Lo} (oral) for humans is $714\,\mu g\,kg^{-1}$. The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value – time-weighted average (TLV – TWA) is $0.05\,mg\,m^{-3}$. The probable lethal dose in humans is less than $5\,mg\,kg^{-1}$.

Chronic Toxicity (or Exposure)

Chronic exposure can lead to profound effects, both in the central and peripheral nervous systems.

Animal

Exposure to sodium fluoroacetate at a concentration of 26 ppm resulted in reversible growth retardation in rats with damage to the testes and sperm in males. In sheep, inhalation of $0.11\,mg\,kg^{-1}\,day^{-1}$ resulted in myocardial damage. Myocardial damage in animals is usually fatal, but may be asymptomatic while the animal is at rest.

Human

Chronic inhalation in humans can result in neurological dysfunction, liver dysfunction, and renal degeneration in humans exposed up to 10 years. The initial symptoms evident are nausea and mental apprehension. Soon following initial symptoms, convulsions, generalized CNS depression, and coma will result. Cardiovascular effects following chronic exposure are usually fatal and are characterized by ventricular arrhythmias.

In Vitro Toxicity Data

Differential outcomes are evident depending on the preparation used to study the *in vitro* effects of sodium fluoroacetate. Mitochondria-free preparations exhibit a K_i value of 22–45 μM for inhibition of aconitase by fluoroacetate. Aconitase bound to mitochondria appears to be much more sensitive to the effects of fluoroacetate: fluoroacetate inhibits aconitase in these preparations in the picomolar concentration range. Inhibition of the tricarboxylic acid cycle in actrocytes results in a depletion of ATP and a concomitant reduction in glutamine synthetase activity, resulting in elevations in glutamate concentration. Reuptake of glutamate is also inhibited due to reductions in ATP-dependent Na^+/K^+ pumps.

Clinical Management

The patient should be moved to fresh air. Decontamination of eyes and skin should be immediate. Treatment for skin and eye contamination should consist of rinsing with copious amounts of water for 10–15 min. For oral exposure, gastric lavage is preferable to emesis and should be prompt. Emesis should be avoided due to the potential for arrhythmias and convulsions. Charcoal should be administered as a slurry to block absorption of sodium fluoroacetate. Although there are no antidotes available, acetamide has been used with some success in a 10% solution in 5% glucose. Solution of calcium gluconate and sodium succinate ($130{:}240\,mg\,kg^{-1}$) has also exhibited some therapeutic benefit. Treatment is largely symptomatic. Respiratory and cardiovascular support is often necessary with significant exposures. Anticonvulsants (barbiturate) and antiarrhythmic (procainamide) agents are useful. Competition with acetate (in the form of acetamide or monoacetin) is recommended. Ethanol appears to be beneficial. Mephentermine is more efficacious than norepinephrine in raising blood pressure. Evidence of fluoroacetate poisoning can be difficult, but may be determined by fluorocitrate concentration in blood or by measuring increasing levels of citrate.

Environmental Fate

There is evidence that leaching and metabolism are the major routes of dissipation. Sodium fluoroacetate that has not undergone degradation is considered mobile by the Environmental Protection Agency (EPA) and has a high risk for movement into the soil and the ground water. Once adsorbed in soil, sodium fluoroacetate can be degraded by halidohydrolase in many microbial and fungal species. The 'half-life' of sodium fluoroacetate in soil is dependent on temperature, weather, initial amount of chemical, and decomposition of the host animal. There have been no reports that sodium fluoroacetate can leach into water and reach levels exceeding that which would be deemed toxic.

Ecotoxicology

Acute oral exposure of sodium fluoroacetate has been shown to be highly toxic to mallard ducks, chukar, ring-necked pheasants, widgeons, golden

eagles, and black vultures. Due to its use as 'toxic collar' predacide, sodium fluoroacetate has been shown to be extremely toxic to coyotes and other small wild rodents. Other nontarget animals which may be affected by sodium fluoroacetate toxicity include birds and other small animals that may feed on the neck of deceased animals which had a toxic collar. Reptiles and amphibians are relatively resistant.

Exposure Standards and Guidelines

The exposure limits (permissible exposure limits) set by the Occupational Health an Safety Administration is 0.05 mg m^{-3} for skin in both general industry and construction industry. This is the same limit (0.05 mg m^{-3}) established by ACGIH (TLV) and the National Institute for Occupational Safety and Health (recommended exposure limit).

See also: Gastrointestinal System.

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http://www.epa.gov – United States Environmental Protection Agency Integrated Risk Information System (IRIS), Sodium Fluoroacetate (CASRN 62-74-8).

Sodium Sulfite

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 7757-83-7
- Synonyms: Anhydrous sodium sulfite; Disodium sulfite; Exsiccated sodium sulfite; Sulftech; Natriumsulfit (German); Sodium sulfite anhydrous; Sodium sulphite; Sulfurous acid; Disodium salt; Sodium salt (1:2)
- Chemical/Pharmaceutical/Other Class: Inorganic salt
- Chemical Formula: Na_2SO_3

Uses

Sodium sulfite is an odorless, solid white powder with a salty sulfurous taste that is soluble in water. It is a reducing agent that is used as a food preservative and antioxidant. Its use is prohibited in meats and other sources of vitamin B_1. Sodium sulfite is also used in the treatment of semichemical pulp in the paper industry, in the treatment of water, as a photographic developer, and in textile bleaching (antichlor). It has also found historical use in the water treatment field as a dechlorinating agent.

Exposure Routes and Pathways

Sodium sulfite is not an 'environmental' pollutant *per se*, but its wide use as a food additive may lead to widespread exposure of the general population to trace amounts through ingestion. This may pose a problem for a small percentage of people who are hypersensitive to this chemical. Exposure to elevated concentrations (i.e., those that might cause abject toxicity) of this compound would only be expected to occur in the workplace, primarily involving sources of production or bulk use as mentioned previously. Because this compound is packaged as a powder, exposure would be expected to occur from airborne dust. Potential exposure routes would thus include skin, inhalation, and possibly involvement of the eye, nose, and throat.

Mechanism of Toxicity

The exact mechanism of toxicity has not been elucidated, although there is a lot of information on how sulfur-based compounds are detoxified by the liver. Sodium sulfite is a mild reducing agent that would most likely cause burning or irritation at the site of exposure or application by altering oxidation–reduction potential and pH.

Sulfites are used widely as antioxidants to keep foods from prematurely spoiling and to keep them looking 'fresh' by preventing oxidation and subsequent 'browning'. Many people, however, are 'sulfite sensitive'. After ingestion of food or beverages containing sulfite, these people may have allergic-type reactions such as asthmatic wheezing, hypotension,

tingling sensations, and flushing of the skin. The mechanism is unclear but probably has to do with an individual-specific chemical stimulation of the immune system, which in turn releases small amounts of vasoactive substances.

Acute and Short-Term Toxicity (or Exposure)

Animal

The median lethal dose (LD_{50}) measured for a mouse was $820\,mg\,kg^{-1}$. The LD_{50} for a rabbit ($2825\,mg\,kg^{-1}$) indicated that sodium sulfite was more than 3 times less toxic to rabbits than to mice. The lowest lethal dose for a cat or dog, administered subcutaneously, was $1300\,mg\,kg^{-1}$, whereas only half that dose was required to have the same effect on a guinea pig or rabbit. The LD_{50} for a mouse, administered intraperitoneally, was similar to the oral route ($950\,mg\,kg^{-1}$).

Human

Concentrated forms (e.g., powders, mixtures) of sodium sulfite may be harmful following exposure by inhalation, ingestion, and skin contact. It is an eye, skin, and respiratory irritant. At the concentrations used as a food additive, sodium sulfite is not toxic *per se* in humans; however, as mentioned previously, it will pose a problem for individuals who are sensitive to this chemical following ingestion. Allergic-type responses include asthmatic wheezing, a feeling of increased warmth and flushing of the skin, hypotension, and tingling sensations. Because some food manufacturers may use sulfites sporadically, it may be difficult for sensitive persons to avoid these additives altogether.

Clinical Management

Persons who are sensitive to sulfites should avoid foods containing this additive (e.g., wines), and those exhibiting severe allergic-type reactions (e.g., difficulty breathing) following a meal or beverage should seek immediate medical attention.

Persons exposed to large quantities of the dust in air should vacate the high-exposure area and seek conventional medical treatment if adverse symptoms are seen or if discomfort persists. As with exposure to any potentially irritating dust, eyes should be irrigated with water immediately following exposure and skin should be thoroughly washed with warm soapy water.

Ecotoxicology

There is very little environmental effects data on sodium sulfite. Water fleas (*Daphnia* sp.) exposed to sodium sulfite for 24–48 h have an LC_{50} between 200 and $300\,mg\,l^{-1}$ (US Environmental Protection Agency ECOTOX database). The pH of the solution, however, can strongly affect how much of the compound is ionized and thus greatly affect the toxicity.

See also: Food Additives.

Further Reading

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Relevant Website

http://toxnet.nlm.nih.gov – Specialized Information Systems, National Library of Medicine, Search for Sodium Sulfite.

Soil Pollution *See* Pollution, Soil.

Solanum Genus

Christopher P Holstege

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- Synonyms: *S. aculeatissimum* (devil's apple, bull nettle); *S. americanum* (black nightshade); *S. carolinense* (horse nettle); *S. dulcamara* (European bittersweet, blue nightshade, woody nightshade, climbing nightshade); *S. eleagnifolium* (white horse nettle); *S. gracile* (bull nettle, wild tomato); *S. melongena* (eggplant); *S. nigrum* (common nightshade); *S. pseudocapsicum* (Jerusalem cherry, natal cherry); *S. rostratum* (Buffalo burr, sandbur, Colorado burr, Texas thistle); *S. seaforthianum* (blue flowered 'potato vine'); *S. sodomeum* (apple of Sodom); *S. tuberosum* (Irish or common potatoes); *S. triflorum* (three-flowered nightshade); *S. villosum* (harry nightshade). Related plants to *Solanum* genus include: *Lycopersicum esculentum* (tomato); *Physalis heterophylla* (ground cherry); *Physalis longifolia* (husk tomato)
- Chemical/Pharmaceutical/Other Class: Solanine is a glycoalkaloid. Solanine is found throughout the plant with highest concentrations in areas of high metabolic activity such as the sprouts, green skin, and stems. Solanine is in highest concentration in the unripe fruit and its presence decreases as the fruit ripens.

Exposure Routes and Pathways

Reports of toxicity are secondary to ingestion of berries or herbage.

Toxicokinetics

The time to peak serum levels of solanaceous alkaloids is variable and depends on the species and amount ingested. Reports suggest peak levels are attained in 4–8 h. Solanine is converted to solanidine by hydrolysis. Solanine is rapidly excreted in urine and feces. The elimination half-life of solanidine is reported to be 11 h.

Mechanism of Toxicity

The mechanism of human toxicity has not been clearly delineated. In animal models, solanaceous alkaloids inhibit cholinesterase activity and demonstrate cardiac glycoside activity. Solanine inhibits hepatic microsomal enzymes and can cause hemolysis.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals may develop acute mental status changes including confusion, indifference to surroundings, prostration, and stupor. Ulcerative stomatitis, conjunctivitis, eczema, and diarrhea have been reported. Treatment focus is on symptomatic and supportive care.

Human

Gastrointestinal and neurological effects predominate in toxicity form in the solanaceous alkaloids. Reported clinical effects include nausea, vomiting, diarrhea, hyperthermia, tachycardia, bradycardia, hypotension, dehydration, blurred vision, mydriasis, salivation, flushing, diaphoresis, muscular cramps, headache, drowsiness, confusion, weakness, hallucinations, delirium, paresthesias, coma, and death.

Chronic Toxicity (or Exposure)

Human

Chronic exposure may produce more pronounced neurological effects and gastrointestinal effects, especially in times of starvation involving malnourished individuals.

Clinical Management

General supportive care is the focus of therapy. There is no antidote. Administration of activated charcoal may decrease absorption of the plant if given within an hour of the ingestion. Intravenous fluid and electrolyte replacement should be administrated as needed. Symptomatic patients should have continuous cardiac monitoring. Symptomatic bradycardia may be treated with atropine. For patients whose blood pressure does not respond to fluid replacement, vasopressors may be needed. Recovery can occur within hours to days.

See also: Plants, Poisonous.

Further Reading

Ceha LJ, Presperin C, and Young E (1997) Anticholinergic toxicity from nightshade berry poisoning responsive to physostigmine. *Journal of Emergency Medicine* 15: 65–69.

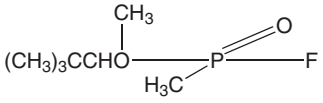
Dalvi RR and Bowie WC (1983) Toxicology of solanine: An overview. *Veterinary and Human Toxicology* 25: 13–15.

Soman

Harry Salem and Frederick R Sidell*

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 96-64-0
- Synonyms: GD; Phosphonoflouridic acid; Zoman; PFMP; G-agent; Methyl-1,2,2-trimethylpropyl ester; Pinacolyl methylphosphonoflouridate; Methylpinacolyloxyfluorophosphine oxide; Pinacolyloxymethylphosphonyl fluoride; Pinacolyl methanefluorophosphonate; Methylflouropinacolylphosphonate; Fluoromethylpenacolyloxyphonphine oxide; Methylpinacolyloxyphosphonyl fluoride; Pinacolyl methylfluorophosphinate; 1,2,2-Trimethylpropoxyfluoromethylphosphine; Nerve gas; Nerve agent
- Chemical/Pharmacetical/Other Class: Soman is a human-made nonpersistent anticholinesterase compound or organophosphate (OP) nerve agent, irreversible cholinesterase inhibitor, and chemical warfare agent. It is a light liquid with a camphor-like odor.
- Chemical Formula: $C_7H_{16}FO_2P$
- Chemical Structure:



Uses

Soman is a nerve agent used in chemical warfare.

Exposure Routes and Pathways

Casualties are caused primarily by inhalation but can occur following percutaneous and ocular exposure, as well as by ingestion and injection. Soman mixes easily with water, and people could be exposed by drinking contaminated water or via dermal contact with contaminated water. People could be exposed by eating contaminated food. Clothing can release soman for ~30 min, which could lead to exposure of other people. Soman vapor is heavier than air, and can sink to low-lying areas.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Toxicokinetics

Soman is absorbed both through the skin and via respiration. The half-life of soman in water at 30°C and pH 7.5 was reported to be 577 min compared to sarin at 30°C and pH 7.6, which was 5 min. Soman consists of a mixture of four stereoisomers:

1. C (−) P (−)
2. C (−) P (+) C (−) – soman
3. C (+) P (−)
4. C (+) P (+) C (+) – soman

The enzyme OP hydrolase hydrolyzes soman, tabun, sarin, and diisopropyl fluorophosphates at approximately the same rate.

Mechanism of Toxicity

Soman and the other nerve agents are organophosphorus cholinesterase inhibitors. They inhibit the enzymes butyrylcholinesterase in the plasma, the acetylcholinesterase on the red blood cell, and acetylcholinesterase at cholinergic receptor sites in tissues. These three enzymes are not identical. Even the two acetylcholinesterases have slightly different properties, although they have a high affinity for acetylcholine. The blood enzymes reflect tissue enzyme activity. Following acute nerve agent exposure, the red blood cell enzyme activity most closely reflects tissue enzyme activity. During recovery, however, the plasma enzyme activity more closely parallels tissue enzyme activity.

Following nerve agent exposure, inhibition of the tissue enzyme blocks its ability to hydrolyze the neurotransmitter acetylcholine at the cholinergic receptor sites. Thus, acetylcholine accumulates and continues to stimulate the affected organ. The clinical effects of nerve agent exposure are caused by excess acetylcholine.

The binding of nerve agent to the enzymes is considered irreversible unless removed by therapy. The accumulation of acetylcholine in the peripheral and central nervous systems leads to depression of the respiratory center in the brain, followed by peripheral neuromuscular blockade causing respiratory depression and death.

The pharmacologic and toxicologic effects of the nerve agents are dependent on their stability, rates of absorption by the various routes of exposure, distribution, ability to cross the blood-brain barrier, rate of reaction and selectivity with the enzyme at specific foci, and their behavior at the active site on the enzyme.

Red blood cell enzyme activity returns at the rate of red blood cell turnover, which is ~1% per day. Tissue and plasma activities return with synthesis of new enzymes. The rates of return of these enzymes are not identical. However, the nerve agent can be removed from the enzymes. This removal is called reactivation, which can be accomplished therapeutically by the use of oximes prior to aging. Aging is the biochemical process by which the agent-enzyme complex becomes refractory to oxime reactivation. The toxicity of nerve agents may include direct action on nicotinic acetylcholine receptors (skeletal muscle and ganglia) as well as on muscarinic acetylcholine receptors and the central nervous system (CNS).

Recently, investigations have focused on organophosphate nerve agent poisoning secondary to acetylcholine effects. These include the effects of nerve agents on γ-amino butyric acid neurons and cyclic nucleotides. In addition, changes in brain neurotransmitters such as dopamine, serotonin, noradrenaline, and acetylcholine, following inhibition of brain cholinesterase activity have been reported. These changes may be due in part to a compensatory mechanism in response to overstimulation of the cholinergic system or could result from direct action of nerve agent on the enzymes responsible for noncholinergic neurotransmission.

Human Toxicity

Toxic effects occur within seconds to 5 min of nerve agent vapor or aerosol inhalation. The muscarinic effects include ocular (miosis, conjunctival congestion, ciliary spasm, and nasal discharge), respiratory effects (bronchoconstriction and increased bronchial secretion), gastro-intestinal effects (anorexia, vomiting, abdominal cramps, and diarrhea), sweating, salivation, and cardiovascular (bradycardia and hypotension) effects. The nicotinic effects include muscular fasciculation and paralysis. The effects on the CNS can include ataxia, confusion, loss of reflexes, slurred speech, coma, and paralysis.

Following inhalation of soman, the median lethal dosage (LCt_{50}) in humans has been estimated to be 70 mg min m^{-3} at a respiratory minute volume of 15 l min^{-1} for ten minutes. For percutaneous liquid the LD_{50} has been estimated to be 350 mg per 70 kg human. The permissible airborne exposure concentration of soman for an 8-h workday or a 40 h work week is an 8 h time-weighted average of 0.00003 mg m^{-3}.

Doses that are potentially life threatening may be only slightly larger than those producing minimal effects. Vapor exposure to the eyes and nose causes miosis and runny nose at ECt_{50} dosages of less than 2 mg min m^{-3}. The median incapacitation dosage (ICt_{50}) of vapor inhalation has been estimated as 35 mg min m^{-3}, while the LCt_{50} is 70 mg min m^{-3}. These vapor exposure durations are from 2 to 10 min. Individuals intoxicated with soman exhibit miosis, visual disturbances, headache and pressure sensation, runny nose, nasal congestion, salivation, tightness in the chest, nausea, vomiting, giddiness, anxiety, difficulty in thinking, difficulty in sleeping, nightmares, muscle twitching, tremors, weakness, abdominal cramps, diarrhea, and involuntary urination and defecation. These effects may progress to convulsions and respiratory failure. Depending on dose, the onset of signs and symptoms may occur within minutes or hours.

Clinical Management

Management of nerve agent intoxication consists of decontamination, ventilation, administration of antidotes, and supportive therapy.

The three therapeutic drugs for treatment of nerve agent intoxication are atropine, pralidoxime chloride, and diazepam.

Atropine, a cholinergic blocking or anticholinergic drug, is effective in blocking the effects of excess acetylcholine at peripheral muscarinic sites. The usual dose is two mg, which may be repeated at 3–5 min intervals. Pralidoxime chloride (protopam chloride; 2-PAM CL) is an oxime used to break the agent-enzyme bond and restore the normal activity of the enzyme. Abnormal activity decreases and normal strength returns to skeletal muscles, but no decrease in secretions is seen following oxime treatment. The usual dose is 1000 mg (iv or im), which may be repeated two or three times at hourly intervals, intravenously or intramuscularly. Diazepam, an anticonvulsant drug is used to decrease convulsive activity and reduce brain damage that may occur from prolonged seizure activity. It is suggested that all three of these drugs be administered at the onset of severe effects from nerve agent exposure, whether or not seizures occur. The usual dose of diazepam is 10 mg (im).

Miosis, pain, dim vision, and nausea can be relieved by topical atropine in the eye. Pretreatment with carbamates may protect the cholinesterase enzymes before nerve agent exposure. It is available in 30 mg tablets and the tablets should be administered every 8 h. When used prior to exposure, it should be followed by atropine and pralidoxime chloride after exposure.

Supportive therapy may include ventilation via an endotracheal airway if possible and suctioning of excess secretions from the airways.

Animal Toxicity

The stereoisomers of soman have different median lethal doses. The C(+)P(+) soman and the C(−) P(+) soman are the least toxic and subcutaneous LD_{50} values $\geqslant 5000$ and $\geqslant 2000\,\mu g\,kg^{-1}$, respectively. The more toxic stereoisomers, C(−) P(−) soman and C(+)P(−) soman, have subcutaneous LD_{50} values of 38 and $99\,\mu g\,kg^{-1}$, respectively. The racemic mixture of soman has a subcutaneous LD_{50} of $156\,\mu g\,kg^{-1}$ in mice.

The cause of death is attributed to anoxia resulting from a combination of central respiratory paralysis, severe bronchoconstriction, and weakness or paralysis of the accessory muscles for respiration.

Signs of nerve agent toxicity vary in rapidity of onset, severity, and duration of exposure. These are dependent on the specific agent, route of exposure, and dose. At the higher doses, convulsions and seizures indicate CNS toxicity.

Following nerve agent exposure, animals exhibit hypothermia resulting from the cholinergic activation of the hypothalamic thermoregulatory center. In addition, plasma concentrations of pituitary, gonadal, thyroid, and adrenal hormones are increased during organophosphate intoxication.

An LCt_{50} of $30\,mg\,min\,m^{-3}$ was reported in rats following a 30-min inhalation exposure to soman. The acute toxicities by other routes of exposure in various animal species are presented in **Table 1**.

Table 1 Acute toxicities of soman in various species by various routes of exposure

Route of exposure/species	*LD_{50} ($\mu g\,kg^{-1}$)*
Percutaneous	
Rat	7800
Subcutaneous	
Chicken	50
Dog	12
Guinea pig	24
Monkey	13
Rabbit	20
Mouse	10
Rat	71
Intramuscular	
Monkey	9.5
Mouse	89
Rat	62
Intraperitoneal	
Chicken	71
Frog	251
Mouse	393
Rat	98
Intravenous	
Cat	15
Rat	44.5
Mouse	35

See also: Nerve Agents; Sarin; Tabun; V-Series Nerve Agents: Other than VX; VX.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

Speed

Henry A Spiller

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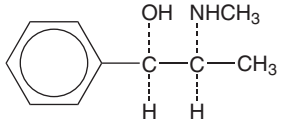
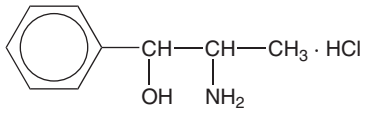
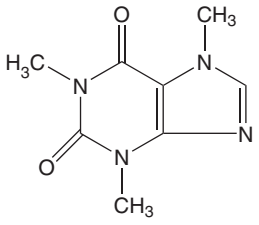
- Representative Chemicals: Ephedrine; Caffeine
- Chemical Abstracts Service Registry Numbers: CAS 299-42-3 (Ephedrine); CAS 58-08-2 (Caffeine)
- Synonyms: Street speed; 'Look alike' drugs; White crosses; Pink hearts; Black beauties; 357s; 357 magnums; Dexies; Robin eggs; Minithins; Stacker 2
- Chemical/Pharmaceutical/Other Class: Speed generally contains one or more agents belonging to the drug class of sympathomimetics
- Chemical Formulas: $C_{10}H_{15}NO$ (Ephedrine); $C_9H_{13}NO$ (Synephrine); $C_8H_{10}N_4O_2$ (Caffeine)
- Chemical Structures:



Ephedrine



Phenylpropanolamine hydrochloride



Caffeine

Uses

Ephedrine is labeled for sale as a bronchodilator. Caffeine is marketed as a minor stimulant to produce greater alertness or reduce drowsiness. Synephrine is available from plant sources as an 'herbal' weight loss supplement.

Background Information

Speed is the nomenclature used for a number of preparations that resemble and are often misrepresented as prescription amphetamines. They are used as substitutes for amphetamines. Speed is commonly composed of ephedrine, caffeine, synephrine, or a combination of these agents. Ephedrine is probably the most frequently encountered component of street speed. Herbal weight loss products containing ephedra, which is made up a number of sympathomimetic alkaloids, were removed from the US market in 2003. Phenypropanolamine, previously marketed as a weight loss supplement and oral decongestant and abused as a 'look alike', was removed from the US market in 2002.

Exposure Routes and Pathways

Oral speed preparations may be in tablet or capsule form. Ingestion is the most common route of intentional or accidental exposure.

Toxicokinetics

Ephedrine, synephrine, and caffeine are all well absorbed from the gastrointestinal tract. Following ingestion of an oral dose, clinical effects are seen within 60 min and persist from 2 to 6 h. Ephedrine is metabolized in the liver by oxidative deamination, demethylation, aromatic hydroxylation, and conjugation. Metabolites of ephedrine include norepinephrine, benzoic acid, and hippuric acid. Ephedrine is resistant to metabolism by monoamine oxidase. Ephedrine, synephrine, and caffeine have wide distribution throughout the body following oral administration. Ephedrine is presumed to cross the placenta and distribute into breast milk.

Ephedrine, synephrine, and caffeine are excreted in the urine. The rate of urinary excretion of ephedrine is dependent on urinary pH. The elimination half-life of ephedrine is 3 h when the urine is acidified to a pH of 5.0 and 6 h when the urinary pH is 6.3.

Mechanism of Toxicity

Sympathomimetic agents frequently found in speed stimulate α-adrenergic and β-adrenergic receptors and also stimulate the release of neuronal norepinephrine. Sympathomimetic drugs stimulate the sympathetic division of the autonomic nervous system. Stimulation of β-adrenergic receptors in the heart initially produces a positive inotropic effect on the myocardium. However, large or frequent doses produce a negative inotropic effect. With prolonged use, ephedrine, in particular, may deplete norepinephrine stores in sympathetic nerve endings, and tachyphylaxis to the cardiac and pressor effects may develop.

Acute and Short-Term Toxicity (or Exposure)

Animal

The clinical effects after overdose include anorexia, diarrhea, dehydration, hyperexcitment, tachycardia, tremor, weakness, seizures, and death.

Human

The clinical effects following overdose of sympathomimetic agents depend on the particular receptor selectivity and consist of α-adrenergic and/or β-adrenergic stimulation. Hypertension is usually the predominating symptom and may be accompanied by tachycardia or bradycardia, depending on the drug involved. The bradycardia is primarily a reflex bradycardia in response to hypertension. Cardiac arrhythmias, hypertensive crisis, and myocardial ischemia are possible effects of excessive exposure to sympathomimetic agents. Anxiety, muscle tremor, central nervous system (CNS) stimulation, seizures, and cerebral hemorrhage may occur. Hypokalemia is a possible transient serum electrolyte finding. Vomiting commonly is seen with caffeine overdose.

Chronic Toxicity (or Exposure)

Animal

No evidence of carcinogenicity has been seen in studies on mice and rats with doses up to 250 ppm ephedrine in diet over 2 years.

Human

Long-term use of large doses of ephedrine (350–2500 mg day^{-1} for 3–20 years) may produce psychotic episodes characterized by paranoia, hallucinations, depression, and bizarre mentation. Following withdrawal of the drug, aberrant mental effects will resolve but reinstitution of ephedrine use may result in a return of the psychotic symptoms. A tolerance may develop with chronic use, allowing larger doses. Tolerance is lost after 4–6 weeks of removal from the drug.

In Vitro Toxicity Data

Mutagenicity studies using the Ames *Salmonella*, Chinese hamster ovary cells, and Syrian hamster liver assays have been negative.

Clinical Management

Basic and advanced life-support measures should be instituted as indicated. Gastric decontamination may be performed depending on the specific drug involved, the patient's symptomatology, and the history of the ingestion. Activated charcoal may be used to adsorb ephedrine, synephrine, and/or caffeine. Careful monitoring of the heart and hemodynamic status should be performed. Hypertension and symptoms of CNS stimulation usually resolve spontaneously with only supportive measures. Antiarrhythmic and antihypertensive agents may be necessary in severe exposures. If treatment of hypertension is necessary, a direct vasodilator such as nitroprusside or nifedipine should be utilized. Treat agitation and seizures as necessary with benzodiazepines. Management of concurrently ingested drugs should be appropriate to the agent involved.

Environmental Fate

Limited data indicate that caffeine has the potential to biodegrade in soil. If released into water, caffeine will not volatilize from water to the atmosphere. It will not bioconcentrate in fish nor will it adsorb to sediment. Limited data indicate that caffeine has the potential to biodegrade in water.

No information is currently available on breakdown in soil groundwater or surface water for ephedrine or synephrine.

See also: Caffeine.

Further Reading

Pentel P (1984) Toxicity of over-the-counter stimulants. *Journal of the American Medical Association* 2: 1898–1903.

Spider, Black Widow

Gary W Everson

- Synonyms: *Latrodectus mactans*; 'Hour glass' spider

Exposure Routes and Pathways

Envenomation occurs subcutaneously due to the small biting apparatus of the spider. Bites occur most frequently on the extremities.

Toxicokinetics

Following a bite, the specific disposition of *Latrodectus* venom is not well understood. The distribution of venom to the central and peripheral nervous system occurs following absorption through the lymphatic system. The onset of muscle cramping and pain ranges from 30 min to several hours. Resolution of symptoms is usually complete within 24–72 h. However, occasionally a longer clinical course is experienced.

Mechanism of Toxicity

Black widow spider venom contains several different protein fractions. However, the high-molecular-weight neurotoxin is the only fraction that is of clinical significance. This neurotoxin acts at the neuromuscular synapse damaging nerve terminals and causing the release and ultimately the depletion

of such neurotransmitters as alpha-aminobutyric acid, norepinephrine, and acetylcholine. Neurotransmitter release is most likely responsible for hypertension, muscle fasciculation, and muscle spasms experienced by victims of a bite. Generalized muscle weakness and labored breathing may develop in severe cases. While the venom of the black widow spider has been characterized as being more potent than that of many poisonous snakes, the small amount of venom injected limits the extent of toxicity.

Acute and Short-Term Toxicity (or Exposure)

Human

Several species of *Latrodectus* exist and all produce a similar clinical course. The severity of an envenomation is dependent on patient age and the presence of any preexisting cardiovascular disease. Fatalities are extremely rare. Infants and the elderly are at greater risk for developing severe symptoms. Initially, the bite is associated with mild local pain and redness limited to the immediate bite site. As the venom causes no tissue damage, little or no swelling occurs. Most frequently, patients present with painful muscle cramping, spasms, and rigidity within a few hours of the bite. In general, the location of the bite determines which muscle groups will be affected. Bites occurring to the upper body commonly affect muscles of the back, shoulders, and chest. Lower extremity bites are associated with abdominal spasms and rigidity. In some cases, the presentation resembles an acute abdomen. Most problematic is the severe pain that commonly accompanies the muscle spasms. Nausea, vomiting, headache, dizziness, diaphoresis, and mild hypertension are other commonly encountered symptoms. Severe clinical manifestations are rare but can include clinically significant hypertension, respiratory insufficiency, and seizures.

Clinical Management

Although life-threatening envenomations are extremely rare, basic and advanced clinical life support should be employed when necessary. Healthy adult patients bitten by black widow spiders often do not develop symptoms significant enough to require medical evaluation. However, the primary complaint of most patients evaluated in the emergency department is moderate to severe pain due to muscle spasms. Therapy is directed toward making the patient as comfortable as possible while monitoring for the development of severe symptoms such as hypertension and labored breathing. Agents commonly employed to treat muscle spasms and pain includes muscle relaxants, narcotic analgesics, and occasionally intravenous calcium. The most effective treatment in reducing muscle spasm and pain appears to be the combined use of intravenous diazepam and a narcotic analgesic, such as morphine sulfate. Titrating to an effective dose is necessary while minimizing adverse effects. Readministration of this combination is usually required until symptoms abate. Intravenous calcium salts, as either calcium chloride or calcium gluconate, have been attempted. However, treatment failure with this treatment is common and is generally not recommended. Occasionally, intravenous calcium is added to the regimen of muscle relaxants and narcotic analgesics. Calcium gluconate, a less concentrated form of calcium than calcium chloride, is often preferred since it is less irritating to blood vessels during administration. The use of *L. mactans* Antivenin or Antivenin (*Latrodectus mactans*) (Black Widow spider antivenin) Equine Origin®, in general, should be limited only to those patients experiencing severe, potentially life-threatening, symptoms. Most studies indicate that the routine use of antivenin is unnecessary and therefore should be discouraged. Since life-threatening symptoms following a black widow spider bite are rare, the benefits of giving the antivenin rarely outweigh the risk of potentially life-threatening anaphylaxis from this horse serum derived antivenin. Therefore, the vast majority of patients will recover fully with only supportive care and the use of muscle relaxants and narcotic analgesics to manage pain. 'High-risk' patients, such as infants, the elderly, or those with significant cardiovascular disease, who exhibit significant toxicity, are potential candidates for receiving antivenin. *Latrodectus* antivenin is derived from horse serum. Both anaphylactic and delayed hypersensitivity reactions have occurred. If antivenin use is indicated, the intravenous administration is the preferred route. One vial of *Latrodectus* antivenin is reconstituted and commonly diluted further in 50 cc of normal saline and administered intravenously over 30 min. A test dose of not more than 0.02 ml, of test material (1:10 dilution of normal horse serum in physiologic saline) should be administered intradermally prior to intravenous infusion of the antivenin to check for hypersensitivity reactions. Wheal formation at the test site indicates the possibility that an allergic reaction may occur and antivenin use should be reconsidered. A negative reaction to the test dose does not necessarily rule out the possibility of an allergic reaction to the antivenin. One should always be prepared for the possibility of anaphylaxis whenever antivenin derived from horse serum is given. The

benefits of giving antivenin in a particular patient should be weighed against the potential risks. Elevation in blood pressure is frequent following black widow spider envenomation but rarely requires treatment with an antihypertensive agent. Tetanus prophylaxis should be provided as necessary.

See also: Spider, Brown Recluse; Spiders.

Further Reading

Clark RF, Wethern-Kestner S, and Vance MV (1992) Clinical presentation and treatment of black widow spider envenomation: A review of 163 cases. *Annals of Emergency Medicine* 21: 782–787.

Gueron M, Ilia R, and Margulis G (2000) Arthropod poisons and the cardiovascular system. *American Journal of Emergency Medicine* 18: 708–714.

Spider, Brown Recluse

Gary W Everson

- SYNONYMS: *Loxosceles reclusa*; Fiddle-back spider; Violin spider

Exposure Routes and Pathways

Envenomation occurs subcutaneously due to the small biting apparatus of the spider. The Brown Recluse (*Loxosceles reclusa*) spider, as its name implies, is found in secluded areas. Bites occur most frequently to the hands and arms while reaching into woodpiles or other well-protected areas.

Toxicokinetics

In humans, the specific disposition of venom is not well understood. The local distribution of venom is enhanced by the presence of hyaluronidase and other spreading factors found in the venom. Systemic absorption of venom components is likely dependent on lymphatic transport. The onset of local symptoms such as redness and pain may develop within a few hours of the bite.

Mechanism of Toxicity

Brown Recluse spider venom contains many diverse protein fractions including spreading factors and enzymes such as hyaluronidase, collagenase, protease, phospholipase, and others. These venom components cause coagulation of blood and, ultimately, the occlusion of small blood vessels at the bite site. This leads to local skin and tissue necrosis due to ischemia. Hemolysis of red blood cells may also occur. The normal inflammatory processes that follow, such as edema and hemorrhage, contribute to the tissue damage caused by the venom. Occasionally, the local tissue necrosis expands as the tissue ischemia spreads from the initial bite site.

Acute and Short-Term Toxicity (or Exposure)

Human

The Brown Recluse spider is one species of the genus *Loxosceles* which is found in the central Midwest: Nebraska south to Texas and eastward to southernmost Ohio and north-central Georgia.

The Brown Recluse spider bite produces symptoms that range from mild local tissue inflammation to wide spread systemic toxicity. The extent of toxicity is dependent on the amount of venom injected and the age and general health of the patient. Although life-threatening symptoms are possible, a localized skin and tissue reaction is much more common. It is important to note that Brown Recluse spider bites often do not progress to a significant necrotic lesion. However, when this lesion is present, the tissue necrosis is usually self-limiting and often responds to general wound management.

There are some brown spiders that somewhat resemble the Brown Recluse to the layperson. Without proper identification by an entomologist, it is often not possible to immediately diagnose a Brown Recluse spider bite. Complicating the diagnosis further is the fact that there are several other species of spider (e.g., *Chiracantheum* species, *Argiope* species, and *Phidippus* species) that can cause a necrotic skin lesion, without systemic complications.

The bite of the Brown Recluse is usually painless and often goes unnoticed initially. The spider is seldom seen. Therefore, most patients do not seek treatment until a necrotic lesion develops. Within several hours of envenomation, local symptoms of redness and pain occur. Within 24 h, a reddish to violet colored blister becomes surrounded by a

blanched, ischemic ring that is bordered by a reddish ring. This represents the often described 'bull's eye lesion'. Over the next several days, the blistered, ischemic area may turn darker and sink below the level of skin due to subcutaneous tissue necrosis. This necrotic reaction may stop or continue to expand, producing a lesion as large as 5–30 cm in diameter. In 7–14 days, the top layer of the blister sloughs off leaving an ulcerated lesion. Depending on the size of the lesion, healing may require several months. The necrosis tends to be more extensive following bites in fatty areas such as the thighs and buttocks. Rarely, systemic involvement may occur. Systemic effects can include fever, chills, weakness, vomiting, muscle pain, generalized rash, seizures, disseminated intravascular coagulation, thrombocytopenia, and hemolytic anemia. Renal failure and death may occur due to widespread hemolysis.

These potentially severe reactions are extremely rare and the vast majority of Brown Recluse spider bites have a relatively benign progression of local symptoms and resolve with supportive care. It should be pointed out that symptoms of localized infection following a bite of any insect or spider may produce localized lesions that might be mistaken for an expanding necrotic spider bite. Cellulitis should be included on the differential diagnosis and ruled out before considering a patient with a necrotic spider bite.

Clinical Management

Since home identification of a Brown Recluse spider is rarely possible, a 'brown spider bite' should be treated as any other bite of an unknown insect or spider. Management should include the application of a topical disinfectant such as 3% hydrogen peroxide or isopropyl alcohol and brief application of a cold compress for pain. Should the bite site develop increased redness and swelling or a local ulcer develop over several days, a physician should evaluate the wound to rule out infection.

Many controversial techniques have been employed in the management of true Brown Recluse spider bites. Unfortunately, no scientific evidence exists which supports an ideal method or methods of management. However, case reports advocate a variety of therapies as potentially useful. Most agree, however, that good local management of the cutaneous lesion is the most important aspect of care. Tetanus prophylaxis should always be included. In general, antibiotics should be withheld unless there is evidence of infection. Local and systemic injections of steroids have also been employed, but research has shown that neither the extent nor the duration of tissue necrosis is affected. Dapsone has been shown to be effective in research done in the animal model. In addition, several case reports have described some success with dapsone in decreasing local pain and preventing further induration and tissue necrosis. Doses have ranged from 50 to 200 mg day^{-1}. This drug appears to decrease the extent of tissue necrosis by inhibiting polymorphonuclear leukocytes, the mediators of the inflammatory response to the bite. However, side effects of dapsone are potentially severe. Hemolytic anemia and liver toxicity have been described.

There appears to be no benefit to early surgical excision of the bite site. Some time is required before a clear boundary is established marking the end of the spread of venom. Excising the necrotic area too soon may leave some venom at the boundary that can produce further tissue necrosis. In cases where the necrotic lesion expands and is unresponsive to local treatment, 'delayed' surgical excision of the wound after 2 or 3 weeks may be indicated.

Although several case reports describe some success in patients following the use of hyperbaric oxygen, no scientific studies have been completed yet to determine the effectiveness of this approach. Management of systemic toxicity is primarily supportive and includes good wound management with emphasis on pain management, wound debridement, and observation for local infection. Also, adequate hydration is important to maintain good urine output. Clotting abnormalities and anemia should be managed with appropriate blood products. The Brown Recluse spider antivenin is still experimental and not commercially available.

See also: Animals, Poisonous and Venomous; Spiders.

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Spiders

Julie Weber

- Representative Genera: *Latrodectus*; *Loxosceles*; *Argiope*; *Chiracanthium*; *Lycosa*; *Phidippus*; *Tegenaria*
- Synonyms: *Latrodectus* species: *L. mactans* (Black Widow); *L. variolus* (Northern Black Widow); *L. hesperus* (Western Black Widow); *L. bishopi* (Red Widow); *L. geometricus* (Brown Widow); *L. hasselti* (Red Back); *L. mactans tredecimguttatus* (European Black Widow). *Loxosceles* species: *L. deserta*; *L. arizonica*; *L. laeta*; *L. rufescens*; *L. unicolor*; *L. recluse*. Necrotizing spiders: *Argiope* (Orb Weaver); *Chiracanthium* (Running or sac spider); *Lycosa* (Wolf spider); *Phidippus* (Jumping spider), *Tegenaria agrestis* (Hobo spider or Northwestern brown spider)

Exposure Routes and Pathways

Envenomation occurs subcutaneously due to the small biting apparatus of the spider. Bites occur most frequently on the extremities. Typically, the victim is bitten while dressing in clothing that has been undisturbed, when rolling over in bed onto a spider, moving stored boxes in an attic or basement or while reaching into woodpiles or other well-protected areas.

Toxicokinetics

In humans, the specific disposition of *Latrodectus* and *Loxosceles* venom is not well understood. Distribution of *Latrodectus* venom to the central and peripheral nervous system occurs following absorption through the lymphatic system. Whereas the local distribution of *Loxosceles* venom is enhanced by the presence of hyaluronidase and other spreading factors found in the venom. Systemic absorption of venom components is likely dependent on lymphatic transport, possibly similar to the *Latrodectus* venom. In *Latrodectus* envenomation, the onset of muscle cramping and pain ranges from 30 min to several hours. Resolution of symptoms is usually complete within 24–36 h. Occasionally, a longer clinical course is experienced with symptoms persisting for several days. In comparison, the onset of local symptoms with *Loxosceles* envenomation may develop within a few hours of the bite with redness and pain, full progression of symptoms and healing may not occur for 1 month or longer.

Mechanism of Toxicity

Latrodectus

Black Widow spider venom contains several different protein fractions. The most significant component of the venom is the neurotoxin, α-latrotoxin. This neurotoxin acts at the presynaptic membrane of the neuronal and the neuromuscular junctions. The binding of the α-latrotoxin results in the opening of nonspecific cation channels, a massive influx of calcium, release of acetylcholine and norepinephrine and decreased uptake of the neurotransmitter. The neurotransmitter release is most likely responsible for hypertension, muscle fasciculations, and spasms frequently experienced by victims of a bite. Later, generalized muscle weakness and labored breathing may develop in severe cases. While the venom of the black widow spider has been characterized as being more potent than that of many poisonous snakes, the small amount of venom injected limits the degree of toxicity.

Loxosceles

The *Loxosceles* venom is complex and contains multiple enzymes including alkaline phosphatase, hyaluronidase, 5-ribonucleotide phosphohydrolase, esterase, and sphingomyelinase D. The venom components cause coagulation of blood and ultimately, the occlusion of small blood vessels at the bite site. This leads to local skin and tissue necrosis due to ischemia. A release of inflammatory mediators resulting in polymorphonuclear leukocyte infiltration is also associated with the local reaction. Hemolysis of red blood cells can occur. Sphingomyelinase D appears to be the major dermonecrotic factor. When calcium and serum amyloid protein are present sphingomyelinase D reacts with sphingomyelin to release choline and *N*-acylsphingosine phosphate stimulating platelet aggregation and release of serotonin. Occasionally, the local tissue necrosis expands as the tissue ischemia spreads from the initial bite site.

Acute and Short-Term Toxicity (or Exposure)

Human

Latrodectus Several species of *Latrodectus* exist and all produce a similar clinical course. The severity

of an envenomation is dependent on patient age and the presence of any preexisting cardiovascular disease. Fatalities are extremely rare. Infants and the elderly are at greater risk for developing severe symptoms. Initially, the bite is associated with mild local pain and redness limited to the immediate bite site. The venom causes no tissue damage, so little or no swelling occurs. Patients most frequently present with painful muscle cramping, spasms, and rigidity that commonly occurs within 15 min to 3 h of the bite. The location of the bite determines which muscle groups will be affected. Bites occurring to the upper body commonly affect muscles of the back, shoulders, and chest. Lower extremity bites are associated with abdominal spasms and rigidity. In some cases, the presentation resembles an acute abdomen. Most problematic is the severe pain which commonly accompanies the muscle spasms. Nausea, vomiting, headache, dizziness, diaphoresis, and mild hypertension are other commonly encountered symptoms. Severe clinical manifestations are rare but can include clinically significant hypertension, respiratory insufficiency, and seizures. Other less common effects that have been reported include ptosis, pulmonary edema, facial edema, encephalopathy, generalized rash, itching, and toxic epidermal necrolysis.

Loxosceles The Brown Recluse spider bite produces symptoms that range from mild local tissue inflammation to widespread systemic toxicity. The extent of toxicity is dependent on the amount of venom injected, the location of the bite, and the age and general health of the patient. Although life-threatening symptoms are possible, a localized skin and tissue reaction is much more common. It is important to note that Brown Recluse spider bites often do not progress to a necrotic lesion. However, when present, the tissue necrosis is usually self-limiting and often responds to general wound management. The bite of the Brown Recluse is usually painless and initially often goes unnoticed. Since the spider is seldom seen, most patients do not seek treatment until a necrotic lesion develops. Local symptoms of pruritus, redness, and pain occur within several hours of the envenomation. Within 24 h, a reddish to violet colored blister becomes surrounded by a blanched, ischemic ring that is bordered by a reddish ring. This represents the often described 'bull's eye' or 'halo' lesion. Over the next several days, the blistered, ischemic area may turn darker and sink below the level of skin due to subcutaneous tissue necrosis. This necrotic reaction may stop or continue to expand, producing a lesion as large as 5–30 cm in diameter. In 7–14 days, the top layer of the blister sloughs off leaving an ulcerative lesion. Depending on the size of the lesion, healing may require several months. The necrosis tends to be more extensive following bites in fatty areas such as the thighs, buttocks, and abdomen. Neck and facial wounds can cause significant edema. Systemic reactions from *Loxosceles* envenomation are infrequent. A systemic reaction typically occurs within 24–96 h. Symptoms can include fever, chills, weakness, vomiting, muscle pain, generalized rash, seizures, disseminated intravascular coagulation, thrombocytopenia, and hemolytic anemia. Renal failure and death may occur due to widespread hemolysis.

Argiope, Chiracanthium, Lycosa, Phidippus, Tegenaria agrestis There are some brown spiders that somewhat resemble the Brown Recluse to the layperson. Without proper identification, it is often not possible to immediately diagnose a brown recluse spider bite. Complicating the diagnosis further is the fact that there are several other species of spiders that can cause a necrotic skin lesion, although not as severe.

Argiope The spider will bite if stressed or provoked. Initial symptoms include sharp pain and swelling. Induration with a surrounding erythema can occur. The bite may cause a necrotic lesion, but no systemic symptoms expected.

Chiracanthium The venom is similar to *Loxosceles*. The bite initially causes a sharp pain and red wheal formation. Several days later, a crust forms at the site with necrotic tissue underneath. Erythema, pruritus, and pain typically surround the bite site and may take one month to heal. Systemic symptoms are rare, but nausea, anxiety, headache, and abdominal cramps have been reported.

Lycosa The initial reaction often consists of erythema, pain, edema with a violaceous discoloration and tenderness. Necrosis is seldom, but may occur. Nausea and light-headedness have been reported.

Phidippus The jumping spider produces a sharp painful bite with redness, pain, edema, and pruritus. The swelling usually subsides within 48 h, but in one report symptoms persisted for 1 week. A small ulcer with eschar may form. No systemic toxicity is expected.

Tegenaria agrestis The initial bite of the hobo spider may be painless and unnoticed. It is the most similar to the brown recluse. Induration surrounded by erythema may be present within 30 min. A blister formation typically follows within 15–35 h. About

half of the reported envenomations may develop an eschar covering a necrotic ulcer. Systemic symptoms reported include headache, lethargy, weakness, nausea, vomiting, memory loss, and visual impairment.

Clinical Management

Latrodectus

Although life-threatening envenomations are extremely rare, basic and advanced clinical life support should be employed when necessary. Normally healthy adult patients bitten by Black Widow spiders often do not develop symptoms significant enough to require medical evaluation. However, the primary complaint of most patients evaluated in the emergency department is moderate to severe pain due to muscle spasms. Therapy is directed toward making the patient as comfortable as possible while monitoring for the development of severe symptoms such as hypertension and labored breathing. Agents commonly employed to treat muscle spasms and pain include benzodiazepines, muscle relaxants, intravenous calcium, and narcotic analgesics. The bite should be cleansed with soap and water. Measures to remove or decrease the spread of venom are ineffective due to the small amount of venom necessary to produce toxicity and its rapid spread to the circulation. Tetanus prophylaxis should be provided as necessary. Diazepam has been found to be effective for the relief of muscle spasms. Methocarbamol may also be effective. Parenteral opioids (codeine, morphine, or meperidine) or a combination of parenteral opioids and sedative-hypnotics such as diazepam or lorazepam are recommended for patients with severe envenomations who are not candidates for antivenin. Cautious use of opioids is recommended as they may be contraindicated by a patient's symptoms (i.e., respiratory difficulty, central nervous system (CNS) depression, seizures, etc.). The efficacy of intravenous calcium has not been firmly established in controlled trials. Calcium gluconate is often preferred and it is less irritating to blood vessels during administration than calcium chloride. It alone though often fails to provide adequate relief of symptoms and is not recommended for symptomatic relief. Use of both intravenous diazepam and a narcotic analgesic appears to be the most effective method in reducing muscle spasm and pain. The use of *L. mactans* antivenin, in general, should be limited only to those patients experiencing severe symptoms. Most studies indicate that the routine use of antivenin is unnecessary and therefore should be discouraged. The vast majority of patients will recover fully with only observation and the use of muscle relaxants and narcotic analgesics to manage pain. 'High risk' patients, such as infants, the elderly, or those with significant cardiovascular disease, are potential candidates for receiving antivenin. *Latrodectus* antivenin is derived from horse serum. Both anaphylactic and delayed hypersensitivity reactions have occurred. If antivenin use is indicated, intravenous administration is the preferred route. Prior to administration of the antivenin the patient should be tested for sensitivity. A small dose should be administered subcutaneously prior to intravenous infusion to check for hypersensitivity reactions. Wheal formation at the test site indicates the possibility of an allergic reaction. While a positive skin test warrants increased caution with antivenin administration, it should not necessarily preclude its use when indicated. A negative reaction to the test dose does not necessarily rule out the possibility of an allergic reaction to the antivenin. One should always be prepared for the possibility of anaphylaxis whenever antivenin derived from horse serum is given. The benefits of giving antivenin in a particular patient should be weighed against the potential risks. One vial (2.5 ml) of *Latrodectus* antivenin is reconstituted and commonly diluted further in 50–100 ml of D_5W or normal saline and administered intravenously over 30 min. Elevation in blood pressure is frequent following Black Widow spider envenomation but rarely requires treatment with an antihypertensive agent.

Loxosceles

Many controversial techniques have been employed in the management of Brown Recluse spider bites. Unfortunately, no scientific evidence exists which supports an ideal method or methods of management. However, case reports advocate a variety of therapies as potentially useful. Most agree, however, that good local management of the cutaneous lesion is the most important aspect of care. Tetanus prophylaxis should always be included. Immobilization, elevation and rest of a bitten extremity may be beneficial as increased activity and metabolic heat production may enhance enzyme activation. Local application of cool compresses has been reported to reduce inflammation and pain, and slow the evolution of lesions. It is postulated that the activity of sphingomyelinase D may be reduced by the cold. Analgesics can be administered for pain. Avoid analgesics and antiinflammatory drugs that affect platelet function. Antipruritics and antianxiety drugs may be administered as needed. In general, antibiotics should be withheld unless there is evidence of infection. Local and systemic injection of steroids has been employed, but research has shown that neither the extent nor the duration of tissue

necrosis is affected. Dapsone has been shown to be effective in research done in the animal model. In addition, several case reports have described some success with dapsone in decreasing local pain and preventing further induration and necrosis. Doses have ranged from 50 to 200 mg day^{-1} in adults. This drug appears to decrease the extent of tissue necrosis by inhibiting polymorphonuclear leukocytes, the mediators of the inflammatory response to the bite. However, side effects of dapsone are potentially severe. Hemolytic anemia and liver toxicity have been described. Dapsone should not be used routinely.

There appears to be no benefit to early surgical excision of the bite site. Some time is required before a clear boundary is established marking the end of the spread of venom. Excising the necrotic area too soon may leave some venom at the boundary that can produce further tissue necrosis. Corrective surgery should be delayed at least 6–8 weeks until the area of necrosis is clearly demarcated.

Although several case reports describe some success in patients following the use of hyperbaric oxygen, it is not considered standard therapy at this time. However, hyperbaric oxygen may provide wound healing benefits in certain patients with vascular insufficiencies. Management of systemic toxicity is primarily supportive and includes the use of steroids to prevent red blood cell hemolysis. Also, adequate hydration is important to maintain good urine output. Clotting abnormalities and anemia should be managed with appropriate blood products. If hemolysis occurs, maintain hydration and urine output to prevent renal failure. Alkalinization of the urine may enhance hemoglobin case excretion. Hemodialysis has no effect on elimination of the venom, but is indicated in the presence of renal failure. The Brown Recluse spider antivenin is still experimental and is not commercially available.

Argiope, Chiracanthium, Lycosa, Phidippus, Tegenaria agrestis

As with the treatment of the *Loxosceles* species, treatment remains controversial. Typically, good local management of the cutaneous lesion is the most important aspect of care. Tetanus prophylaxis should be updated. Immobilization, elevation and rest of a bitten extremity may be beneficial. Analgesics can be administered for pain. Antipruritics and antianxiety drugs may be administered as needed. In general, antibiotics should be withheld unless there is evidence of infection. The use of intralesional, intramuscular or oral corticosteroids is not of proved efficacy, although some case reports have shown a good outcome with the use of a corticosteroid.

Miscellaneous

There are five representative species of the widow spider in the United States. The species are the Black Widow (*L. mactans*, *L. hesperus*, *L. variolus*), Red Widow (*L. bishopi*), and the Brown Widow (*L. geometricus*). The five species of *Loxosceles* documented to produce necrotic bites are *L. arizonica*, *L. deserta*, *L. laeta*, *L. reclusa*, and *L. rufescens*. The *L. reclusa* is responsible for most cases of clinical significance of necrotic arachnidism.

There is no routine test for the diagnosis of trivial or cutaneous arachnidism. The use of a passive hemagglutination inhibition test has been used successfully to identify venom from Brown Recluse spider bites in animal studies. This test has not yet been used for diagnostic purposes in human trials and is not routinely available to clinicians.

See also: Animals, Poisonous and Venomous; Spider, Black Widow; Spider, Brown Recluse.

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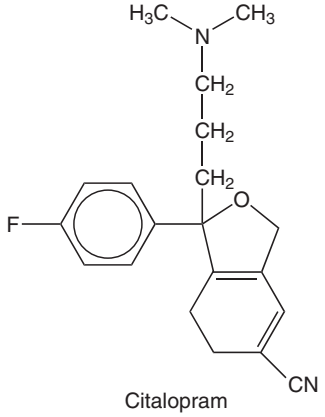
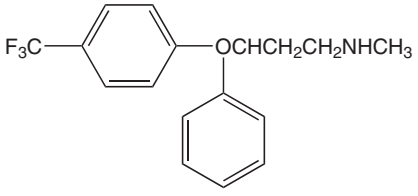
SSRIs (Selective Serotonin Reuptake Inhibitors)

Samantha E Gad

- Trade Names and Chemical Abstracts Service Registry Numbers:
  Citalopram hydrobromide (CAS 59729-32-7) Celexa® (Lundbeck)
  Flouxetine hydrochloride (CAS 59333-67-4) Prozac® (Lilly)
  Fluvoxamine maleate (CAS 61718-82-9) Luvox® (Solvay)
  Paroxetine hydrochloride (CAS78246-49-8) Paxil® (SmithKline-Beecham)
  Sertraline hydrochloride (CAS 79559-97-0) Zoloft® (Pfizer)
  Escitalopram oxalate (CAS 219861-08-2) Lexapro® (Forest)
  These are the six main selective serotonin reuptake inhibitors (SSRIs); others are under development and possibly in use in small populations or foreign countries.
- Therapeutic Class: Antidepressant
- Routes of Administration: Oral
- Chemical Structures: Escitalopram is the *S*-isomer, the active isomer of citalopram's racemic mixture



Citalopram



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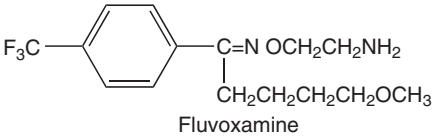

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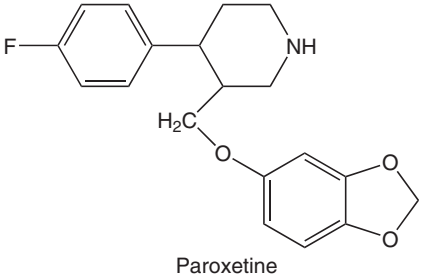
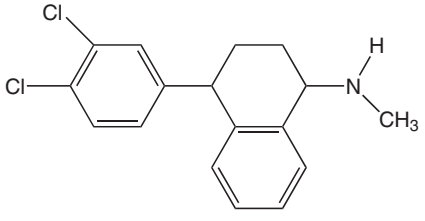


Paroxetine



Sertraline

Uses

Obsessive compulsive disorder, panic disorder, generalized anxiety disorder, bulimia nervosa, social anxiety disorders, post-traumatic stress disorder, dementia, dysthymia, premature ejaculation. Citalopram (investigational) is used for dementia, smoking cessation, ethanol abuse, OCD in children with diabetic neuropathy. Sertraline and Sarafem (contains fluoxetine) are also used to treat premenstrual dysphoric disorder.

Mechanism of Action

SSRIs selectively inhibit the reuptake of serotonin (5-hydroxytryptamine (5-HT)) into nerve endings in the central nervous system (CNS). This allows for an increasing concentration of serotonin at the synapse, enhancing serotonergic neuronal transmission. If depression represents some inadequacy in transmission between the nerves in the brain, as is believed, regulating transmission may go some way toward reversing this inadequacy. Dysfunction of serotonin in neurons is thought to play a major role in a wide variety of diseases, including major depression. It is believed that increasing the availability of serotonin will improve the clinical signs of depression. Serotonin is implicated in brain functions such as perception of pain, sleep, appetite, thermal regulation, reproductive function, balance, gut regulation, sensory interpretation, higher cognitive function, and motor function.

SSRIs have no direct effect on the reuptake of noradrenaline, dopamine, or GABA. Unlike tricyclic antidepressants (TCAs), they have no significant affinity for a1-adrenergic, H1-histamine, and muscarinic receptors. The selectivity of SSRIs may account for the lower incidence of some adverse effects such as sedation or thustatic hypotension, and anticholinergic effects. Seretonin is a neurotransmitter in the human brain; serotonin containing neurons are highly localized in specific clusters in the brainstem and spinal cord. From these sites, the cells send out axons that end in serotonin-containing terminals innervating the diverse areas throughout the brain.

SSRIs affect many postsynaptic serotonin receptors (e.g., 5-HT 1A, 5-HT 1D, 5-HT 2A, 5-HT 2C, and 5HT-3), therefore affecting many neural systems. With the exception of fluoxetine, none of the currently approved SSRIs have metabolites with clinically relevant effects on any of the neural sites. However, every SSRI that has been studied has metabolites with approximately the same activity as the parent drug for the inhibition of specific CYP enzymes and contributes to the effects mediated by this action.

SSRIs appear safer in overdose than most other classes of antidepressants. SSRIs are not associated with clinically significant anticholinergic side effects, or cardiotoxicity.

Pharmacokinetics

Citalopram is distributed in human breast milk. Escitalopram (*S*-citalopram) enters the breast milk. These should only be used during pregnancy if the potential benefit to the mother outweighs the possible risk to the fetus (**Table 1**).

Known Major Drug Interactions

All SSRIs have common 5-HT agonistic effects and because of this, SSRIs have common interactions and side effects. SSRIs are potent inhibitors of serotonin reuptake by CNS neurons and may interact with other drugs such as monoamine oxidase inhibitors (MAOIs) or circumstances which cause serotonin release. A minimum 2 weeks wash-out period should be observed between stopping a MAOI and starting an SSRI. Conversely, a MAOI should not be started for at least 1 week after an SSRI has been stopped, 5 weeks after fluoxetine, and 2 weeks for paroxetine and sertraline. Escitalopram and citalopram are hypersensitive to each other.

Combining SSRIs with the following drugs has caused a drug–drug interaction; the SSRI(s) involved are in italics:

- 3,4-Methylenedioxymethamphetamine (MDMA): The psychological effects of MDMA ('Ecstasy') are markedly reduced by the concurrent use of *citalopram*. It seems likely that other SSRIs will also reduce or block the effects of MDMA. An isolated report describes a neurotoxic reaction in a man on citalopram when he took unknown amounts of MDMA.
- 5-HT agonists (Triptans): The SSRIs normally appear not to interact with the triptans, but there are a few rare cases of dyskinesias and there is some evidence to suggest that the serotonin syndrome may occasionally develop.
- Amphetamines: SSRIs may increase the sensitivity to amphetamines; amphetamines may increase the risk of serotonin syndrome.
- Antiepileptics: These lower the convulsive threshold.
- Antipsychotics: Seven patients developed delirium when given *fluoxetine*, *paroxetine*, or *sertraline* with benztropine in the presence of perphenazine

Table 1 Pharmacokinetics of SSRIs

SSRI	*Initial dose (mg qAM)*	*Maintenance dose (mg day^{-1})*	*Half-life (h)*	*Metabolite half-life*	*Bioavailability (%)*	*Peak plasma level (h)*	*Plasma level*[a] (ng ml^{-1})	*In vitro potency IC_{50}*[b]	*5-HT uptake inhibition (%)*	*% Protein-bound*	*Metabolism*
Citalopram (Celexa™)	20	20–60	35	*S*-Desmethyl-citalopram: 59 h	80	4	≅85 (260 nM)	1.8 (14)	≅60	80	Extensively hepatic
Fluoxetine (Prozac™)	10–20	10–80	Initial: 24–72; Chronic: 96–144	Norfluoxetine: 4–16 days	72	6–8	≅200 (660 nM)	6.8 (3.8)	≅80	95	Hepatic to norfluoxetine
Fluvoxamine (Luvox™)	50	50–300	16	N/A	53	3	≅100 (300 nM)	3.8	≅70	80	Extensive to inactive metabolites
Paroxetine (Paxil®™)	10–20	20–50	21	N/A	>90	5	≅40 (130 nM)	0.29	≅80	95	Extensive by cytochrome P450 to catechol and other inactive metabolites
Sertraline (Zoloft®)	25–50	100–200	26	*N*-Desmethyl-sertraline: 62–104 h	88	5–8	≅25 (65 nM)	0.19 (NA)	≅80	98	Extensive to desmethyl-sertraline
Escitalopram (Lexapro®)	10	10–20	27–32	*S*-Desmethyl-citalopram: 59 h	80	5				56	Hepatic, metabolized to *S*-didesmethyl-citalopram

[a] Plasma level for fluoxetine represents total fluoxetine plus norfluoxetine given the comparable effect of each on 5-HT uptake pump. Plasma levels are a total of both enantiomers for citalopram and fluoxetine.

[b] Value for parent drug; value for the respective major metabolite is in parentheses; no data available for sertraline metabolite.

or haloperidol. Other patients remained symptom-free.

- Aspirin (and benorilate): *Citalopram* may increase the risk of bleeding.
- Artemether with lumefantrine: Avoid concomitant use of *citalopram*.
- Astemizole: The makers of astemizole contraindicate the concurrent use of SSRIs (because of possible increased astemizole serum levels) and those proarrhythmic drugs which might additively prolong the QT interval and thereby increase the risk of serious arrhythmias.
- β-Blockers (carvedilol and metoprolo): *Citalopram* and *fluvoxamine* may increase the levels of some β-blockers.
- Barbituates: They lower the convulsive threshold.
- Benzodiazepines: *Sertraline* and *fluvoxamine* may inhibit the metabolism of alprazolam and diazepam, resulting in elevated serum levels potentially causing sedation and psychomotor impairment.
- Benztropine: Seven patients developed delirium when given *fluoxetine*, *paroxetine*, or *sertraline* with benztropine in the presence of perphenazine or haloperidol. Other patients remained symptom-free.
- Buspirone: An isolated report describes the development of the serotonin syndrome with buspirone and *citalopram*; the same may happen with *fluvoxamine*. Busprone with *fluoxetine* can be effective, but some adverse reactions have been reported. *Fluvoxamine* may possibly reduce the effects of buspirone.
- Carbamazepine: Some, but not all, reports indicate that carbamazepine serum levels can be increased by *fluoxetine* and *fluvoxamine*. Toxicity may develop. *Sertraline* normally appears not to affect carbamazepine, but sertraline levels may be reduced by carbamazepine. Isolated cases of Parkinson-like and serotonin syndrome have occurred with *fluoxetine* and carbamazepine, while an isolated case of pancytopenia has been reported with *sertraline* and carbamazepine. The metabolism of citalopram may be increased.
- Carvedilol: Serum concentrations may be increased.
- Cilostazol: Inhibitors of cytochrome P450 isoenzyme CYP3A4 are predicted to increase the serum levels of cilostazol or its active metabolite (all SSRIs potentially affected).
- Cimetide: Cimetide causes a moderate rise in the serum levels of *sertraline*. It may inhibit the metabolism of citalopram. Cimetide may reduce the first-pass metabolism of *paroxetine*, resulting in elevated *paroxetine* serum concentrations.
- Cisapride (propulsid): Do not take *fluvoxamine*.
- Clozapine: *Fluoxetine*, *paroxetine*, *sertraline*, and possibly *citalopram* can raise serum clozapine levels. Particularly large increases can occur with *fluvoxamine*. Toxicity has been seen in some patients.
- Coumarins: Anticoagulant effect possibly enhanced by *citalopram*.
- CYP2D6 substrates (desipramine, nortriptyline, haloperidol, thioridazine, flecainide, codeine, propanolol, metprolol): SSRIs can inhibit *in vitro* and *in vivo* the hepatic isoenzymes 2D6 of the cytochrome P450 system (CYP2D6), which is involved in the oxidative metabolism of numerous drugs. SSRIs can cause a significant increase in the serum concentrations of these drugs.
- Cyclosporine: *Fluoxetine* may increase the serum levels of cyclosporine (and possibly tacrolimus)
- Cyproheptadine: Reports say that cyproheptadine can oppose the antidepressant effects of *fluoxetine*, *fluvoxamine*, and *paroxetine*.
- Dextromethorphan: Some SSRIs inhibit the metabolism of dextromethorphan; visual hallucinations have occurred; it may cause serotonin syndrome
- Digitalis glycoside: Neither *citalopram* nor *fluvoxamine* appear to interact with digoxin, but an isolated report describes increased serum digoxin levels attributed to the use of *fluoxetine*.
- Digoxin: *Fluoxetine* may increase serum levels of digoxin.
- Dihydroergotamine: Three isolated cases of the serotonin syndrome have been seen in patients on *paroxetine* with imipramine, amitriptyline, or *sertraline* when given dihydroergotamine.
- Disulfram (Antabuse): Do not take with *Zoloft oral concentrate* which contains alcohol and may cause a reaction.
- Erythromycin: An isolated report describes the development of what was thought to be serotonin syndrome in a 12-year-old child taking *sertraline* when erythromycin was added.
- Haloperidol: Serum may be increased slightly by *sertraline*. *Fluoxetine*, *paroxetine* and *fluvoxamine* may inhibit the metabolism of haloperidol and cause extrapyramidal symptoms.
- Human menopausal gonadotropin-CoA reductase inhibitors: *Sertraline*, *paroxetine*, *fluvoxamine*, and *fluoxetine* may inhibit the metabolism of lovastatin and simvastatin resulting in myosis and rhabdomyolysis; although its inhibition is weak, these combinations are best avoided.
- Isoniazid: No important interaction appears to occur between isoniazid and the SSRIs or nefazodone. However, adverse reactions have been seen

during concurrent use but they are thought unlikely to have been due to an interaction.

- Lamotrigine: Toxicity has been reported following the addition of *sertraline*.
- Linezolid: Hyperpyrexia, hypertension, tachardia, confusion, seizures, and deaths have been reported with agents that inhibit MAO (serotonin syndrome), *escitalopram*, and *citalopram*.
- Lithium: There is increased risk of CNS effects; lithium toxicity is reported.
- Loop diuretics: *Sertraline*, *paroxetine*, and *fluvoxamine* may cause hyponatremia; additive hyponatremic effects may be seen with combined use.
- Macrolide antibacterials: An isolated case report describes what appeared to be acute *fluoxetine* intoxication in a man brought about by the addition of clarithromycin.
- MAOI: This is contraindicated owing to potential risk of serotonin syndrome. Hyperpyrexia, hypertension, tachycardia, confusion, seizures, and deaths have been reported. A minimum 2 week's wash-out period should be observed between stopping a MAOI and starting an SSRI. Conversely, a MAOI should not be started for at least 1 week after an SSRI has been stopped (at least 5 weeks for *fluoxetine*; at least 2 weeks for *paroxetine* and *sertraline*).
- Meperidine: Combined use theoretically may increase the risk of serotonin syndrome.
- Methadone: Methadone serum levels may rise if *fluvoxamine* is added, possibly resulting in increased side effects. *Sertraline* may also increase methadone levels, but no interaction appears to occur with *fluoxetine*.
- Mesoridazine: *Fluoxetine* and *paroxetine* may inhibit the metabolism of mesoridazine, resulting in increased plasma levels and increasing the risk of QT_c interval prolongation. This may lead to serious ventricular arrhythmias, such as torsade de pointes-type arrhythmias, and sudden death. Wait at least 5 weeks after discontinuing these SSRIs prior to starting mesoridazine.
- Methylphenidate: Metabolism of *citalopram* may be inhibited.
- Metoprolol: *Escitalopram* and *citalopram* may increase plasma levels of metoprolol.
- Moclobemide: Concurrent use with *escitalopram* or *citalopram* make cause serotonin syndrome.
- Nefazodone: Concurrent may cause serotonin syndrome.
- NSAIDs: There is increased risk of bleeding when NSAIDs are used with escitalopram.
- Olanzapine: *Fluvoxamine* causes a rise in serum olanzapine levels.
- Perhexiline: Three case reports describe an increase in perhexiline serum levels with toxicity due to the concurrent use of *fluoxetine* or *paroxetine*.
- Phenothiazines: *Sertraline* may inhibit metabolism of thioridazine or mesoridazine, potentially leasing to malignant ventricular arrhythmias.
- Phenytoin: Phenytoin serum levels can be increased in some patients by *fluoxetine*. Toxicity may occur. There are also isolated reports of phenytoin toxicity with the concurrent use of *fluvoxamine* and *paroxetine*. Phenyltoin and *sertraline* do not normally interact; nevertheless, two patients have shown increased serum phenytoin levels.
- Pimozide (Orap): Do not take *fluvoxamine*.
- Primidone: When used with escitalopram, convulsive threshold is decreased.
- Propafenone: Serum concentrations and/or toxicity may be increased by *fluoxetine* and *fluvoxamine*.
- Quinidine: Serum concentrations may be increased with *fluvoxamine*.
- Risperidone: *Paroxetine* inhibits the metabolism of risperidone resulting in elevated risperidone levels; it may cause extrapyramidal symptoms.
- Ritonavir: Plasma concentrations of SSRI are possibly increased. It may cause serotonin syndrome in HIV patients when used with *citalopram*.
- Selegiline: Concurrent use has been reported to cause mania, hypertension, and in some cases serotonin syndrome. As a MAO type-B inhibitor, the risk of serotonin syndrome may be less than with nonselective MAO inhibitors.
- Sibutramine: May increase the risk of serotonin syndrome. (Manufacturers recommend avoiding concomitant use.)
- Sumatriptan (and other serotonin agonists): Concurrent use may result in toxicity; weakness, hyperreflexia, and incoordination. This combination may also increase the risk of serotonin syndrome; it also includes naratriptan, rizatriptan, and zolmitriptan.
- SRIs and SSRIs: Concurrent use with other reuptake inhibitors may increase the risk of serotonin syndrome.
- St. John's wort (*Hepericum perforatum*): Four patients on *sertraline* and one on nefazodine developed symptoms diagnosed as serotonin syndrome when St. John's wort was taken concomitantly. Another patient on St. John's wort developed severe sedation after taking a single dose of *paroxetine*.
- Sympathomimetics: Concurrent use may increase the risk of serotonin syndrome.

- Tacrine: *Fluvoxamine* inhibits the metabolism of tacrine; use alternative SSRI.
- Tacrolimus: *Fluvoxamine* may inhibit the metabolism of tacrolimus.
- TCAs: SSRIs inhibit the metabolism of TCAs resulting in elevated serum levels; if necessary, a low dose of TCA should be used.
- Theophylline: *Fluvoxamine* inhibits the metabolism of theophylline. *Paroxetine* may also inhibit the metabolism of theophylline.
- Thioridazine (Mellaril): Dangerous, even fatal, irregular heartbeats may occur when taken with *fluoxetine*, *fluvoxamine*, or *paroxetine*. These may inhibit the metabolism of thioridazine, resulting in increased plasma levels and increasing the risk of QT_c interval prolongation. This may lead to serious ventricular arrhythmias, such as torsade de pointes-type arrhythmias, and sudden death. Wait at least 5 weeks after discontinuing these prior to starting thioridazine.
- Tramadol: Five reports describe the development of the serotonin syndrome in patients on *fluoxetine*, *paroxetine*, or *sertraline* when tramadol was added. Another patient developed hallucinations with tramadol and *paroxetine*. Other reports suggest that the SSRI/tramadol combination is therapeutically valuable and normally safe. Also, carefully monitor concomitant use with *fluvoxamine*.
- Trazodone: Concurrent use may cause serotonin syndrome. SSRIs may inhibit the metabolism of trazadone resulting in increased toxicity.
- Tryptophan: It causes agitation and nausea.
- Valproic acid: *Fluoxetine* may increase serum levels of valproic acid.
- Venlafaxine: Combined use may increase the risk of serotonin syndrome.
- Warfarin: *Sertraline*, *fluoxetine*, *fluvoxamine*, and *paroxetine* may alter the hypoprothrombinemic response to warfarin.
- Zolpidem: A case of delirium has been reported when used in combination with *paroxetine*.
- *Herbal*: Avoid valerian, St. John's wort, SAMe, kava kava, and gotu kola; these may increase CNS depression.

Side Effects

Drowsiness, tremor in the upper extremities, lightheadedness, nausea, and vomiting are the most common symptoms. Other symptoms include tachycardia (occasionally bradycardia), hypo/hypertension, dilated pupils, agitation, dry mouth, and sweating. Citalopram also has shown effects of insomnia, somnolence, and erostomia.

Side effects are similar for all SSRIs, but have different degrees of severity. Data from the Committee on Safety of Medicines showed more reports of suspected reactions (including discontinuation reactions) to *paroxetine*, and of gastrointestinal reactions to *fluvoxamine* and *paroxetine* than to the other SSRIs during the first 2 years of marketing. Prescription–event monitoring revealed a higher incidence of adverse events related to *fluvoxamine* than its comparators. There were higher incidences of gastrointestinal symptoms, malaise, sedation, and tremor during treatment with fluvoxamine and of sedation, tremor, sweating, sexual dysfunction, and discontinuation reaction with paroxetine. It has been shown that *sertraline* caused significantly more sexual dysfunction and libido problems than did *fluvoxamine*. *Fluoxetine* caused more side effects than did *sertraline* in geriatrics. Escitalopram had low discontinuation rates due to its high tolerability.

Discontinuation of SSRIs can cause adverse events including dizziness, insomnia, nervousness, nausea, and agitation. See Discontinuation Syndrome under the section Human Toxicology (Acute) for more information.

SSRIs can inhibit hepatic isoenzymes 2D6 of the cytochrome P450 system (CYP2D6), which is involved in the oxidative metabolism of numerous drugs.

Serotonin Syndrome

Antidepressants are considered to have additive effects, therefore combined use is not recommended. Inhibitors of serotonin reuptake by CNS neurons may interact with other drugs or circumstances which cause serotonin release. The enhancement of the serotonergic effects may produce a life-threatening serotonin syndrome. Drugs which can increase the serotonin level when taken in combination with SSRIs include: TCAs, MAOIs, reversible inhibitors of monoamine oxidase, carbamazepine, lithium, or serotoneric substances. These drugs should not be coadministered with SSRIs and they may increase the risks of developing a serotonin syndrome.

Serotonin syndrome is characterized by the presence of at least three of the following symptoms: mental status changes, agitation, myoclonous, hyperreflexia, sweating, shivering, tremor, diarrhea, motor incoordination, muscle rigidity, and fever. Severe complications may occur, including severe hyperthermia, rhabdomyolysis, disseminated intravascular coagulation, convulsions, respiratory arrest, and death.

No prospective studies have been performed to evaluate the treatment of serotonin syndrome, and

treatment strategies are primarily based on case reports. Nonspecific serotonin receptor antagonists such as methyseride and cyproheptadine have been used successfully. Propanolol, which blocks serotonin 1A receptors, has also been used. Benzodiazepines can reduce the muscular rigidity. Dantrolene, a direct skeletal muscle relaxant, has also been found to be useful. The efficacy of these agents is yet to be evaluated.

Animal/Nonclinical Toxicology

Citalopram: Animal reproductive studies have revealed adverse effects on fetal and postnatal development at a dose higher than the human therapeutic dose.

Escitalopram: Teratogenic effects have been reported in animal studies.

Human Toxicology (Acute)

Ingestion: Nausea, vomiting, abdominal pain, diarrhea, tremor, confusion, agitation, drowsiness, insomnia, flulike syndromes, blurred vision, and in rare cases, seizures and coma. Significant cardiovascular toxicity is unusual (except with citalopram). Effects include mild hypo- or hypertension, tachycardia, and ventricular dysrhythmia. Escitalopram also has been shown to cause dermatologic, gastrointestinal, sexual, respiratory, and other miscellaneous adverse reactions.

More serious toxicity may be expected with high doses or coingestion, e.g., TCAs and MAOIs, which may result in a life-threatening serotonin syndrome.

Discontinuation Syndrome

SSRIs can cause adverse effects after withdrawal, either from a reduction in dosage or from the abrupt cessation of the drug. The most frequent symptoms include vertigo, dizziness, paresthesia (shocklike sensations, tingling and burning sensations); less frequently, symptoms include irritability, anxiety, headache, orthostatic hypotension, and sleep disturbances. The symptoms usually occur within 48 h after stopping the SSRI and they last $\sim$2 weeks.

Human Toxicology (Chronic)

Ingestion: A case of sertraline abuse has been reported with high doses, almost daily, for a period of 6 months. Effects include relaxation, euphoria, then intense excitement, marked tremor, and visual and auditory hallucinations.

Patients have survived large overdoses of each of the compounds, but there is concern over six fatalities following overdoses of citalopram.

Clinical Management of Overdose

Pure SSRI overdoses usually have a fairly benign course. However, a few deaths are reported in the literature, most involving a coingestion and/or very high doses.

Treatment is symptomatic and supportive, with diazepam for sedation if necessary. Cardiac monitoring is recommended in symptomatic cases. Coma and convulsions may occur in large overdose. If the patient presents within 2 h, a dose of activated charcoal should be given (50 g for adults; $1\,g\,kg^{-1}$ for children). Observation of vital signs and neurological status for 6 h is recommended.

Treatment of the serotonin syndrome in more severe intoxications or where there is a coingestion may require more aggressive measures such as establishment of an airway, ventilation, administration of intravenous fluids, and control of seizures and hyperthermia.

Intensive supportive care is rarely required. Measures that may be required based on the clinical presentation include endotracheal intubation and assisted ventilation if coma is present, intravenous fluid resuscitation if hypotension is present, pharmacological control of seizures, cooling if hyperthermia is present.

Gastrointestinal decontamination by administration of a single oral dose of active charcoal may be indicated. Gastric lavage followed by activated charcoal should be advocated in patients who have ingested large doses and/or when there has been a significant coingestion. There is no effective method known to enhance elimination.

See also: Food and Drug Administration, US; International Conference on Harmonisation.

Further Reading

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Staphylococcus aureus

Melanie J Karst

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Description

Staphylococcus aureus is a spherical coccoid which on microscopic examination appears in pairs, short chains, or bunched grape-like clusters. *S. aureus* forms a fairly large yellow colony on rich agar. The organisms are Gram-positive facultative anaerobes that grow by aerobic respiration or by fermentation yielding primarily lactic acid. The bacterium is capable of producing many highly heat-stable protein toxins.

Sources of Exposure

Staphylococci exist in air, dust, sewage, water, milk, and food or on food equipment, environmental surfaces, humans, and animals. Normally, this species lives in the human oropharynx, nose, large intestine, vagina, and on the skin without causing harm. However, if a breach in the skin or mucosal barrier occurs, *S. aureus* gains access to nearby tissues or the bloodstream where it can colonize and cause disease. The relationship between *S. aureus* and its human host, then, is dynamic in nature, capable of quickly shifting from mutualistic or commensualistic to parasitic.

Pathogenesis

S. aureus causes a variety of suppurative (pus-forming) infections and toxinoses in humans. It may cause superficial skin lesions (boils and styes); infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections such as osteomyelitis and endocarditis. *S. aureus* is associated with nosocomial infections of surgical wounds and infections with indwelling medical devices. *S. aureus* can cause toxic shock syndrome by releasing pyrogenic exotoxins into the bloodstream.

Food Poisoning

Human intoxication is caused by ingesting enterotoxins produced in food by some strains of *S. aureus*, usually because the food has not been kept hot enough (60°C, 140°F, or above) or cold enough (7.2°C, 45°F, or below). Foods that are frequently incriminated in staphylococcal food poisoning include meat and meat products; poultry and egg products; salads such as egg, tuna, chicken, potato, and macaroni; bakery products such as cream-filled pastries, cream pies, and chocolate éclairs; sandwich fillings; and milk and dairy products. Any foods that require considerable handling during preparation and that are kept at warmer temperatures after preparation are frequently involved in staphylococcal food poisoning.

Staphylococcal food poisoning is one of the most common types of food poisoning in the United States. The true incidence of staphylococcal food poisoning is unknown due to lack of information from victims, misdiagnosis of the illness, and inadequate collection of samples for laboratory analyses. A toxin dose of less than 1.0 μg in contaminated food will produce symptoms of staphylococcal intoxication. This toxin level represents a *S. aureus* population exceeding 100 000 organisms per gram.

Mechanism of Toxicity

S. aureus expresses many virulence factors: (1) surface proteins that promote colonization; (2) invasins that promote spread of bacteria in tissues; (3) surface characteristics that inhibit phagocytic engulfment (capsule, protein A); (4) biochemical properties that enhance their survival in phagocytes (carotenoids, catalase); (5) membrane-damaging toxins (hemolysins, leukotoxin, leukocidin); and (6) exotoxins that damage host tissue (toxic shock syndrome toxins, exfoliation toxin). The toxic shock toxin is a 'superantigen' that stimulates T-cells and the release of

cytokines causing symptoms mimicking endotoxic shock.

Diagnosis of Human Infection/Illness

Staphylococcal skin infections can usually be diagnosed by their appearance without laboratory testing. Serious staphylococcal infections require samples of blood or infected fluid for culture and diagnosis of which antibiotics should be used. Some strains are resistant to many antibiotics. Methicillin-resistant *S. aureus* is resistant to nearly all antibiotics and is increasingly common.

Proper interviews with the victims and analyzing epidemiological data are essential in the diagnosis of staphylococcal foodborne incidents. The most conclusive evidence is the linking of an illness with a specific food or detection of the toxin in a food sample.

Analysis of Foods

The staphylococcal toxin must be separated from food constituents and concentrated to detect trace amounts. The toxin is then identified by specific precipitation with antiserum as follows: (1) the selective adsorption of the enterotoxin from an extract of the food onto ion exchange resins and (2) the use of physical and chemical procedures for the selective removal of food constituents leaving the enterotoxin in solution. More recently rapid methods based on monoclonal antibodies (e.g., enzyme-linked immunosorbent assay, reverse passive latex agglutination) have been developed for detecting very low levels of enterotoxin in food.

Nature of the Disease

Staphylococcus bacteria are one of the most common causes of skin infection. There are many kinds of staphylococcal skin infections. The least serious is folliculitis, an infection of a hair root that produces a slightly painful pimple at the base of a hair. Most of these infections are minor (pimples and boils); however, *Staphylococcus* bacteria may also cause serious infections. Impetigo causes shallow, fluid-filled blisters and may itch or hurt. *Staphylococcus* skin abscesses are warm, painful, collections of pus below the skin surface and staphylococcus cellulitis is a spreading infection that develops under the skin producing pain and redness. More serious skin infections include toxic epidermal necrolysis and scalded skin syndrome in newborns.

The onset of symptoms of staphylococcal food poisoning is usually rapid and in many cases acute. The severity depends on the individual's susceptibility to the toxin, the amount of toxin ingested from contaminated food, and the general health of the person. The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration. In more severe cases headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur. All people are believed to be susceptible to this type of bacterial intoxication; however, the intensity and severity of symptoms may vary. Death from staphylococcal food poisoning is very rare but may occur among sensitive populations such as the elderly, infants, and immunocompromised individuals.

Clinical Management

Infections can usually be treated with penicillinase-resistant B-lactams. Infections acquired in the hospital are often antibiotic resistant strains and can only be treated with vanomycin. Vaccines are not currently available.

When contaminated food is ingested, the toxins, not the bacteria, produce the illness. Since this food poisoning is not an infectious disease antibiotics are of no value. Most cases do not require hospitalization but fluid replacement may be required. Prevention of staphylococcal food poisoning by cleanliness of food preparation areas, proper refrigeration, and good hand washing is the most effective control strategy.

See also: Food and Drug Administration, US; Food Safety and Toxicology; Gastrointestinal System; Skin.

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Statistics

Shayne C Gad

Introduction

Statistics are an essential (and required by regulation) tool in all aspects of toxicology. They provide a means of objectively evaluating our tests of belief; for example, experiments. Increasing amounts and types of toxicological data are being generated each year. As a result, problems of data analysis have become more complex and toxicology has drawn more deeply from the well of available statistical techniques. Statistics has also changed and grown during the last 35 years, to some extent, at least, because of the parallel growth of toxicology. These simultaneous changes have led to an increasing complexity of data analysis. Before turning to the specific issues related to using statistics in interpreting toxicological data, it is important to discuss some of the basics of statistics.

Functions of Statistics

Statistical methods may serve to do any combination of three possible tasks. The most familiar is hypothesis testing, that is, determining if two (or more) groups of data differ from each other at a predetermined level of confidence. A second function is the construction and use of models which may be used to predict future outcomes of chemical–biological interactions. This is most commonly seen in linear regression or in the derivation of some form of correlation coefficient. Model fitting allows us to relate one variable (typically a treatment or 'independent' variable) to another. The third function, reduction of dimensionality, continues to be less commonly utilized than the first two. This final category includes methods for reducing the number of variables in a system while only minimally reducing the amount of information, therefore making a problem easier to visualize and understand. Examples of such techniques are factor analysis and cluster analysis. A subset of this last function, discussed later under 'descriptive statistics', is the reduction of raw data to single expressions of central tendency and variability (such as the mean and standard deviation (SD)).

There is also a special subset of statistical techniques that is part of both the second and third functions of statistics. This is data transformation, which includes such things as the conversion of numbers to log or probit values.

Data

Each measurement made, each individual piece of experimental information gathered, is called a datum. Generally, multiple pieces of information are gathered and analyzed at one time, and the resulting collection is called data.

Data are collected on the basis of their association with a treatment (intended or otherwise) or as an effect (a property) that is measured in the experimental subjects of a study, such as body weights. These identifiers (i.e., treatment and effect) are termed variables. The treatment variables (those that the researcher or nature control, and that can be directly controlled) are termed independent, while our effect variables (such as weight, lifespan, and number of neoplasms) are termed dependent variables; their outcome is believed to be dependent on the 'treatment' being studied.

All the possible measures of a given set of variables in all the possible subjects that exist are termed the population for those variables. Such a population of variables cannot be truly measured; for example, to achieve this it would be necessary to obtain, treat and measure the weights of all the animals of a particular type; for example, Fischer-344 rats, that were, are, or ever will be. Instead, studies are performed on a representative group, a sample. If the sample of data is appropriately collected and of sufficient size, it serves to provide good estimates of the characteristics of the parent population from which it was drawn.

Data can be classified into different types. **Table 1** shows one such classification.

The nature of the data collected is determined by three considerations. These are the source of the data

Table 1 Types of variables (data) and examples of each type

Classified by	*Type*	*Toxicological example*
Scale		
Continuous	Scalar	Body weight
	Ranked	Severity of a lesion
Discontinuous	Scalar	Weeks until the first observation of a tumor in a carcinogenicity study
	Ranked	Clinical observations in animals
	Attribute	Eye colors in fruit flies
	Quantal	Dead/alive or present/absent
Frequency distribution	Normal	Body weights
	Bimodal	Some clinical chemistry parameters
	Others	Measures of time to incapacitation

(the system being studied), the instrumentation and techniques being used to make measurements, and the design of the experiment. The researcher has some degree of control over each of these, the least over the system; for example, biological organism (he or she normally has a choice of only one of several models to study), and the most over the design of the experiment or study. Such choices, in fact, dictate the type of data generated by a study.

Statistical methods are based on specific assumptions. Parametric statistics, those most familiar to the majority of scientists, have more stringent underlying assumptions than do nonparametric statistics. Among the underlying assumptions for many parametric statistical methods (such as the analysis of variance) is that the data are continuous. The nature of the data associated with a variable (as described previously) imparts a 'value' to that data, the value being the power of the statistical tests which can be employed.

Continuous variables are those that can at least theoretically assume any of an infinite number of values between any two fixed points (such as measurements of body weight between 2.0 and 3.0 kg). Discontinuous variables, meanwhile, are those which can have only certain fixed values, with no possible intermediate values (such as counts of five and six dead animals, respectively).

Limitations on the ability to measure constrain the extent to which the real-world situation approaches the theoretical, but many of the variables studied in toxicology are in fact continuous. Examples of these are lengths, weights, concentrations, temperatures, periods of time, and percentages. For these continuous variables, the character of a sample may be described using measures of central tendency and dispersion that are most familiar: the mean, denoted by the symbol $\bar{X}$ and also called the arithmetic average, and the SD, denoted by the symbol σ and calculated as being equal to

$$\sigma = \sqrt{\frac{\sum X^2 - (\sum X)^2/N}{N-1}}$$

where X is the individual datum and N is the total number of data in the group.

Contrasted with these continuous data, however, are discontinuous (or discrete) data, which can only assume certain fixed numerical values. In these cases the choice of statistical tools or tests is, as will be seen, more limited.

Descriptive Statistics

Descriptive statistics are used to summarize the general nature of a data set. As such, the parameters describing any single group of data have two components. One of these describes the location of the data, while the other gives a measure of the dispersion of the data in and about this location. Often overlooked is the fact that the choice of which parameters are used to generate these pieces of information implies a particular type of distribution for the data.

Most commonly, location is described by giving the (arithmetic) mean and dispersion in terms of the SD or the SEM. The calculation of the first two of these has already been described. If the total number of data in a group is N, then the SEM would be calculated as

$$\text{SEM} = \frac{\text{SD}}{\sqrt{N}}$$

The use of the mean with either the SD or SEM implies, however, that there is reason to believe that the sample of data being summarized is from a population that is at least approximately normally distributed. If this is not the case, then it is more appropriate to use a set of statistical descriptions which do not require a normal distribution. These are the median, for location, and the semiquartile distance, for a measure of dispersion. These somewhat less familiar parameters are characterized as follows.

Median When all the numbers in a group are arranged in a ranked order (i.e., from smallest to largest), the median is the middle value. If there is an odd number of values in a group, then the middle value is obvious (in the case of 13 values, e.g., the seventh largest is the median). When the number of values in the sample is even, the median is calculated as the midpoint between the $(N/2)$th and the $((N/2)+1)$th number. For example, in the series of numbers 7, 12, 13, 19 the median value would be the midpoint between 12 and 13, which is 12.5.

The SD and the SEM are related to each other but yet are quite different.

The SEM is quite a bit smaller than the SD, making it very attractive to use in reporting data. This size difference is because the SEM actually is an estimate of the error (or variability) involved in measuring the mean values of samples, and not an estimate of the error (or variability) involved in measuring the data from which mean values are calculated. This is implied by the central limit theorem, which makes three major assumptions.

- The distribution of sample means will be approximately normal regardless of the distribution of

values in the original population from which the samples were drawn.
- The mean value of the collection equals the mean of all possible sample means, so the collection mean can be estimated from the sample means.
- The SD of the collection of all possible means of samples of a given size, called the SEM, depends on both the SD of the original population and the size of the sample.

The SEM should be used only when the uncertainty of the estimate of the mean is of concern, which is almost never the case in toxicology. Rather, toxicology is concerned with an estimate of the variability of the population for which the SD is appropriate.

Semiquartile Distance When all the data in a group are ranked, a quartile of the data contains one ordered quarter of the values. Typically, we are most interested in the borders of the middle two quartiles Q_1 and Q_3 which together represent the semiquartile distance and which contain the median as their center, are of most interest. Given that there are N values in an ordered group of data, the upper limit of the jth quartile (Q_j) may be computed as being equal to the $((jN-1)/4)$th value. After using this formula to calculate the upper limits of Q_1 and Q_3, it is possible to compute the semiquartile distance (which is also called the quartile deviation, and as such is abbreviated as QD) with the formula $\mathrm{QD} = (Q_3 - Q_1)/2$.

For example, for the 15-value data set 1, 2, 3, 4, 4, 5, 5, 5, 6, 6, 6, 7, 7, 8, 9, the upper limits of Q_1 and Q_3 can be calculated as

$$Q_1 = \frac{1(15+1)}{4} = \frac{16}{4} = 4$$

$$Q_3 = \frac{3(15+1)}{4} = \frac{48}{4} = 12$$

The fourth and 12th values in this data set are 4 and 7, respectively. The semiquartile distance can then be calculated as

$$\mathrm{QD} = \frac{7-4}{2} = 1.5$$

There are times when it is desired to describe the relative variability of one or more sets of data. The most common way of doing this is to compute the coefficient of variation (CV), which is calculated simply as the ratio of the SD to the mean, or

$$\mathrm{CV} = \frac{\mathrm{SD}}{\bar{X}}$$

A CV of 0.2% or 20% thus means that the SD is 20% of the mean. In toxicology the CV is frequently between 20% and 50% and may at times exceed 100%.

Applying Statistics to Toxicology

To successfully apply statistics to toxicology it is critical to understand the biological dimensions of a problem, as well as the unique characteristics of toxicological data analysis. These characteristics include the following:

1. The need to work with a relatively small sample set of data collected from the members of a population (laboratory animals) that are not actually the population of interest (i.e., humans or a target animal population).
2. The need to frequently deal with data resulting from a sample that was censored on a basis other than the investigator's design. By censoring, of course, is meant that not all desired data points were collected. This censoring could be the result of either a biological factor (the test animal being dead or too debilitated to manipulate) or a logistic factor (equipment being inoperative or a tissue being missed in necropsy).
3. The conditions under which experiments are conducted are extremely varied. In pharmacology (the closest cousin to at least classical toxicology), the possible conditions of interaction of a chemical or physical agent with a person are limited to a small range of doses via a single route over a short course of treatment to a defined patient population. In toxicology however, all these variables (dose, route, time span, and subject population) are limited only by the investigator.
4. The timeframes available to solve toxicological problems are limited by practical and economic factors. This frequently means that there is no time to repeat a critical study if the first attempt fails. So a true iterative approach is often not possible.

The training of most pathologists in statistics remains limited to a single introductory course which concentrates on some theoretical basics. As a result, the armentarium of statistical techniques of most toxicologists is limited and the tools that are normally employed (*t*-tests, chi-square, analysis of variance, and linear regression) are neither fully developed nor well understood.

To appreciate the biological dimensions of analyzing data and the difference between biological significance and statistical significance, it is useful to

consider the four possible combinations of these two different types of significance as shown in the table given below:

Biological significance	*Statistical significance*	
	No	*Yes*
No	Case I	Case II
Yes	Case III	Case IV

Cases I and IV give no problems, for the answers are the same statistically and biologically. But cases II and III present problems. In case II (the 'false positive'), we have a circumstance where there is a statistical significance in the measured difference between treated and control groups, but there is no true biological significance to the finding. This is not an uncommon happening, for example, in the case of clinical chemistry parameters. This is called a type I error by statisticians, and the probability of this happening is called the α level. In case III (the 'false negative'), there is no statistical significance, but the differences between groups are biologically and toxicologically significant. This is called a type II error by statisticians, and the probability of such an error happening by random chance is called the β level. An example of this second situation is when there are few of a very rare tumor type in treated animals. In both of these latter cases, numerical analysis, no matter how well done, is no substitute for professional judgment. Along with this, however, it is critical to have a feeling for the different types of data and for the value or relative merit of each. Note that the two error types interact, and in determining sample size it is necessary to specify both α and β levels. **Table 2** demonstrates this interaction in the case of tumor or specific lesion incidence.

The reasons that biological and statistical significance are not identical are multiple, but a central one is certainly causality. Through this consideration of statistics, it should be kept in mind that just because a treatment and a change in an observed organism are seemingly or actually associated with each other does not 'prove' that the former caused the latter. Though this fact is now widely appreciated for correlation (e.g., the fact that the number of storks' nests found each year in England is correlated with the number of human births that year does not mean that storks bring babies), it is just as true in the general case of significance. Proof that treatment causes an effect requires an understanding of the underlying mechanism and proof of its validity. At the same time, it is important to realize that not finding a good correlation or suitable significance associated with a treatment and an effect likewise does not prove that the two are not associated, that a treatment does not cause an effect. At best, it gives a certain level of confidence that under the conditions of the current test, these items are not associated.

Bias and Chance

Any toxicological study aims to determine whether a treatment elicits a response. An observed difference in response between a treated and control group need not necessarily be a result of treatment. There are, in principle, two other possible explanations: *bias*, or systematic differences other than treatment between the groups, and *chance*, or random differences. A major objective of both experimental design and analysis is to try to avoid bias. Wherever possible, treated and control groups to be compared should be alike in respect of all other factors. Where differences remain, these should be corrected for in the statistical

Table 2 Sample size required to obtain a specified sensitivity at $p<0.05$ treatment group incidence

Background tumor incidence (%)	*p*[a]	*Required sample size*									
		Incidence rate (%)									
		0.95	*0.90*	*0.80*	*0.70*	*0.60*	*0.50*	*0.40*	*0.30*	*0.20*	*0.10*
0.30	0.90	10	12	18	31	46	102	389			
	0.50	6	6	9	12	22	32	123			
0.20	0.90	8	10	12	18	30	42	88	320		
	0.50	5	5	6	9	12	19	28	101		
0.10	0.90	6	8	10	12	17	25	33	65		
	0.50	3	3	5	6	9	11	17	31	68	
0.05	0.90	5	6	8	10	13	18	25	35	76	464
	0.50	3	3	5	6	7	9	12	19	24	147
0.01	0.90	5	5	7	8	10	13	19	27	46	114
	0.50	3	3	5	5	6	8	10	13	25	56

[a] Power for each comparison of treatment group with background incidence.

analysis. Chance cannot be wholly excluded, as identically treated animals will not respond identically. While even the most extreme difference might in theory be due to chance, a proper statistical analysis will allow the experimenter to assess this possibility The smaller the probability of a 'false positive', the more confident the experimenter can be that the effect is real. Good experimental design improves the chance of picking up a true effect with confidence by maximizing the ratio between 'signal' and 'noise'.

Hypothesis Testing and Probability (*p*) Values

A relationship of treatment to some toxicological end point is often stated to be 'statistically significant ($p < 0.05$)'. What does this really mean? A number of points have to be made. First, statistical significance need not necessarily imply biological importance, if the end point under study is not relevant to the animal's well-being. Second, the statement will normally be based only on the data from the study in question and will not take into account prior knowledge. In some situations, for example, when one or two of a very rare tumor type are seen in treated animals, statistical significance may not be achieved but the finding may be biologically extremely important, especially if a similar treatment was previously found to elicit a similar response. Third, the *p*-value does not describe the probability that a true effect of treatment exists. Rather, it describes the probability of the observed response, or one more extreme, occurring on the assumption that treatment actually had no effect whatsoever. A *p*-value that is not significant is consistent with a treatment having a small effect, not detected with sufficient certainty in this study. Fourth, there are two types of *p*-value. A 'one-tailed' (or one-sided) *p*-value is the probability of getting by chance a treatment effect in a specified direction as great as or greater than that observed. A 'two-tailed' *p*-value is the probability of getting, by chance alone, a treatment difference in either direction which is as great as or greater than that observed. By convention, *p*-values are assumed to be two-tailed unless the contrary is stated. Where, which is unusual, it is possible to rule out in advance the possibility of a treatment effect except in one direction, a one-tailed *p*-value should be used. Often, however, two-tailed tests are to be preferred, and it is certainly not recommended to use one-tailed tests and not report large differences in the other direction. In any event, it is important to make it absolutely clear whether one- or two-tailed tests have been used.

It is a great mistake, when presenting results of statistical analyses, to mark, as do some laboratories, results simply as significant or not significant at one defined probability level (normally $p < 0.05$). This practice does not allow the reader any real chance to judge whether or not the effect is a true one. Some statisticians present the actual *p*-value for every comparison made. While this gives precise information it can make it difficult to assimilate results from many variables. One recommended practice to mark *p*-values routinely using plus signs to indicate positive differences (and minus signs to indicate negative differences): $+++$ $p < 0.001$, $++$ $0.001 \leqslant p < 0.01$, $+$ $0.01 \leqslant p < 0.05$, $\pm$ $0.05 \leqslant p < 0.1$. This highlights significant results more clearly and also allows the reader to judge the whole range from 'virtually certain treatment effect' to 'some suspicion'. Note that using two-tailed tests, bracketed plus signs indicate findings that would be significant at the conventional $p < 0.05$ level using one-tailed tests but are not significant at this level using two-tailed tests. This 'fiducial limit' ($p < 0.05$) implies a false-positive incidence of one in 20, and though now imbedded in regulation, practice, and convention, was somewhat an arbitrary choice to begin with. In interpreting *p*-values it is important to realize they are only an aid to judgment to be used in conjunction with other available information. A $p < 0.01$ increase might be due to chance when it was unexpected, occurred only at a low dose level with no such effect seen at higher doses, and was evident in only one subset of the data. In contrast, a $p < 0.05$ increase might be convincing if it occurred in the top dose and was for an end point that was expected to show an increase from known properties of the chemical or closely related chemicals.

Multiple Comparisons

When a *p*-value is stated to be < 0.05, this implies that, for that particular test, the difference could have occurred by chance less than one time in 20. Toxicological studies frequently involve making treatment-control comparisons for large numbers of variables and, in some situations, also for various subsets of animals. Some statisticians worry that the larger the number of tests the greater is the chance of picking up statistically significant findings that do not represent true treatment effects. For this reason, an alternative 'multiple comparisons' procedure has been proposed in which, if the treatment was totally without effect, then 19 times out of 20 all the tests should show nonsignificance when testing at the 95% confidence level. Automatic use of this approach cannot be recommended. Not only does it make much more difficult to pick up any real effects, but also there is something inherently unsatisfactory

about a situation where the relationship between a treatment and a particular response depends arbitrarily on which other responses happened to be investigated at the same time. It is accepted that in any study involving multiple end points there will inevitably be a gray area between those showing highly significant effects and those showing no significant effects, where there is a problem distinguishing chance and true effects. However, changing the methodology so that the gray areas all come up as nonsignificant can hardly be the answer.

Estimating the Size of the Effect

It should be clearly understood that a *p*-value does not give direct information about the size of any effect that has occurred. A compound may elicit an increase in response by a given amount, but whether a study finds this increase to be statistically significant will depend on the size of the study and the variability of the data. In a small study, a large and important effect may be missed, especially if the end point is imprecisely measured. In a large study, on the contrary, a small and unimportant effect may emerge as statistically significant.

Hypothesis testing tells us whether an observed increase can or cannot be reasonably attributed to chance, but not how large it is. Although much statistical theory relates to hypothesis testing, current trends in medical statistics are toward confidence interval estimation with differences between test and control groups expressed in the form of a best estimate, coupled with the 95% confidence interval (Q). Thus, if one states that treatment increases response by an estimated 10 units (95% CI 3–17 units), this would imply that there is a 95% chance that the indicated interval includes the true difference. If the lower 95% confidence limit exceeds 0, this implies that the increase is statistically significant at $p < 0.05$ using a two-tailed test. One can also calculate, for example, 99% or 99.9% confidence limits, corresponding to testing for significance at $p < 0.01$ or $p < 0.001$.

In screening studies of standard design, the tendency has been to concentrate mainly on hypothesis testing. However, presentation of the results in the form of estimates with confidence intervals can be a useful adjunct for some analyses and is very important in studies aimed specifically at quantifying the size of an effect.

Two terms refer to the quality and reproducibility of the measurements of variables. The first, accuracy, is an expression of the closeness of a measured or computed value to its actual or 'true' value in nature. The second, precision, reflects the closeness or reproducibility of a series of repeated measurements of the same quantity.

If all of the measurements of a particular variable are arranged in order as points on an axis marked as to the values of that variable, and if the sample were large enough, the pattern of distribution of the data in the sample would begin to become apparent. This pattern is a representation of the frequency distribution of a given population of data; that is, of the incidence of different measurements, their central tendency, and dispersion. The most common frequency distribution, and one that will be discussed throughout this article, is the normal (or Gaussian) distribution. The normal distribution is such that two-thirds of all values are within 1 SD of the mean (or average value for the entire population) and 95% are within 1.96 SD of the mean. Symbols used are μ for the mean and σ for the SD. Other common frequency distributions, such as the binomial, Poisson and chi-square, are sometimes encountered.

Statistical Principles in Experimental Toxicology

Toxicological experiments generally have a twofold purpose. The first purpose is to answer the question whether or not an agent results in an effect on a biological system. The second purpose is to determine how much of an effect is present. It has become increasingly desirable that the results and conclusions of studies aimed at assessing the effects of environmental agents be as clear and unequivocal as possible. It is essential that every experiment and study yield as much information as possible, and that the results of each study have the greatest possible chance of answering the questions it was conducted to address. The statistical aspects of such efforts, so far as they are aimed at structuring experiments to maximize the possibilities of success, are called experimental design.

The four basic statistical principles of experimental design are replication, randomization, concurrent (local) control and balance. In abbreviated form, these may be summarized as follows:

1. *Replication*. Any treatment must be applied to more than one experimental unit (animal, plate of cells, litter of offspring, etc.). This provides more accuracy in the measurement of a response than can be obtained from a single observation, as underlying experimental errors tend to cancel each other out. It also supplies an estimate of the experimental error derived from the variability

among each of the measurements taken (or replicates). In practice, this means that an experiment should have enough experimental units in each treatment group (i.e., a large enough *N*) so that reasonably sensitive statistical analysis of data can be performed. The estimation of sample size is addressed in detail later in this article.

2. *Randomization*. This is practiced to ensure that every treatment shall have its fair share of extreme high and extreme low values. It also serves to allow the toxicologist to proceed as if the assumption of 'independence' is valid. This assumption is that there is no avoidable (known) systematic bias in obtaining data.
   Random allocation of animals to treatment groups is a prerequisite of good experimental design. If not carried out, it is not possible to be sure whether treatment versus control differences are due to treatment or to 'confounding' by other relevant factors. The ability to randomize easily is a major advantage animal experiments have over epidemiology.
   While randomization eliminates bias (as least in expectation), simple randomization of all animals may not be the optimal technique for producing a sensitive test. If there is another major source of variation (e.g., sex or batch of animals), it will be better to carry out stratified randomization (i.e., carry out separate randomizations within each level of the stratifying variable).
   The need for randomization applies not only to the allocation of the animals to the treatment, but also to anything that can materially affect the recorded response. The same random number that is used to apply animals to treatment groups can be used to determine cage position, order of weighing, order of bleeding for clinical chemistry, order of sacrifice at terminations and so on.
3. *Concurrent control*. Comparisons between treatments should be made to the maximum extent possible between experimental units from the same closely defined population. Therefore, animals used as a control group should come from the same source, lot, age, and so on as test group animals. Except for the treatment being evaluated, test and control animals should be maintained and handled in exactly the same manner.
   While historical control data can, on occasion, be useful, a properly designed study demands that a relevant concurrent control group be included with which results for the test group can be compared. The principle that like should be compared with like, apart from treatment, demands that control animals should be randomized from the same source as treatment animals. Careful consideration should also be given to the appropriateness of the control group. Thus, in an experiment involving treatment of a compound in a solvent, it would often be inappropriate to include only an untreated control group as any differences observed could only be attributed to the treatment–solvent combination. To determine the specific effects of the compound a comparison group given the solvent only, by the same route of administration, would be required.
   It is not always generally realized that the position of the animal in the room in which it is kept may affect the animal's response. An example is the strong relationship between incidence of retinal atrophy in albino rats and closeness to the lighting source. Systematic differences in cage position should be avoided, preferably via randomization.
4. *Balance*. If the effects of several different factors are being evaluated simultaneously, the experiment should be laid out in such a way that the contributions of the different factors can be separately distinguished and estimated. There are several ways of accomplishing this using one of several different forms of design, as will be discussed later.

In addition, there are a number of facets of any study which may affect its ability to detect an effect of a treatment. These relate to either minimizing the role of chance or avoiding bias.

Choice of Species and Strain

Ideally, the responses of interest should be rare in untreated control animals but should be reasonably readily evoked by appropriate treatments. For example, some species or specific strains, perhaps because of inappropriate diets, have high background tumor incidences which make increases both difficult to detect and difficult to interpret when detected.

Sampling

Sampling – the selection of which individual data points will be collected, whether in the form of selecting which animals to collect blood from or to remove a portion of a diet mix from for analysis – is an essential step upon which all other efforts toward a good experiment or study are based.

There are three assumptions about sampling which are common to most of the statistical analysis techniques that are used in toxicology. These are that the sample is collected without bias, that each member of a sample is collected independently of the others,

and that members of a sample are collected with replacements. Precluding bias, both intentional and unintentional, means that at the time of selection of a sample to measure, each portion of the population from which that selection is to be made has an equal chance of being selected. Ways of precluding bias are discussed in detail in the section on experimental design.

Independence means that the selection of any portion of the sample is not affected by and does not affect the selection or measurement of any other portion.

Finally, sampling with replacement means that in theory, after each portion is selected and measured, it is returned to the total sample pool and thus has the opportunity to be selected again. This is a corollary of the assumption of independence. Violation of this assumption (which is almost always the case in toxicology and all the life sciences) does not have serious consequences if the total pool from which samples are selected are sufficiently large (say 20 or greater) so that the chance of reselecting that portion is small anyway.

There are four major types of sampling methods: random, stratified, systematic, and cluster. Random is by far the most commonly employed method in toxicology. It stresses the fulfillment of the assumption of avoiding bias. When the entire pool of possibilities is mixed or randomized (procedures for randomization are presented in a later section), then the members of the group are selected in the order that are drawn from the pool.

Stratified sampling is performed by first dividing the entire pool into subsets or strata, then doing randomized sampling from each strata. This method is employed when the total pool contains subsets that are distinctly different but in which each subset contains similar members. An example is a large batch of a powdered pesticide in which it is desired to determine the nature of the particle size distribution. Larger pieces or particles are on the top, while progressively smaller particles have settled lower in the container and at the very bottom, the material has been packed and compressed into aggregates. To determine a timely representative answer, proportionally sized subsets from each layer or strata should be selected, mixed and randomly sampled. This method is used more commonly in diet studies.

In systematic sampling, a sample is taken at set intervals (such as every fifth container of reagent). This is most commonly employed in quality assurance or (in the clinical chemistry laboratory) in quality control.

In cluster sampling, the pool is already divided into numerous separate groups (such as bottles of tablets), and small sets of groups (such as several bottles of tablets) are first selected and then a few members from each set are selected. The result is a cluster of measures. Again, this is a method most commonly used in quality control or in environmental studies when the effort and expense of physically collecting a small group of units is significant.

In classical toxicology studies sampling arises in a practical sense in a limited number of situations. The most common of these are:

1. Selecting a subset of animals or test systems from a study to make some measurement (which either destroys or stresses the measured system, or is expensive) at an interval during a study. This may include such cases as doing interim necropsies in a chronic study or collecting and analyzing blood samples from some animals during a subchronic study.
2. Analyzing inhalation chamber atmospheres to characterize aerosol distributions with a new generation system.
3. Analyzing diet in which test material has been incorporated.
4. Performing quality control on an analytical chemistry operation by having duplicate analyses performed on some materials.
5. Selecting data to audit for quality assurance purposes.

Dose Levels

This is a very important and controversial area. In screening studies aimed at hazard identification it is normal, in order to avoid requiring huge numbers of animals, to test at dose levels higher than those to which man will be exposed, but not so high that marked toxicity occurs. A range of doses is normally tested to guard against the possibility of an inappropriate selection of the high dose as the metabolic pathways at the high doses may differ markedly from those at lower doses and, also, to ensure no large effects occur at dose levels in the range to be used by humans. In studies aimed at risk estimation, more and lower doses may be tested to obtain fuller information on the shape of the dose–response curve.

Number of Animals

This is obviously an important determinant of the precision of the findings. The calculation of the appropriate number depends on: (1) the critical difference, that is, the size of the effect it is desired to detect; (2) the false-positive rate, that is, the probability of an effect being detected when none exists

(equivalent to the 'α level' or 'type I error'), (3) the false-negative rate, that is, the probability of no effect being detected when one of exactly the critical size exists (equivalent to the 'β level' or 'type II error'), and (4) some measure of the variability in the material.

Tables and/or formulas relating numbers of animals required to obtain values of critical size, α and β are available in many statistics texts and software is also available for this purpose. As a rule of thumb, to reduce the critical difference by a factor of n for a given α and β the number of animals required will have to increased by a factor of n^2.

Duration of the Study

It is obviously important not to terminate the study too early for fatal conditions, which are normally strongly age-related. Less obviously, going on for too long in a study can be a mistake, partly because the last few weeks or months may produce relatively few extra data at a disproportionate cost, and partly because diseases of extreme old age may obscure the detection of tumors and other conditions of more interest. For nonfatal conditions, the ideal is to sacrifice the animals when the average prevalence is $\sim 50\%$.

Stratification

To detect a treatment difference with accuracy, it is important that the groups being compared are as homogeneous as possible with respect to other known causes of the response. In particular, suppose that there is another known important cause of the response for which the animals vary, so that the animals are a mixture of hyper- and hypo-responders from this cause. If the treated group has a higher proportion of hyperresponders it will tend to have a higher response even if treatment has no effect. Even if the proportion of hyperresponders is the same as in the controls, it will be more difficult to detect an effect of treatment because of the increased between animal variability.

Given that this other factor is known, it will be sensible to take it into account in both the design and analysis of the study. In the design, it can be used as a 'blocking factor' so that animals at each level are allocated equally (or in the correct proportion) to control and treated groups. In the analysis, the factor should be treated as a stratifying variable, with separate treatment–control comparisons made at each level, and the comparisons combined for an overall test of difference. This is discussed later, where the factorial design is addressed as one example of the more complex designs that can be used to investigate the separate effect of multiple treatments.

Statistics and Experimental Protocols in Toxicology

It is now routine to develop exhaustively detailed protocols for an experiment or study prior to its conduct. *A priori* selection of statistical methodology (as opposed to the *post hoc* approach) is as significant a portion of the process of protocol development and experimental design as any other and can measurably enhance the value of the experiment or study. Prior selection of statistical methodologies is essential for proper design of other portions of a protocol such as the number of animals per group or the sampling intervals for body weight. Implied in such a selection is the notion that the toxicologist has both an in-depth knowledge of the area of investigation and an understanding of the general principles of experimental design, for the analysis of any set of data is dictated to a large extent by the manner in which the data are obtained.

A second concept and its understanding are essential to the design of experiments in toxicology, that of censoring. Censoring is the exclusion of measurements from certain experimental units, or indeed of the experimental units themselves, from consideration in data analysis or inclusion in the experiment at all. Censoring may occur either prior to initiation of an experiment (where, in modern toxicology, this is almost always a planned procedure), during the course of an experiment (when they are almost universally unplanned, resulting from such as the death of animals on test), or after the conclusion of an experiment (when data are excluded because of being identified as some form of outlier).

In practice, *a priori* censoring in toxicology studies occurs in the assignment of experimental units (such as animals) to test groups. The most familiar example is in the common practice of assignment of test animals to acute, subacute, subchronic, and chronic studies, where the results of otherwise random assignments are evaluated for body weights of the assigned members. If the mean weights are found not be comparable by some preestablished criterion (such as a 90% probability of difference by analysis of variance) then members are reassigned (censored) to achieve comparability in terms of starting body weights. Such a procedure of animal assignment to groups is known as a *censored randomization*.

The first precise or calculable aspect of experimental design encountered is determining sufficient test and control group sizes to allow one to have an adequate level of confidence in the results of a study

(i.e., in the ability of the study design with the statistical tests used to detect a true difference, or effect, when it is present). The statistical test contributes a level of power to such a detection. Remember that the power of a statistical test is the probability that a test results in rejection of a hypothesis, H_0 say, when some other hypothesis, H, say, is valid. This is termed the power of the test 'with respect to the (alternative) hypothesis H'.

If there is a set of possible alternative hypotheses, the power, regarded as a function of H, is termed the *power function* of the test. When the alternatives are indexed by a single parameter θ, simple graphical presentation is possible. If the parameter is a vector θ, one can visualize a power surface.

If the power function is denoted by $\beta(\theta)$ and H_0 specifies $\theta = \theta_0$, then the value of β(II), the probability of rejecting H_0 when it is in fact valid, is the significance level. A test's power is greatest when the probability of a type II error is the least. Specified powers can be calculated for tests in any specific or general situation.

Some general rules to keep in mind are:

- The more stringent the significance level, the greater the necessary sample size. More subjects are needed for a 1% level test than for a 5% level test.
- Two-tailed tests require larger sample sizes than one-tailed tests. Assessing two directions at the same time requires a greater investment.
- The smaller the critical effect size, the larger the necessary sample size. Subtle effects require greater efforts.
- Any difference can be significant if the sample size is large enough.
- The larger the power required, the larger the necessary sample seize. Greater protection from failure requires greater effort. The smaller the sample size, the smaller the power; that is, the greater the chance of failure.
- The requirements and means of calculating necessary sample size depend on the desired (or practical) comparative sizes of test and control groups.

This number (N) can be calculated, for example, for equal-sized test and control groups, using the formula:

$$N = \frac{(t_1 + t_2)^2}{d^2} S$$

where t_1 is the one-tailed t-value with $N-1$ degrees of freedom corresponding to the desired level of confidence, t_2 is the one-tailed t-value with $N-1$ degrees of freedom corresponding to the probability that the sample size will be adequate to achieve the desired precision, S is the sample SD, derived typically from historical data and calculated as:

$$S = \sqrt{\frac{1}{N-1} \sum (V_1 - V_2)^2}$$

There are a number of aspects of experimental design which are specific to the practice of toxicology. Before discussing the step-by-step development of experimental designs, these aspects should first be considered.

1. Frequently, the data gathered from specific measurements of animal characteristics are such that there is wide variability in the data. Often, such wide variability is not present in a control or low-dose group, but in an intermediate dosage group variance inflation may occur. That is, there may be a large SD associated with the measurements from this intermediate group. In the face of such a set of data, the conclusion that there is no biological effect based on a finding of no statistically significance effect might well be erroneous.
2. In designing experiments, it is important to keep in mind the potential effect of involuntary censoring on sample size. In other words, though a study might start with five dogs per group, this provides no margin should any die before the study is ended and blood samples are collected and analyzed. Just enough experimental units per group frequently leaves too few at the end to allow meaningful statistical analysis, and allowances should be made accordingly in establishing group sizes.
3. It is certainly possible to pool the data from several identical toxicological studies. One approach to this is meta-analysis, considered in detail later in this chapter. For example, if an acute inhalation study was performed where only three treatment group animals survived to the point at which a critical measure (such as analysis of blood samples) was taken, there would not be enough data to perform a meaningful statistical analysis. In such a case, the protocol could be repeated with new control and treatment group animals from the same source. At the end, after assurances that the two sets of data are comparable, the data from survivors of the second study could be combined (pooled) with those from the first.
4. Another frequently overlooked design option in toxicology is the use of an unbalanced design, that is, of different group sizes for different levels of treatment. There is no requirement that each group in a study (control, low dose, intermediate

dose and high dose) have an equal number of experimental units assigned to it. Indeed, there are frequently good reasons to assign more experimental units to one group than to others, and, all the major statistical methodologies have provisions to adjust for such inequalities, within certain limits. Most commonly in the unbalanced design larger groups are assigned to either the highest dose, to compensate for losses due to possible deaths during the study, or to the lowest dose to give more sensitivity in detecting effects at levels close to an effect threshold or more confidence to the assertion that no effect exists.

5. A common problem is the existence of an undesired variable that is influencing the experimental results in a nonrandom fashion. Such a variable is called a confounding variable; its presence, as discussed earlier, makes the clear attribution and analysis of effects at best difficult, and at worst impossible. Sometimes such confounding variables are the result of conscious design or management decisions, such as the use of different instruments, personnel, facilities, or procedures for different test groups within the same study. Occasionally, however, such confounding variables are the result of unintentional factors or actions, in which there is, as it is called, a lurking variable. Such variables almost always arise as the result of standard operating procedures being violated: water not being connected to a rack of animals over a weekend, a set of racks not being cleaned as frequently as others, or a contaminated batch of feed being used.
6. Finally, some thought must be given to the clear definition of what is meant by experimental unit and concurrent control.

The experimental unit in toxicology encompasses a wide variety of possibilities. It may be cells, plates of microorganisms, individual animals, litters of animals, and so on. The importance of clearly defining the experimental unit is that the number of such units per group is the *N*, which is used in statistical calculations or analyses and critically affects such calculations. The experimental unit is the unit, which receives treatments and yields a response which is measured and becomes a datum.

A true concurrent control is one that is identical in every manner with the treatment groups except for the treatment being evaluated. This means that all manipulations, including gavaging with equivalent volumes of vehicle or exposing to equivalent rates of air exchanges in an inhalation chamber, should be duplicated in control groups just as they occur in treatment groups.

The goal of experimental design is statistical efficiency and the economizing of resources. The single most important initial step in achieving such an outcome is to clearly define the objective of the study: get a clear statement of what questions are being asked.

For the reader who would like to further explore experimental design, there are a number of more detailed texts available which include more extensive treatments of the statistical aspects of experimental design.

Experimental Design Types in Toxicology

There are four basic experimental design types used in toxicology. These are the randomized block, latin square, factorial design, and nested design. Other designs that are used are really combinations of these basic designs, and are very rarely employed in toxicology. Before examining these four basic types, however, we must first examine the basic concept of blocking.

Blocking is, simply put, the arrangement or sorting of the members of a population (such as all of an available group of test animals) into groups based on certain characteristics which may (but are not sure to) alter an experimental outcome. Such characteristics, which may cause a treatment to give a differential effect, include genetic background, age, sex, overall activity levels and so on. The process of blocking then acts (or attempts to act), so that each experimental group (or block) is assigned its fair share of the members of each of these subgroups.

Remember that randomization is aimed at spreading out the effect of undetectable or unsuspected characteristics in a population of animals or some portion of this population. The merging of the two concepts of randomization and blocking leads to the first basic experimental design, the randomized block. This type of design requires that each treatment group have at least one member of each recognized group (such as age), the exact members of each block being assigned in an unbiased (or random) fashion.

The second type of experimental design assumes that it is possible to characterize treatments (whether intended or otherwise) as belonging clearly to separate sets. In the simplest case, these categories are arranged into two sets which may be thought of as rows (for, say, source litter of test animal, with the first litter as row 1, the next as row 2, etc.) and the secondary set of categories as columns (for, say, ages of test animals, with 6–8 weeks as column 1, 8–10 weeks as column 2 and so on). Experimental units are then assigned so that each major treatment

(control, low dose, intermediate dose, etc.) appears once and only once in each row and each column. If the test groups are denoted as A (control), B (low), C (intermediate), and D (high), such an assignment would appear as shown in the table below:

Source litter	*Age (weeks)*			
	6–8	*8–10*	*10–12*	*12–14*
1	A	B	C	D
2	B	C	D	A
3	C	D	A	B
4	D	A	B	C

The third type of experimental design is the factorial design, in which there are two or more clearly understood treatments, such as exposure level to test chemical, animal age, or temperature. The classical approach to this situation (and to that described under the latin square) is to hold all but one of the treatments constant; and at any one time to vary just that one factor. Instead, in the factorial design all levels of a given factor are combined with all levels of every other factor in the experiment. When a change in one factor produces a different change in the response variable at one level of a factor than at other levels of this factor, there is an interaction between these two factors which can then be analyzed as an interaction effect.

The last of the major varieties of experimental design are the nested designs, where the levels of one factor are nested within (or are subsamples of) another factor. That is, each subfactor is evaluated only within the limits of its single larger factor.

See also: Carcinogenesis; Toxicity Testing, Carcinogenesis.

Further Reading

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Lee PN and Lovell D (1999) Statistics for toxicology. In: Ballantyne B, Marrs T, and Syversen T (eds.) *General and Applied Toxicology*, 2nd edn., pp. 291–302. New York: Grone's Dictionaries.

Sokal RR and Rohlf FJ (1995) *Biometry*, 3rd edn. New York: W.H. Freeman and Company.

Glossary

Some frequently used statistical terms and their meanings

Term	Meaning
95% confidence interval	A range of values (above, below or above and below) the sample (mean, median, mode, etc.) has a 95% chance of containing the true value of the population (mean, median, mode). Also called the fiducial limit equivalent to the $p < 0.05$
Bias	Systemic error as opposed to a sampling error. For example, selection bias may occur when each member of the population does not have an equal chance of being selected for the sample
Degrees of freedom	The number of independent deviations, normally abbreviated *df*
Independent variables	Also known as predictors or explanatory variables
p-value	Another name for significance level; normally 0.005
Power	The effect of the experimental conditions on the dependent variable relative to sampling fluctuation. When the effect is maximized, the experiment is more powerful. Power can also be defined as the probability that there will not be a type 11 error $(I - \beta)$. Conventionally, power should be at least 0.07
Random	Each individual member of the population has the same chance of being selected for the sample
Robust	Having inferences or conclusions little effected by departure from assumptions
Sensitivity	The number of subjects experiencing each experimental condition divided by the variance of scores in the sample
Significance level	The probability that a difference has been erroneously declared to be significant, typically 0.005 and 0.001 corresponding to 5% and 1% chance of error
Type I error (false positives)	Concluding that there is an effect when there really is not an effect. Its probability is the α level
Type II error (false negatives)	Concluding there is no effect when there really is an effect. Its probability is the β level

Stoddard Solvent

Richard D Phillips

- Representative Chemicals: A mixture of saturated aliphatic and alicyclic C_7–C_{12} hydrocarbons with a content of 15–20% (by weight) of aromatic C_7–C_{12} hydrocarbons. The C_9–C_{11} hydrocarbons are most abundant, constituting $\geqslant$80% (by weight) of the total
- Chemical Abstracts Service Registry Numbers: CAS 8052-41-3 (Stoddard Solvent); CAS 64742-48-9 (White Spirit Type 3); CAS 64742-82-1 (White Spirit Type 1); 64742-88-7 (White Spirit Type 0); 64741-92-0 (White Spirit Type 2)
- Synonyms: White spirit; Petroleum spirits; Solvent naphtha

Uses

Stoddard Solvent is used mainly in paints and varnishes, in cleaning products, and as a degreasing and extraction solvent. It may also be used in some types of photocopier toners, printing inks, and adhesives.

Background Information

There are different grades of Stoddard Solvent depending upon the type and level of posttreatment. These treatments include hydro-desulfurization, solvent extraction, and hydrogenation. These successive treatments result in lower and lower aromatics in the final product.

Exposure Routes and Pathways

Humans are predominately, exposed to Stoddard Solvent through the inhalation of vapor. Exposure can occur through the skin if one comes into contact with Stoddard Solvent or a product containing it.

Toxicokinetics

Studies in humans and animals have shown that Stoddard Solvent is readily absorbed through the lungs. In general, the aromatic components are likely to be more completely absorbed due to their higher blood/gas solubility. It is expected that volatile components or metabolites of Stoddard Solvent that have low blood solubility would be most easily excreted in exhaled breath. Aromatic components would be expected to be excreted primarily in urine as metabolites.

Mechanism of Toxicity

Stoddard Solvent is a slight to severe skin irritant depending on the exposure condition and duration. This is related to the defatting properties of the solvent. Little is known regarding the specific mechanisms of action for systemic toxicity to Stoddard Solvent. Its effect on the nervous system at high exposure levels may be due to its general solvent properties and anesthetic effect of hydrocarbons in general.

Acute and Short-Term Toxicity (or Exposure)

Animal

Stoddard Solvent possesses low acute toxicity for mammals. Thus, an LC_{50} for rats was not achieved with 8 h exposure to 8200 mg m^{-3} (1400 ppm).

Stoddard Solvent was determined to be a slight to moderate irritant.

In short- and long-term toxicity studies on Stoddard Solvent, the central nervous system (CNS), respiratory system, liver, and kidney were generally found to be the target of Stoddard Solvent toxicity.

Several inhalation studies with Stoddard Solvent have shown that male rats develop hyalin droplet nephropathy which is believed to be associated with the α-2u-globulin in male rats. Stoddard Solvent did not cause developmental toxicity based on studies in rats.

Human

The odor threshold of Stoddard Solvent is quite low, and vapors can be detected at levels of 0.5–5 mg m^{-3}. Tolerance of the odor may be developed.

Eye irritation has been reported in connection with acute exposure down to a level of 600 mg m^{-3} (100 ppm). At higher levels, respiratory irritation and more pronounced eye irritation occur. Acute CNS systems such as headache, 'drunkenness', dizziness, and fatigue have been reported in several cases of occupational exposure.

Ingestion of Stoddard Solvent has been reported to produce gastrointestinal irritation with pain, vomiting, and diarrhea as well as damage to the gastrointestinal tract.

Owing to its low viscosity and low surface tension, Stoddard Solvent poses a risk of aspiration into the lungs following oral exposure. A few milliliters of solvent aspirated into the lungs are able to

produce serious bronchopneumonia and 10–30 ml may be fatal.

Prolonged dermal exposure to Stoddard Solvent, for example, resulting from wearing clothes that have been soaked or moistened by white spirit for hours, may produce irritation and dermatitis.

There are a number of reports associating exposure to Stoddard Solvent in painters with neurological effects. However, in many of these instances, the exposure to Stoddard Solvent and associated confounding exposures are not clear. Many of the reported effects are also subjective in nature and the role of Stoddard Solvent is difficult to decipher.

Chronic Toxicity (or Exposure)

Animal

The National Toxicology Program recently conducted chronic inhalation studies with Stoddard Solvent in rats and mice. The reports are still in review, and details of the draft are summarized here. Groups of 50 male and 50 female rats and mice were exposed to Stoddard Solvent by inhalation at concentrations of 0, 138 (male rats), 550, 1100, or 2200 $mg\,m^{-3}$ (female rats and mice), 6 $h\,day^{-1}$, 5 $day\,week^{-1}$ for 105 weeks. For rats, survival in the top exposure concentration groups of males and females was significantly less than that of the chamber controls.

At 2 years, adrenal medulla tumors occurred with positive trends in male rats, and the incidences in the 550 and 1100 $mg\,m^{-3}$ groups were significantly increased. Also, a slightly increased incidence of renal adenomas occurred in the 1100 $mg\,m^{-3}$ group. Nonneoplastic lesions related to Stoddard Solvent exposure occurred in the kidney of male rats.

Survival of exposed mice was similar to that of the chamber controls. Mean body weights of exposed female mice were greater than those of the chamber controls. The incidences of hepatocellular adenoma occurred with a positive trend in female mice, and the incidence of multiple hepatocellular adenoma in female mice exposed to 2200 $mg\,m^{-3}$ was significantly increased.

In Vitro Toxicity Data

Stoddard Solvent was tested for mutagenicity in *Salmonella typhimurium* and found to be negative with and without 59 metabolic activation.

Clinical Management

Gastric emptying by either lavage or emesis is contraindicated since there is a danger of pulmonary aspiration and subsequent pneumonitis. If a person is overexposed to vapor of Stoddard Solvent, the victim should be moved to fresh air as quickly as possible. If acute effects of central nervous system depression are present, the appropriate treatment may be indicated.

Washing with soapy water is suggested following dermal contact, and ocular washing with water following eye contact.

Environmental Fate

The transport and partitioning of Stoddard Solvent is dependent on the environmental fate of its hydrocarbon components.

Sorption to organic matter in soil or water is a major partitioning process for all hydrocarbon classes (alkanes, cycloalkanes, and aromatics) with partitioning to the soil-vapor phase being relatively unimportant. At low concentrations, the aromatic constituents of Stoddard Solvent, particularly the alkyl benzenes, are more water soluble than alkanes and cycloalkanes and may dissolve in infiltrating water with a minimum of volatilization. As such, they may be transported through soil into the underlying groundwater, although sorption to soil organic matter will retard this leaching process. For saturated deep soils that contain no oxygen and little organic matter, the model predicts that some (20%) aromatic hydrocarbons will not undergo biodegradation, but will be dissolved in the soil-water phase, and subsequently will be transported to underlying groundwater.

If a release of Stoddard Solvent exceeds the sorptive capacity of the soil, large quantities of Stoddard Solvent may move through the soil with gravity as bulk fluid and enter the groundwater. At the soil/groundwater interface, the soluble components can dissolve in the water, while insoluble components with specific gravities of less than 1 will float on top of the water table and move horizontally along the soil/water interface.

Alkanes are likely to be sorbed to organic matter in the soil and are, therefore, unlikely to be dissolved in water moving through soil. However, some of these compounds may volatilize more quickly than they will bind to organic matter. Most aliphatic hydrocarbons have low water solubilities, but those with higher water solubilities are likely to be dissolved in water and may be transported through soil more rapidly, although the extent may be reduced by sorption to organic matter or volatilization.

No information was found on the bioaccumulation potential of Stoddard Solvent in either aquatic or terrestrial ecosystems. However, the potential for bioaccumulation of Stoddard Solvent in either

ecosystem is dependent on the bioaccumulation potential of the individual hydrocarbon components. In general, lower molecular weight alkanes do not tend to bioaccumulate, aromatics may have a moderate tendency to bioaccumulate, and the higher molecular weight alkanes, such as cycloalkanes, tend to bioaccumulate. However, these bioaccumulation tendencies may be offset by the metabolic capabilities of the organisms toward hydrocarbons.

Ecotoxicology

The few studies on the aquatic toxicity of Stoddard Solvent and related hydrocarbon mixtures indicate moderate toxicity to freshwater and marine organisms. The toxicity is probably due to the dissolved fraction and leads to 96 h LC_{50} values of the order of 0.5–5.0 mg l^{-1}.

These results are likely to overestimate the effects of Stoddard Solvent in the field, given its volatility and lowered bioavailability following sorption to soil/sediment.

Further Reading

Agency for Toxic Substances and Disease Registry (ATSDR) (1995) *Toxicological Profile for Stoddard Solvent*, US Department of Health and Human Services.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic substances and Disease Registry. Toxicological Profile for Stoddard Solvent.

Strontium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-24-6
- Chemical/Pharmaceutical/Other Class: Alkaline earth metal
- Chemical Formula: Sr^{2+}

Uses

Strontium compounds are used in making ceramics and glass products, pyrotechnics, paint pigments, fluorescent lights, and medicines.

Strontium can also exist as several radioactive isotopes, the most common is ^{90}Sr. Strontium-90 is formed in nuclear reactors or during the explosion of nuclear weapons. Radioactive strontium generates beta particles as they decay. One of the radioactive properties of strontium is half-life, or the time it takes for half of the isotope to give off its radiation.

It is used in fireworks, red signal flares, and tracer bullets. It also can be added to alloys of tin and lead to add hardness and durability, and it can be used as a deoxidizer in copper and bronze. In addition, it can be used as an igneous coloring agent, a material for the cathode of vacuum bulbs, a material for condensers and optical glass, and as a lead and iron removing agent. Small amounts of strontium are added to molten aluminum to improve the castability of the metal, making it more suitable for casting items that have been traditionally made from steel. The radioactive form (^{89}Sr) is used as an antineoplastic (radiation source). Strontium compounds are used as agricultural chemicals.

Background Information

Strontium is a naturally occurring element found in rocks, soil, dust, coal, and oil. Naturally occurring strontium is not radioactive and is referred to as stable strontium. Stable strontium in the environment exists in four stable isotopes, ^{84}Sr (read as strontium 84), ^{86}Sr, ^{87}Sr, and ^{88}Sr. Twelve other unstable isotopes are known to exist. Its radioactive isotopes are ^{89}Sr and ^{90}Sr. Strontium is chemically similar to calcium. It was discovered in 1790. The isotope ^{90}Sr is a highly radioactive poison, and was present in fallout from atmospheric nuclear explosions and is created in nuclear reactors. Atmospheric tests of nuclear weapons in the 1950s resulted in deposits and contaminations. ^{90}Sr has a half-life of 28 years and is a high-energy beta emitter. Its common cationic salts are water soluble; it forms chelates with compounds such as ethylenediaminetetraacetic acid; strontium coordination compounds are not common. Powdered metallic strontium may constitute an explosion hazard when exposed to flame.

Exposure Routes and Pathways

Oral, ingestion, and inhalation are possible exposure routes. Radiation also penetrates the body.

Toxicokinetics

Strontium tends to replace calcium in bone. Radioactive isotopes of strontium, mainly ^{90}Sr, released into the environment due to nuclear accidents may contribute significantly to the internal radiation exposure of members of the public after ingestion of strontium with contaminated foodstuffs. The committed radiation dose is significantly dependent on the fraction of the ingested activity that crossed the gut wall; sodium alginate is a potent agent for reducing strontium absorption with high efficiency and virtually no toxicity. The data obtained show that the uptake of ingested strontium from milk was reduced by a factor of nine when alginate was added to milk.

Mechanism of Toxicity

Its inherent toxicity and that of its compounds resembles that of calcium. The state of calcium nutrition of exposed individuals is a major determinant of toxicity. The radioactive isotope, when ingested or inhaled, is processed by the body and resides in bones. Strontium ionizes molecules in the body by the emission of beta particles. It increases the risk of cancer.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute poisoning in laboratory animals leads to excess salivation, vomiting, colic, and diarrhea. In rats, death is due to respiratory failure; in cats, it is due to cardiac arrest.

Human

No toxic effects from industrial use of nonradioactive strontium have been recorded.

Chronic Toxicity (or Exposure)

Animal

Leukemia and cancers of the bone, nose, lung, and skin have been observed in laboratory animals exposed to radioactive strontium.

Human

Leukemia has been seen in humans exposed to relatively large amounts of radioactive strontium. The International Agency for Research on Cancer has determined that radioactive strontium is a human carcinogen. ^{89}Sr has been explored as an anticancer treatment, for example, for prostate cancer, and has been used as palliative treatment for patients with bone pain from osseous metastases. Excellent clinical responses for bone pain treatment have been observed (acceptable hematologic toxicity; and clinical results rival those of external beam radiation therapy).

Environmental Fate

Stable strontium is a dust in air. It eventually settles over land and water. Stable strontium dissolves in water and moves deeper in soil to underground water.

Ecotoxicology

Strontium-90 pollutes water and soil at some reprocessing plants. Atmospheric contamination can occur from nuclear fallout. A study in the United States has concluded that high concentrations of strontium in eggshells of some passerine birds may be associated with lower hatching success.

See also: Aluminum; Carcinogenesis.

Further Reading

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Mora MA (2003) Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona. *Environmental Pollution* 125: 393–400.

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Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Diseases Registry. Toxicological Profile for Strontium.

http://risk.lsd.ornl.gov – US Oak Ridge National Laboratory. Toxicity summary for Strontium-90 (from the Risk Assessment Information System).

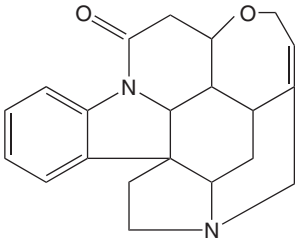
Structure–Activity Relationship *See* Toxicity Testing, Modeling.

Strychnine

Fermin Barrueto Jr.

This article is a revision of the previous print edition article by Janice Reeves and Carey Pope, volume 3, pp. 201–202,

- Chemical Abstracts Service Registry Number: CAS 57-24-9
- Synonyms: Kwik-Kil; Mouse-Rid; Mouse-Tox; Strychnos
- Chemical/Pharmaceutical/Other Class: Naturally occurring alkaloid
- Chemical Formula: $C_{21}H_{22}N_2O_2$
- Chemical Structure:



Uses

Strychnine is used chiefly in poison baits for rodents and sometimes birds.

Exposure Routes and Pathways

The primary pathways for unintentional or intentional exposure to strychnine are inhalation and ingestion. Ocular and dermal exposures can also occur.

Toxicokinetics

Strychnine is rapidly absorbed from the gastrointestinal tract, nasal mucosa, and parenteral sites. It is readily metabolized in the liver by microsomal enzymes. The highest concentrations of strychnine are found in the liver, kidneys, and blood. About 15% will appear unchanged in the urine within 24 h. Strychnine has an elimination half-life of $\sim$10 h.

Mechanism of Toxicity

The exact mechanism of strychnine's action in the nervous system is unclear but it is thought that the inhibitory action of the neurotransmitter glycine at Crenshaw cell-motor axon synapses is blocked by strychnine. This essentially decreases excitatory thresholds and produces tetanic convulsions in response to sensory stimuli. While the main locus for strychnine's neurotoxicity is the spinal cord, the medulla also appears affected. Effects on other organ systems appear to be secondary to these actions in the central nervous system (CNS). Strychnine competitively blocks the binding of glycine to membranes isolated from spinal cord.

Acute and Short-Term Toxicity (or Exposure)

Animal

Strychnine is a compound of high acute toxicity. The oral LD value in rats is $\sim 15\,mg\,kg^{-1}$. Parenteral routes of exposure are more toxic; LD_{50} values in laboratory rodents range from 1 to $4\,mg\,kg^{-1}$.

Human

Within 15–30 min after ingestion of strychnine, the patient will experience restlessness, apprehension, heightened acuity of perception, hyperreflexia, and muscle stiffness of the face and legs. Violent convulsions can follow these symptoms or occur in the absence of these previous symptoms. As poisoning progresses, the convulsions become more violent and the intervals between convulsions become shorter. The LD (oral) for humans has been estimated to be $30\,mg\,kg^{-1}$. Toxicity has been reported at 0.1 mg per 100 ml in blood concentrations.

Chronic Toxicity (or Exposure)

Animal

Toxicity of strychnine varies by sex in rats. Twenty-eight day feeding studies showed that males were able to tolerate up to $8\,mg\,kg^{-1}\,day^{-1}$ without adverse effects; females were able to tolerate $2.5\,mg\,kg^{-1}\,day^{-1}$.

Human

Significant cumulative toxicity has not been described because both detoxification and excretion are comparatively rapid.

In Vitro Toxicity Data

Strychnine is frequently used as a research tool due to its glycine inhibitory effects.

Clinical Management

Treatment is symptomatic and supportive with emphasis on controlling neuromuscular hyperactivity. For patients exposed to strychnine fumes, the patient should be moved to fresh air, and eyes and skin should be decontaminated immediately with water. For patients with strychnine ingestions, emesis is not recommended because of the violent convulsive activity and increased risk of aspiration. Activated charcoal should be used immediately to minimize absorption. Once convulsions have been controlled, efforts to correct fluid, electrolyte, and acid–base abnormalities caused by repeated convulsions should be made.

Environmental Fate

Strychnine has been used as a rodenticide and pesticide for decades. It is believed that strychnine may undergo direct photolysis in the atmosphere, on soil surfaces, and in surface water.

See also: Pesticides.

Further Reading

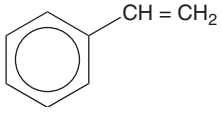
Flood RG (1999) Strychnine poisoning. *Pediatric Emergency Care* 15: 286–287.

Oberpaur B, Donoso A, and Claveria C (1999) Strychnine poisoning: An uncommon intoxication in children. *Pediatric Emergency Care* 15: 264–265.

Styrene

Ralph J Parod

- Chemical Abstracts Service Registry Number: CAS 100-42-5
- Synonyms: Ethenylbenzene; Phenylethylene; Vinylbenzene
- Chemical/Pharmaceutical/Other Class: Hydrocarbon
- Chemical Structure:



Uses

Styrene is produced by the alkylation of benzene with ethylene followed by catalytic dehydrogenation. It is used in the manufacture of general-purpose and high-impact polystyrene plastics (~50%), expanded polystyrene (~7%), copolymer resins with acrylonitrile and butadiene (~7%) or acrylonitrile only (~1%), styrene–butadiene latex (~6%) and synthetic rubber (~5%), unsaturated polyester resins (~6%), and as a chemical intermediate.

Exposure Routes and Pathways

The closed system techniques currently used in styrene monomer and copolymer resin production limit worker exposures to a time-weighted average (TWA) exposure of generally <10 ppm. However, in open systems used to manufacture some polyester reinforced plastics (e.g., shower stalls and boats), worker exposures to styrene and resins via inhalation and to a lesser extent dermal contact may pose a health hazard. It is unlikely that the residual styrene monomer contained in consumer products (200–800 $mg\,kg^{-1}$) poses a significant health risk to the general public due to the slow (0–0.03% $year^{-1}$) and dispersive nature of its release from these sources. Styrene is present in ambient air due to emissions from industrial production, coal-fired power plants, cigarette smoke, and gasoline engine exhaust. Styrene is also present in certain cheeses and fish products where it is produced by microorganisms acting on natural or added substances in these foods, and in several raw agricultural products that have not contacted styrenic based storage containers. The total exposure of the general population to styrene from these background sources is 0.3–0.8 $\mu g\,kg^{-1}\,day^{-1}$ with inhalation being the major route of exposure.

Toxicokinetics

Styrene is absorbed by all routes of exposure. Absorption through the respiratory tract is rapid and the major route of human exposure. Once absorbed, styrene is rapidly distributed throughout the body. Studies in rats and mice indicate that styrene or its metabolites are distributed to the liver, kidneys, heart, subcutaneous fat, lung, brain, and spleen. In both species, fat contained the highest concentration of styrene, suggesting that fat may act as a modest reservoir for these compounds. While there are qualitative similarities in the metabolism of styrene among species, quantitative differences have been noted.

Mice, rats and rabbits exhibit a much greater capacity than humans to metabolize styrene (via cytochrome P450) to styrene-7,8-epoxide in both the respiratory tract and liver. In these species, styrene-7,8-epoxide is inactivated by metabolism to hippuric acid (via epoxide hydrolase) and hydroxyphenylethyl mercapturic acid (via glutathione *S*-transferase). The latter pathway is much more prevalent in rodents than humans. In humans, styrene is metabolized via cytochrome P450 to styrene-7,8-epoxide, which is rapidly metabolized via epoxide hydrolase to mandelic acid ($\sim$60%) and phenylglyoxylic acid ($\sim$25%), with very minor amounts of 4-vinylphenol, hippuric acid, and the glucuronide of styrene glycol. Metabolism occurs primarily in the liver and to a lesser extent in extrahepatic tissues (e.g., kidney, intestine, and lung). Approximately 90–97% of the styrene absorbed by humans is eliminated as urinary metabolites. Urinary elimination of the primary metabolites is biphasic, with half-lives of 4–9 and 17–26 h (mandelic acid) and 10 and 26 h (phenylglyoxylic acid). Only a small fraction of the absorbed dose is eliminated in expired air or urine as the parent compound.

Mechanism of Toxicity

There has been a general belief that styrene-7,8-epoxide is responsible for the carcinogenicity and nasal toxicity associated with styrene. However, more recent data indicate that other mechanisms may be operative or even predominant (i.e., site-specific metabolism of styrene to ring-oxidized metabolites that are toxic to the lung and induce lung cell proliferation (e.g., 4-vinylphenol), depletion of pulmonary glutathione). Such effects occur to a greater extent in mice than rats and are thought to lead to cytotoxicity, cell proliferation, and the slow development of noninvasive tumors in the mouse lung. These stresses are even less prevalent in humans than rats, suggesting that humans are less susceptible to the development of lung tumors than mice. Work in this area is ongoing. The mechanism for the neurotoxic effects of styrene has not been established.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} for styrene in male and female rats is $\sim 5000\,mg\,kg^{-1}$. The LC_{50} in rats exposed to styrene for 4 h is 2770 ppm; the LC_{50} in mice exposed to styrene for 2 h is 4940 ppm. Repeated daily exposures over a period of weeks to months to 500–1300 ppm styrene can result in irritation of the eyes and upper respiratory tract, ototoxicity, and central nervous system (CNS) depression. Liver toxicity noted in mice given daily exposures to 200 ppm styrene over a period of two weeks was associated with the depletion of liver glutathione levels. Studies of clastogenicity have been negative.

Human

Acute inhalation exposures may result in irritation of the nasal mucosa and eyes ($\geqslant$50 ppm), irritation of the skin, and CNS depression ($>$100 ppm). Symptoms of CNS depression include nausea, drowsiness, and ataxia. The disagreeable odor of styrene, which is detectable at $\sim$0.04–0.3 ppm, serves as a good warning aid. However, olfactory fatigue may occur at high concentrations.

Chronic Toxicity (or Exposure)

Animal

In male mice, lifetime inhalation exposures to styrene at concentrations of 40, 80, or 160 ppm (but not 20 ppm) significantly increased the incidence of bronchiolar adenomas (benign) but not bronchial carcinomas. In female mice similar exposures resulted in a significant elevation in the incidences of bronchiolar adenomas (benign) at 20, 40, or 160 ppm (but not 80 ppm) and bronchiolar carcinomas at 160 ppm. The tumors occurred late in life and were found in the terminal bronchioles originating most likely from Clara cells. In contrast, lifetime exposure of rats to between 50 and 1000 ppm styrene did not cause tumors in the lungs or any other tissue. These chronic exposures also resulted in toxicity to the nasal epithelium of mice and rats at the lowest concentrations studied. Styrene has not been shown to cause reproductive or developmental toxicity. Chronic inhalation exposures to multiple species that produced clear evidence of toxicity did not affect the reproductive organs of treated animals. Inhalation exposures up to 500 ppm styrene did not affect either fertility or reproduction in a rat two-generation reproduction study or development of the nervous system in second generation offspring. In addition, inhalation of 300–600 ppm styrene by pregnant rats and rabbits was not embryo- or fetotoxic.

Human

Styrene exposures between 50 and 100 ppm have been associated with neurological effects including decrements in color discrimination, nerve conduction, and neurobehavioral performance. These changes appear to be transient, with improvement

occurring between 1 and 24 months postexposure. Styrene is unlikely to be toxic to the human nasal epithelium since, unlike in rodents, this tissue does not metabolize detectable amounts of styrene and contains metabolic pathways capable of efficiently eliminating metabolites if formed. Epidemiological studies have not provided a clear link between styrene exposure and adverse pregnancy outcomes. Regarding cancer, epidemiological studies have been performed in three industrial settings, including the reinforced plastics industry where styrene exposures tend to be the higher and are less confounded by other chemicals. On balance, the data do not suggest a causal association between styrene exposure and any form of cancer. Increased frequencies of chromosomal aberrations in peripheral lymphocytes have been reported in some but not all studies of workers from the reinforced plastics industry, but the potential relationship of these effects to styrene exposure is still under debate.

In Vitro Toxicity Data

In vitro studies of mutagenicity and chromosomal aberrations in bacterial and mammalian cells have generally produced negative results, although positive results have sometimes been observed in the presence of metabolic activation.

Clinical Management

Acute exposures are likely to be associated with CNS depression and, at very high doses, pulmonary irritation. Removal from exposure and ventilatory support are the initial priorities. Alert individuals ingesting >2 or $3\,mg\,kg^{-1}$ should be given syrup of ipecac. Because hydrocarbon pneumonitis is a significant risk with styrene ingestion, intubation should precede lavage in those individuals at risk of aspiration due to a reduced level of alertness.

Environmental Fate

Styrene is a liquid and will partition to the atmosphere when released to the environment due to its volatility. In the atmosphere, styrene is rapidly eliminated due to its reaction with hydroxyl radicals (7 h half-life) or tropospheric ozone (10 h half-life). Water does not provide a significant sink for styrene due to its low water solubility ($300\,mg\,l^{-1}$), rapid volatilization from water to air (half-life of 1–3 h), and biodegradation (15 days half-life). In soil, styrene rapidly volatilizes from the surface (1 min half-life) but more slowly from deeper strata. Styrene biodegrades in soil and sediment with half-lives of 30 and 300 days, respectively.

Ecotoxicology

Although of limited relevance to real world exposures, a series of guideline studies have evaluated the intrinsic ecotoxicity of styrene under conditions that minimized volatilization. In these investigations that incorporated analytical verification, the 96 h LC_{50} value for fish was $10\,mg\,l^{-1}$ (fathead minnow); the no-observed-effect level (NOEL) was $4\,mg\,l^{-1}$. For the freshwater invertebrates, the 48 and 96 h LC_{50} values were $4.7\,mg\,l^{-1}$ (daphnids) and $9.5\,mg\,l^{-1}$ (amphipods), respectively; the NOELs were 1.9 and $4.1\,mg\,l^{-1}$, respectively. For green algae, the 96 h EC_{50} was $0.72\,mg\,l^{-1}$ and the NOEL was $0.063\,mg\,l^{-1}$; the effects noted were algistatic, not algicidal. Styrene is infrequently detected in surface and drinking water around the world; and when it is detected, levels are typically $<0.01\,mg\,l^{-1}$. For earthworms in soil, styrene has a 14 day LC_{50} of $120\,mg\,kg^{-1}$ and a NOEL of $44\,mg\,kg^{-1}$.

Other Hazards

Styrene is explosive in the range of 1.1–6.1% and has a vapor density of 3.6.

Exposure Standards and Guidelines

International occupational exposure limits (OELs) generally range between 20 and 100 ppm as an 8 h TWA; short-term exposure limits (STELs), typically a 15 min TWA, range between 40 and 250 ppm. The US Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) have established an 8 h TWA OEL for styrene of 100 and 20 ppm, respectively; the ACGIH STEL is 40 ppm. OSHA and the styrene industry have an enforceable voluntary agreement to keep exposures under 50 ppm. Styrene has been judged possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer. Styrene is not listed in the 10th edition of the Report on Carcinogens published by the US National Toxicology Program (NTP); however, styrene-7,8-oxide is listed by NTP as reasonably anticipated to be a human carcinogen. The National Institute for Occupational Safety and Health lists 700 ppm styrene as being immediately dangerous to life or health.

See also: Respiratory Tract.

Further Reading

Alexander M (1997) Environmental fate and effects of styrene. *Critical Reviews in Environmental Science And Technology* 27: 383–410.

Cohen JT, Carlson G, Charnley G, *et al.* (2002) A comprehensive evaluation of the potential health risks associated with occupational and environmental exposure to styrene. *Journal of Toxicology and Environmental Health, Part B* 5: 1–263.

Cushman JR, Rausina GA, Cruzan G, *et al.* (1997) Ecotoxicity hazard assessment of styrene. *Ecotoxicology and Environmental Safety* 37: 173–180.

Subchronic Toxicity *See* Toxicity, Subchronic.

Sudan Grass

Julie Weber

- SYNONYMS: *Sorghum sudanense*; *Sorghum vulgare* var *sudanense*; *Holcus sudanensis*; Grass sorghum; Shattercane; Sudan; Family Gramineae (Poaceae)

Uses

Sudan grass is native to Sudan. It is cultivated and naturalized widely in the United States, but is most common in the southern states. Although common to the southern states, it is also grown from southern Texas to Minnesota and North Dakota in the central grassland regions. Sudan grass only develops fibrous roots and does not become a noxious weed. As seedlings, virtually all species of sorghum resemble one another and are frequently confused with young corn plants. The sorghum species are used for forage, hay, silage, and grain.

Exposure Routes and Pathways

Ingestion of any part of the plant would be the common route of exposure. The green, aerial portions, especially the leaves, stems, roots, and canes are toxic.

Toxicokinetics

Limited information on absorption is available. After ingestion, the onset of symptoms of cyanide toxicity is expected in 30 min to 2 h; however, symptoms could be delayed. The delay in symptoms may be due to the rate at which the hydrocyanic acid is yielded when hydrolyzed by the enzymes during digestion. The major route for detoxification of cyanide is the conversion to thiocyanate by an enzymatic reaction catalyzed by rhodanese, an enzyme widely distributed in tissues, with the highest concentration in the liver. The rhodanese system can detoxify large amounts of cyanide but cannot respond quickly enough to prevent fatalities.

Mechanism of Toxicity

The sorghum group contains the cyanogenic glycoside dhurrin. The glycoside itself is harmless. When the plant is torn, chewed, or damaged, the glycosidase comes in contact with and hydrolyzes the glucoside to an α-hydroxynitrile aglycone and glucose. The aglycone further dissociates to *p*-hydroxybenzaldehyde and hydrocyanic acid. Hydrolysis is complete in 10 min at 20°C. The reaction takes place slowly in an acidic pH, but an alkaline medium hastens the process. A delay in symptoms after ingestion would be explained by a slow hydrolysis when transportation occurs from the acidic stomach medium to the alkaline medium of the duodenum. Released and absorbed hydrocyanic acid forms a stable complex with ferric iron and cytochrome oxidase; inhibiting the activity of the enzyme and aerobic metabolism. Cells with cytochrome oxidase are unable to utilize the available oxygen and suffer from hypoxia. Therefore, the nerve cells can no longer obtain oxygen and the respiratory center ceases to function.

Forage sorghum can accumulate levels of nitrates that can also produce poisoning. The nitrite ion readily reacts with hemoglobin in red blood cells, oxidizing it to methemoglobin which cannot transport oxygen.

Another disease syndrome of ataxia/cystitis/teratogenesis has been seen especially in horses with a slight variation of effects in cattle and possibly sheep. Both cyanide and nitriles are suspected, but the specific cause is unknown.

The last syndrome reported sporadically in sheep is a primary photosensitization. The toxin responsible has not been identified.

Acute and Short-Term Toxicity (or Exposure)

Animal

Livestock cyanide poisoning may result from plant consumption. All animal species are susceptible to the toxic effects of cyanide. Ruminants appear to be more susceptible to cyanogenic glycosides in plants because the favorable conditions prevailing in the rumen facilitate rapid hydrolysis and absorption. Mucous membranes of the eyes and mouth may appear congested. Gastric contents, if examined immediately, have a characteristic benzaldehyde odor, resulting from benzaldehyde production from aglycone breakdown of certain cyanogenic glycosides. Potential symptoms expected are hyperpnea to dyspnea, excitation, gasping, staggering, paralysis, prostration, tremors, convulsions, coma, and death. Cherry red blood may be noted.

Nitrate intoxication is a serious problem for livestock where sorghums are used for forage. In ruminant digestion, nitrates are converted to nitrites after ingestion of plants containing high levels of nitrates. The nitrites begin to increase in the rumen, peaking in 3–4 h. The nitrites are $\sim$10 times more toxic and are the more immediate cause of poisoning. Symptoms of nitrite toxicity include discoloration of the mucous membranes, depression, rapid respiration, ataxia, apprehension, severe dyspnea, trembling, and weakness with a chocolate brown discoloration of the blood.

Some environmental factors that increase the cyanogenic potential of the plant are high nitrogen and low phosphorus in soil, periods of drought that wilt plants or delay growth and age of plants (young growth has the highest potential for toxicity). Many years of selective breeding have resulted in hybrids having lower potential for developing hydrogen cyanide.

Human

Sudan grass poisonings in humans are not reported. Symptoms are expected to be similar to those exhibited in animals. Patients would be treated as for other cyanogenic plant ingestions.

Chronic Toxicity (or Exposure)

Animal

Animals eating Sudan grass containing low levels of cyanogenic glycosides for prolonged periods (usually several weeks or months) may be at risk to develop chronic cystitis, ataxia, and teratogenicity. Mares and cows that chronically eat Sudan grass with low levels of the cyanogenic glycoside are at risk to develop ataxia, urinary incontinence (dribbling urine), and abortion. The offspring may develop musculoskeletal deformities. Sublethal doses of nitrate also may induce abortion because nitrates readily crosses the placenta and causes fetal methemoglobinemia and death. Chronic ingestion of nitrate in the diet is suspected of affecting vitamin A metabolism, thyroid function, reproduction, and milk production.

Clinical Management

In symptomatic patients, decontamination should be deferred until other basic and advanced life-support measures have been instituted. Cyanide toxicity usually progresses so quickly that treatment may not be available in a time period to be effective. Induction of emesis is not recommended. Subtoxic amounts do not require emesis; moreover, the potentially rapid progression of clinical course contraindicates it. Activated charcoal may be effective if administered soon after the ingestion. The cyanide antidote kit should only be administered in those persons with significant symptoms (impaired consciousness, seizures, acidosis, and unstable vital signs). The mainstay of treatment in ruminants is sodium thiosulfate. Sodium nitrite may be used to enhance the effects of the sodium thiosulfate. Arterial blood gases, electrolytes, serum lactate and pyruvate, hemoglobin, glucose, creatinine, whole blood cyanide levels, and methemoglobin levels should be monitored and treated as necessary. Hyperbaric oxygen can be used for those with severe symptoms not responding to normal supportive and antidotal treatment.

Miscellaneous

Sudan grass is an annual grass with stems up to 9 ft tall that branch from the base. Leaf blades are broad to narrow up to 0.5 in. wide and 12 in. long; panicles open. The plant flowers in a 12 in. long, erect, loose panicle that is approximately half as wide as it is tall. It also forms a grass or grain-like glume around seed buds with bristle-shaped tips.

See also: Cyanide.

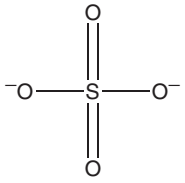
Further Reading

Hall AH and Rumack BH (1986) Clinical toxicology of cyanide. *Annals of Emergency Medicine* 15: 1067–1074.

Sulfates

J R Clarkson, Lu Yu, and Lance Fontenot

- Representative Chemicals: Barite ($BaSO_4$); Epsomite ($MgSO_4 \cdot 7H_2O$); Gypsum ($CaSO_4 \cdot 2H_2O$); Magnesium ($MgSO_4$); Sodium ($NaSO_4$); Calcium ($CaSO_4$); Lead ($PbSO_4$); Barium ($BaSO_4$); Strontium ($SrSO_4$)
- Chemical Abstracts Service Registry Number: CAS 14808-79-8
- Synonym: Sulfate anion
- Chemical Formula: SO_4^{2-}
- Chemical Structure:



Uses

Sulfates and sulfuric acid products are used in the production of fertilizers, chemicals, dyes, glass, paper, soaps, textiles, fungicides, insecticides, astringents, and emetics. They are also used in the mining, wood-pulp, metal, and plating industries, in sewage treatment, and in leather processing. Aluminum sulfate (alum) is used as a sedimentation agent in the treatment of drinking water. Copper sulfate has been used for the control of algae in raw and public water supplies.

Sulfate, a soluble, divalent anion (SO_4^{2-}), is produced from the oxidation of elemental sulfur, sulfide minerals, or organic sulfur. Sulfate is ubiquitous in the environment because of the abundance of sulfur on earth. Anthropogenic sources of sulfate include the burning of sulfur-containing fossil fuels, household wastes including detergents, and industrial effluents from tanneries, steel mills, sulfate-pulp mills, and textile plants. Sulfate is also used in pickle liquor (sulfuric acid) for steel and metal industries, as a feedstock or reagent in manufacturing processes, in some fertilizers, and exists as an end-product in the form of copper sulfate in its use as a fungicide and algicide.

Exposure Routes and Pathways

Humans may be exposed to sulfate from a variety of sources that include drinking water, food, ambient air, occupational settings, and consumer products. Ingestion of drinking water containing sulfate is the most common exposure route. Exposure through inhalation is not very important, although it may be an irritant to mucous membranes, such as eyes, nose, and respiratory tract. Dermal exposure is also not considered a major exposure route because sulfates are poorly absorbed through the skin.

Toxicokinetics

Absorption of sulfate from the intestine depends on the amount of sulfate ingested and the type of cation associated with the sulfate. Inorganic sulfate distributed in blood and serum levels are affected more by ingestion of dietary protein, and less by drinking water containing high concentrations of sulfate. Inorganic sulfate does not accumulate in tissues. Inorganic sulfate is incorporated into several types of biomolecules, such as glycoproteins, glycosaminoglycans, and glycolipids. Metabolism in the body could be affected by the presence of drugs, such as acetaminophen. Sulfates are usually eliminated in the urine as free unbound form or as conjugates of various chemicals. In humans, ~30–62% of an oral dose is excreted in the urine within 24–72 h, whereas rats excrete ~56–90% of the dose. At high sulfate doses that exceed intestinal absorption, sulfate is excreted in the feces. There are no data in literature that indicate that sulfate is accumulated, even when there is chronic ingestion of above-normal sulfate levels.

Mechanism of Toxicity

The cathartic effect of sulfate is mainly due to the osmotic activity of unabsorbed sulfate salts in the intestine. The laxative effect that results from sulfate is an osmotic diarrhea. Whether or not this laxative effect occurs depends on the amount of sulfate and other osmotically active materials that are present in the intestines; these materials include magnesium, sodium, and some sugars.

An osmotic-induced diarrhea ceases once the osmotically active gastrointestinal contents are excreted. In the case of sulfate, adults appear to adapt within 1 or 2 weeks and are no longer affected by the sulfate in their drinking water supply. However, dehydration may be the consequence of these same osmotic forces; therefore, populations such as infants or the elderly consuming formula or powdered nutritional supplements that have been mixed with water containing high concentrations of sulfate may be more sensitive. Persons with renal diseases may be more sensitive to effects of high sulfate ingestion because excess sulfate is excreted through the kidney.

Since humans appear to develop a tolerance to drinking water with high sulfate concentrations, chronic exposures do not appear to produce the same laxative effect as seen in acute exposures. While it is not known when this acclimation occurs in adults, researchers believe that acclimation occurs within 7–10 days.

Acute and Short-Term Toxicity (or Exposure)

Data in humans and animals suggest that acute oral exposures to sulfate exert a laxative effect (loose stool) and sometimes diarrhea (unusually frequent or unusually liquid bowel movements) following exposures to high concentrations. However, these effects are not observed for longer-term exposures. This may be because of acclimation to sulfate over time.

High sulfate concentrations do not appear to exert adverse reproductive or developmental effects. There is little to no data available on the mutagenic or teratogenic effects of sulfate.

Animal

The oral LD_{50} values of ammonium sulfate and potassium sulfate in the rat are 3000–4000, 2140, and 6600 mg kg^{-1}, respectively.

The oral LD_{50} of sodium sulfate in the mouse is 5989 mg kg^{-1}. Sulfate administered to young pigs at 1800 mg l^{-1} in drinking water for 28 days developed loose and watery stools. A study on the effect of inorganic sulfate on bowel function in piglets reported that concentrations greater than 1200 mg l^{-1} increased the incidence of diarrhea. Concentrations greater than 1800 mg l^{-1} resulted in a persistent diarrhea. No adverse developmental effects were observed following the administration of 2800 mg kg^{-1} day^{-1} of sulfate to pregnant ICRISIM mice on gestation days 8–12. No reproductive effects were observed following the ingestion of drinking water containing up to 5000 mg l^{-1} of sulfates by ICRISIM mice and 3298 mg l^{-1} of sulfates by Hampshire × Yorkshire × Duroc pigs. On the basis of these studies, sulfate does not appear to be a reproductive or a developmental toxicant.

Human

Most data on human responses to sulfate are based on short-term exposures that are obtained from controlled settings (i.e., studies and experimental trials). The risk of adverse health effects to the general population is limited and acute, and such effects occur only at high drinking water concentrations (>500 mg l^{-1}, and in many cases >1000 mg l^{-1}). The data from human studies demonstrated that sulfate induces a laxative effect following acute exposures of concentrations greater than 500 mg l^{-1}. However, the severity of the laxative effect may depend on the sulfate salt, as well as the dose administered. Subpopulations sensitive to sulfate ingested through drinking water include formula-fed infants, the elderly, or invalids who use powdered nutritional supplements, and visitors who are not acclimated to high sulfate concentrations in drinking water.

In a case-control investigation to assess the association between infant diarrhea and ingestion of water containing elevated sulfate levels, a total of 274 mothers of infants born in 19 South Dakota counties with high sulfate concentrations in tap water were identified and interviewed using a telephone questionnaire or in person. No significant association existed between exposure to sulfate from tap water and subsequent diarrhea in infants. The average sulfate concentration in drinking water for cases was 416 versus 353 mg l^{-1} for controls. In another study to determine the effects of high sulfate concentrations in transient populations, there were no statistically significant differences in the mean number of bowel movements among dose groups, and there was also no apparent trend in the percentage of subjects that reported diarrhea during the exposure period.

Chronic Toxicity (or Exposure)

Animal

Data from animal studies on the reproductive, developmental, and carcinogenic effects are available for long-term exposures to sulfate. In a 90 day study, rats administered mineral waters containing up to 1595 mg l^{-1} of sulfate showed no soft feces or diarrhea, indicating rapid acclimation. High sulfate concentrations do not appear to exert adverse reproductive or developmental effects. Following the ingestion of drinking water containing up to 5000 mg l^{-1} of sulfates by mice and pigs, no reproductive effects were observed. Furthermore, no adverse developmental effects were observed following the administration of 2800 mg kg^{-1} day^{-1} of sulfate to pregnant mice.

Human

Since humans appear to develop a tolerance to drinking water with high sulfate concentrations, chronic exposures do not appear to produce the same laxative effect as seen in acute exposures. Some reports have shown that chronic exposure to high sulfate concentrations in drinking water does not have laxative effects in human.

A survey conducted in North Dakota showed a slight increase in the percentage of people who reported that their drinking water had a laxative effect when the drinking water contained 500–1000 $mg\,l^{-1}$ sulfate compared to the percentage of people who reported a laxative effect from drinking water that contained $<500\,mg\,l^{-1}$. Sixty-eight per cent of people who consumed water with 1000–1500 $mg\,l^{-1}$ reported a laxative effect. Analysis of data from North Dakota showed that drinking water containing $\geqslant 750\,mg\,l^{-1}$ sulfate was associated with a self-reported laxative effect whereas drinking water containing $\leqslant 600\,mg\,l^{-1}$ was not. These data were reanalyzed and found that most people experienced a laxative effect when they drank water that contained $>1000\,mg\,l^{-1}$ sulfate.

Clinical Management

The available toxicological data indicate that sulfate may cause adverse health effects in humans and animals. Sulfate has a laxative effect in high doses, but adverse health effects arc temporary and recovery is rapid. Subpopulations sensitive to sulfate ingested through drinking water include formula-fed infants, the elderly, or invalids who use powdered nutritional supplements, and visitors who are not acclimated to high sulfate concentrations in drinking water. Persons with renal diseases may also be more sensitive to effects of high sulfate ingestion.

Environmental Fate

Sulfates are discharged into water from mines and smelters, and from kraft pulp and paper mills, textile mills, and tanneries. Atmospheric sulfur dioxide, formed by the combustion of fossil fuels and by metallurgical roasting processes, may contribute to the sulfate content of surface waters. Sulfur trioxide, produced by the photolytic or catalytic oxidation of sulfur dioxide, combines with water vapor to form dilute sulfuric acid, which falls as 'acid rain'. The environmental fate and transport of sulfate are inextricably linked to the physical and chemical processes active in the earth's sulfur cycle.

Ecotoxicology

With respect to the propagation of fish and wildlife, there is no recommended ambient water quality criterion for the protection of aquatic life for sulfate because sulfate is not generally considered a significant ecological concern, except perhaps where it is a dominant component of total dissolved solids, when sulfate would contribute significantly to excessive salinities (greater than $1000\,mg\,l^{-1}$). There are several sources where published ecotoxicological data are available. These include US Environmental Protection Agency's (EPA) Aquatic Information Retrieval System, the Hazardous Substances Databank, and published scientific literature. Reported chronic toxicity effect levels for sulfate range from 361 to $1488\,mg\,l^{-1}$. The acute toxicity threshold is assumed to be $450\,mg\,l^{-1}$.

Exposure Standards and Guidelines

The US EPA established a Secondary Maximum Contaminant Level for sulfate of $250\,mg\,l^{-1}$, based on taste properties.

A US EPA health-based advisory for acute effects (absence of laxative effects) of 500 mg of sulfate per liter is recommended. In situations, where the water contains high concentrations of total dissolved solids and/or other osmotically active ions, laxative-like effects may occur if mixed with concentrated infant formula or powdered nutritional supplement; therefore, an alternate low-mineral-content water source is advised. Infants are more susceptible to diarrhea water loss than adults because of differences in gastrointestinal structure and function.

The Association for the Advancement of Medical Instrumentation suggests a maximum concentration of $100\,mg\,l^{-1}$ of sulfate in water used for dialysis.

In case of sulfuric acid, time-weighted average is $1\,mg\,m^{-3}$, whereas National Institute for Occupational Safety and Health/Occupational Safety and Health Administration establish $80\,mg\,m^{-3}$ to be immediately dangerous to life and health.

See also: Gastrointestinal System; Pollution, Air; Respiratory Tract; Sulfur Dioxide; Sulfuric Acid.

Further Reading

US Environmental Protection Agency (1992) Drinking Water Criteria Document for Sulfate, Final Report.

US Environmental Protection Agency (1999) Health Effects from Exposure to High Levels of Sulfate in Drinking Water Study. EPA 815-R-99-001.

US Environmental Protection Agency (1999) Health Effects from Exposure to Sulfate in Drinking Water Workshop. EPA 815-R-99-002.

US Environmental Protection Agency (2003) Contaminant Candidate List Regulatory Determination Support Document for Sulfate. EPA-815-R-03-16.

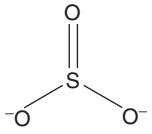
Relevant Website

http:// www.epa.gov – US Environmental Protection Agency (2003) Drinking Water Advisory: Consumer Acceptability Advice and Health Effects Analysis on Sulfate, EPA 822-R-03-007.

Sulfites

Shayne C Gad

- Representative Chemicals: Sulfur dioxide, SO_2; Sodium metabisulfite, $Na_2S_2O_5$; Sodium bisulfite, $NaHSO_3$; Sodium sulfite, Na_2SO_3; Potassium metabisulfite, $K_2S_2O_5$; Potassium bisulfite, $KHSO_3$
- Chemical Abstracts Service Registry Number: Sulfite (CAS 14265-45-3)
- Synonyms: Sulfite; Sulfite anion
- Chemical/Pharmaceutical/Other Class: Inorganic sulfites and bisulfites
- Chemical Structure:



Uses

Inorganic sulfites and bisulfites (such as sodium sulfite, Na_2O_3S) are used in photography, the bleaching of wool, the preserving of foods (e.g., meats and egg yolks), beverages, and medications. They act as effective antioxidant compounds and are also used for pulp making. Their preservative properties include controlling microbial growth, preventing browning and spoilage. Under the (US) Federal Food, Drug, and Cosmetic Act, sulfites are permitted for use as preservatives in food. Like other ingredients, sulfites must be declared in the ingredient statement when added to a food product. In addition, sodium sulfite, ammonium sulfite, sodium bisulfite, potassium bisulfite, ammonium bisulfite, sodium metabisulfite, and potassium metabisulfite are inorganic salts that function as reducing agents in cosmetic formulations. All except sodium metabisulfite also function as hair-waving/straightening agents. In addition, sodium sulfite, potassium sulfite, sodium bisulfite, and sodium metabisulfite function as antioxidants in cosmetics. All except ammonium sulfite are widely used in hair care products.

Background Information

Endogenous sulfite is generated as a consequence of the body's normal processing of sulfur-containing amino acids. In addition, as discussed below, sulfite can be produced by neutrophils. Sulfites occur as a consequence of fermentation and also naturally in a number of foods and beverages. As food additives, sulfating agents were first used in 1664, and approved for use in the United States in the 1800s. Sulfite is also noted as a water treatment additive, for example, to control oxygen levels in power plant boiler water. Further, sulfur dioxide is a common air pollutant, and may enter the body via inhalation. Sulfur dioxide has been reported to react with water in the ambient air and in the respiratory tract's mucous membranes to form sulfite and bisulfite ions.

Exposure Routes and Pathways

The pathways of exposure are oral, dermal, and through inhalation.

Toxciokinetics

Sulfites that enter mammals via ingestion, inhalation, or injection are metabolized by sulfite oxidase to sulfate.

Mechanism of Toxicity

Although the physiological basis for sulfite sensitivity is still poorly understood, clinical observations have established that certain medical conditions are associated with a predisposition to sulfite hypersensitivity. Approximately 500 000 individuals in the United States ($<0.05\%$ of the population) are at risk because they are asthma sufferers, who are steroid-dependent or who have airway hypersensitivity. Completed studies suggest that sulfite in the form of sulfur dioxide is the agent that causes the physiological response. It is hypothesized that sulfur dioxide causes bronchoconstriction, and that sulfur dioxide acts on tracheobronchial receptors to induce a cholinergic reflex. Inhaled sulfur dioxide elicited a stronger reaction in sulfite oxidase-deficient rats than endogenously accumulated sulfites and *S*-sulfocysteine (a reaction product of sulfite with cystine residues in proteins).

Acute and Short-Term Toxicity (or Exposure)

Animal

In oral-dose animal toxicity studies, hyperplastic changes in the gastric mucosa were the most common findings at high doses. Ammonium sulfite aerosol had an acute LC_{50} of $>400\,mg\,m^{-3}$ in guinea pigs. A single exposure to low concentrations of a

sodium sulfite fine aerosol produced dose-related changes in the lung capacity parameters of guinea pigs. A 3 day exposure of rats to a sodium sulfite fine aerosol produced mild pulmonary edema and irritation of the tracheal epithelium. Severe epithelial changes were observed in dogs exposed for 290 days to $1\,mg\,m^{-3}$ of a sodium metabisulfite fine aerosol. These fine aerosols contained fine respirable particle sizes that are not found in cosmetic aerosols or pump sprays. None of the cosmetic product types, however, in which these ingredients are used are aerosolized. Sodium bisulfite (tested at 38%) and sodium metabisulfite (undiluted) were not irritants to rabbits following occlusive exposures. Sodium metabisulfite (tested at 50%) was irritating to guinea pigs following repeated exposure.

In rats, sodium sulfite heptahydrate at large doses (up to $3.3\,g\,kg^{-1}$) produced fetal toxicity but not teratogenicity. Sodium bisulfite, sodium metabisulfite, and potassium metabisulfite were not teratogenic for mice, rats, hamsters, or rabbits at doses up to $160\,mg\,kg^{-1}$. Generally, sodium sulfite, sodium metabisulfite, and potassium metabisulfite were negative in mutagenicity studies. Sodium bisulfite produced both positive and negative results. In evaluating the positive genotoxicity data obtained with sodium bisulfite, the Cosmetic Ingredient Review Expert Panel established by the Cosmetic, Toiletry & Fragrance Association noted that the equilibrium chemistry of sulfurous acid, sulfur dioxide, bisulfite, sulfite, and metabisulfite suggests that some bisulfite may have been present in the genotoxicity tests involving the other ingredients and vice versa. Thus, the genotoxicity data were concluded to not give a clear, consistent picture of the genotoxic potential of these chemicals. Further, the bisulfite form is used in very low concentrations (0.03–0.7%) in most cosmetic products except wave sets. In wave sets, the pH ranges from 8 to 9 where the sulfite form would predominate. Skin penetration would be low due to the highly charged nature of these particles and any sulfite that did penetrate would be converted to sulfate by the enzyme sulfate oxidase. As used in cosmetics, therefore, these ingredients would not present a genotoxicity risk. The Cosmetic Ingredient Review Expert Panel concluded that sodium sulfite, potassium sulfite, ammonium sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite, and potassium metabisulfite are safe as used in cosmetic formulations.

Human

Clinical oral and ocular-exposure studies found no adverse effects for the sulfites used in cosmetics. Sodium sulfite was not irritating or sensitizing in clinical tests. These ingredients, however, may produce positive reactions in dermatologic patients under patch test conditions.

Sulfite-induced bronchospasm (sometimes leading to asthma) was first noticed as an acute sensitivity to metabisulfites, which were sprayed on restaurant salads (and salad bars) and used in wine. Emergency room admissions confirm that ingestion of sulfites can lead to asthmatic attacks, rashes, and abdominal upset. An alert physician observed that six patients, who had been admitted to the emergency room, had consumed the same brand of salsa. Two of the patients had asthma flare-ups, two experienced coughing and tightness of the throat, and two required mechanical ventilation. It was discovered that the offending salsa had a sulfite content of 1800 ppm, well above the level of $\sim$700 ppm found in other brands of salsa. One of the patients, fully aware of her sensitivity to sulfites, thought it was safe to eat the salsa because it was improperly labeled as 'fresh'.

The US Food and Drug Administration Center for Food Safety and Applied Nutrition (CFSAN) has monitored reports of adverse reactions to sulfites since 1980. As of June 1999, CFSAN has received 1132 consumer complaints describing adverse reactions thought to be due to the ingestion of foods with sulfites. Out of 799 reports with adequate information about the intensities of the reaction, 388 (48.6%) were classified as severe.

Recently, it has been shown that sulfite is actively produced from neutrophils by stimulation with the bacterial endotoxin, lipopolysaccharide (LPS), and that the serum sulfite concentration is increased in a rat model of sepsis induced by systemic injection of LPS. The serum concentration of sulfite was determined in patients with acute pneumonia, and was significantly higher than that in control subjects. Further, serum sulfite was serially determined before and after antibiotic therapy, and the levels were significantly reduced during the recovery phase compared with those during the acute phase. Moreover, neutrophils obtained from three patients during the acute phase of pneumonia spontaneously produced higher amounts of sulfite *in vitro* than those obtained after recovery. There was a close positive correlation between serum sulfite and C-reactive protein in patients with pneumonia. These findings suggest that serum sulfite increases during systemic inflammation in humans, and that sulfite may act as a mediator in inflammation.

See also: Food Additives; Food and Drug Administration, US; Sensitivity Analysis.

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Relevant Website

www.cfsan.fda.gov – US Food and Drug Administration. Sulfites: An Important Food Safety Issue.

Sulfonylureas *See* Hypoglycemics, Oral.

Sulfur Dioxide

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7446-09-5
- Synonyms: Sulfurous anhydride; Sulfurous oxide
- Chemical/Pharmaceutical/Other Class: Oxidant gas
- Chemical Structure: O═S═O

Uses

Sulfur dioxide (SO_2) is a colorless and nonflammable gas with a pungent odor. It is used commercially to preserve fruits and vegetables and as a disinfectant in food production. It is also used in bleaching straw and textiles. Liquid SO_2 can be produced by pressurizing the gaseous form and then used as a solvent. Sulfur dioxide is also a primary component of air pollution.

Exposure Routes and Pathways

Contact with mucous membranes (eyes and nose) and inhalation are possible routes of exposure.

Toxicokinetics

Absorption is dependent on the level of exposure. Sulfur dioxide is soluble in both water and biological tissues. It is readily absorbed and distributed throughout the body. Most inhaled SO_2 is detoxified by sulfite oxidase in the liver. It is excreted through urine and through exhalation in expired air, although elimination from the respiratory tract is slow. Sulfur dioxide absorbed into the body may persist 1 week after exposure.

Mechanism of Toxicity

On moist skin or mucous membranes, SO_2 is converted to sulfurous acid, a direct irritant. This mechanism accounts for its ability to cause inflammation, burning sensation, and tissue damage (described below) in the eyes, throat, nose, and other respiratory tissues experiencing direct contact. Bronchoconstriction and other related effects may be mediated by release of leukotrienes, prostaglandins, or other inflammatory factors. How SO_2 causes any of the other systemic and clastogenic effects reported below is unclear. Some evidence suggests that free radicals and oxidative stress may play a role, and that metabolites of SO_2 (sulfites) may be responsible for clastogenicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

In rats, SO_2 accelerates aging and produces heart, lung, and kidney damage. The inhalation LD_{50} is $2520\,\text{ppm}\,\text{h}^{-1}$ in rats. The LD_{50} is 3000 ppm per 30 min in mice.

Human

Sulfur dioxide is irritating to the eyes, mucous membranes, and respiratory tract. High levels of exposure produce cardiac arrest. Moderate exposure produces pulmonary edema. Low exposure results in systemic acidosis. Individuals who have hyperactive airway disease, including asthma, may be particularly sensitive.

Chronic Toxicity (or Exposure)

Animal

No significant adverse reproductive or developmental effects have been noted in animal studies.

A 2 year cancer bioassay in mice that employed a single dose of 500 ppm for 5 min day^{-1}, 5 days week^{-1} for the duration of the study reported seeing an increase in lung tumors. This study is not considered adequate to conduct a meaningful assessment of cancer risk from SO_2 in human populations, and its relevance to human risk is not known.

Human

Lung function can be altered, but there is no clear epidemiologic evidence that chronic exposure to SO_2 has more serious (e.g., cancer-causing) effects in respiratory tissues in exposed populations. Workers and others known to have had significant exposures to SO_2 have manifested evidence of clastogenicity (e.g., chromosome aberrations and sister chromatid exchange in lymphocytes); however, the clinical significance of this is not clear and the possibility that this was a result of exposure to other agents cannot be ruled out.

In Vitro Toxicity Data

Sulfur dioxide produced weak increases in micronuclei after activation in a plant assay (*Tradescantia* spp.). Mammalian (cow, ewe) oocytes exposed *in vitro* had higher levels of chromosome aberrations when exposed to SO_2 without metabolic activation. The *Saccharomyces cerevisae* yeast test for gene mutations was also positive without activation.

Clinical Management

If skin or eye exposure occurs, the affected areas should be flushed with water for ~15 min. If ingested, the stomach contents should be diluted with water or milk. Gastric lavage or emesis should not be attempted. Pain should be treated without numbing the central nervous system. Open airways and steady blood pressure should be maintained. Prednisolone (2 mg kg^{-1} day^{-1}) should be given for 10 days.

Exposure Standards and Guidelines

Occupational/Occupational Safety and Health Administration: The Lowest Lethal Concentration (LLC) is 1000 ppm per 10 min and the permissible exposure limit/time-weighted average is 5 ppm per 8 h.

Environmental Protection Agency/environmental: The National Ambient Air Quality Standards annual arithmetic mean standard is 0.03 ppm; the 24 h limit is 0.14 ppm.

International Agency for Research on Cancer carcinogen classification: 3 (possible human carcinogen, based on a single mouse study employing only one air concentration; see details above).

See also: Absorption; Pollution, Air; Respiratory Tract.

Further Reading

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Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Sulfur Dioxide.

http://www.epa.gov – SO_2 – How Sulfur Dioxide Affects the Way we Live and Breathe (from the US Environmental Protection Agency).

Sulfuric Acid

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 7664-93-9
- Synonyms: Acid mist; Dipping acid; Hydrogen sulfate; Sulfur acid; Sulfuric
- Chemical/Pharmaceutical/Other Class: Strong inorganic acid; Corrosive agent
- Chemical Formula: H_2SO_4

Uses

Sulfuric acid is a highly reactive compound and is extensively used in industry as a chemical intermediate and as a component of many industrial and commercial products. For example, it is used in fertilizers, lead-acid batteries, pigments and dyes, and as an industrial reagent in the paper, petroleum, and metal industries. It is also used in pharmaceuticals, as a food additive, and in toilet bowl cleaners.

Background Information

Sulfuric acid can be found in many strengths and formulations. Toxicological and chemical properties of sulfuric acid solutions are dependent on the sulfuric acid content of the solution. For example, solutions containing less than 10% sulfuric acid are highly irritating, while solutions containing more than 10% sulfuric acid are corrosive. Sulfuric acid solutions used in industry can be up to 98% in concentration, while consumer products such as toilet bowl cleaners may contain up to 8% sulfuric acid.

Exposure Routes and Pathways

Sulfuric acid is corrosive to living tissue. It can damage and destroy living cells and tissue upon contact. Therefore, pathways of exposure include all surface tissues (skin, eyes, mucus membranes) as well as internal surfaces exposed to outside elements such as the digestive and respiratory tracts.

Toxicokinetics

Sulfuric acid can cause tissue damage and destruction upon contact. Therefore, it is not absorbed into the systemic circulation. The reaction of sulfuric acid with body tissues produces irritation and chemical burns at the site of contact. Ingestion of sulfuric acid can cause internal tissue damage and associated secondary systemic effects such as gastrointestinal hemorrhaging, ischemia, hypoxia, shock, and necrosis.

Mechanism of Toxicity

Sulfuric acid is a highly reactive chemical. It can react with cells and tissues upon contact. Damage caused by sulfuric acid can range from tissue irritation to chemical burns and necrosis. Signs and symptoms of exposure include tissue damage at point of contact. Tissue injury appears within seconds of exposure and can continue for hours and even days if not properly treated. The tissue damage extent and severity is dependent on the dose received, exposure interval, and strength (molar concentration) of the sulfuric acid solution. Highly concentrated sulfuric acid solutions (usually found in industrial chemicals) are more dangerous than diluted acid solutions (as those found in consumer products).

The mode of action of sulfuric acid is the same in humans and animals. Therefore, acute and chronic effects are expected to be the same for animals and humans.

Acute and Short-Term Toxicity (or Exposure)

The major hazard associated with exposure to sulfuric acid is direct irritation and corrosion of internal and external tissue surfaces. Signs and symptoms associated with potential routes of exposure include:

- *Inhalation:* Nose and throat irritation, coughing, sneezing, difficulty in breathing, and pulmonary edema. Death may result from esophageal edema (caused by chemical burns to the esophagus) or sudden circulatory collapse (caused by generalized lung tissue destruction).
- *Ingestion:* Throat irritation, difficulty in swallowing, hemorrhaging, perforation, and necrosis of digestive tract.
- *Skin:* Damage can range from dermatitis and irritation to necrosis and scarring. Extensive and severe chemical burns can be life threatening.
- *Eye:* Eyes are especially susceptible to acid burns. Signs and symptoms in increasing severity include: irritation, lacrimation, conjunctivitis, corneal burns and perforation, visual loss, and perforation of the eye.

Chronic Toxicity (or Exposure)

Chronic exposure to diluted solutions of sulfuric acid can produce chronic tissue damage. Adverse effects

seen following chronic exposure are usually due to repeated and sustained tissue damage and repair. Signs and symptoms associated with chronic exposure include: decreased lung capacity and function, recurrent respiratory infections, bronchitis, and possibly cancer. The International Agency for Research on Cancer has determined that chronic, occupational exposure to sulfuric acid mist may cause cancer of the upper respiratory tract. Repeated ingestion of dilute sulfuric acid solutions may cause perforation of teeth enamel and gastritis.

Clinical Management

Basic life support measures should be implemented and further absorption prevented by removing contaminated clothing and washing the affected area. If ingested, the esophagus and digestive tract may be irritated and may be burned. Therefore, a careful examination should be made and gastric lavage performed only if the esophagus is not damaged and it is believed that lavage may be effective at removing the ingested material.

Medical examination should look for signs of skin, eye, esophagus, and lung damage. Patients should be monitored and treated in an intensive care unit. Monitor vital signs and blood chemistry at least once a day. Institute life support as needed.

Environmental Fate

Sulfuric acid is found in nature in the vicinity of volcanoes. It is also used in industry for manufacturing numerous consumer products. Therefore, the chemical may be released to the environment as a waste product or from unintentional, accidental releases. If released to soil, it will dissolve in soil moisture and migrate with either soil moisture or groundwater flow. If released to water, it will dissolve or create sulfate salts. Dissolved sulfuric acid will react with calcium and magnesium to produce sulfate salts. Sulfuric acid can contribute to the 'weathering' of soil and rocks by reacting with calcium and carbonates contained in soil and rocks.

Exposure Standards and Guidelines

The US Occupational Safety and Health Administration has established $1\,mg\,m^{-3}$ as the 8 h time-weighted average permissible level for sulfuric acid in workplace air.

Miscellaneous

Special precautions must be taken when working with sulfuric acid. Personnel handling this chemical must follow industrial hygiene and health protection requirements for handling potentially corrosive substances. At a minimum sulfuric acid exposure should be minimized through the use of engineering controls, work practices, and personal protective equipment, including impervious and disposable gowns and gloves as well as eye and respiratory protection. In addition, working areas and working instruments must be especially designed for handling potentially harmful substances.

See also: Acids; Corrosives; Great Smog of London.

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Sulfuric Acid.

Surfactants *See* Surfactants, Anionic and Nonionic; Surfactants, Perfluorinated; Detergent.

Surfactants, Anionic and Nonionic

Gerald L Kennedy

This article is a revision of the previous print edition article by Anne E Bryan, volume 3, pp. 209–210, © 1998, Elsevier Inc.

- Representative Chemicals:
  - Anionic: Alkyl aryl polyester sulfates and sulfonates (Triton); Alkyl aryl sodium sulfonates; Alkylated sodium phosphates, sulfates (sodium lauryl sulfate, Tergitol) or sulfonates; Linear alkyl benzene sulfonates, soaps, and diethanolamine oleate
  - Nonionic: Synthetic detergents (Joy, Cascade); Alkyl ethoxylates; Alkyl phenoxy polyethoxy ethanols (lgepal); Glyceryl stearate; Pluronics polyoxyethylene sorbitols (Tween), and polysorbates
- Chemical/Pharmaceutical/Other Class: Surfactants (detergents)

Uses

These materials are used primarily for removing dirt from either soft or hard surfaces as in washing clothes or dishes, removing wax from floors, or removing grease from metals. Although the classes are based on the ionization of the surface active moiety at neutral pH (anionic, nonionic, or cationic), within a given class a wide range of chemical structures and toxicities is recognized. This section will consider what is felt to be the most generally applicable toxicities of these classes but reminds the reader that each of the chemicals might have its own particular toxicities and potencies.

Exposure Routes and Pathways

Most of these chemicals (or products) will come in contact with the skin in their normal intended use. However, the primary route for poisonings is the accidental ingestion of these chemicals by small children; so, special care needs to be taken to minimize this potential. Most of these agents are irritants to the eye (reference, soap in the eye), so contact with ocular tissues should be avoided.

Inhalation is a less likely route of contact but, as these materials are irritating to mucous membranes, contact with tissues of the upper respiratory tract (nose) will be irritating.

Toxicokinetics

The materials are not readily absorbed from the skin or gastrointestinal tract. The specifics of adsorption, distribution, metabolism, and excretion vary with the individual surfactants hence further generalization is unwarranted.

Mechanism of Toxicity

Detergents are not readily absorbed and most reported toxicities come from contact with surface tissues such as skin and eye. Hand dishwashing detergents tend to be less irritating than machine dishwashing agents. Where alkyl chain length vary, those of shorter chain length tend to be more irritating (and somewhat more toxic) when given orally.

Relatively little systemic toxicity has been reported except in poisoning situations. The irritating properties of these chemicals, to some extent, serve as a warning mechanism to avoid the exposed organism against further contact.

Acute and Short-Term Toxicity (or Exposure)

Animal

As in the human overexposure cases, gastrointestinal irritation and ocular irritation would be expected in animal tests. Oral doses required to produce mortality in rats are generally very high, in the greater than 1–2 $g\,kg^{-1}$ range. For example, the oral LD_{50} for sodium lauryl trioxyethylene sulfate is 1.8 $g\,kg^{-1}$. Dogs and monkeys appear less sensitive to oral doses than rodents perhaps because they tend to vomit more readily. With alkyl polyethylene glycol ether, monkeys showed emesis following single oral doses above 5 $g\,kg^{-1}$ along with signs of central nervous system depression. Again, in general these materials are irritants that are low in acute toxicity and show systemic responses only under highly exaggerated exposure conditions.

Human

Skin irritation has been encountered after prolonged occupational dermal contact. Skin dryness, irritation, and contact dermatitis have all been seen after varying degrees of exposure. Eye exposure to most anionic and nonionic detergents results in momentary eye irritation with no permanent eye damage. Eye exposure to low-phosphate detergents, which tend to be more alkaline may produce eye injury.

If ingested (and depending on the amount), nausea, vomiting, and diarrhea are the most common manifestations of toxicity. Persistent effects rarely result but dehydration, electrolyte imbalance (most notable hypochloremic metabolic acidosis) have been reported.

In the workplace, occupational asthma has been reported. Aspiration may result in upper airway edema and considerable respiratory distress. Again, low phosphate detergents will produce oral, esophageal, and respiratory tract burns due to their alkaline nature.

Chronic Toxicity (or Exposure)

Animal

Systemic responses have been shown to occur only after treatment with high doses for relatively long periods of time. Sodium lauryl trioxyethylene sulfate fed to rats at 0.5% (50 000 ppm) for 2 years showed no gross anatomical, biochemical, or tissue histopathologic lesions (including no increase in tumors). Mice painted twice weekly applications of a 5% aqueous solution developed no skin tumors. Again, the effects of concern with these chemicals tend to be short term relating to their irritation properties.

Human

There is no useful experimental data on the long-term effects of these chemicals in man. Clearly the fast-acting short-term irritation effects would be expected to allow exposed individuals to be aware of and restrict long-term contact (not necessarily repeated contact as in the use of detergents, dishwashing agents, etc.).

In Vitro Toxicity Data

In general, the surfactant properties of these chemicals tend to make *in vitro* testing difficult both qualitatively and quantitatively. The physical characteristics of surfactants tend to keep the molecule at the water/oil, water/air interface thus making meaningful contact with the *in vitro* organ/tissue systems difficult. Both mechanistic-related effects and noneffects, can result from attempts to test these chemicals in *in vitro* aqueous systems hence caution is advised when attempting to conduct, evaluate, or interpret such information.

Clinical Management

For oral exposure, immediate dilution with either water or milk should be employed. Spontaneous emesis frequently occurs (if not, it is unlikely that significant ingestion has occurred). Patients should be observed for signs of esophageal or gastrointestinal tract irritation or burns. If inhaled, move the patient to fresh air and monitor for respiratory distress. If coughing or difficulty breathing develops, evaluate the patient for respiratory tract irritation, bronchitis, or pneumonia. Oxygen and assisted ventilation can be used in extreme cases. Bronchospasms which occur rarely (and again following significant exposure) can be treated with $\beta 2$ agonists and oral or parenteral corticosteroids. Following eye exposures, irrigate the eye with copious amounts of room temperature water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, seek the attention of a physician. Dermal exposures should be treated by removal of contaminated clothing and jewelry and washing the exposed area with copious amounts of water. As before, persistent irritation or pain should be referred to health care professionals.

In summary, ingestion of nonionic or anionic detergents alone is not generally serious. Ingestion of automatic dishwater soaps or low-phosphate detergents, which are more alkaline, may result in burns of the mouth, pharynx, and esophagus. Ingestion of hard soap bars is generally associated with emesis and mild diarrhea. Eye contact injuries may occur with these agents causing varying degrees of damage.

Environmental Fate

The hydrocarbon chains of these materials generally tend to break down in the environment relatively quickly. The business end of the molecule behaves differently hence no overarching statements can be made. Sodium lauryl sulfate can readily be removed from aqueous systems (filtering and aeration) and is readily biodegraded (42 of 45 Pseudomonas strains could degrade alkyl sulfates to the saturated and unsaturated fatty acid). In sewage, sludge, seawater, and by selected organisms from 75% to 100% degradation is reported for sodium lauryl sulfate.

Ecotoxicology

It is difficult to characterize the effects of surfactants as a class on environmental organisms. However, there appears to have been relatively little impact on the health of environmental organisms as a result of exposure to these chemicals. Again using sodium lauryl sulfate as an example, concentrations of from 1.2 to 600 $\mathrm{mg\,l^{-1}}$ are required to inhibit the activity

of microorganisms in sludge. In bacteria, effective concentration 50% (EC_{50}) of from 43 to $>9000\,mg\,l^{-1}$ have been reported. In invertebrates, EC50 values range from 1 to $118\,mg\,l^{-1}$. Thirty-eight different fish species have been studied for their acute lethality response and values range from 0.4 to $560\,mg\,l^{-1}$ with most of the species responding $\sim 5\,mg\,l^{-1}$. Algae appear to be a more sensitive organism with EC_{50} values ranging from 0.02 to $7\,mg\,l^{-1}$.

Other Hazards

Although not a hazard, these chemicals, as surfactants, produce foam at the air/water interface and direct discharge to waterways results in unsightly build-up of foam on/around the water.

See also: Sensory Organs; Shampoo; Skin.

Further Reading

Anon. (1991) Environmental and Human Safety of Major Surfactants. Volume 1: Anionic Surfactants. Part 1. Linear Alkylbenzene Sulfonates. Part 2. Alcohol Ethyl Sulfates. Part 3. Alkyl Sulfates. Part 4. Alpha Olgin Sulfonates. Volume 2: Nonionic Surfactants, Alcohol Ethoxylates and Alkylphenol Ethoxylates. Government Reports Announcements & Index (GRA&I).

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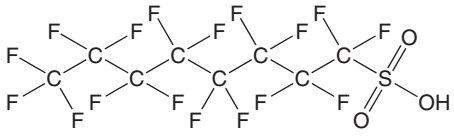
Surfactants, Perfluorinated

John Newsted and Paul Jones

- Representative Chemicals: Perfluorooctane sulfonic acid (PFOS); Perfluorooctanesulfonyl fluoride (POFS); *N*-Methylperfluorooctane sulfonamidoethanol (N-MeFOSE); *N*-Ethylperfluorooctane sulfonamidoethanol (N-EtFOSE)
- Chemical Abstracts Service Registry Numbers: Perfluorooctanesulfonate, acid (CAS 1763-23-1); Perfluorooctanesulfonyl fluoride (CAS 307-35-7); *N*-Methylperfluorooctane sulfonamidoethanol (CAS 24448-09-7); *N*-Ethylperfluorooctanesulfonamidoethanol (CAS 1691-99-2)
- Synonyms: Perfluorooctane sulfonic acid; 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-Octanesulfonic acid; Heptadecafluoro-1-octanesulfonic acid; Perfluorooctylsulfonic acid
- Chemical/Pharmaceutical/Other Class: Perfluorinated surfactants consist of a broad class of fluorinated chemicals of differing structures, physical–chemical properties, and modes of toxic action. The focus in this article is on sulfonyl-perfluorinated surfactants
- Chemical Formulas:
  - Perfluorooctane sulfonate: $C_8F_{17}SO_3H$
  - Perfluorooctanesulfonyl fluoride: $C_8F_{17}SO_2F$
  - *N*-Methylperfluorooctanesulfonamidoethanol: $C_8F_{17}SO_2N(CH_3)CH_2CH_2OH$
  - *N*-Ethylperfluorooctanesulfonamidoethanol: $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- Chemical Structure:



PFOS

Uses

Perfluorinated surfactants are fully fluorinated organic compounds that due to their unique chemical properties can be used in a variety of industrial processes and products. Some characteristics of these chemicals are the ability to repel water and oil, reduce surface tension, catalyze oligomerization and polymerization, and maintain their properties under extreme conditions. Sulfonyl-based perfluorochemicals (PFOS) have been used in a variety of products that can be divided into three main categories: surface treatments (carpet and textile protection), paper protection (grease, oil, and water resistance), and performance chemicals (fire fighting foams, mining surfactants, electronic etching baths). However, due to the presence of PFOS in biota, in sites remote from production, and in human blood, PFOS has been voluntarily withdrawn from commercial production.

Exposure Routes and Pathways

Occupational exposure to perfluorinated chemicals (PFCs) may occur through inhalation of and dermal

contact with these compounds at workplaces where they are produced or used. Environmental monitoring data indicate that the general population may be exposed to PFCs such as PFOS via ingestion of contaminated fish and drinking water, and by dermal contact with products containing PFCs. The use of PFOS in food packaging as water and grease repellents also serves as a source of exposure to these compounds.

Toxicokinetics

PFOS is well absorbed from the digestive tract while dermal absorption appears to be limited. No quantitative data are available on absorption of PFOS via inhalation. Once absorbed, PFOS is bound to protein and is distributed primarily in blood and liver. Significant enterohepatic circulation of PFOS has been reported in several species. PFOS is not known to undergo further metabolism but other fluorochemicals such as perfluorooctanesulfonyl fluoride (POSF) and ethylperfluorooctane sulfonamidoethanol (Et-FOSE) may undergo metabolism to PFOS. Elimination from the body is slow with PFOS being found in both urine and feces. In addition, PFOS has also been shown to traverse the placenta and expose the fetus *in utero*. PFOS is also distributed into the milk of lactating females. The estimated serum half-life in humans is $\sim$1428 days (or 4 years).

Mechanism of Toxicity

The mechanisms governing the toxicity of PFOS to biological systems are still under investigation. Potential modes of action that have been identified include competition with fatty acids for carrier protein sites, cholesterol synthesis, and bioenergetics. Other studies suggest that PFOS may alter peroxisomal fatty acid β-oxidation.

Acute and Short-Term Toxicity (or Exposure)

Animal

PFOS has shown moderate acute toxicity in rats (LD_{50} of 251 mg kg^{-1}). In a 90 day repeat-dose–response study with rats, exposure to PFOS (6 mg kg^{-1} day^{-1}) resulted in hepatotoxicity and mortality. Adverse signs of toxicity included hepatic vacuolization and hepatocellular hypertrophy, gastrointestinal effects, hematological abnormalities, weight loss, convulsions, and death. Postnatal deaths and other developmental effects have been reported at low doses in offspring in a two-generation reproductive toxicity study with rats. At the highest dose (3.2 mg kg^{-1} day^{-1}) all pups died within a day after birth while $\sim$30% of the F1 pups died after 4 days in the 1.6 mg kg^{-1} day^{-1} group. The no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) for the second-generation offspring (F2 pups) were 0.1 and 0.4 mg kg^{-1} day^{-1}, respectively. In a developmental study with rabbits exposed to PFOS, maternal toxicity was evident at 1.0 mg kg^{-1} day^{-1}. Developmental effects due to PFOS included reduced fetal body weight and reduced ossification of the sternum, hyoid, metacarpals, and pubis. The LOAEL for developmental effects was 2.5 mg kg^{-1} day^{-1} and the NOAEL was 1.0 mg kg^{-1} day^{-1}.

Chronic Toxicity (or Exposure)

Animal

Potential carcinogenicity of PFOS has been examined in a dietary 2 year bioassay with rats. In male and female rats exposed to 20 mg kg^{-1}, there was a significant increase in the incidence of hepatocellular adenomas while in females there was also an increase in hepatocellular carcinomas. In addition, there was a significant increase in thyroid follicular cell adenomas and carcinomas in males in a recovery group from the 20 mg kg^{-1} treatment group.

Human

PFOS has been detected in the serum of occupational and general populations. In occupational surveys, PFOS serum levels have been found to range from 0.1 to 12.83 ppm with an average of 1.32 ppm. In the general population the range of PFOS serum concentrations was from 4.3 to 1656 ppb. The mean PFOS level was determined to be 30–53 ppb. Occupational surveys have also shown some association between PFOS exposure and human health. In a survey of males, PFOS serum concentrations of 1.69–10.06 ppm were associated with increased serum triglycerides, alkaline phosphatase, total bilirubin, and alanine aminotransferase. Serum triiodothyronine was higher and the thyroid hormone binding ratio was lower in workers with the greatest PFOS serum levels. In a mortality study of workers, mortality risks for most cancer types and nonmalignant causes were not elevated. However, an increased risk of neoplasms of the male reproductive system and bladder cancer was associated with workers with the highest and longest exposures to fluorochemicals.

In Vitro Toxicity Data

PFOS was not mutagenic in *Salmonella* tests. PFOS also did not induce chromosomal aberrations in

human lymphocytes or micronuclei in bone marrow of mice. PFOS did inhibit gap junctional intercellular communication in a rat liver and dolphin kidney cell line, an effect that was both rapid and reversible.

Environmental Fate

As a result of the production and use of perfluorooctane sulfonic acid and its precursors, PFOS has been released to the environment through variety of waste streams. The environmental partitioning behavior of PFCs is unusual in that PFOS-based substances are both oleophobic and hydrophobic. As a result, an octanol/water partitioning (K_{ow}) coefficient for PFOS has not been determined. PFOS is persistent in the environment and does not hydrolyze, undergo direct or indirect photolysis, or biodegrade to any significant degree. While PFOS has low volatility, several PFOS precursors are considered volatile, including EtFOSE and MeFOSE alcohols. If released to soil, sediment or sludge, PFOS is expected to adsorb strongly to organic and inorganic components. Due to these properties, PFOS is expected to persist in soils, sediments, and sludge. If released into water, PFOS is expected to remain in the water compartment unless it is assimilated into organisms or adsorbed onto particulate matter and potentially deposited into sediments. Volatilization from water surfaces or biodegradation is not expected to be important fate processes. PFOS has the potential to bioaccumulate in aquatic organisms. Laboratory-based bioconcentration factors for PFOS range from 56 to over 1000 while field-based bioaccumulation factors range from 830 to 125 000. The field-based bioaccumulation factors for PFOS may be overestimated due to metabolism of accumulated perfluorinated derivatives of PFOS.

See also: Fluorine; Surfactants, Anionic and Nonionic.

Further Reading

OECD (2002) Hazard assessment of perfluorooctane sulfonate (PFOS) and its salts. ENV/JM/RD(2002)17/FINAL.

Olsen GW, Burris JM, and Mandel JH (2003) Human donor liver and serum concentrations of perfluorooctane sulfonate and other perfluorochemicals. *Environmental Science and Technology* 37: 888–891.

Seacat AM, Thomford PJ, Hansen KJ, *et al.* (2003) Subchronic dietary toxicity of potassium perfluorooctane sulfonate in rats. *Toxicology* 183: 117–131.

Synergism *See* Chemical Interactions.

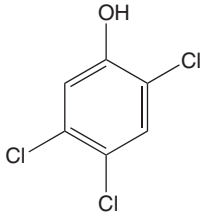
BLANK

T

2,4,5-T

Lynn Weber

- Chemical Abstracts Service Registry Number: CAS 93-76-5
- Synonyms: 2,4,5-Trichlorophenol
- Chemical/Pharmaceutical/Other Class: Chlorinated phenoxyacetic acids. A closely related compound is 2,4-D (2,4-dichlorophenoxyacetic acid)
- Chemical Structure:



Uses

2,4,5-T is manufactured for use as a broad-spectrum herbicide. Its use in the United States has been suspended.

Background Information

A combination of the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T (Agent Orange), was used by the US military during the Vietnam War for defoliation. Long-term health consequences from exposure to Agent Orange, in particular to dioxin contaminants, have been suspected.

Exposure Routes and Pathways

Exposure to 2,4,5-T is by the oral, inhalation, and dermal routes.

Toxicokinetics

2,4,5-T is absorbed dermally in humans. Radioactivity was found in all tissues examined as well as in milk and fetuses after single oral administration of 0.17–41 mg kg^{-1} [^{14}C]2,4,5-T to pregnant rats. 2,4,5-T is eliminated largely unchanged. No metabolites are known, although it is possible that a small amount is eliminated as a glucuronide conjugate. The volume of distribution after a single oral dose of 5 mg kg^{-1} varies as follows: in humans, 0.079 l kg^{-1}; in rats, 0.14 l kg^{-1}; and in dogs, 0.22 l kg^{-1}. 2,4,5-T is bound extensively to the plasma protein, which could limit renal clearance of the herbicide; 2,4,5-T is also bound to renal cortex microsomal and cytosol fractions. 2,4,5-T given orally to volunteers (100–150 mg) was readily absorbed and gradually eliminated from blood plasma, showing a first-order elimination rate; more than 80% of a dose was excreted in urine in intact form within 72 h. Clearance of 2,4,5-T from plasma and body of dogs, mice, and humans is slower than that in rats.

Mechanism of Toxicity

The effect of 2,4,5-T was investigated in *in vitro* studies with sublethal concentrations of 2,4,5-T, which showed an inhibitory effect on calcium-dependent ATPase. *In vivo* exposure to various sublethal concentrations of 2,4,5-T during 96 h caused a significant inhibition of microsomal calcium-dependent ATPase. 2,4,5-T inhibits renal anion transport. Exposure of cells to 2,4,5-T resulted in a dose-dependent inhibition of DNA synthesis. Also, 2,4,5-T was shown to combine with choline to form 2,4,5-T-acetylcholine, a false neurotransmitter that inhibited muscle contraction. Since the false neurotransmitter could be formed at muscarinic as well as nicotinic synaptic sites, this interference with cholingeric neurotransmission may at least partially explain myotonia, ventricular fibrillation, and fetal growth retardation reported after 2,4,5-T exposure. Finally, 2,4,5-T is a well-known peroxisome proliferator agent, particularly in rodent liver.

Acute and Short-Term Toxicity (or Exposure)

Animal

2,4,5-T in pure form is considered to be of relatively low toxicity.

Oral LD_{50}s were as follows: mouse, 389 mg kg^{-1}; rat, 500 mg kg^{-1}; guinea pig, 381 mg kg^{-1}; dog, $>$100 mg kg^{-1}. The percutaneous LD_{50} in rats was $>$5000 mg kg^{-1}. Single oral doses of 100 mg kg^{-1} body weight of 2,4,5-T fed to pigs caused anorexia, vomiting, diarrhea, and ataxia; at autopsy, hemorrhagic enteritis and congestion of liver and kidney were found. 2,4,5-T (containing no detectable 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) affected chromosomes of bone marrow cells of mongolian gerbil (*Meriones unguiculatus*) that received five consecutive daily intraperitoneal injections by causing significant increases in chromatid gaps, chromatid breaks, and fragments after total doses of 250 mg kg^{-1} or more but not after 150 mg kg^{-1} or less.

The no-effect levels for embryotoxicity for commercial 2,4,5-T were as follows: rat, 25 mg kg^{-1} day^{-1}; mouse, 20 mg kg^{-1} day^{-1}; hamster, 40 mg kg^{-1} day^{-1}; and monkey, 40 mg kg^{-1} day^{-1}.

Human

2,4,5-T in pure form is considered to be of relatively low toxicity. Limited data are available on exact toxic doses. Intravenous injection of up to 28 mg kg^{-1} of 2,4-D has been well tolerated, while a dose of 50 mg kg^{-1} produced toxicity. Death has resulted following ingestion of 80 mg kg^{-1}.

Common findings after acute ingestion included miosis, coma, fever, hypotension, emesis, tachycardia, and muscle rigidity. Complications may include respiratory failure, pulmonary edema, and rhabdomyolysis. Ingestions cause burning of the mouth, esophagus, and stomach. Irritation of skin, eyes, nose, and throat may also occur. Tachycardia is common. Cardiac arrhythmias occurred in one suicide case. Pulmonary edema has been reported. Respiratory paralysis and bradypnea are common in large ingestions. Vertigo, headache, malaise, and paresthesias have been reported occasionally in occupational handlers. Higher doses may produce muscle twitching and spasms, followed by profound muscle weakness and unconsciousness. Individual idiosyncrasies may be involved in reported neuropathies. Rhabdomyolysis may occur. Myotonia (stiffness of legs) has been observed in severely poisoned persons. Vomiting and diarrhea have been reported. Elevated LDH, SGOT (AST), and SGPT (ALT) (LDH, lactate dehydrogenase; SGOT, serum glutamic oxaloacetic transaminase; AST, aspartate aminotransferase; SGPT, serum glutamic pyruvic transaminase; ALT, alamine aminotransferase) have been reported. Albuminuria, hemoglobinuria, and azotemia may occur. Acute exposure may cause irritation of the skin. Chloracne from chlorodioxin contaminants in 2,4,5-T has been reported in heavily exposed workers.

Chronic Toxicity (or Exposure)

Animal

2,4,5-T itself is not believed to be carcinogenic or teratogenic in animals; these effects, produced by technical grades of the chemical, are believed to be due to the dioxin that is present as an impurity. In 2 year feeding trials no effect was observed in rats receiving 30 mg kg^{-1} diet or in 90 day trials in beagle dogs at 60 mg kg^{-1} diet.

Human

2,4,5-T itself is not believed to be carcinogenic or teratogenic in humans; these effects, produced by technical grades of the chemical are believed due to the dioxin that is present as an impurity.

Chronic 2,4,5-T exposure may reduce metabolic rate and subsequently lead to perinatal growth retardation. There may be an association of 2,4,5-T exposure with hydantidiform mole formation. However, the effects of 2,4,5-T and dioxin (an impurity in 2,4,5-T preparations) cannot be distinguished from each other in most studies.

Classification of Carcinogenicity

Evidence in humans is limited; overall summary evaluation of carcinogenic risk to humans is group 2B: the agent is possibly carcinogenic to humans. Recent studies conducted to clarify the carcinogenic potential found that although the closely related 2,4-D is mutagenic in a yeast test, cultured mammalian cells, and *in vivo* treated mice, 2,4,5-T appears to exhibit little or no mutagenicity.

Clinical Management

These herbicides can be measured in plasma and urine by high-performance liquid chromatography. Chlorophenoxy compounds do not affect blood cholinesterase activities.

Emesis may be indicated in recent substantial ingestion unless the patient is or could rapidly become obtunded, comatose, or convulsing. It is most effective if initiated within 30 min. For activated charcoal/cathartic, a charcoal slurry, aqueous or mixed with saline cathartic or sorbitol, should be administered. A baseline complete blood count (CBC), electrolytes, and renal and hepatic function test should be obtained. Urine should be tested for protein, RBCs, and myoglobin. Urine output should be monitored. LDH, SGOT (AST), and alkaline phosphatase should

be followed to detect liver injury, and creatine phosphokinase (CPK) should be followed to detect muscle damage. Urine pH, arterial pH, and bicarbonate should be measured to detect acidosis. Respiratory depression, hypotension, and metabolic acidosis should be treated. Adequate urine flow should be maintained with intravenous fluids if victim is dehydrated. The patient should be monitored closely for cardiac arrhythmias, hyperthermia, and seizures.

If exposed via inhalation, the victim should be moved to fresh air and monitored for respiratory distress. If cough or difficulty in breathing develop, evaluation for respiratory tract irritation, bronchitis, or pneumonitis should be performed. Humidified supplemental oxygen (100%) should be administered with assisted ventilation as required.

Exposed eyes should be irrigated with copious amounts of tepid water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should be seen in a health care facility.

If clothing is contaminated, it should be removed and discarded. Affected skin should be washed vigorously, including hair and nails; soap washings should be repeated.

Environmental Fate

Released 2,4,5-T exhibits mobility that ranges from high in sandy soils to slight in organic-rich soils and generally will not persist beyond one growing season. With a pK_a of 2.88, 2,4,5-T will be found in the dissociated form in most compartments of the environment and will be bound to humic acids, to sediment, or to fine droplets in air. Volatilization and bioaccumulation are not expected to be significant.

Ecotoxicology

Exposure to 2,4,5-T reduces fecundity and impairs larval development in honeybees (100–1000 ppm). It has also been reported to reduce arthropod counts in sprayed forested areas by up to 50%. Gill Ca-ATPase activity was reduced by 2,4,5-T exposure in rainbow trout (*Oncorhynchus mykiss*) and juveniles were more susceptible than adults. Studies conducted in birds (Japanese quail and mallard ducks) report liver abnormalities, proliferation of bile canaliculi, anorexia, wasting, and decreased fecundity. Many of these effects are likely attributable to dioxins that may also be present in 2,4,5-T preparations.

Exposure Standards and Guidelines

The chronic reference dose for 2,4,5-T is $0.01\,mg\,kg^{-1}\,day^{-1}$ whereas the acceptable daily intake is $0.03\,mg\,kg^{-1}\,day^{-1}$.

See also: Chlorophenoxy Herbicides; 2,4-D (2,4-Dichlorophenoxy Acetic Acid); Pesticides; Pollution, Water.

Relevant Website

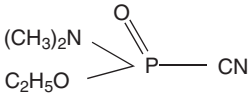
http://extoxnet.orst.edu – Extension Toxicology Network, Oregon State University.

Tabun

Harry Salem and Frederick R Sidell*

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 77-81-6
- Synonyms: GA; Ethyl *N*,*N*-dimethylphosphoramidocyanidate ethyl dimethylphosphoramidocyanidate; Dimethylaminoethoxy-cyanophosphine oxide; Dimethylamidoethoxyphosphoryl cyanide; Ethyldimethylaminocyanophosphonate; Ethyl ester of dimethylphosphoramidocyanidic acid; Ethyl phosphorodimenthylamidocyanidate; G agent; Nerve gas; Nerve agent
- Chemical/Pharmaceutical/Other Class: Tabun is a non-persistent anticholinesterase liquid, or organophosphate (OP) nerve agent, colorless to brown, with a faint almond odor
- Chemical Formula: $C_5H_{11}N_2O_2P$
- Chemical Structure:



Uses

Tabun is a nerve agent used in chemical warfare.

*The views of the authors do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

Exposure Routes and Pathways

Casualties are caused primarily by inhalation but can occur following percutaneous and ocular exposure as well as by ingestion and injection.

Toxicokinetics

Tabun is absorbed both through the skin and via respiration. Nerve agents inhaled as vapors or aerosols enter the systemic circulation resulting in toxic manifestations from seconds to 5 min following inhalation.

The enzyme organophosphate (OP) hydrolase hydrolyzes tabun, sarin, soman, and diisopropyl fluorophosphates at approximately the same rate.

Mechanism of Toxicity

Tabun and other nerve agents are organophosphorus cholinesterase inhibitors. They inhibit the enzymes butyrylcholinesterase in the plasma, acetylcholinesterase on the red blood cell, and acetylcholinesterase at cholinergic receptor sites in tissues. These three enzymes are not identical. Even the two acetylcholinesterases have slightly different properties, although they have a high affinity for acetylcholine. The blood enzymes reflect tissue enzyme activity. Following acute nerve agent exposure, the red blood cell enzyme activity most closely reflects tissue enzyme activity. However, during recovery, the plasma enzyme activity more closely parallels tissue enzyme activity.

Following nerve agent exposure, inhibition of the tissue enzyme blocks its ability to hydrolyze the neurotransmitter acetylcholine at the cholinergic receptor sites. Thus, acetylcholine accumulates and continues to stimulate the affected organ. The clinical effects of nerve agent exposure are caused by excess acetylcholine.

The binding of nerve agent to the enzymes is considered irreversible unless removed by therapy. The accumulation of acetylcholine in the peripheral and central nervous systems leads to depression of the respiratory center in the brain, followed by peripheral neuromuscular blockade causing respiratory depression and death.

The pharmacologic and toxicologic effects of the nerve agents are dependent on their stability, rates of absorption by the various routes of exposure, distribution, ability to cross the blood–brain barrier, rate of reaction and selectivity with the enzyme at specific foci, and their behavior at the active site on the enzyme.

Red blood cell enzyme activity returns at the rate of red blood cell turnover, which is $\sim 1\%$ per day. Tissue and plasma activities return with synthesis of new enzymes. The rates of return of these enzymes are not identical. However, the nerve reactivation can be accomplished therapeutically by the use of oximes prior to aging. Aging is the biochemical process by which the agent-enzyme complex becomes refractory to oxime reactivation. The toxicity of nerve agents may include direct action on nicotinic acetylcholine receptors (skeletal muscle and ganglia) as well as on muscarinic acetylcholine receptors and the central nervous system.

Recently, investigations have focused on organophosphate nerve agent poisoning secondary to acetylcholine effects. These include the effects of nerve agents on y-amino butyric acid neurons and cyclic nucleotides. In addition, changes in brain neurotransmitters such as dopamine, serotonin, noradrenaline, acetylcholine following inhibition of brain cholinesterase activity have been reported. These changes may be due in part to a compensatory mechanism in response to overstimulation of the cholinergic system or could result from direct action of nerve agent on the enzymes responsible for noncholinergic neurotransmission.

Human Toxicity

Following inhalation exposure, the median lethal dosage (LCt_{50}) in humans has been estimated to be 135 mg min m^{-3} at a respiratory minute volume (RMV) of 15 l min^{-1} for a duration of 0.5–2 min and 200 mg min m^{-3} at a resting RMV of 10 l min^{-1}. For percutaneous vapor, the LCt_{50} is estimated to be between 20 000 and 40 000 mg min m^{-3}, while for liquid tabun, the percutaneous human LD_{50} is estimated to be l–1.5 g per human. The permissible airborne exposure concentration of tabun for an 8 h workday or a 40 h workweek is an 8 h time-weighted average of 0.0001 mg m^{-3}. The number and severity of signs and symptoms following tabun exposure are dependent on the quantity, rate, and route of entry. Very small doses to the skin may cause local sweating and tremors with few other effects. Individuals intoxicated with tabun display approximately the same sequence of signs and symptoms regardless of the route of exposure. Signs and symptoms following vapor exposure include runny nose, tightness of chest, dimness of vision and miosis (pinpoint pupils), difficulty in breathing (dyspnea), drooling and excessive sweating, nausea, vomiting, cramps, involuntary defecation and urination, twitching, jerking, staggering, headache, confusion, drowsiness, coma, and convulsions. Death follows cessation of respiration. Death following inhalation and liquid in the eye occurs from 1 to 10 min following exposure. If skin absorption is sufficient to be lethal, death may occur within 1 or 2 min or be delayed for 1 or 2 h.

Clinical Management

Management of nerve agent intoxication consists of decontamination, ventilation, administration of antidotes, and supportive therapy.

The three therapeutic drugs for treatment of nerve agent intoxication are atropine, pralidoxime chloride, and diazepam.

Atropine, a cholinergic blocking or anticholinergic drug, is effective in blocking the effects of excess acetylcholine at peripheral muscarinic sites. The usual dose is 2 mg, which may be repeated at 3–5 min intervals. Pralidoxime chloride (protopam chloride; 2-PAM CL) is an oxime used to break the agent-enzyme bond and restore the normal activity of the enzyme. Abnormal activity decreases and normal strength returns to skeletal muscles, but no decrease in secretions is seen following oxime treatment. The usual dose is 1000 mg (i.v. or i.m.), which may be repeated two or three times at hourly intervals, intravenously or intramuscularly. Diazepam, an anticonvulsant drug is used to decrease convulsive activity and reduce brain damage that may occur from prolonged seizure activity. It is suggested that all three of these drugs be administered at the onset of severe effects from nerve agent exposure, whether or not seizures occur. The usual dose of diazepam is 10 mg (i.m.).

Miosis, pain, dim vision, and nausea can be relieved by topical atropine in the eye. Pretreatment with carbamates may protect the cholinesterase enzymes before nerve agent exposure. Pyridostigmine bromide is available as a pretreatment for nerve agent exposure. It is available in 30 mg tablets; tablets should be administered every 8 h. When used prior to exposure, it should be followed by atropine and pralidoxime chloride after exposure.

Supportive therapy may include ventilation via an endotracheal airway if possible and suctioning of excess secretions from the airways.

Table 1 Acute toxicities of tabun in various species by various routes of exposure

Route of exposure/species	*Toxicities*
Inhalation (10 min exposure)	LCt_{50} ($mg\,min\,m^{-3}$)
Guinea pig	3 930
Cat	2 500
Rat	3 040
Rabbit	8 400
Dog	4 000
Monkey	2 500
Mouse	450
Percutaneous	LD_{50} ($mg\,kg^{-1}$)
Rat	18
Rabbit	2.5
Dog	30
Monkey	9.3
Mouse	1.0
Guinea pig	35
Intravenous	LD_{50} ($\mu g\,kg^{-1}$)
Cat	47
Rat	66
Rabbit	63
Dog	85
Mouse	150
Intraperitoneal	LD_{50} ($\mu g\,kg^{-1}$)
Rat	490
Mouse	604
Subcutaneous	LD_{50} ($\mu g\,kg^{-1}$)
Dog	284
Rat	162
Rabbit	375
Mouse	250
Monkey	70
Guinea pig	120
Hamster	245
Intramuscular	LD_{50} ($\mu g\,kg^{-1}$)
Chicken	118
Monkey	34
Mouse	440
Rat	800
Oral	LD_{50} ($\mu g\,kg^{-1}$)
Rat	3 700
Dog	200
Rabbit	16 300

Animal Toxicity

Tabun is similar in action to sarin (GB); however, it is about have as toxic as sarin by inhalation and is more irritating to the eyes at low concentrations.

Small doses of nerve agents in animals can produce tolerance. They have also been demonstrated to produce neuropathies, myopathies, and delayed neurotoxicity in addition to their classical cholinergic effects. In rats, acute administration of nerve agents in subconvulsive doses produced tumors and hindlimb adduction. In animals, nerve agents can also cause behavioral as well as cardiac effects.

The cause of death is attributed to anoxia resulting from a combination of central respiratory paralysis, severe bronchoconstriction, and weakness or paralysis of the accessory muscles for respiration.

Signs of nerve agent toxicity vary in rapidity of onset, severity, and duration of exposure. These are dependent on the specific agent, route of exposure, and dose. At the higher doses, convulsions and seizures indicate central nervous system toxicity. Following nerve agent exposure, animals exhibit hypothermia resulting from the cholinergic activation of the hypothalamic thermoregulatory center. In addition, plasma concentrations of pituitary, gonadal, thyroid, and adrenal hormones are increased during organophosphate intoxication.

Table 1 lists the LCt_{50} ($mg\,min\,m^{-3}$) values reported following the inhalation of tabun as well as acute toxicities by other routes of exposure in various animal species.

See also: G-Series Nerve Agents; Nerve Agents; Sarin; Soman; V-Series Nerve Agents: Other than VX; VX.

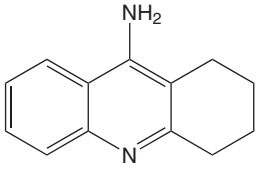
Relevant Websites

http://sis.nlm.nih.gov – Specialized Information Services, Division of the National Library of Medicine.
http://www.bt.cdc.gov – Centers for Disease Control and Prevention. Department of Health and Human Services, USA.

Tacrine

Ramesh C Gupta

- Chemical Name: 1,2,3,4-Tetrahydro-9-aminoacridine
- Chemical Abstracts Service Registry Number: CAS 321-64-2
- Synonym: Cognex
- Chemical and Physical Properties: Tacrine has an empirical formula of $C_{13}H_{14}N_2 \cdot HCl$ and a molecular weight of 234.73. Tacrine hydrochloride represents a white solid that is readily water soluble and soluble in organic solvents. This formulation has a melting point of 283–284°C and a bitter taste.
- Chemical Structure:



Uses

In the 1950s, tacrine was used experimentally to reverse cholinergic coma in animals. In the 1960s, tacrine was used to reverse the effects of phencyclidine-like drugs. It was also marketed for many years as a respiratory stimulant. In 1993, the Food and Drug Administration approved tacrine for the treatment of symptoms of mild to moderate Alzheimer's disease.

Exposure Routes and Pathways

Tacrine is therapeutically indicated by the oral route.

Pharmacokinetics

Tacrine is rapidly absorbed with a bioavailability of between 10% and 30%. Tacrine is about 55% bound to plasma proteins and has a clinical half-life of about 3–6 h following a single oral dose. In the body, tacrine can be metabolized to up to seven different products.

Mechanism of Toxicity

Tacrine has numerous mechanisms of action. The putative principle mechanism of action of tacrine for Alzheimer's disease is reversible inhibition of acetylcholinesterase (AChE), which thereby slows the breakdown of the chemical messenger acetylcholine (ACh) in the brain. In addition, tacrine blocks the sodium and potassium channels.

Acute and Short-Term Toxicity (or Exposure)

Animal

Tacrine causes elevation of serum enzymes indicative of liver cell damage with acute exposures. Studies also suggest that the neurotoxic actions of tacrine in the brain occur due to an increase in ACh levels, resulting in the overstimulation of muscarinic receptors.

Human

Tacrine can cause mild hepatotoxicity, which is self-resolving on discontinuation. Other side effects, including those related to cholinergic effects, are nausea, emesis, diarrhea, abdominal pain, dyspepsia, rhinitis, myalgia, tremors, and excessive urination. Overdose symptoms include seizures, muscle weakness, low blood pressure, severe nausea, vomiting, fast and weak pulse, irregular breathing, and slow heartbeat.

Chronic Toxicity (or Exposure)

Animal

Rats receiving tacrine at the doses of $10\,mg\,kg^{-1}$, i.p., twice daily for 4 days show signs of excess cholinergic stimulation. Tacrine at the doses of 7.5 or $10\,mg\,kg^{-1}$, i.p., two or three times daily for 4 days also induces myopathy in the diaphragm and leg muscles (soleus, gastrocnemius, and plantaris) of rats. As has been shown for other AChE inhibitors, tacrine-induced myopathy appears to result from increased ACh levels at the neuromuscular junction resulting in

the excessive stimulation of nicotinic ACh receptors on muscle cells. In addition, tacrine causes excessive production of free radicals in muscle cells, which can be attenuated by nitric oxide synthase inhibitors.

Human

Hepatotoxicity is the limiting side effect in tacrine therapy. About 50% of those patients given tacrine show elevated serum alanine aminotransferase (ALT) levels indicating some degree of hepatotoxicity. In almost all cases, these changes are noted within the first 12 weeks of treatment. Jaundice is a rare finding.

Clinical Management

In patients experiencing mild liver toxicity, it is often possible to continue at a lower dose or stop and then resume therapy at a lower dose. The addition of lecithin appears to reduce the severity of benign hepatic reaction. Other side effects are generally treated symptomatically.

See also: Anticholinergics; Cholinesterase Inhibition; Liver.

Further Reading

Jeyarassasingam G, Yeluashvili M, and Quilk M (2000) Tacrine, a reversible acetylcholinesterase inhibitor, induced myopathy. *Neuroreport* 11: 1173–1176.

Monteith DK, Theiss JC, Haskins JR, and de la Iglesia FA (1998) Functional and subcellular organelle changes in isolated rat and human hepatocytes induced by tetrahydroaminoacridine. *Archives of Toxicology* 72: 147–156.

Talc

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 14807-96-6
- Synonyms: Nytal 200; Nytal 400, TY 80, Mussolinite; Magnesium silicate hydroxide; Talcum; French chalk
- Chemical/Pharmaceutical/Other Class: Silicate
- Chemical Formula: $Mg_3Si_4O_{10}(OH)_2$

Uses

Talc is a primary ingredient in talcum powder, and is used in some antiperspirants and deodorants, and in cosmetics. It also can be used as a pigment in paints, primers, and enamels as well as a filler for paper, rubber, soap, and in household putty.

Exposure Routes and Pathways

Inhalation, dermal, perhaps some oral, and publications of uptake application to the genital area (uptake via the vagina). Potential occupational exposures include cosmetic workers, paint makers, paper makers, pottery makers, rubber cable coaters, rubber tire makers, talc millers, talc miners, and talc powder makers. Consumer exposures involve various talc-containing products.

Toxicokinetics

The toxic effects of talc are dependent on the route, dose, and properties of the talc involved. Talc commonly contains other minerals, including in some instances several forms of asbestos and silica. The lung deposition and the effects of subchronic exposure to talc were studied in rats and mice. Mathematical models simulating chronic talc exposure utilizing the data were used to predict long-term accumulations of talc in rodent and human lungs. Lungs from other animals were removed and examined for histopathological changes. No clinical signs of toxicity were seen. Talc accumulated in the lungs in a dose-dependent manner, and no exposure related lung lesions other than slight diffuse increases in the number of free macrophages containing talc particles within the alveolar spaces of rats and mice exposed to the highest doses were seen. Talc lung burdens of 2–3 $mg\,g^{-1}$ were predicted to occur in rats and mice exposed to 17 $mg\,m^{-3}$ talc for 2 years. In human lungs, equilibrium talc lung burdens of $\sim$2 $mg\,g^{-1}$ were predicted to occur after 4 years of exposure to 2 mg/m^{-3} talc, the occupational exposure threshold limit value (TLV). It was concluded that the predicted long-term talc lung burdens are significantly lower than those obtained experimentally, that this could reflect impaired pulmonary clearance, and that caution should be exercised when predicting lung burdens from short-term exposures to talc concentrations of 2 $mg\,m^{-3}$

or greater. Other studies have suggested that inert particles of talc can travel from the perineum (i.e., the general region between the anus and the genital organs) to the ovaries.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute inhalation exposure can cause coughing, dyspnea, sneezing, vomiting, and cyanosis. Talc, which is water insoluble, dries up the mucous membranes of the tracheobronchial trees. This results in impairment of ciliary function. Inhaling large quantities of talc can result in obstruction of the small airways in addition to drying the mucous membranes, leading to respiratory distress syndrome, or death. Clinical studies of intravenous (IV) drug abusers have shown that IV injections of pills containing psychoactive agents and talc as a binder can result in microemboli forming in small pulmonary arteries, arterioles, and capillaries. This can result in granuloma formation, impaired pulmonary function, and death. IV injection of talc-containing formulations has been shown to predispose users to infections. Talc can induce severe granulomatous reactions when introduced into wounds or the operative field. See below for genotoxicity information.

Chronic Toxicity (or Exposure)

Animal

Long-term mouse and rat inhalation studies of talc found some evidence of carcinogenic activity of talc in male rats based on an increased incidence of benign or malignant pheochromocytomas of the adrenal gland. There was clear evidence of carcinogenic activity of talc in female rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign or malignant pheochromocytomas of the adrenal gland. There was no evidence of carcinogenic activity of talc in male or female mice exposed to 6 or 18 mg m^{-3}. Hamsters, 4 weeks old, were exposed to an aerosol of talc baby powder for 3, 30, or 150 min day^{-1} for 5 days a week for 30 days. Two further groups of hamsters, 7 weeks old, were exposed to talc aerosol for 30 or 150 min day^{-1} for 300 days or until death. No primary neoplasm was found in the respiratory system of any hamster. The incidence of alveolar cell hyperplasia was 25% in the groups exposed to aerosol for 30 and 150 min day^{-1} for 300 days, compared with 10% in the control group. See below for genotoxicity and for other carcinogenicity information.

Human

Talc produces fibriotic pneumonitis. Four distinct forms of pulmonary disease caused by talc have been defined:

- Talcosilicosis is caused by talc mined with high silica content mineral. Findings in this form are identical with those of silicosis.
- Talcoasbestosis closely resembles asbestosis and is produced by crystalline talc, generally inhaled with asbestos fibers. Pathologic and radiographic abnormalities are virtually identical with those of asbestosis, including calcifications and malignant tumor formation.
- Talcosis, caused by inhalation of pure talc, may include acute or chronic bronchitis as well as interstitial inflammation; radiographically, it appears as interstitial reticulations or small, irregular nodules, typical of small airway obstruction.
- The fourth form, due to IV administration of talc, is usually associated with abuse of oral medications and production of vascular granulomas manifested by consolidations, large nodules, and masses.

Clinical and epidemiologic studies have suggested the existence of an association between ovarian carcinoma and talcum powder and deodorant sprays applied to the genital area. Talc particles have been detected in histologic sections of ovarian carcinomas; however, the results of epidemiologic investigations have varied, finding risks increased twofold to no significant risk detected. One recent review concluded that the concerns that cosmetic talc might be carcinogenic lack persuasive scientific support for the following reasons:

- These concerns are based on some epidemiological studies whose results were barely significant statistically and of questionable biological importance. (Their results lacked dose–response relationships, and were inconsistent and ambiguous. Further whether inanimate talc particles can translocate from the perineum to the ovaries, a precondition if they were to cause ovarian cancer, remains unresolved.)
- The results of the inhalation study in animals, which has raised concerns, "cannot be considered as relevant predictors of human risk" according to a panel of experts and other experts.
- The elevated incidence of lung cancer in pottery workers occurred several decades ago by exposure to air levels that now cannot be allowed to occur, and the exposures were to a multitude of industrial dusts.
- There is a lack of scientific support that pure cosmetic or pharmaceutical-grade talc poses a real risk

under consumer conditions. Talc is not genotoxic, is not carcinogenic when injected into ovaries of rats, does not cause cancer decades after pleurodesis, and induces apoptosis *in vitro* in human mesothelioma cells but not in normal mesothelial cells. There is no credible evidence of a cancer risk from inhalation of cosmetic talc by humans.

In Vitro Toxicity Data

The genotoxicity of talc has been determined using *in vitro* cell systems previously developed for testing asbestos fibers. The talc samples used consisted of particles of respirable size in order to test the effect of particles likely to be deposited in the lung. Genotoxicity was tested in cultures of rat pleural mesothelial cells using genotoxicity assays for unscheduled DNA synthesis and sister chromatid exchanges. The effects were compared with those obtained with negative controls (attapulgite and anatase) and positive controls (chrysotile and crocidolite asbestos). In contrast to asbestos, none of the talc samples, or the negative controls, induced enhancement of unscheduled DNA synthesis and sister chromatid exchanges in treated cultures in comparison with the untreated cultures.

Exposure Standards and Guidelines

Talc is listed as an A4 chemical (not classifiable as a human carcinogen) by the American Conference of Governmental Industrial Hygienists (ACGIH). Further, the ACGIH threshold limit value, 8 h time-weighted average (TWA) is $2\,mg\,m^{-3}$ (for talc containing no asbestos fibers; particulate matter containing no asbestos and $<1\%$ crystalline silica; respirable fraction). The (US) Occupational Safety and Health Administration permissible exposure limit, 8 h TWA is 20 million particles per cubic feet of air (mppcf) (for talc not containing asbestos, and containing less than 1% quartz).

See also: Asbestos; Cosmetics and Personal Care Products.

Further Reading

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Gertig DM (2000) Prospective study of talc use and ovarian cancer. *Journal of the National Cancer Institute* 92: 249–252.

Nasreen N (2000) Talc induces apoptosis in human malignant mesothelioma cells *in vitro*. *American Journal of Respiratory and Critical Care Medicine* 161: 595–600.

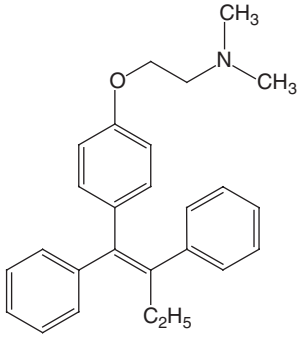
Oberdörster G (1995) The NTP talc inhalation study: A critical appraisal focused on lung particle overload. *Regulatory Toxicology and Pharmacology* 21: 233–241.

Wehner AP (2002) Cosmetic talc should not be listed as a carcinogen: Comments on NTP's deliberations to list talc as a carcinogen. *Regulatory Toxicology and Pharmacology* 36: 40–50.

Tamoxifen

Teresa Dodd-Butera and Molly Broderick

- Chemical Abstracts Service Registry Number: CAS 10540-29-1
- Synonyms: Nolvadex; Tamoxifen citrate
- Chemical/Pharmaceutical/Other Class: Nonsteroidal antiestrogen
- Chemical Structure:



Uses

Tamoxifen is appropriate for use as a palliative treatment in patients with an estrogen receptor-positive tumor. Additionally, it is used to prevent disease recurrence. Tamoxifen is the only agent approved by the United States Food and Drug Administration for breast cancer reduction and is generally administered for 5 years.

Exposure Routes and Pathways

The common mode of exposure to tamoxifen is through ingestion. It is available in oral dosage form (10 and 20 mg tablets) and may also be inhaled. Vapors may produce explosive dust clouds. Hazardous products include carbon monoxide, carbon dioxide, and nitrous oxide.

Toxicokinetics

Tamoxifen is absorbed orally, with peak concentrations in 4–7 h, biphasic decline in plasma concentration, and a terminal half-life of 7 days. The predominant metabolite is *N*-desmethyltamoxifen, which has a half-life of 14 days. However, a minor metabolite, 4-hydroxytamoxifen, is also generated. Both of these are further metabolized to 4-hydroxy-*N*-desmethyltamoxifen. *In vitro* studies showed that erythromycin, cyclosporin, nifedipine, and kiltiazem competitively inhibited formation of the latter metabolite. Steady-state levels are achieved after approximately 4 weeks of treatment. Tamoxifen is enterohepatically recirculated and excreted primarily in the stool.

Mechanism of Toxicity

Tamoxifen competitively blocks estradiol binding to the estrogen receptor.

Acute and Short-Term Toxicity (or Exposure)

Animal

Tamoxifen produced impairment of fertility and conception in female rats. No genotoxic potential was found in conventional *in vivo* and *in vitro* tests.

Human

Adverse reactions include nausea, vomiting, and hot flashes. Vaginal bleeding, menstrual irregularities, and skin rash occur with less frequency. Hypercalcemia, edema, anorexia, depression, and thromboembolic events are uncommon but have been reported.

Chronic Toxicity (or Exposure)

Animal

Studies in rats found a significant increase in hepatocellular cancer at doses higher than that administered to humans.

Human

Tamoxifen increases the risk of two types of cancer that can develop in the uterus: endometrial cancer, which arises in the lining of the uterus; and uterine sarcoma, which arises in the muscular wall of the uterus. Women taking tamoxifen had three times the chance of developing a pulmonary embolism, deep vein thrombosis, and increased chance of stroke. Women taking tamoxifen appear to be at increased risk for developing cataracts. Other eye problems, such as corneal scarring or retinal changes, have been reported. Tamoxifen may cause fetal harm when administered to a pregnant woman. It is unknown whether or not this drug is excreted in human milk when women taking tamoxifen are breastfeeding.

Clinical Management

Acute overdosage in humans has not been reported. If very high doses are administered, which manifest acute neurotoxicity and prolonged QT interval on an electrocardiogram, symptomatic treatment and cessation of the drug is required.

See also: Estrogens I: Estrogens and Their Conjugates.

Further Reading

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Goldfrank L, Flomenbaum N, Lewin N, *et al*. (eds.) (2002) *Goldfrank's Toxicologic Emergencies*, 7th edn. New York: McGraw-Hill Medical Publishing.

Klaassen C (ed.) (2001) *Casarett & Doull's Toxicology: The Basic Science of Poisons*, 6th edn. New York: McGraw-Hill.

White IN (2003) Tamoxifen: Is it safe? Comparison of activation and detoxication mechanisms in rodents and in humans. *Current Drug Metabolism* 4(3): 223–239.

Relevant Website

http://ntp-server.niehs.nih.gov – National Toxicology Program, Department of Health and Human Services, Tenth Report on Carcinogens: Tamoxifen.

Tannic Acid

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 1401-55-4
- Synonyms: Digallic acid; Chinese tannin; Gallotannic acid; Galloylglucose; Glycerite; Digalloyl glucose; Tannin; Tannins
- Chemical/Pharmaceutical/Other Class: Plant polyphenol

Uses

Tannic acid is used as a fixative of dyes and as a chemical intermediate and reagent in the manufacture of inks, rubber, and imitation horns and tortoise shells. Tannic acid is also used to clarify beer and wine; in photography; as a chemical reagent in analytical laboratories; and in pharmaceutical preparations.

Background Information

Tannic acid is a naturally occurring plant polyphenol and can be found in practically all aerial plant tissues.

Exposure Routes and Pathways

The most significant route of exposure for tannic acid is via ingestion. However, inhalation and dermal exposure may also occur in industrial settings.

Toxicokinetics

Tannic acid is not consistently absorbed from intestinal mucosa or from the skin. Enhanced absorption rates can be seen in denuded skin and mucus membranes. Tannic acid can cause hardening of the gastrointestinal mucosa. This hardening can result in reduced gastrointestinal absorption of nutrients as well as of xenobiotics. Tannic acid has been experimentally shown to be able to reduce the carcinogenic potency of some amine derivatives and polycyclic aromatic hydrocarbons in laboratory animals. Tannic acid's anticarcinogenic properties appear to be mediated through the modulation of enzymes involved in xenobiotic metabolism.

Mechanism of Toxicity

Tannic acid causes centralobular liver necrosis following absorption from gastrointestinal tract, mucus membranes, or from denuded skin surfaces. Liver metabolism of tannic acid requires methyl-group donors. Therefore, methyl-group donors can be depleted following excessive tannic acid absorption.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals dosed with tannic acid by oral administration presented hemorrhagic gastritis, colic, jaundice, hemolytic anemia, necrosis of gastric mucosa, nephritis, and liver alterations. The LD_{50} in rats and mice has been reported to be 5 and $6\,g\,kg^{-1}$, respectively.

Tannic acid proved to be lethal to bovines after daily dosage of 50 g for 16 days. However, a daily dose of 25 g for 28 days did not produce observable adverse effects.

Human

Tannic acid is moderately toxic by the inhalation and ingestion exposure pathways. Acute, high-dose ingestion and absorption may cause nausea, vomiting, constipation, abdominal pain, and liver damage. Severe intoxications may result in centralobular liver necrosis.

Chronic Toxicity (or Exposure)

Animal

Foraging animals consuming oak tree leaves may consume potentially toxic doses of tannic acid. Excessive chronic consumption has been shown to decrease iron and thiamin absorption as well as decreased growth rate in juvenile animals.

In a chronic toxicity study, male and female rats were injected subcutaneously with an aqueous solution of tannic acid every fifth day for 290 days. Some dosed animals presented hepatomas and/or cholangiomas at the end of the study (after 388 days). Although tumor incidence in the control group was rare, no clear dose–response was evident in the tannic acid treated animals. In another study, no liver damage was observed in seven male rats fed tannic acid at a dose of $60\,mg\,kg^{-1}$ body weight per day during 152 days.

Human

An unusually high incidence of esophageal cancer has been noted in areas of South Africa where a sorghum rich in tannins is consumed. A positive relation has been observed between the tannin content of the sorghum and the incidence of esophageal cancer.

Clinical Management

Basic life-support measures should be implemented. Further absorption can be prevented by removing contaminated clothing and washing the affected area. If ingested, activated charcoal may be given to reduce absorption. A careful examination should be performed and gastric lavage instituted only if esophagus is not damaged and it is believed that lavage may be effective at removing the ingested material. If inhaled, respiratory distress should be monitored and oxygen administered or assisted ventilation given as needed.

There is no specific treatment for tannic acid toxicity. Supportive and symptomatic treatment is recommended. Liver function should be monitored in patients with gastrointestinal symptoms.

Environmental Fate

Tannins and tannic acid occur naturally in plants. Essentially all wood and plant tissue contain tannins. Therefore, biodegradation is expected to be the major environmental fate process for tannic acid.

Ecotoxicology

Tannic acid given in the diet of chicks at a concentration of 0.5% caused growth rate reductions. Tannic acid doses as high as 5.0% resulted in 70% mortality in dosed chicks.

Tannic acid given orally to rabbits produced hemorrhagic gastritis. Horses given doses ranging from 50 to 300 g by stomach tube presented colic and jaundice with hemolytic anemia. Upon autopsy, some horses presented necrosis of gastric mucosa, degeneration of heart muscle, nephritis, and liver changes.

See also: Gastrointestinal System; Plants, Poisonous.

Further Reading

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Krajka-Kuzniak V and Baer-Dubowska W (2003) The effects of tannic acid on cytochrome P450 and phase II enzymes in mouse liver and kidney. *Toxicology Letters* 143(2): 209–216.

Rossoff IS (2002) *Encyclopedia of Clinical Toxicology.* Boca Raton, FL: The Parthenon Publishing Group.

Relevant Website

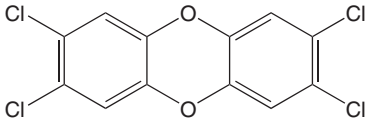
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Tannic Acid.

Taste *See* Sensory Organs.

TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin)

Robert Kapp

- Chemical Abstracts Service Registry Number: CAS 1746-01-6
- EINECS No: 217-122-7
- Synonyms: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; 2,3,7,8-TCDD; 2,3,7,8-Tetra polychlorinated dibenzo-*p*-dioxin; 2,3,7,8-Tetrachlorodibenzo(*b*,*e*)(1,4)dioxin; 2,3,7,8-Tetrachlorodibenzo-1,4-dioxin; 2,3,7,8-Tetrachlorodibenzodioxin; Dibenzo(*b*,*e*)(1,4)dioxin, 2,3,7,8-tetrachloro-; Dibenzo-*p*-dioxin, 2,3,7,8-tetrachloro-; Dioxin; Dioxin (herbicide contaminant); Dioxine; TCDBD; Tetrachlorodibenzo-*p*-dioxin; Tetrachlorodibenzodioxin; Tetradioxin
- Related Compounds: Dioxins is a general term that is used to describe a group of hundreds of chemicals that are found in the environment and are derived from polychlorinated dibenzodioxins. All dioxins contain two benzene rings joined by two oxygen atoms. The polychlorinated dibenzofurans are a closely related family of compounds. There are ~75 known polychlorinated dibenzo-*p*-dioxins and ~135 dibenzofurans. Since the environment has many of the different dioxins at extremely low levels, chemical analysis is difficult.
- Chemical/Pharmaceutical/Other Class: Chlorinated dioxins
- Chemical Structure:



Uses

TCDD has no commercial uses but is found as a contaminant. TCDD is present in certain herbicide and fungicide formulations such as 1,4,5-T and in pentachlorophenols. As noted above, it is an unwanted contaminant created by incineration and was a contaminant in Agent Orange.

Background Information

TCDD is a colorless to white crystalline solid. The molecular formula is $C_{12}H_4Cl_4O_2$. The molecular weight is 322. Dioxin began accumulating in the environment about 1900 when Dow Chemical discovered a way to split NaCl into sodium and

chlorine atoms. Subsequently, Dow and other chemical manufacturers began attaching chlorine atoms to various petroleum hydrocarbons, which has produced a vast array of pesticides, solvents, and plastics. When these materials are manufactured or are burned in an incinerator, they release dioxin into the environment. Other sources of dioxins include sawmills, wire and scrap metal reclamation incinerators, cement kilns, cofiring wastes, transformer fires, wood stoves, fireplaces, and agricultural burning. When released into the air, many dioxins may be transported long distances. When dioxins are released into wastewater, some are broken down; however, most attach to soil and settle to the bottom sediment in a relatively stable form. During the Vietnam War, a broadleaf defoliant called Agent Orange was used by the US Troops to destroy enemy food crops and areas of cover in the jungle. Agent Orange was a 50–50 mix of two chemicals, known conventionally as 2,4,D ((2,4-dichlorophenoxy)acetic acid) and 2,4,5,T ((2,4,5-trichlorophenoxy)acetic acid). The combined product was mixed with kerosene or diesel fuel and dispersed by aircraft, vehicle, and hand spraying. An estimated 19 million gallons of Agent Orange were used in South Vietnam during the war. Agent Orange used in Vietnam was later found to be contaminated with TCDD. Hence, the personnel who were dispersing Agent Orange were possibly exposed to high levels of TCDD. While many believe that man is the sole source of dioxins, dioxins have been found worldwide in very remote areas leading others to believe that some of the primary sources are not yet known.

TCDD has an important history in the field of toxicology. In the 1970s, dioxin-contaminated oil was spread along roadways in the community of Times Beach, MO, in the United States to control dust. In December of 1982, the Meremac River flooded Times Beach and contaminated the entire town with dioxin. The US government bought the entire area and initiated cleanup as a Superfund site. A total of 265 000 tons of contaminated dirt was incinerated. Today, Times Beach, MO, no longer exists. Other incidents including potential health effects on veterans exposed to dioxin in Agent Orange have highlighted the history of this most toxic synthetic chemical known to man. An extensive number of medical and scientific studies on Agent Orange and dioxin have been conducted. The consensus of those studies suggests that even in military personnel exposed to the pesticide, there are no defined, consistent adverse health effects, even though many Vietnam veterans are at risk for a variety of health problems due to their military experience in Vietnam in general.

Exposure Routes and Pathways

The primary pathways for TCDD exposure appear to be inhalation and ingestion. Eating meat, fish, and dairy products makes up more than 90% of the intake of dioxins. Close proximity to an uncontrolled hazardous waste site or working in industries involved in producing pesticides containing dioxins can be sources of inhalation exposure for the general public and workers alike. Skin exposure can occur through contact with contaminated soils.

Toxicokinetics

Dioxins are absorbed by inhalation, oral, and dermal routes of exposure. Absorption is less efficient by the dermal than by the inhalation and oral routes. Absorption is vehicle-dependent and congener-specific. Hepta- and octachlorinated congeners exhibit decreased absorption. Dioxins can be carried in the blood by serum lipids and lipoproteins. Liver and fat are the major storage sites for dioxins. Tissue deposition is congener-specific, dose-dependent, and influenced by factors including route of exposure and age. Dioxins are slowly metabolized by microsomal enzymes to polar metabolites that undergo conjugation with glucuronic acid and glutathione. The major route of excretion is feces with smaller amounts being excreted in the urine. In mammals, lactation is an effective route of elimination. Dioxins can induce xenobiotic metabolizing enzymes. The induction of these enzymes (such as the CYP1A1 in the mouse) increases the metabolic processing of lipophilic chemicals to water-soluble derivatives, facilitating their elimination in the urine. TCDD is a poor substrate for detoxification enzymes and it tends to persist in the body for long periods of time.

Mechanism of Toxicity

Much of the activity initiated by the presence of small amounts of dioxin occurs at the Ah receptor, which is important to the body's ability to detoxify foreign substances. The dioxin molecule binds to the Ah receptor forming the 'receptor–dioxin complex'. TCDD's toxic actions depend on the formation of this complex. Once the complex is formed it moves to the cellular DNA (with the aid of a translocating protein, ARNT) where it activates genes to a number of biotransformation-related enzymes or other genes involved in growth and division of cells. Examples of enzymes induced by activation of the Ah receptor include CYP1A1, CYP1A2, glutathione transferase, NADPH quinine oxidoreductase, UDP-glucuronosyltransferase abd aldehyde dehydrogenase. If the

dioxin remains bound to the receptor, the receptor remains on the DNA so that enzymes are produced continuously. Once dioxin enters the body, a small amount is metabolized and usually eliminated. The majority of the dioxin, however, bioaccumulates in the body fat. As the fat is metabolized, stored dioxin is slowly released and excreted primarily in feces. The approximate half-life of dioxin in humans is estimated to range from 6 to 10 years.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals given a single oral dose of 0.1–25 $\mu g\,kg^{-1}$ have shown hyperkeratosis, facial alopecia, inflammation of the eyelids, increased liver weight, lipid accumulation, thymic atrophy, and histopathologic changes in the liver and thymus. There is a wide species difference in acute toxicity of TCDD, with oral LD_{50} ranging from 0.6 to 5000 μg TCDD kg^{-1}. Additional acute effects include the stimulation of aminolevulinic acid synthetase, which is a rate-limiting enzyme in porphyrin and heme synthesis.

Human

Acute exposure of humans to excessive amounts of dioxins has caused chloracne, liver toxicity, skin rashes, nausea, vomiting, and muscular pains.

Chronic Toxicity (or Exposure)

Animal

Animals exposed to chronic doses of dioxin have shown altered immune systems, thymic atrophy, with changes also noted in the spleen, lymph nodes, and bone marrow. Rodent reproductive studies have noted signs of developmental toxicity, however, including cleft palates and kidney abnormalities at 0.125–3.0 μg TCDD g^{-1} body weight. Impairment of reproduction including decreased fertility, litter size, number of pups alive at birth, postnatal survival, and postnatal body weight of pups was noted at doses of 0.01 μg TCDD $kg^{-1}\,day^{-1}$. TCDD also caused immunological deficits expressed by decreased thymus to body weight ratios in nursing newborn rats exposed through mother's milk. Male rats fed dosages of 0.001 μg TCDD per kilogram of body weight per week for 78 weeks showed ear duct carcinoma, lymphocytic leukemia, kidney adenocarcinoma, malignant peritoneal histiocytoma, skin angiosarcoma, and carcinomas of the tongue and nasal turbinates. Female rats fed dosages of 0.1 μg TCDD $kg^{-1}\,day^{-1}$ developed carcinomas of the liver as well as squamous cell carcinomas of the lung, tongue, and nasal turbinates. Male and female rats fed dosages of 0.5 μg TCDD $kg^{-1}\,week^{-1}$ for 2 years developed neoplastic nodules in the liver and thyroid adenomas.

Human

Chronic exposure to dioxins has resulted in splenic and testicular atrophy, elevated gamma-glutamyl transpeptidase levels, elevated cholesterol levels, and abnormal neurologic findings. Additional effects include enzyme induction, diabetes, and endocrine changes. The chronic, noncancer reference exposure level of $3.5 \times 10^{-6}\,\mu g\,m^{-3}$ is listed for TCDD or 2,3,7,8-equivalents by the California Air Pollution Control Officers Association Air Toxics 'Hot Spots' Program.

The human reproductive data available on TCDD are inconclusive. A study based on the accidental exposure of the population of Seveso, Italy, found no mutagenic, teratogenic, or fetotoxic effects in 30 elective abortions. Carcinogenicity studies examining humans exposed to TCDD have been inconclusive because of the small sample sizes and the concomitant exposures to other substances. Notwithstanding, TCDD is listed with the International Agency for Research on Cancer as Group 1 – carcinogen to humans. It is also listed by the Environmental Protective Agency (evidence that dioxin may have the potential to cause cancer from a lifetime exposure at levels above the maximum contaminant level); the National Toxicology Program (K – known to be a human carcinogen); the National Institute for Occupational Safety and Health (Ca – potential carcinogen with no further categorization); and the German MAK Commission (4 – substances with carcinogenic potential for which genotoxicity plays no role).

Clinical Management

Upon ocular exposure to TCDD, the eye should be immediately washed well with plenty of tap water. Skin exposed to TCDD should be immediately washed with soap and water. Get immediate medical attention in the event of ingestion or inhalation of TCDD.

Environmental Fate

Dioxins are ubiquitous environmental contaminants in air, water, and soil. Lower levels are found in less industrial regions compared to areas with heavy industry. Heptachloro- and octachloro-isomers are most common. Environmental fate of the dioxins involves volatilization, atmospheric distribution, wet

and dry deposition, photolysis, bioaccumulation, and biodegradation. Dioxins strongly adsorb to soils and sediments and are generally immobile. Photolysis of many dioxins is relatively robust. The half-life of TCDD was estimated to be 9–15 years on the surface, but 25–100 years below the surface. Dioxins can bioaccumulate in aquatic and terrestrial biota. Dioxin concentrations in urban air are around $2\,\mathrm{pg\,m^{-3}}$, with octachloro- and heptachlorodibenzodioxins being predominant. Urban-air concentration of 2,3,7,8-TCDD in the United States was estimated at <0.04–$0.18\,\mathrm{pg\,m^{-3}}$ but it is typically not detectable in air samples from rural communities. TCDD concentration can be much higher around contaminated sites. Because of tight adsorption to sediment, conventional water treatment appears to be effective in removing dioxins. TCDD has not been detected in drinking water. Concentrations of 2,3,7,8-TCDD in most soils are <12 ppt but considerably higher levels can be found in contaminated soils. 2,3,7,8-TCDD and other dioxins have also been detected in sediments of industrialized water bodies throughout the United States. The most frequently detected dioxin in fish tissues is 1,2,3,4,6,7,8,-heptachlorodibenzodioxin, which was found in fish tissues at 89% of the sites. Fish collected near pulp and paper mill operations using chlorine had the highest levels of 2,3,7,8-TCDD.

Ecotoxicology

Relatively little is known about the effects of dioxins in invertebrates. Controlled laboratory studies with dioxin-contaminated sediments reported no effects on amphipod mortality. Some studies have shown reduced reproductive success in worms and snails. Some invertebrates have been shown to express Ah receptors, but these receptors do not appear to bind dioxins, thus invertebrates are less sensitive to dioxin toxicity.

Fish exposed to dioxins exhibit alterations in development, reduced feeding, lethargy, and 'head-up' swimming. In general, dioxins are more toxic in the early-life stages. Toxicity in fish is higher for congeners containing 4–6 chlorines. Concentration of dioxins in fish eggs has been demonstrated. Deformities in chicks of cormorants, terns, and other fish-eating species from the Great Lakes area in the United States were correlated with dioxin contamination, a correlation between TCDD-toxic equivalents and reduced egg hatching, embryotoxicity, structural deformities, and altered parental behavior. These effects in birds may, however, have been due to polychlorinated biphenyls (PCBs) and not dioxins.

Controlled studies have shown some birds to be susceptible to dioxins, showing reduced egg production, embryotoxicity, and cardiovascular and brain malformations. Mink eating contaminated fish show listlessness, anorexia, reduced red blood cell counts, and enlarged spleens, livers, and lungs. Again, the contamination by PCBs often makes the discrimination between dioxins and PCBs difficult.

Exposure Standards and Guidelines

There is no reference dose for dioxins. The acceptable daily intake is 1–$10\,\mathrm{pg\,kg^{-1}\,day^{-1}}$. A threshold limit value for TCDD has not been established.

See also: Dioxins; Pesticides; Polychlorinated Biphenyls (PCBs).

Further Reading

Cole P, Trichopoulos D, Pastides H, Starr T, and Mandel JS (2003) Dioxin and cancer: A critical review. *Regulatory Toxicology and Pharmacology: RTP* 38: 378–388.

Young AL (2004) TCDD biomonitoring and exposure to Agent Orange: Still the gold standard. *Environmental Science and Pollution Research International* 11: 143–146.

Relevant Websites

http://europa.eu.int – The European Union. Many documents available on Dioxin Exposure and Health.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for TCDD.

Teflon *See* Perfluorooctanoic Acid (PFOA).

Tellurium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 13494-80-9
- Synonyms: Aurum paradoxium; Mettalum problematum
- Chemical/Pharmaceutical/Other Class: A rare metal with many properties similar to selenium and sulfur
- Chemical Formulas: Te^{4+}; Te^{6+}; Te^{2-}

Uses

The largest use for tellurium is as an additive to free-machining steel. Tellurium is used as a catalyst, in semiconductors and in 'daylight' vapor lamps. It is also used in the manufacturing of rubber and certain metal alloys and as a coloring agent in chinaware, porcelains, enamels, glass, and for producing black finish on silverware.

Background Information

Tellurium is one of the rarest elements on earth, and was discovered in 1782.

Exposure Routes and Pathways

Tellurium is ingested with foods such as nuts, fish, and certain dairy products. Many fatty foods contain tellurium, and some plants, like garlic, accumulate tellurium from the soil. Neither drinking water nor ambient air contains significant amounts of tellurium. Skin contact is not a significant exposure pathway.

In industrial settings, inhalation may be a significant exposure pathway. Airborne concentrations of tellurium are higher than the vicinity of metallurgical industries. Like selenium, tellurium is obtained as a by-product of copper, lead, and zinc refining. It is produced mainly from the tailings of bismuth.

Toxicokinetics

Tellurium is poorly absorbed from the gastrointestinal tract. Tellurous acid (soluble tellurium) can be absorbed through the skin, although ingestion or inhalation of fumes presents the greatest industrial hazard. A metallic taste in the mouth may result from excessive absorption. Tellurium concentrates in a variety of organs, primarily in the bones and kidneys, followed by the liver and the adipose tissue. Tellurium is metabolized in the body by the reduction to tellurides, and can then be biotransformed to dimethyltelluride (a reaction similar to the biotransformation of selenium), which is volatile and can be exhaled. Most tellurium is excreted in urine and bile. The characteristic sign of absorption is the garlic-like odor in the breath and sweat from dimethyltelluride.

Mechanism of Toxicity

Tellurium has a low toxicity in its elemental form, but dimethyltelluride is formed in the body. Tellurium caused highly synchronous primarily demyelination of peripheral nerves, related to the inhibition of squalene epoxidase, which blocks cholesterol synthesis. The sequence of metabolic events in sciatic nerve following tellurium treatment initially involves inhibition of the conversion of squalene to 2,3-epoxysqualene, and that this block in the cholesterol biosynthesis pathway results, either directly or indirectly, in the inhibition of the synthesis of myelin components and breakdown of myelin. The efficacy of garlic as a lipid-lowering agent has been recognized, but the biochemical mechanisms underlying this action are currently unknown. It is possible that organic tellurium compounds, which are found in high concentration in fresh garlic buds, may contribute to this action by inhibiting squalene epoxidase, the penultimate enzyme in the synthetic pathway of cholesterol. Weanling rats fed a diet rich in tellurium develop a demyelinating polyneuropathy due to inhibition of this enzyme in peripheral nerves. Chronic exposure to small amounts of tellurium found in garlic might reduce endogenous cholesterol production through inhibition of hepatic squalene epoxidase and so reduce cholesterol levels. Tellurium may also contribute to the characteristic odor of garlic.

Acute and Short-Term Toxicity (or Exposure)

Animal

The mouse, guinea pig, rabbit, and rat oral LD_{50} values ranged from 20 to 83 mg kg^{-1}. The temporal relationships of blood nerve barrier breakdown, to metabolic and morphological changes in tellurium neuropathy were investigated in rats fed diets containing 0% or 1.3% tellurium. Animals were observed for clinical signs of toxicity and were killed 12,

24, 48, 72, or 96 h after starting the diet. Tellurium-treated rats developed a garlic odor within 48 h and usually developed hind-limb paresis within 72 h. Progressive increases in blood–nerve barrier permeability occurred between 24 and 72 h in rats given tellurium; however, the blood–brain barrier was not affected by tellurium. Tellurium induced increased numbers of intracytoplasmic lipid droplets, intracytoplasmic membrane delimited clear vacuoles, and cytoplasmic excrescences within myelinating Schwann cells after 24 h, axon demyelination after 48 h, and endoneurial edema after 72 h. Cholesterol synthesis was sharply inhibited after 12 h, and squalene began accumulating in sciatic nerve segments at that time. It was concluded that the initial Schwann cell injury seen in tellurium neuropathy may be due to factors other than blood–nerve barrier breakdown and vasogenic endoneurial edema. Breakdown of the blood–nerve barrier could have a synergistic effect on tellurium induced Schwann cell injury.

Human

The toxicity of tellurium is dependent on the oxidation state. The tellurites $(TeO_3)^{2-}$, are the most toxic compared to tellurates $(TeO_4)^{2-}$, or elemental tellurium. Only a few cases of nonoccupational poisoning to tellurium have been reported so far, and toxic effects are rare. Severe poisoning results in respiratory depression and circulatory collapse. After occupational exposure, the main symptoms and signs include loss of appetite, dryness of the mouth, suppression of sweating, a metallic taste in the mouth, and the garlic odor of the breath, sweat, and urine. Acute toxicity from inhalation results in the relatively nonspecific symptoms of nausea, sweating, and loss of sleep in some and drowsiness in others. Kidney damage and fatty degeneration of the liver have been noted in severe cases. In two fatal cases, cyanosis and garlic breath were prominent before coma and death, and fatty degeneration and edema were noted in both cases.

Chronic Toxicity (or Exposure)

Animal

Acute oral parenteral tellurium intoxication in animals results in restlessness, tremor, diminished reflexes, paralysis, convulsions, somnolence, coma, and death. Hematuria was prompt and occurred in all animals. Exposure of weanling rats to a diet containing elemental tellurium results in a peripheral neuropathy characterized by segmental demyelination and minimal axonal degeneration. It is noteworthy that functional recovery occurred despite continual administration of tellurium. One of the earliest ultrastructural abnormalities in tellurium neuropathy is an increased number of cytoplasmic lipid droplets in myelinating Schwann cells. The earliest biochemical abnormality observed in tellurium neuropathy is an inhibition of cholesterol synthesis at the squalene epoxidase step. This leads to an accumulation of squalene within the nerve.

After exposure to 3300 ppm tellurium in the diet for 5 months, rats were markedly impaired in their ability to learn a sequence of behavioral tasks. The administration of 500–3000 ppm tellurium through the diet to pregnant rats resulted in a high incidence of hydrocephalic offspring. Neonatal rats exposed to tellurium via the mother's milk from the day of birth until killing at 7, 14, 21, or 28 days of age developed Schwann cell and myelin degeneration in the sciatic nerves at each age studied. In the central nervous system (CNS), hypomyelination of the optic nerves was demonstrated at 14, 21, and 28 days of age.

The tissue response to tellurium and tellurium dioxide particulates retained in rat lungs following endotracheal introduction was studied. Rats were administered single endotracheal injections of tellurium, tellurium dioxide, or sodium chloride, and were sacrificed 180 days later. Micropathological investigations revealed no difference in the lung tissue of the control and treated rats, except for those resulting from expected defensive mechanisms against any foreign material in the lungs. Black deposits were observed in the lung tissue of treated rats, indicating an inability of the clearance mechanisms of the lungs to remove all of the injected particulates within the 180 day study period. No evidence of a fibrotic tissue response was observed. Many internal organs of the rats treated with tellurium and tellurium dioxide had a bluish tint, however, this discoloration was not accompanied by specific lesions. The 180 day study period was an insufficient time period to draw any conclusions regarding the absence of tumorigenic potential of these compounds.

The peroxidation related effects of tellurium on the brain were studied in rats. Rats were given drinking water containing tellurium tetrachloride at a concentration of $100\,mg\,l^{-1}$ and were killed after 7, 21, or 35 days of exposure. Blood, liver, kidney, and brain samples were analyzed for tellurium. Exposed rats accumulated relatively high concentrations of tellurium. Blood had the highest tellurium concentrations, with an increasing trend according to the exposure period. Liver also showed a rapid increase, while the kidneys and brain had a continuous accumulation. Appreciable neurochemical effects were seen after the brain content exceeded $2\,nmol\,g^{-1}$. Succinic dehydrogenase activity was

above the control range after 21 days, while creatine-kinase activity decreased or remained stable. Brain glutathione content was above the control range at 35 days, possibly as a result of attempts to counteract peroxidative effects associated with mitochondrial damage. The initially low uptake of tellurium in the brain may have been due to a blood–brain barrier. Once incorporated into the nervous system, accumulation apparently occurred because of the long half-life of tellurium.

The developmental toxicity of tellurium was evaluated in rats and rabbits by means of standard segment II-type studies. Groups of pregnant rats were fed a diet containing 0, 30, 300, 3000, or 15 000 ppm of tellurium on days 6 through 15 of gestation (microscopic detection of sperm in a smear of vaginal contents considered as day 0), and artificially inseminated rabbits were fed a diet containing 0, 17.5, 175, 1750, and 5250 ppm of tellurium during days 6 through 18 of gestation (day of insemination considered as day 0). Signs of maternal toxicity were observed during the treatment period in a statistically significant and dose-related manner at dietary concentrations of 300 ppm and greater in rats and 1750 ppm and greater in rabbits. Exposure of these pregnant rats and rabbits to tellurium had no effect upon reproduction as measured by pregnancy rate, litter size, dead or resorbed implantations, or fetal sex ratio. Both skeletal (primarily skeletal maturational delays) and soft tissue malformations (primarily hydrocephalus) were noted in the offspring of pregnant rats exposed to the highest levels (3000 and 15 000 ppm) of tellurium. Rabbit fetuses of the highest dosage group (5250 ppm) had a slightly elevated evidence of skeletal delays and nonspecific abnormalities. Since maternal toxicity was observed at dosages that did not affect the developing conceptus, there were no indications of unique developmental susceptibility upon exposure of pregnant rats or rabbits to tellurium.

Human

Chronic exposure may lead to garlic breath, metallic taste, decreased sweating, dry mouth, fatigue, lassitude, anorexia, and nausea.

Clinical Management

Vitamin C (ascorbic acid) reduces the characteristic garlic breath; however, it may also adversely affect the kidneys when an excess amount of tellurium is present. BAL (British antilewisite; 2,3-dimercaptopropanol) is contraindicated since it enhances the toxicity of tellurium. There are no available treatments for poisoning.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average (TWA), for tellurium and its compounds is $0.1\,mg\,m^{-3}$. The (US) Occupational Safety and Health Administration (OSHA) permissible exposure limit, 8 h TWA, is $0.1\,mg\,m^{-3}$ for tellurium and compounds (as tellurium). The (US) National Institute for Occupational Safety and Health (NIOSH) recommended exposure level, averaged over a 10 h work day, is $0.1\,mg\,m^{-3}$ for tellurium compounds (as tellurium) except tellurium hexafluoride and bismuth telluride, and the NIOSH immediately dangerous to life or health (IDLH) value is $25\,mg\,m^{-3}$ (as tellurium).

See also: Metals; Selenium.

Further Reading

Dart RC (2004) *Medical Toxicology*, 3rd edn. Baltimore, MD: Williams and Wilkens.

Goyer RA, Klaassen CD, and Waalkes MP (1995) *Metal Toxicology*. San Diego, CA: Academic Press.

Taylor AC (1996) Biochemistry of tellurium. *Biological Trace Element Research* 55: 231–239.

Relevant Website

http://www.intox.org – The International Programme on Chemical Safety (IPCS).

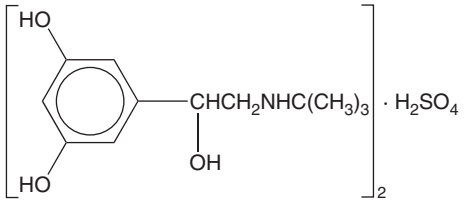
Teratology *See* Developmental Toxicology.

Teratology Testing *See* Toxicity Testing, Developmental.

Terbutaline

Henry A Spiller

- Chemical Abstracts Service Registry Number: CAS 23031-25-6
- Synonym: Brethine
- Chemical/Pharmaceutical/Other Class: A selective β_1 agonist
- Chemical Formula: $C_{12}H_{19}NO_3$
- Chemical Structure:



Uses

Terbutaline is used as a bronchodilator and for the prevention of premature labor. Unlabeled use includes treatment of hyperkalemia.

Exposure Routes and Pathways

Ingestion is the most common route of accidental and intentional exposure to terbutaline. Inappropriate overuse of the inhalation aerosol may also occur. Terbutaline is available as an inhalation aerosol, as tablets (2.5 and 5 mg), and as a solution for subcutaneous injection (1 mg ml^{-1}).

Toxicokinetics

Taken orally, terbutaline is poorly and incompletely absorbed from the gastrointestinal tract, with ~47% of the unchanged drug found in the feces. Administered subcutaneously, it is well absorbed with peak serum levels in 20 min. The bioavailability and biotransformation of terbutaline depend greatly on route of administration. With oral dosing there is significant first-pass biotransformation by sulfate and glucuronide conjugation in the liver and gut wall, with only 15% of absorbed terbutaline available as unchanged drug. With inhalation and parenteral dosing, the majority of the drug is available as unchanged terbutaline. The volume of distribution is 1.47 l kg^{-1}. The percentage of protein binding is 15%. With oral dosing terbutaline is eliminated primarily as sulfate (70%) and glucuronide (30%) conjugates. Approximately 10–15% is cleared in the urine as unchanged drug. With parenteral and inhalation exposure, the majority of the drug is cleared in the urine as unchanged terbutaline (68% and 60%, respectively). The elimination half-life is 12–20 h.

Mechanism of Toxicity

The primary mechanism of terbutaline is the stimulation of adenylcyclase, which catalyzes cyclic adenosine monophosphate (AMP) from adenosine triphosphate (ATP). In the liver, buildup of cyclic AMP stimulates glycogenolysis and an increase in serum glucose. In skeletal muscle, this process results in increased lactate production. Direct stimulus of sodium/potassium ATPase in skeletal muscle produces a shift of potassium from the extracellular space to the intracellular space. Relaxation of smooth muscle produces a dilation of the vasculature supplying skeletal muscle, which results in a drop in diastolic and mean arterial pressure (MAP). Tachycardia occurs as a reflex to the drop in MAP or as a result of β_1 stimulus. β_1-Adrenergic receptors in the locus ceruleus also regulate norepinephrine-induced inhibitory effects, resulting in agitation, restlessness, and tremor.

Acute and Short-Term Toxicity (or Exposure)

Animal

Clinical effects of agitation, tremors, and tachycardia may occur. No specific information on a minimal toxic dose was available.

Human

The toxic events of terbutaline overdose follow its β_1-adrenergic agonist activity. The effects of terbutaline overdose are usually mild and benign; however, they can be prolonged. Cardiovascular effects are usually limited to a sinus tachycardia and widened pulse pressure. Although there may be a drop in diastolic pressure, the systolic pressure is maintained by increased cardiac output from the tachycardia. Evidence of myocardial ischemia after terbutaline overdose has been infrequently reported. Transient hypokalemia may occur, caused by a shift of extracellular potassium to the intracellular space. A transient metabolic acidosis can be seen due to increased lactate production. Restlessness, agitation, and tremors are common in terbutaline overdose.

Chronic Toxicity (or Exposure)

Animal

Terbutaline is used in veterinary practice for the management of bronchoconstriction. Toxic effects

are commonly related to beta stimulation (e.g., tachycardia, hypertension).

Human

Toxic effects are an extension of terbutaline's pharmacologic activity. Common symptoms include hypertension, tachycardia, arrhythmias, central nervous system stimulation, gastrointestinal effects, and transient electrolyte changes (e.g., hypokalemia).

In Vitro Toxicity Data

Studies in rat alveolar type II cells have demonstrated that terbutaline stimulates sodium influx as well as potassium and chloride release via cAMP ;accumulation.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Terbutaline overdoses rarely require treatment beyond gastrointestinal decontamination. Activated charcoal effectively binds terbutaline. The hypokalemia produced reflects a transient shift in potassium location rather than a true deficit of potassium. Therefore, only rarely is there a need for external replacement therapy. A conservative approach to the tachycardia is recommended. In the rare event of complications, intravenous propranolol rapidly and effectively reverses the symptoms of terbutaline poisoning.

Environmental Fate

No information is currently available on breakdown in soil, groundwater, or surface water.

See also: Potassium.

Further Reading

Blake PG and Ryan F (1989) Rhabdomyolysis and acute renal failure after terbutaline overdose. *Nephron* 53: 76–77.

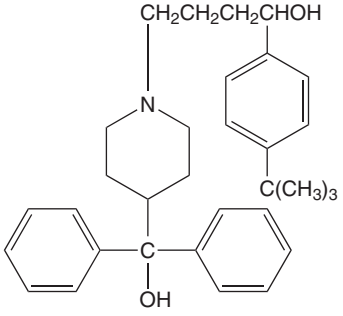
Heath A and Hulten BA (1987) Terbutaline concentrations in self poisoning: A case report. *Human Toxicology* 6: 525–526.

Terfenadine

Elizabeth J Scharman

This article is a revision of the previous print edition article by Carla M Goetz, volume 3, pp. 218–219, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 50679-08-8
- Synonyms: α-[4-(1,1-Dimethylethyl)phenyl]-4-(hydroxydiphenylmethyl)-1-piperidinebutalol; Seldane® (former brand name in United States); Teldane®; Teldanex®
- Chemical/Pharmaceutical/Other Class: An H-1 receptor antagonist; Piperidine derivative
- Chemical Formula: $C_{32}H_{41}NO_2$
- Chemical Structure:



Uses

Terfenadine is indicated for the symptomatic relief of seasonal allergic rhinitis. The Food and Drug Administration removed terfenadine from the US market in 1998.

Exposure Routes and Pathways

Ingestion is the route of both accidental and intentional exposures to terfenadine.

Toxicokinetics

Approximately 70% of an oral dose of terfenadine is absorbed rapidly with peak plasma concentrations occurring in 2 h. The onset of action is 1–2 h, is maximal in 3–4 h, and lasts over 12 h. The first-pass metabolism of terfenadine is 99%. Terfenadine is metabolized by the cytochrome P450 IIIA4 (CYP3A4) microsomal enzyme system to an acid metabolite that is active and a dealkylated metabolite that is inactive. *In vitro*, the acid metabolite has demonstrated ~30% of the H-1 blocking activity of the parent compound. The volume of distribution of terfenadine is undetermined; high concentrations are found in the liver, lung, and gastrointestinal tract. Terfenadine is 97% protein bound and penetrates the

blood–brain barrier poorly. Approximately 60% and 40% of an oral dose of the drug is excreted in the feces and urine, respectively, principally as metabolites. The half-life of terfenadine is 8.5 h. The elimination of the acid metabolite is biphasic with an initial half-life of 3.5 h and a terminal half-life of 6 h.

Mechanism of Toxicity

Terfenadine binds to peripheral H-1 receptors. Receptor affinity for muscarinic, α, and β-adrenergic receptors is low. Poor penetration of terfenadine across the blood–brain barrier limits central nervous system effects. Therefore, terfenadine is classified as 'nonsedating' and lacks anticholinergic side effects. However, accumulation of the parent drug, terfenadine, results in prolongation of the QT interval by blocking the delayed rectifier potassium current in the heart. Prolongation of the QT interval can lead to torsade de pointes and death.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity in dogs has been reported. Vomiting and lethargy are often reported. Central nervous system excitation leading to seizures, ataxia, agitation, and hyperthermia are possible. In one case, tachycardia and premature ventricular contractions were documented.

Human

Cardiac effects seen include prolongation of the QT interval, arrhythmias (e.g., torsades de pointes, ventri tachycardia, ventricular fibrillation), and cardiac arrest. Symptoms seen in patients with torsades de pointes include dizziness, syncope, palpitations, and sudden death. Seizures have occurred following overdose.

Chronic Toxicity (or Exposure)

Animal

Carcinogenicity studies in mice and rats over >18 months at doses of 150 mg kg^{-1} day^{-1} were negative.

Human

Accumulation of the parent drug and resultant QT prolongation may occur following a overdose, a drug interaction that limits metabolism of terfenadine (e.g., concomitant administration with erythromycin or other macrolide antibiotic or with the azole derivatives ketoconazole or itraconazole), or significant hepatic dysfunction that limits metabolism of terfenadine. Patients with preexisting cardiac disease or those with electrolyte abnormalities are also at increased risk for cardiac toxicity.

In Vitro Toxicity Data

Mutagenicity studies using the Ames *Salmonella* and mouse micronucleus assays have been negative.

Clinical Management

Terfenadine is adsorbed by activated charcoal and charcoal may be considered for substantial recent ingestions. There is no antidote for terfenadine overdose. Terfenadine therapy should be discontinued and standard supportive therapies should be utilized as clinically necessary. Close electrocardiographic monitoring should be instituted for a minimum of 24 h. Torsades de pointes may be treated with electrical cardioversion if the patient is hemodynamically unstable. Otherwise, magnesium, isoproterenol, and/or atrial overdrive pacing may be used to manage this arrhythmia.

See also: Cytochrome P-450.

Further Reading

June RA and Nasr I (1997) Torsades de pointes with terfenadine ingestion. *American Journal of Emergency Medicine* 15: 542–543.

Monahan BP, Ferguson CL, and Killeavy ES (1990) Torsades de pointes occurring in association with terfenadine use. *Journal of the American Medical Association* 264: 2788–2790.

Terrrestrial Ecotoxicology *See* Ecotoxicology, Terrestrial.

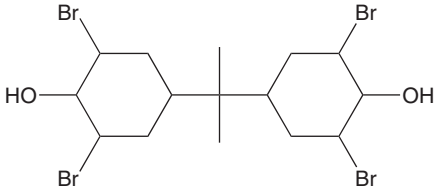
Terrorism *See* Bio Warfare and Terrorism: Toxins and Other Mid-Spectrum Agents.

Testosterone *See* Androgens.

Tetrabromobisphenol A

Paul Jones, Katie Coady, and John Newsted

- Representative Chemicals: 2,2′,6,6′-Tetrabromobisphenol A; 3,3′,5,5′-Tetrabromobisphenol A
- Chemical Abstracts Service Registry Number: CAS 79-94-7
- Synonyms: 2,2′,6,6′-Tetrabromo-4,4′-isopropylidenediphenol; 2,2-Bis(3,5-dibromo-4-hydroxyphenyl) propane; 4,4′-Isopropylidene bis(2, 6-dibromophenol); Saytex 111; Saytex RB-100; Bromdian; FG 2000, Fire Guard 2000; Firemaster BP4A; 4,4′-(1-Methylethylidene) bis(2,6-dibromo)phenol; Tetrabromodian; Tetrabromodiphenyl propane
- Chemical/Pharmaceutical/Other Class: Brominated phenolic
- Chemical Formula: $C_{15}H_{12}Br_4O_2$
- Chemical Structure:



Uses

Tetrabromobisphenol A (TBBPA) is primarily used as a reactive flame retardant in epoxy resin circuit boards. Both hydroxyl groups on TBBPA can be reacted with epichlorohydrin under basic conditions to form the diglycidyl ether, which is widely used in epoxy resin formulations. TBBPA is also used in polycarbonate and ether polyester resins and is used as a chemical intermediate for the synthesis of: tetrabromobisphenol A allyl ether, -bis(2-hydroxyethyl ether), -carbonate oligomer, and -diglycidyl ether. TBBA is also used as a flame retardant in plastics, paper, and textiles, and as a plasticizer in adhesives and coatings.

Exposure Routes and Pathways

Occupational exposure to TBBPA can occur through inhalation of dusts and dermal contact at workplaces where TBBPA is produced or used. Monitoring data indicate that the general population may also be exposed to TBBPA via inhalation of ambient air and dermal contact. Other possible exposure routes include ingestion of drinking water that has been stored in polycarbonate containers and from the consumption of fish and shellfish.

Toxicokinetics

In a study with rats, TBBPA was readily absorbed from the gastrointestinal tract, metabolized in the liver and excreted via the bile to the gut. Of the dose administered to the rats, ~90% was excreted in the feces as parent TBBPA. Three glucoronide conjugates of TBBPA were identified in the feces but only accounted for a small amount of the administered dose. Urine was a minor route for excretion of TBBPA. The half-life of TBBPA in rats was estimated to be less than 3 days with the longest half-lives in fat and testes. The shortest half-lives were in liver and kidneys. In a study of occupationally exposed Swedish workers, the half-life for elimination from the serum was 2.2 days indicating a rapid elimination from the body.

Mechanism of Toxicity

Studies of the effects of TBBPA on the function of biological membranes showed that it resulted in hemolysis of human erythrocytes and the uncoupling of oxidative phosphorylation in rat mitochondria. In addition, TBBPA exposure resulted in the inhibition of calcium accumulation in isolated mitochondria that was associated with an increase in potassium release and latent ATPase activity. These studies suggest that the primary activity of TBBPA *in vitro* is to change the permeability of biological membranes disrupting normal ion transport and respiration of cells. TBBPA has also been shown to weakly induce liver microsomal enzymes *in vitro*. In the E-Screen assay, TBBPA expressed weak receptor-mediated estrogenic activity with an estrogenic potency ~5–6 orders of magnitude lower than that of the native ligand, 17β-estradiol. TBBPA has been shown to bind to human transthyretin *in vitro* with a 10 times greater potency than thyroxin, the natural ligand. However, in a study with pregnant rats, TBBPA did

not bind to transthyretin and did not alter thyroid hormone concentration in the exposed animals. The differences between *in vitro* and *in vivo* studies may have been due to toxicokinetic factors that altered the effective concentrations at the site of action. In female rats, an intragastric dose of 250 mg kg^{-1} for 28 days resulted in the alteration of several serum enzymes including several indicators of porphyrogenic action. These results suggest that TBBPA is capable of disturbing heme metabolism in rats.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of TBBPA in traditional test animals is low. The oral LD_{50} for rat and mouse is >5 and 10 g kg^{-1} body weight and the dermal LD_{50} in rabbits is >2.0 g kg^{-1} body weight. In rats, the inhalation LC_{50} of TBBPA dust is >15.5 mg l^{-1}. In dermal tests, TBBPA was not irritating and gave no sensitization reaction. Upon dermal exposure on abraded skin of up to 2500 mg kg^{-1}, a slight erythema was seen in rabbits. Application of up to 100 mg of TBBPA in the eyes of rabbits did not result in corneal damage, iris irritation, or conjunctival discharge indicating that this compound is not an eye irritant. In rats fed up to 1000 mg kg^{-1} of TBBPA in the diet for 28 days, no effects were observed on mortality, body weight, and feed consumption, and no gross pathological lesions, or histopathological changes were seen. In a 90 day oral study with rats, exposure to 1000 mg kg^{-1} did not induce adverse effects on body weight, and no changes in hematology, clinical chemistry, urinalysis, or histopathology were observed. In an oral, 90 day study with mice, a dietary dose of 700 mg kg^{-1} did not cause any adverse effects while 2200 mg kg^{-1} resulted in decreased body weight, increased spleen weight and reduced concentration of red blood cells, serum proteins and serum triglycerides.

Chronic Toxicity (or Exposure)

Animal

TBBPA administered to rats via gavage on gestation days 0–19 produced no signs of toxicity including no abnormalities in the offspring. TBBPA, orally administered on gestation days 6–15, was more toxic to the conceptus than to the dams. The embryo/fetal no-effect level was 2.5 mg kg^{-1} day^{-1} while the maternal no effect-level was >25 mg kg^{-1} day^{-1}. At $\geqslant 10$ mg kg^{-1} day^{-1}, dose-dependent effects on conceptuses were observed and included fetal malformation, reduced fetal body weight, and delayed skeletal ossification. In a study with Firemaster BP4-A, concentrations up to 10 g kg^{-1} in rats exposed on gestation days 6–15 were not toxic to the dams or conceptus. No carcinogenicity studies have been reported to date.

Human

No skin irritation or sensitization has been observed in humans exposed to TBBPA. No epidemiological or other data are available on effects of TBBPA in humans. To date, no carcinogenicity or long-term studies have been reported.

In Vitro Toxicity Data

TBBPA tested negative for mutagenicity in the Ames assay using five *Salmonella typhimurium* strains (TA1535, TA1537, TA97, TA98, and TA100) with and without metabolic activation. TBBPA caused an effect on induction of intragenic recombination in two *in vitro* mammalian cell assays. TBBPA reduced CD25 (IL-2 receptor-α chain), an inducible receptor chain essential for proliferation of activated T cells in an *in vitro* immunotoxicity assay. However, observed immunosuppression of T cell proliferation was thought to be mediated through the Ah receptor.

Environmental Fate

TBBPA is expected to adsorb to suspended solids and sediment based on an estimated K_{oc} value of 56 000. Volatilization from water surfaces is not expected, based upon an estimated Henry's law constant of 7.0×10^{-11} atm m^3 mol^{-1}. Bioconcentration factor values ranging from 20 to 3200 suggest the potential for bioconcentration is moderate to high in aquatic organisms. In fish, a half-life of less than one day has been observed while in oysters it was less than 5 days. If released to air, TBBPA is expected to exist solely in the particulate phase (based on an estimated vapor pressure of 1.8×10^{-11} mmHg at 25°C) and may be removed from the air by wet and dry deposition. TBBPA is expected to be immobile in soil based on its estimated K_{oc}. Volatilization of TBBPA from moist and dry soil surfaces is not expected to be an important fate process given its Henry's law constant. Biodegradation of TBBPA in three different soils under anaerobic conditions resulted in 44–91% of the parent material with only 0.03–0.35% of the compound being recovered as carbon dioxide. Thus, under anaerobic conditions TBBPA is expected to undergo rapid primary degradation and slow mineralization in soils. A similar biodegradation process

was also observed in a sediment/water microbial test system where mineralization was slow. Photodegradation of TBBPA in water is seasonally dependent with half-lives of 10.2 days (spring), 6.6 days (summer), 25.9 days (autumn), and 80.7 days (winter). Cloud cover increased the times by a factor of 2. The main photodegradation product of TBBPA in the presence or absence of hydroxyl radicals was 2,4,6-tribromophenol. TBBPA has persistence half-life values in the range 44–179 days in soil, 48–84 days in water, and 1–9 days in air.

Exposure Standards and Guidelines

TBBPA is listed in the Environmental Protection Agency Toxic Substances Control Act under Section 8(b) and as a result all manufacturers, importers and processors of TBBPA are required to report all health and safety studies that they have conducted. As particulates not otherwise regulated, the Occupational Safety and Health Administration permissible exposure level time-weighted average (TWA) is $15\,mg\,m^{-3}$. As particulates not otherwise specified, the American Conference of Governmental Industrial Hygienists threshold limit value TWA is $10\,mg\,m^{-3}$.

See also: Bisphenol A; Chlorophenols.

Further Reading

Darnerud PO (2003) Toxic effects of brominated flame retardants in man and wildlife. *Environment International* 29: 841–853.

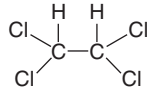
Hakk H and Letcher R (2003) Metabolism and toxicokinetics and fate of brominated flame retardants – a review. *Environment International* 29: 801–828.

Tetracholordibenzo-*p*-Dioxin, 2,3,7,8- *See* TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin).

Tetrachloroethane

Robert Kapp

- Related Compounds: Carbon tetrachloride (CAS 56-23-5); Perchloroethylene (CAS 127-18-4); Trichloroethylene (CAS 79-01-6); Trichloroethane (CAS 79-00-5); Pentachlorophenol (CAS 87-86-5)
- Chemical Abstracts Service Registry Number: CAS 79-34-5
- Synonyms: 1,1-Dichloro-2,2-dichloroethane; Acetosal; Acetylene tetrachloride; Bonoform; Cellon; Dichloro-2, 2-dichloroethane; Ethane, 1,1,2,2-tetrachloro-; TCE (ambiguous); Tetrachloroethane; Tetrachloroethane (VAN); Tetrachlorure d'acetylene (French); Westron; s-Tetrachloroethane
- Chemical/Pharmaceutical/Other Class: Chlorinated aliphatic solvent; Haloalkane
- Chemical Formula: $C_2H_2Cl_4$
- Chemical Structure:



Uses

1,1,2,2-Tetrachloroethane has been used primarily as an intermediate in the synthesis of other chlorinated hydrocarbons such as trichloroethylene, tetrachloroethylene and 1,2,-dichloroethylene. It was also used as a solvent in cleaning and degreasing metals, in paint removers, in photographic films, as an extractant for oils and fats, and in pesticides. Recently, however, the production of 1,1,2,2-tetrachloroethane as an end product has significantly decreased due to replacement with less toxic solvents.

Exposure Routes and Pathways

A primary pathway for tetrachloroethane exposure is by inhalation since tetrachloroethane can be found at low levels in both indoor and outdoor air. The material has not been reported in food or soil; however, in rare instances, tetrachloroethane has been found in public water supplies. Since production of tetrachloroethane has stopped, exposure of most workers would be limited. Minimal exposure could occur from breathing in vapors or touching the material during accidental spills in the workplace.

Toxicokinetics

1,1,2,2-Tetrachloroethane is well absorbed from the gastrointestinal and respiratory tracts. It is also absorbed through the skin upon dermal exposure.

When introduced via the oral or inhalation routes, tetrachloroethane is metabolized primarily to trichloroethanol, trichloroacetic acids that are subsequently broken down to glyoxylic acid, oxalic acid and carbon dioxide and are excreted chiefly as metabolites in the breath and urine. A small amount is expired in the breath as carbon dioxide and as the parent compound.

Mechanism of Toxicity

Generally, 1,1,2,2-tetrachloroethane is considered the most toxic of the common chlorinated hydrocarbons. It is a small lipophilic molecule, well-absorbed and distributed throughout tissue compartments by passive diffusion processes. Its metabolic fate involves both oxidative and reductive reactions, which are related to the mechanisms by which halocarbons are activated to proximate toxins. The presence of the terminal dichloromethyl moiety can convey toxicity because these moieties are hydroxylated to reactive acyl intermediates that bind to proteins and exert toxic effects. Both dichloro- and trichloroacetic acids are known to cause proliferation of peroxisomes, which could elicit a hepatotoxic response. On the other hand, studies investigating the reductive metabolism of 1,1,2,2-tetrachloroethane found a cytochrome P450-mediated reaction, which included lipid peroxidation and dehalogenation of 1,1,2,2-tetrachloroethane. The principal pathway of degradation involves hydrolytic cleavage of the carbon-chlorine bonds and oxidation to dichloroacetaldehyde hydrate, dichloroacetic acid and eventually glyoxylic acid. This glyoxylic acid is then metabolized to oxalic acid, glycine, formic acid, and carbon dioxide. The hepatic and carcinogenic effects of 1,1,2,2-tetrachloroethane may result from the oxidative and reductive pathways that produce direct- or indirect-acting toxins.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of 1,1,2,2-tetrachloroethane in experimental animals is slight to moderate. This material is a skin, eye, and respiratory irritant. The oral LD_{50} has been reported from 250 to 1000 mg kg^{-1} in the rat. Exposure to 1000 ppm for 4–6 h caused death in rats. Dermal and ocular irritations were reported in rabbits exposed to 580 ppm of 1,1,2,2-tetrachloroethane. There are limited short-term data available; however, single low-level inhalation exposure effects have been reported to include hepatic congestion, fatty degeneration, histological changes, alterations in levels of enzymes and elevated DNA synthesis.

Human

Based upon the limited data from acute and subchronic studies, the liver appears to be the most sensitive target organ.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to 1,1,2,2-tetrachloroethane has resulted in increased incidence of liver tumors in mice after 78 weeks. Tumor increases are limited to one species and other data are incomplete and suggest an epigenetic mechanism. The Gene-Tox data collectively indicate that 1,1,2,2-tetrachloroethane is a weak genetic toxin. Reproductive toxicity studies revealed atrophy of the seminal vesicles and decreased spermatogenesis after 10 days' exposure to 2 ppm 1,1,2,2-tetrachloroethane; however, there was no effect on the mating index of the males after 38 days of similar exposure to the material. Similarly, males rats exposed to 2 ppm 1,1,2,2-tetrachloroethane for 4 h per day over a 9 month period showed no effects on any of the mating indices normally examined nor were there any abnormalities in the offspring.

Human

A group of army workers exposed to 1,1,2,2-tetrachloroethane in a gaseous form showed a slight increase in the incidence of death due to genital cancers, leukemia, and lymphomas when compared to nonexposed workers. The data in this study were suspect since the specific exposure levels were not measured and since the increases were small and there were other confounding factors. Because of these factors, the authors concluded that the data were inconclusive as to whether or not 1,1,2,2-tetrachloroethane causes cancer.

The Environmental Protection Agency has classified 1,1,2,2-tetrachloroethane as group C (possible human carcinogen: limited evidence of carcinogenicity in animals in the absence of human data). The International Agency for Research on Cancer classifies the material as group 3 (not classifiable as to carcinogenicity to humans). The National Institute for Occupational Safety and Health classifies 1,1,2,2-tetrachloroethane as Ca (potential occupational carcinogen, with no further categorization). The American Conference of Governmental Industrial Hygienists (ACGIH) classifies the material as A3 (confirmed animal carcinogen with unknown

relevance to humans); while the Federal Republic of Germany Maximum Concentration Values in the Workplace (MAK) classifies the material as 3B (substance for which *in vitro* test or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories). 1,1,2,2-Tetrachloroethane is listed in Schedule 2 of the COSHH (Control of Substances Hazardous to Health Regulations). Its use in the United Kingdom is banned for diffusive applications such as surface or fabric cleaning except for R&D and analysis.

Clinical Management

Upon ocular exposure, the eye should be generously washed with tap water. Refer for medical attention. In case of dermal exposure, contaminated clothing should be removed and the skin should be rinsed with tap water. 1,1,2,2-Tetrachloroethane ingestion should be referred for medical attention. Vomiting should not be induced. Upon inhalation, the victim should be removed to fresh air and given artificial respiration if indicated. The affected individual should be referred for medical attention.

Environmental Fate

1,1,2,2-Tetrachloroethane has the potential to leach to groundwater. In surface water, it volatilizes with a half-life of ~6 h. Hydrolysis also occurs. Adsorption to sediment and bioconcentration in aquatic organisms is not significant.

Exposure Standards and Guidelines

The Occupational Safety and Health Administration permissible exposure limit is 5 ppm (time-weighted average (TWA)) on skin while the ACGIH threshold limit value is 1 ppm (TWA) on skin.

Miscellaneous

1,1,2,2-Tetrachloroethane is a volatile, synthetic, colorless to pale-yellow liquid with a pungent, chloroform-like odor.

See also: Trichloroethane; Trichloroethylene.

Further Reading

Reid JB (2001) Saturated halogenated aliphatic hydrocarbons two and four carbons. In: Bingham E, Cohrssen B and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 5, pp. 136–142. New York: Wiley.

US Department of Health and Human Services (1996) *Toxicological Profile for 1,1,2,2-Tetrachloroethane*. Atlanta, GA: Public Health Service, Agency for Toxic Substances and Disease Registry.

Tetrachloroethylene

Richard A Parent

This article is a revision of the previous print edition article by R A Parent, T R Kline, and D E Sharp, volume 3, pp. 220–221, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 127-18-4
- Synonyms: Tetrachloroethene; 1,1,2,2-Tetrachloroethylene; Perchloroethene; Perchloroethylene
- Chemical/Pharmaceutical/Other Class: Chlorinated olefinic hydrocarbon
- Chemical Formula: C_2HCl_3

Uses

Perchloroethylene (PERC) is manufactured by direct chlorination of ethylene or a petroleum hydrocarbon stream. It has been used extensively in the dry cleaning industry for metal cleaning and degreasing, for processing and finishing textiles, as an extraction solvent, in chemical processing, as heat exchange fluid, as a grain fumigant, for fluorocarbon manufacturing processes, and in typewriter correction fluid. PERC is a clear liquid of high volatility. Its vapor is almost six times as dense as air and has a chloroform-like odor detectable from 5 to 50 ppm in air.

Exposure Routes and Pathways

Major human exposure has been in the dry cleaning industry and in industries employing degreasing procedures. In addition, inhalation of contaminated urban air (especially near point sources such as dry cleaners), drinking contaminated water from contaminated aquifers, and drinking water distributed in pipelines with vinyl liners may offer additional exposure opportunities.

Toxicokinetics

Comparison of the urinary trichloro-compound levels with tetrachloroethylene in the environment revealed that while the metabolite levels increased essentially parallel to PERC concentrations up to 100 ppm, leveling off was apparent in the metabolite excretion when the exposure to PERC was more intense (e.g., more than 100 ppm), indicating that the capacity of humans to metabolize this chlorinated hydrocarbon is rather limited. A tentative calculation indicated that at the end of an 8 h shift with exposure to tetrachloroethylene at 50 ppm (time-weighted average, TWA), 38% of the PERC absorbed through the lung would be exhaled unchanged, $<2\%$ would be metabolized to be excreted in the urine, while the rest would remain mostly in the fat stores of the body to be eliminated later.

Absorption typically takes place through inhalation of the volatile solvent but may also take place through dermal exposure and ingestion of contaminated drinking water. During PERC exposure, urinary metabolite levels of trichloroethanol, total trichloro compounds, and trichloroacetic acid increased until the atmospheric concentration of the solvent reached 50–100 ppm. Little increase in these metabolites occurred at higher solvent concentrations indicating a saturation of metabolic capability.

Metabolism is saturable and relatively slow with only a small percentage of the administered dose excreted as metabolites, the major one being trichloroacetic acid. Following exposure to PERC, trichloroacetic acid, and trichloroethanol have been found in the urine of humans and animals. Additionally, oxalic acid, dichloroacetic acid, and ethylene glycol have been reported in the urine of exposed animals. Other reported biotransformation products include inorganic chlorine and *trans*-1,2-dichloroethylene in expired air.

Once in the bloodstream, PERC tends to distribute to body fat. In human tissue at autopsy, ratios of fat to liver concentrations are greater than 6:1. An autopsy after a fatal PERC exposure revealed an eightfold greater concentration in the brain compared with blood. PERC reached near steady-state levels in the blood of human volunteers within 2 h of continuous exposure.

The respiratory half-life for elimination of PERC has been estimated at 65–70 h and is a result of the very slow elimination of PERC from fat stores. The half-life of elimination of trichloro metabolites of PERC is estimated as being 144 h. This long half-life of elimination has serious implications with regard to the accumulation of PERC during chronic or multiple exposure situations.

Mechanism of Toxicity

PERC is metabolized to trichloroacetic acid and other trichloro metabolites in the liver. Trichloroacetic acid has been shown to produce peroxisome proliferation in mice. This may have implications for the apparent increase in liver tumors in mice. PERC also has been shown to distribute rapidly to the central nervous system (CNS) and is known to have an affinity for the lipophilic cellular membranes in the brain.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values in mice are reported to be between 6 and 8.5 g kg^{-1} body weight and between 2.4 and 13 for rats. The inhalation LC_{50} value for rats during a 6 h inhalation exposure is reported to be 4100 and 2978 ppm for mice.

Human

PERC defats the skin leading to dermatitis. It is irritating to the eyes, skin, and mucous membranes. Excessive exposure can produce CNS effects including depression, dizziness, disorientation, seizures, headache, vertigo, and unconsciousness. Psychoses, hallucinations, and distorted perceptions have been reported from inhalation exposures. Concentrations producing unconsciousness have also produced proteinuria and hematuria. Liver and kidney damage has been noted in cases of acute exposure to PERC which is also considered to be a mucous membrane and upper respiratory system irritant at levels approaching 100 ppm in the atmosphere. Ingestion of PERC may result in nausea, vomiting, and bloody stools, while inhalation of other compounds in this class has been noted to sensitize the myocardium to catecholamines. Acute inhalation exposure has been reported to cause CNS depression, alcohol intolerance, liver necrosis, kidney injury, malaise, headache, dizziness, fatigue, tinnitus, visual field reduction, sensory disturbances, lightheadedness, sweating, a staggering gait, inebriation, mental dullness, and death by anesthesia. Cardiac arrhythmias, peripheral neuropathies, proteinuria, hematuria, and oliguric renal failure have also been associated with PERC exposure.

Direct eye contact may cause pain, lacrimation, and burning. Dermal exposure can cause dermatitis, erythema, burns, and vesiculation.

At 50 ppm, the apparent odor threshold for PERC, and at 100 ppm few physiological effects are noted after 8 h of exposure. At 200 ppm definite odor is

perceived and faint to moderate eye irritation and minimal lightheadedness are noted. At 400 ppm a strong unpleasant odor is perceived along with definite eye irritation, slight nasal irritation, and definite incoordination after 2 h of exposure. At 600 ppm, it has a very strong but tolerable unpleasant odor and causes definite eye and nasal irritation, dizziness, loss of inhibitions after only 10 min of exposure. At 100 ppm the odor is very intense and irritating with marked irritation of the eyes and respiratory tract and considerable dizziness after a 2 min exposure and at 1500 ppm, the odor is almost intolerable with gagging, irritation almost intolerable to eyes and nose with complete incoordination within minutes to unconsciousness within 30 min.

Chronic Toxicity (or Exposure)

Animal

PERC produces leukemia in rats. Although PERC is known to induce peroxisome proliferation in mouse liver, this did not correlate well with tumor formation in the liver. Fetotoxicity and developmental abnormalities have been seen in animal experiments.

Mice receiving single doses of PERC did not show chromosomal aberrations in bone marrow cells nor did a positive mutagenic response result from the host-mediated assay using Salmonella strains in mice. Renal tubular effects have been noted in mice and dogs treated orally with PERC and both liver and kidney changes have been noted in rats inhaling PERC.

As a result of National Toxicology Program (NTP) bioassays, PERC has been reported to produce hepatocellular carcinomas in B6C3F1 mice of both sexes when administered by gavage. An NTP inhalation study also showed hepatocellular carcinomas in B6C3F1 mice and renal cell adenomas and adenocarcinomas and mononuclear cell leukemias and renal tubular cell neoplasms in Fisher 344 rats.

In cats and dogs, PERC increased the vulnerability of the ventricles to epinephrine-induced extrasystoles, bigeminal rhythms and tachycardia. PERC is considered an animal carcinogen

Human

Studies of chronic exposure of those working in dry cleaning plants have reported some CNS effects, some liver function abnormalities, renal dysfunction, and some definite central and peripheral neurotoxicity. Other effects from chronic exposure to PERC include cardiac arrhythmias, reduced color perception, impaired memory, peripheral neuropathy, impaired vision, confusion, disorientation, fatigue, personality changes, and agitation.

Exposure to PERC has been reported to elevate risks of esophageal cancer, non-Hodgkin's lymphoma and cervical cancer in several epidemiological studies and PERC has been classified by the International Agency for Research on Cancer (IARC) as a probable human carcinogen. Dry cleaners chronically exposed to PERC have shown early signs of renal damage and dysfunction. Chronic exposure to PERC may cause arrhythmias, defatting dermatitis, impaired memory, numbness in the extremities, peripheral neuropathy, and impaired vision. Chronic occupational exposure has resulted in hepatitis, confusion, disorientation, muscle cramps, fatigue, and agitation. Some epidemiological studies suggest an increased incidence of liver, esophageal, and urinary tract tumors, and leukemia in humans but the data is inadequate to come to any firm conclusions. Other studies of occupationally exposed workers suggest that there are increased cancer risks for lung, cervix, skin, liver, esophagus, urinary tract, and for leukemia. IARC has classified PERC as a probable human carcinogen based on positive findings in animals and suggestive, although inconclusive, findings in humans.

Scleroderma, an autoimmune disease involving the vascular system, has been associated with exposure to chlorinated ethylene compounds related to PERC, but the reports are not definitive.

In Vitro Toxicity Data

Few positive results have been noted for *in vitro* mutagenicity assays but one test using L5178Y mouse lymphoma cells demonstrated a positive response. Attempted cell transformation using the BALB/3T3 mouse cell line failed to produce a positive response as did an Ames test using TA98, TA100, TA1535, and TA1537. Chromosomal abnormalities have been reported in the circulating lymphocytes of some exposed workers but conflicting results are obvious in the literature. In some studies, factory workers have also failed to show effects of exposure on chromosomal aberrations and sister chromatid exchanges (SCEs).

Clinical Management

Those exposed to PERC regularly should be monitored for kidney and liver function. Current exposure can be monitored by analysis of exhaled PERC. For acute ingestion, emesis is not recommended because of the potential for CNS depression. Gastric lavage should be considered if the quantity of PERC ingested is life threatening but should be performed within 1 h of ingestion. Activated charcoal may be considered and endotracheal intubation and ventilatory

assistance with supplemental oxygen should be considered if CNS depression of the respiratory system is noted. Monitor level of consciousness, EKG, adequacy of respirations and oxygen saturation as well as renal and hepatic function tests. Careful EKG monitoring may aid in early detection of arrhythmias.

For inhalation exposures, move the patient to an uncontaminated atmosphere and administer oxygen as indicated. Insure a patent airway. Treat bronchospasm with inhaled $\beta 2$ agonists and oral or parenteral corticosteroids. Again monitor the level of consciousness, EKG, oxygen saturation, liver, and renal functions carefully. Cardiac sensitization has occurred with other compounds in this class so EKG monitoring should be carried out carefully. Epinephrine or other β-adrenergic agents should be immediately available should arrhythmias occur.

Environmental Fate

When released into the environment, PERC exists as a vapor and will be degraded by photochemically produced hydroxy radicals and the half-life for this reaction is estimated to be ~ 96 days. Since PERC only absorbs UV light weakly, direct photodegradation is not thought to be an important pathway. If released into the soil, PERC is quite mobile and is frequently found in groundwater. Volatilization from dry soil and water are thought to be important pathways of dispersion into the environment. Biodegradation in soil under aerobic and anerobic conditions is thought to proceed slowly. Anerobic biodegradation of PERC produces mainly trichloroethylene but traces of dichloroethylenes and vinyl chloride may also be found.

Other Hazards

Adolescents and others have used PERC to attain an inhalation 'high' by 'huffing' the fumes in a paper bag saturated with PERC from typewriter correction fluids. A clinical observation described as 'degreasers flush' has been repeatedly noted in those exposed to chlorinated solvents in combination with alcohol consumption. Thermal decomposition of PERC results in the production of hydrogen chloride gas and phosgene. Smoking or welding in a PERC-contaminated environment will produce these toxic gases which could result in life-threatening pulmonary edema.

Exposure Standards and Guidelines

- Federal drinking water standard (Environmental Protection Agency 11/93): $5\ \mu g\ l^{-1}$.
- Maine drinking water standard: $3\ \mu g\ l^{-1}$.
- Occupational Safety and Health Administration (OSHA) permissible exposure limit, (8 h TWA): 100 ppm.
- OSHA short-term exposure limit: 15 min 200 ppm.
- American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value, (8 h TWA): 100 ppm.
- Biological exposure index (BEI – ACGIH) and end of shift: 5 ppm in exhaled air.

The National Institute for Occupational Safety and Health recommends that PERC be regulated as a potential human carcinogen with lowest possible exposure level.

The IARC has classified PERC as being probably carcinogenic to man (2A) based on sufficient evidence in animals but limited evidence in humans.

The ACGIH classifies PERC as an animal carcinogen (A3).

Miscellaneous

PERC is a colorless liquid with an ether-like odor. It boils at $\sim 121^{\circ}C$ and has a liquid density of ~ 1.6. It is lipid-soluble with a distribution between octanol/water of 3.4 and has a solubility of 0.015 g $(100\ ml)^{-1}$ in water. Its vapor density is 5.7 and, consequently, it settles in low areas when released in quantity into the environment. PERC quickly desensitizes the olfactory nervous system but can be recognized in air at ~ 4.7 ppm but is generally thought to have an odor threshold of ~ 50 ppm. A worker may be exposed to high concentrations of PERC without smelling it.

See also: Peroxisome Proliferators; Pollution, Water.

Further Reading

Beliles RP (2002) Concordance across species in the reproductive and developmental toxicity of tetrachloroethylene. *Toxicology and Industrial Health* 18(2): 91–106.

Relevant Websites

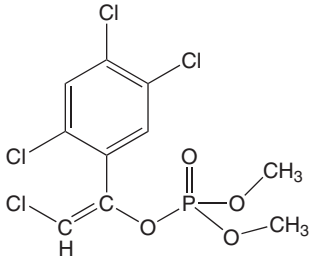
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Tetrachloroethylene.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Tetrachloroethylene.

Tetrachlorvinphos

Subramanya Karanth

- Chemical Abstracts Service Registry Number: CAS 22248-79-9
- Synonyms: Gardona; Stirophos; Rabon; Rabond
- Chemical/Pharmaceutical/Other Class: Organophosphate (vinyl phosphate) insecticide
- Chemical Formula: $C_{10}H_9Cl_4O_4P$
- Chemical Structure:



Uses

Tetrachlorvinphos is commonly used as a feed additive to control flies in livestock and as dusts, sprays, dips, and collar ingredient to control ticks and fleas on domestic pets. It is extensively used in poultry. In horses formulations are commonly used as 'feed-through' larvicide. In addition, tetrachlorvinphos is also used in the control of nuisance and public health pests.

Exposure Routes and Pathways

Dermal absorption and inhalation of dusts are the common routes of exposure for tetrachlorvinphos.

Toxicokinetics

Tetrachlorvinphos is readily absorbed through the gastrointestinal tract following oral exposure. Major metabolites following oral exposure in rats and dogs include desmethyl tetrachlorvinphos, 2,4,5-trichlorophenylethandiol glucuronide, and 2,4,5-trichloromandelic acid. Metabolism and excretion of radioactive tetrachlorvinphos in rats is rapid and majority of radioactivity appears between 0 and 24 h of exposure in urine and feces.

Mechanism of Toxicity

Like other organophosphorus insecticides, tetrachlorvinphos exerts toxicity by inhibiting the enzyme acetylcholinesterase (AChE). AChE inhibition results in accumulation of the neurotransmitter acetylcholine in the cholinergic synapse leading to overstimulation of postsynaptic receptors and cholinergic toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity studies in laboratory animals have shown that tetrachlorvinphos is one of the least toxic organophosphorus insecticides with an oral LD_{50} of $>2000\,mg\,kg^{-1}$ in laboratory animals. General signs of acute poisoning include salivation, diarrhea, urination, lacrimation, sweating, blurred vision, miosis, bradycardia, increased bronchial secretions, emesis, headache, dizziness, anxiety, lethargy, seizures, and depression of respiratory and cardiovascular centers.

Human

Like other organophosphorus insecticides, tetrachlorvinphos can cause cholinesterase inhibition in humans. Tetrachlorvinphos is classified as a skin sensitizer.

Chronic Toxicity (or Exposure)

Animal

Carcinogenicity studies in rats have shown that high doses of tetrachlorvinphos produce adrenal, thyroid, and hepatocellular carcinoma. Experiments in hens have shown that tetrachlorvinphos does not cause delayed neurotoxicity.

Human

Tetrachlorvinphos is classified as a possible human carcinogen. It is not registered for use on any food or feed crop. Human dietary exposure is mainly secondary through livestock uses. Industrial and agricultural workers who are involved in handling and applying tetrachlorvinphos are at a higher risk of exposure.

In Vitro Toxicity Data

In vitro studies have shown that tetrachlorvinphos is not mutagenic in bacteria and is a weak inducer of chromosomal aberrations in human lymphocytes. Studies with plasma and erythrocyte cholinesterases from different species have revealed that the

nontarget enzyme butyrylcholinesterase can be remarkably more sensitive to tetrachlorvinphos than the target enzyme acetylcholinesterase.

Clinical Management

General decontamination procedures should be followed immediately in case of tetrachlorvinphos poisoning. For skin decontamination, the contaminated area should be washed with water using soap and shampoo. If eyes are contaminated, they should be flushed with plenty of water repeatedly for 10–15 min. Contaminated clothing should be removed and a clear airway ensured. In case of ingestion, oral secretions should be removed and gastrointestinal decontamination started. Activated charcoal ($1\,g\,kg^{-1}$, $\sim 5\,ml\,g^{-1}$) may be used if the poisoning is detected within 60 min. Atropine treatment should be initiated immediately to counteract muscarinic effects. Atropine (adults and children >12 years: 2–4 mg; children <12 years: 0.05–0.1 mg) treatment should be repeated every 15 min until oral and bronchial secretions are controlled and atropinization is achieved. The duration and dosage of atropine treatment should be slowly reduced as the condition of the patient improves. Pralidoxime should be administered slowly at the recommended dosage (adults and children >12 years: 1–2 g; children <12 years: 20–50 mg by intravenous infusion in 100 ml saline at $\sim 0.2\,g\,min^{-1}$). This dosage can be repeated at every 1–2 h intervals initially and at 10–12 h intervals later depending on the condition of the patient.

Environmental Fate

Tetrachlorvinphos is nonpersistent in the environment. Based on the current use pattern, risks of contamination of ground or surface water by tetrachlorvinphos are minimal.

Ecotoxicology

Studies in birds indicate that tetrachlorvinphos is practically nontoxic to birds while it is highly toxic to fish and other aquatic organisms. It is also considered to pose minimal risk to wildlife.

See also: Acetylcholine; Cholinesterase Inhibition; Neurotoxicity; Organophosphates; Veterinary Toxicology.

Further Reading

Karanth S and Pope C (2003) *In vitro* inhibition of blood cholinesterase activities from horse, cow and rat by tetrachlorvinphos. *International Journal of Toxicology* 22: 429–433.

Vinggaard AM, Hnida C, Breinholt V, and Larsen JC (2000) Screening of selected pesticides for inhibition of CYP19 aromatase activity *in vitro*. *Toxicology In Vitro* 14: 227–234.

Relevant Website

http://www.epa.gov – US Environmental Protection Agency.

Tetrahydrofuran

Sree L Jasti

This article is a revision of the previous print edition article by Edward Kerfoot, volume 3, pp. 222–224, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 109-99-9
- Synonyms: Cyclotetramethylene oxide; Diethylene oxide; THF; Tetramethylene oxide
- Chemical/Pharmaceutical/Other Class: Substituted epoxide
- Chemical Formula: C_4H_8O
- Chemical Structure:

Uses

Tetrahydrofuran is a solvent used in natural and synthetic polymers and resins such as polyvinyl chloride and vinylidene chloride copolymers. It is also used in the manufacture of lacquers, glues, paints, and inks.

Exposure Routes and Pathways

Industrial exposures to tetrahydrofuran are most likely to occur by inhalation with skin and eye contact possible. Accidental ingestion is also possible. Based on physical and chemical properties, production, use patterns, and environmental monitoring levels in the low ppb range, the environmental exposure potential is expected to be low.

Toxicokinetics

When healthy volunteers were exposed by inhalation to 100 or 400 ppm tetrahydrofuran in air, the percentage of expired tetrahydrofuran was 25–35%. The elimination half-life of tetrahydrofuran was 30 min in individuals exposed to 200 ppm for 3 h. Some tetrahydrofuran is absorbed in the nasal cavity due to its solubility and inspiratory flow rate. Tetrahydrofuran uptake in the nasal tissue is dependent on its reaction with tissue substrates. Some tetrahydrofuran can be metabolized in the nasal cavity. Tetrahydrofuran blood concentrations were higher at 1 h postexposure than immediately after cessation of exposure. *In vitro* studies indicated that tetrahydrofuran was first hydroxylated by microsomal enzymes. High concentrations (10^{-2} mol l^{-1}) of tetrahydrofuran inhibited the *in vitro* activity of rat hepatic cytochrome P450 by 80%. Tetrahydrofuran has been noted to enhance the toxic action of a number of compounds and stimulate the rapid absorption of reactive metabolites. Some of the tetrahydrofuran is excreted in the exhaled breath, while the various metabolites of tetrahydrofuran are excreted in the urine.

Additional metabolism studies have shed some light on a proposed pathway for tetrahydrofuran conversion to CO_2. *In vitro* metabolism studies with hepatic microsomes from rats, mice or humans identified γ-hydroxybutyric acid as a metabolite, a compound which is a potential intermediate in the formation of succinic acid. *In vitro* data demonstrated that liver macrodomes in mice have a greater inherent capacity to metabolize tetrahydrofuran than human or rat macrodomes; however, no data are available to confirm this *in vivo*. *In vivo* studies identify CO_2 as the major terminal metabolite. It has been found that in both rats and mice the metabolic pathway is increasingly saturated at high doses although there is some indication of species differences; however, experimental losses of CO_2 in the rat study make it difficult to interpret the data. Based on the *in vitro* and *in vivo* metabolism data, tetrahydrofuran undergoes oxidative metabolism to γ-butyrolactone, which is further metabolized to γ-hydroxybutyric acid, and then to the endogenous compound, succinic acid. Succinic acid, in its ionized form (succinate), undergoes a series of reactions through the citric acid cycle leading to the release of CO_2. Recent *in vivo* studies in mice have also provided evidence of P450 induction, that is, both ethoxyresorufin-*O*-deethylase activity and pentoxyresorufin-*O*-dependentylase activity, suggesting that tetrahydrofuran may be metabolized by CYP 1A/2B isoforms. Tetrahydrofuran is readily absorbed through multiple routes in animals, is systematically distributed and rapidly metabolized and excreted, suggesting that tetrahydrofuran does not bioaccumulate.

No physiologically based pharmacokinetic (PBPK) models are available for tetrahydrofuran in animals. Based on human volunteer studies, a PBPK model for tetrahydrofuran was developed which predicts rapid elimination of tetrahydrofuran from the body. The human PBPK model predicts that repeated inhalation exposure of 200 ppm would yield end of the work shift levels of tetrahydrofuran of 5.1 ppm in breath, 57 mol l^{-1} in the blood, and 100 mol in the urine.

Mechanism of Toxicity

Irritation of the upper respiratory tract is attributed to the solubility of tetrahydrofuran in the mucous membranes causing irritation of the sensory nerve endings. The direct action of tetrahydrofuran on the skin and eyes is the result of irritation of these tissues. Carcinogenic responses in male rat kidney and female mouse liver are through nongenotoxic mechanisms. Tetrhahydrofuran enhances tumor formation in male rat kidneys and female mouse liver via induction of cell proliferation. The induced cell proliferation, in the female mice liver was associated with an increased cytochrome P450 content. Increased cell proliferation in male rat kidney was coupled with α-2U-globulin accumulation in the renal cortex, indicating a mechanism for tumor formation, which is rodent specific and may not be relevant for human health risk assessment.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in rats for tetrahydrofuran is 2.3 ml kg^{-1}. A single 4 h inhalation study of tetrahydrofuran in rabbits at 100–12 000 ppm produced a transient dose-related decrease of tracheal ciliary activity. Concentrations of tetrahydrofuran $\geqslant$25 000 ppm produced anesthesia. Tetrahydrofuran was irritating to rabbit skin when applied topically in solutions exceeding a 20% concentration. No lasting acute adverse effects on neurological endpoints were seen with THF exposures of 0, 500, 2500, or 5000 ppm for 6 h in rats except for sedation. The no-observed-effect level (NOEL) for the acute neurotoxicity in rats was 500 ppm.

Human

Exposure to tetrahydrofuran has been reported to cause irritation of the skin, eyes, and respiratory

tract. Individuals exposed to high concentrations of tetrahydrofuran have complained of nausea, dizziness, tinnitus, headache, and central nervous system (CNS) depression. Narcosis has been observed in humans exposed to tetrahydrofuran at ~25 000 ppm. The probable oral lethal dose in humans is estimated to be between 50 and 500 mg kg^{-1}.

Chronic Toxicity (or Exposure)

Animal

Male rats exposed to 5000 ppm tetrahydrofuran for 12 weeks at 4 h day^{-1} showed signs of systemic intoxication, skin and respiratory tract irritation, liver function disturbance, and abnormalities in glucose metabolism. Some rats exhibited slight respiratory tract irritation at 200 ppm. In another study, male rats exposed to 200, 1000, or 2000 ppm for 18 weeks at 6 h day^{-1} demonstrated an increase in muscle acetylcholinesterase activity in a concentration-dependent manner. Rats exposed to 200 ppm tetrahydrofuran, 4 h day^{-1}, 5 days week^{-1}, exhibited damage to the nasal and tracheal epithelium. At 1000 ppm tetrahydrofuran, severe damage to the same structures was observed. No lasting adverse effects on neurological endpoints, except for sedation, were seen with tetrahydrofuran exposures in rats after subchronic exposures of 0, 500, 1500, or 3000 ppm, 6 h day^{-1}, 5 days week^{-1}. The NOEL for subchronic neurotoxicity in rats was 500 ppm.

Rats and mice were exposed for 6 h day^{-1}, 7 days week^{-1} on gestation days 6–19 for rats and 6–17 for mice at 600, 1800, or 5000 ppm tetrahydrofuran. Pregnant mice that inhaled 5000 ppm tetrahydrofuran died, while those exposed to 1800 ppm were sedated. Some treatment-related effects were reduced fetal body weight and reduced ossification of the sternebrae. The maternal no-observed-adverse-effect level (NOAEL) for both species was 1800 ppm; the NOAEL for developmental toxicity was 1800 ppm in rats and 600 ppm in mice. A two-generation drinking water reproductive study in rats at 0, 1000, 3000, and 9000 ppm resulted in a NOAEL for fertility and reproductive performance of 9000 ppm. A NOAEL of 3000 ppm was also established for general systemic toxicity of parental generations, $F_1$and F_2 litters and developmental toxicity. Developmental effects were reduced pup growth and delayed eye opening.

The National Toxicology Program (NTP) completed prechronic oral gavage and inhalation studies of tetrahydrofuran in male and female Fischer 344 rats and B6C3F1 mice. No chronic gavage study was performed. However, NTP completed an inhalation bioassay with tetrahydrofuran in male and female Fischer 344 rats and B6C3F1 mice, which resulted in a significantly increased incidence of hepatocellular neoplasms in female mice and a positive trend for increased incidence of renal tubule epithelial adenoma or carcinoma (combined) in male rats.

Male and female Fischer 344 rats exposed for 2 years to inhalation concentrations of 200, 600, and 1800 ppm tetrahydrofuran showed marginally increased incidences of renal tubule epithelial adenoma in male rats at the mid- and high- concentration exposures. The combined occurrence of renal tubule epithelial adenoma and carcinoma exhibited a positive trend. The incidence of combined tumors in mid and high dose males exceeded the historical control range. No tumors were observed in female rats. NTP concluded that tetrahydrofuran showed some evidence of carcinogenic activity in male rats but no evidence in female rats. The mode of action for the kidney tumors observed in male rats is via a nongenotoxic mechanism with associated increases in cell proliferation and evidence that supports a role of α-2U-globulin.

Male and female B6C3F1 mice exposed for 2 years to inhalation concentrations of 200, 600, and 1800 ppm tetrahydrofuran showed increased incidences of hepatocellular neoplasms (adenoma and carcinoma) in high dose females (85%) that were significantly greater than chamber controls (34%) and exceeded the historical control range (3–54%). The incidences of hepatocellular neoplasms in male mice (low dose 62%, mid-dose 60%, and high dose 36%) were not significantly different than chamber controls (70%). The historical control range in males is 11–60%. The lower incidence of combined neoplasms in high dose males was attributed to their lower survival rate. NTP concluded that tetrahydrofuran exhibits no evidence of carcinogenicity in male mice but showed clear evidence of carcinogenic activity in female mice. The mode of action for the liver tumor formation is via a nongenotoxic mechanism with evidence of an associated cell proliferation and cytochrome P450 induction.

Human

No information could be found on the effects of chronic exposure of tetrahydrofuran in humans.

In Vitro Toxicity Data

Tetrahydrofuran was not mutagenic in *Salmonella typhimurium* TA100 at 50 µl per plate and it failed to induce sex-linked recessive lethals in *Drosophila melanogaster* by ingestion or injection. In cultured

Chinese hamster ovary cells, there was no indication of induction of chromosomal aberrations or sister chromatid exchanges. The weight of evidence from several studies indicates that tetrahydrofuran is nongenotoxic.

Clinical Management

Those exposed to tetrahydrofuran by inhalation should be monitored for respiratory tract irritation, bronchitis, or pneumonitis. Humidified supplemental 100% oxygen should be administered. Following ingestion, milk or water should be used to dilute the tetrahydrofuran in the stomach. A charcoal slurry with saline cathartic should be administered. Gastric lavage may be indicated. Treatment of CNS depression is symptomatic. Renal and hepatic function should be monitored. Exposed eyes should be irrigated with copious amounts of water for at least 15 min. If irritation, pain, swelling, lacrimation, or photophobia persists, the patient should be seen in a healthcare facility. After dermal exposure, the affected skin should be washed thoroughly with soap and water. If irritation persists, a healthcare facility should be contacted.

Environmental Fate

Tetrahydrofuran is a liquid at room temperature and boils at 66°C. The fugacity model predicts that tetrahydrofuran will be found in the environment where it is released. Photodegradation by hydroxyl radicals in air is estimated to be rapid and the hydroxyl radical reaction half-life is estimated at 7.3 h. Tetrahydrofuran released to water could partition to the water compartment and readily biodegrade, but not hydrolyze. Tetrahydrofuran has a very low bioaccumulation potential as evidenced by its low octanol/water partition coefficient.

Ecotoxicology

Tetrahydrofuran is essentially nontoxic to aquatic organisms. The 96 h LC_{50} value in static acute fish (*Pimephales promelas*) was 2160 mg l^{-1}; 24 h LC_{50} in *Daphnia magna* was 5930 mg l^{-1}; and the 8 days no-observed-effect concentration (NOEC) in algae, *Schenedesmus quandricauda* was 3700 mg l^{-1}. An NOEC of 216 mg l^{-1} was established in a fish early life stage test with the Fathead minnow.

Other Hazards

Tetrahydrofuran is a flammable liquid with a flash point of 6°F and explosive limits ranging from 2% (lower) to 11.8% (upper). It is incompatible with strong oxidizers and lithium-aluminum alloys. Peroxides may accumulate upon prolonged storage in the air.

Exposure Standards and Guidelines

Occupational exposure limits generally range between 50 and 600 ppm internationally and are expressed as an 8 h time-weighted average (TWA), with 200 ppm being most commonly used. The US Occupational Safety and Health Administration, the American Conference of Governmental Industrial Hygienists, and the National Institute for Occupational Safety and Health (NIOSH) have established an 8 h threshold limit value (TLV)TWA of 200 ppm and a 15 min TLV short-term exposure limit of 250 ppm based on irritation and narcosis. NIOSH lists a concentration of 20 000 ppm tetrahydrofuran as immediately dangerous to life and health.

See also: Respiratory Tract; Sensory Organs.

Further Reading

Chhabra RS, Elwell MR, Chou B, Miller RA, and Renne RA (1990) Subchronic toxicity of tetrahydrofuran vapors in rats and mice. *Fundamental and Applied Toxicology* 14: 338–345.

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Tetranitromethane

Ruth Custance and Cathy Villaroman

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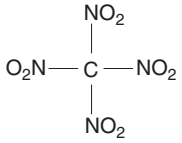
- Chemical Abstracts Service Registry Number: CAS 509-14-8
- Synonyms: TNM; Tetan
- Chemical/Pharmaceutical/Other Class: Aliphatic nitro compounds
- Chemical Formula: CN_4O_8
- Chemical Structure:



Uses

Tetranitromethane is used as an oxidizer in rocket propellants, as a diesel fuel additive, and as an explosive. It is used as a biochemical agent to nitrate tyrosine proteins. It is also used as an organic reagent for detecting the presence of double bonds, and as a mild nitrating reagent, reacting with tyrosine residues in proteins and peptides.

Exposure Routes and Pathways

Tetranitromethane is an oily liquid with a vapor pressure less than that of water. It occurs as an impurity in 2,4,6-trinitrotoluene (TNT). The primary routes of potential human exposure are inhalation and dermal contact. Historically, exposure to tetranitromethane presumably occurred during the manufacture and use of TNT.

Toxicokinetics

No data exist regarding the absorption of tetranitromethane; however, based on toxicity reported in humans and animals, it is clear that it is readily absorbed by the oral route and through inhalation. Rats administered single oral doses exhibited dose-related methemoglobinemia at 90 min, suggesting that the metabolism of tetranitromethane results in the formation of nitrites. Methemoglobinemia was not observed following intravenous or inhalation exposures, suggesting that the blood effects seen in oral studies resulted from nitrase reduction in the gut. No data are available regarding the distribution of absorbed tetranitromethane. No elimination data are available for this compound.

Mechanism of Toxicity

The mechanism of toxicity for tetranitromethane is not known. Methemoglobinemia formation reported following oral administration may be a result of reduction of tetranitromethane in the gut. Nasal lesions observed in lifetime inhalation studies of rats and mice were attributed to the significant irritating properties of the material.

Acute and Short-Term Toxicity (or Exposure)

Animal

Tetranitromethane is a strong irritant of the eyes and respiratory tract in animals. Tetranitromethane is highly toxic to mice and rats by the oral and inhalation routes. In rats with 4 h exposure, an inhalation LC_{50} of 17.5 ppm and an oral LD_{50} of 130 mg kg^{-1} have been reported. The corresponding values in mice are 54.4 ppm and 375 mg kg^{-1}. Effects of overexposure in animals include eye and respiratory irritation, pulmonary edema, lung injury, bronchopneumonia, liver and kidney injury, and in cats, methemoglobinemia. The treatment also affected the body weight of rats and mice and survival of the male animals in the high exposure group.

Human

Tetranitromethane is a strong irritant of the eyes and mucous membranes, which can subsequently cause runny nose, tearing, burning, and redness of the eyes. Other symptoms from acute exposure include coughing, difficult breathing, chest pain, dizziness, and mild skin burns. Workers exposed to the heated tetranitromethane have complained of irritation of the eyes and respiratory tract.

Chronic Toxicity (or Exposure)

Animal

Rats exposed to tetranitromethane at a concentration of 6.4 ppm for 6 h day^{-1}, 5 days week^{-1} for 6 months died; autopsy revealed lung damage. In other lifetime inhalation studies, tetranitromethane caused nasal lesions indicative of chronic irritation of the nasal cavity in rats and mice. In addition, in a National Toxicology Program inhalation bioassay,

tetranitromethane caused increased incidences of alveolar and bronchiolar neoplasms in rats and mice and lung carcinoma in rats.

Human

Chronic exposure to TNT may damage the liver and kidneys with repeated exposure potentially causing low blood cell count (anemia) and nervous system damage. Other symptoms from long-term exposures include headache, drowsiness, chest pain, and respiratory distress. Salivation, shortness of breath, coughing, and pulmonary edema have also been reported. Exposure to high levels can interfere with the ability of the blood to carry oxygen, causing headaches, fatigue, dizziness, and a blue color to the skin and lips (methemoglobinemia). Deaths due to methemoglobinemia and respiratory failure have been reported following exposure to crude TNT.

No adequate human studies have shown a relationship between exposure to tetranitromethane and human carcinogenicity. However, based on sufficient evidence of carcinogenicity in experimental animals, tetranitromethane was classified as possibly carcinogenic to humans (group 2B).

In Vitro Toxicity Data

Tetranitromethane was positive when tested with and without metabolic activation in *Salmonella typhimurium*. In addition, sister chromatid exchanges were induced in cultured Chinese hamster ovary cells when tested without or with a metabolic activation system and chromosome aberrations were also induced. Tetranitromethane also induced DNA single-strand breaks in an *in vitro* assay using primary rat hepatocytes.

Clinical Management

If contact with the liquid occurs, affected areas should be flushed thoroughly with water for at least 15 min. The victim should be observed for burns or resulting irritation. In case of inhalation, the victim should be moved to fresh air, an airway established, and respiration maintained as necessary. The patient should be monitored for irritation and pulmonary edema. If ingestion occurs, emesis should be induced if the victim is conscious. Gastric lavage may be indicated if the victim is unconscious or convulsing. Treatment for methemoglobinemia and/or monitoring for possible liver and kidney injury may be required.

Other Hazards

Tetranitromethane is an oxidizer that may react with a wide variety of materials including organics, brass, zinc, cotton, sodium, pyridine, toluene, aluminum, and finely powdered metals. It is considered heat, friction, and shock sensitive. It may also decompose or react with other chemicals violently.

Exposure Standards and Guidelines

The occupational exposure standards and guidelines for tetranitromethane include the following:

- American Conference of Governmental Industrial Hygienists threshold limit value of 5 ppb ($40\ \mu g\ m^{-3}$).
- US National Institute for Occupational Safety and Health recommended exposure level of 1 ppm as a 10 h time-weighted average, and an immediately dangerous to life and health value of 4 ppm.
- US Occupational Safety and Health Administration permissible exposure limit of 1 ppm ($8\ mg\ m^{-3}$).

Miscellaneous

Tetranitromethane is a colorless to pale yellow, oily liquid with a pungent odor. It is an oxidizer that is highly explosive in the presence of impurities. Tetranitromethane is the primary volatile contaminant of TNT, comprising up to 0.12% of the crude material.

See also: Respiratory Tract.

Further Reading

NTP (1990) National Toxicology Program. Technical Report Series No. 386. Toxicology and Carcinogenesis Studies of Tetranitromethane (CAS 509-14-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NIH Publication No. 90-2841. 207 pp. National Toxicology Program, Research Triangle Park, NC, and Bethesda, MD.

Relevant Websites

http://chem.sis.nlm.nih.gov

http://www.osha.gov – Occupational Safety and Health Administration (OSHA). *Occupational Safety and Health Guideline for Tetranitromethane*. September 1996.

http://ehp.niehs.nih.gov – Tetranitromethane: Tenth Report on Carcinogens.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Tetranitromethane.

Tetrodotoxin

Elizabeth J Scharman

- Chemical Abstracts Service Registry Number: CAS 4368-28-9
- Synonyms: TTX; Tarichatoxin; Tetrodontoxin; Fugu poison; Maculotoxin (MTX); Spheroidine
- Chemical Formula: $C_{11}H_{17}N_3O_8$

Background Information

Tetrodotoxin is a nonprotein, water-soluble, heat-stable neurotoxin found in fish from the order Tetraodontiformes whose suborders include the Tetrodontoidei (pufferfish and porcupine fish) and Moloidei (sunfish). Tetrodotoxin is thought to be identical to tarichatoxin found in selected North American and Japanese newts (e.g., California newt *Tarichatorosa*), salamanders of the family Salamandridae, Central American frogs (genus *Atelopus*), the goby (*Gobius criniger*), some shellfish (e.g., trumpet shell, *Babylonia japonica*), starfish (*Astropecten polyacanthus*), some species of ribbonworm, the flatworm, crab *Atergatus floridus*, horseshoe crab, some species of red calcareous alga, and the Australian blue-ringed octopus.

Exposure Routes and Pathways

Exposure occurs through ingestion of flesh, viscera (e.g., liver, gonads), or skin containing tetrodotoxin. The viscera contain the highest concentration.

Toxicokinetics

Tetrodotoxin is readily absorbed from the gastrointestinal tract. Effects can occur within 10 min to 4 h. The toxin can also be absorbed through the skin.

Mechanism of Toxicity

Tetrodotoxin is believed to be synthesized by a bacterial or dinoflagellate species. Tetrodotoxin blocks axonal transmission by lowering the conductance of sodium at nodes of Ranvier. It is a selective sodium channel blocker that can block nerve and muscle conduction; action potentials are blocked while resting membrane potentials and resting membrane resistance are not affected. Tetrodotoxin does not affect the presynaptic release of acetylcholine or acetylcholine's effects on the neuromuscular junction. Vomiting occurs because the toxin can act directly at or near the chemoreceptor trigger zone. Respiratory depression is caused by either a specific action of tetrodotoxin on the brain's respiratory center or because paralysis of respiratory nerves and muscles occurs.

Acute and Short-Term Toxicity (or Exposure)

Human

A dose of 1–2 mg of purified tetrodotoxin can be lethal; however, because the concentration of tetrodotoxin varies greatly among species, a toxic quantity of pufferfish or other tetrodoxin containing species is not well defined. Paresthesia of the lips and tongue begins shortly after ingestion. Facial and extremity paresthesias and numbness follow. Diaphoresis, hypersalivation, dysphagia, vomiting, diarrhea, and abdominal pain occur early in the course of toxicity as do lightheadedness, dizziness, headache, ataxia, and weakness. Weakness develops first in the hands and arms and then in the legs. Hypoventilation and speech difficulties occur followed by ascending flaccid paralysis with respiratory depression. If ventilation is maintained, victims may remain conscious even though they are paralyzed. Hypotension, dysrhythmias, and seizures may develop. Death occurs within 4–6 h; usually from respiratory muscle paralysis. The prognosis is stated to be good if the patient survives the first 24 h.

In Vitro Toxicity Data

Tetrodotoxin is an important research tool because of its unique voltage gated sodium channels blocking properties. Applications include assessment of pain, basic physiology of nerve generation and organization, understanding of hearing, to bladder pain.

Clinical Management

No antidote is available. Tetrodotoxin is adsorbed by activated charcoal. Treatment is symptomatic and supportive with special attention to airway management and cardiac support.

See also: Marine Organisms; Shellfish Poisoning, Paralytic.

Further Reading

CDC (1996) Tetrodotoxin poisoning associated with eating puffer fish transported from Japan–California. *Morbidity and Mortality Weekly Report* 45: 389–391.

King BR, Hamilton RJ, and Kassutto Z (2000) 'Tail of newt': An unusual ingestion. *Pediatric Emergency Care* 16: 268–269.

Noguchi T and Ebesu JSM (2001) Puffer poisoning: Epidemiology and treatment. *Journal of Toxicology: Toxin Reviews* 20: 1–10.

Texas City Disaster

Paramasivam Srinivasan

The Texas City Disaster is generally considered to be one of the most significant industrial accidents in US history. The following provides a brief summary of the event.

Incident Narrative

The clear spring morning of April 16, 1947, was a morning that many citizens of Texas City thought was the end of the world. The French Liberty ship, SS *Grandcamp*, bearing a cargo of ammonium nitrate fertilizer (over 2300 tons) destined for war-torn Europe, caught fire in the Texas City harbor. The bright orange color that came out of the black smoke caught the attention of passers by. Many people, including children, gathered to watch firefighters putting out the fire. The crowd did not have any idea that potentially explosive materials were stored in the ship.

There was neither a full-scale emergency response plan nor a tug boat to tow the dangerously burning *Grandcamp* away from the port. A little after 9.00 a.m., the Texas City Disaster (as it is popularly known) occurred as the ship *Grandcamp* exploded. A great column of smoke shot up to an estimated 2000 ft, followed in ~10 s, by another even more violent shockwave. Within minutes of the second blast, the Monsanto Chemical Plant was in flames that resulted from broken lines and shattered containers. Entire buildings collapsed and people were trapped inside. Fire spread to the refineries that made up the Texas City industrial complex.

Another catastrophic event happened when a miniature tidal wave (resulted due to the bay water being thrown away by the explosion) swept everything in its path of 150 ft inland and over the docks. Fear mounted throughout the night of April 16, 1947, as the another firefighter the High Flyer, loaded with ammonium nitrate and sulfur, burning all day long, exploded at early morning 1.10 a.m. on April 17, 1947. The fire from High Flyer destroyed a concrete warehouse and a grain elevator and triggered even more fires.

The losses from the disaster were unprecedented. Nearly 600 deaths in a town of ~16 000 was a terrible toll. Not a single family could be found that did not suffer a death, an injury, or severe property damage.

Ammonium Nitrate Characteristics

The chemical compound ammonium nitrate, the nitrate of ammonium with chemical formula NH_4NO_3 is commonly used in agriculture as a high-nitrogen fertilizer. It is a crystalline powder, varying in color from almost white to brown. As a strong oxidizing agent, it has applications as a component of explosives. Ammonium nitrate decomposes into gases including oxygen when heated (nonexplosive reaction); however, ammonium nitrate can be induced to decompose explosively by detonation.

Ammonium Nitrate Involved in Texas City Explosion

The ammonium nitrate involved in the Texas City explosion was brown in color and in small pellets or grains about the size of medium grains of sand. It was packed in six-ply moisture-proof paper bags, two of which were impregnated with some material, apparently an asphaltic compound.

Causes for the Explosion

During the time frame of occurrence of this explosion, little was known regarding the hazards of ammonium nitrate to anyone handling or storing this commodity. The false security engendered in the handling of ammonium nitrate, which was such a major factor in this disaster, was caused by the improper labeling of the paper bags. No instruction was printed on the bags concerning the handling of the material nor was it labeled as being a hazardous chemical. The storage of ammonium nitrate pending

shipment either by ship or railroad had not received the attention it deserved.

Selected web information related to the Texas City Disaster event says whether or not the fire originated from smoking. Smoking in piers or on docks must be considered as a common source of ignition and always be prohibited regardless of the cargo being handled. The use of open lights in these same areas should carry the same restriction as smoking regulations.

Health and Safety Measures

The lessons learned from the Texas City Disaster event from health and safety viewpoint are:

- Anyone dealing with or handling ammonium nitrate should be fully advised of the hazardous nature of the chemical and fully instructed about the proper methods of storage and handling. Proper labeling of the containers is of utmost importance.
- Material should be stored only in masonry or fireproof sprinkled buildings on skids or pallets on concrete floors with at least 1 ft clearance from walls.
- Storage should preferably be in separate fire divisions from highly combustible commodities or well segregated from not so highly combustible commodities such as sulfur, flour, sugar, compressed cotton, and charcoal.
- Intimate contact with metals such as cadmium, zinc, copper, tin, and lead must be avoided.
- A minimum clearance of 5 ft should be maintained between ammonium nitrate and other chemicals.
- Any ship with hazardous material such as ammonium nitrate as cargo entering a port must notify the port facility who in turn should notify the chief of the fire department immediately.
- Fire departments combating ammonium nitrate fires should use only water in large quantities (applied gently so as not to scatter the material) as an extinguishing agent.
- Fire in ammonium nitrate usually generates large quantities of oxides of nitrogen gases which are extremely toxic and therefore all personnel entering the fire area must wear masks approved for use in such locations.

See also: Ammonium Nitrate; Cadmium; Copper; Lead; Tin; Zinc.

Further Reading

Stephens HW (1997) *The Texas City Disaster, 1947*, 1st edn. Austin, TX: University of Texas Press.

Relevant Websites

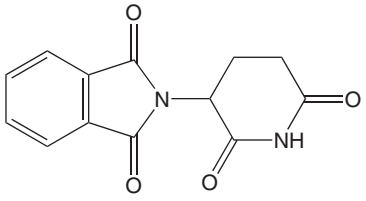
http://www.local1259iaff.org – The Texas City Disaster. April 16, 1947.

http://www.tsha.utexas.edu – Handbook of Texas Online. Texas City Disaster.

Thalidomide

S Rutherfoord Rose

- Chemical Abstracts Service Registry Number: CAS 50-35-1
- Synonyms: K-17; 2-Phthalimidoglutarimide; *N*-Phthalylglutamic acid imide; *N*-(2,6-Dioxo-3-piperdyl)-phthalimide; Talimol®; Sedalis®; Kevadon®; Distavil®; Thalomid®; NSC-66847
- Chemical/Pharmaceutical/Other Class: Thalidomide is a piperidinedione derivative
- Chemical Formula: $C_{13}H_{10}N_2O_4$
- Chemical Structure:



Uses

Thalidomide was formerly used as a sedative/hypnotic. It was approved in 1998 by the US Food and Drug Administration for use as an immunosuppressant in the treatment of erythema nodosum leprosum. It is also used in the treatment of graft-versus-host disease (Orphan drug status in the United States), macular degeneration, oral ulcers in AIDS patients, and reflex sympathetic dystrophy associated with chronic pain syndromes.

Background Information

Thalidomide was first marketed as a sedative/hypnotic agent in Germany in 1956 and in the United Kingdom 2 years later. It was subsequently withdrawn from the market in 1961 after more 12 000 reports of fetal abnormalities, particularly phocomelias.

Exposure Routes and Pathways

Exposures have only been reported by ingestion.

Toxicokinetics

All available data are derived from therapeutic dosing. Peak plasma levels occur 2–6 h following oral doses. Bioavailability in animals varies from 67% to 93%. It is a nonpolar compound that is extensively bound to plasma proteins and has a volume of distribution of ~120 l in healthy adults. There are conflicting data on whether thalidomide undergoes hepatic metabolism. Less than 1% of a dose is excreted unchanged in the urine, suggesting that elimination is largely nonrenal. The serum elimination half-life is ~8 or 9 h after a single oral dose.

Mechanism of Toxicity

Thalidomide has significant teratogenic effects in humans, and it also affects the central and peripheral nervous systems through unknown mechanisms. Evidence of a toxic arene oxide metabolite is unsubstantiated. Thalidomide likely inhibits neutrophil chemotaxis and monocyte phagocytosis, inhibits free radical formation, and alters the ratio of helper and suppressor T-cells. Reduced formation of tissue necrosis factor-α may be at least partially responsible for the antiinflammatory effects of thalidomide.

Acute and Short-Term Toxicity (or Exposure)

Human

In addition to phocomelias, other teratogenic effects include eye and ear abnormalities, esophageal and duodenal atresias, and defects in internal organs such as the heart and kidneys. Congenital defects of the kidneys and nervous system may persist throughout life. Very large doses taken with ethanol have been associated with transient hypotension. Bradycardia has been rarely reported with therapeutic use. Acute toxicity appears infrequent.

Chronic Toxicity (or Exposure)

Animal

Teratogenic effects of thalidomide are well described in several animal models. Pregnant cats have tolerated doses of $500\,mg\,kg^{-1}\,day^{-1}$ of thalidomide with no fetal toxicity evident in offspring. Many rat strains have had no teratogenic effects seen at doses of $4000\,mg\,kg^{-1}\,day^{-1}$ during pregnancy.

Human

Adverse reactions include dose-related peripheral neuropathy (primarily sensory), nausea, vomiting, constipation, dry mouth, headache, and erythematous rashes. Dose-related central nervous system depression is relatively common. Thalidomide is contraindicated in pregnancy and in women of childbearing age.

In Vitro Toxicity Data

Ames *Salmonella*, *Drosophila*, and male mouse sperm morphology assays of thalidomide have been negative; mutagenicity tests using *Allium cepa* and *Vicia faba* have been positive.

Clinical Management

Patients with thalidomide overdose should receive supportive care with attention to airway maintenance. There are no antidotes and no data to support measures to enhance elimination of thalidomide. Hypotension should be treated if needed with intravenous fluids, positioning, and pressors as needed.

See also: Neurotoxicity.

Further Reading

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Nasca MR, Micalli G, Cheigh NH, *et al.* (2003) Dermatologic and nondermatologic uses of thalidomide. *Annals of Pharmacotherapy* 37(9): 1307–1320.

Tseng S, Pak G, Washenik K, *et al.* (1996) Rediscovering thalidomide: A review of its mechanism of action, side effects, and potential uses. *Journal of the American Academy of Dermatology* 35: 969–979.

Relevant Website

http://www.nlm.nih.gov – US National Library of Medicine. Thalidomide: Potential Benefits and Risks. Current Bibliographies in Medicine 97-4. August 1997.

Thallium

Shayne C Gad

- Selected Compounds: Thallium nitrate ($TlNO_3$); Thallium sulfate (Tl_2SO_4)
- Chemical Abstracts Service Registry Number: CAS 7440-28-0
- Synonym: Ramor
- Chemical/Pharmaceutical/Other Class: Metal
- Chemical Formulas: Tl^+; Tl^{3+}

Uses

Thallium is a by-product of iron, cadmium, and zinc refining. It is used in metal alloys, imitation jewelry, optical lenses, artists' pigments, semiconductors, ceramics, and X-ray detection devices. It has limited use as a catalyst in organic chemistry. In the past, thallium (chiefly thallium sulfate) was used as a rodenticide and insecticide. Its use as a rodenticide was outlawed in 1965 due to its severe toxicity (a source of accidental and suicidal human exposures). Medicinally, it has been used as a depilatory and in the treatment of venereal disease, skin fungal infections, and tuberculosis.

Background Information

It was discovered in 1861, and occurs in the earth's crust at 0.7 ppm. Thallium is a heavy metallic element that exists in the environment mainly combined with other elements (primarily oxygen, sulfur, and the halogens) in inorganic compounds.

Exposure Routes and Pathways

Industrial poisoning from thallium is a special risk in the manufacture of fused halides for the production of lenses and windows. Humans may be exposed to thallium by ingestion, inhalation, or dermal absorption. However, the general population is exposed most frequently by ingestion of thallium-containing foods, especially homegrown fruits and green vegetables. Thallium is a waste product of coal combustion and the manufacturing of cement, and inhalation of contaminated air near emission sources or in the workplace may also contribute to thallium exposure of some individuals.

Toxicokinetics

Thallium and thallium salts are readily absorbed by virtually all routes, with gastrointestinal exposure being the most common route to produce toxicity. Thallium also crosses the placenta freely. Thallium enters cells by a unique process governed by its similarity in charge and ionic radius to potassium. Unlike potassium, however, once thallium enters the cells, it is released slowly. It can concentrate in the liver and kidneys. Since it is soluble at physiological pH, it does not form complexes with bone. Most thallium is excreted in the urine, but it is excreted slowly and can be detected months after exposure.

Mechanism of Toxicity

Thallium's mechanism of toxicity is related to its ability to interfere with potassium ion functions. Thallium interferes with energy production at essential steps in glycolysis, the Kreb's cycle, and oxidative phosphorylation. Other effects include inhibition of sodium–potassium–adenosine triphosphatase and binding to sulfhydryl groups.

Acute and Short-Term Toxicity (or Exposure)

Animal

Among animal species, the toxicity of thallium acetates, nitrates, and sulfates varies. In rats, the LD_{50} ranges from 15 to 30 mg kg^{-1}. The oxide is slightly less toxic (LD_{50}, 70 mg kg^{-1}).

Human

Unlike exposure to most metal salts, gastrointestinal symptoms of thallium toxicity are relatively minor, and constipation is more characteristic than diarrhea. The major manifestations of toxicity consist of a rapidly progressive, ascending, extremely painful sensory neuropathy and alopecia. Other potential symptoms of overexposure are nausea, diarrhea, abdominal pain, and vomiting; seizure, tremor and psychosis. Thallium is one of the most toxic of all metals. It is a cumulative poison with an estimated lethal dose of 8–20 mg kg^{-1} in humans. It is difficult to predict the out come of thallium poisoning. With high exposure, death results very soon.

Hair loss throughout the body is common and begins a little over a week after exposure. Gastrointestinal symptoms include abdominal pain and

bleeding and ulceration of the colon. Neurological signs appear within a few days of exposure.

Thallium crosses the placental barrier and can be active in the last trimester of pregnancy. Loss of hair, and nail deformation are noted in exposed newborns. Loss of vision plus the other signs of thallium poisoning have been related to industrial exposures.

Chronic Toxicity (or Exposure)

Animal

Thallium has not been shown to be carcinogenic, although rats that were chemically exposed developed papillomas and exhibited inflammatory proliferation in the forestomach. Thallium causes malformation in chicks; however, teratological studies in animals produced ambiguous results.

Human

Regardless of the entry route, the major symptoms of thallium poisoning are gastrointestinal stress, neurological problems, and hair loss. Pain develops, fingers become numb, motor weakness is noted, and lower limbs may become paralyzed. The eyes become inflamed and retrobulbar neuritis with some loss of central vision follows. Intraocular hemorrhage, formation of cataracts, and optic nerve atrophy can occur.

Myocardial damage with EKG changes can result, and hypotension followed by hypertension can occur. Although thallium can concentrate in the kidneys, renal damage occurs in some cases (it is not generally extensive).

Clinical Management

For acute exposure, ipecac should be administered and lavage performed. The use of single- or multiple-dose activated charcoal is supported by *in vitro* binding experiments and some animal data, and charcoal hemoperfusion may be a useful adjunct. Forced potassium diuresis appears to be harmful. Hemodialysis is also recommended with potassium administration. Since calcium metabolism is disturbed, supplementary calcium is indicated. The use of traditional metal chelators such as dimercaprol (British antilewisite) and penicillamine is not supported by the available evidence. In fact, the use of penicillamine may lead to redistribution of thallium into the central nervous system. Multiple animal studies have found evidence of enhanced elimination and improved survival with Prussian blue; however, despite the fact that many humans have been treated with Prussian blue, the data presented are insufficient to judge its true efficacy. Despite this, one publication notes that Prussian blue's safety profile is superior to that of other proposed therapies, and that it should be considered the drug of choice in acute thallium poisoning.

Environmental Fate

Thallium is quite stable in the environment, since it is neither transformed nor biodegraded. Thallium is bioaccumulated and biomagnified.

Compounds of thallium are generally soluble in water and the element is found primarily as the monovalent ion (Tl^+). Thallium tends to be sorbed to soils and sediments, and to bioconcentrate in aquatic plants, invertebrates, and fish. Terrestrial plants can also absorb thallium from soil. Thallium may be bioconcentrated by organisms from water. The (US) Environmental Protection Agency has identified several 'National Priorities List' sites polluted by thallium.

Ecotoxicology

Environmental concerns are growing, mostly because thallium is a waste product of coal combustion and the manufacturing of cement. Thallium poisoning has been observed in many wildlife populations of the Great Lakes basin. Major releases of thallium to the environment are from processes such as coal-burning and smelting, in which thallium is a trace contaminant of the raw materials, rather than from facilities producing or using thallium compounds.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value, 8 h time-weighted average, for thallium (elemental and soluble compounds) is 0.1 mg m^{-3} with a skin exposure warning.

See also: Metals; Sensory Organs.

Further Reading

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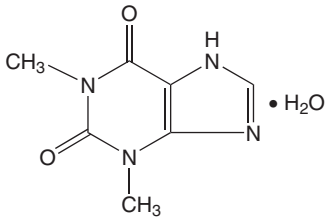
Theophylline

Henry A Spiller

- Chemical Abstracts Service Registry Number: CAS 58-55-9
- Synonyms: 1,3-Dimethylxanthine; Anhydrous theophylline; Elixophyllin SR; Somophyllin; Theophyl; Theolair; Slo-Bid; Slo-phyllin; Theodur. Aminophylline is the ethylenediamine salt of theophylline
- Chemical/Pharmaceutical/Other Class: A naturally occurring methylxanthine derivative structurally related to caffeine
- Chemical Formula: $C_7H_8N_4O_2$
- Chemical Structure:



Uses

Theophylline is used as a bronchodilator in the treatment of asthma and reversible bronchospasm associated with chronic bronchitis and emphysema. Unlabeled use includes treatment of sleep apnea in neonates.

Background Information

Theophylline is a potent methylxanthine. Methylxanthines are widely distributed in the plant world in plants such as *Thea sinensis*, *Coffea Arabica* (coffee), and *Theobroma cacao* (coco, chocolate).

Exposure Routes and Pathways

Ingestion of sustained-release products is the most common route of both accidental and intentional exposure to theophylline. Theophylline is available in oral and intravenous dosage forms. Aminophylline is available in oral, rectal, and intravenous dosage forms.

Toxicokinetics

In therapeutic oral dosing, theophylline is well absorbed, producing peak serum levels in 2 h. However, overdose with the commonly available sustained-release formulations produces a delayed absorption pattern, with peak levels as late as 16 h postingestion. The matrices of these sustained-release formulations may agglutinate, with the potential to form pharmacobezors, further altering and delaying the absorption phase. In adults and children, theophylline is metabolized in the liver by oxidation and *N*-demethylation, producing 3-methybcanthine, 1,3-dimethyluric acid, and 1-methyluric acid. In premature neonates, minimal biotransformation occurs, with the main metabolite being caffeine. The average volume of distribution is $0.45\,l\,kg^{-1}$. Protein binding is 40%. The elimination half-life varies by age. The average half-lives by age are as follows: adults, 6 or 7 h; children 6 months to 13 years, 3.5–4 h; children less than 6 months, 7 h; and neonates, 20 h.

Mechanism of Toxicity

The mechanism of action is multifactorial. Suggested theories of action include increased cellular cyclic adenosine monophosphate levels via inhibition of phosphodiesterase, increased turnover of monoamines in the central nervous system, inhibition of prostaglandins, and antagonism of adenosine receptors. Theophylline causes a release of endogenous catecholamines. There is a positive inotropic and dose-dependent chronotropic response. Hypokalemia, hypercalcemia, and hyperglycemia are caused by a mechanism regulated by the beta-adrenergic system. Methylxanthines are weak diuretics by inhibition of renal tubular sodium resorption. Antagonism of adenosine receptors may play a role in the seizures seen with theophylline.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxicity would be expected from ingestion of pharmaceutical sources. Methylxanthines are not commonly used in animals. Limited information on toxicity exists. Tachyarrhythmias, hypotension, and seizures have been seen.

Human

Theophylline has a narrow therapeutic index, with 12–25% of overdose patients developing serious or life-threatening symptoms. Age >60 years and chronic use are risk factors for increased morbidity and mortality.

In acute overdose, peak serum levels $>100\,\mu g\,ml^{-1}$ may be predictive of arrhythmias and seizures. The use of sustained-release formulations and the presence of pharmacobezors in the gut may make it difficult to determine peak serum levels. Sinus tachycardia is the most common cardiac sign of theophylline toxicity. Ventricular and supraventricular tachycardia, ectopic beats, hypotension, and cardiac arrest may occur. Metabolic acidosis, hypokalemia, hypercalcemia, and hyperglycemia may be seen. Tremulousness and agitation frequently occur. Intractable seizures may occur in severe intoxications, probably secondary to adenosine receptor antagonism in the brain. Onset of seizures is a poor prognostic indicator. Persistent vomiting is commonly seen and may interfere with attempts at therapy.

Chronic Toxicity (or Exposure)

Animal

Rats and mice fed theophylline daily over 2 years found no evidence of carcinogenic activity at doses up to $75\,mg\,kg^{-1}$.

Human

In chronic overdose, peak serum levels $>40\,\mu g\,ml^{-1}$ are suggestive of increased risk of serious toxicity. The first sign of chronic theophylline toxicity may be development of seizures. Sinus tachycardia is another common cardiac finding in theophylline poisoned patients. Ventricular and supraventricular tachycardia, ectopic beats, hypotension, and cardiac arrest may occur. Metabolic acidosis, hypokalemia, hypercalcemia, and hyperglycemia may be seen. Onset of seizures is a poor prognostic indicator.

In Vitro Toxicity Data

Mutagenicity studies using sister-chromatid exchange and *Allium cepa* models were positive; studies using the Ames *Salmonella* assay have been negative.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Activated charcoal effectively adsorbs theophylline and should be employed in both acute and chronic overdoses. Multiple-dose activated charcoal (MDAC) has been shown to significantly increase drug clearance and reduce serum theophylline levels. If persistent vomiting interferes with the administration of MDAC, antiemetics and ranitidine may be effective. Ventricular dysrhythmias may respond to lidocaine. Tachyarrhythmias as well as ventricular dysrhythmias unresponsive to lidocaine may respond to beta blockers or verapamil. Beta blockers may control dysrhythmias, as well as reverse hypotension and hypokalemia. However, beta-blockers should be used with caution in persons with a history of asthma. Seizures should be treated with benzodiazepines or phenobarbital. Intractable seizures may require midazolam or pentobarbital. Phenytoin is ineffective in theophylline-induced seizures. Extracorporeal removal (ECR) may improve outcome if instituted before the onset of life-threatening symptoms. Hemoperfusion with a charcoal cartridge has been used on conjunction with hemodialysis to further enhance drug extraction when available. ECR should be considered in acute overdose patients with levels $>100\,\mu g\,ml^{-1}$, patients older than 60 years with levels $>50\,\mu g\,ml^{-1}$, and chronic overdose patients with levels $>40\,\mu g\,ml^{-1}$. Due to the routine use of sustained-release theophylline preparations, early serum level measurements may not be representative of the peak level. Repeated assessment of theophylline blood levels is required.

Environmental Fate

Screening tests for biodegradability indicate that theophylline may be biodegradable in soil and water. The adsorption of theophylline to suspended solids and sediments in water and to soil should be unimportant. The estimated bioconcentration factor indicates that bioconcentration of theophylline in aquatic organisms should not be important.

See also: Caffeine.

Further Reading

Emerman CL, Devlin C, and Connors AF (1990) Risk of toxicity in patients with elevated theophylline levels. *Annals of Emergency Medicine* 19: 643–648.

Olson KR, Benowitz NL, and Woo OF (1985) Theophylline overdose: Acute single ingestion versus chronic repeated overmedication. *American Journal of Emergency Medicine* 3: 386–394.

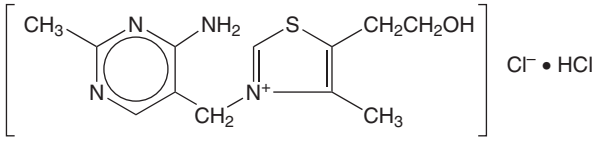
Olson KR, Kearney TE, and Dyer JE (1993) Seizures associated with poisoning and drug overdose. *American Journal of Emergency Medicine* 11: 565–568.

Thiamine

Diana Ku

This article is a revision of the previous print edition article by Denise L Kurta, volume 3, p. 230, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 67-03-8
- Synonyms: Vitamin B_1; Aneurine hydrochloride; Thiamine hydrochloride; Thiadoxine; Thiamin; Vitamin B_1 hydrochloride
- Chemical/Pharmaceutical/Other Class: Water-soluble vitamin
- Chemical Formula: $C_{12}H_{17}ClN_4OS$
- Chemical Structure:



Uses

Thiamine is a nutritional supplement used during periods of deficiency known as beriberi and its manifestations such as Wernicke–Korsakoff syndrome. Thiamine needs increase during diseases of the small intestine, malabsorption, congenital metabolic dysfunction, liver disease, alcoholism, and during pregnancy and lactation. Supplementation of thiamine for treatment of Alzheimer's disease, congestive heart failure, and cataracts has been investigated; however, evidence is unclear as to its benefits at this time.

Background Information

In 1912, Cashmir Funk isolated thiamine from rice husks and coined the term 'vitamine' because they were required for life ('vita') and because thiamine contained nitrogen ('amine'). The original term 'vitamine' was changed to 'vitamin' when scientists identified and purified all the vitamins and discovered that they did not all contain the element nitrogen.

Exposure Routes and Pathways

Routes of exposure are oral, intravenous, and intramuscular. Dietary sources include cereal grains, the hull of rice, yeast, peas, beans, pork, and beef.

Toxicokinetics

Thiamine is readily absorbed from the gastrointestinal tract mainly in the duodenum. It is hepatically metabolized and widely distributed to almost all body tissues. Thiamine is renally excreted almost entirely as metabolites. Excess thiamine (beyond the daily body need) is excreted unchanged and as metabolites in the urine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity not expected.

Human

Acute toxic effects are not expected even with doses of 50–100 times the recommended daily allowance; however, hypersensitivity reactions have been reported.

Chronic Toxicity (or Exposure)

Animal

It would be unlikely for animals to be given chronic thiamine overdoses. Very large parenteral doses of thiamine have produced neuromuscular and ganglionic blockage in animal studies.

Human

Chronic large doses of more than $3\,g\,day^{-1}$ may cause headache, irritability, insomnia, weakness, tremors, ulcers, and tachycardia.

In Vitro Toxicity Data

There are no reports of congenital anomalies among children born to mothers who used large doses of pyridoxine during pregnancy.

Clinical Management

Acute ingestions seldom require treatment. Chronic excessive use should be discontinued and any toxic effects treated symptomatically.

See also: Dietary Supplements.

Further Reading

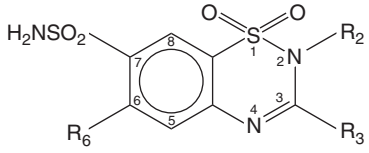
Rodriguez-Martin JL, Qizilbash N, and Lopez-Arrieta JM (2001) Thiamine for Alzheimer's disease. *Cochrane Database of Systematic Reviews* 2: CD001498.

Thomson AD (2000) Mechanisms of vitamin deficiency in chronic alcohol misusers and the development of the Wernicke–Korsakoff syndrome. *Alcohol & Alcoholism* 35(Suppl 1): 2–7.

Thiazide Diuretics

Elizabeth J Scharman

- Chemical Abstracts Service Registry Numbers:
  - Bendroflumethiazide: CAS 73-48-3
  - Benzthiazide: CAS 91-33-8
  - Chlorothiazide: CAS 58-94-6
  - Chlorthalidone (phthalimidine derivative; similar to thiazides in structure and pharmacology): CAS 77-36-1
  - Hydrochlorothiazide: CAS 58-93-5
  - Hydroflumethiazide: CAS 135-09-1
  - Indapamide (an indoline; similar to thiazides in structure and pharmacology): CAS 26807-65-8
  - Methyclothiazide: CAS 135-07-9
  - Metolazone (quinazoline derivative; similar to thiazides in structure and pharmacology): CAS 17560-51-9
  - Polythiazide: CAS 346-18-9
  - Quinethazone (quinazoline derivative; similar to thiazides in structure and pharmacology): CAS 73-49-4
  - Trichlormethiazide: CAS 133-67-5
- Synonyms:
  - Bendroflumethiazide: Bendrofluazide; Benzydroflumethiazide; Naturetin®
  - Benzthiazide: Benzothiazide; Exna®
  - Chlorothiazide: 6-Chloro-7-sulfamoyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide; Diuril®
  - Chlorthalidone: Chlorphthalidolone; Hygroton®
  - Hydrochlorothiazide: HCTZ; 3,4-Dihydrochlorothiazide; Esidrex®; Oretic®
  - Hydroflumethiazide: Trifluoromethylhydrothiazide; Dihydroflumethiazide; Diucardin®
  - Indapamine: *N*-(3-Sulfamyl-4-chlorobenzamido)-2-methylindoline; Lozol®
  - Methyclothiazide: 6-Chloro-3-chloromethyl-2-methyl-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide; Enduron®
  - Metolazone: 2-Methyl-3-*o*-tolyl-6-sulfamyl-7-chloro-1,2,3,4-tetrahydro-4-quinazolinone; Mykrox® (rapid and complete absorption); Zaroxolyn® (slow and incomplete absorption)
  - Polythiazide: 6-Chloro-3,4-dihydro-2-methyl-7-sulphamoyl-3-(2,2,2-trifluoroethylthiomethyl)-2*H*-benzo-1,2,4-thiadiazine-1,1-dioxide; Renese®
  - Quinethazone: 7-Chloro-2-ethyl-6-sulfamoyl-1,2,3,4-tetrahydro-4-quinazolinone; Hydromox®
  - Trichlormethiazide: 3-Dichloromethylhydrochlorothiazide; Hydrotrichlorothiazide; Metahydrin®; Naqua®
- Chemical/Pharmaceutical/Other Class: Diuretic; Antihypertensive
- Chemical Formulas:
  - Bendroflumethiazide: $C_{15}H_{14}F_3N_3O_4S_2$
  - Benzthiazide: $C_{15}H_{14}ClN_3O_4S_3$
  - Chlorothiazide: $C_7H_6ClN_3O_4S_2$
  - Chlorthalidone: $C_{14}H_{11}ClN_2O_4S$
  - Hydrochlorothiazide: $C_7H_8ClN_3O_4S_2$
  - Hydroflumethiazide: $C_8H_8F_3N_3O_4S_2$
  - Indapamine: $C_{16}H_{16}ClN_3O_3S$
  - Methyclothiazide: $C_9H_{11}Cl_2N_3O_4S_2$
  - Metolazone: $C_{16}H_{16}ClN_3O_3S$
  - Polythiazide: $C_{11}H_{13}ClF_3N_3O_4S_3$
  - Quinethazone: $C_{10}H_{12}ClN_3O_3S$
  - Trichlormethiazide: $C_8H_8Cl_3N_3O_4S_2$
- Chemical Structure:



Uses

Thiazide diuretics are used in the management of edema, the management of hypertension, the treatment of nephrogenic diabetes insipidus, and the prophylaxis of renal calculus formation.

Exposure Routes and Pathways

Ingestion is the most common route of both accidental and intentional exposures to the thiazide diuretics. Thiazides and related diuretics are available in oral dosage forms. Chlorothiazide is also available in a parenteral dosage form.

Toxicokinetics

Thiazides are absorbed in varying degrees from the gastrointestinal tract. Bendroflumethiazide is ~100% absorbed, benzthiazide 25%, chlorothiazide 10–21%, chlorthalidone 65%, hydrochlorothiazide 65–75%, hydroflumethiazide 50%, indapamide 93%, and metolazone 40–65%; absorption data are unavailable for the other drugs in this class. Plasma levels do not correlate with diuretic effects. The onset of diuretic action occurs at 2 h with the exception of metolazone whose onset is 1 h, indapamide 1–2 h, and

chlorthalidone 2–3 h. Peak diuretic effects occur within 2 h for indapamide and metolazone, 2–6 h for chlorthalidone, 4–6 h for benzthiazide and hydrochlorothiazide, 4 h for bendroflumethiazide, chlorothiazide, and hydroflumethiazide, and 6 h for methyclothiazide, polythiazide, quienthazone, and trichlormethiazide. Following intravenous administration of chlorothiazide, the onset of action occurs in 15 min with a peak effect occurring in 30 min. The diuretic duration of action is 2 h for chlorothiazide (intravenous), 6–12 h for bendroflumethiazide, chlorothiazide (oral), and hydrochlorothiazide, 12–18 h for benzthiazide, 24–72 h for chlorthalidone, 12–24 h for hydroflumethiazide, 24 h for methyclothiazide and trichlormethiazide, 12–24 h for metolazone, 24–48 h for polythiazide, and 18–24 h for quinethazone. The antihypertensive effects of these agents may not appear for 3–4 days with maximum effect being delayed for 3–4 weeks. The volume of distribution for chlorothiazide is $0.2\,l\,kg^{-1}$, chlorthalidone $3.9\,l\,kg^{-1}$, and hydrochlorothiazide $0.83\,l\,kg^{-1}$. Thiazides cross the placenta and are excreted into breast milk. Bendroflumethiazide is 94% protein bound, chlorothiazide 20–80% protein bound, chlorthalidone 75% protein bound, hydrochlorothiazide 64% protein bound, hydroflumethiazide 74% protein bound, indapamine 79% protein bound, metolazone 95% protein bound, and polythiazide 84% protein bound. Thiazides are primarily excreted unchanged in urine. The half-life of bendroflumethiazide is 3 h, chlorothiazide 1.5 h, chlothalidone 44 h, hydrochlorothiazide 2.5 h, hydroflumethiazide 2–17 h (biphasic), indapamide 14–18 h, metolazone (Mykrox®) 8–14 h, polythiazide 25.7 h, and trichlormethiazide 2.3 h. The antihypertensive effect of these agents may persist for a week after therapy is discontinued.

Mechanism of Toxicity

Thiazides and the related diuretics inhibit the transport of sodium in the early distal tubules, which results in the enhanced elimination of sodium, chloride, and water. Potassium and sodium bicarbonate elimination is also enhanced; calcium excretion is decreased, uric acid is retained. Glomerular filtration rate is decreased. The antihypertensive effects may be the result of direct arteriolar dilation but the full mechanism has not been identified.

Acute and Short-Term Toxicity (or Exposure)

Animal

Chlorothiazide is used therapeutically in dogs and cattle. Hydrochlorothizide is used therapeutically in dogs, cats, and cattle. Toxic effects are similar to those seen in humans.

Human

Determination of toxicity is based on observation as there is no milligram per kilogram toxic dose established. Ingestion of amounts exceeding maximum daily doses has been tolerated in children. Overdose may result in diuresis with accompanying fluid and electrolyte loss, lethargy, and coma. Clinical effects seen, which are secondary to the fluid and electrolyte loss, include hypotension, tachycardia, contraction alkalosis, muscle weakness, headache, and dysrhythmias.

Chronic Toxicity (or Exposure)

Animal

Syrian golden hamsters fed hydrochlorothiazide up to $4\,mg\,kg^{-1}\,day^{-1}$ for 6 months developed increased cholesterol and trigyceride levels. Dogs given doses up to 200 mg hydrochlorothiazide daily for 9 months all developed enlarged parathyroid glands.

Human

Side effects that may occur include hypokalemia, hypomagnesemia, hyponatremia, hyperglycemia, hyperuricemia or gout, anorexia, orthostatic hypotension, anorexia, nausea, and photosensitivity.

In Vitro Toxicity Data

Mutagenicity studies using Ames *Salmonalla* assays have been negative; studies of sister chromatid exchange and mouse lymphoma line assays have been positive.

Clinical Management

Most cases of unintentional thiazide overdoses can be managed safely at home as serious effects are not expected. Thiazides and related agents are adsorbed by activated charcoal and it may be used for substantial recent exposures. Because cathartics can also cause fluid and electrolyte losses, their use should be avoided. Fluid status, electrolytes, and EKG should be monitored. Standard supportive therapies with attention to replacement of fluid and electrolyte losses should be utilized as clinically necessary. No antidote is available. Drug levels are not readily available and are not helpful in assessing toxicity.

See also: Kidney.

Further Reading

Farge D, Turner MW, and Roy DR (1986) Dyazide-induced reversible acute renal failure associated with intracellular crystal deposition. *American Journal of Kidney Diseases* 8: 445–449.

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Thioacetamide

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 62-55-5
- Synonyms: Acetothioamide; Ethanethioamide; Thiacetamide; TAA
- Chemical Formula: C_2H_5NS

Uses

Thioacetamide is used as a replacement for gaseous hydrogen sulfide in qualitative analysis.

Exposure Routes and Pathways

Inhalation, dermal, oral, and ocular exposures are possible. Occupational exposure to thioacetamide may occur through inhalation and dermal contact with this compound at workplaces.

Toxicokinetics

Thioacetamide is readily absorbed through the skin. The highest levels of radioactivity were observed in the liver following oral administration of [^{3}H]thioacetamide (in diet) to male rats. Approximately 80% of [^{35}S]thioacetamide was excreted in the urine of rats within 24 h of intravenous administration.

Mechanism of Toxicity

Thioacetamide acts as an indirect hepatotoxin and causes parenchymal cell necrosis. It can be metabolized *in vivo* to acetamide, which itself is carcinogenic. Acetamide is then hydrolyzed to acetate. Thioacetamide-induced liver necrosis has been explained by a scheme that includes the metabolic conversion of thioacetamide to its S-oxide, followed by the further metabolism of thioacetamide S-oxide to a reactive intermediate that can either bind to liver macromolecules or be further degraded to acetamide and polar products. Examples of thioacetamide's biochemical effects in the liver include glucose-6-phosphate dehydrogenase being induced within days after rats are treated with thioacetamide, and the level of urea product is decreased as are the activities of hepatic carbamyl phosphate synthetase, ornithine transcarbamylase, and arginase. Thus, thioacetamide can produce marked disturbances in the urea cycle in the liver. Further, thioacetamide administered to rats leads to functional disturbances in mitochondria isolated from livers after 24 h, and the maximum respiratory activity of the mitochondria is also depressed, mitochondrial Ca^{2+} content is significantly increased, and the Ca^{2+} transport behavior of the hepatic mitochondria is altered. The results are indicative of structural alterations of the inner mitochondrial membranes. The potential role of thioacetamide in the initiation phase of carcinogenesis may be associated with an increase in nucleoside triphosphate activity in cell nuclear envelopes with a corresponding increase in RNA transport activity. Alterations in the transport phenomenon of nuclear RNA sequences are considered an early response to carcinogens.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rat oral $LD_{50} = 301\,mg\,kg^{-1}$; mouse intraperitoneal $LD_{50} = 300\,mg\,kg^{-1}$; death is delayed after dosing, even with massive doses. Hepatic necrosis and cirrhosis is observed with toxic doses.

Human

Inhalation may cause irritation of the respiratory tract characterized by rhinitis, tracheitis, and pulmonary edema. High concentrations may result in central nervous system depression and death from respiratory paralysis. Skin contact may cause irritation and ocular contact may be associated with palpebral edema, keratitis, and corneal defects. Ingestion may cause nausea, vomiting, headache, convulsions, and unconsciousness. Several people developed degenerative changes in their livers from drinking the juice of

oranges that had been immersed for 2–5 s in thioacetamide to prevent growth of molds.

Chronic Toxicity (or Exposure)

Animal

There is sufficient evidence of carcinogenicity in animals. Repeated dietary administration has produced liver cell tumors in mice and bile duct and liver tumors in rats. Cirrhosis has also been observed in both rats and mice. Thioacetamide is a developmental toxin.

Human

Prolonged exposure by inhalation may result in headache, irritability, nausea, and vomiting. Repeated contact with skin may cause dermatitis and prolonged ocular contact may cause conjunctivitis. Falls within group 2B (possibly carcinogenic to humans) according to the International Agency for Research on Cancer; however, no data are available in humans. Thioacetamide is among the group of Reasonably Anticipated to be Human Carcinogens; according to the US National Toxicology Program's 10th Report on Carcinogens. An oral thioacetamide-induced model of rat cholangiocarcinoma (CCA) has been developed that recapitulates the histologic progression of human CCA. CCA is a lethal disease, afflicting many thousands the world over. Human CCA develops through a multistep progression model, preceded by the onset of dysplasia in the cholangiolar ductal epithelium. The thioacetamide animal model is useful because its multistep process leading to cancer in the biliary tree will enable the study of genetic changes in human CCA and may serve as a powerful preclinical platform for therapeutic and chemoprevention strategies.

In Vitro Toxicity Data

Thioacetamide induced an increase in sex-linked recessive mutations in *Drosophila*. It was nonmutagenic in the *Salmonella*/Ames mutagenicity assay, and in the *Escherichia coli* recombination assay. Protein synthesis in mouse hepatoma (MH-134), but not in L-929 cells, was enhanced by adding thioacetamide.

Clinical Management

Basic and advanced life-support measures should be utilized as necessary. Gastric decontamination may be accomplished by lavage or emesis. Sodium bicarbonate solution should be used to reduce acidity. The use of amyl nitrite by inhalation for 15–30 s of every minute may be indicated in severe poisonings.

Environmental Fate

Thioacetamide's production and use as a substitute for hydrogen sulfide in the laboratory may result in its release to the environment through various waste streams. If released to air, thioacetamide's estimated vapor pressure indicates it will exist solely as a vapor in the ambient atmosphere. Vapor-phase thioacetamide will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 18 h. Thioacetamide was not biodegraded by activated sludge after 5 days, and therefore may be resistant to biodegradation in the environment. Hydrolysis is not expected since amides hydrolyze very slowly at environmental conditions. An estimated bioconcentration factor for thioacetamide suggests the potential for bioconcentration in aquatic organisms is low.

See also: International Agency for Research on Cancer; Liver.

Further Reading

Arni P (1989) Review on the genotoxic activity of thioacetamide. *Mutation Research* 221: 153–162.

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Yeh CN, Maitra A, Lee KF, Jan YY, and Chen MF (2004) Thioacetamide-induced intestinal-type cholangiocarcinoma in rat: An animal model recapitulating the multistage progression of human cholangiocarcinoma. *Carcinogenesis* 25: 631–636.

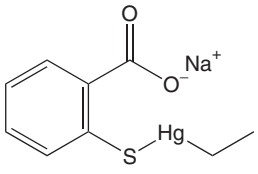
Relevant Website

http://www.cdc.gov – US National Institute for Occupation Safety and Health (NIOSH). Thioacetamide (International Chemical Safety Cards).

Thiomerosal

Arezoo Campbell

- Chemical Abstracts Service Registry Number: CAS 54-64-8
- Synonyms: Sodium ethylmercury thiosalicylate; Merthiolate; Thimerosal
- Chemical Formula: $C_9H_9HgNaO_2S$
- Chemical Structure:



Uses

Thiomerosal is used as a preservative in vaccines and ophthalmic solutions.

Background Information

Thiomerosal (sodium ethylmercury thiosalicylate) is used as a preservative in vaccines to protect from bacterial and fungal contamination. There is currently a controversy as to whether the use of this organic ethylmercury compound is an etiological factor in autism. It is estimated that children treated with these mercury-containing vaccines are exposed to levels of the metal far beyond those considered safe by the US Food and Drug Administration standards. By the age of 2, children can receive as much as 237 mg of mercury through vaccination. This has led to a 2002 class action lawsuit against the manufacturer of thiomerosal by parents with autistic children or children who have not yet developed any symptoms but were exposed to high concentrations of mercury contained in the preservative. Recent studies discount a causal relation between exposure to thiomerosal-containing vaccines and autism. The risk of autism is not increased in children treated with vaccines containing the preservative compared to children who were vaccinated with thiomerosal-free formulations. Furthermore, in Denmark, the use of the preservative was discontinued after 1992. After removal of thiomerosal, there was a continued rise in new cases of autism. Even with these new emerging data, the potential link between autism and mercury poisoning remains inconclusive.

Exposure Routes and Pathways

The most common route of entry is by intravenous injection since the compound is used as a preservative in vaccines.

Toxicokinetics

Thiomerosal is metabolized to ethylmercury and thiosalicylate. Toxicologists have assumed that ethylmercury poisoning is similar to the toxicity of methylmercury. However, ethylmercury cannot bypass the blood–brain barrier as easily as methylmercury. The entry of methylmercury into the brain relies on an active transport system. Ethylmercury on the other hand is a larger molecule and cannot use this system. Furthermore, it is more rapidly decomposed. Because of these limitations, when the same dose of both mercurial compounds is administered, the concentrations of methylmercury are greater in the brain when compared to ethylmercury. Due to the limited entry of the latter into the brain, this compound is more likely to cause damage to the spinal cord, myocardium and skeletal muscle.

Mechanism of Toxicity

Not much is known about the toxic effects of ethylmercury and most toxicologists have assumed that the toxic changes would be similar to that caused by methylmercury. These alterations in turn are very complex and depend on duration of exposure, dose, and the age of the individual. Mercury salts have a strong affinity for sulfhydryl groups and this is likely to play a role in effecting their neurotoxicity. Some *in vitro* studies indicate that oxidative stress leading to lipid peroxidation and DNA damage may also underlie the mechanism of toxicity.

Acute Toxicity (or Exposure)

Animal

There are not many studies addressing the potential toxicity of ethylmercury in animal models. Exposure of rats to ethylmercury results in patchy damage to the granule cells in the cerebellum while the Purkinje cells are generally spared.

Human

In a case report of four patients who were exposed to ethyl mercury, toxicity was seen in the brain, spinal motor neurons, peripheral nerves, skeletal muscles, and myocardium. Several case studies of accidental occupational exposure have also been documented. The most common signs of ethylmercury toxicity are paraesthesia, dysarthria, and constriction of the visual field. However, none of the symptoms of ethylmercury toxicity are specific and death is a common outcome if exposure levels are high.

Chronic Toxicity (or Exposure)

Human

Accidental exposure to massive doses of methylmercury occurred in Japan and Iraq. The former was due to contaminated seafood while the latter was due to contaminated grain. In Japan, neurotoxicity was found in humans after extended periods of fish consumption. Slow onset of symptoms resulted in a high incidence of severe, largely irreversible damage to the central nervous system. Postmortem analysis of the brain of individuals exposed to high levels of the mercury compound showed neuronal cell loss and an increase in glial cell numbers in the cortex. The cerebellar granule cells were also damaged.

In Vitro Toxicity Data

Treatment of cell cultures containing both neuronal and glial cells derived from fetal rat brain with low concentrations of both organic and inorganic mercury compounds leads to cell death. In cell cultures derived from a more mature fetal stage, the organic form of mercury was more toxic and showed specific neuronal toxicity. Below the cytotoxic concentration of mercury ($>1\,\mu mol\,l^{-1}$), pronounced gliosis was observed. In a human fetal hepatic cell line, exposure to low concentrations of inorganic mercury led to lipid peroxidation and single-strand breaks in the DNA. This suggests that oxidative stress may play a role in mercury-induced cytotoxicity.

Clinical Management

Diagnosis of mercury intoxication can be confirmed by measuring levels of the metal in serum. The toxic effects are largely irreversible. In severe cases, death is the major outcome.

See also: Dimethylmercury; Methylmercury.

Further Reading

Magos L (2001) Review on the toxicity of ethylmercury, including its presence as a preservative in biological and pharmaceutical products. *Journal of Applied Toxicology* 21: 1–5.

Nelson KB and Bauman ML (2003) Thiomerosal and autism? *Pediatrics* 111: 674–679.

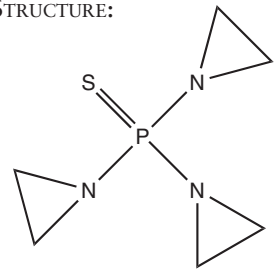
Relevant Website

http://www.fda.gov – The FDA's Center for Biologics Evaluation and Research, search for Thiomerosal.

Thiotepa

Marcia D Howard

- Chemical Abstracts Service Registry Number: CAS 52-24-4
- Synonyms: Triethylene thiophosphoramide; *N*,*N*′,*N*″-Triethylenethiophosphamide; *N*,*N*′,*N*″-Triethylenethiophosphoramide; *N*,*N*′,*N*″-Tri-1,2-ethanediylphosphorothioic triamide; *N*,*N*′,*N*″-Tri-1,2-ethanediylthiophosphoramide; *N*,*N*′,*N*″-Triethylenephosphorothioic triamide; *N*,*N*′,*N*″-Triethylenethiophosphortriamide; 1,1′,1″-Phosphinothioylidynetrisaziridine; Girostan; Ledertepa
- Chemical/Pharmaceutical/Other Class: Aziridine; Alkylating antineoplastic agent, Insect sterilant
- Chemical Formula: $C_6H_{12}N_3PS$
- Chemical Structure:



Uses

Thiotepa is used in the treatment of bladder, ovarian, and breast cancer as well as a component of experimental high-dose chemotherapy regimens. Thiotepa is also used as an insect sterilant.

Exposure Routes and Pathways

Thiotepa may be absorbed through inhalation, ingestion, dermal contact, or eye contact. However, the general public is not likely to be exposed due to its limited use in cancer therapy.

Toxicokinetics

Absorption of thiotepa from the gastrointestinal tract is incomplete while absorption through serous membranes (e.g., pleura and bladder) and from intramuscular injection sites is variable. Distribution of thiotepa occurs rapidly and extensively to tissues. Thiotepa is rapidly converted to its primary metabolite, triethylenephosphoramide (TEPA) by hepatic mixed function oxygenases. TEPA becomes the predominant form of thiotepa within 5 min of

administration of the drug. The plasma half-life of thiotepa is 1–3 h while TEPA has a half-life of 3–24 h. Thiotepa is excreted in urine (60% within 72 h). Less than 10% of the drug or its primary metabolite (TEPA) appears in the urine with the remainder of the drug either metabolized, interacting with biological molecules or undergoing spontaneous chemical degradation. Toxicokinetics are similar for adults and children when conventional doses ($80\,mg\,m^{-2}$) are administered. Protein binding (as determined by ultrafiltration) is reported to be less than 40% under physiological conditions.

Mechanism of Toxicity

Thiotepa is a phase nonspecific polyfunctional alkylating agent (i.e., more than one reactive ethylenimine group). It is chemically and pharmacologically related to nitrogen mustards. Thiotepa (and TEPA) form DNA crosslinks that lead to a reactive metabolite. The aziridine ring opens after protonation of the ring nitrogen. As an alkylating agent, thiotepa interferes with DNA replication and RNA transcription, ultimately leading to the disruption of nucleic acid function. Alkylation causes breaks in DNA and cross-linking of the twin strands. One of the principle bond disruptions occurs when the N-7 position of guanine is alkylated, severing the link between the purine base and sugar, which liberates an alkylated guanine.

Acute and Short-Term Toxicity (or Exposure)

Animal

The reported LD_{50} values in rats are $8\,mg\,kg^{-1}$ (intraperitoneal); 9–$15\,mg\,kg^{-1}$ (intravenous, iv); $2.3\,mg\,kg^{-1}$ (oral), and $7.8\,mg\,kg^{-1}$ (subcutaneous, sc). Reported LD_{50} values are $11\,mg\,kg^{-1}$ (intraperitoneal, ip); $14.5\,mg\,kg^{-1}$ (iv); $38\,mg\,kg^{-1}$ (oral), and $16.5\,mg\,kg^{-1}$ (sc) for mice.

Human

Direct vesicant effects, which can occur with active alkylating agents, can damage tissue at the site of injection as well as cause systemic toxicity. Toxic side effects of thiotepa include nausea, vomiting, fever, anorexia, headache, neutropenia, thrombocytopenia, and variable anemia. Although allergic reactions are rare, hives and skin rashes are occasionally noted. Other side effects include myelosuppression and to a lesser extent, mucositis. Cardiac dysrhythmias and hypotension as well as pulmonary edema or pneumonitis may also occur. Toxicity is generally dose-related and occurs particularly in rapidly growing tissues such as bone marrow, GI tract, and gonadal tissue. Target organs are bone marrow, kidneys, liver, heart, lungs, spleen, blood systems, eyes, gastrointestinal tract, and reproductive systems. It is contraindicated during the first trimester of pregnancy.

Chronic Toxicity (or Exposure)

Animal

Thiotepa is believed to be carcinogenic in both male and female rats and mice. Malignant tumors developed in rats treated weekly with $1\,mg\,kg^{-1}$ body weight (iv). The minimum ip teratogenic dose (TD) in pregnant mice is $1\,mg\,kg^{-1}$ body weight.

Human

Thiotepa is a carcinogen in humans (group 1). Chronic exposure to thiotepa may cause skin depigmentation and allergic reactions as well as effects seen with acute exposure.

Clinical Management

Poisoned patients should be treated for the symptoms of the poisoning and not the drug itself. For dermal exposures, the skin should be immediately flushed with water and all contaminated clothing isolated. Affected skin areas should be thoroughly but gently washed with soap and water. Contact should also be made with a hospital or poison control center (even if the victim has no visible symptoms) and the victim immediately transported to the hospital after washing the affected areas.

For ocular exposure to the compound, the victim should be checked for contact lenses and if present, should be removed. The eyes should be flushed with copious amounts of water or normal saline for 20–30 min while the hospital or poison control center is notified. No ointments, oils, or medications should be instilled into the victim's eyes without specific instructions from a physician.

If the chemical is ingested, first aid will depend on the victim's state of consciousness. If the exposed person is conscious and not convulsing, one or two glasses of water should be given to dilute the chemical and the poison control center or hospital should be immediately called. Generally, it is not recommended that vomiting be induced outside of the care of a medical doctor due to the possibility of aspiration of the chemical into the lungs. However, if the victim is conscious, not convulsing, and medical care is not readily available, induction of vomiting should

be considered due to the high toxicity of the chemical. The victim should be immediately taken to the hospital. If the victim is convulsing or is unconscious, nothing should be administered by mouth. It should be ensured that the victim's airway is open; the victim should be made to lie on his or her side with the head lower than the body and should be immediately transported to the hospital. However, vomiting should not be induced. Bone marrow toxicity with overdosage may be limited by blood transfusion.

See also: Nitrogen Mustard.

Further Reading

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Relevant Websites

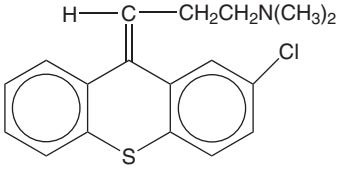
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Thiotepa.
http://www.cancer.org – American Cancer Society.

Thiothixene *See* Thioxanthenes.

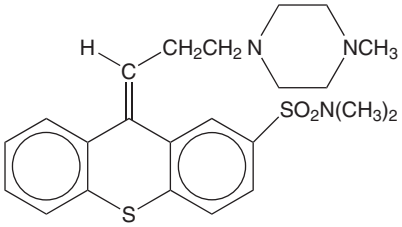
Thioxanthenes

Douglas J Borys

- Representative Compounds: Chlorprothixene; Thiothixene; Flupenthixol; Zuclopenthixol
- Chemical Abstracts Service Registry Numbers: Chlorprothixene (CAS 113-59-7); Thiothixene (CAS 5591-45-7); Flupenthixol (CAS 2413-38-9); Zuclopenthixol (CAS 53772-83-1)
- Synonyms:
  - Chlorprothixene: 2-Chloro-9-[3-(dimethylamino)propylidene]-thioxanthene; Paxyl; Taractan
  - Thiothixene: Thioxanthene-2-sulfonamide; *N*,*N*-Dimethyl-9-[3-(4-methylpiperazin-1-yl)propylidene]; Navane
  - Flupenthixol: 2-[4-[3-(*E*,*Z*)-2-(Trifluoromethyl)-9*H*-thioxanthen-9-ylidene]propyl]piperazin-1-yl]-ethanol dihydrochloride; Depixol
  - Zuclopenthixol: (*Z*)-4[3-(2-Chloro-9*H*-thioxan-9-ylidene)propyl]-1-piperazine ethanol; Clopixol
- Chemical/Pharmaceutical/Other Class: Neuroleptic agent; Antipsychotic; Major tranquilizer
- Chemical Structures:



Chlorprothixene



Thiothixene

Uses

Thioxanthenes are used in the treatment of psychosis, including schizophrenia, senile psychosis, pathological jealousy, and borderline personality disorder. Other uses include the treatment of pain, postoperative neuralgia, sedation, anxiety neurosis, childhood behavior problems, and depression. The maximum therapeutic daily oral dose for chlorprothixene, flupenthixol, and thiothixene is 600, 224, and 60 mg respectively; the maximum intramuscular dose of each is 200 mg day^{-1}, 100 mg weekly, and 30 mg day^{-1}, respectively. Some thioxanthenes and thioxanthenones have shown signs in mice and *in vitro* assays of possible human therapeutic potential against tumors, and some thioxanthenes have been shown to have cytotoxic and antimicrobial activities.

Background Information

In 2002, the American Association of Poison Control Centers' Toxic Exposure Surveillance System reported

5224 human exposures to phenothiazines, thioxanthenes, and other neuroleptic medications. Of those exposures 3691 were in adults and 808 in children. Unintentional and intentional exposures accounted for 43.7% and 47.8% of the exposures, respectively. There were 417 (8.0%) adverse drug reactions reported.

Exposure Routes and Pathways

Thioxanthenes are available in injectable and oral dosage forms. The primary exposure pathway is intentional ingestion by adults; accidental ingestion by small children also does occur.

Toxicokinetics

Thioxanthenes are readily but incompletely absorbed due to first-pass metabolism in the gut wall. Oral bioavailability ranges from 40% to 50%. Peak absorption occurs in 1 or 2 h. Thioxanthenes are extensively metabolized in the liver through glucuronic acid conjugation, *N*-dealkylation, and sulfoxidation. Thioxanthenes are widely distributed throughout the body, including the central nervous system (CNS). They are highly protein bound (>99%), with a volume of distribution ranging from 11 to 23 l kg^{-1}. The main metabolites are excreted in both the urine and feces. There is some enterohepatic circulation. The elimination half-life ranges from 8 to 12 h. The thioxanthenes and their metabolites can be excreted through breast milk.

Mechanism of Toxicity

Thioxanthenes work primarily by blocking postsynaptic dopamine-mediated neurotransmission by binding to dopamine (DA-1 and DA-2) receptors. In addition to significant antidopaminergic action, the thioxanthenes also possess weak anticholinergic and serotonergic blockade, moderate α-adrenergic blockade, quinidine-like effects, and depress the release of most hypothalamic and hypophyseal hormones. Thioxanthenes may also inhibit presynaptic dopamine auto receptors.

Acute and Short-Term Toxicity (or Exposure)

Animal

Sublethal doses produce ataxia and respiratory paralysis, while lethal doses produce convulsions. The oral LD_{50} data are in the range of 400 mg kg^{-1} or greater for mice and rats administered thioxanthenes.

Human

Clinical signs of toxicity frequently reported include extrapyramidal effects, sedation, coma, and rarely seizures, acute renal insufficiency, hypotension, and cardiac arrhythmias. Other adverse reactions following therapeutic use include dysphoria, photosensitivity, anorexia, nausea, vomiting, constipation, diarrhea, and dyspepsia. The extrapyramidal reactions induced by thioxanthenes will result in increased motor activity of the head, face, and neck. Neuroleptic malignant syndrome has been reported after therapeutic use and acute intoxication. The most commonly reported dystonic reactions include akathisias, stiff neck, stiff or protruding tongue, and tremor. Anticholinergic effects, including dry mouth, blurred vision, and tachycardia, may occur. Cardiac effects include prolonged Q–T interval and mild hypotension. Hypokalemia has also been noted. Patients receiving thiothixene should avoid undue exposure to sunlight. These drugs have been implicated in the etiology of acquired hemophilia.

Leukopenic and thrombocytopenic effects of thioxanthenes may result in an increased incidence of microbial infection, delayed healing, and gingival bleeding. When a thioxanthene is used concomitantly with other CNS depressants, caution should be taken to avoid overdosage. Prior administration of thioxanthenes may decrease the pressor response to phenylephrine because of the α-adrenergic blocking action of thioxanthenes. Hypersensitivity reactions, including rash, pruritus, urticaria, photosensitivity, and rarely anaphylaxis, have been reported in patients receiving thiothixene.

Chronic Toxicity (or Exposure)

Human

Tardive dyskinesias (TDs) are involuntary movements of the tongue, lips, face, trunk, and extremities that occur in patients treated with long-term dopaminergic antagonist medications. TDs can be differentiated from acute movement disorders that commonly occur in the same patient groups; the acute movement disorders resulting from exposure to dopamine antagonists are commonly termed extrapyramidal syndromes.

In Vitro Toxicity Data

Efflux-related multidrug resistance (MDR) is a significant means by which bacteria can evade the effects of selected antimicrobial agents. Two geometric stereoisomers of flupentixol, with intrinsic antimicrobial

activity, were studied using strains of *Staphylococcus aureus* possessing unique efflux-related MDR phenotypes, and the results suggest that the mechanism by which thioxanthenes inhibit efflux by proton motive force-dependent pumps may involve an interaction with the pump itself and, to a lesser extent, a reduction in the transmembrane potential.

Clinical Management

Treatment consists of gastric decontamination, hydration, and aggressive supportive care; all basic and advanced life-support measures should be implemented. Gastric decontamination should be performed. Syrup of ipecac is contraindicated. Lavage may be performed and activated charcoal administered, if within 60 min of an acute ingestion. Thioxanthenes are readily absorbed by activated charcoal. Dystonic reactions respond to intravenous benztropine or diphenhydramine. Oral therapy with diphenhydramine or benztropine should be continued for 2 days to prevent recurrence of the dystonic reaction. For patients suffering from neuroleptic malignant syndrome treatment consists of dantrolene sodium, diphenhydramine, and oral bromocriptine in conjunction with cooling and other supportive measures. Arrhythmias should be treated with lidocaine or phenytoin. Diazepam is the drug of choice for seizures while phenytoin is the drug of choice to prevent recurrence. Fluid challenge alone will frequently correct hypotension. Hemodialysis and hemoperfusion have not been shown to be effective.

See also: Tricyclic Antidepressants.

Further Reading

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Ginestet D (2002) L'essor de la psychopharmacologie et son impact sur la psychiatrie (The development of psychopharmacology and its impact on the psychiatry). *Annales Médico-psychologiques, revue psychiatrique* 160: 767–771.

Grohmann R, Engel R, Hippius H, and Ruther E (2000) Adverse effects of the comedication of neuroleptics and antidepressants. *European Neuropsychopharmacology* 10(Suppl. 3): 112–113.

Kaatz GW, Moudgal VV, Seo SM, and Kristiansen JE (2003) Phenothiazines and thioxanthenes inhibit multidrug efflux pump activity in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* 47: 719–726.

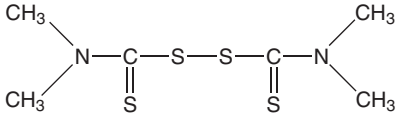
Ravn JF (1970) The history of thioxanthenes. In: Ayd FJ Jr. and Blackwell B (eds.) *Discoveries in Biological Psychiatry*, pp. 180–193. Philadelphia: J.B: Lippincott Company.

Stewart AJ, Manson LM, Dasani H, *et al.* (2000) Acquired haemophilia in recipients of depot thioxanthenes. *Haemophilia* 6: 709–712.

Thiram

Mona Thiruchelvam

- Chemical Abstracts Service Registry Number: CAS 137-26-8
- Synonyms: Tetramethylthiuram disulfide; Bis(dimethyldithiocarbanoyl)disulfide; Arasan; Fermide; Fernacol; Vancide
- Chemical/Pharmaceutical/Other Class: Dimethyl dithiocarbamate
- Chemical Formula: $C_6H_{12}N_2S_4$
- Chemical Structure:



Uses

Thiram is used as a seed protectant and to protect fruit, vegetable, ornamental, and turf crops from a variety of fungal diseases. It is also used as an animal repellent to protect fruit trees and ornamentals from damage by rabbits, rodents, and deer. Thiram has been used in the treatment of human scabies, as a sunscreen and as a bactericide applied directly to the skin or incorporated into soap. Thiram is available as dust, flowable, wettable powder, water-dispersible granules, and water suspension formulations and in mixtures with other fungicides. It has other applications including use as an accelerator and vulcanizing agent for synthetic and natural rubber, an activator in plastics manufacturing and as a chemosterilant in plastic film dry-wound dressing. It is also used as a wood preservative and lubricant oil additive.

Exposure Routes and Pathways

Thiram is a broad-spectrum fungicide and is found in most home-garden formulations whereby day-to-day human exposure can occur. Its use in various industries leads to multiple avenues for occupational exposures. The highest exposures occur in workers utilizing and/or manufacturing this compound. Exposure to thiram can occur via inhalation, ingestion, and eye or skin contact.

Toxicokinetics

Thiram is absorbed via the skin, mucous membranes, respiratory, and gastrointestinal tracts. Thiram is rapidly absorbed from the gastrointestinal tract. Thiram and other dimethyldithiocarbamates are metabolized to diethyldithiocarbamic (DDC) acid, diethylamine, and carbon disulfide. DDC is rapidly absorbed by the gastrointestinal tract and further metabolized by hepatic enzymes. A portion of the acid is excreted unchanged or as glucuronide conjugate. Further metabolism can result in the formation of dimethylamine and carbon disulfide residues.

Mechanism of Toxicity

Thiram and other dithiocarbamates are metabolic poisons. The acute effects of thiram are very similar to that of carbon disulfide, supporting the notion that the common metabolite of this compound is responsible for its toxic effects. The exact mechanism of toxicity is still unclear, however it has been postulated that the intracellular action of thiram involves metabolites of carbon disulfide, causing microsome injury and cytochrome P450 disruption, leading to increased heme-oxygenase activity. The intracellular mechanism of toxicity of thiram may include inhibition of monoamine oxidase, altered vitamin B_6 and tryptophan metabolism, and cellular deprivation of zinc and copper. It induces accumulation of acetaldehyde in the bloodstream following ethanol or paraldehyde treatment. Thiram inhibits the *in vitro* conversion of dopamine to noradrenalin in cardiac and adrenal medulla cell preparations. It depresses some hepatic microsomal demethylation reactions, microsomal cytochrome P450 content and the synthesis of phospholipids. Thiram has also been shown to have moderate inhibitory action on decarboxylases and, in fish, on muscle acetylcholinesterases.

Thiram also leads to thyroid dysfunction. This effect is thought to be a result of metabolic release of sulfur in follicular cells, causing inhibition of tyrosine iodination and ultimately hormone synthesis. Thiram induces alcohol intolerance similar to that of ant-abuse (disulfiram) either through its ability to inhibit acetaldehyde dehydrogenase or through the formation of a quaternary compound with ethanol.

Acute and Short-Term Toxicity (or Exposure)

The acute toxicity of thiram is rather low both in humans and experimental animals. Thus acute poisoning is highly unlikely unless large amounts are ingested. Thiram is an irritant of the eyes, mucous membranes, and skin and can elicit signs of neurotoxicity with acute high exposures.

Animal

In general, thiram is not very toxic unless high levels of exposure occur. The oral LD_{50} in rats is $560\,mg\,kg^{-1}$, and the lowest lethal dermal dose in rabbits is $1\,g\,kg^{-1}$. In contact with the skin and eyes of exposed rabbits, thiram caused irritation. In rabbits and guinea pigs, this substance has been shown to cause skin sensitization. Rats, cats, and rabbits survived a 4 h exposure to thiram dust at concentrations that ranged from 500 to $6225\,mg\,m^{-3}$. Animals killed by single oral doses of thiram showed patchy demyelination in the central nervous system, initially in the cerebellum and medulla. Thiram ($300\,mg\,kg^{-1}$) elicited convulsions and calcification in the cerebellum, hypothalamus, and medulla oblongata in rats.

Animals sacrificed after a single oral dose showed hyperemia and focal ulcerations of the gastrointestinal tract. Single dermal applications of 1000–$2000\,mg\,kg^{-1}$ to rats and 500–$1000\,mg\,kg^{-1}$ to rabbits produced only slight skin irritation.

Human

Since the acute toxicity of thiram is relatively low as is with most dithiocarbamates, acute intoxication in humans is unlikely to occur unless large amounts are ingested. Thiram can be absorbed from the gastrointestinal tract, through the skin and by inhalation of dust and fine spray mist. Inhalation can irritate the nose and throat causing coughing and wheezing. High exposure can lead to headache, dizziness, confusion, fatigue, nausea, and vomiting. Contact with thiram can irritate and burn the skin and eyes. Thiram has been given a toxicity rating of 4 and the probably lethal dose for humans is 50–$500\,mg\,kg^{-1}$. Alcohol, regardless of the route of exposure to thiram, can increase thiram toxicity and contributes to most systemic poisonings.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to thiram produces toxicity to several different organ systems in addition to those affected following acute exposure.

In an 80 day feeding study in rats 5 mg kg^{-1} day^{-1} in males and 6 mg kg^{-1} in females were found to be the no-effect levels. Paralysis and atrophy of the hind legs of females was observed at 67 mg kg^{-1} day^{-1}. In a dietary study where male rats were fed thiram at doses of 30, 58, and 132 mg kg^{-1} day^{-1} for 13 weeks, a dose-dependent reduction in body weight and food consumption was observed. In an 80 week study, male rats were fed 5, 20, and 52 mg of thiram per kg per day, and females 6, 26, or 67 mg kg^{-1} day^{-1}, resulting in a dose-dependent decrease in body weight and food consumption in males starting at 5 mg kg^{-1} and in females starting at 26 mg kg^{-1}. There were no treatment-related mortalities and moderate to severe clinical signs of toxicity were observed only among the females in the highest dosage group. In a 1 year dietary study in dogs the no-effect level was found to be 4.0 mg kg^{-1} day^{-1}. In a 2 year feeding study in rats the no-effect level was found to be ~4.9 mg kg^{-1} day^{-1}. At 2500 ppm there was 100% mortality within 17 weeks. General weakness, ataxia, and occasional paralysis were observed at 300 and 1000 ppm but there was no treatment-related mortality. Thiram caused an increase in squamous epithelial metaplasia in the thyroid and fatty infiltration in males. There was a reduction in incidences of spontaneous nephritis in both sexes.

Thiram is classified as an equivocal tumorigen with no known carcinogenic effects. No clear carcinogenic effect was demonstrated in mice given the maximum tolerated doses in a 77 week feeding study.

Thiram was shown to be teratogenic in rats (400 mg kg^{-1}, p.o. on days 6–15 of gestation), in mice (250 mg kg^{-1}, p.o. on days 6–15 of gestation), and in hamsters (250 mg kg^{-1}, p.o. on days 7 or 8 of gestation). The pattern of fetal defects was not well defined, with many changes linked to retardation of growth. In hamsters the combined effects of thiram and the solvent dimethyl sulfoxide were possibly synergistic. In mice, simultaneous co-administration of L-cysteine tended to abolish the teratogenic effect of thiram.

Thiram was found to have adverse effects on reproduction and to be embryotoxic in mice, rats, and hamsters at high doses that are toxic to adults. In a three-generation dietary study in rats administered 100 mg kg^{-1}day^{-1}, no adverse effects on reproduction or fetal development were noted. In a single generation study in rats, thiram (50 mg kg^{-1} day^{-1}, from gestation day 16 to postpartum day 21), caused reduced pup growth and survival. These effects were prevented when the pups were transferred to untreated lactating dams. In an inhalation study in rats, thiram (3.8 mg m^{-3} of air for 6 h per day, 5 days per week for 4.5 months) caused reproductive defects: prolonged estrous cycles, decreased conception rates, decreased fertility and reduced fetal weights. In mice, thiram (132 mg kg^{-1} day^{-1}, p.o. for 13 weeks) caused male infertility and 96 mg kg^{-1} day^{-1} for 14 days delayed estrous cycles. These adverse effects were reversed when treatment ceased.

In another chronic study, eight out of 24 female rats fed 67 mg kg^{-1} day^{-1} for 80 weeks developed severe signs of neurotoxicity including ataxia and ascending paralysis; degeneration of axis-cylinders and presence of macrophages in the bundle of the sciatic nerve were observed.

Human

In humans, thiram is an eye, nose, and throat irritant, a central nervous system toxicant, and a skin sensitizer. Volunteers given daily thiram doses of 0.5 g day^{-1} for several weeks showed no adverse effects. One worker who treated seeds with thiram for a 10 h period, during which he was estimated to have received a substantial exposure, died 4 days later. Several members of a group of workers exposed to thiram during the planting of trees reported experiencing eye, nose, and throat irritation, headache, and skin problems.

Workers chronically exposed to thiram and concurrently ingesting alcohol have developed skin reactions without any systemic effects. In these cases, the skin becomes red, flushed, and itchy. In susceptible individuals, thiram can cause dermatitis even without concomitant alcohol ingestion, and sensitization of the skin has occurred on the hands, forearms, and feet of exposed individuals. The International Agency for Research on Cancer notes that studies from the USSR report thyroid gland enlargement, one case of thyroid cancer, and seven cases of thyroid abnormalities in a group of 105 workers exposed to thiram at unspecified concentrations for more than 3 years.

The use of thiram in the manufacture of many rubber and plastic products (e.g., shoes) and as a fungicide in recreational areas (e.g., golf courses and bowling greens) presents considerable opportunity for exposure of sensitive individuals to the

compound. Thiram is considered to be a borderline allergen, requiring several exposures to produce sensitization.

In Vitro Toxicity Data

In vitro systems have been developed to try and understand the mechanism of action of thiram alone and in the presence of other potentiating compounds. The genotoxic, cytotoxic, and neurotoxic effects of thiram have been studied using a variety of primary cultures as well as cell-lines. Lymphocytes exhibited sister chromatid exchanges and micronuclei with exposure to thiram. Thiram caused single-strand DNA breaks in testicular cells *in vitro*. Other studies indicate that thiram can be both clastogenic and mutagenic.

Clinical Management

Thiram can be absorbed into the body by inhalation, though the skin, and by ingestion. If swallowed, large amounts of water should be ingested, only if person is conscious, and vomiting induced immediately. If thiram dust is inhaled, the exposed individual should be moved to fresh air, away from the contamination site. If skin contact occurs, all contaminated clothing should be removed and the area exposed should be washed with copious amounts of water and soap. If the product is present in the eyes, the eyes should be flushed with large amounts of water for at least 15 min.

Environmental Fate

Thiram is of low to moderate persistence. It is only slightly soluble in water ($30\,mg\,l^{-1}$) and has a strong tendency to adsorb to soil particles, and thus is not expected to contaminate groundwater. The soil half-life for thiram is reported to be 15 days. Thiram degrades more readily in acidic soils and in soils high in organic matter. Thiram has been shown to persist up to 2 months in sandy soil but disappeared within 1 week from compost soil. The major metabolites of thiram in soil are copper dimethyldithiocarbamates, dithiocarbamate, dimethylamine, and carbon disulfide. In soil, thiram can be degraded by microbial action or by hydrolysis under acidic conditions. In water, thiram is rapidly broken down by hydrolysis and photodegradation, especially under acidic conditions.

Ecotoxicology

Thiram is generally of low toxicity to most wildlife. It is moderately toxic to birds. The reported dietary LC_{50} of thiram in Japanese quail is greater than 5000 ppm.

Thiram is highly toxic to fish. The LC_{50} for the compound is $0.23\,mg\,l^{-1}$ in bluegill sunfish and $0.13\,mg\,l^{-1}$ in trout. Thiram does not bioconcentrate in aquatic organisms.

Exposure Standards and Guidelines

- Occupational Safety and Health Administration: $5\,mg\,m^{-3}$ ceiling.
- American Conference of Governmental Industrial Hygienists: $1\,mg\,m^{-3}$ time-weighted average (TWA).
- National Institute for Occupational Safety and Health: $1\,mg\,m^{-3}$ recommended TWA threshold limit value: 1 mg (Mn) m^{-3}.

See also: Dithiocarbamates; Pesticides.

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Thorium and Thorium Dioxide

Shayne C Gad

- Representative Compounds: Thorium dioxide (ThO_2); Thorium disulfide (ThS_2)
- Chemical Abstracts Service Registry Number: CAS 7440-29-1
- Chemical/Pharmaceutical/Other Class: Actinide metals
- Chemical Formula: Th^{4+}

Uses

Discovered in 1829, thorium is a naturally occurring radioactive metal with no stable isotopes. It is about as abundant as lead. Soil commonly contains an average of about six parts of thorium per million parts (ppm) of soil. Thorium occurs in the minerals thorite, thorianite, orangite, and yttrocrasite, and in monazite sand. Rocks in some underground mines may also contain thorium in a more concentrated form. After these rocks are mined, thorium is usually concentrated and changed into thorium dioxide or other chemical forms. Thorium-bearing rock that has had most of the thorium removed from it is called 'depleted' ore or tailings. Thorium is present in nuclear reactor fuels, and is used in the manufacture of incandescent gas-light mantles, welding electrodes and ceramics, as a hardener in magnesium alloys, and as a chemical catalyst. In addition, it is used in sun lamps, photoelectric cells, and in target materials for X-ray tubes. Thorium is present in fires and explosions caused by thorium metal powder and has been recovered as a by-product of uranium production.

Exposure Routes and Pathways

Ingestion of liquid, inhalation of dust or gas, and percutaneous absorption are the routes of exposure. Occupational exposure to thorium and thorium compounds may occur through handling of various thorium salts in the fabrication of thorium ingots, in handling thorium salts in various industrial uses, in the fume from welding with thoriated tungsten electrodes, in the casting and machining of thorium alloy parts, and from fires and explosions caused by thorium metal powder. As thorium is a naturally occurring background element, the general population may be exposed daily to thorium and thorium compounds through dermal and other contact.

Toxicokinetics

Thorium is poorly absorbed from both the lung and digestive tract, and 70% of the thorium reaching the blood is translocated to the bone, 4% to the liver, and 16% to all other organs and tissues of the body. Thorium accumulates in the liver, spleen, lymph nodes, and bone marrow, leading to long-term exposure with a diversity of cells. Thorium is retained the longest when it has entered the body in the form of an insoluble compound. Transferrin plays a major role in the transport and cellular uptake of thorium. Thorium can be displaced from transferrin by an excess of iron, but it is not known whether thorium and iron bind to the same sites on the transferrin molecule. Tissue distribution and retention are highly dependent upon dose and route. Most of the absorbed dose goes to the reticuloendothelial system, liver, spleen, and bone marrow. Thorium is excreted slowly and primarily via bile to feces. Thorium can also be eliminated via exhalation of radioactive thoron daughter gas.

Mechanism of Toxicity

Binding with bone and other glucoproteins and, in some cases, an interaction with zinc. Thorium oxide is radioactive. As noted above, thorium accumulates in the liver, spleen, lymph nodes, and bone marrow, leading to long-term exposure with a diversity of cells.

Acute and Short-Term Toxicity (or Exposure)

Animal

No deaths were reported in animals following inhalation exposure, and high exposure levels were necessary to produce death in animals following oral exposure, since gastrointestinal absorption is very poor.

Human

Acute exposure results in dermatitis. Thorium oxide has a TD_{Lo} of $1\,g\,kg^{-1}$.

Chronic Toxicity (or Exposure)

Animal

It is a carcinogen and developmental toxin. Animal studies have shown that breathing in thorium may result in lung damage. Other studies in animals

suggest drinking massive amounts of thorium can cause death from metal poisoning.

Human

Studies of thorium workers have shown that breathing thorium dust may cause an increased chance of developing lung disease and cancer of the lung or pancreas many years after being exposed.

Clinical Management

The removal of thorium from the body has been achieved by the use of chelating agents, for example, ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid.

Environmental Fate

Thorium's usage may result in release of thorium compounds to the environment through various waste streams. As noted above, thorium is also found naturally. Thorium compounds are expected to exist in the particulate phase based on their low vapor pressures and may be removed from the air by wet and dry depositions.

See also: Lead.

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Three Mile Island

John Sorensen

Introduction

The accident at the Three Mile Island Unit 2 (TMI-2) nuclear power plant near Middletown, Pennsylvania, on Wednesday, March 28, 1979, was the most serious in a commercial nuclear power plant in the United States even though it led to no deaths or injuries to plant workers or members of adjacent communities. It did have major implications for the nuclear industry because it resulted in major changes in the regulatory requirements involving emergency response planning, reactor operator training, radiation monitoring, human factors engineering, radiation protection, and many other areas of nuclear power plant operations. It also caused the US Nuclear Regulatory Commission (NRC) to tighten and heighten its regulatory oversight.

The accident at TMI-2 was initiated at 4 AM by a minor malfunction, or transient, in the non-nuclear part of the reactor. The main feed-water pumps stopped running, caused by either a mechanical or electrical failure, which prevented the steam generators from removing heat. This minor event would evolve into a series of automated responses in the reactor's coolant system, and during all of this, the relief valve on top of a piece of equipment called 'the pressurizer' would become stuck in an open position. Misreading of the plant conditions by the operators over a 2 1/4 h period before the relief valve was closed and the turning off of an automatic emergency cooling system caused the reactor core to become partially uncovered and severely damaged. The major consequences of the accident would unfold over the next week and it would take a month to bring the reactor to a cold shutdown.

Emergency Response

During the first several days of the accident, communications between the NRC Incident Response Center in Bethesda, Maryland, and the site were problematic and making it extremely difficult for the NRC to obtain up-to-date information from the plant and utility. Communications were so poor that by Friday morning the NRC management personnel still did not have a clear understanding of conditions at the site. As a result, the NRC recommended an evacuation to the state on the basis of poor and incomplete information. A general evacuation was never officially ordered. Communications did not

improve until Harold Denton, designated the sole source of information, arrived at the TMI site and communicated directly with NRC headquarters, the Governor's office, and the White House.

The suggestion by the NRC of a possible large-scale evacuation out to 20 miles was quite different from the planning requirements imposed by the NRC and Pennsylvania before the accident. The 5 mile emergency plans were developed according to a Pennsylvania requirement for emergency planning within a 5 mile radius of nuclear power plants. At TMI-2, although the radiation releases were significantly lower than the design-basis accident, evacuation was being considered for distances much greater than 5 miles.

On mid-morning of Friday, March 30, the governor's press secretary told reporters that there was no need for evacuation and that people in a 10 mile vicinity of the plant remain inside for a while. The only official warning to the public to evacuate came at ~12:30 PM, on Friday, when then Governor Thornburgh advised pregnant women and preschool age children to leave the area within a 5mile radius of TMI until further notice. He also ordered schools to close. The advisory to pregnant women and preschool children was lifted on April 9.

On Saturday and Sunday, other NRC officials believed there was an imminent danger of an explosion of a hydrogen bubble that had formed within the reactor vessel, and the possibility of a large evacuation was again a major subject of discussion. By Monday, the hydrogen bubble had been substantially reduced. Harold Denton announced on Tuesday, April 3, that the bubble had been eliminated.

Human Exposure to Radiation

It is estimated that between March 28 and April 15, the collective dose (total population dose) resulting from the radioactivity released to the population living within a 50 mile radius of the plant was ~2000 person-rems. The estimated annual collective dose to this population from natural background radiation in this area is ~240 000 person-rems. Thus, the increment of radiation dose to persons living within a 50 mile radius due to the accident was somewhat less than 1% of the annual background level.

The maximum estimated radiation dose received by any one individual in the off-site general population (excluding the plant workers) during the accident was 70 millirems. Estimates are that the average dose to ~2 million people in the area was only about 1 millirem. To put this into context, exposure from a full set of chest X-rays is ~6 millirems. Compared to the natural radioactive background dose of ~100–125 millirems per year for the area, the average dose to a person living within 5 miles of the nuclear plant was calculated to be ~10% of annual background radiation and probably was less. The maximum dose to a person at the site boundary would have been less than 100 millirems.

On the basis of scientific knowledge, the radiation doses received by the general population as a result of exposure to the radioactivity released during the accident were so small that there will be no detectable additional cases of cancer, developmental abnormalities, or genetic ill-health as a consequence of the accident at TMI.

In the months following the accident, many questions were raised by members of the public and interest groups about possible adverse effects from radiation on human, animal, and plant life in the TMI area. Thousands of environmental samples of air, water, milk, vegetation, soil, and foodstuffs were collected by a number of groups monitoring the area. These samples showed that very low levels of radionuclide could be attributed to releases from the accident. However, comprehensive investigations and assessments have concluded that in spite of serious damage to the reactor, most of the radiation was contained and that the actual release had negligible effects on the physical health of individuals or the environment and no adverse effects could be directly correlated to the accident.

Public Response

The governor's warning was the only official warning issued by the government. People in the vicinity of the plan were bombarded by media coverage of the events. This coverage suggested that a major evacuation was imminent. As a result, many people decided to evacuate despite the limited recommendation by the governor. It is estimated that 144 000 people within a 15 mile radius evacuated. It is also estimated that some people in the 15–40 mile radius also evacuated. Major reasons for evacuating were concern over the hydrogen bubble or conflicting information. The main reasons for not evacuating were that people had to work or they were waiting for an official evacuation order.

Most people within the 15 mile radius evacuated on Friday, March 30. A much smaller number evacuated on Thursday, Saturday, and Sunday. Most people had returned to their homes by Thursday, April 5, well before the lifting of the governor's order.

Conclusion

Following the TMI accident President Carter formed a commission to investigate the accident and make recommendations about needed changes in the

nuclear power industry. Six months later the 12 member commission issued its findings, recommending fundamental changes in the organization, procedures, practices, as well as the nuclear industry. Radical changes in the way the industry was regulated ensued. These regulations still continue to evolve as the industry matures.

See also: Chernobyl; Cuyahoga River; Radiation Toxicology, Ionizing and Nonionizing.

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Relevant Website

http://www.nrc.gov – Fact Sheet on the Accident at Three Mile Island.

Threshold Limit Value *See* Occupational Exposure Limits.

Thyroid Extract

Greene Shepherd

This article is a revision of the previous print edition article by Bridget Flaherty, volume 3, pp. 236–237, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 8028-36-2
- Synonyms: Dry thyroid; Desiccated thyroid; Thyreoidin; Thyroid Strong, Thyroglobin
- Chemical/Pharmaceutical/Other Class: Natural hormone that provides a mixture of levothyroxine (T4) and liothyronine (T3)

Uses

Thyroid extract is used in the treatment of hypothyroidism, myxedema, and cretinism. It is also used as a diagnostic agent and for suppression of pituitary thyroid-stimulating hormone. Synthetic derivatives are preferred because of uniform potency.

Exposure Routes and Pathways

Ingestion is the route of exposure in both accidental and intentional exposures.

Toxicokinetics

Thyroid extract is partially absorbed from the gastrointestinal tract. Up to 79% of a therapeutic dose is absorbed. Approximately 99% is protein bound. Thyroid extract contains both levothyroxine (T4) and liothyronine (T3). T4 is deiodinated in the liver, kidney, and tissues to form active T3 and inactive T2. The half-life of T4 is 5.3–9.4 days. T3 has a half-life of 2.5 days.

Mechanism of Toxicity

Thyroid hormones are necessary for metabolism, growth, and development. The main effect of thyroid hormones is increased metabolic rate, increased oxygen consumption, and increased metabolism of carbohydrates. Because the mixture contains both T3 and T4, systemic toxicity will be evident within a few hours and may be quite prolonged. Synthetic products that contain only T4 can have a latent period of several days before the development of significant symptoms.

Acute and Short-Term Toxicity (or Exposure)

Animal

Animals that ingest thyroid extract are at risk for thyroid toxicity. Signs of toxicity include vomiting, diarrhea, tachycardia, tachypnea, a decreased level of consciousness, and restlessness. Animal preparations frequently have higher concentrations that human preparations do.

Human

Ingestion of small amounts of thyroid extract produces few symptoms, if any. Symptoms of thyroid toxicity include increased heart rate, nausea, vomiting, diarrhea, restlessness, and fever. The development of thyrotoxicosis with acute exposure is rare except in very large overdoses (grams of thyroid extract containing several milligrams of T3 and/or T4).

Chronic Toxicity (or Exposure)

Animal

Thyroid extract has been used in veterinary practice. Current practice involves synthetic levothyroxine.

Toxicity is related to excessive thyroid hormone (manifested as polyuria, polydipsia, nervousness, aggressiveness, tachycardia, hyperthermia).

Human

Chronic overdosing is more likely to cause thyrotoxicosis than an acute overdose. Thyrotoxicosis should be suspected in patients exhibiting tachycardia, cardiac arrhythmias, hypertension, tremors, and seizures. Coma and circulatory collapse can be seen in severe cases of thyrotoxicosis. This is especially dangerous in patients with cardiac conditions. Deaths have also occurred in healthy adults that have used thyroid extract to lose weight.

In Vitro Toxicity Data

Studies of *in vitro* and *in vivo* models of hyperthyroidism have documented substantial impact on rat liver function. Recent developments have suggested that these findings are likely due to induction of apoptosis via a mitochondria-mediated pathway or pathways.

Clinical Management

Basic and advanced life-support measures should be utilized as needed. Activated charcoal is an effective method of gastric decontamination for large ingestions that present soon after exposure. EKG and blood pressure monitoring should be utilized in severe cases. Measurements of T3 and T4 levels should be obtained frequently in large ingestions until levels have normalized. Propranolol (a nonselective beta antagonist) can be used to treat hypertension, tachycardia, and cardiac arrhythmias. Extracorporeal means of elimination are ineffective in most cases due to extensive protein binding.

See also: Levothyroxine; Liothyronine.

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Ticks *See* Lyme Disease.

Times Beach

Pertti J Hakkinen

The Town of Times Beach, Missouri, USA, gained international attention in 1982 when the US Environmental Protection Agency (US EPA), acting upon recommendations from the US Centers for Disease Control (now the Centers for Disease Control and Prevention), closed down the town after discovering dangerous levels of dioxin. The roads to the town were blocked off, and the site was patrolled around-the-clock by security guards. The contamination occurred because the town and other local towns, businesses, farmers, and churches had, in the early 1970s, hired a waste oil recycler to spray dioxin-contaminated used oil on local roads and parking lots to control dust. The dioxin was an unwanted chemical by-product of certain manufacturing processes, and the recycler had mixed dioxin wastes from a chemical plant into the oil.

Times Beach was one of the most extensive cleanups in US EPA Superfund history. The cleanup effort officially began when, in 1983, the US EPA added the site to the first Superfund National Priorities List (NPL) for further investigation and long-term cleanup actions. After the site was listed, the US EPA permanently relocated >2000 people and tore down all of the homes and businesses.

Cleaning the Times Beach site was a massive estimated $200 million effort that included installation of a temporary incinerator to burn the contaminated soil, and the erection of a 15 ft high barrier around the incinerator to protect that area from regular flooding by the Meramec River. Contaminated soils were dug up, burned, and the resulting waste ash was buried on site. Cleanup of the site was completed by the end of 1997 by the US EPA and Syntex Agribusiness, the company that assumed responsibility for the site's cleanup. More than 265 000 tons of dioxin-contaminated soil from the site and 27

nearby areas that had been sprayed with dioxin-contaminated waste oil had been cleaned. The US EPA and the State of Missouri worked closely with Syntex during cleanup to ensure that the restoration made the site suitable for productive use. It is now a home to an extensive bird sanctuary and migratory bird waterways as a result of the cleanup. The migratory bird waterways were created by allowing some of the soil excavation pits to fill with rain water. Further, in 1999, a new 500-acre State park commemorating the famous US highway named Route 66 opened on the site.

See also: Dioxins.

Relevant Website

http://www.epa.gov – Details of 'Times Beach' and 'Superfund Successfully Responds in Times Beach' can be found in the US EPA website.

Tin

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-31-5
- Synonym: Stannum
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formulas: Sn^{2+}; Sn^{4+}

Uses

Tin is found in many forms, including stannous oxide (SnO), triethyltin $(Sn(C_2H_3))_3$, and triphenyltin hydroxide $(Sn(C_6H_6)OH)$. Tin compounds have many uses, including protective coatings, tin plate, and cans. Alloys such as bronze, brass, and solders also contain tin. Minor uses include dyes, ceramics, flame retardants, and pigments. Stannous fluoride is often used in toothpaste to prevent cavities. Organotin compounds have been used as antifouling agents, as pesticides, and as stabilizers in plastics.

Background Information

Tin has a long, colorful history. This metal was discovered first in Thailand over 2000 years ago. Early craftsmen discovered that bronze – a noncorrosive metal that is extremely hard and strong enough to be used for spears, swords, arrows, and other especially important objects at that time – could be produced by smelting tin with copper. Tin is also the primary constituent of pewter. Long ago, people developed the belief that trace amounts of tin seemed to help prevent fatigue and depression, and that drinking out of tin cups could help combat these ailments. Tin toys and tin roofs have also enjoyed great popularity in the past.

Exposure Routes and Pathways

Inhalation, dermal contact, and ingestion are all potential pathways of exposure to the different forms of inorganic and organic tin one might encounter in the environment. Human exposure to tin is primarily by ingestion of food, especially canned food products. Occupational exposure to tin may be significant in some industrial environments. In industrial areas, tin is inhaled from polluted air. Organotin compounds, typically found mostly in water, can be absorbed dermally. Stannous fluoride can be swallowed from toothpaste. Elemental tin may be ingested with food. Large amounts of tin must be ingested before levels of absorbed tin are detectable.

Ambient environmental levels of tin are generally quite low, except in the vicinity of pollution sources. The Environmental Protection Agency has previously identified tin in just 11 of 1177 evaluated hazardous waste sites.

Toxicokinetics

Inorganic tin compounds are not easily absorbed from the gastrointestinal tract. Inhaled tin first resides in the lungs and then is transferred to the liver and kidneys. Absorbed compounds are carried by the red blood cells. Inorganic tin is mainly excreted in urine. Organic tin compounds are more easily absorbed from the gastrointestinal tract and skin, concentrated in the blood and urine, and excreted in the bile. Most of an administered dose is excreted within 48 h.

There is little information on the effect of tin on enzymes. Organic tin compounds can inhibit the hydrolysis of adenosine triphosphate, resulting in uncoupling of oxidative phosphorylation.

Mechanism of Toxicity

All organic tin compounds inhibit mitochondrial oxidative phosphorylation (hydrolysis of adenine triphosphate) and brain glucose oxidation and are toxic. Very little data are available on inorganic tin.

Acute and Short-Term Toxicity (or Exposure)

Animal

Organic tin can be corrosive. In general, however, tin is considered to have relatively low acute toxic effects.

Human

Organic tin compounds are classified as eye, skin, and respiratory irritants. Inhaled tin particles lead to a mild pneumoconiosis known as stannosis. Orally absorbed inorganic tin produces nonspecific symptoms, including nausea, vomiting, diarrhea, muscle twitching, and even paralysis. Organic tin compounds are much more toxic than inorganic tin compounds.

Chronic Toxicity (or Exposure)

Animal

Although tumorigenic in rat implant studies, there is no conclusive evidence that tin compounds are mutagenic, carcinogenic, or teratogenic. Absorbed tin concentrates in the kidneys, liver, and bone of experimental animals.

Human

Major target organs are the nervous system, respiratory system, gastrointestinal system, and kidneys. Tetraethyltin is converted to triethyltin, which is a potent skin irritant and neurotoxin. It produces depression with loss of memory and aggressive behavior. It also produces cerebral edema and en cephalopathy. Triphenyltin is an immunodepressant. Some organic tin compounds are unusually toxic to the central nervous system.

Clinical Management

Supportive measures must be taken; there is no specific antidote or chelating agent for tin. Administration of water (for dilution) after ingestion of a tin compound may be helpful. Emesis is not recommended.

Ecotoxicology

Tin is a naturally occurring element found in environmental media in inorganic compounds. Tin may be released to the environment from natural and anthropogenic sources. The most significant releases of tin are from burning of fossil fuels and industrial production and use of tin. Tin compounds are generally only sparingly soluble in water and are likely to partition to soils, sediments, and possibly to aquatic organisms. Photodegradation of organotins may occur at relatively slow rates. Organotin compounds may be significantly bioconcentrated by aquatic organisms. Tin has been historically used in antifouling paints and coatings for the bottom of boats, but this has been discontinued due to its extreme toxicity to marine organisms.

A bioconcentration factor (BCF) relates the concentration of a chemical in plants and animals to the concentration of the chemical in the medium in which they live. It was estimated that the BCFs of inorganic tin were 100, 1000, and 3000 for marine and freshwater plants, invertebrates, and fish. Marine algae can bioconcentrate stannic tin by a factor of 1900. The BCF of tributyltin was estimated to be 473, but measured BCFs were always higher. Bioconcentration factors for bis(tributyltin)oxide with marine oysters were measured as 2300–11 400. Seven-day BCFs were derived for seven organotin compounds for muscle, liver, kidney, and vertebra tissue of carp. The BCFs ranged from 12 to 5012; the highest factors were found for tributyltins. However, these factors were not based on steady-state conditions, and may be low estimates. No information was obtained on the food chain and biomagnification of inorganic or organic tin.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value (TLV) – time-weighted average for tin metal, tin oxide, and inorganic compounds (except SnH_4) is $2\,mg\,m^{-3}$. The TLV for tin oxide is $1\,mg\,m^{-3}$. The TLV for organic tin compounds is $0.1\,mg\,m^{-3}$ with a skin exposure warning.

See also: Metals; Neurotoxicity; Organotins; Pollution, Water.

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Relevant Websites

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http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Tin.

Tissue Repair

Udayan M Apte and Harihara M Mehendale

Introduction

Tissue repair (TR) refers to compensatory regeneration of a tissue followed by surgical, mechanical, or chemical-induced injury resulting in restoration of structure and function of the tissue. TR is characterized by cell division to increase the number of cells, differentiation, and specification of the newly divided cells, angiogenesis, that is, regeneration of blood vessels to restore blood supply, and regeneration of extracellular matrix (ECM), which holds the tissue together. TR is a complex and comprehensive process that encompasses various aspects of tissue rebuilding and is governed by an intricate molecular signaling (**Figure 1**). Not all the tissues in the body are capable of stimulating TR. The so-called 'postmitotic' tissues such as muscles and nervous tissue cannot undergo TR while other tissues such as skin, liver, kidney, and lungs are capable of undergoing a TR upon surgical resection, mechanical, or chemical injury.

TR following chemical-induced injury has its special characteristics. Unlike surgical resection, chemicals cause injury at a slower pace and the progression of injury takes place long after the offending chemical is removed from the body. In most cases chemicals are metabolized in the body by various drug-metabolizing enzymes (DMEs) to generate reactive metabolites. These highly reactive metabolites bind and inactivate macromolecules in the cell necessary for cellular functions leading to cell death. Initiation of injury in the tissue leads to stimulation of compensatory TR, directed toward replacing the dead cells. Thus, the extent of TR following chemical injury often depends on the dose of the offending toxicant, the extent of injury, the toxicokinetics of the toxicant (half-life), and exposure time (acute versus chronic exposure). Additionally, TR is governed by a number of other factors

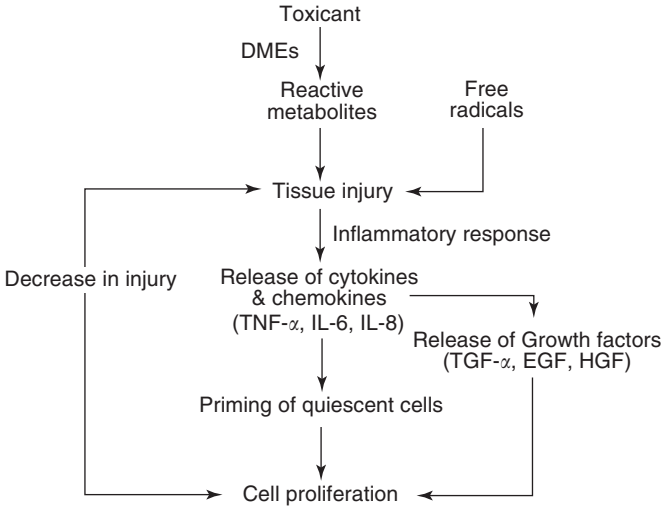

Figure 1 Schematic representation of stimulation of tissue repair. Chemicals and drugs are metabolized in the tissue to their reactive metabolites, which initiate injury. An inflammatory response follows the injury stimulating release of cytokines and chemokines, which prime the quiescent cells to enter cell cycle. Additional stimulation to complete cell division comes from growth factors. As cell proliferation increases, the dead tissue is replaced by viable cells and injury regresses.

such as the age, species and strain difference, nutrition, and presence of disease.

The Cells Involved in Tissue Repair

There are three main types of cells in the body depending upon their regenerative capacity – labile, stable, and permanent. The labile cells are under continuous active division and replace the cells that are lost from the body. Examples of labile cells include the epithelia of ducts, hematopoietic stem cell, and epidermis. Injury to labile cells is rapidly repaired due to an aggressive TR response.

The stable cells have a long life span and divide at a very slow rate. The parenchymal cells of the most solid glandular organs such as liver and kidney are stable cells. They are capable of undergoing rapid division upon injury to the organ to replace the dead cells. Regeneration following injury in the tissues that contain stable cells requires having at least some healthy cells remaining to undergo proliferation and regeneration. Liver is a classic example of such type of tissue where, under normal conditions, less than 1% cells are in division. Upon partial hepatectomy or acute chemical-induced injury, the healthy hepatocytes are stimulated for division resulting in complete restoration of tissue mass and liver function. In case of a chronic injury, the repeated injury caused by the chemicals results in failure of repair mechanisms leading to scar formation (fibrosis), cirrhosis and may develop into cancer.

The central nervous system and the cardiac and skeletal muscles are permanent tissues that do not divide in postnatal life. Due to the inability in proliferation, injury to such tissue results in a scar formation and a permanent loss of function. Extensive investigations are underway to compensate the inability of proliferation in these tissues by using stem cells.

Tissue Repair in Specific Organs

The main organs that exhibit capacity to regenerate following chemical-induced injury (and surgical/mechanical injury) include liver, kidney, lungs, skin, and gastric mucosa. The regeneration of skin has been mainly studied using various models of wound repair and are not a major form of toxicant-induced injury. TR following toxic injury in true sense is observed mainly in liver, kidney, lungs, and gastrointestinal tract, out of which, liver remains to be the most widely studied organ.

Tissue Repair in Liver

The remarkable capacity of liver to regenerate upon surgical resection or toxicant-induced injury has been studied extensively and is still at the center of research in hepatobiology. Liver regeneration has been known since ancient times as indicated by the myth of Prometheus. According to the myth, Prometheus, a Greek god, stole fire from Zeus and gave it to the humans. Zeus punished Prometheus for this by subjecting Promethues's liver to be eaten by an eagle. Each night the eagle would eat Prometheus's liver, which would grow back the next morning, only to be eaten again by the eagle, thus subjecting Prometheus to an eternal torture. Thus, the myth of Prometheus can be viewed as an evidence that ancient humans knew about the regenerative capacity of the liver.

Two-third partial hepatectomy (PH) is the main model used to study liver TR. Surgical removal of 70% tissue mass of liver results in stimulation of a massive regeneration response. Hepatocytes are generally in a differentiated, nondividing stable state. Upon PH, hepatocytes dedifferentiate and enter cell cycle and proliferate. Substantial cell division is observed within 2 days following PH along with regeneration of ECM, angiogenesis, and regeneration of hepatobilliary ducts. In experimental animals such as rats subjected to PH, the complete tissue mass is replaced within seven days following PH.

In the last two decades extensive information has been gathered about TR following chemical-induced liver injury. Liver is a prime target for a variety of chemicals including pharmaceutical drugs, mainly by virtue of its role as a major site of drug metabolism in the body. Hepatocytes are the main reservoirs of DMEs, which metabolize drugs and toxicants to more water-soluble metabolites. During this process, sometimes, toxic metabolic intermediates arise, which attack the macromolecules of hepatocytes directly, or by generating free radicals, resulting in injury and death of the hepatocytes. In response to such drug-induced injury, liver TR is stimulated, which opposes progression of injury by replacing the dead cells and restoring the structure and function of liver. Liver TR following chemical-induced toxicity holds a great clinical significance since acute liver failure (ALF) induced by drugs and toxicants is a prevalent clinical condition. It is known that more than 800 pharmaceutical drugs and chemicals are associated with ALF. In such conditions, ability of the patient to stimulate an effective TR may have a lasting effect on the final outcome (survival versus death) of the ALF. Extensive research is being conducted to develop regenerative therapies against drug-induced hepatotoxicity and ALF.

A number of models have been used to study the TR following chemical-induced injury in

experimental animals. Liver TR has been studied in rodents following liver injury induced by carbon tetrachloride (CCl_4), acetaminophen, thioacetamide, galactoasmine, trichloroethylene (TCE), allyl alcohol, and lipopolysaccharide (bacterial endotoxin). The major finding of studies with a diverse group of chemicals and a number of different animals models is that TR plays an important role in determining survival following toxicant exposure. It is observed that animals treated with nonlethal doses of toxicants develop relatively lower liver injury, and recover as soon as TR replaces the dead cells. In contrast, animals exposed to very high, lethal doses exhibited inhibition of TR resulting in progression of injury and death.

Tissue Repair in Kidney

Due to the filtration and excretory function, kidney receives extensive blood supply, which makes it a prime target for toxicant induced injury. Kidney damage due to toxicants leads to necrosis of tubular cells, resulting in renal failure and death. The renal tubular epithelium is known to regenerate following toxicant- or ischemia-induced renal injury. The renal tubule or nephron is the functional unit of kidney and exhibits regiospecific differences in regenerative capacity in its different structures. The glomeruli do not exhibit regenerative capacity and thus, glomerular damage is irreversible. In contrast, the tubular epithelium exhibits extensive ability for TR, and as a result, the damage to tubular epithelium is completely reversible. Similarly, the cortical tubules exhibit much higher regenerative capacity as compared to the medullary tubules.

A number of toxicants and drugs are known to induce renal injury resulting in acute renal failure, a prevalent clinical condition. These include antibiotics such as gentamycin, anticancer agents such as cisplatin, radiocontrast agents such as diatrizoates, heavy metals such as mercury, and metabolites of environmental toxicants such as *S*-(1-2-dichlorovinyl)-L-cysteine (DCVC, a metabolite of potent carcinogen TCE, which is a liver toxicant itself). Renal TR has been studied in great detail using various toxicants including DCVC, cisplatin, and folic acid and these studies indicate that TR plays a significant role in survival following exposure to renal toxicants.

Tissue Repair in Lung

Lung is a complex tissue formed of a variety of histologically different cell types. Lung is damaged by a number of airborne toxicants such as ozone, arsenic, asbestos and isocyanates. Many of the lung toxicants are important from the occupational health point of view. Lung exhibits extensive regenerative capability, especially in the epithelium of trachea and bronchi, which regenerate rapidly after abrasive action of airborne toxicants. Lung injury initiates an inflammatory response (which is also true in the case of all other organs), following which the alveolar type II pneumocytes undergo rapid proliferation and migration, finally differentiating into alveolar type I pneumocytes. The type I pneumocytes repopulate the damaged areas and restore the epithelial lining.

Tissue Repair and Dose Response

One of the most impressive characteristics of TR following chemical-induced toxicity is its ability to follow a dose response (**Figure 2**). It has been observed that increasing doses of toxicant induce TR in increasing order, until a threshold is reached.

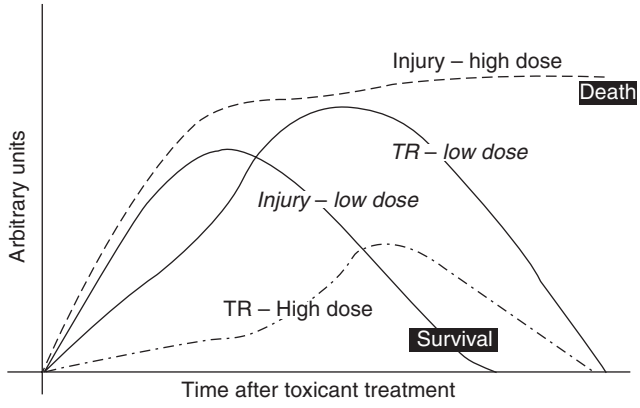

Figure 2 Schematic representation depicting that tissue repair follows dose response. Tissue repair (TR) increases as the dose of the offending chemical increases, until a threshold dose (high dose in this graph), where TR is inhibited resulting in progression of injury and animal death.

Doses higher than the threshold dose inhibit TR leading to progression of injury and death. The extent of TR is, in most cases, governed by the extent of injury. At very high (and mostly lethal) doses, the molecular signaling that stimulates repair is inhibited resulting in inhibition of tissue repair. It is known that TR follows such dose response following administration of individual toxicants and chemical mixtures and is universally observed in all the organ systems.

Factors Affecting Tissue Repair

Studies with a diverse group of chemicals, various organ systems and various animal models such as mice, rats, guinea pigs, hamsters, and dogs have revealed that TR following toxicant exposure depends upon a number of factors such as age, species and strain difference, nutrition and disease condition.

Under experimental conditions, age difference seems to affect the ability of animal to mount an effective TR. It has been observed that neonatal animals are capable of stimulating a prompt and robust TR as compared to adult animals. This is due to the fact that most of their organ systems are still developing and rapid cell division is underway in those organs. Interestingly, old animals such as 12- and 24-month-old rats, exhibit increased ability to stimulate TR following toxicant-induced injury as compared to young adults (3 months old). Certain species and strains exhibit higher TR following toxicant treatment as compared to others. Gerbils have much sluggish TR as compared to rats while F344 rats have higher TR as compared to Sprague–Dawley rats.

Nutritional status changes the ability of animal to stimulate repair. In general, it is observed that high glucose levels inhibit TR while animals fed diet supplemented with fatty acids increases TR response. Interestingly, caloric restriction is known to stimulate TR in various organs including the liver and gastrointestinal tract. The modulation of molecular signaling that underlies the TR by these various nutritional components is central to their unique effects on TR.

Diabetes, known to inhibit the wound repair process, has also been shown to inhibit TR following toxic injury. Diabetes represents a special condition where the extent of injury does not determine the extent of TR since the ability of the animal to mount TR following injury is hampered by the disease condition. In diabetic rats, no matter how low the injury might be, it progresses due to inability of the animal to stimulate repair leading to organ failure.

Molecular Signaling Involved in Tissue Repair

TR is a complex process involving proliferation of parenchymal cells, regenerations of ECM, angiogenesis and reorganization of tissue and is governed by an equally complex network of molecular signaling. Studies with PH in liver, unilateral and five-sixth nephrectomy, chemical-induced liver and kidney injury, and wound repair have collectively generated extensive information about the signal transduction pathways involved in TR. Generally, the first cells to sense the injury are the resident macrophages of the organ such as Kupffer cells in the liver. These macrophages release a variety of proinflammatory cytokines and chemokines, which stimulate and attract the neutrophils and monocytes to the site of injury. Recent evidence suggests that the chemokines and cytokines also stimulate proliferation of other healthy parenchymal cells. These cytokines include tumor necrosis factor-α, interleukin-6 (IL-6), IL-8, and others. It is now known that these cytokines prime the resting nondividing parenchymal cells to enter into cell cycle and undergo division. The primed cells are further stimulated by various growth factors such as transforming growth factor-α (TGF-α), hepatocyte growth factor, and epidermal growth factor. These growth factors stimulate the cells via their cell surface receptor and an intricate intracellular network of kinase enzymes and nuclear transcription factors such as nuclear factor-κB, and AP-1 to complete the cell division. The regeneration of ECM is known to be governed by a complex balance between the ECM-degrading matrix metalloproteases and TGF-β. The role of nuclear receptors such as peroxisome proliferators-activated receptor, retinoid-x-receptor, liver-x-receptor, etc. has also been demonstrated. One of the main mysteries of TR is the timely termination of cell division, a process important in maintaining the critical balance between compensatory repair and unregulated cancerous growth. The molecular signaling involved in the termination of TR is not completely clear, though TGF-β has been implicated in this process.

Significance of Tissue Repair

Extensive evidence gathered from experiments with a number of diverse toxicants, animal models and interventional experiments indicates that TR plays a critical role in the final outcome (survival versus death) of the chemical-induced injury. An effective, timely, and robust TR results in regression of injury, restoration of structure and function of the tissue,

while inhibition of TR leads to progression of injury, and organ failure resulting in animal death. This is important in clinical settings where therapies directed toward stimulation of TR of a patient can lead to survival. Similarly, early detection of factors stimulating TR (such as increase in growth factors) may provide doctors a good prognostic marker helpful in deciding future treatment. In risk assessment area, assessment of TR may indicate additional information about the outcome of toxic exposure and have significant impact on setting guidelines of toxicant exposure.

See also: Diabetes, Effect of Toxicity; Dose–Response Relationship; Kidney; Liver.

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Titanium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-32-6
- Synonyms: Titanate; T40
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Ti^{n+} ($n = 2, 3, 4$)

Uses

Titanium metal is lightweight and has high strength; thus, it is used in aircraft and other structures where cost is not a major factor. It also resists corrosion, making it especially useful in surgical implants and prostheses. Titanium fibers are used as an asbestos substitute. Titanium's most widely used compound, titanium dioxide, is used as a white pigment in paints and plastics and as a food additive to whiten flour, dairy products, and candies. It is also used in cosmetics and sunscreen formulations.

Exposure Routes and Pathways

Ingestion is the primary exposure pathway. Corn oil, butter, and white wheat products are perhaps the main sources of titanium. In industrial settings, inhalation is an important pathway. Titanium is not absorbed dermally.

Toxicokinetics

Approximately 3% of ingested soluble titanium is absorbed. The lungs are the main depot for inhaled titanium; it tends to remain in the lungs for long periods. Titanium is also found in the kidneys, the liver, and some fat tissue. Titanium crosses the blood–brain and placental barriers. Absorbed titanium is excreted in the urine.

Acute and Short-Term Toxicity (or Exposure)

Human

Titanium dioxide appears to be relatively nontoxic. Ti has been used extensively in food products without apparent adverse effects. Upper airway irritation is the principle sign of acute overexposure. Increased pulmonary dust disposition may lead to alveolar cell hyperplasis and fibrosis.

The liquid titanium tetrachloride is corrosive to skin and membranes of the eye. This may be due to liberation of hydrochloric acid on hydrolysis.

Some people may be hypersensitive to titanium.

Chronic Toxicity (or Exposure)

Animal

Titanium is neither mutagenic nor carcinogenic. Titanocene (an organic compound), however, induced fibrosarcoma when injected intramuscularly in rats. This same compound was carcinogenic against the Ehrlich ascites tumor in mice. There have been reports of tumors induced with the pure metal. Titanium dioxide did not induce tumors when administered orally; however, a few lung tumors were detected after titanium dioxide dust was inhaled by rats.

Certain titanium compounds may be nephrotoxic and hepatoxic to animals.

Human

Generally, titanium dioxide is considered physiologically inert by all routes; however, if relatively high concentrations of titanium dioxide dusts are inhaled, toxicological actions are noted. A weak fibrosis of the lung tissue occurs but is not fatal.

Clinical Management

Because of the low toxicity of titanium dioxide, there have not been any reports of therapy. Generally, titanium dioxide is biologically nonreactive when administered orally or intravenously.

Exposure Standards and Guidelines

Titanium is classified as a nuisance particulate with an ACGIH (American Conference of Governmental Industrial Hygienists) threshold limit value time-weighted average of 10 mg kg^{-1}.

Miscellaneous

Titanium was discovered by the Reverend William Gregor in 1791, and is named after the 'Titans' of Greek mythology.

See also: Metals; Toxicity Testing, Inhalation.

Further Reading

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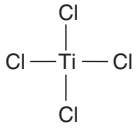
Relevant Website

http://www.inchem.org – Titanium (Environmental Health Criteria 24) from the International Programme on Chemical Safety. Titanium Dioxide (Summaries and Evaluations from the International Agency for Research on Cancer).

Titanium Tetrachloride

Robert Kapp

- Related Compounds: Titanium dichloride (CAS 10049-06-6); Titanium trichloride(CAS 7705-07-9); Titanium dioxide (CAS 13463-67-7); Titanium sulfate (CAS 13693-11-3)
- Chemical Abstracts Service Registry Number: CAS 7550-45-0
- Synonyms: Tetrachlorotitanium; Titanic chloride; Titanium chloride; Titanium chloride ($TiCl_4$); Titanium tetrachloride; Titantetrachlorid (German); Titaantetrachloride (Dutch)
- Chemical/Pharmaceutical/Other Class: Inorganic halide
- Chemical Formula: $TiCl_4$
- Chemical Structure:



Uses

Titanium tetrachloride is used as an intermediate in the production of titanium metal, titanium dioxide, and titanium chloride pigments, as a polymerization catalyst, in the manufacture of iridescent glass and faux pearls, and with ammonia to produce smoke screens. It is also used as a catalyst in many organic syntheses in the chemical industry. Titanium tetrachloride was formerly used with potassium bitartrate as a mordant in the textile industry, and with dyewoods in dyeing leather.

Background Information

Titanium tetrachloride is a colorless to light yellow liquid with a pungent odor.

Exposure Routes and Pathways

Exposure to titanium tetrachloride is primarily occupational with titanium industry workers having the highest potential for exposure. Titanium tetrachloride enters the environment in the air primarily

from factories that use the material in various chemical processes, or as a result of accidental spills. The material reacts quickly with water in the air to form hydrochloric acid, titanium hydroxide, and titanium oxychlorides. Titanium tetrachloride in its pure form is not present in water, soil, food, or air, except in manufacturing sites that use or manufacture the material. Because titanium tetrachloride breaks down rapidly, it is unlikely that anyone outside the workplace would be exposed to it. Minimal exposure could occur from breathing vapors or touching the material during accidental spills in the workplace.

Toxicokinetics

No human or animal studies were found on adsorption, distribution, metabolism, or excretion of titanium tetrachloride. Because of the nature of titanium tetrachloride, it is suggested that the major route of exposure is via inhalation, with the lungs as the major target organ. Dermal exposure can also result where accidental spills have occurred. It has been shown that titanium dioxide was present in the lungs of workers occupationally exposed to titanium tetrachloride.

Mechanism of Toxicity

The instability of titanium chloride leads to its hydrolysis, giving off heat and producing hydrochloric acid among other materials. The hydrochloric acid is partially responsible for the corrosive effects noted following titanium tetrachloride exposure. The hydrolysis of titanium tetrachloride occurs in several steps, which produces titanium oxide hydrate that can absorb the hydrochloric acid vapors and carry them into deeper parts of the lungs. There are no studies done on the mechanism of toxicity following ingestions of titanium tetrachloride. Following dermal exposure to titanium tetrachloride, the hydrochloric acid produced in the reaction with water is responsible for the serious thermal and acid burns that occur.

Acute and Short-Term Toxicity (or Exposure)

Animal

The dermal LD_{50} in the rabbit is $3160\,mg\,kg^{-1}$, while the inhalation LD_{50} in the rat is $400\,mg\,l^{-1}$. Eye injury, including corneal opacity, necrotic keratitis, and conjunctivitis, occurred in rats acutely exposed to titanium tetrachloride vapors. Acute animal tests in rats and mice have demonstrated titanium tetrachloride to have high to extreme acute toxicity via inhalation.

Human

Titanium tetrachloride is a severe skin, eye, and respiratory irritant and corrosive. It can also severely irritate the mucous membranes and the lungs. Inhalation of high levels of titanium tetrachloride can be fatal due to the ensuing lung injury from the hydrochloric acid produced. Acute (short-term) exposure may result in constriction of various sections of the upper respiratory tract in humans.

Chronic Toxicity (or Exposure)

Animal

The major effect of exposure of experimental animals to titanium tetrachloride was an increase in the incidence of rhinitis in the respiratory tract with increasing levels of concentration of titanium tetrachloride (0.1, 1.0, and $10.0\,mg\,l^{-1}$). Tracheitis also increased with duration of exposure and concentration of the material. Gross pathology and histology revealed compound-related changes in the thoracic lymph nodes and the lungs of the treated animals, and the severity of alveolar hyperplasia was noted as increased at increasing concentrations. In addition, high dose animals had a significant increase in neutrophils and a concomitant decrease in lymphocytes. Lesions described as lung squamous cell carcinoma and keratinizing squamous cell carcinoma were observed in rats in a 2 year chronic inhalation study performed in 1986. A 1994 reevaluation of the slides from this study by a group of pathologists concurred that these lesions should have been more properly characterized as either squamous metaplasia or proliferative keratin cysts. Based upon this new evaluation, titanium tetrachloride was not found to be carcinogenic in rats.

Human

Pleural thickening and decreased pulmonary function have been associated with chronic exposure to titanium tetrachloride in titanium metal workers. Chronic inhalation exposure may result in upper respiratory tract irritation, chronic bronchitis, cough, bronchoconstriction, wheezing, chemical pneumonitis, or pulmonary edema in humans. Because titanium tetrachloride rapidly hydrolyzes upon contact with water, the negative findings from the limited studies performed are insufficient to reach any conclusion about titanium tetrachloride's ability to induce genotoxic effects.

Epidemiological studies conducted on titanium tetrachloride are inadequate to determine whether this material can cause carcinomas in occupationally

exposed workers or not. No adequately conducted definitive studies on either humans or animals could be located with respect to the potential of titanium tetrachloride to produce reproductive or developmental effects. The (US) Environmental Protection Agency (EPA) has not established a reference dose or a reference concentration for titanium tetrachloride.

Clinical Management

Upon ocular exposure, the eye should be generously washed with tap water. Refer for medical attention. In case of dermal exposure, contaminated clothing should be removed and the skin should be rinsed with tap water. Titanium tetrachloride ingestion should be referred for medical attention and vomiting should not be induced. Upon inhalation, the victim should be removed to fresh air and given artificial respiration if indicated. The body should be placed in a half-upright position. Refer for medical attention.

Environmental Fate

Environmental exposure to titanium tetrachloride is unlikely because it hydrolyzes rapidly upon contact with moist air to form a vapor of hydrochloric acid, titanium dioxide, and titanium oxychloride.

Exposure Standards and Guidelines

The American Industrial Hygiene Association recommended Workplace Environmental Exposure Level: 8 h time-weighted average is $500\,mg\,m^{-3}$. Titanium tetrachloride is not listed as a carcinogen by the (US) Environmental Protection Agency, the International Agency for Research on Cancer, the (US) National Institute of Environmental Health Sciences National Toxicology Program, the (US) Occupational Safety and Health Administration, and the American Conference of Governmental Industrial Hygienists. The (US) Agency for Toxic Substances, and Disease Registry has calculated a chronic inhalation minimal risk level (MRL) of $0.0001\,mg\,m^{-3}$ based on respiratory effects in rats. The MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. Exposure to a level above the MRL does not mean that adverse health effects will occur. The MRL is intended to serve as a screening tool.

See also: Alkyl Halides.

Further Reading

Murthy LI, Dankovic DA, and Murthy RC (2001) Titanium, zirconium, and hafnium. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 2, pp. 687–688. New York: Wiley.

Roy PK, Bhatt A, and Rajagopal C (2003) Quantitative risk assessment for accidental release of titanium tetrachloride in a titanium sponge production plant. *Journal of Hazardous Materials* 102: 167–186.

Relevant Websites

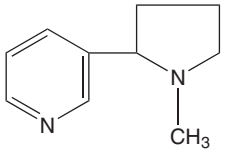
http://www.epa.gov – US Environmental Protection Agency (EPA), Air Toxics. Titanium Tetrachloride.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Titanium Tetrachloride.

Tobacco

C Lynn Humbertson

- Chemical Abstracts Service Registry Number: CAS 54-11-5 (nicotine)
- Synonyms: *Nicotiana tabacum* (cultivated tobacco); *Nicotiana rustica*; Methylpyridylpyrrolidine
- Description: Tobacco products contain dried tobacco leaves, which are used to take advantage of the psychoactive effects of the alkaloid nicotine. Snuff has a pH of 7.8–8.2. Cigarettes are acidic. Chewing tobacco has alkali added and is basic
- Chemical Formula: $C_{10}H_{14}N_2$ (nicotine)
- Chemical Structure:



Nicotine

Uses

Tobacco products do not have a therapeutic use and can produce physiologic addiction. Commonly used products include cigarettes, chewing tobacco, snuff,

and cigars. Tobacco enemas have been used to treat intestinal parasites. Nicotine is used as a pesticide.

Background Information

The tobacco plant is a tall annual that belongs to the Nightshade or Solanaceae family. The Nightshade family includes at least 2400 species, including crop plants, poisonous plants, herbs, shrubs, trees, and perennial flowering plants. Commonly known members of this family include: tomato, potato, eggplant, tobacco, bittersweet, and petunia. Nightshades include plants that contain medicinally active and highly toxic constituents. These include alkaloids (such as nicotine, atropine, and scopolamine), belladonna, and mandrake.

Tobacco includes numerous species that are grown all over the world. The species *Nicotiana tabacum* is known as common tobacco. Common tobacco is what is primarily used in cigarettes. It is native to the West Indies, Mexico, and South America. The two varieties of common tobacco that account for most of what is grown in the United States are the large-leaf 'burley' and 'bright-leaf' tobacco.

Naturally occurring active constituents in common tobacco include alkaloids, organic acids, and nicotine. The nature and concentration of the active constituents vary with the species variety and strain of plant as well as growing conditions.

Exposure Routes and Pathways

Tobacco is smoked, nasally insufflated, or chewed to make the nicotine bioavailable for absorption.

Toxicokinetics

The absorption of the nicotine in tobacco is incomplete after ingestion. Rectal administration via an enema may bypass the first-pass metabolism and result in higher serum levels and toxicity. Snuff is well absorbed nasally. Cigarette tobacco contains 15–20 mg nicotine per gram of tobacco and cigars contain 15–40 mg nicotine. Cigarette butts contain 25% of the total cigarette nicotine content. By the 1980s, cigarettes contained 15 mg tar and 1.3 mg nicotine. Snuff is made from powdered tobacco leaf and contains from 4.6 to 32 mg g^{-1} nicotine in the moist material; dry snuff contains 12.4–15.6 mg g^{-1}.

Peak plasma levels occur 15–30 min after ingestion and 2–10 min after smoking cigarettes. Nicotine undergoes a large first-pass effect during which the liver metabolizes 80–90%. Smaller amounts are metabolized in the lungs and kidneys. The metabolites include isomethylnicotinium ion, nornicotine, cotinine, and nicotine-1-*N*-oxide. Protein binding ranges from 4.9% to 20%. The presence of significant amounts of nicotine in the gastrointestinal tract after intravenous dosing suggests that passive diffusion or enterohepatic circulation occurs. The apparent volume of distribution in animals is $\sim 1\,l\,kg^{-1}$. In one clinical study, it was $2\,l\,kg^{-1}$ in smokers and $3\,l\,kg^{-1}$ in nonsmokers. Nicotine passes into breast milk in small quantities. Nicotine and its metabolites are excreted in the urine. At a pH of 5.5 or less, 23% is excreted unchanged. At a pH of 8, only 2% is excreted in the urine. Nicotine can be found in the urine of nonsmokers.

Mechanism of Toxicity

Tobacco smoke includes ~4000 chemical species with varying potential which cause adverse effects. Nicotine is stimulating to the autonomic nervous system ganglia and neuromuscular junction. The most prominent effects relate to stimulation of the adrenal medulla, central nervous system (CNS), cardiovascular system (release of catecholamines), gastrointestinal tract (parasympathetic stimulation), salivary and bronchial glands, and the medullary vomiting center. There is subsequent blockade of autonomic ganglia and the neuromuscular junction transmission, inhibition of catecholamine release from the adrenal medulla, and CNS depression.

Acute and Short-Term Toxicity (or Exposure)

Animal

A dose of 10 mg kg^{-1} (buccally) is fatal in dogs. Symptoms include initial hyperexcitability, hyperpnea, salivation, vomiting, diarrhea, then depression, incoordination, and paralysis.

Human

Nicotine is highly toxic. Ingestion of more than one cigarette or three cigarette butts, one cigar, or a pinch of snuff is toxic. Symptoms begin within 30–90 min of ingestion and persist for 1 or 2 h after mild exposure and 18–24 h after severe intoxication. Vomiting usually occurs within minutes of absorption, which helps to decrease the severity of intoxication. Abdominal pain and delayed diarrhea are possible. CNS symptoms include headache, dizziness, agitation, incoordination, convulsions, and/or coma. Cardiovascular effects seen include initial hypertension followed by hypotension, tachycardia, then bradycardia, and cardiac arrhythmias. Respiratory symptoms include initial tachypnea followed by

dyspnea, increased bronchial secretions, respiratory depression, cyanosis, and/or apnea. Infants are especially sensitive to the effects of tobacco.

Chronic Toxicity (or Exposure)

Human

Exposure to tobacco in several forms is associated with an increased risk of cancer; in addition, several active ingredients, such as nicotine, have been demonstrated to be addictive. Tobacco smoke is a significant indoor air pollutant. It includes ~4000 components, some regulated as human carcinogens. Second-hand smoke is a particular concern for children's health and is not only associated with an increase in lifetime risk of cancer, but an increased risk of developing respiratory conditions such as bronchitis, pneumonia, and asthma.

Chronic use of snuff has caused oropharyngeal cancer. Tobacco and alcohol ambylopia is seen in chronic smokers who are malnourished and alcoholic. Green tobacco sickness occurs in young workers who do not smoke but work with wet, uncured tobacco. Withdrawal symptoms can occur when use of a tobacco product is stopped.

The occurrence of various cancers and decreased cardiovascular function are increased with tobacco use. These effects may also occur via passive inhalation of cigarette or cigar smoke.

In Vitro Toxicity Data

Tobacco has tested positive in the Ames assay. Tobacco grown in some areas has been reported to show differing results in the Ames assay. A recent study reported tobacco smoke aerosols generated at temperatures greater than 400°C to be positive in the Ames assay (activated with rat liver S9) (strains TA98 and TA100). Aerosols generated at lower temperatures did not test positive in the same study.

Clinical Management

If ingested, syrup of ipecac-induced emesis should be avoided since seizures or lethargy can occur rapidly. Activated charcoal should be administered. Seizures should be treated with diazepam or phenytion. Atropine can be used to control signs of excess parasympathetic stimulation. If hypotension does not respond to intravenous fluids, dopamine or norepinephrine may be indicated. Antacids should be avoided since nicotine has greater absorption in an alkaline media. Vital signs and level of consciousness should be monitored closely. Further care is symptomatic and supportive. Nicotine laboratory determination is only of diagnostic value and does not direct therapy.

Ecotoxicology

Growth and production of tobacco and tobacco products use significant natural resources, from land to materials such as wood used to dye and cure tobacco. This has led to deforestation in some areas of the world with subsequent erosion and flooding of agricultural lands.

The common tobacco plant depletes soil nutrients (e.g., nitrogen, phosphorus, and particularly, potassium) at a higher rate than most food and cash crops (e.g., cotton, coffee). One of the reasons for tobacco's high uptake of soil nutrients is the practice of 'topping' the plants to increase the growth of leaves and increased nicotine content contributes to the increased uptake of soil nutrients.

Other Hazards

Use of cigarettes and matches is the leading cause of deaths from fires in the United States.

Dried tobacco should not be used in animal feed. Pets and livestock should not drink water that has been in contact with tobacco or tobacco products (e.g., ashtrays or puddles where tobacco is being harvested and processed) as the water may contain high levels of nicotine. There is some controversy over risks associated with pregnant sows ingesting tobacco leaves. Grazing on tobacco is not recommended until risks are better understood.

See also: Carcinogenesis; Developmental Toxicology; International Agency for Research on Cancer; Neurotoxicity; Nicotine; Tobacco Smoke.

Further Reading

Conti-Fine BM, Navaneetham D, Lei S, and Maus AD (2000) Neuronal nicotinic receptors in non-neuronal cells: New mediators of tobacco toxicity? *European Journal of Pharmacology* 393(1–3): 279–294.

Relevant Websites

http://www.who.int – World Health Organization (2000) Final Report: Advancing Knowledge on Regulating Tobacco Products Oslo, Norway, 9–11 February 2000.

http://www.ash.org.uk – Clive Bates, Martin Jarvis, Tobacco additives and Gregory Connolly (1999) Cigarette engineering and nicotine addiction. Action on Smoking and Health. London (14 July 1999).

http://www.oehha.ca.gov – Office of Environmental Health Hazard Assessment (OEHHA) California. Health Effects of Exposure to Environmental Tobacco Smoke.

http://www.hhs.gov – The Health Consequences of Smoking: A Report of the Surgeon General (2004).

Tobacco Smoke

Robert Kapp

Chemical

Cigarettes, cigars, and pipes produce tobacco smoke during the combustion process. Tobacco smoke is an aerosol, formed when tobacco is incompletely burned during the smoking of cigarettes, pipes, or cigars. Temperatures in burning cigarettes range from ambient to ~950°C, depending on the amount of oxygen present.

During the burning of tobacco (itself a complex mixture), thousands of chemical substances are generated. These compounds are typically classified into a particulate phase (trapped on a glass-fiber pad, and termed 'TPM' (total particulate matter)) and a gas/vapor phase (which passes through such a glass-fiber pad). 'Tar' is a mathematically derived material determined by subtracting the weight of the nicotine and water from the TPM. Typical smoke components include nicotine (CAS 54-11-5) (mostly in the particulate phase), and carbon monoxide (CAS 630-08-0) (gas phase). However, several components of tobacco smoke (e.g., hydrogen cyanide (CAS 74-90-8), and formaldehyde (CAS 50-00-0)) do not fit neatly into this rather arbitrary classification. Analysis of tobacco smoke has yielded a number of toxicologically significant chemicals and groups of chemicals, including polycyclic aromatic hydrocarbons, tobacco specific nitrosamines, aldehydes, hydrogen cyanide, nitrogen oxides, benzene (CAS 71-43-2), toluene (CAS 103-38-3), phenols, and aromatic amines. The radioactive element polonium-210 (CAS 013981-52-7) and benzopyrene (a carcinogen (CAS 50-32-8)) are also known to occur in tobacco smoke.

Cigarettes are designed and produced with various tar yields. The Federal Trade Commission designated a test to measure the amount of 'tar', nicotine, and carbon monoxide using smoking machines. Tobacco companies use terms such as 'full flavor', 'medium', 'mild', 'light', and 'ultra light' to describe the strength of the taste of cigarettes. These terms are commonly referred to as 'descriptors' and facilitate smokers' ability to distinguish among different product offerings. Descriptors are generally used as a point of comparison for a cigarette brand in order to distinguish it from other brands on the market.

Some researchers report that smokers of 'light' cigarettes inhale as much 'tar' and nicotine as from full-flavor brands. Volume 13 of the National Cancer Institute's Smoking and Tobacco Control Monograph Series concluded "that people who switch to low-'tar' or 'light' cigarettes from 'full flavor' cigarettes are likely to inhale the same amount of cancer-causing toxins and they remain at high risk for developing smoking-related cancers and other diseases." Generally, as 'tar' yield increases, the amounts of individual constituents increase. Several studies indicate that across brands, the relative proportions of constituents remain similar.

Physical

The particles in tobacco smoke are liquid aerosol droplets (~20% water), with a mass median aerodynamic diameter that is submicrometer (and thus, fairly 'lung-respirable' by humans). The droplets are present in high concentrations (some estimates are as high as 10^{10} droplets per cm^3). Most cigarettes today contain a filter, consisting principally of cellulose acetate although other materials have been used (e.g., charcoal granules, paper, etc.) The filter can reduce 'tar' and nicotine smoke yields up to 50% by several different mechanisms, with an even greater removal rate for other classes of compounds (e.g., phenols). Selective filtration of the vapor-phase components of tobacco smoke is conceptually much simpler than selective removal of the permanent gas or particulate components. Cigarette filters containing charcoal granules (either as a cavity filter or embedded into the cellulose acetate) appear to be effective in reducing concentrations of such toxicologically important tobacco smoke components such as 1,3-butadiene (CAS 106-99-0) and acrolein (CAS 107-02-8), but are completely ineffective in reducing other such toxicologically important components such as carbon monoxide.

Toxicology

Cigarette smoking causes lung cancer, heart disease, chronic obstructive pulmonary disease, emphysema, and other serious diseases in smokers. Smokers are

far more likely to develop serious diseases, like lung cancer, than nonsmokers. Given the high prevalence of smoking (at least in the Western world), smoking tobacco is considered the single most preventable cause of human disease. Public health officials have concluded that secondhand smoke from cigarettes causes disease, including lung cancer and heart disease, in nonsmoking adults, as well as causes conditions in children such as asthma, respiratory infections, cough, wheeze, otitis media (middle ear infection), and sudden infant death syndrome. It has also been shown that serum immunoglobulin levels and T killer cell activity decrease in smokers. Immunological studies in tobacco-smoke exposed animals have demonstrated suppression of antibody responses and enhanced susceptibility to murine sarcoma virus and influenza virus. There are currently no validated animal models to predict human disease from smoking. Animal inhalation carcinogenicity studies with tobacco smoke are negative. Tobacco smoke is mutagenic in most *in vitro* assays with and without metabolic activation. Tobacco smoke condensate promotes the formation of papillomas in the 'mouse skin painting assay'. Tobacco smoke is not teratogenic in animal studies; however, it is recognized as producing low birth weight in humans. In humans, the number of alveolar monocyte/macrophage cells (MOs) has been shown to increase several-fold in smokers versus nonsmokers. This increase may be a result of an increased production of IL-1 by the alveolar MOs, which results in an influx of polymorphonuclear cells and peripheral blood mononuclear cells into the lung. While these MOs appear to be in an activated state, they manifest decreased phagocytic and bactericidal activity.

Mechanisms

Despite the strength of the epidemiological associations, the actual mechanisms by which smoking can cause so many diseases remain largely unknown. A major problem in establishing the mechanism is the inability to reproduce the human diseases in animal models. In particular, many attempts have been made to produce lung cancer in animals exposed to tobacco smoke by the inhalation route, without success. A recent review stated that "significant increases in the numbers of malignant tumors of the respiratory tract were not seen in rats, mice, hamsters, dogs or nonhuman primates exposed for long periods of time to very high concentrations of mainstream cigarette smoke." It is only by collecting the 'tar' and repeatedly painting this on to mice that tumors are produced, and these tumors (along with the test material and the target organ) are very different from those tumors exhibited by smokers. While there is no direct information to indicate that nicotine is responsible for any of the major diseases associated with smoking, nicotine does accelerate heart rate, elevate blood pressure, and constrict blood vessels within the skin. These are considered to be the result of stimulation of the ganglionic sympathetic nervous system.

Addiction

The overwhelming medical and scientific consensus is that cigarette smoking is addictive. It is not clear exactly which components of the smoke are responsible for the addiction. Scientific data indicate that nicotine contributes to the addiction; however, other factors may be involved in the addiction process. It is believed that nicotine may exert an effect by binding to a subset of cholinergic receptors that are located at the neuromuscular junction and in the central nervous system where psychoactive and addictive properties reside. In addition, nicotine is associated with alterations of electroencephalographic recordings in humans. Nicotine replacement therapy is not completely successful in aiding in quitting smoking on the one hand and very large numbers of smokers are able to spontaneously stop smoking on the other. Hence, the specific etiology of tobacco addiction remains unknown.

Future Considerations

Given the major health problems that result from smoking, and the high prevalence of people who are unwilling to quit, there is a need for the development of potentially reduced exposure products, or PREPs, as described in a recent publication from the National Academy of Sciences. Cigarette manufacturers have responded in a number of ways including changes in the blend composition, novel filter designs to assist in selective filtration, and the complete removal of burning tobacco as the heat source for the release of flavorful components of tobacco smoke. Animal models for smoke-induced disease are needed to further validate these novel products as reduced-risk products. Additionally, validated biomarkers of effect are needed to more precisely predict the effects in humans.

See also: Carcinogenesis; Cardiovascular System; Developmental Toxicology; Immune System; International Agency for Research on Cancer; Neurotoxicity; Respiratory Tract.

Further Reading

Davis DL and Nielsen MT (1999) *Tobacco Production, Chemistry and Technology.* Ames, IA: Iowa State University Press. ISBN 0632047917.

Institute of Medicine (2001) *Clearing the Smoke: Assessing the Science Base for Tobacco Harm Reduction*. ISBN 0-309-07282-4.

Relevant Websites

http://www.hhs.gov – The Health Consequences of Smoking: A Report of the Surgeon General, 2004.

http://www.cdc.gov – National Center for Chronic Disease Prevention and Health Promotion – Tobacco Information and Prevention Source.

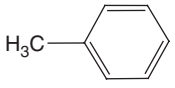
http://cfpub2.epa.gov – Respiratory Health Effects of Passive Smoking (also known as Exposure to Secondhand Smoke or Environmental Tobacco Smoke –ETS).

http://www.oas.samhsa.gov – Results from the 2002 National Survey on Drug Use and Health: National Findings Department of Health and Human Services.

Toluene

Stephen R Clough

- Chemical Abstracts Service Registry Number: CAS 108-88-3
- Synonyms: Methylbenzene; Phenylmethane; Toluol (DOT); Antisal 1a; Methacide; Methylbenzol; NCI C07272; Toluen (Dutch); Toluen (Czech); Tolueno (Spanish); Toluolo (Italian); Tolu-sol
- Chemical/Pharmaceutical/Other Class: Aromatic hydrocarbon
- Chemical Formula: C_7H_8
- Chemical Structure:



Uses

Most toluene is added to automobile or aviation gasoline mixtures (benzene $\geqslant$ xylene $\geqslant$ toluene) to increase octane ratings. Toluene is an excellent organic solvent and is used extensively in the manufacture of benzene derivatives, caprolactam, saccharin, medicines, dyes, perfumes, TNT, toluenediisocyanates (polyurethane resins), toluene sulfonates (detergents); as a solvent for scintillation counting; in paints and coatings, gums, resins, rubber; and as a diluent and thinner in nitrocellulose lacquers, plastic toys, and model airplanes. Toluene is also used extensively in the production of glues and is responsible for the narcosis and permanent brain damage seen in 'glue sniffers'.

Exposure Routes and Pathways

Because toluene is fairly volatile, exposure for humans would occur principally by inhalation. It has a human odor threshold of $\sim 0.1\,\mathrm{mg\,m^{-3}}$ (~ 26 ppb). Dermal exposure may also be significant, especially in an industrial setting, where skin may be exposed for long periods of time. Oral exposure is the least probable route and would occur primarily as a result of accidental poisoning or suicide.

Toxicokinetics

Toluene is readily absorbed from the lung and gastrointestinal tract, although studies in animals suggest absorption occurs more slowly in the gastrointestinal tract. Slow absorption also occurs through skin. Studies of humans and animals indicate that inhaled toluene distributes to tissues that are high in fat content (e.g., body fat, bone marrow, and brain) or well supplied with blood (e.g., liver). It seems reasonable to assume that similar distribution would occur for other routes of exposure.

In both humans and animals, toluene is rapidly excreted as both the unchanged compound in expired air and as a metabolite in the urine. Toluene is converted in the liver to water-soluble hippuric acid and conjugated cresols, which are then excreted in the urine. This conversion has been demonstrated in man and animals exposed via inhalation, although it is expected to occur for other exposure routes as well. Another excretion route for toluene is exhalation of the unchanged chemical. This excretion route might be expected to operate for all exposure routes but be more effective for exposures via inhalation.

Mechanism of Toxicity

Although the exact biochemical mechanism of toxicity has not been identified for toluene, it is known that the primary toxic effect of toluene is dysfunction of the brain and central nervous system (CNS-narcosis). The main function of neurons is to conduct electrochemical signals to one, several, or thousands of other cells. The normal physiology of these neurons is, in turn, largely dependent on the integrity of the cell membrane, which polarizes and depolarizes during the transmission of these signals. Thus, the most probable mechanism of toxicity is the unique

sensitivity of the cell membranes of neurons to the solvent-like property of toluene, which disrupts the normal transmission of nerve impulses.

Acute and Short-Term Toxicity (or Exposure)

Animal

The acute toxicity of toluene in laboratory animals is very low. The oral rat LD_{50} is $\sim 5\,g\,kg^{-1}$ and the inhalation LC_{50} in mice is ~ 400 ppm over 24 h.

Human

Much of the information on toxicity of toluene to humans comes from studies of solvent abuse (such as glue sniffing) and during exposure in the workplace (e.g., painters and printers). Interpretation of the data can be difficult due to the fact that these individuals are simultaneously exposed to mixtures of other chemicals. Both acute experimental and occupational exposures to toluene in the range of 100–1500 ppm ($\sim$325–5600 mg m^{-3}) have elicited dose-related CNS alterations, such as fatigue, confusion, and incoordination, as well as impairments in reaction time and perceptual speed. At 200–500 ppm, headache, nausea, eye irritation, loss of appetite, a bad taste, lassitude, and incoordination are reported but not accompanied by significant laboratory or physical findings. For high acute exposures ($\sim$30 000 ppm), initial lightheadedness and exhilaration is followed by progressive development of narcosis and CNS depression.

Chronic Toxicity (or Exposure)

Animal

Toxicity to the embryo or fetus and teratogenic effects have been rarely observed in animal studies. These effects were seen in only one experiment in which the dose was high enough to be toxic to the mother as well. More frequently, when maternal toxicity was not present, fetal toxicity or teratogenicity was not found. Growth inhibition of rat pups born during inhalation exposure to toluene through two generations has been observed.

CIIT conducted a 2 year inhalation toxicology study in Fischer 344 rats exposed to atmospheric toluene. The concentrations used were 30, 100, or 300 ppm (113, 377, or 1130 mg m^{-3}) for 6 h per day, 5 days per week. The only finding was a dose-related reduction in hematocrit values (number of red blood cells) in female rats exposed to 100 and 300 ppm toluene. This is not considered a significant toxic effect. Therefore, a no-observed-adverse-effect level was set at the highest exposure level – 300 ppm (equivalent to 29 mg kg^{-1} day^{-1}).

National Toxicology Program (NTP) also conducted a 2 year inhalation study in mice and rats (doses of 600 or 1200 ppm in rats and 120, 600, and 1200 ppm in mice, 6.5 h day^{-1}, 5 days per week). Lesions of the nasal cavity (in rats) and abnormal growth (hyperplasia) of the bronchial lining (in mice) were seen, but no deaths and significant body weight changes were observed during the course of the study. There was no evidence of cancer induction in this study.

In a recent study by NTP, rats and mice were given oral doses of toluene, ranging from 312 to 5000 mg kg^{-1}, 5 days per week for 13 weeks. General toxic effects, which included decreased movement or prostration, tearing and salivation, and body tremors were seen in both species at 2500 mg kg^{-1}. A few animals died at this dose. There were changes in organ weights and microscopic pathologic changes of several organs at 1250 mg kg^{-1} in rats. Organ weight changes, but not pathologic changes, were seen at 2500 mg kg^{-1} in mice but not at lower doses. The only adverse effect seen at the lowest dose was increased liver and kidney weights at 625 mg kg^{-1} in rats.

Human

After long-term exposure, blood abnormalities, psychomotor disorders, changes in the lens of the eye, immune system changes, kidney effects, menstrual disorders, and birth defects have been observed in some, but not all, studies of workers or abusers, and the possible confounding effect of mixed chemical exposure is mentioned in most. Liver effects, which figure prominently in animal studies, have not been observed in occupationally exposed individuals. Based on epidemiological studies, there is no evidence that toluene can cause cancer in humans. Although there was no evidence of cancer in the CIIT or NTP studies, and most mutagenicity tests have been negative, US Environmental Protection Agency (EPA) considers the data inadequate to classify toluene relative to its carcinogenicity; it is rated D (not classified, inadequate evidence in animals) in the current weight-of-evidence system.

Clinical Management

Persons who have been overcome by toluene fumes or gases should be removed from the area of exposure to fresh air. Should breathing become labored or shallow, medical intervention (e.g., artificial respiration) may be necessary. Following accidental or intentional ingestion, vomiting should not be induced and prompt medical attention should be

obtained. Liquid toluene spills on exposed skin should be immediately dried with an absorbent towel and then washed with soap and water.

Environmental Fate

Automobile emissions contribute the majority of toluene that is found in the atmosphere. Toluene is the most prevalent aromatic hydrocarbon in the air, with levels ranging from 0.14 to 59 ppb. Toluene has also been detected in surface water and treated wastewater effluents at levels generally below $10\,\mu g\,l^{-1}$. Toluene is readily biodegradable and will not bioconcentrate or bioaccumulate within a food web. In a study of edible aquatic organisms, 95% of the tissues sampled had levels <1 ppm.

Ecotoxicology

According to the US EPA ECOTOX aquatic toxicity database, the saltwater organism that is the most sensitive to toluene is the pink salmon, with respective acute (48 h) and chronic (96 h) LC_{50}s of 6190 and $6410\,\mu g\,l^{-1}$. For freshwater organisms, the most sensitive organism for both an acute (48 h) and a chronic (96 h) exposure is the rainbow trout, with respective LC_{50} values of 6780 and $5800\,\mu g\,l^{-1}$. These organisms are exposed in a laboratory setting and the concentrations of toluene used are many, many times higher than what would be anticipated to occur in natural waters.

Exposure Standards and Guidelines

Under US EPA current guidelines for risk assessment, the acceptable exposure dose for humans (or reference dose) is $0.2\,mg\,kg^{-1}\,day^{-1}$. For an average human weighing 70 kg, this dose is equivalent to $\sim$1/2000th of an ounce.

Under the Safe Drinking Water Act, the maximum contaminant level (MCL) is the standard criterion for drinking water and the maximum contaminant level goal (MCLG) is the goal. The MCL and MCLG for toluene in drinking water are $1000\,\mu g\,l^{-1}$, based on health protective limits developed from the CIIT study. The Occupational Safety and Health Administration recommends workplace air concentrations do not exceed 100 ppm; the American Conference of Governmental Industrial Hygienists recommends 50 ppm (based on potential skin exposure).

Miscellaneous

Toluene is a clear, flammable liquid with a sweet odor that is widely used in both the chemical and the pharmaceutical industries. In terms of production, it is the 24th highest volume chemical in the United States. It is derived mainly from petroleum refining and only a small percentage of that produced is used directly.

See also: Pollution, Air; Pollution, Air Indoor; Pollution, Water; Sensory Organs; Skin.

Further Reading

Filley CM, Halliday W, and Kleinschmidt-DeMasters BK (2004) The effects of toluene on the central nervous system. *Journal of Neuropathology & Experimental Neurology* 63(1): 1–12.

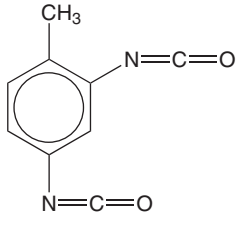
Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Toluene.

Toluene Diisocyanate

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 584-84-9
- Synonyms: Toluene 2,4-diisocyanate; 2,4-Diisocyanatotoluene; TDI; Nacconate 100
- Chemical Formula: $CH_3C_6H_3(NCO)_2$
- Chemical Structure:



Uses

Toluene diisocyanate is used in the production of polyurethane foams, elastomers, and dyes. It is a cross-linking agent for nylon-6.

Exposure Routes and Pathways

The respiratory and dermal routes of exposure are of most concern.

Toxicokinetics

Toluene diisocyanate has rapid linear absorption via inhalation with persistence in tissues at low levels for up to 2 weeks. Absorption of toluene diisocyanates by inhalation is reflected by high acute toxicity following such exposure. Little information is available on the distribution of toluene diisocyanates in mammals. Reaction of toluene diisocyanate with serum albumin yields protein conjugates. Toluene diisocyanate is hydrolyzed into 2,4-diaminotoluene in man.

Mechanism of Toxicity

Toluene diisocyanate is a cross-linking agent and both a pulmonary and dermal sensitizer. Most toxicity occurs with repeated exposure.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rat oral $LD_{30} = 6.17\,g\,kg^{-1}$. It is irritating to the gastrointestinal tract upon ingestion. The RD_{50} (50% respiratory depressive concentration) in mice is 0.4 ppm. The LC_{50} (inhalation) is 14 ppm per 4 h in rats and 10 ppm per 4 h in mice.

Human

Toluene diisocyanate is a strong irritant to the eyes, skin, gastrointestinal tract, and respiratory system. It is a lacrimating agent and a strong dermal and pulmonary sensitizer. It can cause euphoria, ataxia, and mental aberrations. Signs and symptoms of acute exposure are nonspecific and include irritation of the nose and throat, shortness of breath, choking, coughing, nausea, vomiting, and abdominal pain. A common response to inhaled toluene diisocyanate is both acute and chronic decrease in ventilatory capacity, that is, decreased FEV1 (FEV = functional exhalation volume), even in the absence of overt signs or symptoms. The onset of signs and symptoms may be delayed and may persist for several days following removal from exposure.

Chronic Toxicity (or Exposure)

Animal

Experimental studies have shown that dermal application of toluene diisocyanate can elicit pulmonary sensitization. International Agency for Research on Cancer lists toluene diisocyanate as an animal carcinogen (from studies using gavage administration).

Human

Pulmonary sensitization is a serious complication following repeated toluene diisocyanate exposures. Signs may become more pronounced with continued exposure over days to months. Initial symptoms are nocturnal dyspnea and/or cough, progressing to bronchitis. In occupational settings, the time from initial employment to the development of asthmatic symptoms has ranged from 6 months to 20 years. Given sufficient exposure, virtually any person may become sensitized: the proportion of individuals with chemical asthma in working populations varied from 4.3% to 25%. Skin sensitization may also occur with repeated exposures. Urticaria, dermatitis, and allergic contact dermatitis have been reported in workers exposed to toluene diisocyanate-based resins. The dermatological symptoms included eczema and erythema. Toluene diisocyanate is a suspected carcinogen, but it is unclear whether it can lead to cancer with inhalation exposures.

Clinical Management

Affected eyes should be irrigated with running water. Contaminated areas of skin should be washed with soap and water. Patients asymptomatic of respiratory effects should receive oxygen and ventilatory support.

Environmental Fate

Ten days after a spill of 13 tons of toluene diisocyanate onto wet forest soil, the area was covered with sand. The soil concentration of toluene diisocyanate and toluenediamine declined from parts per thousand to parts per million from 10 days to 12 weeks after the spill. Six years later, only polyureas were found. Under controlled conditions, 5 kg of toluene diisocyanate was covered with 50 kg of sand and 5 kg of water and samples taken from the top and bottom of the sand. After 24 h, $<6\%$ toluene diisocyanate remained. Toluene diisocyanate is rapidly hydrolyzed

in aquatic environments. Elimination of toluene diisocyanate from the atmosphere is by reaction with hydroxyl radicals and by dry deposition.

Ecotoxicology

Little information is available on the ecotoxicology of toluene diisocyanate.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value for toluene diisocyanate is 0.005 ppm. The reference exposure level for toluene diisocyanate is 0.01 ppb.

See also: Respiratory Tract; Sensitivity Analysis.

Further Reading

Bingham E, Cohrssen B, and Powell CH (2001) *Patty's Toxicology*, 5th edn., vol. 4, pp. 1439–1443. New York: Wiley.

Bolognesi C, Baur X, Marczynski B, *et al.* (2002) Carcinogenic risk of toluene diisocyanate and 4,4′-methylenediphenyl diisocyanate: Epidemiological and experimental evidence. *Critical Reviews in Toxicology* 31: 737–772.

Collins MA (2002) Toxicology of toluene diisocyanate. *Applied Occupational and Environmental Hygiene* 17: 846–855.

Dart RC (2004) *Medical Toxicology*, 3rd edn. Philadelphia, PA: Lippincott.

Relevant Website

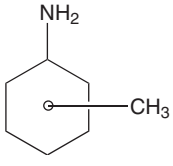
http://www.intox.org – Toluene Diisocyanate (UKPID Monograph from the International Programme on Chemical Safety).

Toluidine

Shayne C Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 3, pp. 251–252, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: *m*-Toluidine (CAS 108-44-1); *o*-Toluidine (CAS 95-53-4); *p*-Toluidine (CAS 106-49-0)
- Synonyms: Aminotoluene; *m*-Toluidine; *o*-Toluidine; *p*-Toluidine; 2-Methylaniline
- Chemical/Pharmaceutical/Other Class: Substituted aromatic
- Chemical Formula: $C_7H_8NH_2$
- Chemical Structure:



Uses

Toluidine is used to produce dyes for textiles and other substances and as an accelerator in vulcanization. It is also used in organic synthesis.

Exposure Routes and Pathways

Inhalation and dermal contact are possible routes of exposure.

Toxicokinetics

In urine, 83.9% of *o*-toluidine is excreted after 48 h. Urinary excretion for *m*-toluidine and *p*-toluidine over a 24 h period is 10%. Hydroxy- and *N*-acetyl derivatives have been identified as urinary metabolites.

Mechanism of Toxicity

Toluidine interferes with enzymes associated with the detoxification process and monooxygenase system. It defats membranes.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toluidine is an irritant primarily due to defatting. It is a mild skin irritant and moderate eye irritant in rabbits. Oral LD_{50}s in rats are 450 mg kg^{-1} for *m*-toluidine, 670 mg kg^{-1} for *o*-toluidine, and 3360 mg kg^{-1} for *p*-toluidine. Oral LD_{50}s in mice are 740 mg kg^{-1} for *m*-toluidine, 520 mg kg^{-1} for *o*-toluidine, and 330 mg kg^{-1} for *p*-toluidine.

Human

o-Toluidine is highly toxic to humans when absorbed through the skin, inhaled as vapor, or absorbed through the gastrointestinal tract. Acute exposure causes methemoglobinemia and central nervous system depression.

Chronic Toxicity (or Exposure)

Animal

In male and female mice exposed to 50 000 ppm *o*-toluidine for 7 weeks, pigment deposition was noted in the spleen, kidneys, and liver. Chronic exposure to *o*-toluidine can cause effects on the spleen, liver, urinary bladder, and blood (methemoglobinemia and reticulocytosis) in laboratory animals. The hydrochloride salt of *o*-toluidine was carcinogenic in rats and mice.

Human

Chronic effects in workers exposed to *o*-toluidine include anemia, anorexia, weight loss, skin lesions, central nervous system depression, cyanosis, and methemoglobinemia. *o*-Toluidine and *p*-toluidine are suspected carcinogens (bladder cancer).

In Vitro Toxicity Data

o-Toluidine has been found genotoxic in the Ames test, sister chromatid exchange, mouse lymphoma assay, and unscheduled DNA synthesis.

Clinical Management

All contaminated areas should be washed, including inside ear canals and under nails. The exposed person should be monitored for methemoglobinemia. If contamination is 30% or less, bed rest is recommended. If contamination is over 30%, the patient should be observed and given oxygen therapy. If contamination is over 50%, the exposed person should be given intravenous glucose solution. If contamination is $\geqslant$60%, methylene blue should be administered.

Environmental Fate

In soil, *o*-toluidine will be eliminated by biodegradation, oxidation, and binding to soil components. In water, toluidine will be eliminated by biodegradation, oxidation, and photooxidation as well as some adsorption to sediment. In the atmosphere, toluidine will photodegrade (half-life about 2 h).

Ecotoxicology

The 24 h LC_{50} in *Medaka* was 60 mg l^{-1}. In fathead minnows, the 96 h LC_{50} was >160 mg l^{-1}. Bioconcentration in aquatic species should not be a concern.

Exposure Standards and Guidelines

The threshold limit value for *o*-toluidine is 2 ppm. The permissible exposure limit is 5 ppm. No reference concentration or reference dose has been established for *o*-toluidine.

See also: Carcinogenesis; International Agency for Research on Cancer; Genetic Toxicology.

Further Reading

ortho-Toluidine (2000) *IARC Monographs on Evaluating Carcinogenesis Risks in Humans* 77: 267–322.

Woo Y and Lai DL (2001) Aromatic amino and nitro-amino compounds. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 4, pp. 1009–1014. New York: Wiley.

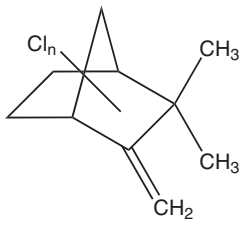
Relevant Website

http://www.inchem.org – *o*-Toluidine (Concise International Chemical Assessment Document Number 7 from the International Programme on Chemical Safety).

Toxaphene

David R Wallace

- Chemical Abstracts Service Registry Number: CAS 8001-35-2
- Synonyms: Chlorinated camphene; Camphochlor; Compound 3956; Melipax; Toxadust, Toxakill, Attac, Anatox; Strobane-T and others
- Chemical/Pharmaceutical/Other Class: Organochlorine insecticide
- Chemical Formula: Toxaphene contains over 670 chemicals
- Chemical Structure:



Uses

Toxaphene is an insecticide that contains over 670 chemicals and can exist as a yellow to amber solid or gas. Heavily used in the United States until 1982, its use was completely banned in 1990. Toxaphene was used primarily to control insects on cotton crops in the southern United States, it has also been used to control pests on livestock and to control unwanted fish in aquatic environments.

Exposure Routes and Pathways

Exposure can occur via oral, inhalation, or dermal routes. Principle exposure appears to have occurred during the manufacturing process, from dust or mists. Therefore, people with the greatest risk of toxaphene exposure were those involved in the manufacturing of toxaphene, cotton farmers, and registered insecticide applicators. It does not have significant solubility in water, but will accumulate in sediment on the lake or river bottom. Individuals can be exposure by breathing air near a toxaphene-contaminated waste site or in agricultural areas where toxaphene was used. Infants and children who consume soil contaminated with toxaphene are also at risk. Since toxaphene will accumulate in the sediment on lake or river bottoms, consumption of fish or shellfish that can concentrate toxaphene residues may pose a higher risk.

Toxicokinetics

Since toxaphene is a mixture of 670 chlorinated camphenes, some have greater toxicity than technical grade toxaphene alone. Toxaphene components A and B have been shown to have 6 and 14 times greater toxicity than the technical mixture, respectively. Component A is a mixture of 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane and 2,2,5-*endo*,6-*exo*,8,9,9,10-octachlorobornane. Component B has been identified as 2,2,5-*endo*, 6-*exo*,8,9,10-heptachlorobornane. Rats dechlorinate toxaphene either by reductive dechlorination or dehydrochlorination. Toxaphene can also be metabolized by NADPH-dependent mixed function oxidases in microsomal preparations.

Mechanism of Toxicity

The neuroexcitatory properties of toxaphene are due to its ability to reduce chloride uptake into neurons, leading to depolarization of the cells and hyperactivity. It is believed that toxaphene acts on the picrotoxin-binding site on the $GABA_A$ receptor. Toxaphene may also impair calcium transport, which will interfere with numerous neuronal pathways and function.

Acute and Short-Term Toxicity (or Exposure)

Short-term exposure to toxaphene above the levels established by the Environmental Protection Agency (maximum contaminant level (MCL)) has been shown to cause effects in the central nervous system (CNS), which include restlessness, hyperexcitability, tremors, spasms, or convulsions.

Animal

Oral administration of toxaphene caused generalized CNS hyperactivity in dogs and rats. Changes in both hepatic and renal function were described in both species.

Human

Short-term exposure of $0.5\,g\,m^{-3}$ for 30 min a day for 10 days caused no discernible effects in individuals inhaling toxaphene vapor. If toxaphene is spilled on skin or clothing, or if exposed skin comes in contact with toxaphene solid, pain and reddening of the tissue may occur. Oral ingestion of toxaphene has produced the most robust toxic responses. Toxaphene is readily absorbed through the intestines and an oral dose of 0.6 g can result in convulsions, nausea, vomiting, a bluish coloration of the skin (resembling cyanosis), and eventually coma or death. The estimated lethal dose for toxaphene in humans following oral ingestion is 2–7 g.

Chronic Toxicity (or Exposure)

Exposure to toxaphene above the MCL has been shown to cause liver and kidney degeneration, excitotoxicity of the CNS, suppression of the immune system and possibly cancer.

Animal

Mice that were fed a diet of technical grade toxaphene showed a significant increase in hepatic carcinomas (98% in the high dose versus only 8% in the control group). There also appeared to be disruption of the endocrine system. Toxaphene increases the hepatic metabolism of estradiol and estrone in rats, thus reducing their effects on the reproductive cycle.

Human

Workers that have been chronically exposed to toxaphene have exhibited genetic changes including

acentric fragments and chromatid exchanges. Whether toxaphene is carcinogenic in humans is unknown, but it can cause cancer in laboratory animals.

In Vitro Toxicity Data

There is no data of carcinogenicity in humans, but toxaphene is classified as 'B2; probable human carcinogen' based on bioassays in laboratory animals and positive mutagenesis results in *Salmonella* assays.

Clinical Management

Blood tests are available to determine the levels of toxaphene. Emesis should not be induced in individuals expected to have suffered from acute exposure as this may trigger potential CNS toxicity. Activated charcoal (25–100 g for adults and 25–50 g for children) should be administered to inactivate unabsorbed toxaphene. Gastric lavage should be used in cases where a lethal dose of toxaphene has been ingested. For seizures and other CNS hyperactivity, a CNS depressant such as an intravenous benzodiazepine (diazepam or lorazepam) or barbiturate (phenobarbital) should be administered. For injury to lungs, ventilation and oxygenation should be maintained. Arterial blood gases should be checked frequently and nonselective adrenergic agonists should not be administered (β-2 agonists result in localized bronchodilation with minimal cardiovascular effects, which may increase the risk for cardiac arrhythmias). Individuals who have experienced ocular contact with toxaphene should have eyes irrigated with copious amounts of water at room temperature. For dermal exposure, extensively wash hair and skin using soap, then alcohol and then soap again. Any contaminated clothing should be discarded.

Environmental Fate

At peak production in 1977, 40 million pounds of toxaphene were being used each year. Toxaphene has a very long half-life in soil and can persist for up to 14 years. Current evidence suggests that it does not leach out of the soil and into ground water; nor is it metabolized by bacteria in the soil. Toxaphene can evaporate and be degraded by photolysis. Run-off from contaminated soil can carry toxaphene into nearby bodies of water where it can be concentrated in fish.

Ecotoxicology

As with many pesticides, toxaphene poses a great concern to wildlife species. In many of the species examined, the LD_{50} value for toxaphene is in the same range as that determined for the rat (80–90 mg kg^{-1}) or below. Therefore, toxaphene could present a significant ecotoxicological problem if released into the environment in significant quantities. Dredging of contaminate sediments could release toxaphene and increase availability for aquatic organisms.

Exposure Standards and Guidelines

The Environmental Protection Agency has established a limit of 0.003 mg l^{-1} of drinking water and also requires that spills in excess of 1 lb be reported. The Occupational Safety and Health Administration (OSHA) has established a permissible exposure limit of 0.5 mg toxaphene per m^3 for an 8 h day/40 h work week. The National Institute for Occupational Safety and Health recommends that toxaphene levels should at the lowest dose/concentration as possible in the workplace due to the potential for toxaphene to be carcinogenic in humans. The American Conference of Governmental Industrial Hygienists recommends a limit equal to that established by OSHA and that 1 mg m^{-3} should not be exceeded over a 15 min period.

See also: Organochlorine Insecticides.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Toxaphene.

http://www.epa.gov – Environmental Protection Agency. EPA Ground water and drinking water: Consumer Factsheet on Toxaphene. United States Environmental Protection Agency, Washington, DC. March 6, 2003.

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Toxaphene.

Toxic Substances Control Act, US

Robert Kapp

- AGENCY: US Environmental Protection Agency (EPA)
- YEAR OF ENACTMENT/AMENDMENTS: Toxic Substances Control Act, 1976; Asbestos Hazard Emergency Response Act Amendment, 1986; Radon Program Development Act Amendment, 1988; Asbestos School Hazard Abatement Reauthorization Act Amendment, 1990; Residential Lead-Based Paint Hazard Reduction Act Amendment, 1992

Background Information

Beginning in the late 1960s and the early 1970s, there was recognition within the United States that existing laws did not adequately regulate the use of toxic chemical substances. One response was that President Nixon created the President's Council of Environmental Quality (CEQ) in 1969, followed by the formation of the EPA in December 1970. In April 1971, the CEQ drafted the first version of the Toxic Substances Control Act, which was debated for several years among Congress, the CEQ, the EPA, numerous chemical industry trade groups and other stakeholders. In the midst of these debates, the Kepone incident in Hopewell, Virginia was publicized on a popular television program, CBS's '60 Minutes', in 1976. Kepone was an insecticide that was being manufactured in a poorly controlled environment near the James River, which lead to an outbreak of severe neurological disorders among workers. The James River was closed to commercial and sport fishing as a result. The national exposure on television coupled with the increased public pressure over controlling exposure to polychlorinated biphenyls led to an agreement in the US Senate-House Conference Committee. The Toxic Substances Control Act (TSCA) (Public Law 94-469) was finalized and signed by President Ford in October 1976.

Overview of the Toxic Substances Control Act

The goal of this legislation was to help control the hazards of chemicals in commercial production in the United States. Specifically, TSCA expanded existing federal authority to regulate manufacturing, disposal, importing, distributing, and processing of all toxic chemicals. Under TSCA all such chemicals must be inspected and approved by the EPA before they enter the market including new and existing chemicals. This Act was originally passed in 1976 and was amended in 1986, 1988, 1990, and 1992.

The 1976 TSCA regulations covered all organic and inorganic chemical substances and mixtures, both synthetic and naturally occurring with the following exceptions: food, food additives, drugs, cosmetics, nuclear materials, tobacco, and pesticides. With respect to the qualified chemicals, the legislation gave EPA the authority to:

1. Require manufacturers and importers to submit information on all new chemical substances prior to manufacture for commercial purposes.
2. Require that manufacturers and processors collect, maintain, and possibly submit specified information on the chemical substances.
3. Regulate both old and new chemical substances that are expected to present or are presenting unreasonable risks to health and the environment.

The TSCA legislation provided the EPA Administrator with the authority to review and evaluate data from new and existing chemicals intended to be sold in commerce with respect to manufacture, processing, distribution, use, or disposal. If the data are insufficient or if the data show that the material or its use can cause adverse health or environmental effects, the EPA Administrator can limit, delay, or completely prohibit the manufacture and distribution of the material. The Act further specifies that the risks of using a particular substance must be compared with the benefits derived from its use. The objective is to create a balance between preventing health or environmental risk and not curbing innovative technology.

TSCA currently has four titles as noted below:

- *Title I – Control of Toxic Substances*: This section includes provisions for testing chemical substances and mixtures; processing notices; regulating hazardous chemicals substances and mixtures; managing imminent hazards; and reporting and retaining information.
- *Title II – Asbestos School Hazard Emergency Response Amendment*: This section was added by the Asbestos Hazard Emergency Response Act (AHERA, Pub. L. 99-519), which was enacted by Congress on October 22, 1986. It authorized EPA to amend its TSCA regulations to impose more requirements on asbestos abatement in schools. AHERA provides for the promulgation of federal regulations requiring inspection for asbestos and

appropriate response actions in schools and mandates periodic reinspection. In addition, it required the EPA Administrator to determine "the extent of the danger to human health posed by asbestos in public and commercial buildings and the means to respond to any such danger."

AHERA was amended in 1990 by the Asbestos School Hazard Abatement Reauthorization Act Amendment (ASHARA, Pub. L. 101-637) to require accreditation of persons who inspect for asbestos-containing material in school, public, and commercial buildings. This amendment also mandated the accreditation of persons who design or conduct response actions with respect to asbestos-containing material in such buildings.

- *Title III – Indoor Radon Abatement*: This change was made on October 28, 1988 (Pub. L. 100-551). The purpose of this legislation was to assist states in responding to the threat to human health posed by exposure to radon. EPA is required to publish and keep current a citizen's guide to radon health risk, and to perform studies of the radon levels in schools and radon contamination in federal buildings.
- *Title IV – Residual Lead-Based Paint Hazard Reduction Act Amendment*: This section was added on October 28, 1992 (Pub. L. 102-550). The purpose of this legislation is to reduce environmental lead contamination and prevent adverse health effects as a result of lead exposure, particularly in children. Provisions include identifying lead-based paint hazards, defining levels of lead allowed in various products, including paint and toys, and establishing state programs for the monitoring and abatement of lead exposure levels, including training and certification for lead abatement workers.

The law is divided into a number of sections, which deal with various issues. The following table outlines the major TSCA Sections:

Section	*Subject*	*40 CFR reference*
4	Authority to require chemical testing	Parts 790–799
5	New chemicals	Part 720
	Premanufacturing notification exemptions (PMN)	Part 723
5(a)	Significant new use rules (SNUR)	Part 721
6, 7	Existing chemicals control	Part 750
8(a)	Chemical use reporting	Parts 740 and 712
8(b)	Inventory reporting guidelines	Part 710
8(c)	Adverse reactions	Part 717
8(d)	Health and safety data reporting	Part 716
12	Export rules	Part 707
13	Import rules	Part 707

At the date of this publication, the US EPA's Office of Pollution Prevention and Toxic Substances enforces TSCA.

Existing Chemicals

TSCA mandates that EPA identify, compile, keep current, and publish the TSCA Chemical Substance Inventory. The Inventory defines what chemicals exist in US commerce for TSCA purposes and not only contains chemical substances that have been manufactured or imported since January 1, 1975, but also includes intermediates used in the manufacture of other chemicals. The TSCA Inventory list currently contains ~80 000 chemicals. EPA can require companies to maintain records and submit reports revealing production and processing, significant adverse reactions, health and safety studies and substantial risk reports. Once an imported substance is found to be on the TSCA Inventory, it is subject to any rule deemed appropriate by EPA. Likewise, if the substance is not on the TSCA Inventory, the manufacturer or importer must comply with the PMN requirements before importation (see 'New Chemicals' below). If deemed necessary, EPA can require testing if there is evidence that a substance presents an unreasonable risk to health or the environment or if a substance is produced in substantial quantity and there is insufficient data to allow proper evaluation. If, in fact, EPA determines a substance presents an unreasonable risk, production can be prohibited, limited, or more substantial labeling can be required. If EPA determines there is an imminent hazard, significant legal action may be pursued including product seizure or recall. Importers of chemical substances must comply with the same regulations including an additional certification requirement. Importers of chemical substance must certify as follows:

- *Negative certification*: The importer must certify that all of the chemicals in the imported product are not subject to TSCA and are regulated under another statute. A negative certification is generally required for imports of pesticides (but not pesticide intermediates), nuclear materials, firearms and ammunition, food, food additives, drugs, registered pesticides, cosmetics, or medical devices.
- *Positive certification*: A positive certification is required for all imports of chemical substances or mixtures (other than articles) subject to TSCA regulations. The importer must certify that all chemicals in the imported product comply with all applicable rules or orders under TSCA and that

they are not offering the product for entity in violation of TSCA or any applicable rule under TSCA.
- *No certification*: No certification is required for chemical substances or mixtures as part of an article (unless required by a rule or order under TSCA), or for tobacco or tobacco products.

Exporters are also required to notify EPA before shipping any product abroad for which test data are required, regulatory action has been proposed or occurred, or action of some sort is pending or relief granted. Substances subject to export notification are listed on the Chemicals on Reporting Rules (CORR).

New Chemicals

Substances not on the Inventory or are not otherwise excluded or exempt are considered 'new' and are subject to a premanufacture notice (PMN). Examples of exclusions would include mixtures, substances subject to another statute, impurities, by-products and nonisolated intermediates. Additional exemptions also include test marketing products, low volume products, polymer exemptions, LoREX (low release and exposure exemption), and R&D substances. By statute, chemical manufacturers must notify the Agency at least 90 days before manufacturing a chemical substance that is not listed on the TSCA Chemical Substance Inventory. However, TSCA does not empower the US EPA to require routine testing of new chemicals to permit a valid evaluation of the potential risks. This has been a limitation in the overall effectiveness of the PMN process. Frequently, very little data accompanies the PMN (50% of submissions present no safety data and 90% have only an LD_{50} and an Ames test); however, the EPA must decide within 90 days if the submitted chemical will pose a health or environmental hazard.

The PMN generally requires the following items:

- chemical identity,
- manufactured amounts,
- number of employees exposed,
- method of disposal,
- categories of use,
- by-products,
- releases to the environment,
- any relevant health or environmental effects data, and
- other 'reasonably ascertainable' data.

If there is a paucity of data and/or the chemical may present an unreasonable risk, EPA has the authority to require various testing to fill data gaps or limit or ban the manufacture or importation of the chemical altogether. However, EPA must review the PMN within 90 days of receipt of the Notification. The EPA assesses the potential risks associated with the manufacture, processing, distribution, use, and disposal of the substance in question. The review period may be extended for 'good cause' under extenuating circumstances. Upon completion of the review, EPA may take regulatory action if the substance in question:

1. may present an unreasonable risk;
2. may enter the environment in substantial amounts; and
3. may result in substantial human exposure.

On the other hand, the EPA may take no action if the data show that there is no substantial exposure or risk. In that case, the substances deemed 'new' are then added to the TSCA Inventory when EPA receives a Notice of Commencement (NOC) from the manufacturer or importer following the completion of the 90 day review period. The NOC must be filed within 30 days of manufacture or import. At this time, the substance in question becomes an 'existing' chemical for regulatory purposes under TSCA and anyone can then begin to manufacture or import the chemical.

Sustainable Futures as a TSCA-Related Voluntary Pilot Project

On December 11, 2002, EPA announced in the US Federal Register, a TSCA-related voluntary pilot project, entitled Sustainable Futures. The goal of this pilot project has been to encourage the application of pollution prevention principles and the development of inherently low hazard new chemicals submitted as PMNs under Section 5 of TSCA. The Agency seeks to gain additional data and experience regarding the pollution prevention, risk reduction, and source reduction benefits of the use of hazard, exposure, and risk screening methodologies such as EPA's Pollution Prevention (P2) Framework in new product development efforts.

To encourage industry participation in this voluntary pilot project, the Agency has provided regulatory flexibility in the form of certain expedited reviews of PMNs. For purposes of this voluntary pilot project, EPA implemented a program leading to the opportunity for simultaneous submissions of Test Market Exemption applications and PMNs on chemical substances for which the submitter demonstrates the application and use of the P2 Framework or other scientifically acceptable hazard and exposure

screening methodologies. This regulatory flexibility has the effect of reducing the time to market for select new chemicals from 90 to 45 days.

In order to qualify for this pilot project, and associated expedited review, companies subject to TSCA Section 5 reporting requirements must demonstrate experience and competence with the P2 Framework or other scientifically acceptable approaches to chemical risk screening. In order to do this, companies need to:

1. take necessary training;
2. use hazard and exposure screening tools and demonstrate to EPA that model results were used to inform corporate decision-making. EPA wants submitters to use these tools to select safer new chemical alternatives to submit as new chemical notifications (and, where appropriate, to identify opportunities to eliminate or control exposures through process controls); and
3. submit 5–10 successful (i.e., not regulated by EPA) PMNs or PMN exemption notices which have been developed using chemical hazard and exposure screening tools. These submissions should also include documentation of chemicals evaluated, models used, endpoints on which decisions were based, and the submitter's perspectives on the extent to which the screening tools provided useful information to compare alternatives and select safer chemicals.

The Federal Register notice provides additional detail relating to the expedited review available under this pilot project and discusses criteria or factors EPA will consider to determine eligibility for the pilot project and associated expedited review.

As indicated above, there is considerable complexity (and potential future change) in the TSCA regulations and the PMN process, and readers would do well to consult the rules and regulations linked to below.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Comprehensive Environmental Response, Compensation, and Liability Act, US; National Environmental Policy Act, US; Toxic Torts.

Relevant Websites

http://www.epa.gov – US Environmental Protection Agency, Office of Pollution Prevention and Toxics (OPPT). Sustainable Futures Project. Substances subject to export notification are listed on the Chemicals on Reporting Rules (CORR) available at this website.

http://www4.law.cornell.edu – Toxic Substances Control (from the US Code).

Toxic Torts

Jack W Snyder*

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Introduction to Toxic Torts

According to Black's Law Dictionary (1990, 6th edn., p. 1489) in American civil law, a tort is a "legal wrong committed upon the person or property independent of contract. It may be either (1) a direct invasion of some legal right of the individual; (2) the infraction of some public duty by which special damage accrues to the individual; or (3) the violation of some private obligation by which like damage accrues to an individual."

During the last half century, a complex form of tort action known as the 'toxic tort' has developed to address some of the challenges of modern industrial society. Typically, the toxic tort is a civil action that seeks damages for injury to person or to property arising from alleged exposure to a toxic substance, emission, or product. Toxic tort claims most commonly are filed in classic civil lawsuits, but toxic tort issues also arise, for example, in workers' compensation claims and in administrative actions for cleanup of hazardous waste sites. In the majority of toxic tort actions, the plaintiff must show: (1) exposure to a toxin, (2) that the toxin caused a compensable injury, and (3) that a compensable injury, in fact, occurred.

Distinguishing Features of Toxic Torts

Toxic tort litigation has several distinguishing characteristics, including issues of exposure, latency, prospective damages, causation, risk, and complex challenges involving expert testimony and multiplicity of parties.

Regarding 'exposure', the plaintiff or claimant alleges knowing or unknowing 'exposure' (e.g., absorption, contact, ingestion, inhalation, implantation, or injection) to one or more environmental (e.g., chemical, biological, radiological, nuclear, or explosive) agents alleged to be 'toxic'. The Toxic Substances Control Act, 15 USCA § 2606(f), defines

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toxicity in terms of 'imminent hazard', which is described as involving "the manufacture, processing, distribution in commerce, use, or disposal of [a substance that] is likely to result in ... injury to health or the environment." Similarly, in the Hazard Communication Standard promulgated by the Occupational Safety and Health Administration (OSHA), a 'health hazard' is defined as "a chemical for which there is statistically significant evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur in exposed employees." 29 C.F.R. § 1910.1200(c).

The environmental agents that appear in toxic tort actions tend to be many of the same agents selected by governmental agencies (e.g., EPA, FDA, CPSC, OSHA, and DOT) for regulation. Agents of concern under TOSCA, 15 USCA § 2603(b) (2) (A), include those causing effects such as 'carcinogenesis, mutagenesis, teratogenesis, behavioral disorders, and cumulative or synergistic effects'. By contrast, under the Hazard Communication Standard, OSHA requires actions to be taken with regard to agents that may be classified as "carcinogens, toxic or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, agents which act on the hematopoietic system, and agents which damage the lungs, skin, eyes, or mucous membranes." 29 C.F.R. § 1910.1200.

Regarding 'latency', in some toxic tort actions, the alleged adverse effects of exposure may not be immediately apparent because the injuries have not yet manifested themselves or because the harm goes undetected for a period of time. Latency often becomes an issue in lawsuits involving cancer, birth defects, and genetic mutations, and statutes of limitation or rules of accrual may be modified to accommodate those situations where the moment of the defendant's action and the discovery of the injury are separated by substantial intervals of time (typically years).

Regarding 'prospective damages', the diseases and illnesses that form the basis of damage claims in many environmental tort actions may develop over long periods, may derive from extended periods of exposure to toxic substances, and often are characterized as disorders whose underlying mechanisms are not well understood. Consequently, plaintiffs may assert claims for 'future harms' that have not yet, and possibly never will, manifest themselves, or whose progression from an early stage is highly speculative. Courts have addressed at least the following proposed kinds of prospective damages:

1. The plaintiff is suffering an existing physical injury that may worsen or develop into or be related to more serious consequences (e.g., asbestosis and possible lung cancer).
2. The plaintiff is not suffering from any existing injury or disease, but due to the exposure to the toxic substance is, or may be, at an increased risk of developing a particular disease in the future.
3. The plaintiff, because of his or her enhanced susceptibility to contracting such a disease, suffers present emotional distress, usually in the form of fear or anxiety about the prospective harm, sometimes accompanied by physical manifestations.
4. The plaintiff, again because of enhanced risk of future serious disease or physical injury, incurs or should incur present and future medical expenses in the nature of surveillance and monitoring costs to ascertain the presence or development of the disorder.

Regarding 'causation', the often lengthy interval between 'exposure' and 'manifestation' increases the challenge for any plaintiff seeking to establish the necessary causal link between a 'toxic' agent and legally cognizable injury. The passage of time increases not only the likelihood of onset of multiple intervening causes, but also the likelihood of developing a condition otherwise known to have significant background incidence and prevalence in the general, nonexposed population. Consequently, it is frequently impossible to determine with any measure of certainty whether a plaintiff's health problem arose from the defendant's product or conduct, or whether that plaintiff would have developed her health problem in the absence of objective evidence of exposure.

Attributes of Causation

In toxic or environmental tort litigation, the plaintiff must prove that the defendant's product was a cause or a substantial factor contributing to her harm. In many cases, however, the evidence of direct causation is difficult to acquire.

Frequently, plaintiff's counsel will rely upon the testimony of experts to prove causation. The basis for their opinions will most likely include epidemiologic studies, case studies, animal studies, and/or *in vitro* studies. These experts will attempt to explain complicated scientific issues to members of a jury who are not trained to assess the reliability of scientific or medical testimony. Because even the most discerning jurors may be 'dazzled' by an expert's credentials and apparent knowledge, some authorities worry that jurors may not derive a 'true' understanding of biomedical thought on a particular subject. Not surprisingly, many judges have struggled with the standards for admissibility of evidence as a way to

limit the 'dazzling' effect and to keep inaccurate or 'junk' science out of the courtroom.

The standards for admissibility of expert testimony to prove causation clearly will continue to impact the future of toxic tort litigation. Therefore, an understanding of the Daubert decision and the continuing debate over the admissibility of expert scientific or medical testimony will benefit anyone dealing with toxic or environmental tort issues.

The Daubert Decision

The Federal Rules of Evidence (FRE) were adopted in 1975. Subsequently most states (at least 37) have adopted their own codified rules of evidence modeled closely on the FRE. For scientific evidence, the most relevant of the Rules are found in Article VII of the FRE in a section known as Opinions and Expert Testimony. Prior to 1993, some federal appellate courts had applied Rule 702 of the Federal Rules of Evidence to medical and scientific experts. (Rule 702 authorizes scientific testimony whenever it will assist the trier of fact to understand the evidence or to determine a fact in issue.) In 1993, in Daubert v. Merrell Dow Pharmaceuticals, Inc., 509 US 579, the Supreme Court of the United States issued an important interpretation of Rule 702. Seven of the nine justices ruled that judges must serve as evidentiary gatekeepers who determine whether proffered evidence is scientifically valid and relevant. The Court suggested several factors for judges to consider in determining whether to admit a particular theory or technique: Is the theory or hypothesis testable? Has it been tested? Has the theory or technique been subjected to peer review and publication? For a particular scientific technique or methodology, what is the known or potential rate of error? What (if any) are the standards that control the technique's operation? To what extent is the theory or technique generally accepted in the scientific community?

Although Daubert involved an interpretation of the Federal Rules of Evidence that binds only federal courts, the decision has influenced many state courts grappling with novel scientific evidence. Experts seeking to testify about scientific or medical matters that are novel or not generally accepted should therefore be prepared to address each of the concerns articulated by the Supreme Court. In addition, experts should remember that:

- In qualifying an expert to offer testimony, courts are typically more concerned with degree of familiarity with the pertinent subject matter than with title or specialty designation.
- Regarding causation analysis, courts often accept testimony from nonphysicians, especially in toxic tort, workers' compensation, and product liability cases.
- In general, experts *cannot* offer legal conclusions or express opinions about the credibility of other witnesses.
- In general, expert testimony that a conclusion is 'possible' does not suffice to meet the standard for admissibility with respect to the party who bears the burdens of production and persuasion. An expert's testimony that a certain thing is possible is no evidence at all. His opinion as to what is possible is no more valid than the jury's own speculation as to what is or is not possible.

A number of problems remain for post-*Daubert* courts. Does *Daubert* apply to jury as well as nonjury trials? Does the decision apply to all, or just novel expert testimony? Does *Daubert* apply only to 'scientific' experts? In appellate courts, how much *de novo* review is warranted? As gatekeepers, judges may need to look at several aspects of an expert's testimony, including the theory or reasoning behind it, the methodology or technique employed, the protocols followed, the data generated, the conclusion reached, or the interpretation of the opinion in a legal context. Some commentators have suggested that *Daubert* seems to indicate that the Federal Rules only require the reasoning or methodology underlying the testimony to be scientifically valid. If either of these aspects of an expert's opinion have the indicia of validity, it would appear that testimony governed by the Federal Rules should be admitted.

But is the *Daubert* approach restricted to assessment of the validity of theory or method, or should courts look behind apparently legitimate reasoning or technique and evaluate the legitimacy of the results as embodied in proper following of protocols, generation of data, and reaching of conclusions? In the same vein, is the apparent *Daubert* distinction between theory and methodology realistic or useful? Perhaps, but courts must recognize that two experts operating under the same generally accepted theory may employ radically different methods, each of which may be generally accepted in one scientific community but not in the other. In those situations, courts must recognize that experts from different disciplines often make certain assumptions that can never by 'scientifically' proved, and that these assumptions may lead legitimate experts to equally logical, but clearly opposite conclusions.

Regarding the indicia of validity and reliability, how should courts weigh the factors enunciated by the *Daubert* Court? How do courts factor the

importance of peer review, publication, testing, rates of error, the existence or lack of standards, and the notions of widespread or general acceptance? The Supreme Court did not state that any one indicator of validity or reliability is essential under the Federal Rules of Evidence. And finally, what is meant by reliability and validity? The scientific and medical literature definition of these terms is quite different from the definitions used by the Supreme Court and legal commentators. In science and medicine, reliability refers to precision or reproducibility, while validity basically refers to accuracy. By contrast, many legal commentators and courts have equated reliability with accuracy or the probability of accuracy, and validity with sound reasoning. Jurists should not be surprised that scientists or physicians may not understand or accept the meanings or connotations that courts and some members of the bar have applied to the terms 'reliability' and 'validity'. Thus, it would appear that uniformity of approach to the admissibility of scientific evidence will not easily be accomplished on the heels of Daubert.

The Categories of Admissibility Standards

Jurisdictions vary drastically in their standards for admission of scientific studies and expert opinions. At least three methods of screening have been characterized, including: (1) the 'pure' Frye approach, (2) the relevance approach, and (3) the discretionary approach.

The Frye Test

Although the Supreme Court stated that the Frye decision did not survive the enactment of the Federal Rules of Evidence, the Frye test remains influential in American courts. The Frye test refers to the standard for admission of scientific evidence applied by the US Court of Appeals for the District of Columbia in Frye v. United States, 293 F. 1013 (D.C. Cir. 1923). In refusing to admit the results of a lie detector test, the court stated in pertinent part:

Just when a scientific principle or discovery crosses the experimental and demonstrable stages is difficult to define. Somewhere in this twilight zone the evidential force for the principle must be recognized, and while the courts will go a long way in admitting expert testimony deduced from a well-recognized scientific principle or discovery, the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field in which it belongs.

Therefore, in Frye jurisdictions, scientific research, studies, techniques, or methods can be admitted into evidence only pursuant to a 'general acceptance' by the applicable scientific community; that is, nonmainstream studies are excluded.

The continued viability of the Frye approach has been a subject of much debate, both before and after Daubert. The purpose of the Frye test was to "prevent...the introduction into evidence of specious and unfounded scientific principles or conclusions based upon such principles." Advocates of this conservative approach argue that it protects the legal system from the 'junk science' that plagues the litigation process. As E.A. Firestone has explained:

> The accumulation of scientific knowledge is an additive process in which short steps of progress are created out of techniques and concepts already in existence. In this manner, a scientist will propose theories to the relevant community with the knowledge, and stoic acceptance, that these facts will be re-examined and redefined by a number of subsequent investigators ... In science, facts arise only as a result of collective acceptance ... good science is defined by consensus, not by credentials. The scientific community rightly treats new theories with skepticism, an attitude which does not reflect on the proponent of the theory but on the training of the scientific community.

In other words, proponents of Frye believe that scientific consensus is a strong indicator of the reliability of a theory.

Some Frye proponents also contend that admission of evidence or opinions unsupported by the scientific or medical community opens the courtroom door to any expert willing to testify on a party's behalf. Consequently, juries may award large verdicts based on evidence which is fundamentally flawed. These awards, in turn, may lead to an 'avalanche of litigation' and 'endless, baseless claims'. Furthermore, recent problems of availability and affordability of insurance have been attributed to controversial scientific and medical opinions admitted into evidence.

By contrast, the critics of Frye proclaim that the test should be abolished because it bans useful groundbreaking studies or theories from the courtroom. Plaintiffs have argued that the people who have been harmed by exposure to toxic substances should not have to wait for proof of others being similarly hurt before they can receive relief. This argument is especially significant in the context of toxic tort litigation, where anecdotal (case) reports have been cited by some experts as evidence that a toxic substance causes a specific type of injury.

Many toxic tort plaintiffs argue against the application of the Frye test. Specifically, as innocent victims, they contend that they should not have to wait several years for adequate epidemiologic studies

to be completed before they seek compensation. Defendants will argue the reverse: that until there is adequate proof of a causal connection between a toxic substance and the problems alleged to have been caused by that agent, they should not be held legally responsible under 'medical certainty', 'scientific certainty', 'general acceptance', or 'consensus' standards. Consequently, conservative standards, on the Frye end of the spectrum, will be difficult for many toxic tort plaintiffs to meet.

Other Standards for Admissibility of Scientific Evidence and Expert Testimony

Critics of Frye saw an opportunity to loosen the admission standards for expert testimony upon the promulgation of the FRE in 1975. The FRE did not mention Frye or the 'general acceptance' test. In fact, according to the court in United States v. Downing, 753 F.2d 1224, 1234 (3d Cir. 1985), "neither the text…nor the accompanying notes of the advisory committee…explicitly set forth the appropriate standard by which the admissibility of novel scientific evidence is to be established."

The arguably more liberal standards permit scientific evidence which is not generally accepted by the medical community to bypass the judge and be presented to the jury. As the court explained in Ferebee v. Chevron Chemical Co., 736 F.2d 1529 (D.C. Cir. 1984), cert. denied, 469 US 1062 (1984):

Judges, both trial and appellate, have no special competence to resolve the complex and refractory causal issues raised by the attempt to link low-level exposure to toxic chemicals with disease. On questions such as these, which stand at the frontier of current medical and epidemiologic inquiry, if experts are willing to testify that such a link exists, it is for the jury to decide whether to credit such testimony.

In federal court, admissibility of scientific evidence and expert testimony depends upon the application of the Federal Rules of Evidence to the facts of any case. After Daubert, these rules generally permit the judge (as 'gatekeeper') to admit evidence which is helpful to the trier-of-fact, reliable, and nonprejudicial.

In practice, application of the liberal standards varies by jurisdiction. The most lenient, or 'let-it-all-in', approach has been implemented by a few federal and state courts. This approach is based upon the theory that "any lack of foundation for an expert's opinion goes to the weight and not the admissibility of the opinion." However, even in these ultra-liberal courts, the party seeking to admit the evidence will still be required to demonstrate helpfulness, reliability, and lack of prejudice.

By contrast, other courts have struggled to reach a compromise between the 'general acceptance' and the 'let-it-all-in' standards. Some have significantly modified the Frye rule to ease the standard for admission of expert testimony, while others have remained quite conservative in their approaches to admissibility. Of note, a 'balancing test' espoused by Judge Weinstein in *In re Agent Orange*, 611 F.Supp. 1223 (D.C.N.Y. 1985), *aff'd*, 818 F.2d 187 (2d Cir.), *cert. denied*, 487 US 1234 (1987) requires the court to determine probativity of evidence by weighing reliability, helpfulness, and relevance against prejudice, confusion, and waste of time. Thus, despite the guidance provided by Daubert, courts do not agree on how to handle expert evidence generally, and on how to apply Frye specifically.

In the first post-Daubert decade, however, the Supreme Court's apparently more liberal approach to admissibility has not had the effect of further opening the courtroom doors to dubious scientific evidence. Indeed, the federal courts now are scrutinizing the evidence under several criteria, not just general acceptance. Such close attention has led to greater exclusion of evidence because each of the Daubert factors offers an opportunity for the court to find deficiencies in the proffered evidence.

Causation in the Trenches

Though there may be some dispute as to what comprises the relevant technical community, 'general acceptance' within that community is usually the beginning and end of the inquiry. Typically, the judge does not ask whether the community is correct in accepting or rejecting the relevant principle, technique, or methodology. By contrast, there are multiple indicia of 'scientific validity' to be examined under Daubert, but it does not appear that any one of these indicia is essential to the court's inquiry. Unfortunately, neither the *Frye* approach nor the Daubert indicia encourage the gatekeeper to undertake the intense, 'behind the scenes' analysis that is required to unearth the basis of expert opinion in many toxic tort cases. Any court that does take a 'hard look' will find that conceptual boundaries of technical 'communities' are increasingly difficult to define, peer review is not a uniform process, publication is easily accomplished as each value-laden community defines itself and creates its own journals, and tests are performed in laboratories that are not certified or accountable.

Importantly, however, there are some science-based criteria that both Frye and Daubert courts can employ in toxic tort cases. These criteria are found in the ontologic framework of scientific

materialism, which displaced vitalism many centuries ago and continues to dominate Western biomedical concepts of disease, causation, and pathogenesis. Realistic views of this ontology hold that the integration of insights derived from epidemiology, basic science, and clinical science remains the best way to generate and test causal hypotheses. To support an opinion with reasonable medical or scientific certainty that exposure to an environmental agent(s) is a cause of a disease, illness, or disorder, the overwhelming majority of scientists, physicians, and epidemiologists demand that the following criteria be satisfied:

1. The prevalence and incidence rate of the disorder should be significantly higher in those exposed to the hypothesized cause than in controls not so exposed (the cause may be present in the external environment or as a defect in host responses).
2. Exposure to the hypothesized cause should be more frequent among those with the disorder than in controls without the disorder when all other risk factors are held constant.
3. In the course of time, the disorder should *follow* exposure to the hypothesized causal agent.
4. A spectrum of host responses should follow exposure to the hypothesized agent along a logical biologic gradient from mild to severe.
5. A *measurable* host response following exposure to the hypothesized cause: (a) should have a high probability of appearing in those lacking this response before exposure, or (b) should increase in magnitude if present before exposure. This response pattern should occur infrequently in persons not so exposed.
6. Experimental reproduction of the disorder should occur more frequently in animals or man appropriately exposed to the hypothetical cause than in those not so exposed; this exposure may be deliberate in volunteers, experimentally induced in the lab, or demonstrated in a controlled regulation of natural exposure.
7. Elimination or modification of the hypothesized cause or of the vector carrying it should decrease the incidence of the disorder.
8. Prevention or modification of the host's response on exposure to the hypothesized cause should decrease or eliminate the disorder.
9. All of the relationships and findings should make biologic and epidemiologic sense.

Importantly, the number of these validation criteria that must be satisfied in the mind of any expert before he or she will render an opinion supporting a causal connection between exposure to agent X and onset of effect (or injury) Y varies from just one to all nine. There is no reliable evidence indicating consistency in the use of these criteria among individual experts or among members of a particular scientific discipline or medical specialty. Furthermore, experts typically do not identify those principles of causation analysis which underlie the basis of their opinions. The failure to make explicit (and to consistently apply) a consensus methodology of causation analysis has created much confusion in workers' compensation, product liability, toxic tort, hazardous waste, and adverse drug reaction litigation.

Even if all of the above validation criteria (for proof of general causation) were met, the alleged causal link between a toxic substance and an objectively verifiable injury merely becomes a possibility. The case-specific causation analysis must now be undertaken. Assuming the causative agent(s) can be identified, the expert must: (a) establish actual and biologically significant exposure; and (b) link the exposure to a reproducible, reasonably well-defined disorder. 'Exposure' does not typically mean 'in the vicinity of' for purposes of scientific causation analysis. There must be some evidence of inhalation, absorption, or ingestion by an individual of measurable quantities of specific substance(s). Assuming some credible evidence of exposure, the expert typically identifies a reproducible, reasonably well-defined, measurable health effect. Not all effects, however, are adverse or detrimental to an individual, and, of course, not all adverse effects constitute disease or disability (in medicine or in law).

Several mistakes of inference should be avoided by those attempting to assign cause-and-effect relationships. For example, although anecdotes and case reports can suggest testable hypotheses, they should not, by themselves, provide a basis for causal inference, especially in the absence of unbiased selection of subjects, examination of patients for other explanations of the adverse event, and measurement of the frequency of the same adverse event in appropriate control patients.

Bias in the selection of experimental subjects should also be avoided. Findings in clinic populations that are not randomly selected may not be representative of (or apply to) the general population. The phenomenon known as Berkson's Paradox (selection bias) is frequently overlooked by those attempting to causally relate environmental exposures to various health problems.

Many people, and some medical and legal professionals, fall into the trap of attributing an adverse effect to a procedure, mishap, or medication simply because the event occurred *sometime after* the performance of the procedure or the administration of

the drug. This fallacy of logic is known as *post hoc, ergo propter hoc*, which loosely translated means 'if condition B temporarily follows situation A, then A must have caused B'. Just because one sees a lot of worms and toads on the sidewalk after a thunderstorm does not mean it has been raining worms and toads.

Interpretations of population means or averages must be made with caution. Some data sets display a Gaussian (bell-shaped) distribution while others manifest biphasic or other distributions. Calculations of averages for the latter population distributions can be misleading, for example, when estimating future costs based on 'average' survival times.

Finally, the role of statistical associations and correlation coefficients in the proof of causality remains controversial. In particular, *meta-analysis*, or the use of formal statistical techniques to provide a 'quantitative synthesis' of a body of separate but similar experiments or studies, has provoked substantial disagreement in many disciplines. Critics of meta-analysis warn that: (a) meta-analysis itself is not an experiment; (b) it is difficult to avoid mixing the results of well-designed studies with poorly designed ones; (c) the investigator never knows if she has included *all* the relevant studies; (d) overlooking unpublished 'negative' studies may produce bias toward 'positive' results; (e) investigators often erroneously assume that exposure conditions are equal among the studies; (f) meta-analysis often abandons quantitative scientific evaluation of the magnitude of some effects; (g) inconsistent use of statistical methods to generate the results of meta-analyses decreases both the ability to extrapolate (decreases external validity) and to undertake comparative risk assessment. The role of meta-analysis as a tool for proof of causation in environmental tort, product liability, and workers' compensation cases remains to be determined.

Causation in the Law

To probe the controversy surrounding occupational disease, workers' compensation, toxic tort, and hazardous substance litigation, one must understand the traditional legal approach to causation. First, alleged wrongful conduct must be a *cause-in-fact* of harm. Proof of 'factual causation' usually involves proof of logical relationships between events linked in a deductive 'causal chain'. Cause-in-fact corresponds to the use of causation in everyday language. It is also called 'but for' or '*sine qua non*' causation, suggesting that the consequences would have been different if the cause-in-fact had not occurred. Courts, however, frequently confront multiple-cause events and experienced jurists recognize that harm is not necessarily the result of antecedent *individual* events. In addition, courts which confront excessively long causal chains must decide where a causal chain should end. Thus, the concept of *proximate cause* evolved to allow a jurist to discriminate between many so-called causes-in-fact and to incorporate policymaking into identification of *the* cause or causes which the legal system holds ultimately responsible for harm.

A third notion of causation – *probabilistic causation* – has also evolved over the last century. Probabilistic causation relies on probabilistic reasoning rather than on simple, deductively derived causal chains. Problems have arisen, however, because, as explained below, probabilistic reasoning serves two analytically distinct purposes in legal proceedings.

Traditionally, a plaintiff has two tasks known as burdens of proof. First, he or she must meet the *burden of production* by providing factual evidence for each element of a particular cause-of-action (e.g., negligence, battery, etc.) Second, he or she has a *burden of persuasion*. That is, she must convince the jury that her version of the facts is worthy of their collective belief with a minimum level of certainty, as defined by a standard of persuasion. The four commonly used standards are: (a) 'beyond a reasonable doubt' in criminal cases; (b) 'by clear and convincing evidence' in some civil cases; and (c) 'more likely than not'; or (d) 'by a preponderance of the evidence' in most civil cases, including workers' compensation, toxic tort, products liability, and occupational disease claims.

Qualitative concepts of probability (as embodied in the above standards) have long and explicitly influenced jury deliberations as to whether or not a plaintiff has met his burden of *persuasion*. By contrast, in conventional personal injury litigation, probability and inductive reasoning have not explicitly played a role in fact-finding *per se*. That is, the facts themselves, defined as elements on which one party has the burden of *production*, are generally deemed true or false – with a probability of either 0 or 1. For example, the light was either red or green, the brakes either did or did not work, or the pedestrian either did or did not fall.

Among the elements of a case which the plaintiff has the burden of proving is causation-in-fact. This element is common to toxic tort, hazardous waste, occupational disease, and conventional traumatic injury claims. As noted above, causation-in-fact *probability* is not an issue in most conventional injury cases. The jury simply decides which version of the facts it believes in an all-or-none, yes-or-no fashion, with no room for intermediate probabilities. Causation evidence is not expressed probabilistically.

This is not so in late twentieth-century environmental claims where, given the frequent impossibility of proving individual causation, statistical causation evidence (expressed probabilistically) is required as a factual estimate of the defendant's contribution to the plaintiff's risk. For example, the issue in a typical trauma case may be whether or not a car could have stopped at a red light. Evidence might be heard on speed, braking ability, and driver reaction time *for that particular vehicle* (car X). The jury then finds that car X either could or could not have stopped. However, in the absence of facts concerning the individual car, undisputed evidence may show that of 100 cars chosen at random, 55 would have been able to stop. As to whether or not plaintiff has met the burden of production, the jury could find either way, depending on how it responds to probabilistic (statistical) evidence. Jury response is, in turn, likely to be influenced by judicial instructions on inferences to be drawn from group-based information.

The jury may believe that 55% of cars could have stopped, but have no idea whether car X is among that group. Thus, the jury would say the plaintiff had not met the burden of production. Alternatively, the jury may believe that 55% of cars could have stopped *and* infer that car X (assuming it is not atypical) more likely than not would have stopped since most cars would have. This finding, however, incorporates a leap of faith from established fact about a population to a conclusion about a particular car.

The propriety of this kind of mental leaping is one of the most controversial aspects of toxic tort and occupational disease cases, where causation often cannot be properly formulated as a yes-or-no fact. Instead, parties rely on evidence of increased risk or enhanced probability of disease which may or may not be attributable to defendant's conduct. The inquiry becomes one of the existence and magnitude of a fact *probability*. Therefore, understanding the dual nature of probability, as both a factual statistical quantity (fact probability) and a measure of strength of belief (belief probability), becomes important. Unfortunately, fact probability and belief probability have not been kept analytically distinct. Courts have 'collapsed' the requirements for burden of production and burden of persuasion into *one* test that blurs plaintiff's twofold task of defining not only the facts or elements to be proved but also the amount of credence to be accorded a fact in support of a finding. When a judge tells a jury that "plaintiff must show that causation is more likely than not," she/he risks confusion. Does she/he mean that the *fact* of causation which plaintiff must prove (burden of production) is not traditional true-or-false (100% vs. 0%) causation but only the existence of a statistical probability of causation greater than 50%? Or does she/he refer to the burden of persuasion guided by a standard of *belief* that causation is 'more likely than not' true; that is, does the jury believe a knowable fact with more than 50% confidence?

Concern over haphazard and unrecognized transfer of 'preponderance of evidence' or 'more likely than not' standards from the burden of persuasion to the burden of factual proof (burden of production) involves more than idle semantics. The adverse effects of failure to undertake a deliberate, two-step probabilistic analysis include: (a) undue preference for particular probabilities of causation found in *one* epidemiologic study, especially when meta-analysis of multiple studies is not possible or available; (b) unrecognized lowering of the burden of production with concomitant stiffening of the burden (standard) of persuasion; (c) inappropriate fixation on simplistic quantitative rules such as the '$>50\%$ likelihood' rule; and (d) poorly reasoned opinions because courts fail to explain exactly how they apply the $>50\%$, 'more-likely than-not' rule.

Courts that apply the rule only to fact probabilities essentially seek a yes-or-no belief in a $>50\%$ fact probability. By contrast, traditional courts that apply the rule only to belief probabilities seek a $>50\%$ belief in a yes-or-no fact. In toxic tort/occupational disease claims where both fact probability and belief probability are issues, there are at least two other approaches. Courts could apply the 'more-likely than-not' standard *jointly*, reducing alleged fact probability by a factor reflecting the jury's doubt about its truth. By contrast, the rule could be applied *sequentially* to require only a $>50\%$ belief in a fact probability which itself may barely exceed the $>50\%$ threshold. It is important to see that joint application stiffens the causation burden-of-production/burden-of-persuasion, while sequential application substantially lessens the causation production/persuasion requirements. The point here is that, regardless of approach, a court that deals with causal indeterminacy characteristic of toxic tort/occupational disease claims should be explicit about what it is doing, especially if defendant's culpability of conduct or duty to prevent risk is factored into determination of the causation issue.

Theories of Liability

The developing law of toxic and environmental torts exhibits a blending of principles from judge-made 'common law' (i.e., court cases) and standards and approaches from regulatory aspects of 'public law' (i.e., statutes and regulations). Some public

law statutes may seek to lessen the burden on plaintiffs for establishing causation (when compared with traditional tort requirements) by creating a presumption that once the plaintiff makes a basic (threshold) showing of evidence, the burden shifts to the defendant to prove that he did not cause the plaintiff's injury…in effect requiring that the defendant prove a negative. Other public law statutes might require the court to accept animal data as evidence of causation when human epidemiologic data are not available. Still other statutes (e.g., Veterans' Dioxin & Radiation Exposure Compensation Standards Act of 1984, 38 USC § 1154) may establish an administrative schedule that provides a fixed amount of compensation to individuals who meet certain criteria. Some statutes, like the Comprehensive Environmental Response Compensation Act (CERCLA), 42 USCA §§ 9601-9675, do not offer remedies for personal injury. Instead, CERCLA creates remedies to be pursued by administrative and judicial action of the government, and in limited circumstances by private parties, in matters related to cleanup of hazardous substances released into the environment.

Toxic tort theories of liability can be organized into three categories: (a) claims against sellers of products, (b) claims related to activities on the land, and (c) miscellaneous torts. Theories associated with claims against sellers of products include breach of express or implied warranty, misrepresentation, fraud, negligence, regulatory duty to disclose (e.g., Hazard Communication Standard, 29 C.F.R. § 1910.1200), and no-fault (strict) product liability based on defective design, defective manufacture, or failure to warn of foreseeable risks or hazards. Theories associated with claims related to activities on the land include trespass, public nuisance, private nuisance, breach of fiduciary duty, and strict liability for unreasonably dangerous, abnormally dangerous, or ultrahazardous activities. Miscellaneous theories include assault, battery, negligence *per se*, intentional or negligent infliction of emotional distress, and violation of the federal Racketeer and Corrupt Organizations Act (RICO), 18 USCA §§ 1961-1968.

Common Law Claims against Sellers of Products

Article 2 of the Uniform Commercial Code, adopted in virtually every state, supplies the basic rules governing claims under express and implied warranties. UCC § 2-313 provides that a seller makes an express warranty by 'affirmation of fact or promise made', by 'description of the goods', or by a 'sample or model', any of which must have been part of the basis of the bargain between the seller and buyer. Breach of an express warranty essentially creates a strict liability because the product need not be shown to be defective under this theory. By contrast, UCC § 2-314 creates an implied warranty of merchantability in a contract of sale in which a seller is 'a merchant with respect to goods of that kind'. To be merchantable, the product must, at a minimum, be 'fit for the ordinary purposes for which such goods are used', be 'adequately contained, packaged, and labeled as the agreement of sale may require', and 'conform to the promises or affirmations of fact made on the container or label if any'. Importantly, a claim for breach of implied warranty of merchantability will not survive if the use of the product was not its 'ordinary use'. Finally, UCC § 2-315 states that "where the seller at the time of contracting has reason to know any particular purpose for which the goods are required and that the buyer is relying on the seller's skill or judgment to select or furnish goods, there is ... an implied warranty that the goods shall be fit for such purpose." For a legally enforceable warranty of fitness for a particular purpose, the seller does not have to be a 'merchant' of the type of goods sold, the seller should know that the buyer will be relying on the seller's skill or judgment, and the particular purpose must be different from the ordinary purpose for which the product is used. The seller's knowledge that the consumer intends to use the product for a certain purpose would not trigger this warranty if that purpose falls within the ordinary range of uses for that product.

Under Section 9 of the Restatement (Third) of Torts, a product seller can be held liable for misrepresentations that are fraudulent, negligent, or innocent. Comment b provides that "one engaged in the business of selling chattels who, by advertising, labels, or otherwise, makes to the public a misrepresentation of a material fact concerning the character or quality of a chattel sold by him is subject to liability for physical harm to a consumer of the chattel caused by justifiable reliance upon the misrepresentation, even though (a) it is not made fraudulently or negligently, and (b) the consumer has not bought the chattel from or entered into any contractual relation with the seller." To prove actual fraud in a toxic tort case, the plaintiff must show (1) a misrepresentation of fact, (2) that the defendant had knowledge of the falsity, (3) that the defendant intended to induce the plaintiff to act in reliance on the factual misrepresentation, (4) plaintiff's justifiable reliance on the misrepresentation, and (5) damage or loss as a result of the plaintiff's reliance.

Negligence began to emerge as a separate cause of action for unintentional torts in the early 1800s

coinciding with the Industrial Revolution in England. The textbook elements of the tort of negligence are: (1) duty, an obligation recognized by the law, requiring the actor to conform to a certain standard of conduct, for the protection of others against unreasonable risks of harm; (2) breach of duty, or the failure to comply with a recognized standard of care; (3) proximate or legal cause, a reasonably close causal connection between the conduct and the resulting injury, which includes both cause-in-fact and certain legal limitations on the extent to which the law will recognize 'cause'; and (4) actual loss or damage to the interests of another. The standard of care defining duty, and the breach of that duty, may be difficult to prove when the interval (latency) between the time of exposure and the manifestation of illness is measured in months to years. Proof of negligence typically requires proof of defendant's knowledge of the hazards at the time of exposure as well as proof of foreseeability of harm to the plaintiff.

Regarding strict liability for design defects, at least three approaches to defining 'defective condition' have emerged. Some courts apply the 'consumer expectation test', derived from comment i of § 402A of the Restatement (Second) of Torts, which defines 'unreasonably dangerous' as "dangerous to an extent beyond that which would be contemplated by the ordinary consumer who purchases it, with the ordinary knowledge common to the community as to its characteristics." However, because this test is difficult to apply in cases involving alleged toxic products, where the expectations of the reasonable consumer may not be clear, other courts have embraced a risk-utility or risk-benefit balancing test, wherein a product will be deemed defective if the danger outweighs the products utility. A somewhat more rigorous approach appears to be adopted by the Restatement (Third) of Torts: Products Liability, comment d to § 2, where the test for a design defect is "whether a reasonable alternative design would, at reasonable cost, have reduced the foreseeable risks of harm posed by the product and, if so, whether the omission of the alternative design by the seller or a predecessor in the distributive chain rendered the product not reasonably safe." Importantly, in *Potter v. Chicago Pneumatic Tool Co.*, 694 A.2d 1319 (1997), the Connecticut Supreme Court rejected an absolute requirement of safer alternative design, concluding that "the feasible alternative design requirement imposes an undue burden on plaintiffs that might preclude otherwise valid claims from jury consideration."

Regarding strict liability for manufacturing defects (e.g., decomposed mouse in a bottle of soda, or excessive amounts of arsenic in a cattle dip), § 2A of the third Restatement invokes liability "when the product departs from its intended design even though all possible care was exercised in the preparation and marketing of the product." This rule of absolute liability is based on a policy of encouraging manufacturers, distributors, and retailers to invest in product safety measures and to raise the level of quality control during production processes.

Regarding strict liability for failure to warn, the seller of a product generally has a duty to disclose only foreseeable risks. However, in Davis v. Wyeth Laboratories, Inc., 399 F.2d 121 (9th Cir. 1968), the court viewed even small foreseeable risks as requiring a warning when it held that a manufacturer of polio vaccine had a duty to warn consumers of the risk that one person in a million would contract polio by receiving the vaccine. In addition, "the manufacturer's status as an expert means that at a minimum he must keep abreast of scientific knowledge, discoveries, and advances and is presumed to know what is imparted thereby. But even more importantly, a manufacturer has a duty to test and inspect his product. The extent of research and experiment must be commensurate with the dangers involved." (See Borel v. Fibreboard Paper Products Corp., 493 F.2d 1076 (5th Cir. 1973)).

Common Law Claims Related to Activities on the Land

Toxic tort cases can involve microscopic substances in air, soil, or water that invade and interfere with a person's possessory interest in property. According to Restatement (Second) of Torts § 158, "one is subject to liability to another for trespass, irrespective of whether he thereby causes harm to any legally protected interest of the other, if he intentionally: (a) enters land in the possession of the other, or causes a thing or third person to do so, or (b) remains on the land, or (c) fails to remove from the land a thing which he is under a duty to remove." The invasion, even by invisible substances, may be on the surface of the land, beneath the land, or in the air above the land. In toxic tort trespass claims, however, courts may be reluctant to proceed in the absence of proof of actual damages.

To bypass some of the historically rigid requirements for proof of trespass, and to avoid numerous privileges that may be asserted as defenses to trespass claims, toxic tort plaintiffs may rely on the theory of nuisance, which is generally defined in the Restatement (Second) of Torts § 821 as "an unreasonable interference with a right common to the general public," or as an interference with the use and enjoyment of one's property. Nuisance may arise

from intentional or negligent conduct, or it may be associated with abnormally dangerous activities. Proof of nuisance does not require physical invasion of property, and the nature of the interest protected (use and enjoyment) is broader than the possessory interest protected by the law of trespass. Two separate doctrines of nuisance have evolved – public nuisance and private nuisance.

A cause of action sounding in public nuisance must allege harm, injury, inconvenience, or annoyance arising out of the invasion of a public interest. The Restatement (Second) of Torts states that analysis of the reasonableness of the challenged interference should ask: (a) whether the conduct involves a significant interference with the public health, the public safety, the public peace, the public comfort, or the public convenience; or (b) whether the conduct is proscribed by a statute, ordinance, or administrative regulation; or (c) whether the conduct is of a continuing nature or has produced a permanent or long-lasting effect, and, as the actor knows or has reason to know, has a significant effect upon the public right. The goal of this legal theory is the protection of community rights, and a private person does not have a claim for damages under public nuisance unless she can establish that she suffered special damage different from that sustained by other members of the general public. Where an injunction is the remedy sought, this requirement may be less stringently imposed by some courts.

By contrast, although no unitary precise definition has emerged, private nuisance is typically defined as an unreasonable nontrespassory interference with a private individual's use and enjoyment of his property. Some courts state that plaintiffs can recover for inconvenience, discomfort, and annoyance in addition to damages for injury to their persons and proprietary interests, while others have limited the scope of private nuisance claims by denying recovery based solely on fear of future injury or on decline in property value.

The recognition and enforcement of fiduciary duties can also play a significant role in toxic tort actions. For example, when property contaminated with hazardous substances, or property in proximity to a hazardous condition is acquired without notice of the condition, plaintiffs may sue real estate brokers who may or may not have known of the existence of the hazard. Under the traditional doctrine of *caveat emptor*, in the absence of outright fraud, a buyer has little recourse against a seller or broker for claims arising out of the defects on the property. However, with increased recognition during the past 30 years of fiduciary relationships between brokers and purchasers, some courts may hold brokers liable when they make representations without determining the actual condition of the property, especially if the buyer inquired about the specific condition, or if the broker had information or a suspicion that should have prompted investigation. Other courts may go further, either imposing broker liability for innocent transmission of misrepresentations by the seller, or imposing on the broker a full duty to investigate. In Strawn v. Canuso, 657 A.2d 420 (N.J. 1995), the Supreme Court of New Jersey went so far as to hold that developers and brokers of new homes have an affirmative obligation to disclose to prospective purchasers the existence of off-site hazards that materially affect the value of the property. These new trends in the law of fiduciaries are based on the inequitable bargaining positions of purchasers of residential property when compared with developers and brokers, and on the differences among the parties in relative access to information about hazards.

Toxic tort plaintiffs may also allege that a defendant's conduct was unreasonably dangerous or ultrahazardous (e.g., storing hazardous chemicals on the property). A plaintiff who relies on this theory does not have to show a lack of due care on the part of the landowner, and does not have to prove fault. Rather, the determination of unreasonable or abnormal danger, and the proof of strict (no-fault) liability, typically require a showing of: (a) the existence of a high degree of risk of harm, (b) a likelihood that the harm that results from it will be great, (c) an inability to eliminate the risk by the exercise of reasonable care, (d) the extent to which the activity is not a matter of common usage, (e) the inappropriateness of the activity to the place where it is carried on, and (f) the extent to which the value of the activity to the community is outweighed by its dangerous attributes. (Restatement (Second) of Torts, § 520.) Importantly, in most courts, no single factor is considered dispositive, and not all of the factors need apply for a finding of abnormal danger. The policy basis for this form of strict liability in toxic tort law is the perceived need to require defendants who place products or services into commerce to pay their way by compensating for any harm they may cause. As of 2004, courts in the United States are split in their decisions as to whether the handling, storage, and/or disposal of hazardous substances necessarily constitutes an abnormally dangerous activity as defined by the Restatement (Second) of Torts.

Miscellaneous Theories of Liability

Assault and battery are two intentional tort causes of action which have been employed in toxic tort cases.

Assault is an intentional, unlawful threat or offer to touch another person under circumstances that create in the mind of the other person a well-founded fear of an imminent battery, coupled with an apparent present ability to complete the attempt. Alternatively, an assault is an act intended to put another person in reasonable apprehension of an immediate battery, accompanied by success in causing such apprehension. The defendant must have been in a position to carry out the threat immediately and he must have taken some affirmative action to do so. By contrast, battery is an intentional harmful or offensive touching or contact with another person. A defendant may be liable for battery where she acts intending to cause such contact or an imminent apprehension of such contact, and the harmful contact indirectly or directly results. In Werlein v. United States, 746 F.Supp. 887 (D.Minn. 1990), *Vacated in part pursuant to settlement agreement*, 793 F.Supp. 898 (D.Minn. 1992), the court defined the standard for battery as requiring the plaintiff to prove that the defendant disposed of the toxic substances intending to cause an offensive or harmful contact, or with the knowledge that such contact was substantially certain to occur. Battery claims are infrequent in toxic tort litigation, but plaintiffs seeking punitive damages may include such a claim in order to prove intentional conduct which is more 'egregious' than mere negligence.

As previously noted, toxic tort cases involve a mixture of common law and statutory law claims. Plaintiffs may attempt to show that a violation of a standard of conduct established by statute or regulation should be viewed as negligence *per se*. In jurisdictions where negligence *per se* is recognized as a basis for a legal claim, the statute giving rise to the claim typically must have been enacted to protect the class of persons of which the plaintiff is a member against the kind of harm that the plaintiff has suffered. The presumption of negligence which flows from judicial recognition of this legal theory, however, has been disfavored in American toxic tort litigation. Numerical standards in statutes or regulations are frequently based on scant (albeit sophisticated) scientific or biomedical data of varying degrees of uncertainty, or data open to multiple reasonable interpretations, or data that are more complete or different at the time the action is brought when compared with data that formed the basis of the standard. Not surprisingly, courts have uniformly held that violations of the Occupational Safety and Health Act may provide evidence of negligence, but do not create legally operative presumptions of negligence. Elliott v. S.D. Warren Co., 134 F.3d 1 (1st Cir. 1998).

In the early part of the twentieth century, the Restatement of Torts concluded that one's interest in freedom from emotional or mental distress was not of sufficient importance to require others to refrain from conduct intentionally designed to cause such distress upon pain of adverse legal consequences. The interest in emotional and mental tranquility was simply one for which the law formerly provided no protection. More recently, according to Prosser and Keeton on Torts, § 12 (1984, 5th edn.), a plaintiff who successfully proves physical personal injury is entitled to compensation for all damages for injury past, present, and future associated with the circumstances giving rise to the action. Consequently, a plaintiff who proves physical injury causally related to exposure to an environmental agent may recover for both the physical injuries and for any associated 'emotional distress'.

Two basic types of claims for emotional distress have been proffered in toxic tort lawsuits. If the defendant's conduct is viewed as extreme and outrageous, courts may allow a claim for intentional or reckless infliction of emotional distress. By contrast, if the defendant's conduct is alleged to be negligent, a claim for negligent infliction of emotional distress may be allowed.

One of the most important issues in any jurisdiction that recognizes claims for emotional distress in toxic tort cases is whether an allegation or some level of proof of physical injury must accompany any such distress claim. For intentional, reckless, or outrageous conduct, the Restatement (Second) of Torts § 46 recognizes claims for intentional infliction of emotional distress, even in the absence of allegation or proof of physical injury or risk of physical injury. By contrast, in the Restatement (Second) of Torts § 436A concludes that "if the actor's conduct is negligent as creating an unreasonable risk of causing either bodily harm or emotional disturbance to another, and it results in such emotional disturbance alone, without bodily harm or other compensable damage, the actor is not liable for such emotional disturbance." The physical harm may be, but is not required to be, caused by the defendant's conduct; physical harm that results from the emotional distress is sufficient to satisfy the requirement. The nature and scope of the physical injury requirement (either leading to or pursuant to the emotional distress) has been addressed by a handful of American courts. For example, in Temple-Inland Products Corp. v. Carter, 993 S.W.2d 88 (Tex. 1999), the Texas Supreme Court held that, in the absence of manifest disease, mere inhalation of asbestos fibers was not a physical injury that would trigger a claim for negligent infliction of emotional distress. Similarly, in

Simmons v. Pacor, Inc., 543 Pa.664 (Pa. 1996), the Pennsylvania Supreme Court held that 'non-impairing, asymptomatic pleural thickening' did not constitute sufficient physical injury to be compensable as a matter of law. Consequently, pleural thickening did not satisfy the physical injury requirement needed to trigger a claim for emotional distress.

In 1993, however, the California Supreme Court broke new ground in toxic tort law. In Potter v. Firestone Tire and Rubber Company, 863 P.2d 795 (Cal. 1993), the court held that landowners (who alleged increased, but unquantified, risk of cancer due to alleged exposure to toxic substances due to the proximity of an adjacent landfill containing 'toxic waste') could nevertheless file a claim for negligent infliction of emotional distress in the absence of physical injury. The court concluded that "the physical injury requirement is a hopelessly imprecise screening device – it would allow recovery for fear of cancer whenever such distress accompanies or results in any physical injury, no matter how trivial, yet would disallow recovery in all cases where fear is both serious and genuine but no physical injury has yet manifested itself." Importantly, the Potter court limited these types of recoveries to plaintiffs proving distress that is reasonable, serious, and based upon a knowledge that the likelihood of developing cancer is more likely than not. Furthermore, claims based on fear of latent disease must be distinguished from claims that seek compensation for the increased risk itself. Most American courts have not as yet recognized or embraced the latter types of claims.

Defenses in Toxic Tort Litigation

Defendants in toxic tort litigation can and do assert a host of defenses in order to avoid liability. Some of these defenses substantively negate a specific cause of action, some limit claims against particular defendants, some arise in the law of procedure, some derive from the plaintiff's culpable conduct, and some arise because public law obligations can preempt the operation of common law. This chapter will conclude with brief descriptions of some of the important defenses that have been asserted in toxic tort cases.

State-of-the-Art

The state-of-the-art defense is asserted in strict liability (failure to warn) claims where the defendant alleges that he did not know, and could not reasonably have known, of the hazards of the product at the time of the plaintiff's exposure. A minority of courts have occasionally rejected this defense, reasoning that the imposition on manufacturers of the costs of failure to discover hazards creates an incentive for them to invest more actively in safety research.

Learned Intermediary

The learned intermediary doctrine is raised when the defendant claims that other parties with superior knowledge had the responsibility to warn the user of the product of its hazards. The third Restatement, § 6(d) (1) retains the learned intermediary doctrine, providing that a commercial seller or distributor of prescription drugs or medical devices is shielded from liability to the ultimate consumer where it has given reasonable warnings of foreseeable harm to "prescribing and other health care providers who are in a position to reduce the risks of harm in accordance with instructions or warnings." Where the manufacturer knows that the physician or other health care provider has a limited decision making role in the therapeutic relationship with patients, then the manufacturer is required to directly warn the patients. Restatement (Third) of Torts: Products Liability, § 6(d) (2) and comment b.

Sophisticated User

Courts allow the sophisticated user defense when the party to whom the product is delivered knows better than the seller the ultimate uses of the product, and the seller provides adequate warning of the hazards of the product to the purchaser. This defense typically arises in cases involving bulk suppliers of chemicals to knowledgeable intermediaries. Where the employer can be shown to be in a position to adequately warn its employees, the existence or the requirement of adequate warnings from the supplier may be irrelevant. In addition, chemical manufacturers may reasonably rely on the industrial user of a chemical to comply with the OSHA Hazard Communication Standard and pass on the manufacturer's warnings to its employees.

Unavoidably Unsafe Products

Some products (e.g., experimental drugs and some prescription drugs) cannot be made safe for the use for which they were intended, but their usefulness may outweigh the risk of harm. Comment k of § 402A of the second Restatement of Torts provides that "such a product, properly prepared, and accompanied by proper directions and warning, is not defective, nor is it unreasonably dangerous." This defense is designed to encourage development of useful and necessary products by allowing the seller to avoid liability associated with the risks of these products.

Sovereign Immunity

In the absence of a statute to the contrary, sovereign immunity generally protects governmental entities from liability in toxic tort actions. Even when a statute waives some aspects of governmental immunity, the statute often contains exceptions. For example, the US Government has waived its sovereign immunity for tort claims in the Federal Tort Claims Act, 28 USCA § 1346(b), which provides that federal district courts shall have exclusive jurisdiction over claims for money damages against the Unites States alleging personal injury, property damage, or wrongful death as a result of a negligent act or omission by a government employee within the scope of employment.

Key exceptions to this waiver of immunity include claims "based upon the exercise or performance or the failure to exercise or perform a discretionary function or duty on the part of a federal agency or an employee of the government, whether or not the discretion be abused." 28 USCA § 2680(a). In general, discretionary function involves judgment or choice by the actor, and the conduct also must be of the sort that the discretionary function exception was designed to protect, so as to prevent "judicial 'second-guessing' of legislative and administrative decisions." United States v. Varig Airlines, 467 US 797 (1984). By contrast, this exception will not apply automatically to all agency activities, and will not apply "when a federal statute, regulation, or policy specifically prescribes a course of action for an employee to follow."

A second exception to the waiver of sovereign immunity arose in the case of Feres v. United States, 340 US 135 (1950), where the Supreme Court held that members of the armed forces cannot bring tort actions against the government for harms that 'arise out of or are in the course of activity incident to service'. This exception was extended in Stencel Aero Engineering Corp. v. United States, 431 US 666 (1977), where the Court said that the government cannot be required to indemnify defendants in an action in which the plaintiff could not have sued the government directly. The Feres doctrine was further extended in Minns v. United States, 155 F.3d 445 (4th Cir. 1998), where the court held that children who claimed severe birth defects were caused by their servicemen-father's exposure to toxic chemicals during the Persian Gulf War were barred from suing the United States.

Government or Military Contractors

Some defendants will argue that the manufacture and provision of toxic chemicals according to government specifications should protect them from toxic tort liability. The accepted elements of the government contractor defense, as outlined in the *Agent Orange Litigation*, 534 F.Supp. 1046 (E.D.N.Y. 1982), include the following: (1) the government must have established the specifications for the product; (2) the product manufactured by the defendant must have met the government's specifications in all material respects; and (3) the government must have known as much or more than the defendant about the hazards to people that accompanied use of the product.

Assumption-of-the-Risk

This defense arises when the defendant can show that the plaintiff had knowledge of the hazard, appreciated the magnitude of the hazard, and voluntarily encountered it.

Comparative Negligence or Fault

Restatement (Third) of Torts § 17(a) provides that "a plaintiff's recovery of damages for harm caused by a product defect may be reduced if the conduct of the plaintiff combines with the product defect to cause the harm and the plaintiff's conduct fails to conform to generally applicable rules establishing appropriate standards of care." These rules clearly vary among jurisdictions, but product misuse, alteration, and/or modification are considered relevant to the determination of comparative responsibility, and evidence of these activities are frequently incorporated into the apportionment assessment.

Statutes of Limitation

These statutes (SOL) are designed to protect defendants from stale claims and to impose a degree of finality on litigation. The period of time within which the plaintiff may assert a claim begins at the time the cause of action *accrues*. In toxic tort litigation, it is not always clear when the action accrues for purposes of triggering the running of the statutory period. At least four approaches have been developed for determining the point of accrual of a toxic tort cause of action.

First, the 'exposure rule' states that the action accrues at the time of last exposure, even if the claimant's illness did not manifest until many years after the final exposure. Since typical statutory periods for commencement of tort claims run between 2 and 4 years, application of the exposure rule effectively bars many claimants who allege development of latent injuries.

Second, the 'judicial discovery rule' states that the judicially imposed date of accrual of a toxic tort cause of action is the earliest date on which the plaintiff knew or should have known the presence of

the injury. Thus, once the potential plaintiff 'discovers' that he may have suffered 'an actionable wrong', the period of the SOL begins to run.

Third, the 'statutory discovery rule' codifies the SOL period, which begins to run at the time of the discovery of the injury or when the injury should have been discovered through the exercise of reasonable diligence. Occasional statutes further provide that if the plaintiff has not discovered the cause of the illness or injury within a stated number of years, the claim will be barred.

Fourth, the 'time of discovery rule' focuses on the point in the disease process at which the potential plaintiff is deemed to have sufficient information to proceed with legal action. Typically, the date of accrual is recognized as the date when the claimant became aware of a potentially compensable injury *and* its potential cause. If the claimant becomes ill, but remains unaware of the precise cause of the illness, then the running of the SOL may be triggered on the date the potential plaintiff acquired knowledge of a 'reasonable possibility' that the particular exposure could be a cause of her injury. In Evenson v. Osmose Wood Preserving Company of America, Inc., 899 F.2d 701 (7th Cir. 1990), the court stated that "a reasonable possibility, while less than a probability, requires more than the mere suspicion possessed by [the plaintiff], a layperson without technical or medical knowledge." Consequently, most potential plaintiffs are not likely to become aware of a 'reasonable possibility' until after hearing a biomedical professional express an opinion regarding that 'reasonable possibility'.

Statutes of Repose

In contrast to statutes of limitation, which bar actions at a specified time period after the cause accrued, statutes of repose bar the institution of an action a specified number of years after a particular event, such as the date of first sale of a product or the date of improvements to real property. After that time, no action can be brought, even though the elements of a claim may not have all yet occurred and only occur years after the period has run. Thus a cause of action may be extinguished before it ever accrues, with the repose conferring immunity upon the defendant. In First United Methodist Church v. US Gypsum Co., 882 F.2d 862 (4th Cir. 1989), the Fourth Circuit Court of Appeals noted that "statutes of repose are based on considerations of the economic best interests of the public as a whole and are substantive grants of immunity based on legislative balances of the respective rights of potential plaintiffs and defendants struck by determining a time limit beyond which liability no longer exists ... as a general rule, a statute of limitations is tolled by a defendant's fraudulent concealment of a plaintiff's injury because it would be inequitable to allow a defendant to use a statute intended as a device of fairness to perpetrate a fraud. Conversely, a statute of repose is typically an absolute time limit beyond which liability no longer exists and is not tolled for any reason because to do so would upset the economic balance struck by the legislative body."

Res Judicata or Claim Preclusion

When a valid and final judgment has been taken on a particular claim, *res judicata*, or claim preclusion, prevents a subsequent action on the same claim. The modern approach to determining what constitutes a claim focuses on 'transaction'. According to the Restatement (Second) of Judgments § 24, "the claim extinguished includes all rights of the plaintiff to remedies against the defendant with respect to all or any part of the transaction, or series of connected transactions, out of which the action arose…[a transaction is] to be determined pragmatically, giving weight to such considerations as whether the facts are related in time, space, origin, or motivation; whether they form a convenient trial unit; and whether their treatment as a unit conforms to the parties' expectations or business understanding or usage."

In toxic tort litigation, the scope of the 'transaction' is not always clear. For example, should the asbestos claimant be able to split his transaction into separate claims for plaques, fibrosis, cancer, and fear or risk of any one or more of those three conditions? If a particular jurisdiction has a discovery statute of limitations and forbids claim splitting, a plaintiff's entire claim would accrue at the time of the first injury. Effectively, a plaintiff could be barred from bringing any future claim long before he even knew about it. Traditional *res judicata* (claim preclusion) rules bar any accrued action arising out of the same circumstances as a claim previously adjudicated. A handful of jurisdictions, however, enable a plaintiff to split claims under limited circumstances, especially when that plaintiff might reasonably be expected to encounter difficulty in trying to prove with reasonable certainty that a disorder will develop in the future. Obviously, the recognition of claims for increased risk of future illness in any particular jurisdiction will depend on the future evolution of both accrual rules and claim-splitting rules.

Preemption

Many of the activities and events underlying private toxic tort actions are also regulated by public laws

embodied in statutes and regulations. Sometimes, the duties defined by public law and common law exist independently, but other times they overlap. Sometimes the overlap creates contradictory, conflicting, or ambiguous obligations, enabling the defendant to assert a defense that the public law obligation preempts the operation of the common law.

In general, the law starts with a presumption against preemption. However, express language in a statute may provide explicit evidence of legislative intent to preempt common law. Hopefully, that language of preemption is clear and unambiguous as to the scope of the preemption. Occasionally, these same statutes will contain other language that attempts to 'save' or retain or reserve all rights under the common law, so as to restrict the scope of the public law preemption and make it easier to interpret the 'express preemption' enunciated in the statute.

By contrast, when the statute is silent on preemption, or the preemptive language is ambiguous or unclear as to the scope of the preemption, courts often undertake an 'implied preemption' analysis in order to determine whether the legislature intended to 'occupy the field' with legislation so sweeping that no room was left for common law, or to determine whether the common law actually conflicts with the scheme of the statute. A court may find an actual conflict where it is not possible to comply with both common law and public law, or the court may decide that common law acts as an obstacle to fulfillment of the statutory objectives.

Toxic Torts and the Future

As noted in previous editions of this chapter, the law of toxic torts continues to develop. Traditional legal rules continue to be strained and stretched. The tension created by the juxtaposition of scientific uncertainty and unsettled law continues to impact toxic tort litigants. The emerging interface of genetic and environmental forces creates new challenges for the proof of causation and injury, and further complicates emerging concepts of latency between exposure and either onset or manifestation of injury. Epidemiological studies continue to vary widely in their attempts and in their ability to detect in populations objectively verifiable effects that can be attributed to chronic, low-dose exposures to various environmental agents. Finally, among the most interesting challenges offered by toxic tort litigation is the persistent need for medical, scientific, legal, and regulatory professionals to improve their capacities to communicate and understand the complex concepts, language, and approaches used by the various participants in this fascinating sphere of human activity and concern.

See also: Clean Air Act (CAA), US; Clean Water Act (CWA), US; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food Quality Protection Act, US; Food, Drug, and Cosmetic Act, US; National Environmental Policy Act; Occupational Safety and Health Act, US; Pollution Prevention Act, US; Resource Conservation and Recovery Act, US; Safe Drinking Water Act, US; Toxic Substances Control Act, US.

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Toxicity Testing, Alternatives

Shayne C Gad

Since the early 1980s, public perception of the value of (and benefits from) animal testing has been strongly influenced by what is now called the animal rights movement. This concern has done a great deal of good because it has caused careful consideration of why and how testing is performed, with significant alterations being made in practices across the range of safety assessment. Its impact has been uneven, however, in the degree of sensitivity of the issue of

animal testing in different industries and organizations. Some organizations no longer perform any animal testing, having it conducted externally if it is required. Others are simply quiet about it.

The guiding principles subscribed to by many in the field are the four Rs. The historical beginnings of this concept date to 1959 when Russell and Burch first proposed what have come to be called the three Rs of humane animal use in research: replacement, reduction, and refinement. These three principles have served as the conceptual basis for reconsideration of animal use in research. To these has been added a fourth principle: responsibility.

Replacement means utilizing methods which do not use intact animals in place of those that do. For example, veterinary students may use a canine cardiopulmonary resuscitation simulator, Resusci-Dog, instead of living dogs. *In vitro* techniques include utilizing cell or tissue cultures, isolated cells, tissue slices, subcellular fractions, transgenic cell cultures, and cells from transgenic organisms. Cell cultures may replace mice and rats in discovering substances poisonous to humans. In addition, using the preceding definition of animal, an invertebrate (e.g., a horseshoe crab) could replace a vertebrate (e.g., a rabbit) in a testing protocol. Further, computer software programs and their associated databases of toxicology-related structure–activity data can be used to do *in silico* modeling to predict the toxicity profile of a new chemical.

Reduction refers to the use of fewer animals. For instance, changing practices allow toxicologists to estimate the lethal dose of a chemical with as few as 1/10 the number of animals used in traditional tests. In biomedical research, long-living animals such as primates may be used in multiple sequential protocols assuming that the protocols are not deemed inhumane or scientifically conflicting. Designing experimental protocols with appropriate attention to statistical inference can lead to either decreases or increases in the numbers of animals used. Through coordination of efforts among investigators, several tissues may be simultaneously taken from a single animal. Reduction can also refer to the minimization of any unintentionally duplicative experiments, perhaps through improvements in information resources.

Refinement entails the modification of existing procedures so that animals are subjected to less pain and distress. Refinements may include administration of anesthetics to animals undergoing otherwise painful procedures; administration of tranquilizers for distress; humane destruction prior to recovery from surgical anesthesia; and careful scrutiny of behavioral indices of pain or distress followed by cessation of the procedure or the use of appropriate analgesics. Refinements also include the enhanced use of noninvasive imaging technologies that allow earlier detection of tumors, organ deterioration, or metabolic changes and the subsequent early euthanasia of test animals.

Responsibility is the fourth R, which was not in Russell and Burch's initial proposal. To toxicologists this is the cardinal R. They may be personally committed to minimizing animal use and suffering and to doing the best possible science of which they are capable, but at the end of it all, toxicologists must stand by their responsibility to be conservative in ensuring the safety of the people using or exposed to the drugs and chemicals produced and used in our society. This is particularly true for medical devices and includes in it the element of ensuring adherence to regulatory requirements and standards.

Toxicology, and particularly the portion of it associated with the assessment of commercial products for safety, is philosophically a conservative scientific practice. If a choice is to be made as to whether to accurately identify human hazard or to over-predict any hazard, with both predictions having a degree of uncertainty, the latter course will be chosen. Likewise, if an evaluation process, which is dated but very familiar is challenged by a new technology which is scientifically, economically, and ethically superior but with which there is no precedent or prior history of use, the former will be selected. Both of these two choices are made with reference to what (1) is regulatorily accepted (i.e., codified in law) and (2) what has to date been the standard in litigation defense. The key assumptions currently underlying safety assessment are (1) that other organisms can serve as accurate predictive models of toxicity in humans, (2) that selection of an appropriate model to use is essential to the accurate prediction of adverse health effects in humans, and (3) that an understanding of the strengths and weaknesses of any particular model is required before the relevance of specific findings to humans can be established. When we refer to models, we usually mean test organism, though in fact the manner in which parameters are measured (and which parameters are measured) to characterize an endpoint of interest is also a critical part of the model (or, indeed, may actually constitute the model). To an increasing degree, both *in vivo* (intact, higher organism) and *in vitro* and *in silico* models are used, though the degree of utilization of *in vitro* and *in silico* models has lagged behind its potential use.

Mechanisms of chemical toxicity are largely identical in humans and animals. Our increased understanding of mechanisms on the molecular and cellular level has caused some of the same people who question the general principle of predictive

value of animal tests to suggest that the state of knowledge is such that *in silico* models or simple biochemical or cell culture systems could always be used in place of intact animals to accurately predict or warn of toxicities in humans. This last suggestion also misses the point that the final expressions of toxicity in humans or animals are frequently the summation of extensive and complex interactions occurring at cellular and biochemical levels. For example, although it was once widely believed (and still is believed by many animal rights activists) that *in vitro* mutagenicity tests would replace animal bioassays for carcinogenicity, this is clearly not the case on either scientific or regulatory grounds. Although there are differences in the responses of various species (including humans) to carcinogens, the overall predictive value of such results (when tempered by judgment) is clear.

Increasingly, alternative models that use other than intact higher organisms are being used in toxicology for a number of reasons. These reasons include desires for specificity of response, use of small quantities of test materials, and expedited development, all of which are particularly important in the biotechnology industries. Well-reasoned use of *in vitro* or other alternative test model systems is essential to the development of a product safety assessment program that is both effective and efficient.

The 'ideal' test to answer a safety assessment question should have an endpoint measurement that provides data such that dose–response relationships can be obtained. Furthermore, any criterion of effect must be sufficiently accurate in the sense that it can be used to reliably resolve the relative toxicity of two test chemicals that produce distinct yet similar responses (in terms of hazard to humans). In general, it may not be sufficient to classify test chemicals into generic toxicity categories. For instance, if a test chemical falls into an intermediate toxicity category but is borderline to the next, more severe toxicity category, it should be treated with greater concern than another test chemical that falls at the less toxic extreme of the same immediate category. Therefore, it is essential for a test system to be able to place test chemicals in an established toxicity category as well as to rank materials relative to others in that category.

The endpoint measurement of the ideal test system must be objective. This is important so that a given test chemical will yield similar results when tested using the standard test protocol in different laboratories. If it is not possible to obtain reproducible results in a given laboratory over time or between various laboratories, then the historical database against which new test chemicals are evaluated will be time or laboratory dependent. If this is the case, then there will be significant limitations on the application of the test system because it could potentially produce conflicting results. From a regulatory point of view, this possibility would be highly undesirable. Along these lines, it is important for the test protocol to incorporate internal standards to serve as quality controls. Thus, test data could be represented utilizing a reference scale based on the test system response to the internal controls. Such normalization, if properly documented, could reduce interest variability.

From a practical point of view, there are several additional criteria that the ideal test should meet. Alternatives to current *in vivo* test systems basically should be designed to evaluate the observed toxic response in a manner as closely predictive of the outcome of interest in humans as possible. In addition, the test should be fast enough so that the turnaround time for a given test chemical is reasonable for the intended purpose (very rapid for a screen and timely for a definitive test). The speed of the test and the ability to conduct tests on several chemicals simultaneously will determine the overall productivity. The test should be inexpensive so that it is economically competitive with current testing practices. Finally, the technology should be easily transferred from one laboratory to another without excessive capital investment (relative to the value of the test performed) or the need for special skills for test implementation.

Table 1 Rationale for using *in vivo* test systems

Evaluate actions/effects on intact animals and assess organ/tissue interactions
Allow either neat chemicals or complete formulated products (complex mixtures) to be evaluated
Yield data on the recovery and healing processes
Are currently predominantly the required statutory tests for agencies worldwide
Afford quantitative and qualitative evaluations using a scoring system that is generally capable of ranking materials according to their relative hazards
Are amenable to modifications to meet the requirements of special situations (such as multiple dosing or exposure schedules)
Allow the use of an extensive available database and have cross-reference capabilities for evaluation of relevance to human situation
Are associated with ease of performance and relatively low capital costs in many cases
Are generally both conservative and broad in scope, providing for maximum protection by erring on the side of over prediction of hazard to humans

The point is that these characteristics of the ideal test system provide a general framework for evaluating alternative test systems in general. No test system is likely to be ideal. Therefore, it is necessary to weigh the strengths and weaknesses of each proposed test system in order to reach a conclusion as to the effectiveness of a particular test.

In recent years, tremendous progress has been made in our understanding of mechanisms of biological action down to the molecular level. This has translated to multiple modifications and improvements to *in vivo* testing procedures, which now give us tests which (1) are more reliable, reproducible, and predictive of potential hazards in humans; (2) use fewer animals; and (3) are considerably more humane than earlier test forms. Since 1971 *in vitro* alternative test systems have been proposed, developed, and validated to at least some extent. Yet the perception persists that little has changed in how safety assessment is performed by or for industry.

In both theory and practice, *in vivo* and *in vitro* tests each have potential advantages, as summarized in **Tables 1** and **2**. It should be noted that the relative

Table 2 Limitations of *in vivo* testing systems that serve as a basis for seeking *in vitro* alternatives for safety assessment tests

May involve complications and/or confounding or masking of findings
May assess only the short-term site of application or immediate structural alterations produced by agents; however, specific *in vivo* tests may only be intended to evaluate acute local effects, so this may be a purposeful test system limitation
Require stringent technician training and monitoring (particularly because of the subjective nature of evaluation)
May not perfectly predict results in humans if the objective is to exclude or identify severe acting agents
Structural and biochemical differences between test animals and humans that make extrapolation from one to the other difficult
Lack of standardization
Variable correlation with human results
Large biological variability between experimental units (i.e., individual animals)
Large, diverse, and fragmented databases that are not readily comparable
Require a comparatively longer time to express/evaluate endpoints
Require comparatively larger quantities of test material
May be conducted using either a single endpoint (e.g., lethality and corrosion) or a so-called 'shotgun' or multiple-endpoint approach (e.g., a 13 week oral toxicity study)
Are the accepted norm for evidence in courts of law for litigation cases

Table 3 Levels of models for toxicity and research

Level/model	*Advantages*	*Disadvantages*
In vivo (intact higher organism)	Full-range of organismic responses similar to target species	Cost Ethical/animal welfare concerns Species-to-species variability
Lower organisms (earthworms, fish)	Range of integrated organismic responses	Frequently lack responses typical of higher organisms Animal welfare concerns
Isolated organs	Intact but isolated tissue and vascular system Controlled environmental and exposure conditions	Donor organism still required Time-consuming and expensive No intact organismic responses Limited duration of viability
Cultured cells	No intact animals directly involved Ability to carefully manipulate system Low cost Ability to study a wide range of variables	Instability of system Limited enzymatic capabilities and viability of system No (or limited) integrated multicell and/or organismic responses
Chemical/biochemical systems	No donor organism problems Low cost Long-term stability of preparation Ability to study a wide range of variables Specificity of response	No *de facto* correlation to *in vivo* system Limited to investigation of a single defined mechanism
In silico (computer) simulations	No animal welfare concerns Speed and low per-evaluation cost	May not have predictive value beyond a narrow range of structures Expensive to establish

Table 4 Possible interpretations when *in vitro* data do not predict results of *in vivo* studies

Chemical is not absorbed at all or is poorly absorbed in *in vivo* studies
Chemical is well absorbed but is subject to 'first-pass effect' in the liver
Chemical is distributed so that less (or more) reaches the target tissue than would be predicted on the basis of its absorption
Chemical is rapidly metabolized to an active or inactive metabolite that has a different profile of activity and/or different duration of action than the parent drug
Chemical is rapidly eliminated (e.g., through secretory mechanisms)
Species of the two test systems used are different
Experimental conditions of the *in vitro* and *in vivo* experiments differed and may have led to different effects than expected. These conditions include factors such as temperature or age, sex, and strain of animal
Effects elicited *in vitro* and *in vivo* by the particular test substance in question differ in their characteristics
Tests used to measure responses may differ greatly for *in vitro* and *in vivo* studies, and the types of data obtained may not be comparable
The *in vitro* study did not use adequate controls (e.g., pH, vehicle used, volume of test agent given, and samples taken from sham-operated animals), resulting in 'artifacts' of methods rather than results
In vitro data cannot predict the volume of distribution in central or in peripheral compartments
In vitro data cannot predict the rate constants for chemical movement between compartments
In vitro data cannot predict the rate constants of chemical elimination
In vitro data cannot predict whether linear or nonlinear kinetics will occur with specific dose of a chemical *in vivo*
Pharmacokinetic parameters (e.g., bioavailability, peak plasma concentration, and half-life) cannot be predicted based solely on *in vitro* studies
In vivo effects of chemical are due to an alteration in the higher order integration of an intact animal system, which cannot be reflected in a less complex system

weight assigned to these advantages will differ depending on the information required and how it is to be used.

Can the proper tests be selected, especially when a decision must be made between using with an existing test system or adopting a new one? What are the available options?

The division between test system models is more complex than *in vivo* and *in vitro*, of course. There is a range of options under *in vitro*, each with its own advantages and disadvantages, as shown in **Table 3**. Each of these levels will need to be considered.

It should be noted that, in addition to potential advantages, *in vitro* systems *per se* also have a number of limitations that can contribute to there not being acceptable models. Findings from an *in vitro* system that either limit their use in predicting *in vivo* events or make them totally unsuitable for the task include there being wide differences in the doses needed to produce effects or differences in the effects elicited. Some reasons for such findings are detailed in **Table 4**.

At the same time, there are substantial potential advantages in using the *in vitro* system. The scientific advantages of using cell or tissue culture in toxicological testing are isolation of test cells or organ fragments from homeostatic and hormonal control, accurate dosing, and quantitation of results. It is important to devise a suitable model system that is related to the mode of toxicity of the compound. Tissue and cell culture has the immediate potential to be used in two very different ways by industry. First, it has been used to examine a particular aspect of the toxicity of a compound in relation to its toxicity *in vivo* (i.e., mechanistic or explanatory studies). Second, it has been used as a form of rapid screening to compare the toxicity of a group of compounds for a particular response. Indeed, the pharmaceutical industry has used *in vitro* test systems in these two ways for years in the search for new potential drug entities. The extension of these approaches to safety assessment is a much more recent occurrence.

Mechanistic and explanatory studies are generally called for when a traditional test system gives a result that is either unclear or for which the relevance to the real-life human exposure situation is unclear. *In vitro* systems are particularly attractive for such cases because they can focus on very defined single aspects of a problem or pathogenic response, free of the confounding influence of the multiple responses of an intact higher-level organism.

See also: Ames Test; Analytical Toxicology; Animal Models; Dominant Lethal Tests; Dose–Response Relationship; Host-Mediated Assay; *In Vitro* Test; *In Vivo* Test; Mouse Lymphoma Assay; Toxicity, Acute; Toxicity, Chronic; Toxicity Testing, Irritation; Toxicity Testing, Modeling; Toxicity, Subchronic; Toxicity Testing, Validation.

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Toxicity Testing, Aquatic

Shayne C Gad

Introduction

Freshwater and marine environments contain complex ecosystems such as ponds, rivers, lakes, and estuaries. Each of these ecosystems contains unique biota that may be represented by several thousand species. These biota, both flora and fauna, are often exposed to a variety of toxicants, including those that result from anthropogenic activities, and in some cases, toxicity and environmental damage can occur. The study of these adverse effects on freshwater and marine biota and on the ecosystems that contain them is called aquatic toxicology.

Aquatic toxicology differs from mammalian toxicology in several aspects. The primary goal of aquatic toxicology is to assess the effect of toxicants on the many diverse populations and communities of plants and animals inhabiting marine and freshwater environments. The biota are cold-blooded and the physical and chemical characteristics of the aquatic environment have a significant effect on their sensitivity to toxicants. The aquatic test species of interest, unlike in mammalian studies, can be used directly. The objective of mammalian toxicology is to assess effects on humans, whose sensitivity to toxicants is less affected by their environment than that of aquatic organisms. The dose of the toxicant used in mammalian toxicology can be measured more accurately, the mechanisms of toxic action are better understood, and the test methods are more established.

Various species of aquatic life, particularly fish, have been used in toxicity experiments for more than 130 years. One of the earliest reported studies was conducted with fish in 1863 and the first proposed standard test species was the goldfish in 1917. Toxicity tests have been conducted with increasing frequency since the 1960s due to the numerous environmental regulations that have been enacted and the increasing availability of standardized test methods, the first of which were published in 1960 for animal test species and in 1970 for algae.

Many test methods are available for aquatic toxicity testing (**Table 1**). They differ in cost, precision, complexity, and the skill needed to conduct them. Nevertheless, their objectives are similar. They are conducted to determine the relative potency among chemicals and the relative susceptibility among different species and life stages and to identify other variables that influence the overall outcome of exposure. Toxicity tests are usually conducted to meet regulatory guidelines for the use and discharge of

Table 1 Available laboratory testing methods for aquatic toxicology

Single-species tests
Trout
Fathead minnows
Daphnia
Bluegills
Algae
Multispecies tests
Experimental streams
Ponds
Microcosms
Bioconcentration tests
Effluent toxicity tests
Sediment toxicity tests
Phytotoxicity tests
Algae
Vascular plants
Duckweed

commercial chemicals such as pesticides. In addition, toxicity results are used to derive national water quality standards to protect aquatic life and to determine the environmental effects of municipal and industrial effluents.

Aquatic toxicologists do not use all the available toxicity tests for any single toxicant. Instead, a tiered approach is used to provide a systematic and comprehensive process for deriving the toxicity data needed to assess the environmental hazard of a chemical. This approach consists of conducting short-term screening tests prior to using predictive studies that are more complex and time-consuming. This sequential evaluation provides an efficient use of resources and tends to eliminate unnecessary testing. The decision points and testing phase depend on the quality and quantity of data needed for the test substance of interest. The types of toxicity tests used in the tiered approach are discussed briefly.

Single-Species Toxicity

Tests: Methodologies

There are two basic types of aquatic single-species toxicity tests: acute and chronic. Acute toxicity tests have been the 'workhorse' of aquatic toxicologists for many years. These tests are relatively simple, take little time, and are cost-effective. A large historical database exists for many chemicals and effluents. Acute toxicity tests are most often used to quickly screen toxicity or to determine the relative sensitivities of different test species. Mortality is the effect monitored during the test duration of 48 h (invertebrates) or 96 h (fish). In a typical acute toxicity test, 5–10 organisms are exposed under static conditions in glass test beakers to five test concentrations. A control is included. The experiments with test concentrations and control are conducted in triplicate. Daily observations are made on survival, and dead organisms are removed.

At test termination, the concentration that kills 50% of the test organisms (LC_{50} value) is determined using probit analysis or graphical interpolation. Unlike in chronic toxicity tests, there is no test solution renewal, the organisms are unfed, and there is no analytical verification of the test concentrations. Furthermore, cumulative, chronic, and sublethal effects of a chemical usually are not evaluated in acute toxicity tests, although frequently behavioral changes and lesions caused by a chemical can be determined.

Chronic toxicity tests are more complex and time-consuming than acute studies and for these reasons are conducted less frequently. The methodologies for these tests differ considerably, unlike for acute tests, because they are designed for the specific life histories of the various test species. Chronic toxicity tests may be for a full-life cycle (egg–egg), partial-life cycle (embryo/larval), and partial-life history (egg–death). Full-life cycle tests are uncommon with fish due to the long durations that are necessary (1 or 2 years). Partial-life cycle tests with fish can be as short as 7 days or as long as 60 days. The early life stage of fish (embryo/larva) is usually the most sensitive period in a fish's life cycle and, consequently, partial-life cycle tests are used as surrogates for the full-life cycle studies. Chronic tests may be conducted for more than one complete life cycle if algal and invertebrate species are used since their life cycles are shorter than those of fishes. Lethal and sublethal effects are monitored in chronic toxicity studies, and these effects include changes in growth, reproduction, behavior, physiology, and histology.

Toxicity tests may be static, continuous-flow, or static renewal based on the toxicant dosing technique. Static and continuous-flow procedures are more widely used in toxicity tests conducted with pure chemicals and animal test species. Chronic toxicity tests conducted with effluents are usually static renewal, and those with algae are static. There is no change or renewal of the test substance with dilution water in a static test. This design is the simplest and least expensive; however, the toxicant concentrations may decrease due to adsorption and biodegradation. The test solutions and dilution water are renewed periodically, usually daily in a static-renewal test. In a continuous-flow test, the dilution water and test substance are continuously or intermittently renewed. The exposure concentrations remain fairly constant and dose–response relationships can be well defined.

A variety of aquatic toxicity test methods have been published for single species and several have been standardized through the efforts of such organizations as the American Society for Testing and Materials, the US Environmental Protection Agency, the American Public Health Association, and the Organization for Economic Co-operation and Development. Test method development is an ongoing process, however, which continues to increase the efficiency of these methods and often results in alternative study designs.

Experimental Conditions

In general terms, toxicity tests are conducted in a laboratory or a room controlled for light and temperature. The test solutions containing the test species are monitored for pH, temperature, dissolved oxygen, and water hardness. Daily observations on lethal and

sublethal effects are made, and several calculations, such as the LC_{50} value, the highest no-observed-effect concentration (NOEC), and the lowest-observed-effect concentration (LOEC), are determined based on the most sensitive effect parameter of interest. Although toxicity tests have similarities, as discussed later, variations among test animals, instrumentation, and methods influence the outcomes and utility of the assessments.

Test Chambers

The types of test chambers used in toxicity tests depend on the test species. Various sizes of beakers, aquaria, jars, bowls, and petri dishes have been used. The test chambers usually are constructed of material such as glass, Teflon, and certain plastics that minimize leaching of toxicants and adsorption of the test substance.

Test Concentrations

Xenobiotic concentrations used in an acute toxicity test are based routinely on results obtained from a pretest or range-finding test. The test concentration range for a chronic test is based on the results of an acute test conducted prior to the chronic test. There are no standard guidelines for conducting these preliminary tests. Generally, 5–10 organisms are exposed to several test concentrations, which are usually an order of magnitude apart. The dilution water and exposure conditions (i.e., water temperature, hardness, and pH) in range-finding tests usually are similar to those in the definitive test.

The test substances used in toxicity tests have been in most cases pure chemical compounds and municipal and industrial effluents. However, toxicity tests are being conducted more frequently with dredged soil materials (prior to ocean disposal), hazardous waste leachates, and contaminated sediments due to increasing regulatory concern for their potential environmental impacts. The test organisms are exposed in the definitive test to give concentrations chosen in a geometric progression. The test concentrations and control are replicated at least threefold. The test compound is added to the dilution water, which may be well water, reconstituted water, dechlorinated tap water, uncontaminated river water, and natural or artificial seawater. The dilution water is well aerated and undesirable organisms are removed before use.

An organic solvent is used to dissolve substances with minimal water solubility. Several have been used, including triethylene glycol, dimethyl sulfoxide, acetone, and dimethyl formamide. The LC_{50} values for these solvents are between 9000 and 92 500 $mg\,l^{-1}$. The concentration of the solvent in the test water should not exceed 0.5 $ml\,l^{-1}$ or should not be more than 1/1000 of the LC_{50} value of the solvent. When an organic solvent is used, a solvent control is included in the study.

Toxicant delivery systems are used to deliver, on a once-through basis, the various test concentrations to the test chambers in continuous-flow toxicity tests. The serial proportional diluter is the most common design used to mix the dilution water with the test substance to produce the desired test concentrations. The construction materials in toxicant delivery systems, like those for the test chambers, should not be rubber, certain plastics, or metallic materials.

The test concentrations are confirmed analytically during chronic toxicity tests. Analyses are performed at least weekly for each test concentration and control for tests of 7 days' duration or longer. In tests of shorter duration, analyses usually are conducted on alternate days. Analytical verification of the test concentrations in range-finding and acute toxicity tests is seldom done, and the results from these tests generally are based on nominal concentrations.

Test Species

Historically, animal test species have been used more frequently than plant species and freshwater species more frequently than marine species. These trends can be seen in **Table 2**.

Most toxicity tests are conducted with single cultured test species such as those listed in **Table 3**. The more commonly used freshwater species, particularly

Table 2 Types of tests and test species used in deriving toxicity data for submissions under the Toxic Substance Control Act Section 4 as of 1988

Test type	*Species*[a]	*Number of tests*
Acute toxicity	Fathead minnow (F)	37
	Rainbow trout (F)	27
	Sheepshead minnow (M)	12
	Daphnids (F)	23
	Midge (F)	12
Partial-life cycle	Fathead minnow (F)	1
	Rainbow trout (F)	7
Full-life cycle	Daphnids (F)	20
	Mysid shrimp (M)	5
Phytotoxicity	Alga (F)	23
	Alga (M)	3
Bioconcentration	Bluegill (F)	1
	Fathead minnow (F)	1
	Rainbow trout (F)	12
	Mussel (M)	1
	Oyster (M)	2

[a] F, freshwater; M, marine.

in tests used for regulatory compliance, are fathead minnows (*Pimephales promelas*), several daphnid species (*Daphnia magna* and *Ceriodaphnia dubia*), and green algae (*Selenastrum capricornutum*). Common marine species are sheephead minnows (*Cyprinodon variegatus*), mysid shrimp (*Mysidopsis bahia*), and a diatom, *Skeletonema costatum*.

The species in **Table 3** were selected based on several criteria, primarily ease of culture, commercial availability, and size. The test species are acclimated for a specific period of time prior to testing to eliminate diseased organisms. Generally, a minimum of 10 animals are exposed in static and static-renewal tests and 20 in a flow-through test for each test concentration and control. The recommended loading density for the test species is between 0.5 and $0.8\,g\,l^{-1}$ in static tests and between 1 and $10\,g\,l^{-1}$ in continuous-flow through tests.

Table 3 Freshwater and marine species used in toxicity tests

Freshwater	*Saltwater*
Fish	Fish
Salmo gairdneri (rainbow trout)	*Cyprinodon variegatus* (sheepshead minnow)
Salvelinus fontinalis (brook trout)	*Fundulus heteroclitus* (mummichog)
Ictalurus punctatus (channel catfish)	*Menidia beryline* (silverside)
Pimephales promelas (fathead minnow)	*Gasterosteus aculeatus* (threespine stickleback)
Lepomis macrochirus (bluegill)	*Leiostomus santhurus* (spot)
Carassius auratus (goldfish)	Invertebrates
Invertebrates	*Acartis tonsa* (copepod)
Daphnia magna (daphnid)	*Neanthes* sp. (polychaeta)
Daphnia pulex (daphnid)	*Callinectes* sp. (crab)
Ceriodaphnia dubia (daphnid)	*Penaeus* spp. (pink shrimp)
Gammarus lacustris (amphipod)	*Palaemonetes* spp. (grass shrimp)
Chrionomus sp. (midge)	*Crassostrea virginica* (oyster)
Physa integra (snail)	*Arvacia punctulata* (sea urchin)
Chambarus sp. (crayfish)	Plants
Plants	Algae
Algae	*Skeletonema costatum* (diatom)
Selenastrum capricornutum (green)	*Thalassiosire pseudonana* (diatom)
Chlorelia vulgaris (green)	*Champia parvula* (red)
Microcystis aeruginosa (blue green)	
Navicula spp. (diatom)	
Vascular	
Lemna minor (duckweed)	
Lemna gibba (duckweed)	
Myriophyllum spicatum (water milfoil)	
Ceratophyllum demersum (coontail)	

Reference toxicants often are used to determine the 'health' of the test species. There is no widely used reference toxicant; several that have been used include dodecyl sodium sulfate (anionic surfactant), sodium chloride, sodium pentachlorophenol, and cadmium chloride.

Sensitivity is a criterion that is used in the choice of a test species. The sensitivity of the species in **Table 3** relative to one another as well as to indigenous flora and fauna in the ecosystem is a matter of contention. There is no single test species and no group of test species consistently most sensitive to toxicants or most reliable for extrapolation to all other organisms. Most toxic effects reported for a variety of test substances have been species-specific. Therefore, acute toxicity tests are conducted first with a variety of freshwater and marine test species to determine the most sensitive plant and animal. These sensitive species then are used in all subsequent chronic testing.

Calculations

The results of acute toxicity tests are reported as the LC_{50} and EC_{50} (concentration that reduces growth 50%) values and their 95% confidence intervals. Probit analysis is the most commonly used statistical method to determine LC_{50} values. Graphical interpolation can be used to estimate the LC_{50} value where the proportion of deaths versus the test concentration is plotted for each observation time.

The NOEC and the LOEC are the usual calculations reported from chronic toxicity tests. The NOEC is the highest concentration in which the measured effect is not statistically different from that of the control. The LOEC is the lowest concentration at which a statistically significant effect occurred. These concentrations are based on the most sensitive effect parameters, that is, hatchability, growth, and reproduction. The statistical procedure for these calculations combines the use of analysis of variance techniques and multiple comparison tests. In some cases, the maximum acceptable toxic concentration (MATC) is reported from chronic toxicity results. The MATC is a concentration (x) that is within the range of the NOEC and LOEC: $\text{NOEC} \geqslant x < \text{LOEC}$. The first-effect concentration can be expressed as the geometric mean of the two terms.

Variability Precision

Toxicity tests conducted with freshwater and marine species are considered relatively precise and reliable based on current information concerning

interlaboratory and intralaboratory comparisons of toxicity results. Generally, the LC_{50} values from acute toxicity tests conducted under similar experimental conditions vary less than threefold. This has been observed for metals, effluents, reference toxicants, and different organic compounds. Coefficients of variation (CV) for acute and chronic toxicity tests conducted with daphnic species and chemicals and effluents are between 27% and 39%. The CV values for several reference toxicants and acute daphnic studies ranged between 10% and 72% and from 47% to 83% for chronic toxicity tests with algae.

Multispecies Toxicity Tests

The results of the 'traditional' acute single-species toxicity tests conducted in the laboratory cannot be used alone to predict effects on natural populations, communities, and ecosystems. The cultural species in laboratory tests are different from those in most ecosystems. Conditions such as the size of the test species, its life stage, and nutritional state can have an effect on toxicity. Furthermore, the experimental conditions in laboratory tests cannot duplicate the complex interacting physical and chemical conditions of ecosystems, such as seasonal changes in water temperature, dissolved oxygen, and suspended solids. In addition to these environmental modifying factors, aquatic life is usually exposed simultaneously to numerous potential toxicants (mixtures). Although the toxicities of binary and ternary mixtures have been evaluated for some chemicals in laboratory toxicity tests, the resultant information has predictive limitations.

Because of the deficiencies of single-species toxicity tests, alternative approaches are being evolved to address the structural and functional processes of an ecosystem. Multispecies tests include the use of laboratory microcosms, outdoor ponds, experimental streams, and enclosures. There are no standardized procedures for these tests. They are conducted with plant and animal species obtained from laboratory cultures and biota collected from natural sources. They can be conducted indoors or outdoors. The toxic effects, in addition to those used for single-species tests, are determined for structural parameters, such as community similarity, diversity, and density, and for functional parameters, such as community respiration and photosynthesis. Effects on these parameters are reported as the NOEC and LOEC.

Sediment Toxicity Tests

In the past, toxicity tests have been conducted primarily with water column-dwelling or planktonic organisms, with the objective of controlling water pollution. However, it has been realized that sediments act as 'reservoirs' for chemicals that can adversely affect benthic aquatic life and, at times, also affect planktonic life. This concern has led to the development of sediment quality criteria to protect aquatic life. Test methods have been developed to support the derivation of these criteria and to support other related regulatory activities (e.g., Superfund site evaluations and ocean disposal of dredged materials).

Most sediment toxicity tests have been conducted in the laboratory with single species of freshwater and marine benthic organisms such as amphipods and midges, but in some cases planktonic species also have been used. Most tests conducted to date have been acute and have been of 10 days' duration or less. Sediment toxicity tests are conducted with the solid phase or the pore water (interstitial water). Methods have been published describing the collection and preparation techniques.

Test guidelines for marine sediment and freshwater sediment also have been reported. Standardized methods are available for freshwater invertebrates and freshwater and marine amphipods.

The availability of reliable test methods for contaminated sediments is relatively recent and the test method development process continues. A variety of issues remain to be solved before these types of studies will be considered as effective as those with planktonic species. Among the more important of these issues are validation of the single-species test results and determination of variations in species sensitivity.

Effluent Toxicity Tests

Toxicity tests are used in the National Pollutant Discharge Elimination System, permitting one to determine the toxicity effects of municipal and industrial effluents and storm water overflows on aquatic life. A summary of the experimental conditions in several of the available test methodologies appears in **Table 4**. The methodologies differ slightly from those used for pure chemicals. For example, the choice of the dilution water and the effluent collection technique are important considerations. In most cases water collected from the receiving water above the outfall is used for dilution, and composite samples of effluent are used. The test species – algae, invertebrate, and fish – are usually exposed to five effluent dilutions for 4–7 days. The tests are static renewal except those for algae, which are static. The calculations reported are the LC_{50} value, the NOEC, and the LOEC, which are expressed as percentage of effluent. The cause(s) of toxicity in the effluent – that is, specific effluent constituents – can be identified using comparative toxicity testing and chemical fractionation techniques.

Table 4 Comparison of several experimental variables in chronic toxicity tests conducted with effluents

Test type	*Duration (days)*	*Number of test concentrations*	*Test species*	*Age of the organism*	*Total test species exposed*	*Number of replicates*	*Temperature (°C)*	*Light intensity ($mE\,m^{-2}\,s^{-1}$)*
Static renewal	7	5	Fathead minnow (freshwater fish)	<24 h	30–60	3–4	25±1	10–20
Static renewal	7	5	Sheepshead minnow (marine fish)	<24 h	30–60	3–4	12±2	10–20
Static renewal	7	7	*Ceriodaphnia dubia* (freshwater invertebrate)	<24 h	10	10	25±1	10–20
Static renewal	7	5	*Mysidopsis bahia* (marine invertebrate)	<24 h	40	8	25–27	10–20
Static	4	5	*Selenastrum capricornutum* (freshwater; green alga)	4–7 days	1 × 104 (initial)	3	25±1	86±8.6
Static	4	5	*Skeletonema costatum* (marine; diatom)	4–7 days	2 × 104 (initial)	3	20±2	60±6

The freshwater invertebrate, *C. dubia*, is a test species commonly used in effluent toxicity evaluations. The *C. dubia* used in a study are obtained from a laboratory culture. Effluent collected within 72 h from the source is used after temperature acclimation. The static-renewal test usually is conducted in a laboratory located off-site from the effluent source but the tests may be conducted on-site using a mobile bioassay facility. The test is conducted at 25°C, 10–20 $mE\,m^{-2}\,s^{-1}$, and under a photoperiod of 16 h light/8 h darkness. Five test concentrations are used that include undiluted effluent (100%) and four dilutions such as 50%, 25%, 12%, and 6%. The effluent is diluted with either a high-quality laboratory water or water collected from the receiving water above the effluent outfall. The control is composed of 100% dilution water. For each test concentration and the control, ten 30 ml plastic test chambers containing 15 ml of the test solution are used. Each test chamber contains one daphnid and daily observations on mortality and production of young are made during the 7 day test. The organisms are fed daily a combination of yeast, trout chow, and algae. Surviving organisms are transferred daily to renewed test solutions. The NOEC and LOEC values are determined based on the adverse effects on survival and reproduction occurring during the 7 day test.

Phytotoxicity

The majority of aquatic toxicity tests have been conducted with animal test species since they once were thought to be more sensitive than plants. This generalization is not supported technically, based on a review of the data for most toxicants. Nevertheless, only recently have phytotoxicity tests been conducted routinely with a limited number of species of algae and vascular plants.

A variety of test methods are available to determine the phytotoxic effects of chemicals and effluents. The freshwater algal species most frequently used has been the microalga, *S. capricornutum*, for which a relatively large database exists. Marine species used include the diatom, *S. costatus*, and the red macroalga, *Champia parvula*.

Acute toxicity tests seldom are conducted with algae. The chronic toxicity tests conducted with microalgae are for 3 or 4 days' duration although exposures can be for less than 1 day if effects on photosynthesis are measured. These static exposures occur in a liquid nutrient-enriched medium under conditions of controlled pH, temperature, and light. Inhibitory and stimulatory effects on population growth are monitored during the exponential growth phase. Five test concentrations and a control are included in each study. The most common calculation reported is the 96 h EC_{50} value but algistatic (that completely stops growth) and algicidal (lethal) concentrations also have been reported. In addition, the SC_{20} (stimulatory) concentration is reported if growth stimulation is observed. The SC_{20} value represents the concentration that increases algal growth 20% above that of the algal population in the control.

Floating and rooted macrophytes are used less frequently in toxicity tests than algae. The

duckweeds, freshwater floating species, are more commonly used than most due to their small size and rapid growth. Several published methods are available describing their use, particularly *Lemna minor* and *L. gibba*. Tests with these species are usually of 4–14 days' duration, during which effects on frond number and chlorophyll content are monitored. The results are expressed as an EC_{50} value and the NOEC. The tests are conducted, as with algae, in a nutrient-enriched medium. The test chambers can be fruit jars, plastic cups, test tubes, and Erlynmeyer flasks. The key research issue that remains to be investigated before the duckweeds are more widely accepted as suitable test species is their sensitivity relative to that of other aquatic plant and animal test species.

The use of rooted macrophytes such as pondweeds (*Potamogeton* spp.), waterweeds (*Elodea* and *Hydrilla*), the water hyacinth (*Eichhornia crassipes*), coontail (*Ceratophyllum demersum*), and water milfoil (*Myriophyllum* spp.) in toxicity tests is less common than that of algae and duckweeds due to their large size and slow growth. There are no standard or commonly used test methods for these species. Consequently, there is a need for their development and validation. The experimental techniques that have been used vary considerably. Recently, seeds from aquatic macrophytic vegetation have been used to assess the toxicities of chemicals and effluents. These studies are usually of 4–7 days' duration, and the effect parameters are seed germination, root elongation, and early seedling growth. The use of whole-plant rooted macrophytes and their seeds in toxicity tests will increase in the future as sediment quality criteria to protect aquatic life and wetlands increase in regulatory importance. However, for this to occur, test method development and validation, as well as determination of species sensitivity, will be needed.

Bioconcentration

A bioconcentration study is conducted to derive information on the ability of an aquatic species to concentrate a toxicant in its tissues. This uptake and accumulation can be hazardous to the organism as well as to other aquatic life utilizing the test species as a food source. Bioconcentration tests are usually conducted with single chemicals and single species of algae, fish, and bivalve mollusks. A variety of fish have been used, including the fathead minnow, bluegill, rainbow trout, sheepshead minnow, and several species of oysters, scallops, and mussels.

There are several test designs that can be used to estimate the bioconcentration potential of a compound. Typically, one group of the test species is exposed to the toxicant for an uptake and depuration phase. A control is included in which the test species is not exposed to the toxicant. In assessing the concentration of the test chemical in the organism, the literature contains examples of measuring total residues and measuring only the parent compound, depending primarily on the methodology used. The uptake phase is usually for 28 days or until a steady state is attained. The depuration period lasts until the concentration in the test species is 10% of the steady-state concentration in the tissue. During both phases, the test water and test species are analyzed daily for the test chemical. All results from a bioconcentration study are based on measured concentrations. The uptake rate, depuration rate, and bioconcentration factor (BCF) typically are reported. The relevance of the BCF value to the survival of the organism and to ecosystem dynamics is an issue that has received and will continue to receive significant scientific attention.

See also: Analytical Toxicology; Biomarkers, Environmental; Ecotoxicology; Effluent Biomonitoring; Environmental Toxicology; Microtox; Photochemical Oxidants; Pollution, Water; Risk Assessment, Ecological.

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Toxicity Testing, Behavioral

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Introduction

Behavioral toxicology, a part of the larger domain of neurotoxicology, uses a wide range of methods to evaluate changes in behaviors of model organisms (in modern toxicology, largely rats and mice) as a means of identifying and studying adverse effects of chemicals on the nervous system. As such, behavioral toxicology tests should be considered functional testing of a complex organ system. Changes in behavior are believed to be sensitive indicators of exposures to substances that result in central nervous system (CNS) toxicity. Three important classes of behavioral neurotoxicants are metals, solvents, and pesticides.

Behavior may be defined as anything an organism does – any move an organism makes. Behavior includes all observable, recordable, or measurable activities of a living organism and reflects genetic, neurobiological, physiological, and environmental determinants and can be used in biomonitoring, the determination of no-observed-effect and lowest-observed-effect concentrations, and the prediction of hazardous chemical impacts on natural populations. The behavior of an organism at any moment is the result of the external environment, the past history of the organism, and the internal environment (e.g., biochemical or electrical processes and hormonal levels) within the organism. The behaviorist studies the functional relationship between the behavior of an organism and variables such as exposure to a chemical. An aspect of the environment that controls behavior in a functional manner is termed a stimulus. A unit of behavior, defined by the experimenter, is termed a response. There are two types of responses: respondent and operant. In addition, either type of response may be unconditioned (unlearned) or conditioned (learned).

Respondent behaviors include such actions as smooth muscle contraction, autonomic responses, glandular secretions, and elicited motor responses such as reflexes. Unlearned respondents are used frequently in observational batteries and include such measures as orienting to stimuli or reflex startle to intense stimuli. The famous experiment of Pavlov, in which dogs learned to salivate at the sound of a bell after numerous pairings of the bell with presentation of food, is an example of a conditioned response. Respondents are paired with an eliciting stimulus in a one-for-one relationship.

Operant responses, on the other hand, have no single eliciting stimulus but occur within the context of many environmental stimuli. The consequences of a certain behavior affect the probability that this behavior will be produced again. Locomotor activity is often given as an example of an unlearned operant because there is no attempt on the part of the experimenter to condition a particular type of response. Ventilatory responses are often some of the first prelethal symptoms exhibited by animals to environmental stressors. Continued, abnormal ventilatory behavior (i.e., rapid or shallow breathing, erratic breathing) can indicate physiological damage that may be irreversible. Such damage could eventually result in decreased survival, growth, or reproduction of the organism, or all of these.

An extremely powerful tool at the disposal of the behavioral toxicologist is that of operant conditioning. Operant conditioning takes advantage of the control that the immediate outcome of behavior has the subsequent frequency in determining of similar behavior. If the outcome of a particular behavior increases its frequency, it is termed a positive reinforcer (i.e., food). If it decreases the frequency, it is called negative reinforcer (i.e., shock). The great strength of this technique is that it may be used to teach a large variety of tasks with wide complexity. Questions can be asked about attention, learning, memory, sensory function, and general well being of the subject. Many techniques discussed in this chapter rely on the principles of operant conditioning. Behavioral assessment would be impossible without a thorough understanding of these principles on the part of the investigator. Behavioral changes are assessed by observational methods, operant techniques, learning and memory tasks or by a combination of these.

The National Institutes of Health have created a handbook, *Methods and Welfare Considerations in Behavioral Research with Animals*, to assist the Institutional Animal Care and Use Committee in the evaluation of protocols that employ various means to manipulate the behavior and health of laboratory animals. The report contains chapters on manipulation of access to food or fluids; experimental enclosures/physical restraint; pharmacological studies; aversive stimuli; social variables; ethological approaches; and teaching with animals. ASTM International has come up with *Standard Guide for*

Behavioral Testing in Aquatic Toxicology. Behavioral testing is often performed on fish, amphibians, and macroinvertebrates.

The behavior of organisms can be divided into motor function, sensory function, learning and memory, and performance on intermittent schedules of reinforcement. These classes are somewhat arbitrary, and virtually all behavioral tests measure more than one of these functions. For example, motor function affects almost all testing, intact sensory function is necessary for learning, performance on intermittent schedules most certainly has a learning component, and so forth. Often, different functions are not separable by one test or type of test; therefore, it is imperative to study several types of behavior to determine the function(s) that is affected. It should be pointed out that none of the procedures described in the following sections should be examined in isolation because all are part of a comprehensive investigation of the potential behavioral effects of a toxicant. Examples of changes monitored include changes in feeding, preference or avoidance, self-protection, social interactions, predation, competition, reproduction, activity level, leaning, performance, or competition.

The examples of tests presented in each section are certainly not exhaustive but were chosen because they are often used or because they promise to contribute substantially to the understanding of behavioral toxicity. The first step in a tier approach is a neurobehavioral observational screen, the tool of choice for initial identification of potentially neurotoxic chemicals. The use of such screens, other behavioral tests methods, or what are generally called clinical observations does, however, warrant one major caution or consideration. That is, short-term (within 24 h of dosing or exposure) observations are insufficient on their own to differentiate between pharmacological (reversible in the short term) and lexicological (irreversible) effects. To so differentiate, it is necessary to either use additional means of evaluation or have the period during which observations are made extended through at least 3 or 4 days.

Screening Batteries

As pointed out previously, one of the agenda that has emerged for behavioral toxicity involves the screening of new chemicals for potential neurotoxicity. The behavioral tests utilized for such purposes are often referred to as apical tests because they require the integrated function of several organ systems, including the nervous system. Such batteries typically have included two behavioral components; a functional observational battery (FOB) and an evaluation of motor activity. **Table 1** depicts the component tests of the FOB, which include an array of measures of both unconditioned operant and respondent behaviors. Such batteries have been shown to exhibit utility for screening potential neurotoxicity, that is, hazard identification and elaboration. Those components of the FOB directed to cholinergic functions exhibited sensitivity to the effects of the cholinesterase inhibitor, carbaryl, whereas few such signs of cholinergic disturbances were evident in the presence of the pesticide chlordimeform which does not inhibit cholinesterase.

Motor activity is frequently included in screening batteries both as a measure of motor function and as an apical test (see **Table 1**). Motor activity, generally considered an unconditioned behavior, exists at some baseline level and is a complex behavior that includes numerous components such as ambulation, rearing, grooming, and sniffing. As discussed previously, toxicants may alter motor activity by affecting any or all of its component behaviors.

From the standpoint of screening and hazard identification, a notable point relating to the interpretation of data from FOB and motor activity studies is whether the effects observed in response to toxicant exposure represent a direct effect of the toxicant on the nervous system or are secondary to changes in other systems since such apical tests rely on the functional integrity of multiple systems. In some circumstances, the fact that the toxic effect is ultimately expressed in behavior may minimize the importance

Table 1 Example of behavioral procedures included in a functional observation battery

Home cage and open field	*Manipulative*	*Physiological*
Posture	Ease of removal	Body temperature
Convulsions	Ease of handling	
Palpebral closure	Palpebral closure	Body weight
Lacrimation	Approach response	
Piloerection		
Salivation	Touch response	
Vocalizations	Finger-snap response	
Time to first step		
Rearing	Tail-pinch response	
Urination		
Defecation	Righting reflex	
Gait	Catalepsy	
Arousal	Hindlimb foot splay	
	Forelimb grip strength	
	Hindlimb grip strength	

of the direct versus indirect source of the effect. It also should be noted in the interpretation of toxicant-induced changes in FOBs and motor activity measures that the concurrent presence of body weight loss or decline in food or water intake does not necessarily indicate that the behavioral changes are the result of malaise or sickness as these measures may change independently of each other. In addition to those procedures mentioned in this entry a standard test battery often includes:

- a simple neurological screen for basic sensory/motor function,
- open-field test for exploratory activity and anxiety-related traits,
- light–dark exploration box for anxiety-related traits,
- an accelerating rotarod test for motor coordination and skill learning,
- prepulse inhibition for sensorimotor gating,
- acoustic startle habituation for sensorimotor adaptation,
- contextual and auditory conditioned freezing for conditioned fear learning,
- Morris water task for spatial learning and memory,
- the hot-plate test for analgesia-related responses,
- locomotor activity testing,
- elevated plus maze,
- water maze escape learning task,
- cocaine-stimulated locomotor activity,
- alcohol consumption, and
- forebrain commissures.

Motor Function Testing

Deficits in motor function are frequently produced in humans as a result of their toxic exposure to a chemical. Heavy metals such as mercury, lead, and manganese; insecticides such as chlordecone (Kepone) or organophosphorus compounds; and air pollutants such as carbon disulfide all produce changes in motor function. Gross assessment of motor function should be performed as part of an initial toxicity screen. Batteries that include observational assessment of muscle tone, body posture, equilibrium, and gross coordination have been suggested. The next level of testing includes such techniques as ability to stay on a rotating rod or quantification of hindlimb splay. The former requires an automated apparatus as well as animal training and practice in order to reduce test variability to acceptable levels. The latter technique is simpler, involving the placement of ink on the paws of rodents after which they are dropped from a specific height. Quantification of hindlimb splay does not require training of the animal and is fast and easy. Another screening procedure for assessment of neuromotor function requires a rodent grasp a bar attached to a strain gauge after which the animal is pulled on manually until it lets go. Assessment of swimming ability is also suitable for incorporation into screening tests. The rodent is placed in a pool of water and such measures as swimming movement, position in the water, and ability to keep the head above water are assessed. These analyses may reveal motor deficits that are not apparent during locomotion on land.

Little work has been focused on more sophisticated tests for assessing neuromuscular function. One promising procedure employs operant techniques to train an animal to depress a lever within a specific force band and time period, thus allowing assessment of the effects of toxic agents on fine motor control or strength.

The test that probably is used most extensively in screening for nervous system toxicity is locomotor activity, in large part because no training is required and activity can be measured rapidly. Locomotor activity represents the functional output of many systems of the body, including but certainly not exclusively motor systems. In addition, although such measurements may appear straightforward, there are many variables that must be considered. Motor activity is not a single activity but consists of many acts, such as horizontal and vertical movement, sniffing, rearing, grooming, and scratching. With some types of measuring devices, even tremor may be monitored. There are, therefore, many methods of monitoring, including scoring the classes of movement by observation, measuring horizontal movement only, measuring vertical displacement with devices that gauge force generated against the floor, and combinations of these measurements. Even within a class of automated devices, there is large variability in the configuration of each apparatus and in the method of measurement. With different kinds of apparatuses, different behaviors can be measured.

When a toxicant is introduced, activity may increase, decrease, or remain unchanged depending on choice of apparatus, age of the animal, the relative novelty and complexity of the environment, and many other variables. Although a change in an animal's activity as a result of its exposure to a toxicant indicates a change in the function of its nervous system, interpretation is not straightforward. The change can be due to the toxicant's primary effect on nervous system function or to its effect on some other system that results in a secondary effect on nervous system function. Certainly, extrapolation from activity measurements in rodents to such phenomena as 'hyperactivity' in children is unwarranted,

both because of lack of consistency in the experimental work and because such syndromes in humans do not consist exclusively, or necessarily, of increases in motor activity.

Sensory Function Testing

Sensory disturbances often result from human exposure to toxic agents, both as vague symptoms reported by the patient and as clearly demonstrable deficits in sensory function. Deficits in visual, auditory, and tactile functions have been reported for a variety of toxicants, including metals (methylmercury and lead), acrylamide, solvents, and pesticides. A variety of techniques, from very simple to extremely sophisticated, have been utilized to assess sensory function in animals exposed to toxicants. Probably the grossest of these is the orienting response, which consists of observing whether the animal turns toward a crude stimulus (e.g., click, light, or touch). Such a procedure is subjective, nonspecific, and insensitive and indicates only the possibility of gross sensory impairment. The auditory startle reflex and discrimination learning tests are often viewed as tests of sensory function. However, there are many other systems involved in these tests; therefore, sensory effects may not be discriminable from motor effects, learning and memory, and attention abilities.

An extremely promising technique for sensory system evaluation is modulation of reflex startle by presentation of a low-intensity stimulus immediately prior to a high-intensity stimulus that elicits the startle response. Such a technique may be used to estimate sensory threshold, and sensory deficits may be differentiated from nonsensory, such as motor, deficits. This technique is, therefore, specific and reasonably sensitive. It has the advantage of being inexpensive and rapid and requires no training of the animal.

Operant training of an animal allows a very detailed evaluation of sensory function. Such techniques are time-consuming and sometimes expensive, but they are useful for careful characterization of toxicant effects for which there is good evidence of sensory impairment. The species chosen for testing must have sensory function as similar to humans as possible. For visual system testing, for example, the rodent is usually not an appropriate model because its visual system differs in fundamental ways from that of humans.

Animals can be trained to report reliably and in great detail about their sensory perception. This is accomplished through 'psychophysical' techniques; that is, sensory function is determined by behavioral means. Such methodology is appropriate for determination of no-effect levels and for detailed characterization of toxic effects. Conditioned suppression is a useful technique for estimating sensory thresholds. A steady baseline rate of responding (such as a lever pressing or licking) is established by use of an intermittent schedule of reinforcement. A test stimulus is presented to an animal several times during its ongoing behavior and signals a specific latency (usually 2 or 3 min) to an unavoidable electric shock. The animal decreases its rate of response (suppresses) during the stimulus in anticipation of the shock, which indicates that the animal detects the stimulus. This technique can be employed to estimate threshold and to detect changes in threshold produced by a toxicant.

Stebbins characterized the thresholds for detection of sound over the range of frequencies normally detectable in the monkey and the effect of an ototoxic agent on these thresholds. This was done by training the monkey to keep its hand in contact with a sensor until it detected the onset of a tone and to break its contact upon detection of the tone. Intensity of the tone was then varied for each frequency tested to determine the intensity at which the monkey was unable to detect the tone. Stebbins was thus able to follow the development of hearing loss produced by an ototoxic antibiotic, from initial high-frequency loss to later low-frequency loss. These changes in hearing in the monkey were correlated with the pattern of receptor loss in the inner ear.

A psychophysical procedure was also used to determine the spatial visual function of monkeys exposed chronically to methylmercury but showed no overt signs of poisoning. In this experiment, the monkey faced two oscilloscopes, one blank and one displaying vertical bars. The monkey had access to two levers, one corresponding to each oscilloscope. The task was to respond on the lever corresponding to the scope on which the bars appeared. The oscilloscope displaying the bars varied randomly from trial to trial. The frequency and darkness of the bars was varied in a systematic manner, allowing a determination of the spatial visual function of each monkey. Monkeys exposed to methylmercury were found to have deficits of high-, but not low-frequency spatial vision. Similar behavioral techniques have been used to characterize visual and somatosensory impairment produced by acrylamide. Such studies demonstrate the power of operant techniques in detection of very subtle sensory deficits, which may be the only discernible effects of a toxicant at low-level exposure.

Learning and Memory Testing

Loss of memory and inability to concentrate are symptoms frequently reported as a result of human

exposure to toxicants such as polychlorinated biphenyls, solvents, methylmercury, and pesticides. Furthermore, developmental exposure may produce mental retardation or learning impairment. It is therefore of great value to test such abilities in animals as markers of toxic effect. There are many techniques available for assessment of learning and memory. Aside from gross screening procedures, this area has probably received the most attention from behavioral toxicologists. Techniques range in complexity from those appropriate for screening to characterization of specific deficits. A screening procedure that is often considered a test of learning is habituation, which is a progressive decrease in reactivity to repeated presentations of a stimulus. Reactivity can be measured in terms of response of the whole organism, as in startle or orienting, or in terms of habituation of a discrete reflexive response, such as blinking. Obviously, habituation must be differentiated from motor effects, fatigue, and sensory adaptation. It is a measure of gross integration of the nervous system and may not involve the higher centers. Often, an incremental repeated acquisition task is used to evaluate the effects of a potential toxicant on learning and a delayed matching to sample task to evaluate effects on memory.

A learned behavior that is obviously of adaptive advantage to an animal is its ability to avoid a substance that it ingested shortly before the onset of an illness or adverse effect. This conditional taste aversion can be used to measure toxicity, for example, by pairing a novel taste (a sugar treat, for example) with administration of a toxicant. If the animal feels ill soon afterward, it will avoid the novel substance in the future. This technique has proved to be sensitive to the effects of neurotoxic agents.

At the next level of sophistication, avoidance procedures (utilizing negative reinforcement) are frequently used. Passive avoidance procedures require the animal (rodent) to refrain from leaving a specific area in order to avoid a shock to the feet. Active avoidance requires the animal to move from a specific area at the onset of a cue in order not to be shocked. These procedures are greatly affected by the baseline level of arousal and ongoing motor activity of the animal. It may often be the case that a toxicant produces an effect on one and not on the other of these avoidance tests or affects the behavior in opposing ways, depending on whether the animal is more or less active than the control animal. These tests, therefore, are considered rather nonspecific.

Discrimination tasks have proved useful in detecting effects of toxicants on learning and memory. The procedure most often employed is termed a 'forced choice' because the animal is presented with two or more stimuli simultaneously and must indicate its choice by some operant response. These tasks are typically one of two types; spatial and nonspatial. With spatial discrimination, the animal must respond to a certain position (i.e., left) in order to be reinforced. A nonspatial task requires responding to a specific stimulus (pattern, color, or direction of a tone) regardless of position. Different operants may be utilized in discrimination testing. For rodents, mazes of various sorts are often employed, whereas for other species (as well as for rodents) operants besides locomotion are utilized.

Primates are often tested in a Wisconsin General Testing Apparatus. The monkey faces a panel on which stimuli are placed. A reinforcement, such as a raisin, is placed in a recessed well under the correct stimulus. The monkey's response consists of displacing one of these stimuli; if the choice is correct the reinforcement is collected. Automated apparatuses are used with all laboratory species. Typically, the response consists of pressing one of several available levers or push buttons in order to signal the choice. Levine developed a technique for rodents in which a photocell beam is interrupted with the nose as an operant. The technique requires no training by the investigator and may be used with young animals.

Discrimination tasks have proved to be sensitive to impairment resulting from exposure to lead. The difficulty of the task may have an important impact on the effects of a toxicant on performance.

Once the task is learned, a discrimination reversal paradigm provides additional information on the animal's learning ability. The previously correct stimulus becomes the incorrect one so that the animal is required to learn a response opposite from the one previously learned. The discrimination reversal paradigm may often be more sensitive to neurotoxicity than simply acquisition of discrimination tasks, as has been found in monkeys exposed to lead early in life.

There are several other means to test spatial orientation or memory that require little or no training of the animal. An apparatus appropriate for use with rodents is the radial arm maze. Typically, this maze consists of a central arena from which radiate a number of arms like spokes of a wheel. The end of each arm is baited with a reinforcement, and the animal simply has to find all the reinforcements within a certain period of time. The most economical strategy is to enter each arm only once. There obviously need not be a memory component to this task, depending on the strategy adopted by the animal (i.e., 'always turn left'). Similarly, motor impairment confounds this task because the number of

reinforcements collected in a specified time is the typical dependent variable. The neurotoxicant trimethyltin has been found to disrupt a rodent's ability to perform this test. A somewhat analogous task used for primates is the Hamilton Search Task. A row of boxes, each containing a reinforcement, is presented to the monkey. The monkey can collect the reinforcement from each box by lifting the lid; again, the most economical approach is to lift each lid only once. This test differs from the radial arm maze in that a delay is instituted between responses during which the boxes are withdrawn from the monkey's reach, thus making memory more likely a component of the performance. (It is possible to institute a delay in the radial arm maze as well, but this is most often not done.) Monkeys exposed to lead postnatally required more trials to learn to perform this task than did their controls.

There are several operant tasks that offer the opportunity to separate an animal's learning from its performance of a known task. Repeated acquisition is such a task and requires the animal to learn a new sequence of lever presses each session. The learning baseline may be more sensitive to disruption by a toxicant than the performance of an already acquired sequence.

A task that tests attention and short-term memory is matching to sample. Monkeys are most typically used for these tasks, although other species are also capable of learning them. In a nonspatial matching-to-sample task, for example, the animal is presented with a stimulus (color, pattern, or object) that is then withdrawn. Following this, a set of stimuli is presented, and the animal indicates which of these is identical in some dimension to the sample stimulus. Delays of various durations may be instituted between the presentation of the sample and test stimuli to test short-term memory. Such tasks have been found to be sensitive to effects produced by lead in monkeys who were exposed to it in early life.

Testing Using Intermittent Schedules of Reinforcement

Performance generated by intermittent schedules of reinforcement has played an important role in behavioral pharmacology and is proving a useful tool in behavioral toxicology. On an intermittent schedule, an animal is not reinforced for every response but for a number of responses according to certain 'rules'. Most intermittent schedules are based on reinforcing the organism as a function of the number of responses emitted, some temporal requirement for emission of responses, or a combination of these. For example, a fixed ratio (FR) schedule requires the animal to emit a fixed number of responses in order to be reinforced. A fixed interval (FI) schedule, on the other hand, requires that a certain fixed length of time elapse before a response is reinforced. Although only one response need be emitted at the end of the interval for reinforcement, the organism typically emits many responses during the interval. Interval schedules generally generate a lower rate of responding than do ratio schedules. The FI schedule generates a characteristic pattern of responding for which a variety of parameters may be analyzed. These parameters are potentially sensitive to disruption by psychoactive agents. Another schedule of some utility in behavioral toxicology is the differential reinforcement of low rate (DRL) schedule in which the animal is required to wait a specified time between responses in order to be reinforced.

Intermittent schedules may also be maintained by negative reinforcement, usually by a brief mild electric shock. The most popular of these is continuous or 'Sidman' avoidance in which each response postpones a shock by a fixed amount of time. By spacing its successive responses within this time interval, the animal may postpone shock indefinitely. This schedule is particularly useful as a comparison to behavior generated by positive reinforcement if a toxicant is suspected of producing anorexia. Simple intermittent schedules such as these have been used fairly widely in behavioral toxicology and have proved to be sensitive to the effects of a number of industrial and environmental toxicants.

Intermittent schedules of reinforcement can be combined to form more complicated schedules such as multiple schedules of reinforcement. For example, if FR and FI schedules are presented to an animal in succession during a single test session, the resulting multiple schedule is termed a multiple FR–FI schedule. Each component of the multiple schedule is independent and occurs in the presence of a different external discrimination stimulus that signals the schedule component in effect. Schedule components are typically presented in an alternating fashion, first one schedule and then the other; this allows the investigator to collect data on both types of behavior almost simultaneously. This schedule in particular has proved to be useful in detecting behavioral toxicity.

Multiple schedules offer the investigator an opportunity to study behavior controlled by different variables, which may be differentially sensitive to the effects of a toxicant. For example, toluene produced a decrease in test animals' response rate in the FR component and an increase in their response rate in the DRL component of a multiple schedule. Furthermore, the relative sensitivity of the two components was different. Similarly, the animals' response in the

FI component of a multiple FR–FI was sensitive to disruption by methyl γ-amyl ketone, whereas their response in the FR component was not. The FI component of the multiple FI–FR schedule was more sensitive to disruption in both monkeys and rodents who sustained developmental lead exposure.

Schedules of reinforcement may be used to monitor toxic effects other than or in addition to direct effects on the CNS. These may include peripheral nervous system toxicity or damage to some other organ systems resulting in general malaise or the animal's feeling 'sick'. For example, acrylamide, an organic solvent that produces a 'dying back' axonopathy, produced decreases in animals' FR response rate. The FR schedule typically produces high response rates and thus may be sensitive to unpaired motor function. Rats exposed to ozone decreased their responding on an FI schedule, which was interpreted as a decrease in their motivation as a result of the general discomfort produced by ozone.

Social Behavior Testing

Animals, particularly mammals, engage in a wide variety of social, sexual, and maternal (or paternal) behaviors that are multidimensional and extremely complex. Despite the obvious importance of social behavior in humans, very little research has been focused on the effects of toxicants on social interactions, and the utility of such interactions in behavioral toxicology is unknown. The reason for this may be the enormous number of variables, which necessitates focusing on only a few parameters to the exclusion of all others. Moreover, many of these behaviors are specific to certain species (e.g., grooming, pup retrieval, and submissive gestures), raising the question of the validity of extrapolation to human behavior.

Each of these components of behavior can be combined into an increasingly complex set of testing paradigms and, as such, then can be used to evaluate a distinct potential toxic event. An example of this is behavioral teratology.

Behavioral Teratology

Behavioral teratology is defined as a separate component of behavioral toxicology primarily in its focus on behavioral modifications resulting from toxic exposures during early development. In general, such studies track the outcome of such exposures over the postnatal and possibly into the juvenile and early adult stages of the life cycle. Outcome measures almost invariably include the development of physical landmarks and reflexes, and also generally include assessment of one or more behavioral functions. Often, attempts are made to evaluate multiple behavioral functions, such as motor function and activity, and sensory capabilities and learning in the same experiment. In addition, testing for species-specific behaviors, such as aggression, play, and vocalization, may be included.

Testing during infancy, postnatal, and juvenile periods of development sometimes requires modifications of procedures that are utilized with adults or even the development of new paradigms. In other cases, behavioral paradigms identical to those used in more mature subjects may be used, albeit with parametric modifications. One example of the former is a procedure that has been widely used in behavioral teratology studies as an assessment of olfactory and motor capabilities and is referred to as 'homing behavior', a behavior used by rat pups to locate the nest should it be displaced. In such a test, a rat pup, the typical experimental subject for most experimental behavioral teratology studies, is placed in the center of a rectangular apparatus in which one side contains clean bedding material and the other side contains bedding from the pup's home cage. The time taken for the pup to orient to or to reach the home cage bedding constitutes the dependent variable of interest. Since this performance depends on both olfactory capabilities and the development of appropriate motor skills, it represents a type of apical evaluation. It has demonstrated that olfactory discriminations can be learned by rat pups. Pairing aversive electric shock with a distinctive odor leads pups to avoid the odor.

See also: Analytical Toxicology; Behavioral Toxicology; Metals; Neurotoxicity; Ototoxicity; Organophosphates; Petroleum Distillates; Psychological Indices of Toxicity.

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Toxicity Testing, Carcinogenesis

Shayne C Gad

Introduction

Carcinogenicity testing in experimental animals is typically done under conditions that maximize the potential for detecting an effect (taking into account issues related to human relevance of the effect observed and the potential for artifacts at excessively high doses) by testing at high doses, with the ultimate aim of extrapolating to human risk at much lower levels. This entry examines the assumptions involved in these undertakings, reviews the aspects of design and interpretation of animal carcinogenicity studies, takes a critical look at low dose extrapolation models and methods, and presents the framework on which risk assessment is based.

The scientific ideal is to evaluate a chemical's carcinogenic potential from human data. While controlled exposure studies for carcinogenesis are not ethical, information can sometimes be obtained from epidemiology studies, particularly occupational studies involving high exposures to a limited number of chemicals. However, it is often difficult to reach definitive conclusions from such epidemiology studies, due to inaccurate exposure measures and/or confounding from other chemical exposures. Exceptions to this general rule are possible for rare cancers (such as angiosarcoma and vinyl chloride), and consistent results in cohorts exposed to the same chemical in different industries (and thus with different confounders) can support an association. In such cases, or in the absence of useful epidemiology data, controlled experimental animal bioassays provide useful information for both hazard identification and dose–response evaluation.

Carcinogenicity studies are the longest and most expensive of the extensive battery of toxicology studies required for the registration of pharmaceutical products in the United States, and in other major countries. In addition, they are often the most controversial with respect to interpretation of their results. These studies are important because, as noted by International Agency for Research on Cancer, "in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents for which there is sufficient evidence of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans."

Bioassay Design

Carcinogenicity bioassays have two primary objectives, though (as will be shown) the second is now more important and (as our understanding of carcinogenesis has increased) is increasingly crowding out the first.

The first objective is to detect possible carcinogens. Compounds are evaluated to determine if they can or cannot induce a statistically detectable increase of tumor rates over background levels, and only by happenstance is information generated which is useful in risk assessment. Most of the earlier studies had such detection as their objective. The current thought is that at least two species must be used for detection.

The second objective for a bioassay is to provide a range of dose–response information (with tumor incidence being the response) so that a risk assessment may be performed. Unlike detection, which requires only one treatment group with adequate survival times (to allow expression of tumors), dose response requires at least three treatment groups with adequate survival. The selection of dose levels for this case will be discussed later. However, given that the species is known to be responsive, only one species of animal need to be used for this objective.

To address either or both of these objectives, three major types of study designs have evolved. First type is a screening assay, such as the classical skin painting study, usually performed in mice. A single, easily detected endpoint (the formation of skin tumors) is evaluated during the course of the study. Though dose response can be evaluated in such a study (dose usually being varied by using different concentrations of test material in volatile solvent), most often detection is the objective of such a study. Though others have used different frequencies of application of test material to vary dose, there are data to suggest that this only serves to introduce an additional variable. Traditionally, both test and control groups in such a test consist of 50–100 mice of one sex (males being preferred because of their very low spontaneous tumor rate). This design is also often used in tumor initiation/promotion studies. Other screening assays include the strain A mouse lung tumor model, and transgenic mouse models that allow detection of mutagenic carcinogens with relatively short exposure periods.

The second common type of design is the original National Toxicology Program (NTP) bioassay. The announced objective of these studies was detection of moderate to strong carcinogens, although the results have also been used in attempts at risk assessment.

Both mice and rats were used in parallel studies. Each study used 50 males and 50 females at each of two dose levels (high and low) plus an equal-sized control group. The NTP has recently moved away from this design because of the recognition of its inherent limitations.

Finally, there is the standard industrial toxicology design, which uses at least two species (usually rats and mice) in groups of no fewer than 100 males and females each. Each study has three dose groups and at least one control. Frequently, additional numbers of animals are included to allow for interim terminations and histopathological evaluations. In both this and the original design, many organs and tissues are collected, processed, and examined microscopically. This design seeks to address both the detection and dose–response objectives with a moderate degree of success.

Selecting the number of animals to use for dose groups in a study requires consideration of both biological (e.g., expected survival rates and background tumor rates) and statistical factors. The prime statistical consideration is reflected in **Table 1**. It can be seen in this table that if, for example, using mice to study a compound that caused liver tumors (with a background or control incidence of 30%), 389 animals per sex per group were used, to be able to demonstrate that an incidence rate of 40% in treatment animals was significant compared to the controls at the $p = 0.05$ level.

Perhaps, the most difficult aspect of designing a good carcinogenicity study is the selection of the dose levels to be used. At the start, it is necessary to consider the first underlying assumption in the design and use of animal cancer bioassays – the need to test at the highest possible dose for the longest practical period.

The rationale behind this design is that although humans may be exposed at very low levels, detecting the resulting small increase (over background) in the incidence of tumors would require the use of an impracticably large number of test animals per group. This point is illustrated in **Table 2**, which shows, for instance, that although only 46 animals (per group) are needed to show a 10% increase over a zero background (i.e., a rarely occurring tumor type), 770 000 animals (per group) would be needed to detect a 0.1% increase above a 5% background. As the dose increases, however, the incidence of tumors (the response) will also increase until it reaches the point where a modest increase (e.g., 10%) over a reasonably small background level (e.g., 1%) could be detected using an acceptably small-sized group of test animals. There are, however, at least two real limitations to the highest dose level. First, the test rodent population must have a sufficient survival rate after receiving a lifetime (or 2 years) of regular doses to allow for meaningful statistical analysis. Typically, the survival should be at least 50% for the study design using 50 animals per sex per dose for rats at 18 months and mice at 15 months, to allow sufficient study sensitivity. Second, it is desirable for the metabolism and mechanism of action of the chemical at the highest level tested to be the same as at the low levels where human exposure would occur. Unfortunately, generally the high-dose level is selected based only on the information provided by a subchronic or range-finding study, but selection of too low a dose will make the study invalid for detection of carcinogenicity and may seriously impair the use of the results for risk assessment.

There are several solutions to this problem. One of these has been the rather simplistic approach of the INUP Bioassay Program, which is to conduct a 3 month range-finding study with sufficient dose levels to establish a level that significantly (10%) decreases the rate of body weight gain. This dose is defined as the maximum tolerated dose (MTD) and is

Table 1 Sample size required to obtain a specified sensitivity at $p < 0.05$ treatment group incidence

Background tumor incidence (%)	*p*[a]	*Required sample size*									
		Incidence rate (%)									
		0.95	*0.90*	*0.80*	*0.70*	*0.60*	*0.50*	*0.40*	*0.30*	*0.20*	*0.10*
0.30	0.90	10	12	18	31	46	102	389			
	0.50	6	6	9	12	22	32	123			
0.20	0.90	8	10	12	18	30	42	88	320		
	0.50	5	5	6	9	12	19	28	101		
0.10	0.90	6	8	10	12	17	25	33	65		
	0.50	3	3	5	6	9	11	17	31	68	
0.05	0.90	5	6	8	10	13	18	25	35	76	464
	0.50	3	3	5	6	7	9	12	19	24	147
0.01	0.90	5	5	7	8	10	13	19	27	46	114
	0.50	3	3	5	5	6	8	10	13	25	56

[a] p = power.

Table 2 Average number of animals needed to detect a significant increase in the incidence of an event (e.g., tumors and anomalies) over the background incidence (control) at several expected incidence levels using the Fisher exact probability test ($p = 0.05$)

Background incidence (%)	*Expected increase in incidence*					
	0.01	*0.1*	*1*	*3*	*5*	*10*
0	46 000 000[a]	460 000	4 600	511	164	46
0.01	46 000 000	460 000	4 600	511	164	46
0.1	47 000 000	470 000	4 700	520	168	47
1	51 000 000	510 000	5 100	570	204	51
5	77 000 000	770 000	7 700	856	304	77
10	100 000 00	1 000 000	10 000	1100	400	100
20	148 000 00	1 480 00	14 800	1644	592	148
25	160 000 000	1 600 000	16 000	1840	664	166

[a] Number of animals needed in each group – controls as well as treated.

selected as the highest dose. Two other levels, generally one-half MTD and one-quarter MTD, are selected for testing as the intermediate and low dose levels. In many early studies, only one other level was used.

The dose range-finding study is necessary in most cases, but the suppression of body weight gain is a scientifically questionable benchmark when dealing with risk assessment. Physiologic, pharmacologic, or metabolic markers generally serve as better indicators of systemic response than body weight. A series of well-defined acute and subchronic studies designed to determine the 'chronicity factor' (i.e., the factor needed to extrapolate from a subchronic study to projected doses for a chronic study) and to study onset of pathology can be more predictive for dose setting than body weight suppression.

Also, the NTP's MTD may well be at a level where the metabolic mechanisms for handling a compound at real-life exposure levels have been saturated or overwhelmed, bringing into play entirely artifactual metabolic and physiologic mechanisms.

Selection of levels for the intermediate and lower doses for a study is easy only in comparison to the selection of the high dose. If an objective of the study is to generate dose–response data, then the optimal placement of the doses below the high is such that they cover as much of the range of the dose–response curve as possible and yet still have the lowest dose at a high enough level that one can detect and quantify a response. If the objective is detection, then having too great a distance between the highest and next highest dose creates a risk to the validity of the study. If the survival in the high dose is too low, yet the next highest dose does not show nonneoplastic results (i.e., cause other than neoplastic adverse biological effects) such as to support it being a high enough dose to have detected a strong or moderate carcinogen, the entire study may have to be rejected as inadequate to address its objective. Statistical guidelines have been proposed (for setting dose levels below the high) based on response surfaces. In so doing they suggest that the lowest dose be no less than 10% of the highest.

Although it is universally agreed that the appropriate animal model for testing a chemical for carcinogenicity would be an animal whose metabolism, pharmacokinetics, and biological responses were most similar to humans, economic considerations have largely constrained practical choices to rats and mice. The use of both sexes of both species is preferred on the grounds that it provides for a greater likelihood of utilizing the more sensitive species, in the face of a lack of understanding of which species would actually be most like humans for a particular agent. Use of the mouse as a second bioassay species is both advocated and defended on these grounds and because of the economic advantages and the species' historical utilization. There are those who believe that the use of the mouse is redundant and represents a diversion of resources while yielding little additional information, citing a 'unique contribution' for mouse data in 273 bioassays of only 13.6% of the cases (i.e., 37 cases). Others question the use of the mouse based on the belief that it gives artifactual liver carcinogenesis results. In addition, in many studies where responses in mice and rats differed, further investigation found that the rat was more pharmacokinetically similar to humans. One suggestion for the interpretation of mouse bioassays is that in those cases in which there is only an increase in liver tumors in mice (or lung tumors in strain A mice) and no supporting mutagenicity findings (a situation characteristic of some classes of chemicals), the test compound should not be considered an overt carcinogen. This last aspect, however, is even more strongly focused on the strain of mouse that is used than on the use of the species itself.

The NTP currently recommends an F1 hybrid cross between two inbred strains, the C57B1/6

female and the C3H male, the results being commonly designated as the B6C3F1. This mouse was found to be very successful in a large-scale pesticide testing program in the mid-1960s. It is a hardy animal with good survival, easy to breed, disease resistant, and has been reported to have a relatively low spontaneous tumor incidence. Usually, at least 80% of the control mice are still alive at a 24 month termination.

Unfortunately, while it was originally believed that the spontaneous liver tumor incidence in male B6C3F1 mice was 13.7%, it actually appears to be closer to 32.1%. The issue of spontaneous tumor rates and their impact on the design and interpretation of studies will be discussed more fully later. Thus, use of a cross of two inbred mouse strains is also a point of controversy. A study has presented data to support the idea that inbred strains have lower degrees of variability of biological functions and tumor rates, making them more sensitive detectors and quantitators. The study also suggests that the use of a cross from two such inbred strains allows one to more readily detect tumor incidence increases. On the other hand, it has been argued that such genetically homogeneous strains do not properly reflect the diversity of metabolic functions present in the human population (particularly functions that would serve to detoxify or act as defense mechanisms).

Study length and the frequency of treatment are design aspects that must also be considered. These are aspects in which the objective of detection and dose–response definition conflict.

For the greatest confidence in a 'negative' detection result, an agent should be administered continuously for the majority of an animal's life span. Many agencies require negative results in valid, well-conducted bioassays in two different species (typically rats and mice) for a chemical to be classified as not carcinogenic. The NTP considers 2 years to be a practical treatment period in rats and mice, although the animals currently used in such studies may survive an additional 6–12 months. The purpose for this approach is to include exposure to the test chemical for a significant percentage of the animal's lifespan, while avoiding a high incidence of early deaths, or a high incidence of background tumors which compromise study sensitivity. Study lengths of 15–18 months are considered adequate for shorter lived species such as hamsters. An acceptable exposure/observation period for dogs is considered to be 7–10 years, an age equivalent to $\sim$45–60 years in humans. For dietary treatments, continuous exposure is considered desirable and practical. With other routes, practical considerations may dictate interrupted treatments. For example, inhalation treatment for 6–8 h day^{-1}, 5 days week^{-1} is the usual practice. Regimens requiring special handling of animals, such as gavage dosing or parenteral injections, are usually on a 5 days week^{-1} basis. With some compounds intermittent exposures may be required because of toxicity, although it is preferred to choose a dose that does not require intermittent exposure. Various types of recovery can occur during exposure-free periods, which may either enhance or decrease chances of carcinogenicity. In view of the objective of assessing carcinogenicity as the initial step, intermittent exposure on a 3–5 days week^{-1} basis is considered both practical and desirable for most compounds.

Following cessation of dosing or exposure, continued observation during a nontreatment period may be required before termination of the experiment. Such a period is often considered desirable because (1) induced lesions may progress to more readily observable lesions, and (2) morphologically similar but noncarcinogenic proliferative lesions that are stress-related may regress. Neoplastic or 'neoplastic-like' lesions that persist long after removal of the stimulus are considered serious consequences from the hazard viewpoint. Many expert anatomical pathologists, however, believe they are able to diagnose and determine the biological nature of tumorous lesions existing at the time of treatment without the added benefit of a treatment-free period.

In determining the length of an observation period, several factors must be considered: period of exposure, survival pattern of both treated and control animals, nature of lesions founds in animals that have already died, tissue storage and retention of the chemical, and results of other studies that would suggest induction of late-occurring tumors. The length of a treatment-free observation period can be as long as 3 months in mice and hamsters and 6 months in rats. An alternative would be to terminate the experiment or an individual treatment group on the basis of survival (e.g., at the point at which 50% of the group with the lowest survival has died).

However, arguments exist against such prolonged treatment and maintenance. These generally revolve around the relationship between age and tumor incidence. As test animals (or humans) become older, the background ('naturally occurring') incidence of tumors increases and it becomes increasingly difficult to identify a treatment effect from the background effect. An analysis of patterns of senile lesions in mice and rats was carried out, wherein the so-called principle of biological confounding is discussed: "If a particular lesion (e.g., pituitary tumor) is part of a larger syndrome induced by the treatment, it is impossible to determine whether the treatment has 'caused' that lesion."

This could lead to a situation in which any real carcinogen would be nonidentifiable. If the usual pattern of old age lesions for a given species or strain of animals includes tumors, then almost every biologically active treatment can be expected to influence the incidence of tumors in a cluster of lesions at a sufficiently high dose.

Reconsidering the basic principles of experimental design, it is clear that one should try to design bioassays so that any carcinogenesis is a clear-cut, single event, unconfounded by the occurrence of significant numbers of lesions due to other causes (such as age). One answer to this problem is the use of interim termination groups. When an evaluation of tumor incidences in an interim sacrifice sample of animals indicates that background incidence is becoming a source of confounding data, termination plans for the study can be altered to minimize the loss of power.

A number of other possible confounding factors can enter into a bioassay unless design precludes them. These include (1) cage and litter effects, which can be avoided by proper prestudy randomization of animals and rotation of cage locations; (2) vehicle (e.g., corn oil has been found to be a promoter for liver carcinogens); and (3) the use of the potential hazard route for man (e.g., dietary inclusion instead of gastric intubation).

Bioassay Interpretation

The interpretation of the results of even the best designed carcinogenesis bioassay is a complex statistical and biological problem. In addressing the statistical aspects, some biological points which have statistical implications need to be reviewed as one proceeds.

All such bioassays are evaluated by comparison of the observed results in treatment groups with those in one or more control groups. These control groups always include at least one group that is concurrent, but because of concern about variability in background tumor rates, a historical control group is also considered in at least some manner.

The underlying problem in the use of concurrent controls alone is the belief that the selected population of animals are subject both to an inordinate degree of variability in their spontaneous tumor incidence rates and that the strains maintained at separate breeding facilities are each subject to a slow but significant degree of genetic drift. The first problem raises concern that, by chance, the animals selected to be controls for any particular study with be either 'too high' or 'too low' in their tumor incidences, leading to either a false-positive or false-negative statistical test result when test animals are compared to these controls. The second problem leads to concern that, over the years, different laboratories will be using different standards (control groups) against which to compare the outcome of their tests, making any kind of relative comparison between compounds or laboratories impossible.

See also: Analytical Toxicology; Animal Models; Carcinogenesis; Carcinogen–DNA Adduct Formation and DNA Repair; Carcinogen Classification Schemes; Dose–Response Relationship; *In Vivo* Test; International Agency for Research on Cancer; Mouse Lymphoma Assay; National Toxicology Program; Risk Assessment, Human Health; Toxicity Testing, Mutagenicity.

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Toxicity Testing, Dermal

Samantha E Gad and Shayne C Gad

Virtually all man-made chemicals have the potential to contact the skin of people. In fact, many (e.g., cosmetics and shampoos) are intended to have skin contact. Also, the most common medical problems in industrial workers are skin conditions, reflecting the large extent of dermal exposure where none is intended. When a large surface area of skin is exposed to contaminated soil or water, skin absorption may be significant. It is also possible for dermal effects to arise from systemic toxicants.

Evaluation of materials for their potential to cause dermal irritation and corrosion due to acute contact has been common for industrial chemicals, cosmetics, agricultural chemicals, and consumer products since at least the 1930s (generally, pharmaceuticals are only evaluated for dermal effects if they are to be administered topically – and then by repeat exposure tests, which will not be addressed here). As with acute eye irritation tests, one of the earliest formal publications of a test method (although others were used) was that of Draize *et al*. in 1944. The methods currently used are still basically those proposed by Draize *et al*. and, to date, have changed little since 1944. These methods have more recently caught the interest of the animal welfare movement, and there are efforts under way to develop alternatives that either do not use animals or are performed in a more humane way. The data generated is somewhat difficult to extrapolate to humans; Draize type tests are also criticized because the distinction between mild and moderate irritants is difficult. The need for alternative methods of testing has become more pressing since a 1993 provision in the 6th amendment to the European Union (EU) Cosmetic Directive (93/35/EEC). This states that it will become illegal to market cosmetic products in EU countries if they contain ingredients or mixtures of ingredients that have been tested on animals, unless there are no valid alternatives to replace the animal tests.

Introduction

Among the most fundamental assessments of the safety of a product or, indeed, of any material that has the potential to be in contact with a significant number of people in our society are tests which seek to predict potential skin irritation or corrosion. Like all the other tests in what is classically called a range-finding, tier I, or acute battery, the tests used here are both among the oldest and are currently undergoing the greatest degree of scrutiny and change. Established test methods for these endpoints use several of the same animal models, but most commonly the rabbit (almost exclusively the New Zealand White) is the test subject. Other species commonly used include mice, rats, and guinea pigs. Hartley, Pirbright, or Himalayan white strains of guinea pigs of 350–400 g are considered to be good respondents. Such tests are not designed to evaluate systemic effects resulting after absorption.

Dermal Toxicity Tests

Testing is performed to evaluate the potential occurrence of two different, yet related, endpoints, irritation and sensitization. The broadest application of these is evaluation of the potential to cause skin irritation, characterized by erythema (redness) and edema (swelling). Severity of irritation is measured in terms of both the degree of these two parameters and how long they persist. Primary irritation, cutaneous sensitization, phototoxicity, and photosensitization are possible types of dermal irritation resulting from dermal application. There are three types of irritation tests, each designed to address a different concern:

1. Primary (or acute) irritation: a localized reversible dermal response resulting from a single application of, or exposure to, a chemical without the involvement of the immune system.
2. Cumulative irritation: a reversible dermal response, which results from repeated exposure to a substance (each individual exposure is not capable of causing acute primary irritation).
3. Photochemically induced irritation: a primary irritation resulting from light-induced molecular changes in the chemical to which the skin has been exposed.

Irritation is generally a localized reaction resulting from either a single exposure or multiple exposures to a physical or chemical entity at the same site. It is characterized by the presence of erythema, edema, and may or may not result in cell death. The observed signs are heat (caused by vessel dilation and the presence of large amounts of warm blood in the affected area), redness (due to capillary dilation), and pain (due to pressure on sensory nerves). The edema often observed is largely due to plasma, which

coagulates in the injured area, precipitating a fibrous network to screen off the area, thereby permitting leukocytes to destroy exogenous materials by phagocytosis. If the severity of injury is sufficient, cell death may occur, thereby negating the possibility of cellular regeneration. Necrosis is a term often used in conjunction with cell death, and it is the degeneration of the dead cell into component molecules which approach equilibrium with surrounding tissue.

There are three major objectives to be addressed by the performance of these tests:

1. Providing regulatory required baseline data: Any product now in commerce must both be labeled appropriately for shipping and be accompanied by a material safety data sheet which clearly states potential hazards associated with handling it. Department of Transportation regulations also prescribe different levels of packaging on materials found to constitute hazards as specified in the regulations. Environmental Protection Agency (EPA) (under FIFRA) also has a pesticides labeling requirement. Similar requirements exist outside the United States. These requirements demand absolute identification of severe irritants or corrosives and adherence to the basics of test methods promulgated by the regulations. False positives (type I errors) are to be avoided in these usages.
2. Hazard assessment for accidents: For most materials, dermal exposure is not intended to occur, but it will occur in cases of accidental spillage or mishandling. Here, it is important to correctly identify the hazard associated with such exposures and be equally concerned with false positives and false negatives.
3. Assessment of safety for use: The materials at issue here are the full range of products for which dermal exposure will occur in the normal course of use. These range from cosmetics and hand soaps to bleaches, laundry detergents, and paint removers. No manufacturer desires to put a product on the market, which cannot be safely used and will lead to extensive liability if placed in the marketplace. Accordingly, the desire here is to accurately predict the potential hazards in humans; that is, to have neither false positives nor false negatives.

Dermal Toxicity Test Design

Table 1 sets forth the current regulatory mandated test designs, which form the bases of all currently employed test procedures. All of these methods use the same scoring scale, the Draize scale, which is

Table 1 Primary dermal irritation test: regulatory mandated test designs for dermal irritation/corrosion

Agency	*Test material*		*Exposure time (h)*	*No. of rabbits*	*Sites per animal (intact/abraded)*	*At end of exposure*	*Occlusion*	*Scoring intervals postexposure*	*Note*
	Solid	*Liquid*							
Department of transportation	Not specified	Not specified	4	6	1/0	Skin washed w/ appropriate vehicle	Yes	4 and 48 h	Endpoint is corrosion in 2 of 6 animals
Environmental Protection Agency	Moisten	Undiluted	24	6	2/2	Skin wiped but not washed		24 and 72 h; may continue until irritation fades or is judged irreversible	Toxic Substance Control Act (TSCA); test also FIFRA
Consumer Product Safety Commission	Dissolve in appropriate vehicle	Neat	24	6	1/1	Not specified	Impervious material	24 and 72 h	Federal Hazardous Substances Act (FHSA)
OECD	Moisten	Undiluted	4	3[a]	1/0	Wash with water or solvent	Semiocclusive	30–60 min, 24, 48, 72 h or until judged irreversible	European Common Market

[a] Additional animals may be required to clarify equivocal results.

Table 2 Evaluation of skin reactions

Skin reaction	*Value*
Erythema and eschar formation	
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Necrosis (death of tissue)	+N
Eschar (sloughing or scab formation)	+E
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised ~1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4
Total possible score for primary irritation	8

presented in **Table 2**. However, though the regulations prescribe these different test methods, most laboratories actually perform somewhat modified methods. New material should first be tested *in vitro* or using animal skin for short exposure times and open application sites (without occlusion). Here, two modifications (one for irritation and the other for corrosion), which reflect prior laboratory experience are recommended.

Performing *In Vivo* Dermal Toxicity Tests

Selection of Animals and Skin Testing Sites

1. A group of at least 8–12 New Zealand White rabbits are screened for the study.
2. All rabbits selected for the study must be in good health; any rabbit exhibiting sniffles, hair loss, loose stools, or apparent weight loss are rejected and replaced.
3. One day (at least 18 h) prior to application of the test substance, each rabbit is prepared by clipping the hair from the back and sides using a small animal clipper. A size No. 10 blade is used to remove long hair and then a size No. 40 blade is used to remove the remaining hair.
4. Six animals with skin sites that are free from hyperemia or abrasion (due to shaving) are selected. Skin sites that are in the telogen phase (resting stage of hair growth) are used; those skin sites that are in the anagen phase (stage of active growth, indicated by the presence of a thick undercoat of hair) are not used.

Study Procedure

1. As many as four areas of skin, two on each side of the rabbit's back, can be utilized for sites of administration.
2. Separate animals are not required for an untreated control group. Each animal serves as its own control.
3. Besides the test substance, a positive control substance (a known skin irritant – 1% sodium laurel sulfate in distilled water) and a negative control (untreated patch) are applied to the skin. When a vehicle is used for diluting, suspending, or moistening the test substance, a vehicle control patch is required, especially if the vehicle is known to cause any toxic dermal reactions or if there is insufficient information about the dermal effects of the vehicle.
4. The intact (free of abrasion) sites of administration are assigned a code number.
   a. Test substance
   b. Negative control
   c. Positive control
   d. Vehicle control

(Note that some tests, such as those required by EPA, and CPSC, see **Table 1**, require abraded sites. For these tests an area of skin should be scratched so as to open the stratum corneum, but not produce blood.)

5. Application sites should be rotated from one animal to the next to ensure that the test substance and controls are applied to each position at least once.
6. Each test or control substance is held in place with a 1 × in., 12-ply surgical gauze patch. The gauze patch is applied to the appropriate skin site and secured with 1in.-wide strips of surgical tape at the four edges, leaving the center of the gauze patch nonoccluded.
7. If the test substance is a solid or semisolid, a 0.5 g portion is weighed and placed on the gauze patch. The test substance patch is placed on the appropriate skin site and secured. The patch is subsequently moistened with 0.5 ml of physiological saline.
8. When the test substance is in flake, granule, powder, or other particulate form, the weight of the test substance that has a volume of 0.5 ml (after compacting as much as possible without crushing or altering the individual particles, such as by tapping the measuring container) is used whenever this volume weight is less than 0.5 g. When applying powders, granules, and the like, the gauze patch designated for the test sample is secured to the appropriate skin site with one of

four strips of tape at the most ventral position of the animal. With one hand, the appropriate amount of sample measuring 0.5 ml is carefully poured from a weighing paper onto the gauze patch that is held in a horizontal (level) position with the other hand. The patch containing the test sample is then carefully placed into position onto the skin and the remaining three edges are secured with tape. The patch is subsequently moistened with 0.5 ml of physiological saline.

9. If the test substance is a liquid, a patch is applied and secured to the appropriate skin site. A 1 ml tuberculin syringe is used to measure and apply 0.5 ml of test substance to the patch.
10. The negative control site is covered with an untreated 12-ply surgical gauze patch (1×1 in.).
11. The positive control substance and vehicle control substance are applied to a gauze patch in the same manner as a liquid test substance.
12. The entire trunk of the animal is covered with an impervious material (such as saran wrap) for a 24 h period of exposure. The saran wrap is secured by wrapping several long strips of athletic adhesive tape around the trunk of the animal. The impervious material aids in maintaining the position of the patches and retards evaporation of volatile test substances.
13. An Elizabethan collar is fitted and fastened around the neck of each test animal. The collar remains in place for the 24 h exposure period. The collars are utilized to prevent removal of wrappings and patches by the animals, while allowing the animals food and water *ad libitum*.
14. The wrapping is removed at the end of the 24 h exposure period. The test substance skin site is wiped to remove any test substance still remaining. When colored test substances (such as dyes) are used, it may be necessary to wash the test substance from the test site with an appropriate solvent or vehicle (one that is suitable for the substance being tested). This is done to facilitate accurate evaluation for skin irritation.
15. Immediately after removal of the patches, each 1×1 in. test or control site is outlined with an indelible marker by dotting each of the four corners. This procedure delineates the site for identification.

Observations

1. Observations are made of the test and control skin sites 1 h after removal of the patches (25 h post-initiation of application). Erythema and edema are evaluated and scored on the basis of the designated values presented in **Table 3**.
2. Observations are again performed 48 and 72 h after application and scores are recorded.
3. If necrosis is present or the dermal reaction is unusual, the reaction should be described. Severe erythema should receive the maximum score (4), and +N should be used to designate the presence of necrosis and +E the presence of eschar.
4. When a test substance produces dermal irritation that persists 72 h postapplication, daily observations of test and control sites are continued on all animals until all irritation caused by the test substance resolves or until Day 14 postapplication.

Evaluation of Results

1. A subtotal irritation value for erythema or eschar formation is determined for each rabbit by adding the values observed at 25, 48, and 72 h postapplication.
2. A subtotal irritation value for edema formation is determined for each rabbit by adding the values observed at 25, 48, and 72 h postapplication.
3. A total irritation score is calculated for each rabbit by adding the subtotal irritation value for erythema or eschar formation to the subtotal irritation value for edema formation.
4. The primary dermal irritation index is an average calculated for the test substance or control substance by dividing the sum of the total irritation scores by the number of observations (3 days $\times$ six animals = 18 observations).
5. The categories of the Primary Dermal Irritation Index (PDII) are as follows (this categorization of the dermal irritation is a modification of the original classification described by Draize *et al.*):

PDII = 0.0 nonirritant
> 0.0–0.5 negligible irritant
> 0.5–2.0 mild irritant
> 2.0–5.0 moderate irritant
> 5.0–8.0 severe irritant

Other abnormalities, such as atonia or desquamation, should be noted and recorded.

Another way of scoring is the Primary Irritation Index (PII). This is found by taking the average of the erythema scores for both abraded and nonabraded sites and adding this to the average of the edema scores both for abraded and nonabraded sites.

Photoirritation

Phototoxicity (photoirritation) is a light induced skin response, similar to an exaggerated sunburn, which can be elicited after a single exposure to a photoactive chemical (topical application, ingestion, or

Table 3 *In vitro* dermal irritation test systems

System	*Endpoint*	*Validation data?*[a]
Excised patch of perfused skin	Swelling	No
Mouse skin organ culture	Inhibition of incorporation of [^{3}H] thymidine and [^{14}C]leucine labels	No
Mouse skin organ culture	Leakage of LDH and GOT	Yes
Testskin: cultured surrogate skin patch	Morphological evaluation (?)	No
Cultured surrogate skin patch	Cytotoxicity	No
Human epidermal keratinocytes (HEKs)	Release of labeled arachidonic acid	Yes
Human polymorphonuclear cells	Migration and histamine release	Yes (surfactants)
Fibroblasts	Acid	
HEKs	Cytotoxicity	Yes
HEKs	Cytotoxicity (MIT)	Yes
HEKs, dermal fibroblasts	Cytotoxicity	Yes
HEKs	Inflammation mediator release	No
Cultured Chinese hamster ovary (CHO) cells	Increases in β-hexosamindase levels in media	No
Cultured C_3 H10T1/2 and HEK cells	Lipid metabolism inhibition	No
Cultured cells		
BHK21/C13	Cell detachment	Yes
BHK21/C13	Growth inhibition	
Primary rat thymocytes	Increased membrane permeability	
Rat peritoneal mast cells	Inflammation mediator release	Yes (surfactant)
Hen's egg	Morphological evaluation	
Skintex; protein mixture	Protein coagulation	Yes
Structure-activity relation (SAR) model	NA[b]	Yes
SAR model	NA	No

GOT: glutamic oxaloacetic transminase; MIT: metabolic inhibition test.
[a] Evaluated by comparison of predictive accuracy (in the sense used here) for a range of compounds compared with animal testing results.
[b] NA, not available.

other). Response to photoirritants includes erythema, edema, vesculation, and pigmentation, which are usually activated by the UV portion of sunlight. Phototoxic potential is not routinely tested in many industrial, household, drug, and cosmetic chemicals that are developed and marketed each year. Photomaximization tests are needed for some chemicals. Leukoderma (depigmentation) due to chemical exposure with compounds, often phenols and or thiols, is known to occur. Also, carcinogenesis and photocarcinogenesis should now be evaluated as recommended by the US National Toxicology Program.

As results vary with species (strains), lamps, detectors, doses, distances, time (exposure chemical to exposure light) chemical routes, endpoint (biological, erythema, edema, ear thickness) a somewhat structured method has been proposed to limit variation. To apply this method, at four skin sites apply 0.05 ml test chemical alone, under radiation, opaque cover, vehicle alone, and positive control at intervals of 5 min to 24 h. Irradiate all skin sites simultaneously for up to 40 min at a distance of up to 15 cm from the source. Adjust the exposure time so that 10 J cm^{-2} UVA and 0.1 J cm^{-2} UVB using:

$$T(\text{min}) = (\text{J cm}^{-2} * 1000)/(\text{mW cm}^{-2} * 60)$$

Evaluate skin at 1, 24, and 48 h and score for erythema, edema (mild, moderate, and severe) and hyperpigmentation in humans.

Testing for Sensitizers

Skin sensitization tests assess the ability of chemicals to affect the immune system, such that a second contact causes a more severe reaction than the first. The antigen involved is presumed to be formed in the bonding of the chemical to body proteins. The antibodies that form to this ligand–protein complex give rise to an allergic reaction with subsequent exposure.

QSAR, statistical, and computational methods are used to determine the possibility that a material is a sensitizer and the potential severity of sensitization. *In vivo* methods are useful to diagnose skin disorders such as drug eruptions, contact dermatitis, immediate contact reactions (contact urticaria), and more. Allergic Contact Dermatitis (ACD) is an inflammatory skin disease, marked by a delayed skin response following skin contact with an allergic chemical. Test groups must be very large to assess this effect. To test for ACD, a test article or sample(s) must be initially exposed to the same skin site/area (induction phase). After a rest period of a week or more (others say over

2 weeks) follow with a challenge exposure of the article (test sample(s)) to a virgin skin site or area. The lesions are scored on the basis of severity and the number of animals responding. Other test methods include those in which the induction phase is conducted by intradermal injection together with Freund's adjuvant and those in which the treatments are all topical and the induction phase is accompanied by intradermal injections of Freund's adjuvant.

Factors Affecting Irritation Responses and Test Outcomes

The results of local tissue irritation tests are subject to considerable variability due to relatively small differences in test design or technique. Well and Scala arranged and reported on the best known of several intralaboratory studies to clearly establish this fact. Though the methods presented previously have proven to give reproducible results in the hands of the same technicians over a period of years and contain some internal controls (the positive and vehicle controls in the PDI) to minimize large variations in results or the occurrence of either false positives or negatives, it is still essential to be aware of those factors that may systematically alter test results. These factors are summarized as follows:

1. In general, any factor that increases absorption through the stratum corneum or mucous membrane will also increase the severity of an intrinsic response. Unless this factor mirrors potential exposure conditions, it may, in turn, adversely affect the relevance of test results.
2. The physical nature of solids must be carefully considered both before testing and in interpreting results. Shape (sharp edges), size (small particles may abrade the skin due to being rubbed back and forth under the occlusive wrap), and rigidity (stiff fibers or very hard particles will be physically irritating) of solids may all enhance an irritation response.
3. Solids frequently give different results when they are tested dry than if wetted for the test. As a general rule, solids are more irritating if moistened (referring to item 1, wetting is a factor that tends to enhance absorption). Care should also be taken regarding moistening agent – some (few) batches of US Pharmacopeia physiological saline (used to simulate sweat) have proven to be mildly irritating to the skin and mucous membrane on their own. Liquids other than water or saline should not be used.
4. If the treated region on potential human patients will be a compromised skin surface barrier (e.g., if it is cut or burned) some test animals should likewise have their application sites compromised. This procedure is based on the assumption that abraded skin is uniformly more sensitive to irritation. Experiments, however, have shown that this is not necessarily true; some materials produce more irritation on abraded skin, while others produce less.
5. The degree of occlusion (in fact, the tightness of the wrap over the test site) also alters percutaneous absorption and therefore irritation. One important quality control issue in the laboratory is achieving a reproducible degree of occlusion in dermal wrappings.
6. Both the age of the test animal and the application site (saddle of the back vs. flank) can markedly alter test outcome. Both of these factors are also operative in humans, of course, but in dermal irritation tests the objective is to remove all such sources of variability. In general, as an animal ages, sensitivity to irritation decreases. For the dermal test, the skin on the middle of the back (other than directly over the spine) tends to be thicker (and therefore less sensitive to irritations) than that on the flanks.
7. The sex of the test animals can also alter study results because both regional skin thickness and surface blood flow vary between males and females.
8. The single most important (but also most frequently overlooked) factor that influences the results and outcome of these (and, in fact, most) acute studies is the training of the staff. In determining how test materials are prepared and applied and in how results are 'read' against a subjective scale, both accuracy and precision are extremely dependent on the technicians involved. To achieve the desired results, initial training must be careful and all-inclusive. Equally as important, some form of regular refresher training must be exercised, particularly in the area of scoring of results. Use of a set of color photographic standards as a training reference tool is strongly recommended; such standards should clearly demonstrate each of the grades in the Draize dermal scale.
9. It should be recognized that the dermal irritancy test is designed with a bias to preclude false negatives and, therefore, tends to exaggerate results in relation to what would happen in humans. Findings of negligible irritancy (or even in the very low mild irritant range) should therefore be of no concern unless the product under the test is to have large-scale and prolonged dermal contact with humans.

Problems in Testing (and Their Resolutions)

Some materials, by either their physicochemical or their toxicological natures, generate difficulties in the performance and evaluation of dermal irritation tests. The most commonly encountered of these problems are due to compound volatility, pigmented material, and systemic toxicity.

Compound Volatility

It is sometimes necessary or desirable to evaluate the potential irritancy of a liquid that has a boiling point between room temperature and the body temperature of the test animal. As a result, the liquid portion of the material will evaporate off before the end of the testing period. There is no real way around the problem; it is thus important to make clear in the report on the test that the traditional test requirements were not met, though an evaluation of potential irritant hazard was probably achieved (because the liquid phase would also have evaporated from a human that it was spilled on).

Pigmented Material

Some materials are strongly colored or discolor the skin at the application site. This makes the traditional scoring process difficult or impossible. One approach is to try to remove the pigmentation with a solvent; if successful, the erythema can then be evaluated. If use of a solvent fails or is unacceptable, another possibility is to (wearing thin latex gloves) feel the skin to determine if there is warmth, swelling, and/or rigidity – all secondary indicators of the irritation response.

Systemic Toxicity

On rare occasions, the dermal irritation study is begun only to have the animals die very rapidly after test material is applied.

In Vivo Study Design Alternatives and Innovations

In vivo alternative approaches to evaluating dermal toxicity are limited to one other dose site and two other species of small animals. These are the guinea pig, mouse ear, and rabbit ear tests. Gilman has previously presented a short overview of these three alternatives, but some additional information has since become available.

Guinea Pig

The response of the guinea pig has been reported as being less severe and more like that of a human, and there have been recommendations that it be the species of choice with the test being performed in the same manner as in the PDI. FIFRA guidelines, indeed, name the guinea pig as an alternative species for the PDI test. However, the rabbit is cheaper and its larger size makes multiple patching more practical than is possible in the guinea pig.

Mouse Ear

The ear of the albino mouse has been proposed as an alternative test system. As originally proposed by the author, the test was performed as follows:

- Ten microliters (liquid) or 10 mg (solid paste) is applied to the dorsal aspect of one ear; the other ear serves as a control.
- Test material is applied topically, daily on four consecutive days.
  Dermal reactions are read on Day 5 as follows:
  0: No visible blood vessels or erythema
  2: Few blood vessels, barely visible; no erythema
  4: Main blood vessels visible on lower half of ear; slight erythema over lower third or base of ear
  6: Main blood vessels more obvious; suggestion of capillary network of tips of main vessels; slight or generalized erythema
  8: Main blood vessels extended to edge of ear; more extensive capillary network between main blood vessels; possibly internal hemorrhage; erythema more pronounced; ear may begin to fold back and lose suppleness
  10: Pronounced blood vessels and extensive capillary network evident; marked erythema; possibly 'frilling' of ear margin
  12: Pronounced blood vessels and extensive capillary network extending to ear margins; severe erythema; frilling and thickening of ear margins; crusting more in evidence
  14: Pronounced blood vessels and severe erythema; obvious thickening of ear; possibly necroses; crusting may extend over whole ear surface.
- Daily differences between control and treated ears for each animal are added. A correction is given for any difference between the control and treated ears initially, divided by 5 and interpreted as follows:
  0–9: Probably not irritating to human skin
  10–15: May be slightly irritating to some users
  Over 15: Likely to prove sufficiently irritating to elicit user complaints at unacceptable levels.

Patrick utilized the mouse ear model in 1985 to evaluate dermal irritants and try to distinguish mechanisms behind irritation. Gad published a paper in

1986 in which a new method for evaluating dermal sensitization was described, but in doing so, they also presented a substantial amount of dermal irritation data arising from a mouse ear model.

Rabbit Ear

Over the years, several people have proposed a dermal irritation evaluation model based on the test material being applied to the inside surface of the rabbit ear. The advantages are that this site does not have to be shaved and the results may not over predict the toxicity as much. Seemingly no formal evaluation of a method based on this site has been performed and published.

The reader should also be aware that there are a variety of cumulative irritancy test designs available, such as the guinea pig immersion test and the 21-consecutive-day occluded patch test in rabbits.

In Vitro Alternatives

The state of development of alternative models for dermal irritation or corrosion is improving rapidly. As was noted previously, though there have been attempts to utilize other animals as models, these have not been well received nor widely adopted – nor do they seem to offer better results. Examples of *in vitro* alternatives are provided in **Table 3**. These and other such alternatives can be divided into a number of categories as can be seen in the following discussion.

Physicochemical Test Methods

Analysis of the physicochemical properties of test substances, including the pH, absorption spectra, partition coefficients, and other parameters, often can be used to assess potential dermal toxicity. According to OECD guidelines, substances with a pH <2 or >11 do not need to be tested for irritancy *in vivo*. The potential effects of acids and bases to produce irritancy have been well established.

Physicochemical analysis has evaluated the particular chemical properties of test substances, which have been identified as key structural components contributing to penetration, irritation, or sensitization. Absence of absorption in the ultraviolet (UV) range also has been used to suggest lack of photoirritant potential. Physicochemical tests are rapid, cost-effective, easily standardized, and transferable to outside laboratories.

Physiologically based pharmacokinetic modeling (PB-PK) accurately describes nonlinear biochemical and physical processes; computer hardware and software based on physiological and pharmacokinetic principles can be used for extrapolation between *in vivo* exposure conditions, doses and species.

For penetration, a partition coefficient of the test sample provides a useful guide. The size of a chemical is also indicative of potential penetration. Many of the physicochemical properties of surfactants have been found to be potential indicators of their action on skin.

Target Macromolecular and Biochemical Systems

Test methods which utilize the analysis of biochemical reactions or changes in organized macromolecules can be used to evaluate toxicity at a subcellular level. Because of their simplicity, they can be readily standardized and transferred to other laboratories to provide yardstick measurements for varying degrees of dermal toxicity.

One *in vitro* irritation prediction method that utilizes nonbiological, nonliving substances can be described as a biomembrane barrier–macromolecular matrix system. This method is known as the Skintex system. The Skintex system makes use of a two-compartment physicochemical model incorporating a keratin/collagen membrane barrier and an ordered macromolecular matrix. The effect of irritants on this membrane is detected by changes in the intact barrier membrane through the use of an indicator dye attached to the membrane. The dye is released following membrane alteration or disruption, which can occur when the synthetic membrane is exposed to an irritant. A specific amount of dye corresponding to the degree of irritation can be liberated and quantified spectrophotometrically. The second compartment within the system is a reagent macromolecular matrix that responds to toxic substances by producing turbidity. This second response provides an internal detection for materials, which disrupt organized protein conformation after passing through the membrane barrier.

Test samples can be applied directly to the barrier membrane as liquids, solids, or emulsions and inserted into the liquid reagent. The results are directly compared to the Draize dermal irritation results.

More that 5300 test samples have been studied in the Skintex system, including petrochemicals, agrochemicals, household products, and cosmetics. The reproducibility with standard deviations of 5–8% is excellent. New protocols applicable to very low irritation test samples and alkaline products have increased the applicability of this method. Skintex validation studies resulting in an 80–90% correlation to the Draize scoring have been reported by S.C. Johnson & Son and the Food and Drug and Safety Center.

Thus far, most *in vitro* irritation methods, including Skintex, have relied heavily on the vast Draize rabbit skin database for validation. As previously discussed, the discrepancies in the information generated by the Draize system raise questions about the applicability of this information to irritation reactions in man.

A new Skintex protocol called the 'human response assay' optimizes the model to predict human irritation. A collaborative study with Dr. Howard Maibach and co-workers at the University of California at San Francisco demonstrated good correlations to human response for pure chemicals with diverse mechanisms of dermal toxicity. Ongoing studies have evaluated pure chemicals, surfactants, vehicles, and fatty acids.

The Skintex test is a rapid, standardized approach with well-refined protocols and an extensive database. The results produced are contiguous with the historical *in vivo* database. However, the method cannot predict immune response, penetration, or recovery after the toxic response.

Cell Culture Techniques

In vitro cytotoxicity tests that indicate basic cell toxicity by measuring parameters such as cell viability, proliferation, membrane damage, DNA synthesis, or metabolic effects have been used as indicators of dermal toxicity.

The most commonly used approaches are the neutral red assay (cell viability and membrane damage), the Lowry (labeled proline), Coomassie blue, and Kenacid blue assays (cell proliferation and total cell protein), the MTT or tetrazolium assay (mitochondrial function), and the intracellular lactate dehydrogenase activity test (cell lysis).

In the neutral red (cell viability) and total protein (cell proliferation) assays, cells are treated with various concentrations of a test substance in petri or multiwell dishes; after a period of exposure, the substance is washed out of the medium. (An analytical reagent is added in the case of protein measurements.) Neutral red is a supravital dye, which accumulates in the lysosomes of viable, uninjured cells, and it can be washed out of cells, which have been damaged. In the protein test, Kenacid blue is added and reacts with cellular protein. Controlled cells are dark blue; killed cells are lighter colored. The IC_{50} (the concentration which inhibits by 50%) is determined; the test can be rapidly performed with automation. However, materials must be solubilized into the aqueous cell media for analysis. For many test materials this will require large dilutions which eliminate properties of the materials which cause irritation.

The MTT test assays mitochondrial function by measuring reduction of the yellow MTT tetrazolium salt to a blue insoluble product. It has been compared with the neutral red technique for testing the cytotoxicity of 28 test substances, including drugs, pesticides, caffeine, and ascorbic acid. With the mouse BALB/c 3T3 fibroblast cell line, for any given cell density the two assays ranked the test substances with a correlation coefficient of 0.939 on the basis of IC_{50} concentrations. The two assays did differ in sensitivity for a few test agents, suggesting that a combination of the two might be most effective.

Some cytotoxicity tests are likely to underestimate the toxicity of chemicals, which are metabolically activated in the body, but this problem can be overcome by the addition of liver enzymes, preferably from a human source to eliminate species differences.

Inhibition of mitogen-stimulated thymidine incorporation in human peripheral blood mononuclear cells has been reported as a method for screening for photosensitizers. Cells from at least three volunteers were used for testing each chemical.

Microorganism Studies

An important method using fungi is Daniels' test for phototoxicity, which utilizes the yeast *Candida albicans* as the test organism. A 1988 study compared favorably the results of this test with the results of photo-patch testing in volunteers for samples from six furocoumarin-containing plants. Many test materials which produce an erythemic response in the photoirritant test are not analyzed as positive in this test. A new test method, Solatex-pi, has demonstrated capability to predict the potential for photoirritation of materials in this class as well as that of other well-known photoirritants. Solatex-pi utilizes the two compartment physicochemical model of Skintex to predict the interactive effects of specific chemicals and UV radiation. Solatex-pi is being validated by Frame and the BGA (Zebet) as an *in vitro* test to predict photoirritants.

Human Tissue Equivalents

Human skin equivalents have been developed by several laboratories. One equivalent, Testskin, consists of human keratinocytes seeded onto a collagen base or collagen-glycosaminoglycan matrix containing human fibroblasts. In many respects, the epidermis which develops resembles epidermis *in vivo*. The tissue culture system survives for several weeks and may be useful in studying skin penetration. Testskin is a commercially produced skin equivalent system marketed by Organogenesis, Inc. (Cambridge, MA);

it is currently being assessed for use in skin penetration studies. Several companies launched studies of Testskin in 1990 and 1991.

Marrow-Tech, Inc. (Elmsford, NY) has also developed a human skin model. Marrow-Tech's skin equivalent consists of (1) a dermal layer of fibroblasts and naturally secreted collagen and (2) an epidermal layer of keratinocytes separated by a dermal–epidermal junction. Whereas Testskin uses bovine collagen, Marrow-Tech's skin model consists solely of human tissue.

Submerged skin co-cultures consisting of NHEK/DF cultured human skin models (neonatal foreskin, fibroblasts, and keratinocytes are grown on a 3-D nylon mesh substrate) are used in a battery of short term cytotoxicity endpoints for prediction of human skin responses to irritants *in vitro*. Several variations with many different endpoints have been developed using human skin models. These methods include, but are not limited to: NHEK/DF, using dermal fibroblasts; NHEK/NR, using neutral red in the cell viability method described previously; and NHEK/SC, which yields more accurate results when the test material is an acid, base, insoluble or neat (EpiDerm is an example of a NHEK/SC model).

All of these skin equivalent methods permit higher concentrations of test samples to be studied. However, dilutions are still necessary when, based on the physical chemistry of the test sample, the chemical structure may be responsible for irritation. Many protocols and endpoints have been evaluated as predictive of eye or skin irritation.

Isolated Tissue Methods

Skin tissues isolated from rats, rabbits, and humans have been monitored *in vitro* to predict penetration and irritation. The rat epidermal slice technique has been validated as a screen for corrosive substances. The electrical impedance changes as the integrity of the stratum corneum is altered. The use of this technique to predict irritancy is being investigated in the United Kingdom. Another method studies enzyme changes when a substance is applied to a slice whose lower surface is bathed in culture medium. Enzyme changes separate irritant and nonirritant chemicals.

Human cadaver skin has also been studied *in vitro*. Human skin shows a higher threshold of sensitivity than does rat skin. The excised or full-thickness slices are also studied in Fran 2 diffusion chambers to evaluate the diffusion or absorption characteristics of test materials. Changes in the amount of a test material at different times and different depths are monitored and are very useful in predicting penetration rates for simple solutions and solvents.

Human Volunteer Studies

Human volunteer studies are widely used to assess skin irritation, penetration, and sensitization. Much knowledge in the dermal toxicology field was previously obtained using human test panels. An advisory committee of the National Academy of Sciences in 1997 outlined a procedure for a human 24 h patch test. This procedure details use of a normal nondiseased skin area at the intrascapular region on the back (up to 10 sites) or the dorsal surface of the upper arm (up to four sites each arm). Using reference material, the procedure is to first test for 0.5–1 h without an occlusive patch. Next, for 4 h, using 1 in. gauze squares with the same amount of test material as in Draize type animal tests, express the dose in $mg\,cm^{-2}$. If using different materials and sizes of occlusive patches, different amounts of test substances are needed. Secure the patch with surgical tape, do not wrap. After a given amount of time, remove the patch, rinse the area with water and mark the test sites. Evaluate after 30 min to 1 h and at 24 h rate using the Draize scale.

Many industries regularly conduct repeat insult patch tests on human volunteers to evaluate topical irritancy. Groups of human volunteers are patched with test substance. One to five concentrations can be tested simultaneously, a wide enough range to yield results relevant to the usage. Cumulative skin irritancy is measured by applying patch applications each day for 3 weeks. Skin irritation is usually assessed visually, but blood flow and skin temperature can be measured objectively by laser Doppler flowmetry, ultrasound Doppler, heat flow disk measurement, sensitive thermocouple devices, or noncontact infrared radiative techniques. In these tests, dose–response curves can be obtained. Skin thickness can be measured with calipers as a measure of edema formation.

Human volunteers are also used in many industries in tests for allergic sensitization by cosmetic substances and formulations. The repeat insult patch test includes an induction phase (repeat applications during 3 weeks) and a 2 week rest period (incubation phase), followed by a challenge to see if sensitization has occurred. A pilot study of 20 human volunteers can be followed by more extensive testing (80–100 subjects). Positive results at more than the 10% level in the human volunteers would suggest a major problem with the formulation. User tests with the sensitized individuals and nonreactive matched control subjects can often determine the importance

of these results to end use. Such a procedure may determine whether the sensitivity is significant under normal conditions of product use. Broader tests can be carried out with 250–500 subjects.

Conclusions

Whole animal tests represent true physiological and metabolic relationships of macromolecules, cells, tissues, and organs and can be used to evaluate the reversibility of toxic effects. However, these tests are costly, time consuming, insensitive, difficult to standardize, and are sometimes poorly predictive of human *in vivo* response.

New *in vitro* test methods target the behavior of macromolecules, cells, tissues, and organs in well defined methods, which control experimental conditions and standardize experimentation. These tests provide more reproducible, rapid, and cost-effective results. In addition, more information at a basic mechanistic level can be obtained from these tests. **Table 3** provides a summary of current test systems.

The challenge of the twenty-first century will be to understand the capabilities and limitations of these methods. Combining information on new molecules obtained from structure–activity relationships with results on macromolecular alterations in Skintex that occur for undiluted molecules may provide more information on dermal toxic effects of particular chemical classes. Combining test methods can provide a greater understanding of the mechanisms of dermal toxicity. Test batteries evaluating cell cytotoxic responses at high dilutions and changes in macromolecules at low dilutions will be more informative than visual scoring of complex events *in vivo*.

See also: Analytical Toxicology; Animal Models; Eye Irritancy Testing; Hypersensitivity, Delayed Type; Organophosphates; Photoallergens; Poisoning Emergencies in Humans; Radiation Toxicology, Ionizing and Nonionizing; Skin; Tissue Repair; Toxicity Testing, Alternatives.

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Toxicity Testing, Developmental

Rochelle W Tyl

Introduction

Human concern with birth defects is as ancient as human awareness. Through the nineteenth century, the prevailing view was that; 'maternal impressions', maternal experience during the pregnancy, directly affected the newborn. Teratology, the study of monsters (terata), was essentially an observational 'art' with perceived supernatural implications. The development of basic concepts of genetics early in the twentieth century provided a scientific basis for causation of congenital defects. The recognition that environmental insult also produced birth defects in mammals inexorably followed, such as ionizing radiation (1907), sex hormones (1917), dietary deficiencies (1933), and chemicals (1948). The supposed safety of the human conceptus was refuted by German measles (rubella) epidemics in Australia in 1941 and in the United States in 1964 that resulted in thousands of children born with cataracts, deafness, and congenital heart disease from infected pregnant mothers.

The thalidomide 'epidemic' in the late 1950s and early 1960s, involving at least 8000 malformed children in 28 countries, confirmed the vulnerability of the human conceptus to environmental insult, especially in the first trimester of pregnancy. It also precipitated worldwide concern for the safety of the unborn and the role of governments to ensure testing of drugs and other chemicals in pregnant mammals.

The early term for the study of birth defects, teratology, has been supplanted by a more general term, developmental toxicology, to enable inclusion of a more diverse spectrum of adverse developmental outcomes (which may be separate and distinct in

etiology or the result of a continuum of response) and to make overt the recognition that specific results of insult in one species may not be the same in other species, including humans.

Developmental toxicity may be currently defined as any structural or functional alteration, caused by environmental insult, which interferes with normal growth, differentiation, development, and/or behavior. The targets for such insult(s) include the fertilized egg or zygote prior to implantation and the establishment of the three primary germ layers – the embryo during the period of major organ formation (i.e., organogenesis), the fetus in the postembryonic period of histogenesis, and the neonate or postnatal offspring, occurring or expressed through the postnatal period until sexual maturity. The expressions of developmental toxicity encompass death, frank structural malformations, functional defects, and/or developmental delays.

Factors Affecting the Vulnerability of the Conceptus

The vulnerability of the conceptus is viewed as due to qualitative or quantitative characteristics of both structure and function: (1) it is composed of a small number of rapidly dividing undifferentiated cells with absent or limited metabolic capabilities to alter or detoxify xenobiotics, repair lesions, etc.; (2) there is a necessity for precise temporal and spatial localization of specific cell numbers and types, as well as specific cell products, for normal differentiation, including programmed cell death; (3) sensitivities of certain cell types to certain insults may be unique to specific periods of cell movement, induction, or differentiation (i.e., transient vulnerability during the period of formation of tissues or organs); and (4) the immunosurveillance system (to provide recognition of 'self' and detection of xenobiotics or lesions) is absent or immature in the prenatal or perinatal individual.

A number of factors influence the teratogenic response. Genetic susceptibility varies among species. For example, aspirin is teratogenic in rodents but not in primates, imipramine is teratogenic in rabbits but not in humans, and thalidomide is teratogenic in primates but not in rodents. Differences also exist among strains. Inbred mouse strains differ radically in their response to many teratogenic agents, e.g., to cortisone induction of cleft palate and cadmium-induced testicular and embryotoxicity. Individuals also vary in their response to teratogenic agents in outbred strains and heterogeneous human populations. The current interpretation is that teratogens act on a susceptible genetic locus or loci which may control disposition of the agent including absorption, metabolism, transport, excretion and/or direct susceptibility of the target tissue or organ, or on genes which control the activation/inactivation of other genes. The teratogen therefore increases the incidence of previously existing malformations; its action must be viewed against the 'background noise' of spontaneous malformation rates, which also vary among species, strains, and individuals. For example, the phocomelic (seal limb) syndrome, induced by thalidomide, occurs at a low rate spontaneously in human populations; $\sim$20–80% of the human fetuses, exposed to the 'appropriate' dose of thalidomide at the 'appropriate' time, developed the malformations.

There is some specificity of agent on the teratological response, with acetazolamide causing perhaps the most specific lesion – right forelimb postaxial ectrodactyly (fourth and fifth digits); at higher doses, other structures are affected. However, there are almost always effects on other systems derived in many cases from different primary embryonic germ layers. The gestational stage of the embryo or fetus at the time of environmental insult appears to be the most critical determining factor. The predifferentiation period, from fertilization to establishment of the three primary embryonic germ layers, has been considered refractory to teratogenic agents (although there are some exceptions such as hypoxia, hypothermia, actinomycin D, and ethylene oxide). This resistance has been explained as due to the small, omnipotent cell population of the pre- and immediately postimplantation embryo. Cell damage or death is either corrected for by the surviving cells, which regulate to produce a normal albeit small term fetus, or the cell loss is so devastating that the embryo dies. Once implantation and establishment of the primary germ layers have occurred, the major period of organogenesis begins – a period of $\sim$10 days in rodents and 58 days in humans. This is the period of maximal susceptibility to teratogenic agents causing structural anomalies. Even within the organogenic period, there are differential susceptibilities of embryonic organ systems to teratogenic agents. For example, administration of an agent on gestational day (gd) 10 in the rat affects eye, brain, heart, and anterior axial skeletal development. The same agent, at the same dose, administered on gd 15, affects palate, urogenital, and posterior axial skeletal system development. These times of specific sensitivity need not correspond to the morphological appearance of the organ or organ system but rather to the time of cell biochemical commitment: the shift of cells from presumptive to determined status.

Once histogenesis has begun, defined as the differentiation of tissue-specific biochemical and morphological characteristics, the conceptus is termed a fetus and is viewed as increasingly refractory to teratogenic agents. However, this is true only of most morphological or structural manifestations. Increasing evidence indicates susceptibility of the fetus to agents causing functional deficits that presumably have a biochemical or microstructural basis. Those systems not yet complete, especially the nervous system, are most vulnerable. For example, vitamin A, lead, and methylmercury cause neurofunctional lesions when administered during this period. In addition, chemicals such as diethylstilbestrol and ethylnitrosourea act during this period to produce a system-specific tumor after a long latency in the postnatal mature animal. However, the only exposure and therefore the initiation of the later carcinogenic event occurs *in utero*, and these agents are therefore called transplacental carcinogens.

The route and duration of administration of the agent are also critical for the development of the teratogenic anomaly. Human industrial exposure is almost always by inhalation or percutaneous absorption of fumes, dusts, aerosols, or vapors. Consumer or other end-use or accidental exposure would be by more varied routes. Experimental evaluations are most useful if they duplicate the human route of exposure for experimental animal models. First-pass organ absorption and metabolism may differ if the exposure is by inhalation to the lung or orally to digestive system and liver, although subsequent transport and organ exposure may yield equivalent metabolite patterns. Most teratology studies usually employ administration of the test compound in the feed, by oral intubation, or by injection into the pregnant animal.

Timing is important. Experimental exposure before implantation or during early organogenesis may result in interference with implantation or in early embryonic death, resulting in no term fetuses to examine. Exposure before peak susceptibility or repeated exposure may induce activating and/or detoxifying enzymes in dam, placenta, and/or fetus. This may result in increased or decreased blood levels of the active metabolite in the dam and, therefore, altered exposure to the fetus. Conversely, these enzymes may be inhibited by accumulation of metabolite(s), again altering blood levels of parent compound and metabolite(s). Other effects of repeated or early exposure may be to alter liver or kidney function, for example, as well as to induce pathological changes in these organs that will affect quantity and quality of compound reaching the fetus. Saturation of protein-binding sites may also occur in the dam to alter transport of essential nutrients, vitamins, elements, hormones, etc. All of these effects may alter disposition parameters and obscure or change any teratological effects of the agent being examined.

Dose range and schedule are also critical. Three or four dose levels are usually employed: A high dose, which is toxic to the maternal organism, perhaps lethal up to 10% of dams, is used essentially to obtain an effect and to establish target organ(s); mid-dose(s), which is embryotoxic or embryolethal and possibly teratogenic; and a low dose which is comparable on a body weight basis to possible human exposure levels or small multiples thereof.

Categories of Teratogenic Agents

Many substances are known to cause malformations in one or more species of mammals. Almost all known human teratogens are drugs, with data generated by drug research companies adhering to US FDA guidelines for reproductive testing of drugs, and there is an awareness that in our drug-permissive society, women consume an average of four drugs, both by prescription and over-the-counter administration, during pregnancy. Various texts and tests have identified from 600 to 1200 drugs as teratogens in animals, only 20 of which are currently documented as human teratogens.

Human teratogenic agents have been discovered initially from anecdotal observations and then more rigorously examined in epidemiological studies and confirmed with animal studies, or they have initially been identified in animal studies with subsequent confirmation by human data. Animal model researchers have suggested that any agent positive in two or more mammalian species must be considered a suspect human teratogen.

Approximately 7% of all live-born humans bear birth defects. This value may be as high as 10% if children are evaluated to age 10 years to include subtle structural or functional deficits such as minimal brain dysfunction or attention deficit disorders. More than 560 000 lives out of $\sim$3 million births per year in the United States are lost through infant death, spontaneous abortion, stillbirths, and miscarriage due presumably to defective fetal development. The relative contributions to human teratogenesis have been estimated as follows: known germinal mutations, 20%; chromosomal and gene aberrations, 3–5%; environmental causes such as radiation, $<$1%; infections, 2% or 3%; maternal metabolic imbalance, 1% or 2%; drugs and environmental chemicals, 4% or 5%; contributions from maternal dietary deficiencies or excesses and

combinations or interactions of drugs and environmental chemicals are unknown. The contribution from unknown sources is 65–70%. The estimated 20–25% pregnancy loss due to chromosomal aberrations may be even higher due to early losses currently diagnosed as late menstrual bleeding. Recovered tissues from spontaneous abortions prior to the thirteenth week of gestation exhibit chromosomal anomalies on the order of 560 per 1000 abortions; the value at term is 5 per 1000. Of the children born alive who subsequently die in the first year of life, ~20% of the deaths are associated with or caused by birth defects, more than any other single factor.

One almost plaintive maxim, sometimes termed Karnofsky's law, states that almost any substance may be teratogenic if given in appropriate dose regimens to a genetically susceptible organism at a susceptible stage or stages of embryonic or fetal development.

Government Regulation

Soon after the worldwide thalidomide disaster in 1966, governmental regulation of the evaluation of test agents for developmental toxicity by formal testing guidelines and rules began when the US FDA established *Guidelines for Reproductive Studies for Safety Evaluation of Drugs for Human Use*. These guidelines were promulgated 'as a routine screen for the appraisal of safety of new drugs for use during pregnancy and in women of childbearing potential'. Three phases or segments were proposed: Segment I, Study of Fertility and General Reproductive Performance, provides information on breeding, fertility, nidation, parturition (birth), neonatal effects, and lactation (see Reproductive Toxicity); Segment II, Teratological Study, provides information on embryotoxicity and teratogenicity; and Segment III, Perinatal and Postnatal Study, provides information on late fetal development, labor and delivery, neonatal viability, and growth and lactation (**Figure 1**).

Segment II testing guidelines are currently followed by US FDA (since 1966), US EPA, Toxic Substances Control Act (TSCA) (since 1985), and Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (since 1982). The US EPA recently revised their reproductive and developmental toxicity testing guidelines (US EPA, OPPTS (Office of Prevention, Pesticides and Toxic Substances), 1998). International regulations also followed suit: Organization for Economic Cooperation and Development (OECD, 1981), Great Britain (1974), Japan (1984), and the European Community (1994). The International Conference on Harmonisation (ICH) also promulgated modified guidelines in 1994, adopted by the European Community, Japan, and the United States (FDA) in 1996.

Considerations for Segment II Studies

The test animals are usually rodents and nonrodents. The rodent of choice is usually the rat and, less often, the mouse. Both species satisfy the need for a small mammalian species with known (and relatively straightforward) husbandry requirements, short pregnancy, high fertility, large numbers of offspring, a low background incidence of spontaneous malformations, and a reasonably well-known embryology. The nonrodent species is usually the rabbit. The rabbit is not a rodent; mice and rats belong to the order Rodentia and rabbits to the order Lagomorpha. The requirement for the use of rabbits is predicated on the awareness that it was the only common test mammal in use in the 1960s which responded to thalidomide and (it is hoped) would have indicated the prenatal risk to humans, and on the need to distinguish between agents with specific or unique specificity (i.e., a rodent-specific teratogen) and those with more universal effects, presumably then also a greater potential risk to human development.

Prenatal development in the Rodentia and Lagomorpha differs in significant ways from that in humans. All three have a chorioallantoic placenta, but the structure differs among species. The human and rat placenta also differ functionally with different secretory patterns of placental lactogen and with the presence in primates of chorionic gonadotropin. What effect, if any, these differences have on placental transport is not fully understood. In addition, rodents and lagomorphs also form a yolk sac placenta immediately after implantation, which is the major (only) mechanism for nutrient processing and transport until gestational day (gd) 11–11.5, and persists as functional, even when the chorioallantoic placenta forms, almost to parturition. Again, what effect this has on embryo and fetal vulnerability is not yet known, although at least one rodent teratogenic agent, trypan blue, appears to act solely on the yolk sac placenta. In multifetal pregnancies, there are differences in blood flow to left and right uterine horns and to implants at ovarian versus cervical ends of the uterine horns. Different fetuses within the same dam have been shown to be at differential risk. In addition, fetal loss is handled differently in test animals: Dead implants are not expelled in a spontaneous abortion as in single-birth mammals but are resorbed *in situ*. It is not uncommon to recover healthy, viable fetuses side by side with large numbers of resorption sites. Maternal, placental, and fetal metabolism of

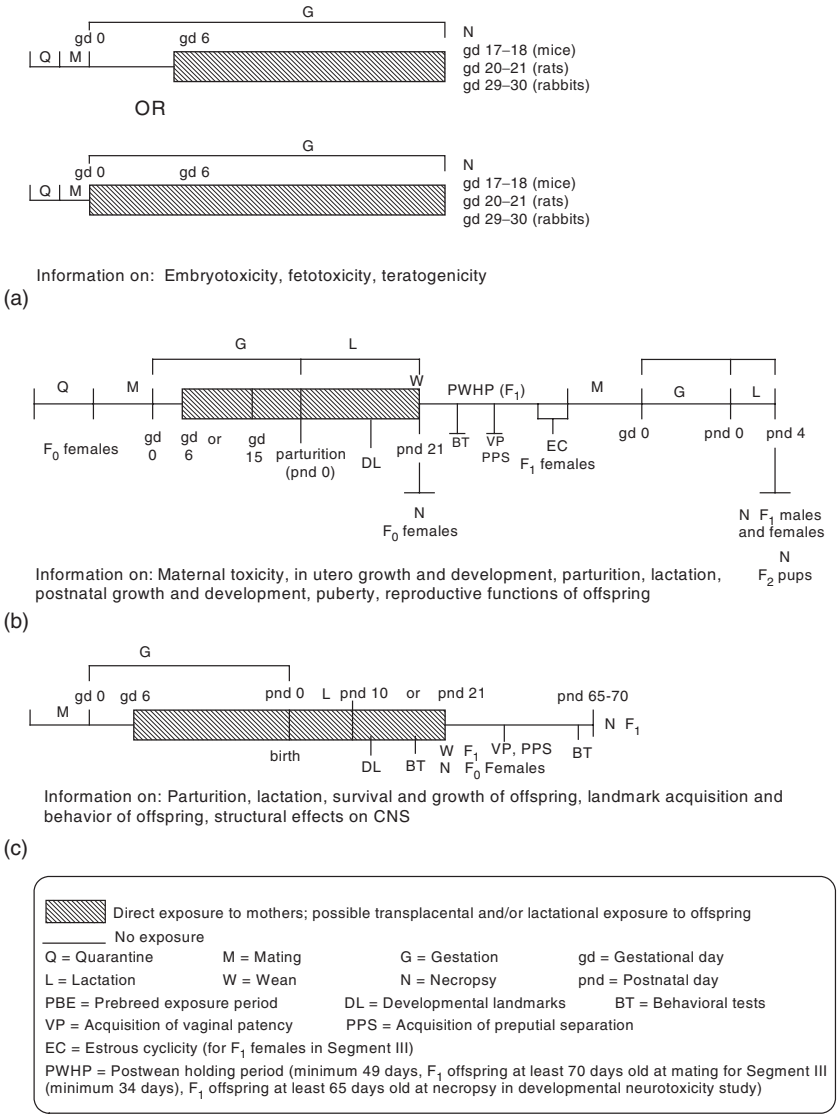

Figure 1 US governmental guidelines for study designs of developmental toxicity assessments in animal models. (a) FDA Segment II – developmental toxicity study; (b) FDA Segment III – perinatal and postnatal study; (c) EPA developmental neurotoxicity study.

xenobiotics may also differ, hence the need for prior characterization, at least, of the test organism's metabolic capabilities of the substance to be tested.

The placenta is both a transport and a metabolizing organ. Transport is accomplished by simple diffusion, facilitated diffusion, active transport across membranes, and by special processes such as pinocytosis, phagocytosis, specific transport molecules, and channels in the 'barrier'. The placenta also contains a full complement of mixed function oxidases located in the microsomal and mitochondrial subcellular fractions capable of induction and metabolism of endogenous and exogenous chemicals.

Metabolism in the test dam and/or fetus and its relevance to the pregnant human is critical. For example, the parent compound may be teratogenic and is metabolized to innocuous products as with diphenylhydantoin, an antiseizure drug used in the treatment of epilepsy. In contrast, the parent compound may be harmless and metabolized to the proximal teratogenic agent as with chlorcyclizine, an antihistamine metabolized *in vivo* to the active teratogen norchlorcyclizine. One of the current hypotheses concerning the mechanism of thalidomide-induced teratogenesis suggests that thalidomide is transmitted to the human fetus and metabolized to a more polar metabolite(s), the putative proximal teratogenic agent(s), which cannot cross the placenta back to the maternal organism for further metabolism and excretion. This sequence may be qualitatively or quantitatively different in the insensitive pregnant rodent. In contrast, imipramine, an

antidepressant, is teratogenic in rabbits in which blood levels of the parent compound stay high. In the human, imipramine is rapidly metabolized by demethylases and is not teratogenic.

Pregnancy *per se* causes many physiological changes, which may alter over the duration of the pregnancy. These changes include alterations in gastrointestinal function which may affect transport and absorption rates of chemicals in the stomach and/or intestine, and ventilatory changes which may modify pulmonary uptake, absorption, and/or elimination of chemicals with a 20–30% increase in maternal oxygen consumption and greater oxygen debt after physical activity (in humans). Changes also occur in the cardiovascular system that alter hemodynamics (there is a 30–40% increase in blood volume and a 33% decrease in erythrocytes in humans) and alter body water compartments that may influence distribution and elimination of chemicals. Plasma components with roles in chemical binding, transport, and disposition also change during pregnancy. Renal elimination is normally enhanced and hepatic metabolism may be modified during pregnancy, also affecting xenobiotic metabolism and/or elimination.

The concern for thorough evaluation of the maternal organism is based on the need to determine, when study results are interpreted, whether maternal toxicity, *per se*, is responsible for the observed embryo/fetal results. A number of fetal malformations in rodents and rabbits have been identified which are observed in the presence of maternal toxicity, regardless of the agent, route, or dose, with the clear implication that maternal toxicity is the cause of developmental toxicity, not the test agent. Mechanistic studies have also implicated the compromised status of the maternal organism as the cause for the adverse embryo/fetal outcome for many drugs. For example, elevation of endogenous corticosteroids, as a result of maternal stress irrespective of the source, or administration of exogenous corticosteroids results in cleft palate in offspring from susceptible strains of mice; hypercapnia (elevated blood CO_2) in mice has been proposed as the cause of forelimb ectrodactyly in mice exposed to acetazolamide, and bradycardia (slowed heart rate) in mice from phenytoin administration has been suggested as the cause of cleft lip/palate in offspring. Renal toxicity from mercuric chloride exposure to the mother may be the cause of hydrocephalus in the offspring. If maternal toxicity *per se*, including even 'stress' from restraint, for example, is the cause of developmental toxicity, then the classification of the test agent as a teratogen may be erroneous.

In addition, information on maternal toxicokinetics and metabolism of the test agent is essential to characterize the conditions under which toxicity to dam or conceptuses is observed; these conditions include evidence of systemic exposure, blood levels of parent compound and/or metabolite(s), identification of metabolites, bioavailability, half-life, and evidence for or against bioaccumulation. This information is necessary to extrapolate results from one route to another, from one species to another, and for human risk assessment. That is, the handling of the test agent by the test species must be characterized so that one can say that, for a specific test agent, maternal and/or developmental toxicity occurs in the presence of the parent compound or identified metabolite(s) at specific blood levels for a specified duration, with the expectation that another species that produces the same metabolite(s) at comparable levels for comparable duration will exhibit the same or similar toxicities. In the absence of metabolic information, one cannot assess whether the test animal is an appropriate surrogate for humans for specific test agents. Histopathology of target organ(s) and organ function tests may also be appropriate.

Standard Segment II Testing Protocol

In brief, the current US EPA and OECD guidelines for the Segment II study consists of exposure of a pregnant rodent species (rats or mice) and a nonrodent species (usually rabbits) to the test agent during organogenesis and fetogenesis, sacrifice of the maternal animals 1 or 2 days prior to the date of expected parturition, Caesarean delivery of the gravid uterus, and thorough evaluation of the fetuses by examination of external, visceral (including craniofacial), and skeletal structures.

The current guidelines call for at least 20 rodent and rabbit litters per group (although the previous guidelines called for 12 rabbit litters and 20 rodent litters) and at least four dose groups (three agent-exposed groups and a concurrent vehicle control group). On gd 0, mated animals are placed on study and randomly assigned or assigned by a randomization procedure (stratified by body weight) into test groups.

The current testing guidelines specify dosing from implantation to term with no postdosing recovery period. Previous testing guidelines specified the period of exposure of the maternal organism to the test agent only during the period of major organogenesis. This corresponds to gd 6–15 for rodents and gd 6 or 7 through 18 or 19 for rabbits. This period of dosing was specifically chosen to preclude efforts on implantation so there would be conceptuses to evaluate and to maximize the chances of inducing and detecting structural changes in the conceptuses

(beginning dosing at fertilization (gd 0) is recommended only if there is evidence that there are no effects on the preimplantation conceptus). The possible effects of the test agent on the reproductive and developmental processes prior to organogenesis are evaluated in Segment I or multigeneration studies. The start of dosing for the previous and current testing guidelines precludes induction of maternal metabolizing enzymes prior to the presence of implanted conceptuses. The previous testing guidelines also allowed for a postexposure recovery period prior to scheduled sacrifice close to term. Variations in the exposure period in the current guidelines include exposure during the entire gestational period (gd 0 to term sacrifice), or exposure beginning prior to gd 0. These latter extended exposure periods may be useful and appropriate if the test agent, or route of administration, results in slow and/or limited systemic absorption and therefore delayed attainment of steady state or maximal blood levels in the maternal organism. In these circumstances, the usual dosing period could result in the conceptuses exposed to less than maximum levels during some or most of organogenesis and the misleading conclusion of little or no developmental toxicity. Extended exposure periods may also be called for if bioaccumulation of or cumulative toxicity from parent compound or metabolites is an important aspect of known or potential human risk.

The guidelines specify the preferred route of administration as gavage (orogastric intubation) to deliver the largest possible bolus dose in order to maximize the potential of the test agent to cause maternal and developmental toxicity, i.e., 'worst case scenario', and to control the delivered dose to the maternal animal. Use of other routes to simulate possible human exposure situations is becoming increasingly popular and is acceptable if scientifically defensible. These alternative routes include dosed feed, dosed water, inhalation by whole body or nose-only exposure, cutaneous application, injection by intravenous, subcutaneous, intraperitoneal, or intramuscular routes, or subcutaneous insertion (for implants or for minipumps for continuous infusion).

Maternal data to be collected from the current Segment II studies include maternal mortality; pregnancy rate; maternal body weights on gd 0 and throughout the dosing period, and at sacrifice; sacrifice weight corrected for the weight of the gravid uterus; body weight changes through gestation, and during the treatment period; feed and/or water consumption when body weights are recorded (water consumption if the test material is administered in the drinking water or is known to affect the kidneys); clinical observations; gravid uterine weight; and weight of other organs (absolute and relative to sacrifice body weight). Additional evaluations of morphological, physiological, and/or biochemical status are suggested, such as histopathology of target organs, more detailed behavioral evaluation, clinical pathology such as hematology, clinical chemistry, and urinalysis, etc.; these could be performed on maternal animals during and after the treatment period or at necropsy. These tests may duplicate those performed in other studies, but pregnant animals may respond quantitatively or qualitatively differently (vide supra), and these data will be critical in interpretation of any observed developmental toxicity.

Reproductive and embryo/fetal data to be collected from Segment II studies at sacrifice include number of ovarian corpora lutea (number of eggs ovulated); number of total, nonviable (resorptions and dead fetuses) and live uterine implantations; and calculation of pre- and postimplantation loss. For litters with live fetuses, data collected include number, sex, and individual fetal body weights, sometimes crown – rump length, anogenital distance, individual fetal external, visceral and skeletal and total malformations, and variations reported by fetus, by sex by litter, and per fetus per litter (male and female fetuses differ in body weights, with males significantly heavier). These procedures thoroughly assess two of the four embryo/fetal end points – death and structural malformations – and also assess developmental delays, but only in terms of delays in growth such as reduced body weight, reduced crown – rump length, and delays in structural development, such as reduced ossification relative to concurrent and historical control fetuses (usually designated as variations), especially in those skeletal districts that ossify late in prenatal development. The current US EPA and OECD guidelines require visualization of both ossified and cartilaginous bone, with the method left up to the performing laboratory. Almost all laboratories perform double-staining of fetuses with alizarin red S for ossified bone and alcian blue for cartilaginous bone to provide information on the status of bones not yet ossified. These techniques aid in the interpretation of a finding as a skeletal malformation or permanent skeletal variation (when there is no cartilage in a short bone or for a missing bone, so no subsequent growth, ossification, or correction would be anticipated) versus a variation of transient delay in ossification (where there is cartilage with anticipated subsequent growth, ossification, and possible correction).

There is apparent potential for extensive remodeling of the skeletal system in the postnatal period; extra ribs become vertebral arches and fused ribs and other skeletal malformations disappear prior to

sexual maturity. This plasticity of the skeletal system may result in revision of the current classification of morphological findings in term fetuses as malformations or variations. The current definition of a malformation specifies a permanent morphological change that is incompatible with or detrimental to postnatal survival, normal growth, and development. Short ribs, extra ribs, fused ribs, alterations in sternebrae (which fuse to form the sternum), alterations in vertebral centra, and arches are currently designated malformations or variations depending on the laboratory; if these changes do not persist, their designation could change. The reverse situation is also true; that is, findings commonly designated as variations, usually delays, in term fetuses may, in fact, sometimes develop into findings designated as malformations in postnatal life. For example, a dilated renal pelvis (reduced renal papilla) may or may not be the precursor of hydronephrosis, and dilated lateral ventricles of the fetal cerebral hemispheres may or may not be the precursor to hydrocephaly in the postnatal organism. With structural evaluations of term fetuses 'frozen in time' in a Segment II study, there is no way to project the postnatal consequences of the initial findings or to identify postnatal consequences of *in utero* exposure. In addition, the term evaluation is based on structure. If the lungs or kidneys, etc., are in the right location and are the right size, shape, and color under a dissecting microscope, they are designated as normal; there is no assessment of microscopic integrity or of function. Additional evaluations of term fetuses should perhaps include biochemical assessment of organ function and histological examination of structure. However, the most important drawback in a Segment II study is the lack of postnatal assessment of the reversibility of detected structural lesions and of the structural and functional sequelae of the prenatal insult; other testing guidelines do evaluate this (see sections Standard Segment III Testing Protocol and Developmental Neurotoxicity Test).

Statistical Analyses of Maternal and Developmental Toxicity Data

As part of protocol development, the choice of statistical analyses should be made *a priori* although specific additional analyses may be appropriate once the data are collected. The unit of comparison is the pregnant female or the litter and not individual fetuses as only the dams are independently and randomly sorted into dose groups. The fetus is not an independent unit and cannot be randomly distributed to groups. Intralitter interactions are common for a number of parameters, for example, fetal weight or malformation incidence. Two types of data are collected: ordinal/discrete data, which are essentially present or absent (yes or no) such as incidence of maternal deaths, abortions, early deliveries, clinical signs, and incidence of fetal malformations or variations; and continuous data such as maternal body weights, weight changes, food and/or water consumption, organ weights (absolute or relative to body or brain weight), and fetal body weights per litter. For both kinds of data, three types of statistical analyses are performed. Tests for trends are available and appropriate to identify treatment-related changes in the direction of the data (increases or decreases), overall tests are performed for detecting significance among groups, and specific pairwise comparison tests (when the overall test is significant) to the concurrent vehicle control group values are the critical end point to identify statistically significant effects at a given dose relative to the concurrent vehicle control group. Continuous data are designated parametric (distributed along a bell-shaped curve) or nonparametric (skewed distribution), with different specific tests employed for the three types of statistical analyses depending on whether the data are parametric or nonparametric.

Risk Assessment

The US government's new approach (2000) to health assessment of agents involves the iterative interaction of four major components: basic scientific research (hazard identification), science-based toxicity/risk assessment (dose–response assessment), exposure assessment, and risk characterization (**Figure 2**). This section relies heavily on the US EPA guidelines for the health assessment of suspect developmental toxicants which describe how the government uses, and plans to use, developmental toxicity data as part of their 'weight-of-evidence' approach to both the hazard identification and the dose–response assessment components of risk assessment.

Standard developmental toxicity studies are performed, under the appropriate governmental toxicity guidelines, for a drug early in the drug discovery period (FDA), for a pesticide prior to registration (as required by EPA FIFRA), or for an industrial chemical (performed on a case-by-case basis under EPA TSCA). These studies provide information on the intrinsic capacity of the test agent to cause developmental toxicity under conditions to maximize the opportunity, that is, hazard identification, and the dosage or dosages at which the developmental toxicity (death, malformation, delays, and/or deficits) is observed, that is, dose–response assessment. Of the three dosage levels employed, the highest dose should

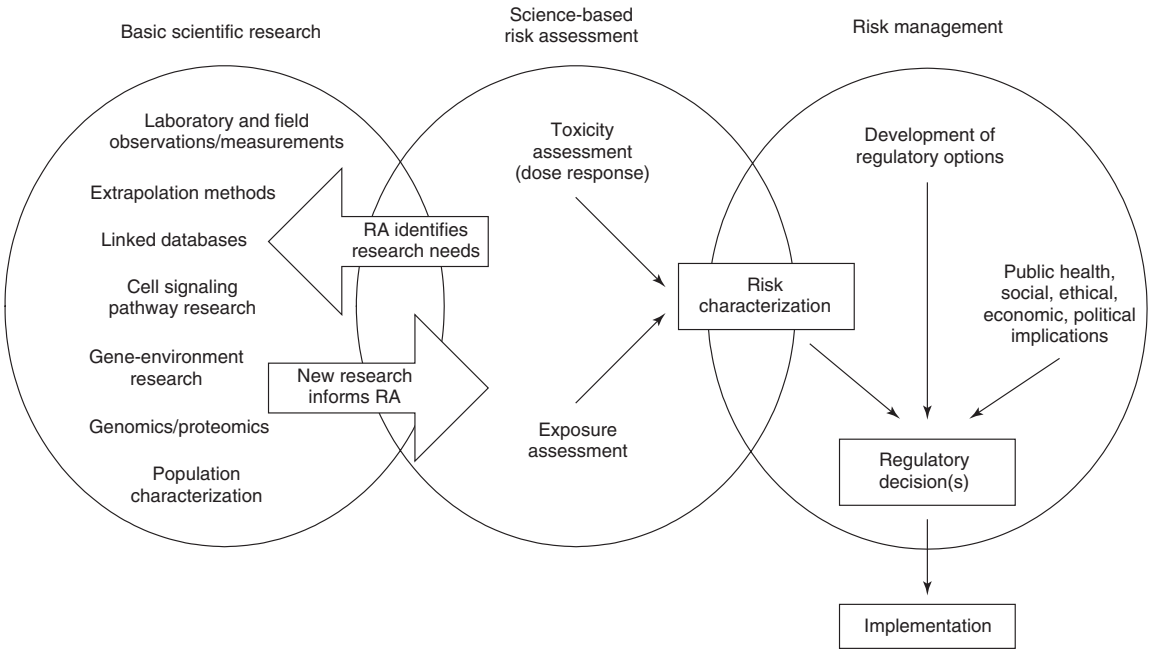

Figure 2 US National Research Council, new risk assessment/risk management paradigm. (Modified from NRC (2000) *Scientific Frontiers in Developmental Toxicity and Risk Assessment.* Washington, DC: National Academy Press.)

result in overt maternal toxicity, including significantly reduced body weight, weight gain, and specific organ toxicity, with maternal mortality up to 10% viewed as acceptable. This dose level should characterize embryo/fetal outcome in a compromised dam/doe and should represent a 'worst-case scenario' for hazard identification. However, the presence of maternal toxicity *per se* confounds the interpretation of observed developmental toxicity since these effects may be due to the status of the dam and not to the test agent *per se* (see previous discussion for maternal toxicity data).

The low dose should be a no-observed-adverse-effect level (NOAEL) for both dams and conceptuses. The NOAEL is defined as the highest dose (or exposure concentration) at which no statistically significant and/or biologically relevant adverse effects are observed in 'any adequate developmental toxicity study'. The middle dose may or may not result in maternal and/or developmental toxicity and should be a lowest-observed-adverse-effect level (LOAEL), defined as the lowest dose or exposure concentration at which a statistically significant and/or biologically relevant adverse effect is observed in 'any adequate developmental toxicity study'. The characteristics of the NOAEL (or the LOAEL) are that (1) it is obviously experimentally derived and therefore dependent on the statistical power of the study (which is in turn dependent on the number of animals employed); (2) it is dependent on the number and sensitivity of the parameters examined; and (3) its presence implies a 'threshold', that is, a dose below which adverse effects would not be observed, again with the same experimental caveats. The attainment of a NOAEL (or LOAEL) is critical for subsequent risk assessment processes since it is used to ultimately extrapolate to human exposure limits. However, the NOAEL is not a characteristic of the population (all rats, all mice, etc.) but only of the group under test and, in a real sense, specific to the species, strain, laboratory, staff, specific time of performance, source and purity of test material, identity of any vehicle, parameters evaluated, etc. The NOAEL also does not provide information on the slope of the dose–response curve (steep or shallow), although it is obviously at the low end of the dose–response continuum. These characteristics are very important since regulators are usually extrapolating from (relatively) high dose levels in animal studies to (relatively) low exposure levels for humans, and the presence and location of the threshold is crucial to risk assessment.

Once a NOAEL (or LOAEL) is provided by the experimental data, the proposed next step by risk assessors is to define a reference dose for developmental toxicity (RfD_{DT}) according to the following equation:

$$RfD_{DT} = \frac{NOAFL/LOAEL}{UF}$$

where UF is uncertainty factors. The RfD_{DT} is defined by the US EPA as an estimate of the daily

human exposure that is likely to be without appreciable risk of adverse developmental effect and is characterized by the use of NOAEL or LOAEL (if NOAEL is unavailable) of most sensitive indicators for most appropriate (if known) and/or most sensitive mammalian species. If the NOAEL is used,

$$\mathrm{RfD_{DT}} = \frac{\text{NOAEL of most sensitive indicator}}{\underline{\text{inter}}\text{species variability (UF; 10)} \times \underline{\text{intra}}\text{species variability (UF; 10)}}$$

The $\mathrm{RfD_{DT}}$ is assumed to be below the threshold for an increase in adverse developmental effects in humans and is used for risk characterization along with human exposure assessments.

A second use for NOAELs (or LOAELs) is in the calculation of a proposed margin of exposure (MOE) for developmental toxicity to be used in risk characterization. The MOE is defined as the ratio of the NOAEL from the most sensitive or appropriate species to the estimated human exposure level from all potential sources. If the MOE is very high relative to the estimated human exposure level, then risk to the human population would be considered low.

The proposed US EPA weight-of-evidence (WOE) scheme for suspect developmental toxicants defines three levels of confidence for data used to identify developmental hazards and to assess the risk of human developmental toxicity: (1) definitive evidence for human developmental toxicity or for no apparent human developmental toxicity, (2) adequate evidence for potential human developmental toxicity or no apparent potential human developmental toxicity, and (3) inadequate evidence for determining potential human developmental toxicity. The scheme may require scientific judgment based on experience to weigh the implications of study design, statistical analyses, and biological significance of the data.

Standard Segment III Testing Protocol

There is growing concern about postnatal sequelae to *in utero* structural and/or functional insult as well as a recognition that exposure to a developing system may result in qualitatively or quantitatively different effects than an exposure to an adult system. In brief, the Segment III study consists of exposure of pregnant rats to the test agent starting at the end of the organogenesis period (gd 15), through the histogenesis period (concepti are termed fetuses), through parturition, and through the lactational period until the offspring are weaned (postnatal day (pnd) 21). The offspring are 'exposed' only from possible transplacental and/or translactational (via the milk) routes (**Figure 1**). There are usually three test material groups and a vehicle control group, with at least 20 litters per group; exposure is by gavage to the dam (to minimize disruption of the mother and her litter). During gestation, the dam is weighed periodically and feed consumption is measured. Dams and pups are weighed, sexed, and examined externally, and feed consumption is measured at birth (pnd 0) and repeatedly during the lactation period (e.g., on pnd 0, 4, 7, 14, and 21). Litters are culled to eight pups on pnd 4. The time of acquisition of developmental landmarks is recorded, such as surface righting reflex, pinna (external ear) detachment, incisor eruption, eye opening (pups are born blind with eyes shut), auditory startle (pups are born deaf with the external ear canal closed), mid-air righting reflex, and testes descent. If the pups are maintained after weaning, then vaginal patency (opening of the vaginal canal) and/or preputial separation (separation of the foreskin from the penile shaft) are monitored as well as motor activity (initial exploratory behavior as well as habituated behavior); learning and memory may also be assessed. This test provides information on the last 'trimester' of pregnancy; delivery; maternal–pup interactions and behaviors, such as pup retrieval, nursing, grooming, nest building, etc.; and on pup postnatal growth and development. At weaning, the dam is sacrificed and the number of uterine implantation scars are counted to obtain information on prenatal loss; pups can be necropsied at wean or beyond, with target tissues examined histologically.

Developmental Neurotoxicity Test

The nervous system – with its long developmental phase, involving proliferation, migration, and differentiation of cells and regions at different gestational and perinatal ages, and its complexity – is one for which there is special concern for postnatal consequences of *in utero* exposure to developing systems. In response, the US EPA has developed a 'standalone' standardized developmental neurotoxicity test to assess 'potential functional and morphologic hazards to the nervous system which may arise in the offspring from exposure of the mother during pregnancy and lactation' (**Figure 1**). When this study design would be employed, that is, the 'triggers' for its requirement, is still not fully established and will (and should) probably be decided on a case-by-case basis. Agents which should be candidates for developmental neurotoxicity or behavioral teratology testing include those that cause central nervous system (CNS) malformations, that are psychoactive,

adult neurotoxicants, hormonally active, and peptides or amino acids. The last agents might be antagonists or agonists of endogenous CNS chemical signalers and could easily cross the blood–brain barrier. Such testing protocols should assess sensory and motor function, neuromotor development, learning and memory, reactivity and/or habituation, reproductive behavior, and other functions such as social or aggressive behaviors.

The study design for the developmental neurotoxicity screen, as currently mandated by the US EPA (OPPTS, 1998), involves performance in rats, at least three agent-exposed groups and one vehicle control group, and at least 20 usable litters in each group. The route of administration should be 'orally by intubation'; other routes of administration are acceptable, on a case-by-case basis, with appropriate justification. If the agent has been previously shown to be developmentally toxic, 'the highest dose for this study shall be the highest dose which will not result in perinatal deaths or malformations sufficient to preclude a meaningful evaluation of neurotoxicity'. If there are no developmental toxicity study data, 'the highest dose shall result in overt maternal toxicity, with weight gain depression not to exceed 20% during gestation and lactation'. The lowest dose should not result in either overt maternal or developmental neurotoxicity, while the intermediate dose(s) must be equally spaced between the highest and lowest doses. With gd 0 designated as the day of copulation, the dosing period extends from gd 6 through pnd 10. Maternal animals are evaluated for body weights and weight gain, functional observational battery (FOB), and clinical signs of toxicity from gd 0 through pnd 21. Live pups are counted and weighed at birth and throughout the lactation and post-wean periods. On pnd 4, litters are culled to yield eight pups (four/sex). Pre- and post-wean developmental landmarks assessed on all appropriate pups include age of vaginal opening and testes descent and/or preputial separation. Motor activity is monitored at multiple pre- and post-weaning times. The period of evaluation for motor activity will include the exploratory phase and the habituation phase. Auditory startle test, including magnitude and habituation of response, and tests to evaluate learning and memory are performed at weaning and at 60 days of age. Necropsy and histopathology requirements include perfusion of pups with fixative *in situ* and specified central and peripheral nervous system tissues examined histologically with 'qualitative, semiquantitative, and simple morphometric analysis'. Additional animals are decapitated, the brains are removed, and regional brain weights are obtained. Current discussions on this testing guideline between the US EPA, pesticide manufacturers, and performing laboratories, especially as used for pesticide registration (now required, rather than triggered, along with an adult neurotoxicity test) center around: (1) direct dosing of offspring pups (if there is no proof that the test chemical and/or metabolites is transferred to the offspring in the milk from the dosed dam, or if there is proof that there is no lactational transfer), including start of direct dosing of pups (and cessation of direct maternal dosing); and (2) timing of neuropathologic evaluations of the brain in pups (current guideline specifies on pnd 11 and at study termination on pnd 60). The assumption by the US EPA is that all brain structures are present by gd 10, but there is evidence that various regions of the brain go through proliferation, differentiation, and scheduled cell death with different timing, so that evaluation on pnd 11 is too early and the natural intra- and inter-litter biological variability in regional brain weights, differentiation, etc., will obscure many treatment-related effects.

This test is perceived as useful in the risk estimation process, to identify specific agents, or classes of agents, for which acceptable exposures in the adult may not be acceptable to the developing organism, to elucidate long-term consequences of pre- and perinatal exposures and results, to determine the relationship of lowest effective (or highest no effect) dose for behavioral effects versus the dose for overt or general toxicity effects, and to identify, for human exposures, those effects which may be important to monitor.

Although the developing nervous system has received the most attention from researchers and governmental regulators, there are many other systems with continuing proliferation and differentiation in the postnatal period. Evaluation of the postnatal sequelae of prenatal exposure has been done for three of these: the renal system, gastrointestinal tract, and immunosurveillance system. Transplacental carcinogenesis, expressed in the adult from late gestational *in utero* exposure, is also well documented in test animal species; diethylstilbestrol is currently the only documented transplacental carcinogen in humans.

Male-Mediated Developmental Toxicity

All of the previously described approaches focus on the maternal–placental–fetal unit as the subject of testing and the object of concern. However, increasing evidence has implicated the male as the cause of any of the classic four end points of developmental toxicity. Human male exposure, such as operating room personnel, to waste anesthetic gases results in

increased incidences of spontaneous abortions, stillbirths, and congenital defects. Male production worker exposure to Oryzalin has been implicated in congenital heart defects in their children. The pesticide DBCP (1,2-dibromo-3-chloropropane) is a human male sterilant. Elevated caffeine consumption in men has been reported to result in spontaneous abortions, stillbirths, and premature births. In animal studies, exposure of the male to methadone, thalidomide, lead, narcotics, alcohol, and caffeine results in malformations in the offspring. Possible mechanisms of male-mediated developmental toxicity include genetic or epigenetic damage to the sperm, the presence of the agent or its metabolite(s) in the semen which may affect the conceptus directly or act on the gravid uterus, or indirect or more systemic actions on the male affecting the hormonal milieu and perhaps libido.

Developmental Toxicity Screening Protocols

Over 80 000 chemicals are listed in the TSCA registry, with 1500–2500 new chemicals added each year; 20 000 chemicals are commonly found in the workplace (NIOSH list) with $<1\%$ tested for reproductive and developmental hazard potential. It is therefore necessary and appropriate to develop fast, inexpensive, sensitive, and accurate methods to prescreen the plethora of chemicals and concentrate resources on those identified by the screening test(s) as potential human health hazards. However, the mechanisms of action of developmental toxicants appear numerous and frustratingly difficult to identify (see section Mechanisms).

A number of approaches have been taken to develop screening protocols, herein arbitrarily classified into *in vivo*, *in vivo/in vitro*, and *in vitro* categories. *In vivo* screens include developmental toxicity range-finding studies, which can also be used to identify (or prioritize) agents which produce developmental toxicity for more rigorous testing, and the so-called Chernoff–Kavlock assay. The assay employs a block design of one dose (the maternal minimally toxic dose; MTD) per chemical for a number of chemicals and a concurrent control group, with 24–50 timed-pregnant animals, usually mice, per group. Dosing is on gd 8–12, with the date of a vaginal plug being designated gd 1. The earliest version of this study design collected maternal weights at the beginning and end of the treatment period and also weight change, with dams allowed to litter. Litters were counted, sexed, weighed, and examined externally on pnd 1 (date of birth) and 3 and discarded. This protocol does not require extensive or intensive technical training in pup visceral or skeletal examinations and assumes that the pups will be their own assay system, that is, if the pups survive and thrive, then they do not exhibit significant toxicity at a dose which is minimally toxic to the dam (the MTD) and they do not bear malformations or variations which preclude or affect normal early postnatal growth and development. Chernoff and Kavlock set up three levels of concern: If there is pre- or postnatal mortality and/or malformations of the offspring, then the test agent has the 'highest priority' for further classic developmental toxicity testing; if the pups exhibit reduced weight gain, then the agent has a 'lower' priority for further testing; and if there is no evidence of developmental toxicity, pre- or postnatally, then the agent has the 'lowest priority' for further testing. The block design described previously provides comparisons among the test agents in the block, all at the MTD, for relative potency with regard to developmental toxicity.

Modifications to the initial protocol include multiple dose levels, dosing during the entire period of major organogenesis, and more thorough evaluation of pups on pnd 3 (including visceral and skeletal examinations) so that this protocol resembles more closely the classic Segment II protocol but with a postnatal component to assess viability and growth.

One *in vivo/in vitro* screening protocol involves administration of the test agent to pregnant rodents, removal (on gd 10 after one or more daily doses to the dam), explantation and culture of embryos for 24–48 h, and evaluation of toxicity and teratogenicity. This protocol allows for the full mammalian complement of metabolizing enzymes in the dam to act on the conceptuses *in utero* and for evidence of early expression of developmental toxicity (limited) to be detected in the explanted embryos *in vitro*. The next step is one whereby explanted rat headfold embryos are cultured for 48 h in human, monkey, or rodent serum after the serum donor had been exposed to the test agent. This protocol utilizes serum containing whatever metabolites, etc. are produced by and transported in the blood of the donor mammal – a condition duplicating the embryonic exposure *in utero*. In a fascinating offshoot of this work, serum from women who were chronic aborters has been used in the embryo culture system to identify missing nutrients and the women were supplemented prior to and during subsequent pregnancies with some early apparent success.

There are a number of fully *in vitro* screens as well, employing mammalian, lower vertebrate, and invertebrate species. Materials used include explanted rat or mouse embryos cultured in rodent serum to which is added the test agent or known metabolites, and

portions of rodents, as intact organs or as dissociated cells (e.g., limb buds, dissected midbrain cells, and palatal cells), explanted and cultured in medium containing test agents and/or metabolites. When explanted embryos (or parts thereof) are exposed to the test agent in culture, they are exposed only to the added test agent since metabolic capability is minimal or absent, so this study paradigm may expose embryos to situations they would not encounter *in utero* and therefore result in false-positive or (worse) false-negative study results. Cloned totipotent stem cell lines from murine embryonal teratocarcinoma or pluripotent lines from neuroblastoma are cultured, exposed to test agents (including those which are 'proteratogens' requiring metabolic activation), and the cultures scored for effects on differentiation. Both tumor lines are capable of extensive differentiation in culture; restriction of this capability is presumed indicative of potential developmental toxicity *in vivo*.

In a novel approach to examine a fundamental property of differentiating cells, cell-to-cell communication, Chinese hamster lung cells or normal embryonic palatal mesenchymal cells in culture are exposed to the test agent and evaluated for disruption of cell-to-cell communication. Cell attachment is another presumed universal cell function during development and therefore a basis for a screen. Ascites or dissociated solid tumor cells are grown in culture in the presence of the test agent and scored for attachment (or inhibition of attachment) to surfaces as a measure of potential developmental toxicity.

Explanted chick embryos at presomite or multiple somite stages or chick embryonic parts are cultured with the test agent incorporated into the culture medium and evaluated for growth and differentiation.

Amphibians are also proposed for use in screening protocols. The FETAX system (Frog Embryo Teratogenesis Assay: Xenopus) involves exposure of early *Xenopus laevis* (African clawed frog) embryos at the notochord stage and/or as late premetamorphic larvae to test agents in the water. A teratogenic index (TI) is proposed to compare relative potencies of test agents and to identify any agents which affect development at doses below which general toxicity is observed; the TI is defined as LC_{50}/ED_{50} (the concentration lethal to 50% of the animals divided by the concentration producing effects in 50% of the animals).

Drosophila melanogaster (the fruit fly) is used in two ways: Larvae are grown on feed containing the test agent, are allowed to pupate, and emerging adults are scored for viability (toxicity) and malformations from alterations in imaginal discs present in the larvae and used to form adult structures; or early primary embryonic cell cultures are grown in medium containing the test agent and are scored for differentiation of embryonic cell types.

Synchronous cultures of *Artemia* sp. (brine shrimp) in seawater or rodent or human serum have also been suggested as a screen, with scoring for survival, growth, and morphological and molecular differentiation after exposure directly to agents or to serum from agent-exposed individuals.

Hydra attenuata (a coelenterate) is the source of the 'artificial embryo' assay. The adult Hydra can be dissociated and the cells pelleted by centrifugation. The cells of the pellet will sort and reaggregate by cell type and redifferentiate into an adult Hydra. The assay consists of pellets (artificial embryos) and adult Hydra exposed to the test agent to determine the lowest effect concentration (or the highest no-effect concentration) of the developing 'embryo', as measured by inhibition of redifferentiation or abnormal differentiation, and of the adult, as measured by mortality or overt damage to adult structures. An A/D ratio is calculated: that is, the ratio of the adult toxicity lowest effect (or highest no-effect) concentration to the developmental toxicity lowest effect (or highest no-effect) concentration. The developers and users of this assay suggest that an A/D ratio ≥ 3 indicates a unique or greater susceptibility of the developing organism relative to that of the adult and therefore a potential of the test agent for mammalian developmental toxicity. They also claim that the A/D ratio is fairly consistent across widely divergent species and therefore predictive of relative risk, although this latter claim has been contested by data from other workers in the field.

The consensus on screening assays appears to be that the *in vivo* protocols are appropriate and useful to prioritize chemicals for subsequent testing, to decide early in the chemical/drug development phase whether to pursue a particular formulation, to evaluate what effect changes in chemical structure have on toxicity, and to 'fill in the blanks' on a chemical series, all relative to the potential for developmental toxicity, including teratogenicity. The *in vivo/in vitro* assay requires the same number of maternal animals to do fully *in vivo* studies, requires sophisticated technical procedures for culturing embryos, and provides for only a limited number of embryological end points due to the limitations on the length of time embryos can be maintained in culture. There does not appear to be an advantage in using these assays as screens.

The *in vitro* assays with mammalian embryos or tissues have two critical limitations. First, the metabolic capabilities of the embryo are very limited and only the embryo is cultured. Any metabolic changes to the parent compound by the maternal organism

and therefore the metabolites to which the embryo would be exposed *in vivo* are totally missing in the explant system. Currently, attempts are being made to provide metabolic capability by coculturing embryos with adult liver cells or cell fractions, which are the major source of metabolism of xenobiotics to obviate the first limitation. Second, the duration of sustained normal growth and development of embryos appears very limited (24–48 h) so that the numbers of structures differentiating and the extent of differentiation are similarly limited. A two-system approach, for example, midbrain plus limb bud micromass culture assay, is an attempt to increase the number of systems evaluated, but it is still very limited relative to the tremendous range of systems developing which may be vulnerable. The *in vitro* assays are very useful in answering research-oriented questions since the age of the embryos (as judged by somite number or other specific morphological signposts) can be precisely controlled, identity and concentration of the test agent are precisely controlled, and early responses can be observed and characterized. They can be used to identify the proximate teratogen by exposing the explanted embryos to specific metabolites which they cannot further transform and to elucidate mechanisms of action of known teratogens at the organ, tissue, cellular, subcellular, or molecular levels early in the toxic response, prior to cell death or demise of the embryo. The utility of nonmammalian (nonvertebrate) assays as predictors of potential mammalian developmental toxicity appears unclear at this time, although the concept of phylogenetically conserved universal processes in embryonic development is attractive and compelling.

Mechanisms

There is no mechanism fully understood for any developmental toxicant causing fetal malformations. Although in many cases the proximate teratogen is known and maternal and/or developmental toxicity is well characterized, what is not known is how the observed effects result in malformation(s). The site(s) of action may be intranuclear, intracellular, at the cell membrane, extracellular, outside of the conceptus, in the placenta, or in the maternal organism. The mode(s) of action may be general or specific, biochemical, physiological, or microstructural. It is also likely that the mechanism(s) will vary from agent to agent. The two extremes in mechanisms, from very specific to very general, may be exemplified by those proposed for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), valproic acid, and ethylene glycol (EG). TCDD produces cleft palate (and hydronephrosis at higher doses) in susceptible mouse strains. The putative mechanism for the induced cleft palate is that TCDD binds to certain epidermal growth factor (EGF) receptors and prevents the normal reduction in expression of certain EGFs in the medial epithelial cells of the palatal shelves just prior to fusion. Therefore, with TCDD, abnormally high levels of certain EGFs apparently continue to stimulate proliferation and differentiation of the cells normally destined to die, and the shelves do not fuse. The suggested mechanism for induction of cleft palate may also explain the induction of hydronephrosis since EGF, TGF-α, Ah, and ARNT play a role in embryonic cell proliferation during normal palatal and urinary tract development and are altered in both the palate and urethral buds in culture from TCDD exposure. Valproic acid causes neural tube defects, including exencephaly (in mice) and spina bifida (in humans). Valproic acid and other weak acid teratogens (of which there are many) reach the mammalian embryo and lower the intracellular pH of the embryonic cells. (The embryonic intracellular pH is more basic than the maternal intracellular pH, especially early in development, and changes over time.) The specificity of the effect probably lies in the sensitivity of the target neural tube. EG causes major malformations, predominantly skeletal, in rats and mice at high oral doses, but not in rabbits and not by nose only, cutaneous, or dosed feed exposures. EG is metabolized to acidic intermediates and forms oxalic acid (oxalate crystals in the kidneys). At high oral bolus doses, the major intermediary metabolite in rodents is glycolic acid (rabbits do not make glycolic acid but do form oxalate crystals in the kidneys). Co-administration of sodium bicarbonate prevents maternal acidosis and ameliorates but does not prevent fetal malformations. Oral administration of glycolic acid in rats produces the same malformations as EG. In embryo culture, either acidosis or sodium glycolate (the salt of the acid) causes malformations *in vitro*. It appears that glycolic acid and maternal metabolic acidosis are both responsible for the teratogenesis produced by large oral bolus doses of EG. The suggested mechanism for valproic acid (and other teratogens which are weak acids) does not explain the specificity and susceptibility of the targets since other weak acid teratogens do not affect the neural tube and many weak acids are not teratogens. Perhaps the most important barrier to understanding the mechanism(s) of abnormal development is that we do not know enough about the mechanism(s) of normal development.

Studies performed initially on *Drosophila* embryos indicated that sequential activation of a hierarchy of regulatory genes occurs during the development of multicellular organisms. These genes regulate the

transcription and translation of genetic information into structures (and functions) by orchestrating a precise temporal and spatial expression of structural genes, which in turn control differentiation, that is, establishment of cell types and organ formation. Many of these genes also appear to play a role in pattern formation during or after gastrulation in vertebrates. The mechanisms of these regulatory genes include genetic and epigenetic control. Genetic mechanisms include the role of genes in establishing the basic embryonic axes (cephalocaudal and dorsoventral), specifying specific embryonic regions, controlling the transition of cells from presumptive to determined in the establishment of the fate of diverse cell types and ultimately specifying directly the differentiated patterns of gene expression, including inter- and intracellular molecules, structure, and functions. Epigenetic mechanisms include the interactions between cells, between cell types, and between cells and the products of other cells. The genetic and epigenetic roles are linked and integrated by so-called second messengers, which translate molecular signals by individual cells into commands to produce specific effects in other cells on cell growth and patterns of gene activity.

Abnormal expression of genes from this regulatory class results in abnormalities in development, which also provide information on normal development and suggest a mechanism(s) of action of xenobiotics. It is clear that cell division, cell migration, and differentiation are directed by regulatory gene classes that control which genes are expressed in which tissues at which times in development. The molecular approach to identifying these fundamental controlling factors of mammalian development may be the most fruitful in the long run in elucidating mechanisms of normal and abnormal development and providing mechanisms of action of developmental toxicants.

See also: Ames Test; Carcinogen–DNA Adduct Formation and DNA–Repair; Chromosome Aberrations; Developmental Toxicology; Dominant Lethal Tests; Dose–Response Relationship; Environmental Hormone Disruptors; Epidemiology; Host-Mediated Assay; Levels of Effect in Toxicological Assessment; Molecular Toxicology–Recombinant DNA Technology; Mouse Lymphoma Assay; Reproductive System, Female; Reproductive System, Male; Risk Assessment, Human Health; Sister Chromatid Exchanges; Toxicity Testing, Reproductive; Toxicology, History of.

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Toxicity Testing, Inhalation

Samantha E Gad and Shayne C Gad

Introduction

Inhalation is, in many senses, simply a particular route of administration in toxicology studies in general and in acute toxicology studies in particular. As will be reviewed here, animal inhalation studies are difficult and complex to perform correctly. The complexity and cost of such studies firmly dictates that they be performed only when there is the substantial likelihood that humans will be exposed to the substance of interest. Human inhalation occurs in three types of settings. In order of decreasing occurrence or importance, these are (1) occupational settings, (2) environmental settings, or (3) therapeutic settings. The toxicity due to human inhalation exposures is often expressed in terms of the inhalation reference concentration (RfC), which is an estimate of a continuous inhalation exposure to the human population that is likely to be without appreciable risk of deleterious noncancer lifetime health effects. Since this estimate includes sensitive population subgroups, it may be considered a conservative estimate.

All inhalation studies can be classified either by the pattern of exposure or by the physical nature of the contaminant. Both of these classifications are important because they dictate equipment, animal selection, and details of study design.

Exposure Pattern

Pattern refers to how (or how much of) a test animal is exposed to the contaminated atmosphere of

interest. In practice, there are only very limited situations in which exposure to a toxicant is purely by inhalation (these are therapeutics cases when a material is administered directly into the nasal or oral cavity of an organism and in an inhalation test system, where nose-only exposure is truly achieved). Rather, both in the real world and in the laboratory, inhalation exposure is accompanied by dermal and oral exposures. How concerned one is with the possible confounding effects of such other routes of exposure on the evaluation of biological outcome dictates selection of the exposure pattern.

The three categories of exposure patterns are nose only, head only, and whole body. There are also minor patterns (lung only and partial lung) that will not be discussed here as they are used only in special research settings for precise delivery of doses of test material directly to the lungs.

In 'nose-only' exposure, which is the least commonly used pattern (particularly in acute tests), the test animal is situated in such a way that only its nasal region (or, for dogs and primates, where a mask is used to administer test compound, only the mouth and nasal region) is exposed to a test atmosphere. This can be achieved by having the animal restrained with only its nose poking through an elastic barrier or with a breathing mask fitted over the nose and mouth region. There is still some small amount of oral exposure in such a system because animals will swallow any material deposited on the surfaces of their mouths or 'cleared' from the nasal region or lungs back into the trachea.

In 'head-only' exposure, the entire animal is inserted into a chamber into which a test atmosphere is introduced. The head is sealed off, using a collar or membrane, so that only the head is exposed. There are a wide variety of chamber designs available such that all common laboratory species can be exposed using this methodology. The head-only approach is especially favored for pharmaceuticals.

There is extensive dermal and oral exposure in animals exposed 'whole body' (particularly oral exposure in rodents and rabbits which carefully 'preen' themselves after an exposure). For gases and vapors, of course, such considerations have minimal impact. The advantages and disadvantages associated with each of these exposure patterns are summarized in **Table 1**.

Physical Nature of the Contaminant

The second method of classifying inhalation studies is in terms of the exposure 'media' – that is, the physical nature of the contaminant atmosphere that is being evaluated. Though these media can be subdivided in a variety of ways, for our purposes the types of possible test media are gases, aerosols, and dusts.

Gases are generally the easiest type of media to use because the contaminants in the test atmosphere are in the gaseous phase. Technically, aerosols include any liquid- or solid-phase material that forms a stable suspension in air. Dusts are solid-phase (contaminant) particles suspended into a gaseous (atmosphere) phase. They are generally the most difficult to use when conducting a study.

Performing Inhalation Exposure Studies

Basic Steps

A technically good inhalation exposure study can be broken into four major parts or basic steps. The following are the four basic steps:

1. generation of a test atmosphere;
2. containment, mixing, and movement of test atmosphere and animals (both before and after exposure);
3. measurement and characterization of what animals have been exposed to; and
4. cleanup and disposal of resulting 'wastes' (gaseous, solid, and liquid).

Mechanics of Exposure

The mechanics of performing acute inhalation exposures to state-of-the-art standards can be complex in their entirety but the individual technical components of the problem are rather simple.

There are four sequential components to an exposure system. These are generation systems, exposure chambers, systems for measuring exposure, and systems for cleaning the effluent air stream.

Generation Systems Optimal generation systems have four major desirable features:

1. uniform rate of sample delivery,
2. uniform character of sample delivered,
3. ability to deliver in desired range of concentrations, and
4. safety of operations.

For each of the types of exposure (vapor, aerosol, and dust), there are a multitude of systems available.

Vapor Generation Vapor generation systems are based on the principle of maximizing the surface area of the liquid, and the temperature (within the limits of chemical stability) and airflow across the surface of the liquid as a means of increasing efficiency.

Table 1 Advantages, disadvantages, and considerations associated with patterns of inhalation exposure

Mode of exposure	*Advantages*	*Disadvantages*	*Design consideration*
Whole body	Variety and number of animals	Messy	Cleaning effluent air
	Chronic studies possible	Multiple routes of exposure: skin, eyes, oral	Losses of test material
	Minimum stress and labor	Variability of 'dose'	Noise, vibration
	Minimum restraint	Difficulties with dispersion and measurement techniques	Even distribution of space
	Controllable environment		Loading
	Large historical database		Sampling and observation
Head only	Good for repeated exposure	Stress to animal	Even distribution
	Limited routes of exposure	Labor in loading/unloading	Pressure fluctuations
	More efficient dose delivery	Seal around neck	Sampling and losses
		Losses can be large	Animal restraint
Nose/mouth only	Exposure limited to mouth and respiratory tract	Stress to animal	Pressure fluctuations
	Efficient use of material	Seal about face	Sampling
	Can pulse the exposure	Effort to expose large numbers	Airlocking
			Losses in plumbing/masks
Lung only	Precision of dose	Technically difficult	Stress to the animal
	One route of exposure	Anesthesia or tracheostomy	Physiologic support
	Efficient use of material	Limited to small numbers	
	Can pulse the exposure	Bypasses nose	
Partial lung	Precision and localization of total dose	Anesthesia	Stress to animal
	Can achieve very high local doses	Placement of dose	Physiologic support
	Unexposed control tissues from same animal	Possible redistribution of material within lung	
		Difficult in interpretation of results	
		Technically difficult	

There are four common generation systems:

- *Tube generators*. Liquid flows along the inside surface of a tube while air is forced over this surface.
- *Wick generators*. A liquid phase is passed up a porous wick while air is forced over it.
- *Bubble generators*. The air is passed through the liquid phase.
- *Special instrument generators*. A turning tube with ridges or sections to increase surface area is warmed while air is forced through it.

Aerosol Generation Liquid aerosols may be difficult to generate; if they are extremely volatile, one may actually end up generating a vapor. Second, denser or more viscous liquids require greater energy to overcome surface tension and form droplets of the desired size. There are four widely used aerosol generation systems:

- spray nozzle,
- ultrasonic generation (uses sound to provide energy to disrupt liquid into droplets),
- spinning disks, and
- nebulizers.

After a stream of test material is generated into airflow, it is mixed (usually in a dueling system of sufficient length, with some turbulence) and then introduced into the exposure chamber system in which test animals are or will be contained.

Dust Generation The following factors need to be considered in dust generation:

- particle size (and size distribution),
- particle shape,
- density of material, and
- concentrations needed (necessary capacity).

Exposure Chambers Technically, chamber exposures can be dynamic or static. In a static chamber exposure, there is no airflow through the system; animals are entered into a closed system that

contains an atmosphere 'precharged' with the desired test material. Static systems are inadequate for anything other than some minor short-term hazard-type assessments. In any other circumstance the animals should be tested with inhalation equipment designed to sustain a dynamic airflow that exceeds at least twice the respiration ventilation volume of all animals in the inhalation device or at least 10 air changes per hour, preferable 12–15 for whole-body chambers, and an oxygen content of at least 19%, with uniform conditions throughout. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas. It is normally not necessary to measure chamber oxygen concentration if airflow is adequate. Food should be withheld during exposure. Water may also be withheld in certain circumstances.

Measurement Systems While animals are being exposed in a chamber, certain characteristics of the measuring system that are critical include:

- accuracy and precision across the range of concentrations (or characteristics) to be measured;
- reproducibility;
- direct measurement of the variable interest (not inference or calculation based on an indirect measurement); and
- automatic measurement at a number of discrete locations in the exposure chamber.

For gases and vapors chemical analysis of the chamber concentration is mandatory and concentrations should be monitored continuously or intermittently depending on the method of analysis. Whenever the test substance is a formulation, the analytical concentration must be reported for the total formulation and not just for the active ingredient. The actual concentrations of the test substance should be measured in the breathing zone. The particle-size distribution of the test aerosol should be determined at least twice during each 4 h exposure to establish the stability of aerosol concentrations. The MMAD particle-size range should be between 1 and 4 μm in the animal's breathing zone and must be calculated. The rate of airflow should be monitored continuously, but recorded at least three times during the exposure. The particular problems, concerns, and instruments associated with each of the separate types of exposure (dust, aerosol, and vapor or gas) are very different. Overcoming all of these problems and concerns in practice is generally impossible. Rather, a number of acceptable compromises are made. For example, the mean of the test atmosphere samples is allowed to vary within $\pm 25\%$ of the concentration tested.

There are a variety of analytical techniques for determining inhalation chamber concentrations and they depend on the nature of the contaminant. These techniques are summarized below:

For vapors

- gas chromatography (direct sampling, extracted samples),
- infrared spectroscopy,
- ion-selective electrodes, and
- ultraviolet–visible spectrophotometers.

For aerosols

- Concentration (gravimetric, forward-scatter detectors, back-scatter detectors, J-attenuation detector, quartz crystal microbalance detector).

For particles (sizing)

- cascade impactors,
- microscopy (fiber morphology), and
- laser/Doppler type.

The most commonly used analytical techniques for analyzing gases and vapors are gas chromatography (GC) and infrared spectroscopy. GC is the most versatile and frequently used analytical technique for monitoring gases and vapors. GC offers chemical separation of components for specific analysis, low detection limits, and rapid turnover of data for feedback control. Infrared spectroscopy works well since most gases and vapors give reasonably intense and unique spectra. The Miran portable gas analyzer is particularly useful for continuous monitoring. Other techniques shown to be useful include the use of ion-specific electrodes, ultraviolet–visible spectrophotometers, and scrubbing colorimeters. As is always the case, frequent calibration of analytical instruments is essential.

Aerosols present a special case in that the investigator needs to measure the mass concentration of the chemical, the chemical composition as a function of particulate size, and the particle-size distribution of the aerosol. No continuous sampling instruments are available to measure both particle-size and chemical concentration. Particle detection can be accomplished using both forward- and back-scatter detectors. A typical back-scatter allows for non-invasive determinations over a range from 6 to $10\,000\,\mathrm{mg\,m^{-3}}$. In the test, the aerosol is drawn through an orifice and articles impact on a surface positioned between a source and a counter.

For particle sizing, many varieties of cascade impactors perform well, although care must be taken to avoid errors introduced by sampling (such as collection in sampling lines). The laser/Doppler-type particle-size device can be used to measure aerodynamic size and low concentrations with a rapid readout. In a system described by Cook, a powerful pulsed laser using temporal analysis of back-scattered light can be used to measure the spatial distribution of particles.

Cleanup Methods The last step or phase in the process of properly conducting an inhalation exposure is cleaning up the air-stream leaving the exposure system before releasing it into the atmosphere, and, of course, then properly disposing of the collected waste products that result from such a cleanup. For acute studies, one has much more flexibility in applying these methods than for longer-term studies in which logistics limit choices. Depending on the nature of the chemical being evaluated, one or a combination of three types of equipment may be utilized. These are filters, incinerators, or scrubbers.

Design of Inhalation Studies

All available information on the test substance should be considered by the testing laboratory prior to conducting the study. Three concentration levels should be used and spaced appropriately to produce a concentration–response curve and permit an estimation of the median lethal concentration. Range-finding studies using single animals may help to estimate the positioning of the test groups so that no more than three concentration levels will be necessary. An acceptable option for pesticide products would be to set the dose levels in correlation with the Environmental Protection Agency Office of Pesticide Programs (OPP) toxicity categories (bracketing). In these cases, the determination of an LD_{50} may not be necessary.

Exposure methods vary in the length of duration with acute (1–4 h exposure), subacute (13 week exposure), subchronic (3–12 month exposure), and/or chronic (multiple years exposure). The observation period should be at least 14 days with an examination at least once each day. Observations should include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or bizarre behavior (e.g., self-mutilation, walking backwards), changes in weight and time of death. Care should be taken when conducting examinations for clinical signs of toxicity that initial poor appearance and transient respiratory changes, resulting from the exposure procedure, are not mistaken for treatment-related effects.

Study Guidelines

EPA's Office of Prevention, Pesticides, and Toxic Substances (OPPTS) provides Health Effects Test Guidelines for use in testing of pesticides and toxic substances and the development of test data that must be submitted to the agency for review under Federal Regulations. The European Organization for Economic Cooperation and Development (OECD) also has published guidelines for inhalation studies.

OPPTS Health Effects Test Guidelines Revised June 1996

	Number	OECD
Acute inhalation toxicity	870.1300	403
Subchronic inhalation toxicity	870.3465	413
Repeated dose inhalation toxicity: 28/14 day		412
Proposed: Acute inhalation toxicity-fixed Concentration Procedure		433

Attempts are being made to have a worldwide testing and classification system for inhalation toxicity testing. At present, however, this has not been achieved. The following table shows the correspondence between the Globally Harmonized Classification System and the EU classification system. Conversion of Classifications for LC_{50} by inhalation using the Globally Harmonized Classification System for Chemical Substances and Mixtures (GHS) and EU classification systems are as follows:

	Classify EU category		
GHS classification	*Vapors*	*Dusts/mists*	*Gases*
Class 1	T+	T+	T+
Class 2	T	T+	T
Class 3	H	T	T
Class 4	H	H	H
Class 5 (unclassified)	U	U	U

T+ = very toxic; T = toxic; H = harmful; U = unclassified.

Acute Studies

Acute studies are generally conducted at a relatively high concentration and are useful in determining the approximate range of toxicity of a chemical. Acute studies can be used as a starting point in the determination of dose levels for longer-term tests. The clinical signs evoked at three high exposures often

allow determination of the nature of the toxic effect induced. The two most common numerical values derived from an acute study are the approximately lethal concentration (ALC) and the LC_{50}. The ALC is defined as the lowest concentration that produces death in at least one of a group of exposed animals, while the LC_{50} is the calculated concentration at which half of the exposed population would be expected to die. Generally, the exposures are conducted for a single 4 or 6 h period and the animals are observed for 14 days after treatment.

OECD Guideline 403 suggests the use of data from substantially similar mixtures to minimize the need for animal testing. In certain cases, it may be possible to get enough information from these data to make preliminary hazard evaluations that may reduce the need for further animal testing. The primary endpoint for Guideline 403 is mortality. Several groups of male and female animals are exposed for at least 4 h to graduated concentrations of the test substance, one concentration being used per group. Subsequently, observations of effects and deaths are made. In practice, many studies are limit tests at the maximum concentration and use only one group, but for full studies exposure concentrations should be sufficient in number, at least three, and spaced appropriately to produce test groups with a range of toxic effects and mortality rates. From such studies, a concentration mortality curve can be generated and an LC_{50} value calculated. Where a vehicle is used to help generate an appropriate concentration of the substance in the atmosphere, a vehicle control group may be used.

Recently, the OECD proposed a new guideline for inhalation testing, Guideline 433: Acute Inhalation Toxicity – Fixed Concentration Procedure. Development of the proposed Inhalation Fixed Concentration Procedure (FCP) will allow the use of a series of fixed concentrations for the determination of acute inhalation toxicity in only one sex (usually females). This will reduce suffering and distress by the animals and, to the extent feasible, reduce the number of animals used. Underpinning the FCP is a belief that the toxic profile of a substance can be characterized with sufficient reliability for most regulatory situations without the need for the identification of a lethal concentration. The primary endpoint for Guideline 433 is the observation of clear clinical signs of toxicity termed 'evident toxicity'. Evident toxicity is a general term describing clear clinical signs of toxicity following exposure to the test substance, such that an increase to the next highest fixed concentration would be expected to result in the development of severe toxic signs and probably mortality.

According to the Guideline, concentrations that are expected to cause marked pain and distress, due to corrosive, class 1 or severely irritant actions, should not be administered. Moribund animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test.

Limit Tests

The highest concentration should result in toxic effects but not produce an incidence of fatalities that would prevent a meaningful evaluation. The intermediate concentrations should be spaced to produce a gradation of toxic effects. The lowest concentration should produce no evidence of toxicity. In the case of potentially explosive test substances, care should be taken to avoid generating explosive concentrations. The limit test is an efficient way to characterize substances of low toxicity, when there is sufficient information available, indicating that the toxic concentration is higher than the limit concentration. Guideline 433 provides a limit test suitable for the design of the main study ($20\,mg\,l^{-1}$, $5\,mg\,l^{-1}$, or 5000 ppm for vapors, dusts/mists, and gases, respectively). A prespecified fixed concentration of $5\,mg\,l^{-1}$ (actual concentration of respirable substance, or where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration) is used for a limit test conducted according to Guideline 403. Also when data on structurally related chemicals are inadequate, a limit test may be considered. In the limit test, a single group of five males and five females is exposed to $2\,mg\,l^{-1}$ for 4 h, or where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration. If no lethality is demonstrated, no further testing for acute inhalation toxicity is needed. If compound-related mortality is produced, further study may need to be considered. The head/nose-only exposure method allows testing of high concentrations as required for limit tests without the need for large quantities of test material.

Procedures for Acute Tests

Groups of female animals are exposed for at least 4 h to graduated concentrations of the test substance, one concentration being used per group. A sighting study is included in the proposed Guideline 433 in order to choose an appropriate starting concentration for a main study and to minimize the number of animals used (see **Table 2**). Prespecified fixed

Table 2 Flowchart for the sighting study – vapors

Sighting study starting concentration: 0.5 mg l^{-1}											
	START										
	1 animal			1 animal			1 animal			1 animal	
	0.5 mg l^{-1}			2 mg l^{-1}			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class 1[a] main study starting (concentration mg l^{-1}):											
	0.5		0.5	2		2	10		10	20	20
Sighting study starting concentration: 2 mg l^{-1}											
				START							
	1 animal			1 animal			1 animal			1 animal	
	0.5 mg l^{-1}			2 mg l^{-1}			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class 1[a] main study starting (concentration mg l^{-1}):											
	0.5	0.5		2	2		10		10	20	20
Sighting study starting concentration: 10 mg l^{-1}											
							START				
	1 animal			1 animal			1 animal			1 animal	
	0.5 mg l^{-1}			2 mg l^{-1}			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class 1[a] main study starting (concentration mg l^{-1}):											
	0.5	0.5		2	2		10		10	20	20
Sighting study starting concentration: 20 mg l^{-1}											
										START	
	1 animal			1 animal			1 animal			1 animal	
	0.5 mg l^{-1}			2 mg l^{-1}			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class 1[a] main study starting (concentration mg l^{-1}):											
	0.5	0.5		2	2		10	10		20	20

Outcome: A, death; B, evident, C, toxicity; no toxicity.
[a] For outcome at 0.5 mg l^{-1} there is an optional supplementary procedure to confirm the GHS classification.

concentrations of 0.5, 2, 10, and 20 mg l^{-1} for vapors, 0.05, 0.5, 1, and 5 mg l^{-1} for dusts/mists, and 100, 500, 2500, and 5000 ppm for gases are used both in the sighting study and the main study. Groups of animals are exposed in a stepwise procedure, with the initial concentration being selected as that expected to produce some signs of evident toxicity. Further groups of animals may be exposed at higher or lower fixed concentrations, depending on the presence of signs of evident toxicity, until the study objective is achieved; that is, the classification of the test substance based on the concentration(s) causing evident toxicity, except when there are no effects at the highest fixed concentration (**Table 3**). It may be necessary to conduct another full acute inhalation toxicity study in the second sex.

Sighting Study

The test substance is administered to single animals in a sequential manner following the flowcharts in **Table 2** for a period of at least 4 h. The sighting study is completed when a decision on the starting concentration for the main study can be made, based on signs of evident toxicity or if a death is seen at the lowest fixed concentration. In the absence of evidence from *in vivo* and *in vitro* data from the same chemical and from structurally related chemicals, the starting concentration will be 0.5 mg l^{-1}, 1 mg l^{-1}, or 2500 ppm for vapors, dusts/mists, and gases, respectively. A period of at least 24 h will be allowed between the testing of each animal. All animals should normally be observed for at least 1 week.

In cases where an animal tested at the lowest fixed concentration level in the sighting study dies or exhibits clear clinical signs of toxicity, the normal procedure is to terminate the study and assign the substance to GHS class 1. If further confirmation of the classification is required, a second animal is tested at the lowest fixed concentration. If this second animal dies, then GHS class 1 will be confirmed and the study will be immediately terminated. If the second animal survives, then a maximum of three additional animals will be tested at this concentration. Because there will be a high risk of mortality, these animals should be tested in a sequential manner with a sufficient time interval between animals to protect animal welfare. If a second death occurs, the testing

Table 3 Flowchart for the main study – vapors

Starting concentration: 0.5 mg l^{-1}											
	START										
	5 animals		5 animals			5 animals			5 animals		
	0.5 mg l^{-1}			2 mg l^{-1} [a]			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class											
1	2		2	3		3	4		4	5	5/Unclassified
Starting concentration: 2 mg l^{-1}											
	START										
	5 animals		5 animals			5 animals			5 animals		
	0.5 mg l^{-1}			2 mg l^{-1}			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class											
1	2	2		3		3	4		4	5	5/Unclassified
Starting concentration: 10 mg l^{-1}											
	START										
	5 animals		5 animals			5 animals			5 animals		
	0.5 mg l^{-1}			2 mg l^{-1} [a]			10 mg l^{-1}			20 mg l^{-1} [a]	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class											
1	2	2		3	3		4	4		5	5/Unclassified
Starting concentration: 20 mg l^{-1}											
	START										
	5 animals		5 animals			5 animals			5 animals		
	0.5 mg l^{-1}			2 mg l^{-1} [a]			10 mg l^{-1}			20 mg l^{-1}	
A	B	C	A	B	C	A	B	C	A	B	C
Classify GHS class main study starting (concentration mg l^{-1}):											
1	2	2		3	3		4	4		5	5/Unclassified

Outcome: A > 2 deaths; B > 1 with evident toxicity and/or 1 death; C, no evident toxicity and no deaths.
[a] Animal welfare override, if this concentration caused death in the sighting study, then no further animals will be tested. Go directly to outcome A.

sequence will be immediately terminated and no further animals will be tested. The classification will be as shown in **Table 2**: class 1 if there are two or more deaths (outcome A), or class 2 if there is one death (outcome B).

Main Study

A total of five animals of one sex will normally be used for each concentration level investigated, in addition to the single animal used in the sighting study. The time interval between exposures at each level is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next concentration should be delayed (initially 3–4 days is recommended) until there is confidence in the survival of the previously tested animals.

The action to be taken following testing at the starting concentration level is indicated by the flowcharts. One of three actions will be required: stop testing and assign the appropriate hazard classification class, test at a higher fixed concentration, or test at a lower fixed concentration. However, a concentration level, which caused death in the sighting study, will not be revisited in the main study. Experience has shown that the most likely outcome at the starting concentration level will be that the substance can be classified and no further testing will be necessary.

Procedures for Subchronic Studies

Subchronic studies generally precede lifetime studies and are conducted to determine what the target organ or organ system might be and what exposure regimen (concentration × time) is required to produce this change. For this purpose, it is common to expose groups ($n = 10$) of male rats to three test concentrations. The highest concentration tested is set at one-fifth the ALC (or the LC_{50} depending on the steepness of the mortality dose–response curve) and the lower two would be one-fifteenth and one-fiftieth of ALC. It is desirable to have not only a concentration–response relationship but also a no-observed-exposure limit and a range of toxic effects. In subchronic tests a concurrent control group is required in addition to a vehicle control group.

Animals should be exposed to the test substance for 6 h day^{-1} on a 7 day per week basis for a period of at least 90 days. Another acceptable metho d is one in which rats are exposed 6 h a day for 5 days, given a 2 day rest period, and are again exposed for 5 days, and this is repeated 10 times. *In vivo* observations, including body weight measurements, are made daily. Following exposure, all rats are subjected to hematological, clinical blood chemistry, and urine analysis evaluations. Half of the rats are sacrificed at that time and complete pathological examinations including histological evaluations are conducted. The remaining rats are held without additional exposures for 2–4 and the parameters altered in rats sacrificed immediately following exposure are evaluated to determine the reversibility of the change(s).

Design of Subchronic Inhalation Study

Test species	Rat
Sex	Male
Number of test groups	3(1/5, 1/15, 1/50 ALC)
Number per group	10
Exposures	6 h day^{-1}, 5 days week^{-}, 2 weeks
Animal sacrifice	Five per group after 10th exposure Five per group after 14 day recovery period

Parameters measured: Growth and *in vivo* responses, clinical pathology, urine analyses, gross pathology with organ weights, microscopic pathology, and chemical index of exposure (where possible).

A variation of this design uses an increasing exposure regimen that continues until severe biologic effects are observed. This provides target organ toxicity data using fewer animals (only one group is treated), but the quantitative aspects can be masked in cases in which chemical buildup in the body occurs or change occurs only after some protective function in the body has been depleted. In both of the subchronic studies, the importance of adequate concurrent control animals needs to be underscored.

In this variation, a satellite group of 20 animals (10 animals per sex) may be treated at the high concentration level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for a posttreatment period of appropriate length, normally not less than 28 days. In addition, a control group of 20 animals (10 animals of each sex) should be added to the satellite study. Animals in the satellite group (if used) scheduled for followup observations should be kept for at least 28 days further without treatment to assess reversibility.

Chronic Studies

Chronic studies are conducted to determine effects of long-term exposures at levels where acute toxicity is not obvious. Chronic exposure patterns generally follow those encountered in the workplace – animals are exposed 6 h day^{-1}, 5 days week^{-1} for their lifetime. For environmental contaminants, continuous exposures of 23 h day^{-1} (allowing 1 h day^{-1} to feed the animals and clean the exposure chambers) for 7 days week^{-1} might be considered more appropriate.

In both chronic study types, exposures are designed to be as constant as possible with minimal deviation from the target or design concentrations.

Dose Quantitation and Effects

In practice, the relationship between dose and lethality for most chemicals is approximately lognormal, but skewed toward hypersensitivity. However, when this frequency population is transformed to a logarithmic abscissa, a normal distribution generally results. When exposure is by inhalation, calculating dose is not straightforward. It is based on knowledge of two variables: How long an animal was exposed and the concentration of material in the atmosphere? What volume of the atmosphere was inhaled by the animal or how much of the material in the inhaled volume was absorbed are not known. As a result, the traditional approach has been to express exposures in terms of concentrations and times of exposure. For most acute exposures, Haber's rule generally holds. That is,

$$ct = K$$

where c is the concentration, t is the time of exposure, and K is the constant specific for that material.

However, in a dynamic exposure situation, the initial concentration in a chamber is clearly lower than the final concentration. It takes time for the atmosphere to equilibrate to a concentration at or very near the desired target concentration. This equilibration time can be calculated as

$$C = (w/b)[1 - \exp(bt/a)]$$

where C is the desired chamber concentration, w is the weight of material introduced per unit time, b is the total airflow through chamber, t is the time, and a is the chamber volume.

A second complication to expressing concentration is that, for gases and vapors, it is properly expressed as parts per million (ppm). Interconversions can be calculated with the formulas

$$\text{mg l}^{-1} = \text{g m}^{-3} \quad \text{mg m}^{-3} = (\text{ppm})\ (\text{MW})/24.5$$

where MW is the molecular weight.

One consideration in model selection is the comparability of doses received to those likely in humans. In the special case of the inhalation route, doses received must be calculated (rather than measured) in a manner somewhat specific to the animal models being employed.

Calculated inhalation dosimetry models, thought not extremely accurate, do have some utility in the cases of (1) comparing toxicity via the inhalation route with toxicity via other routes, (2) risk assessment models and calculations, and (3) interspecies calculations and extrapolations.

These calculations are performed using the formula

$$E = [\mathrm{RF} \times \mathrm{TV} \times C \times 60 \times T]/1000$$

where E is the total maximum possible exposure, RF is the respiratory frequency (per minute), TV is the tidal volume in milliliter, C is the concentration of test agent in milligrams per liter, and T is the daily exposure time in hours. Note that this formula can also be used to compare total doses received over different lengths of exposure. If exposure is repeated over a period of several days, the result of the previous calculation is also multiplied by the number of days of exposure.

Values to be used in this equation for the laboratory species commonly used in inhalation studies and humans are as follows:

Species	*RF*	*TV*	*Hourly exposure*
Rat	85.5	0.86	4.4118 liters × *C*
Mouse	109	0.18	1.1772 liters × *C*
Guinea pig	90	1.8	9.720 liters × *C*
Rabbit	49	15.8	46.452 liters × *C*
Human	11.7	750.0	526.5 liters × *C*

Using this model, the values obtained will be the maximum average limits for the dose received. A number of factors that are not included will affect the actual values, generally serving to reduce the actual values of doses received. These factors include the following:

1. There will be variations in individual animals (and in the same animal at different ages, weights, and states of exercise) in RF and TV.
2. If the material is a particulate or is water insoluble, the degree of deposition and clearance (respectively) in and from the lungs will vary.
3. The degree of absorption from the lungs into the body will vary from compound to compound.

Additional Methods

Intratracheal instillation of materials is a popular alternative to inhalation exposure of animals for studying substances absorbed through the lungs. The advantages of this type of exposure include: very small amounts of test agent are needed (a safety feature in terms of handling and containment of the chemical), extensive chambers are not required, and the complex technical support needed to generate and maintain experimental exposure conditions is avoided. These factors make this type of study very inexpensive to conduct. Furthermore, the dose can be delivered very precisely to the respiratory tract tissues. However, dose distribution to the respiratory tract tissues does not accurately simulate an inhaled dose and, hence, does not reflect the real-life response very closely. Inhalation of airborne toxins generally results in a relatively well-distributed dose throughout the respiratory system. Intratracheal instillation tends to lead to a less uniform deposition and to favor the lower portions of the lung due to gravimetric settling of material. Rats and hamsters were exposed to radioactive particles and the distributions following both inhalation and instillation were examined. The resulting distributions were strikingly different with instillation producing heavy deposits in the medium-sized bronchi. Instilled materials seldom reached the alveoli, whereas inhalation led to considerable deposition in the small airways. High local concentrations following instillation can lead to localized tissue damage which would not be seen following more uniform deposition. The use of this technique then is basically limited to situations in which tissue reactions (both of an acute (inflammation) and chronic (neoplasia and fibrosis) nature) to a variety of materials are to be compared side by side.

Other inhalation toxicology test methods that are less invasive or potentially harmful include:

- noninvasive lung function tests in spontaneously breathing animals (now mandatory under ICH S7A for new pharmaceuticals before they go into humans);
- bronchoalveolar lavage (BAL);
- cell and/or organ cultures of nose, trachea, and lungs; and
- lung slices of different species including man.

See also: Analytical Toxicology; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Levels of Effect in Toxicological Assessment; Occupational Toxicology; Pollution, Air; Respiratory Tract; Toxicity, Acute; Toxicity, Chronic; Toxicity, Subchronic.

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http://www.oecd.org – OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 39. See also OECD Guideline for Testing of Chemicals: Proposal for a New Guideline: 433; Acute Inhalation Toxicity – Fixed Concentration Procedure.

Toxicity Testing, Irritation

Pertti J Hakkinen

Several entries in this encyclopedia address eye and skin toxicology, and the testing approaches used to assess irritation as an endpoint. The most progress in the development of nonanimal procedures has been for the assessment of local toxicity, for example, eye and skin corrosion and irritation, and many alternatives to animal testing are now available to assess the eye and skin corrosion and irritation potentials of chemicals. Work on the development and validation of additional alternatives to animal testing methods is continuing internationally.

The European Centre for Validation of Alternative Methods (ECVAM) and other organizations have developed and/or recommended tiered testing approaches and strategies to assess eye and skin corrosion and irritation potential. These tiered approaches use *in vitro* methods and other nonanimal approaches, for example, structure–activity relationship (SAR) models. A tiered testing strategy is now

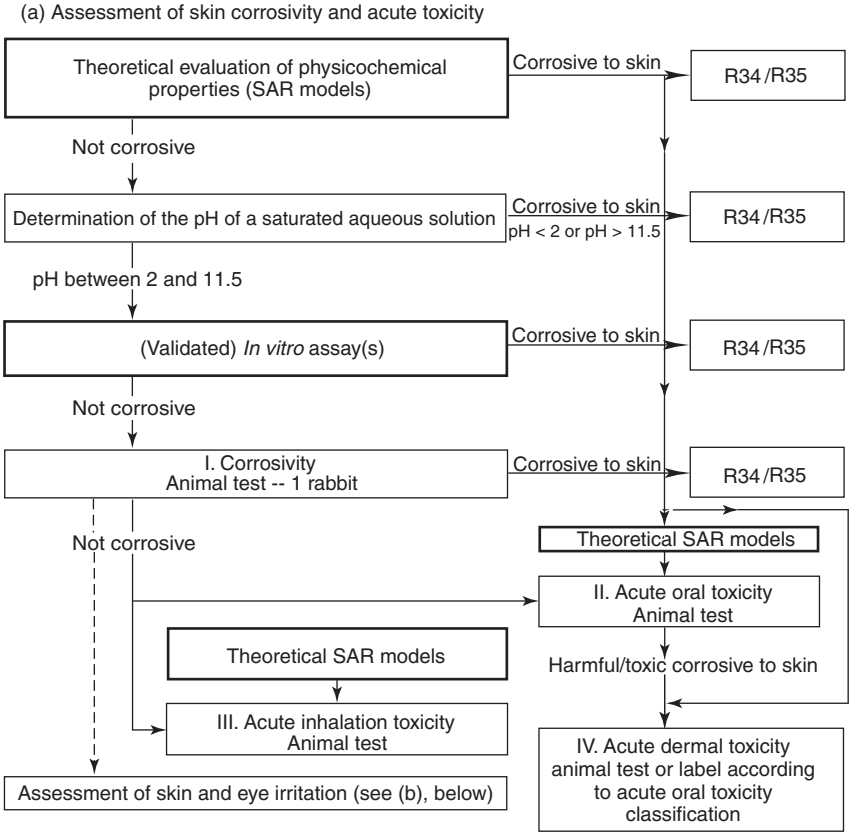
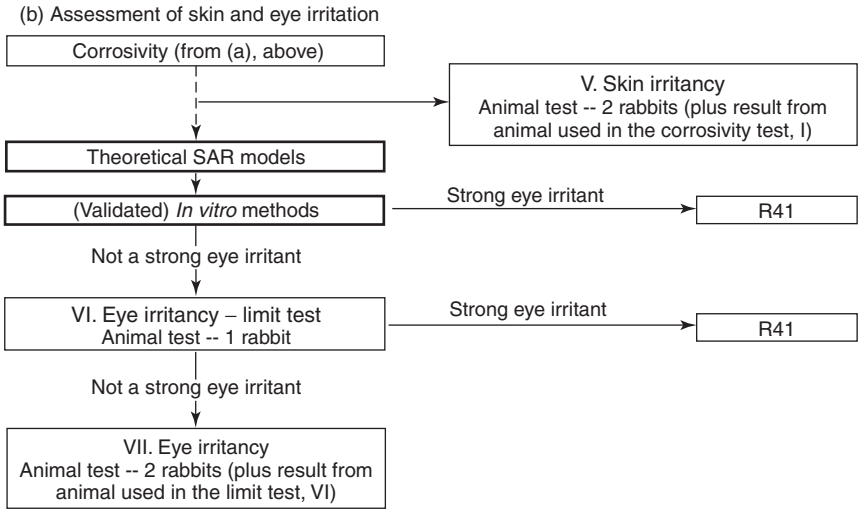


Figure 1 Example of a testing strategy for acute toxicity, corrosivity, and irritancy. (From the European Commission, Institute for Health and Consumer Protection, European Centre for Validation of Alternative Methods (ECVAM) (1995). The Integrated Use of Alternative Approaches for Predicting Toxic Hazard. The Report and Recommendations of ECVAM Workshop 8.)



Strategy for the intoduction of alternative methods (SAR models, *in vitro* tests) into acute toxicity, corrositivity, and local irritancy testing, developed following evaluation of data submitted to the Chemicals Department of the German Federal Institute for Health Protection of Consumers (I. Gerner, BgVV, Germany).
I–VII: Animal tests conducted according to EU/OECD test guidelines.
EU risk phrases: R34 = causes burns; R35 = causes severe burns; and R41 = risk of serious damage to eyes.
= *alternative approaches*

Figure 1 Continued

recommended by the Organisation for Economic Cooperation and Development (OECD), and the European Union (EU) has also adopted this approach. For confirmation of absence of local irritating effects, limited animal testing, however, may still be necessary to obtain the required certainty for the classification and labeling process and to demonstrate the absence of irritating effects. Further, some human eye and skin testing may be very useful in the safety evaluation of cosmetics, and for other consumer products and materials that come in contact with the skin or eyes.

Examples of these tiered testing approaches and strategies are shown in **Figure 1**.

In addition to the tiered testing strategies noted above for assessment of eye and skin effects, a tiered testing and assessment strategy for respiratory toxicity testing *in vitro* was proposed by ECVAM in 1996. This approach and strategy included assessment of irritation potential and other endpoints of cell injury. This included checking the existing data available for the test material itself, or on related substances, followed by acquiring knowledge on the physicochemical properties of the test material, followed by the use of computer modeling techniques (if available) to try to predict the likely toxic effects and target sites. 'First-phase' *in vitro* tests could then follow to identify likely target cells, using tracheal rings, lung slices, alveolar macrophages, or other types of cells. Cell morphology should be determined and crude assessments of the cellular energy status could be undertaken – the results may make it possible to do (semi)quantitative ranking studies of toxic potency. A second phase of *in vitro* tests could then be conducted on the basis of results obtained in the first phase of *in vitro* testing, choosing from tests using airway epithelial ciliated or nonciliated cells, Clara cells; Type II cells, alveolar macrophages; or other type(s) of cells.

See also: Animal Models; European Union and Its European Commission; Eye Irritancy Testing; In Vitro Test; In Vivo Test; Organisation for Economic Cooperation and Development; Respiratory Tract; Safety Testing, Clinical Studies; Toxicity Testing, Modeling; Toxicity Testing, Alternatives; Toxicity Testing, Dermal; Toxicity Testing, Inhalation.

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Relevant Websites

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http://altweb.jhsph.edu – Under the management of the Johns Hopkins Center for Alternatives to Animal Testing (CAAT), a diverse group of organizations serve on the Altweb Project Team, many of which maintain their own websites that provide key links from and to AltWeb. The intent of Altweb is to be "the online clearing house for resources, information, and news about alternatives to animal testing" and to serve as the most comprehensive resource on animal alternatives for scientists, educators, veterinarians, and individuals throughout the world.

Toxicity Testing, Modeling

Charles A Pittinger, Andrew Worth, Joanna Jaworska, and Joanne Shatkin

Structure–activity relationships (SARs) and quantitative structure–activity relationships (QSARs), are theoretical models that can be used to predict the physicochemical, biological, and environmental properties of substances. An SAR is an (qualitative) association between a chemical substructure and the potential of a chemical containing the substructure to exhibit a certain biological property or effect. A QSAR is a mathematical model that quantitatively relates a quantitative numerical measure of chemical structure (e.g., a physico-chemical property) to a physical property or to a biological effect (e.g., a toxicological endpoint).

QSARs are a tool used in the absence of available data for prioritization, classification and screening level risk assessment. In addition, QSARs are rapid, inexpensive, can offer a consistent approach, and help to focus on priorities in data gathering. A broad range of QSAR models can readily fill data gaps for assessing chemicals, particularly for fundamental physical – chemical properties, in an expedient and cost-effective manner. QSAR-based evaluations can provide a systematic and consistent approach to chemical evaluations involving large numbers of chemicals. Certain (Q)SARs and other types of theoretical models have gained broad acceptance by regulatory institutions and the private sector. Predictive models can reduce costs, time, and concerns related to conducting toxicity bioassays, for example, for animal welfare reasons, there is considerable pressure to minimize the reliance on animal testing to obtain the information on chemical effects. Input data requirements are generally modest and increasingly available through public and computerized databases.

The Broad Universe of QSARs

An SAR model qualitatively compares structurally similar chemicals for which a measured toxicological or environmental property or endpoint ('the

activity') is available to estimate the same property/endpoint for an analogous, untested chemical. In QSAR models, the endpoint is quantitatively related to a series of structurally similar chemicals (which are often related). The relationship may be continuous or categorical, and is typically developed by regression methods, classification methods (e.g., discriminant analyses and decision trees), or neural networks.

The broadest array of QSAR models is available for endpoints related to physical and chemical properties of chemicals, such as solubility, hydrophobicity, adsorptivity, and volatilization. Fewer are available for biological processes such as biodegradability and toxicity to nonmammalian organisms. Still fewer are QSARs for predicting mammalian and/or human health effects. This is due to several factors. Prediction of physical – chemical parameters often relies upon relatively well-characterized physical and chemical principles and processes. These may require less complex mechanistic understanding than biological processes involving enzyme kinetics and physiological interactions among multiple organs and organ systems (e.g., adsorption, distribution, metabolism and elimination (ADME) pathways). In addition, physical – chemical endpoints typically have a longer history of study, such that more homogeneous data sets are available for a broader range of chemicals.

QSAR Applications for Chemical Screening, Prioritization, and Regulatory and Corporate Decision-Making

QSAR predictions are used by regulatory authorities and private corporations and institutions in three major contexts: priority-setting, hazard classification and labeling, and screening for health and ecological risks of chemicals. Regulatory uses of QSARS include: (1) supporting priority setting of chemicals; (2) guiding experimental design of regulatory tests or testing strategies; (3) providing mechanistic information; (4) grouping of chemicals into categories based on similarity; (5) filling a data gap needed for classification and labeling; and (6) filling a data gap needed for risk assessment. Each application carries unique considerations for QSAR, with the most stringent considerations placed upon QSARs used for 'high regulatory impact', for example, risk assessments under mandated regulatory programs.

QSAR Reliability and Validity

The reliability of QSAR results estimates varies both with application and endpoint. Environmental fate endpoints based on predictions of physical/chemical parameters are the most common and best validated uses of QSAR, and results are relatively well accepted. Systemic toxicity endpoints are more complex, as toxicity is the net expression of multiple biological processes including ADME. Reliability of QSAR predictions for human health endpoints is generally regarded as less than those for non-mammalian (e.g., fish and invertebrates) toxicity endpoints, partly due to the limited availability of high quality data availability. (Nonmammalian species such as fish and aquatic invertebrates (e.g., *Daphnia, Ceriodaphnia*) and algae are typically less expensive to test, and the bioassays carry fewer legal and ethical concerns.)

In order to be considered for regulatory use, it is widely agreed that QSARs need to be assessed for scientific validity. Importantly, QSARs are generally not used as the sole information source upon which to base regulatory decisions. Empirical data are considered first, if available, and have greater reliability than QSAR predictions, unless there are explicit reasons to consider the data erroneous. Because of the inherent uncertainty in QSAR predictions and the need for conservatism in screening level management decisions, they are not recommended in decisions to support reduced concerns, to demote priorities, or to remove a chemical from a regulatory list of chemicals of concern. QSAR predictions are considered in the context of the weight of evidence from multiple sources (e.g., empirical bioassay data, monitoring, epidemiology, etc.). The collective evidence is often weighed on a case-by-case basis by trained experts, applying the best professional judgment.

Risk assessments with 'high regulatory impact' (e.g., enforceable standards such as new chemical registrations or litigation over contaminated sites) must be legally defensible, transparent and unbiased. Many commercial QSAR packages (e.g., TOPKAT, DEREK, MCASE) maintain proprietary and confidential training sets, algorithms, and software. As such, they can be challenged as legally indefensible in a court of law, due to lack of transparency. QSAR packages developed by public organizations, such as the US Environmental Protection Agency's (EPA's) EFAST and EPISUITE models, are typically more transparent. In many cases, they apply the same or similar peer-reviewed databases and algorithms used in commercial QSAR packages. There is currently a need for well-validated, public domain QSAR models for broad regulatory applications and decision support systems.

A number of principles for judging the validity of QSARs were proposed at an international workshop in Portugal in 2002, and hence have come to be

known as the 'Setubal Principles for QSAR Validity'. They include:

- *Endpoint Criteria*. QSARs should be associated with a well-defined endpoint with clear relevance to priority setting, risk assessment, or classification.
- *Descriptor Transparency Criteria*. QSARs should be associated with unambiguous structural descriptors and algorithms supported by available databases. Input data for some QSARs can be generated using outputs of other QSARs, thus propagating additional uncertainty in results. Risk management decisions based on model outputs may be perceived as sound, when in fact, key underlying assumptions may be flawed.
- *Mechanism Criteria*. QSARs should ideally have a physico-chemical or biological basis and toxicological pathway. Because mechanisms of chemical metabolism and intoxication in mammals are not known for the majority of chemicals, the validity feasibility of assessing toxicity endpoints is limited for many chemicals. Many experts contend that health endpoint QSARs should ideally only be applied to chemicals with a mechanism of action consistent with the domain of the training set. Interpolation within the domain is the best use; extrapolation beyond it may lead to spurious, indefensible results. Some models (e.g., TOPKAT) provide cautionary indications for predictions of chemical activity beyond the domain of the training set.
- *Applicability Domain Criteria*. The applicability domain of a (Q)SAR is the physico-chemical, structural, or biological space, knowledge or information on which the training set of the model has been developed, and for which it is applicable to make predictions for new compounds. Ideally, QSARs should only be used to make predictions within the applicability domain of the training set (i.e., interpolation versus extrapolation). Many chemicals and chemical classes do not conform to current QSAR models, as they extend beyond the domain inherent in the training data sets. These include polymers, reaction products, mixtures and inorganics.
- *Validation Criteria*. QSARs should include a measure of the goodness of fit as well as results from external validation of the QSAR, using independent data beyond the training set. The uncertainty and variability of underlying test data limits the precision, accuracy, and reliability of many QSAR predictions. Hence, large uncertainty factors are commonly applied to QSAR predictions requiring comparisons to protective human health benchmarks.
- *Transparency Criteria*. QSARs should be accompanied with full access to the data sets as well as the methods and quality assurance used to generate the data. The apparent sophistication, user-friendliness and flexibility of many publicly available computer-based models may at times convey a false sense of accuracy and a broader range of applicability than the underlying databases and algorithms would justify. Even well validated, technically robust assessment models can be subject to misuse.

QSARs for Predicting Physical – Chemical Properties of Chemicals

Physical – chemical QSAR models are available to predict a range of chemical properties including: melting point, boiling point, water solubility, biodegradability, vapor pressure, Henry's law constant, sediment adsorptivity, octanol–water partition coefficient, and half-life in the environment. These and other parameters can be readily predicted by EPISUITE (see Relevant Websites section), and enables batch data entry based on Chemical Abstract Service (CAS) numbers or SMILES notations.

QSARs for Predicting Environmental Fate and Transport

The physical – chemical parameters predicted in the QSAR models above are often used to estimate fate and transport of chemicals in the environment, a critical aspect of exposure analysis in chemical risk assessments. QSAR model predictions of physical and chemical properties of chemicals, together with empirical data, can be used as inputs to more sophisticated environmental fate models to predict chemical concentrations in source waters and drinking waters. An Organisation for Economic Co-operation and Development (OECD) website (see Relevant Websites section) lists predictive models for environmental fate and exposure pathways, including human health routes of exposure. Models such as EFAST (e.g., the Exposure and Fate Assessment Screening Tool, at the EPA website) are capable of incorporating multiple parameters in predicting fate and transport processes including wastewater treatment from point-source emissions, fate in the environment, concentration at drinking water intakes, atmospheric deposition, land runoff, soil leaching, groundwater migration, etc.

In addition, a wide range of QSAR models to predict biodegradability or persistence are summarized at the OECD website. QSARs models for

biodegradation are more limited in scope and accuracy than packages that predict physical – chemical parameters. The 'PBT Profiler' (Persistent, Bioaccumulative and Toxic) is a public domain QSAR package developed through the US EPA. EPISUITE and the PBT Profiler include estimates of biodegradability based on chemical similarity. More sophisticated but narrower models such as CATABOL (see Relevant Websites section) require mechanistic understanding of enzyme-mediated processes, similar to those available for predicting toxicity. CATABOL is an expert system software that predicts microbial biodegradation pathways and mineralization extent – key factors to determine environmental exposure of chemicals. Features of CATABOL are: (1) capability to predict aerobic biodegradation pathway and assess persistence of metabolites; (2) probabilistic assessment of the extent of biodegradation based on the entire pathway (not, as with other models, the parent structure alone); and (3) online documentation of $\sim$900 microbial transformations.

QSARs for Predicting Ecological Effects

QSARs for the prediction of toxicity to aquatic organisms, including fish, invertebrates and algae, are relatively well developed for a broad range of chemical classes. More than 100 SARs for 55 chemical classes are available in a free, downloadable model called ECOSAR from the EPA website, based on test data and assumptions from test data. Aquatic toxicity endpoints include; reproduction, growth and mortality, such as acute toxicity to fish, invertebrates, and algae. The PBT Profiler also estimates chronic toxicity to fish by means of the ECOSAR model; it compares the fish chronic value to maximum water solubility, in order to estimate potential for aquatic risk.

QSARs for Predicting Human Health Effects

A variety of QSAR models have been developed for human health endpoints and 'packaged' into user-friendly commercial or public-use programs. Human health hazard endpoints commonly predicted by QSAR models include: mutagenicity, carcinogenicity, teratogenicity, neurotoxicity, reproductive and developmental toxicity, skin/eye sensitization and irritation, and systemic toxicity. The more popular commercial QSAR packages for human health include The Open Practical Knowledge Acquisition Toolkit (TOPKAT), Multicase (MCASE), and the Deductive Estimation of Risk from Existing Knowledge (DEREK). Two general types of models can be distinguished: statistically based models such as TOPKAT, and rule-based models such as MCASE. Concise characterizations of these and other QSAR packages appear on the OECD website.

International Uses of QSARs by Regulatory Authorities

United States

A number of US regulatory agencies currently employ QSARs broadly in chemical screening, prioritization and decision-making. The New Chemicals Program in the US EPA's Office of Pollution Prevention and Toxic Substances (OPPTS) uses a variety of methods to make predictions that include QSAR, nearest analog analysis, chemical class analogy, mechanisms of toxicity, chemical industry survey data, and professional judgment. The models are used to identify possible chemicals of concern, for which additional experimental data may be requested from the notifying company through the OPPTS Sustainable Futures Program, new chemical submitters may 'fast track' the review process by use of the Pollution Prevention Framework, incorporating The PBT Profiler. EPA's Office of Pesticides Programs (OPP) is exploring the use of QSARs for nonactive (i.e., 'inert') components of pesticide formulations. The EPA has used QSAR on a discretionary basis under the Hazardous Waste Identification Rule to screen and prioritize some 4000 chemicals for PBT characteristics.

The TSCA Interagency Testing Committee (ITC) uses QSAR predictions, in combination with empirical data and professional judgment, to maintain a 'Priority Testing List'. Finally, ongoing investigative programs in EPA's Office of Research and Development (ORD) are in place to develop, validate and apply QSARs for various health, ecological, and exposure-related endpoints. ORD's National Center for Environmental Assessment is using TOPKAT to predict health effects associated with drinking water disinfection by products, among others. ORD's National Health and Environmental Effects Research Laboratory is developing receptor-binding QSAR models to predict endocrine disruption. ORD's National Center for Environmental Research Star Grant Program supports academic research in QSAR development.

European Union

The European Union (EU), like the US EPA, supports the use of QSARs for screening new chemicals. The EU's Technical Guidance Document provides extensive guidance for the use of QSARs (see Further

Reading section). Under the current EU legislation for New and Existing Chemicals, the use of QSARs is limited, probably because there has been disagreement in the scientific and regulatory communities over the applications of QSARs, and the extent to which QSAR estimates can be relied upon. However, under the future REACH (Registration, Evaluation and Authorisation of CHemicals) system, proposed by the Commission's White Paper on a Future Chemicals Policy, it is anticipated that QSARs will be used more extensively, in the interests of time- and cost-effectiveness and animal welfare.

The EU's Global Harmonised System of Classification and Labeling similarly recognizes the need for QSAR predictions of chemical properties. Within the EU, the Danish EPA has applied 'validated QSAR models' (i.e., TOPKAT, MCASE, and EPIWIN) to screen some 47 000 discrete organic chemicals included in the European Inventory of Existing Chemical Substances (EINECS) Directory of 100 116 chemicals. They identified 20 624 substances deemed to require classification for one or more of these 'dangerous properties': acute oral toxicity, dermal sensitization, mutagenicity, carcinogenicity, and danger to the aquatic environment. They have also developed a physical – chemical properties prediction database for some 166 000 chemicals.

Further, The European Commission's Joint Research Centre (JRC), Institute for Health and Consumer Protection in Ispra, Italy is partnering in several initiatives to establish an international framework for the development, validation, and implementation of QSAR models that are useful for regulatory purposes. Within the JRC, the work involves the European Chemicals Bureau (ECB) and the European Centre for the Validation of Alternative Methods (ECVAM, in addition to external partners, such as the OECD.

Canada

The Canadian Environmental Protection Act, 1999 (CEPA 1999) requires the Ministers of the Environment and Health to 'categorize' the substances on the Canadian Domestic Substances List (DSL). The DSL contains ~23 000 substances that are subject to categorization (i.e., prioritization). Generally the data selection process involves a search of the scientific literature and databases for quality experimental data for persistence, bioaccumulation potential and 'inherent toxicity' to humans and nonhuman species. If acceptable data are not found, QSARs or other models are used to estimate the persistence, bioaccumulation, and aquatic toxicity of substances based on structure and physical – chemical properties.

Other Countries

Other countries such as the Netherlands and the United Kingdom are also developing adopting and using developing QSAR models for the screening and assessment of new and/or existing chemicals. Models in use by these countries can be identified on the OECD website.

See also: Toxicity Testing, Alternatives.

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Relevant Websites

http://www.oecd.org – Organisation for Economic Cooperation and Development (OECD). Database on Chemical Risk Assessment Models.
http://www.epa.gov – Environmental Protection Agency (EPA) website. Freely distributed EPISUITE QSAR program. SARs for 55 chemical classes are available in a free, downloadable model called ECOSAR. The New Chemicals Program in the US EPA's Office of Pollution Prevention and Toxic Substances (OPPTS).
http://www.oasis-lmc.org – Website for PBT Profiler, a public domain QSAR package.
http://btu6.btu.bg – CATABOL: An expert system software that predicts microbial biodegradation pathways and mineralization extent.
http://www.accelrys.com – The Open Practical Knowledge Acquisition Toolkit (TOPKAT): A popular commercial QSAR package for human health.
http://www.multicase.com – Multicase website. Provides MCASE, a QSAR package.
http://www.chem.leeds.ac.uk – University of Leeds website. Provides Deductive Estimation of Risk from Existing Knowledge (DEREK), a popular QSAR package
http://ecb.jrc.it – The European Union's Technical Guidance Document.
http://www.unece.org – United Nations Economic Commission for Europe. The European Union's Global Harmonized System of Classification and Labeling of Chemicals.

Toxicity Testing, Mutagenicity

Robin C Guy

Background Information

Genotoxicity studies are used to aid in the detection of compounds that may lead to genetic damage and frequently to cancer. A significant proportion of chemical carcinogens have been shown to cause DNA damage. There are, however, other carcinogens, such as hormones, some metals, inert physical agents, and some other chemicals, that are believed to cause cancer by mechanisms other than interaction with DNA. Such carcinogens are not usually detected in tests for genotoxicity.

There have been numerous advances in genetic toxicology research that have led to assays to identify compounds that may cause genetic changes. Genetic damage is often classified into three groups, with different types of genotoxicity tests detecting different types of damage. The first group consists of gene mutations, including deletions or insertions of a few base pairs. The second group consists of chromosomal rearrangements, deletions or breaks (clastogenicity), as well as loss or gain of whole chromosomes (aneuploidy) or chromosomal segments. The third group consists of premutagenic damage, such as DNA adducts or DNA strand breaks, or changes reflecting cellular responses to damage, such as unscheduled DNA synthesis. The first two types of damage result in a permanent genetic change that can be transmitted to daughter cells after cell division, while the damage in the third group may be repaired prior to cell division (or the assay may measure evidence of that repair). The term genotoxicity refers to both mutation induction and DNA damage, while mutagenicity refers specifically to mutation induction at the gene and chromosome levels. Many of these assays have been widely used and a large amount of historical data exist both for individual laboratories and in the published literature.

The selection of the most appropriate assay to meet a specific requirement is dependent on a number of factors. These include the following:

- type of genetic alteration that is essential to detect;
- metabolic capability of the test system in relation to the structure of the chemical to be tested;
- proposed use of the test material and the anticipated level of exposure and distribution;

- predictive value of the assay in terms of mutagenicity and carcinogenicity;
- available expertise and facilities; and
- regulatory requirements, when appropriate.

Genetic Toxicology Testing Battery

Genetic toxicology tests are applied so that data are generated on the activity of a compound until a point is reached where an assessment of the probable mutagenic and possible carcinogenic hazard can be made with an acceptable degree of confidence. Since no single assay has proved capable of detecting mammalian mutagens and carcinogens with an acceptable level of precision and reproducibility, it is common practice to perform the assays in a battery, or specific series, of tests.

The International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use and the US Food and Drug Administration (FDA), Center for Food Safety & Applied Nutrition ('Toxicological Principles', *Redbook*, 2000) have published recommendations for a genetic toxicology battery and detailed information on how to conduct those studies. For specific guidance on the conduct of genetic toxicology tests, consult the Organization for Economic Cooperation and Development, US Environmental Protection Agency (EPA), ICH S2B, and US FDA *Redbook* guidelines.

ICH, FDA, and other organizations, such as the US EPA, have developed similar recommendations for the standard battery of genetic toxicity tests, although there are some differences regarding the second test. The battery includes testing for gene mutations in bacteria, testing for gene mutations in mammalian cells *in vitro* using a system that can also detect structural chromosome aberrations, and an *in vivo* test for chromosome aberrations. Note that, due to the specificity of testing methods, it is essential that the battery includes assays that detect both gene mutations and chromosomal aberrations.

The recommendations are following:

- a test for gene mutations in bacteria. This assay detects frameshift and point mutations that involve substitution, addition, or deletion of one or a few DNA base pairs; and
- an *in vitro* test with cytogenetic evaluation of chromosomal damage using mammalian cells. This test detects structural chromosomal aberrations; or
- an *in vitro* mouse lymphoma thymidine kinase with or without gene mutation assay. This assay detects point mutations, as well as large deletions, translocations, mitotic recombination/gene conversion, and aneuploidy. Because it can detect both point mutations and chromosome aberrations, the mouse lymphoma assay is preferred by ICH and FDA over an *in vitro* cytogenetics test, and is listed as the second component of the battery by EPA; and
- an *in vivo* test for chromosomal damage using mammalian hematopoietic cells. These *in vivo* tests detect structural chromosomal aberrations in the case of the mammalian bone marrow chromosome aberration test, and structural damage to chromosomes or damage to the mitotic apparatus in the case of the mammalian erythrocyte micronucleus test.

The assays that comprise the primary test battery are further described in the rest of this section. Other assays may be utilized to elucidate any observed effects, and are described in the next section.

The assay for gene mutations in bacteria is also known as the Ames bacterial reverse mutation test. This assay is relatively simple to perform, reproducible, and gives reliable data on the ability of a chemical to interact with DNA and produce mutations. Because of the ease of use, this assay is often used as an initial screening evaluation. Strains of *Salmonella typhimurium* and *Escherichia coli* are used that require supplementation with amino acids (histidine or tryptophan, respectively) for growth, due to specific mutations in the genes for the synthesis of these amino acids. Defined reverse mutations restore the ability of the bacteria to synthesize the specific amino acid. Different tester strains require different point mutations or frameshift mutations to revert to the wild-type phenotype (which is able to synthesize the amino acid), allowing the researcher to identify the type of mutation caused by the chemical. Procaryotes are very simple organisms, and a positive result in a bacterial assay does not necessarily indicate that the compound will induce similar effects in eukaryote cells. Similarly, a negative result does not invariably mean that the compound lacks mutagenic activity in eukaryotic cells or in intact mammals.

The mouse lymphoma assay is the preferred mammalian mutation assay, as it measures heritable genetic damage *in vitro* arising by means of several mechanisms in living cells and is capable of detecting chemicals that induce either gene mutations or heritable chromosomal events, including genetic events associated with carcinogenesis. (Note that the term 'heritable' mutations refers to mutations that can be inherited by the next generation of cells,

not necessarily mutations in germ cells.) The cell line used is the L5178Y mouse lymphoma cell. In these cell lines, the most commonly used genetic end points measure mutation at the thymidine kinase (TK) locus on the mouse chromosome 11b. In the early 1950s, while attempting to induce tumors in female DBA/2 mice by painting them with 3-methylcholanthrene, Dr. Lloyd W. Law at the National Cancer Institute isolated the L5178Y cell line. Later, in 1958, Dr. G. Fischer at Yale University was successful in getting the L5178Y cells to grow *in vitro*, using a semidefined medium (Fischer's medium). In the early 1970s, Clive *et al*. developed the mouse lymphoma forward mutation assay, which screens for mutations conferring resistance to the pyrimidine analog trifluorothymidine. Resistant mutants are visible as colonies in soft agar, with large colonies being due to gene mutations and small colonies reflecting chromosome deletions or other large chromosomal changes.

Either of the two common tests for chromosomal damage (clastogenicity) *in vivo* can be part of the standard testing battery. *In vivo* assays have a clear advantage over *in vitro* assays, since they include an evaluation of the effects of metabolism and other physiological functions and interactions. The chromosome aberration assay involves a direct observation of structural changes in chromosomes analyzed at the first mitotic division after exposure. This assay requires an experienced cytogeneticist to microscopically evaluate metaphase chromosome spreads, typically of bone marrow cells. The micronucleus assay is an *in vivo* or *in vitro* assay for the detection of chromosome damage using mammalian hematopoietic cells. Micronuclei are formed in polychromatic erythrocytes due to breakage of chromatin or chromosomes, from spindle fiber or chromosome abnormalities, or from an entire chromosome that may have lagged behind in anaphase. The micronucleus assay has the advantage of requiring less time and specialized skill than the metaphase analysis involved in evaluating chromosome aberrations.

Table 1 General genetic toxicology assays

Assays for gene mutations
Salmonella typhimurium reverse mutation
Escherichia coli reverse mutation
Gene mutation in mammalian cells in culture, including evaluation of mutations at
Hypoxanthine-guanine phosphoribosyl transferase (*hprt*)
A transgene of xanthine–guanine phosphoribosyl transferase (*xprt*)
Thymidine kinase (*tk*)
Drosophila sex-linked recessive lethal
Gene mutation in *Saccharomyces cerevisiae*
Mouse spot test
Assays for chromosomal aberrations
In vitro cytogenetic
In vivo cytogenetic
Micronucleus
Dominant lethal
Heritable translocation
Mammalian germ cell cytogenetic
Assays for DNA effects
In vitro DNA damage and repair, including DNA adducts, single-strand breaks
Unscheduled DNA synthesis
Mitotic recombination in *Saccharomyces cerevisiae*
Sister chromatid exchange

Additional Genetic Toxicology Assays

Although a great deal of effort was put into battery harmonization, there is no universal agreement on the best combination of tests for all purposes. There are other mutagenicity tests in use that may also be useful (**Table 1**). The choice of additional test(s) or protocol modification(s) depends on various factors. Compounds that contain structural alerts for genotoxicity are usually detectable in the standard test battery. However, compounds bearing structural alerts that have given negative results in the standard test battery may require some additional testing. When the standard test battery produces negative results with a chemical that falls within a class known to require special test conditions, then additional testing with appropriate test modifications should be performed. There are compounds for which standard *in vivo* tests do not provide additional useful information. This includes compounds that toxicokinetic or pharmacokinetic data indicate are not systemically absorbed and therefore are not available for the target tissues in standard *in vivo* genotoxicity tests. In cases where sufficient target tissue exposure cannot be achieved, it may be appropriate to base the evaluation only on *in vitro* testing. Alternatively, *in vivo* genotoxicity studies are often conducted using intraperitoneal injection to avoid complications related to poor absorption. Additional genotoxicity testing may be conducted to help determine the mechanism of action if the material was clearly negative in the standard test battery but was positive in carcinogenicity bioassay(s). Additional testing can include modified conditions for metabolic activation in *in vitro* tests or can include *in vivo* tests measuring genetic damage in target organs of tumor induction.

In addition to the mouse lymphoma assay discussed above, several other assay systems are available for evaluating gene mutations in eukaryotic

cells. These systems include gene mutation assays in a variety of mammalian cell systems, gene mutation assays in yeast, the sex-linked recessive lethal test in *Drosophila*, and *in vivo* gene mutation tests, such as the mouse spot test (a somatic cell-specific locus test) and assays in transgenic animals.

Cell lines used for *in vitro* mammalian cell gene mutation assays include the CHO, AS52, and V79 lines of Chinese hamster cells and TK6 human lymphoblastoid cells. In these cell lines, different spectra of genetic events are detected. The most commonly used genetic end points measure mutation at the genes for hypoxanthine–guanine phosphoribosyl transferase (*hprt*), a transgene of xanthine–guanine phosphoribosyl transferase (*xprt*), or thymidine kinase (*tk*). The *tk* and *xprt* genes are autosomal and appear to allow for the detection of genetic events (e.g., chromosomal exchange events) that are not detected at the *hprt* locus, which is located on the X-chromosome. This is because genetic damage that involves vital genes adjoining the *hprt* locus on the X-chromosome is likely to be lethal to the cell, while damage to vital genes in an autosomal cell will be compensated for by intact genes on the homologous chromosome (which lack functional *tk* or *xprt*). Also, the lack of a homologous chromosome in the case of the *hprt* gene may preclude mutations that arise via homologous recombination.

In vivo tests for gene mutations have historically been limited to evaluation of visible mutations (e.g., coat color) or evaluation of specific biochemical changes. However, the development of transgenic mice and rats has revolutionized the field of *in vivo* mutagenesis assays, making it theoretically possible to evaluate mutations in any tissue. The transgenic rodents contain a foreign DNA sequence, commonly the *E. coli lac* genes. After the animal is exposed to the chemical, the transgene is recovered from the animal tissue, and mutants are evaluated. For example, the *lac* genes are readily packaged into phage lambda, and mutant plaques can be identified based on color phenotype on appropriate media.

The same chromosome aberration assay described above can be used to detect numerical chromosome changes (i.e., aneuploidy) in bone marrow cells. Chemicals that cause chromosome damage in germ cells can be detected using *in vivo* assays, either the dominant lethal assay, a test for structural chromosome aberrations in spermatogonia, or a more preferred test, the mouse heritable translocation assay.

A number of assays are available that can detect premutagenic DNA damage and cellular responses to effects on DNA. The initiation of enzymatic repair of the damage involves degradation of the damaged part of the DNA and subsequent synthesis of a new, short strand of DNA to replace the degraded area. This synthesis is termed unscheduled DNA synthesis (UDS). The UDS assay may be conducted as an *in vitro* assay in primary or cultured mammalian cells or as an *in vivo/in vitro* assay. The sister chromatid exchange assay (SCE) detects reciprocal exchanges, at homologus loci, of DNA between two sister chromatids of a duplicating chromosome. Although this is a cytogenetic effect, SCEs are considered to be general indicators of mutagen exposure, analogous to DNA damage and repair assays, due to the uncertainties about the mechanism of their formation. Single-strand breaks in DNA are also indicative of DNA damage, and may be formed by chemical exposure or by the cell's response to the chemical. Mitotic crossing over (the exchange of segments of DNA between genes or between a gene and its centromere) and mitotic gene conversion (transfer of segments of DNA within a gene) can be investigated in the yeast, *Saccharomyces cerevisiae*. While these two end points occur at a low level in untreated cells, they occur at an increased level in yeast cells exposed to DNA-damaging agents, partially as part of the DNA repair response. DNA adducts provide direct evidence that a chemical interacts directly with DNA, but they are not proof of mutagenicity, since an adduct may be repaired prior to the formation of a mutation. Different adducts may have different rates of repair, so the most common adduct produced by a chemical may not be the most important for prediction of mutagenesis or carcinogenesis.

Factors to Consider in the Conduct of Assays

To ensure that the results of an assay are valid, specific criteria have been determined. Care must be taken to follow the published procedures to ensure that the genetic structure of the test organisms meets the requirements of the particular assay. The test material must be able to reach the molecular target (e.g., DNA) in the cell in its reactive form.

Many mutagenic materials are not able to interact with DNA until they have undergone some degree of enzyme-mediated biotransformation. Tests conducted *in vitro* generally require the use of an exogenous source of metabolic activation. This metabolic activation system simulates the metabolic characteristics of a mammal under *in vivo*

conditions. Therefore, a typical assay should determine the chemical's mutagenic potential in the absence and presence of an exogenous metabolic activation system (S9), a rat liver homogenate prepared from the livers of rodents treated with enzyme-inducing agents such as Aroclor 1254. Concurrent testing should be done with negative (solvent) and appropriate positive controls both in the presence and in the absence of S9.

For all studies, care should be taken to avoid conditions that would lead to results not reflecting authentic mutagenicity. Positive results that do not reflect authentic mutagenicity may arise from changes in pH, osmolality (including very high concentrations of the test article), extended exposure to S9, or high levels of cytotoxicity.

Cultures of established cell lines or cell strains should be used. Mammalian cell lines should be determined to be mycoplasm-free and should be karyotyped. This is to ensure that they will respond in the expected fashion in the experimental system being used.

Regulatory agencies and testing laboratories have developed standard criteria for accepting many of the established genotoxicity assays. Negative controls should exhibit some minimal level of viability (e.g., plating efficiency for cell lines), and mutagenicity in the negative control should be within accepted limits. Appropriate positive controls should be used to test both the activity of the S9 and the specific cell line or strain, and should produce a response above some specified minimum level. The test chemical should be evaluated up to sufficiently high doses, or limits of solubility or cytotoxicity, taking into account the caveat about excessive cytotoxicity mentioned above. The sample size and number of replicates should be sufficient for adequate sensitivity, and, for *in vivo* studies, it is desirable to show that the test compound reaches the target tissue.

Once the overall assay conduct has been determined to be acceptable, the assay is evaluated for a positive response. A variety of aspects of the response to the test chemical are considered in evaluating the response. These include the magnitude of the response, the statistical and biological significance, reproducibility (both in replicates within the assay and in repeated independent assays), and the presence or absence of a dose–response. Based on these considerations, an overall evaluation is made.

Interpretation

The carcinogenic process is an extremely complex system. It is apparent from the relative simplicity of short-term assays that they cannot copy all of the stages in the carcinogenic process and are frequently assumed to detect only the event leading to the initiation phase, that is, the ability to induce a mutagenic or clastogenic DNA lesion. Although the short-term assays provide useful qualitative information, considerable caution is required in their interpretation in terms of carcinogenic activity. Results of the mutagenicity tests should be considered together with data from other toxicity tests and pharmacokinetic studies.

See also: Chromosome Aberrations; Environmental Protection Agency, US; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Genetic Toxicology; Good Laboratory Practices (GLP); Harmonization; Host-Mediated Assay; Immune System; International Conference on Harmonisation; Micronucleus Assay; Mouse Lymphoma Assay; National Institutes of Health; National Toxicology Program; Organisation for Economic Cooperation and Development; Redbook; Sister Chromatid Exchanges; Toxicity Testing, Alternatives.

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http://www.bgvv.de – Working Group on Genetic Toxicology Documents (from the German Federal Institute for Risk Assessment).

Toxicity Testing, 'Read Across Analysis'

Pertti J Hakkinen

Testing requirements often mandate that certain sets of toxicological information have to be provided for new substances (e.g., 'base sets' or 'Screening Information Data Sets'). While the data typically come from animal studies and *in vitro* alternatives to animal testing, they can also come from use of modeling, and from human clinical or epidemiological data. A further source of information can be derived from 'read across' evaluations or analyses of the data sets available for structurally similar substances. The 'read across' approach has been accepted by some regulatory authorities, and is based on the understanding that substances with similar physicochemical property profiles will generally have similar toxicity profiles.

The focus of the read across evaluation approach is on interpolation rather than extrapolation, and the rationale and data sources for the read across evaluation should be documented. For example, a read across table of data could have the related chemicals as columns, and the various types of toxicology tests and their results as the rows under each substance. Reading across the columns will highlight the amount and types of data for the group of substances. The read across evaluation will also find any gaps in the data set for a specific chemical that might be judged by the reviewer(s) to be filled by data relevant to those data gaps for the similar substances.

The read across evaluation can reduce the extent of testing required for substances within the group. There is, of course, expert judgment involved. In addition, thoroughness and skill are needed in making sure all relevant substances and their available data sets have been identified for the read across. Another issue is that the read across data should be developed and presented according to the guidelines of the relevant regulatory or other organization. Further, it should be indicated whether the data for each study for each substance were developed by current, established testing protocols and, if so, which guideline (e.g., those from the Organisation for Economic Cooperation and Development)? If established testing protocols were not used in some instances, the protocols used to conduct the studies should be at least described to the extent that they can be reviewed to understand their design, strengths, and limitations as the read across evaluation is performed.

A number of issues should be considered when assessing the toxicological properties of a new substance and similar substances by read across evaluation. These issues include assessing the similarity of the purity and impurity profiles of the new substance and the similar substances. This is important since there should be no toxicologically meaningful differences in the purities or impurities on a scale that would be likely to influence the overall toxicity. Further, the physicochemical properties of the new substance should be compared with the similar substances. This includes the physical form, molecular mass, water solubility, partition coefficient, and vapor pressure. In addition, the likely toxicokinetics of the substances, including the possibility of different metabolic pathways, should be considered.

An example of a read across table of information is shown below. Substances X_1, X_2, X_3, X_4, X_5, X_6, X_7, and X_8 are structurally similar substances. For this example, the main structure could be CH_3–C_x–CH_3, with the only difference being the length of the C_x section of the molecule. X_1 would be one carbon, X_2 would be two carbons, etc., and thus X_2 and X_4 would be the closest in structure to X_3, and X_4 and X_6 would be the closest in structure to X_5. Note that acute oral toxicity data have been identified for X_3 and X_4, while X_5 is supported by read across from X_4 to X_6, with data also identified for X_1 and X_8. For the *in vivo* genetic toxicity (i.e., micronucleus test), no data are available for X_3, X_4, and X_5; however, these substances are supported by read across from X_2 to X_6, with data also available for X_1 and X_8. Analysis of these data sets might lead to a judgment that a basic level of acute, genetic, repeat dose, and reproductive toxicity information is available for all of these substances, either directly or by read across to the other structurally similar substances.

Example of a Read Across table of available studies

Type of toxicity study	*Substance X_3*	*Substance X_4*	*Substance X_5*	*Substances similar in structure to X_3, X_4, and X_5*
Acute toxicity				
Oral	✓	✓	RA	✓ (for substances X_1, X_6, and X_8)
Dermal	RA	RA	RA	✓ (for substances X_1, X_2, X_6, and X_7)
Inhalation	✓	✓	✓	✓ (for substances X_1, X_2, X_6, and X_8)
In vitro genetic toxicity				
Bacterial	✓	✓	RA	✓ (for substances X_1, X_6, and X_8)
Cytogenetics	✓	✓	✓	✓ (for substances X_1 and X_8)
In vivo genetic toxicity				
Micronucleus	RA	RA	RA	✓ (for substances X_1, X_2, X_6, and X_8)
Repeat dose toxicity	✓	✓	RA	✓ (for substances X_1, X_6, X_7, and X_8)
Reproductive toxicity screen	✓	✓	RA	✓ (for substance X_6)

✓ = One or more studies available
RA = Read Across

See also: High Production Volume (HPV) Chemicals; Toxicity Testing, Alternatives; Toxicity Testing, Modeling.

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Toxicity Testing, Reproductive

Rochelle W Tyl

Introduction

It is currently estimated that ~15% of couples are clinically infertile (no conception after 1 year of unprotected intercourse); ~30% of the infertility is attributable to the male partner, 20% to the female partner, 20% to a combination of problems in both partners, and another 30% is not explained by diagnosis of adverse conditions in either partner. Once conception has occurred, up to 80% of human pregnancies may be lost, most in the first trimester. Reproductive toxicity may be defined as an adverse effect on any aspect of male or female reproductive structures or functions, on the developing offspring, or on lactation, which would interfere with the development of normal offspring through sexual maturity, in turn capable of normal reproduction. This definition includes aspects of developmental toxicity, including teratogenesis, and developmental neurotoxicity. This entry focuses on the male and female mammalian reproductive systems, chemicals that affect the status and functions of these systems, and tests currently utilized to detect such effects.

Male and Female Reproductive Systems

The reproductive system in the embryo consists of paired gonadal ridges in the dorsal midline containing all components but the gonial cells; the gonial cells differentiate during embryogenesis, external to

the embryo in the yolk sac, and migrate into the embryo and into the gonadal ridges along prescribed routes. The gonial cells in transit number in the hundreds; once they arrive, they proliferate, and the gonad develops into sex-specific structures.

Male System

The mammalian male reproductive system consists of the testes and associated structures: epididymides, vas deferens, accessory sex glands (seminal vesicles, prostate, Cowper's (bulbourethral) glands, and preputial glands), and intromissive organ (the penis) (**Figure 1**). The testis consists of two major compartments: the seminiferous tubules and the interstital compartment. The seminiferous tubules contain the spermatogonial cells, which differentiate into the spermatozoa (sperm), and the Sertoli cells, which provide support and nutrition to the developing sperm, produce androgen-binding protein to bind the male sex hormone testosterone (and produce a glycoprotein in the conceptus and neonate which suppresses female development), and which maintain the blood–testis barrier. The interstitial compartment contains Leydig cells which produce testosterone and other androgens for transport within and outside the testis.

The androgens control spermatogenesis, the growth and activity of accessory sex glands, and external masculinization. Interestingly, 17-β estradiol (the endogenous potent estrogen), made locally in the male brain, determines male-specific behaviors. *In utero*, androgen production by the fetal testis early in development (e.g., weeks 4–6 in humans) is essential for sexual differentiation of the gonads triggering male development and repressing female development (along with a product of the Sertoli

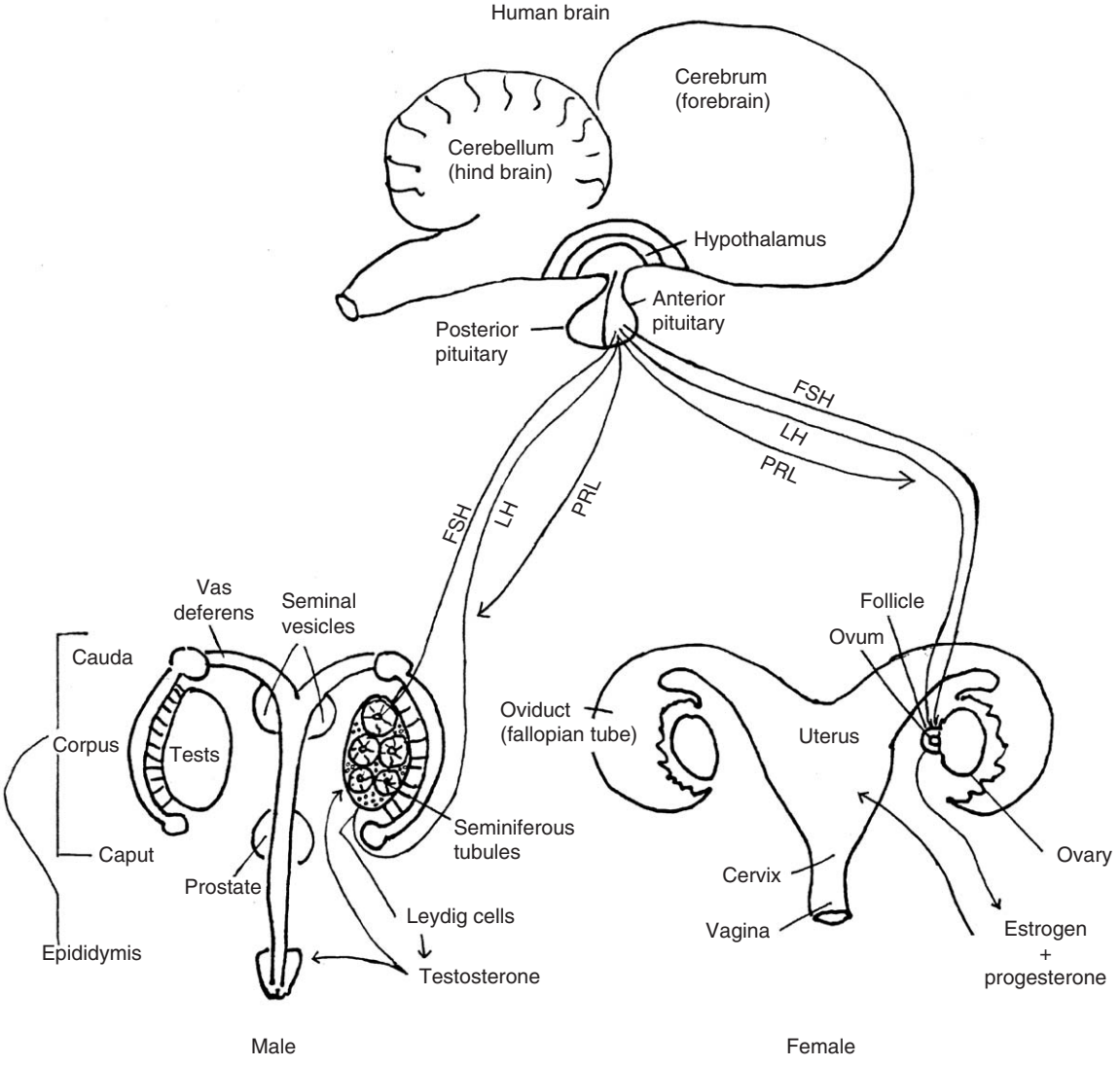


Figure 1 Male and female reproductive systems with hormonal controls from the brain (hypothalamus) and anterior pituitary gland.

cells). The developmental process in the indifferent gonad in the male is triggered by the activation of a gene on the Y chromosome that causes the interstitial cells in the gonad to differentiate into Leydig cells and produce testosterone. This in turn triggers the cascade of gene activations to direct development of male-specific organs. The predominant masculinizing hormone *in utero* is dihydrotestosterone, produced locally in the testis from testosterone by the enzyme 5-α-reductase. The increased presence of testosterone during puberty in males triggers the development of male secondary sex characteristics and the initiation of spermatogenesis.

The postnatal control of testicular function is via the hypothalamus–pituitary–testis axis (**Figure 1**). In the hypothalamus of the forebrain, neuroendocrine neurons secrete gonadotrophic hormone-releasing hormone (GnRH) into the anterior pituitary gland. Here GnRH stimulates release into the blood of the two gonadotrophic hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), named for their first-discovered roles in the female reproductive system. Prolactin (PRL) is also released into the blood from the anterior pituitary under control of dopamine from the median eminence in the forebrain. FSH and LH act on the testis: FSH stimulates the Sertoli cells to enhance sperm production, and LH acts on the Leydig cells to stimulate synthesis of testosterone, which in turn is required in high concentrations in Sertoli cells for sperm production. PRL acts to enhance the effects of LH on the testes.

Spermatogenesis (the process which produces mature sperm) in the testis begins at puberty in mammalian males, when high blood levels of FSH and LH are attained (**Figure 2**). The spermatogonial cells (diploid) at the periphery of each seminiferous tubule undergo repeated mitoses; one 'daughter' cell in each cell division replaces the original spermatogonial cell, and the other daughter cell becomes a primary spermatocyte and begins the process toward production of sperm. Each primary spermatocyte undergoes meiosis I (the first reduction division) to form two secondary spermatocytes; each secondary spermatocyte undergoes meiosis II (the second reduction division) to form two spermatids. Each spermatid undergoes a differentiation process, termed spermiogenesis, to produce a mature sperm (haploid); this process involves compaction of the DNA into the headpiece of the sperm, covered by an acrosomal cap (used to penetrate the egg); formation of a midpiece with mitochondria (to fuel the swimming function of the flagellum); and formation of a flagellum (tail) to drive the sperm. The process produces, from one primary spermatocyte, four functional sperm. The sperm are passively moved from the center (lumen) of each seminiferous tubule into the epididymis where the sperm acquire the capacity for movement and fertilization – the capacitation process. The epididymides, seminal vesicles, and prostate secrete fluids to nourish and capacitate the sperm, to provide the hydrodynamic force for ejaculation, and to neutralize the acidic environment of the female's reproductive tract. Large numbers of sperm are produced in waves, with 10 000–10 million ejaculated per time in males. The process of spermatogenesis takes ~ 5 weeks in mice, 8 weeks in rats, and 10 weeks in humans and occurs continuously from puberty until death.

Female System

The female reproductive system consists of the ovaries, oviducts (fallopian tubes), uterus, cervix, and vagina (**Figure 1**). The ovary consists of oocytes, follicles (containing thecal and granulosa cells) where the oocytes develop, and support cells. *In utero* development of female internal and external structures is by 'default'. In the absence of testosterone, dihydrotestosterone, and Müllerian-inhibiting substance (all made in the testes), male reproductive anlagen regress and female anlagen differentiate into the appropriate female reproductive structures. The prenatal female (at least in rodents) does not make 17β-estradiol in either her ovaries or adrenal glands. The follicles do produce estrogen and progesterone during the reproductive period of the female (in humans from puberty to menopause).

The control of ovarian function, beginning at puberty, is via the hypothalamus–pituitary–ovary axis (**Figure 1**). As with the male, cells in the hypothalamus secrete GnRH in the female that acts on the anterior pituitary to release FSH and LH in a cyclical pattern (PRL is also released). FSH and LH act on the ovary; FSH stimulates the growth of follicles which in turn secrete estrogen and progesterone to prepare the uterus for implantation of the fertilized egg (zygote), and LH, in a mid-cycle surge, triggers the rupture of the follicle and release of the ovum (ovulation). PRL plays a role in the maintenance of the corpus luteum (the collapsed follicle after ovulation which produces estrogen and progesterone), the rupture of the follicle, and in lactation.

Oogenesis (the process that produces mature ova) begins at puberty in mammalian females (**Figure 2**). Note that the series of mitoses of oogonial cells occurs only *in utero*; all the primary oocytes a female will have ($\sim 500\,000$ in humans, most of which will die) are present in her ovaries prior to her birth. *In utero*, each primary oocyte proceeds through the second (of four) phases of meiosis I and waits until puberty and the onset of the cyclical release of FSH

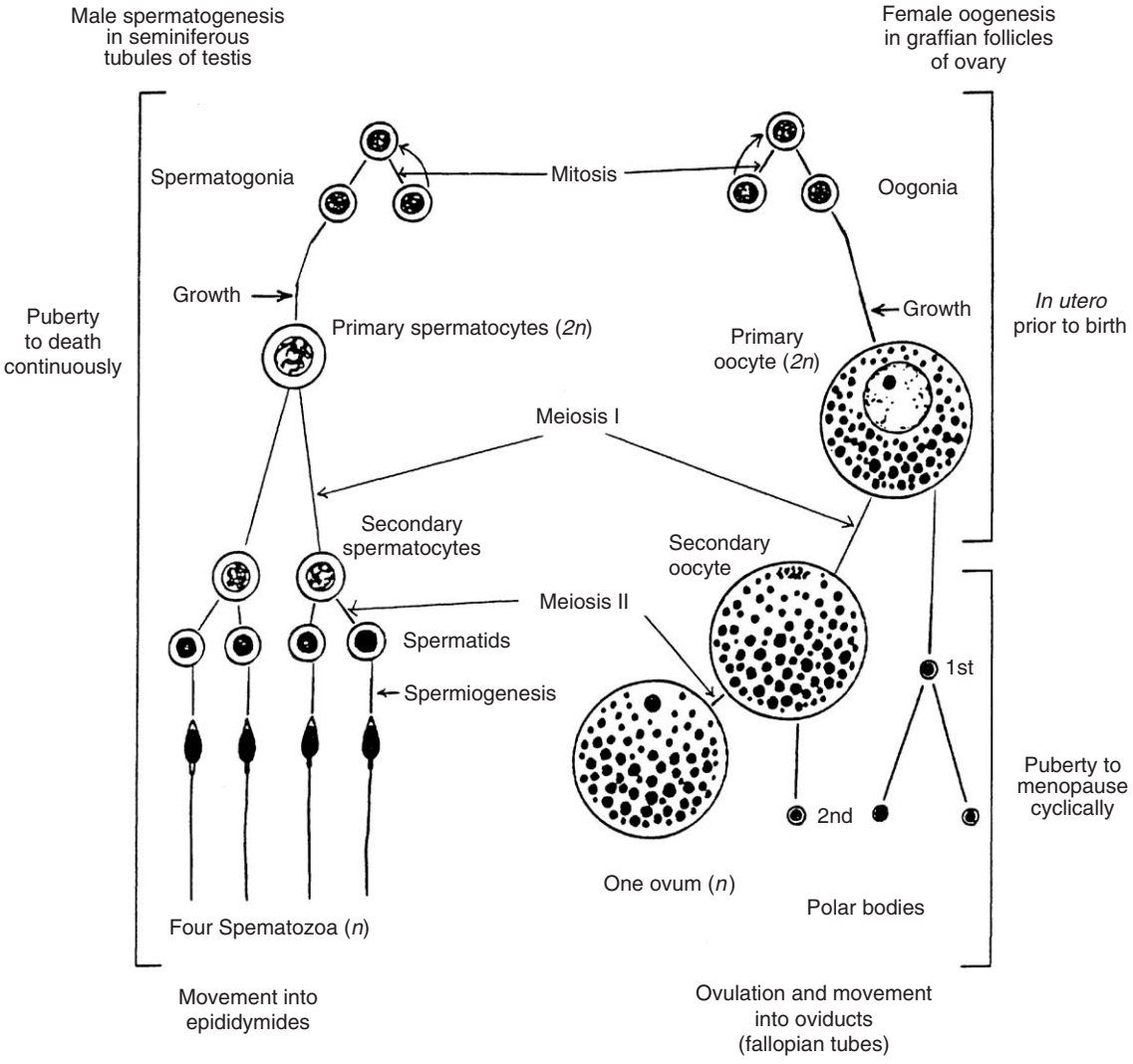


Figure 2 The process of male spermatogenesis and female oogenesis.

and LH. Beginning at puberty, a number of follicles begin the process of oogenesis during each cycle. In each follicle, the primary oocyte completes meiosis I to produce a large secondary oocyte and a very small first polar body. The secondary oocyte (and in some species, the first polar body) undergoes meiosis II to produce a very large ootid (haploid) and a second polar body (if the first polar body divides, it produces two secondary polar bodies). The objective of the 'lopsided' division, and the functions of the layers of accessory nurse cells which surround the developing oocyte in the follicle, is to produce a very large ovum, filled with nutrients and preformed genetic blueprints to sustain the early embryo. In response to the LH surge, the most advanced follicle(s) ruptures (the other ova die in a process termed atresia) and the mature ovum (or ova) is released into the oviduct; the collapsed follicle becomes a corpus luteum with endocrine functions (producing estrogen and progesterone to sustain the uterine lining). One or more eggs is ovulated during each cycle (up to 24 in rodents). The associated uterine cycles (termed estrous cycles in mammals with little or no postovulation luteal phase, and termed menstrual cycles in mammals with a long luteal phase; e.g., humans) build up and then shed the uterine lining if the egg or eggs are not fertilized and last a few days (rat and mouse) to several weeks (dogs, humans, and horses). Estrus, at the time of ovulation of the egg(s), corresponds to the period of female receptivity (high levels of circulating estrogens and LH surge) in species that undergo periodic 'heats'. In humans, there are ~ 500 ova ovulated from puberty to menopause.

Fertilization and Offspring Development

The mature ovum is released from the ruptured follicle and is drawn into the oviduct. The egg is

transported from the ovary to the uterus by movement of cilia at the opening of the oviduct, muscle contractions of the oviduct, and subsequent fluid movement; fertilization takes place in the oviduct. Meanwhile, the uterus, in response to preovulatory estrogen production from the follicle, increases blood flow and the uterine lining (endometrium) begins to thicken. After ovulation, the actions of progesterone (and estrogen) complete the growth of the endometrium including increased blood supply, formation of microvilli, increased synthesis of proteins, and other sources of nutrition.

Fertilization consists of penetration of the ovum by a sperm and union of the two haploid nuclei (one from each gamete); early cell divisions then begin (cleavage). The fertilized egg (zygote) continues to travel down the oviduct into the uterus and implants in the receptive uterine lining. The time from fertilization to implantation is relatively short and comparable among mammalian species: 5 (mice) to 8 (humans) days. Implantation is accomplished by the invasive destruction of the uterine lining by the outermost extraembryonic cells of the conceptus (trophoblast cells) and ultimate intimate association of these cells with the maternal uterine blood vessels; the uterine lining heals over the conceptus and the exchange of nutrients and wastes begins at the site of the future placenta. Once implantation is complete, the conceptus proper begins to differentiate into outermost (ectoderm), innermost (endoderm), and middle (mesoderm) cell layers and the major organ systems begin to form. This period of organogenesis lasts ~10 days in rodents and 58 days in humans. At the end of organogenesis (signaled by the closure of the secondary palate), the conceptus is termed a fetus and the fetal period of histogenesis (differentiation of cells and tissues within systems) begins. It lasts until parturition (birth), ~7 days later in rodents and 7 months later in humans. Delivery occurs after ~19–22 days of pregnancy in rats and 270 days in humans; the perinatal period involves adjustments of the offspring to air breathing, nursing, and rapid growth, development, and learning in a gravity-based environment, aided by required postnatal care by the mother (maternal behaviors such as pup retrieval, milk production and delivery, grooming, and teaching).

Considerations for Reproductive Toxicity Evaluations

The male reproductive system is at risk during fetal development *in utero*, postnatally during puberty, and during the male reproductive lifetime with targets including the processes and structures involved in primary sex differentiation (testes and accessory organs), secondary sex characteristics and sexual behaviors (e.g., libido), and performance (e.g., erection and ejaculation). The traditional endpoint of concern is the production of normal numbers of normal (genetic, chromosomal, and structural) sperm.

Identification of reproductive toxicants is made from clinical workups on men in infertility clinics or undergoing drug treatments, predominantly for cancer, from epidemiologic studies on general populations (environmental exposures), or on worker populations (industrial exposures in production plants or users of chemicals such as pesticides and commodity chemicals), and from animal studies (usually in rodents). Animal studies allow for more invasive examinations such as histopathology of the testes, close scrutiny of mating behavior, and mating to proven breeders. They can be used to confirm or extend initial observations in humans or to initially identify a potential reproductive toxicant. One epidemiologic study of male workers exposed to dibromochloropropane (DBCP) was apparently triggered by the men talking at work breaks about their wives' failure to conceive and a request from the workers to Occupational Safety and Health Administration for an investigation. DBCP proved to be a testicular toxicant that affected spermatogenesis in the male workers; however, data on rats exposed to DBCP with the same testicular findings were, in fact, available in the literature 15 years prior to the worker concerns. One additional unique characteristic of male reproductive toxicity is that if effects are limited to postspermatogonial cells, then the effect is transient (limited to the time when these cells become sperm and are ejaculated) and subsequent waves of spermatogenesis from the intact spermatogonial cells are not affected.

Since the definition of normal sperm includes normalcy of the haploid genetic complement as well as normal sperm structure, numbers, and functions (i.e., ability to swim in the female's reproductive tract, penetrate the ovum, and join its genetic material with the egg's haploid genetic material), analyses of sperm parameters are performed in all three categories of investigations: clinical, epidemiologic, and animal testing. Routinely, sperm numbers, motility (viability), and morphology are ascertained. The interpretation of the human worker reproductive data is usually confounded by, among many factors, job experience, exposures to multiple materials, lifestyle (e.g., smoking, drinking, 'recreational' use of drugs, and hobbies), age, health status, diet, status of spouse, number and ages of children, socioeconomic class,

educational level, and ethnic/religious factors. Exposure data for workers are usually very poor (e.g., workplace air concentrations are not precise for individuals or job descriptions/titles, and measurements are not frequent and not long term (over the job or employee working lifetime)). Exposure data for nonworking environments (i.e., contaminated foodstuffs, water, soil, or air) may be even worse.

The female reproductive system is also at risk during fetal development *in utero*, postnatally during puberty, and during her reproductive lifetime until menopause (cessation of ovulation). The traditional end points of concern are ovulation of a normal ovum, fertilization, uterine status, implantation and prenatal development, parturition, and lactation involving nursing (appropriate quality and quantity of milk) and other maternal behaviors.

Identification of reproductive toxicants in females is made from clinical workups (as with males), from epidemiologic studies in general populations, and to a lesser degree on worker populations (the industrial workforce in chemical production and end use has been traditionally male). Animal studies are also very important to initially identify an agent or to confirm and extend initial findings in women. The risk to women's reproductive status may be transient (limited to the pregnancy at risk) or permanent, if effects are to the primary oocytes, since no additional oocytes will be made during her reproductive lifetime. The confounders for male reproductive risk assessments are essentially the same for female reproductive risk assessments. The cyclical nature of the female's reproductive activity (e.g., hormone levels, ovulation, and uterine lining buildup and shedding) makes both human and animal research more difficult; synchronized test animal populations (by estrous cyclicity) are very useful in identifying some effects on reproductive structures and functions.

Categories of Reproductive Toxicants

Reproductive toxicants can be categorized by type of agents, for example, physical agents such as ionizing radiation; pharmaceuticals such as therapeutic drugs, especially those used in treatment of cancers (which target DNA and therefore act as mutagens and/or target cell proliferation and therefore affect spermatogenetic and oogenetic cell divisions); recreational drugs/drugs of abuse; pesticides (which are obviously biologically active); industrial chemicals (including solvents and commodity chemicals); environmental chemicals (contaminating air, water, soil, and foodstuffs); and naturally occurring toxicants such as phytoestrogens (in soy and clover), plant toxins (e.g., mushrooms and herbs) and animal toxins (from invertebrates such as certain shellfish, spiders, and insects and from vertebrates such as certain fish, frogs, toads, and snakes).

Categorization of reproductive toxicants by function or mechanism would include agents acting as mutagens (causing changes within and between genes), clastogens (causing changes in parts of chromosomes, including chromosome breakage), cytotoxins (killing cells in general or specific cell types such as gonial cells, Leydig cells, Sertoli cells, etc.), mitotic/meiotic poisons (interfering with cell division, e.g., by damage to the assembly/dissociation of spindle fibers which control the movement of chromosomes), agonists or antagonists of endogenous hormones (e.g., environmental estrogens/antiestrogens and androgens/antiandrogens), inhibitors of hormone synthesis, transport, or degradation, and neurotoxicants (affecting central nervous system control of reproduction). Toxic effects on other systems (e.g., the thyroid, liver, kidneys, adrenal glands, etc.) may also affect reproduction.

Governmental Regulations

The thalidomide disaster in the late 1950s and early 1960s resulted in over 8000 children in 28 countries with major drug-specific malformations. The US governmental agencies recognized that it was only extraordinary luck and Dr. Frances Kelsey of the FDA which averted huge numbers of children in the United States being affected (the manufacturers were not permitted to market the drug in the United States); there were no mandated testing procedures in place at the time which would have identified the risk. In response, in 1966, Dr. E. I. Goldenthal, Chief of the Drug Review Branch, FDA, sent a letter to all corporate medical directors establishing *Guidelines for Reproductive Studies for Safety Evaluation of Drugs for Human Use*. These guidelines were promulgated "as a routine screen for the appraisal of safety of new drugs for use during pregnancy and in women of childbearing potential." Three phases or segments were proposed which have since been modified by the International Conference on Harmonisation (ICH) and adopted by the regulatory bodies of the European Union, Japan, and the United States. Segment I, Study of Fertility and Early Embryonic Development to Implantation (**Figure 3a**), was designed to provide information on breeding, fertility, preimplantation survival, nidation (implantation), and early postimplantation survival and growth in rats. It involves exposure of weanling males for at least one full spermatogenic cycle (10 weeks) and/or of adult females for at least two ovulation cycles (2 weeks) and then a mating period,

with males necropsied after the mating period. Females continue exposure through gestation with necropsy of the parental females and their litters during gestation to identify pregnancy rate and pre- and early post-implantation loss. One rarely used alternative is to necropsy one-half of the dams and their litters on gestational day (gd) 13–15 (with exposure ceasing on gd 6), and to necropsy the other half of the females and their litters at weaning at the end of lactation (postnatal day (pnd) 21) to identify *in utero* losses, postnatal losses, and growth and development of the offspring. Acquisition of developmental landmarks such as surface righting, pinna (external ear) detachment, pilation, auditory startle, eye opening, incisor eruption, mid-air righting reflex, negative geotaxis, testis descent, etc., is noted. The offspring maintained beyond weaning allow ascertainment of acquisition of puberty (time of vaginal patency in females, preputial separation in males) and, if appropriate, motor activity, learning and memory, and mating competence. Segment III Study of Pre- and Postnatal Development (also see Toxicity Testing, Developmental), as initially designed, involved exposure of the maternal animal after major organogenesis is over (gd 15) through the 'last trimester' of rodent pregnancy, through parturition and lactation of their litters until weaning (pnd 21). One male and one female F_1 offspring/litter are selected to be

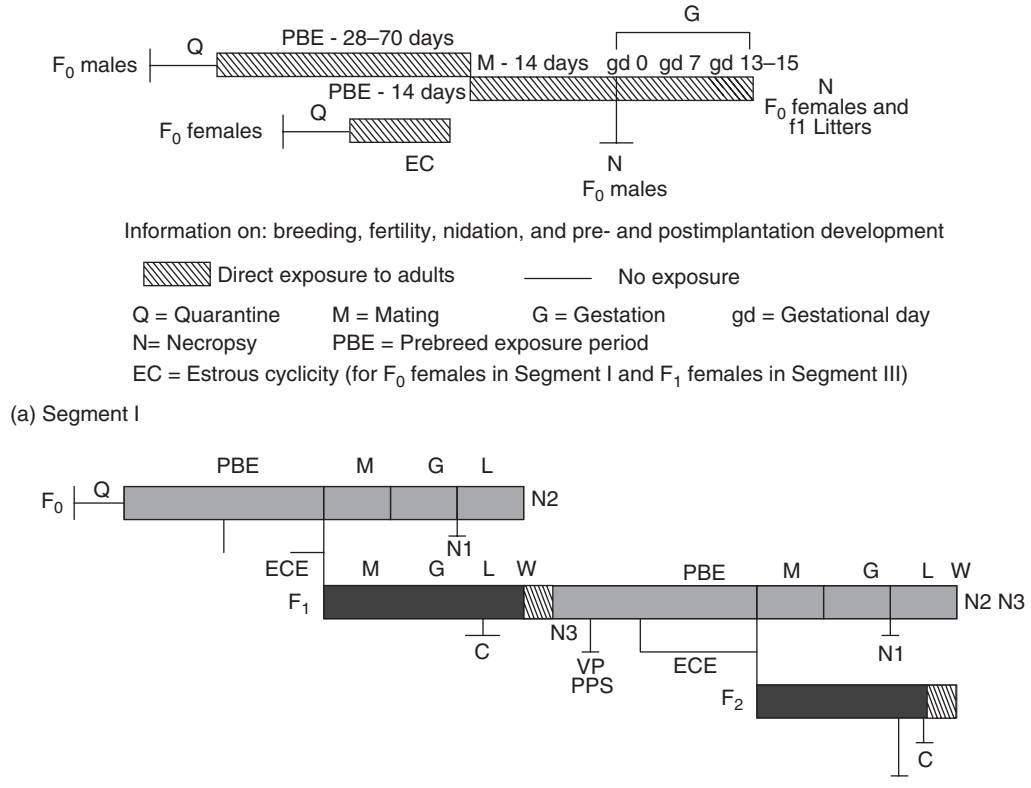
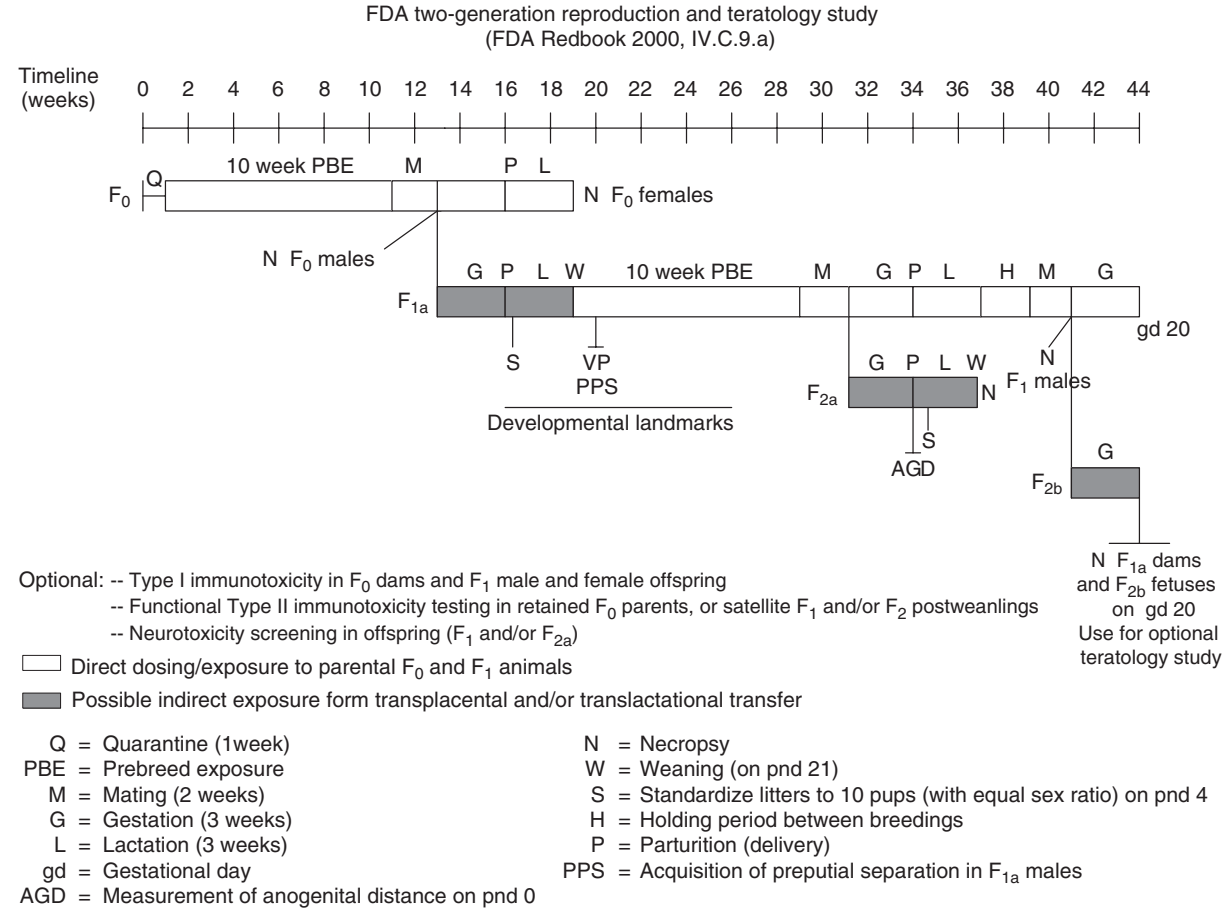


Figure 3 US government guidelines for study designs of reproductive toxicology. (a) Segment 1 (US FDA); (b) two generation (US EPA: TSCA, and FIFRA; OECD).



(c) Reproductive toxicity study design (two-generation, two litters/generation)

Figure 3 (Continued) (c) two generation (US FDA).

retained (with no direct dosing) to adulthood, with acquisition of developmental landmarks, puberty, behavioral, and other functional tests performed (see above for alternative Segment I postnatal evaluations). The retained F_1 offspring are then mated (1:1) within groups. The F_1 males are necropsied after mating, and the F_1 females and their F_2 litters are necropsied on pnd 4. Except for the Segment I alternative, this is the only FDA segment which evaluates postnatal consequences of *in utero* exposure, but it only addresses exposures that occur after major organogenesis is complete (see ICH Guideline 4.1.2). (Segment II studies are discussed under Toxicity Testing, Developmental.)

A two-generation reproductive toxicity study, used by the Environmental Protection Agency (EPA; OPPTS, TSCA, FIFRA) and OECD (**Figure 3b**), involves a long prebreed exposure (10 weeks in rats, 8 weeks in mice) of both sexes (designated F_0) begun after weaning, with continuing exposure during mating, gestation, and lactation (with F_0 males necropsied usually after mating), selection of offspring (designated F_1) and prebreed exposure, mating, gestation, and lactation of F_1 parents and F_2 offspring. The F_1 generation is the major focus of this protocol since it is the only generation that receives exposure from the time its members were gametes through their time of reproductive performance; treatment-related effects on structures and functions of the reproductive system would be discernible in the F_1 animals by this protocol. The new (1998) EPA test guidelines include prebreed estrous cyclicity and stage of estrus at demise for F_0 and F_1 females, andrology (epididymal sperm number, motility and morphology, testicular homogenization-resistant spermatid head counts to calculate daily sperm production (DSP) and efficiency of DSP) in F_0 and F_1 males, organ weights and histopathology of selected organs at adult necropsies, 'triggered' anogenital distance in F_2 newborns, and acquisition of puberty in F_1 postwean animals. A third reproductive toxicity protocol promulgated by FDA involves a three-generation, two litter per generation study design (**Figure 3c**). It is similar to the two-generation study, except that F_0 animals, after they produce the

first litters (designated F_{1a}) are rebred (within groups to different partners) to produce F_{1b} offspring. Usually the F_{1a} offspring are retained for prebreed exposure and generation of F_{2a} and F_{2b} offspring. (F_{1b} offspring are terminated at weaning, with representative animals, usually 10/sex/group, necropsied.) The F_{2a} animals are usually retained for prebreed exposure and generation of F_{3a} and F_{3b} offspring. The last breed of F_{2a} animals to produce the F_{3b} litters can be executed like previous breeds, with the offspring terminated at weaning, or the F_{2a} mothers can be necropsied on gd 20 (prior to expected parturition) and the F_{3b} fetuses evaluated for developmental toxicity (examination of external, visceral, and s keletal morphological development *in utero*).

The ICH has recently (1994) promulgated harmonized testing guidelines for reproductive toxicity in five study designs adopted by the European Union, Japan, and the United States in 1996 (**Figure 4**).

The first study design (**Figure 4a**), termed Study of Fertility and Early Embryonic Development (4.1.1), is similar to an FDA Segment I study except that male prebreed exposure is for 4 weeks and female exposure begins at mating and extends only to gd 6 (at the time of implantation). F_0 males are sacrificed after gd 6 and F_0 females are sacrificed at midpregnancy (gd 15) or just prior to term (gd 20). It is designed to assess the effects of exposure during prebreed (males), mating (both sexes), and the preimplantation period (females) on *in utero* reproductive indices.

The second study design (**Figure 4b**), termed Study for Effects on Prenatal and Postnatal Development (4.1.2), assesses exposure to the parental female from implantation (gd 6) through weaning of her litter (pnd 21) with selected offspring pups retained for mating (to produce F_2 litters). This design is similar to an FDA Segment III study, except that the treatment exposures begin at implantation and encompass the periods of major organogenesis and fetogenesis, with evaluation of postnatal consequences of *in utero* and lactational exposures.

The third study design (**Figure 4c**), titled Study for Effects on Embryo–Fetal Development, is essentially an FDA Segment II study with exposure of the mother during organogenesis of her offspring *in utero* (gd 6–15).

Additional study designs (**Figure 4d** and **e**) are essentially combinations of the first two or three designs.

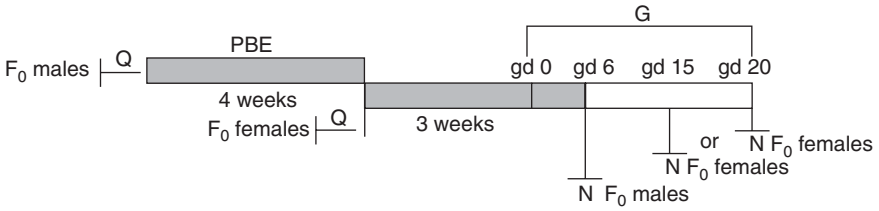
Obviously, specific studies are also designed to investigate a specific endpoint and/or agent.

Statistical Analyses

Statistical analyses of continuous data which distribute according to a bell-shaped curve (e.g., adult body weights, weight changes, feed consumption, pup body weights, and percentage male pups) employ parametric methods, including analysis of variance, tests for homogeneity of variances, tests for dose-related trends, and pairwise comparisons to the concurrent control group values. Continuous data which do not distribute as discussed previously are examined by nonparametric methods to identify trends and pairwise comparisons. Nominal (noncontinuous) data such as reproductive indices (e.g., mating, fertility, fecundity, and incidence of adult clinical signs) and survival indices are also analyzed for trends and pairwise comparisons. The unit of analysis for all tests is the male, the female, or the litter.

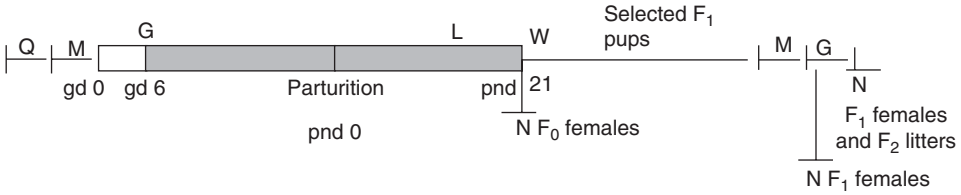
Risk Assessment

The US government's new approach (2000) to health assessment of agents involves the iterative interaction of four major components: basic scientific research (hazard identification), science-based toxicity/risk assessment (dose–response assessment), exposure assessment, and risk characterization (**Figure 5**). Animal studies usually provide hazard identification and dose–response assessment. Animal studies usually employ a route of administration which delivers a bolus dose for a worst-case scenario (i.e., gavage) or which duplicates the known or potential human route of exposure, usually dosed feed or dosed water for end-use consumer exposure, or inhalation or cutaneous routes (for industrial exposures). The highest dose in these studies which produces no effects is designated the no-observed-effect level (NOEL) or the no-observed-adverse-effect level (NOAEL). The NOEL/NOAEL is then divided by one or more uncertainty or safety factors to obtain the 'acceptable daily intake' value (ADI; EPA: FIFRA) or the reference dose (RfD; EPA: TSCA). These doses (ADI or RfD) are defined as an estimate of the daily human exposure that is likely to be without appreciable risk of adverse effect. The uncertainty factors typically include 10 for extrapolation from animal models to humans and 10 for the diversity of human populations (to protect the most sensitive human subpopulations) for a total of 100. (A third factor of up to 10 is proposed for pesticides to protect infants and children.) Another uncertainty factor (usually 10) is commonly used to cover possible lifetime exposures in humans versus the exposure period in the test animal species, especially for ADIs, for a total of 1000 (to protect against chronic, long-term exposure in the diet from food, water, etc. contaminated with pesticide residues). Exposure assessment is then performed for human exposure for all sources. A value termed margin of exposure (MOE) is defined as the



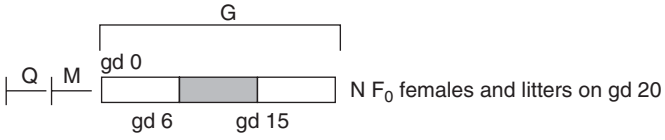
Assess: Maturation of gametes, mating behavior, fertility, preimplantion, implantation

(a) Study of fertility and early embryonic development, (4.1.1) rodent (see segment I)



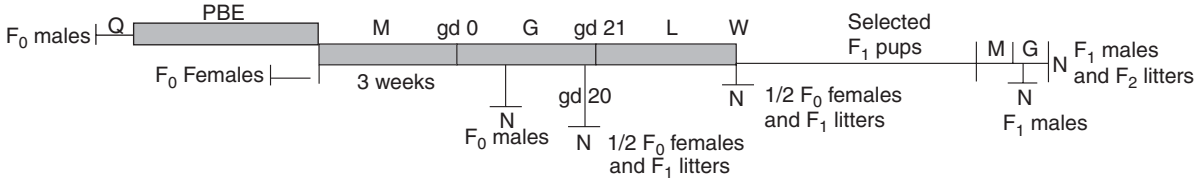
Assess: Toxicity relative to nonpregnant females, prenatal and postnatal development of offspring, growth and development of offspring, functional deficits (behavior, maturation, reproduction)

(b) Study for effects on prenatal and postnatal development, including maternal function (4.1.2) rodent (see segment III)



Assess: Toxicity relative to nonpregnant females, embryofetal death, altered growth of offspring *in utero*. Sructure changes of offspring *in utero*

(c) Study for effects on embryo–fetal development (4.1.3) rodent and non-rodent (see segment II)



(d) Single-study design (4.2) rodents (combine 4.1.1 and 4.1.2)

4.1.1 with 4.1.2 1/2 F_0 females and F_1 litters necropsied on gd 20
1/2 F_0 females and F_1 litters necropsied on pnd 21
(retained selected F_1 pups followed through mating gestation of F_2 liters)

(e) Two study design (4.3) rodents

Q = Quarantine
PBE = Prebreed exposure
M = Mating
G = Gestation
L = Lactation
W = Wean
N = Necropsy
gd = Gestational day
pnd = Postnatal day

Direct exposure to adults

Figure 4 International Conference on Harmonisation guidelines on detection of toxicity to reproduction for medicinal products. (a) Study of fertility and early embryonic development (4.1.1); (b) study for effects on prenatal and postnatal development, including maternal function (4.1.2); (c) study for effects on embryo–fetal development (4.1.3); (d) single-study design (4.2); (e) two-study design (4.3).

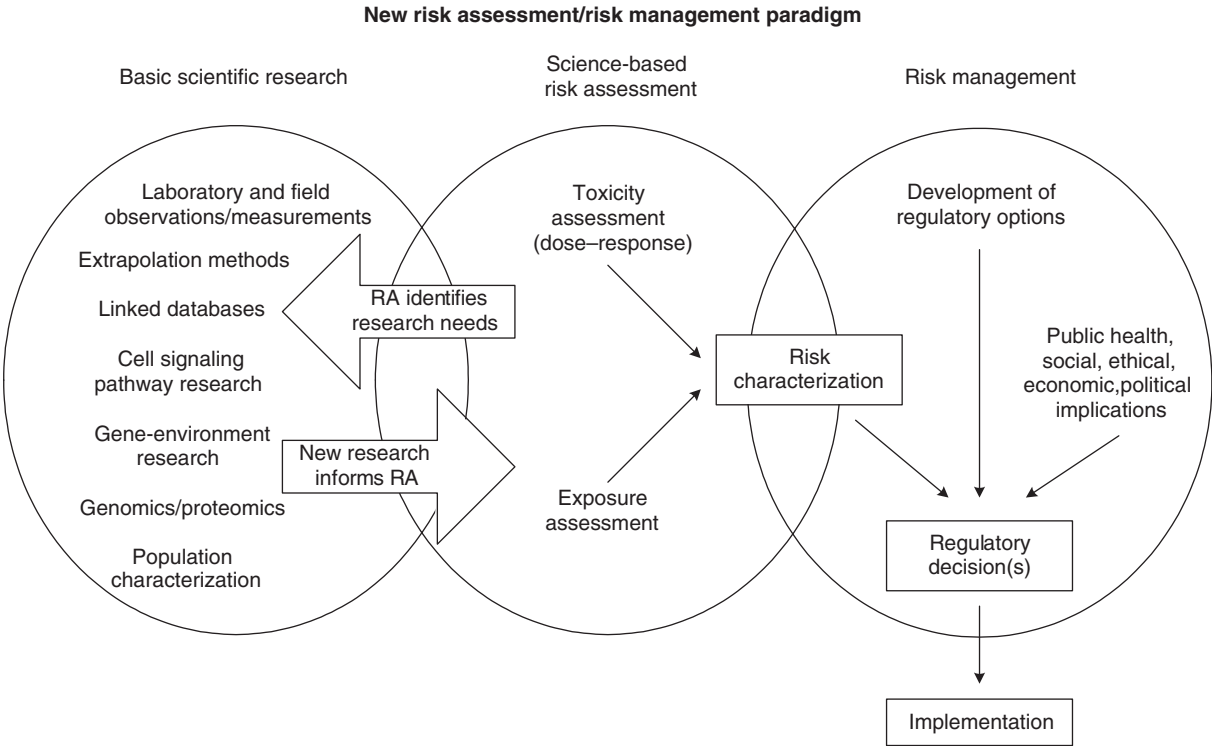


Figure 5 US National Research Council, new risk assessment/risk management paradigm. (Modified from National Research Council (2000) *Scientific Frontiers in Developmental Toxicity and Risk Assessment*, from National Academy Press.)

ratio of the NOAEL from the most sensitive test species to the human estimated exposure from all possible sources. If the MOE is very large, the risk to humans is perceived as low.

The EPA also employs a weight-of-evidence scheme to factor in the levels of confidence for the data from various animal and human studies/reports and to emphasize human data over animal data.

See also: Androgens; Developmental Toxicology; Dose–Response Relationship; Endocrine System; Levels of Effect in Toxicological Assessment; Neurotoxicity; Radiation Toxicology, Ionizing and Nonionizing; Reproductive System, Female; Reproductive System, Male; Risk Assessment, Human Health; Risk Characterization; Toxicity Testing, Developmental.

Further Reading

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Tyl RW (2002) Section 16. Male reproductive toxicology. Chapter 16.1, *In vivo* models for male reproductive toxicology, and Chapter 16.2, Guidelines for mating rodents. In: Maines MD *et al.* (ed.) *Current Protocols in Toxicology*, vol. 2, pp. 16.1.1–16.1.15 and 16.2.1–16.2.11. New York: Wiley.

Toxicity Testing, Sensitization

Robin C Guy

Background Information

Skin sensitization (allergic contact dermatitis, allergic sensitization) can occur following exposure by the dermal or inhalation routes and presents problems to significant numbers of humans, both in the occupational field and in the general population. In the human, the responses may be characterized by itching, redness, swelling, skin lesions, or a combination of these. In other species the reactions may differ and only redness and swelling may be seen. Allergic reactions are of various types, but all involve at least

one exposure to initiate the process of sensitization, as they are immunologically mediated cutaneous reactions to a substance. Determination of the potential to cause or elicit skin sensitization reactions (allergic contact dermatitis) is a significant factor in evaluating a substance's toxicity. Early identification of any allergic potential is a prudent measure for identifying possible hazards to a population exposed repeatedly to a test substance and to ensure that appropriate methods of control can be applied.

The sensitization test selected should identify substances with significant allergenic potential and minimize false-negative results. Several animal models have been developed for identification of sensitizers, including

- Draize sensitization test;
- Freund complete adjuvant (FCA) technique;
- Buhler test;
- open epicutaneous test;
- Maurer optimization test;
- split adjuvant technique;
- guinea pig sensitization (Buehler);
- guinea pig maximization (Magnusson–Kligman);
- mouse ear swelling test;
- mouse local (auricular) lymph node assay; and
- photosensitization (photoallergy) models including:
  - photo-mouse ear swelling test (photo-MEST); and
  - photo-local lymph node assay (photo-LLNA)

In the traditional Draize test in guinea pigs, potent sensitizers are identified but moderate sensitizers in humans may give false-negative reactions in guinea pigs. Use of adjuvant in the guinea pig maximization test (GPMT) or the split adjuvant technique gives a lower probability of missing a sensitizer.

Sensitization testing is determined by examining the reaction to the challenge exposure and comparing this reaction with that of the initial induction exposure. In general, the test animals are initially exposed to the test material by intradermal or epidermal application. This step is the induction exposure, an experimental exposure of a subject to a test substance with the intention of inducing a hypersensitive state. The induction period follows. This is a rest period of at least 1 and possibly 2 weeks following the induction exposure. At this time, a hypersensitive state/immune response may develop. After the induction period, the animals are exposed to a challenge dose, which is an experimental exposure of the previously treated subject to the same test substance. At specific time points after the challenge dose, the extent and degree of the skin reaction to the challenge exposure are compared with those demonstrated by control animals that undergo vehicle treatment during induction and then receive the challenge exposure.

Experimental Design

The GPMT of Magnusson and Kligman (which uses adjuvant) and the nonadjuvant Buehler test are given preference over other methods and these procedures are described in detail.

Guinea Pig Maximization Test

Briefly, the GPMT uses intradermal injection with and without FCA for induction, followed on days 5–8 by topical irritation/induction, followed by topical challenge for 24 h on days 20–22. Readings are made ~24 h after removal of the challenge dose, and again after another 24 h. If the results are equivocal, the animals may be rechallenged 1 week later.

Animals A minimum of 10 animals are used in the treatment group and at least 5 animals in the control group. When fewer than 20 test and 10 control guinea pigs have been used, and it is not possible to conclude that the test substance is a sensitizer, testing in additional animals to give a total of at least 20 test and 10 control animals is strongly recommended.

Dose Levels The concentration of test substance used for each induction exposure should be well tolerated systemically and should be the highest to cause mild to moderate skin irritation. The concentration used for the challenge exposure should be the highest nonirritant dose. The appropriate concentrations can be determined from a pilot study using two or three animals.

Induction: Intradermal Injections *Day 0 treated group*: Three pairs of intradermal injections of 0.1 ml volume are given in the shoulder region, which is cleared of hair so that one of each pair lies on each side of the midline:

- *Injection 1*: A 1:1 mixture (v/v) of FCA/water or physiological saline.
- *Injection 2*: The test substance in an appropriate vehicle at the selected concentration.
- *Injection 3*: The test substance at the selected concentration formulated in a 1:1 mixture (v/v) of FCA/water or physiological saline.

In injection 3, water-soluble substances are dissolved in the aqueous phase prior to mixing with FCA. Liposoluble or insoluble substances are suspended in

FCA prior to combining with the aqueous phase. The concentration of test substance shall be equal to that used in injection 2. Injections 1 and 2 are given close to each other and nearest the head, while injection 3 is given toward the caudal part of the test area.

Day 0 control group: Three pairs of intradermal injections of 0.1 ml volume are given in the same sites as in the treated animals:

- *Injection 1*: A 1:1 mixture (v/v) of FCA/water or physiological saline.
- *Injection 2*: The undiluted vehicle.
- *Injection 3*: A 50% w/v formulation of the vehicle in a 1:1 mixture (v/v) of FCA/water or physiological saline.

Induction: Topical Application *Days 5–7 treated and control groups*: Approximately 24 h before the topical induction application, if the substance is not a skin irritant, the test area, after close-clipping or shaving is painted with 0.5 ml of 10% sodium lauryl sulfate in vaseline, in order to create a local irritation.

Days 6–8 treated group: The test area is again cleared of hair. A filter paper (2 cm × 4 cm) is fully loaded with the test substance in a suitable vehicle and applied to the test area and held in contact by an occlusive dressing for 48 h. The choice of the vehicle should be justified. Solids are finely pulverized and incorporated in a suitable vehicle. Liquids can be applied undiluted, if appropriate.

Days 6–8 control groups: The test area is again cleared of hair. The vehicle only is applied in a similar manner to the test area and held in contact by an occlusive dressing for 48 h.

Challenge: Topical Application *Days 20–22 treated and control groups*: The flanks of treated and control animals are cleared of hair. A patch or chamber loaded with the test substance is applied to one flank of the animals and, when relevant, a patch or chamber loaded with the vehicle only may also be applied to the other flank. The patches are held in contact by an occlusive dressing for 24 h.

Observations of Treated and Control Groups

- Approximately 21 h after removing the patch the challenge area is cleaned and closely-clipped and shaved or depilated if necessary.
- Approximately 3 h later (~48 h from the start of the challenge application) the skin reaction is observed and recorded according to the grades shown in **Table 1**.

Table 1 Magnusson and Kligman grading scale for the evaluation of challenge patch test reactions

Grade	*Reaction*
0	No visible change
1	Discrete or patchy erythema
2	Moderate and confluent erythema
3	Intense erythema and swelling

- Approximately 24 h after this observation a second observation (72 h) is made and once again recorded.

Rechallenge If it is necessary to clarify the results obtained in the first challenge, a second challenge (i.e., a rechallenge), where appropriate with a new control group, should be considered ~1 week after the first one. A rechallenge may also be performed on the original control group.

Clinical Observations All skin reactions and any unusual findings, including systemic reactions, resulting from induction and challenge procedures should be observed and recorded. Other procedures, for example, histopathological examination, the measurement of skin fold thickness, may be conducted to clarify doubtful reactions.

Buehler Test

Briefly, the standard Buehler test uses three 6 h dermal patches, one per week, to the same shaved site. After a 2 week rest period, the test animals and half of the control animals receive another 6 h patch at another site. Then, the test animals are tested again 7–15 days later. Reactions are graded according to a five-point scale. If the results are equivocal, the animals may be rechallenged 1 week later.

Animals A minimum of 20 animals is used in the treatment group and at least 10–20 animals in the control group.

Dose Levels

- The concentration of test substance used for each induction exposure should be the highest to cause mild irritation. The concentration used for the challenge exposure should be the highest non-irritating dose. The appropriate concentration can be determined from a pilot study using two or three animals.
- For water-soluble test materials, it is appropriate to use water or a dilute nonirritating solution of

surfactant as the vehicle. For other test materials 80% ethanol/water is preferred for induction and acetone for challenge.

Induction: Topical Application *Day 0 treated group*:

- One flank is cleared of hair (closely clipped). The test patch system should be fully loaded with test substance in a suitable vehicle (the choice of the vehicle should be justified; liquid test substances can be applied undiluted, if appropriate). The test patch system is applied to the test area and held in contact with the skin by an occlusive patch or chamber and a suitable dressing for 6 h.
- The test patch system must be occlusive. A cotton pad is appropriate and can be circular or square, but should be ~4–6 cm^2. Restraint using an appropriate restrainer is preferred to assure occlusion. If wrapping is used, additional exposures may be required.

Day 0 control groups: One flank is cleared of hair (closely clipped). The vehicle only is applied in a similar manner to that used for the treated group. The test patch system is held in contact with the skin by an occlusive patch or chamber and a suitable dressing for 6 h.

Days 6–8 and 13–15 treated and control groups: The same application as on day 0 is carried out on the same test area (cleared of hair if necessary) of the same flank on days 6–8, and again on days 13–15.

Challenge *Days 27–29 treated and control groups*:

- The untreated flank of treated and control animals is cleared of hair (closely clipped). An occlusive patch or chamber containing the appropriate amount of test substance is applied, at the maximum nonirritant concentration, to the posterior untreated flank of treated and control animals.
- When relevant, an occlusive patch or chamber with vehicle only is also applied to the anterior untreated flank of both treated and control animals. The patches or chambers are held in contact by a suitable dressing for 6 h.

Observations of Treated and Control Groups Approximately 21 h after removing the patch the challenge area is cleared of hair; ~3 h later (~30 h after application of the challenge patch) the skin reactions are observed and recorded according to the grades shown in the GPMT (**Table 1**); ~24 h after the 30 h observation (~54 h after application of the challenge patch) skin reactions are again observed and recorded.

Rechallenge If it is necessary to clarify the results obtained in the first challenge, a second challenge (i.e., a rechallenge), where appropriate with a new control group, should be considered ~1 week after the first one. The rechallenge may also be performed on the original control group.

Clinical Observations All skin reactions and any unusual findings, including systemic reactions, resulting from induction and challenge procedures should be observed and recorded. Other procedures, for example, histopathological examination, measurement of skin fold thickness, may be carried out to clarify doubtful reactions.

Additional Procedures for the GPMT and the Buehler Test

- The young adult guinea pig is the preferred species.
- Blind reading of both test and control animals is recommended.
- Removal of the test material should be accomplished with water or an appropriate solvent, without altering the existing response or the integrity of the epidermis.
- In a properly conducted test, a response of at least 30% in an adjuvant test and at least 15% in a nonadjuvant test should be expected for mild to moderate sensitizers. Preferred substances are hexylcinnamic aldehyde (CAS 101-86-0), mercaptobenzothiazole (CAS 149-30-4), benzocaine (CAS 94-09-7), dinitrochlorobenzene (CAS 97-00-7), or DER 331 epoxy resin. There may be circumstances where, given adequate justification, other control substances may be used.
- Depending on the test selected, animals may be used as their own controls, but usually there will be a separate group of vehicle-treated animals that are exposed to the test substance only after the induction period, whose reactions are compared to those of the animals that have received both induction and challenge exposures. Control groups which provide the best design should be used. Some cases may best be served by both naive and vehicle control groups.
- Periodic use of a positive control substance with an acceptable level of reliability for the test system selected is recommended.
- The dose level will depend on the test method selected. In the Buehler test, the concentration of the induction dose should be high enough to cause

mild irritation, and the challenge dose should use the highest nonirritating concentration. In the GPMT, the concentration of the induction dose should be well tolerated systemically, and should be high enough to cause mild to moderate skin irritation; the GPMT challenge dose should use the highest nonirritating concentration.
- If the formulation intended for the final product is used in testing, the concentration of a substance suspected of causing sensitization can be increased 10-fold in the formulation during induction as a safety factor in conducting sensitization studies; the original formulation should be used during challenge. The amount of the test substance applied to the guinea pig during challenge should not exceed the highest amount which is nonirritating in naive guinea pigs in a 24 h irritation study.

Alternatives to Guinea Pig Tests

The MEST or the mouse LLNA (auricular) may be used as screening tests to detect moderate to strong sensitizers. Historically, the naive guinea pig has been used as the model for evaluating drug and chemical hypersensitivity in sensitization tests since its response level is higher than that of other animals and similar to that of humans. The high cost associated with guinea pig studies and an increasing awareness of animal welfare issues have led to the development of more cost-effective and humane models.

Mouse Ear Swelling Test

Briefly, the MEST procedure involves weighting mice and anesthetizing, if necessary, so that the dorsal thorax of each mouse may be shaved. On the first day of dosing, the test article, a positive control, and the respective vehicles are applied to their respective sites on the shaved area. Following application, the animals are restrained long enough to allow the vehicle to start to volatilize. These procedures are repeated on days 2 and 3. Mice are then rested during days 4–7. On day 8, the pretreatment thickness of both ears on each mouse is measured.

Following this measurement, mice are challenged with the test article, vehicle, positive control, or the positive control vehicle on both sides of each ear. The same ears are then measured 24 and 48 h after challenge. Recorded raw data include pretreatment measurements, 24 and 48 h posttreatment measurements of the thickness of two sites on the right ear of all mice. The percent ear swelling is calculated as follows: ((mean thickness of both ears (24 or 48 h posttreatment)/mean thickness of both ears measured before treatment) $\times$ 100) $-$ 100. The percent ear swelling for the test article is compared to the percent ear swelling for the vehicle for significance and dose response.

Mouse LLNA

The LLNA may be used as an alternative to the GPMT or the Beuhler assay. The LLNA measures the response of the lymph nodes to a substance. Sensitization is mediated by lymphocytes, the pivotal cell type in the immune system. When susceptible individuals are exposed to a chemical allergen, those lymphocytes that are able to recognize it as a foreign substance divide and increase in number. It is this increase in the number of chemical allergen-responsive lymphocytes that renders the individual sensitized; the stimulation of lymphocyte division is, therefore, a central event in sensitization.

Advantages of the LLNA include:

- The ability to test colored materials that may otherwise be contraindicated, as it may interfere with any irritation scoring of the skin.
- An adjuvant is not needed.
- It is not necessary to clip or shave the fur from the mice, as must be done more than once during the guinea pig tests.
- Guinea pig sensitization tests require 6–8 weeks and, therefore, take a long time to complete.

Disadvantages of the LLNA include:

- Weaker sensitizers, as detected in the GPMT, were usually not detected.
- It is not recommended for metallic compounds (e.g., metals, metal salts, and organometallic materials) and high-molecular weight proteins.
- It is not very suitable for materials that do not sufficiently adhere to the ear for the treatment period. Particular care should be taken to ensure that hydrophilic materials are incorporated into a vehicle system that wets the skin and does not immediately run off. Therefore, very aqueous vehicles or test materials and runny liquids are to be avoided.

Briefly, the LLNA procedure involves the application of a test material to the backs of the ears of four or five young adult (6 to 16-week-old) female mice per concentration. Each mouse is treated for three consecutive days, and then rested for 2 days. On the sixth day after the start of dosing, the mice are euthanized and their lymph nodes are excised and examined. A test substance that causes a stimulation index (SI) of three or greater, meaning a threefold proliferation of lymph node cells in the test mice, at

one or more concentrations is considered to have skin-sensitizing activity. However, the magnitude of the SI should not be the sole factor determining the biological significance of a skin sensitization response. A quantitative assessment must be performed by statistical analysis of individual animal data in order to provide a more complete evaluation of the test substance. Factors to be considered in evaluating the biological significance of a response or outcome of the test include the results of the SI determinations, statistical analyses, the strength of the dose–response relationship, chemical toxicity, solubility, and the consistency of the vehicle and positive control responses.

Human Testing

More subjects are necessary for sensitization studies in humans than in animal studies because of the larger variation in immune responses and the need to use lower concentrations of the test material in human exposure. Usually, the sensitization of a material is assessed in a preliminary study with 20–25 subjects, and then expanded to a main study with up to 200 subjects. The sample size of test subjects in the main study must be large enough so that the results are valid for the population for which the preparation is intended. It may be of interest to use a particular component at a level 10 times the concentration in the finished product for induction. For the challenge dose, a nonirritating concentration should be used. In addition, the test conditions should reproduce the actual use of the final product, that is, the materials tested should contain all of the ingredients. In general, sensitization procedures are similar to the procedures described above:

- Multiple applications (24–48 h) of occlusive patches for the induction phase.
- A total of 9–15 applications are made over a 3 week period.
- Induction is followed by a 10–14 day rest phase to allow for development of latent sensitization.
- Subjects are then challenged by application of the test material at a different site for 48 h.
- Responses are evaluated 1, 24, 48, and 96 h after application of the patches.
- If results are positive, a second challenge 2 weeks after the original challenge may be conducted. At this time a particular component suspected as the sensitizer may be tested alone at different concentrations to confirm identification of the sensitizing component of the product.

Evaluation must distinguish between primary irritation responses, which may disappear within a couple of days, and sensitization responses which may develop more slowly, persist longer, and are characterized by pruritis, erythema, edema, papules, vesicles, bullae, or a combination of these. Further identification of sensitization reactions may involve microscopic examination of skin biopsy samples. Some issues which may be encountered in human studies include reactions early in the induction phase which may be indicative of preexisting sensitization to the test material, or a delayed response at 192 h instead of at 48 or 96 h. Follow-up of subjects not completing the study may yield valuable information on the adverse effects of a preparation.

See also: Environmental Protection Agency, US; Federal Insecticide, Fungicide, and Rodenticide Act, US; Food and Drug Administration, US; Immune System; National Institutes of Health; National Toxicology Program; Organisation for Economic Cooperation and Development; Toxicity Testing, Alternatives; Toxicity Testing, Dermal; Toxicity Testing, Irritation.

Further Reading

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Relevant Websites

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Toxicity Testing, Validation

Leon H Bruner, G J Carr, M Chamberlain, and R D Curren

Introduction

Toxicologists have developed an array of animal-based tests that are used to assess the toxicity of chemicals and mixtures of chemicals during the last half century. These methods have been adopted by regulatory agencies throughout the world to provide data that are ultimately used to protect public health and to warn chemical users of potential health dangers. For the most part, these methods have provided adequate information for the protection of plant workers and the general public.

The use of animals for routine toxicity testing is now questioned by a growing segment of society. If currently used animal tests are to be successfully replaced, it is important to demonstrate that the alternative methods provide chemical hazard data equivalent to that now available from animal-based tests. Additionally, in order for toxicologists to take the best advantage of new technologies that are constantly evolving, it is important that the validation process be conducted in a manner that efficiently and definitely characterizes the performance of new test methods.

The performance of numerous alternative methods has been assessed in validation programs, and these programs have provided a great deal of information about the validation process and the utility of the alternative methods. Additionally, there has been significant discussion on the theoretical and practical aspects of the validation process. This review summarizes some of the important lessons that have been acquired during this work, and to provide recommendations of design factors that should be considered in future validation programs. The role of validation studies in obtaining objective measures of alternative method performance is reviewed. Additionally, a multistep process is presented that may guide the design, execution, and evaluation of validation studies. Finally, factors that must be considered when the relevance of an alternative method is assessed are reviewed. The validation of alternative methods for eye irritation testing is used as a specific example to illustrate important points associated with the validation process.

The Definition of Validation

Validation has been defined as 'the process by which the reliability and relevance of an alternative method is established for a particular purpose'. The discussion below begins with what is meant by reliability and relevance from a test user's point of view, and provides a perspective on how these elements may be assessed in the validation process.

Reliability

Toxicologists must rely on results obtained from an alternative method if it is to serve as a replacement for an *in vivo* toxicity test. Two measures of alternative method performance must be known in order to define reliability from a test user's point of view. First, a toxicologist must know it is possible to consistently reproduce the data obtained from the alternative method over long periods of time. A test that does not provide the same results on the same test substance repeatedly would not be useful in the safety assessment process. Second, it must be possible to consistently predict *in vivo* toxicity endpoints at a known level of accuracy and precision. These measures of reliability are objective endpoints that can be measured experimentally. The part of the validation process that provides the data needed to confirm the reliability of an alternative method as proposed by its developers is the validation study.

Relevance

In practical terms, the assessment of relevance addresses the following question: Given the information known about the alternative method, are the data provided by the assay good enough to allow its acceptance as a replacement for a given *in vivo* test? In order to answer this question, all of the available information related to performance, operation, and mechanistic basis of an alternative method and the *in vivo* toxicity test it is intended to replace must be thoroughly reviewed. The benefits and risks associated with the adoption of the new method must also be defined. Once this information is available, it must be synthesized in a manner that allows those involved in a validation process to render a judgment that the performance of the alternative method is acceptable or not as a replacement for the *in vivo* toxicity test.

Based on the preceding discussion, it is clear that the processes used to establish the reliability and relevance of an alternative method are distinct. The confirmation of alternative method reliability is an objective process, since it provides data measured in the laboratory during a validation study. The assessment of relevance is a subjective process, since it is based on the evaluation and integration of

information and requires judgment. Since these processes are distinct, they will be reviewed separately.

Confirming Alternative Method Reliability in a Validation Study

As noted already, the tool used to obtain the data that provides objective measures of an alternative method's reliability is the validation study. Experience shows that conducting validation studies is complex. In order to provide a clear review of the steps that need to be considered, the discussion is organized around the flow chart depicted in **Figure 1**, beginning with a consideration of the information that must be available about the performance of an alternative method before it is included in a validation study.

Step 1. Define the Performance Measures to be Confirmed in the Validation Study (Figure 1)

In order to more easily design and ultimately interpret the results of a validation study, it is important to define two factors that define the reliability of an alternative method before the study starts. These factors are reproducibility and predictive capability of the alternative method. It is of critical importance that these performance factors are clearly stated before a validation study starts. When these performance characteristics are defined beforehand, they provide critical information needed to design the study so that it includes the appropriate number of laboratories, an acceptable set of test substances, and the appropriate range of toxicity. They also provide benchmarks that can be used to set the criteria that an alternative method must meet in order to be considered reliable. If the data obtained from the study meet or exceed these predefined performance criteria, then it confirms that the alternative method performs as described by its developers. If the method fails to perform at a level equivalent to the criteria set at the start of the study, then its performance cannot be confirmed.

Preliminary evidence of an alternative method's reproducibility is usually generated during its initial development. This information may be further supported by data obtained from formal method development programs that involve the collaboration of several laboratories.

Evidence demonstrating the predictive capability of an alternative method is usually also generated early in its development. This evidence is obtained by evaluating a subset of test substances of known toxicity in the alternative method. The results from the alternative method are directly compared with the *in vivo* toxicity data from each test substance. If this comparison

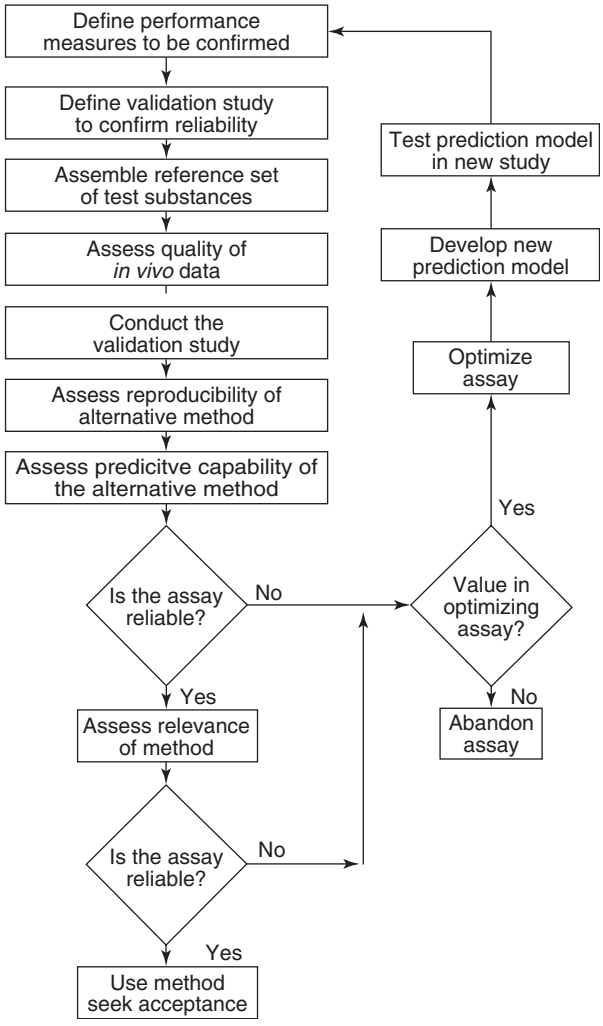

Figure 1 The validation process. The flow chart depicts a eries of steps that may be used as a guide to design and conduct a validation program. The steps proceeding down the left side of the chart represent the actual validation process. The steps proceeding up the right side of the chart depict the steps associated with improving the performance of the alternative method and defining another prediction model prior to inclusion of the method in a new validation study. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

reveals the existence of a definable relationship between the two data sets, it indicates that the alternative method may be useful for predicting *in vivo* toxicity.

An example of a definable relationship between alternative method and *in vivo* test data is illustrated in **Figure 2**. This plot shows that the results from a hypothetical alternative method are directly related to the level of toxicity measured *in vivo*. In this case, the relationship may be described in terms of the standard equation for a line, $y = mx + b$, where m is the slope of the regression line, and b represents the value of the y intercept of the regression line. If this algorithm is true for all test materials, then any result

x, from this alternative method could be incorporated into the algorithm, $y = mx + b$, to obtain an output, y, that represents the prediction of toxicity *in vivo*. Other nonmathematical approaches, like binary classification schemes, can be used to define the relationship between two tests.

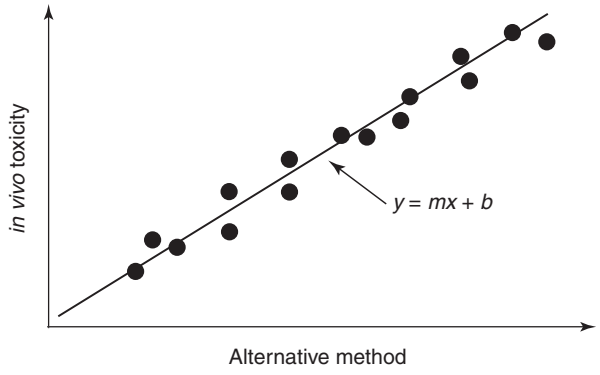

Figure 2 Plot showing a hypothetical relationship between a specific toxic endpoint measured *in vivo* and corresponding results from an alternative method. In order for an alternative method to be useful, there must be a consistent and definable relationship between toxicity measured *in vivo* and corresponding results in the alternative method. In this case, the relationship may be described in terms of a mathematical algorithm, $y = mx + b$. If this algorithm is true for all test materials, then any result, x, from this alternative method could be input into the algorithm $y = mx + b$, to obtain an output, y, which represents the prediction of toxicity *in vivo*. Such algorithms can be incorporated into prediction models that translate the results from an alternative method into a prediction of toxicity *in vivo*. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

Since such algorithms constitute models that convert the results from an alternative method into a prediction of toxicity observed *in vivo*, they have been called 'prediction models'. A prediction model is essential because it defines exactly how an alternative method is used to predict *in vivo* toxicity. Therefore, if an assay does not have an adequate prediction model, there is no way to confirm the assay's reliability. Described next in detail are the key elements that make up an adequate prediction model.

A prediction model is adequate when it defines three elements (**Figure 3**). These elements include a definition of all the possible results that may be obtained from an alternative method (inputs), an algorithm that allows a conversion of each result into a prediction of the *in vivo* toxicity endpoints (outputs), and a description of the types of test materials for which the prediction model may be used.

A prediction model must define all of the possible results that may be obtained from the alternative method. This is important since there are many different types of data available from typical alternative methods. Examples of data types include quantitative data, censored data, qualitative data, descriptive data, default values, and nonqualified

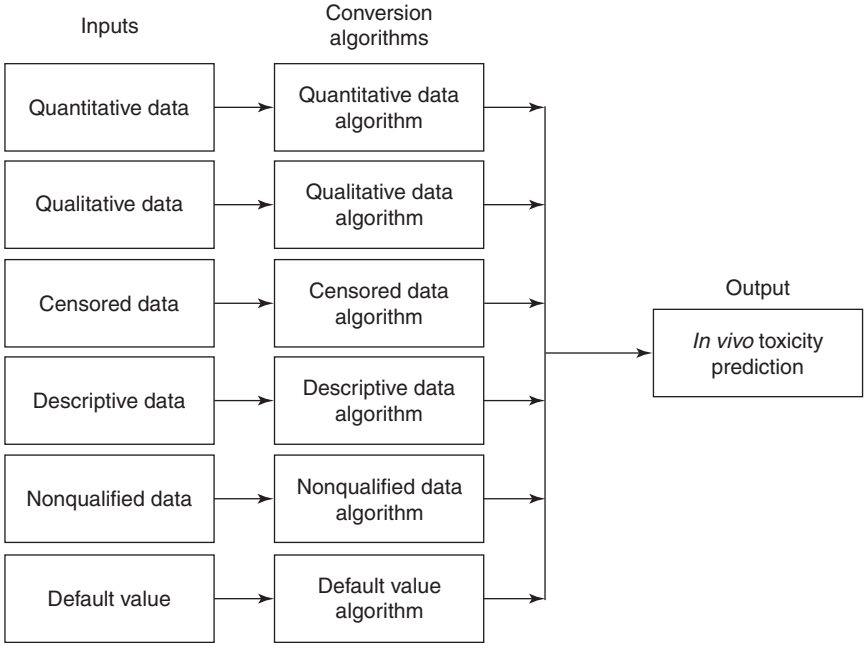

Figure 3 Elements of the prediction model. In order for an alternative method to predict toxicity *in vivo*, there must be a way to convert the results of the alternative method into a correct prediction of toxicity *in vivo*. The description of how to perform this conversion is called the prediction model. The conversion process followed must be input into the conversion algorithm that will lead to a prediction of toxicity as an output. A prediction model must define each of the data types available from the alternative method, an algorithm useful for converting the results of the alternative method into a prediction of toxicity, and the chemical classes, product categories, and physical forms for which the prediction model is valid. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

data. 'Quantitative data' are specific numerical values obtained as endpoints from the assays. These data are most commonly ED_{50} values, but may be any other numerical values specifically measured in the assay. 'Censored data' occur in assays that have maximum or minimum obtainable quantitative data. The result '$>10\,000\,\mu g\,ml^{-1}$' is a specific example of a censored datum. Censored data usually occur due to technical restraints that limit the dynamic range of a particular procedure. 'Qualitative data' represent a classification of the effects caused by the test substance on the target of the assay. A result described as 'irritant', 'moderate irritant', or 'nonirritant' is an example of qualitative data. 'Descriptive data' are written phrases that characterize an observed effect of the test substance in the test system. The result 'test substance causes coagulation of the chorioallantoic membrane' is an example of descriptive data. 'Default values' result when two or more outputs obtained from an alternative method at the same time are combined to make a prediction. For example, two results from an alternative method at the same time are combined to make a prediction. For example, two results from an alternative method such as 'the ED_{50} is greater than $10\,000\,\mu g\,ml^{-1}$' and 'no denaturation of proteins' may be combined to give the default value of 'not irritating'. 'Nonqualified data' represent values obtained from an assay that cannot be used due to some kind of technical incompatibility of the test substance with the assay. For example, the buffering capacity of the tissue culture medium might neutralize an acid test substance. If the toxic effect of the test substance depends on its acidity, the result obtained from testing the material should not be considered an accurate indicator of the test substance toxicity. Other types of data in addition to these examples may be available. If so, each type must be defined in the prediction model.

Second, a prediction model must adequately define the conversion algorithms that translate each alternative method result into a prediction of the toxicity *in vivo* (**Figure 3**). The example illustrated in **Figure 2** depicts an alternative method where the quantitative data algorithm, $y = mx + b$, is used to predict an *in vivo* toxicity, y, given any alternative method result, x. The conversion algorithms do not necessarily need to be mathematical equations. For example, algorithms may describe how to convert the alternative method data into classifications that fit a particular *in vivo* toxicity test classification scheme. No matter what approach is used, each algorithm must provide an unambiguous description of how to arrive at a prediction of *in vivo* toxicity given any possible result obtained from an alternative method. Any reasonably trained individual should be able to perform this translation. In addition to providing a prediction, it is important that the prediction model provide an indication of the variability associated with any prediction.

Third, the prediction model should define the chemical classes, product categories, and physical forms of test substance for which it is valid. For example, a particular alternative method may be useful (or validated) *only* for predicting the toxicity of surfactant-containing liquids. If so, these limitations must be defined.

Step 2. Design a Validation Study to Test the Validity of the Prediction Model (Figure 1)

Once the performance measures of an alternative method have been defined in terms of reproducibility and predictive capability, the next step in the process is to design a validation study that will test whether or not the alternative method actually performs as described. The design of a validation study is crucial to its success, not only in terms of testing reliability but also in retaining credibility and gaining acceptance by regulatory agencies. The factors that need to be considered include how the validation study will be managed, the nature and competence of the participating laboratories, the protocols and standard operating procedures (SOPs) to be used, how test substances will be coded and distributed, how data will be collected and analyzed, how well laboratories comply with the principles of good laboratory practice (GLP), and what data will be needed in order to confirm the reliability of the alternative method.

Management Structure In order to be successful, large and complex validation studies must have a well-defined management structure. This structure is required in order to assure the study principles and overall design is followed as agreed by the sponsors of the study. Responsibilities of managers and participating laboratories should be defined to assure the program accomplishes the mandates outlined in the goals of the study.

Participating Laboratories Ideally, all participating laboratories should be independent in order to ensure the integrity of separate data sets. If more than one laboratory is in the same large organization, the laboratories should be able to demonstrate local management structure and operational as well as financial independence. In the case of commercial enterprises, the design of the study should ensure that their participation is as unbiased as any other. If it is necessary for technical staff to undergo a period of

training to ensure use of common methods, such training should be undertaken and documented.

Establishment of Common Protocols and Standard Operating Procedures It is essential that all factors relevant to the conduct of the alternative method that may affect the results, the collection of data, and interpretation of the alternative method results be clearly defined before the study begins. These are best documented in the study protocol and SOPs that define the alternative methods. In order to assess the adequacy of the SOPs, they should be examined to determine if they contain three key elements. First, each SOP must have a detailed step-by-step description of how to conduct the assay. Enough details need to be provided such that any appropriately trained and competent laboratory technician need use only this document as the guide to run the assay. Second, the SOP must indicate the steps used to calculate the endpoint of the assay and the number of replicates necessary. Any data transformation or algorithms applied to the data should be clearly documented and consistently applied across all laboratories conducting a particular assay. Third, the protocol must specifically describe the prediction model being tested in the validation study.

Test Material Selection, Coding, and Distribution The reference set of test substances (RSTS) included in the study should be commensurate with the prediction model being tested in the validation study (discussed in detail in step 3). The substances should be obtained with a specification stating source, purity, and whether there are any contaminants. These specifications should be identical to those of the material actually used to generate the *in vivo* data. If identical substances are not available, the potential effect that such discrepancies may have on test substance toxicity must be assessed. In the case of formulated products, the ingredients and their levels in the product should be identified so that the formulation can be made again if necessary. Commercial sources of all single substances should be stated so that substances of the same or similar specification may be purchased in the future. Since testing usually occurs under conditions where participants do not know the identity of the reference substances, procedures need to be established to distribute substances under a randomly generated code. The system established to code and distribute the test samples should be evaluated to assure that the coding is done correctly and that participants do not have access to the codes. Each laboratory should receive substances under different codes in order to assure that results generated in each laboratory are independent.

Data Collection and Analysis The methods for the submission of results from the participants should be established to assure that all the necessary information has been provided to the study statistician. The mechanisms used to assure there are no errors in transcription should also be established in order to ensure that all data are accurately entered into the analysis.

Good Laboratory Practice Acceptance of results and conclusions from a validation study may be compromised if the principles of GLP are not applied during the study. While this is unlikely to be an issue for industrial laboratories, it may be a more important concern in academic laboratories where adherence to GLP traditionally has been less of a concern. All efforts should be made to ensure that the principles of GLP were adhered to in all participating laboratories. This in large part can be achieved by determining whether the participants followed common protocols, SOPs, and data reporting procedures.

Step 3. Assemble an RSTS Appropriate for Confirming the Reliability of the Alternative Method (Figure 1)

The next step in the process is to assemble an RSTS appropriate for assessing the reliability of the alternative method. The factors that need to be considered are the chemical classes, physical form, distribution of toxicity, and the number of materials that need to be included.

Chemical Classes Included in the RSTS The chemical and physical forms of the substances included must be consistent with the stated prediction model. For example, if the prediction model indicates that the alternative method is valid for assessing the eye irritation potential of mild, moderate, and severely irritating liquid, surfactant-based formulations, then the RSTS should contain liquid surfactant-based substances of the relevant class that cover a range of toxicity from mild to severe. Quantitative structure-activity relationships may be useful in helping selection of relevant test chemicals.

Distribution of Toxicity in the RSTS The toxicity of the substances in the RSTS should be distributed as uniformly as possible across the range of interest. This is important because a nonuniform distribution of test substance toxicity in an RSTS may not allow an effective assessment of alternative method performance. Potential effects of nonuniform test substance distribution are illustrated in **Figure 4**. The ideal situation is shown in **Figure 4b**. In this example, the test substances are uniformly distributed across the range of possible toxicity. If such results were

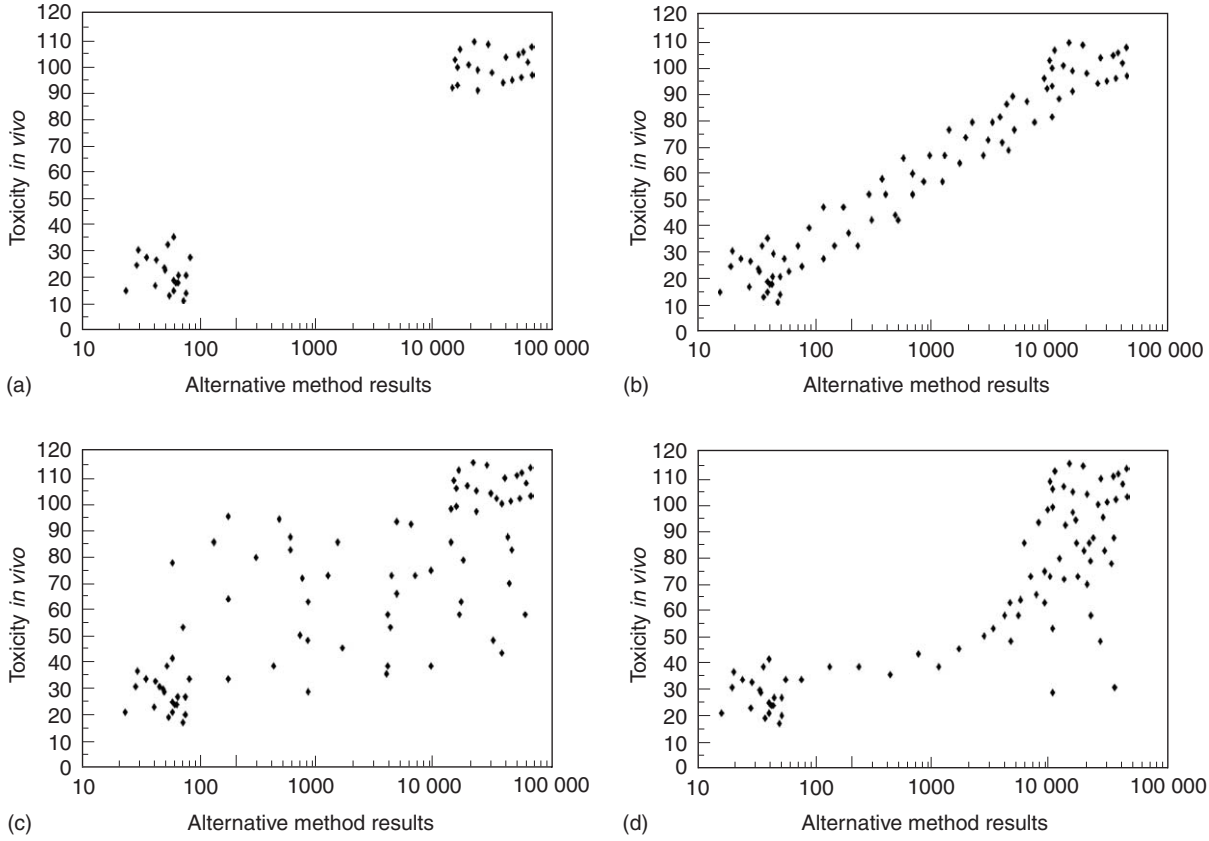

Figure 4 The effects of nonuniform data distributions in the reference set of test substances (RSTS). This series of figures illustrates why the irritancy of the materials in the RSTS must be uniformly distributed across the range of toxicity of interest. (a) includes test substances that are not uniformly distributed across the range of toxicity observed in the *in vivo* method. In this case, it is impossible to determine whether the method is useful for predicting the toxicity of moderately toxic materials. If moderately toxic materials were to be evaluated in the assay shown in part (a), it may be found that the performance of the alternative method is similar to that shown in part (b), (c), or (d). The ideal situation is shown in part (b) where there is a useful relationship between the *in vivo* and alternative method results across the entire range of toxicity assessed. The less satisfactory outcomes shown in parts (c) and (d) are also possible. The only way to determine whether the relationship between the alternative method results and *in vivo* toxicity is useful is to assess an RSTS with a uniform distribution of toxicity across the entire range of interest. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

obtained from an alternative method, it would strongly suggest that it could be used to predict toxicity across the full range of possible responses. In **Figure 4a**, the substances in the RSTS are not uniformly distributed, but rather are either mildly or strongly toxic. The problem with such an RSTS is that it is impossible to determine whether the method is useful for predicting the toxicity of the moderately toxic materials. In fact, recent work has shown that the distribution of toxicity included in an RSTS can have profound effects on the performance statistics obtained from a validation study. If the distribution of toxicity used in a validation study were indeed similar to that shown in **Figure 4a**, it is good test performance. However, when materials of mild and moderate toxicity are tested, the measures of performance may be considerably poorer. It may be found that the performance is more similar to those shown in **Figure 4c** or **d**. Alternative methods performing similar to the former two examples are less useful than one similar to **Figure 4b**. The best way to distinguish between the possible outcomes illustrated in **Figure 4b–d** is to evaluate an RSTS having a uniform distribution of toxicity across the entire range of interest.

Number of Test Substances in the RSTS The number of test substances included in the RSTS must also be evaluated. Although it has been suggested that an RSTS should contain up to 250 substances, requiring such a large number is impractical for several reasons. First, it has proven extremely difficult to identify such a large set of test substances that have been evaluated in a common toxicity test procedure. Hence, it is unlikely that a set containing 250 substances can be assembled without conducting additional *in vivo* testing. Second, experience has shown that the cost of conducting a validation study

using such a large RSTS is prohibitive. Third, there is a diminishing returns phenomenon after the RSTS reaches a certain size.

In order to gain a better understanding of how many test substances would be acceptable, a computer simulation based on the Draize eye irritation test was used to investigate the effects of changing sample size on the precision of future predictions of an *in vivo* test result from an alternative method test score. For this simulation, it was assumed that the relationship between a hypothetical alternative method response, X, and a corresponding eye irritation response, Y, has the linear form:

$$Y = (1.1)X \qquad (1)$$

where we restricted the alternative response X to be in the range (0–100), so that the *in vivo* response Y is on the usual maximum average scores (MAS) scale (0–110).

Values for X were chosen with a uniform distribution across the range of interest. Corresponding Y values were then calculated using eqn (1). Since there is inherent variability in both alternative method and *in vivo* data, random error was added to the X and Y values, respectively. This was achieved through the use of independent beta distributions scaled to give a specified coefficient of variation. The coefficient of variation applied to the alternative method response, X, was maintained constant on the full range 0–100. The coefficient of variation applied to the *in vivo* response, Y, was based on the distance of a particular Y value from the closest end of the 110 point Draize scale. This was done because the variability in eye irritation scores decreases as the score approaches either extreme of the Draize irritation scale. Data were available to provide a basis for assigning a level of variability to the Y values. MAS were computed using data given in the original publication. The coefficient of variation for the MAS was also calculated for each test substance. The degree of variation among the laboratories conducting the Draize eye irritation test on the same substances was strikingly large, ranging between 40% and 60% for a six-animal rabbit eye irritation test. The variability in alternative method data is typically less than the *in vivo* test, with coefficients of variation (CV) ranging between 10% and 25% (**Table 1**).

Data sets were generated containing hypothetical RSTS sample sizes of 10, 20, 50, 100, or 250,

Table 1 Intralaboratory reproducibility

Eye irritation test, alternative method	*Positive control*	*n*	*Endpoint units*	*Mean*	*SD*	*CV* (%)
Bovine corneal opacity[a]	Acetone	119	Units	156.50	18.800	12.0
Bovine corneal opacity	Ethanol	44	Units	54.30	9.000	16.0
Bovine corneal opacity	Imidazol	20	Units	113.60	17.400	15.3
MICROTOX[b]	Phenol	123	$\mu g\,ml^{-1}$	20.10	3.900	19.4
Silicon microphysiometer[b]	SLS	163	$\mu g\,ml^{-1}$	78.60	12.200	15.5
Neutral red uptake[b]	SLS	191	$\mu g\,ml^{-1}$	4.24	0.920	21.7
SIRC plaque forming assay[c]	SLS	205	$\mu g\,ml^{-1}$	24.50	4.170	17.0
Neutral red release[d]	Triton X-100	26	$mg\,ml^{-1}$	0.20	0.038	19.0
CORROSITEX[e]	NaOH	44	Minutes	11.74	1.120	9.5
ZK1200 topical application[f]	SLS	44	% viability	45.40	11.800	26.0

Note: The coefficient of variation following multiple runs using the indicated positive control test substances in one laboratory is shown. The overall average of the intralaboratory CVs listed is ~17%. *n* is the number of times the assay has been conducted with the indicated positive control material; units indicates the measurement units obtained from the alternative method; mean indicates the average value obtained for all the indicated runs; SD is the standard deviations calculated associated with the mean of the alternative method sources; CV is the coefficient of variation (mean/SD); SLS is sodium lauryl sulfate.

[a] As described by Gautheron P, Dukic M, Alix D, and Sina JF (1992) Bovine corneal opacity and permeability test: An *in vitro* assay of ocular irritancy. *Fundamentals of Applied Toxicology* 18(3): 442–449.

[b] As described by Bruner LH, Kain DJ, Roberts DA, and Parker RD (1991) Evaluation of seven *in vitro* alternatives for ocular safety testing. *Fundamentals of Applied Toxicology* 17(1): 136–149.

[c] As described by North-Root H, Yackovich F, Demetrulias J, Gacula M Jr., and Heinze JE (1982) Evaluation of an *in vitro* cell toxicity test using rabbit corneal cells to predict the eye irritation potential of surfactants. *Toxicology Letters* 14(3–4): 207–212; and North-Root H, Yackovich F, Demetrulias J, Gacula M Jr., and Heinze JE (1985) Prediction of the eye irritation potential of shampoos using the *in vitro* SIRC cell toxicity test. *Food and Chemical Toxicology* 23(2): 271–273.

[d] As described by Reader S, Blackwell V, O'Hara R, *et al.* (1989) A vital dye release method for assessing the short term cytotoxic effects of chemicals and formulations. *Alternatives to Laboratory Animals* 17: 28–37.

[e] As described by Gordon VC, Harvell JD, and Maibach HI (1993) Dermal Corrosion: The CORROSITEX system: A DOT accepted method to predict corrosivity potential of test materials. *Alternative Methods in Toxicology* 10: 37–42.

[f] As described by Osborne RM, Perkins MA, and Roberts DA (1995) Development and intralaboratory evaluation of an *in vitro* human cell-based test to aid ocular irritancy assessments. *Fundamental and Applied Toxicology* 28: 139–153.

each having defined levels of error added to the *X* and *Y* terms. The 95% confidence interval for the prediction of a single future observation of a MAS of 55 (95% CI_{pred}) and the standard deviation of the 95% CI_{pred} values were then calculated for each data set.

The effects of the RSTS size on the precision of a prediction derived from an alternative method are summarized in **Table 2**. Each of the tabled 95% CI_{pred} values and standard deviations are based on 1000 simulations conducted for each sample size. In the first case (ideal conditions, **Table 2**) the imposed variation is relatively low. As expected, the 95% CI_{pred} values are relatively narrow, ranging from ± 7 to ± 16. As the number of test substances included in the RSTS increases from 10 to 250, the 95% CI_{pred} decreases slightly and the standard deviations of the 95% CI_{pred} decrease by about fourfold.

In the second case (typical conditions, **Table 2**), the CVs applied to the data are more consistent with those observed in the Draize test and currently available alternative methods. Under these circumstances, the 95% CI_{pred} is significantly wider, ranging from approximately 54–28 depending on the sample size and imposed variation. Again, the prediction intervals tend to be narrower as the sample size increases, but this improvement is not substantial when $n > 20$. Also, the standard deviation of the 95% CI_{pred} decreases approximately four- to eightfold.

These simulations therefore indicate that the width of the 95% CI_{pred} does not improve with larger RSTS sizes. Rather, the real benefit of increasing sample

Table 2 Ninety-five percent confidence intervals for the prediction of an *in vivo* eye irritation score of 55 from an alternative method (95% CI_{pred})

Coefficient of variation		*Sample size n = 10*		*Sample size n = 20*		*Sample size n = 50*		*Sample size n = 100*		*Sample size n = 250*	
Alternative method	*In vivo*	*95% CI_{pred}*	*SD* of 95% CI_{pred}*	*95% CI_{pred}*	*SD of 95% CI_{pred}*	*95% CI_{pred}*	*SD of 95% CI_{pred}*	*95% CI_{pred}*	*SD of 95% CI_{pred}*	*95% CI_{pred}*	*SD of 95% CI_{pred}*
Ideal conditions											
0.05	0.05	8.4	2.4	7.5	1.4	7.1	0.8	7.0	0.5	7.0	0.3
0.10	0.10	16.3	4.5	14.9	2.9	14.2	1.5	13.9	1.1	13.8	0.7
Typical conditions											
0.10	0.40	34.0	9.2	30.5	5.0	28.9	2.9	28.3	2.1	27.9	1.3
0.10	0.50	41.8	11.6	37.1	6.4	34.7	3.9	34.0	2.6	33.6	1.6
0.10	0.60	48.5	12.9	43.3	7.7	40.7	4.5	39.9	3.0	39.6	2.0
0.20	0.40	41.8	10.6	37.5	6.1	34.9	3.4	34.5	2.4	34.3	1.5
0.20	0.50	46.8	12.4	43.0	7.3	40.0	4.1	39.4	2.9	39.1	1.8
0.20	0.60	53.1	14.2	47.9	8.1	45.6	4.6	44.7	3.2	44.3	2.0
0.40	0.40	56.4	14.2	50.5	8.0	48.0	4.5	46.9	3.2	46.5	2.0
0.40	0.50	60.4	15.4	54.7	8.9	51.7	5.0	50.6	3.3	50.1	2.1
0.40	0.60	66.0	16.0	59.3	9.2	56.0	5.2	54.8	3.6	54.3	2.3

Note: The mean 95% CI_{pred} is shown for different numbers of materials in the reference set of test substances (RSTS). The 95% CI_{pred} for a predicted *in vivo* score of 55 were obtained from computer simulations designed to assess the effect of changing the size of the RSTS on the uncertainty in predictions obtained from an alternative method. The variability in the 95% CI_{pred} is indicated as the standard deviation of the 95% CI_{pred}. Each of the values shown is based on 1000 runs of the simulation. This simulation shows that the 95% CI_{pred} is relatively wide given the variability associated with the *in vivo* eye irritation test and current alternative methods. For example, if an alternative method having a CV = 0.2 predicts a maximum average score of 55, the 95% CI_{pred} is ± 40 if the CV = 0.5 for the *in vivo* data and the RSTS $n = 50$.

References: Gautheron P, Dukic M, Alix D, and Sina JF (1992) Bovine corneal opacity and permeability test: An *in vitro* assay of ocular irritancy. *Fundamentals of Applied Toxicology* 18(3): 442–449; Bruner LH, Kain DJ, Roberts DA, and Parker RD (1991) Evaluation of seven *in vitro* alternatives for ocular safety testing. *Fundamentals of Applied Toxicology* 17(1): 136–149; North-Root H, Yackovich F, Demetrulias J, Gacula M Jr., and Heinze JE (1982) Evaluation of an *in vitro* cell toxicity test using rabbit corneal cells to predict the eye irritation potential of surfactants. *Toxicology Letters* 14(3–4): 207–212; and North-Root H, Yackovich F, Demetrulias J, Gacula M Jr., and Heinze JE (1985) Prediction of the eye irritation potential of shampoos using the *in vitro* SIRC cell toxicity test. *Food and Chemical Toxicology* 23(2): 271–273; Reader S, Blackwell V, O'Hara R, *et al.* (1989) A vital dye release method for assessing the short term cytotoxic effects of chemicals and formulations. *Alternatives to Laboratory Animals* 17: 28–37; Gordon VC (1992) Utilization of biomacromolecular *in vitro* assay systems in the prediction of *in vivo* toxic responses. *Lens and Eye Toxicity Research* 9(3–4): 211–227; Gordon VC and Bergman HC (1987) Eytex: An *in vitro* method for evaluation of ocular irritancy. *Alternative Methods in Toxicology* 5: 87–90; and Osborne RM, Perkins MA, and Roberts DA (1995) Development and intralaboratory evaluation of an *in vitro* human cell-based test to aid ocular irritancy assessments. *Fundamental and Applied Toxicology* 28: 139–153.

size is that the 95% CI_{pred} estimation is more precisely defined. This is because increasing the sample size improves estimation of the confidence interval endpoints. This is because statistical theory assures that in arbitrarily large samples the estimated endpoints converge to the true endpoint values.

The simulations also indicate that the overall width of the 95% CI_{pred} is limited by the variability in the *in vivo* response. Low levels of variability in the populations are needed in order to predict individual responses both accurately and precisely. High levels of variability in individual predictions cannot be overcome by simply increasing the size of the RSTS. The quality of the *in vivo* data, therefore, is more important than the quantity of substances included in a validation study.

Step 4. Evaluate the Quality of the *In Vivo* Toxicity Data (Figure 1)

The quality of the *in vivo* data available for the substances in the RSTS must be reviewed. This is important. If the quality of the *in vivo* data is poor, then the results of comparison between the *in vivo* data and the alternative method results will be difficult to assess definitely.

It is difficult to obtain a set of test substances that has consistent, high-quality toxicity data. The difficulty arises because many different schemes are used for measuring and classifying toxicity endpoints. The situation is made worse by the fact that there is no consistent source for the information that currently exists. This has meant that toxicity data used in validation studies have often come from many laboratories that have used different protocols and produced different kinds of data.

Some of these problems have been addressed through the efforts of organizations such as the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). This organization established a committee that developed a reference data bank containing 55 chemicals with *in vivo* eye irritation data that were generated using tests conforming to OECD (Organisation for Economic Co-operation and Development) Guideline 405. The ECETOC criteria provide a useful example of an approach that can be taken to assemble sets of test substances for which there is a uniform quality of *in vivo* data. The criteria the test substances had to meet in order to be included in the eye irritation data bank are shown in **Table 3**. If it is not possible to identify a set of test substances where the data meet such a set of standards, then it may be necessary to generate new *in vivo* data. If that is not possible, then all the shortcomings associated with the *in vivo* data in the RSTS must be documented and factored into the overall assessment of the performance of the alternative method at the end of the study. ECETOC have also prepared reference chemicals data banks for skin and respiratory sensitizes, and for skin irritation and corrosion.

Step 5. Conduct the Validation Study (Figure 1)

Once an adequate RSTS has been assembled and characterized, the next step is to test each of the materials in the alternative method. Many logistical issues need to be carefully monitored during this phase of typical large validation studies, and a few are particularly important. First, careful communication between the participants is essential. Those involved in validation studies must never underestimate the possibility for misunderstanding. Second, preliminary runs of the alternative methods using a small subset of test substances are particularly useful in helping to identify and solve start-up problems

Table 3 Acceptance criteria for *in vivo* data

All test materials should be defined entities available at a known high level of purity or specification
Each material should be chemically stable
In vivo data generated recently (since 1981 when GLP were introduced)
Good laboratory practices followed in generation of *in vivo* data
Studies carried out according to OECD Guideline 405:
At least three rabbits evaluated per test material
A volume of 0.1 ml or the equivalent weight of test substance was instilled into the conjunctival sac
Topical anesthesia was not used (Durham *et al.* 535–541)
Observations made at least at 24, 48, and 72 h
Enable reversibility/irreversibility to be assessed
Scoring done using the scheme of (Draize *et al.* 377–390) so that corneal opacity and area affected, iris inflammation, conjunctival redness, swelling, and discharge data are available for each test substance at each time point evaluated
Chemicals tested undiluted, except where testing materials undiluted would likely lead to severe effects

Note: The *in vivo* data available for materials in the reference set of test substances should meet a minimum quality standard before they are used in a validation program. An example of a set of criteria established for the selection of test substances is provided in the ECETOC Eye Irritation Chemicals Data Bank (ECETOC, 1992). The factors considered important by the Technical Committee who prepared the ECETOC Technical Report are listed here. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

that invariably occur. Third, managers of validation studies must carefully monitor the progress being made throughout the study to assure the program proceeds as planned.

Step 6. Assess the Reproducibility of the Alternative Method (Figure 1)

Once the testing of the RSTS materials in the alternative method is completed, the next step in the validation process is to assess the reproducibility of the data generated by the participating laboratories. This analysis is important because it provides the half of the data needed to confirm the reliability of the alternative method.

Many validation studies use a nested or hierarchical design (**Figure 5**). These studies usually involve several laboratories that independently conduct the same alternative method on all the substances in an RSTS. There are four sources of variability in such studies. These include variation in the test substances, variation within experiments within a laboratory (intraexperiment variability), variation between experiments within a laboratory (intralaboratory variability), and variation between laboratories (interlaboratory variability). Reviewed next is the nature and importance of each. The differences between chemicals are ignored in this discussion since they can generally be minimized with well-controlled test article distribution and storage. Attention is concentrated on the variability in results obtained by testing a single chemical in a number of different laboratories.

Definitions (Figure 5) During a validation study, each participating laboratory generally carries out a number of separate executions of an alternative method on each material in the RSTS. Each of these independent executions is a 'repeat experiment'. Often, within each repeat experiment, duplicate, triplicate, or quadruplicate measures are obtained. These are 'replicate measurements'. The mean of results from several repeat experiments gives the 'laboratory mean', and the mean of several laboratory means gives the 'overall laboratories mean'. The importance of variation in the replicate measures, repeat experiments, and overall laboratories mean and how these measurements should be assessed is described in the following sections.

Intraexperiment Variability (Figure 5) The intraexperiment variability is evaluated by examining the variation in the replicate measures obtained within a given repeat experiment. This value is most useful for workers within a laboratory, since it is an indicator of the performance of a particular assay on a specific day. While useful for internal monitoring, this value does not provide a particularly good indication of how an assay performs over time. This is because replicate measures are obtained under conditions where sources of variability such as different technical staff, preparation of the test substances, preparation of cell cultures, and preparation of media are tightly controlled. Because of this control, results obtained from a group of replicate measures best represent a 'precise' estimate of test variability at the particular time under the particular conditions when the test was run. It is not necessarily an 'accurate' reflection of a test's performance over multiple runs. Thus, the performance of an assay over time within one laboratory is best measured at the level of the repeat experiment.

Intralaboratory Variability (Figure 5) Intralaboratory variability is assessed by evaluating the results obtained from repeat experiments conducted on the same substance in the same laboratory over a reasonable

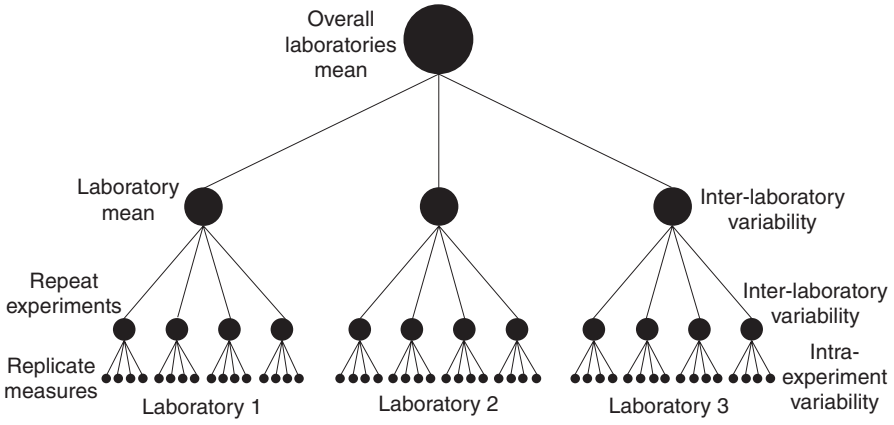

Figure 5 Hierarchical design of a validation study. The different levels of repeated measures obtainable from assays tested in a validation study are shown. The descriptions on the left side indicate the names of the endpoints at each level, and the descriptions of the right side indicate the variability term associated with each level. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

period of time. This assessment will be most representative if it is performed using data from several repeat experiments conducted completely independent of each other in terms of substances, batches of chemicals, and possibly even the technical staff who performed the work.

What are the limits of interlaboratory variation that should be considered acceptable for a given alternative method? The consideration of this question must be done on a case-by-case basis. The example illustrated in **Figure 6** shows the results obtained from a test substance that has a laboratory mean score of 100 (bold horizontal line) in an alternative method that produces scores ranging between 0 and 200. Two diagonal lines indicate the upper and lower 95% confidence limits for the laboratory mean when the alternative method CV ranges from 0% to 50%. As the CV increases, the width of the 95% confidence interval for the laboratory mean score increases. Eventually this interval becomes so wide that the results obtained from the test become meaningless relative to the entire response range of the alternative method. As the CV reaches 20% in this example, the width of the 95% confidence interval is ± 40 (dotted horizontal lines, **Figure 6**). Because the ± 40 covers 40% of the total range of possible responses from the alternative method, if the CV is consistently greater than 20%, a good case could be made that this alternative method is not acceptably reproducible. On the other hand, if the range of responses from the alternative method covers several orders of magnitude, a CV of 20% might not cause concern. This is because an alternative method response in the range of 100 ± 40 may correspond to only a small percentage of the total possible range of responses *in vivo*. Ultimately, the toxicologist who uses the alternative method for making decisions in a safety assessment must define the level of uncertainty in a prediction that is acceptable for the purpose at hand.

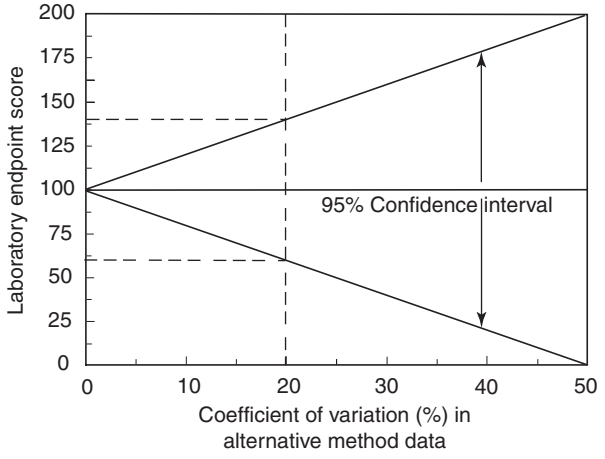

Figure 6 The effect of varying the coefficient of variation in the width of the 95% confidence interval is shown. The alternative method illustrated in this example has a laboratory mean score of 100. As the CV increases from 0% to 50%, the width of the confidence interval increases. In this specific example, when the CV = 20%, the 95% confidence interval is ± 40. An acceptable level of variability for an alternative method depends on the range of responses obtained from the alternative method and the effect of this variability on the precision of *in vivo* toxicity predictions. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

Interlaboratory Variability (Figure 5) Interlaboratory variability gives an assessment of how well results can be reproduced across several independent laboratories. This level of reproducibility measurement is of greatest importance when assessing the reliability of a toxicity test to be used for regulatory purposes since regulators will receive results generated from any number of laboratories across the world. This value can be determined by evaluating results derived from the same alternative method protocol on the same test substances across several laboratories. An approach often used to assess interlaboratory reproducibility is to calculate the correlation coefficient that relates the results from different laboratories. Although this statistic provides useful information, it has some limitations. For example, the results of an alternative method in one laboratory may differ across the entire range of results by an order of magnitude compared to a second laboratory. Thus, even though the laboratories did not duplicate each other's results (i.e., poor interlaboratory reproducibility), the data will be highly correlated. Correlation, therefore, should be used with caution when comparisons between laboratories are required. Satisfactory evidence of interlaboratory variability could also be obtained by considering the CV obtained from the results of all the participating laboratories (i.e., the CV associated with the overall laboratories mean). As noted in the discussion of intralaboratory variation, the acceptability of a particular CV must be assessed on a case-by-case basis. The effect of the variability on the uncertainty in predictions must also be considered. This issue is discussed later relative to the assessment of alternative method relevance.

Number of Participating Laboratories The number of laboratories that need to be included in a validation study in order to obtain a precise assessment of reproducibility is dependent on the level of variability associated with the alternative method being evaluated. This is illustrated in **Figure 7**. The figure shows the relationship between the width of the 95% confidence interval for the overall laboratories mean and the number of laboratories included in a determination of the overall laboratories mean. Each curve shows the results obtained when the CV of the

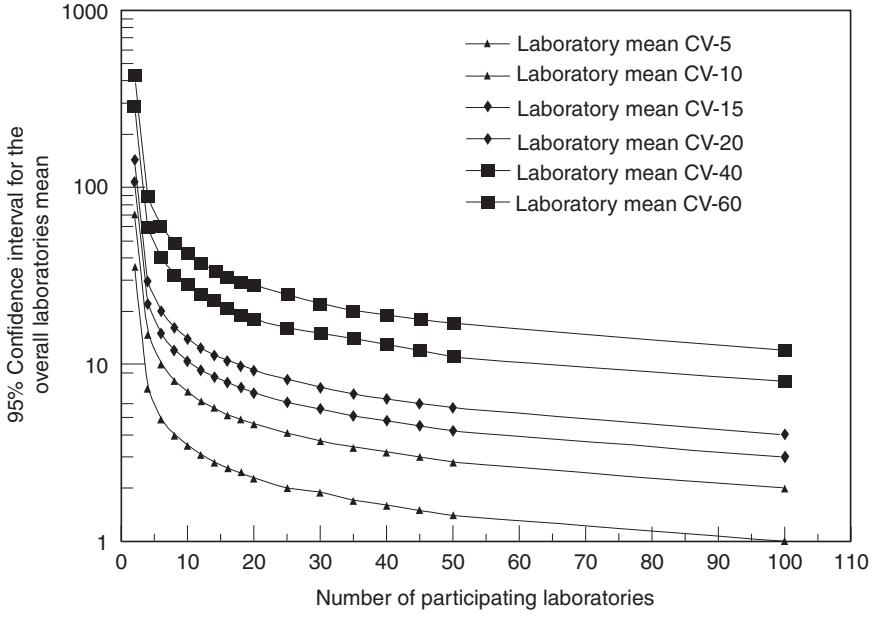

Figure 7 Number of laboratories in an interlaboratory variability assessment. This figure shows the relationship between the width of the 95% confidence interval for the overall laboratories mean and the number of laboratories participating in a validation study. Each curve shows the results obtained when the CV of the laboratory means range from 5% to 60%. The curves were calculated using a model that assumes that the true laboratory mean score is 100. As the laboratory mean CV decreases, and as the number of laboratories included in the evaluation increases, the width of the 95% confidence interval of the overall laboratories mean becomes narrower. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

laboratory means range from 5% to 60%. These curves were calculated using a model that assumed the true laboratory mean score is 100 and that, on average, each laboratory is able to obtain this value. These calculations demonstrate that as the variability in the laboratory means decreases, and as the number of laboratories included in the study increases, the 95% confidence interval for the overall laboratories mean becomes narrower. The 95% confidence interval is especially wide when tests are highly variable (i.e., laboratory means CVs > 20%). This is particularly true when the number of participating laboratories is low (note the ordinate in **Figure 7** is a log scale). For example, the width of the 95% confidence interval for the overall laboratories mean of 100 is ± 59 if the four laboratories have a laboratory mean CV = 40%. If only two laboratories participate, the 95% confidence interval is so wide (± 287) that such results would have to be viewed with considerable caution. The choice of how many laboratories to include in a validation study ultimately becomes a trade-off between reducing the variability in the estimate of interlaboratory variation versus the cost of including more laboratories. Certainly there is a diminishing returns aspect to number of laboratories used. The largest benefits occur with smaller sizes: there is a large benefit to using three instead of two, and five instead of three.

Step 7. Assess the Predictive Capability of the Alternative Method (Figure 1)

The next step in the process is to assess the predictive capability of the alternative method. This may be done by confirming that alternative method data input into the predefined prediction models provides outputs that predict *in vivo* toxicity at the level of both accuracy and precision defined at the beginning of the study. Once the data are available from the validation study, each result from the alternative method should be converted by the algorithm(s) in the prediction model into a prediction of *in vivo* toxicity. Then the predicted toxicity should be directly compared with the actual toxicity of each test substance. If the method predicts toxicity within the limits defined by the prediction model, then it would provide strong evidence supporting the predictive capability of the assay. If the results from the alternative method poorly predict *in vivo* toxicity, then there would be little evidence supporting the utility of the method.

If the conclusion reached is that the method is reliable in terms of reproducibility (see step 6) and predictive capability, then the results of the validation study may be used in the next part of the validation process that is the determination of relevance (discussed next). If the methods are shown not to be reliable, then two courses of action may be followed (**Figure 1**). If it appears there is merit

in further developmental work (test method optimization), then additional research should be undertaken. When the assay is adequately modified and/or a new prediction model is developed, it may be evaluated in a subsequent validation study. Alternatively, the method may be abandoned if it is apparent that additional effort is unlikely to be fruitful.

Assessing Alternative Method Relevance

Once the reliability of an alternative method has been confirmed in a validation study, its relevance as a replacement for an *in vivo* toxicity test must be assessed. As noted earlier, the assessment of relevance addresses the question: Is the performance of the method good enough to allow its acceptance as a replacement for a given *in vivo* test? Answering this question requires assembly and review of as much information as possible about the performance of the alternative method and the *in vivo* test it is intended to replace. This review process must ultimately allow the formulation of a judgment of whether the alternative method is acceptable or not for its intended use. Reviewed next is the information that must be considered in order to judge the relevance of an alternative method, and provide recommendations on how to establish objective benchmarks that can be used to help make this judgment.

The factors that must be considered in defining the relevance of an alternative method include an assessment of: (1) the best performance that can be expected from an alternative method given the performance characteristics of both the alternative method and the *in vivo* test it is intended to replace, (2) the performance of the *in vivo* test being replaced, and (3) the supplemental data available for use in conjunction with the alternative method during a safety assessment. Each of these factors is reviewed next.

Assessing the Best Performance that can be Expected from an Alternative Method

Ideally, alternative method results should provide nearly perfect predictions of the toxic endpoints measured in the *in vivo* method. However, there are important technical factors that prevent this ideal from being reached. One of the most important is the variability in the *in vivo* and alternative method data. If perfect prediction is unrealistic, then what is the best performance that can be expected from an alternative method? One approach that can be taken to answer this question is to use computer simulations based on the known performance characteristics of the *in vivo* test and the alternative method to create a picture that describes how the results from the validation study will appear if the prediction model is true. The results of these simulations can be used as benchmarks for objectively judging the acceptability of the alternative method that was measured in the validation study. In order to provide a practical example of this process, one can again return to the assessment of eye irritation alternatives. In order to assess the performance that may be expected from an alternative method evaluated in a validation program, a computer simulation based on the simple linear relationship of eqn (1) was used:

$$Y = (1.1)X$$

The effect of variability on the overall performance of the method was assessed by adding an error term to X and Y in each run of the simulation as described earlier (see step 3). After a large number of data points were simulated for each set of alternative method and *in vivo* test CVs, the Pearson's correlation coefficient was calculated in order to determine the correlation between the X and Y values. A second set of X values ranging from 0 to 40 were also run to simulate results for eye irritation scores that might be observed with a more restricted set of test substances such as cosmetics products.

Results from the simulations are summarized in **Table 4** and **Figure 8**. Each of the correlations shown in **Table 4** is based on 10 000 simulated responses. The effects on the size of the Pearson's correlation coefficient due to error imposed on the *in vivo* alternative method responses are shown under several conditions. In general, it is known that a tight linear relationship with nearly perfect correlation will exist when there are negligible levels of error in either X or Y. The expected level of correlation will be reduced as error is introduced into either X or Y. In the first case (ideal conditions, **Table 4**, **Figure 8**a), the imposed variation is set relatively low. As expected, Pearson's correlation coefficients are large, ranging between 0.97 and 0.99. Furthermore, restricting the alternative method results to the least irritating portion of the Draize scoring scheme ($X = 0$–40) has little effect on the correlation coefficients. In the second case (typical conditions, **Table 4**, **Figure 8b**), the CVs applied to the data (*in vivo* $CV = 50\%$, alternative method $CV = 20\%$) are consistent with those observed in the Draize test. Under these circumstances, the correlation is still high (> 0.8). Importantly, restricting the range of alternative method responses to the least irritating materials ($X = 0$–40) results in a decrease in the correlation coefficient to an approximate range of 0.6–0.7. Setting the imposed CV for the alternative method at 0.4 further decreases the correlation

Table 4 Expected Pearson's correlation coefficients when the error *in vivo* and alternative method data are considered

Imposed coefficient of variation		*Expected Pearson's correlation coefficient*	
Alternative method	*In vivo*	*Full range* ($x = 1$–100)	*Restricted range* ($x = 1$–40)
Ideal conditions			
0.05	0.05	0.994	0.990
0.10	0.10	0.975	0.960
Typical conditions			
0.20	0.40	0.860	0.719
0.20	0.50	0.828	0.652
0.20	0.60	0.803	0.608
0.40	0.40	0.719	0.604
0.40	0.50	0.690	0.542
0.40	0.60	0.672	0.504
Theoretical best conditions			
0.00	0.40	0.930	0.787
0.00	0.50	0.891	0.706
0.00	0.60	0.862	0.647
Alternative method equivalent to *in vivo* method			
0.40	0.40	0.719	0.604
0.50	0.50	0.635	0.490
0.60	0.60	0.543	0.403

Note: Computer simulations were used to assess the effects of variability in eye irritation test and alternative method data on the correlation coefficients expected between the data sets. The model used in the simulation assumed that the algorithm, $y = (1.1)x$, describes the relationship between the *in vivo* and alternative method data. Values for $x = 0$–100 were used to simulate responses across the entire Draize eye irritation scale. The simulations were conducted with test substances having the full range of response ($x = 1$–100) and for a restricted range representing the least irritating part of the eye irritation scale ($x = 1$–40). Each result is based on 10 000 runs of the simulation. Results are shown for the simulations where the variability is set relatively low (ideal conditions), and where the variability was set at a level consistent with performance of currently available alternative methods and the *in vivo* test (practical conditions). Additionally, simulations were conducted where the variability was set at zero for the alternative method (theoretical best conditions) and where the variability of the alternative method was set equivalent to the eye irritation test (alternative method equivalent to *in vivo*). The results of these simulations demonstrate that variability in the data sets can have a significant effect on the performance of the alternative method in predicting the *in vivo* response. Thus, the effect of variability must be taken into account when the performance of an alternative method is assessed. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

coefficients (**Table 4**). If an alternative method could be perfected technically, its CV would approach 0. Third case shows the effect of setting the CV = 0 (theoretical best conditions, **Table 4**, **Figure 8c**). Under these conditions, the expected Pearson correlation coefficients range between 0.85 and 0.95 when $X = 0$–100. When the alternative method results are restricted to the least irritating substances ($X = 0$–40) the correlation coefficients are lower, ranging from $\sim$0.65–0.80. Finally, in the fourth simulation, the alternative method CV was set to a level equivalent to that observed in the Draize eye irritation test. Under these conditions, the simulations estimate that the correlation coefficients will range from $\sim$0.5–0.7 when all levels of alternative method response ($X = 0$–100) are included (alternative method equivalent to the *in vivo* method, **Table 4**, **Figure 8d**). When the alternative method results are restricted to the least irritating range ($X = 0$–40), the coefficients are lower, ranging from 0.4 to 0.6.

It is important to note that these simulation studies were conducted under idealized conditions. The underlying assumption is that the relationship between the *in vivo* and alternative method data is linear. Also, the number of substances included in the simulations was large (10 000). Hence, the results of the simulations shown in **Table 4** and **Figure 8** represent a long-run average of the most optimistic correlations. In practical terms, even if all of the assumptions were true, the observed correlation might be higher or lower than the long-run average. Other deviations from the conditions assumed in these simulations, such as nonlinearity or nonuniform distribution of responses, can also be expected to reduce the level of correlation. Since it is unlikely the results from an alternative method are so simply related to a particular *in vivo* toxicity, it can be expected that validation studies will result in lower correlation coefficients, even for those alternative methods that may actually be reasonably predictive of the *in vivo* response.

Once completed, the results of the simulations may be used as benchmarks to objectively compare against the actual results obtained in the validation study. If the data from the validation study meet or exceed the simulated benchmarks, a strong case could be made that the method performs at an acceptable level. For example, if an eye irritation alternative method has a prediction model algorithm of $Y = (1.1)X$ and a CV = 20%, and if the *in vivo* test has a CV = 50%, then the best performance that may be expected would appear as follows: the relationship between the *in vivo* and alternative method data would look similar to that shown in **Figure 8b**, the correlation coefficients would be within the range of $\sim$0.6–0.8 (**Table 4**), and 95% CI_{pred} for a MAS prediction of 55 would be in the range of ± 40 ($n = 50$, **Table 2**). A method performing at these levels should be considered a reasonable performer. If the alternative method performance was less than such a

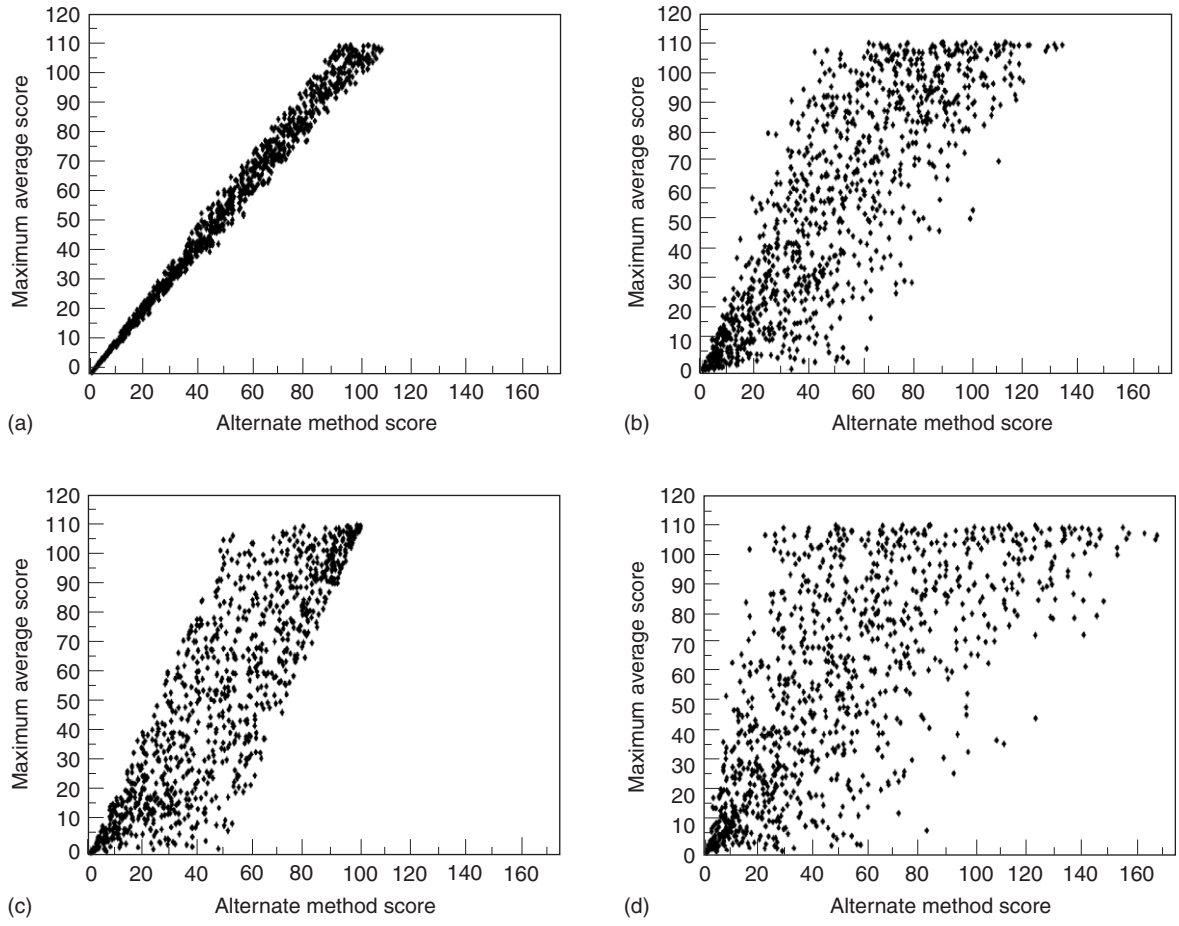

Figure 8 Effects of variability in the Draize test and alternative methods on correlation. Computer simulations were used to assess the effects of variability in the eye irritation test and alternative method data on the relationship between the two data sets. (a) The CVs applied to both the *in vivo* and alternative method data were 5%. (b) The CVs applied to the *in vivo* and alternative method data were 50% and 20%, respectively. (c) The CVs applied to the *in vivo* and alternative method data were 50% and 0%, respectively. (d) The CVs applied to the *in vivo* and alternative method data were 50% and 40%, respectively. Reproduced from *Toxicology In Vitro* 10: 479–501, 1996, Bruner, L. © Proctor & Gamble.

simulated benchmark, then there would be little evidence supporting its relevance.

Assess the Performance of the *In Vivo* Test that will be Replaced

Once estimates of the best possible performance of the methods are defined, another useful benchmark that can be used to assess the relevance of an alternative method is to compare its performance against that of the *in vivo* toxicity test it will replace. If the capability of the alternative method to predict an *in vivo* toxicity endpoint was at least equivalent to the capability of the *in vivo* test to predict its own result, then it would provide strong evidence supporting the relevance of the alternative method.

The capability of an *in vivo* method to predict results across multiple laboratories can be determined if its performance characteristics are known. If these data do not exist, then the interlaboratory variability terms needed to define the performance characteristics can be obtained by testing a common set of substances in several independent laboratories. If an alternative method is capable of predicting toxicity at a level equivalent to or better than the test it is intended to replace, then it would provide strong evidence supporting the contention that the alternative method can substitute for the *in vivo* toxicity test. Returning to the specific example of assessing the validity of eye irritation alternatives, the results of simulations that have conducted to assess the capability of the Draize test to predict results across laboratories are similar to those obtained from the simulations presented in **Tables 2** and **4** and **Figure 8**b. Thus, if the capability of the Draize test to predict eye irritation responses is used as the criterion for judging the relevance of eye irritation alternatives, then methods that perform similar to or better than those illustrated under typical conditions in **Tables 2** and **4** and **Figure 8**b should be considered adequate performers.

Assessing Supplemental Data Available for use in Conjunction with the Alternative Method During a Safety Assessment

Once the performance of the alternative method is compared against the appropriate benchmarks (such as the computer simulations described earlier), there are other factors that need to be considered when assessing the relevance of an alternative method. One factor considered particularly important is the mechanistic basis supporting the alternative method. Having an understanding of the mechanistic basis is important because it increases the probability that predictions of *in vivo* toxicity by the alternative methods are correct. Unfortunately, the science of toxicology has not progressed to the point that all toxic mechanisms are well understood. Thus, if alternative methods are to be accepted in the foreseeable future, they will be used under conditions where a full understanding of mechanisms is not available. Therefore, it is important to consider the approaches that the developers of an alternative method recommend following in order to compensate for this lack of knowledge.

Toxicologists who use alternative methods in the safety assessment process generally utilize three approaches to help decrease the uncertainty of the predictions when mechanistic understanding is weak. The first is to restrict the use of an alternative method to the same chemical classes that were used to develop the prediction model. This is important because similar materials are more likely to act by the same mechanisms of action. If the materials tested diverge significantly from those used to develop the prediction model, then the reliability of the predictions will decrease. This will occur because the divergent materials may exert effects through different toxic mechanisms that should perhaps be tested in different alternative methods that are sensitive to different chemical parameters and that use different prediction models.

The second approach commonly used to decrease the uncertainty of predictions is to compare the results obtained from an unknown test substance with one or two similar benchmark substances tested in the alternative method at the same time. If a material is intermediate in toxicity between two well-known benchmark standards in the alternative method, then it provides evidence that the material will be intermediate in toxicity *in vivo*. This approach provides greater confidence in a prediction than can be derived from testing isolated test substances on an absolute scale without any reference to other materials.

The third approach used to decrease the uncertainty associated with the predictions is to define specific limitations on the use of the assay. For example, developers may recommend restricting the use of a particular method to specific physical forms of test materials (liquids only or solids only) or for predicting limited ranges of irritancy (mild irritancy or severe irritancy only). These limitations may depend on many factors, especially specific technical incompatibilities associated with testing certain kinds of substances.

Next, it is important to consider whether the width of the 95% CI_{pred} from the alternative method is small enough to provide an acceptably precise prediction of *in vivo* toxicity. The analysis presented earlier demonstrated that the 95% CI_{pred} from an alternative method might be large. A benchmark that can be used to assess the acceptability of a large 95% CI_{pred} could be derived from an examination of the precision of toxicity measurements obtained from the *in vivo* toxicity test. If an alternative method provides predictions as precise as those obtained from the *in vivo* method it is intended to replace, then it would provide evidence that the 95% CI_{pred} alternative method is acceptable (for more information on the utility of the 95% CI_{pred}).

The margin of safety provided by a prediction from an alternative method compared to that obtained from the *in vivo* method must be considered. *In vivo* tests, such as the Draize eye irritation test, significantly overpredict the human response. This has been considered important because use of an overpredictive method decreases the probability of false-negative results that may be associated with a highly variable test. Establishing an acceptable margin of safety for an alternative method will depend on finding an appropriate balance between the risks to humans associated with possible underprediction due to variability versus the losses associated with a higher incidence of false-positive results that invariably results from setting more stringent cut-offs.

Finally, it is important to consider the experience that has been gained in use of the alternative method outside formal validation programs. Although the quality of data from other sources may be variable and not collected under blind conditions, it may provide additional useful insights into an alternative method's performance. If these additional data are consistent with the results obtained from a validation study, it would provide further evidence supporting the relevance of the alternative method.

Once all of this information is assembled and assessed, the participants in the validation study must render a final judgment on whether or not the method is relevant for the stated purpose. If (1) the measurement of method reliability from a validation study, (2) the actions taken to compensate for lack of mechanistic understanding, the performance of the method relative to calculated benchmarks, (3) the

width of the 95% CI_{pred}, the margin of safety, and (4) the breadth of experience toxicologists have with the method are judged to be adequate, this would provide strong evidence supporting the relevance of the alternative method.

If the alternative method is judged both reliable and relevant at the end of this process, then the new assay should be considered validated. Once validated, the alternative method may be used routinely in the safety assessment process and should be considered for acceptance by regulatory authorities (**Figure 1**). If the alternative method is judged not relevant, then it should not be used or considered for acceptance by regulatory authorities. The reasons for the rejection should be clearly stated so that the deficiencies can be identified and resolved in follow-up research if such work is likely to be fruitful.

Discussion

The development of an alternative method begins with the creation of a test followed by generation of a database that supports its utility. This work provides the preliminary evidence that a method is reproducible and has predictive capability. Once available, this information can be used to construct a prediction model that describes how to convert the results from an alternative method into predictions of toxicity *in vivo*. When a method has been technically advanced to this point, it may be assessed in the validation process.

Relative to the development of valid toxicity tests, the assessment of a toxicity test's validity is a relatively simple matter. Validation is relatively simple when the studies are designed to test the performance of an alternative method relative to performance criteria established prior to the start of the study. Defining a prediction model prior to the commencement of the study allows those evaluating an alternative method to construct a clear picture of what the results from a valid assay will look like before the study begins. When the results from the validation study become available, objective comparisons can be made between the predetermined picture and the actual study results. If the results are consistent with this picture, it provides strong evidence that the alternative method is reliable. If the results do not fit the picture, it provides evidence that either further developmental work on the alternative method is needed, or that the method should be abandoned. Such an approach has the advantage that it allows an objective evaluation of the data, while avoiding post hoc data fitting that does not provide definitive answers on alternative method validity.

Once the reliability of an alternative method has been confirmed in a validation study, the next step in the process is to review the relevance of the alternative method. This requires thorough consideration of all the performance data related to both the alternative method and the *in vivo* test it will replace. Ultimately, those conducting a validation program must take this information and render a judgment on whether the performance is good enough to allow replacement of the alternative method.

See also: Toxicity Testing, Alternatives; Toxicity Testing, Irritation; Toxicity Testing, Modeling.

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Toxicity, Acute

Donald J Ecobichon

By definition, acute toxicity studies are conducted to determine the total, adverse, biological effects caused during a finite period of time following the administration of single, frequently large doses of a chemical, physical (dusts, fibers), or biological (proteins, vaccines, genetically modified foods) agents, or some form of energy (radiation, ultraviolet light). The objectives of such studies are to discover any adverse health effect that could be attributed to the agent under investigation, including any immediate biochemical, physiological, and/or morphological changes, any delayed changes suggesting some secondary injury to body organs/tissues, as well as the death of the animal. In general, the effects observed in the experimental subjects, usually animals, are directly related to the amount of the poisonous substance administered. It is a common misconception that acute toxicity studies are designed only to express the potency of an agent in terms of the median lethal dose (LD_{50}), a value representing the estimated dose causing death of 50% of the population of test subjects exposed under the defined conditions of the test. Nothing could be further from the truth; acute toxicity studies encompass a number of experiments. The usual battery of acute toxicity tests is shown in **Table 1**.

Since information concerning the toxicity of the agent must be obtained before humans are exposed to it, this necessitates the use of animal models as surrogates or substitutes for the human. Several strains of rodents (mice, rats) are used routinely to determine the acute toxicity of new agents. Experiments are

Table 1 A battery of tests for the evaluation of acute toxicity

Test	*Description*	*Study period*
Lethality	LD_{50} (LC_{50}) or an estimated value	24 h
	Surviving animals – close observation permits determination of duration of toxicity; recovery; development of secondary toxicity; changes in hematology, blood chemistry, urinalysis; changes in organ and tissue function	14 days
Primary irritation	Skin	
	Exposure	4–24 h
	Evaluation	24, 48, 72 h
	Eye	
	Exposure	1.0 s
	Evaluation	1.0, 24, 48 and 72 h
Sensitization	Repeated (5 days week^{-1}) dermal application	14 days
	Rest period of 10–14 days	
	Challenge dose at days 28–30	
	Evaluation	24, 48, 72 h
Photoallergic and phototoxic reactions	Repeated treatment (oral, iv, dermal) for 10–14 days	
	Rest period of 14–21 days	
	Retreatment with UV light on shaved skin patch	
	Evaluation	24, 48, 72 h

conducted using both males and females of the species because of known sex differences in response(s) to various agents. Indeed, an extensive body of data has been built up over several decades using rodent species, thereby permitting chemical-to-chemical, inter- and intraspecies comparisons. It is desirable to have acute toxicity data from nonrodent (rabbit, guinea pig, dog, monkey) species, particularly if the results from the mouse and rat differ greatly, suggesting distinct species differences in response(s). In contrast, should similar toxicity be seen in a number of experimental animal species, the same toxicity might be produced in the human at some, as yet unknown, dosage to the agent.

The agent should be administered via the route by which the species might be expected to obtain the toxicant. In general, the usual routes of exposure for the human include ingestion, inhalation, or by contact with the skin. Accidental exposure to industrial or home products might also include having them splashed into the eyes or onto the skin. However, if an agent being tested is a drug, exposure might require intravenous, intramuscular, or subcutaneous routes of administration.

Why are acute toxicity studies necessary? With any new agent, there will be workers exposed to relatively high concentrations during its manufacture, handling, packaging, and use. Accidents may occur not only in the workplace but during the transportation (ship, rail, truck) of the agent, with exposure of bystanders in the immediate vicinity of the accident and risk to personnel involved with the accident or cleanup of the spillage (e.g., police, firefighters, emergency response teams, sanitation crews). There is also the potential of accidental and/or intentional exposure of the product user, members of his/her family, neighbors, children, and pets. It is essential to know just how toxic or nontoxic the agent may be under the most bizarre circumstances of exposure (e.g., ingesting or inhaling the agent or getting it on the skin). How much is safe? How little is too much? The information obtained from acute toxicity studies can be found printed in a material safety data sheet (MSDS) required by law to be prepared for each and every product manufactured and to be available to the public, to industries using the products, and to health and safety professionals.

Determination of the Lethal Dose

The LD_{50} is a statistical estimate of the acute lethality of an agent administered to a specified sex, age, and strain of a species of animal. The value provides a measure of the relative toxicity of an unknown agent compared to other agents administered by the same route to the same species, strain, age, and sex of the animal. As listed in **Table 1**, the LD_{50} value, an indicator of lethal potency, is frequently the first biological safety test determined for a new chemical, the agent being administered via the route by which the human might acquire a high concentration, and animal mortality being assessed in the 24 h period after treatment. Traditional LD_{50} tests have been replaced by abbreviated test protocols that minimize animal use. Given that people might acquire the chemical by different routes, it might be necessary to carry out two experiments, choosing two of the three possible routes (ingestion, inhalation, dermal) of administration in anticipation of quite different values. Although accurate determinations of the lethal potency are no longer required (designing experiments in which 60 to 100 animals might be used in the classical determination of the LD_{50}), some insight into the potency, even a rough estimate of the range of acute toxicity, is essential. Regulatory agencies are still concerned about massive spills and the impact of these on the health of local populations. The Bhopal incident revealed just how little information was available on the toxicity associated with inhalation, dermal, and ocular exposure to methyl isocyanate. While criticism has been leveled that the LD_{50} values are just numbers, they are valid predictors of acute toxicity, albeit only of mortality and not of morbidity or long-term adverse health effects.

Animal rights activists have repeatedly challenged the need for the LD_{50} determination, in terms of the inflicting of injury to the test animals and the needless waste of large numbers of animals to obtain a number that is only a rough estimate. A properly designed study will yield much more information than just 'a number'. While, by definition, 50% of the animals will die, close observation of these animals during the first 12 h period after treatment may reveal several biological clues to possible mechanisms by which the toxicant may be causing an effect, clues that are valuable to the clinical toxicologist in attempts to alleviate human suffering. However, 50% of the animals will survive the treatment and these survivors are a repository of biological effects elicited by the test agent. These effects are studied over the next 14 day period to assess the short or long duration of toxicity; the rapid or slow recovery; the appearance of any additional, delayed, or secondary toxic effects; changes in hematology, serum biochemistry, and urinalysis; and changes in organ/tissue function (liver, kidney, and nervous system) measured by relatively noninvasive techniques, all without having to destroy the animals. When the animals are euthanized at 14 days

after treatment, organs/tissues will be available for detailed microscopic examination to correlate observed biological effects and/or injury with possible morphological changes. Thus, the animals surviving the toxic insult are a veritable treasure trove of information concerning the mechanism(s) of the chemical-induced toxicity.

Given the spectrum of observed and measured biological effects, one important aim of the acute toxicity study is to develop a quantitative relationship between the intensity of a measured response or adverse health effect and the concentration(s) of agent administered. Assuming that the dosage of agent can be 'delivered' to the test animals accurately with minimal variability, this leaves interanimal variability in response, one major reason why the classical LD_{50} determination uses 8–10 animals per treatment group. The biological responses and variability are usually presented in graphic form, the *x*-axis representing the range of dosage while the *y*-axis reflects the biological response in some quantitative manner (**Figure 1**). From such graphs, a dosage-related appearance of target organ toxicity may be determined, some organs/tissues responding to low levels of the agent (**Figure 1a**), others responding only at elevated concentrations (**Figure 1b**). In addition, the slope of the dose–effect relationship for each organ/tissue can be determined, indicating whether or not small changes in dosage produce marked biological changes (a steep slope, potent agent; **Figure 1a**), or the reverse, where large increases in dosage are accompanied only by weak to modest changes in responses (a shallow slope, weakly toxic agent, **Figure 1c**).

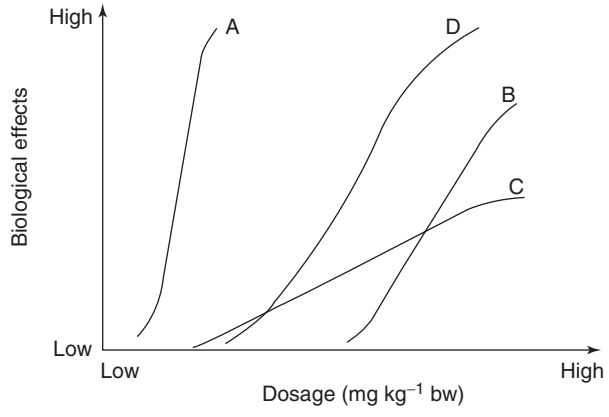

Figure 1 Theoretical dose–effect relationships illustrating possible, different target organ (A, B, C, D) responses over a wide range of agent concentrations, indicating the importance of the slope of the relationship to predict whether or not a small or large change in dosage is required to induce marked, moderate, or weak biological effects.

Range-Finding for the Lethal Dose

With a view toward reducing, refining, and replacing animal testing procedures, the 'three Rs' initiative, a simplified range-finding (or up-and-down) procedure using, at most, six animals has been developed. An arbitrarily selected, initial dosage (mg kg^{-1} body weight) is administered to a pair of suitable animals, with subsequent close observation for effects over a predetermined time period. If little or no toxicity is observed, the second pair of animals receives a dosage 50% (1.5/l.0) higher than the initial dosage. If no toxicity is observed, the second pair of animals receive a dosage double (2.0/1.0) the original dosage. If, however, the second level (1.5/1.0) is lethal to one or both animals, some concentration between the original and second dosage is given to the third pair of animals. If the originally chosen dosage causes severe intoxication and mortality, the dosage administered to the second pair will be downscaled to the order of 50% or 66% of the initial dosage.

How close do the above 'estimates' relate to the actual LD_{50}? Studies have shown that the approximate lethal dose for 86% of those chemicals tested in this manner were within 30% of the known LD_{50} values determined by the classical approach. The method is not infallible: for example, some 14% of chemicals were outside this range; and no dose–response or slope information can be obtained. International agreement has been reached that the up-and-down procedure could replace the conventional acute toxicity test for the purposes of hazard classification and label (including color-coding) production.

Primary Irritation Studies

Regulatory agencies demand the preparation of MSDSs for each chemical manufactured and sold. These must include testing for ocular and dermal irritancy as well as dermal hypersensitivity, sensitization, and phototoxicity. Such information is also required to meet regulations for packaging and labeling, hazard classification, and transportation. Products freely available for purchase by the public, including cosmetics, pesticides, cleaners, detergents, polishes, waxes, health care products (soaps, bath gels, shampoos, creams, mouthwashes) must receive the same assessment.

The Skin Test

Testing procedures for dermal irritation evolved from the product safety tests of the early 1940s using a variety of animal species, rat, rabbit, guinea pig, and dog, as surrogates for the human, with the addition of miniature swine and rhesus monkeys at later

Table 2 Factors governing the selection of animal species for dermal irritation testing

Physical properties of the test agent (liquid, powder, emulsion)
Solubility of the test agent in aqueous or organic-based solutions
Inherent toxicity of the test agent (e.g., low toxicity requiring application of large volumes)
Known species sensitivities of test animal dermis compared to that of the human skin from earlier experience
Application of different concentrations of test agent and the need for comparable control sites where the dermis is similar
Application of the test agent to intact and abraded dermis, the latter having slight damage to the stratum corneum (dead cell layer) by mechanical means to mimic the loss of the protective barrier through abrasions and cuts

Table 3 *In vitro* methods for testing dermal toxicity

Test system	*Biological endpoint*
Corrositex™	Corrosion
Skintex™	Corrosion
Testskin™	Irritation
Epiderm™	Phototoxicity
Episkin™	Percutaneous absorption
Melanoderm™	DNA damage
Immunoderm™	Immunological parameters
Epiderm-FT™	
Skin™ 2K1350	
Transcutaneous electrical resistance assay – mouse skin disk	Corrosion

Source: Data from various chapters in Salem H and Katz SA (eds.). (2003) *Alternative Toxicological Methods*. Boca Raton: CRC Press.

dates. As has been observed for humans, absorption of test agents through the skin varied considerably from region to region on the body surface. The choice of test species may be dictated by a number of factors such as those shown in **Table 2**.

The test substance (liquid, solid, paste, emulsion) is usually applied to a shaved test area (6.0 cm^2 or 1.0 $in.^2$) of skin on the back in a uniform layer, the site being covered by a gauze patch taped in place to prevent the animal from licking the material off the site during normal grooming. The trunk may be wrapped with an impervious plastic sheet, this practice being particularly useful when multiple sites are treated. At the end of the test period, usually 24 h, the coverings are removed, the residual test agent being wiped off with gauze wetted with warm soapy water, and the test areas are evaluated for (1) edema (puffiness, swelling) and (2) erythema (redness, inflammation). This evaluation is repeated at 48 and 72 h after treatment. The Organization for Economic Cooperation and Development have introduced a 4 h rabbit covered patch test in place of the 24 h study.

While highly subjective, a scoring system has been developed to measure visually the degree of puffiness (edema) and redness (erythema). The scoring system assigns a number (0–4) for each toxicological endpoint, ranging from none through very slight, slight, moderate, to severe effects. Average numerical scores for the primary irritation index are obtained from the results on five or six animals per treatment level over the test period (24, 48, or 72 h) to provide a dose–response relationship concerning the potency of the test agent to cause irritation (edema, erythema) as well as the rate of recovery from effects. This information provides guidance to regulatory agencies for cautions/warnings to be printed on product labels.

In Vitro Irritation – Skin

Concerns relating to pain and suffering as well as the numbers of animals used in the classical dermal irritation test have resulted in much effort to develop rapid, *in vitro*, test systems suitable for screening out highly irritant or corrosive chemicals, assessing hypersensitivity, sensitization, and phototoxicity. Unfortunately, the validation criteria of reliability, reproducibility, and relevance (predictability, biological basis for stated purpose) have not been attained and, consequently, no tests have been validated by national or international regulatory agencies. A list of such test systems and their biological endpoints is presented in **Table 3**. A few of these tests are considered as 'stand alone' indicators of corrosivity, thereby eliminating the need for further confirmatory animal studies.

Most commercial *in vitro* test systems are human tissue equivalents – three-dimensional tissue culture models of human skin at an air–liquid interface forming a reconstructed, differentiated dermis and a functional stratum corneum, usually composed of keratinocytes grown on a collagen matrix with fibroblasts, and/or melanocytes or Langerhans cells. The skin-like layer is suspended in a two-chamber cell, the test agent being added to the upper chamber either in solution or applied directly to the moist surface (stratum corneum). Samples of medium from the lower chamber can be removed for analysis or the 'skin' can be stained specifically for microscopic study of cell damage. The chemical or biological endpoints examine cell viability and membrane integrity (Neutral Red dye penetration, tetrazolium dye metabolism, protein leakage), cell proliferation and protein synthesis (Coomassie Blue or Kanacid Blue dyes), release of inflammatory mediators (cytokines, prostaglandins), and mediators of apoptosis (p53, p21, caspases) (**Table 4**). Isolated dermis from human cadavers or from swine can be used in double-chamber systems in a similar manner to that described for skin equivalents. Eventually, a battery of *in vitro* tests will be approved by

Table 4 Biological endpoints for testing skin toxicity

Skintex system: cell-free complex mixture of vaious macromolecules, with physicochemical changes occurring on interaction with irritants, resulting in changes in light transmission
Cell cultures: microorganisms and mammalian cell lines, examining Neutral red dye penetration Protein leakage, colorimetric assay Coomassi Blue or Kenacid Blue for cell proliferation protein synthesis
Testskin: human tissue equivalents (human kerationcytes on collagen or collagen – glycosaminoglycan matrix with human fibroblasts) forming an epidermis for examining Dye penetration (Neutral Red) Dye exclusion Cellular damage by agents Chemical penetration Cell growth and development Cellular metabolism
Isolated tissues: prepared for a double chamber; agent applied to the top and sampled from the bottom fluid to test penetration through the skin; can use candaver skin or isolated animal skin (mouse, rat, swine)

regulatory agencies, the guidelines requiring the submission of a spectrum of results from tests having well defined biological endpoints.

The Eye Test

Damage to the eye is an all-too-common consequence of an accidental splashing of industrial chemicals, home and health care products, pesticides, solvents, etc., resulting in painful and frequently permanent injury. The Draize eye test, first described in 1944, has become a target of animal welfare groups, antivivisectionists, and concerned scientists who claim that it is not required, is inhumane, causes unnecessary pain to the test animal, generates a subjective result even with the available detailed scoring systems used, and is prone to interlaboratory variability in results such that the test is meaningless. The thought of knowingly placing some highly irritating agent in an animal's eye and causing pain is abhorrent. In fact, if the dermal irritancy test is positive, there is little scientific basis for carrying out the eye test, since the agent will almost certainly be positive in the eye. Hence, dermal irritancy tests will screen out the highly toxic agents. However, between the highly damaging, strong acids or bases, and completely innocuous agents, lie a wide variety of seemingly neutral, slightly acidic or basic soaps, detergents, shampoos, cosmetic creams, and lotions, all of which may show minimal effects on the skin but still be irritating if accidentally introduced into the eye. Literally thousands of products must be tested annually. For the sake of occupational, bystander, and consumer safety, these products must still be subjected to an eye irritation test.

The basic ocular irritation test in the rabbit will be described in detail in another section, but it is important to point out that the number of test animals can be reduced from the usual six at each exposure level to two or, at most, three animals per dose without sacrificing much accuracy. Many test series have shown 88–91% accuracy with two animals per treatment group. The agent, instilled in the pouch formed by the lower eyelid, is held in place for 1 s and then released. The treated eye is not washed, allowing the animal's own tear secretions to flush out the material. The untreated eye serves as a control. Both eyes are examined at 1, 24, 48, and 72 h after treatment, the irritation (or damage) to the cornea, the conjunctiva, and the iris being scored numerically in a subjective manner. The test is open in that the experiment can be terminated at 72 h if there is no evidence of irritation, but observed effects can be assessed for a longer time period.

In Vitro Irritation – Eye

Considerable effort has been made by industry, national and international regulatory bodies to replace the eye irritancy test with suitable *in vitro* assays for such toxicity endpoints as cytotoxicity, corneal opacity, and inflammation, replacing the subjective nature of the assessment with objective and quantitative measurements (**Table 5**). What may evolve from the myriad of test tube and cell culture assay systems and *in vitro*, isolated eye or corneal test systems undergoing development and validation at the present time is one or more battery of test, none of them giving a complete answer to the question, but each contributing some quantifiable information for a selected endpoint. These test batteries will be used as screening devices to identify the strong-to-moderate irritants and the nonirritants, leaving those agents showing suspicious or equivocal results to be tested in animals. These *in vitro* test systems will aid significantly in reducing the number of animals subjected to the eye test, but they will never totally replace it.

None of the *in vitro* alternative 'eye' tests has proven applicable as a valid replacement for the Draize eye irritation test or has been acceptable for regulatory purposes (**Table 5**), though some are considered either reliable or reproducible. The most frequently used test has been the *ex vivo* bovine cornea opacity and permeability assay. The newer human corneal equivalents system, an *in vitro* culture of immortalized human corneal cells that develops into

Table 5 *In vitro* methods for testing eye toxicity

Cytotoxicity
Immortalized mammalian cell lines such as HeLa, V79, human keratinocytes and mouse fibroblasts and canine kidney cells to study
Dye uptake/exclusion – viable cells
Dye penetration – cell integrity
Dye penetration – membrane damage
Opacity
Eyetex assay: formation of high-molecular-weight protein aggregates causing reduced light transmission
Isolated bovine cornea and permeability (BOCP): prepared in a two-compartment chamber; chemical-induced damage causing changes in light transmission through the cornea; e.g.,
– an increase signifying corneal cell loss
– a decrease indicating opacity
Fluorescein dye uptake assessing cell damage
Inflammation
Bovine corneal cup method: inflammatory response releasing chemotactic factors and then reacted with neutrophils
Bovine corneal cup assay: inflammatory response releasing specific mediators (histamine, serotonin, prostaglandins, leukotrienes, thromboxanes) that can be collected in the bath medium and quantitated by chemical assay
Rat vaginal tissue assay: similar to bovine corneal cup assay with release of specific mediators
Fertile chicken egg chorioallantoic membrane (CAM) assay: scoring for vascular changes in the membrane blood vessels with fluorescein dye as well necrotic damage

all three elements of the cornea (stratified epithelium, stroma with keratinocytes, endothelial cell layer), may prove exceedingly useful as it mimics key physical, morphological, and physiological properties of the cornea.

Skin Sensitization Studies

Dermal reactions are seen whereby exposure to a certain chemical causes little effect following initial contact with it but, with repeated (daily, weekly, or even once a month) exposure of the skin, an effect, usually an erythema or red spot, is seen that occurs earlier in time, is more severe, and persists for a longer duration. Subsequent exposures, even though weeks or years apart, result in what appears to be an allergy-like, delayed reaction at the site of exposure or even on parts of the body where no exposure has occurred. The pattern of development of this skin condition, frequently found in the workplace, is suggestive of an allergy.

It is known that certain chemicals, upon penetrating the skin, act as antigens, reacting with immature, dermal, dendritic cells called 'Langerhans cells', which process the antigen while migrating to the drainage lymph nodes where they interact with T-cells to stimulate lymphocyte proliferation. The new lymphocytes are 'primed' effector cells capable of recognizing this new antigen (or allergen). With time, the entire immune system becomes 'alerted' to this antigen, the antibodies responding to its presence even at a much later date to cause a contact hypersensitive reaction or allergic contact dermatitis.

While there are a number of animal models for dermal sensitization studies, all based on the Draize test, the two most frequently accepted and used by regulatory agencies are the guinea pig maximization test (GPMT) and the Buehler assay (BA). In both, repeated, daily low doses of the test agent are injected intradermally or applied topically on closely shaved skin over a 14 day period (induction phase). This treatment period encourages the development of an immunological response as described above. Following a suitable 10–14 day resting period, a challenge dose, usually a lower concentration than was used as a sensitizing dose, is applied to a fresh, untreated site (elicitation phase). The severity of the responses of the animals (erythema, edema) is scored as described earlier over a period of 24, 48, and 72 h after the challenge dose. A greater irritation (edema, erythema) after the challenge dose is indicative of chemical sensitization. The GPMT and BA differ in that Freund's complete adjuvant is used in the former, and the latter test requires 21 days of sensitization exposure, the challenge dose being administered on day 28.

With time (28 days), the number of animals required (20 guinea pigs), costs, plus discomfort and/or pain to the animals, more rapid tests using fewer animals have been sought to screen chemical antigens. The mouse local lymph node assay requires that the test agent be applied for three consecutive days to the dorsal portion of the ears. On day 6, a radiolabeled compound (^{3}H-methylthymidine, ^{125}I-iododeoxyuridine) is injected in the tail vein and, 5 h later, the draining lymph node of each ear is excised, macerated, and incorporation of the radiolabel into proliferating lymphocytes is measured by β-scintillation or gamma counting for comparison with controls. A second test, the mouse ear swelling test, is conducted by application of the suspected antigen to the abdomen or back followed, in a few days, by a challenge dose applied to the ears. The endpoint analysis is the measurement of edema, the thickness of the swollen ears being assessed with calipers.

Photoallergic and Phototoxic Reactions

These skin conditions, found in the workplace and in some cases of therapeutic treatment, involve the interaction of certain wavelengths (275–325 nm) of ultraviolet (UV) light that can penetrate skin to the

depth of the subdermal blood capillaries and a host of drugs (salicylates, sulfonamides, tetracyclines, thiazides, phenothiazines, chlordiazepoxide, cyclamates, hexachlorophene, griseofulvin) and chemicals (coal tar derivatives, dyes, etc.), resulting in the formation of highly reactive intermediates. In the phototoxic situation, current theories suggest that, in the presence of light of suitable wavelength (UV < 320 nm), the chemical molecules are converted into reactive intermediates that can cause direct local cellular toxicity displayed as delayed erythema and hyperpigmentation (urticaria, rash), followed by a desquamation (shedding or scaling) of the skin (eczema). With photoallergic skin reactions, the light-induced activation of the chemical results in the strong binding of some reactive intermediate to cellular and blood plasma proteins to produce antigens that will stimulate antibody formation and recognition by the complete immune system.

The guinea pig or rabbit are species of choice for studying UV light induced chemical toxicity in the skin. In most cases, small amounts of the test agent will be administered orally or by intravenous injection for 10–14 days. Following a resting period of 14–21 days, the animals will receive the same dosage of test agent via the same route of administration, with exposure to light of an appropriate wavelength on an area of closely shaved skin, scoring the edema and erythema by the subjective numerical system described above. Such animal studies are complicated by the necessity of finding the correct wavelength of UV light to activate the particular chemical being tested, the narrower the band on either side of the specific wavelength, the more intense the biological effect that will be seen. More frequently, one sees screening tests for photoallergy and phototoxicity being included in toxicity data submissions since, as the test systems and diagnostic techniques improve, more of these toxicities are being detected in the workplace, the home, and in patients receiving certain medications.

A number of *in vitro* test systems have undergone assessment for detecting phototoxicity. None, other than the 3T3 mouse fibroblast – neutral red dye uptake (NRU) – have yielded reproducible results correlating well with the *in vivo* data. Having been validated, the 3T3NRU method is ready for regulatory acceptance. However, only the European Community has proposed that this test become the standard method for testing UV light absorbing cosmetic ingredients for phototoxic potential.

See also: Eye Irritancy Testing; *In Vitro* Test; *In Vivo* Test; LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50); Photoallergens; Skin; Toxicity, Chronic.

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Toxicity, Chronic

Donald J Ecobichon

Chronic toxicity studies may be defined as those involving the characterization of adverse health effects following the long-term, repeated administration of a test substance over a significant portion of the lifespan of the test animal species. In general, the term usually denotes a study conducted for longer than 3 months. However, depending upon the test species being used, a 24–72 h exposure period could represent a chronic study for aquatic insects between hatching and flying, a span of some months for birds, etc., or a number of years if dogs or monkeys were to be used. Originally, in regulatory terms, a chronic mammalian study signified a duration of 2 years, ~70–80% of the lifespan of a laboratory rodent. However, the length of chronic studies has been shortened in the past decade and currently stands at 6 months duration. This reduction was based partly on results from 286 repeated dose toxicity studies in

which new findings were noted after 6 months for only seven compounds. All significant findings were detected within 6 months for 91% of studies in rats, 98% of those in dogs, and 87% of investigations using monkeys. To accommodate for animals with longer lifespans or the shortening of the study duration, the dosage regimen is usually adjusted upward so that the level obtained in a lifetime will be acquired in the shorter time interval. There are, of course, major problems inherent in overloading the animals' physiological capacity to distribute, biotransform, and excrete the excessive amounts administered. These difficulties lead to interference in function and to secondary toxic effects seen in other organs.

In chronic studies, it is essential to distinguish between a study defining the shape and nature of the dose–effect relationship for some or any toxicological end point, and one in which the primary objective is to evaluate the presence or absence of a particular toxicological effect, for example, neurotoxicity or carcinogenesis.

Experimental Design

With the longer duration of chronic studies and the labor-intensive nature of the investigations involving the employment of a number of individuals to care for the animals, to obtain samples of biological fluids (blood, urine), to carry out various analyses (hematology, blood biochemistry, urinalysis, quantitation of tissue residues of test agent, etc.) and to prepare and examine histological slides of various body tissues, careful attention must be paid to the design of the study. Such investigations can become very expensive particularly if they have to be repeated because of some oversight, a mistake made, or the appearance of unexpected toxicity. It is important to develop an experimental protocol based on the following questions:

1. How many animals will be acquired?
2. How many dosage levels should be used?
3. When does the 'lesion' or toxicity begin?
4. How rapidly does the toxicity progress toward signs and symptoms?
5. Does the toxicity disappear (rapidly, slowly or never) when exposure is stopped?
6. How long should the study be conducted?
7. How can the main 'theme' of the study be retained (or regained) when other, unexpected toxicity is observed, including excessive mortality within a single treatment group, etc.?

A piece of paper and a pencil are the most valuable tools, at this stage of the study, to develop responses to the question 'what if' this or that might happen during the investigation and attempting to anticipate what might happen with repeated administration of the agent. Study designs should be as open-ended as possible, retaining flexibility to react to the unforeseen, unpredicted events, as well as to those that are anticipated. An example of an experimental protocol is shown in **Figure 1**.

All too frequently, chronic studies are carried out according to the guidelines of national or international regulatory agencies rather than according to good scientific principles. The regulatory guidelines are only there to guarantee a minimum of requirements, information, results, etc., standardized or harmonized within and between national and international governmental bodies.

There may be scientific justification in carrying out a study in a particular manner not commonly ascribed to by such agencies. Few agencies would react

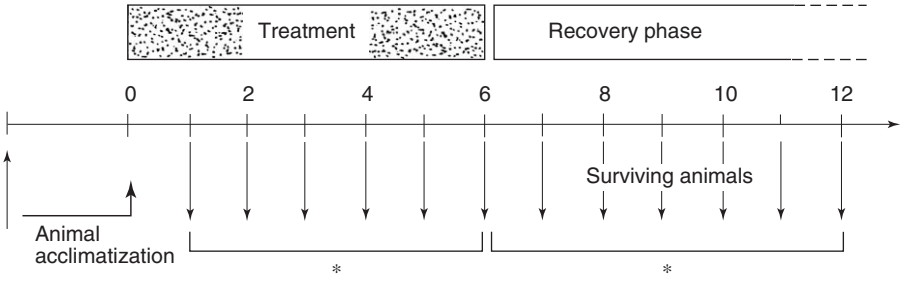

Figure 1 The design of a chronic (6 months, 180 days) study, the planning chart enabling the investigator to determine the total number of animals required based on the number of dosage levels and the number of treated animals required for euthanasia at each selected time interval (30 days). Periodic selection of representative subgroups of each population (controls, low, intermediate, and high levels of test agent) would permit both a dosage- and time-related study of the development of toxicant-related lesions as well as changes in physiological and/or biochemical tests of organ and tissue function/injury. Included in the design is a post-treatment recovery phase open to a further 6 months to assess the permanence or reversibility of the toxicant effects. *Animals (representative subgroups) killed at predetermined time intervals for physiological, biochemical, and morphological study.

unfavorably to a design defended by valid scientific principles.

The most difficult task in developing the protocol for a chronic toxicity study is the selection of an appropriate range of dosages to be used, based on limited knowledge about the effects of long-term exposure of an animal model to the test agent. Some prior knowledge of the shape of the dose–effect relationship is required, most frequently obtained from subchronic studies conducted beforehand. The objective is to use dosages of the test agent, administered by inhalation, in the diet, in drinking water, or by oral gavage, that will cause adverse health effects within the study period but will not cause excessive mortality. The maximum tolerated dose (MTD) is frequently used as the highest dose. This exposure level is being defined as the highest dose that causes no more than a 10% decrease in body weight, and does not produce mortality, clinical signs of toxicity or pathologic lesions that would be predicted to shorten the animal's natural lifespan. Usually, two lower levels, an intermediate (MTD/4) and a low (MTD/8) level will be selected in anticipation of observing a gradation in both appearance and severity of effect(s). Suitable numbers of control, untreated animals must be carried throughout the study, resulting in a four-dose design.

The MTD has caused considerable controversy when used in chronic toxicity studies. Many scientists believe that the highest dose should be above the MTD to be certain of eliciting some quantifiable deleterious effect(s), for example, to demonstrate that the model 'works'. Other scientists feel just as strongly that the highest dose should be lower than the MTD. In these scenarios, either a dose-related increase in adverse health effects or little or no toxicity may be detected independent of minimal body weight changes. The latter scenario poses a number of problems in interpreting the results for regulatory purposes. In other study designs, the lower dose levels may be fractions of the selected highest dose, either equally spaced as is shown in **Figure 2** (50% and 25%, respectively, of the highest dose chosen) or unequally spaced (20% and 1.0% of the highest dose chosen).

A basic principle of toxicology is that there is a correlation between biological effects and the level(s) of exposure, a dose–effect relationship. With a three-dose design (0, X/2, X), straight line relationships can always be determined. However, this may not reflect the true situation whereas, with a four-dose design, the usual curvilinear (concave or convex) relationship will be seen. Prior to embarking on the chronic study, an initial trial period of 2 or 3 weeks duration should be carried out at the selected dosage range, even to the point of conducting dose-dependent kinetic and

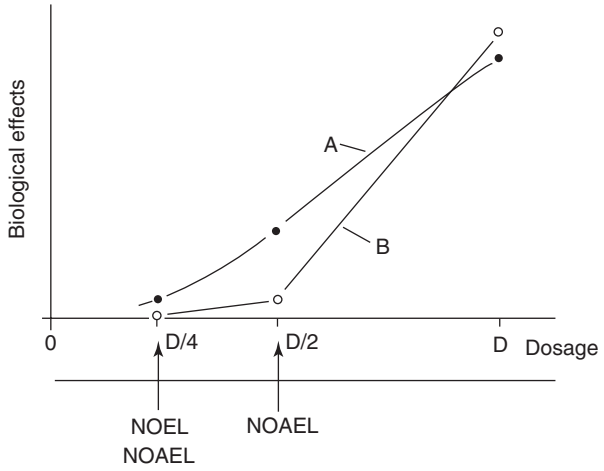

Figure 2 An experimental design showing unequally spaced dosages and theoretical results used for extrapolation to estimate the no-observed-adverse-effect level (NOAEL) or the no-observed-effect level (NOEL). In curve A, some slight degree of toxicity was observed at the lowest dose administered, permitting only the estimation of an NOAEL. In curve B, no toxicity was observed at the lowest dose, and slight toxicity at the intermediate dose, permitting the estimation of a NOEL and a NOAEL.

tissue distribution studies on a few animals at each treatment level in order to assess whether or not the test animals will tolerate the dosages selected. Adjustments to the preselected dosages can be made at this time without compromising the remainder of the study. Frequently, some downward adjustment of the highest dose may be necessary when excessive toxicity is observed.

The duration of the study may be dictated by guidelines from certain national or international regulatory bodies. As was indicated earlier, formerly, chronic studies were of 2 years duration. The exorbitant costs involved plus the concept that the same toxicity could be detected and quantitated in a shorter period of time by giving higher dosages resulted in a reduction in the duration to a 6 month time period. This is still a contentious issue among toxicologists, many maintaining that 6 months is only a fraction (20%) of the lifespan of a rodent and that toxicity may not appear until the animal is older than 12 months, when geriatric dysfunction begins to occur.

In earlier chronic studies, the animals were allowed to proceed until obvious toxicity was seen or the animals became moribund, these animals being killed in a humane manner for study. However, by that time, the toxicity was well advanced and, of course, the question of when the toxicity began or subtle changes in organ function occurred, the usual signals of impending toxicity could not be answered. Such questions can only be answered by the periodic (every 30 days) selection of representative subgroups from each treatment group and from control animals

for euthanasia and an in-depth study of biochemical, physiological, and morphological indices of toxicity (**Figure 1**).

Such an approach does not preclude the periodic sampling of blood from animals or the collection of urine and feces for analysis using techniques that are not life threatening. Such a protocol will permit the investigator to identify pretoxic changes in organ function and morphology as well as to determine when, in a dose-dependent manner, toxicity appears initially, both as obvious signs and symptoms and as morphological changes.

How does one know whether or not toxicity persists following termination of the exposure or that the signs and symptoms disappear slowly or quickly? Are there any long-lasting effects? Is the tissue damage reversible or irreversible? To answer these questions, additional animals should be incorporated into the study protocol so that, at the end of the treatment period, there is a reasonable population of animals remaining, sufficient to participate in a recovery phase study. Once again, small representative subgroups from each treatment group and controls will be killed and subjected to detailed study at predetermined time intervals (e.g., at 30, 60, and 90 days).

How many animals will be needed to provide biochemical, physiological, and morphological data for each of the previously mentioned, planned intervals of subgroup selection (during and after treatment), as well as for the unexpected toxicity and mortality that almost certainly will be encountered if the study is being conducted properly? What constitutes a representative subgroup? If one accepts the premise that five animals of each sex, selected at each time interval for euthanasia, are representative of the population being studied, the number of animals required can be calculated quickly. A larger number of controls, for example, 10 animals, would be required at each interval so that variability in the population as a whole can be monitored. Ten to 15 additional animals should be included in each treatment group in anticipation that some mortality may occur during treatment. Thus, for a 6 month chronic toxicity study having three dosage levels plus control animals, with subgroups being killed at 30 day intervals during treatment and at 30 day intervals over a 3 month recovery phase, an investigator might consider a minimum of 150 male and 150 female animals undergoing treatment with 90 control animals of each sex, a total of 480 animals. The number of animals could be reduced by spacing out the time intervals of subgroup selection but perhaps at the risk of missing some subtle change in one or more parameters being assessed, thereby not recognizing the appearance of the toxic effect(s).

A wide range of biochemical and physiological parameters should be monitored throughout the entire study, both during and after treatment (**Figure 3**). The moribund animals or those killed at preselected intervals will undergo an extensive morphological examination, both gross and microscopic, in order to identify possible organs or tissues where the test agent may exert an effect. Changes in body weight, food, and water consumption can provide information concerning the tolerance/aversion of the test animal to the agent in the food or water. A reduction in body weight, particularly if it is dose-related, over the study period is a simple but effective indicator of the animals' well-being; any sharp deviation will alert the investigator to a possible chemical-related event. If the animal does not feel well, it will not eat sufficient food to maintain normal growth and development, this being particularly critical in small rodents that have an elevated basic metabolic rate. Taste aversion to the test agent can be identified quickly by measuring the amount of food or water ingested in a 24 h period. A spectrum of biological markers–general tests of hematology, blood serum biochemistry and urinalysis–should be planned before the experiment is started, picking parameters that, if they are seen to change, will point in a meaningful way to some organ/tissue that may be affected by the test agent. All of these tests should be broad enough in scope to detect the unexpected as well as the anticipated toxicity. During this prolonged treatment period, various noninvasive tests of neurological competence (behavioral, sensory perception, motor function, learning skills) can be conducted in addition to organ (liver, cardiac, pulmonary) function tests that will pose minimal risk to the animals' health. At euthanasia, the animals will be dissected and a wide range of organs will be removed, fixed, sliced, and stained appropriately for light and electron microscopic examination. An attempt will be made to correlate morphological changes or damage with the biochemical and physiological changes observed and quantified.

Carcinogenicity Studies

Chronic studies include one additional end point of toxicity; any carcinogenicity related to exposure to the test agent. Traditionally, and mainly because tumor formation is seen in older animals, carcinogenicity studies in rodents are conducted for an 18 or 24 month period for mice and rats, respectively, separate from the shorter-term, 6 month chronic studies. The dosage range used for carcinogenic assessment is lower than that used for chronic toxicity

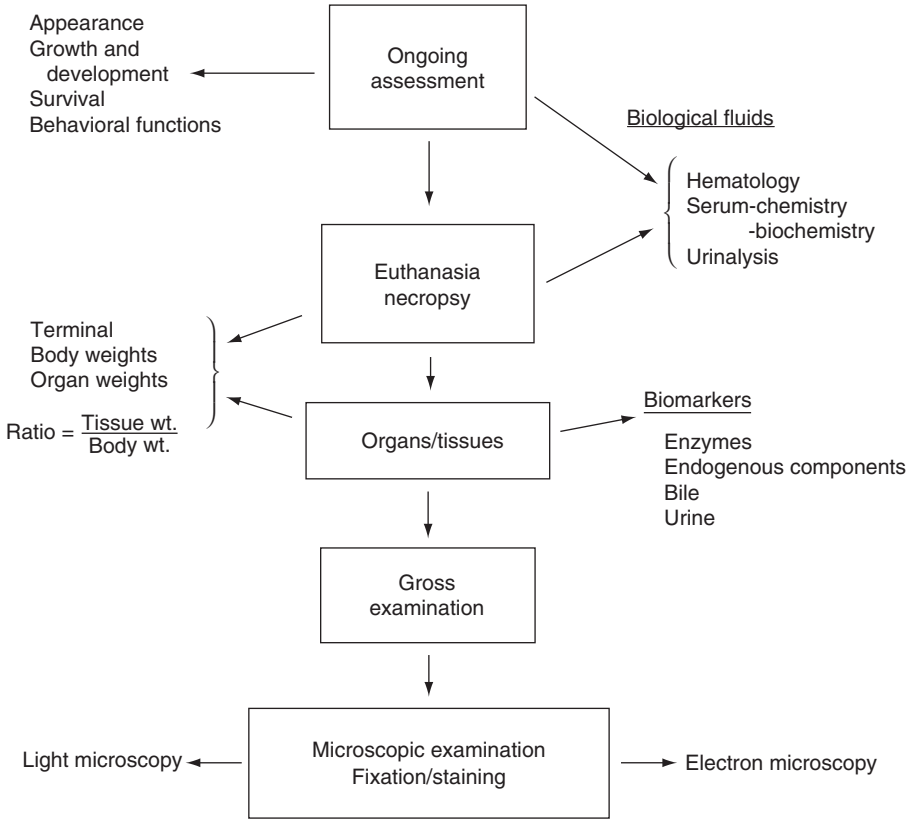

Figure 3 A flow chart depicting the various parameters or end points of toxicity to be monitored during the chronic study, both during and after treatment, as well as those studied following euthanasia and necropsy. The routine, periodic assessment of chosen parameters may detect the onset of impending toxicity, proving invaluable for the detection of developing lesions and as predictors of target-organ toxicity.

because the end point is tumor formation, independent of any other sort of toxicity.

Considerable controversy has arisen among scientists over the selection of the highest dosage to be used in carcinogenic studies. One faction suggests a value known as the MTD–the highest dose of the test agent during a chronic study that can be predicted not to alter the animals' normal longevity from effects other than carcinogenicity. This dosage level is also known as the 'minimally toxic dose'. With this dosage, selected from a chronic study as a reference point, two lower dosage levels, equally or unequally spaced, can be calculated so that, hopefully, the lowest level of exposure that will cause tumor formation can be determined. Another faction claims that doses near the MTD may cause significant cell mortality and/or compensatory changes (mitogenesis) that would make the damaged organ/tissue more susceptible to tumor formation, and that these levels are far higher than the human would encounter. Other investigators recommend that the top dose be some appropriate multiple of the expected human exposure. While this controversy has not been settled, retrospective review of a large number of chemical-induced carcinogenicity studies has revealed that two-thirds of the carcinogens would have been detected even if the estimated MTD had not been included but that, in many studies, some site-specific carcinogenic effects would not have been observed. Among the remaining one-third of the studies, $\sim 80\%$ had elevated rates of site-specific tumors at lower doses as well. Most carcinogenic effects observed at the highest dose were also present at reduced incidences at lower doses (MTD/4, MTD/2), although the results might or might not be statistically significant. The choice of dosages for long-term carcinogenicity studies will remain a contentious issue.

In the future, genetically engineered, transgenic animals may be used in carcinogenicity studies because these strains are more highly susceptible to early induction of cancer within 6–9 months. The 1997 International Conference on Harmonisation agreement gave study sponsors the option to replace one of the two species required for current carcinogenicity assessment with a short- or medium-term alternative model, usually a transgenic mouse. A number of mouse strains, for example, TgAC, TgrasH2, P53 $+/-$, and XPA $-/-$ P53 $+/-$, have

been examined, the data being reviewed recently. The replacement of an 18 month mouse carcinogenicity study with an alternative assay requires scientific justification for the selection of the model. To date, data obtained for TgrasH2 together with P53 +/− appear to provide the best combined coverage for detecting carcinogens. However, these various transgenic models are just beginning the arduous process of experimentation and validation.

Interpretation of Results

The main objective of any chronic study is to supply a database which will provide assurance to public concerns about the safety of chemicals found in the human environment, the sources usually being air, food, and water, and the results of such studies being used in safety evaluation, risk assessment, and risk management decisions. The objective of long-term toxicity testing, usually in rodents, is to assess the potential chronic toxicity of a chemical, including carcinogenicity, effects that would not be evident in subchronic studies. Pertinent to the studies is the development of a dose–effect relationship that ranges from no observed effect through minor changes to overt toxicity and the determination of dosages at which these observations occur. These values will be used by regulatory agencies to determine safe levels of exposure stated as a maximum allowable concentration, recommended maximum levels, reference dose, virtually safe dose, tolerance or acceptable daily intake, etc.

End points of toxicity and the severity of observed adverse health effects obtained from a four-dosage range study may be represented by arbitrarily determined dosage values such as the lowest-observed-adverse-effect level (LOAEL), the no-observed-adverse-effect level (NOAEL), or the no-observed-effect level (NOEL) (**Figure 2**). These values, of course, must be derived at the end of the chronic toxicity study based on the observations. It is unlikely that all three values would be obtained from a study. Usually, one of these indices might be determined with a degree of reliability in the estimated value. Either the NOAEL or NOEL can be used by regulatory bodies to establish reasonable, estimated values for the indices mentioned in the previous paragraph.

See also: Toxicity, Subchronic.

Further Reading

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Toxicity, Subchronic

Donald J Ecobichon

While acute exposure to high concentrations of chemicals can occur in any environment (the outdoors, the home, the workplace), individuals are more frequently exposed over much longer periods of time to agents at levels lower than those that might prove fatal. Acute exposure studies will not identify those adverse health effects, both immediate and/or delayed, that might arise as a consequence of long-term, lower level exposure. The simulation of such exposure requires the development of more carefully designed experiments in which larger numbers of animals are used, lower levels of the potential toxicant are administered by a suitable route of exposure, over a longer time period and a number of preselected biochemical, physiological, and morphological endpoints of toxicity are monitored and quantified throughout the study period. In general, a subacute or subchronic study is one conducted over a 21–90 day period, using surrogate animal species to mimic conditions anticipated to be found in human exposure.

These long-term studies are designed to examine the nature of the toxic effects from lower dosages at the organ, tissue, and cellular level in order to determine possible mechanisms of toxicant action. The repeated administration/exposure to the agent will permit examination of possible cumulative effects as body burdens of the agent and/or biotransformation products (metabolites) are acquired with time. Subchronic

studies will allow the investigator to ascertain the variation in response(s), by making close observations of a continuum of biological changes and/or unique events occurring over a wide range of dosage levels in both sexes and ages of different animal species. This will permit the identification of the appropriate dosage at which biochemical, physiological, and morphological changes, both macroscopic and microscopic, occur in relation to the level and/or duration of exposure. The last objective of such studies is to be able to predict the long-range adverse health effects in the test animal species, using the results to extrapolate whether or not toxicity might be expressed in the human at some, as yet unknown, level of exposure.

The old adage holds true that 'the more species of animals in which the same biological response(s) to an agent can be produced, the greater is the chance that, at some dosage, the same effect might occur in the human'. Invariably, subchronic studies are conducted in at least two species – one rodent species (with a choice of the mouse, rat, or possibly the hamster) and a nonrodent species (frequently the dog (purebred beagle), the rabbit, or occasionally, a strain of monkey (rhesus or macaque)). With such diverse species being studied, distinct variations in response(s) related to physiological (distribution, storage, excretion) or biochemical (e.g., biotransformation rate, type of metabolites formed) differences should be anticipated, thus, it is hoped, permitting a better appreciation of how the human might respond.

Experimental Design

The design of subchronic studies is extremely important not only because of the longer time period involved, but also because such studies are labor-intensive, involving a number of people in caring for the animals, obtaining blood samples, carrying out analyses on samples (hematology, blood chemistry, urinalysis) or examining morphological specimens (e.g., preparing and staining slides of tissue sections, light and electron microscopic evaluation). Since these studies become very expensive, it is important to set up an experimental design before the studies are begun, asking the following questions:

1. How many animals are required?
2. How many dosage levels should be used?
3. When does the 'lesion' or toxicity begin?
4. How rapidly does the toxicity progress toward signs and symptoms?
5. Does the toxicity disappear (rapidly, slowly, or never) when exposure is stopped?
6. How can the main 'theme' of the study be retained (or required) when other, unexpected toxicity is observed, including excessive mortality within a single treatment group, etc.?
7. How long should the study be conducted?

Any study design should be open-ended, allowing for unforeseen and unpredicted events that frequently appear in long-term studies as well as those events predicted. A piece of paper and a pencil are the most valuable tools at this stage of the design, asking the question 'what if' this or that might happen during the study. A simple experimental design is shown in **Figure 1**.

Having selected the agent for study, the first question is what dosage range will be used, and how many dosage levels will be necessary. Generally chosen from

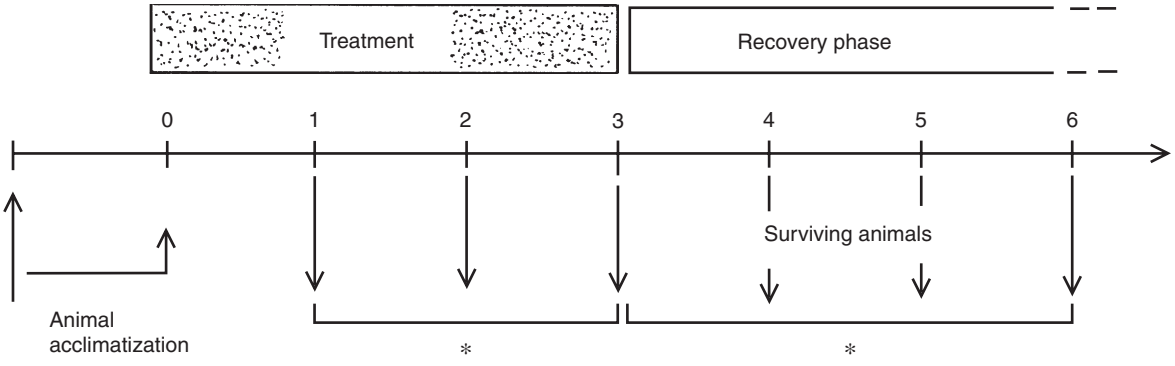

Figure 1 The design of a subchronic (3 month, 90 day) study: the planning chart enables the investigator to determine the total number of animals required based on the number of dosage levels and the number of treated animals required for euthanasia at each selected time interval (30 days). Periodic selection of representative subgroups of each population (controls, low, intermediate, and high levels of test agent) would permit both a dosage- and time-related study of the development of toxicant-related lesions as well as changes in physiological and/or biochemical tests of the organ and tissue function/injury. Included in the design is a post-treatment recovery phase open to a further 90 days to assess the permanence or reversibility of the toxicant effects. *Animals (representative subgroups) killed at predetermined time intervals for physiological, biochemical, and morphological study.

the dosages used in acute toxicity studies, one should always use three dosage levels, a high level guaranteed to elicit toxicity in the animal model and two lower (intermediate and low) dosages in the hope that a gradation in the appearance and severity of toxicity will be observed. Comparable control (untreated) animals must be carried through the study as well. A major objective of the study is to establish a relationship between biological effects and a level of exposure, for example, a dose–effect relationship which may be linear, or more likely, will be curvilinear.

The duration of the study may be dictated by guidelines from specific regulatory agencies. In general, subchronic feeding studies, particularly if the agent may be incorporated into the diet, drinking water, or given by oral gavage, are of 90 days duration. Inhalation or dermal exposure studies may be from 21 to 90 days in duration.

It will be unlikely that the investigator can predict when toxicity will begin to appear based on the acute toxicity results. One can allow the subchronic study to proceed until sick animals are observed before killing them in a humane manner but, by that time, toxicity will be in an advanced state. The question of when toxicity begins to appear can only be answered by the periodic (every 7 or 30 days in 21 or 90 day studies, respectively) selection of representative subgroups from each treatment group for euthanasia and in-depth study of biochemical, physiological, and morphological indices of toxicity. Such a design allows the investigator to identify pretoxic changes in organ function and morphology as well as to determine when, in a dose-dependent manner, toxicity appears.

If exposure is stopped, does toxicity persist or do the signs and symptoms slowly or quickly disappear? Is the tissue damage reversible or irreversible? By incorporating additional animals into the treatment groups at the beginning, there is a good chance that, at the end of the treatment period, there will be sufficient animals surviving to permit a recovery phase to be studied, again, selecting small, representative subgroups for euthanasia and detailed study at predetermined time intervals (30, 60, 90 days).

How many animals are needed to cover all the eventualities mentioned above – three dosage levels, periodic euthanasia of subgroups during treatment and after termination of exposure, possible expected and unexpected toxicity, some mortality among the animals, related or unrelated to treatment, etc.? What constitutes a representative group or subgroup? How many untreated control animals should be included? If one accepts the premise that five animals of each sex, selected at each time interval for euthanasia, are representative of the population under study, then the number of animals required can be calculated quickly with a few (10–15) additional animals being included for 'safety'. By the time the study is under way, the animal cost is the least expensive item in the investigation. Thus, one should not be reticent at including more animals. For a 90 day feeding study, an investigator would conservatively consider 150 male and 150 female rodents to adequately protect the study from the vagaries of Murphy's Law (if anything can happen, it will), including adequate numbers of control and 'spare' animals undergoing treatment.

A wide range of parameters can and should be measured during the entire study. Some, as simple as body weight, growth/development, food and water consumption are noninvasive and pose no risk to the animals. A change in body weight, particularly in small rodents having normally high basal metabolic rates, is a simple yet effective indicator of general well-being. A sharp decrease alerts the investigator to perhaps a chemical-related appetite depression, although the effect may be as simple as an aversion to the taste of the test agent in the diet rather than toxicity. Frequently, one can see a nice gradation in growth curves between the control animals and those in the three treatment groups. A spectrum of biological markers – general tests of hematology, blood serum chemistry, urinalysis – should be planned before the start of the study along with other specific physiological and biochemical markers. These parameters should be based on anticipated target organ toxicity but, of course, remaining broad enough in scope to detect the unexpected toxicity as well. Such a scheme is shown in **Figure 2**. During the treatment period, various noninvasive tests of neurological competence (behavior, sensory perception, motor function, learning skills) can be conducted along with liver, kidney, cardiac, and pulmonary function tests that pose minimal risk to the survival of the test animal.

Information Management

As you can appreciate, with over 300 animals in a subchronic study being monitored periodically for any adverse health effect, one can accumulate literally thousands of continuous and terminal data points on biochemical, physiological, and morphological parameters which become a significant burden to the investigator and the staff. Management of this data is crucial. Most of the data flow control has become automated on computer.

Good Laboratory Practices (GLP) regulations insist on the appropriate management of animal data so that quality assurance/quality control (QA/QC) personnel can, at any time, select an animal number

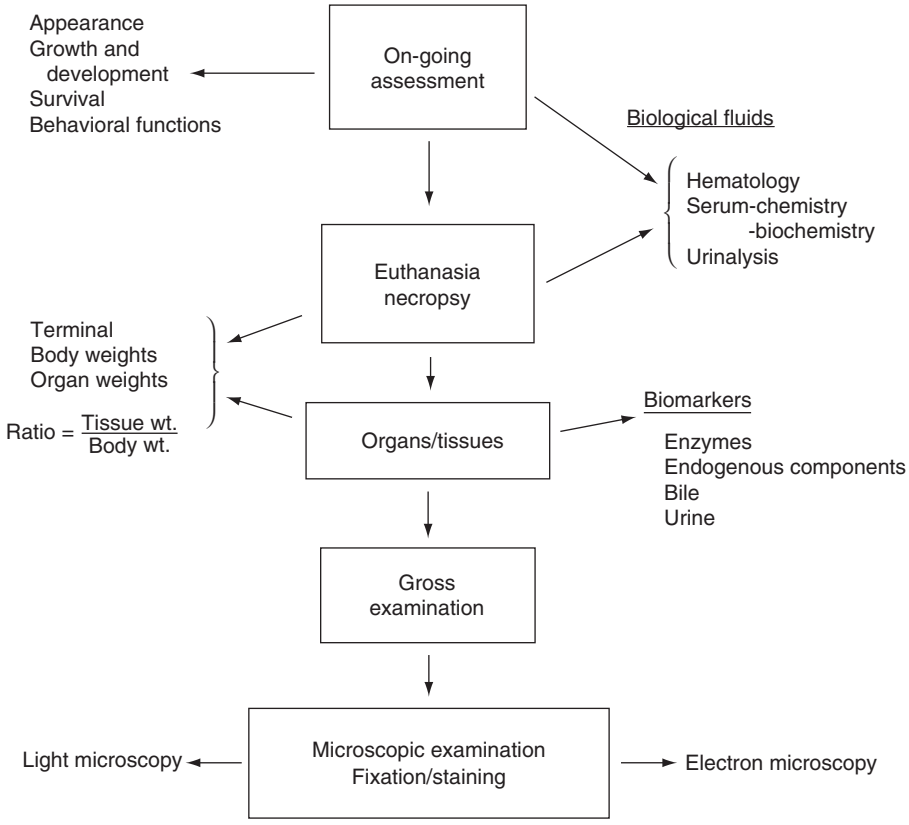

Figure 2 A flow chart depicting the various parameters or endpoints of toxicity to be monitored during the subchronic study, both during and after treatment, as well as those studied following euthanasia and necropsy. The routine, periodic assessment of chosen parameters may detect the onset of impending toxicity, proving invaluable for the detection of developing lesions and as predictors of target-organ toxicity.

and track it and its data throughout the study until it either dies or is killed. The laboratory carrying out the study will have a QA/QC staff while the company for whom the study is being done will have its QA/QC personnel check the study in progress and at the completion of the report before it is submitted to another QA/QC evaluation carried out by the regulatory agency.

Interpretation of Results

Depending upon the eventual use of the chemical, the results of a well designed and conducted subchronic study may provide all of the information required for the agent, for example, for a drug that will be used for only a limited time period of treatment. However, if exposure is anticipated to be longer or that the individual may be exposed to the agent (pesticides, food additives, industrial chemicals, etc.) for a lifetime, the results of the subchronic study may only justify making a decision on the need for additional or more extended and perhaps specific studies to determine more clearly the toxicological profile of the agent. In the time period of the study, some preliminary evidence may have been obtained to show that, if treatment had persisted for a longer time at the same or at lower dosages, some specific target organ toxicity might have become manifest. In such a situation, the subchronic study results have at least established a dosage range for administration over a significant proportion of the animal species lifespan (e.g., the characteristic chronic toxicity study).

Changes observed in body weight gain, organ weight, hematological and biochemical data, organ function, etc. should be subjected to trend analysis with those parameters measured in control animals. These findings should be correlated with the pathological and histopathological data. Since a basic tenet of toxicology is that there should be some correlation between observed biological effect(s) and the concentrations of test chemical, much effort is expended in establishing a trend in a dose–effect relationship for each parameter being measured.

Subchronic studies are of limited value for predicting the toxic effects of lifespan exposure. The nature and degree of toxicity vary, the sensitivity performance, and metabolic capability of organs/tissues change with aging and the spontaneous

occurrence of other diseases. No predictions can be made from subchronic exposure concerning the mutagenic, teratogenic, or carcinogenic potential of the test agent, and any effects on reproduction can only be related to primary effects on the testes and ovaries.

Given the known uncertainties which arise from qualitative and quantitative differences as well as similarities in toxicological effects observed in animals and man, interpretation of the dose-related effects must be done cautiously. If a no-observed-adverse-effect level of dosage can be determined from the experiment, this may be used to establish values for acceptable daily intake or a reference dose for setting tolerances of additives in food, for residue levels of unintentional contaminants, or for acceptable levels of exposure (threshold limit values, maximum acceptable concentrations) to chemicals in the workplace. However, beyond these indices, extrapolation to what might occur during a lifetime of exposure is extremely risky.

See also: Toxicity, Chronic.

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Toxicokinetics *See* Pharmacokinetics/Toxicokinetics.

Toxicology

Gabriel L Plaa

All chemical or physical agents can affect living organisms. Some of these effects can be beneficial while others can be adverse to the well-being of the organism. 'Toxicology', in its broadest sense, is the science that concerns itself with the adverse effects of chemical or physical agents on living organisms. There are two important elements in the definition of toxicology: the first is the aggressor substance (the chemical or physical agent), and the second is the target (the living organism affected). Since the target is a living organism, toxicology, as a consequence, is a biological science. The science of toxicology, however, draws heavily from the knowledge acquired in other sciences: chemistry, physics, physiology, biochemistry, pathology, pharmacology, immunology, genetics, molecular biology, mathematics, statistics, etc. Although the aggressor substance may be a physical agent (e.g., an electromagnetic field), the major concern of modern toxicology deals with chemicals (medicinal products, drugs of abuse, occupational chemicals, pesticides, industrial effluents, hazardous wastes, etc.). Biological targets include humans as well as other species. In our egocentric fashion, we humans place much emphasis on ourselves as potential biological targets, but we must not forget that chemicals can have an important impact on other biological targets. While humans are considered a target of particular interest, other terrestrial and aquatic species are of considerable importance as potential biological targets. Toxicological problems worthy of societal concern are not limited only to those that affect human beings.

Toxicology is a very broad science. Toxicity studies are essential to the safe use of chemical substances in various aspects of our lives. In medicine, one must know the adverse effects of therapeutic agents as well as their beneficial utility. In the workplace, chemicals used as solvents, components of a process, or as intermediates must be handled safely. In agriculture, the safe use of pesticides, feed additives, or growth regulators as well as the problem of food residues are major considerations. Industrial effluents and their impact on the environment are societal preoccupations. Identification of chemically induced diseases and their prevention is an important public health undertaking. Regulatory controls essential for the safe use of chemicals require a broad and detailed understanding of toxicology.

Subdivisions of Toxicology

There are a number of subdivisions to the science of toxicology, and these vary according to the particular interests of the toxicologist concerned. No single classification system of categorization is entirely satisfactory. About 35 years ago, however, T.A. Loomis divided the science of toxicology into three

major subdivisions: environmental, economic, and forensic. These subdivisions were in large part based on how humans would come in contact with potentially harmful chemicals. Generally, the scheme is still valid today.

Environmental toxicology, according to Loomis, is concerned primarily with the harmful effects of chemicals that are encountered by humans because of the presence of chemicals in the atmosphere, or in the occupational setting, or through recreational activities, or by ingestion as food residues. Environmental toxicology is the branch of toxicology that deals with the incidental exposure to chemicals that appear basically as contaminants of air, food, or water. This characterization of environmental toxicology is still appropriate today, although toxicologists are also interested in the impact of chemical substances on species native to various parts of the environment.

Economic toxicology, according to Loomis, deals with the potentially harmful effects of chemicals that are intentionally administered to living organisms for the purpose of achieving a specific beneficial effect. Here we find drugs developed for medicinal therapeutic purposes in human or veterinary medicine, chemicals developed for use as pesticides or insecticides, or substances designed as food additives. The term 'economic' used by Loomis stems from the work of Adrian Albert, who coined the phrase 'selective toxicity' to describe the use of chemicals by one species (humans) to eliminate an undesirable species, such as insects. In this context, humans were called the 'economic species' and the insect the 'uneconomic species'. Regardless of the terminology, economic as used by Loomis denotes that the potentially toxic chemical in question is being developed for some specific purpose, and we are interested in the undesirable effects that may accompany the beneficial effect.

Loomis categorizes forensic toxicology as the subdivision of toxicology that deals with the medical and legal aspects of the harmful effects of chemicals on humans. Therefore, here one finds those aspects of toxicology related to the diagnosis and treatment of chemical intoxications. The legal aspects of the subdivision pertain to cause-and-effect relationships between exposure to an aggressor agent and the adverse consequences observed in humans. We are very familiar with certain aspects of forensic toxicology, like the operation of a motor vehicle while under the influence of alcohol, or the use of performance enhancing drugs in sporting events. The detection and quantification of chemicals in biological fluids or tissues is a very important phase of forensic toxicology.

Scope and Activities of Toxicology

While Loomis' three-category scheme covers the broad use of applied toxicological information, it does little to denote the wide scope that represents the activities of toxicologists. In 1987, E. Hodgson and P.E. Levi formulated another set of characteristics in an attempt to cover the scope of the many activities encompassed by the discipline. They chose to organize toxicology into five broad categories, each with a number of subcategories:

A. Mechanisms of toxic action – all events leading to adverse effects at the level of the organ, cell, or molecular function

1. biochemical toxicology (enzymes, receptors, molecular events, etc.);
2. behavioral toxicology (peripheral and central nervous system, endocrine system etc.);
3. nutritional toxicology (influence of diet on the expression of toxicity);
4. carcinogenesis (chemical and biochemical events that lead to cancer);
5. teratogenesis (effects on embryonic and fetal developmental processes);
6. mutagenesis (effects on the genetic material and inheritance of these defects); and
7. organ toxicity (effects at the level of organ function).

B. Measurement of toxicants and toxicity – these include the use of analytical chemistry, bioassays, and applied mathematics

1. analytical toxicology (identification and assay of toxic chemicals in biological material);
2. toxicity testing (use of living systems to estimate toxic effects);
3. toxicological pathology (branch of pathology dealing with the effects of toxic substances);
4. structure–activity study (relationship between chemical structure and toxicity);
5. biomathematics and statistics (determination of significance, risk estimates); and
6. epidemiology (occurrence of toxicity).

C. Applied Toxicology – Applications as they occur in the field

1. clinical toxicology (diagnosis and treatment of human poisoning);
2. veterinary toxicology (diagnosis and treatment of poisoning of animals);
3. forensic toxicology (medicolegal aspects of clinical poisonings, including analytical detection);

4. environmental toxicology (movement of toxicants in the environment and food chain, effects on various species); and
5. industrial toxicology (deals with the occupational environment).

D. Chemical use classes – includes the toxicological aspects of the development of new chemicals for commercial use

1. agricultural chemicals (pesticides, targeted species);
2. clinical drugs (adverse effects of pharmaceutical agents);
3. drugs of abuse (chemicals taken for psychological effects that cause dependency and toxicity);
4. food additives (food preservatives, facilitate food processing);
5. industrial chemicals (solvents, degreasers, intermediates, etc.);
6. naturally occurring substances (includes phytotoxins, mycotoxins, and inorganic minerals); and
7. combustion products (generated from fuels and other industrial chemicals).

E. Regulatory toxicology – concerned with laws and regulations and their enforcement

1. legal aspects (government agencies); and
2. risk assessment (definition of risk, risk–benefit considerations).

Early History of Toxicology

Obviously, the earliest humans gathered toxicological information through experience, and trial-and-error. Animal venoms and plant poisons eventually were used for killing other animals or humans. Over time, an art of poisoning developed, including the training of professionals. (Much of what follows is based on a chapter written by J.F. Borzelleca in the third edition of A.W. Hayes' *Principles and Methods of Toxicology*, 1994.) The interested reader is encouraged to consult this comprehensive work for more detail.

Poisons, antidotes, and case histories are found in early Egyptian writings (Ebers Papyrus, ~1500 BC); toxic agents were used by Egyptians in the administration of justice. Additional lists of poisons and antidotes appear in early Chinese (Shen Nung, ~2700 BC) and Hindu (the Riga-Veda, ~1500 BC) writings. The contributions of Hippocrates (~400 BC) and Diocles (~350 BC) in ancient Greece described rational methods for the treatment of poisoning. Theophrastus (~350 BC) is said to be the first to recognize the adulteration of food. The Roman physician Celsus' treatise (~40 BC), *De Medicina*, continues Hippocratic teaching and contains a separate section on poisons and antidotes; *De Medicina* gained worldwide importance, since it was the first medical work published (~1500 AD) after the invention of the printing press.

Important writings came from Avicenna of Persia (~1000) and Maimonides, court physician to Saladin and rabbi of Cairo (~200). These texts exerted an enormous influence for nearly 500 years. Finally, Paracelsus (~1525), a Swiss physician, made the important declaration that "all things are poisons...solely the dose determines that a thing is not a poison." This concept is the cornerstone of modern toxicology.

Toxicology was brought to other areas of human endeavor. An Italian physician, Ramazzini (~1700), is credited with bringing toxicology to the workplace with his works on health problems related to the occupational setting. He is considered the founder of occupational medicine. The application of analytical chemistry to food and drug safety was introduced by the works of Accum (~1800) in *A Treatise on Adulterations to Food*, and *Culinary Poisons*. Finally, Orfila's (~1815) classic work on toxicology combined forensic and clinical toxicology with analytical chemistry; it is said to be the first book devoted entirely to toxicology. The father of experimental physiology, C. Bernard, used toxic chemicals (~1850) as laboratory tools to understand mechanisms involved in normal physiological processes. As such, he contributed greatly to the understanding of mechanisms of action of toxic substances.

Modern toxicology, which is over 100 years old, is both an experimental and an applied science. It is a predictive science that has evolved remarkably since the time of Orfila, particularly in the last 60 years. Recent additions to the discipline include safety evaluation and risk assessment. Toxicology will be influenced greatly by expanding knowledge in immunology and genetics in years to come.

See also: Behavioral Toxicology; Developmental Toxicology; Ecotoxicology; Environmental Toxicology; Food Additives; Forensic Toxicology; Information Resources in Toxicology; Molecular Toxicology–Recombinant DNA Technology; Occupational Toxicology; Radiation Toxicology, Ionizing and Nonionizing; Toxicology, Education and Careers; Toxicology, History of.

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Toxicology in the Arts, Culture, and Imagination

Philip Wexler

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Toxicology's merit as an interdisciplinary science with a growing research base and extensive technical literature is undisputed. It has a long history in practice as well. Early man sought to expose himself to food and other substances, both natural and concocted, which would enhance his well-being. Would-be suicide victims aside, he sought to avoid those products which were harmful to him. Dangerous substances did have their uses, though, against enemies and crop pests, or for hunting. Toxicology also has a long-established legal and regulatory framework, aiming at the sensible management and use of chemicals to benefit society while minimizing their harm.

Less appreciated, though, is the extensive role toxicology has played in our collective imagination. There has always been, and always will be, a fascination with the actions of poisons and how people use them, usually for nefarious ends. Poisoning has been seen as tragic, humorous, subversive, and edgy, given one's perspective. The criminal mind has served as a fertile ground for artists and writers throughout the ages. Real and imagined stories about poisons keep us transfixed over the campfire, make us shudder as we read, and chill our bones as we watch movies and theatre. The language of toxicology serves as a rich source of metaphor in our daily lives. Outside the laboratory, away from the regulatory and judicial systems, or the research library, toxicology continues to infiltrate our thoughts.

Literature, Poetry, Drama, and Legend

Because certain stories, real or imagined, have been expressed in a multitude of genres, there is some arbitrariness in this article in classifying a story as literature or art or music if it fits into two or more categories. One will discover, for example, that many of the stories taken up by representational art, as discussed in the next section, could just as well have been treated in this section as part of the verbal domain.

Though historically a king of Babylonia, Gilgamesh has served as a source of legends. The 2000 BC Sumerian *Epic of Gilgamesh*, for example, refers to scorpions as the guardians of hell:

> Those who guard the gate are
> Poison scorpions
> Who terrorize all, whose spells bring death.

Greek mythology is littered with toxic tales. One of the many examples is Achilles' humiliating death toward the end of the Trojan War. Paris let loose a poison arrow which lodged in his heel, the one vulnerable spot on his body. Poison arrows were used extensively by Odysseus and other warriors in the Homeric cycle and legends penned by other classical Greek authors and playwrights.

The Jewish and Christian bibles are splendid sources of toxicological metaphor and allusion. Witness *Job* 6:

> The arrows of the Almighty are in me,
> my spirit drinks in their poison;
> God's terrors are marshaled against me.

And *Jeremiah* 9:

> Therefore, this is what the LORD Almighty, the God of Israel, says: "See, I will make this people eat bitter food and drink poisoned water."

Here is one of the Lord's admonitions to his people in Deuteronomy: "Lest there should be among you man, or woman, or family, or tribe, whose heart turneth away this day from the LORD our God, to go and serve the gods of these nations; lest there should be among you a root that beareth gall and wormwood." Wormwood oil is a potent poison. Wormwood itself, as well as other herbs, is used to distill absinthe, a potent alcoholic beverage, particularly popular in the nineteenth century.

Early literary adaptations of poisoning imagery, before the advent of synthetic pharmaceuticals and industrial chemicals, revolved around food, drink, and poisonous and venomous animals. Chaucer's *Pardoner's Tale* concerns three drunken men on a quest to destroy Death as revenge for a friend who died of the plague. An old man directs them to an

oak tree where he says they can find Death. Surprisingly, they discover, instead, a treasure. The youngest of the three is sent to town for wine for all of them to celebrate. He returns with the wine, poisoned though, in order to murder his companions and keep the treasure for himself. Before he can accomplish his plot, they stab him and he dies. The remaining two celebrate with the poisoned wine and die as well. "Thus ended been thise homicides two,/ And eek the false empoysoner also." Thus, circuitously, they found Death indeed.

William Blake's two poems *The Chimney Sweeper* and *A Poison Tree* are inspired by toxicological imagery. In the latter, anger directed toward a foe grows into a veritable poison tree, cultivated with ill-intentioned care by the poem's persona until it bears a bright shiny apple that seemingly concentrates his wrath. This foe,

> … into my garden stole
> When the night had veiled the pole;
> In the morning glad I see
> My foe outstretched beneath the tree.

Shakespearean drama is rife with daggers, swordplay, and poisonings. Claudius murdered Prince Hamlet's father by pouring poison in his ear. The slew of additional poisonings at the end of the play – Gertrude, Hamlet, Laertes, and Claudius, all are victims – is formidable. Cleopatra's suicide by an asp in Shakespeare's play is one of the many adaptations of this event in literature and art. The Witches' brew of Macbeth with its "Eye of newt and toe of frog, wool of bat and tongue of dog" is anything if not a highly toxic potion.

Romeo intentionally imbibes a fatal draught when he learns, mistakenly, that Juliet is dead:

> Arms, take your last embrace! and, lips, O you
> The doors of breath, seal with a righteous kiss
> A dateless bargain to engrossing death!
> Come, bitter conduct, come, unsavoury guide!
> Thou desperate pilot, now at once run on
> The dashing rocks thy sea-sick weary bark!
> Here's to my love!
>
> *[Drinks.]*
>
> O true apothecary!
> Thy drugs are quick. Thus with a kiss I die.

We have also the Merchant's "If you poison us, do we not die …." King Lear, near the eponymic play's conclusion, already befuddled but sensible, it appears, of the waywardness of his ungrateful daughters, and his own blindness to Cordelia, tells her

> … I pray, weep not:
> If you have poison for me, I will drink it.

Countless more possibilities unfold in Shakespeare if one considers poisoning as a metaphor. One can argue, for example, that Iago poisons Othello's mind, Lady Macbeth may be said to be poisoning her own consciousness, and so on.

In John Keats' (1795–1821) extended reverie, *Ode to a Nightingale*, the poet likens the vivid oneness with nature he seems to achieve through the bird's song to the effects that could be mimicked, though not as successfully, with a designer drink –

> My heart aches, and a drowsy numbness pains
> My sense, as though of hemlock I had drunk,
> Or emptied some dull opiate to the drains
> One minute past, and Lethe-wards had sunk.

Later in the nineteenth century, poisonous plants play a pivotal role in Nathaniel Hawthorne's *Rappaccini's Daughter.* The year 1896, a peak year of productivity for British poet A.E. Housman, saw publication of *Terence This is Stupid Stuff*. It revolves around Mithradates, King of ancient Pontus, who is said to have consumed minute quantities of poison daily in order, presumably, to build up immunity –

> There was a king reigned in the East:
> There, when kings will sit to feast,
> They get their fill before they think
> With poisoned meat and poisoned drink
> He gathered all the springs to birth
> From the many-venomed earth:
> First a little, thence to more,
> He sampled all her killing store;
> And easy, smiling, seasoned sound,
> Sate the king when healths went round.
> They put arsenic in his meat
> And stared aghast to watch him eat;
> They poured strychnine in his cup
> And shook to see him drink it up:
> They shook, they stared as white's their shirt:
> Them it was their poison hurt.
> – I tell the tale that I heard told.
> Mithridates, he died old.

Betrayed by his son, later in life, it is said, Mithradates attempted to take his own life with poison, but did not succeed (because of the above), and ordered a mercenary to kill him in a chemical-free manner.

A.R. Ammons won his second National Book Award in 1993 for his book-length poem, *Garbage*, in which he writes

> toxic waste, poison air, beach goo, eroded
> roads draw nations together, whereas magnanimous
>
> platitude and sweet semblance ease each nation
> back into its comfort or despair …

Andrew Hudgins in the title poem of his 2003 book, *Ecstatic in the Poison*, reminisces about the fog sprayed by DDT trucks when he was a child –

> The white clouds tumbled down our streets
> pursued by spellbound children
> who chased the most distorting clouds,
> ecstatic in the poison.

Poisoning makes good drama. Witness *Arsenic & Old Lace* – a play by Joseph Kesselring that achieved its greatest success in the Frank Capra film version about two elderly matrons who are ultimately less than cordial to certain gentlemen visitors. Aunt Martha is very forthcoming with the recipe for the drinks they supply:

> For a gallon of Elderberry wine, I take one teaspoon full of arsenic, then add half a teaspoon full of strychnine and then just a pinch of cyanide.

The genre of mysteries and its shining lights, Agatha Christie, Dorothy Sayers (*Strong Poison*), Edgar Allen Poe, Arthur Conan Doyle, Raymond Chandler, as well as its mediocre adherents, dip into poisoning plots for their stories. If one is uncertain of what to prepare for dinner, it stands to reason that the only solution is to consult Ebenezer Murgatroyd's *Cooking to Kill; The Poison Cookbook*, a humorous offshoot of the culinary crime genre. And if you are of a literary bent yourself, consider *Deadly Doses: A Writer's Guide to Poisons*, which is aimed at writers who want to incorporate poisons into their literary endeavors. The reader of this how-to text will learn toxicity ratings of poisons, their effects and symptoms, reaction times, antidotes and treatments, all in the interest of making a more accurate and gripping tale.

In *The Poison Belt*, a non-Sherlock Holmes science fiction story, Conan Doyle speculates about a cloud of poisonous gas that will destroy the human race, and the world with it.

Fairy tales and children's literature are other sources that have borrowed from the toxicology lexicon. The fairy tale of the brothers Grimm, *The Poor Boy in the Grave*, concerns a boy who, fixed upon taking his own life because of inadvertently offending his master, feasts on what he falsely believes to be poison. Realizing his mistake he takes another bottle that he is sure is poison. This instead turns out to be strong Hungarian wine. He drinks enough of it, though, to die, conveniently laying himself in a newly dug grave just before his demise. "The dose," as Paracelsus sharply observed, "makes the poison."

In *Snow White*, the evil queen, in a rage, formulates ingredients to poison an apple which she presents to Snow White, causing her to fall into a deathlike sleep. When the bit of apple is removed from her mouth, she is reanimated.

As already noted in the context of Shakespeare, toxicology makes a splendid metaphor. The traditional Five Poisons of Buddhist thought are greed, anger, ignorance, jealousy, and pride. In 2004, during the initial trial of the former Tyco chief executive on charges of grand larceny and more, a juror submitted a note stating that "The atmosphere in the jury room has turned poisonous", and we can be assured that this did not refer to pollution measurable by any analytical instrument. The judge ultimately declared a mistrial.

Much of nonfiction literature has also appropriated terms such as 'toxic' and applied them in novel senses. Consider the books, *Toxic Parents, Toxic Faith, Toxic People, Divorce Poison, The Book of Poisonous Quotes, Toxic Co-Workers, Toxic Emotions at Work, Toxic Work, Toxic Relationships and How to Change Them*, and even *Toxic In-Laws*. We have not begun to exhaust the possibilities.

Not to be neglected, on the verbal front, are jokes and other intentional and unintentional instances of toxicological humor. One variant (substitute pastor, doctor, lawyer, etc., for Rabbi and it still works) of the "My Wife is Poisoning Me" joke goes like this:

> There's this man. He goes to his Rabbi. "Rabbi, I need help. Something terrible is happening to me. I don't know where to turn." "Good grief," answers the Rabbi, "what is the cause of your distress?" "My wife is trying to poison me." Shocked, the Rabbi asks, "Is it possible it's in your imagination?" "No, I'm certain, she wants to poison me." The Rabbi rests his hand on the man's shoulder. "You settle down. I'll get to the root of this. It must be a misunderstanding. Let me talk to her." Several days later the Rabbi pays a visit to the man. "Well, I met with your wife. In fact," he details, rolling his eyes, "we spoke for three entire hours. Would you like my frank advice"? "Yes, please," the man begs, desperate. The Rabbi takes a deep breath, looks him straight in the eye, and says, "Take the poison."

Visual Arts

Just as primitive man learned quickly to determine which natural substances around him were beneficial and which were harmful, these are among the subjects he sought to depict in early art. There has been some speculation that rock paintings in the Sahara Desert dating back as many as 7000–9000 years ago are representations of hallucinogenic mushrooms, perhaps Psilocybe and Amanita. Some of these scenes show such mushrooms arrayed around dancers in ecstatic states.

Representations of the Minoan snake goddess have appeared in many forms, including a famous faience

sculpture from Knossos, Greece, *c.* CE 1600, now part of the collection of the Archaeological Museum in Herakleion. With elaborate floor-length skirts and exposed breasts, she holds a snake in each of her extended arms. Although there seems to be no definitive interpretation of the meaning of such snakes in Minoan religion and culture, one hypothesis suggests that they are, in fact, the poisonous asp viper.

A Magical Stela of dark stone, from the 30th Dynasty of Egypt (CE 360–343), now in the Metropolitan Museum of Art's collections is described in this way:

> On the part below the central figure panel, rows of hieroglyphs record thirteen magic spells to protect against poisonous bites and wounds and to cure the illnesses caused by them … A victim could recite or drink water that had been poured over the magic words and images on the stela.

Indeed, in Egyptian mythology, Selket was the goddess of scorpions and magic. She is typically painted with a scorpion on her head and was said to be a protector from venomous bites.

As in literature, Greek and Roman legends provided much material for art. Hercules in his Second Labor decapitates the Hydra's multiple heads and dips his arrows in the creature's venom. There are various depictions of the beheading, ranging from a *c.* 525 BC painting on a vase in the J. Paul Getty Museum in Malibu to John Singer Sargent's oil on canvas in the Museum of Fine Arts, Boston. Other art shows victims, such as the Centaurs Chiron and Pholus, of the venom-tipped arrows.

Alcohol may be fine in moderation, but in excess it is another story, and sometimes the line between one and the other is blurred. Dionysus, the Greek god of wine (and his Roman counterpart, Bacchus) and his followers, were the subjects of numerous paintings on walls and sarcophagi, as well as of mosaics. Hogarth's print, Gin Lane, on the other hand, leaves little doubt about which end of the virtue or vice spectrum inebriation inhabits.

In medieval times it was thought that the legendary unicorn's single horn could neutralize poisons. Such a scene is portrayed in *The Unicorn is Found* tapestry, one of the famous series completed 1495–1505 in the Netherlands, and now in the Cloisters Collection of the Metropolitan Museum of Art. Serpents were believed to pollute waters with their poisons. In this tapestry the hunters watch as the unicorn cleanses a poisoned stream with his horn.

In Rembrandt van Rijn's *Artemesia*, alternately titled *Sophonisba Receiving the Poison* in the Prado Museum, the robustly figured heroine, theatrically bathed in golden light, is about to accept a goblet of poison from her servant.

There are numerous depictions in art of legends and historic events memorializing poisoning. *The Death of Socrates* by Jacques Louis-David (1787, Oil on Canvas, Metropolitan Museum of Art) shows the philosopher bravely accepting his fate, the cup of hemlock, as he is surrounded by his anguished and grieving followers. In William Henry Margetson's *Cleopatra*, the Egyptian seductress's servants prepare her for death as a basket with figs and snakes sits at her feet.

Although the thrust of this paper considers toxicology as interpreted within the Western tradition, a brief aside to the wealth of artifacts from other cultures is warranted. In Japan, Yotsuya Oiwa was the wife of a masterless samurai known as Tamiya Iemon who is complicit in her poisoning. The poison leaves her face gruesomely disfigured. She goes insane and dies, but returns in assorted forms to wreak vengeance on her unfaithful husband. Her grotesque image returns over and over to haunt Iemon. The story has been the subject of a famous Japanese play and numerous artworks including woodblock prints and netsuke. In Yoshitoshi's print of the story, the face of Oiwa leers from a half-burned paper lantern.

The Five Poisons, unrelated to the Buddhist metaphorical poisons discussed earlier, is a motif consisting of the centipede, lizard, scorpion, snake, and toad. They are incorporated into Chinese folk art, embroidered on clothing, appliquéd to luggage, engraved on amulets, and otherwise used. Customarily this group is not viewed negatively, but serves, on the contrary, as a charm for neutralizing evil.

Moving into the modern realm, there has been an entire genre of psychedelic paintings, most common perhaps in the 1970s, in which artists (or those who claimed to be) created work while under the influence of hallucinogenic agents such as LSD.

Jean Michel Basquiat, very influential in the grafitti movement in the late seventies, burst upon the contemporary art scene in the 1980s. He collaborated with Andy Warhol on a painting in acrylics and oilstick on canvas, littered with many skulls and a few crossbones and announcing 'Poison' and 'Caution'. Basquiat died before reaching the age of 30 from a drug overdose.

Fred Tomaselli recently was featured in a solo museum show, a 10 year retrospective, of his work. Tomaselli has constructed a sizeable body of fairly abstract art combining acrylic, photographs, hallucinogenic plants, synthetic pharmaceuticals, and therapeutic herbs.

In 2002, using Adobe PhotoShop 6, the artist Scott Blake made an intriguing self-portrait from images of

ecstasy pills he downloaded from the Web through the DanceSafe site. DanceSafe promotes health and safety within the rave and nightclub community. Masanta is a young Japanese artist whose work has been exhibited widely, and who uses Photoshop, Illustrator, Macromedia Fontographer, pen, acrylic, and mixed media. One of her constructions, displayed on the virtual Museum of Computer Art, appears to be an orange candelabra not only supporting flames, but in flames itself, with a small female apparition at its center and apex, against a background of turquoise and tiny pearls, and with the word 'Poison' in billowy pink letters across the lower foreground.

Video and Cartoons

Arsenic and Old Lace was already mentioned above among the literary arts as a famous toxicology-oriented play. It found, of course, another life in the memorable 1944 film version directed by Frank Capra. Toxic themes have been adopted by many movies.

D.O.A., a 1950 film noir starring Edmund O'Brien, concerns a man who's been poisoned and has only a few days to live. He needs to find out who is trying kill him, and why.

Godzilla, or more properly *Gojira* (1954), was very influential in Asian and monster cinema genres. Godzilla is said to have been an ancient dinosaur of sorts whose underwater habitat was disrupted by nuclear testing and subsequently became irradiated himself, the outcome of a toxicological experiment gone awry.

The Young Poisoner's Handbook, inspired by an actual mass murderer, is a dark comedy set in England in the 1960s. It concerns a young teenager who is a budding chemist and proclaims, "I want to be the greatest poisoner the world has ever seen."

The series of movies beginning with *The Toxic Avenger* is the brainchild of Troma Productions. These raunchy films feature the mop boy at a local health club who falls into a vat of hazardous chemicals, making him hideously deformed and at the same time bestowing upon him superhuman powers.

The winner of numerous awards in 2003, Bill Domonkos' short film, *The Fine Art of Poisoning*, uses animation and music by Jill Tracy to layer nightmare visions in the creation of a strange, unsettling world.

Only slightly touched upon here are the countless literary works, films, plays, and other artworks that consider addictions of one kind or another – alcoholism, drug abuse, tobacco use. Think of the decades in which holding, lighting, smoking, or caressing a cigarette was standard fare, even considered romantic, sexy, and cool, in the movies. This use of cigarette as prop may be less pervasive in contemporary works, but it has hardly disappeared. Consider Otto Preminger's 1955 film, the *Man with the Golden Arms*, starring Frank Sinatra in a powerful look at drug addiction, and its many more explicit successors. There is also a veritable cornucopia of material on alcoholism – for example, *Days of Wine and Roses* with Jack Lemmon and Lee Remick from 1958, *Leaving Las Vegas*, released in 1995 and featuring Nicholas Cage and Elisabeth Shue, and everything earlier, in between the two, and since.

Cartoons and animation, too long relegated to kiddy fare, have come into their own and been recognized on their own artistic merit. There is a surfeit of cartoons exploiting issues related to the environment, hazardous chemicals, toxic waste, etc. Many websites compile and offer access to these, which are typically copyright. CartoonStock is one among many.

In the 1935 Mickey Mouse short, *Mickey's Garden*, our protagonist and Pluto attempt to bug-proof their vegetable garden. Exposure to insecticide causes the pair to hallucinate that they shrink down to the size of the bugs.

Among the many villains encountered by Batman was Poison Ivy, formerly a female botanist who, because of experiments gone awry, developed a deadly touch and simultaneously became immune to all poisons. She is a specialist in the use of plant toxins, may use a cross-bow and vine whip and, sometimes, poisoned darts. Poison perfumes and lipstick are also in her arsenal. Uma Thurman played the role in the nonanimated and critically panned film, *Batman and Robin*.

Eric Pigor's Toxic Toons incorporates poisons into generally ghoulish, grotesque, and gross (and he would surely be proud to have them described as such) images and animations. In a similar vein, Jacob Wexler's celebrated Ruin Dog single-panel cartoon (**Figure 1** bottom panel) is symbolic of toxicology not as a science but as a poster child for its most depraved practitioners and victims.

Television, especially series, and made for TV movies have employed countless poisoning motifs. The genres are typically mysteries or shows featuring detectives or police, or set in the courtroom and hospital.

No discussion on entertainment today would be adequate if it did not mention video games, whether via computer or console. By some accounts, television viewing hours are declining considerably, with much of the loss applied to a gain in video game usage, especially among young men. Although hand-to-hand combat, weapons that shoot, detonate,

Figure 1 Toxicological iconography – traditional (skull and crossbones/JollyRoger) and contemporary (ruin dog).

slash, pierce, radiate, or burn are the overwhelming favorites, poisons are not totally absent. Poison Claws and Poison Rods, for example, are among the cornucopia of weapons available to players in *Final Fantasy 11*, part of a role-playing game series extraordinaire. In *Everquest*, rogues can use blinding poisons, dizzy poisons, feeble mind poisons, flesh rot poisons, system shock poisons, etc.

The long awaited and exemplary first-person shooter, Half-life 2, for PC, released in late 2004 brings the headcrabs, evil creatures which gain control of others by attaching themselves to their head. A new variant, the black headcrab, can deliver a potent poison. It is lethal but will drastically reduce a victim's health and potentially turn him a poison zombie. Socrates must surely be turning over in his grave.

Music

Can music be poisonous? Perhaps. There is a greater challenge in trying to find kinships between music, especially music which is not vocal or at least programmatic, and toxicology. Music not designed to tell a story is referred to as 'absolute' music, and although one can try to make a case that a Beethoven symphony or a Hindemeth sonata is rife with toxicological tendencies, such connections can be far-fetched and are, regardless, a matter of personal interpretation. A nonvocal programmatic piece such as *Mars: The Bringer of War*, a segment of Gustav Holst's suite, *The Planets*, while intended by the composer to be suggestive of the battlefield, leaves it to the imagination to determine what the implements of destruction may be – rifles, tanks, poison gas?

Music that explicitly tells a story or has a message, by virtue of its lyrics, is a more reliable source of toxicological lore and legend. The high drama of much of the standard nineteenth century opera fare opens the floodgates of poisonous mischief. Consider that Donizetti composed an entire opera based on *Lucrezia Borgia*. Major characters in Verdi's operas *Nabucco*, *Simon Boccanegra*, and *Luisa Miller* all succumb to poison. In the same composer's *Il Trovatore*, the heroine Leonora promises to give herself to her enemy Count Luna if he will release her true love, Manrico. Leonora takes poison in order not to have to fulfill her pledge.

The legend of *Tristan & Isolde* has been variously interpreted, probably to greatest musical acclaim in Wagner's opera of the same name. A key plot element revolves around what is mistakenly believed to be poison. Isolde, in love with Tristan but spurned by him, and unwilling to be part of an arranged marriage with his uncle, conspires to poison herself and her would-be lover. Her maid substitutes a love potion for the poison and the rest is history, not to mention great drama and music.

Even ballet has not been immune from toxic influences. *La Bayadere*, with music by Leon Minkus, and choreographed by Lucien Petipa in its 1877 premiere, involves a love triangle. Nikiya, the temple dancer and abandoned third of the triangle, succumbs to the bite of a poisonous snake sent to her in a basket of flowers by her rival, Gamzatti, and the latter's prospective father-in-law, the Rajah.

In 1910, Calvin Lee Woolsey, during ragtime's heyday, composed, in a minor key, the *Poison Rag*. Herbert Ingraham is responsible for the *Poison Ivy Rag* in 1908. It is easy to hear the playful piano trill suggestive of itching in Ingraham's rag.

Fast forwarding to 1959, we reach the Coaster's hit, *Poison Ivy*:

> Measles make you bumpy
> And mumps'll make you lumpy
> And chicken pox'll make you jump and twitch
> A common cold'll fool ya
> And whooping cough'll cool ya
> But poison ivy, Lord'll make you itch!!

The Rolling Stones also recorded this several years later.

The grisly black humor of Stephen Sondheim's *Sweeny Todd, the Demon Barber of Fleet Street*, while not explicitly incorporating poisoning, certainly captures its spirit. One can envision considerable offstage food poisoning by minimal stretching of the lyrics of *The Worst Pies in London* and *A Little Priest*.

Among the many popular songs with toxicology leanings are *Poison in the Well* (10 000 Maniacs), *Poison* (Alice Cooper), *Church of the Poison Mind* (Culture Club), *Poison* (Laurie Anderson), *Toxic* (Britney Spears, in which she sings "I'm slipping under/With a taste of poison paradise/I'm addicted to you/Don't you know that you're toxic/And I love what you do"), and *Sweet Toxic Love* (Boy George).

Toxic Audio, the a capella group, has given new meaning to the flexibility and versatility of the human voice. Their production, *Loudmouth*, played on Broadway in 2004. One suspects that the 'toxic' in their name refers more to their intoxicatingly refreshing sound than to anything negative about their music.

The heavy-metal band Poison, peaked in the late 1980s and early 1990s. The band named System of a Down's 2001 album is titled *Toxicity* –

> More wood for their fires, loud neighbors,
> Flashlight reveries caught in the headlights of a truck,
> Eating seeds as a past time activity,
> The Toxicity of our city, of our city …

Dr. Carl Winter, a respected extension toxicologist with the University of California at Davis and director of its FoodSafe program has another, somewhat related musical life. He parodies popular songs, rewriting lyrics, composing and recording musical arrangements, and producing the music, and it is all with a toxic twist. Among his 'greatest hits' are *I Sprayed it on the Grapevine* to the tune of *I Heard it through the Grapevine*, *Rat Number 49* to the tune of *Love Potion Number 9*, and *You Better Wash your Hands* to the tune of *I Want to Hold Your Hand*.

More Cultural Miscellany

The perfume industry may seem an unlikely province to inspire the toxic imagination, but the market is ripe. Witness Christian Dior. Its women's fragrance, Poison, created in 1985, is a blend of amber, honey, berries, and spices. The year 1994 saw a follow-up with Tendre Poison, combining florals, mandarin, vanilla, and sandalwood. Hypnotic Poison, as an eau de toilette spray, was launched in 1998. It blends bitter almond, caraway, jasmine, moss, wood, and vanilla. Advertising copy has depicted it as "Mysterious and mesmerizing, extravagant and bewitching, audacious and profoundly feminine, the fragrance is an unsettling harmony, a fusion of contrasting olfactory facets. Recommended for romantic use." It has also been described as 'temptation in a bottle', 'a magic potion for modern times', and 'disturbing, sensual, and bewitching, to take you beyond impulse and beyond fantasy. ' It may seem ironic to identify anything suggestive of poison with romance, but in the context of perfume, there is an allure to danger. As an aside, there is also a Dior purse spray called Addict. One wonders that a scent called Risk has not yet been developed. Versions of these various 'Poison' fragrances come as lotions, shower gel, and in other forms.

Alcoholic beverages, in immoderate doses, are well-documented toxic agents. Drinkers often, of course, perceive the effects that they yield as beneficial. Names of straight and mixed drinks vary from the buoyant (and even erotic) to the rather diabolical. Consider the following mixed drinks – Choke and Puke (a combination of Jack Daniel's, Jose Cuervo Gold, and gin, with grenadine as the mixer), Cocaine Shooter (with blackberry brandy, vodka, and grapefruit juice as the mixer), and Death Wish (a combination of Wild Turkey 101, peppermint schnapps, and 151 Rum, also using grenadine as the mixer). The potent Icelandic potato-based vodka known as Brennevin used to be called Black Death and had an ominous-looking skull on its label. Armida Winey, of Sonoma County throws its hat into the ring of beverages influenced by the lure of toxicology with a Zinfandel cleverly named 'Poizin', described as 'the wine to die for'.

Street names of abused drugs are a testament to the verbal creativity of the addicted. Flamethrowers, for example, are cigarettes laced with cocaine and heroin. Heroin itself is the subject of many pseudonyms – while most of them are derivative of the drug's potency (e.g., thunder, red eagle, raw fusion, Rambo), some have clear negative connotations – poison, dead on arrival, brain damage.

The graphic symbol that seems to be universally understood to apply to poisons is the skull and crossbones (**Figure 1**, top panel). Traditionally associated with European and American pirate flags and known in English as the Jolly Roger, it has long symbolized death and signified that those who dare approach the object or persons associated with it had better beware. Beginning in the late nineteenth century, the skull and crossbones was regularly embossed on glass bottles of poisons. This image was also appropriated by one of the branches of the Nazi SS.

The Pittsburgh Poison Center created the graphically vivid Mr. Yuk symbol to educate the public about poison prevention. The round sticker currently has a frowning green face with its tongue out and includes the US national toll-free poison help phone number. Millions upon millions of these have been distributed worldwide through the years. Curiously enough, certain research has shown that these stickers do not necessarily serve as a deterrent to poisoning but may have the opposite effect, attracting children, in particular, to the products on which they are placed, and some poisoning centers have, therefore, stopped distributing them.

Public health posters have been used by various organizations and in a variety of settings to influence behavior conducive to a healthy society. The International Programme on Chemical Safety, for instance, created several posters in the 1980s related to environmental health. One, showing children in the process of opening a bottle of insectide, was designed to promote poison prevention. Another, also utilizing children, this time eating ice cream and an apple, states "Food Additives and Pesticides Should be Used with Care."

To delve more deeply into the linguistic ramifications of 'toxicology' and 'poison', or indeed of any words, one would do well to consult The Rosetta Edition of Webster's Online Dictionary. In it, one would discover that 'ilmu racun' means toxicology in Indonesian, that 54 6F 78 69 63 6F 6C 6F 67 79 is hexadecimal for toxicology, that some rhyming words are gynecology, mycology, and oncology, and that there are an estimated 300 searches executed per

day on the word 'toxicology' across the major English-language search engines.

Conclusion

This entry has barely scratched the surface of the ways in which toxicology, often without our realizing it, continues to hold a spell over us. Enter the word 'toxic' or 'poison' or some such term on Google or another Web search engine. It is likely you will not be surprised by the amount of retrieval, but you may be by the proportion of sites that use the word purely in a figurative and sometimes self-contradictory sense. Toxicology lives in fact and as metaphor. The terminology and spirit of toxicology and poisoning has infiltrated our own vocabulary and the way we view the world. Clearly something about it will not let go of our psyches, so we might as well just roll with the (poison) punches and enjoy it.

See also: Ancient Warfare and Toxicology; Notorious Poisoners and Poisoning Cases; Hemlock, Poison; Toxicology, History of.

Further Reading

Martinetz D and Lohs K (1987) *Poison: Sorcery and Science, Friend and Foe*. Germany: Edition Leipzig.
Mayor A (2003) *Greek FirePoison Arrows and Scorpion Bombs: Biological and Chemical Warfare in the Ancient World*. Woodstock, NY: Overlook Press.
Mithrada: Newsletter of the Toxicological History Society.

Relevant Websites

http://www.nlm.nih.gov – Visual Culture and Public Health Posters (from the National Library of Medicine).
http://arthursclassicnovels.com – Doyle C. *The Poison Belt* a non-Sherlock Holmes science fiction story.
http://www.dancesafe.org – DanceSafe website.
http://www.cartoonstock.com – CartoonStock website.
http://www.toxictoons.com – Eric Pigor's Toxic Toons website.
http://www.websters-online-dictionary.org – The Rosetta Edition of Webster's Online Dictionary.
http://moca.virtual.museum – Virtual Museum of Computer Art.
http://foodsafe.ucdavis.edu – See link to the page containing details of the music of Dr. Carl Winter, a respected extension toxicologist with the University of California at Davis.

Toxicology, Education and Careers

Susan J Borghoff

Toxicology and Education

Toxicology is the study of harmful effects of agents on people, animals, and other living organisms. One aspect of toxicology is to evaluate the likelihood that adverse effects will occur under specific chemical exposure scenarios; referred to as risk assessment. Toxicology is a combination of biology and chemistry, with elements of physical and computational sciences, that leads to a wide variety of career opportunities. The field of toxicology provides the excitement of science and research while contributing to the well-being of current and future generations.

The objective of this entry is to provide information to individuals considering a career in toxicology and some guidance and resources as to the education and training that is required. Various careers in toxicology will be discussed in the following section and opportunities for education and training outlined.

Much of the information provided in this section was obtained from the book *Resource Guide to Careers in Toxicology* developed by the Society of Toxicology. A complete reference for this guide is presented in the Further Reading section. A copy of this *Resource Guide* can be obtained from the Society of Toxicology. Individuals serious about a career in toxicology are encouraged to obtain a copy.

Why Consider a Career in Toxicology?

Challenges

Chemicals are an essential component of the high standard of living we enjoy. The challenge to toxicologists is to ensure that products or by-products of modern living are not endangering our health or environment. With a career in toxicology the individual can contribute toward finding solutions to important challenges such as protecting public health and the environment.

Opportunities

A wide variety of career opportunities exists in toxicology. Toxicologists participate in basic research

studying mechanisms by which chemicals exert their toxicological or cancer-causing effects. Research toxicologists use the most advanced techniques in molecular biology, chemistry, and the biomedical sciences. Many toxicologists work in the chemical, pharmaceutical, and consumer products industries to test and ensure that their products and workplaces are safe. There are also many toxicologists that work within government at the state and federal levels to develop and enforce laws to ensure that chemicals are produced, used, and disposed of safely. Toxicologists are also involved with monitoring both water and air for levels of specific chemicals and biological agents known to cause adverse health effects to people and the environment. In academia, toxicologists train future toxicologists as well as conduct research to understand the mechanisms by which chemicals cause toxic effects in living organisms. Clinical toxicologists help to diagnose patients with diseases caused by toxic substances and often work at hospitals or Poison Control Centers. Forensic toxicologists help establish the cause of death or identify important toxicity clues that can be used to solve a crime, whereas the occupational toxicologist conducts studies to understand conditions of chemical exposure or work practices that may place the worker at unacceptable risk.

Attractive Salaries and Professional Advancement

There continues to be a high demand for toxicologists within all sectors of employment – government, industry, and academia. Salaries are especially competitive for the advanced trained toxicologist. Specific information on salaries for toxicologists in various workplace settings and with various levels of experience and education can be found in a survey published by Gad Consulting Services in Raleigh, NC. The Fifth Triennial Toxicology Salary Survey is currently posted on the Society of Toxicology website (see Relevant Websites section). A reference for this survey can be found in the Further Reading section.

What Do Toxicologists Do?

Research

Research in toxicology is conducted at basic and applied levels. Basic research may involve studying the biochemical or molecular mechanism by which a chemical causes an adverse effect on various cellular processes. Knowledge gained through toxicology has improved our fundamental understanding of basic life processes. Applied research is more directed and is expected to yield direct social or commercial benefit. Examples of applied research are studies to identify chemicals that selectively kill certain pests or studies to determine whether a particular industrial process is responsible for a specific disease identified in a population of workers. Toxicologists working in applied areas also conduct studies directly related to determining whether or not a chemical is toxic to laboratory animals and by inference, toxic to people.

Research in toxicology is generally conducted in various specialty areas such as carcinogenesis, reproductive and developmental toxicology, neurotoxicology, immunotoxicology, respiratory toxicology, dermal toxicology, endocrine or genetic toxicology. The specialty areas may also focus on various organ systems such as the liver, kidney, eye, skin, or on different species of plants or animals. Since researchers are studying the effects of substances on living organisms, they work with various systems ranging from whole organisms (*in vivo*) to isolated cell suspensions or cell cultures (*in vitro*), to imaginary systems based on computer simulation or modeling of living organisms (*in silico*).

Product Safety Evaluation

Drugs, agricultural products, and other chemicals are introduced into society every day. Toxicologists in product safety evaluation are continuously developing better ways to evaluate the potential adverse effects of chemicals and physical agents and to determine the dose at which adverse responses occur. Many industries employ toxicologists to evaluate the safety of their products. For drugs, food additives, cosmetics, agricultural chemicals and other classes of chemicals, federal laws require that the manufacturer provide adequate testing of the product before it is approved for use. Tests to determine whether a chemical has the potential to cause cancer, birth defects, reproductive effects, neurological toxicity, or other adverse effects are commonly conducted by the manufacturer. Toxicologists involved in product safety evaluation have the responsibility to ensure that these tests are designed, conducted, and interpreted in a scientifically sound manner. The information gathered from these studies is reviewed by toxicologists in various regulatory agencies such as the Food and Drug Administration or the United States Environmental Protection Agency to ensure that the products will not present an unreasonable risk to human health or the environment.

Teaching

Toxicologists employed in colleges and universities are frequently involved in teaching courses in toxicology. Many colleges and universities are developing new courses at both the undergraduate and graduate

Table 1 Partial listing of academic institutions that support training programs in toxicology at the graduate level (grouped by geographical location)

Mid-Atlantic
Clemson University
Duke University
North Carolina State University
University of Kentucky
University of Louisville
University of North Carolina at Chapel Hill
Vanderbilt University
Virginia Commonwealth University
Virginia-Maryland Regional College of Veterinary Medicine
North central
Indiana University
Iowa State University
Michigan State University
Purdue University
University of Cincinnati
University of Illinois at Urbana-Champaign
University of Kansas Medical Center
University of Michigan
University of Nebraska
University of Wisconsin-Madison
Wayne State University
Wright State University
Northeast
Dartmouth College
Johns Hopkins University School of Hygiene and Public Health
Massachusetts Institute of Technology
New York University
Northeastern University
Rutgers University
St. John's University
State University of New York at Buffalo
University of Albany
University of Connecticut
University of Maryland
University of Pittsburgh Graduate School of Public Health
University of Rochester School of Medicine and Dentistry
University of the Sciences in Philadelphia
Northwest
Oregon State University
University of Washington
South central
Louisiana State University Medical Center
Mississippi State University
Texas A&M University
Texas Tech University
University of Arkansas for Medical Sciences
University of Mississippi
University of Oklahoma Health Sciences Center
University of Texas at Austin
University of Texas Health Science Center of Houston
University of Texas Medical Branch at Galveston
Southeast
Florida A&M University
University of Alabama at Birmingham
University of Florida
University of Georgia
Southwest
Colorado State University
San Diego State University
University of Arizona
University of California, Berkeley
University of California, Davis
University of California, Irvine
University of California, Los Angeles
University of California, Riverside
University of Colorado Health Sciences Center
University of New Mexico
University of Utah
Utah State University

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levels to provide students with a background in the science of toxicology. A partial list of Universities that support toxicology training programs at the graduate level is presented in **Table 1**. Details of each of these programs can be obtained through the online version of the fifth edition of the *Resource Guide to Careers in Toxicology*. Many other academic institutions do not have a specific graduate program in toxicology but employ toxicologists to participate in curriculum development and teaching in more basic programs such as chemistry and biology. Thus, opportunities exist to teach toxicology in small colleges as well as major universities.

Public Service and Regulatory Affairs

There has been tremendous growth in public awareness of chemical hazards over the past two decades which has resulted in the passage of many laws governing the production, use, and disposal of chemicals. Many local, state, and federal agencies employ toxicologists to assist in the development and enforcement of their laws. An increasingly important area of toxicology is public communication of chemical risks. Toxicologists employed by regulatory agencies may often be called on to examine the scientific basis for regulatory actions or to assist in communicating to the public the reasons regulatory actions are or are not taken in particular situations. There are many private consulting firms with expanding expertise in toxicology that can now provide such services to local and state health departments, public utilities, and private industries. Thus, many employment opportunities in the private sector are available to the toxicologist interested in assisting public agencies and private industries in resolving many public health and environmental problems.

Clinical and Forensic Toxicology

Clinical toxicologists are health professionals concerned with disease caused by exposure to toxic agents. Generally, clinical toxicologists are physicians, pharmacologists (e.g., individuals with a Doctor of Pharmacology degree), and veterinarians who receive specialized clinical training in toxicology. These individuals are engaged in the diagnosis and treatment of poisoned patients. Poisoning may result from accidental, deliberate, environmental, or occupational exposure to a toxicant. Forensic toxicologists interact with clinical toxicologists to establish analytical chemical methods for the detection of toxic agents in tissue samples from poisoned patients. Research performed by clinical and forensic toxicologists has led to the recognition of new chemical hazards and the development of novel therapies for poisoning. Clinical and forensic toxicologists may be found in academia (medical centers), industry, and other places in which health professionals are employed.

Occupational Toxicology

Occupational toxicologists conduct studies to understand the conditions of chemical exposure and develop work practices that reduce health risks to the worker. They work in all sectors: industry, academia, and government. Their efforts are focused on obtaining knowledge of the relationship between workplace exposure to a chemical, and the health effects that are of concern to workers.

Who Employs Toxicologists?

Industry

The 'Job Market Survey' reported on the SOT website (see Relevant Websites section), reveals that in North America, the chemical, pharmaceutical, and support industries account for ~47% of the toxicologists employed. Product development, product safety evaluation, and regulatory compliance generate a large job market for toxicologists. These industries employ toxicologists trained at all levels of education, including those holding bachelor, master, and doctoral degrees. Many companies have their own research programs in product safety evaluation, whereas others may contract their work to specific organizations that specialize in contracted research studies.

Academia

Academic institutions account for ~21% of all employed toxicologists. Most have advanced degrees and are conducting basic research. There has been an increase in the number of programs in toxicology at many academic institutions because of a need for toxicologists with doctoral-level training. Most of these opportunities are within Medical Schools and Schools of Public Health at major universities. Increasingly, smaller colleges are beginning to employ toxicologists to teach toxicology within their basic biology, chemistry, and engineering programs.

Government

The government employs ~15% of toxicologists. Although most government jobs are with federal regulatory agencies, many states employ toxicologists with masters or doctoral degrees. While most of the toxicologists employed by the federal government are involved in the development and enforcement of laws related to the toxicity of materials, a number of federal agencies employ toxicologists to conduct both basic and applied research in toxicology.

Consulting Firms

The professional service industry is a growing employer of toxicologists and currently accounts for ~15% of toxicologists. Many graduates of baccalaureate and master's programs in toxicology are finding employment with consulting firms. In the consulting field, experienced individuals provide professional guidance and advice to local public agencies, industries, and attorneys involved in problems with toxic chemicals. Consulting is a rapidly growing activity for the experienced toxicologist.

Research Foundations

Private nonprofit research foundations provide opportunities for research in toxicology to ~4% of the toxicologists. Numerous public and private research foundations employ toxicologists to conduct research on specific problems of industrial or public concern. Toxicologists at all levels of education might find employment with these research foundations.

Preparing for a Career in Toxicology

For those individuals who are in the midst of their college education, careful planning of undergraduate courses will enhance graduate education opportunities in toxicology and other biomedical sciences. For those who have already received an advanced degree such as a PhD, an MD, or a DVM in a biomedical science other than toxicology, careers can be focused toward toxicology through postdoctoral clinical or research training.

Undergraduate and Graduate Training

Planning Depending on career aspirations, a bachelor's degree may not be sufficient for achieving career

goals. Although there are some employment opportunities in toxicology for those with a bachelor's degree, the breadth of career choices and opportunities for advancement are much greater for those with post baccalaureate degrees. Acceptance into graduate programs in toxicology generally requires a strong academic record and evidence of research and/or leadership abilities. Most graduate toxicology programs have specific prerequisites for admission. The primary requirement is a baccalaureate degree in a relevant field of study such as biology, chemistry, environmental health, or other science-related field. Persons graduating with these degrees can seek employment at the technical support level at many research institutions. Additional upper level courses in biochemistry and physiology will often increase the competitive advantage for graduate school admissions. As the ability to be an effective communicator becomes increasingly important for toxicologists, course work in scientific writing and public speaking is also useful. Performance on the Graduate Record Examination (GRE) is often evaluated by graduate admissions committees and the exam should be prepared for in advance. Many programs require GRE scores on both the General Test and on the Subject Test if it is given in an undergraduate major such as biology or biochemistry. The GRE should be taken at least 5 months prior to the time one plans to begin graduate study. Individual graduate programs should be consulted in advance to determine specific admission requirements.

In addition to a strong academic record, demonstration of basic laboratory research skills enhances the chance of admission. Laboratory courses in chemistry and biology are an important part of an undergraduate education and help develop research skills. Cooperative work-study programs enhance those skills by placing students in a research setting during the semester. Summer internships in a research laboratory are another approach to enhancing laboratory skills. Research internships provide interested undergraduate science majors with a stimulating research experience in toxicology. These internships are available in academic and industrial research laboratories across the country. More information on research internships in toxicology can be obtained by contacting the Society of Toxicology or searching the Peterson's Guide (see Relevant Websites section).

Selection of an Appropriate Toxicology Program Identifying a graduate training program and mentor most appropriate for a particular individual requires some advance planning. First, individuals should establish a potential career plan. By considering the various subspecialties in toxicology such as neurotoxicology, chemical carcinogenesis, teratology, inhalation toxicology, computational modeling and risk assessment, a specific field of research that is of particular interest to the student can be identified. Although such a choice early in the education process does not commit one to this direction, careful assessment helps in deciding which programs are most likely to meet your needs. Talking with toxicologists in local universities, industries, and governmental agencies is helpful in selecting a training program and in deciding on a future career direction.

The admission requirements of the graduate program should be identified well in advance since these requirements must be met prior to the time of beginning the program. Requirements vary among programs and from the general requirements described previously. Details of the specific requirements of toxicology graduate programs can be obtained by referring to the *Resource Guide to Careers in Toxicology*, which is available on the SOT website.

Financial Assistance University financial assistance is often available through research and teaching assistantships, fellowships, traineeships, and grants. Inquires should be made to the prospective institution, program, and mentor as to the availability of grants and financial aid. The National Institutes of Health, other federal institutions such as the Environmental Protection Agency, private foundations, and the Society of Toxicology are all potential sources of financial support.

Resource Guide to Careers in Toxicology The *Resource Guide to Careers in Toxicology* contains descriptions of a large number of very diverse academic programs in toxicology located throughout the United States (**Table 1**). Geographic considerations may be important to some individuals and may substantially limit the number of potential toxicology programs of interest to those individuals. Review of this document in the early stages of planning a career in toxicology is one of the most important steps that individuals can take in planning their toxicology education. The listing for each toxicology program includes program website address, degrees offered, areas of program strengths and contact information. The online version of the *Resource Guide* has links to these listed programs. Most of the websites for toxicology departments include a list of faculty and a synopsis of their research interests.

When a specific program website is accessed there will be a description of the program along with an outline of the prerequisites for admission. This is a very important section and provides clear direction as to the types of college courses needed to be accepted

into toxicology graduate programs. It is also useful to know at an early point other information required by the toxicology program. For example, most programs require official college transcripts, GRE scores, a letter of intent, and letters of recommendations. The letter of intent describes why the individual wants to be admitted into the graduate program and general career goals. Recommendations are generally from individuals who know the applicant on a professional or academic level. Examples of appropriate references are teachers, advisors, or employers.

The final section in each program description is the curriculum. The description of the curriculum includes the degrees that are offered by the particular toxicology program, the areas of specialization for the degrees, required courses, optional course work, and specific dissertation requirements. The graduate curriculum for a doctorate in toxicology often includes courses in biochemistry, physiology, anatomy, histology, pathology, pharmacology, and statistics. The academic program can also include areas such as analytical methods, carcinogenesis, mutagenesis, teratogenesis, comparative toxicology, molecular mechanisms of toxicology, and organ-specific toxicity. Some programs may also include course work in such fields as statistics, computer science, computational modeling, immunology, and pharmacokinetics.

Because the doctorate in toxicology is a scholarly degree, the student is required to conduct a program of original research that extends over a period of 2 or more years. Part of this research requirement is the completion of a dissertation. This document is written by the student and includes an introduction or literature survey, a statement of the hypothesis underlying the dissertation, methods, results (including figures, graphs, and tables), and a discussion. By conducting original research, the student can understand and experience the application of observation and analysis to specific problems in toxicology. In keeping with the tradition of the doctorate degree, defense of the graduate research thesis is expected. The final section of the program description provides the name and address of a person who can be contacted for more information and applications for admission and financial assistance.

Postdoctoral Training

Graduate students who are interested in a career at an advanced level in toxicology (i.e., conducting basic research, leading research groups and projects, or teaching) typically need to go through some additional postdoctoral training. This training may last from 2 to 4 years beyond the doctoral degree, and typically consists of an in-depth, independent research project. Most institutions that offer a doctoral degree in toxicology or support advanced research in toxicology have postdoctoral programs. These programs are highly tailored to the individual researcher and his/her postdoctoral mentor.

Even students who have not gone through a graduate program in toxicology can enter into a career in toxicology at this junction. Since a postdoctoral fellowship usually has highly specific requirements, students with specialized training in fields such as molecular biology, genetics, computational modeling, chemical engineering, medicine, and many others may be able to find a toxicology-related research topic that requires their specialty.

See also: Academy of Toxicological Sciences; American Academy of Clinical Toxicology; American Board of Toxicology; American College of Medical Toxicology; American College of Toxicology; European Society of Toxicology; Information Resources in Toxicology; International Union of Toxicology; National Center for Toxicological Research; Society for Environmental Toxicology and Chemistry; Society of Toxicology; Toxicology Forum; Toxicology in the Arts, Culture, and Imagination.

Further Reading

Gad SC (2002) *Fifth Triennial Toxicology Salary Survey.* Currently listed on the Society of Toxicology's website.

Society of Toxicology. *Resource Guide to Careers in Toxicology*, 4th edn. Reston, VA: Society of Toxicology. (Available from the Society of Toxicology Offices, Society of Toxicology, 1821 Michael Faraday Drive, Suite 300, Reston, VA 20190-5332, USA. Tel: +1-703-438-3115.)

Relevant Websites

http://www.toxicology.org – Society of Toxicology website.
http://www.petersons.com – Peterson's Guide.

Toxicology, History of

Katherine D Watson

> All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy.
>
> Paracelsus

The science dealing with the harmful effects of chemical agents on biological systems is called toxicology, from the Greek word *toxikon*, a bow (to shoot poisoned arrows) or a poison in which to dip arrowheads. A poison is generally defined as a substance that is capable of destroying life or causing illness when introduced into, or absorbed by, a living system in small quantities. Since 1900, toxicology has undergone continuous expansion and development by assimilating knowledge and techniques from many branches of the physical and biological sciences. Historically, attempts to both kill and cure with chemically active preparations (poisons and drugs) have led to the evolution of toxicology, and today it is a discipline of diverse application and widespread importance.

It is likely that the history of toxicology is as old as the history of the human race: early humans must have learned to discriminate between things that were good to eat and those that were not. In exploring their environment and searching for food, they observed the healing or harmful effects of plants and minerals, and that the bites of certain insects and reptiles caused illness or death. It was a natural progression to use injurious substances for hunting, in warfare, and for homicide. Arrow poisons were developed by ancient peoples in all parts of the world (with the possible exception of Australia and New Zealand), and many are still in use. Among the best known are the 'calabash curares' (derived mainly from varieties of *Strychnos* in South America), reptile poisons (venoms) from toads and salamanders in Central and South America, and ouabain, from African varieties of *Acocanthera* and *Strophanthus*.

Toxicology in the Ancient World

The earliest use of arrow poisons probably occurred in the Mesolithic Age, when arrows first began to appear. It is possible that Masai hunters who lived in Kenya 18 000 years ago may have used poison; evidence from other sites in Africa indicate later use (3000–1700 BC), and in ancient Egypt and Nubia poisoned arrows appear to have been used during the period 3100–300 BC.

In China, arrow poisons have been known to the Han and other peoples for at least 2500 years. They were used for both hunting and warfare, and documentary evidence indicates that the principal source of poison was *Aconitum*, the tubers of which yield aconitine. The same poison was also used in ancient India, where it was called visha and derived from a plant known as Bish. The hymns of the *Rg Veda* and *Atharva Veda* (1200–900 BC) show that poisoned arrows were used in war, and that the tubers of *Aconitum* were the major poison source. Later Buddhist and Sanskrit writings indicate the continued use of poisoned arrows and reveal that a second source of poison was decomposing snakes.

Among the peoples of the ancient Middle and Near East, the Egyptians, Assyrians, Sumerians, and Hebrews all had some knowledge of poisons, from which they developed a primitive pharmacology. Much of their experience was bound up with mysticism and the supernatural, and many details remain unclear. The Bible, where references are mostly to venoms (as in the Midrash and Talmud), does not contain a list of poisons or allude to their deliberate use. The Hebrew people most likely acquired information about poisons in Egypt, where they established a vibrant community after the destruction of Jerusalem in the sixth century BC.

Egyptian knowledge of poisons appears to have been highly advanced. The first pharaoh (or king), Menes, is said to have cultivated and studied poisonous and medicinal plants in about 3000 BC. Following his reign, information about animal, vegetable, and mineral poisons was accumulated in Egypt. The discovery in 1872 of the Papyrus Ebers, written about 1550 BC, revealed the extent of this knowledge. It is a compilation of medical prescriptions derived from much earlier sources and includes 829 prescriptions, of which 72% are quantified.

The text lists many possibly active drugs, including: sulfate, oxide, and other salts of lead used as astringents and demulcents; pomegranate and acanthus pith as vermifuges; sulfate and acetate of copper; magnesia, lime, soda, iron, and niter; oxide of antimony, sulfide of mercury; peppermint, fennel, absinth, thyme, cassia, coriander, carraway, juniper, cedar wood oil, turpentine, and many other essential oils; gentian and other bitters; mandrake, hyoscyamus, opium with other hypnotics and anodynes; linseed, castor oil, squills, colchicum, mustard, onion, nasturtium, tamarisk, frankincense, myrrh, and yeast.

Toxicology in Ancient Greece

The literature of ancient Greece contains many references to poisons and their use, none more famous than Plato's account of the death of Socrates. Condemned to death for impiety and corruption of youth, the Athenian philosopher swallowed a fatal dose of hemlock in 399 BC. This was the state method of execution, the poison being derived from the tubers of *Conium maculatum* (the 'spotted hemlock' or 'poison hemlock'); for quicker effect, it may have been mixed with opium. Other poisonous plants known to the Greeks included aconite, hellebore, mandrake, and henbane. The writings attributed to Hippocrates (460 to *c.* 375–350 BC), the 'father of medicine', included ~400 drugs of mainly plant origin and contained suggestions for managing poisoned patients primarily by limiting the absorption of toxic agents. During the reign of Attalus III (138–133 BC), poisonous plants were cultivated and used in experiments on condemned prisoners.

Rulers lived in fear of poison, and Mithridates VI Eupator, king of Pontus from 120 to 63 BC, spent years searching for a universal antidote to all poisons; he has been called the first experimental toxicologist. After investigating individual venoms, poisons, and antidotes, he combined all of the effective substances into one antidote, which he took daily to obtain, reportedly successfully, immunity to poison. His formula, called Mithridatium, survived in various forms until the nineteenth century. A variant derived from poisonous reptiles became known as theriac, and was equally long-lived in European pharmacopoeias.

Theriac became famous as a result of its association with the earliest extant work on poisonous animals, the *Theriaca* of Nicander of Colophon (second century BC). His poems describe venomous animals (snakes, scorpions, spiders, insects, and myriapods) and their bites as well as poisonous plants and prescribe specific remedies to counteract the effects of these poisons. Nicander's work was widely influential: successive Greek and Roman authors took much of their information on toxicology from him; he was read and cited for many centuries.

Following the work of Nicander, which included the beginnings of a scheme for identifying toxic agents by means of the symptoms they produce in human victims, a system of toxicology was developed between the second century BC and the first century AD. The Roman naturalist and historian Pliny the Elder (23/24–79 AD) described the biological effects of poisonous plants and animals in his *Historia Naturalis*. A contemporary, Pedanius Dioscorides, developed a classification scheme for poisons based on their origin (animal, vegetable, mineral) that remains convenient to this day. Dioscorides studied the medicinal properties of plants and minerals, and provided descriptions of ~600 plants and 1000 simple drugs, with the diseases they might cure, in his *Materia Medica*, the leading text in pharmacology for 16 centuries.

Mineral poisons were also well known in the ancient world. In particular, the ores and compounds of arsenic, antimony, copper, mercury, and lead were familiar to many cultures. Pseudo-Dioscorides detailed the poisonous effects of arsenic (meaning sometimes the sulfide, sometimes the white oxide), litharge (red lead or lead oxide), cinnabar (mercuric sulfide), and white lead (lead acetate). Hippocrates, Nicander, Dioscorides, Galen, and Paul of Aegina wrote clinical accounts of lead poisoning, of which there were occasional epidemics, and miners were known to be at risk from the fumes created by smelting processes.

The chronicles of ancient Greece contain few references to criminal poisoning, but the fact that Hippocrates required his students to swear that they would 'give no deadly medicine to anyone if asked, nor suggest any such counsel' implies that it existed. Suicide by poison was fairly common; the state gave permission and provided a lethal dose of hemlock. In the Roman Republic, however, criminal poisoning reached epidemic proportions as documented by Livy (59 BC–17 AD) in his *History of Rome*.

At the time of the civil wars in Rome, poisoning had become so common that the dictator Sulla issued the *Lex Cornelia* in 82 BC. This was the first legislative attempt to prevent poisoning, and it carried harsh penalties: banishment and confiscation of property if an offender was of noble birth, exposure to wild animals if of low status. Later interpretations extended the law to careless preparers of drugs. Despite this edict, however, homicidal poisoning continued to plague Rome, where a class of professional poisoners arose and practiced their skills with impunity. During the first century AD, the worst offenders were members of the ruling family, particularly Nero and his mother Agrippina, who used a variety of poisons, probably aconite, henbane, belladonna, arsenic, and poisonous fungi.

Islamic Toxicology

The death of Galen (~216 AD) marked the beginning of the transition of Western (i.e., Greek) medicine into monastic medical practice that resided in the hands of monks and was a part of their divine mission. As a result, the study of toxicology as a system of knowledge came to a halt in the Christian world

and did not reappear until the rise of the school of Salerno in twelfth-century Italy. Following the rise of Islam in the seventh century, scholarship shifted to Muslim centers, where Arab and Persian physicians dominated medical learning. They discovered Greek medicine through translations made from Byzantine manuscripts.

Several Indian medical texts containing information about poisons were available in translation and, together with the works of Greek authors, became key sources of information for Arab toxicologists. The most complete Arabic works on toxicology still extant are the *Book on Poisons* of ibn Jabir, the *Paradise of Wisdom* of al-Tabari, and the *Book on Poisons* of ibn Wahshiya – all dating to the ninth century AD. The *Canon* of ibn Sina, or Avicenna, and the *Treatise on Poisons and Their Antidotes* of Moses Maimonides (1135–1204) were particularly well known in medieval European universities and medical schools where works written in Greek and Arabic were made available in Latin translation after the eleventh century.

The physicians and alchemists of the Islamic world were the first to note the toxic properties of corrosive sublimate (mercuric chloride), and ibn Sina described the foul odor exhaled by victims of mercury poisoning. The replacement of arsenic trisulfide by white arsenic (arsenic trioxide) in poisonous preparations had a profound influence on the history of toxicology, as it became one of the most versatile and widely used poisons ever known. The medical works of Maimonides, a Jewish philosopher and physician in the service of the Sultan of Egypt, are still seen as modern in their approach to illness. The first part of his book on poisons described the effects of the bites of snakes and other animals, while the second part addressed poisoning with vegetable and mineral substances. He included advice on treating poisoned patients: he advised drawing animal poisons from the wound (sucking, cupping glasses, plasters) and employing antidotes (including theriac and Mithridatium); to treat poisoning by vegetable and mineral substances, he suggested inducing vomiting and purging. Some of his suggestions – suitable diet, keeping the patient awake, applying sedatives to the affected spot or internally – hold true today. The compositions of some of his medicinal recipes and their use according to the age of the patient are also relevant today.

Toxicology in the Middle Ages and Renaissance

One century later, Petrus of Abano (1250–1316), wrote *De Venenis* based on Greek and Arabic works. In it, he classified poisons as vegetable, mineral, and animal, and listed all known poisonous agents with their symptoms and treatment. He also suggested methods for avoiding the ingestion of poison and for neutralizing it if taken. Poison was frequently used for murder and political assassination in Italy in the later Middle Ages and Renaissance. Schools of poisoning arose in Rome, Naples, and Florence. In Venice, the records of the infamous Council of Ten listed the names of intended victims and the fees paid to poisoners for their services. By the seventeenth century, the activities of Italian poisoners had been redirected from political toward social, marital, and financial objectives. In Naples, Giulia Toffana (*c.* 1635–1719) sold arsenical solutions and supposedly poisoned over 600 people; in Rome, Hieronyma Spara conducted a similarly lucrative business (*c.* 1659), her clients being primarily young married women. Both were executed for their crimes.

Italian refinements to the 'art' of poisoning are said to have been introduced to France by Catherine de Medici in the sixteenth century. Favored poisons included arsenic mixed with the decomposition products of an animal to which it had been administered (corrosive sublimate was sometimes substituted), cantharides, and mixtures of arsenic, aconite, belladonna, and opium. Poisoning became a public menace, and in 1662 Louis XIV issued a decree forbidding apothecaries to sell poisons to anyone unknown to them and requiring purchasers to sign a register. A series of scandals soon brought about the downfall of professional poisoners. In 1679, the Chambre Ardente was appointed to investigate suspected poisoning cases, and within 3 years it had brought charges against 442 people. Of those executed, the most notorious was Catherine Deshayes, known as La Voisin: she was convicted of many murders, including those of 2000 infants.

The 'Affaire des Poisons' represented the culmination of the professional poisoners in France, but the fact that the crimes were brought to light owed more to the use of torture to extract confessions than to the ability of doctors or chemists to detect and identify poisons. It was not until the nineteenth century that experimental toxicology developed sufficiently to make such identification possible, but the foundations of this progress were laid much earlier, during the sixteenth century. The key figure in the change from reliance on traditional lore to reliance on objective investigation in science and medicine was Paracelsus (1493–1541), a controversial but influential physician, alchemist, and scientist. Although his science was mixed with mysticism and astrology, his contributions to medicine were revolutionary. Paracelsus rejected the medical theories of the

Greco-Arabic classics, insisted on the value of experimentation (including the use of animal tests), and developed the idea that minerals and chemicals could have medicinal applications (iatrochemistry). His use of mercury preparations in the treatment of syphilis led to accusations of poisoning, to which Paracelsus replied by writing the *Third Defense*. It contains the following important statement:

What is there that is not poison? All things are poison and nothing (is) without poison. Solely the dose determines that a thing is not a poison.

Consequently, toxicologists give credit to Paracelsus for this basic tenet of toxicology, dose-dependency.

Toxicology in the Eighteenth and Nineteenth Centuries

Another concept originated by Paracelsus, that chemicals have effects on specific organs of the body (target-organ toxicity), was developed by Felice Fontana (1730–1805). In his experimental studies of the venom of the European viper, Fontana discovered that the symptoms of poisoning caused by a bite were attributable to the direct action of venom on the blood. His findings contributed to the ongoing debate about whether drugs and poisons acted through the nerves, or by a process of absorption and transport in the blood. This debate stimulated chemical and physiological research throughout the seventeenth and eighteenth centuries. Together with advances in the analytical chemistry of animal and plant substances, and a mounting acceptance of animal experimentation, this contributed to the development of experimental toxicology as a distinct scientific discipline during the nineteenth century.

François Magendie (1783–1855), the first great experimental physiologist of the nineteenth century, laid the foundation for the systematic study of the mechanisms by which poisons act in the body with his investigation of the Javanese arrow poison *Upas tieuté*, later shown to contain strychnine. His pupil, Claude Bernard (1813–78), studied the nature of the action of curare on neuromuscular transmissions, effectively using a poison as an instrument for resolving important physiological problems. In addition, Bernard suggested that carbon monoxide poisoning occurs as a result of tissue asphyxiation caused by an irreversible combination with hemoglobin, preventing the effective transport of oxygen to body tissues. Another of Magendie's students, James Blake (1815–93) performed research on the relationship between the chemical structure of a drug and its biological activity, supporting the concept of target-organ toxicity. Additional research on structure–activity relationships was conducted in Britain, perhaps the most sophisticated being that of Alexander Crum Brown (1838–1922) and Thomas Fraser (1841–1920) on organic alkaloids. The successes of the experimental method in physiology, combined with advances in analytical chemistry, stimulated the development of pharmacology. The complementary nature of toxicological and pharmacological research during the nineteenth century was embodied in the work of the Germans Rudolf Kobert (1854–1918), who studied the digitalis glycosides and the ergot alkaloids, and Louis Lewin (1850–1929), an expert on narcotics, alcohols, poisonous gases, and arrow poisons.

The chemical approach to the study of poisons was pioneered by a man long considered the founder of modern toxicology, Mathieu Joseph Bonaventura Orfila (1787–1853). Orfila put toxicology on a firm quantitative basis by introducing new, primarily chemical, experimental methods for proving lethal intoxications – replacing diagnoses made solely on the basis of observed features. A trained chemist and physician, he performed experiments on thousands of dogs, the basis for his monumental work: *Traité des poisons tirés des règnes minéral*, *végétal et animal*, *ou toxicologie générale*, *considérée sous les rapports de la physiologie*, *de la pathologie et de la médecine légale*, published in 1814–15. The book examined the physiological and pathological effects of poisons, the symptoms of poisoning, antidotes, the chemical properties of poisons, and analytical methods for detecting them. This was the first systematic attempt to correlate chemical and biological information concerning known poisons and was unique in combining the use of postmortem examination with analytical chemistry.

As the leading medicolegal expert of his time, Orfila made considerable contributions to legal (forensic) medicine such as the discovery that poisons are absorbed from the gastrointestinal tract and accumulate in tissues specific to each poison. Previously, a chemist or a physician who found nothing in the stomach would not have examined the other organs of the body. In Britain, the development of forensic toxicology was stimulated by one of Orfila's pupils, (Sir) Robert Christison (1797–1882), who wrote *A Treatise on Poisons in relation to medical jurisprudence*, *physiology and the practice of physic* – the first textbook of its kind written in English. He regarded toxicology as the principal branch of medical jurisprudence, its object being to unite evidence from four sources (pathology, chemistry, physiology, and visible symptoms) to detect crime.

The works of Orfila and Christison, which were widely read and translated, laid the foundation for the development of forensic toxicology in the

nineteenth century. Orfila was the first (1839) to extract arsenic from human organs other than gastrointestinal tissue; in 1840, his analysis of organ samples resulted in the conviction of Marie Lafarge for the murder of her husband. The method used was based upon Scheele's discovery (1775) that when zinc and acid act on arsenic salts, a gaseous compound (arsine) is evolved, that, when burned, deposits metallic arsenic. This qualitative procedure was modified by Berzelius to permit quantitative evaluation of metals. Three years later, Fresenius and von Babo devised a method for quantitating all mineral poisons, using wet ashing with chlorine. Other quantitative methods were developed soon afterward.

Newer methods of chemical analysis led to the isolation of the major alkaloids from crude drug preparations. By 1833, aconitine, atropine, codeine, hyoscyamine, morphine, nicotine, and strychnine had been isolated from plants. Color tests for alkaloids were developed between 1861 and 1882; by 1890 quantitative analysis methods became available. Physiological tests for alkaloids, particularly strychnine, first used in 1856, were employed well into the twentieth century. Tests for alcohol, devised by Lieben (iodoform crystal test, 1870) and others, were later perfected for the quantitative analysis of alcohol in body fluids and tissues. Qualitative tests for carbon monoxide in the blood were developed about this time and in 1880, Fodor developed a palladium chloride reduction method to quantitate carbon monoxide in blood.

Textbooks of forensic medicine and toxicology proliferated throughout the nineteenth century. In Britain, the work of Christison was complemented by that of Alfred Swaine Taylor (1806–80), an eminent medico-legal expert who wrote texts that incorporated legal precedents and judicial rulings. These became standard references for over a century; the most recent, thirteenth edition, of *The Principles and Practice of Medical Jurisprudence* appeared in 1984. In 1848, O.H. Costill wrote the first book in the United States pertaining to the symptoms and treatment of poisoning: *A Practical Treatise on Poisons*. In 1867, Theodore Wormley (1826–97) published the first American text devoted exclusively to the experimental detection of poisons in organic mixtures, *The Microchemistry of Poisons*. Soon after, John Reese produced a similar book (*Manual of Toxicology*, 1874), which he followed up a decade later with *A Text Book of Medical Jurisprudence and Toxicology* (1884). During the late nineteenth and early twentieth century, a great amount of toxicological data was presented in the thorough textbooks of German scientists, particularly Kobert (*Compendium der praktischen Toxikologie*, 1887) and Lewin (*Gifte und Vergiftungen*, 1929). Lewin is especially remembered as the author of a toxicologist's view of world history: *Die Gifte in der Weltgeschichte* (1920).

Toxicology in the Twentieth Century

The early part of the twentieth century marked the beginning of the development of the modern science of toxicology. However, the most rapid growth of the discipline occurred after the Second World War, as the production of organic molecules for use as drugs, pesticides, and industrial chemicals began to increase at an exponential rate. Today, toxicology is concerned with the many chemicals that may cause toxicity in the outdoor, indoor, and occupational environments. Modern toxicology utilizes skills and knowledge derived from pathology, pharmacology, physiology, biochemistry, chemistry, and statistics to quantitate the effects of chemicals on living tissue.

Research on anesthetic gases during the nineteenth century facilitated the development and use of poisonous war gases in the twentieth. This led to attempts to counteract the effects of chemical warfare agents and other toxic compounds, particularly arsenicals, introduced by Paul Ehrlich (1854–1915) for the treatment of syphilis. This resulted in the synthesis of the first specific chemical antidote, British anti-Lewisite (BAL), in 1945 by R.A. Peters, L.A. Stocken, and R.H.S. Thompson in Oxford. Studies on the mechanistic bases for toxicity were applied to the synthesis of effective insecticides. For example, during the 1940s, the Swiss chemist Paul Müller discovered a compound, now known as DDT, that poisons insects on contact.

With the increasing use of synthetic drugs and chemicals, toxicology assumed an important role in public health: the protection of workers and the public from the adverse effects of chemical exposure. In Britain, the systematic application of scientific techniques to the detection and control of food and drug adulteration arose largely as a result of the work of the Society of Public Analysts. In the United States, concerns about the adulteration of foods led to the passage of the Food and Drug Act in 1906. This law, created under the impetus of H.W. Wiley (1844–1930), the head of the Bureau of Chemistry of the US Department of Agriculture, influenced food safety legislation worldwide. Since that time numerous laws, in the United States and elsewhere, have been established to minimize public encounters with harmful chemicals in the environment and consumer products. To carry out these laws, toxicologists are needed to provide accurate safety assessments of new and existing chemicals; particularly to establish the

dose–response relationship for both short- and long-term toxicity.

The principal method used for assessing the safety of drugs, pesticides, food additives, and other chemicals is animal testing, which can usually reveal the range of potential toxic effects. Over the past 50 years testing guidelines have been developed and modified. For example, the thalidomide catastrophe of 1961 led to significant changes in existing tests for reproductive and developmental effects. It is recognized that laboratory studies cannot always establish the full toxicity of the test agent in humans, and safety factors are used to compensate for limitations in testing protocols and the possible differences in response between humans and the test species. In addition, increasing social disquiet in regard to animal testing and animal welfare has led to the development of alternative methods in toxicology, particularly *in vitro* assays, which can sometimes serve as substitutes for live animal testing.

Modern toxicology may be divided into six principal areas of application: regulatory, occupational, environmental, clinical, forensic, and analytical. In the United States, the eminent regulatory toxicologist Arnold J. Lehman (1900–79) was instrumental in strengthening the commitment of the Food and Drug Administration (FDA) to toxicology. In 1955, he and his staff at the FDA published *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics,* the first agency guidelines for toxicological studies. These guidelines have been very influential in the subsequent approaches adopted by agencies in the United States and elsewhere to assess and manage risks from chemicals in the environment and in foods and consumer products. Lehman's statement that "anyone can become a toxicologist in two easy lessons, each of which takes 10 years" has achieved the status of an adage among practicing scientists.

Occupational and environmental toxicologists study and monitor the causes, conditions, and effects of exposure to chemicals in the workplace and environment. In some cases, the same chemical may show toxicity in both the industrial and environmental setting: for example, lead and other heavy metals. In addition, toxicologists have shown that some chemicals that have beneficial effects on human health, for example, DDT, may also have adverse effects ecologically. Public awareness of this dichotomy was heightened as a result of the publication of *Silent Spring* in 1962. The author, Rachel Carson, touched off a heated debate about the links between industrialization and pollution when she claimed that "we have put poisonous and biologically potent chemicals indiscriminately into the hands of persons largely or wholly ignorant of their potentials for harm." Although highly controversial, the book strongly stimulated the study of chemical effects on ecosystems and the development of more stringent regulation of environmental contaminants.

When cases of intoxication occur, the need for clinical toxicology becomes apparent: physicians are expected to make a correct diagnosis and implement appropriate treatment, which may involve delaying the absorption of the poison and/or enhancing its elimination. Today, accidental and intentional self-poisoning contribute significantly to morbidity and mortality in many countries, most as a result of household chemicals, drugs, pesticides, solvents, and carbon monoxide. The establishment of poison control centers, the first of which opened in Chicago in 1953, has facilitated the compilation of information on the ingredients of pharmaceuticals and other industrial products and their toxicity, and has led to the creation of sophisticated information distribution systems. The ultimate aim is to quickly and accurately supply information to aid the diagnosis, treatment, and prevention of poisoning. Similar centers have been set up in many other countries.

Since poisons continue to be significant causes of death and disease, forensic and analytical toxicology remain important sciences. Both employ some of the same methods and techniques but for different ends. Forensic toxicology is concerned with intentional and accidental poisonings in relation to law, while analytical toxicology deals with the detection, identification, and measurement of poisons and their metabolites in both biological and environmental matrices. Before the advent of spectroscopic and chromatographic methods in the early 1950s, chemical techniques for separating and identifying the increasing number of synthetic chemicals were time consuming and lacking in sensitivity. Analytical capabilities have increased greatly in recent decades, permitting rapid tests for a variety of compounds. For example, gas and high-performance liquid chromatography, together with immunoassay techniques, now allow quantitation of most organic drugs. Low concentrations of metals can be quantitated using mass spectrometric, electrochemical, radiochemical, and spectrophotometric methods.

During the past three decades, toxicology research has been increasingly devoted to a quantitative assessment of the probable health risks posed by chemicals to which humans might be exposed. In a society that is increasingly risk averse, toxicological information is heavily relied upon by regulatory agencies for prioritizing and managing environmental health concerns. Environmental regulations and the associated

risk assessments have, in effect, become the driving force behind the practice of toxicology in the United States and, more recently, in the European Union. Toxicity testing requirements are in fact remarkably similar across international boundaries.

Advances in risk assessment depend on a growing foundation of scientific knowledge, particularly increased understanding of toxicology at the molecular level. This has resulted from advances in molecular biology such as methods for the sequencing of nucleic acids as well as biochemical (enzyme-oriented) approaches to the study of the metabolism of drugs and environmental toxicants. These advances have contributed to a better understanding of the nature, site, and mechanism of action of toxicants. Once the mechanism of toxicity of a compound is understood, it may be possible to design a replacement chemical that retains desirable properties but is less toxic. The role played by genes in metabolic activation and detoxification constitutes another leading area of research in modern toxicology.

Currently, a great deal of attention is being focused on two new areas of toxicology – toxicogenetics and toxicogenomics. Toxicogenetics is the study of variations in human heritable make-up related to differences in susceptibility to the adverse effects of exogenous agents. Research on toxicogenomics, on the contrary, focuses on changes in gene expression resulting from exposure to xenobiotics. It is expected that advances in these complementary areas of study will lead to greater ability to make individual-based rather than population-based predictions of adverse effects in humans as a consequence of exposures to potentially toxic substances. Such predictions will reduce the need to utilize arbitrary safety factors in risk assessment and risk management.

The development of toxicology as a recognized scientific discipline has proceeded at a rapid pace since the end of the Second World War. One of the most important consequences has been the establishment of training programs and the founding of scientific journals and societies. Graduate education in North America and Western Europe reflects the multidisciplinary nature of toxicology, as it is administered by a variety of university departments, including medicine (human and veterinary), pharmacy, pharmacology, and chemistry. The modern toxicologist is thus a specialist in one or more branches of the field, as it becomes increasingly difficult for one individual to be qualified in all aspects of the science. This specialization is mirrored in the very many national and international organizations and journals that are dedicated to toxicology and related subjects. Toxicologists remain united, however, in their ultimate objective, which is understanding the basis of the morbidity and mortality that occurs in humans and other living systems as a result of exposures to toxic substances.

See also: Animals, Poisonous and Venomous; BAL (British Antilewisite); Food and Drug Administration, US; Toxicology.

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Toxicology, Intuitive

Pertti J Hakkinen

Humans have always been intuitive toxicologists via the use of the senses of sight, taste, and smell to try to detect harmful or unsafe food, water, and air. Relying only on these senses is inadequate to assess all potential dangers, and complicating matters is that even the best scientific approaches used in human risk assessment depend on extrapolations and judgments when assessing animal and other toxicology data. This has led to the study of the intuitive elements of expert and public risk judgments involved with

exposure assessment, toxicology, and risk assessment, usually called the study of 'intuitive toxicology'.

The studies of intuitive toxicology have surveyed toxicologists (e.g., members of the Society of Toxicology) and others about a wide range of attitudes, beliefs, and perceptions regarding risks from chemicals. These have included basic concepts, assumptions, and interpretations related to the effects of chemical concentration, dose, and exposure on risk, and the value of animal studies for predicting the effects of chemicals on humans. The chemicals covered in these studies have included those used in pesticides, food additives, industrial chemicals, household cleaning agents, and prescription and nonprescription drugs.

Two statements studied repeatedly in intuitive toxicology studies are:

- Would you agree or disagree that the way an animal reacts to a chemical is a reliable predictor of how a human would react to it?
- If a scientific study produces evidence that a chemical causes cancer in animals, then we can be reasonably sure that the chemical will cause cancer in humans.

Noteworthy findings from studies of intuitive toxicology in the United States, Canada, and the United Kingdom include:

- The public is more likely than toxicologists to think chemicals pose greater risks.
- The public finds it difficult to understand the concept of dose–response relationships.
- The public is much more likely than toxicologists to think the results of animal carcinogenicity studies can be applied to humans.
- Much disagreement between toxicologists about how to interpret various results, for example, a study in the United States noted, "among the most important findings in this study was…the high percentage of toxicologists who doubted the validity of the animal and bacterial studies that form the backbone of their science."
- Fewer toxicologists in industry than in university or government jobs agreed that animal carcinogens could reasonably be expected to cause cancer in humans.
- Technical judgments of toxicologists were also found to be associated with factors such as gender, age, and the level of agreement with various 'worldview' statements.

The demographic information for experts gathered in these studies has included the highest academic degree earned, fields of study, age, sex, race, health, organizational affiliation, and current position at work. The demographic information for the lay public gathered as part of the studies has included education, age, sex, marital status, race, children, health, present employment status, career, and annual household income.

As highlighted above, studies of intuitive toxicology have yielded a number of intriguing findings, and have highlighted some important risk communication and other challenges. Large differences in responses to intuitive toxicology questions and statements can exist between toxicologists and laypeople. Further, meaningful differences exist between toxicologists working in industry, academia, and government, including sharp divisions in their opinions about the ability to predict a chemical's effect on human health when basing the prediction on results from animal studies. Although these studies have identified misconceptions that experts should try to clarify for the public, the results also suggest that disagreement among experts, especially as perceived by the news media and the public, can play a key role in controversies over chemical risks.

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Trans Fatty Acids

Pertti J Hakkinen

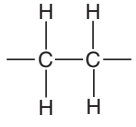
Trans fatty acids, also called trans fat, occur in foods when manufacturers use hydrogenation, a process in which hydrogen is added to vegetable oil, to turn the oil into a more solid fat. Trans fat is often found in the same foods as saturated fat, including vegetable shortening, some margarines, crackers, candies, cookies, snack foods, fried foods, baked goods, salad dressings, and other processed foods. In addition, a small amount of trans fat is found naturally, primarily in some animal-based foods.

The Types of Fatty Acids

There are three main types of fatty acids: saturated, monounsaturated, and polyunsaturated. All fatty acids are chains of carbon atoms with hydrogen atoms attached to the carbon atoms. A saturated fatty acid has the maximum possible number of hydrogen atoms attached to every carbon atom. Fatty acids missing a pair of hydrogen atoms in the middle of a chain, leaving two carbon atoms connected by a double bond rather than a single bond, are 'unsaturated'. A fatty acid with one double bond is called 'monounsaturated' and fatty acids having more than one gap are called 'polyunsaturated'.

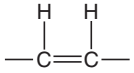
The fat in foods contains a mixture of saturated, monounsaturated and polyunsaturated fatty acids. In foods of animal origin, a large proportion of fatty acids are saturated. In contrast, in foods of plant origin and some seafood, a large proportion of the fatty acids are monounsaturated and polyunsaturated. The structures of saturated and unsaturated chemical bonds are shown below.

Saturated fat
(i.e., saturated fatty acid)



Carbon–Carbon
single bond

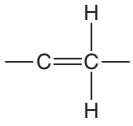
Unsaturated fat
(i.e., unsaturated fatty acid)



Carbon–Carbon
double bond

The hydrogenation used to make trans fats increases the shelf life and flavor stability of foods containing these fats. Usually, the hydrogen atoms at a double bond are positioned on the same side of the carbon chain. However, partial hydrogenation reconfigures some double bonds and the hydrogen atoms end up on different sides of the chain. This type of configuration is called 'trans' ('across' in Latin). The structure of a trans unsaturated chemical bond is shown below.

Trans fat
(i.e., *trans* fatty acids)



Hydrogen atoms are on opposite sides of the chain of carbon atoms at the carbon–carbon double bond.

the Food and Drug Administration's (FDA's) regulatory chemical definition for trans fatty acids is all unsaturated fatty acids that contain one or more isolated (i.e., nonconjugated) double bonds in a trans configuration. Under the Agency's definition, conjugated linoleic acid would be excluded from the definition of trans fat.

The Health Impact

Scientific reports have confirmed the relationship between trans fat and an increased risk of coronary heart disease. Trans fat, like saturated fat and dietary cholesterol, can raise low-density lipoprotein (LDL or 'bad' cholesterol) levels in the blood. An elevated LDL cholesterol level increases the risk of developing coronary heart disease. Unlike saturated fat, trans fat also lowers high-density lipoprotein (HDL or 'good') cholesterol in the blood.

US Food and Drug Administration Actions

The Food, Drug, and Cosmetic Act (FD&C Act) provides the FDA with statutory authority to require food and nutrition labeling. Two sections of the FD&C Act provide the legal authority, including Section 403(a) requiring foods to be adequately labeled and that material facts be disclosed to consumers, and Section 403(q) (2) (A) giving the authority to require additional nutrients to be included in nutrition labeling if such information will "assist consumers to maintain healthy dietary practices." A goal of the Nutrition Facts panel part of labeling is to provide consumers with more information to make

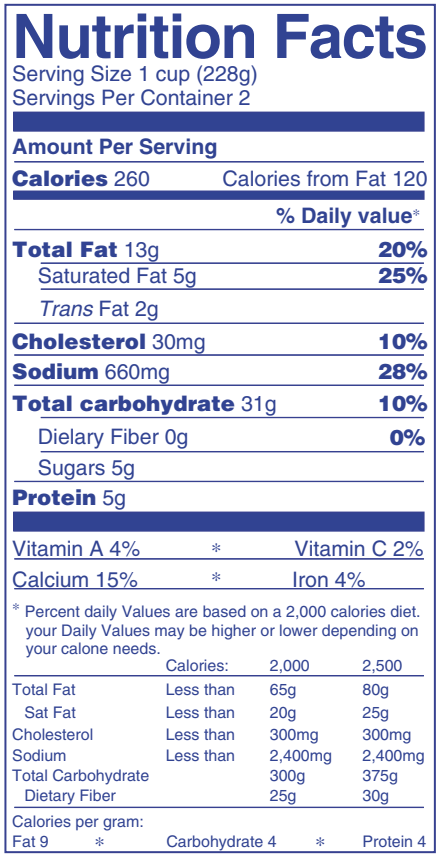
healthier food choices that could lower their consumption of trans fat as part of a heart-healthy diet.

When the 1993 Nutrition Labeling and Education Act regulations were finalized, FDA did not require trans fat to be listed on the Nutrition Facts panel because the scientific evidence was not conclusive about the relationship between trans fat intake and increased blood cholesterol levels. In 1994, the Center for Science in the Public Interest, a consumer advocacy organization, filed a petition (amended in July 1998) with FDA requesting that the agency take steps to require trans fat to be listed on nutrition labels. In response to that petition, FDA issued a proposed rule in the Federal Register on November 17, 1999, proposing to amend the regulations to require that trans fat be listed on nutrition labels. In response to comments and evolving science, FDA reopened the comment period on December 5, 2000 and November 15, 2002. FDA received over 1650 letters in response to the November 1999 proposal, over 45 letters in response to the December 5, 2000 notice reopening the comment period, and over 25 letters in response to the November 15, 2002 proposal and notice to reopen the comment period.

FDA reviewed the scientific evidence and recommendations of various scientific bodies, including the Institute of Medicine, National Academies of Science, an expert panel for the National Cholesterol Education Program, and the Advisory Committee on the Dietary Guidelines for Americans 2000. On July 9, 2003, the FDA issued a regulation requiring manufacturers to list trans fatty acids on the Nutrition Facts panel of the labels of foods and some dietary supplements. The new requirement will mean that manufacturers of most conventional foods and some dietary supplements will have to list in the Nutrition Facts panel the trans fat content of the product, in addition to the information about its overall fat content and saturated fat content. Dietary supplement manufacturers will need to list trans fat, as well as saturated fat and cholesterol, on the Supplement Facts panel when their products contain more than trace amounts (0.5 g) of trans fat. Examples of dietary supplements that may contain trans fat are energy and nutrition bars.

The new information is the first significant change on the Nutrition Facts panel of the labeling since it was established in 1993. Food manufacturers have until January 1, 2006, to list trans fat on the nutrition label. The FDA estimates that by 3 years after that date, trans fat labeling will have prevented from 600 to 1200 cases of coronary heart disease and 250–500 deaths each year, and that the changes in regulations will save between US$900 million and $1.8 billion each year in medical costs, lost productivity and pain and suffering. However, while the relationship between trans fat and an increased risk of coronary heart disease has been confirmed, no studies, reports, or expert panels have provided a reference value for trans fat or any other information that the FDA believed to be sufficient to establish a daily reference value. Thus, FDA does not intend to include a percent daily value (%DV) in the Nutrition Facts panel.

The FDA final rule on trans fat requires that the amount of trans fat in a serving be listed on a separate line under saturated fat on the Nutrition Facts panel (see below). However, trans fat does not have to be listed if the total fat in a food is less than 0.5 g per serving and no claims are made about fat, fatty acids, or cholesterol content. If it is not listed, a footnote will be added stating that the food is "not a significant source of trans fat." Further, food manufacturers are allowed to list amounts of trans fat with less than 0.5 g as 0 (zero) on the Nutrition Facts panel. As a result, consumers could see products that list 0 g trans fat on the label, while the ingredient list will have 'shortening', 'partially hydrogenated vegetable oil', or 'hydrogenated vegetable oil' on it. This means the food contains very small amounts (less than 0.5 g) of trans fat per serving.



Nutrition Facts

Serving Size 1 cup (228g)
Servings Per Container 2

Amount Per Serving

Calories 260 Calories from Fat 120

% Daily value*

Total Fat 13g **20%**
Saturated Fat 5g **25%**
Trans Fat 2g
Cholesterol 30mg **10%**
Sodium 660mg **28%**
Total carbohydrate 31g **10%**
Dielary Fiber 0g **0%**
Sugars 5g
Protein 5g

Vitamin A 4% * Vitamin C 2%
Calcium 15% * Iron 4%

* Percent daily Values are based on a 2,000 calories diet. your Daily Values may be higher or lower depending on your calone needs.

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 * Carbohydrate 4 * Protein 4

In addition, in partnership with the (US) National Heart, Lung and Blood Institute of the National Institutes of Health, health and consumer organizations, and trade associations, FDA wants to educate consumers on the importance of lowering their intake of saturated and trans fats by developing consumer education materials for its nutrition and food labeling website. Further, FDA is interested in establishing new nutrient content and health claims about trans fat, as well as possibly having footnote or disclosure statements on the label that could enhance consumer's understanding about saturated fat, trans fat, and cholesterol in order to help them make heart-healthy food choices. Finally, FDA plans to conduct a study on whether nutrient content claims could be made for products with either 'reduced' or 'zero' trans fatty acids in a way that would not mislead consumers when there are significant amounts of saturated fat present.

See also: Food and Drug Administration, US.

Further Reading

Kris-Etherton PM and Etherton TD (2003) The impact of the changing acid profile of fats on diet assessment and health. *Journal of Food Composition and Analysis* 16: 373–378.

Relevant Websites

http://www.cfsan.fda.gov – US Food and Drug Administration Federal Register Final Rule: *Trans* Fatty Acids in Nutrition Labeling, Nutrient Content Claims, and Health Claims. Guidance on How to Understand and Use the Nutrition Facts Panel on Food Labels, July 11, 2003.

http://www.fda.gov – US Food and Drug Administration. FDA Acts to Provide Better Information to Consumers on Trans Fats.

Transgenic Animals

Kartik Shankar and Harihara M Mehendale

Understanding the molecular mechanisms of toxicity is a central part of advancing the processes of drug development and regulatory decision-making. Due to the complex nature of the varied interactions that leads to eventual toxic outcome, it is a daunting challenge in most cases to identify the role of a single gene (or signaling thereof) in mediating an adverse outcome. Genetic manipulations of organisms have been a powerful and staple technique in the biosciences for almost two decades. Mutant *Drosophila* lacking specific genes have been employed to identify the roles of specific genes. However, the use of these techniques in rodent models to identify mechanisms of toxicity is relatively new. Currently, transgenic rodents (specially mice) are being used for a number of reasons including, understanding roles of specific metabolizing enzymes, receptors, transcription factors, regulatory sequences of promoters to just name a few. Although transgenic manipulations of rodents can broadly involve a large number of genetic manipulations the two most commonly used manipulations by far are deletion of a gene (gene knockout or 'null') or expression (or overexpression) of a particular gene. The animal model of choice in biomedicine remains the mouse (*Mus musculus*).

Creating a Knockout Animal

The cursory protocol of creating a gene knockout mouse for a particular gene begins with creating a gene-targeting construct that is transfected into embryonic stem (ES) cells to silence a particular gene. ES cells that have successfully included the recombination are injected into a blastocyst, which is implanted in a pseudo-pregnant female. After a series of back and self-crosses, a mouse homozygous for the mutation is generated. A variety of methods exist to selectively target genes including targeted gene deletion, gene trapping, and the Cre/*lox*P recombinase system.

A protocol of creating a gene knockout mouse is given below:

1. Prepare gene-targeting construct.
2. Transfect embryonic stem cells.
3. Select for transfectants.
4. Confirm recombination.
5. Introduce ES cells into blastocyst.
6. Transfer blastocyst into pseudo-pregnant female.
7. Breed chimeric mouse.
8. Back and self-crosses to gene homozygous knockout.

Applications in Toxicology

In toxicology, the main utility of transgenic or gene-knockout mice has been to understand the

mechanisms of toxicity of chemicals. A classic example is the elucidation of the mechanism of the bioactivation of acetaminophen. Acetaminophen is a commonly use analgesic, antipyretic drug with high potential for liver toxicity at supra-pharmacological doses. Although, it has been clear that a reactive intermediate is necessary for toxicity, the P450 isozymes involved are were not clear. Studies from Lee *et al.* unequivocally demonstrated that mice lacking CYP2E1 were remarkably resistant to acetaminophen-induced liver injury. Further mice lacking both P450s, CYP2E1 and CYP1A2 were even more resistant to acetaminophen toxicity than CYP2E1 null mice alone. Mechanisms of not only drug metabolism/bioactivation pathways but also information about critical molecular factors involved in mediating or mitigating toxicity has been uncovered using transgenic and knockout animals.

See also: Mechanisms of Toxicity.

Further Reading

Gonzalez FJ and Kimura S (1999) Role of gene knockout mice in understanding the mechanisms of chemical toxicity and carcinogenesis. *Cancer Letters* 143: 199–204.

Lee SS, Buters JT, Pineau T, Fernandez-Salguero P, and Gonzalez FJ (1996) Role of CYP2E1 in the hepatotoxicity of acetaminophen. *The Journal of Biological Chemistry* 271: 12063–12067.

Wolf CR and Henderson CJ (1998) Use of transgenic animals in understanding molecular mechanisms of toxicity. *The Journal of Pharmacy and Pharmacology* 50: 567–574.

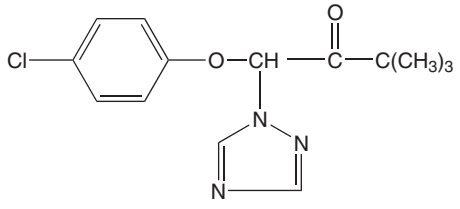
Zaher H, Buters JT, Ward JM, *et al.* (1998) Protection against acetaminophen toxicity in CYP1A2 and CYP2E1 double-null mice. *Toxicology Applied Pharmacology* 152: 193–199.

Triadimefon

Marcia D Howard

This article is a revision of the previous print edition article by Todd A Bartow, volume 3, pp. 371–372, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 43121-43-3
- Synonyms: 1,2,4-triazole; 1H-1,2,4-triazole, 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazolyl)-2-butanone; 1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone; Acizol; Amiral; Bayleton; Bay MEB 6447
- Chemical/Pharmaceutical/Other Class: Systemic triazole fungicide
- Chemical Formula: $C_{14}H_{16}ClN_3O_2$
- Chemical Structure:



Uses

Triadimefon is a fungicide used to control powdery mildews, rust and other fungal pests on cereals, fruits, vegetables, turf, shrubs, and trees.

Exposure Routes and Pathways

Exposure to triadimefon may occur via inhalation, dermal contact and absorption, eye contact, and by ingestion of contaminated foods.

Toxicokinetics

Triadimefon is absorbed through dermal, inhalation or oral exposure routes. Triadimefon has a half-life of 2.5 h in blood plasma. Metabolism occurs in the liver resulting mostly in the formation of triadimenol and glucuronic acid conjugates. In mammals, 83–98% is excreted unchanged in the urine and feces within 2–3 days following oral administration. Metabolism of triadimefon occurs more rapidly in male rats compared to females. Radioactivity in male rats was found mainly in feces but more equally distributed between the urine and feces in females.

Mechanism of Toxicity

Triadimefon binds to hepatic cytochrome P450 and inhibits microsomal enzyme activities. It inhibits sterol demethylation and thus sterol synthesis. Fungi sensitive to triadimefon utilize ergosterol as the primary sterol, the production of which is inhibited. It is also thought that triadimefon may have actions similar to those caused by indirect-acting dopamine agonists.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute effects on the central nervous system may occur. Minimum irritation to the conjuctiva was observed in rabbits with irritation resolved within 1 day. Triadimefon is a skin sensitizer according to primary dermal sensitization studies. There was slight dermal irritation in rabbits. Acute dermal toxicity LD_{50} is $>2000\,mg\,kg^{-1}$ for rats and rabbits. However, absorption following dermal exposure occurs more rapidly in young rats compared to adults. LD_{50} values are reported to be 90–1500 $mg\,kg^{-1}$ (rats), $500\,mg\,kg^{-1}$ (rabbits and dogs), and $1000\,mg\,kg^{-1}$ (mice) following acute oral exposure. Values for LC_{50} following a 4 h exposure were similar in rats and mice ($0.48\,mg\,l^{-1}$).

Human

Mild toxicity generally results from oral or dermal exposure. Triadimefon may cause dermal sensitization and moderate irritation of the eyes.

Chronic Toxicity (or Exposure)

Animal

The no-observed-effect level (NOEL) in dogs was 100 ppm ($2.5\,mg\,kg^{-1}\,day^{-1}$) in a 2 year feeding study. Studies in rats and rabbits suggest that triadimefon has little or no teratogenic potential. In rats, the teratogenic NOEL is $50\,mg\,kg^{-1}\,day^{-1}$. Both maternal toxicity and reproductive NOELs were 300 ppm in rats three-generation reproductive toxicity studies. Responses to low or moderate doses of the toxicant in rats, mice and dogs included decreased body weight, increased cholesterol levels, and increased liver weights.

In both male and female rats, the neurobehavioral NOEL was $2\,mg\,kg^{-1}$ based on hyperactivity and increased motor activity. Triadimefon has been shown to increase motor activity and stereotyped response in a manner resembling psychomotor stimulants by potentiation of dopaminergic transmission. One study noted enhanced locomotor and stereotypic behavioral patterns in Sprague–Dawley rats treated with repeated triadimefon exposure ($100\,mg\,kg^{-1}$ every other day for 14 days). Furthermore, this study suggested that chronic exposure to triadimefon may involve effects of dopamine uptake in the striatum and nucleus accumbens.

Human

No deleterious effects or symptoms are anticipated from chronic exposure under normal usage.

In Vitro Toxicity Data

An *in vitro* study by Vinggaard and colleagues in 2000 reported that triadimefon was able to inhibit aromatase activity in human placental microsomes ($IC_{50} = 32\,\mu mol\,l^{-1}$).

Clinical Management

Eyes should be flushed with copious amounts of water for 15 min. Further medical attention should be sought if irritation persists or develops after flushing. For dermal exposure, contaminated clothing should be removed and the affected area washed with soap and water. A physician should be consulted if irritation develops or persists. In cases of poisoning by ingestion, a physician should be contacted or poison control should be called immediately. Vomiting should be induced after administering one to two glasses of water to the victim. Vomiting should not be induced, nor anything given orally if the person is unconscious. If suffering from inhalation exposure, the exposed individual should be removed to fresh air or an uncontaminated area. Artificial respiration (e.g. cardiopulmonary resuscitation) should be administered if the victim is not breathing. Medical treatment should be sought as soon as possible.

Environmental Fate

Triadimefon is reported to have a half-life in soil of a few weeks to a few months. Its primary metabolite is more persistent.

Ecotoxicology

Triadimefon toxicity in wild birds is thought to be low while acute toxicity to aquatic invertebrates is moderate. LD_{50} values for quail and ducks ranged from 2 to $4\,g\,kg^{-1}$. Triadimefon is not toxic to bees.

Exposure Standards and Guidelines

The reference dose for triadimefon is 0.04 $mg\,kg^{-1}\,day^{-1}$ while the acceptable daily intake is $0.03\,mg\,kg^{-1}\,day^{-1}$.

See also: Biocides; Neurotoxicity; Pesticides.

Further Reading

Hill D, Ikaiddi M, Mazzio E, and Soliman KFA (2000) The neurochemical basis for the behavioral effects of

triadimefon. *Annals of the New York Academy of Sciences* 914: 336–353.
Vinggaard AM, Hnida C, Breinholt V, and Larsen JC (2000) Screening of selected pesticides for inhibition of CYP19 aromatase activity *in vitro*. *Toxicology In Vitro* 14: 227–234.

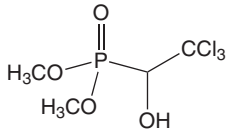
Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Triadimefon.

Trichlorfon

Ramesh C Gupta

- Chemical Name: *O,O*-dimethyl-1-hydroxy-2,2,2-trichloroethylphosphonate
- Chemical Abstracts Service Registry Number: CAS 52-68-6
- Synonyms: Chlorofos; Dipterex; Dylox; Dyrex; Dyvon; Neguvon; Proxol; Tugon and Wotex. The common name for trichlorfon in Great Britain is trichlorphon, in Turkey is dipterex, and in Russia is chlorofos.
- Chemical and Physical Properties: Trichlorfon is an organophosphate compound, which has an empirical formula of $C_4H_8Cl_3O_4P$ and a molecular weight of 257.44. It is a racemic mixture of two isomers. Trichlorfon is a pale clear, white or yellow crystalline powder, melting point 75–84°C, boiling point 100°C, vapor pressure 7.8 mmHg at 20°C, and is stable at normal temperatures and pressure. At higher temperatures and pH less than 5.5, trichlorfon decomposes to form dichlorvos (*O,O*-dimethyl-*O*-(2,2-dichlorovinyl) phosphate, DDVP). It is readily soluble in chloroform and methylene chloride, and less soluble in water, benzene, and diethyl ether.
- Chemical Structure:



Uses

Trichlorfon was introduced in 1950, and has been used as an insecticide since 1952. It is available in the form of dust, granular, emulsifiable concentrate, soluble powder, injectable solution, tablets, and fly bait. Trichlorfon is a broad-spectrum insecticide that is particularly used against *Diptera*. It is used to control a variety of other insects in field, vegetable, and fruit crops, and forestry. In domestic animals, trichlorfon is used for the control of internal and external parasites. It has selective insecticidal action. Under the generic name metrifonate, trichlorfon is used as an anthelmintic in the treatment of schistosomiasis. Trichlorfon was shown in a number of clinical trials to have efficacy in treating Alzheimer's disease and was pending drug certification, but the application for this use was subsequently withdrawn due to pulmonary toxicity. But currently, it is labeled as a mutagen, carcinogen, and possibly teratogen, and therefore used as an investigational agent.

Exposure Routes and Pathways

Poisonings have occurred after oral, dermal, and inhalation exposure to trichlorfon.

Toxicokinetics

In humans and animals, the absorption, distribution, and excretion of trichlorfon is rapid. The biological half-life of trichlorfon in mammalian blood is estimated to be ~30 min. It eliminates from the blood stream within a matter of 1.5–4 h, and only low levels are detected after 8–24 h. In mice, ~70–80% of an oral dose of trichlorfon gets excreted within the first 12 h. Trichlorfon undergoes transformation via dehydrochlorination to form dichlorvos in all biological fluids and tissues. The major metabolites of trichlorfon found *in vivo* are demethyl trichlorfon, demethyl dichlorvos, dimethyl hydrogen phosphate, methyl hydrogen phosphate, phosphoric acid, and trichloroethanol. The excretion of trichlorfon and its metabolites occurs primarily via the urine.

Mechanism of Toxicity

Trichlorfon is primarily an indirect inhibitor of AChE, that is, it is converted in the body to the active chemical inhibitor dichlorvos. In fact, trichlorfon is considered to be a slow release cholinesterase inhibitor, transformed nonenzymatically to dichlorvos. This leads to irreversible AChE inhibition by phosphorylation, primarily at the synapses of the nervous system and at the neuromuscular junctions. Dichlorvos is

estimated to be at least 100 times more potent than trichlorfon as a cholinesterase inhibitor. As a result, signs and symptoms due to trichlorfon overexposure develop after a latent period and may even continue to increase after exposure has been discontinued.

Acute and Short-Term Toxicity (or Exposure)

Animal

Trichlorfon is moderately toxic for laboratory animals by ingestion or dermal absorption. The oral LD_{50} for trichlorfon in rats is 150–649 mg kg^{-1}, 300–1370 mg kg^{-1} in mice, 97 mg kg^{-1} in cats, 400 mg kg^{-1} in dogs, 420 mg kg^{-1} in guinea pigs, and 160 mg kg^{-1} in rabbits. The dermal LD_{50} of trichlorfon is 2000–5000 mg kg^{-1} in rats, and from 1500 to >2100 mg kg^{-1} in rabbits. The LC_{50} for trichlorfon in rats is 1300 mg m^{-3}. Oral acute toxicity studies conducted on rats, dogs, monkeys, rabbits, and guinea pigs revealed that trichlorfon poisoning caused the usual OP-cholinergic signs attributed to the accumulation of ACh by virtue of AChE inhibition. Exposure with very high doses of trichlorfon is known to produce neurotoxicity. Interestingly, trichlorfon may cause delayed symptoms beginning 1–4 weeks after an acute exposure, which may or may not have produced immediate symptoms. In such cases, numbness, tingling, weakness, and cramping may appear in the lower limbs and progress to incoordination and paralysis. Improvement may occur over months or years, but some residual impairment will remain.

Human

Several cases of acute trichlorfon poisoning from suicidal, accidental, or occupational exposure have occurred. Signs and symptoms of intoxication include those characteristic of AChE inhibition, such as weakness, exhaustion, excessive salivation, sweating, vomiting, chest pain, miosis, and muscle spasms. In severe cases, convulsions and unconsciousness develop, and death ensues from respiratory failure. In some cases, victims surviving because of medical interventions developed a delayed polyneuropathy with weakness of the lower limbs after a few weeks of exposure.

Chronic Toxicity (or Exposure)

Animal

Repeated or prolonged exposure to trichlorfon, like with other organophosphates, may result in the same effects as with acute exposure, including delayed symptoms. With 45 mg kg^{-1} day^{-1} trichlorfon administered to dogs for 3 months, serum cholinesterase was reduced to 60% of normal. A dietary level of ~10.5 mg kg^{-1} day^{-1} for 12 weeks produced a similar effect. During a 60 day testing period with repeated doses of trichlorfon at 100 mg kg^{-1} day^{-1}, cholinesterase activity was reduced to 50–75% of normal levels. Trichlorfon produced no pathological changes in rats that were fed 500 mg trichlorfon per kg diet for 1 year. In a 16 week study on rats, a 4 year study on dogs, and a 26 week study on monkeys, no-observed-effect levels (NOELs) of 100 mg kg^{-1} diet, 50 mg kg^{-1} diet, and 0.2 mg kg^{-1} body weight (based on plasma, erythrocyte or brain AChE activity), respectively, were observed. Inhalation exposure of rats over a 3 week period indicated a NOEL of 12.7 mg m^{-3}.

Exposure of laboratory animals (rats, mice, and hamsters) to trichlorfon at higher doses during the gestation period caused adverse effects on reproduction. An increased number of embryonic deaths, a decreased number of live fetuses and an increased number of fetal abnormalities were observed in rats given a single oral dose of 80 mg kg^{-1} body weight, p.o., on the 13th day of pregnancy. During a three-generation study conducted on rat reproduction, a dietary level of 3000 ppm trichlorfon or 150 mg kg^{-1} day^{-1} resulted in a marked decrease in the rate of pregnancy, and underdeveloped rat pups at birth, none of which survived to weanling. A dietary dose of 50 mg kg^{-1} day^{-1} reduced the number of pups per litter, as well as the weight of individual pups; however, a dietary level of 300 ppm (about 15 mg kg^{-1} day^{-1}) had no detectable effect on reproduction.

Trichlorfon and its active metabolite dichlorvos can cross the placenta and produce fetal abnormalities. Trichlorfon was found to be teratogenic when given to pregnant rats through a stomach tube, at a dose level of 480 mg kg^{-1} day^{-1}, on days 6–15 of pregnancy, but not when administered only on days 8 or 10 of pregnancy. Teratogenic effects have also been found in hamsters given 400 mg kg^{-1} day^{-1} on days 7–11 of pregnancy. In a three-generation study, rats fed dietary doses of trichlorfon as high as 150 mg kg^{-1} day^{-1} did not show any evidence of teratogenesis. It is important to note that these exposure levels are within the LD_{50} range.

Trichlorfon produced mutations in mice when it was given in the maximum tolerable single dose or repeated smaller doses. Trichlorfon has been shown to produce carcinogenic effects in rats given oral doses of 186 mg kg^{-1} or intramuscular doses of 183 mg kg^{-1} for 6 weeks. However, no evidence of carcinogenicity in rats given trichlorfon orally or intraperitoneally for 90 days.

In Vitro Toxicity Data

In vitro studies suggest that trichlorfon or its degradation products can be mutagenic in bacterial and mammalian cell assays.

Clinical Management

The diagnosis of a trichlorfon or related organophosphate intoxication should be confirmed as soon as possible by the determination of cholinesterase activity in venous blood. If dermal exposure occurs, decontamination procedures include removal of contaminated clothes and washing of the skin with soap or with a sodium bicarbonate solution. Extensive eye irrigation with water or saline should be performed. In the case of ingestion of a liquid formulation of trichlorfon, which may contain hydrocarbon solvents, avoid inducing emesis because of the risk of aspiration pneumonia. Instead, the stomach should be emptied, as soon as possible, by careful gastric lavage using 5% sodium bicarbonate (with a cuffed endotracheal tube already in place). Artificial respiration should be applied, if necessary. In a severe poisoning case, as early as possible, administer 2 mg of atropine sulfate intravenously and 1000–2000 mg of pralidoxime chloride or 250 mg of obidoxime chloride intramuscularly or intravenously. Repeated doses of 2 mg of atropine sulfate should be given until muscarinic receptor associated effects are completely subsided. For children, the doses are 0.04–0.08 mg of atropine sulfate per kg body weight, and 250 mg of pralidoxime chloride per child or 48 mg of obidoxime chloride per kg body weight. In adults, a single dose of diazepam (10 mg, i.p. or s.c) is found to be beneficial.

Environmental Fate

Trichlorfon rapidly degrades in the environment.

Ecotoxicology

Trichlorfon is highly toxic to birds, as the oral LD_{50} is $37\,mg\,kg^{-1}$ in wild birds, $36.8\,mg\,kg^{-1}$ in mallards, $22.4\,mg\,kg^{-1}$ in bobwhite quail, $59.3\,mg\,kg^{-1}$ in California quail, $95.9\,mg\,kg^{-1}$ in male pheasant, and $23\,mg\,kg^{-1}$ in rock doves. Signs of poisoning, such as regurgitation, imbalance, trembling, ataxia, and wingbeat convulsions, occur as early as within 10 min after exposure. Death usually occurs within 30 min to 3 h of exposure.

Trichlorfon is also highly toxic to both cold and warm water fish, and its acute toxicity is between 1.67 and 180 ppm. The 24 h LC_{50} for striped bass is 10.4 ppm. The 48 h LC_{50} for rainbow trout is 3.2 ppm, and the 96 h LC_{50} for fathead minnow is 180 ppm. Trichlorfon does not bioaccumulate.

Exposure Standards and Guidelines

The maximum permissible concentration of trichlorfon in air is $0.5\,mg\,m^{-3}$. The US Environmental Protection Agency (EPA) has set an acute population adjusted dose (PAD) for trichlorfon at $0.01\,mg\,kg^{-1}\,day^{-1}$. The chronic PAD is set at $0.2\,\mu g\,kg^{-1}\,day^{-1}$. Currently, none of the organizations – Occupational Safety and Health Administration, the National Institute for Occupational Safety and Health, or the American Conference of Governmental Industrial Hygienists – has established any occupational exposure limits for trichlorfon.

See also: Organochlorine Insecticides; Organophosphates; Pesticides.

Further Reading

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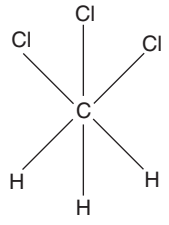
Hayes WJ and Laws ER (1990) *Handbook of Pesticide Toxicology, Vol. 3, Classes of Pesticides*. New York: Academic Press.

National Institute for Occupational Safety and Health (NIOSH) (1981–1986) Registry of Toxic Effects of Chemical Substances (RTECS). Cincinnati, OH.

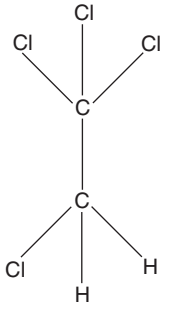
Trichloroethane

Robert Kapp

- Chemical Abstracts Service Registry Numbers: 1,1,1-Trichloroethane (CAS 71-55-6); 1,1,2-Trichloroethane (CAS 79-00-5)
- Synonyms:
  - 1,1,1-Trichloroethane: 1,1,1-TCE; Aerothene TT; Baltana; Chloroform, methyl-Chloroethene; Chlorten; Ethane, 1,1,1-trichloro-; Inhibisol; Methylchloroform; Methyltrichloromethane; Tafclean; Trichloroethane; Trichloromethylmethane; Alpha-Trichloroethane
  - 1,1,2-Trichloroethane: 1,2,2-Trichloroethane; Ethane, 1,1,2-trichloro-; Trojchloroetan(1,1,2); Vinyl trichloride; Vinyltrichloride; Beta-Trichloroethane
- Related Compounds: Tetrachloroethane (CAS 79-34-5); Carbon tetrachloride (CAS 56-23-5); 1,1-Dichloroethane (CAS 107-06-2); 1,2-Dichloroethane (CAS 75-34-3)
- Chemical/Pharmaceutical/Other Class: Chlorinated hydrocarbons
- Chemical Formula: $C_2H_3Cl_3$
- Chemical Structures:



1,1,1-Trichloroethane



1,1,2-Trichloroethane

Uses

1,1,1-Trichloroethane is a solvent used for resins, oils, waxes, tars, paints, and glues. It is widely used as a degreasing agent in the manufacture of metals and plastics, silicon chips, and other electronic parts. It is used in pesticides, textile processing, cutting fluids, aerosols, lubricants, cutting oil formulations, drain cleaners, spot cleaners, printing inks, and stain repellents.

1,1,2-Trichloroethane is used primarily as a chemical intermediate in the production of 1,2-dichloroethene and vinylidine chloride. It is also used in adhesives, lacquers, coating formulations, for chlorinated rubbers and polyesters, as a solvent for fats, oils, waxes, resins, and in the production of Teflon tubing.

Exposure Routes and Pathways

A primary pathway for trichloroethane exposure is by inhalation of contaminated air both indoors (from building materials, aerosols, cleaning products, paints, or metals degreasing agents) and outdoors near industrial sites or accidental releases. Other routes of exposure include ingesting contaminated water or food or through the skin upon dermal contact.

Toxicokinetics

Human data on both forms of trichloroethanes indicate that they are both rapidly and extensively absorbed upon inhalation, dermal, or gastrointestinal exposure. Animal studies show that 1,1,1-trichloroethane is metabolized slowly, but it is distributed by the blood to virtually all tissues and organs with a preference to fatty tissues. In humans and animals the principal pathway of elimination is by exhalation of the unchanged material via the lungs. The biological half-life is estimated to be 8.7 h. Only very limited studies on distribution and elimination were available for 1,1,2-trichloroethane; however, it is likely that these mechanisms are very similar to that of 1,1,1-trichloroethane.

Mechanism of Toxicity

1,1,1-Trichloroethane is oxidized to 2,2,2-trichloroethanol and trichloroacetic acid by the cytochrome P450 mixed-function oxidase system, which are excreted in the urine while unchanged 1,1,1-trichloroethane, carbon dioxide, and acetylene are excreted in expired air. It is estimated that less than 7% of 1,1,1-trichloroethane is absorbed and metabolized by any exposure route and the toxicokinetic behavior is qualitatively identical across all species. Less than 1% of 1,1,1-trichloroethane remains in the human body after 9 days. 1,1,2-Trichloroethane is metabolized to

chloroacetic acid, *S*-carboxymethylcysteine, and thiodiacetic acid. Thiodiacetic acid and *S*-carboxymethylcysteine are formed following glutathione conjugation while chloroacetic acid is the metabolite formed by hepatic cytochrome P450. This reaction is thought to proceed through acyl chloride. Acyl chlorides and free radicals that are formed from both 1,1,1-trichloroethane and 1,1,2-trichloroethane are believed to bind nucleic acids and proteins causing various cytoxic, mutagenic, and carcinogenic effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute exposure to 1,1,1-trichlororethane is considered to be of a low-order toxicity with a rat oral LD_{50} of 10.3–12.3 g kg^{-1}. High concentrations cause disturbances of the central nervous system (CNS), cardiovascular effects, and irritation of the skin, mucous membranes, and eyes.

Studies on exposure of experimental animals to 1,1,2-trichloroethane have reported effects on the liver kidney and CNS from inhalation exposure and ingestion. Acute exposure to 1,1,2-trichloroethane is considered to be moderately to highly toxic with a rat oral LD_{50} of 580 mg kg^{-1}.

Human

Exposure of humans to high concentrations of 1,1,1-trichloroethane can produce severe CNS depression, respiratory arrest, decrease blood pressure, as well as lung, kidney, and liver damage. 1,1,1-Trichloroethane has been intentionally inhaled to alter mood or consciousness. Deaths have been attributed to abuse of this solvent. It is mildly irritating to the skin and eyes of both humans and experimental animals.

Exposure of humans to high concentrations of 1,1,2-trichloroethane via the skin has caused burning sensations and transient whitening of the skin. No other acute human exposure data are available.

Chronic Toxicity (or Exposure)

Animal

Chronic exposure to 1,1,1-trichloroethane has resulted in some liver damage and neurological effects in experimental animals. US Environmental Protection Agency (EPA) has not established a reference concentration (RfC) or a reference dose (RfD) for this material.

Some liver and immune effects have been noted in chronic oral studies in experimental animals exposed to 1,1,2-trichloroethane.

Human

US EPA's IRIS gives 1,1,1-trichloroethane a class D (not classifiable as to human carcinogenicity) carcinogenic potential.

US EPA's IRIS lists 1,1,2-trichloroethane as a class C (possible) human carcinogen. This is based upon hepatocellular carcinomas and pheochromocytomas in one strain of mice. Carcinogenicity was not shown in rats.

Reproduction/Developmental Effects

There are no data in humans or in experimental animals indicating any potential for reproductive or developmental effects from exposure to either 1,1,1- or 1,1,2-trichloroethane.

Carcinogenicity

There are no epidemiology studies on both compounds regarding the potential for carcinogenic effects in humans. The carcinogenicity categories for these materials are presented in the table below:

Agency	*1,1,1-Trichloroethane classification*	*1,1,2-Trichloroethane classification*
EPA	D	C
IARC	3	3
ACGIH	A4	A3
NIOSH	NL	Ca
MAK	NL	3B

US EPA Classification C = Possible human carcinogen: limited evidence of carcinogenicity in animals in the absence of human data; US EPA Classification D = Not classifiable as to human carcinogenicity: inadequate human and animal evidence of carcinogenicity or no data available; IARC (International Agency for Research on Cancer) Classification 3 = Unclassifiable as to carcinogenicity in humans; ACGIH (American Conference of Governmental Industrial Hygienists) Classification A3 = Confirmed animal carcinogen with unknown relevance to humans; ACGIH Classification A4 = Not classifiable as a human carcinogen; NIOSH (US National Institute for Occupational Safety and Health) Classification Ca = Potential occupational carcinogen, with no further categorization; MAK (Federal Republic of Germany Maximum Concentration Values in the Workplace) Classification 3B = Substances which are suspected of being germ cell mutagens because of their genotoxic effects in mammalian somatic cells *in vivo*; in exceptional

cases, substances for which there are no *in vivo* data but which are clearly mutagenic *in vitro* and structurally related to known *in vivo* mutagens.

Clinical Management

Upon ocular exposure, eyes should be generously washed with tap water; medical attention should be sought. In case of dermal exposure, contaminated clothing should be removed and the skin should be rinsed with soap and tap water. Upon ingestion, the mouth should be rinsed and vomiting should be induced in conscious victims only; medical attention should be sought. Upon inhalation, the victim should be removed to fresh air and given artificial respiration if indicated; medical attention should be sought.

Environmental Fate

1,1,1-Trichloroethane tends to be stable in the atmosphere and is transported considerable distances. The rate of degradation is increased by the presence of chlorine radicals and nitrogen oxides. In water, its primary loss is by evaporation into the atmosphere. At a vapor pressure of 23 mmHg at 25°C, 1,1,2-trichloroethane is expected to exist almost entirely in the vapor phase in the ambient atmosphere. It will gradually degrade by reaction with photohemically produced hydroxyl radicals.

Exposure Standards and Guidelines

The ACGIH threshold limit value (TLV), time-weighted average (TWA), for 1,1,1-trichloroethane is reported to be 350 ppm or 1910 mg m^{-3}. The ACGIH TLV short-term exposure limit (STEL)/TLV ceiling (CEIL) is reported to be 450 ppm or 2460 mg m^{-3} on skin.

The ACGIH TLV, TWA, for 1,1,2-trichloroethane is reported to be 10 ppm or 55 mg m^{-3} on skin. The ACGIH TLV, STEL/CEIL, is reported to be 10 ppm or 45 mg m^{-3} on skin. The NIOSH recommended exposure limit, TWA, is also 10 ppm or 45 mg m^{-3} on skin.

See also: Pollution, Water.

Further Reading

Bruckner JV, Kyle GM, Luthra R, *et al.* (2001) Acute, short-term, and subchronic oral toxicity of 1,1,1-trichloroethane in rats. *Toxicological Sciences* 60(2): 363–372.

Wang RY (2004) Hydrocarbons In: Dart RC (ed.) *Medical Toxicology*, 3rd edn., pp. 1329–1340. Philadelphia: Lippincott Williams and Wilkins.

Relevant Website

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profiles for 1,1,1-Trichloroethane and 1,1,2-Trichloroethane.

Trichloroethylene

Richard A Parent

This article is a revision of the previous print edition article by R A Parent, T R Kline, and D E Sharp, volume 3, pp. 372–374, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 79-01-6
- Synonyms: Trichloroethene; 1,1,2-Trichloroethene; TCE; TRI; Trichlor; Acetylene trichloride; Ethylene, trichloro; Ethylene trichloride; Triclene
- Chemical/Pharmaceutical/Other Class: Chlorinated olefinic hydrocarbon
- Chemical Formula: C_2HCl_3

Uses

Manufactured by chlorination of acetylene or other two carbon hydrocarbons or by dehydrohalogenation of tetrachloroethane, trichloroethylene (TCE) may contain one of numerous additives for stabilization. This chlorinated solvent has been used extensively as a degreasing and metal cleansing agent, as a heat exchange liquid, as a diluent in paints and adhesives, in textile processing and in the manufacture of organic chemicals and pharmaceuticals, as a cleaning solvent, for dyeing of polyester textiles, as an extractant for caffeine from coffee, in typewriter correction fluid, and as a solvent for insecticides and other chemicals. It is a volatile liquid whose vapor is heavier than air. The vapor has a chloroform-like odor detectable at ~21 ppm in air.

Exposure Routes and Pathways

The two main sources of human exposure to TCE are the environment and the workplace, although home use of products containing TCE is not uncommon. Background levels of TCE can be found in the

outdoor air we breathe (30–460 ppt) and in many lakes, streams, and underground water used as sources of tap water for homes and businesses. An important source of environmental release of TCE is evaporation into the atmosphere from work performed to remove grease from metal (degreasing).

Toxicokinetics

Absorption of TCE following inhalation exposure in humans is characterized by an initial rate of trichloroethylene uptake that is quite high. Retention of inhaled TCE has been measured at 37% and 75% of the amount inhaled. Absorption of TCE following oral exposure in both humans and animals is rapid and extensive. In animal studies, absorption from the gastrointestinal tract has been measured at 91–98%, and peak TCE blood levels are attained within a matter of hours. Dermal absorption of TCE in both humans and animals is slow, but dermal absorption studies are complicated by the fact that pure liquid TCE can act to defat the skin and thereby enhance its own absorption.

TCE is extensively metabolized (40–75% of the retained dose) in humans to trichloroethanol, glucuronides, and trichloroacetic acid (TCA). Saturation of metabolism has not been demonstrated in humans up to an exposure concentration of 300 ppm. Mathematical models predict, however, that saturation of metabolism is possible at TCE concentrations previously used for anesthesia (i.e., 2000 ppm). Although the liver is the primary site of TCE metabolism in animals, there is evidence for extrahepatic metabolism of trichloroethylene in the kidneys and lungs.

The distribution of TCE in rats following exposure to 200 ppm, 6 h day^{-1} for 5 days was studied. Seventeen hours after exposure on Day 4, there were relatively high levels of TCE in the perirenal fat (0.23 nmol g^{-1}) and in the blood (0.35 nmol g^{-1}) and virtually no TCE in the other tissues. Following exposure on Day 5, tissue levels in brain, lungs, liver, fat, and blood reached a steady state within 2 or 3 h.

In humans, ~11% of TCE is eliminated through the lungs, whereas more than 50% of the absorbed dose is metabolized and excreted in the urine as TCA and other metabolites. Elimination is relatively slow in humans, with TCA being detected in the urine of exposed individuals up to 12 days postexposure suggesting a cumulative process, probably related to storage in fatty tissue. The biological half-life of urinary metabolites of TCE in humans is ~41 h.

Mechanism of Toxicity

Extended exposure (e.g., occupational exposure) to a chlorinated solvent like trichloroethylene typically results in signs of central nervous system (CNS) disturbance and hepatotoxicity. Administration of this chemical to mice induces neoplasms in the liver, as is typical of virtually all the chlorinated hydrocarbons. TCE is readily converted to TCA and other metabolites including the corresponding alcohol. TCA acts as a peroxisome proliferator, and hepatic neoplasms in mice may arise through this mechanism. Glutathione adducts of TCE are thought to be converted to the reactive metabolite in the kidneys through the action of β-lyase. These processes may account for nephrotoxicity exhibited in rats. TCE induces liver tumors in mice and the contributing metabolites are thought to include trichloroacetate, chloral hydrate, and dichloroacetate. Peroxisome proliferation is thought to be involved in the mechanism of formation of mouse liver tumors. TCE is lipophilic and easily crosses the blood–brain barrier resulting in the observed CNS effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute toxicity data indicate that trichloroethylene is relatively nontoxic by the inhalation and oral routes. In mice, LC_{50} values ranged from 7480 to 49 000 ppm, whereas in rats the range was 12 500–26 300 ppm. By the ingestion route, acute LD_{50} values in dogs, cats, rats, mice, and rabbits ranged from ~2000–8000 mg kg^{-1}. Inhalation and oral studies indicate that the bone marrow, CNS, liver, and kidneys are the principal targets of TCE in animals. Effects on the liver and kidney include enlargement with hepatic and biochemical and/or histological alterations. Other reported effects include indication of impaired heme biosynthesis and other hematological alterations in rats exposed by inhalation and immunosuppression in orally exposed mice.

Inhalation studies with mice and rats indicate that TCE is a developmental toxicant. Fetotoxicity is expressed mainly as skeletal ossification anomalies and other effects consistent with delayed maturation. Oral studies with rats and mice showed no trichloroethylene-related effects on fertility or other indicators of reproductive performance. No definitive teratogenic effects have been reported regarding exposure to TCE.

Autoimmune responses to TCE in susceptible mice were demonstrated including significant increases in antinuclear antibodies and total serum globulin among other parameters. Some indications of an autoimmune response have also been reported in Brown Norway rats.

Human

TCE is readily absorbed through ingestion, inhalation, and dermal exposure, the latter producing a defatting effect if contact is prolonged, resulting in erythema and vesiculation followed by exfoliation. The liquid solvent is also an eye irritant producing pain and irritation but apparently no permanent injury. Exposure to high vapor concentrations of TCE has been reported to result in irritation of the mucous membranes of the eyes, nose, and throat, conjunctivitis, rhinitis, and pharyngitis. Exposures exceeding 100 ppm in air are reported to result in restlessness, peripheral neuritis, impaired concentration, irritability, euphoria, lightheadedness, dizziness, depression, reversible trigeminal degeneration, psychic disturbances, cranial nerve deafness, alterations in electrical patterns in the brain, bronchoconstriction, fatal cardiac arrhythmias, and renal and hepatic damage. In combination with alcohol, trichloroethylene exposure produces a vasodilation which has been described as 'degreasers flush'. Optic neuritis, hallucinations, and gastrointestinal changes have been reported after ingestion of trichloroethylene. These symptoms are often accompanied by nausea and vomiting, as well as major cardiovascular effects including hypotension, conduction defects, myocardial injury, and cardiac arrhythmias. The latter has been reported to be the cause of death in some individuals who have been exposed to high levels of TCE, but usually death is preceded by coma and subsequent hepatic or renal failure. Occasional sudden deaths have been attributed to ventricular fibrillation. The estimated oral dose in humans to cause death is reported to be 3–5 ml kg^{-1} while the lowest reported concentration in air to produce unconsciousness in adult humans is 3000 ppm. Reversible trigeminal nerve degeneration and psychic disturbances have also been reported.

Chronic Toxicity (or Exposure)

Animal

Chronic inhalation exposure to TCE produced lung and liver tumors and leukemia in mice and Leydig cell tumors in rats. Chronic oral exposure to TCE produced increased incidences of hepatocellular carcinomas in mice and marginally significant increased incidences of renal adenocarcinomas in rats. TCE was neither embryotoxic nor teratogenic in Sprague–Dawley rats or Swiss Webster mice. Since there was a question about some impurities in these bioassays, the National Toxicology Program treated F344 rats and B6C3F1 mice with epichlorohydrin-free TCE producing renal tubular cell neoplasms in rats and increased incidences of hepatocellular carcinomas in male and female mice and hepatocellular adenomas in female mice.

Human

There is limited evidence in humans for the carcinogenicity of trichloroethylene in humans. There are reports of increased risks of multiple myeloma, non-Hodgkins lymphoma, and cancer of the biliary passages, but the studies are limited. There is sufficient evidence, however, in experimental animals for the carcinogenicity of TCE; therefore, TCE has been classified as being a probable human carcinogen. TCE in drinking water has been associated with leukemia in women and children and, in a study of the Tucson water supply, low birth weight was also reported.

Prolonged TCE exposure has been associated with impairment of peripheral nervous system function, persistent neuritis and temporary loss of tactile sense and paralysis of the fingers after direct solvent contact. Chromosomal effects have been reported in those involved in the use of TCE for degreasing and symptoms of systemic lupus erythematosis have been reported after chronic TCE exposure. In addition, organic dementia has been noted after occupational exposure to TCE and there have been some reports of an association between exposure and scleroderma, an autoimmune disease.

In Vitro Toxicity Data

Mutagenic responses generally occurred with metabolic activation only, suggesting the involvement of metabolites of TCE. The mutagenic potential of pure trichloroethylene is unclear; however, the limited information available suggests that TCE would be a weak mutagen. Both positive and negative findings showing frameshift and base pair mutations in *Saccharomyces cerevisiae* and reverse mutations in *Escherichia coli* K12 have been reported. Fisher rat embryo cells were transformed by TCE producing foci which, when transplanted into host animals, grew as undifferentiated fibrosarcomas at the site of inoculation. However, attempts to induce morphological transformations in the BALB/3T3 mouse cell line were not successful and an attempt to produce increased unscheduled DNA synthesis in a rat hepatocyte primary culture also failed.

Clinical Management

After oral ingestion, emesis is not recommended due to potential for CNS depression and cardiovascular instability. Gastric lavage may be appropriate shortly

after ingestion and activated carbon may also be administered. Cardiovascular monitoring should be instituted and treated as appropriate. For inhalation exposure, the patient should be moved to an uncontaminated environment and monitored for respiratory symptomatology. Bronchospasm if present may be treated with a $\beta 2$ agonist and corticosteroids. Oxygen should be administered and ventilation assist applied when appropriate. Seizures may result and should be addressed using diazepam.

Environmental Fate

In the atmosphere, TCE will be degraded by reaction with photochemically produced hydroxyl radicals. Its half-life in the atmosphere is ~ 7 h. In soils, TCE is expected to have a high mobility since its average K_{oc} is 101 but volatilization is expected to be an important fate process in both wet and dry soils. Under anaerobic conditions, TCE is slowly degraded by reductive dechlorination leading to dichloroethylene and vinyl chloride, among other decomposition products. Studies in aquifers have reported half-lives ranging from 35 days to 6 years. TCE has a bioconcentration factor ranging from 4 to 39 suggesting moderate to low bioconcentration in aquatic organisms. Estimated volatilization half-lives in a model river and lake are 3.5 h and 5 days, respectively.

Other Hazards

TCE decomposes at high temperatures forming toxic gases including hydrogen chloride, chlorine, and phosgene. Welding or smoking in a TCE-contaminated environment may result in inhalation of these toxic gases. TCE pools in low-lying areas when released to the environment. It converts to vinyl chloride monomer under anaerobic conditions in the environment.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value for TCE is 50 ppm (8 h time-weighted average) and the short term exposure limit is 100 ppm for a 15 min period.

The Occupational Safety and Health Administration's permissible exposure limit is 100 ppm with an acceptable ceiling exposure concentration of 200 ppm.

The National Institute for Occupational Safety and Health considers TCE as a potential occupational carcinogen and recommends an exposure limit of 25 ppm for a 10 h day.

The International Agency for Research on Cancer states that there is sufficient evidence for the carcinogenicity of TCE in experimental animals and classifies it as being probably carcinogenic to humans (Category 2B).

ACGIH classifies TCE as not being suspected as a human carcinogen (Category A5) but this classification was made in 1993.

TCE is designated as a hazardous substance under Section 311(b) (2) (A) of the Federal Water Pollution Control Act and is further regulated by the Clean Water Act Amendments of 1977 and 1978.

The US Environmental Protection Agency Federal Drinking Water Standard for TCE is $5\,\mu g\,l^{-1}$.

Miscellaneous

TCE is a volatile chlorinated organic solvent having excellent solvent characteristics. It is a colorless liquid having a boiling point of 87.2°C and an ethereal odor. As a liquid it has a vapor density of ~ 1.5 and is miscible in oils and other lipophilic organic solvents. Its vapor pressure is 69 mmHg at 25°C and a vapor density of 4.53 causing the vapors to 'pool' in lower elevations. TCE has an odor threshold of ~ 21 ppm in air and $\sim 10\,mg\,l^{-1}$ in water.

See also: Pollution, Water.

Further Reading

Bruening T and Bolt HM (2000) Renal toxicity and carcinogenicity of trichloroethylene. *Critical Reviews in Toxicology* 30(3): 253–285.

World Health Organization (WHO) (1985) Environmental Health Criteria 50: Trichloroethylene.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Trichloroethylene.

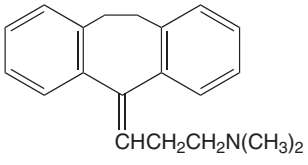
http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Trichloroethylene.

Tricyclic Antidepressants

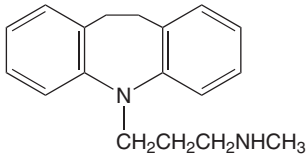
Fermin Barrueto Jr.

This article is a revision of the previous print edition article by Michael J Hodgman, volume 3, pp. 375–376, © 1998, Elsevier Inc.

- Representative Chemicals: Imipramine; Amitriptyline; Doxepin; Desipramine; Nortriptyline
- Chemical/Pharmaceutical/Other Class: The tricyclic antidepressants are a group of drugs that have a three-ring molecular core and share a similar pharmacologic effect, inhibiting the reuptake of biogenic amines at central presynaptic terminals
- Chemical Structures:



Amitriptyline



Desipramine

Uses

Tricyclic antidepressants are used to treat depression. They are also used for treatment of enuresis in children, chronic pain syndromes, neuropathic pain, the fibromyalgia syndrome, and chronic headaches.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Several tricyclic antidepressants are also available in injectable form.

Toxicokinetics

The tricyclic antidepressants are well absorbed following oral ingestion. Large ingestions will be more slowly absorbed because of the anticholinergic effects and resulting decreased gut motility. There is extensive first-pass metabolism that limits oral bioavailability.

Tricyclic antidepressants are highly lipid soluble and bind extensively to tissue and plasma proteins. The volume of distribution ranges from 10 to 50 $l\,Kg^{-1}$.

The half-life of various tricyclic antidepressants ranges from 10 to 50 h. Less than 5% of these drugs appear unchanged in the urine.

The tricyclic antidepressants are extensively metabolized by the liver and partially enterohepatically recirculated. They undergo demethylation, hydroxylation, and glucuronide conjugation. The demethylated metabolites of the tertiary amine tricyclic antidepressants are pharmacologically active. Drugs that induce hepatic microsomal enzymes speed the metabolism of tricyclic antidepressants.

Mechanism of Toxicity

There are six main properties that tricyclic antidepressants have: antihistaminic, GABA antagonism, Na^+ channel blockade, peripheral alpha antagonism, inhibition of the reuptake of biogenic amines, and anticholinergic properties. The cardiac toxicity of tricyclic antidepressants is mostly related to their quinidine-like sodium channel blockade leading to prolongation of the QRS complex. This leads to altered conduction, slowing of both depolarization and repolarization, and decreased inotropy. This can lead to ventricular dysrhythmias, bradydysrhythmias, and asystole. Decreased inotropy, peripheral alpha blockade, and dysrhythmias can all contribute to hypotension and shock. The antihistaminic properties are largely responsible for the sedation seen in overdose. The anticholinergic affects produce tachycardia, which is further exacerbated by the alpha antagonism and, together with the GABA antagonism, cause central nervous system (CNS) excitation and seizures.

Acute and Short-Term Toxicity (or Exposure)

Animal

Toxic effects are similar to those seen in humans.

Human

Early signs of tricyclic antidepressant toxicity are due to anticholinergic effects and include tachycardia, mydriasis, dry mouth, low-grade fever, diminished bowel sounds, CNS excitation, and delirium. More serious toxicity is manifested by coma, respiratory depression, seizures, and cardiovascular toxicity including conduction disturbances, hypotension, ventricular arrhythmias, and asystole. Seizures cause hyperthermia, rhabdomyolysis, and metabolic acidosis. Clinical deterioration can be rapid and catastrophic in patients with tricyclic antidepressant overdose. Death most often occurs due to dysrhythmia and circulatory collapse. The typical therapeutic dose of a tricyclic antidepressant is 2–4 $mg\,kg^{-1}\,day^{-1}$. Doses of 15–20 $mg\,kg^{-1}$ are potentially lethal. Therapeutic drug levels for most tricyclic antidepressants range from 100 to

260 ng ml^{-1}. Toxicity can be seen at levels only modestly elevated, although severe symptomatology is usually associated with levels >1000 ng ml^{-1}. However, drug levels are not useful in predicting toxicity, complications, or patient management. Electrocardiogram changes include sinus tachycardia, a rightward deviation of the terminal vector of the frontal plane QRS complex to >120°, intraventricular conduction disturbances with a prolongation of the QRS duration >100 ms, and T wave changes. A QRS duration >100 ms is associated with a 32% chance of seizure and a QRS >160 ms is associated with a 50% chance of a ventricular dysrhythmia. In distinction to the tricyclic antidepressants, newer cyclic antidepressants such as the bicyclics and dibenzoxazepines are less cardiotoxic but are associated with an increased risk of seizures.

Chronic Toxicity (or Exposure)

Animal

Pregnant rats were given imipramine 5 mg kg^{-1} day^{-1} during pregnancy. Basal body temperature was higher in female offspring compared to male offspring. Puppies given intravenous imipramine showed fewer arrhythmias and caused less of a fall in blood pressure compared to puppies who were administered intravenous amitryptilline or doxepin.

Human

Tricyclic antidepressants have a wide range of uses in humans and seem to be generally well tolerated. Patients taking therapeutic doses chronically have rarely developed blood dyscrasias (e.g., agranulocytosis, thrombocytopenia). Common adverse effects include CNS effects (CNS depression, confusion, aggression, nightmares, tremor, seizures, etc), anticholinergic effects (e.g., dry mouth, urinary retension), and gastrointestinal effects (e.g., nausea, vomiting, weight gain).

In Vitro Toxicity Data

Some studies have implied that several tricyclic antidepressants may be able to stimulate tumor growth in animals or humans with existing tumors. However, recent research using human and murine *in vitro* models have not been able to support these findings.

Clinical Management

If the patient is seen early postingestion (e.g., within 60 min), gastric decontamination by lavage may be considered. Because of the risk of rapid CNS depression, ipecac should be avoided and airway protection by endotracheal intubation should be aggressively considered. Activated charcoal should also be given. Flumazenil and physostigmine should be avoided. Sodium bicarbonate administration is beneficial in treating cardiac toxicity and hypotension though it is not clear if the effects are a consequence of sodium administration or alkalinization of the serum. In case of signs of impaired conduction (QRS >100 ms), ventricular arrhythmias, or hypotension, alkalinize serum to pH 7.45–7.55. Sodium bicarbonate should be used by bolus injection (1–2 mEq kg^{-1} of $NaHCO_3$) followed by continuous infusion to maintain target pH. Ventricular dysrhythmias unresponsive to alkalinization should be treated by standard ACLS methods, avoiding the class Ia antidysrhythmics (quinidine, procainamide, and disopyramide). Hypotension is multifactorial and treatment should include volume resuscitation if not contraindicated, serum alkalinization, and pressor support if needed. Norepinephrine may be the more effective pressor as the hypotension is caused by both negative inotropy and vasodilation. Central hemodynamic monitoring should be useful in this setting. Seizures should be treated using benzodiazepines. For uncontrolled seizures, paralysis is indicated as the associated acidosis and hyperthermia will aggravate cardiac toxicity. Hemodialysis and hemoperfusion are not effective treatment modalities. Only patients free of any signs of toxicity during the first 6 h, with the exception of a resolved tachycardia, can be considered medically clear at that time.

Further Reading

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McCabe JL, Cobaugh DJ, Menegazzi JJ, and Fata J (1998) Experimental tricyclic antidepressant toxicity: A randomized, controlled comparison of hypertonic saline solution, sodium bicarbonate and hyperventilation. *Annals of Emergency Medicine* 32: 329–333.

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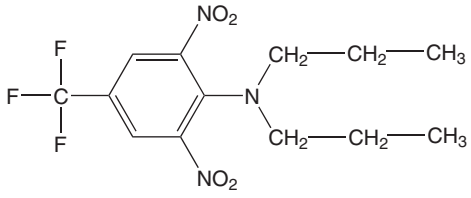
Weld FW and Bigger JT (1980) Electrophysiological effects of imipramine on ovine cardiac purkinje fibers and ventricular muscle fibers. *Circulation Research* 46: 167–175.

Trifluralin

David R Wallace

- Chemical Abstracts Service Registry Number: CAS 1582-09-8
- Synonyms: Agreflan; Elancolan; Nitran; Olitref; Synfloran; Trefancocide; Treflan; Trifloran; Trifluraline; Trikepin; Tristar
- Chemical/Pharmaceutical/Other Class: Herbicide
- Chemical Formula: $C_{13}H_{16}F_3N_3O_4$
- Chemical Structure:



Uses

Trifluralin is a herbicide, first approved in 1963, for control of annual grasses and broadleaf weeds on a variety of crops. Trifluralin is registered for nonfood uses, including residential use. Trifluralin comes in a variety of formulations and is applied as a soil-incorporated treatment.

Exposure Routes and Pathways

Trifluralin can enter the body by inhalation of contaminated air or absorption through the skin or by diet if contaminated plants or animals are consumed.

Toxicokinetics

The predominant metabolic pathway appears to be hydroxylation of alkyl groups or *N*-dealkylation. To a lesser extent, a cyclized compound, benzimidazole, and the reduction products of nitro groups or amines, are also possible metabolic products.

Mechanism of Toxicity

Technical trifluralin has low acute toxicity, whereas solvents often used for the emulsification of trifluralin have been shown to be irritating to the eyes and skin. An active component of trifluralin toxicity is the volatile nitrosamine, *N*-nitroso-di-*n*-propylamine, which may be the active compound in trifluralin toxicity.

Acute and Short-Term Toxicity (or Exposure)

Trifluralin poses the greatest risk following acute exposure. It can be a strong irritant following inhalation, oral, or dermal routes of administration. As noted above, however, this irritation may be primarily due to the solvents used for emulsification. Generally, trifluralin is well tolerated and relatively safe.

Animal

Animal studies have shown that trifluralin is moderately toxic to rats exposed by inhalation, oral ingestion, or dermal contact. Dogs appear to be more sensitive to the actions of trifluralin and exhibited weight loss, hematological changes, and increase liver weights.

Human

Trifluralin is classified as a Toxicity Category IV (practically nontoxic) agent for acute oral toxicity and dermal irritation; and Toxicity Category III (slightly toxic) for acute dermal toxicity, acute inhalation toxicity, and eye irritation. Ocular irritation is characterized by increased lacrimation, photophobia, and redness. Conjunctivitis can continue for 5–7 days.

Chronic Toxicity (or Exposure)

Trifluralin is irritating to the eyes and produces mild skin irritation after prolonged exposure. Central nervous system and respiratory depression are observed following lethal doses of trifluralin.

Animal

There have been reports of increased incidence of urinary tract tumors and thyroid tumors in rats exposed chronically to trifluralin. Trifluralin is structurally similar to ethalfluralin, which is a known carcinogen in rats, and formulations contain *N*-nitroso-di-*n*-propylamine, an omnipresent contaminant, also a known carcinogen. Trifluralin has been classified as a group C (possible carcinogen) due to evidence of increased combined malignant and benign urinary bladder tumors in female rats and renal pelvis carcinomas in male rats. There is also evidence for an increased incidence of thyroid and follicular cell tumors in male rats.

Human

The risk of carcinogenicity to the general population is low. The primary risk group consists of individuals

who will come directly into contact with trifluralin on a long-term, daily basis.

In Vitro Toxicity Data

Trifluralin is strongly mutagenic in plants, producing a 3 to 4 times increase in spontaneous mitosis and chromosomal aberrations. A commercial trifluralin formulation induced chromosomal aberrations in bone marrow, embryonic cells, and male germ cell line in mice. Aneuploidy was induced in several lower eukaryotes.

Clinical Management

Emesis should not be induced due to the potential for central nervous system and respiratory depression. Instead, trifluralin should either be diluted with water or milk, or activated charcoal should be administered. In severe cases gastric lavage should be initiated.

Environmental Fate

Trifluralin is relatively nonmobile and is persistent in soil. Due to the lack of mobility of trifluralin and the fact that annual average surface water concentrations are not likely to exceed the lifetime health advisory level, the threat from drinking water is minimal. There may be some risk of run-off contamination.

Ecotoxicology

Effects of trifluralin on birds and mammals in their natural habitat on an acute basis are very low. Acute risk to endangered species is a potential concern. For aquatic animals, trifluralin is considered moderately to highly toxic and poses acute risk to endangered species.

Exposure Standards and Guidelines

The Environmental Protection Agency (EPA) has estimated that an exposure of $0.0075\,\mathrm{mg\,kg^{-1}\,day^{-1}}$ or less over a lifetime would not result in noncancer endpoints of toxicity. The EPA uses mathematical modeling to estimate the probability of a person developing cancer from drinking water containing specified amounts of trifluralin. Based on these estimates, if a person drank water containing $5\,\mu\mathrm{g\,l^{-1}}$ over their lifetime, that person would have no more than a 1:1 000 000 chance of developing cancer as a result of this exposure.

See also: Nitrosamines.

Further Reading

Environmental Protection Agency (EPA) R.E.D. FACTS: Trifluralin. EPA-738-F-95-035, Washington, DC, April 1996.

Relevant Websites

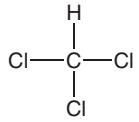
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Trifluralin.
http://www.epa.gov – Environmental Protection Agency, Technology Transfer Network Air Toxics Website: Trifluralin.
http://www.weblakes.com – Lakes Environmental Software. Air Toxic Index: Trifluralin Factsheet.

Trihalomethanes

Shayne C Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 3, pp. 376–377, © 1998, Elsevier Inc.

- Representative Compounds: Bromoform; Dichlorobromomethane; Dibromochloromethane
- Chemical Abstracts Service Registry Number: CAS 67-66-3 (Chloroform; representative compound)
- Synonyms: Carbonyl chloride; Chloroformyl chloride; Trichloromethane; Freon-20 or HCF C-22; THM
- Chemical/Pharmaceutical/Other Class: Halogenated hydrocarbons
- Chemical Formula: $CH(halogen)_3$
- Chemical Structure:



Trichloromethane

Background Information

Trihalomethanes are by-products of the chlorination process. Almost all of the chloroform used in the

United States has been for chlorofluorocarbon refrigerant production, although these uses are being discontinued due to concerns about ozone depletion. Chloroform was once used as an anesthetic.

Exposure Routes and Pathways

Ingestion, inhalation, and dermal contact are possible routes of exposure.

Toxicokinetics

Trihalomethanes are absorbed readily through the skin, breathing, or ingestion, and then distributed primarily to stomach, liver, and kidneys. Elimination of the parent compounds is primarily by the lungs. Chloroform undergoes more conjugation than other trihalomethanes. The metabolism of chloroform is well understood. Approximately 50% of an oral dose of 0.5 g was metabolized to carbon dioxide in humans. Metabolism was dose dependent, decreasing with higher exposure. Approximately 38% of the dose was converted in the liver and $<17\%$ was exhaled unchanged from the lungs before reaching the systemic circulation. Metabolism studies indicated that chloroform was, in part, exhaled from the lungs or was converted by oxidative dehydrochlorination of its carbon–hydrogen bond to form phosgene. This reaction was mediated by cytochrome P450 and was observed in the liver and kidneys. Covalent binding of chloroform to lipids can occur under anaerobic and aerobic conditions; binding to protein occurs only under aerobic conditions. Chloroform can induce lipid peroxidation and inactivation of cytochrome P450 in rat liver microsomes under anaerobic conditions. Evidence that chloroform is metabolized at its carbon–hydrogen bond is provided by experiments using the deuterated derivative.

Mechanism of Toxicity

Chloroform inhibits the function of kidney tubules. It increases nitrogen in blood urea, renal concentrating ability, and glomerular filtration rate. It also increases the metallothionein concentration and reduces the level of cytochrome P450. P450 oxidation also contributes to metabolic release of carbon monoxide. Glutathione is a cofactor of this process. Toxic intermediates such as phosgene, a conjugate by-product of chloroform, may bind covalently with proteins and lipids, contributing to toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Rat oral $LD_{50} = 444–2000\,mg\,kg^{-1}$ (limited research finds similar toxicity levels for all trihalomethanes).

Human

Chloroform is an irritant to skin and mucous membranes. The fatal human dose is estimated to be 10 ml. Central nervous system depression (narcosis) is a prominent response to high levels of chloroform.

Chronic Toxicity (or Exposure)

Animal

Chloroform is a proven carcinogenic in the liver, kidneys, and/or intestines of rodents. It inhibits kidney function and increases chromosomal aberrations. Chloroform is also a developmental toxicant.

Human

Chloroform produces liver and kidney damage and central nervous system depression. Carcinogenic properties are suspected but not confirmed.

Clinical Management

Respiratory therapy should be administered. If ingested, emesis should be induced. Blood pressure and normal urinary output should be maintained. A high carbohydrate diet can assist in restoring normal liver function. Epinephrine should not be used.

Environmental Fate

Chloroform exists almost entirely as a vapor in the atmosphere. Chloroform is effectively eliminated by wet deposition but can reenter the atmosphere following subsequent volatilization. Long-range transport within the atmosphere is possible. Chloroform is eliminated from surface waters primarily by volatilization. Chloroform is not expected to substantially adsorb to organic matter in surface water. Chloroform does not appreciably bioconcentrate in higher aquatic organisms. Chloroform has a moderate potential to concentrate in some aquatic plants.

In surface soil, chloroform is volatilized. Remaining chloroform travels through the soil, as confirmed by detection of chloroform in groundwater. In air, chloroform is degraded through reactions with free radicals. In water and soil, chloroform is degraded under both aerobic and anaerobic conditions. Hydrolysis and direct photolysis are not significant.

Ecotoxicology

The 96 h LC_{50} in channel catfish and rainbow trout is 75 and 44 ppm, respectively. In fathead minnows and bluegills, 96 h (static conditions) LC_{50} values were 129 and 100.0 mg l^{-1}, respectively. A 96 h (static conditions) LC_{50} in *Daphnia* was 29 mg l^{-1}.

Exposure Standards and Guidelines

The threshold limit value – time-weighted average for chloroform is 10 ppm. The permissible exposure limit is 50 ppm. No reference concentration has been set.

See also: Metallothionein; Neurotoxicity; Phosgene; Pollution, Water.

Further Reading

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Komulainen H (2004) Experimental cancer studies of chlorinated by-products. *Toxicology* 198: 239–248.

Relevant Website

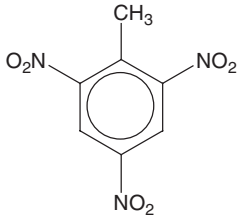
http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Trihalomethanes.

Trinitrotoluene

Shayne C Gad

This article is a revision of the previous print edition article by Shayne C Gad and Jayne E Ash, volume 3, p. 377, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 118-96-7
- Synonyms: TNT; 2,4,6-Trinitrotoluene; Methyltrinitrobenzene
- Chemical/Pharmaceutical/Other Class: Aromatic nitro compound
- Chemical Formula: $C_7H_5N_3O_6$
- Chemical Structure:



Uses

Trinitrotoluene (TNT) is used as a high explosive. It is also an intermediate in the production of dyes and photographic chemicals.

Exposure Routes and Pathways

Ingestion, inhalation, and dermal contact are possible routes of exposure.

Toxicokinetics

TNT is readily absorbed through skin, especially when skin is moist. It is excreted in urine more than in feces; some is found in bile. The major biotransformation reaction is nitroreduction and, to a lesser extent, oxidation. The main metabolite formed by nitroreduction seems to be 4-amino-2,6-dinitrotoluene (4-ADNT). Other metabolites include 2-amino-4,6-dinitrotoluene (2-ADNT), 2,4-diamino-6-nitrotoluene, and 2,6-diamino-4-nitrotoluene. The metabolites are excreted in the urine as glucuronide conjugates and in the free form. Ring oxidation products of TNT such as trinitrobenzylalcohol, trinitrobenzoic acid, and simultaneous oxidation and reduction metabolites such as 2,6-dinitro-4-amino-benzylalcohol and 2,6-dinitro-4-amino-*m*-cresol are of less importance. Untransformed TNT is also excreted in the urine. ADNT and TNT concentrations were found in workers in explosives factories. 4-ADNT excretion was reported to be complete within 3–4 days after exposure. However, another study reported detectable urine concentration of ADNT in explosives workers even after 17 days away from the workplace.

Mechanism of Toxicity

TNT increases UDPglucuronsyltransferase in the liver and kidneys. It increases renal epoxide hydrolase activity. Animal studies have suggested covalent binding between TNT and macromolecular proteins including serum albumin, hemoglobin (Hb), hepatic and renal proteins, and possibly lens protein. The Hb adduct was dose dependent. Macromolecular binding is likely to be correlated with toxic effects;

however, it is unclear if a cause and effect relationship can be established. Formation of organic nitro radicals was also hypothesized based on hemolysis in glucose 6 phosphate dehydrogenase (G6PD)-deficient TNT workers. G6PD is a limiting factor in the maintenance of cellular glutathione, which protects against oxidative damage. TNT was also found to be oxidized oxyhemoglobin, resulting in methemoglobin formation.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} values are $795\,mg\,kg^{-1}$ in rats and $660\,mg\,kg^{-1}$ in mice.

Human

TNT stains the skin orange and yellow. It can cause dermatitis, irritation of eyes, nose, throat, and skin. High exposures may cause weakness, anemia, headaches, liver, or central nervous system damage.

Chronic Toxicity (or Exposure)

Animal

TNT can cause anemia, methemoglobinemia, splenomegaly (dogs, 6 month oral study) in animals. Also, note that TNT is a mutagen in animals.

Human

Chronic exposure may cause cataracts, cyanosis, jaundice, methemoglobin aplastic anemia or hepatitis. TNT is a possible human carcinogen.

Clinical Management

Methylene blue should be administered with oxygen therapy. Contaminated skin or eye should be irrigated with large amounts of water.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value is $0.5\,mg\,m^{-3}$ of air, the Occupational Safety and Health Administration permissible exposure limit is $63\,mg\,m^{-3}$, and the National Institute for Occupational Safety and Health recommended exposure limit is $0.5\,mg\,m^{-3}$.

See also: Pollution, Water.

Further Reading

Bingham E, Cohrssen B, and Powell CH (eds.) (2001) *Patty's Toxicology*, vol. 4. New York: Wiley.
Dart RC (2004) *Medical Toxicology*, 3rd edn. Philadelphia: Lippincott.
IARC (1996) 2,4,6-Trinitrotoluene. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 65: 449–475.

Tungsten

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-33-7
- Synonym: Wolfram
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: W^{6+}

Uses

Tungsten compounds (e.g., tungsten oxides and sulfides) are used as catalysts, and to increase hardness, toughness, elasticity, and tensile strength of steel. Tungsten metal and alloys are also used in filaments, for example, incandescent lamps. Tungsten (e.g., organic tungsten) is used in dyes and pigments. Tungsten carbides are used in cutting and forming tools, for example, in rock drills and machine tools.

Background Information

Tungsten was discovered in 1781. The average tungsten concentration in the earth's crust is $\sim$0.006%. Tungsten occurs naturally as tungstate, mainly in compounds such as wolframaites and scheelites.

Exposure Routes and Pathways

Occupational exposure to tungsten compounds may occur through inhalation of dust and dermal contact. The production and use of tungsten compounds as catalysts, and in cutting and forming tools, filaments, and dyes and pigments may result in the release of tungsten to the environment through various waste

streams; however, only small concentrations of tungsten have been released into the atmosphere, primarily by industrial emissions and nuclear fall-out. If released to air, most tungsten compounds have low vapor pressures and are expected to exist solely in the particulate phase in the ambient atmosphere.

Toxicokinetics

Amount ($\sim$70%) of inhaled tungsten is cleared within 4 h. Absorbed portions are distributed primarily to the liver and kidneys, skeleton and skeletal muscles.

Mechanism of Toxicity

Reported inhalation effects are probably due to cobalt in exposures, a competitive inhibitor of molybdenum utilization.

Acute and Short-Term Toxicity (or Exposure)

Animal

The LD_{50} of tungsten metal powder in rats is $5\,g\,kg^{-1}$ body weight. Fifty milligrams of tungsten dust introduced into the trachea of rats resulted in proliferation of the intraalveolar septa. Chamber exposures of animal to tungsten dust produced only minor changes.

Human

Eye, skin, and respiratory irritations.

Chronic Toxicity (or Exposure)

Animal

Intratrachael instillation or inhalation of dusts (of tungsten or tungsten carbide) into rodents causes only minor lung damage. When tungsten coils were implanted into the subclavian artery of rabbits, the mean tungsten levels rose from $0.48\,\mu g\,l^{-1}$ prior to the implantation to $12.4\,\mu g\,l^{-1}$ 4 months postimplantation. The study concluded that tungsten coils corrode and lead to a steady increase in serum tungsten levels starting as early as 15 min after implantation; however, despite the markedly elevated serum tungsten levels 4 months after implantation, the degradation of tungsten coils was not associated with local or systemic toxicity.

Human

Diffuse pulmonary fibrosis ('hard metal disease'), loss of appetite, nausea, cough, blood changes. No known mutagenicity, carcinogenicity, or developmental/reproductive toxicity.

Clinical Management

Dimercaprol may be useful as a chelating agent.

Ecotoxicology

Tungsten inhibits molybdenum utilization, which is essential for the induction of nitrate reductase.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) has an 8 h time-weighted average (TWA) of $5.0\,mg\,m^{-3}$ and a 15 min short-term exposure limit (STEL) of $10.0\,mg\,m^{-3}$ for tungsten metal and insoluble tungsten compounds. ACGIH has a TWA of $1\,mg\,m^{-3}$ and an STEL of $3\,mg\,m^{-3}$ for soluble tungsten compounds. In addition, the US National Institute for Occupational Safety and Health recommends a 15 min STEL of $3.0\,mg\,m^{-3}$ for tungsten and insoluble compounds, and a TWA of $5\,mg\,m^{-3}$ for tungsten and a TWA of $1\,mg\,m^{-3}$ for soluble tungsten compounds.

See also: Metals; Toxicity Testing, Inhalation.

Further Reading

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Kraus T, Schramel P, Schaller KH, *et al.* (2001) Exposure assessment in the hard metal manufacturing industry with special regard to tungsten and its compounds. *Occupational and Environmental Medicine* 58: 631–634.

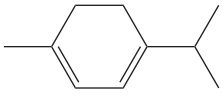
Lagarde F and Leroy M (2002) Metabolism and toxicity of tungsten in humans and animals. *Metal Ions in Biological Systems* 39: 741–759.

Peuster M, Fink C, Wohlsein P, *et al.* (2003) Degradation of tungsten coils implanted into the subclavian artery of New Zealand white rabbits is not associated with local or systemic toxicity. *Biomaterials* 24: 393–399.

Turpentine

Vijay M Vulava

- Representative Chemicals: Turpentine is primarily composed of $C_{10}H_{16}$ terpene hydrocarbons such as α-pinene, β-pinene, limonene, 3-carene, and camphene. It may also contain other acyclic, monocyclic, or bicyclic terpenes, oxygenated terpenes, and anethole
- Chemical Abstracts Service Registry Number: CAS 8006-64-2
- Synonyms: Gum spirits; Turps; Gum thus; D.D. turpentine; Wood turpentine; Oil of turpentine; Rectified turpentine oil; Spirits of turpentine; Sulfate wood turpentine; Sulfate turpentine; Gum turpentine; Steam-distilled turpentine; Turpentine oil; G 4134
- Chemical/Pharmaceutical/Other Class: Terpene
- Chemical Formula: $C_{10}H_{16}$ (approximate formula)
- Chemical Structure (Generalized Structure):



Uses

Turpentine is commonly used in several food and chemical products. Some of the flavoring agents (e.g., menthol) used in candy, baked goods, and chewing gum have the same chemicals that make up turpentine. Turpentine is also used in perfumery, sprays, deodorizers, and stimulating ointments. It is commonly used in the manufacture of synthetic pine oil, insecticides, beta-pinene resins, disinfectants, and human and veterinary medicines. It is also used in the preparation of shoe, stove, furniture, and other polishes; manufacture of synthetic camphor and menthol, cleaning materials, inks, putty, mastics, cutting and grinding fluids, paint thinners, degreasing agents, and paints. Turpentine is often used as a solvent thinner for paint, varnishes, waxes, resins, fats, oils, lacquers, and rubber. It is a starting component in the production of a variety of volatile bases.

Exposure Routes and Pathways

Turpentine can affect humans and animals by multiple routes – inhalation, ingestion, or dermal contact. Exposure to turpentine vapors has been reported to cause irritation of eyes, skin, and upper respiratory tract in addition to headache, dizziness, and nausea. Other effects include depression of central nervous system (CNS), kidney problems, and bladder irritation. Inhalation exposure to turpentine can also result in asthmatic symptoms such as cough, hoarseness, rhinorrhea, wheezing, and conjunctivitis. Exposure to turpentine can be fatal to small children.

All terpenes, a key component of turpentine, are local irritants. Ingestion of terpenes usually produces gastrointestinal signs and symptoms while aspiration causes pulmonary toxicity. Terpene absorption, which may begin in oral cavity, is associated with alteration in mental status, ranging from coma to seizures. Renal toxicity has also been reported. Following ingestion, pine oil (a popular terpene product) may be concentrated in the lungs, resulting in chemical pneumonitis without evidence of aspiration.

Toxicokinetics

Terpenes are oxidized by cytochrome P450, conjugated principally with glucuronic acid in the liver, and are excreted by the kidney. Part of inhaled turpentine is eliminated unchanged in expired air and in urine, but most is metabolized and excreted in urine conjugated with glucuronic acid. The excretory product of turpentine has a characteristic odor of violets.

Mechanism of Toxicity

Turpentine is readily absorbed through the gastrointestinal and respiratory tracts and skin. Turpentine, as a lipophilic substance, accumulates in fatty tissues. In animal studies, the highest concentrations of turpentine following inhalation by rats were found in the spleen, kidneys, brain, and peripheral and perinephric fat. Liver microsomal epoxide hydrase and uridine diphosphoglucuronosyl transferase activities were elevated during chronic turpentine exposures. While some turpentine may be eliminated unchanged through the lungs, most turpentine and its metabolites are eliminated through the urinary tract as glucuronides.

Acute and Short-Term Toxicity (or Exposure)

The signs and symptoms of acute inhalation exposure to turpentine may include irritation of the skin, eyes, mucous membranes, and upper respiratory

tract; salivation, cough, chest pain, and shortness of breath; confusion, headache, dizziness, nausea, anxiety, painful urination, bloody urination, or decreased urine output. The signs and symptoms of turpentine ingestion include a burning sensation in the mouth and throat; nausea, vomiting, diarrhea, and abdominal pain; excitement, confusion, ataxia, stupor and seizures; fever; and increased heart rate.

Animal

Turpentine is an eye, mucous membrane, and skin irritant and a CNS depressant in animals. The oral LD_{50} in rats is 5760 mg kg^{-1} and the LC_{50} in the same species is 12 g m^{-3} for 6 h. Cats exposed to a 540–720 ppm concentration of turpentine exhibited signs of immediate eye and mucous membrane irritation and had mild convulsions; at a concentration of 1440 ppm, they developed paralysis within 150–180 min. No adverse effects were noted in dogs exposed to 180 ppm for 3.5 h day^{-1} for 8 days; however, raising the concentration to 818 ppm and exposing the dogs for 3.5–4.5 h caused nausea, lack of coordination, mild paralysis, and weakness. Exposure of rats to a 12–20 mg l^{-1} (2150–3600 ppm) concentration for 1–6 h and of mice to a 29 mg l^{-1} (5200 ppm) concentration for 2 h produced seizures and apnea; at autopsy, however, no pulmonary lesions were noted in these animals. Injection of turpentine into rabbits' eyes produced shrinkage of the orbit and corneal opacification. In one study, dermal application of turpentine produced skin tumors in rabbits but not in mice; in another experiment, however, painting the skin of mice with 240 g kg^{-1} turpentine did cause tumors.

Human

Turpentine is a skin, eye, mucous membrane, and upper respiratory tract irritant in humans. It may also cause skin sensitization and adverse effects to CNS, gastrointestinal, and urinary tract. The lowest estimated oral dose reported to be lethal in humans is 441 mg kg^{-1}. Exposure to a 75 ppm concentration for 3–5 min irritates the nose and throat, and exposure to a 175 ppm concentration irritates the eyes and may be considered intolerable by human volunteers. Ingestion of turpentine causes a burning pain in the mouth and throat, nausea, vomiting, diarrhea, abdominal pain, excitement, ataxia, confusion, stupor, seizures, fever, and tachycardia and may cause death due to respiratory failure. Toxic glomerulonephritis and bladder irritation, with hematuria, albuminuria, oliguria, and dysuria, have been associated with overexposure to the vapor of turpentine in the past; however, the more purified form of turpentine now in use appears to have decreased the incidence of or eliminated turpentine-induced nephritis. Splashes of the liquid in the eye produce severe pain and blepharospasm; conjunctival redness and temporary corneal erosion may also occur, but these effects are reversible.

Chronic Toxicity (or Exposure)

Chronic effects associated with occupational exposures to turpentine include cerebral atrophy, behavioral changes, anemia, bone marrow damage, glomerulonephritis, and dermatitis. Urinary disturbances, albuminuria, and urinary casts were observed in workers exposed to paints and varnishes. However, renal damage associated with occupational exposures to turpentine was transient and reversible.

A number of epidemiology studies have been completed that were associated with the pulp and paper industry. Cancers considered included lung, lymphoproliferative diseases (Hodgkin's disease, multiple myeloma, leukemia, lymphosarcomas), and cancers of the digestive organs. These studies were confounded by other possible chemical exposures. Without job-exposure matrices, it is difficult to pinpoint exposures to specific chemicals and corresponding risks of developing cancers. Workers exposed to terpenes (a principal component of turpentine) for longer than 5 years may also be at greater risk of developing lung cancer.

Chronic skin exposure to turpentine may produce a hypersensitivity reaction, with bullous dermatitis and/or eczema. A case–control study of workers in particle-board, plywood, sawmills, and formaldehyde glue factories demonstrated a statistically significant association between chronic exposure (longer than 5 years) to terpenes (the principal component of turpentine) and the development of respiratory tract cancers.

Clinical Management

Degree of exposure should be considered when determining initial treatment. If eyes are exposed to turpentine or a solution containing turpentine, the eyes should be flushed with large amounts of water for a minimum of 15 min, lifting the lower and upper lids occasionally. Medical attention will be required as soon as possible. Upon skin exposure, the contaminated skin should be washed with soap and water. If irritation persists or a large skin area was affected, medical attention will be required.

A victim of turpentine vapor inhalation should be moved quickly to fresh air and provided medical

care. If the victim is not breathing, cardiopulmonary resuscitation should be performed; if breathing is difficult, supplemental oxygen should be provided. Careful attention to the airway, including an attempt to prevent emesis, is important. It is best to administer nothing by mouth. All precautions should be taken to minimize the victim's risk of vomiting and further aspiration.

If turpentine or a solution containing turpentine is ingested, the victim should be given copious amounts of water to dilute stomach contents. Emesis induction is contraindicated because of the initial risk of aspiration and the risk of CNS depression or seizure development before ipecac syrup can produce vomiting. If gastric decontamination is considered, the airway must be stabilized to minimize the risk of aspiration secondary to the victim's vomiting. Because a major complication of hydrocarbon ingestion is aspiration, the use of gastric decontamination should be reserved for only cases of large intentional ingestions or those involving an increased risk of systemic toxicity. Absorption of some toxic terpenes, such as camphor, is so rapid as to make any attempt at gastric emptying ineffective. Seizures may be managed with benzodiazepines. In all cases, professional medical help will be required. The victim should be kept warm and quiet until medical help arrives.

Environmental Fate

Turpentine is a natural product and is completely biodegradable. It is immiscible in water and miscible in organic solvents such as alcohol, ether, chloroform, and glacial acetic acid. Below the solubility limits, turpentine does not represent a hazard to biological wastewater-treatment plants. However, the biological and chemical oxygen demand for turpentine is exceptionally high and therefore effluent discharges are regulated. Environmental releases of turpentine may occur at production facilities where faulty equipment or spills occur. Facilities are required to use best management practices to reduce the amount of turpentine released to the air during turpentine production processes. Turpentine released into the environment either evaporates rapidly or is completely degraded by natural processes within a few days. The rate of degradation depends on the concentration of turpentine, temperature, availability of air, and presence of bacteria. Turpentine has been ranked as having zero potential as an ozone depleting substance or for global warming. Turpentine has a specific gravity of $0.86\,g\,cm^{-3}$ and hence floats on water. However, there is strong potential for it to seep deep into soil and subsurface environment when discharged in large quantities at the surface. This leads to eventual contamination of groundwater sources.

Ecotoxicology

Several animal studies involving a variety of exposure pathways to turpentine indicate extreme toxicity to small mammals. Inhalation of turpentine vapors resulted in acute toxicity in rats with symptoms including salivation, weakness, incoordination, bloody nasal discharge, paraplegia, ataxia, tremor, convulsions, tachypnea, decreased tidal volume, coma, and death due to sudden apnea. High-level exposures cause irritation of the skin, nose, and mucosal membranes. CNS depression is accompanied by an increased respiration rate and decreased tidal volume. Systemic effects include damage to the kidney and liver. Hyperplasia was demonstrated within 48 h of a single cheek painting in the hamster cheek pouch model. Thirty percent turpentine in acetone elicits a moderate degree of skin irritation free from ulcer formation.

Turpentine is also toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment. Coating action of resins can destroy water birds, plankton, algae, and fishes. Penaeid shrimp given abdominal injections were highly sensitive to turpentine. Turpentine-induced cellular inflammatory response was fibrous scar tissue in all tissues. Early gill and hepatopancreas tissue destruction, and extensive heart and abdominal tissue destruction was also observed.

Other Hazards

Turpentine should be stored in a cool, dry, well-ventilated area in tightly sealed containers. Turpentine can undergo auto-oxidation in contact with air and can generate heat that may spontaneously ignite in a confined space. Containers of turpentine should be protected from physical damage and should be stored separately from strong oxidizers (especially chlorine), heat, sparks, and open flame. Only nonsparking tools may be used to handle turpentine. To prevent static sparks, containers should be grounded and bonded for transfers. Because containers that formerly contained turpentine may still hold product residues, they should be handled appropriately.

Toxic gases and vapors (such as carbon monoxide and the partial oxidation products of terpenes) may be released in a fire involving turpentine. Turpentine attacks some coatings and some forms of plastic and rubber.

The odor threshold for turpentine is 200 ppm of air. Because this value is above the US Occupational Safety and Health Administration (OSHA) current permissible exposure limit (PEL) of 100 ppm, turpentine is considered to have inadequate warning properties. The eye irritation threshold for turpentine is 175 ppm.

Exposure Standards and Guidelines

- The current OSHA PEL for turpentine is 100 ppm ($560\,mg\,m^{-3}$) as an 8 h time-weighted average (TWA) concentration.
- The US National Institute for Occupational Safety and Health (NIOSH) has not issued a recommended exposure limit (REL) for turpentine; however, NIOSH concurs with the PEL established for this substance by OSHA. NIOSH estimates that turpentine concentration of 800 ppm ($4457\,mg\,m^{-3}$) is immediately dangerous to life or health (IDLH).
- The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned turpentine a threshold limit value (TLV) of 100 ppm ($560\,mg\,m^{-3}$) as a TWA for a normal 8 h workday and a 40 h workweek. The OSHA and ACGIH limits are based on the risk of irritation associated with exposure to turpentine.
- The value of the maximum contact with skin in countries such as Australia, Austria, Belgium, France, Germany, New Zealand, Singapore, The Netherlands, The Philippines, Turkey, and Vietnam is a TWA of 100 ppm ($560\,mg\,m^{-3}$).
- In UK and Finland, the following values are used: long-term exposure limit 8 h TWA of 100 ppm ($560\,mg\,m^{-3}$) and short-term exposure limit of 15 min at 150 ppm ($840\,mg\,m^{-3}$).

See also: Camphor; Food Additives; Fragrances and Perfumes.

Further Reading

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UN *See* Food and Agriculture Organization of the United Nations.

Uncertainty Analysis

Virginia Lau

Uncertainty analysis provides an evaluation of the key parameters that contribute to the uncertainty (e.g., variability and imprecision) involved in performing a risk assessment. Also known as probabilistic risk assessment, it provides information that enables decision makers to better understand the strengths, weaknesses, and assumptions inherent in the assessment and to evaluate the conclusions of the risk assessment accordingly. The result of the uncertainty analysis is a distribution of risks that a population may be potentially exposed to and thus, may be used by the risk manager to better understand the implication of the conclusions derived from the risk assessment and to support scientifically based and economically feasible hazardous waste management decisions.

Uncertainty analysis has become a popular method for assessing the uncertainty associated with risk estimates calculated for specific receptors under designated routes of exposures. Two types of uncertainty analysis are typically performed in risk assessment: qualitative and quantitative. Qualitative uncertainty analysis literally describes and lists the parameters or assumptions likely to produce the highest uncertainties and quantifies whether the risks have been over- or underpredicted. Although the qualitative analysis is simple to perform, it generally addresses the overall uncertainty of the assessment in vague and general terms and yields very imprecise results. Quantitative uncertainty analysis, on the other hand, is the mathematical investigation of the uncertainty in the risk output performed by varying the input parameters such that the relative contribution of each parameter is determined. This method has numerous advantages over the qualitative analysis since (1) it provides precise results that are for the most part reproducible and consistent; (2) with the advent of many commercially available software, the analysis is straightforward and easy to perform; and (3) it does not require mathematics beyond that commonly used in risk assessments.

In most risk assessments, calculations are based on deterministic values (i.e., all of the variables are treated as known constants). Many of these values are actually estimates of either an average, high-end, or conservative worst-case condition used in place of a range of values that would better characterize a population or condition. For example, one value may be used to represent the body weight of all the individuals in a population that is composed of people of different ages, sexes, and sizes. Clearly, the deterministic values commonly used in risk assessments represent only a portion of the overall available data.

The current practice in risk assessment of using multiple point estimates that are either high end or worst case to calculate risk often results in a compounding of conservatism intended to significantly overestimate risk. The uncertainty associated with each parameter commonly used to calculate risk usually has not been fully characterized. Major sources of uncertainty associated with risk assessment include:

- Incomplete information on the potential adverse health effects that can be caused by a chemical in the species, at the dose, and over the anticipated length of time the exposure might occur.
- Natural variability (e.g., uncertainty about the range of sensitivity in the population of interest).
- Measurement and sampling errors (i.e., errors resulting from direct measurement due to instrument and observation variations).
- Bias (i.e., difference between the estimated value and true value).

- Judgmental error (i.e., estimated value based on professional opinion).
- Randomness.
- Unpredictability (i.e., systems that exhibit extreme sensitivity).
- Disagreement (i.e., differences in opinion based on expert opinion).
- Approximations (i.e., simplification of the real world).

The uncertainty associated with certain exposure parameters may decrease once probability distributions are used that describe the parameter of interest (e.g., body weight) in place of point estimates as inputs into the calculations. This methodology is described as stochastic modeling and utilizes the full range of data available by selecting random variables from a defined probability distribution. There are three modeling methods used to propagate uncertainty: analytical methods using mathematical statistics, the delta method, and Monte Carlo analysis. The analytical and delta methods are only used for analysis with limited complexity and thus are not discussed further. It should be noted that although increasing the complexity of the uncertainty model may initially improve its accuracy (i.e., consistent and reproducible results), uncertainty within the analysis increases with complexity as less characterized parameters are included in the model.

The Monte Carlo method is a well-established approach used in characterizing uncertainty that can be used to incorporate ranges of data (distributions) into calculations. The Monte Carlo method involves choosing values from a random selection scheme drawn from probability density functions based on a range of data that characterize the parameters of interest. Monte Carlo analysis can be selectively used to generate input parameters and mixed with point estimates, as appropriate, to calculate risk.

The use of this method has become increasingly popular as the availability of commercial software that allows the probability distribution functions to be input directly into a computer spreadsheet have become available. Once the probability distribution is incorporated into a spreadsheet cell, each time the spreadsheet is recalculated, a new value for the random variable is selected from the distribution and used in the calculations. The key to appropriately using this method is to run the entire simulation (choosing random samples from each distribution) hundreds to thousands of times. After each selection, a new representative parameter is generated and can be used as the basis for an estimate of exposure or risk. When the method is used to calculate risk or exposure, the results of all risk estimates are summarized in a histogram which provides risk assessors a full possible distribution of risk based on probability.

The Monte Carlo analysis is performed using the following steps:

- Standard spreadsheet calculation results are entered for all chemicals and pathways to be modeled following the methods used for deterministic calculations. For each of the random variables, discrete or continuous probability density functions are placed in the appropriate cells.
- Any correlations among the exposure parameters must be identified. For example, body weight and skin surface area would be positively correlated such that when a high body weight is selected a corresponding high skin surface area should likewise be used. It is important to identify these correlations so that individual simulations avoid selecting values of two different random variables that are not representative of an individual (i.e., high body weight and low skin surface area). In addition, variability in some exposure assumptions should likewise be accounted for in the toxicity metric. All other variables are assumed to be independent and are not correlated with any other parameter selected.
- The simulation should be run thousands of times to fully sample from each distribution. The summaries of the risk estimates can include statistical tables and histograms of resulting risks and intermediate calculations.

The most difficult aspect of performing a Monte Carlo analysis is estimating the probability distributions underlying many of the variables used. Because it is not immediately obvious what distributions best characterize the exposure parameters for a particular population, the risk assessor must carefully evaluate the available data and choose the appropriate distributions based on the level of information known. A sensitivity analysis may provide additional insight into any distribution selected by indicating the significance of the parameter in affecting the conclusions. There are several general rules used in uncertainty analysis in determining the most unbiased distribution for a specific parameter. The data should initially be tested using a robust goodness-of-fit test (i.e., chi-square test, Shapiro–Wilk test, or Kolmogorov–Smirnov test) to determine if the data are normally or lognormally distributed. If only a range of values is known for a variable, a uniform distribution is the least biased assumption. If the range and mode of values are known, a triangular distribution could be used although it may result in

values being selected more from the extremes than would be expected. A beta distribution can be selected when estimates of the mean, lower bound, and upper bound are available. If the data cannot be adequately described by a standard distribution, the empirical data may be 'bootstrapped' into the simulation in which the model randomly selects individual data points from the data provided. It should be noted that the use of full distribution functions (e.g., lognormal or normal) is not entirely accurate since it is impossible to have mass values beyond physical plausibility within the extreme tails of these distributions although these values may be selected during the analysis (i.e., body weights that are negative, zero, or infinite). Users of packaged software have the flexibility of describing the exposure distributions in terms of parametric and nonparametric functions including cumulative percentiles, bootstrapped values, and moments to limit the values selected from a distribution to within the physical realm.

Uncertainty analysis should account for and characterize the variability inherent in most data sets. In certain cases (e.g., use of Monte Carlo analysis), it is used to more accurately represent a parameter (e.g., body weight) that influences the calculation of risk. Quantitative analysis provides information that enhances understanding and implications of the risk assessment. In the case of human health risk assessment, the risk assessor attempts to quantify the likelihood an individual in a population will develop cancer or other adverse effect due to contact with a chemical at a particular dose level over a specified exposure period. Uncertainty analysis allows the risk assessor to more accurately account for the differences in the population being evaluated (e.g., body weight, exposure duration, ingestion rates, and other exposure parameters) that potentially impact the overall estimate of risk and the conclusions that can be made based on the assessment. It does not, however, address the uncertainty or validity of the methodologies used to develop the parameter distributions or test the underlying uncertainty model itself.

See also: Hazard Identification; Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization; Risk Communication; Risk Management; Sensitivity Analysis.

Uncertainty Factors

Michael Dourson

Health organizations throughout the world utilize a 'safe' dose concept in the dose–response assessment of noncancer toxicity. This safe dose has often been referred to by different names, such as acceptable daily intake (ADI), tolerable daily intake (TDI) or tolerable concentration (TC), minimal risk level (MRL), reference dose (RfD), and reference concentration (RfC). The approaches used by various health organizations share many of the same underlying assumptions, judgments on critical effect, and choices of uncertainty (or safety) factors.

There is enormous variability in the extent and nature of different databases for risk assessment. For example, in some cases, the evaluation must be based on limited data in experimental animals; in other cases detailed information on the mechanism of toxicity and/or toxicokinetics may be available. In some cases the risk evaluation can be based on effects data in exposed human populations; however, few chemicals have been adequately studied in humans to accurately identify a safe dose directly. Therefore, scientists typically rely on existing human epidemiologic and animal laboratory data to estimate safe doses for humans. In estimating a safe dose for a given chemical, scientists first review all toxicity data, judge what constitutes an adverse effect, and determine the critical effect. The critical effect is the first adverse effect that occurs as dose or concentration increases. Not all effects are adverse effects, and the judgment of what constitutes an adverse effect is sometimes difficult.

Scientists then determine the appropriate uncertainty (or safety) factors to apply to the no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) for the critical effect, based on considerations of the available toxicity, toxicodynamic, and toxicokinetic data. Uncertainty factors (UFs) used in the estimation of safe doses are necessary reductions to account for the lack of data and inherent uncertainty in these extrapolations. Other areas of uncertainty include extrapolations of subchronic-to-chronic exposure, LOAEL to NOAEL, and use of an incomplete database. The major assumptions underlying each of these UFs are described in **Table 1**.

The various major areas of uncertainty are described briefly below. These areas represent most of the considerations of estimating safe doses from differing toxicity databases. However, this list may

Table 1 Major assumptions for individual uncertainty factors[a]

Factor	*Assumption*
Interhuman variability	Assumes that there is variability in response from one human to the next and that this variability may not have been detected in the study, usually due to small sample size; may also assume that subpopulations of humans exist that are more sensitive to the toxicity of the chemical than the average population
Animal to human	Assumes that results seen in experimental animals are relevant to humans and that humans are more sensitive than animals at a given $mg\,kg^{-1}\,day^{-1}$ dose or $mg\,m^{-3}$ concentration; this UF may also account for assumptions about specific toxicokinetic and toxicodynamic properties
Less than chronic to chronic	Assumes that an effect seen at subchronic exposures will be seen at lower doses after chronic exposures; may also assume that effects may only be seen after an experimental group is exposed chronically
LOAEL to NOAEL	Assumes that the chosen LOAEL is reasonably close to the projected NOAEL in an experiment, and that the use of this uncertainty factor will drop the LOAEL into the range of the expected NOAEL
Incomplete data	Assumes that the critical effect can be discovered in a reasonably small selection of toxicity studies

[a] This list of assumptions is not exhaustive.

not be exhaustive. **Table 2** shows these areas of uncertainty and how different expert bodies use them.

Interhuman Variability

Whenever possible, data on humans is used to conduct noncancer risk assessment, thereby avoiding the problems inherent with interspecies extrapolation. If sufficient data on sensitive individuals exist, the safe dose can be estimated directly, that is, without the need of an UF. If adequate data on sensitive humans do not exist, an uncertainty is encountered that must be addressed, most often with a 10-fold factor. This UF assumes that variability in response from one human to the next occurs and that this variability may not have been detected in the study, usually due to small sample size. This factor may also assume that subpopulations of humans exist that are more sensitive to the toxicity of the chemical than the average population.

Some groups use data on differences in dynamics and kinetics among humans for this UF. This concept is based on the work of Renwick and is described more fully below.

Animal to Human

If adequate toxicity data on humans do not exist, then experimental animal data are used as the basis of the assessment, and an UF of 10 is routinely applied to the NOAEL. The basic assumptions for this UF are that the results seen in experimental animals are relevant to humans, that toxicokinetic and toxicodynamic differences exist among species, and that humans are more sensitive than animals at a given milligram per kilogram per day dose or milligram per meter cube concentration. Researchers have tried to quantify this area of uncertainty by investigating the ratios between animals and humans. Also ongoing is physiologically based pharmacokinetic modeling as applied to this area of uncertainty.

Some groups use data on differences in dynamics and kinetics between humans and common laboratory animals, such as rats, mice, and dogs, for this UF. This concept is based on the work of Renwick, and is also described in detail below.

Less-than-Chronic Studies to Chronic

The subchronic-to-chronic UF is based on the assumption that an effect seen at shorter durations will also be seen after a lifetime of exposure, but at lower doses. This factor also assumes that effects may only be seen after an experimental group is exposed chronically. In fact, several investigators have examined subchronic-to-chronic ratios of NOAELs and LOAELs, and the average differences between subchronic and chronic values are only 2–3, while some small percentage of chemicals has ratios that exceed 10-fold. Data suggest that the routine use of a 10-fold default factor for this area of uncertainty should be examined closely. For example, short-term (2 weeks) and subchronic (90 days) NOAELs are often available for comparison, which can give an indication of the possible differences in the subchronic NOAEL and the expected chronic NOAEL. When such data are not available, a 10-fold UF may not be unreasonable, but it should be considered as a loose upper-bound estimate to the overall uncertainty.

LOAEL to NOAEL

If an LOAEL exists on which to base the estimation of a safe dose, the uncertainty in the NOAEL must be addressed. Analysis of several sets of data suggests

Table 2 Description of typical uncertainty and modifying factors in the development of 'safe' doses for several groups[a]

		Agency				
Uncertainty factors (UFs)[b]	Guidelines[c] from Health Canada, the International Programme on Chemical Safety (IPCS), the Netherlands National Institute for Public Health and the Environment (RIVM), the US Agency for Toxic Substances and Disease Registry (ATSDR), and the US Environmental Protection Agency (EPA)	Health Canada	IPCS	RIVM	ATSDR	EPA
Interhuman variability	Generally use when extrapolating from valid results from studies of prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among humans and is thought to be composed of toxicokinetic and toxicodynamic uncertainties	1–10	10 (3.16×3.16)	10	10	10
Animal to human	Generally use when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to humans, and is also thought to be composed of toxicokinetic and toxicodynamic uncertainties	1–10	10 (2.5×4)	10	10	10
Less than chronic to chronic	Generally use when extrapolating from less than chronic results on experimental animals or humans. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs or LOAELs to chronic NOAELs or LOAELs			10	Not available/ not used (NA)[d]	$\leqslant 10$
LOAEL to NOAEL	Generally use when extrapolating an LOAEL to an NOAEL. This factor is intended to account for the experimental uncertainty in developing a safe dose from an LOAEL, rather than an NOAEL			10	10	$\leqslant 10$
Incomplete data	Generally use when extrapolating from valid results in experimental animals when the data are 'incomplete'. This factor is intended to account for the inability of any single study to adequately address all possible adverse outcomes	1–100	1–100	NA	NA	$\leqslant 10$
Modifying factor	Generally use upon a professional assessment of scientific uncertainties of the study and database not explicitly treated above (e.g., the number of animals tested)	1–10	1–10	NA	NA	0 to $\leqslant 10$

[a] *Sources*: Dourson (1994), Jarabek (1995), IPCS (1994), Meek *et al.* (1994), Pohl and Abdin (1995), and Rademaker and Linders (1994).

[b] The maximum uncertainty factor used with the minimum confidence database is generally 10 000.

[c] Professional judgment is required to determine the appropriate value to use for any given UF. The values listed in this table are nominal values that are frequently used by these agencies.

[d] ATSDR develops MRLS for specified durations of exposure, and generally does not extrapolate among durations. Therefore, an uncertainty factor for extrapolation between subchronic and chronic exposures is not used.

that a factor of 10 or lower is adequate and that use of data does support a lower factor with certain chemicals. Such a result is not surprising, since experiments are seldom designed with doses in excess of 10-fold apart, leading to the common belief that these ratios depend more on dose spacing than inherent toxicity. The choice of dose spacing, however, often reflects the judgment on the likely steepness of the dose–response slope, with steeper slopes resulting in tighter dose spacing.

Incomplete Data

If data are only available from one chronic study on which to base the estimation of a safe dose, the question may be asked whether data from chronic studies in other species or data from different types of bioassays (such as reproductive or developmental toxicity) would yield lower NOAELs. If so, an uncertainty exists that must be addressed. The default approach to address this uncertainty is by dividing by a 3- or 10-fold UF, based on the assumption that the critical effect can be discovered in a reasonably small selection of toxicity studies. This area of uncertainty has been investigated through the comparisons of NOAELs of different types of studies.

Data-Derived or Compound Specific Adjustment Factors (CSAF)

The science supporting the use of UFs has evolved considerably over the past years. Increased knowledge of inter- and intraspecies sensitivity, mechanism of action, and detailed evaluation of databases has led to improvements that allow for the incorporation of more scientific data into the dose–response assessment of noncancer toxicity, and permit the use of factors other than the standard default values.

Renwick examined the nature of the UFs generally applied for intraspecies and interspecies extrapolations. He proposed the division of each of these UFs into subfactors to allow for separate evaluations of differences in toxicokinetics and toxicodynamics. The toxicokinetic considerations include absorption, distribution, metabolism, and excretion of a toxic compound, and therefore address differences in the amount of the parent compound or active metabolite available to the target organ(s). The toxicodynamic considerations are based on variations in the inherent sensitivity of a species or individual to chemical-induced toxicity, and may result from differences in host factors that influence the toxic response of a target organ to a specified dose. The advantage to such a subdivision is that components of these UFs can be addressed where data are available (e.g., if data exist to show similar toxicokinetic handling of a given chemical between laboratory animals and humans, then the interspecies extrapolation factor would need to account only for differences in toxicodynamics).

Renwick examined in detail the relative magnitude of toxicokinetic and toxicodynamic variations between and within species. Results suggested that toxicokinetic differences were generally greater than toxicodynamic differences. Thus, he proposed that the 10-fold overall UF be subdivided into factors of 4 for kinetics and 2.5 for dynamics. The International Programme on Chemical Safety (IPCS) has adopted the principles set forth by Renwick, but has suggested that while the UF for interspecies extrapolation be subdivided unequally into four-fold (toxicokinetics) and 2.5-fold (toxicodynamics), the UF for intraspecies extrapolation should be split evenly (3.16-fold for both kinetics and dynamics) (see **Figure 1**).

This equal subdivision of the human variability factor was supported by a subsequent, more extensive analysis of appropriate kinetic parameters for 60 compounds in humans and concentration–effect data for 49 compound-related effects.

The quantitative toxicokinetic and toxicodynamic data used to inform interspecies and interindividual extrapolations in dose/concentration–response assessment was initially referred to as 'data-derived UFs'. But the new nomenclature of 'chemical-specific

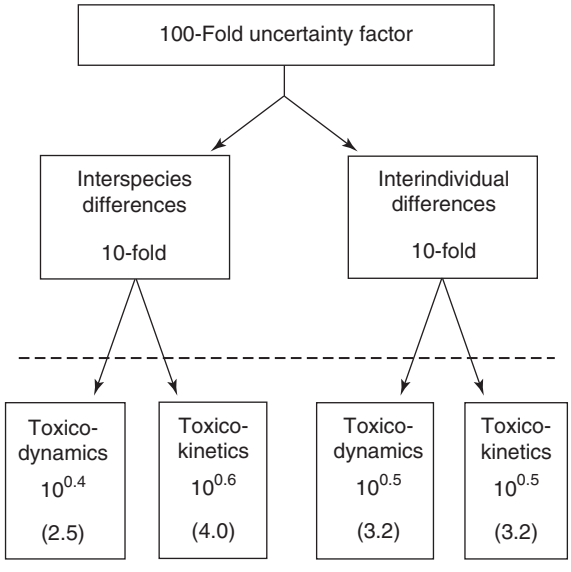

Figure 1 Subdivision of the 100-fold uncertainty factor showing the relationship between the use of uncertainty factors (above the dashed line) and proposed subdivisions based on toxicokinetics and toxicodynamics (IPCS, 1994, based on Renwick, 1993). Actual data should be used to replace the default values if available. (Reproduced with permission from IPCS.)

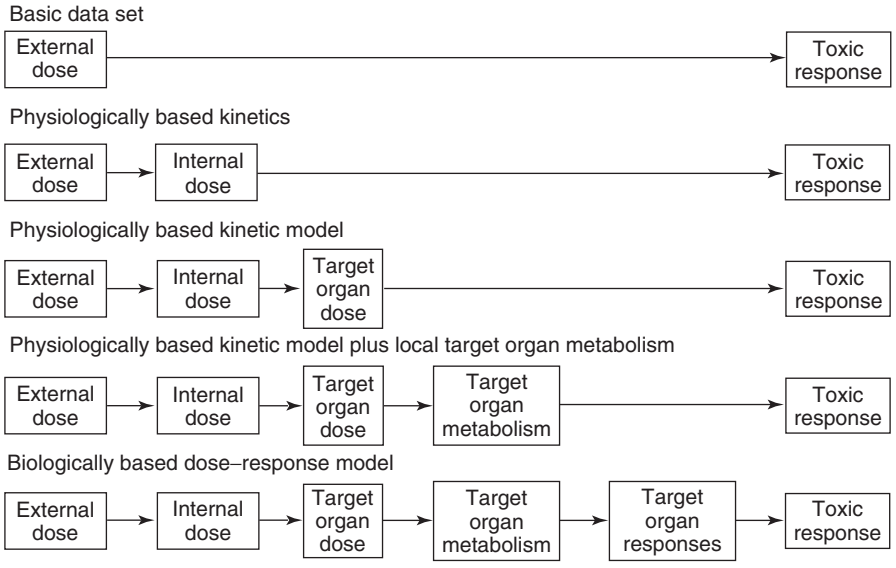

Figure 2 The relationship between external dose and toxic response for specific compounds. (Reproduced from Renwick AG, Dorne JLCM, and Walton K (2001) Pathway-related factors: The potential for human data to improve the scientific basis of risk assessment. *Human and Ecological Risk Assessment* 7: 165–180, with permission.)

adjustment factors' (CSAFs) has been adopted because it more accurately describes the nature of the refinement to the usual default approach. Also, it avoids confusion with factors that are based on an analysis of data for a group of chemicals sharing a common characteristic, that is, 'categorical' default factors such as those based on common physical/chemical characteristics or pathways of metabolism, which are sometimes referred to as data-derived factors, and which are not chemical-specific.

It is acknowledged that for many substances there are few data to serve as a basis for development of CSAFs. Indeed, currently, relevant data for consideration are often restricted to the component of uncertainty related to interspecies differences in toxicokinetics. While there are commonly fewer appropriate, relevant data at the present time to address the three other components considered here, namely interspecies (animal to human) differences in toxicodynamics, interindividual (human) variability in toxicokinetics, and interindividual (human) variability in toxicodynamics, it is anticipated that the availability of such information will increase with a better common understanding of its appropriate nature. Application of the approach even in the absence of data is considered to be informative, therefore, since it focuses attention on gaps in the available information that, if filled, would permit development of more appropriate measures of dose/concentration–response.

It should be recognized that CSAFs represent part of a broader continuum of increasingly data-informed approaches to account for interspecies differences and human variability, which range from default ('presumed protective') to more 'biologically based predictive' (**Figure 2**). The approach along this continuum adopted for any single substance is necessarily determined principally by the availability of relevant data. The extent of data available is, in turn, often a function of the economic importance of the substance.

The development of CSAFs may not always be possible or even necessary. For example, if the margin between the no- or lowest-effect level or bench mark concentration/bench mark dose (BMC/BMD) and anticipated human exposure is very wide, the generation of the more sophisticated data necessary to replace part of a default UF would not warrant the necessary experimentation in animals and humans and the associated resource expenditure. However, where this margin is small, development of additional chemical-specific quantitative data may be justified to refine the dose–response analyses and scientific credibility of the outputs, such as ADIs, TDIs, margins of exposure, or margins of safety.

Summary

As risk assessment scientists continue to accumulate and develop knowledge of toxicokinetics, toxicodynamics, mechanisms of toxicity, and temporal effects of critical effects for various chemicals, evaluations become increasingly more accurate and detailed. Moreover, the science behind the use of UFs has progressed considerably. Increased understanding of

inter- and intraspecies sensitivity, mechanisms of action, and detailed evaluation of databases can support the use of data-derived or CSAFs, which ultimately results in a risk assessment with greater confidence.

See also: Benchmark Dose; Chemical-Specific Adjustment Factor (CSAF); Environmental Protection Agency, US; International Programme on Chemical Safety; Risk Assessment, Human Health; Uncertainty Analysis.

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http://www.who.int – International Programme on Chemical Safety, World Health Organization.

Uranium

Fletcher F Hahn and Raymond A Guilmette

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 7440-61-1
- Selected Compounds: Ammonium diuranate, $(NH_4)_2U_2O_7$ (CAS 7783-22-4); Uranium dioxide, UO_2 (CAS 1344-57-6); Uranium octaoxide, U_3O_8 (CAS 1344-59-8); Uranium tetrafluoride, UF_4 (CAS 10049-14-6); Depleted uranium, U (CAS 7440-61-1)
- Chemical/Pharmaceutical/Other Class: Metals

Uses

The primary use for uranium is in nuclear power reactors and in weapons. Low-enriched metal or ceramic UO_2 fuel pellets (enriched in fissile U-235) are produced for commercial power reactors. Smaller quantities of high-enriched fuel are produced for shipboard power reactors and weapons manufacture. Depleted uranium, a by-product of the enrichment process, is used for armor-piercing ammunition for the military, for counter balances and weights, and for radiation shielding. A small amount of uranium is used in specialty chemicals and catalysts.

Background Information

The chemical toxicity of uranium compounds is well known when compared to the toxicity of most other compounds. In 1824, a treatise described uranium salts as 'feeble poisons' when given by mouth to animals. In the late 1800s, uranium salts were used as homeopathic therapeutic agents in humans, primarily for treatment of diabetes. In the early 1900s, the renal toxicity of uranium became apparent in humans, and its use as a therapeutic agent ceased.

Toxicity studies of uranium compounds were initiated during World War II as nuclear weapons were developed. Initially the occupational exposure limits for lead were used for uranium. From studies in animals, however, it became clear that the amount of soluble uranium salts depositing and remaining in the lung or bone would never constitute a sufficient radiation hazard to override the chemical toxicity to the kidney. Based on these studies in animals a threshold concentration of $3\,\mu g\,U\,g^{-1}$ kidney and a limiting air concentration of $50\,\mu g\,m^{-3}$ were recommended for soluble uranium. Subsequently, all exposure standards for uranium have been based on the renal concentration of $3\,\mu g\,U\,g^{-1}$ kidney. Numerous calls for a reduction in this standard have been made based on more recent studies in animals and epidemiologic studies of individuals living in areas with high concentrations of uranium in the drinking water.

Exposure Routes and Pathways

Inhalation and ingestion are the primary ways for uranium to enter the body. Uranium is ubiquitous in the environment and is present in all soils and rocks in the form of a variety of minerals. Trace amounts of uranium are found in all foods. The intake of uranium in food in the United States ranges from 1 to $4\,\mu g\,day^{-1}$. Drinking water is also a source of uranium and may contribute more than food to the human intake. The quantity of uranium in drinking water varies widely. People in the United States ingest between 0.2 and $2\,\mu g\,U\,day^{-1}$ from water. Air concentrations of uranium also vary widely, but typically are low, ranging from 0.01 to $0.2\,ng\,m^{-3}$, resulting in estimated intakes of $0.002–0.020\,\mu g\,U\,day^{-1}$. Industrial processes, such as mining or milling of uranium ore and nuclear manufacturing facilities, can increase the uranium concentrations present in the air, resulting in potential occupational exposures to uranium.

Toxicokinetics

Three isotopes of uranium, all radioactive, occur together in natural uranium: U-238, U-234, and U-235. The chemistry of these isotopes, which is identical, determines the reactions of the isotopes within the environment as well as their transport and reactions within the body. All the isotopes of uranium emit primarily α particles. The U-238 isotope is the longest lived with a half-life of 4.5 billon years and constitutes more than 99% of the mass of natural uranium and half of its radioactivity. Uranium-234, a decay product of U-238, is responsible for nearly all the remainder of the radioactivity of natural uranium. A small amount (0.7% by weight) of fissionable U-235 is present in natural uranium.

Depleted uranium (DU) is a by-product of the enrichment process in which about 70% of the U-235 in natural uranium is separated from U-238. The remaining uranium, DU, contains about 0.2% U-235 by weight and emits about 60% of the radioactivity of natural uranium. US military specifications designate that DU contain less than 0.3% U-235.

Mixtures of uranium oxides ('yellow cake') are produced in the processing of uranium ores and can result in occupational exposures in uranium mill workers. Exposure to uranium tetrafluoride and hexafluoride is a potential for workers in the uranium enrichment industry.

After deposition in the lung or in wound sites, the movement or translocation of uranium depends primarily on the solubility of the uranium compound. Relatively insoluble compounds may be retained in the lung or a wound with a half-life of years. After absorption to blood from the lungs, a wound site or intestines, uranium is deposited systemically or is excreted by the kidneys. A substantial fraction of the metal ions filtered by the kidneys is retained in the renal tubules before it is passed into the urinary bladder. Over 90% of the uranium remaining in systemic tissues at one day is excreted with half-life ranging from 2 to 6 days and the remainder with half-lives ranging from 30 to 340 days. After a few days, most of the remaining uranium in the body is found in the kidneys, skeleton and, in the case of insoluble compounds, the site of entry (lung or wound).

Mechanism of Toxicity

The kidneys are considered to be the target organ for uranium chemical toxicity. Upon entering the bloodstream, $\sim$40% of the uranium in plasma is complexed with transferrin. The remaining 60% is in stable low molecular weight complexes with carbonate or bicarbonate, that are filtered though the renal glomeruli. As the glomerular filtrate passes through the proximal tubules, the complexed

uranium dissociates with decreasing pH. This dissociation liberates the reactive uranyl ion, which can interact with other complexing species in the filtrate or with components of the proximal tubular membrane. Some of the uranium that remains complexed in the lumen, and a portion of the freed uranyl ions, may traverse the length of the tubules and enter the bladder, thus resulting in a high rate of urinary excretion of uranium soon after exposure. Heavy metals, including uranium, have a great affinity for ionic sites of the brush border membrane of the proximal tubule. The suggested mechanism for renal damage is that the binding of uranium to these cells may alter cellular permeability to sodium, which, in turn, interferes with the transport of glucose, amino acids, and phosphates, resulting in the increased release of these compounds into the urine.

Acute and Short-Term Toxicity (or Exposure)

Renal Effects in Humans

Health effects related to immediate kidney injury are of primary concern following inhalation or ingestion of large quantities of soluble uranium compounds. Acute nephrotoxicity, manifest by acute renal failure with severe oliguria, proteinuria, and increased nonprotein nitrogen has been described in occupationally exposed workers. Transient biochemical effects from lower-level exposures include proteinuria, albuminuria, enzymuria, and the appearance of casts in the urine. Because these biochemical changes typically resolve within a few days, it is possible that individuals exposed to uranium resulting in low kidney burdens will not have detectable renal effects if they are not assessed within a few days of the exposure. Protracted biochemical changes noted are similar to the transient changes, the principal difference being the duration of the change. These changes were not necessarily irreversible. Following recovery from acute effects, individuals typically have no persistent effects.

Pulmonary Effects in Humans

The acute pulmonary toxicity of inhaled uranium is dependent on the chemical form of the uranium. Uranium hexafluoride is the only uranium compound that has been associated with acute effects after inhalation. Two accidents involving uranium hexafluoride have resulted in the deaths of three workers in the US uranium processing industry. However, the lethal effects were due to liberated hydrogen fluoride rather than the uranium.

Chronic Toxicity (or Exposure)

Renal Effects in Humans

Two situations are of primary concern for chronic toxicity of uranium, inhalation of yellowcake or ore concentrate by uranium process workers, and ingestion of drinking water by the general population. These exposures are chronic, lasting for years. Uranium millers, exposed over a period of years to aerosols of yellowcake, had calculated kidney concentrations up to $\sim 1\,\mu g\,U\,g^{-1}$. Biochemical indicators of renal effects, but not clinical symptoms, were noted in some of these workers. The lowest estimated kidney concentrations of uranium reported to result in renal effects were related to high concentrations ($\sim 80\,\mu g\,l^{-1}$) of uranium in the drinking water seen in two studies. In both of these studies, biochemical indicators of subtle renal effects of undetermined significance were seen. The length of time of the exposure, however, may be an important factor. The groups with the longer exposure times have the greatest effects.

Pulmonary Effects in Humans

Based on epidemiologic studies of workers in the uranium processing industry, the chronic exposure to aerosols of uranium compounds has not been related to chronic pulmonary health effects, such as chronic obstructive pulmonary disorder or lung cancer.

In Vitro Toxicity Data

Soluble compounds of uranium are genotoxic in cultured cells. They have caused micronuclei, chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells and chromosomal aberrations in human peripheral lymphocytes.

Clinical Management

For treatment when acute nephrotoxicity is possible, alkalinization of the urine is important to increase urinary excretion of uranium. Systemic chelating agents, such as calcium or zinc salts of diethylenetriaminepentaacetic acid (Ca-DTPA or Zn-DTPA), although recommended in some publications, have not been shown to be useful in increasing the excretion of uranium.

Environmental Fate

Uranium is naturally occurring in the environment with an average abundance in the earth's crust of $\sim 2\,mg\,kg^{-1}$ (range $0.1–20\,mg\,kg^{-1}$). It is more

abundant than silver or gold. Concentrations of uranium in water, food, and soil are variable (typically 0.1–5 $\mu g\,l^{-1}$ in water; 0.1–2 $mg\,kg^{-1}$ in soil and 0.01–2 μg in food) and depend largely on the presence of uranium in soil or rocks or proximity of industries that may introduce uranium into the environment. Extreme concentrations (up to a factor of 100 times the typical ranges noted) may be found in certain geologic environments or where uranium has been concentrated, such as mine tailings. Uranium concentrations in surface and ground waters, as well as many bottled mineral waters, commonly exceed current drinking water standards.

Exposure Standards and Guidelines

- Water: Environmental Protection Agency National Primary Drinking Water Standard – 30 $pCi\,l^{-1}$ (20 $\mu g\,l^{-1}$) proposed.
- Air: Occupational Safety and Health Administration permissible exposure level time-weighted average (corrected rule) – soluble 0.05 $mg\,m^{-3}$; insoluble 0.25 $mg\,m^{-3}$.

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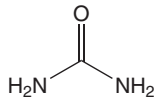
Urea

Midhun C Korrapati and Harihara M Mehendale

- Chemical Abstracts Service Registry Number: CAS 57-13-6
- Synonyms: Aqua Care; Carbamide; Keratinamin
- Chemical/Pharmaceutical/Other Class: Diuretic, osmotic agent, and dermatological agent
- Chemical Formula: N_2H_4CO
- Chemical Structure:



Uses

Urea is used as an osmotic to treat problems like high pressure in the eye ball (glaucoma). It is also used as a diuretic and as a topical dermatological agent in treating psoriasis, and other dry, scaly conditions.

Exposure Routes and Pathways

Exposure to urea may occur through inhalation and dermal contact with this compound at workplaces where urea is produced or used, especially to workers applying urea fertilizers. General population may be exposed to urea via ingestion of food, drinking water, and dermal contact with products containing urea.

Toxicokinetics

Urea is a nonionizable compound that readily traverses mammalian membranes, probably along with water, through the pores.

Mechanism of Toxicity

The primary mechanism of toxicity appears to be inhibition of the citric acid cycle. It leads to blockade of electron transport and a decrease in energy

production and cellular respiration, which leads to convulsions.

Acute and Short-Term Toxicity (or Exposure)

Animal

Intravitreal injection of 0.2 ml of 10 M solution into vitreous humor of rabbits has caused inflammation, chorioretinitis, and degeneration of retina. Urea mixed with soy meal is particularly dangerous, as urease in the latter leads to formation of ammonia. Poisoning of cattle may also be caused by urea as fertilizer and spread unevenly on pasture lambs given $2\,g\,kg^{-1}$ of urea died in 90–200 min. Adult sheep given same dose exhibited almost continuous convulsions after 165 min.

Human

Adverse reactions include headache, nausea, vomiting, disorientation, and transient confusion. Urea causes redness and irritation of skin and eyes.

Chronic Toxicity (or Exposure)

Animal

Urea was tested for mutagenicity in the Salmonella/microsome preincubation assay using the standard protocol approved by the National Toxicology Program. Urea was tested at doses of 0.10, 0.33, 1.0, 3.3, and 10 mg per plate in as many as five *Salmonella typhimurium* strains (TA1535, TA1537, TA97, TA98, and TA100) in the presence and absence of rat or hamster liver S-9. Urea was negative in these tests and the highest ineffective dose tested in any *Salmonella typhimurium* strain was 10 mg per plate.

Human

Urea is found to be mutagenic in humans.

Clinical Management

Urea is effectively eliminated by the kidney. If normal renal function exists, diuresis will ensue. Patients should have adequate hydration. When diuresis is extensive electrolytes should be monitored. Patients should be moved to fresh air when exposed through inhalation. Respiratory distress should also be monitored. If cough or difficulty in breathing develops, patient should be evaluated for respiratory tract irritation, bronchitis, or pneumonitis. Administer oxygen and assist ventilation as required. Bronchospasm should be treated with inhaled $\beta 2$ agonist and oral or parenteral corticosteroids. When dermally exposed, contaminated clothing should be removed and exposed area should be washed thoroughly with soap and water. A physician should examine the area if irritation or pain persists.

Environmental Fate

Terrestrial Fate

Urea is expected to have very high mobility in soil. Urea is not expected to volatilize from dry soil surfaces based upon its vapor pressure. Various field and laboratory studies have demonstrated that urea degrades rapidly in most soils. Urea is rapidly hydrolyzed to ammonium ions through soil urease activity, which produces volatile gases, that is, ammonia and carbon dioxide. However, the rate of hydrolysis can be much slower depending upon the soil type, moisture content, and urea formulation.

Aquatic Fate

Urea is not expected to adsorb to suspended solids and sediment. Volatilization from water surfaces is not expected. Urea is rapidly hydrolyzed to ammonia and carbon dioxide in environmental systems by the extracellular enzyme, urease, which originates from microorganisms and plant roots.

Atmospheric Fate

According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere, urea, which has a vapor pressure of 1.2×10^{-5} mmHg at 25°C, will exist in both the vapor and particulate phases in the ambient atmosphere. Vapor-phase urea is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 9.6 days.

Ecotoxicology

The toxicity threshold for *Scenedesmus quadricauda* (green algae) is $>10\,000\,mg\,l^{-1}$. Toxic effect is multiplication inhibition of cell division. Toxicity threshold for *Entosiphon sulcatum* (protozoa) is $>29\,mg\,l^{-1}$ and the toxic effect is inhibition of cell multiplication.

Exposure Standards and Guidelines

Residues of urea are exempted from the requirement of a tolerance when used as a stabilizer, inhibitor in accordance with good agricultural practices as inert

(or occasionally active) ingredient in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest. This action promulgates standards of performance for equipment leaks of volatile organic compounds (VOCs) in the Synthetic Organic Chemical Manufacturing Industry (SOCMI). The intended effect of these standards is to require all newly constructed, modified, and reconstructed SOCMI process units to use the best demonstrated system of continuous emission reduction for equipment leaks of VOC, considering costs, nonair quality health and environmental impact and energy requirements. Food and Drug Administration requirements: substance added directly to human food affirmed as generally recognized as safe.

Workplace environmental exposure level: 8 h time-weighted average 10 mg m^{-3}.

See also: Volatile Organic Compounds (VOC).

Further Reading

Environment Canada (1985) Technological Information for Problem Spills: Urea.

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WHO/IPCS (1993) Toxicological Evaluation of Certain Food Additives and Contaminants. WHO Food Additives Series 32.

Urethane

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 51-79-6
- Synonyms: Ethyl carbamate; Ethylurethane; Urethan
- Chemical/Pharmaceutical/Other Class: Carbamates
- Chemical Formula: $C_3H_7NO_2$

Uses

Urethane is used as a solvent for various organic materials, pesticides, and fumigants. It is also used as a chemical intermediate in the production of cross-linking agents in textiles and pharmaceutical industry. Urethane was once used as an anesthetic in veterinary medicine. Its veterinary use was discontinued in 1948 when its carcinogenic properties were revealed.

Exposure Routes and Pathways

Occupational exposure to urethane may occur through inhalation of dust particles and dermal contact with this compound at workplaces where urethane is produced or used. In addition to industrial worker exposure, urethane is unintentionally formed during the manufacture of certain consumer beverages. Urethane has been found predominantly in bourbons, sherries, fruit brandies, whiskeys, and wines. The general population may thus be exposed to urethane via ingestion of fermented foods and alcoholic beverages.

Toxicokinetics

Urethane can be excreted in the urine unchanged (0.5–1.7% of dose). Urethane can also be metabolized by the liver cytochrome P450 system. The urinary metabolites of urethane include *N*-hydroxy urethane, acetyl-*N*-hydroxyurethane, ethyl mercapturic acid, and *N*-acetyl-*S*-ethoxycarbonyl cysteine.

Mechanism of Toxicity

Urethane is activated in the liver into a carcinogenic metabolite. The activation of urethane by cytochrome P450 involves two sequential reactions. First, urethane is dehydrogenated to vinyl carbamate followed by epoxidation to form vinyl carbamate epoxide. The former is believed to be the ultimate carcinogenic metabolite of urethane.

Acute and Short-Term Toxicity (or Exposure)

Animal

Developmental defects have been produced in offspring of rats and hamsters treated *in utero* with urethane. Some of the malformations noted included eye, skeletal, neuronal tube defects, and cardiac malformations.

Human

Signs and symptoms of overexposure in humans include vomiting, anorexia, drowsiness, nausea,

vomiting, and dizziness. Exposure to high concentrations has been reported to produce hemorrhages, kidney and liver injury and, in severe cases, coma.

Chronic Toxicity (or Exposure)

Animal

Chronic administration of urethane through the oral, inhalation, subcutaneous, and intraperitoneal routes has produced cancer in mice, rats, and hamsters. Urethane exposure in laboratory animals has produced an increased incidence of spontaneous lung adenomas in susceptible mice strains.

Human

Chronic overexposure in humans has been reported to produce damage to the blood and bone marrow as well as to the liver. No data exist that link human exposure to urethane and cancer. Nonetheless, given its carcinogenic effects in animals, the International Agency for Research on Cancer has labeled urethane as a possible human carcinogen.

Clinical Management

There is no specific treatment for urethane toxicity. Supportive and symptomatic treatment is recommended.

Environmental Fate

Urethane may be released to the environment in various waste streams from its production and use in the preparation and modification of amino resins, as a solubilizer and cosolvent for pesticides and fumigants, as an intermediate in the production of pharmaceuticals, as an antineoplastic agent, and as a reagent in biochemical research. If released to the atmosphere, urethane is expected to exist solely as a vapor in the ambient atmosphere. Vapor-phase urethane will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals with an estimated half-life of 2.2 days. If released to soil, urethane is expected to have very high mobility. Volatilization from moist soil surfaces is not expected to occur. Biodegradation of urethane in soil may be important. If released into water, urethane is not expected to adsorb to suspended solids and sediment in the water column. Volatilization from water surfaces is not expected. The potential for bioconcentration in aquatic organisms is low based on an estimated bioconcentration factor (BCF) of 0.45. Purely aliphatic carbamates are expected to be resistant to hydrolysis under environmental conditions; hydrolysis half-lives of 3300 and 330 years at pHs 7 and 8, respectively, were estimated for urethane. Urethane was judged easy to biodegrade in river die-away tests. Other biodegradation studies using activated sludge indicate urethane may biodegrade slowly.

Other Hazards

Urethane is combustible. When heated it emits toxic nitrogen oxide fumes.

Exposure Standards and Guidelines

Urethane is classified as a Group 2B carcinogen (probable human carcinogen) and hazardous air pollutant. Special precautions must be taken when working with urethane. Personnel handling urethane must follow industrial hygiene and health protection requirements for handling potentially carcinogenic substances. At a minimum, urethane exposure should be minimized through the use of engineering controls, work practices and personal protective equipments, including impervious and disposable gowns and gloves as well as eye and respiratory protection. In addition, working areas and working instruments must be especially designed for handling potentially harmful substances.

See also: Carcinogenesis; Dithiocarbamates.

Further Reading

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Urethane.

V

Validation of Toxicity Testing *See* Toxicity Testing, Validation.

Valium *See* Diazepam.

Valley of the Drums

Pertti J Hakkinen

A site in Brooks, Kentucky, USA, is called the Valley of the Drums. It was one of the earliest and most serious hazardous waste sites in the United States. The Valley of the Drums involved a vast quantity of illegally disposed material, and discovery of this site helped lead the US Congress to create the Superfund law.

The property was owned by Arthur Taylor until 1977, and the 13 acres of the Valley of Drums were used as a refuse dump, drum recycling center, and chemical dump from 1967 to 1977. Another 10 acres of the tract owned by Mr. Taylor was not part of the Valley of the Drums. The paints and coatings industries of the Louisville, Kentucky area were the primary waste generators using the Valley of the Drums.

In 1967, the Kentucky Department of Natural Resources and Environmental Protection (KDNREP) identified the Valley of the Drums site as an uncontrolled dump location for hazardous waste.

Some of the drums were emptied into open pits and trenches, cleaned, and recycled. Other drums were buried on site and, during the later years of operation, many drums were stored on the surface. Mr. Taylor was eventually stopped from burning the chemical waste, and soil from nearby hillsides was used to cover the pits and trenches. Thousands of drums were also stored on the surface of the site, and investigation found four or five major cells of buried wastes containing chemical liquids, sludges, and crushed drums.

The KDNREP first documented releases of hazardous chemicals from the Valley of the Drums in 1975, and they pursued legal actions against Mr. Taylor until his death in late 1977. Throughout the history of his operating the site from 1967 to 1977, Mr. Taylor never applied for the required state permits. A KDNREP investigation of the property revealed that over 100 000 drums of waste were delivered to the site. When it rained, the deteriorating drums leaked, and drainage overflowed into Wilson Creek, a tributary of the Ohio River. In 1979, large amounts of chemicals were carried into Wilson Creek by the spring snow melts. In January of 1979, at the request of KDNREP, the US Environmental Protection Agency (EPA) responded to the releases under the authority of Section 311 of the Clean Water Act. The open pits which had been used for burning solvents had been covered over before EPA's involvement.

The initial drum inventory conducted in 1979 found 17 051 drums on the surface, including 11 628 empty ones. The EPA analyzed the property and creek and found $\sim$140 chemical substances. The chemicals found most often and in the highest concentrations were xylene, methyl ethyl ketone, methylene chloride, acetone, phthalates, anthracene, toluene, fluoranthene, alkyl benzene, vinyl chloride, dichloroethylene, and aliphatic acids. Polychlorobiphenyls were detected in low concentrations and several metals including barium, zinc, copper, strontium, magnesium, and chromium were detected in concentrations exceeding background levels.

In 1980, KDNREP contacted six Responsible Parties who identified and removed some of the waste remaining on the surface of the site. Through these response activities and voluntary removal of wastes by the known generators, a majority of the drums on

the surface were removed. Actions by EPA intended to prevent further releases of chemicals into the creek included the construction of interceptor trenches and a temporary water treatment system, securing leaking drums, and segregating and organizing drums of the site.

In 1981, US EPA again inspected the site and discovered deteriorating and leaking drums and discharges of chemicals into Wilson Creek occurring again. EPA responded by upgrading the treatment system and removed the remaining several thousand drums on the surface of the site for recycling or disposal; however, some waste remained buried on the site. The area became Kentucky's first federal Superfund site in 1983.

In 1986 and 1987, the US EPA took additional remedial action to contain the site from any further impact to the surrounding environment. Overall, over $2.5 million has been spent to clean up the Valley of the Drums. The Valley of the Drums undergoes periodic scheduled reviews by US EPA and the Army Corp of Engineers to determine, if the cleanup measures that have been taken are still judged to be adequate. The reviews have found that the remedies put into place in 1987, including covering the site with a containment cap, were effective, and that a remarkable cleanup job has been accomplished. In addition to 5 year reviews, the site is also monitored regularly to make sure that tree roots or other foreign objects are not damaging the cap, and to sample water in several wells that were installed near the cap to check whether contaminated water is leaving the site.

See also: Environmental Protection Agency, US; Hazardous Waste.

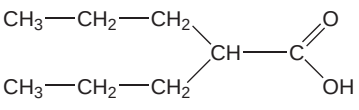
Relevant Website

http://www.epa.gov – US EPA Record of Decision System (RODS) Website on EPA/ROD/R04-86/009. 1986. EPA Superfund Record of Decision: A.L. Taylor (Valley of Drums) EPA ID: KYD980500961 OU 01, Brooks, KY, 06/18/1986. The US EPA website also has information on the history of the Valley of the Drums.

Valproic Acid

Dennis J Naas

- Chemical Abstracts Service Registry Numbers: CAS 99-66-1; CAS 76584-70-8 (Sodium valproate)
- Synonyms:
  - Valproic acid: 2-Propylpentanoic acid; Dipropylacetic acid (n-DPA); 2-Propylvaleric acid; Di-n-propylacetic acid; Depakene
  - (Semi)sodium valproate: Divalproex sodium; Sodium hydrogen bis(2-propylpentanoate); Depakote; Mylproin
  - Other proprietary names: Epilim; Convulex; Depakin; Depakine; Deprakine
- Chemical/Pharmaceutical/Other Class: Anticonvulsant
- Chemical Structure:



Uses

Valproic acid is used therapeutically as an anticonvulsant. It is a synthesized, simple, branched-chain carboxylic acid that is chemically unrelated to other anticonvulsants. Valproic acid and valproate are used in a variety of absence and generalized seizure disorders.

Exposure Routes and Pathways

Toxicity results from acute or chronic ingestion of tablets, capsules, or elixir.

Toxicokinetics

Peak plasma levels occur 1–4 h for capsules and syrup and 3–4 h for delayed-release capsules and tablets. Absorption is delayed but not diminished in the presence of food. Bioavailability appears to be complete. The majority of a dose undergoes hepatic glucuronidation or oxidation. At least two metabolites, 2-propyl-2-pentenoic acid and 2-propyl-4-pentenoic acid, have anticonvulsant activity. Biotransformation can be enhanced by enzyme-inducing drugs (e.g., primadone, carbamazine, phenobarbital, and

phenytoin), but there is no apparent autoinduction. The apparent volume of distribution is 0.2 or $0.3\,l\,kg^{-1}$ (but $\sim 1\,l\,kg^{-1}$ for the free, unbound portion), with high concentrations found in areas containing gamma-aminobutyric acid (GABA). Plasma protein binding is 90–95% at therapeutic concentrations but decreases as plasma levels increase. The therapeutic level in plasma is $50–150\,\mu g\,ml^{-1}$.

Less than 3% of a dose is excreted unchanged in the urine or through the feces. The elimination half-life from plasma is 10–15 h when valproic acid is used alone, but interaction with other anticonvulsant drugs can reduce the half-life to 4–10 h. It may be much longer in hepatic-impaired individuals, the elderly, and young children.

Mechanism of Toxicity

The anticonvulsant properties of valproic acid (and/or its metabolites) are likely attributable to enhancement (decreased metabolism or decreased re-uptake in brain tissues) of GABA activity. Valproic acid may also inhibit platelet aggregation.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} in rats is $675\,mg\,kg^{-1}$.

Human

In most cases, overdoses with valproic acid are relatively well tolerated. Most patients will have nausea and/or vomiting, and a mild degree of lethargy. Miosis and confusion can also occur. Rare cases of seizures, coma, cerebral edema, hypotension, and cardiorespiratory arrest have been reported. The incidence of these effects is unknown, but they are likely dose related. Significant depression of consciousness has been associated with ingestions exceeding $200\,mg\,kg^{-1}$. Recovery has occurred following ingestion of 25 g. A fatality was reported in a 15-year-old with a plasma concentration of $1914\,\mu g\,ml^{-1}$ and cardiorespiratory arrest occurred 20 h postingestion. Transient elevation of hepatic transaminases, acute pancreatitis, and hyperammonemia has been noted after acute overdose.

Chronic Toxicity (or Exposure)

Animal

Developmental toxicity, manifest as skeletal abnormalities and neural tube defects, have been observed in rodents treated during gestation.

Human

The first confirmed report of an infant with congenital defects after valproic acid exposure during pregnancy appeared in 1980. The mother took 100 mg of valproic acid daily throughout gestation, and delivered a growth-retarded infant with facial dysmorphism and heart and limb defects. The infant expired at age of 19 days. Since this initial report, several studies and case reports have described newborns with malformations after *in utero* exposure to either valproic acid monotherapy or combination therapy. The most serious abnormalities observed with valproic acid (or sodium valproate) exposure are defects in neural tube closure. The absolute risk of this defect is $\sim$1–2%, about the same risk for a familial occurrence of the anomaly. No cases of anencephaly have been associated with valproic acid. Exposure to valproic acid between the 17th and 30th days after fertilization must occur before the drug can be considered a cause of neural tube defects. Other predominant defects involve the heart, face, and limbs. A characteristic pattern of minor facial abnormalities has been attributed to valproic acid. Cardiac anomalies and cleft lip/palate occur with most anticonvulsants, and a causal relationship with valproic acid has not been established.

Hepatotoxicity is a concern. During the first few months of therapy, transient elevation of hepatic transaminases occurs in an average 11% (up to 40%) of patients. Fulminant hepatic failure will develop in 1 in 5000–10 000 patients. In these cases there is hepatic necrosis, steatosis, and a Reye's syndrome-like illness. Fatal hepatic injury is most likely in children less than 2 years old and in those patients on multiple-drug therapy.

In Vitro Toxicity Data

Valproic acid is an *in vitro* developmental toxicant (rodent whole embryo culture system).

Clinical Management

The majority of patients with acute overdose have a benign course and needs supportive care alone. The gut should be decontaminated with oral doses of activated charcoal. Gastric lavage can be considered after ingestion of life-threatening quantities if it can be done soon after ingestion (generally within 1 h). Airway management should be provided after severe overdose. Ipecac is not recommended due to its potential for central nervous system depression. If hypotension develops, isotonic fluids should

be infused. Measures to enhance elimination are not justified despite testimonials from case reports. Patients requiring treatment in the emergency department should be tested for valproic acid plasma concentration, complete blood count, liver function, and perhaps for the presence of other anticonvulsant drugs. Valproic acid therapy should be discontinued in patients with elevated hepatic enzymes or serum ammonia. There is no known antidote; however, one case report describes a positive response to naloxone in a child with a serum level of 185 $\mu g\,ml^{-1}$.

See also: Benzodiazepines; Carbamazepine; Developmental Toxicology; Phenytoin.

Further Reading

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Sztajnkrycer MD (2002) Valproic acid toxicity: Overview and Management. *Journal of Toxicology. Clinical Toxicology* 40(6): 789–801. (Erratum in: (2003) *Journal of Toxicology. Clinical Toxicology* 41(4): 215.)

Vanadium

Shayne C Gad

- Chemical Abstracts Service Registry Number: CAS 7440-62-2
- Chemical/Pharmaceutical/Other Class: Transition metals
- Chemical Formulas: V^{2+}; V^{3+}; V^{4+}; V^{5+}

Uses

Vanadium is used as an alloying addition to steel, iron, titanium, copper, and aluminum, with the primary use in the steel industry. Vanadium is also used as a target material for X-rays, as a catalyst for the production of synthetic rubbers, plastics, and chemicals, and in ceramics. Vanadium is an element of pharmacological and nutritional significance; for example, it has increasing therapeutic use in diabetes, and is emerging as a potent anticarcinogenic agent.

Background Information

Vanadium was discovered in 1830. It is present at 0.01% in earth's crust. Vanadium is released naturally into the air through the formation of continental dust, marine aerosols, and volcanic emissions. The natural release of vanadium into water and soils occurs primarily as a result of weathering of rocks and soil erosion. Anthropogenic sources include the combustion of fossil fuels, particularly residual fuel oils, which constitute the single largest overall release of vanadium to the atmosphere. Deposition of atmospheric vanadium is also an important source both near and far from industrial plants burning residual fuel oils rich in vanadium. Other anthropogenic sources include leachates from mining tailings, vanadium-enriched slag heaps, municipal sewage sludge, and certain fertilizers. Natural releases to water and soil are far greater overall than anthropogenic releases to the atmosphere.

Exposure Routes and Pathways

The general population is exposed to background levels of vanadium primarily through ingestion of food. Workers in industries processing or using vanadium compounds are commonly exposed to higher than background levels via the inhalation pathway. A 1980 estimate by the National Institute for Occupational Safety and Health indicates that in 1980 about 5319 people were exposed to vanadium pentoxide in their workplace. Exposure through inhalation may also be of importance in urban areas where large amounts of residual fuel oil are burned. Other populations possibly exposed to higher than background levels include those ingesting foodstuffs contaminated by vanadium-enriched soil, fertilizers, or sludge. Populations in the vicinity of vanadium-containing hazardous waste sites may be exposed under these circumstances.

Toxicokinetics

In humans, 0.1–1% of orally administered vanadium is absorbed through the gut. Lung and gut absorption increases with the solubility of the vanadium compound. Vanadium pentoxide is ~100% absorbed by inhalation. Vanadium is not absorbed through the skin. When absorbed, 60% of the vanadium is excreted by the kidneys within 24 h of administration. Vanadium can pass through the blood–brain barrier.

Mechanism of Toxicity

In the consolidated form, vanadium metal and its alloys may pose no particular health or safety hazard; however, the toxicity of vanadium alloys may be a function of other components of the alloy. Vanadium compounds have been proven to be associated with the pathogenesis of some human diseases and also in maintaining normal body functions. Salts of vanadium interfere with many enzyme systems, for example, ATPases, protein kinases, ribonucleases, and phosphatases. Vanadium may also be an essential trace element, contributing to glucose balance; however, the importance of this element as a micronutrient is yet to be unequivocally accepted. Vanadium deficiency has been associated with disturbances in physiological functions, for instance, thyroid, glucose, and lipid metabolism. Vanadate (VO_3^-) mimics the action of insulin in target tissues and is a potential inhibitor of the sodium pump. Vanadium toxicity is enhanced by dietary zinc. Several genes are regulated by this element or by its compounds, including those for tumor necrosis factor-alpha, interleukin-8, activator protein-1, ras, c-raf-1, mitogen activated protein kinase, p53, and nuclear factors-kappaB.

When inhaled, vanadium is toxic to alveolar macrophages and therefore may impair pulmonary resistance to infection and clearance of particulate matter. An increase in inflammatory cells of the nasal mucosa has been observed in workers exposed to vanadium.

Acute and Short-Term Toxicity (or Exposure)

Animal

Inhalation of vanadium in animals results in lung irritation, coughing, wheezing, chest pain, atrophic rhinitis, and conjunctivitis. Pulmonary edema has been observed in animals after exposure to some vanadium compounds. The acute oral toxicity of vanadium is low: In mice, $1000\,mg\,kg^{-1}$ causes catarrhal gastritis. Acute oral exposure in rats results in distress, hemorrhagic exudates from the nose, diarrhea, hind limb paralysis, labored respiration, convulsions, organ congestion, fatty degeneration of the liver and kidney, focal hemorrhage of the lung and adrenal cortex, and death. Rat (oral) $LD_{Lo} = 225\,mg\,kg^{-1}$ over 5 days.

Human

In general, vanadium has a very low oral and dermal toxicity and a moderately low toxicity by the inhalation route. The toxicity of vanadium increases with its valence state, with vanadium pentoxide being the most toxic of the vanadium compounds. Vanadium fumes are more toxic than vanadium dust. Acute inhalation exposure has resulted in lung irritation, coughing, wheezing, chest pain, nosebleeds, atrophic rhinitis, pharyngitis, epistaxis, tracheitis, asthma-like diseases, irritation of the eyes, and a metallic taste in the mouth. Symptoms generally disappear within 2 weeks of exposure. A quantity of $2–10^4\,mg\,m^{-3}$ has resulted in mild to moderate respiratory effects and no systemic effects in humans.

Acute oral exposure results in abdominal cramping, diarrhea, black stools, and a greenish-black coating on the tongue. Skin exposure may result in dermatitis, allergic skin lesions, and a green discoloration of the skin. A fatal dose may result in central nervous system depression with tremors, headache, and tinnitus.

Chronic Toxicity (or Exposure)

Animal

Chronic inhalation and oral exposure to vanadium in laboratory animals has resulted in kidney and liver changes, decreased erythrocyte count and hemoglobin levels, and increased reticulocyte count in peripheral blood. Chronic oral exposure to vanadium has caused an increase in minor birth defects and fetal death in pregnant rats. Vanadium has not been found to cause mutagenic, carcinogenic, teratogenic, or reproductive effects in short-term studies. Among laboratory animals, rabbits and guinea pigs are particularly susceptible.

Human

Systemic symptoms of exposure to vanadium are extremely rare but could include peripheral vasoconstriction of the lungs, spleen, kidneys, and intestines. Chronic exposure to vanadium may result in arrhythmias and bradycardia.

Clinical Management

Irrigate exposed skin and eyes with copious amounts of tepid water (with soap for exposed skin). After inhalation exposures, move to fresh air and monitor for respiratory distress. Administer 100% humidified supplemental oxygen with assisted ventilation as required. If coughing or breathing difficulties are noted, the patient should be evaluated for irritation or bronchitis, including chest X-rays and determination of blood gases. For ingestion exposures, emesis may be indicated for recent, substantial ingestion.

Activated charcoal may be considered, depending on the form of vanadium ingested. Chelation is not usually indicated since systemic effects are rare.

Ecotoxicology

It is unlikely that there is bioaccumulation or biotransformation.

Miscellaneous

Vanadium can react violently with bromine trifluoride, chlorine, lithium, and oxidants; for example, powdered vanadium can explode in contact with chlorine.

See also: Metals; Zinc.

Further Reading

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Mukherjee B, Patra B, Mahapatra S, *et al.* (2004) Vanadium – An element of atypical biological significance. *Toxicology Letters* 150: 135–143.
Rydzynski K (2001) Vanadium, niobium, and tantalum. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 3, pp. 1–74. New York: Wiley.

Relevant Website

http://www.who.int – Vanadium Pentoxide and Other Inorganic Vanadium Compounds (Concise International Chemical Assessment Document 29 from the International Programme on Chemical Safety).

Vanillin

Lu Yu

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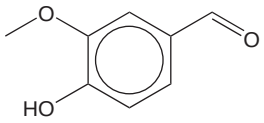
- Chemical Abstracts Service Registry Number: CAS 121-33-5
- Synonyms: *m*-Anisaldehyde; Vanillic aldehyde; Lioxin; 3-Methoxy-4-hydroxybenzaldehyde; *p*-Hydroxy-*m*-methoxybenzaldehyde; Lioxin; Vanillaldehyde; Vanillic aldehyde; 2-Methoxy-4-formylphenol; 3-Methoxy-4-hydroxybenzaldehyde; Vanilla; Protocatechualdehyde, methyl-; Zimco; *p*-Vanillin; Methylprotcatechuic aldehyde; Methylprotocatechuic aldehyde
- Chemical/Pharmaceutical/Other Class: Essential oil
- Chemical Formula: $C_8H_8O_3$
- Chemical Structure:



Uses

Vanillin is used in flavorings for food, perfumes, and pharmaceuticals (flavor, antidepressant drugs); it is a source of L-dopa; reagent in analytical chemistry; and formulating insect attractants.

Exposure Routes and Pathways

Occupational exposure to vanillin may occur through inhalation and dermal contact at workplaces where vanillin is produced or used. The general population may be exposed to ethyl vanillin via dermal contact with perfumes and ingestion of food products that contain vanillin as a flavor additive.

Toxicokinetics

In rats fed with vanillin at 100 mg kg^{-1}, most of the metabolites were excreted in the urine within 24 h. The majority of administrated vanillin will be excreted as vanillic acid. Glucurovanillin and some other forms of conjugates of vanillin were also excreted.

Mechanism of Toxicity

Vanillin in solution is an acid. It has an irritating action on eyes, gastrointestinal tract, and mucous membranes of the respiratory tract.

Pharmacologically, vanillin can accelerate bile secretion. Vanillin is capable of effectively minimizing methotrexate-induced chromosomal damage. Vanillin is an anticlastogenic agent; it has also been demonstrated to inhibit gene mutations in both bacterial and mammalian cells. Vanillin enhances or suppresses chemical-induced cytotoxicity, mutations, and chromosome aberrations.

Acute and Short-Term Toxicity (or Exposure)

Animal

Vanillin is a weak dermal sensitizer in guinea pigs and mice. Treatment with vanillin caused eye irritation in rabbits. Rat LD_{50} values are 1580 mg kg^{-1} (oral), 1500 mg kg^{-1} (subcutaneous), and 1160 mg kg^{-1} (intraperitoneal). The mouse LD_{50} is 475 mg kg^{-1} (intraperitoneal). Guinea pig LD_{50} values are 1400 mg kg^{-1} (oral) and 1190 mg kg^{-1} (intraperitoneal). Oral ingestion at high doses causes hyperpnea, muscular weakness, dyspnea, collapse, and circulatory failure in rats.

Human

Vanillin is a weak human sensitizer. It has induced skin sensitization in humans, and it was also reported to have highly irritating action on the eyes and mucous membranes of the respiratory tract. Ingestion of vanilla has provoked intolerance reaction; it is pharmacologically active and may cause depressed blood pressure, increased respiratory rate, and even death due to cardiovascular collapse. Probable oral lethal dose to human is 500 mg kg^{-1} for a 70 kg person.

Chronic Toxicity (or Exposure)

Animal

Repeated administration in rats may induce tissue effects at various sites. Growth depression, enlargement of liver, kidney, and spleen were reported in rats after administration of vanillin at 50 000 ppm for 91 days. Administration of vanillin at 64 mg kg^{-1} day^{-1} for 10 weeks caused growth depression, damage to myocardium, liver, kidney, lung, spleen. Vanillin has not been shown to cause cancer in animals. No excess of lung tumor was observed in mice given an intraperitoneal dose of 3.6–18 g kg^{-1} over 24 weeks.

In Vitro Toxicity Data

Vanillin was found to directly suppress the *in vitro* antisheep RBC antibody response at a noncytotoxic dose (200 µg per culture). Vanillin induced chromosomal damage in human cells treated in culture, but showed no genotoxic activity in mice treated orally or in hamster cells in culture. There was also no evidence of mutagenic activity in bacterial (including Ames test) or in yeast.

Clinical Management

The patient should be moved from the source of exposure. If there is respiratory distress, an airway should be established. Patients should be closely observed for esophageal or gastrointestinal tract irritation, or signs of respiratory insufficiency. Eyes should be gently flushed with water immediately after exposure. Activated charcoal should be administered if vanillin is ingested. Early removal of ingested vanillin by cautious gastric lavage should be considered if there is significant gastrointestinal tract irritation or if life-threatening amount of vanillin has been ingested. Skin burns should be covered with dry sterile dressing after decontamination. Further treatment is needed for patients who develop a dermal hypersensitivity reaction.

Environmental Fate

Vanillin's production and use as a flavoring agent in foods and in perfumery may result in its release to the environment through the waste stream. It is also a naturally occurring compound in vanilla beans and may be released to the environment through decay of plant material. If released into the air, vanillin will exist as a vapor and may be degraded by reaction with photochemically produced hydroxyl radicals with a half-life of 14 h. In the soil, vanillin is expected to be highly mobile; volatilization from soil surface is estimated to be less and it degrades rapidly. When vanillin is released into water, it exists in the ionized form at environmental pH, and is not expected to adsorb to suspended solids and sediments in water. Volatilization from the water surface is also expected to be low. Vanillin has a low potential to bioaccumulate in aquatic organisms.

Other Hazards

Some synthetic fragrant substances and intermediate products are flammable. Violent reactions occur when a small amount of vanillin was added to thallium trinitrate trihydrate (up to 50%) in 90% formic acid. When heated to decomposition it emits acrid smoke and irritating fumes.

Exposure Standards and Guidelines

There are exposure standards for vanillin. US Environmental Protection Agency (EPA) promulgated a model Health and Safety Data Reporting Rule, which requires manufacturers, importers, and processors of listed chemical substances and mixtures to submit to US EPA copies and lists of unpublished

health and safety studies. Vanillin is included in this list.

See also: Consumer Products; Limonene.

Further Reading

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Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Vanillin.

Vein *See* Blood.

Venoms and Poisons from Animals *See* Animals, Poisonous and Venomous.

Vesicants *See* Blister Agents/Vesicants.

Veterinary Toxicology

Wilson K Rumbeiha and Frederick W Oehme

This entry focuses on understanding and managing chemically induced disorders in domestic animals. Approximately 10% of veterinary practice is devoted to the diagnosis and treatment of poisonings. The species treated range from small domestic animals (i.e., cats and dogs) to food-producing animals (i.e., dairy cattle, beef cattle, and swine), to horses, pet birds, zoo animals, and, occasionally, wild game (i.e., rabbits and fish).

Species Differences

Small animals react to chemicals more or less the same way as do humans because they are all monogastrics. Ruminants (i.e., cattle and sheep), however, react differently than the monogastrics; they have evolved a unique digestive tract structure and microbial flora, which play a major role in the fermentation of the forage ingesta. The ruminant's microflora are usually capable of metabolizing toxic chemicals. For example, cattle are more susceptible to nitrate poisoning than are horses, whereas dogs and cats are very resistant to nitrate poisoning. Cattle are very susceptible to nitrate poisoning because their digestive tract microbes will convert nitrates to the proximate toxic metabolite, nitrite.

Dogs, because of their relatively small gastrointestinal microbial population, are resistant to nitrate poisoning. The horse may succumb to nitrate poisoning because of the presence of microorganisms in the cecum in its posterior digestive tract. However, by the time nitrate reaches the cecum, more than 70% will have been absorbed; little will be available for biotransformation into the toxic nitrite ion. Horses, therefore, require threefold higher nitrate concentrations to be poisoned than do cattle.

Physiological differences among species will markedly alter the susceptibility to toxicants. Birds are more sensitive to toxic vapors and gases than mammals. Canaries have been used in mines to test for the presence of poisonous gases because their elaborate respiratory system causes them to succumb to lower concentrations of toxic gases than would endanger humans.

Biochemical differences also contribute to differential susceptibility between and within species. Cats are more susceptible to acetaminophen poisoning than other domestic animals. The cat's glucuronyl

transferase activity for conjugating acetaminophen is much lower than that of other domestic species, and feline hemoglobin is more susceptible to oxidation than that of other animals. Therefore, cats given what would be considered a therapeutic dose of acetaminophen in humans die of methemoglobinemia. Biochemical differences are also found within the same species. For example, the Bedlington terrier is much more susceptible to copper poisoning than other species of dogs. Most biochemical differences are of genetic origin.

Adequate comprehension of the variance in toxicity from chemicals in the domesticated species requires an understanding of the anatomy, physiology, and biochemistry of the affected animals. Other general factors that affect the toxicity of chemicals must also be considered when dealing with clinical toxicities in domestic animals. These factors include the animal's age, sex, health, nutritional status, environment, and concurrent exposure to other chemicals.

The effects of these and other factors in modifying the outcome of poisoning can be of vital significance in determining its outcome and also point to appropriate management options. There is a vast amount of literature in this area.

Common Toxicoses in Food-Producing Animals

Food-producing species are cattle, swine, and small ruminants. Swine differ from other animals in this category in that they have a simple stomach (monogastric), whereas the other animals have a compound stomach. Most of the toxicants affecting other animals also affect food-producing animals, but some toxicants are peculiar to or predominantly seen in food-producing animals. Toxicoses frequently encountered in ruminants include nonprotein nitrogen toxicoses; copper, lead, or arsenic poisoning; mycotoxicoses; nitrite poisoning; plant poisoning; and blue-green algae poisoning. In swine, salt poisoning, mycotoxicoses, organic arsenicals, plant poisoning, and gases generated in swine confinement operations are often is involved in toxic episodes.

Nonprotein Nitrogen Compounds

Nonprotein nitrogenous sources include urea, biuret, and ammoniated feeds. These compounds are cheap sources of the nitrogen required by the animals for protein synthesis. Nonprotein nitrogen poisoning is a common problem and is often seen in animals not gradually introduced to diets containing these compounds. It is an acute fatal condition characterized by bloating, intense abdominal pain, ammonia breath, frequent urination, and frenzy. Often several animals are affected.

In ruminant animals, the rumen microflora normally convert urea to ammonia, and the ammonia is rapidly utilized by the liver for protein synthesis. However, in cases of excess ammonia production, the blood ammonia concentration builds up to toxic levels very fast and induces central nervous system (CNS) derangement. Therefore, in addition to the gastrointestinal signs, the animals will show fulminating CNS signs. Treatment of the condition involves giving a weak acid such as vinegar and plenty of cold water orally. The rationale for giving cold water and acetic acid is to slow down the action of urease, the enzyme responsible for converting urea to ammonia, which requires body core temperature and pH for optimal function. The cold water lowers the temperature and the acetic acid lowers the pH. Infusions of calcium and magnesium solutions are administered to alleviate tetany.

Another source of nonprotein nitrogen (urea) poisoning in ruminants is the accidental ingestion of nitrogen-based fertilizers such as ammonium phosphate. Occasionally, cattle break into drums or bags of fertilizers containing these nitrogen-based compounds. The prognosis is grave in most cases if several animals are affected. If only a few valuable animals are affected, a rumenotomy can be performed. Although small ruminants (e.g., sheep and goats) have the same anatomical predisposition to suffer from nonprotein nitrogen poisoning, they are rarely involved, probably because they are not usually fed rations containing these compounds.

Nitrate–Nitrite

Excessive exposure of ruminant animals to nitrates causes nitrite toxicity, an acute rapidly fatal disease. The most common source of nitrates in ruminants is through consumption of forage that was grown on heavily fertilized fields and has accumulated high levels of nitrates. All common animal feeds, such as sorghum, alfalfa, and milo, can accumulate excessive amounts of nitrates. Another common source of dietary nitrates is contaminated drinking water. Nitrates are highly water-soluble and underground water can become contaminated from heavily fertilized fields. Runoff from fertilized fields is another source of contamination in surface pools and ponds. Nitrate from different sources is additive.

Nitrates are reduced to nitrites by rumen microflora. In normal circumstances the nitrite ion is rapidly utilized for ammonia synthesis, but in cases of excessive acute intake of nitrate, the rapidly formed nitrite ion is absorbed into the bloodstream. In blood

the nitrite ion reacts with hemoglobin to form methemoglobin. Methemoglobin is incapable of oxygen transport, and the animal compensates for the anoxia by increasing its respiratory rate. Therefore, affected animals will be hyperventilating, have brownish mucous membranes, and be weak. Chronic intake of nitrates has been reported to cause reproductive problems such as abortion, but experimental results regarding this claim are currently inconclusive. Besides reacting with methemoglobin, the nitrite ion also replaces iodine in the thyroid gland, thereby interfering with the function of the thyroid hormone.

Treatment of nitrate/nitrite poisoning involves intravenous infusion of 1% methylene blue at a rate of $1.5\,mg\,kg^{-1}$ body weight and withdrawal of the offending feed.

Copper–Molybdenum

Sheep are more susceptible to copper poisoning than are cattle, but cattle are more sensitive to molybdenum poisoning than are sheep. The *in vivo* relationship between copper and molybdenum is well understood. Excess copper induces molybdenum deficiency and vice versa. The most frequent cause of copper poisoning in sheep is by uninformed farmers feeding cattle feed to sheep. Copper from different sources is additive. Copper is an essential element for cattle and is usually added to their feeds; however, molybdenum is not considered essential and is therefore not added. Cattle feeds therefore have high copper concentrations and no molybdenum; feeding this ration to sheep upsets the normal 6:1 copper:molybdenum ratio *in vivo*.

Copper toxicity in sheep is an acute condition that develops after a chronic copper intake. During the chronic phase copper is stored in the liver until a critical concentration is reached. Stressful conditions, such as transportation or insufficient feed or water intake, will trigger a massive hepatic release of copper and cause a hemolytic crisis. Affected sheep have hemoglobinuria, are weak, and die acutely. The massive release of hemoglobin can block the renal tubules, inducing renal failure. The prognosis is poor for animals already showing clinical signs. Chelation therapy using D-penicillamine is recommended for the exposed animals not showing clinical signs.

In cattle, molybdenosis is characterized by a foamy diarrhea which may be bloody. Affected cattle also have depigmented hair. Molybdenosis is a subacute to chronic condition and occurs when the copper:molybdenum ratio is 2:1 or less. The condition shows geographical distribution and occurs in areas deficient in copper or having an excess of molybdenum (e.g., parts of California, Oregon, Nevada, and Florida). Treatment of this condition involves copper supplementation in the feed.

Lead

Despite awareness regarding the dangers of lead poisoning in humans and domestic species, it is surprising that lead poisoning is the most frequently encountered toxicity in food-producing animals. Lead poisoning is more commonly seen in cattle than in other food-producing species. Young animals are mostly affected because of their curiosity and because they are indiscriminate in their feeding habits. There are several sources of lead in cattle. Discarded junk automobile batteries, paint, and leaded water pipes are the most common sources. Quite often uninformed owners will discard or store old batteries in farm environments and cattle will chew on them. Discarded leaded pipes, especially those used around oil wells, are a common source of lead poisoning.

Lead interferes with heme synthesis and causes renal and CNS lesions in food-producing animals. Affected animals are initially anorectic. They may then become belligerent, blind, and have periodic seizures at the terminal stages of the poisoning. Once the CNS signs have set in, the prognosis is grave but treatment with chelating agents may be of value. Chelating agents include calcium disodium-EDTA (calcium disodium salt of ethylenediaminetetraacetic acid) and 2,3-dimercapto-1-propanesulfonic acid.

Arsenic

Arsenic poisoning is second to lead as the most frequently reported heavy metal toxicant in food-producing animals. Arsenic is present in the environment in two forms: inorganic and organic arsenicals. Inorganic arsenic is often incorporated into pesticides, which are the most common sources of arsenic poisoning in cattle. Inorganic arsenicals are also used as herbicides and cattle sometimes are exposed by eating grass clippings from recently sprayed forage.

Inorganic arsenic poisoning is a rapidly developing and fatal disease. Affected animals show severe gastrointestinal irritation without CNS involvement. They have severe abdominal pain and hemorrhagic diarrhea and are depressed. Usually, these signs appear 24–36 h after exposure to the inorganic arsenic.

Phenylarsonic arsenicals are less toxic to mammals than the inorganic arsenicals. Phenylarsonic compounds are usually incorporated in swine (and poultry) feed for disease control and to improve weight gain. Examples of these compounds include arsenilic acid, 3-nitroarsenilic acid, and 4-nitroarsenilic acid. Organic arsenicals are also available as trivalent and

pentavalent compounds, and the trivalent forms are more toxic than the pentavalent compounds.

These phenylarsonic compounds are peripheral nervous system toxicants. They cause demyelination of peripheral nerve fibers leading to ataxia and paralysis of hindquarters. The condition occurs frequently in swine kept on feed containing 1000 ppm arsenic for at least 3–10 days or 250 ppm arsenic for 20–40 days. Therefore, unlike inorganic arsenic poisoning, which is an acute form of the disease, poisoning by phenylarsonic compounds is an insidious condition. In addition, organic arsenic is commonly involved in swine toxicities because of its incorporation in swine feeds, whereas inorganic arsenic poisoning is more commonly seen in cattle.

Treatment of inorganic arsenic poisoning involves decontamination procedures and use of the antidote BAL (British anti-lewisite compound; 2,3-dimercaptopropanol). Use of demulcent to coat the gastrointestinal tract and the use of antibiotics is also recommended. Organic arsenic poisoning treatment involves only withdrawal of the feed involved, with recovery occurring in 3–5 days. Severely affected pigs should be culled.

Selenium

Selenium poisoning is a regional problem occurring in areas where the selenium content in soil is high. Selenium is then absorbed and concentrated by selenium-accumulating plants such as the *Astragalus* species. Cattle, sheep, goats, and swine are exposed by consuming these plants.

Acute selenium poisoning occurs when animals consume plants containing more than 10 000 ppm. This is characterized by sudden death or labored breathing, abnormal movement and posture, frequent urination, diarrhea, and death. Because plants containing high selenium concentrations are unpalatable, they are rarely consumed by animals. Therefore, acute selenium poisoning is rare. However, chronic selenium poisoning is relatively common. Chronic consumption of plants containing as low as 50 ppm can cause chronic selenium poisoning. Affected animals are anorexic, have impaired vision, wander, salivate excessively, are emaciated and lame, and lose hair.

Removal of animals from pastures whose forages contain high selenium is the recommended cure but may be unsuccessful if the condition has persisted for several days or more.

Mycotoxins

Some of the mycotoxins of veterinary interest are aflatoxins, deoxynivalenol (DON), diacetoxyscirpenol (DAS), T-2, zearalenone, ochratoxins, and fumonisin B_1. Mycotoxins are especially a common problem in warm climates where high temperatures and relative humidity support fungal growth and favor mycotoxin production. All food-producing animals are susceptible and clinical signs will depend on the mycotoxins is involved. Usually only one mycotoxin is involved because several species of fungi (e.g., *Fusarium, Penicillium,* and *Aspergillus*) coexist and often produce more than one type of mycotoxin.

The common sources of aflatoxins for food-producing animals include corn and oats. When aflatoxins are ingested in parts-per-million quantities, acute death can occur. The affected animals show severe gastrointestinal pain and hemorrhage. Aflatoxins are severe hepatotoxicants; therefore, hepatomegaly and jaundice may be observed in severe subacute cases. Quite often, however, aflatoxin poisoning is an insidious condition due to the chronic intake of parts-per-billion aflatoxin concentrations over a prolonged period of time. Clinical signs include poor weight gain, decreased milk production, and poor reproductive performance, including abortions. Virtually every organ function is affected by aflatoxins. The immune system of the affected animals is also impaired, and animals may more easily succumb to infectious diseases.

Toxicity due to T-2 has been reported in North America and other parts of the world, including Germany, Hungary, France, and South Africa. It is less common than aflatoxin toxicity. T-2 mycotoxins act by interfering with the blood clotting mechanism. Affected animals have gastrointestinal bleeding and will pass bloodstained feces. The animals will perform poorly (i.e., have low weight gain, decreased milk production, and decreased food intake). T-2 is also an immunosuppressant. All food-producing animals are susceptible to T-2 mycotoxicosis.

Zearalenone is an estrogenic mycotoxin that usually causes toxicity in swine that consume contaminated corn. Prepubertal swine are mostly affected. Affected females show swelling of the vulva and excessive straining, which may cause vaginal prolapses. In male animals, zearalenone will cause decreased libido. There is no effective treatment apart from withdrawing the feed containing the mycotoxin.

Other mycotoxins, including DAS, DON, and ochratoxin, are not of major economic importance although they can be toxic to food-producing animals. DAS causes necrosis and erosion of the oral mucous membranes. Consequently, affected animals exhibit feed refusal and have impaired growth. DON (also called 'vomitoxin') induces vomiting and feed

refusal in swine. Ochratoxins cause renal problems, including hydronephrosis, especially in swine.

Ergot poisoning is occasionally encountered in livestock fed grain screenings contaminated with *Claviceps purpurea*. The active constituents are ergotoxin and ergotamine, which are vasoactive compounds. These compounds cause vasoconstriction of the peripheral vessels, especially those of extremities, causing necrosis and gangrene of hooves, ears, and tails. Abortions and agalactia have been reported in cattle fed ergot-contaminated feed. Therapy consists of discontinuation of the source of the toxicant and antibiotic therapy to prevent secondary bacterial infections in the necrotic tissues.

Fumonisin B_1 is produced by *Fusarium moniliforme*, a fungus that predominantly grows worldwide on corn. Fumonisin B_1 causes pulmonary edema and respiratory distress in swine. Numerous deaths have been reported in swine fed fumonisin-contaminated corn screenings.

The most practical treatment for mycotoxicoses consists of withdrawal of the contaminated feed from the herd and supportive care for the affected animals.

Blue-Green Algae

Blue-green algae poisoning occurs in late summer and early fall when the algae forms a scum on top of ponds or other stagnant waters. Because of husbandry practices, cattle are most frequently involved. Blue-green algae poisoning has been reported in North America, South Africa, and Britain. Algae of genus *Anabaena* are most frequently involved.

There are two distinct syndromes in blue-green algae poisoning: the neurotoxic effects and the hepatotoxic syndrome. The neurotoxic disorder is peracute, and cattle drinking water containing the neurotoxic principle Anatoxin A can die within a few minutes and usually are found quite close to the pond or water (algae) source. On the other hand, the hepatotoxic principle causes an acute type of poisoning characterized by lethargy and jaundice. Death may occur 2 or 3 days after drinking contaminated water.

Because of the peracute nature of the blue-green algae-induced neurological syndrome, there is hardly time for treatment and the prognosis is universally grave. Treatment of animals affected with the liver syndrome of blue-green algae poisoning involves appropriate supportive therapy.

Toxic Gases

Toxic gases are of primary concern in closed animal housing, especially in swine operations. Because of the intensive swine confinement operations with buildings designed to save on energy, toxic gases can accumulate in swine houses and result in serious health consequences to animals and caretakers in cases of ventilation failure. These toxic gases are generated from the decomposition of urine and feces, respiratory excretion, and the operation of fuel-burning heaters.

The most important gases are ammonia, hydrogen sulfide, carbon monoxide, and methane. A number of vapors, which represent the odors of manure decomposition, such as organic acids, amines, amides, alcohols, carbonyls, and sulfides, are also produced. Respirable particles, which may be loaded with endotoxins, are also a major health problem in swine confinement operations.

Ammonia is highly soluble in water and will react with the mucous membranes of the eyes and respiratory passages. At 100 ppm or greater ammonia concentrations, toxicosis will produce excessive tearing, shallow breathing, and clear or purulent nasal discharge. The irritation of the respiratory tract epithelium leads to bronchoconstriction and shallow breathing.

Hydrogen sulfide poisoning is responsible for more animal deaths than any other gas. At concentrations of 250 ppm and above, hydrogen sulfide causes irritation of the eyes and respiratory tract and pulmonary edema. Hydrogen sulfide concentrations above 500 ppm cause strong nervous system stimulation and acute death. In order to prevent hydrogen sulfide poisoning, manure pits should not be agitated when pigs are on the premises, and proper ventilation should be in place.

Carbon monoxide is produced by incomplete combustion of hydrocarbon fuels. Poisoning by carbon monoxide is caused by operating improperly vented space heaters or furnaces in poorly ventilated buildings. Carbon monoxide binds to hemoglobin forming carboxyhemoglobin, thereby reducing hemoglobin's oxygen carrying capacity and subsequently causing hypoxia. Concentrations of carbon monoxide $\geqslant$250 ppm cause hyperventilation, respiratory distress, and stillbirths.

Methane is a flammable and colorless gas produced from organic wastes through bacterial action. It serves to displace oxygen in respirable air, thus producing oxygen starvation if present in high concentrations.

Nitrogen dioxide is a very poisonous gas that is responsible for causing silo fillers disease in humans. The gas is also toxic to animals. Nitrogen dioxide is produced during the first few weeks after silage has been cut and put into the silo. The highest nitrogen dioxide concentrations are reached during the first 48 h after filling the silo. Nitrogen dioxide dissolves in water to form nitric acid, which is very corrosive

to the respiratory tract epithelium and the lungs. Nitrogen dioxide concentrations as low as 4 or 5 ppm can cause respiratory system disturbances.

Exposure to sulfur dioxide concentrations of 5 ppm or greater causes irritation and salivation in swine. The gas is soluble in water, forming the more toxic sulfuric acid. It is the sulfuric acid that causes eye and nasal irritation, and in severe cases it produces hemorrhage and emphysema of the lungs.

The effect of these toxicants singly or in combination is to produce a hypofunctional respiratory system. Affected animals are also predisposed to respiratory tract infections. The end result is significantly retarded performance and productivity decreases in the affected animals. It is therefore important to ensure that proper animal housing is provided with adequate ventilation in all seasons of the year to provide animals with a healthy breathing and a highly productive environment.

Toxic Plants

Plant poisoning is very common in areas where open grazing is practiced, such as in the Great Plains of the United States, where plant poisoning is widely reported during spring and fall grazing seasons. The wide range of toxic plants and their variations in growth environments produce risks that can affect almost all body systems, depending on the plant consumed, its level of maturity, and the soil and environmental characteristics in which it is growing.

Body systems and organs most prominently affected by plants include the digestive tract, the liver, kidneys, and nervous system, the heart and blood, the skin, and the reproductive tract and its functioning. It is important to realize, however, that toxic plants rarely affect only one body system or organ and thus may generate a complex pattern of effects in any one poisoned animal. Toxicity of a given plant can vary widely depending on the prevailing natural conditions. It is therefore not surprising that a given toxic plant may be toxic under certain conditions (e.g., during stressful drought conditions) but safe during other times.

Sodium Chloride (Salt)

Salt poisoning/water deprivation is frequently encountered in swine operations but can also occur in feedlot cattle. The causes of this condition are twofold. Most commonly, the pigs will be on a ration containing a recommended concentration of sodium chloride, but management failures or changes can favor conditions that cause salt poisoning to occur. These poor management conditions include the sudden absence of water, which can be caused by frozen water in winter or the breakdown of water supplies. The other possibility is the accidental addition of excessive amounts of salt or sodium-containing materials to the ration.

Salt poisoning has also been reported in swine operations even when the management situation is appropriate; the only change was that the animals had been moved into a new housing facility, as occurs with weaning. In those situations, the animals are not used to the watering facilities in the new buildings and they do not know how to obtain the water; thus, they go without water while continuing to feed on the normal salt-containing ration.

Clinically, salt poisoning is a neurological disorder, and the syndrome is rather acute. Affected pigs will spin on their hindquarters and fall down convulsing. The pigs will also show a characteristic rhythmic pattern of seizures which occurs cyclically every 3–5 min. Many pigs are usually affected at the same time. The condition is corrected by the provision of adequate but restricted amounts of water made available gradually.

Common Toxicoses of Poultry

Even chickens, ducks, and turkeys are affected by poisonings. There is also much concern and interest in the toxicities seen in wild birds, especially those kept in zoos, as well those kept as pet birds in households. This discussion will emphasize the toxicoses encountered in poultry.

Drugs and Medications

Sulfonamides have been used as coccidiostats in poultry for several decades. Although sulfonamides possess inhibitory action against coccidiosis and other pathogenic agents, they can be toxic and have particularly been shown to be so to poultry. In poultry, sulfonamide toxicity is characterized by blood dyscrasia and renal and liver dysfunctions. Feeding chickens a mash containing as low as 0.2% sulfonamides for 2 weeks is toxic.

Clinically affected birds have ruffled feathers; are depressed, pale, and icteric; and have poor weight gain and a prolonged bleeding time. In laying birds, sulfonamides cause a marked decrease in egg production, thin rough shells, and depigmentation of brown eggs. The temperature of affected birds is often elevated. At postmortem, hemorrhages are found in the skin, muscles (especially those of thighs and breast), and in the internal organs. Once these effects are noticed, the concentration of sulfonamides in the ration should be evaluated and the feed involved withdrawn.

Other chemotherapeutic agents sometimes involved in poisoning poultry are the other coccidiostats, such as nicarbazine, zaolene, and nitrophenide, and the ionophore monensin. As little as 0.006% nicarbazine in the diet causes mottled yolks, and at 0.02% there is depressed rate of growth and reduced feed efficiency. Feeding 0.025% nicarbazine to day-old chicks for 1 week resulted in the chicks becoming dull, listless, weak, and ataxic.

Feeding zaolene at twice the recommended level of 0.025% will cause nervous signs and depress growth and feed efficiency. The nervous signs include stiff neck, staggering, and falling over when the birds are excited.

Nitrophenide possesses marked electrostatic properties and, therefore, sticks to the walls of a feed mixer. The last bits of feed in the feed mixer will normally contain a high concentration of nitrophenide and that elevated concentration has caused disturbances in posture and locomotion, retarded growth, and increased mortality in chickens. Postural disturbances include a tilted position of the head, tremor of the neck, and difficulty in maintaining the righting reflex.

In general poultry are more resistant to monensin toxicity than other species, but there have been reports of monensin toxicity in turkeys accidentally fed rations containing 250 ppm monensin. There is a big difference in susceptibility to monensin poisoning among various species of poultry. Chickens and turkeys less than 2 weeks old are more resistant than older birds, but keets (young guinea fowl) seem more susceptible than their adults and the young of other species. For example, diets of 200 ppm monensin were not toxic for poults, whereas 100 ppm was toxic for keets.

Cresol

Cresol was a commonly used disinfectant in poultry houses but has been gradually withdrawn and replaced by less toxic disinfectants. Nevertheless, in some regions and countries cresol is still being used. Cresol poisoning in the chicken usually occurs at 3–6 weeks of age. Affected chicks are depressed and have a tendency to huddle. There are respiratory problems such as rales, gasping, and wheezing. With prolonged cresol exposure some chicks will develop edema of the abdomen.

Sodium Chloride (Salt)

All poultry and pigeons are susceptible to salt poisoning. Young birds are more susceptible than adults. Although both acute and chronic forms of salt poisoning can occur, the chronic form is more commonly encountered and is due to prolonged ingestion of feed containing high salt content. Levels of 0.5% and above in drinking water or 5–10% in feed cause death in baby chicks.

Signs of salt poisoning in poultry include anorexia, thirst, dyspnea, opisthotonos, convulsions, and ataxia. Increased water consumption may be the most significant early indicator of hazardous exposure to salt in poultry.

Insecticides

Chlorinated hydrocarbon insecticides and organophosphate compounds are used regularly around poultry houses to control external parasites. Commonly used organochlorine insecticides include chlordane, dieldrin, DDT, heptachlor, and lindane. Occasionally, birds get exposed by gaining access to sprayed grounds such as golf courses.

Chlordane causes chicks to chirp nervously, rest on their hocks, and lie on their sides. The birds then become hyperexcitable as the condition progresses. In adult birds there is reduced food consumption, decreased body weight, and a fall in egg production.

Consumption of seeds dressed with dieldrin has been a source of exposure in wild birds. Affected birds are listless and have coordination problems while lighting; severely poisoned cases have nervous signs characterized by lateral movements of the head and tremors of the head and neck. Birds die in violent convulsions.

DDT toxicity in chickens is characterized by hyperexcitability and fine tremors in severe cases. Moderate cases are characterized by loss of weight, molting, and reduced egg production.

Lindane in the form of a dust is frequently used around chickens. Adult chickens poisoned by lindane stop eating, manifest opisthotonos and flapping of wings, have clonic muscle spasms, and die in a coma.

The organophosphate compounds commonly involved include diazinon, malathion, and parathion. Diazinon is applied to chicken premises, but this compound is very toxic to ducklings. When used at rates recommended for chickens, 100% mortality has resulted from use of this compound on 1- or 2-week-old ducklings. Experimental studies suggest that goslings are three times more sensitive than ducks, chickens, and turkeys. Poisoned birds are unable to stand, salivate profusely, and manifest tremors of the head and neck. Brain cholinesterase levels in birds that die of organophosphate poisoning are on the average 69% less than those of controls.

Other organophosphate compounds commonly used on chicken premises include dichlorvos, malathion, and parathion. Birds poisoned by these

compounds manifest signs similar to those seen in diazinon poisoning. Other effects that may be seen include depression, ataxia, and reluctance to move; paralysis and lacrimation; gasping for breath; and development of diarrhea, crop stasis, and dyspnea.

In general ducks are more sensitive to organophosphate poisoning than are chickens, and care should be exercised when using these products on premises holding ducks.

The carbamate insecticide sevin is a widely used poultry insecticide. This compound is relatively safe, but deaths have been reported in turkey poults kept on premises where the product has been excessively applied at 10 times the recommended rate. The clinical signs are similar to those caused by organophosphate insecticides.

Heavy Metals

Lead poisoning is not as common in domestic poultry as in wild birds, but it is the most common toxicity reported in the avian species. Lead shot has caused losses in waterfowl populations throughout North America. All birds are susceptible to lead poisoning, but most losses are reported in waterfowl because their feeding habits predispose them to the ingestion of lead pellets from shotguns and other sources.

Characteristic signs of lead poisoning are related to CNS derangement, such as ataxia, depression, paralysis of the wings, and convulsions. In some cases the birds presented are anemic, emaciated, regurgitating, and weak. Green diarrhea has often been reported in affected birds.

Yellow phosphorus is a highly toxic element that is still used as a rodenticide. Poultry and wild birds can be intoxicated by consumption of bait intended for rodents. Firework fragments also are a common source of poisoning in free-ranging birds. Affected birds are depressed and anorectic, have increased water consumption, and manifest diarrhea, ataxia, paralysis, coma, and death.

Rodenticides

In addition to the metal yellow phosphorus being used as a rodenticide, other rodenticides are potentially toxic to poultry and other birds. The clinical signs caused by these rodenticides in birds are similar to those observed in other animals.

Birds occasionally consume baits containing anti coagulant rodenticides. The more potent second-generation rodenticide-containing baits, such as brodifacoum, are especially dangerous to birds. These coumarin anticoagulants act by interfering with vitamin K utilization, causing bleeding because of depletion of vitamin K-dependent clotting factors. Poisoned birds bleed from their nares and subcutaneously and have oral petechiations. Quite often the birds are also weak and depressed from the resulting anemia or may be found dead due to stress superimposed on the anemic condition.

Of special interest are secondary intoxications due to free-ranging birds consuming carrions of animals that died of rodenticide poisoning. Strychnine and sodium monofluoroacetate are other rodent control compounds that are involved because they cause acute death in the primary victims and are thus present in high concentrations in carrions.

Strychnine-poisoned birds show clinical effects within 2 h of ingesting the product. The birds become apprehensive and nervous and have violent tetanic convulsions, which cause them to become exhausted and to die of hypoxia. Sodium monofluoroacetate causes overstimulation of the CNS and myocardial depression. Cardiac failure is the cause of death and occurs within 1 h of consuming the product or contaminated carcass.

Mycotoxins

Mycotoxicoses are common problems for the poultry industry in warm moist climates and in developing countries in the tropics. Aflatoxins are the most commonly involved mycotoxins. Poultry are normally exposed by consumption of contaminated feed, especially corn. Some developing countries lack the resources to adequately screen contaminated corn. In other instances poultry feed is made from the poor-quality (and contaminated) corn that has been rejected for human consumption.

Aflatoxicosis in poultry can be either acute or chronic in nature, depending on the exposure dose. Ducklings are more susceptible to aflatoxin than are turkeys, pheasant, or chickens. In acute cases, affected birds become lethargic, their wings droop, and they manifest nervous signs such as opisthotonos; they die with their legs rigidly extended backward. Chronic dietary consumption of 2.5 ppm aflatoxin causes a significant drop in weight gain and egg production.

Perhaps more important is the increased susceptibility of the affected flock to infection because chronic consumption of aflatoxin-containing feed lowers the immunity of the birds. Aflatoxicosis is therefore a disease of serious economic consequences to the poultry industry in developing countries both through lowered productivity and because of death of affected birds.

Ergot poisoning has been reported in areas where rye is commonly used as poultry feed. In acute

ergot poisoning the birds' combs are cold, wilted, and cyanotic. The animals are weak, thirsty, and have diarrhea. In severe cases the birds go into convulsions, become paralyzed, and die. Ochratoxins have been reported to cause renal toxicity in poultry.

Common Toxicoses in Dogs and Cats

Dogs and cats are commonly poisoned by pesticides, herbicides, household products such as antifreeze, and drugs such as acetaminophen applied by humans to their pets. By far the most common toxicities in these small animals involve various insecticides and the overzealous use of these products by owners attempting to control fleas and ticks on their pets.

Insecticides

The insecticides most commonly involved in poisoning dogs and cats are the organophosphates and carbamates, pyrethroids, chlorinated hydrocarbons, and diethyltoluamide (DEET). The organophosphate and carbamate insecticides have a common mode of action, which is the inhibition of acetylcholinesterase. Acetylcholinesterase is the enzyme that breaks down acetylcholine, a neurotransmitter in autonomic ganglia and at cholinergic nerve endings. The inhibition of acetylcholinesterase by organophosphate and carbamate compounds causes acetylcholine to accumulate at nerve synapses and to produce persistent firing of cholinergic nerve fibers. Affected animals are overexcited and have increased respiratory rates, excessive salivation, and muscle tremors.

Treatment of animals poisoned by organophosphate compounds involves the administration of atropine and prolidoxime. Cases involving carbamates may be treated with only atropine because of the rapid biological detoxification of carbamates. The organophosphate and carbamate compounds have a relatively high acute toxicity compared to chlorinated hydrocarbons but have a lower residual activity. As such, organophosphate compounds have largely replaced the chlorinated hydrocarbons for insecticide use because of environmental concerns.

The chlorinated hydrocarbons were among the first synthetic insecticide compounds to be used but have fallen into disfavor because of their persistence in the environment. Typical examples of chlorinated hydrocarbon insecticides are DDT, lindane, and toxaphene. The toxicity of these compounds in small animals is characterized by severe CNS effects, including ataxia and convulsions. Small animals usually get poisoned by being accidentally sprayed or by drinking chlorinated hydrocarbon insecticide concentrates intended for spraying on crops. Although most of these insecticides are banned or their use highly restricted in Western countries, they are still widely applied in developing countries. Thus, chlorinated hydrocarbon insecticide poisonings still occur in the developing countries.

Another group of insecticides commonly involved in small animal poisonings are plant product derivatives – pyrethrins and their synthetic congeners, the pyrethroids. These products are currently enjoying a resurgence because of their selective insecticidal properties and absence of environmental persistence. These compounds are mainly metabolized in the body by liver glucuronidation. The cat is the most sensitive domesticated animal to pyrethrin toxicity because of the low activity of the glucuronide conjugating system in this species. Young cats, less than 6 weeks of age, are the most sensitive.

Pyrethroid compounds formulated with the insect repellant DEET were responsible for numerous deaths in cats and dogs in the past decade. Pyrethroids interfere with sodium channels in nerves causing them to fire repetitively. Clinical signs of pyrethroid poisoning in small animals include ataxia, excitement, and muscle fasciculations and tremors. There is no antidote for pyrethrin poisoning, but symptomatic treatment, such as decontamination procedures and sedation, usually results in full recovery.

Rodenticides

Rodenticide poisoning is commonly seen in all small animals. Rodenticides are widely used around farmhouses to control rodents, such as rats and mice, which destroy property and farm produce. Several classes of rodenticides are currently in use, including the anticoagulant rodenticides (warfarin and its second-generation cousin brodifacoum), zinc phosphide, strychnine, compound 1080, and arsenicals. Small animals get poisoned by either consuming baits directly or through consumption of the carrion of animals that have died of rodenticide poisoning. The clinical signs seen will vary with the compound involved and, in the majority of cases, dogs (because of their indiscriminate eating habits) are involved.

Strychnine and anticoagulant rodenticides are the most frequently reported offenders. Strychnine poisoning in dogs is a rapidly developing syndrome characterized by tonic–clonic seizures. These signs result from strychnine competitively blocking the inhibitory neurons in the nervous system. The animals start showing clinical effects within 20 min to 1 h of ingesting strychnine and, if the animal has ingested a

sufficient amount, death from anoxia occurs acutely. Anoxia results from paralysis of the respiratory muscles. Treatment of strychnine poisoning is symptomatic and involves general decontamination procedures, use of sedatives such as phenobarbital and diazepam, maintenance of adequate urine output, and respiratory support. The sedatives control the seizures and allow the vital muscles to relax and maintain their lifesaving functioning.

The anticoagulant rodenticides have been in use for many decades. Because of the long time required for them to take effect, some strains of rats have become genetically resistant to the so-called first-generation anticoagulant rodenticides such as warfarin. This has led to the introduction of second-generation rodenticides such as brodifacoum. Unlike the first-generation rodenticides, which took at least 24–48 h to take effect, the second-generation rodenticides act relatively acutely, and clinical signs can be evident within a few hours and have a long residual action.

These anticoagulant rodenticides act by inhibiting vitamin K-dependent blood coagulation factors (VII, IX, and X), by decreasing prothrombin synthesis, and by directly damaging blood capillaries. Animals poisoned by the anticoagulant rodenticides are weak, have swollen joints because of bleeding into the joint cavities, may hemorrhage from the nostrils, and may pass bloodstained feces. Treatment of anticoagulant rodenticide poisoning involves whole blood transfusions if the bleeding and resulting anemia is severe and vitamin K_1 injections for several days. Early intervention requires general decontamination procedures to limit further rodenticide absorption, especially in the case of exposure to second-generation rodenticides, followed by prolonged vitamin K_1 therapy.

The toxicity of zinc phosphide results from the phosphine gas, which is produced by acid hydrolysis of the pesticide in the stomach. Animals with partially filled stomachs are more sensitive to zinc phosphide poisoning than those with empty stomachs because of the greater acid secretion precipitated by the presence of food. The generated phosphine gas is absorbed systemically and exerts its effects in the lungs. Poisoned animals exhibit respiratory difficulties because of the buildup of fluids in the lungs. The cause of death is respiratory failure. Supportive therapy, including respiratory support, is recommended in cases of zinc phosphide poisoning, but the prognosis is poor because no effective antidote is available.

Compound 1080 (sodium monofluoroacetate) is a very lethal toxicant which acts by blocking the Embden–Meyerhoff pathway, thereby depriving vital cells of energy. Fluoroacetate is metabolized to fluorocitrate, which inhibits mitochondrial aconitase. This blocks adenosine triphosphate production. Affected animals are initially uneasy, then they become excitable and will run in various directions in a frenzy, and finally they will fall into seizures and die of anoxia. There is no antidote and, once clinical signs develop, poisoned animals will almost always die within a few hours.

Cholecalciferol is a rodenticide that has been introduced relatively recently and that has been reported to be frequently involved in poisonings of dogs. The compound alters calcium homeostasis by promoting calcium absorption from the gut and also by mobilizing calcium from bone for tissue deposition. Consequently, poisoned animals have increased levels of blood calcium. The calcium is then subsequently deposited in soft tissues like the kidneys, digestive tract mucosa, lungs, heart, liver, and muscle. Mineralization of soft tissues interferes with normal function of these organs.

Clinically, the animals do not show signs until 24–48 or more hours after ingestion of the bait. The affected animals are depressed, have reduced urine production, and the urine is of low specific gravity. Severely poisoned animals have hematemesis, azotemia, and cardiac arrhythmias. Animals with renal impairment are more susceptible to cholecalciferol poisoning than those with normal renal function. Cholecalciferol poisoning requires protracted treatment, which may require as long as 3 weeks in severe intoxications. Appropriate treatment consists of fluid therapy to assist the kidneys in removing the excess calcium, corticosteroids to minimize inflammation, and calcitonin to enhance calcium resorption into the bone. Pamidronate disodium is the new antidote for this poison.

Several other rodenticides can cause poisoning in small animals but do so less frequently because these rodenticides are used less often. Red squill and thallium have been used as rodenticides for many years. Red squill acts as a cardiotoxicant and causes death by cardiac arrest. It also produces convulsions and paralysis. Thallium is a general systemic toxicant. It has a high affinity for sulfhydryl groups throughout the body. Thallium causes cracking at the corners of lips and also causes hair loss. α-Naphthylthio-urea causes death by inducing lung edema and subsequently leading to anoxia. White phosphorus is a hepatorenal toxicant. Animals poisoned by white phosphorus have severe abdominal pain, hepatomegaly, and signs of hepatic insufficiency, such as prolonged bleeding and hypoglycemia.

Cases of rodenticide poisoning in small animals should always be regarded as emergencies. General decontamination procedures, such as inducing

vomiting with hydrogen peroxide or apomorphine, the use of activated charcoal to bind the unabsorbed toxicant(s), and/or enterogastric lavage, should almost always be employed to minimize absorption and the resulting hazard from the toxicant(s).

Herbicides

Herbicides are not often involved in small animal toxicity despite their frequent use around farms and the continual possibility of exposure. However, toxicity in dogs from consumption of herbicide concentrates during mixing is occasionally reported. The triazine herbicides act by inhibiting plant photosynthesis and are generally safe products for mammals. The LD_{50} value of these compounds is at least $1900\,mg\,kg^{-1}$ body weight in the rat. Therefore, toxicity in dogs can only practically occur following the ingestion of large volumes of concentrates. In experimental situations, triazine herbicide-poisoned dogs can become either excited or depressed, develop motor incoordination, and may proceed to have clonic–tonic spasms.

Some inorganic arsenic compounds are also used as herbicides. These inorganic arsenicals are general protoplasmic poisons and are therefore hazardous to both plant and animal life. Affected dogs almost always vomit, have severe abdominal pain, and develop bloody diarrhea. The vomitus may contain mucous shreds and blood from erosion of the gastric and intestinal epithelium.

Paraquat, although restricted from use in Western countries, is a highly toxic herbicide that is still readily available in developing tropical countries. Upon intake, paraquat is rapidly metabolized in the liver and the lungs with the production of secondary oxygen radicals. It is these radicals that cause injury to tissues and especially do so to the lungs. Poisoned animals die acutely of respiratory failure.

Unlike other animals, the dog appears sensitive to chlorphenoxy herbicides, such as 2,4-D; the dog's oral LD_{50} to 2,4-D is $100\,mg\,kg^{-1}$ body weight. Ventricular fibrillation is the cause of death in severely poisoned dogs. Ingestion of sublethal doses induces stiff extremities, ataxia, myotonia, paralysis, coma, and subnormal temperatures.

Chlorates are herbicides that are often used along roadsides. They are rapidly metabolized in the liver to the chlorate ion, which induces methemoglobinemia in both cats and dogs. Cats, however, because of the greater susceptibility of their hemoglobin molecule to oxidation, are more susceptible to chlorate poisoning than dogs.

Organophosphate herbicides (e.g., glyphosate and merphos) are weak cholinesterase inhibitors and are moderately toxic to dogs and cats. Carbamate herbicides are not inhibitors of acetylcholinesterase but are also moderately toxic to dogs. The LD_{50} of most of the carbamate herbicides is at least $5000\,g\,kg^{-1}$ body weight.

Household Chemicals

Antifreeze is the household product most commonly involved in small animal poisonings. The active ingredient in antifreeze is ethylene glycol. The characteristic sweet taste of this compound makes it attractive to small animals. Ethylene glycol is metabolized in the liver by alcohol dehydrogenase to glycolic acid and then to oxalate. Glycolic acid contributes to acidosis, which is characteristic of ethylene glycol poisoning. The oxalic acid binds calcium from blood to form calcium oxalate, which is filtered by the glomerulus into renal tubules where it precipitates into crystals which cause blockage of the tubules. Consequently, severely affected animals have renal failure characterized by anuria and uremia. The binding of blood calcium to the oxalic acid produces hypocalcemia, which, if severe, can also cause death.

Ethylene glycol poisoning is treated by giving ethanol if the animal is presented within 4 h of suspected ingestion and by giving quantities of fluids containing sodium bicarbonate to facilitate flushing the calcium oxalate crystals from the kidneys and also to correct the acid–base imbalance. Alcohol dehydrogenase, the enzyme which reduces ethanol to acetic acid and water, prefers ethanol to ethylene glycol and, in the presence of both substrates will metabolize ethanol, leaves ethylene glycol to be excreted unchanged in urine. 4-Methylpyrazole is newly reported to have effective antidotal properties against ethylene glycol poisoning.

Household products, such as sink cleaners, dishwashing detergents, and toilet cleaners, are also common causes of poisonings in small animals. The majority of cleaning detergents are corrosive compounds, which contain strong alkali, acids, or phenolic compounds. These compounds act as contact poisons, causing coagulative necrosis of the tissues they come in contact with. Following ingestion of these products, the dog or cat will vomit, have severe abdominal pain, and may develop diarrhea. The animal's vomitus and feces may be bloody. Consuming animals may show other signs depending on the specific ingredients of the ingested products. For example, products containing phenolic derivatives will cause acidosis and hepatotoxicity.

In general, treatment following ingestion of household products is symptomatic and involves the administration of adsorbents such as activated

charcoal, gastrointestinal protectants such as Peptobismol, and the correction of any systemic disturbances (such as acidosis) which may accompany the poisoning. Animals should also be provided abundant glucose and a high protein diet.

Garbage

Garbage poisoning is a frequently encountered problem in small animals. This condition is also referred to as enterotoxicosis or endotoxemia, depending on whether poisoning is due to bacterial infection or due to bacterial endotoxins. Dogs and cats not well fed and/or not closely supervised when allowed to roam will eat garbage. Cats may also be affected, but only rarely so, because they are discriminate eaters. The bacteria most commonly involved are coliforms, staphylococci, salmonellae, and occasionally *Clostridium botulinum*. Enterotoxemia-affected animals often develop a bacteremia after eating infected carrion. Clinical signs normally appear at least 24–48 h after ingestion of the infected material.

The condition is characterized by anorexia, vomiting, severe abdominal pain, fever, and a bloody diarrhea. Endotoxemia poisoning is due to bacterial endotoxins, which are normally present in bacterial cell walls. The clinical signs are generally indistinguishable from those of enterotoxemia, except that in the latter there is no bacteremia; this can be evaluated by performing a blood culture. Although rare in occurrence, botulism is a rapidly developing fatal disease resulting from ingesting bones contaminated with *C. botulinum*. In small animals the disease is characterized by an ascending paralysis. At first there is muscle weakness and incoordination in the hindlimbs. As the paralysis progresses anteriorly, dyspnea and convulsions develop.

Garbage poisoning is rarely a severe condition in small animals because the animals usually vomit and thereby reduce the amount of toxicant ingested. However, in severe cases veterinary attention will be required. If the cat or dog is presented early after ingestion, general decontamination procedures should be performed to reduce absorption. Antiinflammatory corticosteroids and antibiotics should be given. Further treatment involves appropriate supportive therapy.

Heavy Metals

Lead and arsenic are the heavy metals most frequently seen in small animal poisonings. Lead poisoning is more commonly reported in the dog than in the cat, but both species are susceptible. The sources of canine lead poisoning include ingested leaded objects, such as lead weights, and paint chips from old houses being renovated. The clinical signs of lead poisoning in the dog involve primarily the CNS. Dogs may also be presented having abdominal pain and diarrhea in addition to the CNS involvement. Lead poisoning is a chronic disease in dogs, but the overt CNS signs may appear suddenly. Lead poisoning also causes blood dyscrasias characterized by reticulocytosis and anemia. Similar clinical signs are elicited in the cat. Treatment consists of giving chelating agents, such as calcium-EDTA, BAL, dimercaptosuccinic acid, or D-penicillamine.

Arsenic is the active ingredient in some insecticides, in rodenticides, and in some industrially used herbicides. Inorganic and aliphatic organic arsenic compounds are rapidly absorbed from the gut, skin, and lungs and are more toxic than the cyclic organic arsenicals used as feed additives. Trivalent arsenic is the proximate toxicant of the pesticide arsenicals which reacts with sulfhydryl groups of proteins throughout the body. It is, therefore, a general poison, inhibiting the sulfhydryl-containing enzymes it comes in contact with. The clinical signs of inorganic arsenic poisoning in dogs include anorexia, severe abdominal pain, bloody diarrhea, and hair loss, as discussed previously for herbicides. Treatment includes thorough decontamination, chelation therapy with BAL, and aggressive supportive therapy.

Toxic Plants and Mushrooms

Although one would not expect dogs and cats to commonly eat plants, plant poisoning is surprisingly often reported in these species. Because of their exploratory behavior, puppies and kittens are most often involved. Boredom and unfamiliarity due to change of environment are some of the predisposing factors that lead to plant ingestions by dogs and cats. Poisonous ornamental plants (e.g., philodendron and rhododendron) and plants growing around fences (e.g., cassia and oak) are often involved. The range of potentially poisonous plants is vast, and the clinical signs are diffuse and similar to those reported for food-producing animals.

Occasionally, dogs or cats will eat poisonous mushrooms or be fed poisonous mushrooms by uninformed owners. *Amanita muscaria* and *A. pantherima* are acutely toxic and induce signs within 15–30 min of ingestion. These two mushroom species cause nervous signs which include salivation, pupillary constriction, muscular spasms, drowsiness or excitement, and, in severe intoxications, coma and death. Ibotenic acid and muscimol are the active chemical components. However, consumption of *A. phaloides*, *A. virosa*, and *A. verna* produces gastrointestinal signs which become evident 6–12 h postingestion. These effects include violent vomition, muscle cramps, diarrhea, and

dehydration. These latter mushrooms also cause hepatic damage that becomes apparent 3–5 days after ingestion. Phalloidin and α- and β-amanitin are the poisonous principles in this group of fungi.

Common Toxicoses in Horses

In comparison to food-producing animals and cats and dogs, horses are less frequently poisoned. The most commonly encountered equine toxicoses are caused by pesticides, snakebites, arsenic, selenium, monensin, cantharidin, and mycotoxins. Most plants that are hazardous to food-producing animals are also toxic to horses, but horses are less frequently affected since owners usually assure the availability of good feed. Horses are very sensitive to monensin and cantharidin poisonings.

Insecticides

The pesticides most frequently responsible for equine poisonings are the organophosphate, carbamate, and chlorinated hydrocarbon insecticides. Both the organophosphates and the carbamates are acetylcholinesterase inhibitors and present clinical pictures similar to those seen in food-producing animals. Affected horses salivate and sweat profusely and have muscle incoordination and ataxia. The chlorinated hydrocarbons are strong CNS stimulants; affected horses become hyperalert, then excited, and, in severe cases, develop convulsions. In almost all instances, the mode of horses being exposed to pesticides is topical.

Monensin

Horses are highly susceptible to monensin poisoning in comparison with the other domesticated animals. Monensin is an ionophore normally added to cattle and poultry feed to provide growth stimulation by enhancing the intestinal absorption of calcium and sodium. Horses are easily poisoned by accidentally consuming cattle or poultry feed containing the recommended amounts of monensin for those species. Affected horses can die suddenly without any other signs. Monensin affects the cardiac and skeletal muscles, and acute cardiac failure is the cause of death.

Blister Beetles

Cantharidin is an irritant toxic agent present in blister beetles. Only a few of the several species of blister beetles contain cantharidin. Blister beetles are abundant in mid-summer and late summer when alfalfa hay containing the beetles is harvested in the central plains of the United States. Horses are poisoned by eating the alfalfa hay containing crushed swarms of blister beetles. Affected horses develop severe colic, abdominal pain, and blood-tinged urine. They will kick at their bellies and roll on the ground; severely affected horses may die of shock. Although recommended treatment involves the use of pain killers, such as banamine hydrochloride, and large volumes of intravenous fluids, there is no effective therapy for affected horses.

Heavy Metals

Lead poisoning in horses is characterized by neurological effects. Affected horses will be either depressed or excited. Colic and diarrhea are also seen. Because of laryngeal nerve paralysis, horses poisoned by lead also present with difficult respirations and a roaring syndrome. Abortions may also occur.

Arsenic poisoning in horses is usually caused by the consumption of foliage which has recently been sprayed with arsenic herbicides. The condition is acute and characterized by intense colic and hemorrhagic diarrhea. As in other animals, inorganic arsenic poisoning does not affect the nervous system, which helps differentiate this poisoning from organophosphate or carbamate poisonings.

Selenium is an essential element but is toxic when excessive quantities are ingested. Exposure of horses is usually through consumption of seleniferous (accumulator or indicator) plants (e.g., *Astragalus* spp.). Exposure to high quantities of selenium over a short time causes diarrhea (which is often foul smelling and contains air bubbles), neurological and cardiovascular effects, and respiratory difficulty. Death in these horses is due to respiratory failure. Chronic exposure to low levels of excessive selenium is characterized by hoof abnormalities at the coronary bands and by discoloration and loss of hair. The hoof deformities are painful and cause lameness.

Toxic Plants

The plant poisonings commonly encountered in horses are those that cause gastrointestinal problems, liver damage, primary or secondary nervous system involvement, and sudden death. Plants such as castor bean and oleander cause colic and diarrhea. Oleander also causes cardiac toxicity. Prolonged ingestion of some plants for several weeks can lead to liver damage and hepatic cirrhosis. This commonly occurs with the hepatotoxic plants *Amsinckia*, *Senecio*, and *Crotolaria*. Liver damage compromises the ability of the horse to detoxify ammonia that accumulates *in vivo,* leading to CNS derangement.

Plants that can cause CNS stimulation include larkspur, locoweed, lupine, water hemlock, and fitweed. Common plants that produce CNS depression are black locust, bracken fern, horsetail, milkweed,

and white snake root. Like ruminants, horses will avoid eating toxic plants because they are usually not palatable. Therefore, consumption of poisonous plants will most often occur during drought conditions or following overgrazing when the animals lack suitable pasture.

Sudden death in horses can be caused by consumption of cyanide-containing plants, such as sorghum. The cyanide ion forms a complex with cytochrome oxidase. This prevents electron transport and the utilization of oxygen by body tissues. As a consequence, the circulating blood is well oxygenated and is bright cherry red in color. This condition is an emergency, and treatment requires the prompt intravenous administration of both sodium thiosulfate and sodium nitrite.

Horses, like other monogastrics, are more resistant to plants capable of causing nitrate–nitrite poisoning than are ruminants. However, horses can modestly reduce nitrates to nitrites in their cecums, but it requires about three times as much nitrate to produce the same toxic effect in horses as in ruminants.

Mycotoxins

Contaminated grains (corn, wheat, and milo) are the sources of mycotoxin exposure for horses. The most commonly involved mycotoxins are aflatoxins, T-2, and fumonisin B_1. Aflatoxins will cause nonspecific effects, such as a poor thriving condition, hemorrhages, and abortions. T-2 is a trichothecene mycotoxin that causes prolonged bleeding times and digestive tract inflammation in affected horses.

A specific mycotoxin which uniquely affects horses is fumonisin B_1, which is produced by the fungus *F. moniliforme*; it has been responsible for causing the condition called equine leukoencephalomalacia. Horses receive the fumonisin by consumption of molded corn. Affected horses become anoretic and depressed after consuming the infested grain for only a few days. The condition then progresses, with the animals becoming blind, walking aimlessly, head pressing, being unable to swallow, and dying in a coma 1–4 days after the initial onset of signs.

Conclusions

The broad discipline of veterinary toxicology has been presented using brief accounts of the common toxicities in different animal species to draw the attention of the reader to similarities and differences in their reactions to toxicants. Because some animals are more sensitive than others receiving the same toxicant, the diagnosis of some poisonings may require the help of toxicologists within the veterinary profession.

This entry was not intended as a detailed reference for diagnosis and treatment of animal poisonings, nor was it meant to be all-inclusive. Rather, it presents the commonly encountered toxicoses in veterinary medicine.

It should be clear that all animals are susceptible to some toxicants and that some toxicants are toxic to all animals (including humans). It is therefore important to be cautious when handling and using chemicals around animals; also, a clean environment must be provided for all animals. Domestic animals particularly are subject to the whims of their owners for hazard-free environments. Animals should be fed well-balanced quality food from reputable sources, and suspect feed should be either avoided or carefully examined for potential toxicants before being given to animals.

It is also vitally important to remember that all chemicals become poisons if the exposure rate is sufficiently high. Therefore, even useful and recommended compounds used routinely around animals (e.g., growth promoters) can be life threatening if used excessively or if given to species for which they were not intended. The susceptibility of sheep to copper-containing cattle feed or the high risk to horses when fed poultry feeds containing monensin are cases in point.

See also: Aflatoxin; Algae; Ammonia; Asbestos; Benzene Hexachloride, Mixed Isomers; Brodifacoum; Carbamate Pesticides; Carbon Monoxide; Castor Bean; Copper; Coumarins; DDT (Dichlorodiphenyltrichloroethane); DEET (Diethyltoluamide); Dichlorvos; Dieldrin; Ethylene Glycol; Hydrogen Sulfide; Lead; Malathion; Methane; Molybdenum; Mushrooms, Coprine; Mushrooms, Cyclopeptide; Mycotoxins; Nitrites; Oleander; Organochlorine Insecticides; Organophosphates; Paraquat; Parathion; Pyrethrins/Pyrethroids; Selenium; Sodium; Strychnine; Sulfur Dioxide; Thallium; Warfarin.

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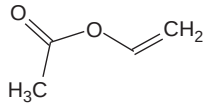
Vinyl Acetate

Heriberto Robles

- Chemical Abstracts Service Registry Number: CAS 108-05-4
- Synonyms: Acetic acid ethenyl ester; Acetic acid ethylene ether; Acetic acid vinyl ester; Ethenyl acetate; Vinyl ethanoate
- Chemical Formula: $C_4H_6O_2$
- Chemical Structure:



Uses

Vinyl acetate is used in the manufacturing of polyvinyl compounds, resins, glues, polymer adhesives, vinyl copolymers, plastics, and latex paints.

Background Information

The International Agency for Research on Cancer has determined that vinyl acetate is possibly carcinogenic. This determination is based on inadequate human epidemiological data and limited evidence of carcinogenicity in laboratory animals.

Exposure Routes and Pathways

Workers handling vinyl acetate may be exposed to it through vapor inhalation and skin contact with its liquid form.

Toxicokinetics

Vinyl acetate is moderately toxic when administered through ingestion, inhalation, and peritoneal injection. At low to moderate doses, it produces irritation at the point of contact. Prolonged dermal exposure may produce severe irritation and skin blistering.

Mechanism of Toxicity

Vinyl acetate is rapidly metabolized in the body to acetaldehyde. *In vitro* studies have revealed similar toxic effects in cell cultures incubated in the presence of either vinyl acetate or acetaldehyde. These results suggest that the acetaldehyde is the ultimate toxic metabolite of vinyl acetate.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute exposures of humans to high concentrations may result in tissue irritation at the point of contact (e.g., skin, eyes, respiratory tract). Signs and symptoms associated with potential routes of exposure include:

- *Inhalation:* Nose and throat irritation with cough and hoarseness. Olfactory fatigue may develop after continuous exposure.
- *Ingestion:* Vinyl acetate seems to have low toxicity when administered by ingestion.
- *Skin:* Skin irritation that may progress to blistering if product is allowed to remain on the skin.
- *Eye:* Eyes irritation and mild (reversible) corneal injury.

Chronic Toxicity (or Exposure)

Animal

Chronic inhalation exposure in rats produces nasal cancer. In addition, thyroid cancers and effects on the male reproductive system have been reported in laboratory animals chronically exposed to vinyl acetate.

Human

Results of an epidemiological study indicated that chronic, occupational exposure to vinyl acetate at concentrations below 22 ppm is not likely to result in irritation of the upper respiratory system. In addition, no chronic adverse effects were reported for chronic exposures to concentrations between 5 and 10 ppm. Chronic exposures to moderate and high doses have been shown to produce damage and

alterations of the central nervous and cardiovascular systems as well as lung and liver damage.

In Vitro Toxicity Data

Vinyl acetate has been shown to be rapidly metabolized to acetaldehyde in cell cultures. The formation of acetaldehyde is believed to be a precursor to the chromosome cell damage, sister chromatid exchange, and DNA crosslinking seen in cell cultures treated with vinyl acetate.

Clinical Management

There is no specific treatment for vinyl acetate toxicity. Supportive and symptomatic treatment is recommended.

Environmental Fate

Vinyl acetate is expected to have a short half-life in environmental media. If released to soil, it will either volatilize or be hydrolyzed in the presence of soil moisture. If released to air, it is expected to degrade by reacting with either hydroxyl radicals or ozone. If released to water, vinyl acetate is likely to either volatilize to the atmosphere or undergo hydrolysis and biodegradation. Biodegradation of vinyl acetate is known to occur under both aerobic and anaerobic conditions.

Exposure Standards and Guidelines

Special precautions must be taken when working with vinyl acetate. Personnel handling this chemical must follow industrial hygiene and health protection requirements for handling potentially carcinogenic substances. A minimum vinyl acetate exposure should be minimized through the use of engineering controls, work practices, and personal protective equipment, including impervious and disposable gowns and gloves as well as eye and respiratory protection. In addition, working areas and working instruments must be especially designed for handling potentially harmful substances.

See also: Polymers.

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Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Vinyl Acetate.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Vinyl Acetate.

Vinyl Bromide

Karl K Rozman

- Chemical Abstracts Service Registry Number: CAS 593-60-2
- Synonyms: Bromoethylene; Monobromoethylene; Bromoethene
- Chemical Formula: C_2H_3Br
- Chemical Structure: $CH_2 = CH–Br$

Uses

Vinyl bromide is not known to occur as a natural product. It is commercially synthesized either by a metal halide catalyzed reaction of acetylene with hydrogen bromide or from ethylene dibromide with potassium hydroxide. Vinyl bromide is primarily used in flame retardant synthetic fibers, as a copolymer with vinyl chloride in films, laminating fibers, and as a rubber substitute.

Exposure Routes and Pathways

Workers may be exposed occupationally via inhalation during manufacture or use.

Toxicokinetics

Vinyl bromide is readily absorbed by all routes of exposure. Like all other volatile organics, the major route of exposure is via inhalation, though low level exposure in the drinking water and through the skin may also occur. Because of its low solubility in water, its absorption through the lungs will be

ventilation-limited. Due to its lipophilicity it will be preferentially sequestered in tissues of high lipid content. Biotransformation is the critical step in its clearance. Vinyl bromide, like its other halogenated analogues, vinyl chloride and vinyl fluoride, will be converted to its epoxide (2-bromoethylene oxide) by mixed function oxidases. This epoxide reacts with both RNA and DNA to form corresponding covalently bound etheno adducts, which are identical chemical moieties after exposure to any of the vinyl halides. A minor metabolite of vinyl bromide is 2-bromoacetaldehyde which is a rearrangement product of the epoxide. This reactive aldehyde avidly binds to protein. Elimination of vinyl bromide occurs by metabolic clearance and by exhalation of the unchanged parent compound. Its biotransformation is saturated at $\sim$250 ppm. Consequently, at higher concentrations first-order clearance ($18\,l\,h^{-1}\,kg^{-1}$) becomes a zero-order process ($40\,\mu mol\,h^{-1}\,kg^{-1}$). Its kinetic half-life below saturation of metabolic clearance is $\sim$1 h. Since adduct formation involves nucleophilic displacement of bromide, serum levels of bromide will rise to steady state concentrations up to metabolic saturation in accordance with the half-life of bromide (3–5 days in rats, $\sim$12 days in humans).

Absorption, distribution, biotransformation, and excretion of vinyl bromide have not been extensively studied in humans but are unlikely to be significantly different from vinyl chloride which has been studied in humans.

Mechanism of Toxicity

The most sensitive endpoint of toxicity of vinyl bromide is angiosarcoma of the liver. The dynamic half-life of the etheno-DNA-adduct is $\sim$30 days, which is much slower than the kinetic half-life and therefore represents the rate-determining step in its carcinogenic action. Thus, the dynamics of angiosarcoma are not driven by the kinetics of vinyl bromide but by the DNA-adduct which will accumulate for 6.64 half-lives ($\sim$200 days) to a very high steady state concentration even if exposure occurs only sporadically within 6.64 dynamic half-lives. Therefore, it must be understood that in the carcinogenicity of vinyl bromide it is not the time-weighted average of exposure that is important but peak concentrations. In agreement with metabolic saturation is the truncation of the angiosarcoma dose response at an inhalation concentration of 250 ppm. Thus, the mechanism of carcinogenicity of vinyl bromide is one of the best understood among the many compounds studied and the human and animal data base is without internal inconsistency.

Acute and Short-Term Toxicity (or Exposure)

Animal

The oral LD_{50} of vinyl bromide was $\sim 500\,mg\,kg^{-1}$. No histopathological changes were found in rats exposed for 7 h to $110\,000\,mg\,m^{-3}$ (25 000 ppm) showing that vinyl bromide is moderately toxic acutely.

In subacute inhalation studies, rats were exposed to $44\,000\,mg\,m^{-3}$ (10 000 ppm) 7 h per day, 5 days per week for 4 weeks; rats, rabbits, and monkeys were exposed to 1100 or $2200\,mg\,m^{-3}$ (250 or 500 ppm) for 6 h and 5 days a week with no change in gross pathology or histopathology, revealing vinyl bromide as a moderately toxic chemical subacutely.

In subchronic studies rats inhaling $8800\,mg\,m^{-3}$ (2000 ppm) vinyl bromide 8 h per day for 5 days a week for 8–15 weeks developed ATP-ase deficient foci in the liver, indicative of its carcinogenic potential, which is also in agreement with its mutagenicity in almost every test system.

Human

Short-term inhalation of high concentrations has been reported to cause loss of consciousness which is not unexpected since all low molecular weight chlorinated aliphatics are known to possess this property. Skin and eye contact with liquid vinyl bromide produced irritation and 'frost-bite' type burn, again, not unexpected of a highly volatile compound.

Chronic Toxicity (or Exposure)

Animal

As expected, based on very strong similarity with vinyl chloride in terms of kinetics and dynamics, vinyl bromide provided a truncated, but up to metabolic saturation, perfect dose response in terms of liver angiosarcoma in both male and female rats inhaling 0, 10, 50, 250, and 1250 ppm (44, 219, 1093, and $5875\,mg\,m^{-3}$) vinyl bromide. Increased tumor incidences were also observed in squamous cell carcinomas of the Zymbal gland and neoplastic nodules and hepatocellular carcinomas.

Human

Effects on reproduction and prenatal toxicity have also not been reported nor mutagenicity or carcinogenicity in human populations. Even though there is no epidemiological evidence that vinyl bromide causes liver angiosarcomas in humans, its close structural analog, vinyl chloride, is a confirmed human carcinogen having caused liver angiosarcomas

in workers first exposed to vinyl chloride prior to 1968. Therefore, and since vinyl chloride, vinyl fluoride, and vinyl bromide all caused angiosarcoma of the liver in all species studied, it can be concluded with certainty that at high enough doses vinyl bromide would also give rise to liver angiosarcomas in humans. Conversely, it can also be concluded from the lack of evidence of liver angiosarcomas in humans that workers were not exposed to cancer-causing concentration/time combinations of vinyl bromide. Since vinyl bromide is commercially available only since 1968, a time at which industrial hygiene practices were already much improved (no liver angiosarcomas in workers first exposed to vinyl chloride after 1968) in fact no angiosarcomas could and should have occurred in vinyl bromide exposed workers. Since humans were shown to be less sensitive to vinyl chloride-induced cancer than were experimental animals, it is highly likely that this would also be the case for vinyl bromide.

Clinical Management

There is no specific antidote known for vinyl bromide. Since most of inhaled vinyl bromide is reexhaled and only a fraction of it is converted to adducts liberating bromide as a metabolite and since this process is saturable, it is unlikely that bromism will ever be a problem in acute or chronic vinyl bromide exposure. Acute inhalation exposure will result in anesthesia which requires immediate removal from enclosed areas and institution of standard emergency room treatment such as general support therapy with oxygenation by nonrebreather mask. Emetics should not be used after ingestion but activated charcoal should be administered. Mouth should be rinsed and up to 200 ml of water or milk should be administered, if patient can swallow. Orotracheal or nasal intubation for airway control may be necessary if patient is unconscious. Decontamination should be done and the skin burns covered with dry sterile dressing. Proparacaine hydrochloride should be used to aid eye irrigation.

Environmental Fate

Vinyl bromide's production and use as a flame retardant for acrylic fiber may result in its release to the environment through various waste streams. It may form in air as a degradation product of 1,2-dibromoethane. Vinyl bromide was detected in fumaroles and lava gas from volcanoes. If released into air, with a vapor pressure of 1030 mmHg at 25°C, vinyl bromide will exist in the gas phase. Gas-phase vinyl bromide will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated at 2.4 days. If coming in contact with soil, vinyl bromide is expected to be adsorbed to suspended solids and to have moderate mobility based upon an estimated K_{oc} of 170. Volatization from moist soil surfaces could be important in the environmental fate of vinyl bromide based upon an estimated Henry's law constant of 1.4×10^{-2} atm m^{-3} mol^{-1}. Estimated volatization half-lives from a model river and a model lake are 1.1 h and 4.1 days, respectively. An estimated bioconcentration factor of 3 suggests that the potential for bioconcentration in aquatic organisms is low.

Exposure Standards and Guidelines

The International Agency for Research on Cancer classified the carcinogenicity of vinyl bromide as having sufficient evidence in experimental animals. The National Toxicology Program Board of Scientific Counselors designated vinyl bromide as reasonably anticipated to be a human carcinogen. Occupational Safety and Health Administration had no formal permissible exposure limit (PEL) for vinyl bromide and accepted the then existing American Congress of Governmental Industrial Hygienists (ACGIH) recommendation of a 5 ppm time-weighted average (TWA) as PEL with final ruling. The National Institute for Occupational Safety and Health has no recommended exposure limit for vinyl bromide. The most recent ACGIH recommendation for vinyl bromide is a threshold limit value-TWA of 0.5 ppm (2.2 mg m^{-3}) with an A2 (suspected human carcinogen) designation. The Environmental Protection Agency's reference concentration for vinyl bromide is 0.003 mg m^{-3}.

See also: Vinyl Chloride.

Further Reading

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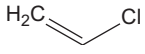
Vinyl Chloride

Robert Kapp

- Chemical Abstracts Service Registry Number: CAS 75-01-4
- Synonyms: Chlorethene; Chlorethylene; Chloroethene; Chloroethylene; Ethene, chloro-; Ethylene monochloride; Ethylene, chloro-; Monochloroethene; Monochloroethylene; Monovinyl chloride; Trovidur; VC; VCM; Vinyl C monomer; Vinyl chloride monomer; Vinyl chlorine
- Related Compounds: Vinyl bromide (CAS 593-60-2); Vinyl fluoride (CAS 72-02-5); Vinylidene chloride (CAS 75-35-4); 1,2-Dichloroethene (CAS 540-59-0); Hexachlorobutadiene (CAS 87-68-3)
- Chemical/Pharmaceutical/Other Class: Vinyl monomers; Halogenated hydrocarbons
- Chemical Formula: C_2H_3Cl
- Chemical Structure:



Uses

Vinyl chloride is produced in the following industrial reactions: (1) the thermal cracking of 1,2-dichloroethane, which is produced by the chlorination and/or oxychlorination of ethylene; and (2) the hydrochlorination of acetylene. The vast majority of vinyl chloride is used for the production of polyvinyl chloride (PVC) and the manufacture of copolymers with monomers such as vinyl acetate or vinylidene chloride. A much smaller proportion of vinyl chloride is used in the production of chlorinated solvents – primarily trichloroethanes.

Exposure Routes and Pathways

The main route of occupational exposure to vinyl chloride is by inhalation that can occur in plastics manufacturing plants. Inhalation exposure to the general public is generally quite limited and probably restricted to accidental releases from hazardous waste sites and landfills. Vinyl chloride has been detected in surface and well waters, sediment and soil samples near manufacturing facilities. Some dietary exposure can occur from leaching from certain PVC materials into packaged foodstuffs.

Toxicokinetics

Vinyl chloride is readily absorbed via all routes of exposure and rapidly distributed throughout the body. Following oral administration in male rats, peak blood concentrations are noted in less than 10 min. Approximately 40% of inhaled vinyl chloride is absorbed while as much as 95% is absorbed upon ingestion. Highest concentrations are found in the liver and kidneys.

Storage of vinyl chloride is limited by the rapid metabolism and subsequent excretion. Vinyl chloride is biotransformed by cytochrome P450-mixed function oxidase systems (CYP 2E1), with the two primary metabolites being chloroethylene oxide and chloroacetaldehyde. These materials are further converted to chloroethanol and monochloroacetic acid. Metabolites are primarily excreted in urine. When rats were exposed to vinyl chloride at 100 ppm for 5 h, ~70% of the absorbed dose was excreted as urinary metabolites within 24 h. The half-life for urinary excretion in rats was ~4 h. With an increase in dose via either inhalation or ingestion, the proportion exhaled increased and urinary and fecal elimination decreased.

Mechanism of Toxicity

The mechanism of noncancer toxicity have not been extensively studied. Some immunological changes have been noted suggesting that one of the reactive metabolites binds to IgG, thus initiating immune responses depositing precipitates that can cause blockage in capillaries. It is also suggested that peripheral nervous system symptoms such as paresthesia, numbness, and pain in the extremities may be a direct result of vinyl chloride exposure or may be due to tissue anoxia because of vascular blockage.

Vinyl chloride is also a human and animal carcinogen associated with an increased incidence of hepatic angiosarcoma. Chloroethylene oxide, chloroacetaldehyde, and monochloroacetic acid all react covalently with DNA and RNA. This alkylation results in highly effective base-pair substitutions that can lead to neoplastic transformation. These reactive metabolites might also interact with chromosomes causing clastogenic effects.

Acute and Short-Term Toxicity (or Exposure)

Animal

Vinyl chloride appears to have a low toxicity when administered by inhalation, with LC_{50} values

reported to be in the 130 000–500 000 mg l^{-1} range. The oral LD_{50}, on the other hand, is reported to be 500 mg kg^{-1}. Vinyl chloride is reported to be slightly irritating to the eyes and respiratory tract at high concentrations. Inhalation can cause headache, nausea, central nervous system (CNS) depression, lung and kidney irritation, inhibition of blood clotting, and cardiac arrhythmias in animals.

Human

Vinyl chloride is a CNS depressant; loss of consciousness can occur following exposure to high concentrations (25 000 mg m^{-3}). Acute exposure to high ambient concentrations can lead to dizziness, light-headedness, nausea, headache, irritability, cognitive problems, paresthesia, and irritation of the eyes and respiratory tract.

Chronic Toxicity (or Exposure)

Animal

Chronic animal studies report increased mortality and weight loss, as well as effects on the liver, kidney, and CNS at levels as low as 1.3 mg kg^{-1} day^{-1}. Animal studies have shown increased testicular damage as well as decreased male fertility in rats exposed to low levels of vinyl chloride for 12 months. In addition, some animal studies have shown decreased fetal weights and increased terata at maternally toxic inhalation exposure levels of vinyl chloride. Animal studies have also reported that inhaled vinyl chloride increases the incidence of angiosarcoma of the liver.

Human

Chronic inhalation or oral exposure to low levels of vinyl chloride may cause liver damage in humans. Some individuals occupationally exposed to high levels of vinyl chloride develop a specific syndrome termed 'vinyl chloride disease'. This is characterized by dizziness, numbness, earache, headache, blurred vision, fatigue, nausea, shortness of breath, Raynaud's phenomenon, loss of weight, changes in bone structure at the ends of the fingers, joint, and muscle pain, and scleroderma-type changes in the skin.

Several unsubstantiated case reports have reported reduced male sexual performance upon occupational exposure to vinyl chloride. There have been mixed epidemiological results with respect to teratogenic effects in human exposure.

Epidemiological studies conducted on humans exposed to inhaled vinyl chloride have shown increases in angiosarcoma of the liver. Hepatocellular carcinoma of the liver as well as some brain tumors have been reported; however, the data are not considered definitive. Vinyl chloride has been reported to be mutagenic and clastogenic in human studies. US Environmental Protection Agency (EPA) has classified vinyl chloride as group A, human carcinogen, and group K – known human carcinogen. International Agency for Research on Cancer classifies vinyl chloride as group 1 – carcinogenic to humans. National Institute for Occupational Safety and Health and Occupational Safety and Health Administration (OSHA) both categorize vinyl chloride as Ca – potential occupational carcinogen. US National Toxicology Program categorizes vinyl chloride as K – known to be a human carcinogen. American Conference of Governmental Industrial Hygienists (ACGIH) categorizes vinyl chloride as threshold limit value (TLV)-A1 – confirmed human carcinogen.

Clinical Management

Upon massive exposure, the primary risks are CNS effects and cardiac arrhythmias; therefore, the evaluation of vital functions and life-support measures should be taken and the victim should be decontaminated and removed from the area to minimize further exposure. Vinyl chloride exposure can irritate the skin, eyes, and mucous membranes, and the liquid can cause frostbite. The affected area should be washed with copious amounts of lukewarm or cold water. Hot water should not be used. Oral ingestion of vinyl chloride is unlikely; however, should that occur, it is suggested that water or milk be administered and, in addition, gastric lavage and administration of activated charcoal can be used as a means to reduce absorption.

Upon ocular exposure, the eye should be generously washed with tap water. Refer for medical attention. In case of dermal exposure, contaminated clothing should be removed and the skin should be rinsed with tap water. Titanium tetrachloride ingestion should be referred for medical attention. Vomiting should NOT be induced. Upon inhalation, the victim should be moved to fresh air and given artificial respiration if indicated. The body should be placed in a half-upright position. Refer for medical attention.

Environmental Fate

Anthropogenic sources are responsible for all of the vinyl chloride found in the environment. Vinyl chloride has been identified in at least 493 of the 1416 hazardous waste sites that have been included on the EPA National Priorities List. Of these sites,

491 are located in the United States and two are located in the commonwealth of Puerto Rico. Most of the vinyl chloride released into the environment is eventually transported to the atmosphere, whereas lesser amounts are transported to the groundwater. Vinyl chloride has been detected in the ambient air in the vicinity of vinyl chloride and PVC manufacturing plants and hazardous waste sites. The compound has also leached into groundwater from spills, landfills, and industrial sources. In the atmosphere, vinyl chloride is eliminated by reaction with photochemically generated hydroxyl radicals (half-life = 1–2 days): products include hydrochloric acid, formaldehyde, formyl chloride, acetylene, chloroacetaldehyde, chloroacetylchloranial, and chloroethylene epoxide. Dry deposition is not an important elimination pathway. In photochemical smog, the half-life is reduced to a few hours. In water, volatilization is the primary elimination process. Half-lives for volatilization from a typical pond, river, and lake have been estimated at 43, 9, and 35 h, respectively. In soil, vinyl chloride can volatilize from soil surfaces or leach into groundwater.

Ecotoxicology

Vinyl chloride can bioconcentrate to a limited extent in aquatic organisms. Biomagnification of vinyl chloride in terrestrial and aquatic food chains does not appear to be important because of its high volatility and the fact that it is readily metabolized by higher-trophic-level organisms. Little is known regarding biomagnification in terrestrial food chains.

Exposure Standards and Guidelines

The reference dose for vinyl chloride is 0.003 mg kg^{-1} day^{-1}, the reference concentration is 0.01 mg m^{-3}. The ACGIH TLV, time-weighted average, is 13 mg m^{-3} and the OSHA permissible exposure level is 2.6 mg m^{-3}.

See also: Carcinogenesis; Liver; Occupational Toxicology; Polymers; Respiratory Tract.

Further Reading

Albertini R, Clewell H, Himmelstein MW, *et al.* (2003) The use of non-tumor data in cancer risk assessment: Reflections on butadiene, vinyl chloride, and benzene. *Regulatory Toxicology and Pharmacology* 37: 105–132.

Lemen RA (2001) Unsaturated Halogenated Hydrocarbons. In: Bingham E, Cohrssen B, and Powell CH (eds.) *Patty's Toxicology*, 5th edn., vol. 2, pp. 247–255. New York: Wiley.

Relevant Websites

http://www.epa.gov – Vinyl Chloride (from the US Environmental Protection Agency's Technology Transfer Network Air Toxics Website).

http://risk.lsd.ornl.gov – Toxicity summary for Vinyl Chloride (from the Risk Assessment Information System (RAIS)).

Vinylidene Chloride

Anna M Fan

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 75-35-4
- Synonyms: 1,1-Dichloroethylene; 1,1-Dichloroethene; Vinylidene dichloride; Sconatex
- Chemical/Pharmaceutical/Other Class: Vinylidene chloride is an unsaturated halogenated hydrocarbon.
- Chemical Formula: $C_2H_2Cl_2$

Uses

The principal use of vinylidene chloride is in the production of polyvinylidene chloride polymers (PVDC). PVDC is used in the food packing industry for making flexible films such as in Saran and Velon wraps and as a barrier coating for paper, cellulose, polypropylene, and other plastics. These polymers are also used in the textile industry for drapery fabric, furniture and automobile upholstery; as flame retardant coatings for fiber and carpet backing; and in piping, coating for steel pipes, and adhesive applications.

Exposure Routes and Pathways

The principal sources of exposure to vinylidene chloride in the environment are ambient air especially near industrial sources and contaminated drinking water. Exposure can occur through inhalation, ingestion, and eye or skin contact. Air releases account for 99% of the total environmental releases. Ambient levels are primarily from emissions from polymer synthesis and fabrication industries, mostly

during manufacture and use, and little during the incineration of polymerized products. Ambient air levels have been reported in the range of 0.005–0.84 ppb, but concentrations were up to 97 ppb at a contaminated waste site. Information on release to soil is limited. Since vinylidene chloride in soil tends to partition into the air or groundwater, soil concentrations are expected to be low. Occupational exposure may occur from inhalation or dermal contact. Low levels have been detected in a number of drinking water supplies in the United States. A US Environmental Protection Agency (EPA) survey reported in 1985 showed that ~3% of the drinking water supplies contained vinylidene chloride with a range of 0.2–0.5 ppb, and an estimated mean of 0.3 ppb. Concentrations of residual vinylidene chloride in household films used for food packaging have been reported at 6.5–10.4 ppm, and in foodstuffs wrapped with commercial films containing residues (average 8.8 ppm), <0.005 to 0.01 ppm.

Toxicokinetics

Vinylidene chloride is well absorbed following oral and inhalation exposure. Dermal absorption is unlikely due to its low molecular weight and hydrophobic nature. After oral exposure to radiolabeled vinylidene chloride, animals showed highest level of radioactivity at 72 h in the liver and kidneys. Following inhalation, highest levels were found at 2 h also in the liver and kidneys.

Biotransformation involves oxidation via the cytochrome P450 system and subsequent detoxification by conjugation with glutathione and cellular macromolecules. The oxidative metabolic pathway is saturable, occurring at an oral dose of ~10–50 mg kg^{-1} in the rat, and at inhalation exposures exceeding 200 ppm. Elimination of vinylidene chloride and its metabolites occurs primarily through the urine and in the expired air at a relatively rapid excretion rate.

Mechanism of Toxicity

The toxicity of vinylidene chloride is related to cytochrome P450-catalyzed metabolism to reactive intermediates that bind covalently to cellular macromolecules. The target organs after acute oral or inhalation exposures are the liver, kidney, and lung. The specific targets are the centrilobular hepatocytes and bronchiolar Clara cells, cell types that are rich in CYP2E1. In rats and mice, vinylidene chloride is oxidized by the liver cytochrome P450 system to the epoxide 2-chloroacetyl chloride and to 2,2-dichloroacetaldehyde. The isozyme CYP2E1 appears to be responsible for the oxidation of vinylidene chloride in the liver and lungs of both species. The epoxide is the principal product, albeit more of the 2,2-dichloroacetaldehyde is produced more in the murine lung than liver. The metabolites then undergo secondary reactions, which are principally conjugation with glutathione and cellular molecules, a detoxification mechanism. The extent of covalent binding is inversely related to the loss of glutathione so that tissue damage increases with a decrease in glutathione level.

Acute and Short-Term Toxicity (or Exposure)

Animal

The following lethal dose (LD) values have been reported:

- Oral LD_{50}, rats 1500 mg kg^{-1}, males; 1800 mg kg^{-1}, females.
- Oral LD_{50}, young or fasted rats 50 mg kg^{-1} (lower glutathione levels).
- LD_{50}, mice 200 mg kg^{-1}, males and females.
- Inhalation LC_{50}, fasted rats 400 ppm, 4 h.
- Inhalation LC_{50}, rats 10 000–15 000 ppm, 4 h.
- Inhalation LC_{50}, mice 40 ppm, males; 200 ppm females; 4 h.

Vinylidene chloride is hepatotoxic, nephrotoxic, and mutagenic in experimental animals. It can affect the developing embryo.

Acute studies in rats showed that the chemical has high toxicity from oral exposure and moderate toxicity from inhalation exposure. For both acute oral and inhalation exposures, young and fasted animals are more sensitive to the acute toxicity of vinylidene chloride than those with access to food, and mice are more sensitive than rats, based on lower median lethal dose values. The principal target organs are the liver and kidney and the most sensitive endpoint is liver damage. Increases in enzyme markers of liver damage (aspartate transminase, AST; alanine transminase, ALT) and histological evidence of centrilobular and midzonal necrosis were observed. Fasting and administration by vehicle that increases the rate of absorption attenuates hepatotoxicity. The liver, kidney, and lung are also the organs most affected by subchronic exposure to vinylidene chloride.

Soft tissue anomalies in rats and skeletal effects in rats, mice and rabbits were observed following exposure to 15–449 ppm vinylidene chloride. Cardiac malformations were observed in fetus from maternal

rats exposed before and during pregnancy in drinking water.

Human

Vinylidene chloride is an eye irritant and can affect the central nervous system (CNS), liver, and kidneys in humans.

Contact with the eyes can cause conjunctivitis and transient corneal injury. Dermal contact causes irritation and there is little effect on the skin if it is allowed to evaporate. Inhalation can cause respiratory effects such as mucous membrane irritation. Exposure to high concentrations (4000 ppm) of vinylidene chloride as seen in workers may show signs of CNS depression with accompanying characteristic signs of intoxication and symptoms including drowsiness, nausea, headache, unsteadiness, or unconsciousness. Inebriation, convulsions, spasms have also been reported.

Chronic Toxicity (or Exposure)

Animal

Consistent with the findings from acute and subchronic exposures, a chronic oral gavage study in rats and mice conducted by the National Toxicology Program (NTP) (1982) also identified liver toxicity as the major toxic effect. Chronic exposures caused effects on the kidneys, liver, CNS, and lungs.

An update in 2004 by US EPA provided an oral reference dose (RfD) of 5×10^{-2} mg kg^{-1} day^{-1}, based on an no-observed-adverse-effect level (NOAEL) of 9 mg kg^{-1} day^{-1} on liver toxicity (hepatocellular midzonal fatty change as the minimal adverse effect) and a lowest-observed-adverse-effect level (LOAEL) of 14 mg kg^{-1} day^{-1} in a 2 year rat chronic and carcinogenicity study via drinking water. The updated evaluation used the benchmark dose (BMD) methodology and calculated a $BMDL_{10}$ of 4.6 mg kg^{-1} day^{-1} for midzonal fatty change in female rats. The BMD_{10} is 4.6 mg kg^{-1} day^{-1}. In this study, vinylidene chloride concentrations were 0, 50, 100, or 200 ppm. The time-weighted average (TWA) exposures were 7, 10, or 20 mg kg^{-1} day^{-1} for males and 9, 14, or 30 mg kg^{-1} day^{-1} for females. There were no significant differences observed among the groups in appearance, demeanor, mortality, body weight, food consumption, water consumption, hematology, urinalysis, clinical chemistry determinations, organ wrights, or organ to body weight ratios. The only treatment-related effect was hepatocellular fatty change and hepatocellular swelling. No exposure related hepatocellular necrosis or neoplastic changes were observed. The minimal hepatocellular swelling was not considered an adverse effect in this study and there were no changes liver weigh, clinical chemistry, or abnormal liver function. The fatty change was considered a minimal effect and might not be considered adverse as there was no evidence of a functional change in the liver of rats and glutathione levels were not reduced. On the other hand, the $BMDL_{10}$ was used to derive the RfD to limit exposure to this $BMDL_{10}$ and protect the liver from more serious damage (fatty liver or necrosis) that could compromise liver function. An uncertainty factor of 10 each was used for interspecies and intraspecies variability.

In a 2 year study by NTP, male and female rats and mice were administered corn oil 5 days week^{-1} for 104 weeks. A nonsignificant increase in adrenal pheochromocytomas was observed in male rats.

Tumor incidence data were used by the US EPA in 1994 for deriving an inhalation slope factor of 1.2 (mg/kg day)$^{-1}$ and a unit risk of 5.0×10^{-5} (μg/m^3)$^{-1}$. An oral slope factor of 6×10^{-1} (mg/kg day)$^{-1}$ and a unit risk of 1.7×10^{-5} (μg/l)$^{-1}$ were derived from the NTP 1982 study.

An update in 2004 by the US EPA determined that quantitative estimate of carcinogenic risk from either oral or inhalation exposure was not applicable. Vinylidene chloride showed equivocal evidence of carcinogenicity by the oral route and suggestive evidence by the inhalation route. Overall, the weight of evidence is not sufficient to justify deriving any unit risk. Male mice developed kidney tumors at one exposure level but not in female mice or male and females rats (i.e., one sex, one species, one exposure level). There were also enzymatic differences (i.e., CYP2E1) between male and female mice, male and female rats, and human kidney cells, and US EPA considered that genotoxicity evidence was limited. Under the draft revised guidelines for carcinogen assessment of 1999, US EPA concluded that vinylidene chloride exhibits suggestive evidence of carcinogenicity but not sufficient evidence to access human carcinogenic potential following inhalation exposure in studies in rodents.

Human

No signs or symptoms of chronic exposure to vinylidene chloride have been reported in humans. However, chronic exposure may lead to liver or kidney damage and the signs and symptoms of such damage may be apparent. There are no adequate human studies available for a quantitative determination of chronic effects. A few existing epidemiological studies involved concomitant exposure to vinyl chloride. There is one epidemiological study

reported in 1976 that investigated the health records of 138 workers exposed to vinylidene chloride in processes not involving vinyl chloride. The subjects had worked in experimental or pilot plant polymerization operations, in a monomer production process as tankcar loaders, or in a production plant manufacturing monofilament fiber. They were designated into three exposure categories: less than 10, 10–24, and greater than 25 ppm. There were no differences between the exposed cohort and the controls in hematology, clinical chemistry, and mortality. The numbers of subjects and endpoints studied were too limited for deriving useful information.

There is no clear evidence that vinylidene chloride exposure poses a carcinogenic risk to humans. Eighteen carcinogenicity or long-term bioassays have been conducted on vinylidene chloride and most were not adequately designed to have the sensitivity for detecting carcinogenic effects. Of the 18 studies, administration was by inhalation in 11 studies, oral route in five studies, skin application in one study, and subcutaneous injection in one study. Only one study showed an increased incidence of tumors (in Swiss mice). There was a significantly increased incidence of kidney adenocarcinomas in the males in the 25 ppm group. The tumors were accompanied by a significant degree of renal pathology (tubular necrosis). However, the relevance of these tumors in Swiss mice to humans is not clearly resolved. In this regard, DNA synthesis associated with tissue regeneration combined with the weak genotoxicity evidence for vinylidene chloride was thought to be a plausible mechanism for the tumors seen in this strain and species only. Additional studies showed that the P450 isozyme (CYP2E1), which is responsible for the metabolism of vinylidene chloride to the predominant reactive species, is not present in the kidneys of nonsusceptible species such as the rat. Also, assay of samples from human kidney showed negative results for the presence of the isozyme. Accordingly, the determination of potential carcinogenicity for humans based on these data is not resolved.

The International Agency for Research on Cancer (IARC) concluded that there is inadequate evidence in humans for its carcinogenicity, that there is limited evidence in experimental animals, and that it is not classifiable as to its carcinogenicity to humans (Group 3).

Clinical Management

The incapacitated worker should be removed from further exposure and appropriate emergency procedures should be implemented (e.g., those listed on the Material Safety Data Sheet, also shown below).

Inhalation. If adverse effects occur, the individual should be removed to an uncontaminated area. Artificial respiration should be given, if not breathing. Immediate medical attention must be sought.

Skin contact. The skin should be washed with soap and water for at least 15 min while removing contaminated clothing and shoes. Medical attention should be sought, if needed. Contaminated clothing and shoes should be thoroughly cleaned and dried before reuse.

Eye contact. The eyes should be flushed with plenty of water for at least 15 min. Then, immediate medical attention should be obtained.

Ingestion. If a large amount is swallowed, medical attention must be obtained.

In Vitro Toxicity Data

Vinylidene chloride has shown positive responses in the bacterial test systems including Salmonella test strains (mutation), *Escherichia coli* (mutation) and *Saccharomyces cerevisiae* (reverse mutation, mitotic gene conversion, aneuploidy) in the presence and absence of metabolic activation. It has produced both negative and positive responses in mammalian and in *in vivo* systems. The positive results were seen in the increase in the incidences of sister chromatid exchange and chromosomal aberration in Chinese hamster cell lines in the presence of an S-9 activation system. Negative results were seen in rat and mouse dominant lethal, micronuclei and chromosomal aberration assays. Following inhalation administration to rats and mice, there was only a minimal increase in DNA alkylation in both species and a minimal increase in DNA repair in kidney of mice. However, tissue damage (kidney nephrosis), an increase in DNA replication and an increase in mitotic figures were observed.

Environmental Fate

Vinylidene chloride is a human-made chemical and is not naturally found in the environment. It can be found from the breakdown of polyvinylidene (PVDC) products, and from the biotic and abiotic breakdown of 1,1,1-trichloroethane, tetrachloroethene, 1,1,2-trichloroethene, and 1,2-dichloroethane. Biotransformation of the chemical in groundwater can form vinyl chloride through reductive dechlorination, which is subsequently mineralized to carbon dioxide. The major transport process from water, soil and sediment is volatilization. Half-lives of

6 days in static pond water and 1 day in mobile river water have been calculated. Vinylidene chloride in soil tends to partition into the air or groundwater. That which is deposited on or near the soil surface is expected to rapidly volatilize into the air, and because soil mobility is quite high it may end up in groundwater. Bioaccumulation is expected to be low.

Atmospheric concentrations are relatively high compared with other environmental compartments because of vinylidene chloride's high vapor pressure and low water solubility. The half-life for the chemical in air has been estimated to be 16 h and 2–3 days. Atmospheric hydroxyl radicals play a major role in its degradation. The major reaction products in air are formaldehyde, phosgene, and hydroxylacetyl chloride.

Ecotoxicology

- Green alga, growth inhibition, 72 h EC_{50} 9.12 mg l^{-1}.
- Bluegill, lethality, 96 h LC_{50} 74 mg l^{-1}.

Little data exist on the effects of vinylidene chloride in the aquatic and terrestrial environments. Bioaccumulation is expected to be low based on the low octanol/water partition coefficient and low water solubility. A bioconcentration factor of 4 and a bioaccumulation factor of 6.9 were reported for fish, and a bioaccumulation factor of less than 13 reported for common carp. Because of the rapid volatilization of the chemical from the aquatic and terrestrial environments and the low concentrations found in surface water (microgram per liter range), no significant risk is expected.

Other Hazards/Sensitive Populations

Persons with higher risk are those who have underlying liver or kidney disease.

Exposure Standards and Guidelines

The World Health Organization (WHO) established a drinking water quality guideline of 0.03 mg l^{-1} in 1993 which is currently under review.

The US EPA has set a federal drinking water standard (called maximum contaminant level, or MCL) and a MCL goal of 7 ppb for vinylidene chloride.

The Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency has established a public health goal of 10 ppb for vinylidene chloride in drinking water. This is based on midzonal hepatocellular fatty changes in female rats observed at all three treatment levels after exposure for 2 years.

The Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency has established a chronic exposure level of 0.02 mg m^{-3} based on liver effects in guinea pigs.

The National Institute for Occupational Safety and Health (NIOSH) has established a recommended exposure limit (REL) for vinylidene chloride of the lowest feasible concentration of 0.4 ppm, the limit of quantitation. NIOSH also considers vinylidene chloride to be a potential human carcinogen based on the risk of potential cancer (liver and kidney tumors in animals).

The American Conference of Governmental Industrial Hygienists (ACGIH) has assigned vinylidene chloride a threshold limit value (TLV) of 5 ppm (20 mg m^{-3}) as a TWA for a normal 8 h workday and a 40 h workweek and a short-term exposure limit (STEL) of 20 ppm (79 mg m^{-3}) for periods not to exceed 15 min. Exposures at the STEL concentration should not be repeated more than 4 times a day and should be separated by intervals of at least 60 min. The limits are based on the risk of renal, hepatic, or other systemic toxicity.

The reportable quantity of vinylidene chloride is 100 lb. If an amount equal to or greater than this quantity is released within a 24 h period in a manner that will expose persons outside the facility, employers are required to notify the National Response Center immediately at (800) 424-8802 or at (202) 426-2675 in Washington, DC. A hazardous substance release is defined by EPA as any spilling, leaking, pumping, pouring, emitting, emptying, discharging, injecting, escaping, leaching, dumping, or disposing into the environment (including the abandonment or discarding of contaminated containers) of hazardous substances. In the event of a release that is above the reportable quantity for that chemical, employers are required to notify the proper Federal, State, and local authorities.

Vinylidene chloride is not currently regulated under the Occupational Safety and Health Administration.

Vinylidene chloride is listed as a hazardous waste under the Resource Conservation and Recovery Act and has been assigned EPA Hazardous Waste No. U078. It is approved for land disposal after treatment and only if the concentration of vinylidene chloride in the waste or treatment residual does not exceed 33 mg kg^{-1}.

Vinylidene chloride is designated as a hazardous substance under the Federal Water Pollution Control Act and further regulated by the Clean Water Act

Amendments of 1977 and 1978. These regulations apply to discharge of this substance.

Miscellaneous

Because of its volatility, determination of vinylidene chloride is best by gas chromatography using a variety of detectors, including flame ionization, electron capture, electrolyte conductivity, and mass spectrometry. The major limitation is interference by other constituents of the media being analyzed. Methods are available for quantifying environmental samples (air, water, soil sediment) and biological samples (breath, food, body tissues).

Volatility should be considered in the estimation of the overall exposure from the presence of vinylidene chloride in water to account for inhalation or dermal absorption due to the use of contaminated tap water.

See also: Vinyl Chloride.

Further Reading

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Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Vinylidene Chloride.

http://www.osha.gov – Occupational Safety and Health Administration (OSHA 2004). Occupational Safety and Health Guideline for Vinylidene Chloride. Occupational Safety and Health Administration, Department of Labor, Washington, DC.

http://www.epa.gov – US Environmental Protection Agency (US EPA) (last revised 8/13/2002, last updated July 8, 2004).

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Vinylidene Chloride.

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Virtually Safe Dose (VSD)

Stephen M DiZio

The various ways that the dose–response relationship may be portrayed includes the simple, intuitive concept of a 'virtually safe dose'. This has its roots in the portrayal of what is termed the 'margin of exposure', a concept itself derived from the pharmaceutical industry when portraying to the physician the ratio between the amount of a drug that produces a harmful effect and that which produces the desired beneficial one.

Margin of exposure is an intuitively simple concept, founded in the premise that any chemical, at some level of exposure, can cause harm. Where that level of exposure, or 'dose' is established, the level of exposure where no harm is present (the 'no-observed-adverse-effect level' or NOAEL) may then be determined. This NOAEL, divided by the dose presented by that chemical due to environmental exposure, represents the margin of exposure. As an example, if chemical 'X' causes mild liver swelling at an oral dose of 10 milligrams (mg) per kilogram (kg) of body weight per day ($mg\,kg^{-1}\,day^{-1}$) when given to a subject for 2 weeks, and no liver problems are found at a dose of $1\,mg\,kg^{-1}\,day^{-1}$, then the NOAEL for that chemical is $1\,mg\,kg^{-1}\,day^{-1}$. If chemical X is present in the public drinking water at 35 μg (0.0035 mg) per liter, and we assume that humans drink 2 l of that water per day, then the environmental exposure is 70 μg per human per day. If that same human weighs ~70 kg, then the human environmental dose is $1\,\mu g\,kg^{-1}\,day^{-1}$, and the margin of exposure is $1\,mg\,kg^{-1}\,day^{-1}/0.001\,mg\,kg^{-1}\,day^{-1}$, or 1000. It has commonly been accepted that a margin of exposure of 1000 or greater is a 'virtually safe dose'.

This, of course, comes with three caveats. The first relates to the experiment where the NOAEL was determined. If animals were the experimental subject, and five animals of each sex were used in the 2 week experiment, then the opportunity of determining an adverse effect at a dose of $1\,mg\,kg^{-1}\,day^{-1}$ (the NOAEL used above) is less than an identical experiment where 100 animals of each sex were used. The second caveat is that harm from a chemical may be time dependent, that an NOAEL found

in a 2 week experiment may (and often is) far greater that one where the animals were exposed on a daily basis for a lifetime. The third caveat is that creatures differ in their sensitivity to chemicals, again intuitively obvious when one realizes that humans are often unharmed when exposed to a single dose of an insecticide, where the insects themselves die. The NOAEL for a mouse may differ from that for a rat, which may differ from that for a human. All these must be taken into account before declaring a level of environmental exposure a 'virtually safe dose'.

In recent years, the concept of a virtually safe dose has been extended to the probability of producing cancer in the exposed subject. Because cancer is assumed to be possible when a subject is exposed to even one molecule of a cancer-causing chemical, then mathematical techniques have been used to extrapolate the probability of cancer occurring in large populations. If, in an experiment where 100 animals per dose are exposed to the cancer-causing chemical Y, and the NOAEL (for cancer) for that experiment is $1\,mg\,kg^{-1}\,day^{-1}$, it is expected that, as stated above, an experiment using 1000 animals per dose would possibly produce a different, and lower, NOAEL. Mathematical techniques that establish a cancer potency for even smaller doses (it is assumed that even one molecule could cause a tumor) are used to extrapolate the dose that could possibly cause cancer in one out of one million exposed subjects. Using these techniques, the United States Environmental Protection Agency, realizing that small amounts of dioxins are found ubiquitously (especially in food), has established a 'virtually safe dose' for these chemicals. This dose is the dioxin level that is assumed to cause cancer in only one out of one million exposed persons. The caveat to the latter is that dose results from an upper bound estimate of cancer potency and the probability of cancer for the exposed population may be far less, or, in fact, zero.

See also: Dose–Response Relationship; Margin of Exposure (MOE); Risk Assessment, Ecological; Risk Assessment, Human Health; Risk Characterization.

Further Reading

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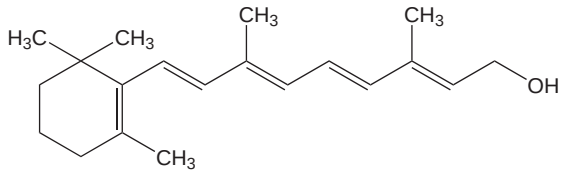
Vision *See* Sensory Organs.

Vitamin A

Allison A Muller

This article is a revision of the previous print edition article by Anne E Bryan, volume 3, pp. 404–405, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 68-26-8
- Synonyms: Retinol; Retinyl esters; Antiinfective vitamin; 3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonaretraen-1-ol
- Chemical/Pharmaceutical/Other Class: Fat-soluble vitamin, derived from retinol
- Chemical Formula: $C_{20}H_{30}O$
- Chemical Structure:



Uses

Vitamin A is used as a dietary supplement and for treatment of deficiency syndromes. It is not an endogenously produced vitamin; thus, it must be provided through dietary or vitamin supplement sources. Vitamin A is essential for normal vision in dim light. Furthermore, it is needed for regulation of all growth and development, for maintaining mucous membrane integrity, and for the reproductive process.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Available forms include capsules, tablets, topical preparations, and intramuscular solutions. Animal livers are rich in vitamin A.

Toxicokinetics

Vitamin A is readily absorbed from the intestine as retinyl esters. Peak serum levels are reached 4 h after ingestion of a therapeutic dose. The vitamin is distributed to the general circulation via the lymph and thoracic ducts. Ninety percent of vitamin A is stored in the liver, from which it is mobilized as the free alcohol, retinol. Ninety-five percent is carried bound to plasma proteins, the retinol-binding protein. Vitamin A undergoes hepatic metabolism as a first-order process. Vitamin A is excreted via the feces and urine. Beta carotene is converted to retinol in the wall of the small intestine. Retinol can be converted into retinoic acid and excreted into the bile and feces. The elimination half-life is ~ 9 h.

Mechanism of Toxicity

The exact mechanism leading to toxicity is not known. Both acute and chronic toxicity may occur. Daily vitamin A requirements range from 1500 international units (IU) to 4500 IU for children, 5000 IU for adults, and 5000 IU for pregnant women.

Acute and Short-Term Toxicity (or Exposure)

Human

Acute toxicity is uncommon in adults. However, very large exposures to vitamin A have resulted in the development of increased intracranial pressure (symptoms described include headache, dizziness, vomiting, visual changes, and bulging fontanel in infants).

Chronic Toxicity (or Exposure)

Human

Toxicity is more frequently seen with chronic ingestion of high doses of 30 000–50 000 IU day^{-1}. Children have developed acute toxicity following ingestion of 300 000 IU, but more frequently vitamin A toxicity in children develops following chronic ingestion of >10 times the recommended daily allowance for weeks to months. Malnutrition and individual tolerance may also be factors in predisposition to toxicity. Signs and symptoms of toxicity include vomiting, anorexia, agitation, fatigue, double vision, headache, bone pain, alopecia, skin lesions, increased intracranial pressure, and papilledema.

Clinical Management

In massive acute overdose, decontamination is advised. If the ingestion is recent (<30 min) and the patient is asymptomatic, syrup of ipecac is indicated. Activated charcoal may be used to adsorb vitamin A. Plasma vitamin A levels can aid in diagnosis but are not clinically useful in treatment. Upon discovery of a potential overdose, exposure to vitamin A should be immediately discontinued. Young children should be monitored for symptoms of increased intracranial pressure. Elevated intracranial pressure should be treated with mannitol, dexamethasone, and hyperventilation as needed. Vital signs and fluid and electrolyte status should be monitored closely. In general, vitamin A toxicity often resolves itself spontaneously within days to weeks following withdrawal of vitamin A. There are no known cases of vitamin A toxicity associated with beta carotene ingestion.

See also: Dietary Supplements; Liver.

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Vitamin B_1 *See* Thiamine.

Vitamin B_2 *See* Riboflavin.

Vitamin B_6 *See* Pyridoxine.

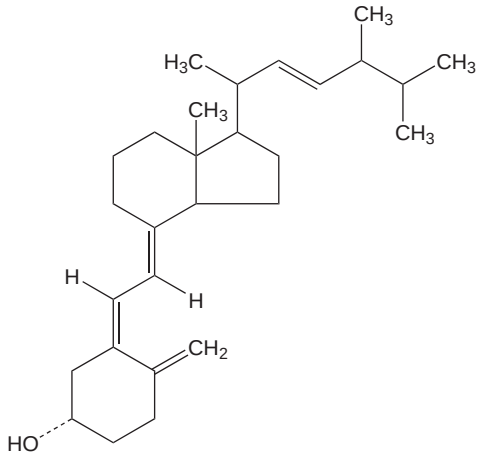
Vitamin B_9 *See* Folic Acid.

Vitamin C *See* Ascorbic Acid.

Vitamin D

Allison A Muller

- Chemical Abstracts Service Registry Number: CAS 50-14-6
- Synonyms: Ergocalciferol (D_2); Cholecalciferol (D_3); α-Calcidol; Calcitriol; Dihydrotachysterol (DHT)
- Chemical/Pharmaceutical/Other Class: Fat-soluble vitamin
- Chemical Structure:



Vitamin D_2

Uses

Vitamin D is a dietary supplement used for the prevention/treatment of deficiency syndromes. It is the only vitamin synthesized by the conversion of 7-dehydrocholesterol to cholecalciferol by exposure to sunlight or shortwave ultraviolet light.

Exposure Routes and Pathways

Ingestion of oral dosage forms is the most common route of exposure in both acute and chronic overdose.

Toxicokinetics

Vitamin D is readily absorbed from the gastrointestinal tract. Cholecalciferol is metabolized in the liver to 25-hydroxycholecalciferol and then to 1-α-25-dihydroxycalciferol in the kidney. This mobilizes stores of calcium from the bone matrix to the plasma. Cholecalciferol is stored in adipose and muscle tissue. The metabolites of vitamin D compounds are excreted primarily in bile and feces.

Mechanism of Toxicity

Excess vitamin D results in hypercalcemia and hypercalciuria, due to increased calcium absorption, bone demineralization, and hyperphosphatemia.

Acute and Short-Term Toxicity (or Exposure)

Animal

No reports of animal toxicity from vitamin D supplements could be found. However, vitamin D has proven fatal to animals when they were exposed to a vitamin D-containing rodenticide.

Human

Acute toxicity is rarely reported. Infants have reportedly tolerated up to 60 000–100 000 IU per kg without ill effect. Chronic toxicity occurs after the recommended daily allowance is excessively exceeded for weeks to months. Common symptoms of toxicity include nausea, flatulence, and diarrhea. Other nonspecific symptoms reported include muscle weakness, fatigue, and bone pain.

Renal failure may also occur due to precipitation of calcium in the kidneys. Vitamin D serum levels may be useful, as well as serum calcium, and alkaline phosphatase levels.

Clinical Management

Exposure to all forms of vitamin D should be stopped. Treatment should be supportive and symptomatic. Hypercalcemia treatment should include a low-calcium diet and prednisone as necessary.

See also: Dietary Supplements; Vitamin A; Vitamin E.

Further Reading

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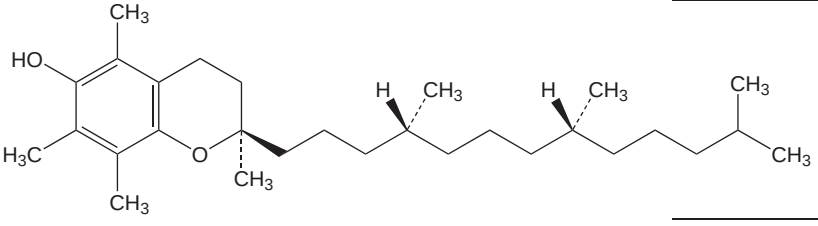
Vitamin E

Allison A Muller

- Chemical Abstracts Service Registry Number: CAS 59-02-9
- Synonyms: Antisterility vitamin; Almefrol; α-Tocopherol
- Chemical/Pharmaceutical/Other Class: Fat-soluble vitamin
- Chemical Formula: $C_{29}H_5O_2$
- Chemical Structure:



Uses

Vitamin E is used as a dietary supplement and for the treatment of deficiency syndromes.

Exposure Routes and Pathways

Ingestion as a supplemental vitamin is the most common route of exposure. Available forms include tablets, capsules, topical preparations, and intramuscular solutions.

Toxicokinetics

Vitamin E is absorbed in the gastrointestinal tract. Bile is necessary for absorption. Vitamin E is metabolized in the liver. The major metabolites of vitamin E are the glucuronides of tocopheronic acid. Vitamin E is distributed to all tissues. Lipid tissues store the vitamin for prolonged periods of time. Up to 30% is excreted in the urine. The half-life after intramuscular injection is ~45 h.

Mechanism of Toxicity

The exact mechanism of toxicity is unknown.

Chronic Toxicity (or Acute Exposure)

Human

The toxidrome of acute and chronic toxicities is no defined. Subjective symptoms include nausea, vomiting, flatulence, fatigue, muscle weakness, headaches, and blurred vision. Controversy exists over whether excessive vitamin E may cause liver and renal damage. The plasma concentration levels for vitamin E vary among individuals.

Clinical Management

Since there is no evidence that acute overdose represents a medical emergency, decontamination is not advised. Once chronic toxicity is suspected, discontinuation of vitamin usage and supportive/symptomatic therapy is recommended.

See also: Dietary Supplements.

Further Reading

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Vitamins *See* Vitamin A; Vitamin D; Vitamin E.

Volatile Organic Compounds (VOC)

S Satheesh Anand and Harihara M Mehendale

Background

Volatile organic compounds (VOCs) also referred to as solvents, are chemicals that evaporate easily at room temperature. VOCs are a class of liquid organic chemicals of variable lipophilicity and volatility. These properties, coupled with smaller molecular size and lack of charge, make inhalation the major route of exposure and provide ready absorption across the lungs, gastrointestinal (GI) tract, and skin. VOCs are frequently used to dissolve, dilute, or disperse materials that are insoluble in water. VOCs are classified according to molecular structure or functional group. These include aliphatic hydrocarbons (many of which are chlorinated – halocarbons), aromatic hydrocarbons, alcohols, ethers, esters, aldehydes, etc. Solvents may be utilized individually or as mixtures containing several ingredients. Most VOCs contribute in varying degrees to the formation of ground levels of ozone. This chapter is devoted to describe the uses, health effects, disposition, and factors affecting disposition and toxicity of VOCs in general. For detailed information, readers are advised to refer individual VOCs described elsewhere in this book.

Uses

Billions of pounds of VOCs are produced and utilized annually and there are hundreds of different VOCs used at present. VOCs are widely used as ingredients in household products including paints, paint strippers, varnishes, wax, cleaning, disinfecting, cosmetic, degreasing, aerosol sprays, cleansers, moth repellents, air fresheners, and automotive products. Drinking water is a common source of solvent exposure due to discharge of solvents from industries and household use and the presence of disinfection by-products, such as chloroform ($CHCl_3$), etc. Office equipment such as copiers and printers, correction fluids and carbonless copy paper, graphics and craft materials including glues and adhesives, permanent markers, and photographic solutions also contain some amount of VOCs. Since VOCs are solvents, they are widely employed as degreasers, and as intermediates in chemical synthesis, fuels, and fuel additives.

Environmental Contamination and Exposure

Widespread use of solvents has resulted in their dissemination throughout the environment. Almost everyone is exposed to VOCs, albeit in minute

amounts. VOCs enter the environment through evaporation. The volatilization occurs when the products containing them are used as intended and also during production, processing, storage, and transport activities. The presence of solvents in drinking water has become a major health concern for over three decades due to their potential carcinogenicity. The predominant VOCs present in water are $CHCl_3$ (water disinfection), trichloroethylene (TCE), and tetrachloroethylene (PERC).

People are exposed to solvents in environmental media by inhalation, ingestion, and skin contact. TCE, PERC, benzene, xylenes, and ethylebenzene are most frequently found in highest concentrations in air. Personal activities (smoking, visiting the dry cleaner's, or service station, etc.) and occupational exposures are believed to be largely responsible for relatively high exposures to other VOCs (benzene, toluene, xylene, PERC, etc.). Toluene and benzene are the most commonly found VOCs in indoor air, whereas $CHCl_3$ is most prevalent in water.

Toxicokinetics

The fundamental goal of toxicokinetics (TK) is to delineate the uptake and disposition of chemicals in the body and the subsequent toxicity. Gaining understanding of how the processes that govern VOCs kinetics vary with dose, species, and even different individuals would greatly reduce the number of assumptions that have to be made in the assessment of health risks from the toxicity data.

Absorption

The majority of systemic absorption of inhaled VOCs occurs in the deep part of the lungs, the alveoli, although some absorption has been demonstrated to occur in the upper respiratory tract. Blood:air partition coefficients (PCs) of VOCs are important determinants of the extent of their uptake. Increases in respiration and cardiac output/pulmonary blood flow enhance pulmonary absorption. The percent intake is initially high, but progressively declines as the VOC accumulates in tissues and the level in the blood returning to the lungs increases. A near steady state or equilibrium will soon be reached with inhalation of a fixed concentration of lipophilic solvents. In laboratory animals, VOCs are well absorbed from the GI tract. Peak blood levels are observed within minutes of oral dosing, although the presence of food in the GI tract can delay absorption. It is now assumed that 100% of an oral dose of most solvents is absorbed systematically. The vehicle or diluent in which a solvent is ingested can affect the absorption and TK of the compound. Administration of many VOCs in corn oil delays and prolongs the absorption when compared to aqueous ingestion. This is because of the 'depot' effect where VOCs are retained longer in the liphophilic vehicle (such as corn oil) before being absorbed. Solvents penetrate the stratum corneum, the skin's barrier to absorption, by passive diffusion. Important determinants of the rate of dermal absorption of solvents include the chemical concentration, surface area exposed, exposure duration, integrity and thickness of stratum corneum, lipophilicity, and molecular weight of the solvent.

Transport and Distribution

The VOCs absorbed from the GI tract largely enter the portal circulation and are subjected to first-pass elimination by the liver and lungs. The most volatile and well-metabolized VOCs are most efficiently eliminated before they enter the arterial blood. The efficiency of the hepatic first-pass elimination is thus dependant upon the chemical as well as the rate at which it arrives in the liver. Pulmonary first-pass elimination, in contrast, is believed to be a first-order process irrespective of the chemical concentration in the blood. VOCs are transported by the arterial blood and taken up according to tissue blood flow and mass and tissue:blood PCs. Hence, the extrahepatic organs receive a greater dose following inhalation exposure. This has been shown in rodents receiving the same systemic dose of CCl_4 by inhalation and gastric infusion. The solvents do not bind to plasma proteins or hemoglobin, but partition into hydrophobic sites in the molecules. Mostly, the partition occurs into phospholipids, lipoproteins, and cholesterol present in the blood. VOCs quickly accumulate in the brain after inhalation, producing in the central nervous system (CNS), effects as profound as those of surgical anesthesia in as little as 1–2 min. Adipose tissue accumulates relatively large amounts of VOCs. The pattern of ingestion of VOCs can significantly influence their TK and health effects. The toxic effects seen after administration of VOCs by gavage in acute, subchronic and chronic treatments were not observed when administered via drinking water. For example, $CHCl_3$ has produced hepatotoxicity and carcinogenecity when administered via oral gavage, however, the same dose did not cause any effects when exposed via drinking water except in Wistar and Osborne–Mendel rats.

The rate of systemic elimination of different solvents varies considerably. The two major routes of systemic elimination are metabolism and exhalation. The rate and extent of metabolic clearance are dose- and compound-dependent. Exhalation is

determined largely by the rate of pulmonary blood flow, the chemical's air:blood PC and the alveolar ventilation rate. The more volatile, lipophilic VOCs are exhaled most readily, since they have higher air:blood PCs. Body fat plays an important role in the elimination of lipophilic solvents. Body fat increases the volume of distribution and total body burden of the solvents. Deequilibration from adipose tissue is prolonged due to slow blood flow and high fat:blood PC.

Metabolism

Biotransformation plays a key role in modulating the toxicities of VOCs. Many VOCs are poorly soluble in water and the metabolism converts them to relatively water-soluble derivatives, which may be more readily eliminated in the largely aqueous urine and/or bile. Metabolism either produces inactive metabolite from the parent compound (detoxification) e.g., toluene metabolized to inactive hydroxyl and carboxyl metabolites or produces active metabolite (bioactivation), for example, benzene is oxidized to toxic quinone metabolites. VOCs undergo multiple metabolic pathways to products of varying toxicity. A variety of factors can influence the prominence of the different pathways and hence alter toxic outcomes. Biotransformation of VOCs is catalyzed largely by cytochrome P450 s in liver. Different isozymes can predominate at different doses of the same chemical, for example, TCE and $CHCl_3$ are primarily metabolized by CYP2E1 at low doses, whereas CYP2B1/2 showed to involve at high doses. CYP2E1 is primarily responsible for oxidation of some 16 halogenated and aromatic hydrocarbons. Phase II enzymes such as glutathione-*S*-transferase (GST) involve mostly in the detoxification of VOCs.

Toxicity

The ability of organic chemicals to cause health effects varies greatly from those that are highly toxic, to those with no known adverse health effects. Eye and respiratory tract irritation, headaches, dizziness, visual disorders, and memory impairment are among the immediate symptoms that some people have experienced soon after exposure to some organics. Many organic compounds are known to cause cancer in animals; some are suspected of causing, or are known to cause cancer in humans. The main determinants of VOCs inherent toxicity are: (1) number of carbon atoms; (2) saturated or unsaturated; (3) configuration, that is, straight-chain, branched-chain, or cyclic; (4) presence of functional group; and (5) level of exposure and length of exposure time. Some class-wise generalizations regarding toxicity can be made. For example, amides/amines tend to be potent sensitizers; aldehydes are particularly irritating; hydrocarbons tend to be cytogenic/mutagenic and many unsaturated, short-chain halocarbons are animal carcinogens. The toxicity of VOCs within the same class may vary dramatically. For example, trichloroethane and TCE are both hydrocarbons, yet the latter is an animal carcinogen, but the former is not.

Central Nervous System toxicity

One of the common physiological effects which is associated with high levels of exposure to VOCs is depression of the CNS activity. VOCs have the capacity to cause general anesthetic effects and may ultimately result in unconsciousness, or death, as the most severe consequence. CNS effects due to VOCs increase with an increase in carbon length, double bonds, halogen substitution and functional groups as these increase the lipophilicity. For example, $CHCl_3$ is more potent than methylene chloride and CCl_4 is the most potent in terms of anesthetic effects. Methanol and ethanol are more potent CNS depressants than methane and ethane.

Membrane and Tissue Irritation

Another important adverse effect with VOCs is potential for membrane and tissue irritation. Because membranes are composed principally of a protein–lipid matrix, VOCs at sufficient concentrations may act to dissolve that matrix or extract the fat or lipid components of the membrane. This defatting process, when applied to the skin, may cause irritation and cell damage and by similar processes, may seriously injure the lungs or eyes. Upon accidental or intentional ingestion, irritation in lungs caused by aspiration predisposes lungs from microbial infection to life threatening clinical pneumonias.

Carcinogenicity

Carcinogenicity by VOCs is a paramount public health concern because most of the VOCs are rodent carcinogens and some of them are known to cause cancer in humans. Benzene and vinyl chloride are known human carcinogens under some intense exposure conditions. The carcinogenesis is attributed to the metabolites of these VOCs. Several other chlorinated VOCs (CCl_4, $CHCl_3$, TCE, PERC, etc.) exhibit varying degrees of carcinogenic potential, notably hepatic tumors in animals. Except for leukemogenic effects from extreme benzene exposure and hepatic angiosarcoma in vinyl chloride workers,

no unequivocal human reports are available that document cancer hazard from exposure to VOCs. However, there are a number of epidemiologic observations regarding cancer and exposure to VOCs such as TCE, $CHCl_3$, PERC, etc. Based on the animal studies or the epidemiological reports, VOCs are classified into the following classes: known carcinogen, probable carcinogen, possible carcinogen, unclassifiable carcinogen, and unlikely carcinogen. Determining the human relevance of tumors observed in high-dose rodent studies is the major challenge. Like other chemicals, the toxicity of VOCs depends on several factors: (1) exposure route; (2) amount or rate of exposure; (3) duration of exposure; (4) individual susceptibility; and (5) interaction with other chemicals.

Other Toxic Effects

Apart from CNS effects, hepato-, nephro-, and cardiotoxicity are caused by VOCs. These effects have been reported in animals following acute and chronic exposures at high doses. However, these effects have been rarely reported in humans in occupational or environmental circumstances.

Solvent Abuse

Many solvents are intentionally inhaled in order to achieve a state of intoxication. Solvent inhalation can rapidly produce euphoria, delusions, and sedation as well as visual and auditory hallucinations. Solvent abuse is a unique exposure situation in that participants repeatedly subject themselves to vapor concentrations high enough to produce effects as extreme as unconsciousness. Solvents can be addicting and are often abused in combination with other drugs. Various solvents are present in a wide variety of household and commercial products, which are relatively inexpensive and readily available to children and adolescents. Nearly all hydrocarbon solvents cause CNS depression, but residual organ damage is chemical-dependent. There is concern that chronic solvent abuse can lead to long-term neurologic and psychological sequelae. Some solvents, such as n-hexane, and methyl-n-butyl ketone, cause peripheral neuropathies. Chronic abuse of VOCs such as toluene, which was previously thought to be innocuous, may be responsible for diffuse cerebral and cerebrallar atrophy. Blood dyscrasias, liver damage, kidney injury, and other toxicities are seen in patients who have abused VOCs known to be injurious to those organs. Death, frequently due to arrhythmogenic effects of high concentration of some VOCs (e.g., 1,1,1-trichloroethane, benzene), is sometimes a consequence of solvent abuse.

Factors Affecting Toxicity of VOCs

A number of factors alter the disposition of VOCs and their toxicity. Following is a brief account of such factors:

Age

Systematic data are lacking on age-dependent susceptibility of humans to solvents. The younger and more immature the subject is, the more different its response from that of an adult. There may be developmental periods or 'windows of vulnerability' when the endocrine, reproductive, immune, nervous, and other organ systems are particularly sensitive to certain chemicals. Maturational changes may also substantially affect the kinetics and ensuing toxicity of solvents and other agents. Systemic absorption of inhaled VOCs may be higher in infants and children than adults because of greater cardiac output and respiratory rates, reduced plasma binding, and increased fat content. Poisonings of premature and newborns exposed to benzyl alcohol, hexachlorobenzene, etc. are attributable to initial deficits in metabolic conjugation capacity. It is generally believed that liver P450 activities are greater in infants and children than in adults. A higher P450 would mean that infants and children are either susceptible or resistant depending upon the VOC. Susceptibility of immature subjects may be age-, organ-, chemical-, and species-dependent.

In the elderly, body fat usually increases substantially at the expense of lean mass and body water. Relatively lipid-soluble solvents accumulate in adipose tissue and slowly released to sites of action, metabolism, and elimination. Diminished cardiac output and renal and hepatic blood flows might affect the disposition of VOCs. There are contradictory reports on age-dependent susceptibility to liver damage. While some reports show that aged rats are more vulnerable to CCl_4 and allyl alcohol toxicity, a recent report shows that aged rats are able to mount higher liver regeneration and survive otherwise a lethal combination of chlordecone plus CCl_4 hepatotoxicity. Other major sources of variability and complexity in responses of geriatric populations to solvents include inadequate nutrition, the prevalence of disease states, and the concurrent use of multiple medications.

Gender

Women may differ from men in their responses to solvents, but the differences do not appear to be too

great. Levels of toluene and TCE in expired air are lower in females, reflecting more fat deposition. The major gender differences in P450-mediated metabolism in rats are not seen in humans or most other mammals. Relatively little is known about potential influences of contraceptive, hormone replacement therapy, or pregnancy on the metabolism of VOCs.

Genetics

A variety of genetic polymorphisms for biotransformation have been found to occur at different frequencies in different ethnic groups. Polymorphisms for xenobiotic metabolizing enzymes may affect the quantity and quality of enzymes and the outcomes of exposures to solvents. It is difficult to separate the influences of genetic traits from those of different lifestyles, socioeconomic status, and geographic settings. Ethnic differences in P450 enzyme expression and phase II biotransformation reactions such as GST appears to be associated with variations in VOCs metabolism. The Caucasians have higher total P450 levels and CYP2E1 activities than the Japanese. Some individuals with a null/null genotype for GST-theta lack the ability to conjugate and detoxify metabolites of methyl chloride and methylene chloride. The prevalence of this genotype ranges from 10% in Mexican Americans to 60–65% in Chinese and Koreans. Increased susceptibility to different cancers has been reported to be associated with certain genetic polymorphisms, which occur with different frequencies in different ethnic groups. Significant variations in allelic distributions for isoenzymes including CYP2E1, 2D6, 1A1, and GSTM1 have been observed in different racial groups.

Cytochrome P450 Inducers

Preexposure to chemicals that induce CYP2E1 and/or CYP2B1/2 can potentiate the toxicity of high doses of solvents that undergo metabolic activation. Induction of these enzymes may be of little consequence for low doses of well-metabolized solvents. Ethanol, acetone, ketones, and nicotine are some of the P450 inducers, which alter the PK of VOCs. Many P450 inducers also alter the inducers of detoxifying enzymes.

VOCs are also capable of inducing P450s and alter their, and/or other VOCs', metabolism. For example, repeated exposure to styrene, increased metabolic clearance, and isopropanol has been shown to potentiate CCl_4 hepatotoxicity.

Cytochrome P450 Inhibitors

A number of compounds inhibit P450s and these compounds are expected to enhance the toxicity of solvents that are metabolically inactivated. Conversely, these compounds should protect solvents that undergo metabolic activation. Compounds such as diallyl sulfide inhibit CYP2E1 and protect animals from some carcinogens. Some solvents such as CCl_4, in the course of their own metabolism, destroy P450s and subsequently decrease their metabolism. A reduction of the biotransformation in humans may occur due to metabolic competition between two solvents. Concurrent exposure to ethylene benzene and *m*-xylene and benzene and toluene in humans caused mutual metabolic inhibition.

Physical Activity

Exercise increases two of the major determinants of VOC uptake, alveolar ventilation, and cardiac output/pulmonary blood flow. Blood flow to liver and kidney is diminished with exercise, so biotranformation of well-metabolized solvents may be decreased.

Diet

Dietary habits can influence the TK and toxicity of solvents in several ways. The mere bulk of food in the stomach and intestine can inhibit systemic absorption of VOCs. Solvents in the GI tract partition into dietary lipids, largely remaining there until the lipids are emulsified and digested. This substantially delays the absorption of VOCs such as CCl_4 and its hepatotoxicity. Increased incidences of cancer have been observed in obese humans possibly due to increase in liver CYP2E1 by ketone body formation. Caloric restriction has clearly been shown to reduce the incidence of cancer. Fasting results in increased P450 activities and reduced GSH, which affect the TK and toxicity of VOCs. Food may contain certain natural constituents, pesticides, and other chemicals, which may enhance or reduce the solvent metabolism.

Diseases

Illness can be a major source of variability in response to solvents. Impaired metabolism and clearance are commonly seen in patients with cirrhosis and hepatitis. Lower levels of CYP2E1, 1A2, and GSH are seen in livers of patients with cirrhosis. The CYP2E1 and 2B1 levels were reported to be higher in a diabetic condition. Thus, diabetes may predispose individuals to increased risk of cell injury by solvents, which undergo metabolic activation. However, the final outcome of tissue injury depends upon whether or to what extent repair of injured tissue may occur. After exposure to high doses of VOCs, tissue repair is known to be inhibited causing continued progression of injury leading to organ failure, regardless of

whether initial injury is low or high. Persons with bacterial infection may be more sensitive to cytotoxic actions of solvents. Exposure of animals to small amounts of endotoxins potentiates liver injury by CCl_4 and other VOCs.

Ethanol

Depending upon the size and time of the dose, ethanol may have two opposing effect on the biotransformation of VOCs. Intake of ethanol (short-term) in moderate amounts has a marked inhibitory effect on the biotransformation of several VOCs such as toluene, TCE, styrene, and *m*-xylene. However, chronic ingestion of alcohol induces the liver P450s.

Drugs

Drugs may affect the TK of VOCs by changing pulmonary as well as peripheral blood flow or by inhibiting or stimulating metabolism. The intake of acetaminophen increased the concentration of toluene concentration in volunteers and acetylsalicylic acid decreases the transformation of *m*-xylene. The extent of protein binding may also be affected by drugs.

Metals

Metals can inhibit the hepatic enzyme system and affect the metabolism of VOCs. Rats pretreated with zinc chloride decreased CYP450 and protected CCl_4 liver damage. The effects of exposure to metals on the metabolism of solvents are more pronounced in acute situations than chronic at high doses.

Risk Assessment

VOCs are found in everything from paints and coatings to underarm deodorant and cleaning fluids. The work environment is typically where the highest exposures occur, mainly via inhalation and dermal contact. An estimated 10 million people are potentially exposed to organic solvents in the workplace. The effects ranging from CNS toxicity to carcinogenicity of VOCs have been reported on animals and humans. Regulatory agencies such as the Environmental Protection Agency, Agency for Toxic Substances and Disease Registry, Occupational Safety and Health Organization, etc. use the results of these studies to determine health advisory levels and set limits on the amount of each VOC that is considered safe for human exposure. Health advisory levels are based on a "no-effect level." The no-effect level is the maximum VOC dose that does not produce a known toxic effect in experiments and is further reduced by an additional safety factors for uncertainties such as high to low dose and animal to human extrapolation.

The current risk assessment practices for individual VOCs are generally considered to be protective of potentially vulnerable subgroups, because of the conservative default assumptions. However, most solvent exposures involve a mixture of chemicals, rather than single compounds. Our knowledge of the toxicity of solvent mixtures is rudimentary relative to the toxicology of individual solvents. While the assumption is frequently made that the toxic effects of multiple solvents are additive, solvents may also interact synergistically or antagonistically. The significant data gap that exists in the toxicity of solvent mixtures can preclude accurate risk assessments.

Conclusions

Nearly everyone is exposed to VOCs in the conduct of their normal daily activities due to their wide spread use. Hence, health effects of VOCs have been extensively studied. The common toxicological effects are CNS effects, irritation, hepato- and nephrotoxicity, and carcinogenecity. The health effects of VOCs vary greatly according to the compound, the level of exposure, and the length of exposure. Most studies to date have been conducted on single chemicals and less is known about the health effects of combined exposure of VOCs. Since human exposure often involve multiple VOCs, additional research is needed to characterize the potential health effects associated with mixed exposure.

See also: Benzene; Chloroform; Toluene; Trichloroethylene.

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Vomiting Agents *See* Arsenical Vomiting Agents.

V-Series Nerve Agents: Other than VX

Harry Salem*

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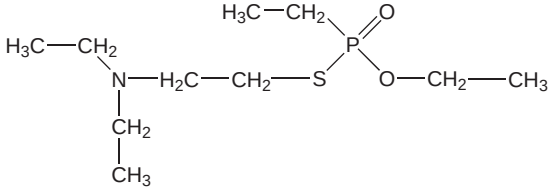
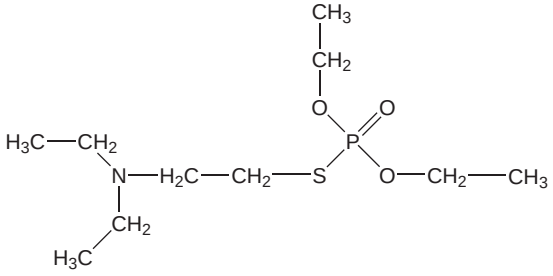
The V-series of nerve agents are less volatile than the G-series but are better able to penetrate the skin. Although they are an inhalation hazard when they are in the air as vapor or aerosol, they are considered more of a percutaneous hazard. This series contains VX, VE, VG (Amiton), VM, VR (RVX, Russian VX), and VS. The V designation is considered to be derived from Victory, Venemous, or Viscous.

Other V-Series Organophosphate Nerve Agents

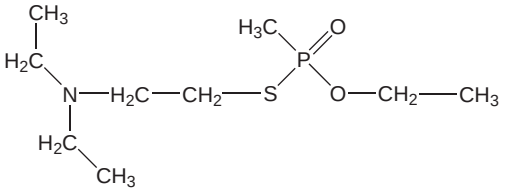
VE – Phosphonothioic acid, ethyl-, *S*-[2-(diethylamino)ethyl]*O*-ethyl ester



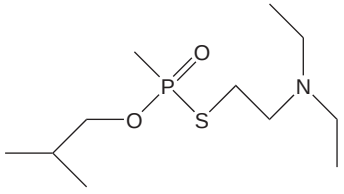
VG (Amiton) – Phosphorothioic acid, *S*-[2-(diethylamino)ethyl]*O*,*O*-diethyl ester



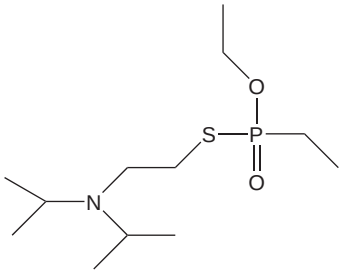
VM – Phosphonothioic acid, ethyl-, *S*-[2-(diethylamino)ethyl]*O*-ethyl ester



VR (RVX, Russian VX) – Phosphonothioic acid, methyl-, *S*-[2-(diethylamino)ethyl]*O*-(2-methylpropyl) ester



VS – Phosphonothioic acid, methyl-, *S*-[2-(diethylamino)ethyl]*O*-(2-methylpropyl) ester



See also: G-Series Nerve Agents; Nerve Agents; Sarin; Soman; Tabun; VX.

Relevant Websites

http://www.bt.cdc.gov – (US) Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry.

http://sis.nlm.nih.gov – (US) National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

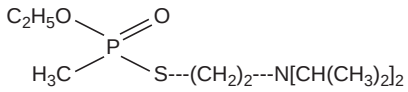
* The views of the author do not purport to reflect the position of the US Department of Defense. The use of trade names does not constitute official endorsement or approval of the use of such commercial products.

VX

Harry Salem and Frederick R Sidell*

Published by Elsevier Inc.

- Chemical Abstracts Service Registry Numbers: CAS 50782-69-9; CAS 51848-47-6; CAS 53800-40-1; CAS 70938-84-0
- Synonyms: Phosphonothioic acid; Methyl-*S*-(2-bis(1-methyl))phosphonothioate; *S*-2-Diisopropylaminoethyl-*O*-ethyl methylphosphonothioate; *S*-2(2-Diisopropylaminoethyl)-*O*-ethyl methylphosphonothioate; *O*-Ethyl-*S*-(2-diisopropylaminoethyl) methylthiolphosphonoate; TX60; Nerve gas; Nerve agent
- Chemical/Pharmaceutical/Other Class: Persistent anticholinesterase compound or sulfonated organophosphorus (OP) nerve agent
- Chemical Formula: $C_{11}H_{26}NO_2PS$
- Chemical Structure:



Uses

VX is a human-made nerve agent used in chemical warfare

Exposure Routes and Pathways

Casualties are caused both by inhalation and by dermal contact. Since VX is an oily liquid with low volatility, liquid droplets on the skin do not evaporate quickly, thus facilitating effective percutaneous absorption. Clothing can release VX for about 30 min after contact with VX vapor, which can lead to the exposure of other people. In addition to inhalation and percutaneous exposure, casualties can also be caused by ocular exposure, ingestion, and injection. Although VX does not mix with water as easily as nerve agents do, it could be released into water and lead to exposures via drinking contaminated water or dermal contact with contaminated water. People can also be exposed by eating food contaminated with VX.

VX is the least volatile of the nerve agents, and can be very persistent in the environment. VX can last for days on contaminated objects and can last for months under very cold conditions. Thus, surfaces contaminated with VX can be a long-term hazard.

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Toxicokinetics

VX is absorbed through the skin and respiratory system. Because it is nonvolatile, it may remain in place for weeks after dispersion and cause casualties. Thus, it is classified as a persistent agent. Although VX does not pose a major inhalation hazard in usual circumstances, by the inhalation route it is estimated to be 10 times as toxic as sarin. It is hydrolyzed by the enzyme organophosphorus (OP) hydrolase.

Mechanism of Toxicity

VX and the other nerve agents are irreversible OP cholinesterase inhibitors. They inhibit the enzymes butyrylcholinesterase in the plasma, the acetylcholinesterase on the red blood cell, and the acetylcholinesterase at cholinergic receptor sites in tissues. These three enzymes are not identical. Even the two acetylcholinesterases have slightly different properties, although they have a high affinity for acetylcholine. The blood enzymes reflect tissue enzyme activity. Following acute nerve agent exposure, the red blood cell enzyme activity most closely reflects tissue enzyme activity. However, during recovery, the plasma enzyme activity more closely parallels tissue enzyme activity.

Following nerve agent exposure, inhibition of the tissue enzyme blocks its ability to hydrolyze the neurotransmitter acetylcholine at the cholinergic receptor sites. Thus, acetylcholine accumulates and continues to stimulate the affected organ. The clinical effects of nerve agent exposure are caused by excess acetylcholine.

The binding of the nerve agent to the enzyme is considered irreversible unless removed by therapy. The accumulation of acetylcholine in the peripheral nervous system and central nervous system (CNS) leads to depression of the respiratory center in the brain, followed by peripheral neuromuscular blockade causing respiratory depression and death. The pharmacologic and toxicologic effects of the nerve agents are dependent on their stability, rates of absorption by the various routes of exposure, distribution, ability to cross the blood–brain barrier, rate of reaction and selectivity with the enzyme at specific foci, and their behavior at the active site on the enzyme.

Red blood cell enzyme activity returns at the rate of red blood cell turnover, which is ~1% per day. Tissue and plasma activities return with synthesis of new enzymes. The rate of return of these enzymes is not identical. However, the nerve agent can be removed from the enzymes. This removal is called reactivation, which can be accomplished therapeutically by the use of oximes prior to aging. Aging is the biochemical process by which the agent–enzyme complex becomes refractory to oxime reactivation. The toxicity of nerve agents may include direct action on nicotinic acetylcholine receptors (skeletal muscle and ganglia) as well as on muscarinic acetylcholine receptors and the CNS.

Recently, investigations have focused on OP nerve agent poisoning secondary to acetylcholine effects. These include the effects of nerve agents on γ-aminobutyric acid neurons and cyclic nucleotides. In addition changes in brain neurotransmitters such as dopamine, serotonin, noradrenaline as well as acetylcholine following inhibition of brain cholinesterase activity have been reported. These changes may be due in part to a compensatory mechanism in response to over stimulation of the cholinergic system or could result from direct action of nerve agent on the enzymes responsible for noncholinergic neurotransmission.

Human Toxicity

Following inhalation of VX, the median lethal dosage (LCt_{50}) in man has been estimated to be 30 mg min m^{-3} at a respiratory minute volume (RMV) of 15 l min^{-1}. Following percutaneous exposure, the LD_{50} was estimated to be 0.315 mg kg^{-1} or 10 mg per 70 kg in humans. The intravenous LD_{50} was estimated to be 0.008 mg kg^{-1} or 0.56 mg per 70 kg humans, and the intramuscular LD_{50} 0.012 mg kg^{-1} or 0.84 mg per 70 kg humans.

The doses that are potentially life-threatening may only be slightly larger than those producing minimal effects. The ECt_{50} for miosis from ocular vapor exposure was estimated to be < 0.09 mg min m^{-3}, and the ECt_{50} for runny nose is also estimated to be < 0.09 mg min m^{-3}. For severe incapacitation for vapor inhalation, the ICt_{50} was estimated to be 25 mg min m^{-3}, while the LCt_{50} was estimated to be 30 mg min m^{-3}.

The permissible airborne exposure concentration for VX for an 8 h workday of a 40 h workweek is an 8 h time-weighted average (TWA) of 0.00001 mg m^{-3}.

These signs and symptoms occur within minutes or hours following exposures. The signs and symptoms following vapor exposure include miosis and visual disturbances, headache and pressure sensation, runny nose and nasal congestion, salivation, tightness in the chest, nausea, vomiting, giddiness, anxiety, difficulty in thinking, difficulty sleeping, nightmares, muscle twitching, tremors, weakness, abdominal cramps, diarrhea, and involuntary urination and defecation. These signs and symptoms may progress to convulsions and respiratory failure. After liquid exposure on the skin, the initial effects are nausea, vomiting, and diarrhea, followed by muscular weakness, seizure, and apnea.

Clinical Management

Management of nerve agent intoxication consists of decontamination, ventilation, administration of antidotes, and supportive therapy.

The three therapeutic drugs for treatment of nerve agent intoxication are atropine, pralidoxime chloride, and diazepam. Atropine, a cholinergic blocking or anticholinergic drug, is effective in blocking the effects of excess acetylcholine at peripheral muscarinic sites. The usual dose is 2 mg, which may be repeated at 3–5 min intervals intravenously (iv) or intramuscularly (im). Pralidoxime chloride (protopam chloride; 2-PAM CL) is an oxime used to break the agent–enzyme bond and restore the normal activity of the enzyme. This is most apparent in organs with nicotinic receptors. Abnormal activity and normal strength returns to skeletal muscles, but no decrease in secretions is seen following oxime treatment. The usual dose is 1000 mg (iv or im). This may be repeated two or three times at hourly intervals (intravenous or intramuscular). Diazepam, an anticonvulsant drug, is used to decrease convulsive activity and reduce brain damage that may occur from prolonged seizure activity. It is suggested that all three of these drugs be administered at the onset of severe effects from nerve agent exposure, whether or not seizures occur. The usual dose of diazepam is 10 mg (im).

Miosis, pain, dim vision, and nausea can be relieved by topical atropine in the eye.

Supportive therapy may include ventilation via an endotracheal airway if possible and suctioning of excess secretions in the airways.

Animal Toxicity

Small doses of nerve agents in animals can produce tolerance in addition to their classical cholinergic effects. In rats, acute administration of nerve agents in subconvulsive doses produced tumors and hindlimb abduction. In animals nerve agents can also cause effects in behavior, analgesia, as well as cardiac effects.

The cause of death is attributed to anoxia resulting from a combination of central respiratory paralysis,

severe bronchoconstriction, and weakness or paralysis of the accessory muscles for respiration.

Signs of nerve agent toxicity vary in rapidity of onset, severity, and duration of exposure. These are dependent on the specific agent, route of exposure, and dose. At the higher doses, convulsions and seizures are indication of CNS toxicity.

Following nerve agent exposure, animals exhibit hypothermia resulting from the cholinergic activation of the hypothalamic thermoregulatory center. In addition, plasma levels of pituitary, gonadal, thyroid, and adrenal hormones are increased during organophosphate intoxication.

The available animal toxicity data are presented as follows:

VX animal toxicity

- Subcutaneous LD_{50} ($mg\,kg^{-1}$)
  - Rat 12
  - Mouse 22
  - Rabbit 14
  - Guinea pig 8400
- Intraperitoneal LD_{50} ($mg\,kg^{-1}$)
  - Mouse 50
  - Rabbit 66
- Intramuscular LD_{50} ($mg\,kg^{-1}$)
  - Chicken 30
- Intravenous LD_{50} ($mg\,kg^{-1}$)
  - Cat 5

See also: G-Series Nerve Agents; Nerve Agents; V-Series Nerve Agents: Other than VX.

Relevant Websites

http://www.bt.cdc.gov – US Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, Chemical Agents.

http://sis.nlm.nih.gov – US National Library of Medicine, Specialized Information Services, Chemical Warfare Agents.

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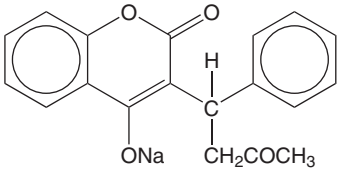
W

Warfarin

Henry A Spiller

This article is a revision of the previous print edition article by Michael J Hodgman, volume 3, pp. 411–412, © 1998, Elsevier Inc.

- Chemical Abstracts Service Registry Number: CAS 81-81-2
- Synonyms: Courmadin; Hydroxycoumarin
- Chemical/Pharmaceutical/Other Class: A synthetic derivative of 4-hydroxycoumarin, the hemorrhagic component of sweet clover
- Chemical Formula: $C_{19}H_{16}O_4$
- Chemical Structure:



Uses

Warfarin is used therapeutically as an anticoagulant. It is also used as a rodenticide.

Background Information

In 1921, during a particularly wet year a large number of cattle died from a hemorrhagic disorder. Investigation by the Wisconsin Alumni Research Foundation (WARF) eventually identified the cause of the disorder in a substance found in the rotting sweet clover used as feed by the cattle. The name warfarin is a combination of the acronym for the original patent holder (WARF) and the suffix from coumarin.

Exposure Routes and Pathways

Ingestion is the most common route of exposure. Warfarin is also absorbed transdermally and by inhalation. It is available in oral and injectable forms. Warfarin rodenticides are typically 0.025–0.050% warfarin by weight.

Toxicokinetics

Warfarin is rapidly and nearly completely absorbed by the oral route. Peak plasma levels typically occur within 2–8 h. Warfarin is highly protein bound: 97–99%. The volume of distribution approximates $0.15\,l\,kg^{-1}$. Warfarin is extensively metabolized by hepatic microsomal enzymes. The primary metabolites are 6- and 7-hydroxy warfarin via oxidation and several warfarin alcohols via reduction. The warfarin alcohols retain weak anticoagulant activity. The metabolites undergo enterohepatic circulation. Approximately 85% of warfarin appears in the urine as metabolites. Less than 1% or 2% appears in the urine unchanged. Warfarin metabolites are also excreted in the stool. The plasma half-life varies widely, from 10 to 80 h; it is typically 36–44 h. The duration of clinical effects can significantly exceed the half-life of warfarin. (Note: There are many drug interactions with warfarin; the reader is referred to a standard pharmacology text for further details.)

Mechanism of Toxicity

Warfarin interferes with the hepatic production of a number of proteins involved in hemostasis. These include the coagulation factors II (prothrombin), VII, IX, and X and also proteins C and S, important modulators of coagulation. Vitamin K is a cofactor for the carboxylation of specific glutamic acid groups in these proteins. This is the final phase in activation of these clotting factors. During carboxylation, vitamin K is oxidized to vitamin K 2,3-epoxide. The cyclical regeneration of vitamin K by vitamin K epoxide reductase allows production to continue. However this step is antagonized by warfarin. As a result, vitamin K stores are depleted and vitamin K is unavailable during the carboxylation phase of the coagulation factor activation. Dysfunctional decarboxycoagulation factors are produced and overall synthesis may be reduced. This leads to impaired coagulation.

Acute and Short-Term Toxicity (or Exposure)

Animal

Mammals and birds vary in their sensitivity to warfarin. Horses are resistant to the coumarins and cats are more sensitive than dogs. Signs of toxicity in animals include anorexia, weakness, vomiting, diarrhea, bleeding, and dyspnea. Toxic effects can be monitored by measurement of the prothrombin time (PT) or one-stage PT. Treatment is as for humans. The recommended dose of vitamin K for dogs and cats is 0.25–1 mg kg^{-1} day^{-1} for 5–14 days. There is minimal to no toxicity in aquatic organisms.

Human

Depletion of preformed circulating coagulation factors must occur before any effect by warfarin is apparent. Factor VII has the shortest half-life (4–6 h) and factor II the longest (60 h) of the vitamin K-dependent coagulation factors. The PT may begin to increase by 24 h and be maximal 36–72 h postingestion. Significant toxicity from single-dose ingestion is uncommon; most instances of toxicity are the result of repeated ingestion over time. The most frequent sites of bleeding are mucocutaneous, genitourinary, and gastrointestinal, although bleeding can occur virtually anywhere. Reported effects secondary to overcoagulation are cardiac tamponade, pulmonary hemorrhage, hemothorax, intracranial hemorrhage, gastrointestinal bleeding with hemocult positive stools, lower back/flank pain, hematuria, and retinal hemorrhage.

The more serious events include massive hemorrhage with shock, intracranial bleeding and stroke, and pericardial tamponade. Plasma warfarin levels are not routinely done. The effect of warfarin is best followed by the PT and International Normalized Ratio (INR). Under therapeutic conditions the INR is maintained at 2.0–3.0, except for prophylaxis after artificial heart valve replacement when it may be 2.0–3.5. Specific assays of factor activity can be measured although this is not usually necessary.

Chronic Toxicity (or Exposure)

Animal

Warfarin is used as a rodenticide. Rats exposed chronically to warfarin develop bleeding and death over time. Birds are relatively resistant to the effects of warfarin. Leghorns exposed over 15 days developed no signs of toxic effects. Some birds need to eat half their body weight with feed containing 0.1% warfarin per kg feed in order to develop anticoagulant effects.

Human

Effects other than those outlined previously have not been described. Warfarin use has been associated with birth defects and spontaneous abortion. Toxicity is more likely to occur and at lower doses with chronic exposure.

In Vitro Toxicity Data

Warfarin has been shown to have no impact on Mtln3 rat mammary carcinoma cell growth *in vitro* at concentrations less than 1 mmol l^{-1}.

Clinical Management

For acute single unintentional ingestions, observation at home with poison center follow-up may be acceptable. For substantial recent ingestions, activated charcoal should be administered. Induced emesis should be avoided in the anticoagulated patient as it may cause an increase in intracranial pressure and potentiate a vascular accident. The PT should be monitored for at least the first 48 h for signs of toxicity. Extreme caution should be used with any invasive procedure in the anticoagulated patient. The airway should be protected if compromised. Volume resuscitation should be provided as indicated by clinical status. With active, uncontrolled, or life-threatening hemorrhage, fresh frozen plasma should be administered to provide preformed clotting factors (at least 4–6 units will be necessary in an adult).

Vitamin K_1 (phytonadione) is a specific antidote for warfarin toxicity. In cases with no active bleeding and an INR < 5.0 withdrawal of the warfarin may be sufficient. Pharmacologic doses of vitamin K antagonize the inhibitory effect of warfarin on clotting factor production. The dose and route of vitamin K administration depends on the clinical setting. For rapid reversal, 5–25 mg should be administered intravenously no faster than 1 mg min^{-1}. In children, 0.6 mg kg^{-1} should be used. Clinical effects may be seen within hours. The response and duration of a single dose of vitamin K is variable and dependent on the severity of the toxicity. The half-life of vitamin K is less than 4 h and repeat doses may be necessary. In less acute settings, vitamin K may be given subcutaneously or orally. The PT should be monitored to follow toxicity and response to treatment. Anaphylaxis has been reported with intravenous vitamin K. Vitamin K_3 (menadione) is not effective therapy. In patients therapeutically anticoagulated, rapid reversal can be dangerous and should be done with caution.

Environmental Fate

No information is currently available on breakdown in soil groundwater or surface water.

Exposure Standards and Guidelines

Threshold limit value time-weighted average, $0.1\,mg\,m^{-3}$ (Occupational Safety and Health Administration); short-term exposure limit, $0.3\,mg\,m^{-3}$.

See also: Blood.

Further Reading

Chai SJ and Macik BG (2002) Improving the safety of warfarin. *Seminars in Hematology* 39(3): 179–186.

Montanio CD, Wruk KM, and Kulig KW (1993) Acute pediatric warfarin (coumadin) ingestion: Toxic effects despite early treatment. *American Journal of Diseases of Children* 147: 609–610.

Wasp *See* Hymenoptera.

Water Pollution *See* Pollution, Water.

Wildlife Toxicology *See* Ecotoxicology, Wildlife.

Wisteria

Ann P Slattery

- Synonyms: *Wisteria floribunda* (Japanese wisteria); *Wisteria sinensis* (Chinese wisteria)

Uses

Wisteria is a woody vine or climbing shrub of North America and Eastern Asia. This invasive vine may grow 30 ft or more only limited by the structure on which it is allowed to grow. The pink, white, or blue fragrant flowers bloom in clusters. The seeds are contained in pea-shaped flat pods. Each pod contains three to five seeds.

Exposure Routes and Pathways

Ingestion of seeds, pods, and flowers is the primary route of exposure. Symptoms have developed following exposure to the smoke of this plant when burned.

Mechanism of Toxicity

Although toxins are identified (wistarine and lectin), clear information about their behavior does not exist. As a saponin-containing compound, it is classified as a gastrointestinal irritant.

Acute and Short-Term Toxicity (or Exposure)

Human

All parts of the wisteria plant are considered toxic, especially the pods and seeds. Although serious poisonings are not common, exposures to as few as two seeds have been known to result in serious effects. Symptoms include oral burning, stomach pain, diarrhea, and vomiting. Gastrointestinal symptoms may appear in 1.5–3.5 h. Confusion, syncope, vertigo, and weakness have been described. Increased white blood cells have also been documented.

Symptoms usually resolve within 24–48 h, but one case reported persistent weakness and vertigo lasting 5–7 days. The mitogenic and blood clotting effects of lectins are not seen in toxic exposures. Exposure to smoke from the burning of this plant is known to cause headaches.

Clinical Management

Initial treatment with gastric lavage is indicated. Support with fluid replacement and antiemetics may be indicated.

See also: Plants, Poisonous.

Further Reading

Rondeau ES (1993) Wisteria toxicity. *Clinical Toxicology* 31(1): 107–112.

Wood Dusts

Alan J Weinrich and Paul Demers

Published by Elsevier Inc.

Characteristics

Tree species are classified either as gymnosperms, which generally have needle-like leaves, or as angiosperms, which generally have broad leaves and are deciduous in temperate climates. In practice, trees usually are classified as softwoods, hardwoods, or tropical woods. Softwoods include the temperate gymnosperms or conifers; hardwoods include the temperate angiosperms; and tropical hardwoods primarily are angiosperms, but also include some gymnosperms that thrive in tropical climates.

Wood is composed primarily of cellulose, hemicellulose, and lignin. In addition to these basic components, wood also contains many organic compounds, known as 'wood extractives'. Wood extractives serve to protect trees from bacteria, fungi, and other potentially harmful agents. They also provide grain and color to the wood. These extractives typically make up 5–30% of the wood mass. Softwoods and hardwoods differ in cellular structure as well as chemical composition and there is great variability between species. Biologically active chemicals found in wood include terpenes, lignans, stilbenes, tannins, flavinoids, and quinines.

Human Exposures

Wood dust exposure is common in many types of work. The greatest exposures occur in secondary wood industries, such as furniture and cabinet manufacturing, wood pattern and model shops, and other manufacturing industries. Finished products are created from dried wood using sanders and other high-energy tools that generate fine, inhalable particulate and require detail work that brings the worker's breathing zone close to the source of these wood dust emissions. Substantial exposures also can occur in primary wood industries, such as logging, lumber mills, and pulp mills, and in construction during preparation of the wood including such activities as cutting and sanding.

Wood dusts are generated during woodworking processes, either by shattering the wood cells or by chipping out whole cells or groups of cells. Shattering generally creates more dust and much finer particle sizes than chipping. In general, operations designed to create a smooth wood surface, such as sanding, result in more shattering of cells than rougher woodworking processes. Processes like sawing performed parallel to the natural grain of the wood are less likely to shatter cells than processes performed perpendicular to the grain. Dusts produced from chipped cells cause less concern for human health because the large particles settle quickly from the air and rarely are inhaled.

The dominant route of human exposure to wood dusts is inhalation. In fact, most significant health effects seem to result from direct contact of the inhaled wood dusts with tissues of the respiratory tract. Because of the wide distribution of wood dust particle sizes, there is potential for deposition throughout the respiratory system. However, the majority deposit in the upper airways, primarily in the nose. This correlates well with observations that the most important health effects, such as upper respiratory symptoms and sino-nasal cancer, occur in the upper airways. While ingestion also is common, no adverse health effects were reported. Dermal contact also occurs routinely, occasionally causing dermatitis.

Exposure Measurements

Airborne wood dust exposure is sampled by collecting airborne dust on a filter and measured gravimetrically. A variety of samplers are in use, differing primarily in the size of the particles they collect. Occupational hygiene authorities recommend measuring airborne wood dust concentrations using 'inhalable' dust samplers because they best collect the range of particle sizes that cause health effects. However, most wood dust exposure data have been obtained using methods commonly referred to as

'total' dust samplers. The term 'total' is a misnomer, because typical North American 'total' dust samples have excluded the largest particles that can enter the nose and upper airways and may be responsible for the most important health effects. Wood dust exposure measurements comparing 'total' dust to inhalable dust measurements have demonstrated that 'total' dust samples underestimated worker exposures by factors ranging from 1.2 to 4.

Toxicity

Respiratory Disease

Many studies have considered the risk of respiratory disease among workers exposed to wood dusts. Adverse effects have been observed in the great majority of these studies, although in many cases workers also may have been exposed to bioaerosols, formaldehyde, isocyanates, and other manufactured chemicals with known respiratory effects. A number of reviews of respiratory disease from wood dust exposure have been published and are noted below in the Further Reading list.

Various studies have noted the following respiratory symptoms:

- decreased mucociliary clearance;
- nasal symptoms, such as obstruction, hypersecretion, or irritation;
- chest symptoms, including cough and dyspnea;
- phlegm;
- wheeze; and
- bronchitis.

These effects have been observed among workers exposed to wood dusts from a variety of tree species in many countries and settings, including lumber mills, furniture manufacturing, and cabinet making.

Workers exposed to wood dusts also have demonstrated decreased lung function

- compared to unexposed controls;
- after a workshift;
- decreased air flow (forced expiratory volume in one second (FEV_1), midflow, or peak flow); and
- both decreased FEV_1 and forced vital capacity (FVC).

Asthma

Case reports and epidemiological studies have attributed asthma to exposure to dusts from many different tree species. Several reviews are available, including those noted below in the Further Reading list.

Dusts from several North American and many exotic woods have been identified as allergenic. Included among these are wood types, such as

- African maple,
- ash,
- cedars,
- oak,
- ebony,
- jacaranda,
- mahogany,
- ramin,
- redwood, and
- walnut.

While many case reports and epidemiologic studies on asthma associated with wood dusts have been published, Western red cedar is the most notable and is the only one that has been studied extensively and has dose–response information available.

In addition to Western red cedar, many other tree species have been identified as causing asthma, based on epidemiologic studies and case reports.

Genotoxicity

Researchers have reported the following evidence of genotoxicity from wood dust exposures.

- Significantly ($p < 0.01$) more chromatid breaks among 13 nonsmoking male plywood workers compared to 15 nonsmoking, age-matched controls.
- Significantly ($p < 0.01$) more micronuclei in the peripheral lymphocytes of 298 match factory workers exposed to poplar and linden dusts compared to 45 waiters, with no apparent dose–response relationship.
- Significantly ($p < 0.05$) more DNA single-strand breaks in peripheral lymphocytes of 24 wooden furniture workers than in the 28 controls.

Carcinogenicity of Wood Dusts

An excess of sino-nasal cancer among wood workers was first recognized in the 1960s in England. Workers involved in furniture manufacturing and cabinet making had a 10–20 times increased risk of nasal cancer, and a 100–500 times increased risk specifically for nasal adenocarcinoma. Many case–control studies conducted in different countries have confirmed these findings, with extremely high relative risks observed in European studies. Although the highest risks have been observed among workers in

the wood furniture industry, excesses also have been observed in other wood-related industries, such as sawmills, cabinet making, and carpentry.

In general, the relative risks observed in North American studies have been considerably lower than those observed in European studies. While the reasons for this disparity are unclear, they may be artifacts of the relatively low power of most of the North American epidemiological studies. However, the consistency with which the differences have been observed suggests there may be other causes. US and Canadian studies observed excess risks for all types of sino-nasal cancer, ranging from 1.5 to 4.4. A pooled analysis of data from four cohorts of US wood workers and British furniture workers appeared to show that the sino-nasal cancer mortality excess was restricted to the British cohort. However, the US studies had relatively low power for detecting a two- or threefold excess risk. A cohort study of Canadian softwood sawmill workers that specifically controlled for exposure to chlorophenol fungicides showed a 1.9 times excess of sino-nasal cancer.

Adenocarcinoma has been highly associated with exposure to hardwood dusts while squamous cell carcinoma has been associated with exposure to dusts from a variety of wood types. Based on interviews with sino-nasal cancer patients, exposures to oak and beech clearly have been associated with excess risk. Birch, mahogany, teak, and walnut exposures are strongly suspected of causing sino-nasal cancer. However, because the mechanisms by which wood dust exposures increase the risk of sino-nasal cancer are not clear, other tree species also may be carcinogenic.

No studies were found that compared the risk of sino-nasal cancer based on quantitative estimates of wood dust exposure. However, several studies observed a dose–response relationship using semiquantitative estimates of wood dust exposure based on job title and industry. Because of the long latency of sino-nasal cancer, the effective exposure period is likely to be at least 20–30 years prior to diagnosis, which equates to the 1950s and 1960s for most studies. Unfortunately, there are very few exposure measurements from that period.

Some Carcinogenicity Classifications for Wood Dusts

American Conference of Governmental Industrial Hygienists (ACGIH®): Certain hardwoods as beech and oak: A1 – known human carcinogens.

Germany: Beech and oak wood dust: Group 1 – confirmed human carcinogens; wood (except beech and oak) dust: Group 3 – possible human carcinogens.

International Agency for Research on Cancer: Group 1 – carcinogenic to humans.

US National Institute for Occupational Safety and Health (NIOSH): Hardwood and softwood: Carcinogens.

Some Occupational Exposure Limits for Wood Dusts

1. ACGIH threshold limit value, Western red cedar, 0.5 mg m^{-3}, inhalable particulate mass, sensitizer; all other species, 1 mg m^{-3}, inhalable particulate mass.
2. Australia: Beech and oak, 1 mg m^{-3}, sensitizer; softwoods, 5 mg m^{-3}, sensitizer (both under review).
3. Germany: TRK (technical exposure limit): 2 mg m^{-3} inhalable fraction of the aerosol; airways and skin sensitizer for Western red cedar.
4. Japan Society for Occupational Health: 'total' dust, 4 mg m^{-3}; respirable dust, 1 mg m^{-3}.
5. Netherlands: Beech and oak, 1 mg m^{-3}.
6. South Africa (Department of Minerals and Energy): Hardwood and softwood, 5 mg m^{-3} ('total' dust) respiratory sensitizer.
7. Sweden: 2 mg m^{-3}; 0.5 mg m^{-3}, if dust has unevaluable impregnated substances.
8. United Kingdom: Hardwood and softwood, 5 mg m^{-3}, respiratory sensitizer.
9. US NIOSH: 1 mg m^{-3}.
10. US Occupational Safety and Health Administration: Particulates not otherwise regulated, 15 mg M^{-3}, 'total' dust; 5 mg m^{-3}, respirable dust.

See also: Carcinogenesis; Epidemiology; Respiratory Tract.

Further Reading

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Workplace Environmental Exposure Levels (WEELs) *See* Occupational Exposure Limits.

World Health Organization *See* Joint FAO/WHO Expert Meetings (JECFA and JMPR).

Wound Healing *See* Tissue Repair.

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X

Xenobiotics

Midhun C Korrapati and Harihara M Mehendale

The term *xenobiotic* (foreign to the body) was coined to cover all compounds that were foreign to the organism under study. In some situations, this is loosely defined to include naturally present compounds administered by alternate routes or at unnatural concentrations. An examination of the fate of foreign compounds in biological systems is a natural outgrowth of man's curiosity about his environment and how it can affect his health as well as the health of other animals and organisms around him. While the majority of studies concern with the fate of man-made chemicals including drugs used as medicines, in humans and animals there are extensive investigations on the fate of organic compounds in plants, animals and microorganisms.

Classification of Xenobiotics

Humans are exposed throughout their lifetime to a large variety of xenobiotics like drugs and nonessential exogenous compounds by ingestion, inhalation, dermal, or by any parenteral routes of exposure that may pose as a health hazard. Drugs taken for therapeutic purposes as well as occupational or accidental exposure to any man-made or naturally occurring chemicals and the vapors of volatile chemicals or solvents pose possible health risks; smoking and drinking involve the absorption of large amounts of substances with potential health effects. Furthermore, the ingestion of natural toxins in vegetables and fruits, pesticide residues in food, as well as cancer causing pyrolysis products from fats and protein formed during the charbroiling of the meat have to be considered.

Various organ systems like nervous system, cardiovascular, respiratory, and gastrointestinal systems are affected by xenobiotics. Xenobiotics are also classified based upon the mechanism by which they cause toxicity. Immunomodulatory, endocrinomodulatory, antiproliferative, and mutagenic agents are some of the examples of such xenobiotics. These effects are the result of biotransformation of these xenobiotics.

Biotransformation of Xenobiotics

Most of these xenobiotics undergo enzymatic biotransformations by xenobiotic-metabolizing enzymes in the liver and extrahepatic tissues, and are eliminated by excretion as hydrophilic metabolites. In some cases, especially during oxidative metabolism, numerous chemical procarcinogens form reactive metabolites capable of binding covalently to proteins or nucleic acids – a critical step to mutagenicity, cytotoxicity, and carcinogenicity. Therefore, insight into the biotransformation and bioactivation of xenobiotics becomes an undisputable prerequisite for the assessment of drug safety and risk estimation of chemicals and drugs.

Detoxification and toxic effects of drugs and xenobiotics have been studied extensively in various mammalian species. Frequently, differences in sensitivity to these toxic effects were observed and can now be attributed to a difference between species in the isoenzyme/isoforms of cytochrome P450 monoxygenases. The level of expression of the CYP450 enzymes is regulated by a variety of endogenous factors such as hormones, sex, age, diseases, and the presence of environmental factors such as inducing agents. Drugs undergo a variety of chemical changes in the animal organism by enzymes of the liver, intestine, kidney, lung, and other tissues, with consequent alterations in the nature of their pharmacologic activity, duration of activity, and toxicity. Thus, the pharmacologic and toxicologic activity of a drug (or xenobiotic) is in many ways the consequence of its metabolism.

The study of xenobiotic metabolism has developed rapidly during the past few decades. These studies have been fundamental in the assessment of drug efficacy, safety, and design of dosage regimens; in the development of food additives and the assessment of potential hazards of contaminants; in the evaluation of toxic chemicals; and in the development of pesticides and herbicides and their metabolic fate in insects, other animals, and plants. The metabolism of many xenobiotics is fundamental to many toxic processes such as carcinogenesis, teratogenesis, and tissue necrosis. Often the same enzymes involved in

drug metabolism also carry out the regulation and metabolism of endogenous substances. The inhibition and induction of these enzymes by drugs and xenobiotics may consequently have a profound effect on the normal processes of intermediary metabolism, such as tissue growth and development.

The increased knowledge of xenobiotics and their fate in the living organisms, along with the need for greater safety evaluation of drugs and chemicals, has resulted in a proliferation of publications and a series of monographs that represent the current state of knowledge of foreign compound metabolism from biochemical and pharmacologic viewpoints.

See also: Carcinogenesis; Common Mechanism of Toxicity; Mechanisms of Toxicity.

Further Reading

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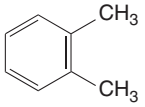
Xylene

Stephen R Clough

- Chemical Abstracts Service Registry Numbers: CAS 1330-20-7 (mixed isomers); 95-47-6; 108-38-3; 106-42-3 (*o*-, *m*-, and *p*-isomers, respectively)
- Synonyms: Dimethyl benzene; Lsylen (Polish); Methyltoluene; NCI C55232; UN1307 (DOT); Violet 3; Xiloli (Italian); Xylenen (Dutch); Xylol; Xylole (German); RCRA Waste No. U239
- Chemical/Pharmaceutical/Other Class: Aromatic hydrocarbon. Xylene is a benzene ring with two methyl substitutions which can occur at the *ortho*, *meta*, and *para* positions giving rise to *o*-xylene (1,2-dimethylbenzene), *m*-xylene (1,3-dimethylbenzene), and *p*-xylene (1,4-dimethylbenzene), respectively. The *m*-xylene form is generally the predominant isomer (44–70%) in commercial mixtures.
- Chemical Formula: C_8H_{10}
- Chemical Structure:



Uses

Xylenes are used as thinners; solvents in paints, inks, rubbers, gums, resins, adhesives, and lacquers; paint removers; and as intermediates in the production of plasticizer (phthalic acid and anhydride) and polyester fibers. They are also used extensively as intermediates in the manufacture of perfumes, dyes, insecticides, and pharmaceuticals. The annual production of mixed xylenes varies between 6 and 12 billion pounds.

Exposure Routes and Pathways

Because xylene is fairly volatile, exposure of humans occurs principally by inhalation and is most likely to occur near its principal sources; that is, chemical plants and refineries, gas pumps, painting, and refinishing operations, and automobiles (e.g., in tunnels). Dermal exposure may also be significant, especially in an industrial setting, where skin may be exposed for long periods of time. Oral exposure is the least probable route and occurs primarily as a result of accidental poisoning or suicide.

Toxicokinetics

Xylene is primarily absorbed through the mucous membranes and pulmonary system. In experimental subjects, ~60% of airborne xylene is absorbed from the lung into the bloodstream. Xylene is also readily absorbed from the gastrointestinal tract and through broken or intact skin. Once absorbed, xylene distributes to many tissues in the body, especially lipid-rich organs, although this occurs to a lesser extent than for benzene. Chemical alteration of xylene occurs in the liver and the lung, where the compound is changed to more water-soluble metabolites (the corresponding *o*-, *m*-, and *p*-toluic acids and/or methylhippuric acid) so it can be easily excreted in the urine. In animals, it has been shown that metabolism is qualitatively different in the lung versus the liver. Greater than 95% of absorbed xylene is excreted as a water-soluble metabolite, with the remaining fraction being exhaled unchanged. Excretion appears to occur rapidly; animal studies indicate complete clearance of the compound in 24 h. Xylene will cross the placenta and enter fetal tissue.

Mechanism of Toxicity

Although the exact mechanism of toxicity has not been determined, it is known that the primary toxic

effect of xylene is dysfunction of the brain and central nervous system (CNS narcosis). The main function of neurons is to conduct electrochemical signals to one, several, or thousands of other cells. The normal physiology of neurons is largely dependent on the integrity of the neuronal cell membrane which polarizes and depolarizes during the transmission of these electric signals. Thus, the most probable mechanism of toxicity results from the unique sensitivity of the cell membranes of neurons to the solvent property of xylene, which disrupts the membrane lipid bilayer and thus the normal transmission of nerve impulses.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute CNS effects in animals, such as exaggerated visual disturbances, are similar to those in humans. The median lethal oral dose in rats is $\sim 3\,g\,kg^{-1}$.

Human

Xylene is an irritant to the eyes, nose, throat, and the gastrointestinal tract. Direct contact with the skin is also irritating and will cause defatting, which may lead to dryness, cracking, blistering, or dermatitis. Xylene appears to be more acutely toxic than other structural analogs, such as benzene or toluene. CNS depression, a typical effect seen in solvent exposures, is the primary toxic effect seen following exposure to xylene. At high air concentrations, xylene may cause the following acute signs in humans: flushing and reddening of the skin, feeling of increased body heat, disturbed vision, dizziness, tremors, salivation, cardiac stress, CNS depression, and confusion. Very high exposures may cause anorexia, nausea, vomiting, and abdominal pain; continued exposure may lead to coma and death, which appear to be due to cardiac fibrillation and/or lung congestion and hemorrhage. Females are reported to be more susceptible to the effects of xylene than males.

Chronic Toxicity (or Exposure)

Animal

The results of early subchronic and chronic studies administering xylene to laboratory animals were biased because many of the effects of the solvent were found to be caused by toxic impurities such as benzene. However, later studies showed that xylene does cause a significant change in blood-forming elements and blood chemistry. A National Toxicology Program (NTP) study of rats and mice exposed orally to mixed xylenes showed that exposure resulted only in decreased body weights in both sexes. This occurred at doses of $1000\,mg\,kg^{-1}\,day^{-1}$ given 5 days a week for 13 weeks. No effects were seen at the next lowest dose, $500\,mg\,kg^{-1}\,day^{-1}$. No carcinogenic effects were observed.

In reproductive studies, effects on the fetus have been seen only at oral doses that were associated with concurrent maternal toxicity. In an inhalation study conducted by Biodynamics, pregnant female rats were exposed to mixed xylenes $6\,h\,day^{-1}$ for 190 days. Toxicity to the fetus was apparent in the group exposed to 500 ppm. Rat pups born to dams exposed to 500 or 250 ppm displayed reduced weight of ovaries, but the effect was transient. Developmental toxicity was seen in another inhalation study in rats.

Human

Effects from chronic exposure to xylene are similar to those from acute exposure but are systemically more severe. Repeated, prolonged exposure to xylene may result in conjunctivitis of the eye and dryness of the nose and throat. Repeated exposure of the skin will cause dryness, flaking, and/or dermatitis. Inhalation may cause CNS effects, such as excitation, then depression characterized by signs such as paresthesia, tremors, apprehension, impaired memory, weakness, nervous irritation, vertigo, headache, anorexia, nausea, and flatulence. Clinical findings may include moderate but reversible changes such as bone marrow hyperplasia, liver enlargement, and kidney nephrosis. Based on the weight of evidence, the US Environmental Protection Agency (EPA) has classified xylene as a class D carcinogen (insufficient evidence to classify human carcinogenicity).

Clinical Management

Persons who have been overcome by xylene fumes or gases should be removed from the area of exposure and exposed to fresh air. Should breathing become labored or shallow, medical intervention (e.g., artificial respiration) may be necessary. Following accidental or intentional ingestion, vomiting should not be induced; stomach lavage should be initiated as soon as possible. Liquid xylene spills on exposed skin should be immediately dried with an absorbent towel; next, the affected area should be washed with soap and water. In cases of eye exposure, the eyes should be irrigated immediately.

Environmental Fate

Xylenes are ubiquitous in the environment and the vast majority ultimately end up partitioning into the

atmosphere. Once in the air, xylene is transformed into other products; for example, substituted aldehydes and phenols. The estimated half-life for the photooxidation of xylenes in the atmosphere is between 0.5 and 1.0 day. Automobile and industrial emissions contribute the majority of xylene found in the atmosphere. Concentrations are lowest in remote areas (average levels of <0.5 ppb) and highest in urban areas (levels ranging from 0.5 to 21 ppb). Xylene is also found in plants and is present in their combustion products; for example, forest fire smoke and tobacco smoke. Xylene has also been detected in surface water and treated wastewater effluents, with average levels below $1\,\mu g\,l^{-1}$. It has been detected in 3% of groundwater and 6% of surface water supplies sampled in a US EPA survey. It is typically found in groundwater impacted by gasoline releases and, when found in concert with benzene, toluene and ethylbenzene, is generally a good indicator of a gasoline spill. Xylene is readily biodegradable and will not concentrate to a great degree.

Exposure Standards and Guidelines

The no-effect level from the NTP study has been used to calculate a safe oral dose for xylene in humans of $2\,mg\,kg^{-1}\,day^{-1}$. This is 10 times higher than the oral reference dose published by the US EPA on its IRIS database, $0.2\,mg\,kg^{-1}\,day^{-1}$ based on the rat no-observed-adverse-effect level of $250\,mg\,kg^{-1}\,day^{-1}$ adjusted for duration of exposure and then divided by an uncertainty factor of 1000. The inhalation reference concentration for xylene is currently $0.1\,mg\,m^{-3}$, derived from a duration adjusted rat lowest-observed adverse-effect level of $39\,mg\,m^{-3}$ divided by an uncertainty factor of 300. Under the Safe Drinking Water Act, the maximum contaminant level (MCL) is the standard criterion for drinking water and the maximum contaminant level goal (MCLG) is the ideal. The proposed MCL and the MCLG for mixed xylenes are both $10\,mg\,l^{-1}$.

The current occupational exposure limit (threshold limit value time-weighted average) recommended by the American Conference of Governmental Industrial Hygienists and enforced by the US government, as an Occupational Safety and Health Administration permissible exposure limit time-weighted average, is 100 ppm ($434\,mg\,m^{-3}$). The ceiling limit is 150 ppm ($651\,mg\,m^{-3}$).

Miscellaneous

Xylene compounds are lighter than water and only slightly soluble ($\sim 130\,mg\,l^{-1}$ for mixed isomer solution).

See also: Benzene; Neurotoxicity; Petroleum Hydrocarbons; Pollution, Air Indoor; Toluene.

Relevant Websites

http://www.epa.gov – US Environmental Protection Agency (2003) Toxicological Review of Xylenes (CAS No. 1330-20-7) in Support of Summary Information on the Integrated Risk Information System (IRIS). EPA 635/R-03/001.

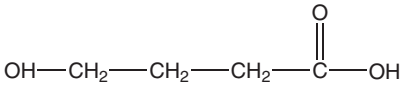
http://risk.lsd.ornl.gov – Oak Ridge National Laboratory (2003) Xylene. Risk Assessment Information System, Oak Ridge, TN.

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Xylene.

Xyrem

Arezoo Campbell

- Chemical Abstracts Service Registry Number: CAS 502-85-2
- Synonyms: Gamma hydroxybutyric acid (GHB); Sodium oxybate; 4-Butanediol, Gamma-butyrolactone (Slang terms: G-riffic; Home Boy; Grievous Bodily Harm; Liquid-X)
- Chemical/Pharmaceutical/Other Class: Drug of abuse
- Chemical Formula: $C_4H_8O_3$
- Chemical Structure:



Uses

Xyrem is used as an oral medication, available in solution form, for the treatment of cataplexy in patients with narcolepsy. Gamma hydroxybutyric acid (GHB) is also used as a drug of abuse.

Background Information

Xyrem is manufactured by Orphan Medical as an oral solution approved in 2002 by the US Food and Drug Administration (FDA) for the treatment of cataplexy associated with narcolepsy. GHB is the active component of Xyrem. It is a metabolite of the neurotransmitter gamma aminobutyric acid (GABA). Because of its role as a central nervous system (CNS) depressant, it causes sleep and ataxia. GHB has been used as a 'date-rape drug' and many hospitalizations and deaths are associated with it. Therefore, until recently, it was classified as a schedule I controlled substance. This meant that GHB had a high potential for abuse and no medical purpose. However, in 2002, because of a series of clinical trials that demonstrated that the drug reduces cataplexy attacks, it was approved for use under the trademark Xyrem. Because it had previously been used as a 'date-rape drug', Xyrem is considered a schedule III controlled substance and will be distributed only in accordance with strict FDA regulations. Illegal use of Xyrem will be punishable under Federal and state laws.

Exposure Routes and Pathways

The gastrointestinal tract is the route of exposure for Xyrem.

Toxicokinetics

GHB is absorbed very quickly. After ingestion, it is detected in the serum after 10 min. The maximum plasma concentration is achieved $\sim$1–2 h after exposure. The elimination of the drug is also very rapid and the elimination half-life is $\sim$1 h. The removal of GHB occurs via expired carbon dioxide and very little ($\sim$4%) of it is eliminated unchanged in the urine. If Xyrem is taken with food, the bioavailability is greatly reduced while the excretion remains the same. GHB can readily cross the blood–brain barrier and the placenta.

Mechanism of Toxicity

Xyrem is a CNS depressant because it is an analog of the inhibitory neurotransmitter, GABA. GHB has high affinity binding sites in the mammalian brain. These are found in the basal ganglia, cortex, midbrain, and the hippocampus. It is endogenously present in the brain and thought to have a function although the specificity of its role in the CNS is at present unknown. The highest concentrations of GHB are in the basal ganglia. GHB dose-dependently decreases the release of enkephalins in the brain of rats. This function appears to be modulated by the nigrostriatal dopaminergic pathway. However, a high affinity receptor for GHB has not been identified. Some of its negative effects may be mediated by the $GABA_B$ receptors. The maximum stimulation of these receptors by GHB is $\sim$69% when compared to the binding of a $GABA_B$ receptor agonist. Therefore, the drug appears to be a weak agonist of the GABA binding site of $GABA_B$ receptors. GHB has been shown to prevent cell damage. The exact mechanism of this protection is unknown. However, it seems to be mediated by antiinflammatory and antioxidative properties. The precise mechanism by which Xyrem produces an effect on cataplexy is also unknown.

Acute and Short-Term Toxicity (or Exposure)

Animal

Intracerebroventricular injection of GHB causes generalized seizures in animals. Therefore, the compound has been used as a model for petit mal epilepsy.

Human

Primary effects of Xyrem are dose related and include CNS depression, amnesia, and hypotonia ($10\,mg\,kg^{-1}$). Exposures in the range of 20–$30\,mg\,kg^{-1}$ cause somnolence, drowsiness, dizziness, and euphoria. At levels of 50–$70\,mg\,kg^{-1}$ common symptoms are bradycardia, nausea, and vomiting. Higher exposures can lead to coma. Xyrem may cause neuropsychiatric side effects even at recommended doses. Oral doses as low as 5 g have caused CNS depression. Concurrent alcohol use can delay the onset of symptoms.

Chronic Toxicity (or Exposure)

Animal

There are no data indicating chronic toxicity due to Xyrem. The potential for abuse of GHB in rhesus monkeys is low. Rats show mild withdrawal symptoms when injected every 3 h for 3–6 days with concentrations GHB that do not cause seizures.

Human

Symptoms of withdrawal similar to other sedatives have been documented in adults using Xyrem daily. Patients who become dependent on the drug will need supportive care for up to 15 days.

Clinical Management

Because GHB is rapidly absorbed, it will not be detected in most routine toxicology screenings. Further, gastric lavage with activated charcoal will not be helpful. Intubation and mechanical ventilation may be needed in patients with CNS depression. There is no antidote for Xyrem intoxication and treatment is based on the symptoms present.

Exposure Standards and Guidelines

Xyrem is a liquid with a GHB concentration of $0.5\,g\,ml^{-1}$. The total daily dose for patients who are taking the medication as an anticataplexy drug should be in the range of 4.5–9.0 g. It should not be taken with alcohol, sedatives, or other CNS depressants.

See also: Drugs of Abuse.

Relevant Website

http://www.fda.gov – Xyrem (sodium oxybate) Information Page.

Y

Yew

Ann P Slattery

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- SYNONYMS: *Taxus baccata*, *Taxus cuspidata*, *Taxus brevifolia*; *Taxus canadensis*; *Taxus floridana*; American Yew; Chinese yew; English yew; Japanese yew; Ground hemlock; Pacific yew; Western yew

Uses

Yews are evergreen shrubs or trees with alternate branch and reddish brown, thin, scaled bark. They are common as ornamental hedging and ground covers. The yew is often brought indoors at Christmas and used as decoration. Aqueous extracts of the yew have been used for years in Native American folk medicine for the cardiotonic, expectorant, antispasmodic, diuretic, and antiseptic properties. Experiments are being conducted on the potential for some extracts to possess central nervous system depressant, analgesic, antipyretic, cytotoxic, and antileukemic properties. Paclitaxel (Taxol) is an antineoplastic agent derived from various *Taxus* species used to treat numerous types of cancer including ovarian, breast, nonsmall cell lung and Kaposi's sarcoma.

Exposure Routes and Pathways

Ingestion of any part of the plant is the common route of exposure. The aril is not toxic. The hard seed and leaves are toxic and have the potential to release taxine. The plant parts are toxic whether green or dry.

Toxicokinetics

Taxine can be absorbed orally or by injection. Inhalation absorption is unlikely because it is not highly volatile. The onset of symptoms may be within 1 h or delayed for several hours. Systemic symptoms are expected within 1–3 h.

Mechanism of Toxicity

The main toxins of the yew species are the alkaloids taxine A and taxine B, which are present in all parts of the shrub except the fleshy part of the berry. These compounds are capable of causing symptoms similar to digitalis poisoning including hypotension, bradycardia, and depressed myocardial contractility and conduction delay. The mechanism appears to involve a block of the distal part of the conduction tissue of the heart, which can result in fatal arrhythmias. Atrioventricular conduction is particularly susceptible to yew alkaloids.

Acute and Short-Term Toxicity (or Exposure)

Human

Serious poisoning is uncommon. Most cases of yew berry ingestions result in no symptoms, because the seed must be chewed to release the taxine. One chewed berry may be potentially fatal in a child. Persons who ingest other parts of the plant or multiple berries should have gastric decontamination performed.

Symptoms initially expected after ingestions of leaves or a chewed seed are dizziness, dry mouth, and mydriasis, which develop rapidly. Nausea, vomiting, and abdominal pain follow these symptoms. A rash may appear, and facial pallor and cyanosis or reddish discoloration of the lips may occur. This is followed by generalized muscle weakness and drowsiness leading to coma. Seizures are also possible.

The primary action of these alkaloids is bradycardia and various other life-threatening arrhythmias with hypotension and decreased respiratory function. Death is due to cardiac and/or respiratory failure. Anaphylactoid reactions have been reported from chewing yew needles. If the seeds are ingested and not masticated, there is a likelihood they will pass without releasing the taxine.

Severe contact dermatitis can result from cutting yew wood. Taxines are water soluble, so drinking teas or water in which leaves are soaking is potentially dangerous.

Chronic Toxicity (or Exposure)

Human

Chronic ingestion of the yew species has revealed liver and kidney fatty degeneration on autopsy.

Clinical Management

Animal

In animals, ingestion of large amounts of any part of the yew often causes sudden death without previous symptomatology or signs of struggle. Survival after yew poisoning is uncommon. Smaller ingestions would be expected to cause gastroenteritis. Clinical signs in a herd of 35 yew-poisoned cattle included lethargy, recumbency, dyspnea, jugular pulsation and distension, and death. Most cattle died within 4 h. EKG changes and seizures were noted in dogs. Toxicity symptoms in a horse included weak pulse, ataxia, lower lip and tail limp, leg muscle trembling, respiratory grunt, collapse, seizures, and death within 15 min.

Induction of emesis should not be attempted. Lavage may be used if possible. Activated charcoal and a cathartic should be administered. Life support and respiratory function should be maintained as needed. Diagnosis of yew poisoning is based on the presence of yew plant in the gut on necropsy.

Lethal toxic doses reported in specific animal species are as follows:

- Horse: 2 g leaves per kg body weight or 100–200 g.
- Sheep: 10 g leaves per kg body weight or 100–200 g.
- Dog: 30 g of leaves.
- Swine: 3 g leaves per kg body weight or 75 g.
- Fowl: 30 g of leaves.
- Oxen: 10 g leaves per kg body weight or 500 g.
- Goats: 12 g leaves per kg body weight.

Surprisingly, deer are able to eat the foliage of *Taxus cuspidata* and apparently suffer no harm.

Human

Basic and advanced life-support measures should be utilized as needed. Several studies have demonstrated that the vast majority of unintentional exposures result in either no effects or only minor gastrointestinal symptoms. However, intentional exposures require management in an emergency department or other critical care environment. There are no antidotes. No laboratory test identifies taxine specifically.

Serious ingestions require cardiac monitoring in an intensive-care setting. Hypotension may be resistant to dopamine and dobutamine. Norepinephrine can also be used. Bradycardia can be treated with atropine and a temporary pacemaker as needed. Digoxin-specific FAB antibody fragments have been used with some success for cardiac conduction abnormalities after a yew exposure. If no contraindication, lidocaine, amiodarone, or procainamide may be used for ventricular dysrhythmias.

See also: Digitalis Glycosides.

Further Reading

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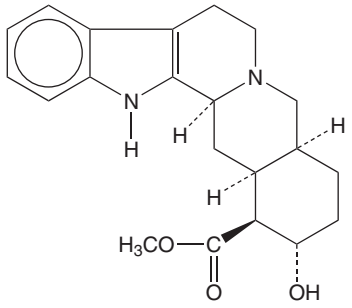
Yohimbine

Rebeca Gracia

- Chemical Abstracts Service Registry Numbers: CAS 146-48-5 (Yohimbine); CAS 65-19-0 (Yohimbine hydrochloride)
- Synonyms: Aphrodine; *Pausinystalia yohimbe* (*Corynanthe yohimbe*); Cotyine; *Rauwolfia serpentina* (roots only); YoYo; Quebrachine; Actibine; Aphrodyne; Dayto Himbin; Revervyl; Reverzine; Yobine; Yocon; Yohimex; Yohydrol (also available in various combination products); (16α,17α)-17-Hydroxyyohimban-16-carboxylic acid methyl ester
- Chemical/Pharmaceutical/Other Class: Rubiaceae family and α-2 antagonist

- Chemical Formula: $C_{21}H_{26}N_2O_3$
- Chemical Structure:



Uses

Yohimbine is used in the treatment of impotence, and as an aphrodisiac and mild hallucinogen. It is also used as a mydriatic and sympatholytic and has been suggested as an antidote to clonidine overdose.

Background Information

Yohimbine has been on the US Food and Drug Administration (FDA) unsafe herb list since March 1977, it is approved for use in veterinary medicine, as a reversal agent for xylazine overdose. Yohimbine has FDA approval as a mydriatic and sympatholytic in humans. It is also available as a herbal supplement without a prescription.

Exposure Routes and Pathways

Ingestion is the most common route of accidental and intentional exposure to yohimbine. The substance is extracted from the bark of the tree *Corynanthe yohimbe*. This tree is found in western Africa. The powder form may also be smoked or steeped into a tea. It is available in an intravenous form for veterinary purposes.

Toxicokinetics

Oral absorption is rapid, with an absorption half-life of 7–11 min. Peak plasma levels occur at 45–60 min. The volume of distribution is highly variable, demonstrated to be 2.24 ± 1 to $1.25\,l\,kg^{-1}$ after oral administration but $0.26\,l\,kg^{-1}$ after intravenous exposure. Yohimbine is excreted via the kidneys. Less than 1% of the unchanged drug was recovered in the urine after 24 h. Yohimbine is rapidly eliminated from the plasma with a half-life of less than 1 h.

Mechanism of Toxicity

Yohimbine is a competitive α-2 antagonist causing increased sympathetic outflow and enhanced release of norepinephrine. In fact, a two- to threefold increase in plasma norepinephrine has been reported after intravenous doses of $0.016\,mg\,kg^{-1}$. Yohimbine may also have effects at α-1 adrenoceptors and, in high concentrations, serotonin and dopamine receptors. It also has been shown to inhibit monoamine oxidase.

Acute and Short-Term Toxicity (or Exposure)

Animal

Yohimbine has US FDA approval to reverse the effects of xylazine in dogs. Toxic effects are similar to those observed in humans. Hypertension, tachycardia, central nervous system stimulation, and antidiuresis may occur.

Human

Although overdoses are rare, oral doses of 15–20 mg have produced hypertension. Oral doses as little as $0.1\,mg\,kg^{-1}$ may produce stimulant effects but reported therapeutic doses vary and up to $100\,mg\,day^{-1}$ has been given. Daily doses of 18 mg divided three times a day are generally well tolerated. Toxic manifestations usually involve tachycardia, diaphoresis, mydriasis, salivation, nausea, vomiting, and facial flushing. Neurological signs include dizziness, anxiety, 'squeezing headache', incoordination, and paresthesias.

Clinical Management

Basic and advanced life-support measures should be performed as needed. Gastric decontamination with activated charcoal may be beneficial if performed within the first hours of ingestion. Treatment is focused on decreasing hypertension and anxiety. Nitroprusside is preferred for severe hypertension although labetalol, nitroglycerin, and phentolamine are possible alternatives. Clonidine may be effective to reverse the α-adrenergic antagonism. Diazepam is useful to decrease anxiety.

See also: Clonidine.

Further Reading

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Z

Zinc

Shayne C Gad

This article is a revision of the previous print edition article by Arthur Furst and Shirley B Radding, volume 3, pp. 423–424, © 1998, Elsevier Inc.

- Representative Chemicals: Zinc chloride ($ZnCl_2$); Zincochromite ($ZnCrO_4$); Zinc sulfate ($ZnSO_4$)
- Chemical Abstracts Service Registry Number: CAS 7440-66-6
- Synonyms: LS6; Blue powder; Merrillite
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: Zn^{2+}

Uses

Zinc is an essential trace element and is commonly ingested as a nutritional supplement. Divalent zinc is one of the most important of the micronutrients. More than 100 enzymes are zinc dependent; for example, carboxypeptidase, carbonic anhydrase (which is responsible for the exchange of carbonic acid in the blood and the exhalation of carbon dioxide), and the alcohol dehydrogenase (which metabolizes alcohol). Deficiency of zinc, especially in newborns, results in impaired growth, loss of hair, skin eruptions, and often impaired or delayed sexual maturation. Many medical problems are also associated with zinc deficiencies (e.g., ulcerative colitis, chronic renal disease, and anemia).

Commercially, zinc is used in galvanized iron and in various alloys (e.g., brass and bronze). It is also used in dry cell batteries, electrical fuses, fungicides, and construction materials (e.g., roofing and gutters). Zinc chloride is used in electroplating, soldering fluxes, burnishing and polishing compounds for steel, and in antiseptic and deodorant solutions. Zinc chloride is used as yellow pigment. Zinc oxide is used in ointments, rubber, and paints (for white pigments).

Exposure Routes and Pathways

Ingestion and inhalation of zinc are possible exposure pathways. Zinc is readily absorbed by most plants and, hence, is found in most foods (especially grains, nuts, legumes, meats, poultry, and most seafood). The concentration of zinc in drinking water depends on the composition of water pipes and vessels. Inhalation is a significant exposure pathway in industrial areas, where zinc levels in air are high.

The concentrations of zinc in various foods and human tissues have also been determined. In a 1980–82 survey of total diet samples, the Food and Drug Administration (FDA) estimated that the average intake of zinc from food (including water) for an adult was $0.23\,mg\,kg^{-1}\,day^{-1}$. The FDA concluded that the daily intake of zinc from the inhalation of ambient air is negligible compared to the daily intake from food. Certain population groups may be exposed to higher concentrations of zinc than the general population. People who work in coal mines, people who work with the refining and smelting of nonferrous metals, and people who live near waste sites and metal smelting operations may be exposed to high levels of zinc. People who consume large amounts of foods high in zinc content, such as oysters and mussels, may also be exposed to high levels of zinc. The higher exposure may not always be manifested as increased body burden in the exposed individuals.

Toxicokinetics

Up to 30% of ingested zinc is absorbed from the small intestine; however, a homeostatic mechanism controls the absorption. Nutritional status also influences zinc absorption; deficiency of pyridoxine or tryptophan somewhat inhibits absorption. Zinc induces a zinc metallothionein, the form in which it is bound to the liver and other tissues. The pancreas is high in zinc, and in males the prostate gland contains the greatest store of zinc. Zinc is excreted in the feces.

Mechanism of Toxicity

Excessive zinc interferes with iron and copper metabolism; the latter leads to copper-deficiency anemia. Salts of strong mineral acids are corrosive to skin and intestine.

Acute and Short-Term Toxicity (or Exposure)

Animal

Acute oral toxicity in rodents exposed to zinc is low, with LD_{50} values in the range 30–600 mg kg^{-1} body weight, depending on the zinc salt administered. Acute effects in rodents following inhalation or intratracheal instillation of zinc compounds include respiratory distress, pulmonary edema, and infiltration of the lung by leukocytes.

Human

Zinc is a skin irritant. It is difficult to ingest too much zinc from foodstuffs. Consumption of beverages stored in galvanized containers or pipes, use of zinc utensils, or ingestion of too many zinc supplements can result in nausea, cramps, vomiting, and diarrhea.

In the industrial setting, inhalation of fumes of zinc, zinc oxide, or zinc chloride leads to pulmonary edema and metal fume fever. Onset occurs within 4–6 h and may be delayed up to 8 h. Symptoms include chills alternating with fever, sweating, and weakness, which can last from 24 to 48 h.

Zinc salts (e.g., zinc chloride and zinc sulfate) are corrosive to the skin and the gastrointestinal tract and can cause acute tubular necrosis and interstitial nephritis.

Chronic Toxicity (or Exposure)

Animal

Zinc is not carcinogenic; however, testicular tumors were induced by direct injection of zinc chloride into the testes of experimental animals (copper chloride produced the same effect).

Human

Since the zinc–copper ratio is important, intake of too much zinc can lead to symptoms of copper deficiency. However, patients have taken 10 times the recommended daily allowance for zinc with no adverse reaction.

Chronic inhalation of zinc compounds can lead to liver damage, which can be fatal.

Clinical Management

Clinical management is supportive. Chelating agents such as British Antilewisite (2,3-dimercaptopropanol) or D-penicillamine are not effective.

Environmental Fate

Zinc enters the air, water, and soil as a result of both natural processes and human activities. Most zinc enters the environment as the result of human activities, such as mining, purifying of zinc, lead, and cadmium ores, steel production, coal burning, and burning of wastes. These releases can increase zinc levels in the atmosphere. Waste streams from zinc and other metal manufacturing and zinc chemical industries, domestic wastewater, and run-off from soil containing zinc can discharge zinc into waterways. The level of zinc in soil increases mainly from disposal of zinc wastes from metal manufacturing industries and coal ash from electric utilities. In air, zinc is present mostly as fine dust particles. This dust eventually settles over land and water. Rain and snow aid in removing zinc from air. Most of the zinc in bodies of water, such as lakes or rivers, settles on the bottom. However, a small amount may remain either dissolved in water or as fine suspended particles. The level of dissolved zinc in water may increase as the acidity of water increases. Some fish can collect zinc in their bodies if they live in water containing zinc. Most of the zinc in soil is bound to the soil and does not dissolve in water. However, depending on the characteristics of the soil, some zinc may reach groundwater. Contamination of groundwater from hazardous waste sites has been noticed. Zinc may be taken up by animals eating soil or drinking water containing zinc. If other animals eat these animals, they will also have increased amounts of zinc in their bodies.

Ecotoxicology

Bivalves and other sessile estuarine organisms are often used as a measure of contamination of estuarine water because they usually contain higher levels of metals than fish. The arithmetic mean concentration of zinc in oysters (*Crassostrea virginica*) from the Mississippi Sound collected in 1988 was 640 mg kg^{-1} (wet weight). In a nationwide mussel watch program, the mean concentrations of zinc in molluscs (*Mytilus edulis*) around the coast of the United States during 1976–88 ranged from 67 to 3700 mg kg^{-1} (dry weight). Although the concentration on a nationwide basis varied depending on sampling sites, the level of zinc showed little evidence of statistically significant change during 1976–88. The mean concentration of zinc in oysters (*Crassostrea virginica*) collected from the US coastline of the Gulf of Mexico during 1986–88 was 2150 mg kg^{-1} (dry weight). In the National Contaminant Biomonitoring Program, the geometric

mean concentration of zinc in various whole fish was 21.7 mg kg^{-1} (wet weight). Of all fish tested (e.g., bloater, sucker, white perch, bass, and catfish.), common carp showed the highest level of zinc. No significant trend in the level of zinc in whole fish was observed during 1978–84. The concentration of zinc in yellow perch (*Perca flavescens*) from six acidic lakes in northwestern New Jersey ranged from 26.1 to 66.2 mg kg^{-1} (dry weight). Although the concentrations of mercury and lead in fish from acidic lakes were higher compared to fish collected from non-acidic lakes, the concentrations of zinc showed no significant difference. Similarly, high concentrations of zinc were not found in white suckers (*Catostomus commersoni*) and brown bullheads (*Ictalurus nebulosus*) collected from two acidic Adirondack lakes in New York.

Exposure Standards and Guidelines

The American Conference of Governmental Industrial Hygienists threshold limit value–time-weighted average (TLV – TWA) for zinc chloride (as fume) is 1 mg m^{-3}. The TLV – TWA for zinc oxide (as fume) is 5 mg m^{-3}.

Miscellaneous

For hundreds of years before being recognized as a distinct element, zinc ores were used to make brass. The pure metal was isolated in India in the thirteenth century. Zinc occurs at 0.02% in the Earth's crust.

See also: Metallothionein; Metals.

Further Reading

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Zatta P, Lucchini R, van Rensburg SJ, and Taylor A (2003) The role of metals in neurodegenerative processes: Aluminum, manganese, and zinc. *Brain Research Bulletin* 62(1): 15–28.

Relevant Websites

http://www.atsdr.cdc.gov – Agency for Toxic Substances and Disease Registry. Toxicological Profile for Zinc.

http://www.inchem.org – Zinc (Environmental Health Criteria 221 from the International Programme on Chemical Safety).

Zinc Oxide

Rebeca Gracia

- Chemical Abstracts Service Registry Number: CAS 1314-13-2
- Synonyms: Chinese white; Zinc white; Flowers of zinc; Philosopher's wool; Calcine; Amalox; Calamine; Felling zinc oxide; Zincite; AZO 22; Emar; Outmine; Pasco
- Chemical/Pharmaceutical/Other Class: Metals
- Chemical Formula: ZnO

Uses

Zinc oxide is used in various pharmaceutical products such as ointments, powders, creams, bandages, gauze, and pastes. It is commonly found in combination with other topical agents. Medicinal-grade zinc oxide is a concentration of no less than 99.5% and calamine is no less than 98%. Zinc oxide is used in industry as an accelerator, rubber reinforcing agent, in paints (white pigment, mold-growth inhibitor), plastics (ultraviolet absorber), feed additive, cosmetics, as a photoconductor, and in piezoelectric devices.

Exposure Routes and Pathways

Exposure to zinc oxide can occur through inhalation, ingestion, and eye or skin contact. Ingestion of zinc oxide ointments is most common in household settings and is generally considered nontoxic due to relatively low product concentrations. Inhalation of zinc oxide in industrial areas, as particulate matter or fumes, may lead to potentially toxic exposures.

Toxicokinetics

Zinc oxide is not absorbed to any significant amount when applied to intact skin. It is absorbed slowly when ingested; only 20–30% of dietary zinc is absorbed from the small intestine. Inhalation may result in minimal systemic absorption. Zinc is widely distributed throughout the body with increased concentrations of zinc metallothionein being found in

the liver, pancreas, muscles, and bone. It is highly protein bound and excess zinc may also be stored in erythrocytes. Zinc is excreted predominately in the feces.

Mechanism of Toxicity

Topical and inhalational exposures to zinc oxide primarily produce irritation. Excessive systemic absorption of zinc may result in altered iron and copper metabolism with resultant toxicity.

Acute and Short-Term Toxicity (or Exposure)

Animal

Topical administration of zinc oxide in animals failed to cause toxicity. The oral LD_{50} in mice is $7950\,mg\,kg^{-1}$. The LC_{50} in mice is $2500\,mg\,m^{-3}$ (duration not provided). Rats exposed to $2500\,mg\,m^{-3}$ for 3–4 h died either during or immediately after the exposure. Guinea pigs exposed to $0.7\,mg\,m^{-3}$ over 1 h showed no change in pulmonary airway resistance, but did demonstrate progressive diminution in lung compliance. Guinea pigs exposed to 5 or $7\,mg\,m^{-3}$ zinc oxide fumes for $3\,h\,day^{-1}$ for 5 and 6 days, respectively, had transient changes in pulmonary function with small airway inflammation and edema. These animals showed reduced total lung capacity, vital capacity, and carbon monoxide diffusion capacity. No adverse effects were observed in guinea pigs exposed to zinc oxide fumes at a concentration of $2.7\,mg\,m^{-3}$.

Chronic Toxicity (or Exposure)

Animal

Zinc oxide administered to rats at $200\,mg\,kg^{-1}\,day^{-1}$ for 21 days prior to mating and throughout pregnancy resulted in increased fetal deaths and reduced fetal body weights. No adverse effects were observed at $100\,mg\,kg^{-1}\,day^{-1}$.

Human

Ingestion of large amounts of zinc oxide may cause nausea, cramps, vomiting, and diarrhea. Zinc oxide is often used in ointments along with vitamins A and D, and toxicity usually develops to these added constituents rather than the zinc oxide. It would require an ingestion of greater than 60 g of a typical ointment to result in vitamin A toxicity. Smaller amounts could also cause gastrointestinal disturbances such as diarrhea due to the emollient base. As much as $2\,g\,kg^{-1}$ of zinc oxide has been tolerated. Zinc oxide dust is an irritant at high concentrations can result in respiratory system effects. Acute inhalation of zinc oxide can result in coughing, substernal pain, upper respiratory tract irritation, rales, chills, fever, nausea, and vomiting. Inhalation of zinc oxide fumes can result in metal fume fever.

In Vitro Toxicity Data

Zinc oxide has been demonstrated to be mutagenic, causing alterations in cell lines.

Human

Prolonged, recurring zinc oxide exposures to the skin may cause papular-pustular eruptions. This skin condition may be referred to as oxide pox. Studies of zinc refinery workers found no correlation between exposures and lung or other types of cancer. Chronic inhalation of zinc compounds has been implicated in cases of fatal liver damage.

Clinical Management

Clinical management is supportive. Gastric decontamination should be considered only in the case of massive ingestions. Normal zinc levels in the blood are between 68 and $136\,\mu g\,dl^{-1}$. Chelating agents such as BAL (British Antilewisite; 2,3-dimercaptopropanol) or calcium EDTA will enhance removal of zinc, but are not likely indicated unless the unusual case of massive chronic exposure. Hemodialysis and other methods of extracorporeal elimination are not necessary.

Environmental Fate

Zinc oxide poses no inherent risk to ecosystems, but it may be dissociated and release a zinc ion that can then result in aquatic toxicity.

Exposure Standards and Guidelines

Occupational Safety and Health Administration permissible exposure limit: $15\,mg\,m^{-3}$ of air for total zinc oxide dust and $5\,mg\,m^{-3}$ for the respirable fraction as an 8 h time-weighted average (TWA) concentration.

National Institute for Occupational Safety and Health recommended exposure limits: $5\,mg\,m^{-3}$ for total zinc oxide dust as a TWA for up to a 10 h workday and a 40 h workweek and a 15 min ceiling of $15\,mg\,m^{-3}$ (based on the risk of metal fume fever).

American Conference of Governmental Industrial Hygienists threshold limit value (TLV): $10\,mg\,m^{-3}$ for total zinc oxide dust (containing no asbestos and $<1\%$ crystalline silica), as a TWA for a normal 8 h workday and a 40 h workweek; TLV – TWA of $5\,mg\,m^{-3}$ and a TLV – STEL (short-term exposure limit) of $10\,mg\,m^{-3}$ for zinc oxide fume (based on providing reasonable control of this nuisance dust).

See also: Cosmetics and Personal Care Products; Zinc.

Further Reading

Meerdink GL, Reed RE, and Perry D (1986) Zinc poisoning from the ingestion of pennies. *Proceedings of American Association of Veterinary Laboratory Diagnosticians* 29: 141–150.

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APPENDIX 1
SELECTED TOXICOLOGY-RELATED INSTITUTIONS

Academy of Toxicological Sciences*

Sachin S Devi and Harihara M Mehendale

This article is a revision of the previous print edition article by David M Krentz and Harihara M Mehendale, volume 1, p. 7, © 1998, Elsevier Inc.

The Academy of Toxicological Sciences was established in 1981 for the purpose of recognizing and certifying toxicologists in order to ensure the competence and experience of professional practitioners whose work affects the public welfare. Recognition and certification are accomplished by the peer-review process, a time-honored mechanism for scientists to evaluate one another. The academy bases certification on formal training, proven ability, and experience. Demonstrated achievement, rather than the potential for achievement, is the substance of the academy's evaluation process. Thus, an individual certified as a fellow in toxicology by the Academy of Toxicological Sciences is a qualified person who actively practices toxicology and who has been evaluated by the peer-review process by the academy according to its bylaws.

*Adapted from information supplied by the Academy of Toxicological Sciences.

Candidates for certification must have broad knowledge of toxicology and demonstrate substantive involvement in toxicological activities. To apply, an applicant submits an application form and supporting documentation to the secretary-treasurer of the academy. The board of directors reviews the credentials of applicants twice a year in the spring and fall. The criteria for certification in toxicology by the academy are divided into three sections: (1) education and training, (2) professional experience, and (3) demonstration of scientific judgment and recognition. Following review by the board of directors, candidates are notified in writing of the board's decision.

Successful candidates are certified as fellows of the academy for a period of 5 years. Every 5 years, each fellow is re-certified by submitting a current *curriculum vitae* for the board's review and vote.

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American Academy of Clinical Toxicology

Christopher P Holstege

This article is a revision of the previous print edition article by Harihara M Mehendale, volume 1, p. 50, © 1998, Elsevier Inc.

History

The American Academy of Clinical Toxicology (AACT) was founded in 1968 by a group of physicians and scientists with the specific goal of advancing the diagnosis and treatment of poisonings. In 1974, the AACT established the American Board of Medical Toxicology (ABMT) to certify physicians in the specialty of clinical toxicology. This subspecialty was recognized by the American Board of Medical Specialties in 1992. In 1985, a second certifying board, the American Board of Applied Toxicology (ABAT) was established for nonphysician peer recognition.

Mission

The AACT was established in 1968 as a not-for-profit multidisciplinary organization uniting scientists and clinicians in the advancement of research, education,

and prevention and treatment of diseases caused by chemicals, drugs, and toxins.

Purpose

The AACT unites scientists and clinicians in the advancement of research, education, and prevention of diseases caused by chemicals, drugs, and other toxins. Today, the AACT is an international organization whose membership comprises clinical and research toxicologists, physicians, veterinarians, nurses, pharmacists, analytical chemists, industrial hygienists, poison information center specialists, and allied professionals.

Objectives

The founders of AACT established the academy to:

- Promote the study of health effects of poisons on humans and animals.
- Unite into one group scientists and clinicians whose research, clinical, and academic experience focus on clinical toxicology.
- Foster a better understanding of the principles and practice of clinical toxicology.
- Encourage development of new therapies and treatment in clinical toxicology.
- Facilitate information exchange among individual members and organizations interested in clinical toxicology.
- Define the position of clinical toxicologists on toxicology-related issues.

Key Activities

The ABAT was established by the AACT to provide special recognition to professionals (other than practicing physicians) who demonstrate exceptional knowledge, experience, and competence in applied clinical toxicology. An examination is administered periodically and is open to AACT members who meet the qualifications. Candidates who pass the examination are awarded the status of Diplomate of the American Board of Applied Toxicology.

Publications

Members receive AACTion, the Academy's newsletter, to keep them current with the organizational activities of the AACT. The Academy seeks to be active on issues that affect the membership and the discipline of clinical toxicology. As the need arises, an *ad hoc* committee is appointed to develop an Academy position paper. A directory that identifies the members of the Academy is available only to AACT members. The Journal of Toxicology – Clinical Toxicology is the official journal of AACT.

Meetings

The AACT, affiliated with many professional organizations, holds annual meetings in conjunction with both the American and Canadian Associations of Poison Control Centers and the American College of Medical Toxicology.

Awards and Grants

AACT offers a Multicenter Research Award, the Lampe-Kunkle Research Award, and the Micromedex International Travel Scholarship.

Related Organizations

The AACT was a charter member of the World Federation of Associations of Clinical Toxicology Centers and Poison Control Centers sponsored by the World Health Organization. The Academy supports the efforts of other toxicology organizations worldwide.

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American Association of Poison Control Centers

Christopher P Holstege

This article is a revision of the previous print edition article by David M Krentz and Harihara M Mehendale, volume 1, pp. 50–51, © 1998, Elsevier Inc.

Mission

The American Association of Poison Control Centers (AAPCC) is a nationwide organization of poison centers and interested individuals. (For a complete listing of poison centers please see the website.)

Purpose

The AAPCC provides a forum for poison centers and interested individuals to promote the reduction of morbidity and mortality from unintentional poisonings through public and professional education and scientific research. It sets standards for poison center operations.

Membership Criteria

There are several membership categories of the AAPCC including US Poison Center Member, Poison Prevention Education Center, Associate Institutional Member, Canadian Associate Institutional Member, Animal Poison Center, Industry Product Surveillance Service of Industry Poison Center, Individual Member, Emeritus Individual Member, and Sustaining Member. Each membership category has its own criteria and related entitlements (see Website).

Key Activities

The activities of the AAPCC include the following:

- Certification of regional poison centers and poison center personnel.
- Interaction with private and governmental agencies whose activities influence poisoning and poison centers.
- Development of public and professional education programs and materials.
- Collection and analysis of national poisoning data.

AAPCC policies and programs are determined by the Board of Directors, composed of the officers of the Association (president, past-president, president-elect, secretary, treasurer) and eight directors. Elected at-large, Board of Directors members serve 3 year terms.

Publications

The AAPCC publishes the following:

- Annual Report of the American Association of Poison Control Centers Toxic Exposure Surveillance System (TESS) published every September in *The American Journal of Emergency Medicine*.
- The Association's newsletter, *The Poison Line*, published six times a year.
- Hosts an online discussion forum called 'Patient Management Guidelines for Poisonings'.

Meetings

Each year, the Association holds a meeting that includes scientific presentations, business meetings, and committee meetings.

Awards and Grants

The AAPCC offers a Recognition Award to individuals who have made significant contributions to poison control and offers Research Awards to educators and specialists in poison information.

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Tel.: 202-362-7217
Email: info@aapcc.org
Toll Free Emergency Number: The AAPCC has established the following national toll-free number for poisoning emergencies: 1-800-222-1222.

American Board of Toxicology

Sachin S Devi and Harihara M Mehendale

This article is a revision of the previous print edition article by David M Krentz and Harihara M Mehendale, volume 1, pp. 51–53, © 1998, Elsevier Inc.

The American Board of Toxicology, Inc. (ABT) certifies individuals in general toxicology through a process that evaluates expert knowledge as demonstrated by education, experience, and passage of a comprehensive written examination. Certified individuals are initially recognized by being designated as Diplomats of the American Board of Toxicology for a period of 5 years.

Other ABT objectives are to encourage the study of the science of toxicology and to stimulate its advancement by promulgation of standards for professional practice. It is ABT policy that Diplomats demonstrate a continual commitment to excellence in the science of toxicology. Successful achievement of these goals as outlined by the Board will result in an individual maintaining recognition as a Diplomat by the ABT.

The ABT has identified three performance criteria by which a Diplomat will be evaluated pursuant to recertification. These criteria are: (1) Active Practice of Toxicology, (2) Continuing Education, and (3) Maintaining Expert Knowledge in General Toxicology. Each Diplomat, at the beginning of the fourth year of their current certification, will be required to apply for recertification. ABT will review activities in each of the three performance areas and notify the Diplomat of acceptable progress or deficiencies that need to be addressed. If, in the opinion of the Board, a Diplomat is not compliant with each of the three criteria at the end of the fifth certification year, that Diplomat may be required to successfully pass the formal certification examination. Diplomats who are compliant with each of the three performance criteria will be certified for an additional 5 years.

Active Practice of Toxicology: Active practice is defined as performing, directing, or managing toxicology activities such as research, testing, teaching, clinical practice, or regulation.

Continuing Education: A successful program of continuing education may encompass a myriad of diverse activities. The study of published texts, periodicals, or scientific journals germane to toxicology is a means by which Diplomats routinely maintain or expand their knowledge of toxicology. Other evidence of a commitment to continued education is attendance at specific programs where toxicology themes are presented in a comprehensive or in-depth manner. Such programs are often held during general or annual meetings of the Society of Toxicology, American College of Toxicology, FASEB, Environmental Mutagen Society, Teratology Society, American Association for Cancer Research, or Chapter Meetings of the Society of Toxicology. Attendance Forum or Target Organ Conferences also provide opportunities to maintain or expand a Diplomat's knowledge of toxicology.

Maintaining Expert Knowledge of General Toxicology: It is held that an objective mechanism is required for the Diplomat and ABT to gauge the success of their efforts to maintain expert knowledge in general toxicology. A recertification examination prepared by the ABT is to serve in this evaluation process. Diplomats will have the opportunity to privately complete the recertification examination during the fourth year of their certification period using their own reference material as needed. The completed examination will be graded by ABT and returned to the Diplomat. The Diplomats will be furnished a comparison of their results with the performance of peers for each subject area. Stimulated by these results the Diplomat would be expected to tailor a continuing education program that addresses those subject areas in which their knowledge appears to have diminished. The ABT may ask a Diplomat to complete specific portions of the recertification exam to assess the success of their focused continuing education program.

Summary of Recertification Process: Each Diplomat maintains a personal file of activities germane to the Active Practice and Continuing Education criteria for certification, that is, name of meeting attended, number of hours, title, topics, faculty, etc. Each Diplomat is required to be recertified every 5 years in order to maintain the Diplomat status. In addition to maintaining active practice of toxicology during the first 3 years, this procedure involves submission of credentials during the fourth year and fulfilling other requirements during the fifth year.

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American College of Medical Toxicology

Christopher P Holstege

The American College of Medical Toxicology (ACMT) is a professional association of physicians with recognized expertise in the field of medical toxicology. The purpose of the ACMT is to advance the science, study, and practice of medical toxicology by fostering the development of medical toxicology in its provision of emergency, consultation, forensic, legal, community, and industrial services. ACMT is a nonprofit organization that is not involved in authorizing or designating any political lobby action. ACMT members elect a Board of Directors (nine members), including executive officers.

History

The ACMT was formerly known as the American Board of Medical Toxicology (ABMT). The ABMT offered specialty certification in medical toxicology at a time when the American Board of Medical Specialties (ABMS) did not recognize subspecialty certification in toxicology. When the ABMS approved formal recognition of medical toxicology as a subspecialty in September 1992, the ABMT discontinued its function as a certifying body. It was reincorporated in September 1993 as the ACMT.

Membership

Active members of the ACMT are physicians who have been certified by the ABMT and/or by the Sub-Board in Medical Toxicology of the ABMS. In addition to active members, the ACMT accepts applications for international and associate membership. International members are physicians licensed to practice medicine in countries outside the United States, who practice medical toxicology as a substantial portion of their professional activities. Associate members are physicians licensed to practice medicine, who have completed a residency training program in a primary medical specialty, and who are enrolled in or have completed a training program in medical toxicology. Active members, international members, and members emeritus of the ACMT who have met additional criteria may be designated as 'Fellow of the American College of Medical Toxicology', and are entitled to use the title 'FACMT'.

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American College of Toxicology

Harihara M Mehendale

Introduction

The American College of Toxicology (ACT) is dedicated to providing an interactive forum for the advancement and exchange of toxicologic information between industry, government, and academia. In the international arena, ACT supports and participates in the efforts of the International Union of Toxicology (IUTOX), which allows ACT to advance these goals on a global scale. The goal of the college is to bring together people having common interests in the broad field of toxicology. This includes not only those individuals involved in toxicology but also those from related disciplines: analytical chemistry, biology, biological statistics, computer science, physiology, toxicokinetics, pathology, teratology, genetic toxicology, molecular biology, experimental psychology, immunology, cancer biology, and animal husbandry. The college recognizes that application of the science of toxicology is multifaceted, encompassing many disciplines. All of these relate to modern toxicology and attempt to address present and future problems. Toxicology can be defined in the classical sense as the scientific study of the effects of toxicants on biological systems. However, the explosion of scientific knowledge has made infinitely more complex the statement of the toxicological problem and understanding the solution. Modern toxicology offers a unique challenge since it involves quantitative interpolation of data from high to low dose

and its eventual extrapolation from simple life forms and animals to humans and the environment. The prediction of toxic effects upon all stages of development and the use of computer models represent frontiers of knowledge in toxicology that in some cases are in their infancy.

ACT also recognizes that the interests and problems of its members are disparate and not only stem from the performance of their responsibilities but also are significantly impacted by government regulations, industrial practices, and societal perception.

ACT is committed to addressing the toxicological issues of the day and those it anticipates will arise in the future. Its interests lie in disseminating information to and among its members so that their combined talents and creative insights may further the practice of their science. To do so, the college brings together the necessary experts to debate and discuss unique and creative approaches to problems that hopefully will better serve the needs and interests of the communities in which we live and the society we serve.

Mission Statement

Mission

The mission of the ACT is to educate and lead professionals in industry, government, and related areas of toxicology by actively promoting the exchange of information and perspective on the current status of safety assessment and the application of new developments in toxicology.

Strategic Objectives

- Focus on interdisciplinary exchange of scientific information, especially as scientific information is used in regulation.
- Sponsor scientific and educational programs in toxicology.
- Present the ideals and opinions to its membership.
- Disseminate information of the results of toxicological research, standards, and practices through the College journal and newsletter.
- Serve in other capacities in which the College can function more efficiently as a group than as individuals.

Activities

Activities include annual meetings and workshops. ACT publishes a newsletter (quarterly) and the *International Journal of Toxicology* (formerly *Journal of the American College of Toxicology*). The *International Journal of Toxicology* publishes fully refereed papers covering the entire field of toxicology, including research in risk assessment, general toxicology, carcinogenicity, safety evaluation, reproductive and genetic toxicology, epidemiology and clinical toxicology, mechanisms of toxicity, new approaches to toxicological testing, and alternatives to animal testing. Reviews and major symposia in the field are included.

Membership

ACT membership is by election after submission of an application and supporting documentation. There are three types of individual membership: full, associate, and student. Full membership is for qualified individuals who have conducted and published original research in toxicology. Associate membership is for individuals with critical interests in toxicology who have not reached full membership status. Student membership is for qualified predoctoral students. Honorary membership and Fellow status are also awarded periodically. Corporate membership is available for corporations, associations, and other organizations that support the activities of the College.

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American Conference of Governmental Industrial Hygienists

Andrew Maier

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History

The American Conference of Governmental Industrial Hygienists (ACGIH®) is a private, not-for-profit, nongovernmental organization headquartered in Cincinnati, OH. The ACGIH has played an important role in the area of occupational health and safety with a history in the field of over 60 years.

Mission and Purpose

The ACGIH is a member-based organization with a mission and purpose to advance worker health and safety through education and the development and dissemination of scientific and technical knowledge.

Membership Criteria

There are over 4000 members of the organization worldwide. Membership categories include regular membership for those who are occupational hygiene, occupational health, environmental health, or safety professionals whose primary employment is with a government agency or an educational institution. Associate memberships are for those who are engaged in the occupational hygiene, environmental health, occupational health, or safety professions, but are not eligible for regular membership. Other membership categories include student, retired, honorary, or organizational.

Key Activities, Publications, Databases, and Services

The ACGIH supports its objectives by developing professional guidelines and technical documents and sponsoring professional conferences and seminars. The ACGIH publishes jointly with the American Industrial Hygiene Association the *Journal of Occupational and Environmental Hygiene*, a monthly peer-reviewed technical journal. In addition, the organization, through the work of its numerous technical committees, publishes professional guidelines and technical documents. An important example of this activity includes the Threshold Limit Values (TLVs®) for Chemical Substances and physical agents and Biological Exposure Indices (BEIs®). These occupational exposure criteria are widely used around the world as the basis for occupational health protection. The organization has published over several hundred other documents on a variety of occupational health and safety topics. The ACGIH also supports its mission through the sponsorship of conferences and seminars, including as a sponsor for the annual American Industrial Hygiene Conference and Exposition (AIHCE), and through focused seminars, workshops, and lectures on topics of current interest.

Related Organizations

- American Industrial Hygiene Association.
- (US) National Institute for Occupational Safety and Health.
- (US) Occupational Safety and Health Administration.

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American Industrial Hygiene Association

Andrew Maier

History

The American Industrial Hygiene Association (AIHA) is a nonprofit organization founded in 1939 and headquartered in Fairfax, VA, USA.

Mission and Purpose

The mission of AIHA is that it 'promotes, protects, and enhances industrial hygienists and other occupational health, safety and environmental professionals in their efforts to improve the health and well-being of workers, the community, and the environment'.

Membership Criteria

The AIHA has ~12 000 members, making it one of the largest international associations of occupational and environmental health professionals. Members include professionals practicing industrial hygiene in industry, government, labor, academic institutions, and independent organizations. A variety of membership categories exist, based on area or practice and level of experience in the field.

Key Activities, Publications, Databases, and Services

The AIHA supports this mission through a variety of different activities and membership services. AIHA publishes jointly with the American Conference of Governmental Industrial Hygienists (ACGIH), a monthly peer-reviewed technical journal – *Journal of Occupational and Environmental Hygiene*. In addition, the organization, through the work of its numerous technical committees, publishes professional guidelines and technical documents. Important examples of this activity include the Emergency Response Planning Guidelines and Workplace Environmental Exposure Level Guides. The emergency and occupational exposure criteria published in these documents are widely used for assessing health risk during emergency exposure situations and for occupational health protection. The organization publishes numerous other documents on a variety of occupational health and safety topics. The AIHA also supports its mission through the sponsorship of conferences and seminars, including as a sponsor for the annual American Industrial Hygiene Conference and Exposition (AIHCE), as well as numerous focused seminars, workshops, and lectures, and online training courses on topics of current interest. AIHA also provides laboratory accreditation programs to help ensure a high standard of quality in the analysis of exposure and bulk material sampling data used in making occupational health decisions.

Related Organizations

- American Conference of Governmental Industrial Hygienists (ACGIH);
- (US) National Institute for Occupational Safety and Health (NIOSH); and
- (US) Occupational Safety and Health Administration (OSHA).

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CIIT Centers for Health Research

Sachin S Devi and Harihara M Mehendale*

The CIIT Centers for Health Research (CIIT) is a private, not-for-profit research organization whose purpose is to advance industry and public interest in the acquisition, evaluation, and dissemination of information on the potential impact of exposure to chemicals and other substances on human health. CIIT's core research program on human health risks is funded by member companies of the American Chemistry Council through the Long-Range

*Compiled from the information provided by CIIT.

Research Initiative (LRI). CIIT also receives funding from federal grants and industry contracts.

CIIT uses a systems biology approach to human health effects research. The hallmark of systems biology is the seamless integration of functional genomics, computational biology, and bioinformatics to guide biomedical research and provide integrative, quantitative tools for hypothesis testing and experimental design. CIIT's LRI-funded research program on human health risks is concentrated in four major areas: (1) toxicological and physiological studies to assess responses of the organism and cell to chemical exposures; (2) high-throughput genomics with *in vivo*, *in vitro*, and *ex vivo* preparations to catalog and evaluate tissue and cell responses; (3) computational analysis of biological data using simulation and bioinformatics to interpret these responses; and (4) quantitative modeling of dynamic systems that predict dose–response behavior of these biological responses under realistic exposures. In 2004, CIIT expanded its endocrine biology program to provide a foundation for human disease research.

CIIT is administratively organized into the Division of Biological Sciences and the Division of Computational Biology. In keeping with the systems biology model, CIIT is also organized into technology-based research centers that support multiple projects and serve as a focus for external funding. The centers target areas relevant to environmental health issues and will change as new issues emerge. CIIT currently has three research centers. The Center for Computational Biology and Extrapolation Modeling develops biologically based computer simulation models and uses them as the basis for human health risk assessments. The Center for Developmental Dosimetry brings together the expertise needed to evaluate the fate of xenobiotics in developing organisms. The Center for Integrated Genomics provides centralized equipment, expertise, and training for studies of gene expression.

CIIT faculty hold advanced degrees in analytical chemistry, biochemistry, biology, cell biology, chemical engineering, environmental engineering, environmental sciences, inhalation toxicology, mathematics, mechanical engineering, molecular biology, pharmacology, physiology, statistics, toxicology, and veterinary biosciences. In the 27 years since CIIT began operations, faculty have established a strong presence in the research community through publication in the peer-reviewed literature and the formal presentation of research results at scientific meetings. CIIT scientists have published more than 3800 scientific documents, including over 1200 research articles. CIIT faculty have served in a number of scientific advisory positions for organizations representing public health concerns over the years. They have contributed to the quality of a wide variety of research publications by serving as peer reviewers and in various editorial capacities. Faculty are also involved in the education of numerous college students in the Research Triangle through adjunct faculty appointments at Duke University, North Carolina State University, and the University of North Carolina at Chapel Hill.

CIIT has a strong commitment to training and education in health effects research. CIIT awards postdoctoral fellowships to scientists who have recently obtained advanced degrees, predoctoral fellowships to PhD students at area universities, and summer internships to undergraduate students. Since 2001, CIIT has been sponsoring workshops to introduce middle and high school teachers to biomedical research. As a result of CIIT's strong commitment to education, alumni of the postdoctoral and predoctoral programs have been major contributors to health effects research. During the 27 years since CIIT began operations, 201 postdoctoral fellows and trainees, 67 predoctoral fellows, and 121 summer interns have participated in CIIT's education programs.

CIIT was founded in 1974 as the Chemical Industry Institute of Toxicology by farsighted leaders of 11 major chemical companies in the United States to address growing concerns about the effects of chemicals on environmental and human health. CIIT began operations in 1976 under the leadership of first President Dr. Leon Golberg, an internationally known toxicologist who had founded and headed the British Industrial Biological Research Association. Dr. Robert A. Neal, who was Director of the Center in Environmental Toxicology and Professor of Biochemistry at Vanderbilt University School of Medicine, was appointed CIIT's second President in 1981. Dr. Roger O. McClellan, President and Director of the Inhalation Toxicology Research Institute in Albuquerque, New Mexico, was recruited to be CIIT's third President in 1988. Dr. William F. Greenlee, Chair of the Department of Pharmacology and Molecular Toxicology at the University of Massachusetts Medical School, was appointed fourth President in 1999 as CIIT entered the final stages of its transition from an institute primarily sponsored by CIIT member companies to an organization receiving its major funding from member companies of the American Chemistry Council. Dr. Greenlee refocused CIIT's research vision on the key human health issues of global concern, restructured CIIT's core research program using a systems biology approach, and developed a multifaceted approach to funding. In keeping with Dr. Greenlee's vision, the institute changed its name in December 2000 to the CIIT Centers for Health Research.

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Consumer Product Safety Commission*

Michael A Babich

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The US Consumer Product Safety Commission (CPSC) is an independent federal regulatory agency created by Congress in 1972. The agency's mission is to protect the public against unreasonable risks of injuries and deaths associated with consumer products. The CPSC has jurisdiction over 15 000 types of products used in and around the home, in schools, and in recreation. Products under the jurisdiction of CPSC include clothing, children's articles, household appliances, home furnishings, cleaners, and consumer fireworks. CPSC is directed by three Commissioners, each of whom is appointed by the President of the United States, with one of the Commissioners nominated by the President to the position of Chairman.

Statutes Administered by CPSC

To carry out its mission, CPSC administers five statutes. They are: (1) the Consumer Product Safety Act (CPSA), (2) the Federal Hazardous Substances Act (FHSA), (3) the Flammable Fabrics Act (FFA), (4) the Poison Prevention Packaging Act (PPPA), and (5) the Refrigerator Safety Act (RSA). Toxicological issues arise most frequently under the CPSA, FHSA, and PPPA. CPSC regulations implementing these statutes may be found at Title 16 of the Code of Federal Regulations (CFR) and are available on the Commission's website.

CPSA regulations include a ban of paint containing more than 0.06% lead, as well as children's products that bear lead-containing paint (16 CFR part 1303). Certain products that contain respirable, free-form asbestos are also banned under the CPSA (16 CFR part 1304).

In 1992, the Commission issued guidelines for assessing chronic hazards under the FHSA, including carcinogenicity, neurotoxicity, reproductive/developmental toxicity, exposure, bioavailability, risk assessment, and acceptable risk (57 FR 46626-46674). The chronic hazard guidelines are intended to assist manufacturers in complying with the FHSA. A summary of the chronic hazard guidelines appears in the CPSC regulations at 16 CFR § 1500.135, and is available on the Commission's website.

In 1998, the Commission issued guidance requesting manufacturers, importers, distributors, and retailers to eliminate lead that may be accessible to children from consumer products (16 CFR § 1500.230). In 1998, the Commission also issued guidance requesting manufacturers to eliminate the use of hazardous liquid chemicals (e.g., methanol, methylene chloride, and petroleum distillates) from children's products, such as rolling balls, necklaces, pens, and liquid timers (16 CFR § 1500.231).

The Labeling of Hazardous Art Materials Act (LHAMA) amended the FHSA to provide additional requirements for arts and crafts materials. Under regulations implementing LHAMA, each producer or repackager of an art material must describe in writing, and submit to the Commission, the criteria used to determine whether an art material has the potential for producing chronic adverse health effects (16 CFR § 1500.14 (b)(8)). The producer or repackager must also submit a list of art materials requiring chronic hazard labeling (16 CFR § 1500.14 (b)(8)(ii)(C)). In addition, the CPSC regulations require art materials to have a statement of conformance and bear an emergency management telephone number (16 CFR § 1500.14 (b)(8)(ii)(C)).

To require child-resistant packaging under the PPPA the Commission must find that special packaging is needed to protect children from serious personal injury or illness from handling, using, or ingesting a substance and that special packaging can be developed and mass produced that will protect the integrity of the product. Chemicals are regulated under the PPPA on a case-by-case basis.

Contacting CPSC

Consumers may contact CPSC to report an unsafe product or product-related injury, find out whether a

*This information has been prepared by CPSC staff; it has not been reviewed or approved by, and may not reflect the views of, the Commissioners. Because this material was prepared by CPSC staff in their official positions, it is in the public domain and may be freely copied or reprinted.

product has been recalled, request injury data, or obtain CPSC publications, including press releases, staff reports, and regulations.

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4330 East-West Highway, Bethesda, MD 20814, USA
Tel.: +1-800-638-2772 (Call to obtain product safety information and other agency information and to report unsafe products. Available 24 h a day, 7 days a week.)
URL: http://www.cpsc.gov

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Further Reading

US Consumer Product Safety Commission (CPSC) (1992) Labeling requirements for art materials presenting chronic hazards; guidelines for determining chronic toxicity of products subject to the FHSA; supplementary definition of 'toxic' under the Federal Hazardous Substances Act; final rules. Federal Register 57: 46626–46674 (1992).

Relevant Websites

http://www.access.gpo.gov – Code of Federal Regulations. Volume 16, Chapter II. Consumer Products.

Department of Defense, US

Ruth Custance

Introduction

The Department of Defense (DoD) is responsible for providing the military forces to prevent war and protect the security of the United States. The DoD includes the Office of the Secretary of Defense, Joint Chiefs of Staff, three Military Departments, nine Unified Combatant Commands, the DoD Inspector General, 15 Defense Agencies, and seven DoD Field Activities. The three Military Departments include the Army, Navy, which includes the Marine Corps, and the Air Force. The Department has an annual budget of ~$370 billion and has ~1.5 million active duty personnel and employs 650 000 civilian employees.

History

The Army, Navy, and Marine Corps were established by the Second Continental Congress in 1775 in support of the American Revolutionary War. The War Department was established in 1789 to administer these military forces. The armed forces were reorganized under a secretary of defense by the National Security Act of 1947, which also created the US Air Force as an independent service from the Army. In 1949, the services were brought together in a single Department of Defense.

Support of basic research within the military establishment has a long history. The Lewis and Clark expedition to explore the Northwest Territory was funded by the Army in 1804. However, prior to World War II, the Army and Navy Departments were conducting little basic research, which resulted in a military force that was not aware of the engineering and scientific opportunities available. During the early stages of World War II, it was realized that new technologies were needed and a large effort was expended to develop such technology as radar for early warning, surveillance, nuclear weapons, homing torpedoes, jet aircraft, rockets, and cryptology. The importance in World War II of these technologies developed from basic research programs caused the

Congress to formalize the DoD support of basic research by establishing the Office of Naval Research (ONR) in 1946. The National Science Foundation (NSF) was established shortly after in 1950, the Army Research Office (ARO) in 1951, the Air Force Office of Scientific Research (AFOSR) in 1952, and following Sputnik, the first spaceship launched into orbit by the Russians, the Defense Advanced Research Projects Agency (DARPA) in 1958. Postwar DoD contributions include the DARPA-NET, which developed into the Internet and Global Positioning System developed by the Naval Research Laboratory. The DoD basic science research budget is targeted more heavily in the area of physical sciences and engineering. Of all the Federal agencies, DoD is one of the largest funders of research in electronics, computers, mathematics, aeronautics, material science, mechanics, and environmental sciences. More than half of the DoD's basic research budget is spent at universities.

Mission

The mission of the DoD is to "support and defend the Constitution of the United States; to provide for the common defense of the nation, its citizens, and its allies; and to protect and advance US interests around the world." To accomplish this mission, the Department maintains trained forces ready to respond to threats to US security.

The DoD has established two corporate-level goals:

- *Goal 1*. Shape the international security environment and respond to the full spectrum of crises by providing appropriately sized, positioned, and mobile forces.
- *Goal 2*. Prepare now for an uncertain future by pursuing a focused modernization effort that maintains US qualitative superiority in key warfighting capabilities. Transform the force by exploiting the Revolution in Military Affairs and reengineer the Department to achieve a twenty-first century infrastructure.

In support of the goals of the DoD, scientific research is conducted under many of the defense agencies and military departments.

Department of Defense Basic Research

The DoD supports a major Basic Research Program across science and engineering fields that are important to defense needs. Historically, DoD research programs have introduced innovative capabilities such as radar, digital computers, cryptology, wireless mobile communications, multimedia connections, lasers and fiber optics in communications and in medicine, composite materials, satellite navigation, and environmental technologies.

Defense research is conducted in the following disciplines (approximate percentages of total funding are shown in parentheses): physics (9%), chemistry (9%), mathematics (7%), computer science (6%), electronics (13%), materials science (8%), mechanics (13%), terrestrial sciences (3%), ocean sciences (13%), atmospheric and space sciences (6.0%), biological sciences (9%), and cognitive and neural science (4%).

The Director of Research reports to the Director of Defense Research and Engineering (DDR&E) in the Office of the Secretary of Defense. The responsibilities of the Director of Research include: Providing leadership, policy guidance, and scientific oversight of basic research and serving as the DoD advocate for the Research (budget category 6.1) Program, which is managed by the Service Research Offices, namely the ARO and other Army organizations, the ONR, and the AFOSR, and by the DARPA, and through smaller research programs in the Ballistic Missiles Defense Organization, the National Security Agency, and the Army Corps of Engineers (COE). The Director of Research coordinates DoD basic research activities with the NSF and other federal departments and agencies, and with interagency groups such as those under the National Science and Technology Council chaired by the President's Science Adviser.

Most of the science and engineering work comprising the Defense Research Program is organized in the following 12 disciplinary areas, which are grouped under five categories:

- The physical sciences
  - Physics
  - Chemistry
- Mathematics and computer science
  - Mathematics
  - Computer science
- Engineering
  - Electronics
  - Materials sciences
  - Mechanics
- The environmental sciences
  - Terrestrial sciences
  - Ocean sciences
  - Atmospheric and space sciences
- The life sciences
  - Biological sciences
  - Cognitive and neural sciences

Some research interests in the Environmental Sciences and Life science disciplinary areas include:

- Terrestrial sciences
  - Weather related behavior of solid earth
  - Hydrodynamic and sedimentary processes for logistics over the shore
  - Pollution prevention and conservation
  - Structures research for survivability of airfields, pavements, buildings
  - Geodesy, seismology, remote sensing, terrain analysis, and modeling
- Ocean sciences
  - Ocean engineering and instrumentation
  - Physical oceanography
  - Marine chemistry, biology, and meteorology
  - Underwater acoustics
  - Littoral underwater visibility and target recognition
- Atmospheric and space sciences
  - Atmospheric effects on electromagnetic propagation
  - Atmospheric sensing and probing
  - Aerosol research
  - Solar and space physics
  - Upper atmospheric and ionospheric research
- Biological sciences
  - Biotechnology for novel materials enhancing survivability and mission effectiveness, such as more sensitive and accurate sensors against biological warfare agents, and protective materials against them
  - Biomolecular processes and materials for biosensors and biodegradation
  - Cellular biology, for example, to improve the healing of wounds sustained in combat
  - Treatment of infectious diseases more common in military service
  - Laser safety and eye protection

The principal points of contact for each discipline are in the Service Research Organizations, the ARO, the ONR, and the AFOSR, each of which is generally organized internally by the 12 disciplinary areas presented above, and the DARPA.

Examples of specific research programs or focus areas within the DoD that relate to toxicology include BioSystems administered under the Under Secretary of Defense Science and Technology, the Army Center for Environmental Health Research (USACEHR), the Armed Forces Institute of Pathology, the Naval Health Research Center, and the Navy Environmental Health Center.

The BioSystems program is responsible for guidance and oversight in the technology areas of Human systems, biomedical, chemical/biological defense, environmental quality, and civil engineering. Research topics include developing risk knowledge, vaccines, and therapeutic agents for infectious disease protection and providing advanced technologies for DoD to operate in an environmentally sound manner, for example, through the Strategic Environmental Research and Development Program, the DoD's corporate environmental research and development program.

The USACEHR developed from the toxicology program that existed as part of the US Army of Biomedical Research and Development Laboratory and is realigning under the Army Medical Research Institute of Chemical Defense. Research programs include participation in the Tri-Service Toxicology, which is responsible for developing the biochemical data needed to characterize the toxicity of materials used by the Armed Forces and to use these data to conduct health-hazard evaluation and risk assessment and the Toxicology Research program and Reproductive Hazards Program, which conducts research in the area of the carcinogenicity, immunotoxicology, and reproductive and development toxicology in fish and other nonmammalian systems.

The Naval Health Research Center Environmental Health Effects Laboratory conducts research in the areas of reproductive toxicology, cardiac toxicology, risk assessment, neurobehavioral toxicology, inhalation toxicology, and environmental and molecular toxicology in support of the Tri-Service toxicology needs. Research topics include evaluating the acute toxicity of jet fuel in occupationally exposed humans, inhalation toxicity of combustion of composite materials, and evaluating neurobehavioral effects at the neuromolecular level resulting from exposure to compounds and stressors such as physical fatigue.

The Navy Environmental Health Center administers the occupational health program for the Department of the Navy. Support is provided to the Naval Facilities Engineering Command in support of the Navy's Installation Restoration Program, the Base Realignment and Closure Program, and other related environmental projects. Specific services include health and safety, health and environmental risk communication, public health assessment and toxicology. The Navy was selected to serve as the lead agent for health and environmental risk communication training for the DoD.

Basic Science Research Funding

Defense research programs are organized within two types of budget categories: Funding Offices (offices

responsible for certain application areas, e.g., ships, space, communications) and Performing Organizations (usually DoD laboratories). The budget category out of which a contract or grant is funded indicates whether the work is considered basic research, applied research, prototype development, manufacturing technology, or something else. Basic Research is known as 'Budget Category 6.1'. Each of the above Service Research Organizations conducts 6.1 research. Three additional organizations within the ARO that support 6.1 research are the Army Research Institute for the Behavioral and Social Sciences (ARI), the Army Medical Command, and the COE.

ARO, ONR, and AFOSR regularly publish brochures and Broad Agency Announcements (BAAs) describing their research interests in general terms. The BAAs, published in the *Commerce Business Daily*, include instructions regarding proposal content and submission, as well as the criteria used to evaluate proposals.

In addition to research within the DoD Service Organizations, the DoD supports research within universities in the University Research Initiative (URI). The URI is a program funded out of the Office of the Director of Research, in the Office of the ODDR&E. It is jointly administered by the ARO, ONR, and AFOSR. The URI consists of several component programs including:

- Multidisciplinary University Research Initiative, which provides funding for research groups from different disciplines working for a common objective.
- The Defense University Research Instrumentation Program, which provides funding for badly needed, relatively expensive instrumentation for scientific and engineering research of special interest to the DoD.
- The National Defense Science and Engineering Graduate Fellowships, which support outstanding students to undertake graduate research in areas of strong interest to DoD.
- The Defense Experimental Program to Stimulate Competitive Research, which funds research in States underrepresented in Federal research support.

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- Defense Technical Information Center
  URL: http://www.dtic.mil
- United States Army Center for Environmental Health Research (USACEHR)
  URL: http://www.usacehr.detrick.army.mil
- The Naval Health Research Center Environmental Health Effects Laboratory
  URL: http://www.navy.al.wpafb.af.mil

Department of Energy, US

Ruth Custance

Introduction

The Department of Energy (DOE) is primarily a national security agency with all of its missions related to its core mission to support national security. The DOE is responsible for energy security, maintaining the safety, security and reliability of the nuclear weapons stockpile, cleaning up the environment from past practices during the Cold War, and advancing science and technology. The DOE has been in existence for ~25 years and operates 24 research laboratories and facilities, and manages the environmental cleanup related to nuclear defense activities conducted over the last 50 years. The DOE has an annual budget of ~$23 billion and employs ~14 500 Federal and 100 000 contractor employees.

History

The founding of the DOE can be traced to the Manhattan Project and the race to develop the atomic bomb during World War II. Following the war, there was much debate in Congress regarding whether control of the atom should be under civilian or military control. The Atomic Energy Act of 1946 settled the debate by creating the Atomic Energy Commission, which took over the Manhattan Project's extensive scientific and industrial complex.

The Atomic Energy Commission was specifically established to maintain civilian government control over the field of atomic research and development. During the early Cold War years, the Commission focused on designing and producing nuclear weapons and developing nuclear reactors for naval propulsion. The Atomic Energy Act of 1954 ended exclusive Government use of the atom and began the growth of the commercial nuclear power industry, giving the Atomic Energy Commission authority to regulate the new industry.

In the 1970s, the Atomic Energy Commission was abolished and the Energy Reorganization Act of 1974 created two new agencies: the Nuclear Regulatory Commission to regulate the nuclear power industry and the Energy Research and Development Administration to manage the nuclear weapon, naval reactor, and energy development programs. As a result of the prolonged energy crisis of the 1970s, the Department of Energy Organization Act joined the Federal Government's agencies and programs into a single agency, the Department of Energy. Established on October 1, 1977, the Department of Energy assumed the responsibilities of the Federal Energy Administration, the Energy Research and Development Administration, and parts and programs from several other agencies.

Mission

Over its 25 year history, the DOE has changed its emphasis and focus as the needs of the Nation have changed. During the late 1970s, the DOE emphasized energy development and regulation. In the 1980s, nuclear weapons research, development, and production took a priority. Since the end of the Cold War, the DOE has focused on environmental cleanup of the nuclear weapons complex, nuclear nonproliferation and nuclear weapons stewardship, reliable energy supplies and delivery, energy efficiency and conservation, and technology transfer.

The DOE's principal tools in the pursuit of its national security mission are science and technology. The DOE has developed great scientific and technical capabilities which have served America in ways never anticipated. Those capabilities will be applied to the overarching mission of ensuring the national security.

The DOE has four strategic goals:

- *Defense strategic goal*: To protect our national security by applying advanced science and nuclear technology to the Nation's defense.
- *Energy strategic goal*: To protect our national and economic security by promoting a diverse supply and delivery of reliable, affordable, and environmentally sound energy.
- *Science strategic goal*: To protect our national and economic security by providing world-class scientific research capacity and advancing scientific knowledge.
- *Environment strategic goal*: To protect the environment by providing a responsible resolution to the environmental legacy of the Cold War and by providing for the permanent disposal of the Nation's high-level radioactive waste.

Office of Science

The Office of Science within the DOE manages fundamental research programs in basic energy sciences, biological and environmental sciences, and computational science. In addition, the Office of Science is the Federal Government's largest single funder of

materials and chemical sciences, and it supports important parts of US research in climate change, geophysics, genomics, life sciences, and science education.

The Office of Science manages research through five interdisciplinary program offices: Advanced Scientific Computing Research, Basic Energy Sciences, Biological and Environmental Research, Fusion Energy Sciences, and High Energy Physics and Nuclear Physics.

The Office of Science also manages the 10 laboratories within the national laboratory system that was created over half a century ago. Five of the laboratories are multiprogram facilities: Argonne National Laboratory, Brookhaven National Laboratory, Lawrence Berkeley National Laboratory, Oak Ridge National Laboratory, and Pacific Northwest National Laboratory. The other five laboratories are single-program national laboratories: Ames Laboratory, Fermi National Accelerator Laboratory, Thomas Jefferson National Accelerator Facility, Princeton Plasma Physics Laboratory, and Stanford Linear Accelerator Center.

The Office of Science also funds research and development projects conducted at the following national laboratories which are overseen by other DOE offices: Idaho Engineering and Environmental Laboratory (DOE's Office of Nuclear Energy, Science and Technology), Lawrence Livermore National Laboratory (DOE's National Nuclear Security Administration), Los Alamos National Laboratory (DOE's National Nuclear Security Administration), National Energy Technology Laboratory (DOE's Office of Fossil Energy), National Renewable Energy Laboratory (DOE's Office of Energy Efficiency and Renewable Energy), and Sandia National Laboratory (DOE's National Nuclear Security Administration).

The Biological and Environmental Research (BER) program within the Office of Science is involved in developing environmental and biomedical knowledge that is needed to identify, understand, anticipate, and mitigate the long-term health and environmental consequences of energy production, development, and use. As the founder of the Human Genome Project in 1986, BER continues to play a major role in biotechnology research and also invests in basic research on global climate change and environmental remediation. DOE's Genomes to Life program will use new genomic data and high-throughput technologies to explore the diverse natural capabilities found in microbes. This research will play an important role in helping solve DOE's mission challenges in energy production and environmental cleanup.

The BER program supports fundamental research in climate change, environmental remediation, genomics, systems biology, and medical sciences. BER funds research at public and private research institutions and at the DOE laboratories. BER supports research facilities used by public and private sector scientists across a range of disciplines: structural biology, DNA sequencing, functional genomics, climate science, the global carbon cycle, and environmental molecular science. Specific long-term goals in scientific advancement that the BER program is committed to, include:

- *Life sciences*: Characterize the multiprotein complexes (or the lack thereof) involving a scientifically significant fraction of a microbe's proteins. Develop computational models to direct the use and design of microbial communities to clean up waste, sequester carbon, or produce hydrogen.
- *Climate change research*: Deliver improved climate data and models for policy makers to determine safe levels of greenhouse gases for the Earth system. By 2013, substantially reduce differences between observed temperature and model simulations at subcontinental scales using several decades of recent data.
- *Environmental remediation*: Develop science-based solutions for cleanup and long-term monitoring of DOE contaminated sites. By 2013, a significant fraction of DOE's long-term stewardship sites will employ advanced biology-based clean up solutions and science-based monitors.
- *Medical applications and measurement science*: Develop intelligent biomimetic electronics that can both sense and correctly stimulate the nervous system and new radiopharmaceuticals for disease diagnosis.
- *Facilities*: Manage facilities operations to the highest standards of overall performance using merit evaluation with independent peer review.

The Life Sciences Division within the BER manages a diverse portfolio of research to develop fundamental biological information and to advance technology in support of DOE's missions in biology, medicine, and the environment. Specific research areas include:

- *Genomes to life research* – to underpin biotechnology solutions for energy, the environment, carbon sequestration, and biothreat defense. This program will develop high throughput, genome-scale technologies needed to understand the workings of biological systems from the nature of multiprotein 'molecular machines' to the regulatory networks that control them to the complex workings of natural microbial communities. A key

aspect is the development of the computational capabilities and systems that will be needed to model complex biological systems. This is a joint program with the Office of Advanced Scientific Computing Research.

- *Human genome research* – to create and apply new technologies and resources in comparative genomics, the use of model systems, and information management for identifying the genes and their regulatory elements within the human genome.
- *Microbial genome research* – to characterize and exploit the genomes and diversity of microbes with potential relevance for energy, bioremediation, or global climate.
- *Low dose radiation research* – to understand and characterize the risks to human health from exposures to low levels of radiation.
- *Structural biology user facilities* – to develop and support DOE national user facilities for use in fundamental structural biology.
- *Structural biology research* – to develop novel technologies for high throughput determination of protein structure and function.
- *ELSI research* – to anticipate and address ethical, legal, and social implications (ELSI) arising from genome research.

The Environmental Remediation Sciences Division (ERSD) within the BER supports research that will provide the fundamental scientific knowledge needed to address the challenging environmental problems that hinder the remediation of contaminated environmental sites and treatment of stored waste and contaminated waters across the DOE complex. The Division is currently made up of two research programs (the Environmental Management Science Program and the Natural and Accelerated Bioremediation Research program), one research laboratory (the Savannah River Ecology Laboratory), and a DOE user facility (the William R. Wiley Environmental Molecular Sciences Laboratory). The goal in bringing these programs together in one Division is to increase their effectiveness through coordination and integration of the research supported in the individual programs.

In addition to the programs run by the ERSD, it is involved in the following multiagency programs:

- Environmental Molecular Science Institutes (NSF-DOE Partnership). The ERSD, together with the Chemical Sciences, Geosciences, and Biosciences Division of the DOE Office of Basic Energy Sciences have teamed with the National Science Foundation to establish several Environmental Molecular Science Institutes (EMSIs). The EMSI program is aimed at increasing the fundamental understanding of molecular-level process in natural environments, including those impacted by human activities. Five-year grants are awarded competitively to universities and National Laboratory partners. NSF funding is used to support the university researchers and DOE funding is used to support National Laboratory participation. ERSD currently supports two EMSIs, one based at the University of Notre Dame and the other based at the State University of New York at Stony Brook.

The Medical Science Division (MSD) within the BER supports fundamental research and technology development in medicine, particularly in the fields of nuclear medicine, imaging sciences, and neurosciences.

The goal of the research and development programs conducted by the MSD is to utilize current advances in science and technology to develop innovative diagnostic and treatment solutions to important human health problems. The DOE is uniquely capable of advanced technological solutions to medical problems because of its unsurpassed expertise in the physical sciences, particularly in physics, chemistry, engineering, and computational sciences.

The current programs of the MSD are an extension of the original charge of the Atomic Energy Commission (AEC), "to exploit nuclear energy to promote human health." From the production of a few medically important radioisotopes in 1947, to the development of production methods for radiopharmaceuticals used in standard diagnostic tests for millions of patients throughout the world, to the development of ultrasensitive diagnostic instruments, for example, the positron-emission tomography scanner, the DOE medical sciences program leads progress in the field on nuclear medicine. Today, the MSD program has incorporated recent developments in radiochemistry, genomic sciences, and structural biology to establish a new era in mapping the human brain, and is using highly specific radiotracers and instruments to more precisely diagnose neuropsychiatric illnesses and cancer.

The DOE National Laboratories have great expertise in development of both large instruments (neutron and light sources, high field magnets, lasers, and supercomputers) as well as very small instruments (microengineering labs on a chip). Coordinated programs in the DOE National Laboratories, universities, and industry are directed to developing an artificial retina to restore sight to the major causes of blindness; development of clinical instruments to

image a moving patient; and using techniques developed in astronomy, visualize cells in the far reaches of the eye without distortion.

In addition, the DOE and Environmental Protection Agency (EPA) collaborate on research and computing resources. For example, the linking of two national supercomputers, will take place under a Memorandum of Understanding (MOU) signed by EPA and the DOE. High performance computing will allow better and faster runs of environmental models such as the Community Multi-Scale Air Quality model, an important tool for states to meet upcoming deadlines for their air quality attainment plans.

Work in computational toxicology, the application of computer-based statistical techniques, and molecular genetics that allow chemical testing based on a chemical's molecular structure and its effects on genes, will also be accelerated by this agreement. Computational toxicology can reduce animal testing and provide better toxicity information for chemicals in a faster manner.

EPA will also benefit under the MOU from access to DOE's Joint Genome Institute. Genomics is a new area of biology, derived from the large-scale DNA sequencing efforts of the human genome, and holds the potential to reveal molecular pieces of the toxicity pathway and improve chemical risk assessments and the evaluation of the health of ecosystems.

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Environmental Protection Agency, US

Patricia M Nance

In July 1970, the White House and Congress worked together to establish the Environmental Protection Agency (EPA) in response to the growing public demand for cleaner water, air, and land. Before the establishment of the EPA, the federal government was not structured to make a coordinated attack on the pollutants that harm human health and degrade the environment. The EPA was assigned the daunting task of repairing the damage already done to the natural environment and to establish new criteria to guide Americans in making a cleaner environment a reality. EPA's mission is to protect human health and to safeguard the natural environment – air, water, and land – upon which life depends.

The EPA employs 18 000 people across the country, including the headquarters offices in Washington, DC, 10 regional offices, and more than a dozen labs. EPA's staff is highly educated and technically trained; more than half are engineers, scientists, and policy analysts. In addition, a large number of employees are legal, public affairs, financial, information management, and computer specialists. The Administrator, who is appointed by the President of the United States, leads EPA. The organizational chart for the EPA is given below.

EPA Offices

Each of the EPA's Offices is responsible for specialized areas involved with the protection of the environment and human health. The Office of the Administrator provides executive and logistical support for the EPA Administrator and the staff offices that directly support the Administrator. The Administrator is responsible to the President, and is assisted by the Deputy Administrator and staff offices. The Office of the Administrator supports the leadership of EPA's programs and activities to protect human health and safeguard the air, water, and land upon which life depends.

The Office of Administration and Resources Management's mission is to provide management, infrastructure, and operations support to EPA's ~150 offices and laboratories nationwide. EPA strives to make its buildings as energy-efficient and sustainable as possible to serve as models of healthy workplaces with minimal environmental impacts. From the award winning Science and Technology Center in Kansas City, Kansas, and New England Regional Laboratory in Chelmsford, Massachusetts, to green power purchases at the Region 2 Office in New York City, EPA facilities are demonstrating the principles of sustainable design.

The Office of Air and Radiation oversees the air and radiation protection activities of the Agency including national programs, technical policies, and regulations.

The American Indian Environmental Office coordinates the Agency-wide effort to strengthen public health and environmental protection in Indian Country, with a special emphasis on building Tribal capacity to administer their own environmental programs.

The Chief Financial Officer manages and coordinates EPA's planning, budgeting, analysis, and accountability processes as well as provides financial management services.

The Office of Enforcement & Compliance Assurance delivers compliance with US environmental laws while inspiring the regulated community to employ methods that focus on pollution prevention.

The Office of Environmental Justice serves as a focal point for ensuring that communities comprised predominately of people of color or low-income populations receive protection under environmental laws.

The Office of Environmental Information is responsible for establishing an innovative center of excellence that advances the creation, management, and use of information as a strategic resource at EPA. The History Office preserves the Agency's institutional memory and provides background information and publications to the public.

The Office of General Counsel provides legal service to all organizational elements of the Agency with respect to Agency programs and activities. The Office of General Counsel provides legal opinions, legal counsel, and litigation support. In addition, the Office assists in the formulation and administration of the Agency's policies and programs as legal advisor.

The Office of Inspector General conducts audits and investigations of Agency programs and operations.

The Office of International Affairs manages Agency involvement in international policies and programs that cut across Agency offices and regions. It provides leadership and coordination on behalf of the Agency and acts as the focal point on international environmental matters.

The Office of Prevention, Pesticides, and Toxic Substances develops national strategies for toxic substance control and promotes pollution prevention and the public's right to know about chemical risks.

The Office of Research and Development is responsible for the research and development needs of the Agency's operating programs and the conduct of an integrated research and development program for the Agency. The Science Policy Council is responsible within the Agency to address and resolve cross-media, cross-program, and cross-disciplinary science policy issues. The Deputy Administrator chairs the Council.

The Office of Solid Waste and Emergency Response provides policy, guidance, and direction for the land disposal of hazardous wastes, underground storage tanks, solid waste management, encouragement of innovative technologies, source reduction of wastes, and the Superfund Program.

The Office of Water is responsible for the Agency's water quality activities including development of national programs, technical policies, and regulations relating to drinking water, water quality, groundwater, pollution source standards, and the protection of wetlands, marine, and estuarine areas.

EPA Activities

EPA leads the nation's environmental science, research, education, and assessment efforts.

Develop and Enforce Regulations

EPA works to develop and enforce regulations that implement environmental laws enacted by Congress. EPA is responsible for researching and setting national standards for a variety of environmental programs, and delegates to states and tribes the responsibility for issuing permits and for monitoring and enforcing compliance. Where national standards are not met, EPA can issue sanctions and take other steps to assist the states and tribes in reaching the desired levels of environmental quality.

Offer Financial Assistance

In recent years, between 40% and 50% of EPA's enacted budgets have provided direct support through grants to State environmental programs. EPA grants to States, nonprofit organizations, and educational institutions support high-quality research that will improve the scientific basis for decisions on national environmental issues and help EPA achieve its goals. EPA provides research grants and graduate fellowships. The Agency supports environmental education projects that enhance the public's awareness, knowledge, and skills to make informed decisions that affect environmental quality. The Agency also offers information for state and local governments and small businesses on financing environmental services and projects. EPA also provides other financial assistance through programs as the Drinking Water State Revolving Fund, the Clean Water State Revolving Fund, and the Brownfields program.

Perform Environmental Research

At laboratories located throughout the nation, EPA works to assess environmental conditions and to identify, understand, and solve current and future environmental problems. Further, it integrates the work of scientific partners such as nations, private sector organizations, academia, and other agencies; and provides leadership in addressing emerging environmental issues and in advancing the science and technology of risk assessment and risk management.

Sponsor Voluntary Partnerships and Programs

The Agency works through its headquarters and regional offices with over 10 000 industries, businesses, nonprofit organizations, and state and local governments on over 40 voluntary pollution prevention programs and energy conservation efforts. These partners set voluntary pollution-management goals; examples include conserving water and energy, minimizing greenhouse gases, slashing toxic emissions, re-using solid waste, controlling indoor air pollution, and getting a handle on pesticide risks. In return, EPA provides incentives like vital public recognition and access to emerging information.

Further Environmental Education

EPA advances educational efforts to develop an environmentally conscious and responsible public, and to inspire personal responsibility in caring for the environment.

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Relevant Website

http://www.epa.gov – US EPA website (for more information about EPA or specific EPA offices).

European Centre for Ecotoxicology and Toxicology of Chemicals

Michael Gribble

History

The European Centre for Ecotoxicology and Toxicology of Chemicals AISBL (ECETOC) is a scientific, nonprofit, noncommercial association founded in 1978. ECETOC is financed by over 45 companies with interests in the manufacture or use of chemicals. A stand-alone organization, it was established to provide a scientific forum in which the extensive specialist expertise of the European industry associated with chemicals, specialty chemicals, pharmaceuticals, agrochemicals, consumer products, and food could be harnessed to research, review, assess, and publish studies on the ecotoxicology and toxicology of chemicals. The main objective of these activities is to identify, evaluate, and minimize any potentially adverse effects on health or the environment that might arise from the manufacture and use of chemicals.

A Scientific Committee (SC) comprising leading industry scientists in the field of health and environmental sciences is appointed to direct and peer-review the work program and outputs of ECETOC. In 2001, leading scientists from academia joined the industry scientists in ECETOC's SC and peer-review panel, reinforcing and extending the range of expertise available to guide and test the ECETOC science program and its outputs.

Vision

ECETOC supports the safe manufacturing and use of chemicals, pharmaceuticals, and biomaterials through sound science.

Mission

ECETOC acts as an independent, credible peer-reviewed technical resource to all concerned with the identification of research needs and provision of scientific rationale for the assessment of health effects and environmental impact, and thereby to

justify industry's license and freedom to operate. Strategic objectives include: (1) promoting the use of sound science in both industry and regulatory decision-making and report on the results; (2) in close consultation with ECETOC members, defining the scope, managing the progress, and interacting with research programs; (3) providing a forum for regulators, academic and industrial scientists for the evaluation of the safe use of chemicals and their associated products; (4) contributing to understanding of the societal issues associated with health assessment and environmental safety of substances; and (5) identifying emerging issues that are of importance to ECETOC member companies.

Membership Criteria

Membership is based on the principle of scientific participation. Any company that is legally constituted according to the laws and customs of its country of origin and has a registered office in a European country can be a Member of the Association, provided it is engaged in the industrial manufacture, processing, or use of chemicals and has appropriate expertise that enables it to contribute to ECETOC's strategic objectives.

Partnerships

ECETOC's relationship with academia was established and reinforced through partnerships in the Task Force activities, collaborative European Commission-sponsored research projects, and in successful joint projects such as symposia organized with the European Environmental Mutagen Society (EEMS).

In 1997, drawing on its networks with leading scientists in academia and regulatory agencies, ECETOC was actively engaged in the founding of the chemical industry's Long-range Research Initiative (LRI). ECETOC continues to provide the essential scientific input to the development and management of many of the program areas funded by European Chemical Industry Council (Cefic).

Key Activities

ECETOC facilitates the networking of suitably qualified industry scientists with relevant skills and expertise, complemented, where appropriate, with experts from academia and/or regulatory agencies. The output includes workshops, technical reports, and monographs, reflecting the current state of the science for the issues under review.

ECETOC operates by coordinating efforts by chemical manufacturers, processors, and users to study and attempt to resolve the ecotoxicological and toxicological problems that may result from the manufacture, processing, and use of chemicals.

ECETOC aims to act as a scientific advisor to organizations such as the Cefic and other industry organizations with related interests. Commercial, political, and advocacy activities are strictly excluded from the modus operandi of the Association.

ECETOC cooperates in a scientific context with intergovernmental agencies, governments, health authorities, and other public and professional institutions with interests in ecotoxicological and toxicological issues relating to chemicals. ECETOC has become a valued partner with the European Commission and with many other regulatory bodies in the development of European Union chemicals legislation. For example, recognizing the ongoing need for improved approaches for evaluating the risks to humans and the environment arising from exposure to chemicals, ECETOC has supported a range of activities of direct relevance to the European Union's REACH (Registration, Evaluation, Authorization of Chemicals) chemicals legislation. REACH-related activities have included a task force on Targeted Risk Assessment, and other task forces have addressed informed testing strategies, appropriate application of human data, and alternative methodologies including quantitative structure–activity relationships.

A Specific Substances program reflects a steady demand from participating companies for ECETOC hosting and peer-review of their consortia-driven projects.

Workshops staged in support of the Environmental program have included the 'Water Framework Directive Awareness Workshop', the 'Availability, Interpretation and Use of Environmental Monitoring Data' Workshop, and the 'Ecological Quality' Workshop. In addition, a Stakeholder Event was held to share the developing methodology and associated web tool for ECETOC's 'Targeted Risk Assessment' focused on improving the eventual workability of the REACH Regulations.

Examples of other workshops include one on the 'Use of Human (Epidemiology) Data in Risk Assessment' workshop in conjunction with World Health Organization's International Programme of Chemical Safety (IPCS), and an 'Influence of Maternal Toxicity on Developmental Toxicity' workshop.

Publications

Technical documents are the major work product of the ECETOC organization and a total of 226 have been published and distributed as of the end of 2003.

The full list is available through the ECETOC Secretariat and originals are available for purchase through the ECETOC website.

ECETOC began building its scientific credentials with the preparation of critical reviews, guidance documents, and issue papers, embracing the fundamental aspects of toxicology and ecotoxicology and their interpretation and extrapolation to effects in humans and the environment. The first monographs, published in the early 1980s, dealt with the complex issue of chemical carcinogens. Other publications have covered topics such as mutagenicity, reproductive toxicity, neurotoxicity, skin sensitization, and respiratory allergy. In parallel, in the environmental sciences, other publications have dealt with aspects such as atmospheric and aquatic phototransformation, biodegradation, and bioaccumulation followed the first report on photodegradation of chemicals in the environment.

By 1990, ECETOC had published over 100 reports and was recognized officially by the WHO IPCS and the International Agency for Research on Cancer (IARC). Liaison with these and other agencies such as the European Commission's European Chemicals Bureau (ECB) and European Centre for Validation of Alternative Methods (ECVAM) confirmed and developed ECETOC's role as a key contributor to the development of sound scientific approaches to the safety assessment and consequential responsible environmental management of chemicals.

Data Bases

Databases are frequently part of the contents of the Technical Documents and some are also being provided electronically on CD. Examples of the databases developed by ECETOC include ones on aquatic toxicity, eye and skin irritation, and skin and respiratory sensitizers.

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European Society of Toxicology

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European Society of Toxicology (EUROTOX) is the Federation of national societies of toxicology in Europe, which together have ~7000 members. In addition, EUROTOX counts some 500 individual members who come from 50 countries, mostly from Western Europe. According to its statutes, EUROTOX aims to foster toxicology, both scientifically and educationally, in all countries of Europe. For this, EUROTOX organizes an annual scientific congress, workshops, and postgraduate training courses. Specific activities are organized by the EUROTOX Specialty Sections.

EUROTOX is actively harmonizing toxicology education and training, having established the European Register of Toxicologists in 1994. It participates in the worldwide recognition of toxicologists as recently started under the auspices of the International Union of Toxicology (IUTOX). Furthermore, EUROTOX honors annually a distinguished European toxicologist by its Merit Award. Important recent research contributions are honored by inviting an outstanding toxicologist to present the Gerhard Zbinden Memorial Lecture at the annual congress.

Young toxicologists are encouraged by the annual Young Scientist Award, which is awarded every year to the best presentation at the EUROTOX Congress. Finally, EUROTOX members (i.e., the individual members and all members of the affiliated national societies) are entitled to attend the scientific meetings at a reduced fee, and are given a discount on the subscription rates of *Archives of Toxicology* (published by Springer-Verlag, Heidelberg).

Historically, EUROTOX has its roots in the European Society for the Study of Drug Toxicity, which was founded in 1962 in Zürich. The first annual scientific meeting was held in 1963 in Paris, during which the Statutes of the new society were adopted. As the Society's interests started to extend into toxicology areas other than drug toxicology, it was

decided to change the name into European Society of Toxicology (EST).

This was done at the scientific meeting in 1974 in Carlsbad. In the late 1970s and early 1980s national toxicology societies grew rapidly, both in number and in membership. Thus, 14 national societies of toxicology (Finland, France, the German Federal Republic, the German Democratic Republic, Hungary, Ireland, Italy, The Netherlands, Norway, Poland, Spain, Sweden, Switzerland, and the United Kingdom) and EST decided to found the Federation of European Societies of Toxicology (FEST), which was done at the EST congress in Kuopio, 1985.

As this soon turned out to create unnecessary duplications, EST changed its Statutes, adopted the name EUROTOX, and EST and FEST merged at the 5th IUTOX Congress in Brighton, 1989. In the years thereafter EUROTOX grew steadily, now encompassing also Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Greece, Latvia, Macedonia, Portugal, Romania, Russia, Slovak Republic, Slovenia, Turkey, and Ukraine.

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European Union and Its European Commission

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The year 2004 was a significant year for the European Union (EU) and its European Commission as the number of EU member countries (or 'Member States') expanded from 15 to 25. The 25 Member States include Austria, Belgium, Cyprus, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom. In addition, other countries such as Bulgaria and Romania may be joining the EU before 2010.

The EU was established as a family of democratic European countries, committed to working together for peace and prosperity. It is a unique international organization in that its member states have set up common institutions to which they delegate some of their sovereignty so that decisions on specific matters of joint interest can be made democratically at the EU level. The EU's beginning came after World War II, with the idea of the EU based on the desire to prevent such killing and destruction from ever happening again.

EU-Wide Institutions

There are five EU-wide institutions, each with a specific role:

- *The European Parliament* (EP) is elected by the people within Member States every 5 years. Parliament's principal roles include examining and adopting European legislation (under the codecision procedure, Parliament shares this power equally with the Council of Ministers), to approve the EU budget, to exercise democratic control over the other EU institutions, and to assent to important international agreements such as the accession of new EU Member States and trade or association agreements between the EU and other countries. The EP has parliamentary committees to deal with particular issues, for example, foreign affairs, budgets, and the environment.
- *The Council of the European Union*, representing the governments of the Member States, is the main legislative and decision-making body in the EU. It brings together the representatives of the Member State governments, which are elected at the national level. It is the forum in which the representatives of Member State governments can assert their interests and reach compromises. The members of the Council meet regularly at the level of working groups, ambassadors, and ministers, or, for deciding the major policy guidelines, at the level of presidents and prime ministers.

The Council, together with the EP, sets the rules for all the activities of the European Community (EC), which forms the first 'pillar' of the EU. It covers the single market and most of the EU's common policies, and guarantees freedom of movement

for goods, persons, services, and capital. In addition, the Council is the main EU institution responsible for the second and third 'pillars', that is, intergovernmental cooperation on common foreign and security policy and on justice and home affairs.

- *The European Commission*, the driving force and executive body of the EU: it is the institution where much the of the EU's day-to-day work is done. It drafts proposals for new European laws, which it presents to the EP and the Council. The Commission makes sure that EU decisions are properly implemented and supervises the way EU funds are spent. It also sees that everyone abides by the European treaties and European law. The president is chosen by the governments of the EU Member States and must be approved by the European Parliament. The other members are nominated by the member governments in consultation with the incoming president and must also be accepted by Parliament.
- *The Court of Justice* ensures compliance with the common rules in the EU, and settles disputes over how the EU treaties and legislation are interpreted. Member State courts must ask the Court of Justice when they are in doubt about how to apply EU rules. Individual persons can also bring proceedings against EU institutions before the Court.
- *The Court of Auditors* controls the sound and lawful management of the EU-wide budget. The funds available to the EU must be used legally, economically, and for the intended purpose.

Other Important EU Bodies

These five institutions described above are flanked by five other important EU bodies, that is, the European Economic and Social Committee (expresses the opinions of organized civil society on economic and social issues), the Committee of the Regions (expresses the opinions of regional and local authorities), the European Central Bank (responsible for monetary policy and managing the euro), the European Ombudsman (deals with citizens' complaints about misadministration by any EU institution or body), and the European Investment Bank (helps achieve EU objectives by financing investment projects).

How Environmental and Safety Needs and Issues are Addressed within the EU

Many environmental and safety issues in Europe could not be tackled without joint action by all EU countries. For example, the EU's European Environment Agency gathers information on the state of the EU environment, enabling protective measures and laws to be based on solid data, and the European Chemicals Agency is being created to work on and implement the EU-wide effort on the human and environmental safety of the uses and exposures to 'existing' and 'new' chemicals called the Registration, Evaluation, Authorisation of CHemicals (REACH).

In all, the EU has adopted over 200 environmental protection directives that are applied in all Member States. Most of the directives are designed to prevent air and water pollution and encourage waste disposal. Other major issues include nature conservation and the supervision of dangerous industrial processes. The EU wants transport, industry, agriculture, fisheries, energy, and tourism to be organized in such a way that they can be developed without destroying natural resources and leading to sustainable development. For example, the EU has cleaner air because of the EU decisions in the 1990s to put catalytic converters into all cars and to get rid of the lead added to gasoline.

The General Product Safety Directive

The safe uses of chemicals in consumer products and articles (e.g., in toys, clothing, and furniture) are covered in part by the General Product Safety Directive (GPSD), revised in 2004. The GPSD includes a rapid exchange (RAPEX) notifications system to quickly share suspected or known safety issues about nonfood consumer products between Member States. The European Commission typically receives several safety alerts each week via RAPEX. Among the dangers presented are the risk of choking and suffocation, electric shock, and fire. The types of products most often found in these alerts are toys, followed by electrical appliances. The EU has a separate rapid alert system on food safety, which also makes weekly summaries of alerts available.

The European Commission's Nonfood Scientific Committees

In 2004, the European Commission reorganized its nonfood scientific committees following the creation of the European Food Safety Authority (EFSA) and the transfer to the Authority of responsibilities for risk assessment on food-related issues previously carried out by some of the scientific committees. The Commission reviewed and refocused the work of its three remaining nonfood scientific committees and created three new committees: Scientific Committee on Consumer Products (SCCP), Scientific Committee

on Health and Environmental Risks (SCHER), and Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). The reorganized committees provide the EU with a more proactive and flexible approach to risk assessment. The restructuring also ensures appropriate cooperation and coordination between these committees and other Community bodies responsible for scientific advice such as the EFSA, the European Agency for the Evaluation of Medicinal Products, and the European Centre for Disease Prevention and Control.

Specifically, the SCCP advises on questions related to the safety of consumer products (other than food). Examples of the type of issues within the scope of the SCCP include the safety and allergenic properties of cosmetic products and issues relating to the safety of toys, textiles, clothing, domestic products, and consumer services such as tattooing. The SCHER examines issues relating to the toxicity and ecotoxicity of chemical, biochemical, and biological compounds whose use may have harmful consequences for human health and the environment. The SCENIHR advises on emerging or newly identified risks and on broad issues requiring a comprehensive assessment of risks to consumer safety or public health. It will also give an opinion on human health issues not covered by other EU risk assessment bodies. Examples of the type of issues within the scope of the SCENIHR include antimicrobial resistance, nanotechnology and other new technologies, medical devices including those incorporating substances of animal and/or human origin, tissue engineering, physical hazards such as noise and electromagnetic fields (from mobile phones, transmitters, and electronically controlled home environments), and methodologies for assessing new risks.

The European Commission's Joint Research Centre

The European Commission's Joint Research Centre (JRC) is a source of independent scientific and technical reference for European policy makers, serving the European Commission, the EP, the Council, and the Member States. The JRC's seven scientific institutes carry out research of direct concern to EU citizens, working with industry, universities, other research institutes, and Member States. The JRC is among the European Commission's 36 Directorates-General (DGs). The DGs are specialized services within the European Commission. Examples of other DGs include the Brussels, Belgium-based Directorate General (the JRC's central coordination and administrative body), the Institutional and Scientific Relations Directorate, the Programme and Resource Management Directorate, and the DG Health and Consumer Protection, with the overall goal of promoting a better quality of life by ensuring a high level of protection of consumers' health, safety, and economic interests as well as of public health. The seven JRC institutes are the Institute for Health and Consumer Protection (IHCP), the Institute for Environment and Sustainability (IES), and the Institute for Protection and Security of the Citizen (IPSC) in Ispra, Italy, the Institute for Reference Materials and Measurements (IRMM) in Geel, Belgium, the Institute for Transuranium Elements (ITU) in Karlsruhe, Germany, the Institute for Energy (IE) in Petten, the Netherlands, and the Institute for Prospective Technological Studies (IPTS) in Seville, Spain.

The JRC's IHCP includes the European Centre for the Validation of Alternative Testing Methods (ECVAM), an international reference center for the development and scientific acceptance of alternative testing methods to replace, reduce, and refine the use of laboratory animals. IHCP also includes the Physical and Chemical Exposure (PCE) Unit providing scientific understanding, information, and assessment tools to support the Commission services in evaluating and quantifying exposure and risk assessments for environmental stressors such as chemicals, biological contaminants, radiation, and noise. The PCE's work includes a new European information system on risks of exposures to chemicals in consumer products and articles (EIS-ChemRisks). This effort serves as a European-wide expert and stakeholders 'network of networks' to systematically exchange and assess information on risks from chemicals released from consumer products/articles. EIS-ChemRisks will support the GPSD and may provide technical support to the relevant aspects of REACH. Further, as part of EIS-ChemRisks, the PCE is establishing a single Web-based gateway to all major European initiatives in the field of human exposure to chemicals contained and released from products/articles. This gateway is being designed to act as an interactive EU-wide information source and a common communication tool for the user society to develop and continuously update reference data and tools, and includes the 'European Exposure Assessment Toolbox' as a set of tools and reference data to enable harmonized exposure assessment procedures within the EU.

In addition, the IHCP includes the European Chemicals Bureau (ECB). Current working areas of the ECB, at least until the new European Chemicals Agency officially begins operation, are collecting information on new and existing chemicals, and providing scientific and technical support to the conception, development, implementation, and

monitoring of EU policies on dangerous chemicals. Further, the ECB supports the development and harmonization of testing methods such as quantitative structure activity relationships, the legal classification and labeling of substances; the management of risk assessment of substances; the notification of new substances; the authorization of biocides; and the information exchange on import and export of dangerous substances.

The European Chemical Substances Information System (ESIS) serves as a portal to the existing chemicals data sets maintained by ECB. ESIS includes information related to the European Inventory of Existing Chemicals (EINECS), the European List of Notified Chemical Substances (ELINCS), High Production Volume Chemicals (HPVCs) and Low Production Volume Chemicals (LPVCs), Classification, and Labeling, IUCLID (International Uniform Chemical Information Data Base) Chemical Data Sets, and the EU's chemical risk assessment process. IUCLID is the basic tool for data collection and evaluation within the EU Risk Assessment Programme, as well as under the OECD Existing Chemicals Programme. The Risk Assessment reports are extensive documents written in first draft by EU member states, and the ECB also mediates meetings that attempt to reach consensus on the conclusions of the risk assessments.

Further, the IHCP's Biomedical Materials and Systems (BMS) Unit conducts applied and exploratory research studies in the area of bioengineering, materials and surface sciences, medical photonics, and nuclear technology for health application. Finally, IHCP's 'Biotechnology and GMOs' unit provides scientific and technical support to EU legislation in biotechnology through the development, validation, and harmonization of detection methods of GMOs (genetically modified organisms) and genetically modified foods.

The European Pollutant Emission Register

Another example of an EU-wide database established by the European Commission is the European Pollutant Emission Register (EPER), developed in 2004 as the first European-wide register of industrial emissions into air and water. Member States have to produce a triennial report on the emissions of industrial facilities into the air and waters, and the report covers 50 pollutants. EPER gives access to information on the annual emissions of thousands of industrial facilities in the Member States as well as Norway. It lets users group information easily, by pollutant, activity (sector), air and water (direct or via a sewerage system), or by country. In addition, it is possible to see detailed data on individual facilities by searching by name or by clicking on a map. Users can also look for the sources of a particular pollutant. The European Commission has made these data publicly accessible on a website hosted by the European Environment Agency (EEA).

Support of Research

The EU has been increasingly active in helping European research to achieve scientific excellence. In a variety of sectors covering the whole spectrum of modern technology, the EU finances projects undertaken by research centers, universities, and industry. The emphasis is on putting research and innovation to work for precise socioeconomic objectives, such as job creation and improved quality of life. Current research priorities include, among others, life sciences, nanotechnology, space, food quality, sustainable development, and the knowledge-based society. The European Commission has multi-year Framework Programmes for Research, for example, the 6th Framework Programme (2003–2007), which supports research in toxicology, risk assessment, and numerous other areas.

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Relevant Websites

http://europa.eu.int – European Commission, See index pages of The European Union at a Glance, EU News, Environment Directorate-General, Food Safety, Health and Consumer Protection Directorate-General, Health and Consumer Protection Directorate-General, Calls for Research Tenders, Risk Assessment Activities.

http://ecb.jrc.it – European Commission, Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances. Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances, and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part 1 (2003).

http://ihcp.jrc.it – European Commission, Institute for Health and Consumer Protection (IHCP) website.

http://www.eper.cec.eu.int – European Pollutant Emission Register (EPER).

Flavor and Extract Manufacturers Association

Gwendolyn L Ball

The Flavor and Extract Manufacturers Association (FEMA), founded in 1909, represents the interests of its members in the US flavor industry including flavor manufacturers, users, ingredient suppliers, and other interested parties. FEMA maintains a strong scientific program to evaluate the safety of flavor ingredients.

Objectives

- To support the FEMA Expert Panel process for the independent evaluation of the safe use of flavor ingredients, using panel members with recognized expertise in areas such as toxicology, pharmacology, pathology, and medicine.
- To serve as an effective advocate for FEMA members in regulatory affairs and in collaboration with other organizations with related interests.
- To effectively communicate with members regarding current issues.
- To provide education and training services.
- To protect the intellectual property of FEMA members by addressing current regulatory issues.

Activities

- Convene the FEMA Expert Panel.
- Interact with national and international groups involved in the evaluation and/or regulation of flavor ingredients, including the Joint FAO/WHO Expert Committee on Food Additives (JECFA).
- Provide guidance to members on technical and regulatory issues.

Publications

- Twenty-one FEMA GRAS (generally recognized as safe) publications, the latest in 2003, with tables of use levels on which the FEMA Expert Panel based its GRAS determinations.
- FEMA GRAS Assessments of individual flavor ingredients made by the Expert Panel published in scientific journals.
- Flavor & Fragrance Ingredient Data Sheets.

Meetings

FEMA holds an Annual Convention in the spring and a Fall Symposium. Workshops and seminars on regulatory compliance and special issues are also held.

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Acknowledgment

Adapted from the FEMA website at http://www.femaflavor.org

Food and Agriculture Organization of the United Nations

Manfred Luetzow*

The Food and Agriculture Organization of the United Nations (FAO) was established on October 16, 1945, with the goal to promote global sustainable development of agriculture, fisheries, forestry, and food production and security, quality, and safety as well as the related socioeconomic issues in the member countries. There are 187 member countries plus the European Community in the organization (as of 2003). The headquarters of FAO is located in Rome and is organizationally divided into eight departments, with five regional, five subregional, five liaison, and more than 100 country offices with functions dealing with regional and in-country

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activities and projects. Five specialized offices within FAO headquarters assist the director-general in directing and managing this, the largest specialized agency of the United Nations system.

Within the FAO headquarters there are two departments with responsibilities that include toxicology-related activities. They are the Economic and Social Department (ES) and the Agriculture Department.

The ES Department, within its Food and Nutrition Division (ESN), houses the Secretariat of the Joint FAO/WHO Food Standards Program established in 1962 and implemented through the Codex Alimentarius Commission (CAC). CAC is an intergovernmental body that meets alternately in Rome and Geneva at the headquarters of the two parent organizations, FAO and World Health Organization (WHO), annually. The aims of CAC are to protect the health of the consumer and facilitate international trade through the harmonization of national legislation and regulations through establishing international codes of practice, general standards for food additives and contaminants, food commodity standards, maximum limits for residues of pesticides and residues of veterinary drugs in foods, food labeling standards, methods of analysis, etc. The preparatory work for these activities is accomplished by the CAC subsidiary bodies, the Codex General Subject and Commodity Committees. Three of these Codex committees are especially important in this connection: the Codex Committee on Food Additives and Contaminants (CCFAC), the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), and the Codex Committee on Pesticide Residues (CCPR).

The Food and Nutrition Division also provides the FAO Secretariat of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). This Committee was established in the mid-1950s by FAO and WHO to assess chemical additives in food on an international basis. The first meeting was held in 1956 in response to recommendations made in 1955 at a FAO/WHO Conference on Food Additives meeting in Geneva. JECFA is managed by a Joint FAO/WHO Secretariat.

When the CAC was formed it decided to utilize the expert scientific advice provided by JECFA on matters relating to the toxicological and specifications activities of food additives. A system was established whereby the Codex Committee on Food Additives and Contaminants, a general subject committee, identified food additives that should receive priority attention, which were then referred to JECFA for assessment before being considered for inclusion in Codex food standards.

Over the years, JECFA's responsibilities have been expanded to include evaluation/assessment of food contaminants, naturally occurring toxins, and residues of veterinary drugs in food. JECFA's advice and evaluations are used by several Codex committees (e.g., CCFAC, CCRVDF). JECFA also provides scientific advice directly to FAO, WHO, and their member states. JECFA cooperates very closely with Codex but is not a component of the CAC.

Specialists invited to serve as members of JECFA are independent scientists who serve in their individual capacities as experts and not as representatives of their governments or employers. They also understand that the discussions at the meetings are confidential. The goal is to establish acceptable daily intakes (ADIs) (or equivalent tolerable intakes) for food chemicals and to develop specifications for identity and purity for food additives or maximum residue limits (MRLs) when veterinary drugs are used in accordance with good practice in the use of veterinary drugs.

FAO and WHO have complementary functions in selecting members for JECFA. FAO is responsible for selecting members to deal with the development of specifications for the identity and purity of food additives and the assessment of residue levels of veterinary drugs in food. WHO is responsible for selecting members to deal with the toxicological evaluations of the substances under consideration. Both FAO and WHO invite members who are responsible for assessing intake.

As of 2004, a total of 63 meetings of JECFA have been held and over 2100 food additives including more than 1500 flavoring agents, ~40 contaminants, and 93 veterinary drugs evaluated. The reports are published in the WHO Technical Report Series. The comprehensive toxicological evaluations, which review the data that serve as the basis for the safety assessments, are published in the WHO Food Additives Series. The specifications for food additives and residue evaluations of veterinary drugs are published in the FAO Food and Nutrition Paper Series.

JECFA meetings are convened twice a year, with one session devoted to the evaluation of food additives and contaminants and the other to the evaluation of residues of veterinary drugs in foods. The meetings are open only to the invited experts and the Joint Secretariat. JECFA can hold hearings during the meeting in which those who have submitted data for evaluation are invited to answer specific questions by the committee to clarify the submission. The JECFA procedures do not permit the committee to discuss the substances under review when the nonmembers are present during these hearings.

JECFA is one body: the discussions are held and decisions made in plenary sessions. The drafting, however, is done in separate groups. In the case of food additives, the FAO experts are responsible for proposing specifications of identity and purity for food additives. The three main objectives of the specifications prepared by the committee are to identify the substance that has been subjected to biological testing, to ensure that the substance is of the quality required for safe use in foods, and to reflect and encourage good manufacturing practice.

Experts invited by FAO also prepare Chemical and Technical Assessments (CTA) for the substances on the agenda to provide the committee with the information on the physical and chemical characteristics of the additive, on the raw material(s) used in commercial production of the additive, and on methods of manufacture by which the raw material(s) is converted into a finished commercial food additive. It is acknowledged that some of these data may be trade secrets. Therefore, such data are held in strict confidence. Furthermore, the CTA includes information on impurities including intermediates, functional use(s) with the technological purpose for using the additive and the levels of use on a commodity basis, reactions and fate in food, and effects on nutrients. In the case of contaminants, FAO experts are responsible for gathering information on their occurrence in food and methods for their analysis.

ADIs for food additives and veterinary drugs and provisional tolerable weekly intakes (PTWIs) for contaminants are proposed by the WHO experts.

In JECFA meetings dedicated to the evaluation of veterinary drug residues, the FAO experts are responsible for proposing MRLs for foods of animal origin based on pharmacokinetic and metabolism studies in experimental animals, target animals, and in humans when available. The Committee will consider the following when proposing MRL:

- Radiolabeled residue depletion studies in target animals from zero withdrawal time to periods beyond the recommended withdrawal time (these studies should provide information on total residues, including free and bound residues, and major residue components in order to select a marker residue and target tissue).
- Unlabeled drug depletion studies for analysis of marker residue in target animals including muscle, liver, kidney, fat, eggs, milk, and honey as applicable (this should include studies with appropriate formulations, routes of application, and species using up to maximum recommended doses).
- Methods for routine analysis that may be used by authorities for the detection of residues in target tissue.
- The ADI proposed by the WHO experts.
- The standard daily food intake of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat, 100 g of eggs, and 1.5 l of milk ('food basket').

Other assumptions and variables may also be involved in determining MRLs, including safety factors used in establishing ADIs, withdrawal times, the contribution of bound residues, and the bioavailability of residues.

A veterinary drug is any substance applied or administered to any food-producing animal, such as meat or milk-producing animals, poultry, fish, or bees, whether used for therapeutic, prophylactic, or diagnostic purposes or for modification of physiological functions or behavior.

The MRL is the maximum permissible concentration of residue resulting from the use of a veterinary drug, expressed in milligram per kilogram or microgram per kilogram on a fresh weight basis. It is based on the type and amount of residue considered to be without toxicological hazard for human health as expressed by the ADI. Consideration is also given to residues of the compound that occur in food or plant origin and/or environment (the same active ingredient may be used as a veterinary drug and pesticide).

Metabolic studies identify and quantify the residues. These studies should simulate the conditions of use of the drug in animal husbandry as closely as practicable. The pharmacokinetics (distribution and elimination) of the residues should be examined between the time of administration of the drug and the time the animals enter the human food supply.

The withdrawal time after administration of a drug is the time during which animals or animal products should not be harvested by fishing, milking, slaughtering, egg collection, etc., for human consumption.

The total residues of a drug in animal-derived food consist of the parent drug, together with all the metabolites and drug-based products that remain in the food after the administration of the drug to food-producing animals. The amount of total residues is generally determined by means of a study using the radiolabelled drug and is expressed as the parent drug equivalent in milligram per kilogram or microgram per kilogram of the food.

The use of veterinary drugs in food-producing animals can result in residues that are neither extractable from tissues nor readily characterized (bound residues). The extractable residues are the

residues extracted from tissues or biological fluids by means of aqueous acidic or basic media, organic solvents, and/or hydrolysis with enzymes to hydrolyze conjugates. The nonextractable residues are obtained by subtracting the extractable residues from the total residues and comprise residues of the drug incorporated through normal metabolic pathways into endogenous compounds (e.g., amino acids, proteins, and nucleic acids) or chemically bound residues derived by interaction of residues of the parent drug or its metabolites with macromolecules. The bioavailable residues are the residues that can be shown, by means of an appropriate method, to be absorbed when fed to experimental (laboratory) animals. In the absence of relevant residue data, it should be assumed that all of the residues are bioavailable and that its potency is equal to that of the most toxic component of the residue.

A marker residue is a residue whose level decreases in a known relationship to the level of total residues in tissues, eggs, or milk. In other words, a marker residue is, or is representative of, the residue of toxicological concern in the target tissue and/or milk/eggs. Identification of a marker residue is important because it is the substance determined for control purposes in the enforcement of MRLs by the national authorities and other parties concerned.

A target tissue is defined within JECFA as the edible animal tissue (muscle, liver kidney, or fat) for which the MRL is recommended and that may be analyzed for purposes of the enforcement of the MRL.

In assessing the safety of veterinary drug residues, the Expert Committee determines the MRL expressed in terms of a named marker residue for target tissues of interest of individually specified animal species. The committee identifies at least two target tissues whenever possible, with one being muscle or fat and the other liver or kidney. Selection of an appropriate target tissue permits regulation of the MRL in international trade in meat (liver and kidney not available) as well as in national control programs.

In summary, when an ADI is established, consideration of the estimated intakes of the relevant foods by human beings allows an assessment to be made of a safe and acceptable residue level for the relevant animal tissue(s). If the levels of residues estimated from supervised trials, when the drug is administered according to good practices in the use of veterinary drugs (only the amount which is necessary to obtain the desired effect is used), are below those considered toxicologically acceptable, then the levels determined by good practice will dictate the acceptable residue level, provided that practical analytical methods are available at that level for routine residue analysis. The committee is reluctant to establish MRLs lower than a level twice that of the limit of quantitation of the previous analytical method.

If the levels of residues found in practice exceed those determined to be acceptable from the toxicological evaluation and consumption data, then drug use in the food-producing animals may need to be modified to reduce residue concentrations in edible tissues to acceptable levels. Possible modifications include extending the withdrawal periods and changing the drug dosage, formulation, or method of application.

When it has been determined that an ADI is unnecessary because the compound of interest is produced endogenously in human beings and animals, then the establishment of an acceptable residue limit is also unnecessary. At the other extreme, when an ADI has not been allocated because, on toxicological grounds, the safety of the compound cannot be assured, then no acceptable residue limit should be established.

The principles outlined here apply to the evaluation of residues of all veterinary drugs. For the establishment of tolerance limits for residues of certain chemotherapeutic agents, however, the antimicrobial properties of the residues must also be taken into account. Antimicrobial properties will become the determining factor in safety evaluation when the toxicity of the substances to be considered is so low that their residues in food could, from the toxicological viewpoint, be tolerated even at the height of therapeutically effective tissue concentrations. At the microbial level, concern for food safety is centered on the question of whether or not residues of antimicrobial agents ingested via food of animal origin pose a danger to human health by exerting a selective pressure on the intestinal flora and thus favoring the growth of microorganisms with natural or acquired resistance.

The committee does not attempt to derive withdrawal times for veterinary drugs that are necessary to ensure that the concentration of residues in food will be below the established MRL. Residue kinetics and withdrawal times depend on various parameters strictly linked to a given veterinary drug including, but not limited to, the pharmaceutical formulation, the concentration of the active ingredient, the dosage, and the route of administration. The determination of the appropriate withdrawal time for a given veterinary drug in order to comply with an assigned MRL is the responsibility of the appropriate national authority. Nevertheless, when determining MRLs, the committee verifies that those that it recommends can be achieved through realistic

withdrawal times and established good practices in the use of veterinary drugs.

To demonstrate how the ADI and MRLs are linked together and how the maximum ingested residue of the parent drug and its equivalents is calculated, the following information is presented as extracted from the Food and Nutrition Paper No. 41/6 and the Technical Report Series No. 851.

Based on the ADI of 0–6 $\mu g\,kg^{-1}$ of body weight for levamisole (parent drug) established by the committee, the permitted daily intake of the parent drug and/or its equivalents is 360 μg for a 60 kg person.

The following factors were considered in estimating the MRLs: the ADI; the parent drug is a suitable residue marker and is 2.4% of the total residues; all the residues in muscle and fat are equivalent to the parent drug; 50% of the residues in liver are bound and 15% of these bound residues are bioavailable; the residues in kidney are qualitatively similar to those in liver; it is assumed that all bioavailable residues in liver and kidney are equivalent to the parent drug; and the residues are similar in cattle, sheep, and pigs.

The committee recommends MRLs of 10 $\mu g\,kg^{-1}$ for muscle, kidney, and fat and 100 $\mu g\,kg^{-1}$ for liver of cattle, sheep, poultry, and swine expressed as the parent drug. Because residues in eggs at recommended dose level, at 1 day withdrawal, are ~1000 $\mu g\,kg^{-1}$, the committee considered that levamisole should not be used in laying hens.

The previous assumptions can be used to calculate maximum theoretical daily intake of levamisole equivalents if a consumer ate the standard meat diet containing concentrations of levamisole at the proposed MRLs. The maximum ingested residue of the parent drug and its equivalents is 397 $\mu g\,day^{-1}$, which consists of 14 $\mu g\,day^{-1}$ of parent drug and 383 $\mu g\,day^{-1}$ of levamisole equivalents. The calculation is shown in **Table 1**.

Considering the inherent uncertainty of the total levamisole equivalents based on levamisole as the marker residue and considering that only a small proportion of the total residues are used to estimate the total levamisole equivalents (397 μg), the committee considered this value to be equivalent to the maximum ADI.

Depending on the subject of the meeting, the outcome of the JECFA meetings will be the ADIs for food additives or veterinary drug residues, PTWIs for food contaminants, MRLs for residues of veterinary drugs, or specifications for identity and purity of food additives. The MRLs and specifications are then discussed by the CCRVDF and CCFAC, respectively, and if found acceptable, forwarded to the CAC for adoption as Codex Maximum Residue Limits and Codex Specifications. The ADIs for food additives and PTWIs for contaminants are used by the CCFAC in the preparation of general standards for food additives and contaminants and in recommending of maximum-use levels of food additives or guideline levels of food contaminants in food. Aside from the Codex committees, the outcomes of the JECFA evaluations are freely available to all parties concerned.

The Agriculture Department, within its Plant Production and Protection Division, includes the FAO Secretariat of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Toxicological and Environmental Core Assessment Groups, otherwise known as the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). JMPR was established following the resolution of the FAO Conference in 1962 by the Codex Alimentarius Commission to recommend MRLs for pesticide residues and environmental contaminants in specific food products, including methods of sampling and analysis to ensure safety of food containing residues.

The JMPR meetings are closed to nonmember participation and are held annually in September, alternately in Rome and Geneva. WHO-invited members are responsible for proposing ADIs for the substances on the agenda. FAO-invited members draft MRLs for substances under evaluation based on findings in supervised field trials conducted in various countries worldwide. The ADI and MRL

Table 1 Calculation of maximum ingested residue of levamisole (parent drug) and its equivalents

Tissue	*Standard intake (kg)*	*MRL ($\mu g/kg^{-1}$)*	*UD (μg)*[a]	*EQ (μg)*[a]		*Total (μg)*
				Free	*Bound*	
Muscle	0.300	10	3	125	0	125
Liver	0.100	100	10	208	31	239
Kidney	0.050	10	0.5	10	2	12
Fat	0.050	10	0.5	21	0	21
Total	0.500		14	364	33	397

[a] UD, unchanged drug; EQ, parent drug equivalents.

proposals are discussed, examined, and the decisions made in the plenary when all the committee members are present. The report of the meeting and the *Evaluations, Part I – Residues* are published in the FAO Plant Production and Protection Paper Series and the *Evaluations, Part II – Toxicology* as a WHO/IPCS publication.

The cooperation between the JMPR and the Codex Committee on Pesticide Residues is close. CCPR identifies those substances which require priority evaluation. After the JMPR evaluation, CCPR discusses the recommended MRLs and forwards them, if they are acceptable, to the Codex Alimentarius Commission for adoption as Codex Maximum Residue Limits.

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www.fao.org – The specifications for food additives, and residue evaluations of veterinary drugs are published in the FAO Food and Nutrition Paper Series. The FAO Secretariat maintains a webpage that provides online access to these publications.

Food and Drug Administration, US

Ankur V Dnyanmote and Harihara M Mehendale

The US Food and Drug Administration (FDA) is the federal agency responsible for ensuring that foods are safe, wholesome, and sanitary; human and veterinary drugs, biological products, and medical devices are safe and effective; cosmetics are safe; and electronic products that emit radiation are safe. Its jurisdiction encompasses most food products (other than meat and poultry), human and animal drugs, therapeutic agents of biological origin, medical devices, radiation-emitting products for consumer, medical, and occupational use, cosmetics, and animal feed. The agency grew from a single chemist in the US Department of Agriculture in 1862 to a staff of ~9100 employees and a budget of $1.294 billion in 2001, comprising chemists, pharmacologists, physicians, microbiologists, veterinarians, pharmacists, lawyers, and many others.

The FDA is also responsible for advancing the public health by helping to speed innovations that make medicines and foods more effective, safer, and more affordable; and helping the public get the accurate, science-based information they need to use medicines and foods to improve their health. The FDA also ensures that these products are honestly, accurately, and informatively represented to the public. Some of the agency's specific responsibilities include:

1. *Biologics*
   - Product and manufacturing establishment licensing
   - Safety of the nation's blood supply
   - Research to establish product standards and develop improved testing methods
2. *Cosmetics*
   - Safety
   - Labeling
   - Drugs
3. *Product approvals*
   - Over-the-counter and prescription drug labeling
   - Drug manufacturing standards
   - Foods
4. *Labeling*
   - Safety of all food products (except meat and poultry)
   - Bottled water
   - Medical devices
5. *Premarket approval of new devices*
   - Manufacturing and performance standards
   - Tracking reports of device malfunctioning and serious adverse reactions
   - Radiation-emitting electronic products
6. *Radiation safety performance standards for microwave ovens, television receivers, diagnostic*
   - X-ray equipment, cabinet X-ray systems (such as baggage X-rays at airports), laser products
   - Ultrasonic therapy equipment, mercury vapor lamps, and sunlamps
   - Accrediting and inspecting mammography facilities
   - Veterinary products

7. *Livestock feeds*
   - Pet foods
   - Veterinary drugs and devices

The FDA is an agency within the Department of Health and Human Services and consists of eight centers/offices, whose webpages can be found in the FDA website:

- Center for Biologics Evaluation and Research (CBER);
- Center for Devices and Radiological Health (CDRH);
- Center for Drug Evaluation and Research (CDER);
- Center for Food Safety and Applied Nutrition (CFSAN);
- Center for Veterinary Medicine (CVM);
- National Center for Toxicological Research (NCTR);
- Office of the Commissioner (OC); and
- Office of Regulatory Affairs (ORA).

For general food safety questions, call the FDA Consumer Hotline at 888-INFO-FDA (888-463-6332). For questions about seafood, call the FDA Seafood Hotline at 800-FDA-4010. Questions involving meat or poultry products can be asked at the US Department of Agriculture's hotline at 800-535-4555. In case of emergencies, call 301-443-1240, which is staffed 24 hours a day.

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Tel: 1-888-463-6332 (1-888-INFO-FDA)
URL: http://www.fda.gov

Relevant Website

http://www.fda.gov – Website of the US Food and Drug Administration.

Intergovernmental Forum on Chemical Safety (IFCS)

Judy A Stober

History

Effective coordination of activities and strong cooperation among governments and organizations working in the field of chemical safety is key for achieving sound chemicals management. The 1992 United Nations Conference on Environment and Development recognized this need, and action was taken in 1994 at the International Conference on Chemical Safety, where the Intergovernmental Forum on Chemical Safety (IFCS) was formed.

Purpose and Structure

The IFCS was established to improve coordination and cooperation by identifying and building consensus on chemicals assessment and management priorities, coordinating, and monitoring international and regional action related to sound chemicals management.

The IFCS is an overarching, participatory forum where governments meet with intergovernmental and nongovernmental organizations to discuss chemical safety issues and provide policy guidance for the sound management of chemicals, to be implemented by national governments and organizations. All questions concerning chemical risks are within the purview of the Forum. The IFCS monitors performance against indicators, and sets the agenda for research regarding chemicals management and new and emerging environmental health issues. The World Health Organization is the administering agency for the IFCS and its Secretariat. IFCS expenses are covered by voluntary contributions from United Nations Member States and other IFCS participants.

The IFCS structure is unique. It places a strong emphasis on the full and open participation of all sectors relevant to the sound management of chemicals. It engages industry, public interest, science, labor, and academia, as well as governments in discussion and debate that is not constrained by the structures and process of formal negotiations. A key benefit of the Forum is that national concerns may be expressed directly by participants, rather than indirectly through governing bodies of major organizations. While decisions taken are not obligatory, they are taken as authoritative commitments by governments and organizations.

Strong regional participation is a focus for IFCS. Each government and sector is asked to have a single

National Focal Point to act as a conduit for communication on IFCS activities (currently, the forum has representatives for 152 countries). Between sessions of the Forum, the Forum Standing Committee (FSC), under the chairmanship of the President of the Forum, monitors the progress on the work of the IFCS and provides advice and assistance on preparations for the next forum session.

The FSC comprises 25 IFCS participants, including five regional Vice-Presidents (Africa, Asia-Pacific, Central and Eastern Europe, Latin America and Caribbean, and Western Europe and Other Groups), and is supported by an Executive Secretariat. *Ad hoc* working groups are established by the Forum or the FSC to undertake specific tasks between forum meetings, such as the preparation of documents for forum meetings.

Forum Meetings

Forum meetings are convened approximately every 3 years. Forum III was held in October 2000 in Salvador da Bahia, Brazil. Forum III conducted a full review of the IFCS. It established the 'Priorities for Action Beyond 2000', which outline realistic and measurable targets set for defined timeframes in the following priority areas: international assessment of chemical risks; harmonizing classification and labeling of chemicals; exchanging information on toxic chemicals and chemical risks; establishing risk reduction programs; strengthening national capabilities and capacities for the management of chemicals; and preventing illegal international traffic in toxic and dangerous products. Participants at Forum III also adopted the 'Bahia Declaration', a statement to reaffirm commitment to the goals for chemical safety set in 1992 at the United Nations Conference on Environment and Development held in Rio de Janeiro, Brazil.

The fourth session of the Forum (Forum IV) was held from 1 to 7 November 2003 in Bangkok, and hosted by the Royal Government of Thailand. At Forum IV, the IFCS 'took stock' of the progress achieved on the commitments and recommendations made in past Forum meetings and set an agenda for action on chemicals management over the next several years.

The theme of Forum IV was 'Chemical Safety in a Vulnerable World'. As such, the Forum focused on populations that are particularly susceptible to health risks due to chemical exposure, such as children. Governments and stakeholders, for example, were asked to prepare initial national assessments on children's health and chemical safety as a basic information tool to identify priority concerns. Improving the provision of information on chemical risks to workers and consumers was also high on the agenda. Forum IV called for new efforts by industry and governments to generate and make available practical information on hazardous chemicals. Furthermore, governments were provided tools and guidance to implement the Globally Harmonized System for the Classification and Labelling of Chemicals – an international hazard communication system with standardized chemical labels and safety information.

Forum IV also examined the problems associated with acutely toxic pesticides; the widening gap in the ability of developing countries to keep pace with developed countries in implementing chemical safety policies and conventions; capacity building for the sound management of chemicals; and the further development of a strategic approach to international chemicals management (SAICM). The SAICM will be founded on the 'Bahia Declaration' and the 'Priorities for Action Beyond 2000'. The SAICM process will identify gaps in chemicals management regimes, obstacles to achieving current targets, and will seek improvements in the current system of international chemicals management.

Forum V will be hosted by Hungary in 2005/2006.

The IFCS website has more information regarding the IFCS, its officers and participants, and Forum meetings.

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International Agency for Research on Cancer

Peter Boyle

Introduction

The mission of the International Agency for Research on Cancer (IARC) is to promote, by way of international collaborative research and other means, improvement of health through reduction of the incidence and mortality from cancer throughout the world by:

- Conducting research into the occurrence, impact, causes, early detection, treatment, and prevention of human cancer.
- Evaluating and disseminating the results of such research.
- Training personnel in relevant scientific and technical skills.

Its unique role in international cancer research depends on its international status, its independence of national political interests, its experience and acceptability as a coordinator of research in developing countries, and its capacity within its own structure to combine epidemiological and laboratory approaches to cancer research.

The agency's research concentrates on the occurrence, causes, treatment, and prevention of cancer. It does not develop cancer control policies or implement cancer control measures except where this is necessary to achieve its research objectives.

Selection of specific activities for the agency's scientific program is based on its mission, considerations of scientific quality, ethical issues, and the known or potential impact on public health of particular cancers or agents that may cause or prevent cancer. The agency's activities have concentrated on three main fields: (1) research into the prevention and early detection of cancer, particularly intervention studies in both developing and developed countries; (2) use of biomarkers of exposure, effects, and susceptibility in epidemiological studies; and (3) studies with potential cancer prevention applications, particularly in developing countries. For the next few years, there will be new research lines, focusing on molecular carcinogenesis, genetics and epidemiology, clinical epidemiology including translational research and treatment outcomes, and on tobacco control and prevention strategies.

In this short review, the general directions of the IARC are briefly presented.

Background

The IARC was established almost 40 years ago by the World Health Assembly on May 20, 1965. The agency was created in a spirit of altruism by several of the wealthier countries of the world to provide capacity for research on important cancer problems wherever they might occur. In the spirit of its creation, the public health-oriented mission of the IARC is to promote, by way of international collaborative research and other means, improvement of health through reduction of the incidence and mortality from cancer throughout the world by conducting research into the causes, early detection, and prevention of cancer; by evaluating and disseminating the results of such research; and by organizing training also for those parts of the world which otherwise would be somewhat deprived of the educational possibilities in cancer research and control. Research into treatment or other aspects of the care does not usually form part of the agency's mission.

Some of the agency's lines of activities are described in the following sections in general terms.

Identification, Elucidation, and Evaluation of Environmental Causes of Cancer

The IARC Monographs Programme on the Evaluation of Carcinogenic Risks to Humans is one of the cornerstones of the agency's activities. During the past few years, it has become increasingly evident that in addition to environmental chemical carcinogens, which have been the traditional target of the IARC monographs, infectious agents contribute significantly to the human cancer burden. In addition, chemical carcinogens and infectious agents may interact in producing their adverse effects on biological systems, leading to an overproportional increase in cancer risk, as exemplified by the combined effects on liver cancer risk of aflatoxin B1 exposure and hepatitis B virus (HBV) infection. Other biological agents that have been evaluated in the monographs series, and found to carry a cancer risk, include schistosomiasis (bladder cancer), liver flukes (cholanglocarcinoma of the liver), *Helicobacter pylori* (stomach cancer), and human papilloma viruses (HPV) (types 16 and 18) (cervix cancer).

The use of tobacco products remains a central focus of the agency's epidemiological work since it is

generally agreed that in industrialized countries, ~30–35% of all known cancers are related to tobacco consumption and, worldwide, one in seven cancers can be attributed to smoking. In addition, the program has now evaluated the carcinogenic hazards associated with 'involuntary smoking' and classified that exposure as carcinogenic to humans. There is no doubt that the high incidence of all cancers in the Indian subcontinent is related to the oral use of tobacco. Subject to the results of a feasibility study now in progress, the agency plans a prospective study of oral tobacco use and cancer in more than 100 000 men and women in Bombay, India.

In addition to environmental factors acting as cancer causing agents, carcinogens may also be formed endogenously; for example, in the context of chronic inflammatory states. One mechanism is the production of reactive oxygen and nitrogen species that may cause tissue and DNA damage. The Endogenous Risk Factors Group focuses on biochemical mechanisms by which oxidative stress produces DNA damage and protein oxidation/nitrosation. These studies will be conducted in a variety of conditions including inflammation and precancerous lesions.

The agency has a long and successful history of achievements in the field of DNA damage and its relationship to mutation and cancer. This has led to the development of highly sensitive methods to assess the extent of interaction between environmental agents and the human genome. Scientists at the agency have succeeded in assessing specific mutations in the tumor-suppressor gene p53 and the Molecular Carcinogenesis Group maintains a database of known p53 mutations.

It is now generally agreed that phenotypic changes occurring in cells during the process of malignant transformation reflect the sequential acquisition of genetic alterations. This applies to all tissues, but the type of oncogene and tumor-suppressor gene and the sequence in which they contribute to tumor progression show a remarkable degree of organ specificity.

IARC scientists have therefore focused their attention on some organ sites (e.g., esophagus, stomach, liver, and cervix) that contribute significantly to the overall human cancer burden. Analyses of genetic alterations associated with tumor progression not only help us to understand the evolution of human cancers but, in some cases, also provide a tool to identify the environmental agent responsible for the initiation of malignant transformation. This has been shown in tumors of the skin (ultraviolet irradiation), liver (aflatoxin B1), and hemangiosarcoma of the liver (vinyl chloride monomer). In the case of stomach cancer, basic laboratory research will be extended into preventive measures, particularly with respect to the role of *H. pylori* in the causation of human stomach cancer. This is similarly true for cancer of the cervix, which in many parts of Central America and Asia remains the most frequent cause of cancer mortality in women. The agency has in the past conducted extensive surveys in different world regions regarding the prevalence of certain types of HPV and their association with cancer of the cervix. It is now conducting a large study on screening modalities for cervical cancer in India and Africa, with a view to establishing the most cost-effective preventive measures in low resource settings.

Mechanisms of Carcinogenesis, Host Factors, and their Interaction with Environmental Agents

The cytochrome P450 enzyme system has been the focus of investigation in several laboratories worldwide since these enzymes participate in the bioactivation of many environmental carcinogens. IARC scientists focus their research on the role of individual patterns of cytochrome P450 isozymes as determinants of genetic susceptibility to environmental carcinogens, in particular tobacco smoke. Over the past few years, evidence has accumulated indicating that individual susceptibility may, at least in part, be related to the individual pattern expression of genes involved in the bioactivation of xenobiotics. This question will also be pursued with respect to the genetic polymorphism of enzymes involved in the detoxification of environmental carcinogens. Individual capacity for DNA repair also appears to play a role in genetic cancer susceptibility. Methodological progress will allow us to launch, in the future, epidemiological projects to analyze the complex relationship between the bioactivation and detoxification of environmental carcinogens, DNA repair, and the production of mutations in critical target cells and transformation-associated genes.

For some human cancers, including brain tumors, our understanding of the etiology is still incomplete. The fraction of cases attributable to radiation or environmental agents is very small. The possibility exists that genetic alterations observed in the evolution of gliomas may be due to endogenous DNA damage rather than interaction with environmental factors. Also, there is strong evidence that germline mutations may play a larger role in brain tumor development than previously anticipated. The Molecular Pathology Group focuses much of its work on the etiopathogenesis of human brain tumors, particularly in children.

A new promising line of research has evolved from the observation that in some human cancers genomic instability may be reflected in microsatellite DNA changes, which commonly originate from replication errors. Effective mismatch repair may be the underlying cause, but so far only a restricted number of fragile microsatellite foci have been identified.

Research on the Prevention of Cancer and Its Consequences

The Gambia Hepatitis Intervention Study is a major effort to determine the role of chronic HBV infection in the evolution of hepatocellular carcinomas. Vaccinated children are being followed-up for serological markers of HBV infection and, later in life, the occurrence of liver tumors. This is a long-term study, but the agency is committed to lead this important work to a successful conclusion.

It is now increasingly possible to identify individuals with a high risk to cancer development; for example, through genetic predisposition, high levels of exposure to environmental carcinogens, or the occurrence of a tumor at a site where second primary tumors are frequent. It is, therefore, necessary to offer these subjects advice and treatment. More targeted screening for early neoplastic lesions is advisable and, in addition, chemoprevention may be a tool to reduce the incidence of malignant transformation and to revert early stages of cancer development. Since cancer prevention is a key element of the agency's mission, IARC scientists would like to be involved in this important research area. This is planned to be done in two ways. Similar to, but distinct from, the monographs series on the *Carcinogenic Risks to Humans*, the *IARC Handbook of Cancer Prevention* series has published a number of volumes concentrating on primary prevention and screening strategies such as: the use of sunscreen, weight control and physical activity, breast cancer screening, and consumption of fruits and vegetables. In addition, a newly created tobacco group will focus on the etiology of tobacco-induced cancers, while producing a new publication series on tobacco control. The agency is intent on establishing itself as the world's leading agency in cancer prevention.

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International Conference on Harmonisation

Robin C Guy

The International Conference on Harmonisation (ICH, formally known as The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) is a joint initiative involving both regulators and industry from Europe, Japan, and the United States as equal partners in the scientific and technical discussions of the testing procedures which are required for product registration and to ensure and assess the safety, quality, and efficacy of medicines. The focus of ICH has been on the technical requirements for medicinal products containing new drugs. The vast majority of those new drugs and medicines are developed in Western Europe, Japan, and the United States and therefore, when ICH was established, it was agreed that its scope would be confined to registration in those three regions.

History

The pharmaceutical industry has become very global, and while a particular pharmaceutical may be sold to worldwide markets, its registration in each country remained a nation-by-nation process. Individual nations' regulatory systems were based on the same fundamental obligations to evaluate the quality, safety and efficacy. However, the detailed technical requirements had diverged over time to such an extent that industry found it necessary to duplicate many time-consuming and expensive test procedures, in order to market new products internationally. Harmonization of regulatory requirements was pioneered by the European Community (EC), in the 1980s, as the EC (now the European Union) moved towards the development of a single market for pharmaceuticals. The success achieved in Europe demonstrated that harmonization was feasible. It was, however, at the WHO International Conference of Drug Regulatory Authorities (ICDRA), in Paris, in

1989, that specific plans for action began to harmonize procedures in Europe, Japan, and the United States. Soon afterwards, the authorities approached IFPMA to discuss a joint regulatory-industry initiative on international harmonization, and ICH was conceived. The founding of the ICH took place at a meeting in April 1990, hosted by the EFPIA in Brussels. Representatives of the regulatory agencies and industry associations of Europe, Japan, and the United States met, primarily to plan an international conference but the meeting also discussed the wider implications and terms of reference of ICH.

Mission, Purpose, Objectives, Goals

The purpose of ICH is to make recommendations on ways to achieve greater harmonization in the interpretation and application of technical guidelines and requirements for pharmaceutical registration in Europe, Japan, and the United States, in order to reduce or preclude the need to duplicate the testing during research and development. The objective of such harmonization is a more economical use of human, animal, and material resources, and the elimination of unnecessary delay in the global development and availability of new medicines whilst maintaining safeguards on quality, safety, and efficacy, and regulatory obligations to protect public health.

Membership Criteria

Six parties to ICH represent the regulatory bodies and research-based industry in the three regions, Europe, Japan, and the United States, where the vast majority of new medicines are currently developed. These Six Parties are directly involved in the decision making process and were selected as they represent the regulatory bodies and the research-based industry in the European Union, Japan, and the United States. However, the Conference, its preparations, and follow-up activities are conducted in an open and transparent manner and the presence of observers from other regulatory authorities and WHO is welcomed as a means of ensuring that the benefits of progress towards harmonization can be utilized worldwide. The Six Parties are as follows:

- *European Commission – European Union (EU)*: The European Commission represents the 15 members of the EU. The Commission works through harmonization of technical requirements and procedures, to achieve a single market in pharmaceuticals, which would allow free movement of products throughout the EU.
- *European Federation of Pharmaceutical Industries and Associations (EFPIA)*: EFPIA is situated in Brussels and has, as its members, Member Associations in 16 countries in Western Europe. Much of the Federation's work is concerned with the activities of the European Commission and the EMEA. Companies in membership of EFPIA are manufacturers of prescription medicines and include all of Europe's major research-based pharmaceutical companies.
- *Ministry of Health, Labor, and Welfare, Japan (MHLW)*: Affiliated institutions include the National Institute of Health Sciences and academia that carries out research and testing on drugs, vaccines, and biologicals.
- *Japan Pharmaceutical Manufacturers Association (JPMA)*: JPMA represents 90 member companies. Membership includes all the major research-based pharmaceutical manufacturers in Japan.
- *US Food and Drug Administration (FDA)*: The US FDA has a wide range of responsibilities for drugs, biologicals, medical devices, cosmetics, and radiological products. The largest of the world's drug regulatory agencies, FDA is responsible for the approval of all drug products used in the United States.
- *Pharmaceutical Research and Manufacturers of America (PhRMA)*: The PhRMA represents the research-based industry in the United States. The Association has 67 companies in membership, which are involved in the discovery, development and manufacture of prescription medicines. There are also 24 research affiliates that conduct biological research related to the development of drugs and vaccines.

Others also are part of the ICH process. They include Observers, the Secretariat, and Administration.

Observers act as links with non-ICH countries and regions. They nominate nonvoting participants to attend the ICH Steering Committee Meetings.

The Observers to ICH are:

- the World Health Organization (WHO);
- the European Free Trade Area (EFTA), represented at ICH by Switzerland; and
- Canada, represented at ICH by Health Canada

Secretariat: The International Federation of Pharmaceutical Manufacturers Association (IFPMA) is a Federation of Member Associations representing the research-based pharmaceutical industry and other manufacturers of prescription medicines in 56

countries throughout the world. IFPMA runs the ICH Secretariat.

Administration: ICH is administered by the ICH Steering Committee, which is supported by the ICH Secretariat. Each of the six sponsors has had two seats on the ICH Steering Committee (SQ, which oversees the harmonization activities.

Additionally, groups are broken down into the Steering Committee (SC) and Expert Working Groups. The SC determines the policies and procedures for ICH, selects topics for harmonization and monitors the progress of harmonization initiatives. The SC meets at least twice a year. The Expert Working Groups are assigned by each of the six parties to designate a Topic Leader for the new topic.

Key Activities

ICH provides an opportunity for regulators and industry worldwide to reach consensus on the steps needed to achieve harmonization of technical requirements and to set out practical and realistic targets for harmonizing requirements where significant obstacles to drug development and the regulatory process have been identified.

There is a five-step process for ICH activities:

1. Consensus building, where an initial draft of a guideline or recommendation, is prepared, then signed off by the Expert Working Group Members. This is then which is submitted to the SC.
2. Start of regulatory action, when the SC agrees that there is sufficient scientific consensus on the technical issues, for the draft guideline or recommendation to proceed to the next stage of regulatory consultation.
3. Regulatory consultation, where the guideline or recommendation leaves the ICH process and becomes the subject of normal wide-ranging regulatory consultation in the three regions. In the EU, it is published as a draft CPMP Guideline; in the United States, it is published as draft guidance in the Federal Register; and in Japan, it is translated and issued by MHLW, for internal and external consultation.
4. Adoption of a tripartite harmonized text occurs when the topic returns to the ICH forum where the SC receives a report regarding the regulatory and industry satisfaction that the consensus achieved at step 2 is not substantially altered as a result of the consultation, the text is adopted by the SC. This adoption takes place on the signatures from the three regulatory parties to ICH affirming that the Guideline is recommended for adoption by the regulatory bodies in the three regions.
5. Regulatory implementation is carried out according to the same national/regional procedures that apply to other regulatory guidelines and requirements, in the EU, Japan, and the United States.

Key Accomplishments

Many procedures, including technical guidelines and format and content of registration applications have been grouped and have successfully been harmonized. These are readily available to the scientific community.

The ICH Topics are divided into four major categories and ICH Topic Codes are assigned according to these categories. The ICH website has summary charts with the status of harmonization of the ICH Topics and Guidelines.

- Q = 'Quality' topics, that is, those relating to chemical and pharmaceutical Quality Assurance. Examples: Q I Stability Testing, Q3 Impurity Testing.
- S = 'Safety' topics, that is, those relating to *in vitro* and *in vivo* preclinical studies. Examples: S I Carcinogenicity Testing, S2 Genotoxicity Testing.
- E = 'Efficacy' topics, that is, those relating to clinical studies in human subject. Examples: E4 Dose–Response Studies, Carcinogenicity Testing, E6 Good Clinical Practices.
- M = Multidisciplinary topics, that is, topics which do not fit uniquely into one of the above categories.
- M1: Medical Terminology.
- M2: Electronic Standards for Transmission of Regulatory Information (ESTRI).
- M3: Timing of Preclinical Studies in Relation to Clinical Trials.
- M4: The Common Technical Document.

Meetings

ICH 1 (i.e., first Conference) was held in Brussels in 1991
ICH 2 was held in Orlando, Florida in 1993
ICH 3 was held in Yokohama, Japan in 1995
ICH 4 was held in Brussels in 1997
ICH 5 was held in San Diego, California in 2000
ICH 6 was held in Osaka, Japan in November, 2003.

Relevant Websites

http://www.ich.org – International Conference on Harmonization (ICH).

http://www.efpia.org – European Federation of Pharmaceutical Industries and Associations (EFPIA).
http://www.mhlw.go.jp – Ministry of Health, Labor and Welfare, Japan (MHLW).
http://www.jpma.go.jp – Japan Pharmaceutical Manufacturers Association (JPMA).
http://www.fda.gov – US Food and Drug Administration (FDA).
http://www.phrma.org – Pharmaceutical Research and Manufacturers of America (PhRMA).
http://www.ifpma.org – International Federation of Pharmaceutical Manufacturers Association (IFPMA).

International Fragrance Association (IFRA)

Audrey Martin

History and Organization

The International Fragrance Association (IFRA) was founded in 1973, in Geneva, to represent the collective interests of the fragrance industry worldwide. IFRA is a Swiss association with a Belgian branch.

IFRA is headed by an Executive Director and a Board of Directors. The General Assembly is the governing body of IFRA. Each national delegation/ordinary member has one vote. The General Assembly meets at least once a year.

The association has a Scientific Director who oversees the technical and scientific aspects of the association.

Mission and Activities

The primary focus of IFRA is the worldwide development and advancement of the fragrance industry. The activities of IFRA cover three major areas: science, regulatory affairs, and communication. This occurs through:

- Development and implementation of a Code of Practice and Safety Standards for the good manufacture and safe use of fragrances, based on broadly recognized scientific principles and utilized worldwide with the final objective to protect the consumer and the environment.
- Close association with RIFM (Research Institute for Fragrance Materials), an independent international nonprofit research institute founded in 1966 for the purpose of developing and maintaining ingredient safety information.[1]
- Promotion of the self-regulatory practices of the industry, in keeping with the idea that the adaptation of worldwide industry rules to new scientific findings can occur more quickly through self-regulation than a change of legislation in different countries on different continents.
- Analysis and review of pending regulations applicable to fragrances, as well as legislative trends in related areas such as cosmetics, intellectual property, chemicals, and occupational health and safety.
- Development and maintenance of open communication and cooperation with national and international government bodies, concerned members of the medical and scientific community, related industries (e.g., cosmetics), and other stakeholders.
- Dissemination of timely and comprehensive information to membership on matters of relevance to the industry.
- Promotion of the merits of fragrances and the role they play in enhancing the quality of life.

Membership

IFRA membership is open to all countries and currently comprises the national associations of fragrance manufacturers from Australia, Europe, the Far East, and North and South America. Since there is no company membership in IFRA, individual fragrance companies belong to IFRA through IFRA's member associations.

Ordinary member associations and their member companies benefit from IFRA membership as follows:

- participation in the work of the IFRA Board and of the IFRA Committees;
- receipt of all information disseminated by the IFRA headquarters, covering a range of critical subjects relevant to fragrance use, safety, and operations;

[1] The scientific foundation of RIFM is based on an international Panel of Experts, made up of toxicologists, pharmacologists, and dermatologists who have no commercial ties to the fragrance industry, and whose work involves the safety evaluation of fragrance materials under conditions of intended use. Their evaluations are (*footnote continued*) based on existing data or, where insufficient data exist, on testing performed by RIFM itself.

- contacts with experts and industry colleagues from all over the world; and
- assistance of IFRA, as an avenue of support to the resolution of complex issues faced by fragrance companies in international commerce.

Publications, Databases, and Services

Code of Practice for the Fragrance Industry: The main thrust of IFRA's scientific activities consists in developing, communicating, and implementing a Code of Practice for the international fragrance industry. The Code of Practice provides Standards of good operating practice and product safety. It was first issued in 1973 and has been followed by fragrance companies worldwide ever since. Amendments and updates are issued on a regular basis. Standards regarding use restrictions are based on safety assessments by the Panel of Experts of RIFM and are carefully reviewed by the IFRA Scientific Committee.

Meetings

Regular meetings of IFRA Committees and Working Groups. For example

- Scientific Committee;
- Environmental Task Force; and
- Communications Working Group.

Regular meetings of joint Committees. For example

- IFRA/Customer Industry Joint Advisory Group;
- IFRA/EFFA Analytical Working Group;
- EFFA/IFRA/IOFI Labeling Group; and
- IFRA/IOFI Committee on Health, the Environment and Workplace Safety.

Related Organizations

COLIPA	European Cosmetic, Toiletry and Perfumery Association
CTFA	US Cosmetics, Toiletry and Fragrance Association
EFFA	European Flavour and Fragrance Association
IOFI	International Organization of the Flavor Industry
RIFM	Research Institute for Fragrance Materials

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International Labour Organization (ILO)

Pertti J Hakkinen

History and Mandate

The International Labour Organization is the United Nations (UN) specialized agency that seeks the promotion of social justice and internationally recognized human and labor rights. It was founded in 1919 at the end of the First World War, during the Peace Conference convened first in Paris and then at Versailles. Two industrialists, Robert Owen of Wales and Daniel Legrand of France, had advocated the need for such an organization in the nineteenth century. After having been put to the test within the International Association for Labour Legislation, founded in Basel in 1901, their ideas were incorporated into the Constitution of the International Labour Organization, adopted by the Peace Conference. The ILO is the only surviving major creation of the Treaty of Versailles that brought the League of Nations into being, and it became the first specialized agency of the United Nations in 1946.

The ILO formulates international labor standards in the form of Conventions and Recommendations setting minimum standards of basic labor rights, that is, freedom of association, the right to organize, collective bargaining, abolition of forced labor, equality of opportunity and treatment, and other

standards regulating conditions across the entire spectrum of work-related issues. It provides technical assistance primarily in the fields of: vocational training and vocational rehabilitation, employment policy, labor administration, labor law and industrial relations, working conditions, management development, cooperatives, social security, labor statistics, and occupational safety and health.

The ILO promotes the development of independent employers' and workers' organizations and provides training and advisory services to those organizations. Within the UN system, the ILO has a unique tripartite structure with workers and employers participating as equal partners with governments in the work of its governing organs. The ILO accomplishes its work through these three main bodies, all of which encompass its tripartite structure:

1. *International Labour Conference*: The member States (currently 177 countries) of the ILO meet at the International Labour Conference in June of each year, in Geneva. Two government delegates, an employer delegate and a worker delegate, represent each Member State and technical advisors accompany them. The Conference establishes and adopts international labor standards, and acts as a forum where social and labor questions of importance to the entire world are discussed. The Conference also adopts the budget of the Organization and elects the Governing Body.
2. *Governing Body*: The Governing Body is the executive council of the ILO and meets three times a year in Geneva. It takes decisions on ILO's policy, and establishes the program and the budget that it then submits to the Conference for adoption. It also elects the Director-General. It is composed of 28 government members, 14 employer members, and 14 worker members.
3. *International Labour Office*: The International Labor Office is the permanent secretariat of the ILO and focal point for the overall activities that it prepares under the scrutiny of the Governing Body and under the leadership of a Director-General. The Office includes the Geneva headquarters and 40 field offices around the world. In addition, experts undertake missions in all regions of the world under the program of technical cooperation. The Office also constitutes a research and documentation center and a printing house issuing a broad range of specialized studies, reports, and periodicals.

Examples of ILO Resources

Books, journals, databases, CD-ROMs, and videos: The ILO is the world's major resource center for information, analysis, and guidance on the world of work. The ILO also is an international publishing house, including publications on social security, occupational safety and health, industrial relations, labor law, training, management development, and other aspects of the world of work. For example, the *ILO Encyclopaedia of Occupational Health and Safety* (the fourth edition is available in a four-volume print version and on CD-ROM) serves as a worldwide reference reflecting the state of the art in occupational health and safety.

In addition, the ILO publishes statistical, legal, and bibliographic materials in both printed and interactive electronic forms. *The Yearbook of Labour Statistics* also exists in the form of a database (LABORSTA). The ILO journal, *International Labour Review*, features current policy analysis on employment and labor worldwide, and the ILO also publishes the magazine *World of Work* and *The Bulletin of Labour Statistics*. As noted below, the ILO publishes other documents, databases, CD-ROMs, and videos.

The ILO Library: The ILO Library provides access to a multilingual collection of over 1.5 million books, reports, journals, national legislation texts and statistical publications, and electronic information sources on all aspects of the world of work. The Library produces LABORDOC, a unique bibliographic database providing international, multilingual coverage of social and labor affairs, available online, as a CD-ROM, and in print (*International Labour Documentation*). The library also publishes the *ILO Thesaurus* (fifth edition, 1998), and develops projects and training courses for labor librarians.

International Institute for Labour Studies: Established in 1960, the ILO International Institute for Labour Studies in Geneva promotes a wider study and public discussion of policy issues of concern to the ILO and its constituents, through systematic interaction between the Organization and the international academic community, and other policymakers. The central theme of its activities is the interaction between labor institutions, economic growth, and equity. The Institute's means of action include research networks, social policy forums, courses and seminars, visiting scholar and internship programs, and publications.

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International Life Sciences Institute – North America

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History, Purpose, and Financial Support

The International Life Sciences Institute (ILSI) is a nonprofit, worldwide foundation established in 1978 to advance the understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment. By bringing together scientists from academia, government, industry, and the public interest/public health sector, ILSI seeks a balanced approach to solving problems of common concern for the well-being of the general public. ILSI receives financial support from industry, governments, and foundations.

Affiliations

ILSI is affiliated with the World Health Organization as a nongovernmental organization, and has specialized consultative status with the Food and Agriculture Organization of the United Nations.

Locations

ILSI is headquartered in Washington, DC. ILSI branches include Argentina, Brazil, Europe, India, Japan, Korea, Mexico, North Africa and Gulf Region, North America, North Andean, South Africa, South Andean, Southeast Asia Region, the Focal Point in China, and the ILSI Health and Environmental Sciences Institute. ILSI also accomplishes its work through the ILSI Research Foundation (composed of the ILSI Human Nutrition Institute and the ILSI Risk Science Institute) and the ILSI Center for Health Promotion.

Publications

ILSI publishes two journals (*Nutrition Reviews* and *Nutrition in Clinical Care*) and a series of newsletters from the parent organization and its branches. All of these are available through the ILSI website. In addition, ILSI Press has published many books and monographs, reflecting the broad range of ILSI's areas of interests. Some recent publications include *Present Knowledge in Nutrition*; *Direct Dosing of Pre-weaning Mammals in Toxicity Testing; Microbial Pathogens and Disinfection By-products in Drinking Water: Health Effects and Management of Risk; Functional Foods – Scientific and Global Perspectives*; and *Similarities and Differences Between Children and Adults: Implications for Risk Assessment*. A catalog of all publications available from ILSI Press can be found on the ILSI website.

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International Organization of the Flavor Industry (IOFI)

Thierry Cachet

History and Organization

The International Organization of the Flavor Industry (IOFI) was founded in 1969 in Geneva to represent the collective interests of the flavor industry worldwide. IOFI is a Swiss association with a Belgian branch, and is headed by an Executive Director and a Board of Directors. The General Assembly is the governing body of IOFI. Each national delegation/ordinary member has one vote. The General Assembly meets at least once a year. The association has a Scientific Director who supervises the technical and scientific aspects of the association.

Mission and Activities

IOFI is the representative of the global flavor industry. Acting directly and through its members, IOFI provides the industry, its customers, government agencies, and consumers with sound scientific

information, education, and training in order to promote the benefits and safe use of flavors. The activities of IOFI cover Science, Advocacy, Communication, and Intellectual Property protection. This is done by (1) serving as an effective advocate for IOFI members by representing industry's interests before global legislative and regulatory bodies, (2) participating in the harmonization of global flavor legislation, (3) providing information and advice to emerging countries on the status of flavor regulation in the United States, Europe, and Japan, (4) collecting confidential information on a worldwide basis on the identity and use levels of flavoring substances, (5) collecting data on safety studies on flavoring ingredients, with the aim of facilitating their evaluation by scientific bodies, and (6) participating in international meetings, hearings, and conferences to help promote the interests of IOFI and the global harmonization of flavor legislation.

IOFI also functions by (1) maintaining good relationships with related associations (e.g., the International Federation of Essentials Oils and Aroma Trades (IFEAT), International Life Sciences Institute (ILSI), and regular communication with the Food and Agriculture Organization (FAO), the World Health Organization (WHO), Codex Alimentarius Commission, the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the Council of Europe), (2) preparing publications on safety and regulatory matters, (3) keeping IOFI's members fully informed on scientific, legal, regulatory, and other relevant matters, through information letters, circular letters, a Code of Practice, and other guidelines, and (4) keeping the Code of Practice and similar IOFI documents updated.

Membership

IOFI Membership is open to all countries and currently comprises the national associations of flavor manufacturers from Australia, Europe, the Far East, and North and South America, such as FEMA (United States), EFFA (European Union), and JFFMA (Japan). Since there is no company membership in IOFI, individual flavor companies belong to IOFI through IOFI's member associations.

Ordinary member associations and their member companies benefit from IOFI membership via (1) participation in the work of the IOFI Board and of the IOFI committees, (2) receiving information disseminated by IOFI, covering a wide range of critical subjects relevant to flavor use, safety, and operations, (3) contact with experts and industry colleagues from all over the world, and (4) assistance of IOFI as an avenue of support to the resolution of complex issues faced by flavor companies in international commerce.

Publications, Databases, Services

Code of Practice for the flavor industry: The main thrust of IOFI's scientific activities consists of developing, communicating, and implementing a Code of Practice for the international flavor industry. The Code of Practice provides Standards of good operating practice and product safety. It was first issued in 1969 and has been followed by flavor companies worldwide ever since. Amendments and updates are issued on a regular basis.

Meetings

Regular meetings of IOFI Committees and Working Groups, for example, the Global Safety Management Committee (GSMC), Technical Experts Committee (TEC), and the Working Group on Methods of Analysis (WGMA). In addition, there are regular meetings of joint Committees, for example, EFFA/IFRA/IOFI Labeling Group and the IFRA/IOFI Committee on Health, the Environment and Workplace Safety (CHEW).

Related Organizations

- European Flavour and Fragrance Association (EFFA).
- International Fragrance Association (IFRA).
- Flavor and Extract Manufacturers Association (FEMA).
- Japan Flavor and Fragrance Materials Association (JFFMA).

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International Programme on Chemical Safety

Lynne Haber

The International Programme on Chemical Safety (IPCS) is a cooperative program of the United Nations Environment Programme (UNEP), the International Labor Organization (ILO), and the World Health Organization (WHO). The central unit of IPCS is based at WHOs Programme for the Promotion of Chemical Safety. The 1992 United Nations Conference on Environment and Development (UNCED) stated that collaboration on chemical safety among UNEP, ILO, and WHO in the IPCS should be the nucleus for international cooperation on environmentally sound management of chemicals. The work of the IPCS is divided into four areas: evaluation of chemical risks to human health; poisons information, prevention and management; chemical incidents and emergencies including public health preparedness, response, prevention and surveillance; and capacity building. The IPCS also has an Interregional Research Unit (IRRU) located at the National Institute of Environmental Health Sciences (Research Triangle Park, NC, USA).

The Intersecretariat Coordinating Committee meets regularly to ensure participation of the three cooperating organizations (COs) in the programme. The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) has been established to promote coordination of the relevant policies and activities of UNEP, ILO, UN Food and Agricultural Organization (FAO), WHO, UNIDO, UNITAR, and OECD.

Overall policy guidance for the work of IPCS is provided by the Programme Advisory Committee, which is composed of 20 external experts who serve as individuals, rather than representing their respective organizations. This committee provides advice on scientific, technical, ethical, administrative, and regulatory aspects of the activities of the IPCS.

Collaborative Structure of the IPCS with Other Organizations

Countries or national agencies wishing to participate actively in the work of the IPCS sign a Memorandum of Understanding (MOU) that specifies the scope and areas of collaboration. The IPCS collaborates closely with the European Commission (EC) and the OECD as well as with several nongovernmental organizations active in the field of chemical safety. These nongovernmental organizations include the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), the CropLife International, the International Life Sciences Institute, the International Organisation of Consumers Unions (Consumers International), the International Union of Pharmacology (IUPHAR), the International Union of Pure and Applied Chemistry (IUPAC), and the International Union of Toxicology (IUTOX).

Many IPCS activities are implemented through a network of governmental and nongovernmental institutions, which are designated as Participating Institutions. These institutions include various types of centers of excellence, usually conducting scientific activities concerning effects of chemicals. A number of specific IPCS projects have their own network of participating centers covering particular project activities. For example, the International Agency for Research on Cancer (IARC) participates in the work of the IPCS in the field of chemical carcinogenicity.

Task Groups and Working Groups function as informal advisory mechanisms for IPCS. *Ad hoc* groups provide advice on specific technical and scientific topics concerning the program's work. Such groups are convened as required by the Coordinator of the IPCS.

Roles and Activity Areas of the IPCS

IPCS Objectives

The two main objectives of the IPCS are to establish the scientific health and environmental risk assessment basis for safe use of chemicals (normative functions) and to strengthen national capabilities for chemical safety (technical cooperation). As a result of a redesign of its program of activities in 2002–03, IPCS is emphasizing the collection of evidence for chemical-related adverse effects and determining the quality of the evidence used in risk assessment, including improved use of observational data from human exposures. IPCS is also emphasizing the use of risk assessment products by individual countries for the support of chemical management activities. Support of WHO normative activities is also being emphasized, including development of WHO drinking water and air-quality guidelines.

IPCS Areas of Activities

In fulfilling its roles, IPCS conducts activities in the following areas:

1. Carries out and disseminates evaluations of the risk to human health and the environment from

exposure to chemicals and produces health- or environment-based guideline values for exposure to the agents evaluated.
2. Promotes the development, improvement, validation, harmonization and use of methods for laboratory testing and ecological and epidemiological studies and other methods suitable for the evaluation of health and environmental risks and hazards from chemicals.
3. Promotes research to improve the scientific basis for health and environmental risk assessment to ensure a sound management of chemicals.
4. Promotes technical cooperation with Member States, in particular developing countries, to strengthen their capabilities and capacities in the area of chemical safety.
5. Promotes effective international cooperation with respect to emergencies and accidents involving chemicals.
6. Supports national programs for prevention and treatment of poisonings.
7. Contributes to the harmonization of classification and labeling of chemicals.
8. Promotes development of the human resources required in the areas above.

IPCS Achievements

Within its eight activity areas, IPCS conducts or supports a broad range of activities, with a variety of target audiences.

Evaluation of Chemical Risks to Human Health and the Environment Since its conception, the IPCS has evaluated an impressive list of commonly used and internationally traded agricultural and industrial chemicals. IPCS has also evaluated radioisotopes, chemicals frequently found as dangerous air and water pollutants, chemicals associated with global atmospheric changes, such as the greenhouse gases and the chlorofluorocarbons, as well as certain natural toxins and certain physical factors, such as noise and low-frequency radiation.

Chemical evaluations published by the IPCS include:

- *Environmental Health Criteria* (EHC) documents are detailed chemical evaluations designed for scientific experts responsible for evaluation of the risk posed by chemicals to human health and the environment, enabling relevant authorities to establish policies for the safe use of these chemicals.
- *Health and Safety Guides* are designed for a wide range of administrators, managers, and decision-makers in various ministries and governmental agencies, as well as in commerce, industry, and trade unions, who are involved in various aspects of using chemicals safely and avoiding environmental health hazards. They are short documents summarizing toxicity information in nontechnical language, and provide practical advice on matters such as safe storage, handling and disposal of the chemicals, accident prevention and health protection measures, first aid and medical treatment in cases of exposure leading to acute effects, and clean-up procedures.
- *International Chemical Safety Cards* (ICSCs) summarize essential data using standard phrases on a single sheet, and are intended for use in the workplace and field. These cards have no legally binding status and are not intended to be used in the regulatory process in any specific country.
- *Concise International Chemical Assessment Documents* (CICADS) are concise documents that provide summaries of the relevant scientific information concerning the potential effects of chemicals upon human health and/or the environment. They are based on selected national or regional evaluation documents or on existing EHCs. Before acceptance for publication as CICADs by IPCS, these documents have undergone extensive peer review by internationally selected experts to ensure their completeness, their accuracy in the presentation of original data, and the validity of the conclusions drawn.

Toxicological evaluations of chemicals associated with food are made jointly with the FAO, through the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). The IPCS also collaborates with the FAO and WHO in the preparation of Pesticide Data Sheets (PDSs). These data sheets contain not only toxicological and risk information but also guidance on first aid and laboratory analysis as well as recommendations to regulatory authorities. IPCS also contributes to development of WHO Guidelines for Drinking-Water Quality. These guidelines are the international reference point for standard setting and drinking-water safety. The guidelines are supported by other publications that explain how they were derived and that assist in implementing safe water activities.

Methodologies for Evaluation of Hazards and Risks IPCS aims at promoting the development, improvement, validation, harmonization, and use of generally

acceptable, scientifically sound methodologies for the evaluation of risks to human health and the environment from exposure to chemicals. IPCS aims to promote global harmonization of approaches to risk assessment through increased understanding, focusing on specific issues, and striving for agreement on basic principles. IPCS notes that such harmonization should not be perceived as standardization but rather as an understanding of the methods and practices used by various countries and organizations so as to develop confidence in an acceptance of assessments that use different approaches. It further involves a willingness to work toward convergence of these approaches or methods as a long-term goal. Progress through all stages of this project will result in efficient use of resources and consistency among assessments, avoiding unnecessary duplication of efforts, and increasing the volume of information made available to countries.

The development and improvement of methods will provide the scientific basis for safe use of chemicals and therefore enhance the capabilities of countries in risk assessment and management. IPCS has published a number of EHC documents on risk methods, including monographs on general principles, evaluation of toxic effects in specific organs, systems, or end points, and consideration of susceptible populations. Other monographs address principles of environmental epidemiology and toxicokinetic studies, and the principles and concepts of using biomarkers in risk assessment.

Emerging Issues in Chemical Safety The work of IPCS on endocrine disruptors is an example of work on emerging issues and falls into two main areas. The Global Inventory of Ongoing Research on Endocrine Disruptors is an Internet-accessible repository of ongoing global research activities on the health and ecological effects of endocrine disruptors, and is designed to facilitate communication and minimize duplication of effort. IPCS also published in 2002 a Global Assessment of the State-of-the-Science of Endocrine disruptors.

Other emerging areas include risk assessment of vulnerable population groups, toxicogenomics, and the integration of human and ecological risk assessment.

Prevention and Management of Toxic Exposures and Chemical Emergencies IPCS develops and supports a number of information resources to aid in this area. The IPCS INCHEM database (see 'Relevant Websites') contains many of the publications of the IPCS, including Pesticide Data Sheets (PDSs), Poisons Information Monographs (PIMs), the full text of Environmental Health Criteria documents, CICADs, Health and Safety Guides, carcinogenicity summaries and evaluations from the International Agency for Research on Cancer (IARC), International Chemical Safety Cards, monographs from JECFA and JMPR, and Screening Information Data Sets (SIDs) for High Production Volume Chemicals. Many of these publications are also available on compact disk (CD-ROM).

IPCS INTOX is an essential tool for poison centers and other organizations concerned with preventing, recording, evaluating, diagnosing, treating, and reporting on chemical emergencies. It includes a databank of consolidated, authoritative information on toxic agents and the management of toxic exposures; an information management tool for poison centers and others dealing with toxic exposures; the gateway to a global electronic network of poison centers and other users of the package; and it provides a forum for collaboration between experts and those responding to emergencies concerning toxic exposures.

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International Society for the Study of Xenobiotics

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Objectives

The purpose of the International Society for the Study of Xenobiotics (ISSX) is

- to facilitate the association of scientists engaged in xenobiotic research and in other related disciplines;
- to disseminate, discuss, and publish results of research and related matters of interest in this field;
- to promote public awareness of the field and its social and environmental implications; and
- to promote education and training in this field.

History

Man's use of xenobiotics dates from antiquity but interest in foreign compound metabolism dates from only the mid-nineteenth century, when the knowledge and techniques of organic chemistry were first applied to its study. For nearly a century thereafter biotransformation was generally equated with 'detoxication' or the elimination of a compound's biological activity.

This view changed in the late 1930s with the discovery that the synthetic azo-dye prontosil owed its life-saving antibacterial activity to its metabolite, sulfanilamide. Since the 1950s, the biological effects of numerous xenobiotic substances have been shown to be due to biotransformation products rather than to effects of the parent compound. The importance of biotransformation and other aspects of the interactions of xenobiotics with biological systems has been continuously reinforced by regulatory agencies worldwide. Their need for scientific knowledge on which to base regulations and safety evaluations for chemicals and drugs provides one important motivation for the study of xenobiotics.

Scientists working in such diverse fields as clinical and basic pharmacology, biochemistry, toxicology, and oncology were drawn into metabolism studies, both in universities and research institutes, and in the pharmaceutical, chemical, agrochemical, food processing, tobacco, and cosmetic industries. In 1981, a small group of scientists, brought together during the 1970s under the aegis of the Gordon Research Conferences on Drug Metabolism, took the bold step of suggesting the organization of an international society to promote the interaction of scientists dedicated broadly to the study of xenobiotics in living systems. Thus, the International Society for the Study of Xenobiotics was formed.

Membership

Currently, ISSX has more than 2700 members in 57 countries. The majority of members work in the areas of pharmacology, toxicology, biochemistry, analytical chemistry, etc. ISSX offers two types of memberships: full member and Graduate Student/Postdoctoral Researcher member. In addition, the Council may award Honorary memberships to persons who are members or nonmembers of the Society in recognition of outstanding and sustained achievement in the field of xenobiotics.

Publications

The Society publishes the quarterly ISSX Newsletter that is distributed to members. The newsletter includes announcements of ISSX meetings, meeting reports, and book reviews, as well as a calendar of future meetings in the field and other items of relevance to the membership. The journal *Drug Metabolism Reviews* has been adopted as the official journal of the Society, and this journal publishes abstracts of all ISSX meetings and full manuscripts from selected speakers. ISSX members may subscribe to several journals offered at reduced subscription rates.

Meetings

The Society organizes an international meeting every 3 years in locations that rotate from North America, to Asia/Pacific, to Europe. During years when an international meeting is not scheduled, each of these three regions of the Society may organize a regional meeting. North American regional meetings are presented every October during years without an international meeting. The schedule for the next several years is as follows:

August 29–September 2, 2004	*International* meeting in Vancouver, BC, Canada
October 23–27, 2005	*Regional North American* meeting in Maui, Hawaii Cosponsored with Japanese Society for the Study of Xenobiotics (JSSX)
June, 2006	*Regional European* meeting in the UK
October, 2006	*Regional North American* meeting in Puerto Rico
Summer 2007	*International* meeting in Sendai, Japan

Awards and Grants

ISSX has a Society awards system that follows the schedule of its meetings. The R.T. Williams Distinguished Scientific Achievement Award and the Frederick J. DiCarlo Distinguished Service Award, which recognize outstanding individuals in each category worldwide, are presented during the Society's international meetings every 3 years. A regional Scientific Achievement Award and regional New Investigator Award are presented at each of the Society's regional meetings.

In addition, Best Poster Awards are presented to recognize superior research in both the predoctoral and postdoctoral research categories at Society meetings.

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International Society of Exposure Analysis

Pertti J Hakkinen

History, Purpose, and Objectives

The International Society of Exposure Analysis (ISEA) was established in 1989 to foster and advance the science of exposure analysis related to environmental contaminants, both for human populations and ecosystems. The membership promotes communication among –all disciplines involved in exposure analysis, recommends exposure analysis approaches to address substantive or methodological concerns, and works to strengthen the impact of exposure assessment on environmental policy.

Membership Criteria

Any individual with a professional interest in exposure analysis is invited to join. The Society seeks broad participation from various disciplines such as exposure assessment, chemistry, biochemistry, risk assessment, biostatistics, physiology, toxicology, epidemiology, ecology, environmental engineering, and others. There are currently several hundred members of ISEA, with a US focus but international representation. Students and international professionals with an interest in exposure assessment are especially encouraged to join.

Membership Benefits

Members get voting privileges in the election of officers and councilors, and Web-based access to the membership directory. In addition, members get a subscription to the *Journal of Exposure Analysis and Environmental Epidemiology* (JEAEE), and Web-based access to current and recent issues of this journal. The JEAEE focuses on manuscripts dealing with measurements, modeling, instrumentation, questionnaires, studies on chemical, biological, and physical principles required to analyze human exposure from single and multiple media and routes, and epidemiological investigations. The ISEA website provides online access to additional information, for example, funding opportunities for research.

Meetings

An annual meeting is held, sometimes in conjunction with the International Society for Environmental Epidemiology.

Awards

Various awards are announced at the annual meeting, including the Jerome J Wesolowski Award ('in recognition of outstanding contributions to the knowledge and practice of human exposure assessment'), the Joan M Daisey Outstanding Young Scientist Award ('to recognize outstanding contributions to the science of human exposure analysis by a young scientist'), and the Constance L Mehlman Award ('in recognition of outstanding contributions in exposure analysis research that helped shape a National or State policy' or 'that provided new approaches for reduction or prevention of exposures').

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International Union of Pure and Applied Chemistry

John H Duffus

The International Union of Pure and Applied Chemistry (IUPAC) aims to advance the worldwide aspects of the chemical sciences and to contribute to the application of chemistry in the service of mankind. As a scientific, international, nongovernmental, and objective body, IUPAC addresses many global issues involving the chemical sciences.

IUPAC was formed in 1919 by chemists from industry and academia. Over nearly eight decades, the Union has fostered worldwide communications in the chemical sciences and has united academic, industrial, and public sector chemistry in a common language. IUPAC is recognized as the world authority on chemical nomenclature, terminology, standardized methods for measurement, atomic weights, and many other critically evaluated data. The Union sponsors major international meetings ranging from specialized scientific symposia to CHEMRAWN meetings with broad societal impact.

IUPAC is an association of various bodies, National Adhering Organizations, which represent the chemists of the member countries. There are 44 National Adhering Organizations, and 20 other countries are linked to IUPAC as Associate National Adhering Organizations. Almost 1000 chemists throughout the world are engaged on a voluntary basis in the scientific work of IUPAC, primarily through projects, which are components of eight Divisions and several Committees. The Divisions of IUPAC are as follows:

I Physical and biophysical chemistry

II Inorganic chemistry

III Organic and biomolecular chemistry

IV Macromolecular

V Analytical chemistry

VI Chemistry and the environment

VII Chemistry and human health

VIII Chemical nomenclature and structure representation

IUPAC and Nomenclature

IUPAC's nomenclature books are used by professional chemists in academia, government, and chemical industry throughout the world. They are commonly identified by the colors of their covers:

- *Gold*: Chemical terminology.
- *Green*: Quantities, units, and symbols in physical chemistry.
- *Red*: Nomenclature of inorganic chemistry.
- *Blue*: Nomenclature of organic compounds.
- *Purple*: Macromolecular nomenclature.
- *Orange*: Analytical nomenclature.
- *Silver*: Nomenclature and symbols in clinical chemistry.

Standards

IUPAC publishes definitive and up-to-date data on atomic weights and isotopic abundances. It also publishes a wide variety of other chemical data of immense value to chemists and chemical engineers:

- International thermodynamic tables of the fluid state.
- Solubility data series – over 70 volumes of data in this series have already been published.
- Stability constants – this database of metal–complex stability constants available on disk contains ~25 000 pieces of data.
- Enthalpies of vaporization of organic compounds.
- Thermodynamic and transport properties of alkali metals.
- Recommended reference materials for achievement of specific physicochemical properties.
- Evaluated kinetic and photochemical data for atmospheric chemistry.

IUPAC is widely involved in establishing standard methods for use in analytical, clinical, quality control, and research laboratories. Some examples are given below:

- Standard methods for the analysis of oils, fats, and derivatives.
- Harmonization of international quality assurance schemes for analytical laboratories.
- Protocol for self-auditing of analytical laboratories for ISO 9000 certification.
- Quality assurance and sampling.
- Standardization of immunoassay determinations.
- Standard methods for the determination of trace elements in body fluids.
- JCAMP-DX, a standard format for the exchange of spectra in computer readable form.
- Experimental thermodynamics: measurement of the transport properties of fluids; solution calorimetry.

Environment

The various Commissions and Committees of IUPAC have undertaken an extensive array of environmental projects. Some examples are given below:

- environmental analytical chemistry;
- environmental particles;
- polymer recycling;
- determination of trace elements in the environment;
- gas kinetic data for atmospheric chemistry;
- glossary of atmospheric chemistry terms; and
- pesticides in surface water.

IUPAC Congress and Other Meetings

IUPAC organizes a biennial Congress, and each year IUPAC sponsors a large number of independently organized symposia that cover a wide range of specialized topics in chemistry. Sponsorship by IUPAC attests to the quality of the scientific program and indicates the host country's assurance that scientists from all countries may participate. IUPAC sponsors a continuing series of conferences on CHEMical Research Applied to World Needs (CHEMRAWN). These meetings focus on topics in chemistry that have sociopolitical impact, such as availability of raw materials, food chemistry, and environmental matters.

IUPAC Division VII – Chemistry and Human Health

Toxicology is one of the concerns of this division. The main activities of the Division covered by three Subcommittees are:

- Nomenclature, Properties, and Units in Laboratory Medicine.
- Medicinal Chemistry and Drug Development.
- Toxicology and Risk Assessment.

All of these contribute to relevant areas of chemical toxicology.

Subcommittee on Nomenclature, Properties, and Units in Laboratory Medicine

In 1995, the predecessor of the present Subcommittee, the Commission on Nomenclature, Properties and Units (C-NPU of IFCC and IUPAC) started publishing a series of papers on a coding system (i.e., a structure or a framework for the pairs of codes and meaning) and a coding scheme (i.e., the pairs of codes and their meaning), for Properties in Laboratory Medicine. 'Meanings' can be descriptions of properties that are measured or observed in laboratory medicine. The codes offer unique and sufficient information about properties and are designed to facilitate the transfer of information between laboratories and the end users of laboratory information. The codes make it possible to translate the data to any language automatically. So far the meanings have been tested for translation into 18 languages, including many of the European languages, Arabic, and Cantonese.

In order to test functionality, the coding scheme has been successfully mapped to the various codes that are presently used in more than 50 medical laboratories in Denmark and Sweden. To accommodate national or local needs special codes can be used. The coding scheme is accessible on the IFCC website. The coding scheme now includes clinical pharmacology and toxicology, and environmental toxicology.

Subcommittee on Medicinal Chemistry and Drug Development

This subcommittee arranges two meetings a year, publishes books, most recently the *IUPAC Handbook of Pharmaceutically Acceptable Salts*, and prepares glossaries to aid communication between chemists working in this area. These glossaries include a 'Glossary of Terms Used in Medicinal Chemistry', which is available on the IUPAC website. The glossary was published in the *Annual Report on Medicinal Chemistry*, which is distributed by the American Chemical Society to over 10 000 medicinal chemists, and also made available on the Internet. This led further to production and publication of a 'Glossary of Terms Used in Computational Drug Design'.

To assist medicinal chemists in their understanding of combinatorial chemistry and to help with the acceptance of a universally understood language, a 'Glossary of Combinatorial Chemistry Terms' was published in *Pure and Applied Chemistry* and subsequently, in the *Journal of Combinatorial Chemistry*. This ensures its use within the American Chemical Society as a standard glossary of terms. Further work is focused on producing an opinion document on the legal implications of patenting virtual libraries. This is a very important issue which has profound implications for research and development in the pharmaceutical industry.

Other glossaries of terms are being prepared, including a Glossary of Drug Metabolism Terms, Glossary of Terms in Pharmaceutical Process Chemistry, and Glossary of Terms in Pharmaceutical Technology.

Training of Medicinal Chemists

A series of papers has been published on training. A syllabus for a short course on medicinal chemistry has also been published, and courses have been initiated in some Latin American countries.

Guidelines for Natural Product Collaborations

To facilitate collaborations, a document of guidelines for this was published in 1996 as IUPAC Recommendations entitled 'Preservation and utilization of natural biodiversity in context of the search for economically valuable medicinal biota'. Two other documents have been published on the subject: a technical report intended to help with drawing up contracts and an article titled 'Medicinal Chemistry in the Development of Societies'.

IUPAC Subcommittee on Toxicology and Risk Assessment

IUPAC Subcommittee on Toxicology and Risk Assessment is open to all those members of Division VII and other chemists (who may be co-opted) who are interested in toxicology.

Its terms of reference are as follows:

(i) To coordinate projects that have been approved by the Division VII Committee and which relate to toxicology and risk assessment.

(ii) To provide a forum for discussing the information content and progress of projects identified under (i) above.

(iii) To provide a forum for initiating new project submissions in the subject area of toxicology and risk assessment that are considered to be suitable activities for Division VII.

(iv) To provide the opportunity for coordination in both experimental and computational approaches to toxicology and risk assessment methods.

(v) To report to the Division VII President and the Division Committee on items (i) to (iii) above.

(vi) To provide a connection with other organizations concerned with toxicology such as the International Union of Toxicology (IUTOX), the International Programme for Chemical Safety (IPCS), the World Health Organization (WHO), the Organization for Economic Cooperation and Development (OECD), the International Labour Organization (ILO), the International Union of Biochemistry and Molecular Biology (IUBMB), the International Union of Immunological Societies (IUIS), the International Union of Pharmacology (IUPHAR), the International Federation of Clinical Chemistry (IFCC), other national and international toxicology and clinical chemistry societies, and chemical industry health and safety groups.

(vii) To provide an opportunity for IUPAC interaction with chemists in the Chemical Industry worldwide in the field of toxicology and risk assessment.

(viii) To broaden the activities of the Division by providing opportunities for other organizations involved in toxicology and risk assessment to work together with Division VII members.

(ix) To offer advice to the Division President and the Committee on matters concerning toxicology and risk assessment in all aspects from the purely chemical to the protection of human health and the natural environment.

One of the main concerns of the Subcommittee on Toxicology and Risk Assessment and its predecessor the Commission on Toxicology has been education of chemists in fundamental principles of toxicology. Activities here have involved the compilation of glossaries, educational modules, and reviews of matters of current concern. These can be found on the IUPAC website at http://www.iupac.org/divisions/VII/VII.C.2/index.html.

The Future of IUPAC

Chemistry historically emerged and developed as an interdisciplinary scientific field, with a broad definition of its borders. Paraphrasing Linus Pauling's definition of the chemical bond "whatever is convenient to the chemist to define as a bond", chemistry can be defined as a discipline encompassing all areas which are of interest to chemists and where molecular science makes significant contributions. The rich and diverse world of modern chemistry encompasses remarkable intellectual accomplishments, scientific creativity and originality and the generation of new knowledge. IUPAC serves international scientific endeavor in the dual function of a basic science and a mission-oriented Union. The Union is in a unique position to contribute to the central interdisciplinary chemical sciences. Strengthening international chemistry, striving towards inspiring high standards of excellence and relevance in academic and industrial research and promoting the service of chemistry to society and to global issues are the visions that shape IUPAC's activities toward the twenty-first century.

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International Union of Toxicology

Paolo Preziosi

The International Union of Toxicology (IUTOX) is an international body consisting of 44 national/supernational toxicological societies from all parts of the world. Its creation reflects the growing awareness of the wide range of toxic threats throughout the world and the need for concerted and coordinated efforts to resolve these problems. This awareness led initially to the creation of special toxicological sections or groups within international or national associations of scientists from various fields. Although the specific toxicological concerns of these groups were different, the basic principles and approaches used to investigate them are strikingly similar, and independent bodies dedicated specifically to the discipline of toxicology were soon established in many countries. The IUTOX was founded on July 6, 1980, in Brussels, Belgium, during the Second International Congress of Toxicology. Its purposes are to promote a full and uniform development of toxicology within and across various scientific disciplines and to provide an international platform for scientists active in toxicological research.

As a result of a recent (2001) change in its By-laws, the IUTOX is now directed by an 11-member executive committee composed of the President Elect, the Vice-President, the Secretary General, the treasurer, the Past President, and five directors. A new executive committee, reflecting the international membership of the Union, is elected every 3 years by a general assembly of the member societies. Each member society is allowed one vote for every 200 registered members, up to a maximum of five votes for those with more than 800 individual members. Standing committees include the Nominating Committee (standing Committee of the Council) and the Membership Committee (standing Committee of the Executive Committee).

The IUTOX has been an associate member of the International Council of Scientific Unions since 1987 and became a full member in 1996. In 1993, it was recognized as an official nongovernmental organization of the World Health Organization (WHO) and, as such, it contributes to the activities of the WHO's International Programme on Chemical Safety (WHO-IPCS) and sends delegates to the recently established International Forum on Chemical Safety.

Every 3 years, the IUTOX organizes an International Congress of Toxicology (ICT) lasting 4 or 5 days with an average attendance of from 800 to 1500 persons. During these congresses, joint symposia on specific questions are organized with other international scientific bodies, such as the International Union of Pharmacology, the International Union of Pure and Applied Chemistry (IUPAC), the WHO-IPCS, the International Agency for Research on Cancer, and the International Council for Laboratory Animal Sciences. Past congresses have been held in Brussels (1980), San Diego (1983), Tokyo (1986), Brighton (1989), Rome (1992), Seattle (1995), Paris (1998), and Brisbane (2001).

The 10th ICT was held in Tampere, Finland, in 2004 and the 11th ICT in Montreal, Canada, has been scheduled for 2007. A Congress of Toxicology in Developing Countries was held in 2003 in China and another is scheduled in 2006 in Croatia.

The IUTOX also sponsors numerous other international meetings (e.g., Joint Meeting of the Italian and French Societies of Toxicology in 1991, the Convention of the International Neurotoxicology Association in 1991, and the Second Nordic Toxicology Congress in 1992). Activities organized in developing countries include the Workshop on Prevention and Management of Poisonings in South America (Montevideo, Uruguay) in 1991, the Workshop on Development of Poison Control Programmes in South America (Montevideo, Uruguay) in 1992 (both were organized in conjunction with the WHO-IPCS), the Seminar and Training Course on Diagnosis, Management and Prevention of Poisoning for Francophone, Sub-Saharan Countries (Dakar, Senegal) in 1995, and the Second and Third Congresses of Toxicology in Developing Countries, held, respectively, in New Delhi, India, in 1991 and Cairo, Egypt, in 1995. A symposium on Inhalation Toxicology in Pilsen was co-organized by the IUTOX and EUROTOX as a satellite meeting to the 1995 EUROTOX Congress held in Prague.

One of the Union's most successful activities has been the development of a program for continuing education (CE) risk assessment. As part of this program, the IUTOX organizes Risk Assessment Summer Schools (RASS), which have been held every 2 years since 1984. The ninth RASS was held in Gozo (Malta) in 2002. These 1 week courses provide training for young toxicologists in strategies and skills associated with chemical risk assessment. The RASS project will be converted into a long-range education program of the IUTOX. IUTOX arranged for two CE courses in 2000, one in Poland and one in Hungary. A number of courses and lectures were held

in recent years including two CE courses in Mexico (1999), a workshop lecture in Egypt (2000), a congress lecture in Brazil (2000), workshop lectures in South Africa (2001), CE courses in Venezuela (2001) and Slovenia (2002), CE course and lectures in China (2001), and a workshop in Chile (2002).

As a member of the International Council for Science (ICSU), the IUTOX organized workshops on environmental estrogens as part of a program to expand upon information contained in the Book *Natural and Anthropogenic Environmental Oestrogens: The Scientific Basis for Risk Assessment*. These workshops were held in Canberra, Australia (1998); New Orleans, USA (1999); Keele, UK (1999); Oslo, Norway (1999); Antalya, Turkey (1999); and Seoul, Korea (2000). Their aim was to increase participants' awareness of controversial issues related to the impact of environmental estrogens on human health and the environment.

In 2001, thanks to a grant received from the ICSU, the IUTOX and the International Union of Nutritional Sciences published a monograph on genetically modified (GM) foods. The objective was to provide the global community with an informative, nonbiased review of the benefits and risks associated with GM foods. To prepare the monograph, a planning workshop was organized with representatives of five other International Unions, several ICSU committees, and various experts on issues surrounding the use of GM foods in developing countries and countries in transition.

IUTOX Member Societies

- *Founding members*: European Society of Toxicology, the British Toxicological Society, the Finnish Society of Toxicology, the French Society of Toxicology, the Italian Society of Toxicology, the Japanese Society of Toxicology, the Netherlands Society of Toxicology, the Norwegian Society of Pharmacology and Toxicology, the Society of Toxicology of Canada, the Indian Society of Toxicology, the Society of Toxicology, USA, the Swedish Society of Toxicology, the Swiss Society of Pharmacology and Toxicology, the Yugoslavian Society of Toxicology (now the Croatian and Slovenian Societies of Toxicology), and the Society of Toxicology of the Democratic Republic of Germany (now the German Society for Experimental and Clinical Pharmacology).
- *Other members*: The American Academy of Clinical Toxicology, the American College of Toxicology, the Argentine Toxicological Association, Asiatox, the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, the Brazilian Society of Toxicology, the Chinese Society of Toxicology, the Croatian Toxicology Society, the Danish Society of Pharmacology and Toxicology, the Egyptian Society of Toxicology, the European Association of Poison Centres and Clinical Toxicologists, EUROTOX, the French Society of Clinical Toxicology, the Hellenic Society of Toxicology, the International Neurotoxicology Association, the Hungarian Pharmacological Society, the Irish Society of Toxicology, the Israeli Society of Toxicology, the Japanese Society for Clinical Toxicology, the Korean Society of Toxicology, the Latin American Association of Toxicology, the Latvian Society of Toxicology, The Mexican Society of Toxicology, the Polish Society of Toxicology, the Russian Society of Toxicology, the Slovenian Society of Toxicology, the Toxicological Society of Thailand, the Spanish Association of Toxicology, the Spanish Society of Toxicology, the Toxicology Society of Taiwan, the Turkish Society of Toxicology, and the Union of Hungarian Toxicologists

The IUTOX has been particularly successful in developing new societies in areas of the world where toxicology has been underrepresented.

It has also played a significant role in the development of the International Assembly for the Recognition of Toxicologists (IART). The IART is a forum whose aims are (1) the development of criteria for recognizing qualified experts in toxicology; (2) assisting toxicology organizations in establishing and implementing these criteria; and (3) promotion of efforts to identify toxicological education and training needs.

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Inter-Organization Programme for the Sound Management of Chemicals

Pertti J Hakkinen

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by a Memorandum of Understanding among International Labour Organization (ILO), Organisation for Economic Co-operation and Development (OECD), Food and Agriculture Organization of the United Nations (FAO), United Nations Environment Programme (UNEP), United Nations Industrial Development Organization (UNIDO), and World Health Organization (WHO). This followed recommendations made by the 1992 UN Conference on Environment and Development in Rio de Janeiro, and in particular its Chapter 19, Agenda 21.

The United Nations Institute for Training and Research (UNITAR) joined the IOMC in 1997. The IOMC vision is to be the preeminent mechanism for initiating, facilitating, and coordinating international action to achieve the World Summit on Sustainable Development (WSSD) 2020 goal for round management of chemicals.

The ILO, OECD, FAO, UNEP, UNIDO, WHO, and UNITAR are the seven Participating Organizations (POs) which contribute to the work of the IOMC as the Inter-Organization Coordinating Committee (IOCC). The areas in which coordination is sought include the international assessment of chemical risks; harmonization of classification and labeling of chemicals, information exchange on chemicals and chemical risks; establishment of risk reduction programs; strengthening of national capabilities and capacities for management of chemicals, prevention of illegal international traffic in toxic and dangerous products; and other areas as agreed by all POs. Planning, programming, implementation, and monitoring of activities undertaken jointly or individually by the POs is carried out by the IOCC. This ensures full consultation among all those involved, with the aim to ensure effective implementation without duplication. The WHO is the administering organization for the IOMC and provides secretariat services to the IOCC.

Technical coordinating groups: Specific coordinating groups have been used to progress IOMC activities, including Harmonization of Chemical Classification Systems, Persistent Organic Pollutants (POP), Stocks of Obsolete Pesticides and Industrial Chemicals, Assessment of Existing Industrial Chemicals and Pollutants, Pollutant Release and Transfer Registers (PRTR), and Chemical Accident Prevention, Preparedness and Response. These groups provide a means for all interested bodies working in the respective areas to consult with each other on program plans and activities, and to discuss ways and means of ensuring that the activities are mutually supportive. Membership in the Coordinating Groups is not limited to intergovernmental bodies, and may involve nongovernmental organizations and appropriate national institutions. The IOMC website provides the terms of reference, memberships, and meeting reports for the Coordinating Groups.

Inventory of activities: The IOCC has established an Inventory of Activities database hosted by OECD. This provides a calender of events, and details of relevant activities of each PO, including a short description of the activities undertaken, with an indication of the relevant program areas of Chapter 19, Agenda 2, to which the work contributes. The title of each activity, the responsible IOMC PO for implementation, any partners involved, the objectives of the work, outputs, geographical coverage, and relevant contact point are also provided.

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National Center for Environmental Health

Sachin S Devi and Harihara M Mehendale

Agency for Toxic Substances and Disease Registry (ATSDR), located in Atlanta, Georgia, is a federal agency created in 1980 by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), or what is more commonly known as Superfund legislation. Congress enacted Superfund as part of its response to two highly publicized and catastrophic events: discovery of the Love Canal hazardous waste site in Niagara Falls, New York, and an industrial fire in Elizabethtown, New Jersey, which set off the release of highly toxic fumes into the air in a densely populated area. Congress also created ATSDR to implement the health-related sections of laws that protect the public from hazardous wastes and environmental spills of hazardous substances.

In 1983, the secretary of the Department of Health and Human Services by administrative order established ATSDR as a separate agency of the Public Health Service. In 1984, amendments to the Resource Conservation and Recovery Act (RCRA) authorized ATSDR to conduct public health assessments at RCRA sites when requested by the US Environmental Protection Agency (EPA), states, or individuals, and to help EPA decide which substances should be regulated and at what levels those substances threaten human health.

In June 1983, ATSDR was formally organized to begin in concert with US EPA, the Centers for Disease Control (now the Centers for Disease Control and Prevention), and the National Institute of Environmental Health Sciences to address CERCLA, one of the most challenging and innovative environmental laws relating to public health.

Following the reauthorization of Superfund in 1986 under the Superfund Amendments and Reauthorization Act (SARA), the agency received major new mandates. SARA broadened ATSDR's responsibilities in the areas of public health assessments, establishment and maintenance of toxicological databases information dissemination, and medical education; new groups within ATSDR were organized to carry out the new tasks. By August 1989, the agency had assumed its current structure.

In October of 2003, The ASTSDR was consolidated with the National Center for Environmental Health (NCEH) of the CDC to form the NCEH/ATSDR.

Agency Mission

The mission of ATSDR is to prevent exposure and adverse human health effects and diminished quality of life associated with exposure to hazardous substances from waste sites, unplanned releases, and other sources of pollution in the environment. ATSDR works closely with state, local, and other federal agencies to reduce or eliminate illness, disability, and death that result from exposure of the public and workers to toxic substances at waste disposal and spill sites.

As the lead agency within the Public Health Service responsible for implementing the health-related provisions of CERCLA, ATSDR was charged with assessing the presence and nature of health hazards at specific Superfund sites, helping to prevent or reduce further exposure and the illnesses that result and expanding what is known about the health effects of exposure to hazardous substances. The newly consolidated NCEH/ATSDR has as its mission to serve the public by using the best science, taking responsive public health actions and providing trusted health information to prevent harmful exposures and disease related to toxic substances.

Range of Agency Activities

The following is a summary of the activities assigned to ATSDR in 1980 under the original Superfund statute:

- Determine the extent of danger to public health from a release or threatened release of a hazardous substance. (This mandate covers the range of public health assessment and other support activities provided to US EPA, states, and other federal agencies at emergency, immediate-removal, and remedial Superfund sites.)
- Conduct periodic surveys and screening programs to determine the relationships between exposure to hazardous substances and illness. (This mandate includes *in vivo* and *in vitro* toxicologic testing, human epidemiologic studies, and establishment of surveillance systems.)
- Establish and maintain a registry of serious diseases and illnesses and registries of all persons environmentally exposed to hazardous substances whenever inclusion of such persons in registries would be scientifically appropriate or valuable for long-term follow-up or specific scientific studies.
- Establish and maintain a comprehensive and publicly accessible inventory of literature on the health effects of hazardous substances.

- When public health emergencies are caused or are believed to be caused by exposure to hazardous substances, assist, consult, and coordinate with private or public health care providers in providing medical care and testing exposed individuals, including collecting and analyzing laboratory specimens as may be indicated by specific exposures.
- Establish and maintain a complete list of areas closed to the public or otherwise restricted in use because of hazardous substance contamination.

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National Center for Toxicological Research

Ankur V Dnyanmote and Harihara M Mehendale

The National Center for Toxicological Research (NCTR), a component of the Jefferson Laboratories of the Food and Drug Administration (FDA), is located in Jefferson, Arkansas. Its mission is to conduct innovative peer-reviewed scientific research focused on FDA regulatory needs. Research findings provide the basis for FDA to make sound science-based risk management decisions that promote the health of the American people.

The NCTR conducts a variety of research, including basic (scientific discovery and knowledge driven), translational (interpretation and/or revision of basic scientific concepts), and applied (developing and applying standards). This research is aimed at studying the biological effects of potentially toxic chemicals or microorganisms, defining the complex mechanisms that govern their toxicity, understanding critical biological events in the expression of toxicity, and developing methods to improve assessment of human exposure, susceptibility, and risk. Customized bioassessment of chemicals of vital interest to the FDA involves the coordination of expertise in the areas of biochemical and molecular markers of carcinogenicity, quantitative risk assessment, transgenics (mimicking responses in animal modes by insertion of human genes into a test animal or tissue culture), neurotoxicology, microbiology, chemistry, and genetic or reproductive/developmental toxicology.

Using its existing strengths in methods development, statistics, analytical chemistry, and spectroscopy, NCTR is developing and standardizing new technologies, such as genomics, proteomics, hepatotoxicology, metabonomics, phototoxicology, and nanotechnology. NCTR's Center for Toxicoinformatics uses software systems and analysis capability to manage and integrate the data from these new technologies with traditional toxicological data.

Perhaps of greater importance to its research accomplishments is the benefit gained by sharing knowledge through collaborations with scientific staff. A major emphasis for NCTR is to conduct research on compounds nominated by the FDA for evaluation by the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP). In addition to these collaborations with the other FDA centers and NTP, NCTR partners with 10 other government agencies, over 25 universities and medical centers (including local, national, and foreign), other research facilities around the world, and several industries to investigate predictive toxicology issues.

The NCTR organizational structure consists of the Office of the Director, encompassing staff offices to implement safety and security functions; the Office of Planning and Resource Management, which coordinates strategic and logistical planning, financial management, as well as administrative services and management; and the Office of Research, which includes eight divisions to conduct mission research.

NCTR employs ~230 full-time federal employees, supplemented by ~250 contractor employees providing animal diet, maintenance, and pathology; computer and information management services; facilities maintenance; onsite occupational health care; and administrative services (e.g., supplies receiving and warehousing, mail delivery, and document reproduction).

NCTR aggressively provides scientific training opportunities in its state-of-the-art research facilities to help increase the limited pool of qualified scientists. NCTR provides coordination and support of an

interdisciplinary toxicological program and regulatory science curriculum at two Arkansas universities and maintains a commitment to science education initiatives, which provide a 'pipeline' from high school to postgraduate training. In 2003, 79 people, 41 of whom represented 21 foreign countries, participated in independent or collaborative research supported by these initiatives.

The NCTR is an FDA-owned facility that houses more than 30 buildings containing over a million square feet of floor space on 496 acres and has $40 million worth of advanced research equipment. Laboratory space consists of 132 general or special purpose research labs, 82 breeding or conventional animal rooms, a nonhuman primate research facility, and 23 specialized laboratories for pathological processing and evaluation. A BioSafety Level 3 laboratory contains seven suites to support animal and microbial bioterrorism research. An onsite housing unit exists to support visiting scientists.

In calendar year 2003, NCTR staff participated in more than 310 scientific protocols and published 234 manuscripts, book chapters, and books (plus abstracts) and 34 final technical reports.

NCTR provides an online scientific journal entitled *Regulatory Research Perspectives*, which highlights some of the latest research topics in the scientific regulatory arena. Another online publication, *NCTR Quarter Page*, highlights special events, Center research activities and staff, and publications in scientific journals. These publications, as well as additional information about the NCTR, are available from the FDA website.

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National Institute for Occupational Safety and Health

Ankur V Dnyanmote and Harihara M Mehendale

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The National Institute for Occupational Safety and Health (NIOSH) is the federal agency responsible for conducting research and making recommendations for the prevention of work-related injury and illness. NIOSH is part of the Centers for Disease Control and Prevention (CDC) in the US Department of Health and Human Services. NIOSH provides national and world leadership to prevent work-related illness, injury, disability, and death by gathering information, conducting scientific research, and translating the knowledge gained into products and services.

The objectives of NIOSH include:

- Conducting research to reduce work-related illnesses and injuries.
- Promoting safe and healthy workplaces through interventions, recommendations, and capacity building.
- Enhancing global workplace safety and health through international collaborations.

NIOSH scientists work in multidisciplinary teams and carry out a focused program of intramural and extramural research to prevent or reduce work-related injury and illness. In 1996, NIOSH and over 500 partners established the National Occupational Research Agenda (NORA), a framework to guide the efforts of the occupational safety and health community in 21 priority research areas. NORA encompasses research areas such as traumatic injury, asthma and chronic obstructive pulmonary disease, hearing loss, and control technologies. These priority areas were identified through extensive input from NIOSH's federal and nonfederal partners. Since 1996, NIOSH has aligned its intramural and extramural research to increase its investment in NORA priority areas.

The Occupational Safety and Health Act of 1970 created both NIOSH and the Occupational Safety and Health Administration (OSHA). OSHA is in the US Department of Labor and is responsible for developing and enforcing workplace safety and health regulations. NIOSH is in the US Department of Health and Human Services and is an agency established to help assure safe and healthful working conditions for working men and women by providing research, information, education, and training in the field of occupational safety and health.

Toll-Free Technical Information Service

The NIOSH 800 number (+1-800-356-4674) is a toll-free technical information service that provides convenient public access to NIOSH and its information resources. The service is available to anyone in the continental United States, Alaska, Hawaii, Puerto Rico, or the Virgin Islands.

Callers may request information about NIOSH activities, order NIOSH publications, or request information about any aspect of occupational safety and health. However, this toll-free number is NOT a hotline for medical emergencies.

The 800-number combines an automated voicemail system with direct access to NIOSH technical information staff and the NIOSH Publications Office. The automated system operates 24 h a day. It provides recorded information on a variety of topics, including directions for ordering NIOSH publications. In addition, callers may speak directly with a technical information specialist or to a publications representative from 9:00 a.m. until 4:00 p.m. (EST). All information is provided free of charge within 10 working days.

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Relevant Website

http://www.cdc.gov – The NIOSH home page is located on this URL. The home page provides access to information about NIOSH and related activities, including NIOSH documents, databases, and other resources. The eNews weblink is a monthly electronic update that highlights the latest news at NIOSH.

National Institute of Environmental Health Sciences

Harihara M Mehendale

The National Institute of Environmental Health Sciences (NIEHS) is located in Research Triangle Park, North Carolina, and is part of the federal National Institutes of Health. Since its creation in 1966, the NIEHS has been the primary source of federal efforts for studying how environmental factors affect human health. The mission of the NIEHS is to define how exposures to environmental agents affect our health, how individuals differ in their susceptibility to these effects, and how these susceptibilities affect over time. Diseases and dysfunctions with an environmental component include cancer, birth defects, infertility, neurological impairments, immune disorders, and lung dysfunctions. Because of the broad scope of its mission, NIEHS research relies on essentially every discipline in the biological, chemical, and physical sciences. It maintains a multidisciplinary intramural research program at its Research Triangle Park facility and supports university-based research and training through a variety of grant mechanisms. Through its participation in the National Toxicology Program, NIEHS has made significant contributions in providing state-of-the-art toxicological characterization for a host of environmentally and commercially important agents. Because of the high quality of these studies, they serve to guide risk assessments both in the United States and abroad.

Basic science supported by NIEHS attempts to identify the environmental components of human disease and to understand the basic molecular mechanisms leading to these disease states. Environmentally related diseases of special interest to the institute are those dealing with women's health, children's health, minorities' health, aging, respiratory disorders, neurological disorders, immune system disruption, and cancer. Cellular processes that hold promise for explaining environmentally related diseases include regulatory genes that serve as targets for environmentally induced effects, cellular communication pathways, the integration of biological processes across organ systems, and the genetic basis of individual susceptibility to environmental agents and the diseases and disorders they cause. The NIEHS is also expanding its clinical research programs to enable it to more readily translate laboratory-based findings into human therapies.

Prevention and intervention efforts are a major focus of NIEHS activities. These efforts include hazard identification and characterization, both through

traditional animal testing and epidemiological studies and through incorporation of mechanistic considerations to arrive at new insights into the molecular basis of toxic effects. The improved understanding of the molecular foundation of environmentally associated effects will enable the institute to strengthen the validity of risk assessment schemes as a means of deciding regulatory policy. An improved understanding of the molecular basis of toxicant action could also lead to innovative molecular prevention and intervention therapies to circumvent clinical manifestations of environmentally caused diseases.

Finally, the NIEHS has devised a communication strategy, which ensures that the findings generated by its basic and applied research reach the groups that need the information. These groups include the lay public and the institute's partners in research, governmental agencies, advocacy groups, and the international community.

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National Institutes of Health

Ankur V Dnyanmote and Harihara M Mehendale

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The National Institutes of Health (NIH) is the principal biomedical research agency of the US Federal Government. Its mission is to employ science in the pursuit of knowledge to improve human health conditions. To accomplish this goal, the Institute seeks to expand fundamental knowledge about the nature and behavior of living systems, to apply that knowledge to extend the health of human lives, and to reduce the burdens resulting from disease and disability. In the quest of this mission, NIH supports biomedical and behavioral research domestically and abroad, conducts research in its own laboratories and clinics, trains promising young researchers, and promotes acquiring and distributing medical knowledge. Focal points have been established to assist in developing NIH-wide goals for health research and training programs related to women and minorities, coordinating program direction, and ensuring that research pertaining to women's and minority health is identified and addressed through research activities conducted and supported by NIH. Research activities conducted by NIH will determine much of the quality of health care for the future and reinforce the quality of health care currently available.

The NIH comprises the Office of the Director and 27 Institutes and Centers. The Office of the Director is responsible for setting policy for NIH and for planning, managing, and coordinating the programs and activities of all NIH components.

The major components of the NIH are

1. National Cancer Institute;
2. National Eye Institute;
3. National Heart, Lung, and Blood Institute;
4. National Human Genome Research Institute;
5. National Institute on Aging;
6. National Institute on Alcohol Abuse and Alcoholism;
7. National Institute of Allergy and Infectious Diseases;
8. National Institute of Arthritis and Musculoskeletal and Skin Diseases;
9. National Institute of Biomedical Imaging and Bioengineering;
10. National Institute of Child Health and Human Development;
11. National Institute on Deafness and Other Communication Disorders;
12. National Institute of Dental and Craniofacial Research;
13. National Institute of Diabetes and Digestive and Kidney Diseases;
14. National Institute of Diabetes and Digestive and Kidney Diseases;
15. National Institute of Environmental Health Sciences;
16. National Institute of General Medical Sciences;
17. National Institute of Mental Health;
18. National Institute of Neurological Disorders and Stroke;
19. National Institute of Nursing Research;
20. National Library of Medicine;
21. Center for Information Technology;
22. Center for Scientific Review;
23. John E. Fogarty International Center;

24. National Center for Complementary and Alternative Medicine;
25. National Center on Minority Health and Health Disparities;
26. National Center for Research Resources; and
27. Warren Grant Magnuson Clinical Center.

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National Library of Medicine/TEHIP

Carlo Nuss

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The National Library of Medicine (NLM), part of the National Institutes of Health (NIH) and located in Bethesda, Maryland, is by far the largest medical library in the world. It provides toxicological information through its substantial holdings of books and journals and, more specifically, through its Toxicology and Environmental Health Information Program (TEHIP). TEHIP, which resides in NLM's Division of Specialized Information Services (SIS) and whose origin dates back to 1967, offers a broad array of web-based, freely available databases and other electronic information resources in toxicology and environmental health; furthermore, it answers queries, sponsors publications, and responds to the information needs of other federal agencies. TEHIP is considered one of the world's major providers of toxicology and environmental health information resources.

TEHIP's primary information resource repository is the TOXNET system of databases. TOXNET comprises databases of summary toxicological information, technical literature, references, chemical nomenclature, and toxic releases. It also provides links to additional sources of information on toxicology, environmental health, and hazardous chemicals. The contents of the TOXNET databases are derived from federal agencies such as the US Environmental Protection Agency, the National Cancer Institute, and NLM itself.

The following paragraphs will summarize TEHIP's free, web-based toxicological information resources. The individual databases of the TOXNET system will be described first. Descriptions of miscellaneous additional electronic resources available through TEHIP will follow.

The TOXNET databases contain various types of information. One group of these databases, sometimes referred to as the 'factual databanks', contain toxicologically oriented records organized by chemical. They are the Chemical Carcinogenesis Research Information System (CCRIS), GENE-TOX, the Hazardous Substances Data Bank (HSDB), the Integrated Risk Information System (IRIS), and the International Toxicity Estimates for Risk (ITER). Following their descriptions, information is given about ChemIDplus (an online chemical dictionary), the Toxics Release Inventory (TRI), TOXNET's bibliographic databases, and other resources.

Factual Databanks

CCRIS

CCRIS is a scientifically evaluated and fully referenced data bank, developed and maintained by the National Cancer Institute (NCI). It contains over 8000 chemical records with carcinogenicity, mutagenicity, tumor promotion, and tumor inhibition test results. Data are derived from studies cited in primary journals, current awareness tools, NCI reports, and other special sources. Test results have been reviewed by experts in carcinogenesis and mutagenesis.

GENE-TOX

GENE-TOX is a toxicology data file created by the US Environmental Protection Agency (EPA) and contains genetic toxicology (mutagenicity) test data, resulting from expert peer review of the open scientific literature, on over 3000 chemicals. The GENE-TOX program was established to select assay systems for evaluation, review data in the scientific literature, and recommend proper testing protocols and evaluation procedures for these systems.

HSDB

HSDB is a toxicology data file that focuses on the toxicology of potentially hazardous chemicals. It covers information on human exposure, industrial hygiene, emergency handling procedures, environmental fate, regulatory requirements, and related

areas. All data are referenced and derived from a core set of books, government documents, technical reports, and selected primary journal literature. HSDB is peer reviewed by the Scientific Review Panel (SRP), a committee of experts in the major subject areas within the data bank's scope. HSDB contains over 4700 extensive and highly structured individual chemical records.

IRIS

IRIS is a toxicology data file that contains data in support of human health risk assessment. It is compiled by the US EPA and contains over 500 chemical records. IRIS data, focusing on hazard identification and dose–response assessment, are reviewed by work groups of EPA scientists and represents EPA consensus. Among the key data provided in IRIS are EPA carcinogen classifications, unit risks, slope factors, oral reference doses, and inhalation reference concentrations.

ITER

ITER is a toxicology data file that contains data in support of human health risk assessments. It is compiled by Toxicology Excellence for Risk Assessment (TERA) and contains over 600 chemical records with key data from the Agency for Toxic Substances and Disease Registry (ATSDR), Health Canada, the Dutch National Institute of Public Health and the Environment (RIVM), the US EPA, and independent parties whose risk values have undergone peer review. ITER provides a comparison of international risk assessment information in a side-by-side format and explains differences in risk values derived by different organizations. ITER data, focusing on hazard identification and dose–response assessment, are extracted from each agency's assessment and contain links to the source documentation.

Chemical Dictionary

ChemID*plus*

ChemID*plus* is a search system that provides access to structure and nomenclature authority files used for the identification of chemical substances cited in NLM databases. ChemID*plus* also provides structure searching and direct links to many biomedical resources at NLM and on the Internet for chemicals of interest. The database contains over 368 000 chemical records, of which over 206 000 include chemical structures, and is searchable by Name, Synonym, CAS Registry Number, Molecular Formula, Classification Code, Locator Code, and Structure.

Toxics Release Inventory

TRI

TRI is an annually compiled series of databases spanning the reporting years 1987–2001, and which contains information on the annual estimated releases of toxic chemicals to the environment. It is based upon data collected by the US EPA. Mandated by the Superfund legislation, TRI's data cover air, water, land, and underground injection releases, as well as transfers to waste sites, and waste treatment methods and efficiency, as reported by industrial facilities in the United States. TRI also includes data related to source reduction and recycling.

Bibliographic Databases

ALTBIB

Technically not part of TOXNET, but supplemental to it, ALTBIB is a bibliographic database on alternatives to the use of live vertebrates in biomedical research and testing. Its intent is to assist in identifying methods and procedures helpful in supporting the development, testing, application, and validation of alternatives to the use of vertebrates in biomedical research and toxicology testing. This bibliographic database, covering the literature for the time period 1992–2001, is produced from MEDLARS database searches, performed and analyzed by TEHIP subject experts.

DART (Developmental and Reproductive Toxicology)

DART is a bibliographic database that covers teratology and other aspects of developmental and reproductive toxicology. It contains over 100 000 references to literature published since 1965. DART/ETIC is funded by the US EPA, the National Institute of Environmental Health Sciences, the National Center for Toxicological Research of the Food and Drug Administration, and NLM.

TOXLINE

TOXLINE is a bibliographic database covering the multidisciplinary literature of toxicology. Its records provide bibliographic information on the biochemical, pharmacological, physiological, and toxicological effects of drugs and other chemicals. TOXLINE contains over 3 million bibliographic citations, most with abstracts and/or indexing terms and CAS Registry Numbers. TOXLINE references are drawn from various sources and are grouped into two parts: TOXLINE Core and TOXLINE Special. TOXLINE Core covers much of the standard journal literature

in toxicology. A search link to TOXLINE Core is available from the TOXLINE interface. It is also directly available via the PubMed system by selecting 'toxicology' as a subset limit. TOXLINE Special complements TOXLINE Core with references from an assortment of more specialized journals, technical reports, and other sources.

Health and Biomedicine Directory

DIRLINE

DIRLINE is a database containing location and descriptive information about a wide variety of information resources including organizations, research resources, projects, and databases concerned with health and biomedicine. This information may not be readily available in bibliographic databases. DIRLINE contains over 17 000 records and focuses primarily on health and biomedicine, although it also provides limited coverage of some other special interests. DIRLINE includes records pertinent to the areas of toxicology, chemical safety, and environmental health. Each record may contain information on the publications, holdings, and services provided.

Additional Specialized Resources

Haz-Map

Haz-Map is an occupational health database designed for health and safety professionals and for consumers seeking information about the health effects of exposure to chemicals at work. Haz-Map links jobs and hazardous tasks with occupational diseases and their symptoms. Chemicals and biological agents in Haz-Map are linked to industrial processes and other activities such as hobbies. Occupational diseases and their symptoms are associated with hazardous job tasks and possible exposure to hazardous agents. Information from textbooks, journal articles, and electronic databases such as HSDB (described above) is classified and summarized to create this database.

Household Products Database

The Household Products Database provides information on the potential health effects of chemicals contained in over 5000 common household products. This database allows scientists and consumers to find out about ingredients in brand-name products. It is designed to help answer the following questions: What chemicals are contained in specific brands and in what percentage? Which products contain specific chemicals? Who manufactures a specific brand? How can the manufacturer be contacted? What are the potential adverse health effects (acute and chronic) of the ingredients in a specific brand? What other information is available about such chemicals in toxicology-related NLM databases? The database allows browsing of product categories and searching of products by type, manufacturer, product ingredient/chemical name, and health effects. The record for each product shows the ingredients as reported in the manufacturer's Material Safety Data Sheet (MSDS) and includes other information such as handling, disposal, and health effects.

Internet Resources in Toxicology and Environmental Health

The Internet Resources section of the TEHIP Website covers topics such as Arsenic and Human Health, Biological Warfare, Chemical Warfare, Children's Environmental Health, Environmental Justice, September 11th World Trade Center Disaster Lingering Airborne Hazards, and Pesticides Used for West Nile Virus Control. For each topic, TEHIP provides numerous links to a variety of related Websites and other electronic resources.

NLM-Tox-Enviro-Health-L Listserv

The NLM-Tox-Enviro-Health-L listserv is an email announcement list whose purpose is to broadcast updates on SIS's resources, services, and outreach in toxicology and environmental health. The NLM-Tox-Enviro-Health-L Archives allow users to search list postings and to modify subscription options. Anyone interested in subscribing to this listserv should send an email to listserv@list.nih.gov.

Review of PDA Applications in Toxicology and Environmental Health

The Review of PDA Applications in Toxicology and Environmental Health was undertaken by TEHIP staff in light of the increasing use of personal digital assistants (PDAs) and specialized PDA software applications in the fields of toxicology and environmental health. The purpose of this Web resource is to make available descriptive reviews of the main technical and content features of selected PDA software applications. Individual reports in the review series are based on free, downloadable demo versions of the software and cover the following topics: General Information, Intended Users, Authorship/Data Source, Contents, Navigation, Requirements, Application Type/Price, Availability, Useful Web Links, and Updates.

Toxicology Tutorials

The Toxicology Tutorials are written at the introductory college student level and are intended to provide a basic understanding of toxicology as an aid for users of the toxicology literature, such as that found in the TOXNET databases described above. Toxicology Tutor I is the first in a set of three tutorials and covers basic principles of toxicology. Toxicology Tutor II covers toxicokinetics, while Toxicology Tutor III deals with the basic toxic mechanisms that operate at the cellular level, including those that interfere with normal biochemical functions. The Toxicology Tutorials are scheduled to be updated and expanded, and will be positioned as a highlight within a new page more broadly concerned with toxicology education.

TOXMAP

TOXMAP is a Web resource that uses maps of the United States to show the amount and location of toxic chemicals released into the environment. Data are derived from the TRI database (described above), which provides information on toxic releases into the environment as reported by US industry. TOXMAP helps users create nationwide or local area maps showing where chemicals are released into the air, water, and ground. It also identifies the releasing facilities, color-codes release amounts for a single year, and provides multi-year chemical release trends, starting with 1987. Users can search the system by chemical name, chemical name fragment, and/or location (such as city, state, or zip code). TOXMAP also overlays map data such as US Census population data.

Tox Town

Tox Town, an interactive guide to commonly encountered toxic substances, is designed to provide the following: information on common locations where one might find toxic chemicals; nontechnical descriptions of chemicals; links to selected, authoritative information about chemicals on the Internet; information concerning environmental impacts on human health; and Internet resources on environmental health topics. Tox Town uses color, graphics, sounds, and animation to add interest to learning about connections between chemicals, the environment, and public health. Tox Town's target audience comprises students above elementary-school level, educators, and the general public. Tox Town also provides some resources in Spanish and has a text version. Tox Town currently offers a 'Town' scene and a 'City' scene; a 'US–Mexico Border Community' scene is under development.

WISER

Wireless Information System for Emergency Responders (WISER) is a system designed to assist first responders in hazardous material incidents. The application provides a wide range of information on hazardous substances, including substance identification support, physical characteristics, human health information, and containment and suppression guidance. WISER features mobile support, comprehensive decision support (including assistance in identifying unknown substances and guidance on immediate actions required to save lives and protect the environment), access to 390 substances derived from HSDB (described above), rapid access to crucial information about them, and an intuitive, simple, and logical user interface.

TEHIP's New Initiatives

Drugs and Lactation Database

The Drugs and Lactation database will be a searchable database of records on drugs and their use during lactation. This database, which is intended to support medical decision-making by healthcare professionals, is envisioned as a comprehensive, evidence-based resource covering possible adverse effects of prescription and nonprescription drugs and diagnostic agents in breast-feeding infants. The Drugs and Lactation database will complement the suite of TOXNET databases and will be linked, where appropriate, to other TOXNET and NLM database records.

ToxSeek

ToxSeek is an experimental toxicology and environmental health meta-search engine that allows users to search diverse Web-accessible databases simultaneously. ToxSeek automatically identifies key concepts in search results and uses these 'Related Concepts' and other information to merge, rank, and intelligently cluster the items retrieved from heterogeneous information sources. ToxSeek also supports a focused drill-down in its 'Concept Clusters', as well as dynamic query modification and multiple spell-checkers for general English, medical terminology, and chemical names.

World Library of Toxicology, Chemical Safety, and Environmental Health

The World Library is envisioned as a Web portal to international information resources in the areas of toxicology, chemical safety, and environmental health. It will be hosted by TEHIP and maintained

in collaboration with national representatives (in-country experts who have volunteered their time and expertise). The World Library will link to credible sources of scientific and consumer information on chemical, biological, and physical (including radiation) hazards, and their effects on human, animal, and ecosystem health. The audience will include many sectors of the international community – research, academic, government, corporate, and non-profit.

More Toxicology Information from other Divisions of the National Library of Medicine

Finally, in addition to the many information resources provided by TEHIP, there are a few relevant toxicological information resources provided by other entities within NLM. One such information resource is PubMed, the world's largest medical database, which contains a great deal of toxicological information accessible via its 'toxicology' subset. As mentioned earlier, this information is equivalent to that accessible via a TOXLINE Core search. MEDLINEplus provides a wide range of consumer health information and thus represents a significant source of toxicological and environmental health information of interest to the general public. ClinicalTrials.gov (http://www.clinicaltrials.gov) provides regularly updated information about federally and privately supported clinical research in human volunteers. More specifically, ClinicalTrials.gov provides information about a trial's purpose, who may participate, locations, and phone numbers for further details. This database, which allows its users to access significant toxicological information, was developed in collaboration with the US Food and Drug Administration.

Contact Details

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Further Reading

Wexler P (2004) The US National Library of Medicine's Toxicology and Environmental Health Information Program. *Toxicology* 198(1–3).

Relevant Website

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine.

National Toxicology Program

Harihara M Mehendale

The National Toxicology Program (NTP), an interagency program, was established in 1978 as a cooperative effort within the US Public Health Service of the Department of Human Services. The four primary objectives of the NTP are to (1) coordinate toxicology research and testing activities within the department, (2) provide information about potentially toxic chemicals to health regulatory and research agencies, scientific and medical communities, and the public, (3) strengthen the science base in toxicology, and (4) develop and validate improved testing methods. In its 25 years, the NTP has become a world leader in designing, conducting, and interpreting various types of assays for toxicity. Through its activities, the NTP provides, directly or indirectly, a large component of the basic scientific data that other federal and state scientific and regulatory agencies, as well as private-sector organizations, use in responding to issues relevant to the effects of chemical and physical agents on human health and the environment. All of the NTP's activities are open to public scrutiny, including communication with all interested parties. The NTP draws strength and direction from the commitment of its scientists to exchanging information openly, maintaining impartiality, and applying rigorous scientific peer review.

The charter agencies of the NTP were the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH), the National Cancer Institute (NCI) of the NIH, the National Institute of Occupational Safety and Health of the Centers for Disease Control and Prevention, and the Food and Drug Administration's National Center for Toxicological Research. In 1981, the

carcinogenesis bioassay program of the NCI was transferred to the NIEHS. Although no longer a core NTP agency, the NCI remains active in the NTP and serves on the NTP Executive Committee. The director of the NIEHS also serves as director of the NTP and the administrative staff for the NTP is located at the NIEHS.

The NTP relies upon advice on its activities and priorities from its advisory committees. The NTP Executive Committee, composed of the heads (or their designees) of federal health regulatory and research agencies, provides primary policy oversight to the NTP. The NTP Board of Scientific Counselors and its two standing subcommittees provide primary scientific oversight for the NTP's intramural and collaborative activities. The Board is a federally chartered advisory committee composed primarily of nonfederal scientists comprising a broad spectrum of expertise and affiliations, including academia, industry, labor, public health, and state and federal governments. The Technical Reports Review Subcommittee of the Board provides peer review for NTP long-term toxicology and carcinogenicity studies and short-term toxicity reports. The Report on Carcinogens Subcommittee of the Board provides external scientific evaluation of substances nominated for listing in or delisting from the Report on Carcinogens, a Congressionally mandated document that lists substances known or reasonably anticipated to be human carcinogens, and to which a significant number of persons in the United States are exposed. The Scientific Advisory Committee on Alternative Toxicological Methods provides external scientific input on priorities and directives related to the development, validation, scientific review, and regulatory acceptance of new or revised toxicological test methods and on ways to foster communication and partnerships with all interested parties.

The NTP's mission is to evaluate agents of public health concern by developing and applying tools of modern toxicology and molecular biology. The program maintains a number of complex, interrelated research and testing programs that provide unique and critical information needed by health regulatory and research agencies to protect human health. The NTP's research and testing program includes chronic bioassays, short-term toxicity studies, mechanistic studies, model development, alternative models, and human studies.

The NTP maintains a balanced research and testing program that provides data on a wide variety of issues important to human health. The NTP continually solicits nominations of substances for study and invites nominations from all interested parties and groups. In particular, the NTP seeks nominations of studies that enhance the predictive ability of future NTP studies, address mechanisms of toxicity, or fill significant gaps in the knowledge of the toxicity of chemicals or classes of chemicals. Nominations undergo several levels of review that include the opportunity for public comment. The NTP strives to balance the selection of substances for study (e.g., occupational exposures, environmental pollutants, food additives, consumer products, and pharmaceuticals). The NTP evaluates selected substances for a variety of health-related effects, among them, general toxicity, reproductive and developmental toxicity, genotoxicity, immunotoxicity, neurotoxicity, metabolism, disposition, and carcinogenicity. In addition, there are special projects focused on AIDS therapeutics and toxicity of superfund chemicals.

As the NTP moves into the twenty-first century, the program is evaluating its key activities and in a focused and concerted effort determining how best to incorporate new scientific technologies of molecular biology, computer science, and genomics into its research and testing strategies and broaden scientific knowledge on the linkage between mechanism and disease. The NTP's vision is to move toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations. The NTP is inviting input on how best to achieve this vision from its federal partners, advisory committees, academia, industry, and the public. The NTP will use this input to develop a framework targeted toward achieving the vision and including the necessary components for implementation, management, and communication of changes in NTP activities. It is envisioned that the acceptance and implementation of this vision in addressing public health priorities will result in better science and ultimately better decisions that protect human health and the environment.

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Occupational Safety and Health Administration

Harihara M Mehendale

With the passage of the Occupational Safety and Health Act of 1970 (OSH Act, P.L. 91-596), the US Congress created the Occupational Safety and Health Administration (OSHA) to "assure so far as possible every working man and woman in the Nation safe and healthful working conditions."

OSHA provides leadership and encouragement to US employers and employees to help them recognize and realize the value of safety and health on the job. The agency's goal is to save lives, prevent injuries and illnesses, and protect the safety and health of American workers.

Since its inception in 1971, OSHA has helped to cut workplace fatalities by more than 60% and occupational injury and illness rates by 40%. At the same time, US employment has doubled from 56 million workers at 3.5 million worksites to more than 115 million workers at 7.1 million sites.

The Labor Department agency employs three strategies to promote safety and health in American workplaces: strong, fair, and effective enforcement; outreach, education, and compliance assistance; and cooperative and voluntary programs.

The OSH Act also encourages states to develop and operate their own safety and health plans. Approved under Section 18 (b) of the Act, these plans must adopt and enforce standards at least as effective as federal requirements.

OSHA offers safety and health training through the OSHA Training Institute in Des Plaines, Illinois, and through 20 Education Centers at 35 sites across the country. Training schedules are available on the agency's website along with interactive software called eTools, which offer step-by-step guidance on many safety and health issues. More than 65 compliance assistance specialists in local OSHA offices are also available to speak to groups, teach workshops, and present seminars on safety and health topics. Consultation programs in each state offer small businesses onsite safety and health guidance from experts.

Cooperative and voluntary programs sponsored by OSHA include Voluntary Protection Programs, the agency's premier partnership; Strategic Partnerships, which emphasizes effective safety and health management systems; SHARP, a recognition program for excellence for small businesses; and Alliances, which promotes safety and health training and sharing of best practices.

Contact Details

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Organisation for Economic Cooperation and Development

Robert Visser*

The Organisation for Economic Cooperation and Development (OECD) is an intergovernmental organization. Its aims and responsibilities are achieving the highest sustainable economic growth and employment; promoting economic and social welfare throughout the OECD region by coordinating policies of its member countries; and stimulating and harmonizing the efforts of member countries in favor of developing countries. At the time of writing, there are 30 member countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Japan, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Republic of Korea, the Slovak Republic, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States. The European Commission also participates in the Organization's work.

*Any opinions expressed in this article do not necessarily represent the opinions of the Organisation for Economic Cooperation and Development (OECD) or its member countries and should therefore be viewed as solely those of the author.

Within the OECD, the governments of these industrialized countries compare and, if they so decide, coordinate their domestic policies. Monitoring international economic trends is one of the Organization's best known activities. However, since the OECD was established in 1960 the number of policy areas in which it functions as a center for cooperation and exchange of views has steadily increased. Since 1971, work in the OECD related to chemical safety has been organized under the Chemicals Programme. The policy direction and priorities of the Chemicals Programme are determined by member country representatives, nominated by each country's government, who take part in the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. The Chemicals Programme is supported by the Environment, Health and Safety Division of the OECD. Technical work on chemicals is carried out for the most part by experts from government, industry, nongovernmental interest groups, and academic institutions in member countries, who attend workshops and other meetings.

The main objectives of the Chemicals Programme are to assist member countries in identifying, preventing, and managing the risks of chemicals; promote the public's right to know about the hazards, exposures, and potential risks of chemicals; prevent unnecessary nontariff distortions in the trade of chemicals; facilitate the optimal use of resources available in governments and in industry for chemicals management; assist member countries in achieving the objectives of the United Nations Conference on Environment and Development's (UNCED's) Agenda 21, Chapter 19, and in the application of the criteria for environmental sustainability, as agreed upon by OECD Environment Ministers in the OECD Environment Strategy for the first decade of the twenty-first century; work with OECD countries and specific nonmembers to prevent globalization of the chemicals industry that would lead to negative impacts on human health and the environment throughout the world; assist member countries in the development of approaches to improve the use of voluntary actions for chemicals management by the industry; and promote the development and implementation in member countries of new and innovative instruments for more holistic approaches to management of the risks of chemicals throughout their life cycle, including those related to the risks of their use in products.

Since the 1970s, the OECD has been in the forefront in developing policies and instruments to control chemicals and chemical products. Work in the Chemicals Programme on the development, updating, and expansion of scientifically valid harmonized methods for testing and assessment helps OECD countries (and, increasingly, non-OECD countries that use these methods) to prevent new chemicals that might present unacceptable risks from entering the market and to assess the safety of chemicals already in use.

Many OECD chemical safety activities are carried out in cooperation with other relevant organizations (Food and Agriculture Organization of the United Nations, International Labour Organization, United Nations Environment Programme (UNEP), United Nations Industrial Development Organization, United Nations Institute for Training and Research, and World Health Organization) through the Inter Organization Programme on the Sound Management of Chemicals and with the United Nations Development Programme and the World Bank.

Mutual Acceptance of Data

One of the most significant achievements of cooperative work in the OECD aimed at the harmonization of national chemical control procedures and policies is the 1981 Council Act on the Mutual Acceptance of Data (MAD) in the Assessment of Chemicals. This Act contains a Decision that "data generated in the testing of chemicals in an OECD member country in accordance with OECD Test Guidelines and OECD Principles of Good Laboratory Practice (GLP) shall be accepted by other member countries for purposes of assessment and other uses relating to the protection of man and the environment." The Council is the OECD's highest authority, in which the governments of all member countries participate. Once member countries have adopted a Council Decision, they are under a legal obligation to implement it. Consequently, when data developed in an OECD country under the conditions set out in this Decision are submitted in other OECD countries to fulfill regulatory requirements, the data cannot be refused and so do not have to be developed over again for notification in each country. (The OECD Test Guidelines and OECD Principles of GLP are described below.)

The intention of this Council Act is to ensure that data generated in the safety testing of chemicals are of high quality and are based on internationally harmonized methods. Such data can then be used to assess chemical hazards and to make decisions on appropriate activities to prevent or reduce risk to human health or the environment. Where the need for duplicative testing is minimized through MAD, testing costs can

be reduced for both governments and industry (it has been calculated that the annual saving resulting from MAD in this respect amounts to ~US$60 million). Test facilities and specialist personnel can be utilized more efficiently, and fewer animals will be used in testing (an important consideration in OECD countries, where animal welfare is an issue of concern). Harmonized safety testing can also help prevent non-tariff barriers which could arise in the trade of chemicals and chemical products as a result of differences in national chemical control regulations.

Since 1997 a procedure through which non-OECD countries can adhere to the MAD system has been embodied in a Council Decision (Council Decision on the Adherence of Non-Member Countries to the Council Acts Related to the Mutual Acceptance of Data in the Assessment of Chemicals C(97)114/FINAL). South Africa is now a full participant. India, Israel, and Slovenia participate on a provisional basis and several important chemicals-producing nonmembers are at the point of also doing this.

The OECD Test Guidelines

The *OECD Guidelines for the Testing of Chemicals* is a collection of standard methods used by professionals in governments, industry, academic institutions, and independent laboratories for safety testing of chemical substances. They cover tests for physical–chemical properties, effects on biotic systems (ecotoxicity), environmental fate (degradation/accumulation), and health effects (toxicity) (see **Table 1**). The Test Guidelines are systematically updated to respond to scientific progress or to address new needs identified by member countries. They are published in two loose-leaf binders, and are also available in a CD-ROM version.

The OECD Test Guidelines began to be developed in the late 1970s, by several expert groups, with the goal of enhancing the validity and international acceptability of test data. They have become recognized in both OECD and non-OECD countries as the authoritative reference tool for testing chemicals in a regulatory context. Over the years, many new Test Guidelines have been developed to address new data requirements in the notification and registration of chemicals and pesticides. Furthermore, OECD Test Guidelines are continuously updated to bring them in line with the state of the art of science. A network of more than 1000 experts has been involved in the OECD work on Test Guidelines.

The Test Guidelines Programme is overseen by National Coordinators, who work to achieve consensus on draft versions of new and revised Test Guidelines. Proposals for new or updated Guidelines can be made by a National Coordinator, the international scientific community, and by the OECD Secretariat. To become effective, any new or updated Guideline must be adopted by member countries as part of the Council Decision on MAD.

Special attention is paid within the Test Guideline Programme to animal welfare issues, in particular the reduction, refinement, and replacement of animal use in the OECD Test Guidelines. In updating and developing Test Guidelines, wherever scientifically justified, test methods that do not require the use of animals or that require fewer test animals than existing methods are incorporated. A number of Test Guidelines have been developed or revised with a view to reducing the number of animals used and introducing a framework of testing that allows alternative methods to be applied first.

In 1996, the OECD established a Special Project on Endocrine Disrupter Testing and Assessment with the objectives of providing information on and coordinating the activities of member countries; developing new and revising existing Test Guidelines to detect endocrine disrupters; and harmonizing hazard and risk characterization approaches.

This activity was launched at the request of the member countries and the Business and Industry Advisory Committee to the OECD to ensure that testing and assessment approaches for endocrine disrupters would not substantially differ among countries. In addition to developing tests for endocrine disrupters in the human health and environmental fields, OECD has also developed a conceptual framework for endocrine disrupter testing, outlining consecutive steps that could be followed.

The OECD Principles of GLP

The OECD Principles of GLP provide quality assurance concepts concerning the organization of test laboratories and the conditions under which laboratory studies are planned, performed, monitored, and reported. The purpose of the GLP Principles is to make certain that test data are reliable. Like the Test Guidelines, the Principles of GLP began to be developed at the end of the 1970s and were established in the 1981 Council Decision on MAD.

In 1989, the OECD Council adopted an Act on Compliance with Principles of GLP. This Act contains a Decision that member countries shall (1) establish national procedures for monitoring compliance with GLP Principles, based on laboratory inspections and study audits; (2) designate national compliance monitoring authorities ('GLP inspectors'); and (3) require the management of test facilities to issue a

declaration, where applicable, that a study was carried out according to GLP Principles. It also contains a Decision that member countries shall, under specific conditions, recognize assurance from other member countries that test data have been generated in accordance with GLP Principles, and that they shall designate authorities for international liaison, exchange relevant information on compliance monitoring procedures, and implement procedures whereby, if good reason exists, information on GLP compliance by a test facility in one member country can be sought by another member country. Annexed to this Act are *Guides for Compliance Monitoring Procedures for GLP*, *Guidance for the Conduct of Laboratory Inspections and Study Audits*, and *Guidance for the Exchange of Information Concerning National Procedures for Monitoring Compliance*.

Information exchange takes place within the OECD on technical and administrative matters related to the application of the GLP Principles and the implementation of the compliance-monitoring procedures. The Working Group on GLP, made up of representatives of national GLP inspectors, oversees the Programme on GLP and develops common positions on the administration of compliance monitoring. One of the Working Group's responsibilities is to find solutions to problems involving the acceptance of compliance monitoring. OECD training courses are held for GLP inspectors, who perform laboratory inspections on behalf of national GLP-monitoring authorities. Several OECD expert groups have met to produce Consensus Documents on the harmonized application and interpretation of the GLP Principles in specific areas, or in relation to specific points.

The *OECD Series on Principles of GLP and Compliance Monitoring*, published in the form of short free-on-demand booklets, includes the GLP Principles, the 1981 and 1989 Council Acts, and the Consensus Documents. At the time of writing, there were 13 booklets in the series.

Within OECD, the inspectors are also undertaking mutual joint visits to review all the national GLP-monitoring programs. Each country is visited by a team, comprising inspectors of three other countries, and the program, inspections, and on-site study audits done by the country which is visited are evaluated and discussed among the inspectors.

Cooperative Investigation of High-Production Volume Chemicals

Much of the work in the Chemicals Programme in the 1970s and early 1980s involved the development of anticipatory policies to prevent chemicals that could present unacceptable risks to human health or the environment from reaching the market. In 1987, however, the OECD Council adopted an Act on the Systematic Investigation of Existing Chemicals. Existing chemicals are the many thousands of industrial chemicals already in use. Adequate safety data or hazard assessments for these chemicals (some of which have been in use for a long time) are often unavailable. This Council Act contains a Decision that member countries "shall establish or strengthen national programmes to systematically investigate existing chemicals, in order to identify those which need to be managed and/or controlled." For the purposes of the Act, systematic investigation could include the following steps: identification of relevant chemicals; priority setting, including collection or estimation of information needed for setting priorities; generation of necessary further information, including testing; and performance of hazard assessments.

The 1987 Council Act was intended to strengthen and harmonize member countries' policies with regard to existing chemicals. To avoid duplication of efforts, and to facilitate sharing the financial and administrative burden (for governments and chemical companies) of investigating these chemicals, the Council decided in a 1990 follow-up Act that member countries shall cooperatively investigate high-production volume (HPV) chemicals in order to identify those which are potentially hazardous; cooperatively select the HPV chemicals to be investigated; agree on a set of data needed to make an informed judgment concerning the potential hazards of each chemical, through collection of available data or by ensuring that testing is undertaken; and cooperatively make an initial assessment of the potential hazards of each chemical using that basic data set. These Decisions are contained in the Council Act on the Cooperative Investigation and Risk Reduction of Existing Chemicals.

Following the adoption of this 1990 Council Act, HPV chemicals are defined as those produced or imported in volumes of at least 1000 tons year^{-1} in at least one OECD country. Governments, in consultation with the chemical industry provide the OECD with information on the chemicals produced in these volumes in their countries. A consolidated OECD Representative List of more than 5000 HPV chemicals is regularly prepared and updated. These chemicals represent an estimated 90–95% of the total volume of chemicals produced in member countries. While there was already adequate information concerning the health and environmental effects of certain chemicals on this HPV list, little or no information of this type was available for many chemicals despite their HPV.

Table 1 OECD Guidelines for the Testing of Chemicals

Section 1: Physical–Chemical Properties
101 UV–VIS Absorption Spectra (original guideline, adopted May 12, 1981)
102 Melting Point/Melting Range (updated guideline, adopted July 27, 1995)
103 Boiling Point (updated guideline, adopted July 27, 1995)
104 Vapour Pressure (updated guideline, adopted July 27, 1995)
105 Water Solubility (updated guideline, adopted July 27, 1995)
106 Absorption–Desorption Using a Batch Equilibrium Method (updated guideline, adopted January 21, 2000)
107 Partition Coefficient (*n*-octanol/water): Shake Flask Method (updated guideline, adopted July 27, 1995)
108 Complex Formation Ability in Water (original guideline, adopted May 12, 1981)
109 Density of Liquids and Solids (updated guideline, adopted July 27, 1995)
110 Particle Size Distribution/Fibre Length and Diameter Distributions (original guideline, adopted May 12, 1981)
111 Hydrolysis as a Function of pH (original guideline, adopted May 12, 1981) (see draft guidelines below)
112 Dissociation Constants in Water (original guideline, adopted May 12, 1981)
113 Screening Test for Thermal Stability and Stability in Air (original guideline, adopted May 12, 1981)
114 Viscosity of Liquids (original guideline, adopted May 12, 1981)
115 Surface Tension of Aqueous Solutions (updated guideline, adopted July 27, 1995)
116 Fat Solubility of Solid and Liquid Substances (original guideline, adopted May 12, 1981)
117 Partition Coefficient (*n*-octanol/water), HPLC Method (original guideline, adopted March 30, 1989)
118 Determination of the Number-Average Molecular Weight and the Molecular Weight Distribution of Polymers Using Gel Permeation Chromatography (original guideline, adopted June 14, 1996)
119 Determination of the Low Molecular Weight Content of a Polymer Using Gel Permeation Chromatography (original guideline, adopted June 14, 1996)
120 Solution/Extraction Behaviour of Polymers in Water (updated guideline, adopted January 21, 2000)
121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge Using High Performance Liquid Chromatography (HPLC) (original guideline, adopted January 22, 2001)
Section 2: Effects on Biotic Systems
201 Alga, Growth Inhibition Test (updated guideline, adopted June 7, 1984)
202 *Daphnia* sp. Acute Immobilisation Test and Reproduction Test (updated guideline, adopted April 4, 1984)
203 Fish, Acute Toxicity Test (updated guideline, adopted July 17, 1992)
204 Fish, Prolonged Toxicity Test: 14 Day Study (original guideline, adopted April 4, 1984)
205 Avian Dietary Toxicity Test (original guideline, adopted April 4, 1984)
206 Avian Reproduction Test (original guideline, adopted April 4, 1984)
207 Earthworm, Acute Toxicity Tests (original guideline, adopted April 4, 1984)
208 Terrestrial Plants, Growth Test (original guideline, adopted April 4, 1984)
209 Activated Sludge, Respiration Inhibition Test (original guideline, adopted April 4, 1984)
210 Fish, Early-Life Stage Toxicity Test (original guideline, adopted July 17, 1992)
211 *Daphnia magna* Reproduction Test (original guideline, adopted September 21, 1998)
212 Fish, Short-Term Toxicity Test on Embryo and Sac-Fry Stages (original guideline, adopted September 21, 1998)
213 Honeybees, Acute Oral Toxicity Test (original guideline, adopted September 21, 1998)
214 Honeybees, Acute Contact Toxicity Test (original guideline, adopted September 21, 1998)
215 Fish, Juvenile Growth Test (original guideline, adopted January 21, 2000)
216 Soil Microorganisms, Nitrogen Transformation Test (original guideline, adopted January 21, 2000)
217 Soil Microorganisms, Carbon Transformation Test (original guideline, adopted January 21, 2000)
Section 3: Degradation and Accumulation
301 Ready Biodegradability
A: DOC Die-Away Test
B: CO2 Evolution Test
C: Modified MITI Test (I)
D: Closed Bottle Test
E: Modified OECD Screening Test
F: Manometric Respirometry Test (updated guideline, adopted July 17, 1992)
302A Inherent Biodegradability: Modified SCAS Test (original guideline, adopted May 12, 1981)
302B Inherent Biodegradability: Zahn-Wellens/EMPA Test (updated guideline, adopted July 17, 1992)
302C Inherent Biodegradability: Modified MITI Test (II) (original guideline, adopted May 12, 1981)
303 Simulation Test – Aerobic Sewage Treatment
303A Activated Sludge Units; 303B Biofilms (updated guidelines, adopted January 22, 2001)
304A Inherent Biodegradability in Soil (original guideline, adopted May 12, 1981)
305 Bioconcentration: Flow-Through Fish Test (updated guideline, adopted June 14, 1996)
306 Biodegradability in Seawater (original guideline, adopted July 17, 1992)
307 Aerobic and Anaerobic Transformation in Soil (original guideline, adopted April 24, 2002)
308 Aerobic and Anaerobic Transformation in Aquatic Sediment Systems (original guideline, adopted April 24, 2002)
Section 4: Health Effects
402 Acute Dermal Toxicity (updated guideline, adopted February 24, 1987)
403 Acute Inhalation Toxicity (original guideline, adopted May 12, 1981)

Table 1 Continued

404 Acute Dermal Irritation/Corrosion (updated guideline, adopted April 24, 2002)
405 Acute Eye Irritation/Corrosion (updated guideline, adopted April 24, 2002)
406 Skin Sensitisation (Updated Guideline, adopted 17th July 1992)
407 Repeated Dose 28-Day Oral Toxicity Study in Rodents (updated guideline, adopted July 27, 1995)
408 Repeated Dose 90-Day Oral Toxicity Study in Rodents (updated guideline, adopted September 21, 1998)
409 Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents (updated guideline, adopted September 21, 1998)
410 Repeated Dose Dermal Toxicity: 21/28-Day Study (original guideline, adopted May 12, 1981)
411 Subchronic Dermal Toxicity: 90-Day Study (original guideline, adopted May 12, 1981)
412 Repeated Dose Inhalation Toxicity: 28-Day or 14-Day Study (original guideline, adopted May 12, 1981)
413 Subchronic Inhalation Toxicity: 90-Day Study (original guideline, adopted May 12, 1981)
414 Prenatal Developmental Toxicity Study (updated guideline, adopted January 22, 2001)
415 One-Generation Reproduction Toxicity Study (original guideline, adopted May 26, 1983)
416 Two-Generation Reproduction Toxicity Study (updated guideline, adopted January 22, 2001)
417 Toxicokinetics (updated guideline, adopted April 4, 1984)
418 Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure (updated guideline, adopted July 27, 1995)
419 Delayed Neurotoxicity of Organophosphorus Substances: 28-Day Repeated Dose Study (updated guideline, adopted July 27, 1995)
420 Acute Oral Toxicity – Fixed Dose Method (updated guideline, adopted December 20, 2001)
421 Reproduction/Developmental Toxicity Screening Test (original guideline, adopted July 27, 1995)
422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (original guideline, adopted March 22, 1996)
423 Acute Oral Toxicity – Acute Toxic Class Method (updated guideline, adopted December 20, 2001)
424 Neurotoxicity Study in Rodents (original guideline, adopted July 21, 1997)
425 Acute Oral Toxicity: Up-and-Down Procedure (updated guideline, adopted December 20, 2001)
429 Skin Sensitisation: Local Lymph Node Assay (updated guideline, adopted April 24, 2002)
451 Carcinogenicity Studies (original guideline, adopted May 12, 1981)
452 Chronic Toxicity Studies (original guideline, adopted May 12, 1981)
453 Combined Chronic Toxicity/Carcinogenicity Studies (original guideline, adopted May 12, 1981)
471 Bacterial Reverse Mutation Test (updated guideline, adopted July 21, 1997)
473 *In Vitro* Mammalian Chromosomal Aberration Test (updated guideline, adopted July 21, 1997)
474 Mammalian Erythrocyte Micronucleus Test (updated guideline, adopted July 21, 1997)
475 Mammalian Bone Marrow Chromosomal Aberration Test (updated guideline, adopted July 21, 1997)
476 *In Vitro* Mammalian Cell Gene Mutation Test (updated guideline, adopted July 21, 1997)
477 Genetic Toxicology: Sex-Linked Recessive Lethal Test in *Drosophila melanogaster* (updated guideline, adopted April 4, 1984)
478 Genetic Toxicology: Rodent Dominant Lethal Test (updated guideline, adopted April 4, 1984)
479 Genetic Toxicology: *In Vitro* Sister Chromatid Exchange Assay in Mammalian Cells (original guideline, adopted October 23, 1986)
480 Genetic Toxicology: *Saccharomyces cerevisiae*, Gene Mutation Assay (original guideline, adopted October 23, 1986)
481 Genetic Toxicology: *Saacharomyces cerevisiae*, Miotic Recombination Assay (original guideline, adopted October 23, 1986)
482 Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells *In Vitro* (original guideline, adopted October 23, 1986)
483 Mammalian Spermatogonial Chromosome Aberration Test (original guideline, adopted July 21, 1997)
484 Genetic Toxicology: Mouse Spot Test (original guideline, adopted October 23, 1986)
485 Genetic Toxicology: Mouse Heritable Translocation Assay (original guideline, adopted October 23, 1986)
486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In Vivo* (original guideline, adopted July 21, 1997)

The objective of the work in OECD is that through cooperative investigation by member countries, thereby efficiently sharing the burden of the enormous task of systematically investigating existing chemicals, adequate safety data for all HPV chemicals become available.

At a minimum, the OECD's Screening Information Data Set (SIDS) should be available. The SIDS is a list of data elements similar to those which governments in most OECD countries require from industry before a new chemical can be marketed. It includes information on the chemical's identity, its physical and chemical properties, the indications on exposure and use, its environmental fate and how the chemical might be disseminated in the environment, as well as toxicological data. All of these data elements are essential if an initial assessment is to be made of a chemical's hazards (see **Table 2**).

In order to fill identified data gaps for a specific chemical, or replace data whose quality is considered insufficient, a SIDS Testing Plan is prepared. The chemicals industry then undertakes the necessary testing on a voluntary basis, in consultation with a sponsor country, which overlooks and manages the process for the chemical under consideration, paying particular attention to the quality of the data. Any test is performed only once, by a single company. In accordance with the 1981 Council Decision on Mutual Acceptance of Data, all member countries will accept the results as long as testing has been done according to the OECD Test Guidelines and Principles of GLP.

Table 2 Screening OECD's Screening Information Data Set (SIDS)

Chemical identity
Physical–chemical data
Melting point
Boiling point
Vapor pressure
Water solubility
Dissociation constant
Exposure information
Sources
Users
Estimates of releases
Estimates of exposure to man and environment
Environmental fate and pathways
Biodegradation
Abiotic degradability
Distribution estimates
Ecotoxicological data
Acute fish toxicity
Prolonged *Daphnia* toxicity
Terrestrial toxicity (if significant terrestrial exposure)
Toxicological data
Acute toxicity
Repeated-dose toxicity
Genetic toxicity
Point mutation
Chromosomal aberration
Reproductive toxicity

When (through a combination of data collection and testing) a complete SIDS has been compiled, the sponsor country provides OECD with a SIDS Initial Assessment Report. The Initial Assessment Report is discussed, in the presence of experts from companies producing the chemical and from public interest groups and trade unions, at a SIDS Initial Assessment Meeting (SIAM). At this meeting a cooperative assessment of the chemical is made and conclusions are drawn on the potential hazard(s) posed by the chemical and recommendations are made on the need for further work. The conclusions present a summary of the hazards of the chemical, written with sufficient detail and clarity so as to be informative and to assist countries with classification work and other hazard-based national decision making; exposure information to put the hazard information into context (e.g., on use in the sponsor country) is also included. The recommendation, based on these conclusions, can be either that the chemical is currently of low priority for further work or that it is a candidate for further work to clarify its potential risk (e.g., that further information is required to clarify concerns identified in the SIDS process, and that post-SIDS testing is recommended).

In principle, follow-up work is left to the member countries and they will decide on what to do, depending on the national exposure situation of member countries in the policy bodies of OECD. Member countries discuss and confirm all conclusions and recommendations made on all chemicals which have undergone a SIDS and draft initial assessments. When full SIDS dossiers and initial assessment reports are finalized, the results are made available worldwide through UNEP Chemicals.

In 1998 the global chemical industry through the International Council of Chemical Associations (ICCA) announced its intention to work with OECD by using the OECD HPV Chemicals List to establish a working list of $\sim$1000 substances as priorities for investigation by industry itself (based on presumed wide dispersive use, production in two or more global regions, or similarity to another chemical meeting either of these criteria). The ICCA set the goal of completing SIDS and draft initial hazard assessments on these chemicals by the end of 2004. The draft initial assessments reviewed by sponsor countries are then considered in OECD by a SIAM. This initiative is an important source of assessments for consideration in the OECD Programme. Industry is encouraged to collaborate with member countries to ensure that the chemicals they select for investigation will be brought forward to the OECD Programme by a government (sponsor country). Member countries are encouraged to work with the chemical industry in order to make the most efficient use of the information compiled through the ICCA initiative in meeting their commitments to investigate a certain proportion of the chemicals on the OECD HPV Chemicals List.

At the time of writing, almost 1000 HPV chemicals had undergone or were undergoing systematic investigation of their potential health or environmental effects. Many more HPV chemicals will be assessed in the near future. The OECD Programme is closely coordinated with the work ongoing in the US Chemicals Right to Know Programme on HPV Chemicals. There is furthermore close cooperation with the European Union (EU); the work undertaken in the EU is fully integrated into that of the OECD.

All data that become available through the OECD Existing Chemicals Programme are made publicly available. It has been agreed that SIDS Initial Assessment Reports will be forwarded to UNEP for publication with the OECD.

The Hazard Assessment Programme

Recently, OECD work has focused on exposure assessment including estimation of emission, monitoring, and modeling. Important products are the OECD Emission Scenario Documents (ESDs).

An ESD is a document that describes the sources, production processes, pathways, and use patterns with the aim of providing a quantified scenario for emissions (or releases) of a chemical from production, formulation, use (industrial use, professional use, private use of chemical substances/preparations), service life (use in articles), and recovery/disposal into water, air, soil, or solid waste. An ESD should ideally include all the following stages: (1) production; (2) formulation; (3) industrial use; (4) professional use; (5) private and consumer use; (6) service life of product/article; (7) recovery; and (8) waste disposal (incineration, landfill). ESDs are used in risk assessment of chemicals to establish the conditions of use and releases of the chemicals, and are the basis for estimating the concentration of chemicals in the environment.

Risk Management Program

The reason for testing and assessing chemicals is ultimately to prevent or reduce their risks. OECD governments, academia, NGOs, and industry work together to identify best practices and new techniques for managing risks, and then develop methodologies that can be used by governments and industry. In addition, if governments agree on the risks posed by a particular chemical, they can work together to take concerted action across OECD countries.

Most of OECD's current work is focused on developing guidance for risk management that applies to the chemical industry as a whole. This includes guidance on conducting socioeconomic analysis; risk communication; tools to help companies screen potentially dangerous chemicals before they are manufactured; and development of environmentally benign chemicals.

Over the last few years, there has been a significant increase in the use of new and innovative approaches for managing risks posed by chemicals. OECD countries have found that traditional 'command and control' techniques are not always the most effective or efficient ways to control risk. One approach that has been of particular interest is the use of 'non-regulatory initiatives'. In order to increase awareness of the range of approaches that can be used, OECD has produced a report which identifies factors that can contribute to, or inhibit, the success of these approaches.

OECD work on facilitating the development of environmentally benign chemicals includes several elements. To start with, effective techniques and approaches in the field of sustainable chemistry were identified. This includes such aspects as recognizing and regarding sustainable chemistry accomplishments; disseminating technical information; promoting the incorporation of sustainable chemistry principles into various levels of chemical education; and promoting the research, discovery, and development of innovative sustainable chemistry technologies. Work is under way on each of these approaches.

OECD has also started a new project to help member countries and others assess and manage the impacts of chemicals throughout their life cycle (i.e., from production of a chemical substance, to distribution, use, recycling and/or recovery, and final disposal). To date, most methodologies for generating and collecting data, conducting risk assessments, and making risk management decisions have focused primarily on the production stage. In this new work also much attention is being given to chemicals in products. This new approach will build off existing methodologies and develop new ones to support a more holistic approach to chemicals management.

Other Activities Related to Environment, Health, and Safety

Other OECD activities in the environment, health, and safety areas are concerned with pesticides; chemical accident prevention, preparation, and response; pollutant release and transfer registers; harmonization of regulatory oversight in biotechnology, and the safety of novel foods and feeds. These activities are closely connected with the work in the Chemicals Programme, and are carried out in cooperation with other parts of the OECD and other international organizations.

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Appendix

ECD Council Acts Related to Chemicals

The OECD Council, under the chairmanship of the OECD Secretary-General, is the focal point of a continuing review by member governments of the work of the OECD. The Council also decides on the

Programme of Work of OECD and its Budget. When appropriate, the Council may also agree by consensus on Decisions which are legally binding under international law. Alternatively, member governments, through the Council, may agree on Recommendations, which are expressions of political will to follow certain policies. Council Decisions and Council Recommendations are known collectively as Council Acts. The following list of Council Acts is relevant to the work of the Chemicals Programme.

- Decision on the Mutual Acceptance of Data in the Assessment of Chemicals (C(81)30/Final).
- Decision of the Council Amending the Decision Concerning the Mutual Acceptance of Data in the Assessment of Chemicals (C(81)30/Final) (C(97) 186/Final).
- Decision on the Minimum Pre-marketing Set of Data in the Assessment of Chemicals (C(82)196/ Final).
- OECD Council Decision on the Minimum Premarketing Set of Data in the Assessment of Chemicals.
- OECD Council Recommendation on the Protection of Proprietary Rights to Data Submitted in Notifications of New Chemicals (C(83)96/Final).
- OECD Council Recommendation on the Exchange of Confidential Data on Chemicals (C(83)97/Final).
- Recommendation on the OECD List of Non-Confidential Data on Chemicals (C(83)98/Final).
- Decision–Recommendation on Further Measures for the Protection of the Environment by Control of Polychlorinated Biphenyls (C(87)2/Final).
- Decision–Recommendation on the Systematic Investigation of Existing Chemicals (C(87)90/Final).
- Decision-Recommendation on Compliance with Principles of Good Laboratory Practice (C(89)87/ Final).
- Decision of the Council on the Exchange of Information Concerning Accidents Capable of Causing Transfrontier Damage (C(88)84/Final).
- Decision–Recommendation Concerning Provision of Information to the Public and Public Participation in Decision-Making Processes Related to the Prevention of, and Response to, Accidents Involving Hazardous Substances (C(88)85/Final).
- Recommendation on the Application of the Polluter-Pays Principle to Accidental Pollution (C(89)88/Final).
- OECD Council Decision–Recommendation on Compliance with Principles of Good Laboratory Practice (C(89)87/Final).
- OECD Decision-Recommendation on the Co-operative Investigation and Risk Reduction of Existing Chemicals (Chemicals (1990) C(90)163/Final).
- Recommendation on Integrated Pollution Prevention and Control (C(90)164/Final).
- Recommendation Concerning Chemical Accident Prevention, Preparedness and Response (C(92)1/ Final).
- Decision of the Council Amending the Annexes to the Council Decision–Recommendation on Compliance with Principles of Good Laboratory Practice (C(89)87/Final) (C(95)8/Final).
- Recommendation of the Council on Implementing Pollutant Release and Transfer Registers (C(96)41/ Final).
- Council Decision on Adherence of Non-Member Countries to the Council Acts Related to the Mutual Acceptance of Data in the Assessment of Chemicals (C(97) 114/Final).

Selected Documents Related to Test Guidelines

- Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides during Agricultural Application (1996).
- Chemicals Testing Monographs No. 1 Guidance Document for the Development of OECD Guidelines for the Testing of Chemicals (1998).
- No 19: Testing and Assessment: Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints (2000).
- The Work of OECD on Testing and Assessment of Endocrine Disrupters (2001).
- Chemicals Testing Monographs No. 33: Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures (2001).
- Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods (2002).

Documents Published in the OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring (Also Available in French and German Translations; Russian Translations are Underway)

- The OECD Principles of Good Laboratory Practice (1998).
- Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice (1995).
- Revised Guidance for the Conduct of Laboratory Inspections and Study Audits (1995).
- Quality Assurance and GLP, Paris (1998).
- Compliance of Laboratory Suppliers with GLP Principles (2000).
- The Application of the GLP Principles to Field Studies, Paris (1999).
- The Application of the GLP Principles to Short-Term Studies (1999).

- The Role and Responsibilities of the Study Director in GLP Studies (1999).
- Guidance for the Preparation of GLP Inspection Reports (1995).
- The Application of the Principles of GLP to Computerised Systems (1995).
- The Role and Responsibilities of the Sponsor in the Application of the Principles of GLP (1998).
- Requesting and Carrying Out Inspections and Study Audits in Another Country (2000).
- The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies (2002).

Selected Documents Related to Hazard/Risk Assessment

- Guidance Document on Emission Scenario Documents (2000).
- OECD/IPCS Database on Hazard-Risk Assessment Methodologies (2001).
- Database on Use and Releases of Chemicals (2001).
- Report of the OECD/UNEP Workshop on the Use of Multimedia Models for Estimating Overall Environmental Persistence and Long Range Transport in the Context of PBTS/POPS Assessment (2002).
- Emission Scenario Documents on Wood Preservatives, Part 1, Part 2, Part 3, Part 4 (2003).

Selected Documents Related to Pesticides

- OECD Guidance Documents for Pesticide Registration (2001).
- OECD Guidance for Industry Data Submissions on Plant Protection Products and Their Active Substances (2001).
- Monograph Guidance – OECD Guidance for Country Data Review Reports on Plant Protection Products and Their Active Substances (2001).
- Survey of Best Practices in the Regulation of Pesticides in Twelve OECD Countries (2001).
- Guidelines for the Collection of Pesticides Usage Statistics Within Agriculture and Horticulture (2002).
- Guidance for Registration Requirements for Pheromones and Other Semiochemicals Used for Arthropod Pest Control (2002).
- Report of the OECD Workshop on Electronic Tools for Data Submission (2003).
- Guidance for Registration Requirements for Microbial Pesticides (2003).

Selected Documents Related to Risk Management

- Proceedings of the OECD Workshop on Non-Regulatory Initiatives for Chemical Risk Management (1997).
- Proceedings for the OECD Workshop on Sustainable Chemistry (1998).
- Guidance for Conducting Retrospective Studies on Socio-economic Analysis (1999).
- Lead Risk Management Activities in OECD Member Countries (1993 to 1998) Part 1.
- OECD Guidance Document on Risk Communication for Chemical Risk Management (2002).
- Technical Guidance Document on the Use of Socio-Economic Analysis in Chemical Risk Management Decision Making (2002).
- Framework for Integrating Socio-Economic Analysis in Chemical Risk Management Decision Making (2002).
- Proceedings of the OECD Workshop on the Integration of Socio-Economic Analysis in Chemical Risk Management Decision Making (2002).

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Further Reading

Current OECD environment, health, and safety activities are described in greater detail in a brochure, *The OECD Environment, Health and Safety Programme*, which is available from the OECD, Environment, Health and Safety Division, 2 rue André-Pascal, 75775 Paris Cedex 16, France, E-mail: ehscont@oecd.org.

Public Health Service, US

C Charles Barton and Alan G Parham

Introduction

The main task of the Public Health Service (PHS) is protecting and advancing the health of our nation's people and contributing to the delivery of health care worldwide. The PHS is a principal part of the Department of Health and Human Services (DHHS) and the major health agency of the Federal Government.

The mission of the PHS is to provide highly trained and mobile health professionals who carry out programs to promote the health of the nation, understand and prevent disease and injury, assure safe and effective drugs and medical devices, deliver health services to Federal beneficiaries, and furnish health expertise in times of war or other national or international emergencies.

In order to fulfill its very broad mission of promoting health in our nation and the world, the PHS has designed programs and created agencies that help control and prevent diseases; conduct and fund biomedical research that will eventually lead to better treatment and prevention of diseases; protect us against unsafe food, drugs, and medical devices; improve mental health and deal with drug and alcohol abuse; expand health resources; and provide health care to people in medically under-served areas and to those with special needs.

The eight major agencies that make up the PHS and that do this work are the Centers for Disease Control and Prevention (CDC), the Agency for Toxic Substances and Disease Registry (ATSDR), the National Institutes of Health (NIH), the Food and Drug Administration (FDA), the Substance Abuse and Mental Health Services Administration (SAMHSA), the Health Resources and Services Administration (HRSA), the Agency for Health Care Policy and Research (AHCPR), and the Indian Health Service (IHS).

The Assistant Secretary for Health, with the assistance of the Surgeon General, heads the PHS, advises the DHHS Secretary on health and health-related matters, and directs the activities of the major PHS agencies. The PHS continues to fulfill its mission to protect and advance the public's health. It has grown from a small collection of marine hospitals to the largest public health program in the world. As part of the DHHS, the PHS consists of the Office of Public Health and Science (headed by the Assistant Secretary for Health and including the Surgeon General), ten Regional Health Administrators, and eight operating divisions.

History

The Public Health Service traces its origins to an Act of Congress signed by President John Adams on July 16, 1798, which provided for the care and relief of sick and injured merchant seamen. These seamen traveled widely, often became sick at sea, and then, away from their homes and families, could not find adequate health care in the port cities they visited or would overburden the meager public hospitals then in existence. Since they came from all the new states and former colonies, and could get sick anywhere, their health care became a national or Federal problem. The Marine Hospital Service (MHS), a loose network of marine hospitals located mainly in port cities, was established by Congress in 1798 to care for these sick and disabled seamen. The earliest marine hospitals created to care for the seamen were located along the East Coast, with Boston being the site of the first such facility; later they were also established along inland waterways, the Great Lakes, and the Gulf and Pacific Coasts.

The Federal Government had only three executive departments then to administer all Federal programs – State, Treasury, and War. The MHS was placed under the Revenue Marine Division of the Treasury Department. Funds to pay physicians and build marine hospitals were appropriated by taxing American seamen 20 cents a month. This was one of the first direct taxes enacted by the new republic and the first medical insurance program in the United States. The monies were collected from ship masters by the customs collectors in different US ports.

Lack of money and any supervisory authority were major problems for the MHS. The demand for medical services far exceeded the funds available. For that reason, sailors with chronic or incurable conditions were excluded from the hospitals and a 4-month limit was placed on hospital care for the rest. Additional funds had to be appropriated constantly from Congress in order to maintain the Service and to build the hospitals. Because of these problems, Congress was forced to act and in 1870 reorganized the MHS from a loose network of locally controlled hospitals to a centrally controlled national agency with its own administrative staff and headquarters in Washington, DC.

Through this reorganization, the MHS became a separate bureau of the Treasury Department under the direction of the Supervising Surgeon, who was

appointed by the Secretary of the Treasury. The title of the central administrator was changed to Supervising Surgeon General in 1875 and to Surgeon General in 1902. Additional money to fund the reorganized Service was appropriated by raising the hospital tax on seamen from 20 to 40 cents per month. The money collected was deposited in a separate MHS fund.

Taxing seamen to fund the MHS was abolished in 1884. From 1884 to 1906 the cost of maintaining the marine hospitals was paid from the proceeds of a tonnage tax on vessels entering the United States, and from 1906 to 1981, when the Public Health Service hospitals were closed, by direct appropriations from Congress.

The reorganization in 1870 created the position of Supervising Surgeon (later Surgeon General) to administer the Service, and John Maynard Woodworth was appointed as the first incumbent in 1871. He moved quickly to reform the system and adopted a military model for his medical staff, instituting examinations for applicants and putting his physicians in uniforms. Woodworth created a cadre of mobile, career service physicians who could be assigned as needed to the various marine hospitals.

The 1870 reorganization also changed the general character of the Service. It became national in scope and military in outlook and organization. Medical officers, called surgeons, were required to pass entrance examinations and wear uniforms. In 1889, the medical officers were given titles and pay corresponding to Army and Navy grades. Physicians who passed the examinations were appointed to the general service, rather than to a particular hospital, and were assigned wherever needed. The goal was to create a professional, mobile, health corps, free as far as possible from political favoritism and patronage, and able to deal with the new health needs of a rapidly growing and industrializing nation.

Beginning with the control of infectious diseases, the scope of activities of the MHS began to expand well beyond the care of merchant seamen in the closing decades of the nineteenth century. Responsibility for quarantine was originally a function of the states rather than the Federal Government, but the National Quarantine Act of 1878 conferred quarantine authority on the MHS. Over the course of the next half a century, the MHS increasingly took over quarantine functions from state authorities.

Beginning in 1891 as immigration increased dramatically in the late nineteenth century, the Federal Government also took over the processing of immigrants from the states. The MHS was assigned the responsibility for the medical inspection of immigrants arriving at sites such as Ellis Island in New York. Commissioned officers played a major role in fulfilling the Service's commitment to prevent disease from entering the country.

To help diagnose infectious diseases among passengers of incoming ships, the MHS established in 1887 a small bacteriology laboratory, called the Hygienic Laboratory, at the marine hospital on Staten Island, New York. That laboratory later moved to Washington, DC, and became the National Institutes of Health, the largest biomedical research organization in the world.

Because of the broadening responsibilities of the Service, its name was changed in 1902 to the Public Health and Marine Hospital Service, and again in 1912 to just the Public Health Service. The Service continued to expand its public health activities as the nation entered the twentieth century. As the century progressed, PHS officers served their country by controlling the spread of contagious diseases such as smallpox and yellow fever, conducting important biomedical research, regulating the food and drug supply, providing health care to underserved groups, supplying medical assistance in the aftermath of disasters, and in numerous other ways.

The PHS Today

Throughout all of the reorganizations which have shaped, defined, and established the PHS in its present place in the Federal Government, and which have spanned nearly two centuries, the PHS has never lost sight of its primary goal – providing health care for those with special needs. From the care of sick and disabled sailors the PHS has extended its activities to other groups with special needs (such as the American Indians, the Alaska Natives, migrant workers, Federal prisoners, and refugees), and to the nation as a whole.

The duties and functions of the PHS have expanded to include disease control and prevention, biomedical research, regulation of food and drugs, mental health and drug abuse, and health care delivery.

Today, the PHS is a part of the DHHS. It consists of the Office of Public Health and Science (headed by the Assistant Secretary for Health), ten Regional Health Administrators, and eight operating divisions. There are more than 6000 officers on active duty. Officers are assigned to all of the PHS Agencies and to a number of agencies outside of PHS, including the Bureau of Prisons, US Coast Guard, Environmental Protection Agency, Health Care Financing Administration, and the Commission on Mental Health of the District of Columbia.

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Further Reading

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Research Institute for Fragrance Materials (RIFM)

Anne Marie Api

History and Organization

The Research Institute for Fragrance Materials, Inc. (RIFM) was formally chartered as a nonprofit, international organization on April 12, 1966.

The headquarters is located in New Jersey, USA, and is directed by a President, who is appointed by the Board of Directors. Responsibilities of the corporation are carried out in close cooperation with the Scientific Director of the association through a scientific and support staff of 20 full- and part-time individuals.

RIFM is administered by a Board of Directors, which meets four times a year and is elected by the general membership during its annual meeting. Membership categories include Active, for those companies primarily engaged in the manufacture and/or sale or distribution of fragrance materials at other than the retail level; Supporting, for those companies at the retail level of consumer products using or consisting of fragrance or fragrance ingredients; and Associate, for those companies engaged as brokers or dealers in the fragrance industry.

All scientific efforts are reviewed by an independent experts panel of academic dermatologists, toxicologists, and environmental scientists. The experts panel uses a decision tree approach to assessing the dermal, systemic, and environmental endpoints. Conclusions of the expert panel on safe use, drawn from critical evaluation of all available hazard data, and exposure information provided by industry, form the basis for standards issued by the International Fragrance Association.

Fragrance materials are prioritized by human health and environmental endpoints and are evaluated by using a group approach. Chemical structure helps to predict transdermal absorption, metabolism and disposition, and functional groups that can influence toxicity. Using the group approach permits some generalizations; 88% are structurally simple, low molecular weight, predominantly semivolatile substances consisting of carbon, hydrogen, and oxygen. The majority of fragrance materials can be assigned to several homologous groups of structurally related materials in which one might reasonable predict some degree of consistency of metabolism and toxicity. These structural homologies allow safety issues to be considered within the context of the information that exists for the structural group as a whole. In many cases existing information for a structural group may obviate the need to submit a particular individual substance to full toxicological testing. In other cases, it may be necessary to test one or more particular members of a structural class to obtain more robust data to solidify assessment of the class as a whole.

Mission and Activities

The primary objectives of RIFM are to: gather and analyze scientific data from industry and open literature, engage in the evaluation and testing of fragrance ingredients, review and evaluate the standards and methods employed by industry for testing on a continuous basis. RIFM has a comprehensive research and testing program in the areas of fragrance allergy, respiratory safety, human health and environmental methodology, group safety evaluations, and user level support. Results of RIFM sponsored studies are published in open, peer-reviewed

scientific literature. In addition to sharing data with official international agencies, RIFM actively works with international industry associations. RIFM also maintains, for its members, the most extensive technical database of human health effects, environmental fate, and product regulations on fragrance and flavor ingredients.

RIFM's stated mission includes being the international scientific authority for the safe use of fragrance materials. The stated mission also includes engaging in research and evaluation of fragrance materials through an independent expert panel; determining safety in use; gathering, analyzing, and publishing scientific information; distributing scientific data and safety assessment judgments to RIFM members, industry association and other interested parties and maintaining an active dialog with official international agencies.

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Further Reading

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Society for Chemical Hazard Communication

Michele R Sullivan

History

The Society for Chemical Hazard Communication (SCHC), originally know as the American Conference on Chemical Labeling (ACCL), was incorporated in 1982 as a nonprofit professional organization, but it had actually started several years earlier. When the Chemical Manufacturers Association (CMA) discontinued the Labeling and Precautionary Information Committee, its members recognized the need for individuals responsible for labeling to have a forum to discuss ideas and keep up to date on new requirements. CMA initially sponsored ACCL and its first four meetings.

The ACCL membership grew from 40 people in 1979 to ~700 in 2003. Over time the 'labeling issues' grew into 'hazard communication issues'. In 1992, ACCL changed its name to SCHC to reflect the expanded issue of hazard communication and the needs of the expanded membership. As the society grew, the structure has become more formal. However, SCHC is essentially a volunteer organization. Today, there is an elected Board of Directors consisting of seven members, the past president, president, vice president, and secretary–treasurer. To accomplish the Society's goals, SCHC has various standing committees: Arrangements, Awards, Exhibit, HazCom Resources, Membership, Newsletter, Nominating, Professional Development, Program, Small Package, and Web. The Board of Directors manages the affairs of the Society and its committees.

The Society

Purpose

SCHC's purpose is to promote effective communication of chemical hazards. The Society is committed to sharing knowledge and resources to ensure a consistent and uniform approach to assessing and communicating chemical hazards on product labels, material safety data sheet (MSDS), and other product literature and documentation by

- Monitoring legislative and standards development.
- Broadening awareness of new developments in research and practice.

- Facilitating understanding and interpretation of regulatory requirements.
- Fostering professional development.
- Providing opportunities for professional networking and exchange of ideas.
- Serving as a primary source of information on international standards regarding hazard communication.

Membership

SCHC is a professional society of individuals who are engaged in the business of hazard communication. The members have a broad range of occupations – chemistry, industrial hygiene, and toxicology are a few examples. Their jobs are also diverse. Many prepare labels and MSDSs for their employers' products. Others train users of hazardous chemicals, act as expert witnesses, or implement government regulations. They work in industry, government, and academia.

SCHC welcomes members who are involved in the field of hazard communication.

Meetings

The Society holds meetings to provide up-to-date information on current developments and education and networking opportunities for its members. Meetings are regularly held in the Washington, DC, area and frequently feature regulatory updates from the federal agencies involved in hazard communication requirements. In addition, other meetings are held at selected cities across the country that will attract membership participation and attendance. As part of its meetings, SCHC allows time for members to network with each other, has a section of the program allotted to member updates, and holds new member luncheons.

The SCHC strives to keep its members aware of the latest developments concerning hazard communication. Topics at meetings include: Internet resources; American National Standards Institute (ANSI) Standards; Environmental Protection Agency, Department of Transportation (DOT), and Occupational Safety and Health Administration (OSHA) updates; and international information. A major topic has been the international harmonization of hazard communication, the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals. The development of the system was completed in 2001, adopted/endorsed by the UN in 2002, and is expected to affect hazard communication globally.

Also of interest to SCHC members are issues that affect their industry and professions. Presentations on these topics include: managing hazard communication programs; and liability in writing MSDSs and labels. Continuing education/maintenance points can be obtained for SCHC meetings.

Members are given the opportunity of participating in a technical poster session at meetings where they can exchange ideas in an informal environment. Ideas are presented as posters in an atmosphere where authors and attendees can mingle. Any topic related to chemical hazard communication, in its broadest sense, may be presented at the poster session. Poster Abstracts are published, distributed at the meeting, and posted on the web. The poster session is an excellent opportunity to present ideas and to receive feedback from other hazard communication professionals.

A computer software and vendor exhibit is also held once a year. This exhibit features vendors of products that aid SCHC members in the creation and maintenance of information for hazard communication. Members can interact at the exhibit with commercial product and service providers who display the latest in hazard communication technology and resources.

Professional Development

The Society's purpose has always been to educate and provide information on hazard communication. Professional development courses were first offered by SCHC in 1990 with a single half-day course. Today, the society offerings have grown to over 25 professional development courses including basic, advanced, and in-depth multiday courses. Continuing education/maintenance points can be obtained for SCHC courses.

Basic professional development courses offered by SCHC are: MSDS and Label Preparation Workshops; Science, Toxicology and Industrial Hygiene for Hazard Communication; and Hazard Determination & Risk Assessment. Regulatory courses include: Canadian & Mexican Hazard Communication; Pesticide & Consumer Product Labeling; Component Disclosure Requirements; European Union Hazard Communication; Transportation Classification & Labeling; HMIS/NFPA Labeling; and International Chemical Control Laws.

Several advanced courses have been developed such as Reproductive & Developmental Toxicology; Endocrine Disrupters, Clinical & Occupational Toxicology; Occupational Exposure Limits; and Life Cycle Assessment.

New courses on topics of current interest include: Hazard Communication for Asia, Pacific Rim & Latin America; Ecotoxicology for Hazard Communication;

and the GHS of Classification and Labeling of Chemicals.

SCHC offers HAZCOM 101, a 2 day course. This course, the first of its kind in the United States, is designed for people who have little formal HazCom training and are recently assigned to hazard communication, MSDS, labeling, or regulatory compliance responsibilities. The curriculum presents basic information, provides reference material, and practical exercises.

The SCHC Professional Development Committee recognizes students who have accumulated 40, 80, and 120 or more hours of SCHC professional development training and instructors and/or course directors who have contributed to 15 or 30 SCHC courses.

Outreach

The Society has a history of collaboration and outreach. The OSHA Hazard Communication Standard (HCS), 29 CFR 1910.1200, was published in 1983. Shortly thereafter, SCHC and OSHA collaborated to educate stakeholders on the new HCS. Jointly sponsored seminars were held on a regional basis with both OSHA and SCHC participating to inform both members and stakeholders about the HCS. Recently, SCHC and OSHA have signed an alliance to provide information and training on hazard communication, MSDSs, and the new GHS of Classification and Labeling of Chemicals. This alliance is another step in the longstanding relationship between SCHC and OSHA to promote effective hazard communication.

Publications

The Society maintains a website. The site contains Society and membership information, SCHC presentations, SCHC newsletters, SCHC meeting material, and professional development courses. There are links to hazard communication and related websites and a list of hazard communication and translation resources. Updates on legislative and standards activity concerning hazard communication are posted.

The *SCHC News* is published for distribution to members. It includes pictures, meeting information, articles, and news of general interest to SCHC members and others interested in hazard communication issues.

SCHC runs an Internet discussion group, known as the Forum. Comments and questions related to hazard communication and SCHC's activities can be posted. Forum topics include: United States of America, Canadian WHMIS, European Union, and Pacific Rim HazCom; general HazCom topics, and SCHC meeting and activity topics. Each discussion forum has a moderator and both the moderator and members participate in the discussions.

SCHC Awards

To recognize contributions to both the Society and the field of hazard communication, SCHC offers three types of awards. Nomination for awards are solicited by the Awards Committee and reviewed by the Board. The *HazCom Lifetime Achievement Award* recognizes individuals who have contributed significantly to the field of hazard communication or to SCHC over an extended period of time. Recipients of the award must have achieved at least one of the following additional criteria: exceptional performance in the field of hazard communication; lasting impact on the practice of hazard communication; and/or broad benefits to hazard communication professionals or users of hazard communication information.

The *Award for Excellence in Hazard Communication* recognizes individuals or groups who have made significant contributions to the field of hazard communication. The individual or group need not be a member of SCHC. Examples of activities meriting this award include: developing 'systems' to address hazard communication matters; publications in journals or periodicals; and formation of panels or organizations addressing hazard communication.

The *SCHC Distinguished Service Award* recognizes individuals who have contributed outstanding services to SCHC beyond their function as committee members or chairs.

Related Organizations

As SCHC's interests broadened to global hazard communication, international speakers were invited to SCHC meetings. A speaker from the United Kingdom (UK) Health and Safety Executive believed that hazard communication in the UK could benefit from a similar organization. SCHC provided infromation about forming a society. In 1994 a group of hazard communication professionals in the UK formed the Chemical Hazards Communication Society (CHCS). (http://www.ches.org.uk).

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Society for Environmental Toxicology and Chemistry

Harihara M Mehendale

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, and others interested in environmental issues such as managers and engineers. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in 1979 to fill this void. Based on growing membership, attendance in meetings, and publications, the forum was needed.

A unique strength of SETAC is its commitment to balance the interests of academia, business, and government. The Society by-laws mandate equal representation from these three sectors for World Council Officers, Board of Directors/Council Members, and Committee members. And although there is no control mechanism, the proportion of members from each of the three sectors has remained nearly equal over the past 24 years.

Like many other professional societies, SETAC publishes an esteemed scientific journal and convenes annual meetings replete with state-of-the-science poster and platform presentations. Because of its multidisciplinary approach, however, the scope of the science of SETAC is much broader in concept and application than that of most other societies.

SETAC is concerned about global environmental issues. Its members are committed to good science worldwide, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. SETAC was founded in North America but membership was open to environmental scientists worldwide. SETAC Europe was organized in 1989, SETAC Asia/Pacific in 1997, and SETAC Latin America in 1999. Members voted overwhelmingly in 2001 to combine these 'geographic units' into one global society to form the SETAC World Council. SETAC meets the professional needs of individuals at local and regional levels throughout all geographic units, throughout national branches and chapters (Argentina, Brazil, United Kingdom, and soon-to-be organized Japan), through regional chapters (16 in North America), and through national-language chapters (Germany, France, and Iberia). International acceptance of the SETAC model continues with widespread interest in Russia and Africa. It is now the job of the SETAC World Council to oversee the myriad SETAC activities around the world and to assure the integrity of the Society.

Membership has increased from 230 Charter Members in October 1980 to nearly 5000 members from 50 US states, 13 Canadian provinces, and more than 60 other countries worldwide. Participants and technical presentations at SETAC annual meetings in North America have increased from 470 delegates and 86 technical presentations in 1980, to 2200 delegates and 1600 presentations in 2003. Annual meetings in Europe began in 1991 with 500 delegates and more than 200 presentations. In 2003, there were 1400 delegates and 1100 presentations. Meetings are also held in Asia/Pacific and Latin America.

Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1982 to a monthly publication of 3094 pages in 2003. SETAC publishes the global newsletter, *SETAC Globe*, peer-reviewed workshop and symposia proceedings, and a variety of technical reports.

Purpose and Goals

SETAC is a nonprofit, professional society established to provide a forum for individuals and institutions engaged in the study of environmental issues, management and regulations of natural resources, education, and research and development.

Environmental toxicology and chemistry, in their broadest sense, embrace components of classical toxicology; physiology; genetics; biology; microbiology; ecology; anatomy; organic, environmental, and analytical chemistry; soil, water, and atmospheric sciences and engineering; and economics.

The purpose of the Society is to

- Promote research, education, training, and development in areas of environmental toxicology and chemistry, and promote the collective application of these sciences to risk assessment.
- Disseminate information on environmental toxicology and chemistry, and participate in the application of these sciences to issues concerned with the technology of risk assessment and risk management.
- Promote the study of concepts and the implementation of programs that can be used for the development of ecologically acceptable practices and principles.
- Provide a forum for communication among professionals in government, business, academia, and other segments of society involved in the use, protection, and management of the environment and in the protection and welfare of the general public.

The goals of the Society are to

- Represent toxicologists, chemists, engineers, and others interested in the environmental sciences at the local, regional, national, international, and global levels.
- Facilitate identification, evaluation, resolution, and communication of environmental problems and issues among SETAC members, and the global community of environmental scientists, engineers, and the general public.
- Organize and conduct local, regional, national, and international meetings, workshops, and symposia for SETAC members and others interested in the environmental sciences.
- Publish, disseminate, and archive a peer-reviewed journal (*Environmental Toxicology & Chemistry*), technical documents, and other materials concerning environmental issues and SETAC affairs.
- Provide scientific information to planners, legislators, managers, regulators, and others to influence the development and application of rational environmental policies, laws, and regulations.
- Assume an active leadership role in the development of environmental education programs and provide educational opportunities for SETAC members, the general public, and others interested in the environmental sciences.
- Provide efficient and effective management and service to SETAC members, and assure continuity of operations.
- Continue to strive for a membership balance among academia, government, and business.
- Encourage the integration of voluntary and professional services in the management operation of SETAC.
- Encourage participation of all members in Society affairs and continue to provide equal opportunity in the governance of SETAC.
- Obtain resources sufficient to accomplish goals.

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Society for Risk Analysis (SRA)

Mike Dourson and Pertti J Hakkinen

History, Purpose, and Objectives

The Society for Risk Analysis (SRA) was established in 1981 as a nonprofit organization to foster and promote: (1) knowledge and understanding of risk analysis techniques and their applications; (2) communication and interaction among individuals engaged in risk analysis; (3) application of risk analysis and risk management techniques to the hazards and risks to which individuals and populations are exposed; (4) dissemination of risk analysis information and concepts to all interested individuals; (5) advancement of the state-of-the-art techniques in all aspects of risk analysis; and (6) integration and interaction of the various disciplines involved in risk analysis.

Membership Criteria

There are currently ~2500 members of SRA, with international representation. Members of SRA include professionals from a wide range of institutions, including federal, state, and local governments, small and large industries, private and public academic institutions, not-for-profit organizations, law firms, and consulting groups. Those professionals include statisticians, engineers, safety officers, policy analysts, economists, lawyers, environmental and occupational health scientists, natural and physical scientists, environmental scientists, public administrators, and social, behavioral, and decision scientists.

There are five classes of members, including active members, student members, retired members, Fellows (see below), and sustaining members (organizations interested in risk analysis).

Membership Benefits and Website Contents

Members get voting privileges in the election of officers and councilors, and web-based access to the membership directory. In addition, members get a subscription to a bimonthly journal, *Risk Analysis*, and a quarterly newsletter, the *RISK Newsletter*. Members have web-based access to current and several recent years of issues of this journal and

newsletter. The SRA website provides online access to additional information, for example, funding opportunities for research, employment opportunities, links to sources of specific risk-related resources (data, models, technical reports, etc.), instructions for subscribing to an Internet (RISKANAL) mailing list, and a glossary of risk analysis terms.

Details about the Journal

The journal *Risk Analysis* provides a focal point for new developments in risk analysis covering a wide range of disciplines. It covers topics of interest to regulators, researchers, and scientific administrators, including research results on health risks, and the engineering, mathematical, and theoretical aspects of risks. This journal focuses on manuscripts dealing with measurements, modeling, instrumentation, questionnaires, and studies of chemicals. In addition, it covers the social and psychological aspects of risk such as risk perception, acceptability, economics, and ethics. All scientific articles in *Risk Analysis* are peer reviewed.

Meetings, Specialty Groups, Sections, and Chapters

Through its meetings and publications, SRA fosters a dialog on health, ecological, and engineering risks and natural hazards, and their socioeconomic dimensions. SRA has helped develop the field of risk analysis and has improved its credibility and viability. The society has a number of chapters and sections around the world and sponsors an annual meeting of the society, usually in December. The annual meeting includes meetings of specialty groups on dose response, economics and benefits analysis, ecological risk assessment, engineering, exposure assessment, food/water safety risk, risk communication, and risk science and law. The sections of SRA include SRA Europe and SRA Japan, and the chapters of SRA are located in various parts of the United States and Canada, and in other parts of the world. The sections and chapters conduct their own meetings at different locations and times; SRA's website contains contact information and summaries of the meetings.

Awards

Various awards are announced at the annual meeting, including the Distinguished Achievement Award (honors any person for extraordinary achievement in science or public policy relating to risk analysis), Outstanding Service Award (honors SRA members for extraordinary service to the Society), Outstanding Risk Practitioner Award (honors individuals who have made substantial contributions to the field of risk analysis through work in the public or private sectors), Chauncey Starr Award (honors individuals age 40 and under who have made exceptional contributions to the field of risk analysis), and the Fellow of the Society for Risk Analysis Award (recognizes and honors up to 1% of the Society's membership, selected based upon substantial achievement in science or public policy relating to risk analysis and substantial service to SRA. Fellows include all former SRA presidents).

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Society of Toxicology*

Sachin S Devi and Harihara M Mehendale

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Objectives

The Society of Toxicology (SOT) is a professional organization of scientists from academic institutions, government, and industry representing the great variety of scientists who practice toxicology in the United States and abroad. SOT promotes the development and integration of innovative basic and applied toxicology to enhance human, animal, and environmental health. Each member must commit to the SOT Code of Ethics. The Society facilitates the exchange of information among its members as well as among investigators in other scientific disciplines. SOT has a strong commitment to education in toxicology and to the recruitment of students and new members into the profession.

*Compiled from the information provided by Society of Toxicology.

History and Organization

SOT was founded in 1961 as a not-for-profit scientific society. The Society is governed by an 11 person elected Council and managed by an administrative office in the Washington, DC area.

The Society's activities are highly diverse and assisted by the efforts of 22 committees and task forces, such as Animals in Research Committee, Career Resource and Development Committee, Education Committee, Regulatory Affairs and Legislative Assistance Committee, and the Task Force for a Chemical/Biological Terrorism Resource Registry.

Membership

Currently, SOT has more than 5250 members in 44 countries. The majority of members are practicing toxicologists and scientists from allied disciplines. The Society offers three kinds of individual memberships: full, associate, and student/postdoctoral. There are 53 companies and other related organizations listed as SOT Affiliates. Undergraduate Affiliate status is available for pre-baccalaureate students. In addition, the Council may award honorary memberships to persons who are not members of the Society in recognition of outstanding and sustained achievement in the field of toxicology.

Specialty Sections

The Society has established 19 specialty sections that may propose sessions for the annual meeting, exchange information via newsletters, present awards, and participate in other scientific activities as subdisciplinary groups. The specialty sections of the Society are Biological Modeling, Carcinogenesis, Comparative and Veterinary, Dermal, Ethics, Legal & Social Issues, Epidemiology and Occupational Health, Food Safety, Immunotoxicology, *In Vitro*, Inhalation, Mechanisms, Metals, Molecular Biology, Neurotoxicology, Regulatory and Safety Evaluation, Reproductive and Developmental Toxicology, Risk Assessment, Toxicologic and Exploratory Pathology, and Women in Toxicology.

Regional Chapters

The SOT has 18 regional chapters that sponsor regular local meetings throughout the year. The purpose of the regional chapters is to foster scientific exchange at a local level, including regional meetings, poster awards for students, newsletters, as well as proposals for the annual meeting. Each regional chapter selects a student who serves on the Student Advisory Committee, the student voice in the SOT.

Publications

Toxicological Sciences, the official SOT journal, is distributed monthly in print and electronically. The journal publishes premier peer-reviewed, hypothesis-driven, original research articles that are broadly relevant to assessing the potentially adverse health effects resulting from exposure of humans or animals to chemicals, drugs, natural products, or synthetic materials. Studies may involve experimental animals or human subjects, or they may focus on *in vitro* methods or alternatives to the use of experimental animals. Sections include original research, reviews, forum articles on policy or research issues, editorials, letters to the editor, and supplementary data guidelines.

The Toxicologist, the abstract issue of *Toxicological Sciences*, is also an official publication of the SOT. Abstracts are from presentations for the symposium, platform, workshop, roundtable, poster, and continuing education sessions at the SOT Annual Meetings.

In striving to be the premier source of information in toxicology, SOT has gathered a diverse array of information for the public and for members on the Society webpage. The Society also publishes the *Communiqué*, the member newsletter, distributed electronically four times a year. The spring Special Edition is also printed.

Meetings

SOT conducts an annual meeting, the largest of its kind in the world. The meeting occurs in March of each year and over 2100 papers are presented on a variety of subjects. Sessions include platform sessions, workshops, and poster sessions. Continuing education courses and symposia sponsored by specialty sections of the Society are regular features at the annual meeting. The abstracts of all presented papers are published annually in *The Toxicologist*.

ToxExpo® provides the opportunity for attendees to see the latest in cutting-edge technology and services available on the market today and meet with vendors face-to-face. Online *ToxExpo*® provides access to products and services all year.

SOT offers occasional Contemporary Concepts in Toxicology workshops, and cosponsors a variety of meetings with other groups and governmental agencies.

Awards and Grants

The Society presents several awards annually that recognize outstanding achievement in the field of

toxicology. SOT also supports travel grants, fellowships, and other student awards. Special awards are presented at the discretion of the Council. SOT also presents a number of sponsored awards.

Related Societies

SOT maintains liaison with numerous affiliated societies, participates in the International Union of Toxicology, and supports intersociety activities and meetings.

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Trade Associations

Patricia M Nance

Trade associations are individuals and companies in a specific business or industry organized to promote common interests. Trade associations are associated with a type of business, type of product, or a specific product. The focus of this article is on trade associations in the United States, and non-US readers are urged to conduct Web searches for trade associations relevant to their chemicals, products, country, region, and continent. (A limited number of international trade associations appear as individual entries in this book, including the European Centre for Ecotoxicology and Toxicology (ECETOC), Flavor and Extract Manufacturers Association (FEMA), International Fragrance Association (IFRA), International Organization of the Flavor Industry (IOFI), and the Research Institute for Fragrance Materials (RIFM).)

The listing below includes various common trade associations related to the field of toxicology, focusing on those relevant to the United States.

American Chemistry Council

The American Chemistry Council represents the leading companies engaged in the business of chemistry. Council members apply the science of chemistry to make innovative products and services that make people's lives better, healthier, and safer. The Council is committed to improved environmental, health, and safety performance through Responsible Care®, common sense advocacy designed to address major public policy issues, and health and environmental research and chemical testing. The business of chemistry is a $450 billion enterprise in the United States and a key element of the nation's economy. It is the nation's largest exporter, accounting for 10 cents out of every dollar in US exports. Chemical companies invest more in research and development than any other business sector.

Chlorine Chemistry Council

The (US) Chlorine Chemistry Council® (CCC), a business council of the American Chemistry Council, is a national trade association based in Arlington, VA, representing the manufacturers and users of chlorine and chlorine-related products. CCC strives to achieve policies that promote the continuing, responsible uses of chlorine and chlorine-based products. Chlorine is widely used as a disease-fighting disinfection agent, as a basic component in pharmaceuticals and myriad other products that are essential to modern life.

Cosmetic, Toiletry, and Fragrance Association

The (US) Cosmetic, Toiletry, and Fragrance Association (CTFA) provides a complete range of services that support the personal care products industry's needs and interests in the scientific, legal, regulatory, legislative, and international fields. CTFA strives to ensure that the personal care products industry has the freedom to pursue creative product development and compete in a fair and responsible marketplace. CTFA represents the industry's interests at the local, state, national, and international levels, promoting voluntary industry self-regulation and reasonable governmental requirements that support the health and safety of consumers.

CTFA has ~600 member companies. Active members are manufacturers and distributors of finished products. Associate members are suppliers of

ingredients, raw materials, packaging, and other services used in the production and marketing of finished products, as well as consumer and trade publications. The association also coordinates educational activities and supports public service programs such as 'Work Your Image!', which in partnership with 'Women Work!' provides women reentering the work force with guidance on hygiene and the importance of a professional appearance in getting and keeping a job.

The CTFA Foundation works with the American Cancer Society and the National Cosmetology Association in implementing 'Look Good...Feel Better', a free, public service program that teaches makeup techniques to women undergoing cancer treatment, helping them to regain their self-confidence and to better cope with the appearance-related side effects of chemotherapy and radiation. CTFA also supports the Cosmetic Ingredient Review (CIR), a program it helped establish in 1976, which assesses the safety of ingredients used in cosmetics in an unbiased, independent forum with an expert panel comprised of world-renowned physicians and scientists.

Halogenated Solvents Industry Alliance, Inc.

The Halogenated Solvents Industry Alliance, Inc. (HSIA) was formed in 1980 by a group of executives in the chlorinated solvents industry to meet the growing challenges of government regulation. HSIA is dedicated to serving the interests of the halogenated solvents industry – interests that include solvent equipment manufacturers, and producers, distributors, and commercial users of halogenated solvents. By working together, the halogenated solvents industry and HSIA protect industry interests and promote the safe and responsible use of chlorinated solvents. From its office in Washington, DC, HSIA represents companies that manufacture, distribute, and use methylene chloride, perchloroethylene, trichloroethylene, and other halogenated compounds. HSIA places great emphasis on staying ahead of and actively participating in the decision-making process. The staff collects and analyzes information about the halogenated solvents and government plans and activities relating to them, and relays that information to HSIA board and committee members.

HSIA communicates with the European Chlorinated Solvent Association (ECSA) and the Japan Association for Hygiene of Chlorinated Solvents (JAHCS). The European Chlorinated Solvent Association was formed over 25 years ago by the leading chlorinated solvent manufacturers in Europe. Like HSIA, the goals of ECSA and JAHCS are to support safe use of chlorinated solvents and to encourage balanced regulation.

International Copper Association, Ltd.

The International Copper Association, Ltd. (ICA) is the leading organization for promoting the use of copper worldwide. ICA increases awareness and usage of copper by communicating the unique attributes that make this sustainable element an essential contributor to the formation of life, to advances in science and technology, and to a higher standard of living throughout the world. The Association's 35 member companies represent ~80% of the world's refined copper output and are among the largest copper producers, copper alloy fabricators, and wire and cable companies in the world. ICA is responsible for guiding policy, strategy, and funding international initiatives and promotional activities. Headquartered in New York, ICA has regional offices in Brussels, Santiago, Shanghai, and Singapore. ICA's programs and initiatives are executed in 24 countries through regional offices, 27 copper promotion centers, and copper fabricating companies. Programs to accomplish the goals of ICA's strategic plan are focused on copper's major end uses. These include wire and cable for the transmission of power and information, plumbing systems for potable water, products for architectural and industrial applications, scientific studies regarding copper's role in human health and the environment, and worldwide communications about the benefits of copper. ICA's mission is "to promote the use of copper by communicating the unique attributes that make this sustainable element an essential contributor to the formation of life, to advances in science and technology, and to a higher standard of living worldwide." The association was formed in 1989 by 24 of the world's leading primary copper producers to coordinate and improve the effectiveness of the international market development, research, and technology activities of the industry. The association evolved from the International Copper Research Association (INCRA), established in 1960.

Nickel Institute

The Nickel Institute, whose members represent over 70% of current world production, generates and communicates knowledge required to support safe and sustainable production, use and reuse of nickel. It was established on January 1, 2004.

For consumers, governments, regulators, and other stakeholders, the Nickel Institute is committed to responding effectively to the growing requests for

nickel-related information. For nickel producers and users it offers research-based, cutting-edge science and technical information.

The Institute provides a single membership and management structure for activities previously undertaken by the Nickel Development Institute (NiDI) and the Nickel Producers Environmental Research Association (NiPERA). NiPERA is an independently incorporated division of the Nickel Institute, continuing as a well-respected provider of peer-reviewed, published information on the human health and environmental science of nickel.

The Nickel Institute continues the use-related technical work of NiDI, but focuses more on nickel issues related to stewardship and sustainable development, especially the generation and use of knowledge about the full life cycle impacts of nickel.

The Institute develops partnerships with organizations representing the interests of the nickel-producing industry's downstream customers and other parts of the nickel life-cycle. The Institute also collaborates with regional and local metals industry organizations.

Soap and Detergent Association

Established in 1926, the (US) Soap and Detergent Association (SDA) is the national, nonprofit trade association representing ~135 manufacturers of household, industrial, and institutional cleaning products; the ingredients used in cleaning products; and finished packaging. SDA is dedicated to advancing public understanding of the safety and benefits of cleaning products, and protecting the ability of its members to formulate products that best meet consumer needs. SDA serves both its members and the public by developing and sharing information about industry products with the technical community, policy makers, child care and health professionals, educators, media, and consumers. SDA members produce more than 90% of the cleaning products marketed in the United States. Membership is open to US, Canadian, and Mexican companies.

Synthetic Organic Chemical Manufacturers Association

The (US) Synthetic Organic Chemical Manufacturers Association (SOCMA) is the leading trade association serving the specialty-batch and custom chemical industry since 1921. Its 300 member companies have more than 2000 manufacturing sites and 100 000 employees. SOCMA members encompass every segment of the industry, from small specialty producers to large multinational corporations, and manufacture 50 000 products annually valued at 60 billion dollars.

Batch chemical manufactures play a key role in the US chemical industry producing intermediates, specialty chemicals, and ingredients that are used to develop a wide range of commercial and consumer products. Thus, SOCMA's member companies manufacture products that are key building blocks and ingredients for a range of other production operations. Specialty chemicals made by many SOCMA members are formulated to meet the detailed specifications of various end users, and usually have unique purposes, such as making nylon fibers stronger or serving as the active ingredient in medicine. Therefore, specialty chemicals are often essential elements in the end-user's manufacturing process.

In batch manufacturing, the raw materials, processes, operating conditions, configuration of equipment, and end products change on a regular basis. Batch producers must respond quickly to new requests by customers, fill small market niches, and participate in the development of new products. The depth and expertise of this industry sector are vital components of the US chemical industry and contribute significantly to US global competitiveness.

Further Reading

Hakkinen PJ, Stoss FW, Behrendt B, and Wexler P (2000) Organizations. In: Wexler P, Hakkinen PJ, Kennedy GL Jr., and Stoss FW (eds.) *Information Resources in Toxicology*, 3rd edn., pp. 439–489. San Diego: Academic Press.

Wukovitz LD (2001) Using internet search engines and library catalogs to locate toxicology information. *Toxicology* 157: 121–139.

Relevant Websites

http://www.socma.com – Synthetic Organic Chemical Manufacturers Association.

http://www.americanchemistry.com – American Chemistry Council.

http://c3.org – Chlorine Chemistry Council.

http://www.ctfa.org – Cosmetic, Toiletry, and Fragrance Association.

http://www.eurocholor.org – European Chlorinated Solvent Association.

http://www.hsia.org – Halogenated Solvents Industry Alliance, Inc.

http://www.copperinfo.com – International Copper Association, Ltd.

http://www.nickelinstitute.org – Nickel Institute.

http://www.nipera.org – Nickel Institute (for health and environment issues only).

http://www.sdahq.org – Soap and Detergent Association.

Toxicology Excellence for Risk Assessment

Jacqueline Patterson

Toxicology Excellence for Risk Assessment (TERA), a nonprofit organization, has a mission to protect public health by developing and communicating human risk assessment values, improving risk methods through research, and educating the public on risk assessment issues. Dr. Michael Dourson, formerly of the United States (US) Environmental Protection Agency (EPA), established TERA in 1995. Project areas include: analyzing toxicity data and developing chemical risk assessments; compiling and distributing a comparative database of human health risk values from leading government agencies and organizations around the world; conducting cutting-edge research to improve risk assessment methods and approaches; convening expert peer review and consultations of risk documentation; and providing general assistance to US, other nonprofit organizations, and the public on human health risk assessment issues.

TERA is a small organization of ~12 scientists with expertise in toxicology, human health risk assessment, noncancer and cancer risk methods, pharmacology, risk communication, environmental science, technical writing, and other disciplines.

Chemical Risk Assessments

TERA scientists analyze available human and animal toxicity data to determine the potential for human health effects from exposure to chemicals. These assessments can include hazard assessments and determination/evaluations of mode of action and weight of evidence determinations for relevance of particular endpoints/effects to humans from environmental or occupational exposures. When adequate data are available, TERA derives noncancer and cancer risk estimates for various routes of exposure. TERA frequently publishes the results of the finalized assessment in peer reviewed journals and posts the assessments on its website.

Risk Assessment Database – International Toxicity Estimates for Risk

TERA created and manages the International Toxicity Estimates for Risk (ITER) database, a comparative database of human health risk values and cancer classifications from leading government agencies and organizations around the world. ITER is a free Internet database of human health risk values for over 600 chemicals. It can be accessed directly from the TERA website and from the (US) National Library of Medicine's TOXNET compilation of databases.

The database includes tabular summary information on the risk estimates and evaluations, along with information on peer review and links to source documents. The format allows for easy comparisons across organizations and, as appropriate, includes an explanation as to why the values may differ. ITER includes risk values and/or cancer classifications from the Agency for Toxic Substances and Disease Registry (ATSDR, United States), Health Canada (Canada), International Agency for Research on Cancer (IARC, World Health Organization member), National Institute of Public Health and the Environment (RIVM (see Relevant Websites section), The Netherlands), and the EPA (United States). In addition, risk values that have undergone independent peer review (see below) are included. Risk estimates and evaluations by additional groups will be added in the future.

Research into Risk Assessment Methods

TERA scientists conduct research to further develop and improve the scientific approaches used to evaluate human health risks from exposures to chemicals and other substances. TERA scientists have led efforts in the development of the reference dose, categorical regression, and use of mechanistic data in risk assessment. Areas being addressed include a chemical's mode of action for toxicity and evaluating the human health relevance of animal data, dose–response modeling to better estimate risk, considerations of special sensitivity of children and other subgroups, refining methods for estimating occupational exposure limits, and application of human health risk approaches to nonchemical exposures.

Peer Review and Consultation Meetings

TERA provides peer consultation and peer review services to meet the needs of public and private sponsors who have developed risk assessment documentation. Work products such as chemical assessments, methodologies, guidance documents, protocols for studies, or research plans are reviewed during expert panel meetings that are open to the public. TERA

works independently of the sponsors and authors of the documents to select an unbiased panel of experts. TERA scientists write reports of each peer review and consultation that summarize the discussions and conclusions of the panels. Risk values and cancer classifications that have been approved by an independent peer review panel may be included on the ITER database (see above).

Technical Assistance and Education

In keeping with its mission and nonprofit status, TERA provides a limited amount of free technical assistance to government and nonprofit organizations. This may include brief reviews of documents, guidance over the telephone, or a written review of an assessment done by the organization. TERA also develops educational materials on health risks of chemicals and risk assessment methods.

Contact Details

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URL: http://www.tera.org

Further Reading

Dourson ML and Patterson J (2003) A 20-year prospective on the development of non-cancer risk assessment methods. *Human and Ecological Risk Assessment* 9: 1239–1252.

Dourson ML, Anderson P, Cartledge D *et al.* (2002) Comparative dietary risk: Balance the risk and benefits of fish consumption. *Comments on Toxicology* (Special Issue) 8: 335–536.

Felter SP, Dourson M, and Patterson J (1997) Assessing risks to human health from chemicals in the environment. In: Calow P (ed.) *Handbook of Environmental Risk Assessment & Management*, ch. 2, pp. 9–23. Oxford: Blackwell Science.

Haber LT, Dollarhide JS, Maier A, and Dourson ML (2001) Noncancer risk assessment: Principles and practice in environmental and occupational settings. In: Bingham E, Cohrssen E, and Powell CH (eds.) *Patty's Toxicology*, 5th edn. New York: Wiley.

Relevant Websites

http://toxnet.nlm.nih.gov – TOXNET, Specialized Information Services, National Library of Medicine. Search for Toxicology Excellence for Risk Assessment.

http://www.tera.org – the process of adding oral risk data to ITER from NSF International, an independent, not-for-profit global leader in providing public health and safety risk management solutions is in progress, in the meantime more information about NSF International can be obtained at http://www.nsf.org.

Toxicology Forum

Latrice Vincent

The Toxicology Forum is an international nonprofit organization devoted entirely to the organization of open dialogs among the various segments of society concerned with problems in toxicology. Meetings are organized at which experts from government regulatory agencies, international health agencies, industry, academia, politicians, and consumers exchange views. The scientific community is aware of the scrupulously balanced approach taken in presentation of the issues at the Forum; for each issue alternative positions are presented. The presentations and comments of all participants are recorded and a transcript is made available. The unique nonadversarial atmosphere of Forum meetings promotes uninhibited, productive discussions unencumbered by a need to arrive at a consensus.

Subjects chosen for particular sessions represent the interests of Forum members. This is an important advantage of membership. Every suggestion is given a thorough review by the program committee, which comprises academic, government, and industry representatives. Because emphasis is given to leading edge problems in toxicology as well as programming flexibility, it is common for topics to be addressed years in advance of other organizations. In an era of increasing international trade, another important feature of Toxicology Forum programs is that they are international in scope. Members include people from the public and private sectors in the United States, Canada, Japan, and several European countries.

A vital part of all Toxicology Forum meetings is a session on emerging issues, recent findings, or decisions of governments. These are an unparalleled source of up-to-date information. Numerous Forum meetings resulted in productive discussions of highly controversial subjects of great importance to industry and government. This kind of leadership promotes change in the directions of toxicological research and

permits debate on the public policy aspects of regulatory reform.

The Toxicology Forum holds two to three meetings per year. The Annual Winter Meeting takes place in Washington, DC, during the month of February. The Annual Summer Meeting is held in July in Aspen, Colorado. The European Meeting is usually held in spring or autumn.

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UNEP Chemicals

Pertti J Hakkinen*

The United Nations Environment Programme (UNEP) works to protect public health and the environment worldwide. UNEP Chemicals is the focus for all of UNEP's activities, and the main catalytic force in the UN system for concerted global action on the environmentally sound management of hazardous chemicals.

The main goals of UNEP Chemicals are to catalyze actions and to promote chemical safety by: providing countries with access to information about toxic chemicals; to assist countries in building their capacities to produce, use, and dispose of chemicals safely; and support global actions that are needed to reduce or eliminate chemical risks, such as the Stockholm and Rotterdam Conventions. To achieve these goals, UNEP Chemicals works closely with governments, UN agencies, intergovernmental organizations (IGOs), and nongovernmental organizations (NGOs). UNEP Chemicals concentrates its activities in several areas.

Rotterdam Convention

The Rotterdam Convention on the Prior Informed Consent (PIC) Procedure for Certain Hazardous Chemicals and Pesticides in International Trade was adopted on 10 September 1998. The Convention was ratified by 50 countries (Parties) and entered into force (e.i.f.) on February 24, 2004. Presently, it provides a legal basis for the implementing of the existing PIC procedure that was operated on voluntary basis since 1989. UNEP and the Food and Agriculture Organization of the United Nations (FAO) jointly serve as the secretariat for the Convention.

*The author would like to acknowledge Dr. Salem Milad, Scientfic Affairs Officer, UNEP Chemicals as a provider of much of the information used in this entry.

The objective of the Convention is to protect human health and the environment from certain hazardous chemicals by promoting shared responsibilities and cooperation among Parties with respect to their international trade and environmentally sound use, by facilitating relevant information exchange, and by providing an agreed process for making national decisions on the import and export of these chemicals, and for distribution of such decisions to Parties. At the present the Convention subjects to the PIC procedure 22 hazardous pesticides and five industrial chemicals. There are provisions for exchanging specified information between Parties, for labelling potentially hazardous chemicals that may be exported and imported, and for informing Parties of any national decision to ban or severely restrict a chemical. Other chemicals will be added to the Convention in the future through a specified process in which a Chemical Review Committee will evaluate candidates for addition, including pesticide formulations, nominated by developing countries or countries with economies in transition, and chemicals or pesticides that have been banned or severely restricted for health and environmental reasons by Parties in at least two geographic regions.

The UNEP/FAO joint Secretariat supports and promotes the Convention by:

- Continuing the implementation of the PIC procedure as outlined.
- Promoting understanding, training, ratification, and facilitating a smooth transition to the implementation of the Convention now that it is in force.
- Convening of the 11th session of the Intergovernmental Negotiating Committee that developed the Convention held in September 2004.
- Preparing for the meeting of the 9th Conference of the Parties (COP-1) of the Convention held in September 2004.

Stockholm Convention

The Stockholm Convention on Persistent Organic Pollutants (POPs) was adopted on May 22, 2001. It has been ratified by 50 Parties (countries) and entered into force (e.i.f.) on May 17, 2004.

The Convention was developed in response to the urgent need for global action to protect human health and the environment from 'POPs' through measures designated to reduce and eliminate their release. These are chemicals that are highly toxic, persistent, bioaccumulate, and move long distances in the environment.

Presently under the Convention, Parties are required to take action on an initial list of 12 specified chemicals, including intentionally produced pesticides and industrial chemicals, and unintentionally produced by-products of industrial and combustion processes. Specific goals are set for POPs, including POPs present in stockpiles and wastes.

UNEP Chemicals provides the Secretariat to the Convention. Its actions in support of the Convention include:

- Creating awareness of the POPs issue, the Convention, its provisions, and implementation actions.
- Assisting countries in developing their National Implementation Plans (NIPs).
- Regional, subregional, and national projects addressing specific issues such as dioxin/furans and PCBs.
- Strengthening institutional structures through Global Environment Facility (GEF) projects related to POPs on national and international levels.
- Preparing for the meeting of the 1st Conference of the Parties (COP-1) of the Convention held in early 2005.

Building National Capacities

The heart of UNEP Chemicals is its capacity building work that includes the following activities:

Capacity building. UNEP Chemicals is expanding and improving access to information and information tools to help countries develop the capability with which to assess and manage chemical risks. The UNEP Chemicals programme of support to governments in improving the management of chemicals has included over 75 workshops and conferences addressing priority issues, including:

- Implementing the Stockholm and Rotterdam Conventions.
- PCB identification and management.
- Dioxin and furan source identification and release estimation.
- Alternative to POPs pesticides.
- Establishing a chemicals information network.
- Best available techniques and best environmental practices.

In addition, a wide range of information products have been issued to assist countries and others responsible for chemical management in ensuring environmentally sound production, use and disposal practices.

National Implementation Plans for Stockholm Convention. The Stockholm Convention requires Parties to develop National Implementation Plans (NIPs) within 2 years of entry into force of the Convention. The NIPs outline the POPs', situation in the country, and the measures to be taken in implementing the Party's obligations under the Convention.

In 2003, 125 Countries had received funding from the Global Environment Facility (GEF) to develop their NIPs. Twelve of these countries are also participating in a pilot project aimed not only at NIPs, but also at the development of generic and technical guidelines for the development of NIPs and the adoption of POP management options. Over twenty-five national regional meetings, workshops, and/or training have been organized for the NIPs and pilot projects.

Strategic Approach to International Chemical Management (SAICM)

The development of a SAICM was mandated by UNEP's Governing Council in 2002, and subsequently endorsed by the World Summit on Sustainable Development. A consultative process engaging all stakeholders will culminate in an international conference around the end of 2005. UNEP Chemicals provides the SAICM secretariat and collaborates with a 10-organization steering committee comprising Inter-Organization Programme for the Sound Management of Chemicals (IOMC) partners, the Intergovernmental Forum on Chemical Safety (IFCS), United Nations Development Programme (UNDP), and the World Bank.

The first session of the Preparatory Committee for Development of a SAICM was held in Bangkok, from 9 to 13 November 2003 ('Prepcom1'). The second session of a SAICM was held in Nairobi, Kenya, from 4 to 8 October 2004 ('Prepcom2'). Background information and meeting documents for SAICM-Prepcom1 and Prepcom2 can be found at UNEP's website.

Mercury. In response to UNEP's Governing Council decision in 2001, UNEP Chemicals undertook an assessment of the risks from mercury in the environment. Following the publication of the 'Global Mercury Assessment' report in December 2002, the UNEP's Governing Council, in February 2003 found that there is evidence of 'significance global adverse impacts from mercury,' and called for further international action to reduce the risks to humans and wildlife. In response, a Mercury Program was established within UNEP Chemicals, which aims to promote national, regional, and global actions to reduce or eliminate as far as possible the use and release of mercury into the environment. As a first step during 2004, UNEP Chemicals began to develop plans to organize a series of workshops for developing countries to help them to identify and understand any mercury problems in their country and implement action to mitigate the problem.

Furthermore, a wide range of other information products has been issued by UNEP Chemicals, often with partner organizations like the International Programme on Chemical Safety (IPCS). For example, data sources about persistent organic pollutants include the UNEP Chemicals' extensive website home page on POPS, and reports on POPS workshops, global destruction capacity for polychlorinated biphenyls, and inventories of dioxins and furans. Other publications cover lead in gasoline, chemicals risk assessment, and the Organisation for Economic Co-operation and Development (OECD) Screening Information Data Sets (SIDS) for high protection volume (HPV) chemicals. The UNEP Chemicals Newsletter is published periodically to give readers an update of activities to promote the environmentally sound management of chemicals: A full listing of publications may be obtained and copies ordered free of charge by contacting UNEP Chemicals.

Contact Details

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United States Pharmacopoeia (USP)

Shayne C Gad

The United States Pharmacopoeia (USP) is a nongovernmental, standards-setting organization that advances public health by ensuring the quality and consistency of medicines, promoting the safe and proper use of medications, and verifying ingredients in dietary supplements. For historic reasons (plastics being used in containers for medicines), the USP also served to set the initial standards and test schemes for medical devices and device materials. USP standards are developed by a unique process of public involvement and are accepted worldwide. In addition to standards development, USP's other public health programs focus on promoting optimal health care delivery and are listed below. USP is a nonprofit organization that achieves its goals through the contributions of volunteers representing pharmacy, medicine, and other health care professionals, as well as science, academia, the US government, the pharmaceutical industry, and consumer organizations.

USP's activities and initiatives revolve around four public health programs: Standards, Dietary Supplement Verification Program, Health Care Information, and Patient Safety.

Standards

Establishing standards is a core USP activity. Currently, USP provides standards for more than 4000 prescription and nonprescription drugs, dietary supplements, veterinary drugs and health care products. These standards are published in the *United States Pharmacopoeia* and *National Formulary* (USP-NF), and are officially recognized in the Federal Food, Drug, and Cosmetic Act (21 U.S.C. ξ 321 *et seq*.). USP also produces Reference Standards, which are an integral part of USP's standards program. In addition, USP offers a Pharmacopoeial Education program that provides continuing educational courses for professionals working in the pharmaceutical industry – helping those who use the USP-NF better understand pharmacopoeial processes, standards, tests, and methods.

USP's standards-setting tradition began in 1817 when Lyman Spalding, a New York physician, responded to a growing desire among his physician

colleagues for standardization names and formulations, which, up to then, had differed from one region of the country to another. Between 1817 and 1820, working with physicians in medical schools and medical societies across the country, Spalding conducted a survey to determine those formulations physicians considered to be the most fully established and best understood. The survey asked what those medicines were called and how they were prepared. Later, Spalding and fellow physicians founded a group that met in the US Capitol's Senate chamber in Washington, DC. By the time the meeting adjourned, the groundwork was laid for the compilation of the Pharmacopoeia of the United States of America – a compendium that standardized the most fully established and best understood medicines of that era.

Today, the *United States Pharmacopoeia* and *National Formulary* contain specifications of strength, quality, purity, packaging, and labeling for more than 3800 prescription drugs, nonprescription drugs, dietary supplements, medical devices, excipients, botanicals, and other products. USP works closely with the Food and Drug Administration (FDA), the pharmaceutical industry, and the health professions to establish authoritative drug standards. These standards are enforceable by FDA and the governments of other countries and are recognized as the hallmark of quality.

Dietary Supplement Verification Program (DSVP)

USP developed this program in response to the increasing concerns expressed about dietary supplements in the marketplace. Through compliance testing and document review, adherence to good manufacturing practices (GMPs), and postmarketing surveillance, DSVP is designed to help ensure that dietary supplement products contain the declared ingredients in the declared quantities.

Health Care Information

USP provides health care professionals and patients with drug information about new and off-label uses of nearly all medicines in the United States and Canada. This drug information is contained in the *USP Drug Information* (DI) database and publications, and it is distributed in association with MICROMEDEX (a division of Thomson Publishing). USP provides oversight and approves drug information content in the USP DI. USP's Health Care Information program also consists of several global initiatives. USP was awarded several grants by the United States Agency for International Development's (USAID) Center for Population, Health and Nutrition (PHN). Currently, USAID is supporting the USP Drug Quality and Information (USP DQI) program, which funds programs in Nepal, Romania, Russia, Senegal, China, Kazakhstan, Mozambique, and the Mekong Delta region.

Patient Safety

The Center for the Advancement of Patient Safety (CAPS) was created in order to broaden USP's work within the patient safety arena. CAPS conducts data analysis and research, seeks grants, develops professional education programs, publishes articles on issues related to medication errors, participates in legislative activities, and proposes standard recommendations and guidelines for the goal of improving patient safety by preventing and reducing medication errors. In addition, USP operates two medication error reporting, tracking, and analysis programs: the Medication Errors Reporting (MER) Program (operated in collaboration with the Institute for Safe Medication Practices) and the MEDMARX^{SM} program. MEDMARX is an Internet-accessible database for hospitals to report and track medication errors anonymously (see Relevant Websites).

Collaborative Ventures

The United States Pharmacopoeia – a trusted leader in drug quality, standards, and information – in cooperation with the US Agency for International Development (USAID) manages the Drug Quality and Information (USP DQI) Program to support delivery of priority interventions for the USAID Center for Population, Health, and Nutrition. USP DQI focuses on ensuring the quality of pharmaceuticals and their informed and appropriate use worldwide.

Contact Details

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Further Reading

Anderson L and Higby GJ (1995) *The Spirit of Voluntarism, A Legacy of Commitment and Contribution: The United States Pharmacopoeia, 1820–1925*. Rockville, MD: USP.

Hicks RW, *et al.* (2004) Selected medication-error data from USP's MEDMARX program for 2002. *American Journal of Health-System Pharmacy* 61(10): 993–1000.

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APPENDIX 2
PUBLIC DOMAIN ONLINE CHEMICAL COMPENDIA – A BRIEF SELECTION

Chemical lists, compiled for regulatory, research, and other purposes, are widespread and may offer insight into potential hazards associated with chemicals. Many of these are subject to frequent change and update. A number of databases and peer-reviewed reports also contain important information on significant chemicals. Web URLs for a select number of these resources (all available at no charge) are presented below. In some cases the link is intended simply to a listing of chemicals, in others it is to peer-reviewed or otherwise substantive and reliable information on chemicals.

- Chemicals Known to the State to Cause Cancer or Reproductive Toxicity – Proposition 65 List (from the state of US California) – http://www.oehha.ca.gov

- CHEMIDplus (from the US National Library of Medicine) – a database with access to structural and nomenclature information for hundreds of thousands of chemicals, including links to toxicity and regulatory data – http://chem.sis.nlm.nih.gov

- Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Priority List of Hazardous Substances – substances that are most commonly found at facilities on the National Priorities List (NPL) and which are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure – http://www.atsdr.cdc.gov

- Environmental Fate Data Base (EFDB) (from Syracuse Research Corporation) – data related to chemical environmental fate, microbial toxicity, biodegradation, etc. – http://www.syrres.com

- European Chemicals Bureau – extensive information on chemicals in Europe – http://ecb.jrc.it

- EXTOXNET – from a consortium of universities, offering a variety of information about pesticides – http://extoxnet.orst.edu

- Hazardous Air Pollutants (from the US Environmental Protection Agency (EPA)) – those pollutants that cause or may cause cancer or other serious health effects, such as reproductive effects or birth defects, or adverse environmental and ecological effects, and which the EPA is required to control – http://www.epa.gov

- INCHEM (from the International Programme on Chemical Safety) – internationally peer-reviewed information on chemicals from intergovernmental organizations – http://www.inchem.org

- Integrated Risk Information System (IRIS) (from the US EPA) – containing information on human health effects that may result from exposure to various chemicals in the environment – http://www.epa.gov (see link to IRIS page in this website) and also via the US National Library of Medicine's TOXNET system at http://toxnet.nlm.nih.gov

- International Agency for Research on Cancer (IARC) Monogrăphs Programme on the Evaluation of Carcinogenic Risks to Humans – http://monographs.iarc.fr

- Minimal Risk Levels (MRLs) for Hazardous Substances (from the US Agency for Toxic Substances and Disease Registry) – An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure – http://www.atsdr.cdc.gov

- Pesticides Database (from Pesticides Action Network (PAN)) – toxicity and regulatory information on an extensive array of pesticides – http://www.pesticideinfo.org

- Pocket Guide to Chemical Hazards (from the US National Institute for Occupational Safety and Health (NIOSH)) – a source of general industrial hygiene information on several hundred chemicals/classes for workers, employers, and occupational health professionals – http://www.cdc.gov

- List of Drinking Water Contaminants and their Maximum Contaminant Level (MCLs) (from the US EPA) – http://www.epa.gov

- National Toxicology Program, US (NTP) – extensive information on numerous chemicals studied by this US interagency group – http://ntp-server.niehs.nih.gov

- Report on Carcinogens (from the US NTP) – includes listings of both, known human carcinogens and reasonably anticipated to be human carcinogens – http://ntp.niehs.nih.gov (click on Report on Carcinogens)

- Right-to-Know Hazardous Substances Fact Sheets (from the New Jersey Department of Health and Senior Services) – information on hazardous substances in the workplace – http://www.state.nj.us

- Risk Assessment Information System (from the Oak Ridge National Laboratory and the University of Tennessee) – includes toxicity profiles on chemicals – http://risk.lsd.ornl.gov
- International Toxicity Estimates for Risk (ITER) (from Toxicology Excellence for Risk Assessment (TERA)) – provides tabular comparisons of risk values from an assortment of agencies and countries – http://www.tera.org and also from the US National Library of Medicine's TOXNET system at http://toxnet.nlm.nih.gov

- Toxicological Profiles (from the US Agency for Toxic Substances and Disease Registry) – detailed information on hazardous substances found at National Priorities List (NPL) sites – http://www.atsdr.cdc.gov

- Toxics Release Inventory (TRI) Chemical Lists (from the US EPA) – includes lists of chemicals subject to reporting requirements under Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA) – http://www.epa.gov and also from the US National Library of Medicine's TOXNET system at http://toxnet.nlm.nih.gov

- TOXNET (from the US National Library of Medicine) – an array of databases with copious information related to many aspects of toxicology, chemical safety, and environmental health – http://toxnet.nlm.nih.gov

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Annexure 1

Abbreviations and Acronyms Used in Toxicokinetics

ADI	Acceptable daily intake
AF	Assessment factor
ALARA(P)	As low as reasonably achievable (practicable) In UK regulations relating to worker exposure In USA goal of risk management (USNRC regulations)
AUC	Area under the concentration-time curve
AUMC	Area under the moment curve
BCF	Bioconcentration factor
BEI	Biological Exposure Indices (ACGIH)
BEM	Biological effect monitoring
BOD	Biochemical oxygen demand
b.w.	Body weight
CMR	Carcinogenic, mutagenic and reproductive (toxicant)
CoMFA	Comparative molecular field analysis
Cyt	Cytochrome
CV	Ceiling value
DNA	Deoxyribonucleic acid
DNEL	Derived no-effect level
EC	Enzyme classification number or effective concentration
EC_n	Median effective concentration to n% of a population
EDI	Estimated daily intake
ED_n	Median effective dose to n% of a population
EEC	Estimated exposure concentration
EQS	Environmental quality standard
EED	Estimated exposure dose
EEL	Environmental exposure level
EMDI	Estimated maximum daily intake
GLP	Good laboratory practice
HSG	Health and Safety Guide (IPCS)
HQ	Hazard quotient
IC	Inhibitory concentration
i.c.	Intracutaneous
i.d.	Intradermal
i.m.	Intramuscular
inhl	By inhalation
i.p.	Intraperitoneal
I-TEF	International Toxicity Equivalency Factor
i.v.	Intravenous
K_M	Michaelis constant
K_{oc}	Organic carbon partition coefficient
K_{ow}	Octanol–water partition coefficient
LADD	Lifetime average daily dose
LC_n	Median concentration lethal to n% of a test population
LC_{50}	See LC_n
LD_n	Median dose lethal to n% of a test population
LD_{50}	See LD_n
LEL	Lowest effect level, same as LOEL
LOEL	Lowest-observed-effect level
LOAEL	Lowest-observed-adverse-effect level
LT_n	Median time for death of n% of a test population
LV	Limit value
MAC	Maximum allowable concentration
MEL	Maximum exposure limit
MF	Modifying factor
MOE	Margin of exposure
MPC	Maximum permissible concentration
MRL	Maximum residue limit
mRNA	Messenger ribonucleic acid
MSDS	Material safety data sheet
MTC	Maximum tolerable concentration
MTD	Maximum tolerable dose, Maximum tolerated dose
MTEL	Maximum tolerable exposure level
NADP(H)	Nicotinamide adenine dinucleotide phosphate (reduced)
ND_n	Median dose narcotic to n% of a population
NEL	No effect level, same as NOEL
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
NSC	Normalized sensitivity coefficients
PBT	Persistent, bioaccumulative and toxic
PEL	Permissible exposure limit
PBPK	Physiologically based pharmacokinetics modeling
$PM_{2.5}$	Particles in air of with a maximum aerodynamic diameter of 2.5 μm
PM_{10}	Particles in air of with a maximum aerodynamic diameter of 10 μm
PMR	Proportionate mortality rate, ratio
p.c.	Per cutim (Latin) = Through the skin
p.o.	Per os (Latin) = By mouth
POW	Octanol–water partition coefficient
PPAR	Peroxisome proliferator-activated receptor
PTWI	Provisional tolerable weekly intake
QSAR	Quantitative structure–activity relationship

3D-QSAR	Three-dimensional quantitative structure–activity relationship
QSMR	Quantitative structure–metabolism relationship
RD	Rate difference
RfC	Reference concentration
RfD	Reference dose
RNA	Ribonucleic acid
RR	Rate ratio
ROS	Reactive oxygen species
SAR	Structure–activity relationship
s.c.	Subcutaneous
SCE	Sister chromatid exchange
SMR	Standard mortality ratio
SMR	Structure–metabolism relationship
SNARL	Suggested no-adverse-response level
STEL	Short-term exposure limit
$t_{1/2}$	Half-life, half-time
TBPK	Toxicologically based pharmacokinetic modeling
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TDI	Tolerable daily intake
TEF	Toxicity equivalency factor
TEQ	Toxicity equivalent
TL_n	See LT_n
TLV	Threshold limit value (ACGIH)
TMDI	Theoretical maximum daily intake
TWA	Time-weighted average
TWAC	Time-weighted average concentration
TWAE	Time-weighted average exposure
TWI	Tolerable weekly intake
UF	Uncertainty factor
V_{max}	Maximum velocity
vPvB	Very persistent and very bioaccumulative

Annexure 2

Abbreviations and Acronyms of Names of International Bodies and Legislation

ACGIH	American Conference of Governmental Industrial Hygienists
ATSDR	Agency for Toxic Substances and Diseases Registry
BCR	Bureau Communautaire de Référence (Bruxelles)
BIBRA	British Industrial Biological Research Association
CCFA	Codex Committee on Food Additives
CCPR	Codex Committee on Pesticide Residues
CDC	Centers for Disease Control and Prevention
CEC	Commission of the European Communities
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act (USA)
CHIP	Classification, Hazard Information and Packaging (UK)
COSHH	Control of Substances Hazardous to Health Regulations (UK)
CPL	Classification, Packaging and Labeling
EC	European Community, European Commission
ECB	European Chemicals Bureau
EEA	European Environmental Agency
EEC	European Economic Community
EINECS	European Inventory of Existing Chemical Substances
ELINCS	European List of New Chemical Substances
EPA	Environmental Protection Agency (USA), same as USEPA
EUROTOX	European Society of Toxicology
EUSES	European Uniform System for Evaluation of Substances
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration (USA)
IAEA	International Atomic Energy Agency
IARC	International Agency for Research on Cancer
ICH	International Conference for Harmonization
ICRP	International Commission on Radiological Protection
ICSU	International Council of Scientific Unions (since 1998, International Council of Science)
IFCC	International Federation of Clinical Chemists
ILO	International Labour Organization
IPCS	International Programme on Chemical Safety, UNEP, ILO, WHO
IRIS	Integrated Risk Information System (USA)
IRPTC	International Register of Potentially Toxic Chemicals, now UNEP Chemicals
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
IUTOX	International Union of Toxicology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
NBS	National Bureau of Standards (USA), now NIST
NIH	National Institutes of Health (USA)
NIOSH	National Institute of Occupational Safety & Health (USA)
NIST	National Institute of Standards and Technology (USA), formerly NBS
NRC	National Research Council (USA)
OECD	Organization for Economic Cooperation and Development
OMS	Organisation Mondiale de la Santé, same as WHO
OSHA	Occupational Safety and Health Administration (USA)
RSC	Royal Society of Chemistry
REACH	Registration, Evaluation, and Authorization of Chemicals (EC)
SCOPE	Scientific Committee on Problems of the Environment (ICSU)
TOSCA	Toxic Substances Control Act (USA)
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency, same as EPA
WHO	World Health Organization, same as OMS

Further Reading

Duffus JH (1993) *Pure and Applied Chemistry* 65: 2003–2122.

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